M.J.M. Hootsmans & J.E. Vermaat 1991

Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems

Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems

Promotor: Dr. W.J. Wolff, hoogleraar Aquatische Ecologie, Landbouwuniversiteit Wageningen

Co-promotor: Dr. W. van Vierssen, hoogleraar Aquatische Ecologie, International Institute for Hydraulic and Environmental Engineering, Delft

MACROPHYTES, A KEY TO UNDERSTANDING CHANGES CAUSED BY EUTROPHICATION IN SHALLOW FRESHWATER ECOSYSTEMS

Proefschrift
ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
Dr. H.C. van der Plas,
in het openbaar te verdedigen
op vrijdag 18 oktober 1991
des namiddags te half drie in de Aula
van de Landbouwuniversiteit te Wageningen

For further inquiries and to order additional copies:

International Institute for
Hydraulic and Environmental
Engineering

P.O. Box 3015
2601 DA Delft
The Netherlands
Phone +31 - 15 - 78 80 21,
Fax +31 - 15 - 12 29 21,
Telex 38099 ihe nl

STELLINGEN

1. Statistische modellen van ecosystemen gebaseerd op correlaties zijn aantrekkelijk door hun relatieve eenvoud maar leiden zelden tot een vergroting van het begrip van de processen die aan die correlaties ten grondslag liggen.
Complexe deterministische modellen gebaseerd op kennis van de belangrijkste causale relaties in een ecosysteem zijn dan ook noodzakelijk indien een werkelijk inzicht in het functioneren van dat ecosysteem gewenst is.
Scheffer, 1990.

2. De vaak geconstateerde onnauwkeurigheid van modeluitspraken gedaan door ecologen moet worden afgezet tegen het feit dat zij voor een goede voorspelling van ecosysteemprocessen ook nog een perfecte weersverwachting nodig hebben.

3. Problemen bij overleg tussen statistici en ecologen berusten vaak op biologisch analfabetisme van de eersten en statistische ongeletterdheid van de laatsten.

4. Als de variatie in fotosynthetische respons tussen ogenschijnlijk identiek plantenmateriaal gemakkelijk groter kan zijn dan 10% dan hoeven we ons minder zorgen te maken over de precisie van de meetapparatuur maar des te meer over voldoende replicatie.
Dit proefschrift.

5. Variatie noopt tot replicatie. Meer monsters nemen uit hetzelfde experimentele aquarium betekent pseudo-replicatie. Meer monsters van dezelfde experimentele lokatie in een meer is echter meestal het enige dat praktisch mogelijk is. De steun vanuit statistische standaardwerken voor dit dilemma is minimaal.
Hurlbert, 1984.

6. De beperktheid van ons huidige inzicht in het functioneren van aquatische ecosystemen wordt schrijnend duidelijk als experimenten verstoord worden door 'ongewenste algenbloei'.
Dit proefschrift.

7. De keuze tussen een pizzeria en een Grieks restaurant is niet te verklaren met een optimal foraging theorie gebaseerd op energie opname criteria.
Krebs & McCleery, 1984.

8. Omvangrijke ingrepen in de visstand van grote, ondiepe, wind-geëxponeerde meren om de helderheid te verbeteren zonder veel aandacht voor sedimentstabilisering door waterplanten kunnen gezien worden als geld in het water gooien.
Meijer et al., 1990.

9. Als Ph.D. onderwijs slechts op experimentele schaal op enkele instituten voor internationaal onderwijs mag worden gegeven, dan miskent dit het belang van gepromoveerden voor de opbouw van goed hoger onderwijs in de Derde Wereld en voor een verminderde afhankelijkheid van westerse kennis.
Pronk, 1990.

10. Het structureren van een afdeling voor wetenschappelijk onderwijs en onderzoek dient het functioneren van het personeel van die afdeling, en niet omgekeerd.

11. Participatie en milieu vormen belangrijke thema's binnen ontwikkelingshulp. Voor beide begrippen geldt echter dat ze niet eenduidig zijn. Dit bemoeilijkt een heldere discussie over hun onderlinge relatie.

12. Het overheidsstreven naar invoering van de TGV is onbegrijpelijk aangezien we al voldoende treinen met grote vertraging hebben.

13. Bij veldwerk in het getijdegebied is ook de maanstand van belang.

14. Duurzaam gebruik van ons milieu is voor het voortbestaan van de menselijke samenleving van essentieel belang maar onbelangrijk voor de aarde.

Literatuur

Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187-211.

Krebs, J.R. & McCleery, R.H., 1984. Optimization in behavioural ecology. In Krebs, J.R. & Davies, N.B. (eds), Behavioural ecology: an evolutionary approach. Second edition, Blackwell, Oxford, pp. 91-121.

Meijer, M.-L., Jagtman, E., Grimm, M.P. & Hosper, S.H., 1990. Toepassing van actief biologisch beheer op grote schaal. H_2O 23: 652-657.

Pronk, J.P., 1990. Een wereld van verschil. Nieuwe kaders voor ontwikkelingssamenwerking in de jaren negentig. SDU, 's-Gravenhage, 385 pp.

Scheffer, M., 1990. Simple models as useful tools for ecologists. PhD Thesis, University of Utrecht, pp. 8-9.

Stellingen van Michiel Hootsmans, behorende bij het proefschrift "Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems"

Wageningen, 18 oktober 1991

STELLINGEN

1. Intensieve samenwerking bij het verrichten van wetenschappelijk onderzoek hoeft geen rem te zijn op de ontplooiing van het individu.

2. De verschillende betekenis die in de statistiek en de plantenfysiologie aan het woord stratificatie wordt verbonden doet vermoeden dat deze vakgebieden zich te gescheiden ontwikkeld hebben.
Steel & Torrie, 1980; Thomas & Vince-Prue, 1984.

3. Daar juist waterplanten een grote plasticiteit in groeivorm vertonen, dient een indeling van waterplanten naar groeivorm met de nodige zorg te gebeuren.
Bradshaw, 1965; Chambers, 1987; Van Wijk et al., 1988; dit proefschrift.

4. Als 'consumers of unknown trophic level' in staat zijn 'to cause the fall of artificial substrates to the bottom', dan noopt dit tot een kritische evaluatie van de trofische relaties in het ecosysteem onder studie, waarbij ook de relatie tussen de onderzoeker en zijn objecten betrokken dient te worden.
Gons, 1982.

5. Als plantaardige organismen hun chlorofylgehalte uitgedrukt per eenheid biomassa (asvrij drooggewicht) binnen korte tijd (uren, dagen) kunnen aanpassen aan de lichtomstandigheden, dan lijkt het wijdverbreide gebruik van chlorofyl a als maat voor de biomassa van onder andere fytoplankton onterecht.
Falkowski et al., 1985; Jiménez et al., 1987; Vermaat & Sand-Jensen, 1987; dit proefschrift.

6. Het gebruik van aquaria of enclosures om bijvoorbeeld perifytonontwikkeling op waterplanten te bestuderen introduceert een complicerende factor. De wanden zijn namelijk een relatief groot extra oppervlak ter kolonisatie. Mogelijke effecten hiervan dienen bij de analyse van dergelijke experimenten betrokken te worden.

7. Het vergelijken van de groei en ontwikkeling van verschillende soorten waterplanten onder experimentele omstandigheden dient met evenveel zorg te gebeuren als het vergelijken van appels met peren.

8. Hoe bevredigend een simulatiemodel de werkelijkheid ook moge beschrijven, de fysieke bevrediging die wordt ondervonden na een dag veldwerk blijft achterwege na een dag rekenen.

9. De stelling 'Als het milieu geschikt is zullen de zeldzame (plante)soorten die daarin thuishoren er zich na verloop van tijd vanzelf wel vestigen' vereist het nodige geduld van de terreinbeheerders gezien de dispersiecapaciteit van deze soorten en de vangkans van de huidige potentieel geschikte terreinen.
Westhoff et al., 1970; Silvertown, 1982; MacArthur & Wilson, 1967.

10. Als werkelijk belang gehecht wordt aan de zogenaamde beklijfbaarheid van samenwerkingsprojecten met als doel institutionele ontwikkeling van de partner-instelling in een ontwikkelingsland, dan moeten vraagtekens gesteld worden bij de zin van projecten die dit doel in 3 jaar of minder moeten bereiken.

11. Ter bevordering van het wandelen en fietsen en ter verbetering van de ecologische infrastructuur verdient het met name op de Waddeneilanden aanbeveling meer aandacht te besteden aan de overheersende windrichting bij de aanleg van heggen en houtwallen langs openbare wegen en paden.

12. Het besef dat het grondig mislukken van meerdere experimenten niet het mislukken van het gehele onderzoek hoeft in te houden is een belangrijke leerervaring van promotieonderzoek.

13. Voor natuurwetenschappelijke onderzoekers (m/v) geldt eerder 'wie wat vindt moet verder zoeken' dan 'wie wat vindt heeft slecht gezocht'.
Kopland, 1972.

14. Wetenschap bedrijven is zowel kunst als kunstje.

Literatuur

Bradshaw, A.D., 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. 13: 115-155.

Chambers, P.A., 1987. Light and nutrients in the control of aquatic plant community structure II. In situ observations. J. Ecol. 75: 621-628.

Falkowski, P.G., Dubinsky, Z. & Wyman, K., 1985. Growth-irradiance relationships in phytoplankton. Limnol. Oceanogr. 30: 311-321.

Gons, H.J., 1982. Structural and functional characteristics of epiphyton and epipelon in relation to their distribution in Lake Vechten. Hydrobiologia 95: 57-70.

Jiménez, C., Niell, F.X. & Algarra, P., 1987. Photosynthetic adaptation of *Zostera noltii* Hornem. Aquat. Bot. 29: 217-226.

Kopland, R., 1972. Wie wat vindt heeft slecht gezocht. Van Oorschot, Amsterdam, 48 pp.

MacArthur, R.H. & Wilson, E.O., 1967. The theory of island biogeography. Princeton University Press, Princeton, New Jersey, USA, 203 pp.

Silvertown, J.W., 1982. Introduction to plant population ecology. Longman, Harlow, UK, 209 pp.

Steel, R.G.B. & Torrie, J.H., 1980. Principles and procedures of statistics, a biometrical approach. Second edition, McGraw-Hill, New York, USA, 633 pp.

Thomas, B. & Vince-Prue, D., 1984. Juvenility, photoperiodism and vernalization. In Wilkins, M.B. (ed.), Advanced Plant Physiology, Longman, Harlow, UK, pp. 408-439.

Van Wijk, R.J., Van Goor, E.M.J. & Verkley, J.A.C., 1988. Ecological studies on *Potamogeton pectinatus* L. II. Autecological characteristics, with emphasis on salt tolerance, intraspecific variation and isoenzyme patterns. Aquat. Bot. 32: 239-260.

Vermaat, J.E. & Sand-Jensen, K., 1987. Survival, metabolism and growth of *Ulva lactuca* under winter conditions: a laboratory study of bottlenecks in the life cycle. Mar. Biol. 95: 55-61.

Westhoff, V., Bakker, P.A., Van Leeuwen, C.G. & Van der Voo, E.E., 1970. Wilde planten, flora en vegetatie van onze natuurgebieden deel 1. Vereniging tot behoud van Natuurmonumenten in Nederland, 's-Graveland, 320 pp.

Stellingen van Jan Vermaat, behorende bij het proefschrift: "Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems"

Wageningen, 18 oktober 1991

VOORWOORD

Dit proefschrift vormt geen uitzondering op de regel dat behalve de auteurs ook vele anderen er aan hebben bijgedragen. Onze dank gaat uit naar onze vrienden, vriendinnen en collega's in Wageningen en Delft en onze familie, voor het begrip, het vertrouwen en het geduld gedurende de afgelopen jaren. Een aantal mensen willen we graag met name noemen.

In de eerste plaats willen we Wim van Vierssen bedanken voor de stimulerende discussies die we al ver voor het begin van dit onderzoek voerden, en voor de gerichte begeleiding gedurende de verschillende fasen van het werk. Het doet ons plezier dat we Wim Wolff bereid hebben kunnen vinden als onze promotor op te treden. Hun beider kritiek op onze manuscripten was van grote waarde, zowel voor de 'grote lijn' als voor de details.

De hulp van André Breukelaar, Ronald Gijlstra en John Beijer op Natuurbeheer was veel meer dan technische ondersteuning, we denken met plezier terug aan de dagen die we met hen in het lab en het veld hebben besteed. Onze directe collega's Roel Knoben en Gerda van Dijk willen we bedanken voor de collegialiteit gedurende onze gezamenlijke tijd op Natuurbeheer. Helaas konden de oorspronkelijke ideeën van de *Potamogeton pectinatus* groep niet volledig gerealiseerd worden. De goede samenwerking met Irmgard Blindow gedurende haar verblijf in Wageningen en daarna was stimulerend. De technische dienst van de Landbouwuniversiteit bedanken we voor het uitvoeren van onze vaak vreemde opdrachten.

De studenten Harriët Bakker, Alvean Fentener van Vlissingen, Karin Raap, Astrid Smit, Jan Smits, Jurgen Vet, Henk de Vries en Bert Wilbrink hebben allen bijgedragen aan dit proefschrift. Nico van de Brink deed dit als werkstudent. We willen ze bedanken voor het verzamelen van al die gegevens onder niet altijd even comfortabele omstandigheden. Zonder hen was het onmogelijk geweest om onze ambitieuze plannen te realiseren.

Wil Segeren, directeur van het IHE, bedanken we voor de ruimte die hij ons gaf om gedurende onze aanstelling op de afdeling EE verder te werken aan de voltooiing van dit proefschrift en voor de financiële ondersteuning van de uitgave ervan. Luis Santamaria Galdon, Michiel Wallis de Vries, Shen Sufeng en Dmitri Solomatin verzorgden de vertaling van onze 'General Abstract' in respectievelijk het Spaans, het Frans, het Chinees en het Russisch. Herman Bouw corrigeerde het Nederlands.

Tenslotte willen we ook elkaar bedanken, voor de ongezouten kritiek en de stimulerende kameraadschap. Zonder daar al te pretentieus over te willen doen, beseffen we dat de samenwerking ons gelukkig bijzonder goed afging en nog steeds gaat. We zijn ervan overtuigd dat deze samenwerking er voor gezorgd heeft dat het geheel toch iets meer is dan de delen.

Michiel Hootsmans
Jan Vermaat

Delft, augustus 1991

CONTENTS

SCOPE OF THE RESEARCH PROJECT 1

J.E. Vermaat & M.J.M. Hootsmans

1. Introduction

Numerous shallow freshwaters have been affected by cultural eutrophication during the last decades (Parma, 1980). This has often been associated with a loss of macrophyte species diversity and production. As a result of the disappearance of macrophytes, faunal species diversity often declines and food-webs and carbon and nutrient cycles are drastically changed (Hall et al., 1970; Kemp et al., 1984; Carpenter & Lodge, 1986). In many of such habitats, the phytoplankton becomes dominated by Cyanobacteria during prolonged times of the year. A recent review on this subject is given by De Nie (1987).

During the last decade, several restoration efforts have been undertaken to improve the water quality and to prevent a further deterioration due to eutrophication. The measures range from dephosphorizing the effluent of water treatment plants (Van Liere et al., 1984) via dredging of sediments rich in phosphorus (Gelin & Ripl, 1978) to whole-scale manipulation (temporary drainage, fish stock changes; e.g. Van Donk & Gulati, 1990).

Still, little is known of the mechanisms behind the observed changes. At least two major hypotheses appear to exist that try to explain macrophyte disappearance, for convenience they are named Model 1 and Model 2 in the following.

Model 1 stresses the importance of a changed fish stock composition and phytoplankton development. Due to increased nutrient availability, phytoplankton biomass increases strongly. Macrophyte biomass decreases due to shading, and this causes a decline in piscivorous fish stocks, e.g. pike (*Esox lucius* L.) in Western Europe. This predatory fish is highly dependent on vegetation for spawning, hiding (both as juvenile and adult) and furthermore is very dependent on sight for successful prey capture. Predation pressure on non-piscivorous fish thus decreases, leading to a strong increase in planktivorous and benthivorous fish like bream (*Abramis brama* L.). This species limits zooplankton densities and is also (when older) a bottomfeeder, stirring up the sediment (Lammens, 1989). The turbidity of the water increases, and the system cascades to a new balance in which phytoplankton dominates.

Model 2 suggests that periphyton development acted as a trigger for macrophyte decline, with a postponed phytoplankton reaction due to allelopathic growth limitation by substances excreted by the macrophytes. Of course, many variations exist on these two themes.

The first model hypothesis is a combination of mechanisms suggested by Hrbaček et al. (1961), Andersson et al. (1978) and Andersson (1984) on the fish-plankton interaction and by Jupp & Spence (1977) on the interaction between phytoplankton and macrophytes. The second model originates from Phillips et al. (1978).

The present study focused on the causes of macrophyte decline in Lake Veluwe, a shallow, man-made and eutro-

phicated lake in The Netherlands. In this lake, well-developed mixed macrophyte stands (Leentvaar, 1961) have been replaced gradually by monospecific stands of *Potamogeton pectinatus* L. during the last decades. The phytoplankton was dominated until quite recently by Cyanobacteria such as *Oscillatoria agardhii* Gom. (Berger & Bij De Vaate, 1983).

Our study is part of a larger research project that lasted from 1985 till 1988. In this project, emphasis was placed on trying to collect as many relevant data as possible within one specific macrophyte community. Laboratory experiments on the dominant macrophyte were combined with field experiments in which relatively large areas of the lake were experimentally manipulated. A conceptual model in which the relationships between macrophytes, periphyton, the water layer and grazing snails are determining the biological dynamics in the lake was taken as a starting point for the research. This model is an expanded version of the model of Phillips et al. (1978), it will be treated more elaborately in the next section of this chapter. The choice for model 2 was because we felt that the macrophyte decline that is the starting point for fish stock changes is not satisfactorily explained by model 1. Often, macrophytes decline also when light limitation due to phytoplankton shading and overall turbidity alone cannot be the cause (Phillips et al., 1978).

The ultimate goals of the entire research project were to determine the optimal strategy for the restoration of a shallow, eutrophic lake, to redevelop its potential biological diversity and at the same time to develop strategies for sustainable management of shallow water bodies risking cultural eutrophication.

In this thesis, a major part of the results from the integrated study are presented. Together with the results from most field experiments, a full account will be published elsewhere (Van Vierssen et al., in prep.).

2. The conceptual model, a working hypothesis

We used the modification of the model of Phillips et al. (1978) suggested by Van Vierssen et al. (1985) as a working hypothesis. This model elegantly describes the relationships between a number of ecosystem compartments. Moreover, the model offers the possibility to hypothesize within a well-described context about the causes and consequences of macrophyte decline after eutrophication. The model was used to derive a set of laboratory and field experiments. A graphic representation is shown in Fig. 1.1. Hough et al. (1989) suggest another modification where non-rooted, floating macrophytes affect submerged macrophytes in a similar way as periphyton. However, floating macrophytes never occurred in Lake Veluwe.

To explain macrophyte decline, the model attributes a decisive role to the periphyton development after eutrophication. Phytoplankton development is supposed to be limited because of allelopathic substances coming from the macrophytes. Because of periphyton shading, macrophytes become light-limited and gradually disappear. As a consequence, the supposedly existing negative influence of allelochemicals from macrophytes on the phytoplankton decreases. Consequently, this leads to blooms all year round, increased turbidity and thus a further decline in macrophytes. Besides, phytoplankton is supposed to be able to produce substances that limit macrophyte growth as well (Van Vierssen & Prins, 1985). Finally, macrophytes will com-

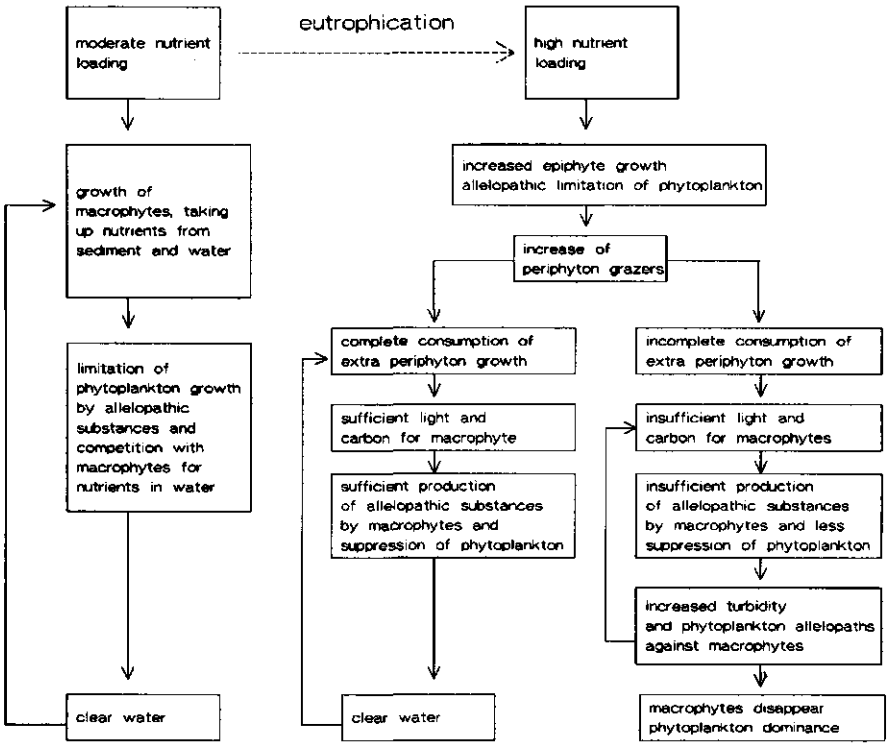


Fig. 1.1 The revised eutrophication model of Phillips et al. (1978).

pletely and persistently disappear from the ecosystem. Sediments are no longer stabilized by a vegetation cover and resuspension of bottom material can contribute significantly to water layer light extinction. With the macrophytes a large variety of animal species, ranging from aquatic invertebrates to amphibians and waterfowl, will disappear or seriously decrease in abundance.

Some of the invertebrate species, e.g. some snails, may be important grazers of periphyton, and may limit the decrease in the amount of available light in spite of the fact that the colonization and growth rates of periphyton are high. Taking into account that most of these snails depend on the macrophytes for their reproduction (deposition of eggs on leaves), their disappearance is tightly connected with that of the macrophytes.

3. Overview of the different research topics

Several research topics were derived from the conceptual model. They were regarded as essential to elucidate the explanatory power of the model. A macrophyte community of *P. pectinatus* was chosen to act as model system, because of its abundance even under the present conditions. This may of course obscure the sensitivity of

the many other macrophyte species that have disappeared. However, it seemed reasonable from a management-oriented point of view to work with a species that is still present in a significant number of Dutch waters. We decided not to study nutrient and carbon competition between microalgae and macrophytes. The first decision was based on our restriction to eutrophic ecosystems where nutrients will probably not be severely limiting. Carbon competition was omitted because it was supposed not to play a significant role in our study lake, Lake Veluwe, regarding the pH fluctuations, total available dissolved inorganic carbon and the fact that *P. pectinatus* is able to use bicarbonate (Sand-Jensen, 1983; Brinkman & Van Raaphorst, 1986).

Despite its abundance and the fact that it is relatively well studied (cf. reference lists to chapters 2-4 in this thesis), several important aspects of the ecology of this cosmopolitan macrophyte species still remain unclear. Observations of a.o. Van Wijk et al. (1988) suggested high phenotypic variability in a number of ecologically significant characteristics. Thus, several aspects of growth and development of the species were studied under laboratory conditions.

A study was done on the plasticity of the species in The Netherlands. Results from experiments with material coming from a brackish ditch on the island of Texel and from the freshwater Lake Veluwe (the field study area) are presented in chapter 2. The growth of *P. pectinatus* from Texel under different combinations of light and temperature was followed for two months in the laboratory (chapter 3). Light response curves of this population were measured, together with an evaluation of the effect of light intensity during growth and plant age on photosynthesis (chapter 4). Additional data on photosynthesis were collected for plants growing in Lake Veluwe under various artificial shading levels (also in chapter 4).

An important interaction in the model is the allelopathic limitation of algal growth by the macrophytes (chapter 5). Up till now, evidence for this interaction is scarce, and not very convincing. Especially *Chara* spp. can be suspected as active in this process (Wium-Andersen et al., 1982). Several species of this genus were tested for allelopathic effects on two species of phytoplanktonic algae. It was tried also to find evidence for allelopathic limitation of algal growth in water samples collected on various places in Lake Veluwe during the growing season. Some data are presented on the occurrence of this interaction in biomanipulation projects in The Netherlands.

Periphyton development is considered to be a crucial trigger starting macrophyte decline. The dynamics of periphyton development under various temperature-light conditions were studied, with special attention for light attenuation aspects (chapter 6). The effect of various grazer species on periphyton biomass and light extinction was evaluated. The consequences of periphyton grazing for the macrophyte were studied also (chapter 7).

To get more insight in the simultaneous operation of various interactions, two experiments were performed in Lake Veluwe. In a short-term enclosure experiment, the effect of a small littoral fish (three-spined stickleback, *Gasterosteus aculeatus* L.) on turbidity and plankton composition was studied (chapter 8). The consequences of turbidity, allelopathy, wave action and fish activity were followed in another enclosure experiment (also treated in chapter 8).

Part of the results, mainly on macrophyte development, was incorporated in a simulation model describing a simpli-

fied, age-and depth-structured vegetation of *P. pectinatus* in dependence of light conditions (chapter 9). The model has been calibrated among others with data that will be published in Van Vierssen et al. (in prep.).

Finally, the results from the research project and various model simulations are evaluated in chapter 10. Here also some implications for vegetation and lake management are discussed.

4. References

Andersson, G., 1984. The role of fish in lake ecosystems - and in limnology. In Bosheim, S. & Nicholls, M., (eds), Nordic Limnology Symposium on interactions between trophic levels in freshwater. Norsk Limnologforening, Oslo, pp. 189-197.

Andersson, G., Berggren, H., Cronberg, G. & Gelin, C., 1978. Effects of planktivorous and benthivorous fish on organisms and water chemistry in eutrophic lakes. Hydrobiologia 59: 9-15.

Berger, C. & Bij De Vaate, A., 1983. Limnological studies on the eutrophication of Lake Wolderwijd, a shallow hypertrophic *Oscillatoria* dominated lake in The Netherlands. Schweiz. Z. Hydrol. 45: 458-479.

Brinkman, A.G. & Van Raaphorst, W., 1986. De fosfaathuishouding in het Veluwemeer (Phosphorus dynamics in Lake Veluwe, in Dutch). PhD thesis, Twente Technical University, 481 pp.

Carpenter, S.R. & Lodge, D.M., 1986. Effects of submerged macrophytes on ecosystem processes. Aquat. Bot. 26: 341-370.

De Nie, H.W., 1987. The decrease in aquatic vegetation in Europe and its consequences for fish populations . EIFAC/CECPI occasional paper no. 19, 52 pp.

Gelin, C. & Ripl, W., 1978. Nutrient decrease and response of various phytoplankton size fractions following the restoration of Lake Trummen, Sweden. Arch. Hydrobiol. 81: 339-367.

Hall, D.J., Cooper, W.E. & Werner, E.E., 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol. Oceanogr. 15: 839-928.

Hootsmans, M.J.M. & Vermaat, J.E., 1985. The effect of periphyton grazing by three epifaunal species on the growth of *Zostera marina* L. under experimental conditions. Aquat. Bot. 22: 83-88.

Hough, R.E., Fornwall, H.D., Negele, B.J., Thompson, R.L. & Putt, D., 1989. Plant community dynamics in a chain of lakes: principal factors in the decline of rooted macrophytes with eutrophication. Hydrobiologia 173: 199-217.

Hrbaček, J., Dvořakova, M., Kořinek, V. & Procházkóva, L., 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton association. Verh. int. Verein. Limnol. 14: 192-195.

Jupp, B.P. & Spence, D.H.N., 1977. Limitation on macrophytes in a eutrophic lake, Loch Leven. I. Effects of phytoplankton. J. Ecol. 65: 175-186.

Kemp, W.M., Boynton, W.R., Twilley, R.R., Stevenson, J.C. & Ward, L.G., 1984. Influences of submerged vascular plants on ecological processes in upper Chesapeake Bay. In Kennedy, V.S., (ed.), The estuary as a filter. Academic Press, pp. 367-394.

Lammens, E.H.R.R., 1989. Causes and consequences of the success of bream in Dutch eutrophic lakes. Hydrobiol. Bull. 23: 11-18.

Leentvaar, P., 1961. Hydrobiologische waarnemingen in het Veluwemeer (Hydrobiological observations in Lake Veluwe, in Dutch). De Levende Natuur 64: 273-279.

Parma, S., 1980. The history of the eutrophication concept and the eutrophication in The Netherlands. Hydrobiol. Bull. 14: 5-11.

Phillips, G.L., Eminson, D.F. & Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4: 103-126.

Sand-Jensen, K., 1983. Photosynthetic carbon sources of stream macrophytes. J. Exp. Bot. 34: 198-210.

Van Donk, E. & Gulati, R.D., (eds), 1989. Biomanipulation in The Netherlands: applications in fresh-water ecosystems and estuarine waters. Hydrobiol. Bull. 23: 1-99.

Van Liere, L., Parma, S., Mur, L., Leentvaar, P. & Engelen, G.B., 1984. Loosdrecht Lakes Restoration Project, an introduction. Verh. int. Verein. Limnol. 22: 829-834.

Van Vierssen, W. & Prins, Th. C., 1985. On the relationship between the growth of algae and aquatic macrophytes in brackish water. Aquat. Bot. 21: 165-179.

Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E., 1985. Waterplanten: bondgenoten bij het waterkwaliteits-beheer? (The role of aquatic macrophytes in water quality management, in Dutch). H_2O 18: 122-126.

Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E. (eds), in prep. Dynamics of a macrophyte-dominated system under eutrophication stress: an integrated approach. Geobotany, Junk, The Hague.

Van Wijk, R.J., Van Goor, E.M.J. & Verkley, J.A.C., 1988. Ecological studies on *Potamogeton pectinatus* L. II. Autecological characteristics, with emphasis on salt tolerance, intraspecific variation and isoenzyme patterns. Aquat. Bot. 32: 239-260.

Wium-Andersen, S., Anthoni, U., Christophersen, C. & Houen, G., 1982. Allelopathic effects on phytoplankton by substances isolated from aquatic macrophytes (Charales). Oikos 39: 187-190.

INTRASPECIFIC VARIATION IN *POTAMOGETON PECTINATUS* L., A CONTROLLED LABORATORY EXPERIMENT

2

J.E. Vermaat & M.J.M. Hootsmans

Abstract

In a controlled laboratory experiment, the question was addressed whether intraspecific variation in *Potamogeton pectinatus* L. has a genotypic component. Two populations from contrasting habitats in The Netherlands were tested, one habitat was the exposed and eutrophic freshwater Lake Veluwe and the other a brackish ditch on the island of Texel. Weight frequency distributions of the natural tuber banks in spring were significantly different, while tuber specific densities (weight per volume) were not.

Plants from four tuber weight classes (up to 100 mg fresh weight) of each population were cultured under identical conditions for two months. A higher proportion of the tubers from Lake Veluwe did not sprout at all, and a higher proportion of the sprouted 'Veluwe' plants did not elongate its stem but retained a 'dwarfed', bushy appearance. Logistic growth curves for the 'normal' plants (i.e. not- 'dwarfed') of the two populations were significantly different for all tested morphometric characteristics. Initial tuber weight did significantly affect the growth curves. After two months, 'normal' plants of the 'Veluwe' population from tubers of equal weight had produced more leaves and photosynthetic area, had a higher total chlorophyll content per unit leaf biomass and a higher proportional chlorophyll b content than the 'Texel' population, but aboveground biomass and plant length was less. Thus, a genotypic component appears to be present in the phenotypic variation in *P. pectinatus*.

A difference in the quality of the initial tuber material may have been present, since the 'Texel' plants had depleted their initial tubers less whilst producing more new biomass. This qualitative difference may have been caused by different environmental conditions during the previous growing season. The presence of an environmental component thus cannot be ruled out completely.

The relatively faster transition through developmental stages, the higher investment in photosynthetic tissue and the relative compactness of the growth form may be of adaptive benefit to the population from Lake Veluwe in its more wind-exposed habitat with high turbidity and a high dislodgement risk.

Contents

1. Introduction

The freshwater macrophyte *Potamogeton pectinatus* L. reportedly shows a considerable variation in its life cycle and morphometric characteristics between populations from different sites (Luther, 1950; Kautsky, 1987; Van Wijk, 1988; Van Wijk et al., 1988). Phenotypic variation between populations may be genotypic and/or environmentally induced. Distinction between the two types is generally investigated by means of controlled laboratory experiments, the determination of chromosome numbers or isoenzyme patterns and reciprocal transplantation experiments (Jefferies, 1984; Verkleij et al., 1983; Van der Aart, 1985; Dennison & Alberte, 1986; Van Wijk et al., 1988).

Kalkman & Van Wijk (1984) and Van Wijk et al. (1988) used the first three approaches for *P. pectinatus*. Variation in chromosome number could not be related to variation between populations (Kalkman & Van Wijk, 1984) and 'isoenzyme patterns were far more complicated' than morphometric differences between populations that were apparent from laboratory culture experiments (Van Wijk et al., 1988). Thus, though isoenzyme variation indicated overall genetic heterogeneity, it cannot be concluded that genotypic variation was the basis of between-population differences in life strategies or life history traits (cf. Venable, 1984; Van der Aart, 1985).

Two comments must be made on the laboratory growth experiments done with *P. pectinatus* by Van Wijk et al. (1988): (a) Though average tuber size of *P. pectinatus* was different for the different populations (Van Wijk, 1988) and tuber size reportedly affects growth of the sprouting plant (Ozimek et al., 1986; Spencer, 1988), Van Wijk et al. (1988) did not quantify tuber size for the different growth experiments. (b) Seasonal day-length fluctuations (Salisbury, 1981; Spencer & Anderson, 1987) and probably red/far red ratios (Morgan & Smith, 1981; Chambers et al., 1985) in the irradiance spectrum affect propagule formation and other aspects of life cycles of several macrophyte species. Van Wijk et al. (1988) did their experiments in glasshouses from March to July and supplemented natural light with Philips HLRG lamps to maintain a photoperiod of 16 h. This clearly must have affected red/far red ratios in the supplemented light and thus may have affected tuber formation in the tested *P. pectinatus* material. Only the pairs of populations that Van Wijk et al. (1988) tested simultaneously have been exposed to similar light climates. It can be concluded that phenotypic differences between populations as observed in the growth experiments of Van Wijk et al. (1988) may also have been caused by a lack of control of initial tuber size or by environmental differences (i.e. light climate). Whether these differences have a genotypic basis thus cannot be concluded yet.

In the present experiment tuber age and environmental conditions were held constant, thus eliminating possible interference of these factors. Further, initial tuber size was controlled to allow for a separate evaluation of a tuber size effect. Two populations were studied from locations more or less representative for the wide spectrum of habitats of *P. pectinatus* (Van Wijk, 1988): (a) a population from a brackish experimental ditch on the island of Texel, hereafter referred to as the 'Texel' population, and (b) a population from the shallow, large and fairly exposed freshwater Lake Veluwe (Van Dijk & Van

Vierssen, 1991; Vermaat et al., 1991), referred to as the 'Veluwe' population.

In this study, the two populations are compared with respect to tuber bank characteristics, plant growth parameters, morphometry, biomass at the end of the growth experiment (various fractions) and chlorophyll content.

2. Material and methods

Tubers were collected in January 1986. This enabled a natural (physiological) winter stratification (Van Wijk, 1983). Also, a tuber bank that is sampled in January has probably been subject to most of the naturally occurring autumn and winter mortality and thus represents the net initial 'inoculum' for the oncoming growing season. Natural tuber bank characteristics were determined on complete field samples.

The collected tubers were stored in the dark at 4°C in small batches of 10 - 30 tubers in separate petri dishes (9 cm diameter) containing tap water ('Veluwe' population) or tap water brought to 0.3% chlorinity with Wimex Meeressalz ('Texel' population). Prior to the experiment, the 'Texel' tubers were acclimated to freshwater by slow dilution of the brackish water in an overflow system for approximately one week at 4°C in the dark. Before planting, fresh weight (fw, 1 mg precision) and volume of individual tubers were determined after blotting dry with tissue paper for 5 seconds. From both tuber stocks a subsample was taken for ash-free dry weight (afdw, 0.1 mg precision) determinations.

We did not presprout (or 'pregerminate') the tubers prior to use, contrary to other authors (Spencer, 1986; Spencer & Anderson, 1987; Van Wijk et al., 1988). Since two natural populations were to be compared, we considered rejection of the unsprouted tubers an unjustified selection from the natural spring tuber stock.

For high precision tuber volume determinations we developed a U-tube device. The U-tube was filled with water, the left arm was a standard titration burette adjustable in height with a binocular microscope height-screw, the right arm was a 1 ml pippette of fixed height. The two tubes were connected with a flexible pvc tube to form the U. Water level in both tubes was read, the tuber added and the water level in the left tube was readjusted to the level before addition of the tuber by screwing the burette up. Then the level in the right tube was read once more. Difference between the two readings from the fixed right tube is the volume of the tuber (precision 0.01 ml).

The tubers were planted in a clay/sand mixture (ratio 1/3) in coffee beakers (135 ml sediment each). For each population 4 aquaria (50*30*30 cm, L*B*H, water depth above the sediment 20 cm) were used containing 30 beakers each. The aquaria were placed in a thermostatted cooling basin that was flushed with well water. Irradiance was held at 200 μE m^{-2} s^{-1} (range within 10%, measured 1 cm below the water surface with a Bottemanne submersible quantum sensor, measuring PAR) with four Philips HPIT metal halide lamps suspended above the four aquaria. Photoperiod was 16 hours and water temperature was 18°C during illumination and 15°C ($\pm$ 1°C) in the dark. The aquaria were filled with tap water that was replenished fortnightly.

Four tuber size classes were distinguished: 0 - 25, 25 - 50, 50 - 75 and 75 - 100 mg fw $tuber^{-1}$. The tubers from the different size classes were distributed randomly over the four aquaria of each

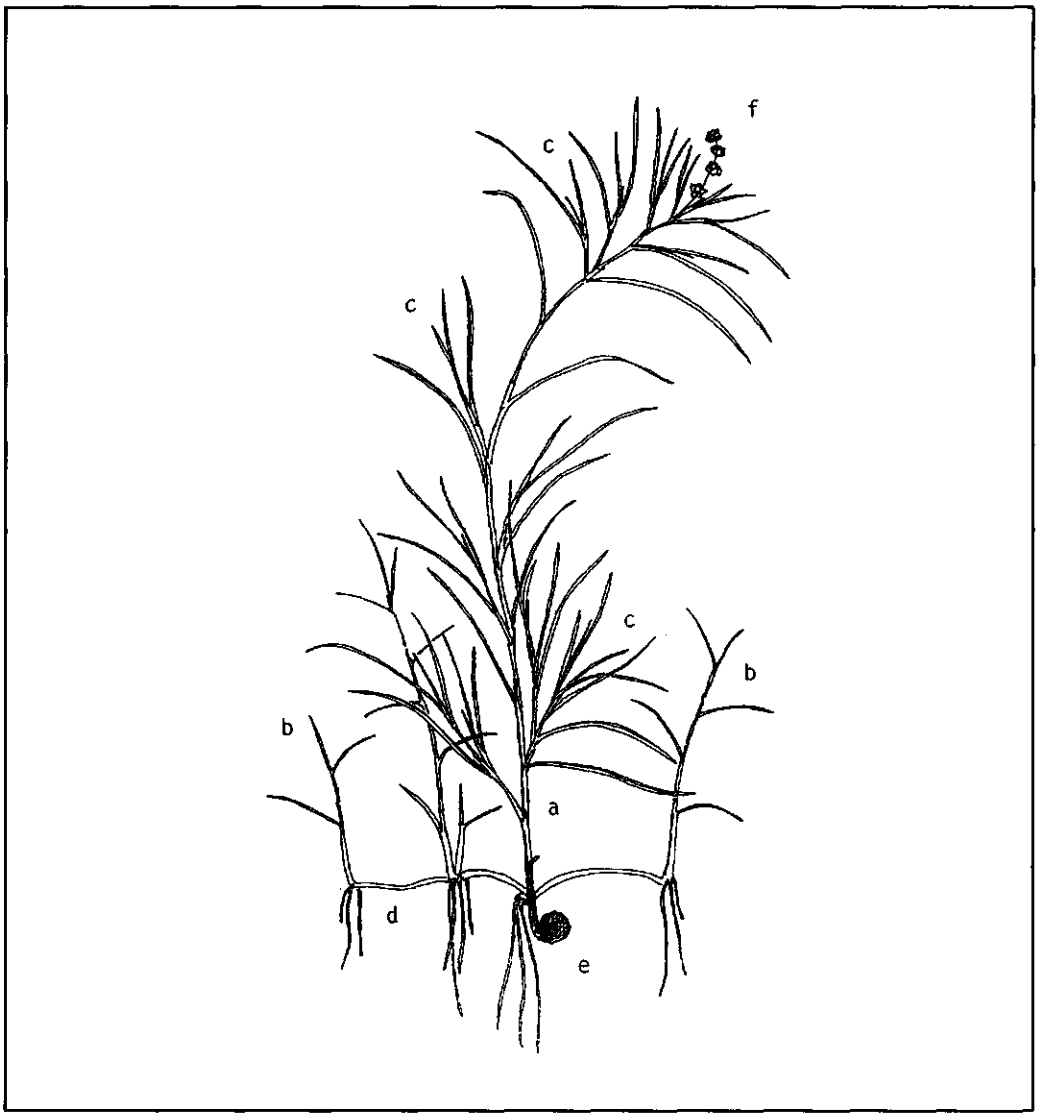


Fig. 2.1. Schematized specimen of *P. pectinatus* indicating: distance a-f = length of main shoot, b = secondary shoots, c = bundles, d = rhizome segment, e = tuber from which the plant has sprouted, f = inflorescence.

population. This range of tuber sizes was chosen because only few tubers heavier than 100 mg fw were available in the 'Veluwe' stock. Also, if an effect of tuber size is present, we assumed that this would be the most apparent for small tubers, i.e. at weights below 100 mg fw. The experiment lasted 52 ('Veluwe' popu-

lation) and 55 days ('Texel' population). Weekly morphometric measurements involved distinction of individual bundles, numbers of leaves per bundle, bundle lengths and lengths of the main shoot (plant length). Fig. 2.1 indicates the discerned morphometric characteristics. A bundle is defined here as a group of leaves enclosing a meristem at the common base. We chose the bundle as a demographic entity or module (White, 1980) mainly for practical reasons: we found it an easily discernable module and it appeared to have considerable stability during its life time (see also Vermaat & Hootsmans, 1991). Morphologically, a bundle should be considered as a shoot in its primary phase and of (fairly) small size. The elongation of the stem internodes between the leaves in these bundles is probably controlled hormonally (apical dominance). Bundle length was measured as the length of the longest leaf in the bundle.

From the weekly morphometric measurements 4 characteristics were used for further analysis: (a) the total number of leaves per plant (hereafter referred to as 'n leaves'), (b) the number of bundles (n bundles), (c) total plant length (i.e. main stem length plus length of the final bundle of the tallest shoot), and (d) sum of shoot bundle lengths (Σ length, i.e. the sum of the length of all bundles on a plant). This last characteristic may be interpreted as an index of photosynthetic area.

A logistic growth curve was fitted for the above morphometric characteristics using a non-linear iterative technique based on the Marquardt algorithm (Conway et al., 1970). To facilitate computation and comparisons, the curves were not computed for every separate plant but for every tuber size class (all plant data pooled per class). We applied the following logistic formula:

$$A_t = K / (1 + q * \exp^{(-rt)})$$

where A_t is size or number (depending on the characteristic) at time t, K is the asymptotic maximum value for A_t, q is an integration constant determining A_t at time zero ($q=K/(A_0-1)$) and r is the instantaneous, 'unrestricted' growth rate (Causton & Venus, 1981; Rodriguez, 1987).

Multiple comparisons among fitted curves and regression lines were performed applying an experimentwise error rate (EER) of 0.05 (with comparisonwise error rates CER adjusted according to the number of comparisons) and the following F statistic:

$$F = \frac{\{RSS_{1+2}-(RSS_1+RSS_2)\} / \{df_{1+2}-(df_1+df_2)\}}{(RSS_1+RSS_2) / (df_1+df_2)}$$

Where RSS stands for residual sum of squares and df for degrees of freedom. The zero hypothesis is that the two sets of data pairs can be described best by one regression line, the alternative is that two lines 'are better'. This is tested with a difference in residual sum of squares in the above F with the formulated degrees of freedom. RSS_{1+2} is the RSS of the regression on the two data sets together, RSS_1 and RSS_2 are the RSS of the separate regressions. Statistical analyses were performed with the SPSS/PC$^+$ package (Norusis, 1986).

At the termination of the experiment the plants were carefully washed free of adhering sediment, divided into aboveground (leaves and stems), belowground (roots and rhizomes) and tuber

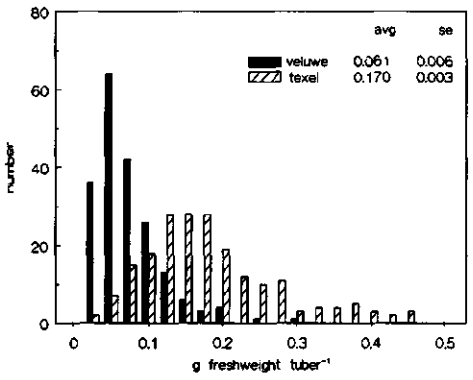


Fig. 2.2. Fresh weight (fw) frequency distributions of tuberbanks of the 'Veluwe' and 'Texel' populations sampled in January 1986. The weight classes have width of 0.025 g fw. Sample sizes were 196 and 202 for 'Veluwe' and 'Texel' respectively. Average (avg) weights and standard errors (se) are indicated in the top right corner.

parts, dried (105°C, 24 h), weighed, ashed (520 - 540°C, 3 h) and weighed again to determine ash-free dry weight (afdw). From every aquarium a sample of 5 to 10 randomly selected leaves from each of 7 to 10 plants was frozen for chlorophyll determination. Fresh weight of this subsample of leaves and the rest of the aboveground material was determined to enable the calculation of chlorophyll concentrations per g afdw of leaf material. Chlorophyll a, b and their phaeopigments were determined in 80% acetone according to Vernon (1960), modified according to Moed & Hallegraeff (1978) to control the pH of the acidified sample.

In one of the four 'Veluwe' aquaria a phytoplankton bloom developed that persisted a few weeks despite extensive flushing. Final plant biomass and chlorophyll content were significantly lower for this aquarium compared to the other three 'Veluwe' aquaria, but no significant differences could be observed with respect to the morphometric growth curves. Therefore, data from this aquarium have been excluded from analyses except for the growth curves.

3. Results

3.1 Tuber bank characteristics

The fresh weight frequency distribution of the natural spring tuber bank of the two populations differed significantly (Fig. 2.2, $p<0.001$, Kolmogorov-Smirnov test for comparison of distributions). The 'Veluwe' sample had a higher kurtosis than the 'Texel sample (i.e. clustering for a given variance, kurtosis values were 4.3 and 0.7, respectively) and a higher skewness (i.e. tails to the end of the distribution, values were 1.7 and 1.0). The same samples were not significantly different in volume/fresh weight relation of the tubers (Fig. 2.3, $p>0.50$). Thus, the samples from the two populations did not differ significantly in specific density of the tubers.

Also the fw/afdw and volume/afdw relations were not significantly different ($p=0.293$ and 0.529 respectively). The afdw/fw relation, however, was significantly different ($p=0.028$). This is probably due to the presence of a few outliers in the 'Veluwe' sample that have a relatively strong influence in this relatively small sample (for 'Veluwe' n = 36, for 'Texel' n = 95) that was used for drying and ashing. Deviation of outliers from the least squares regression is different when the dependent variable is changed to be the independent and vice versa. Consequently, this may have effect on the RSS and thus on significance tests. We there

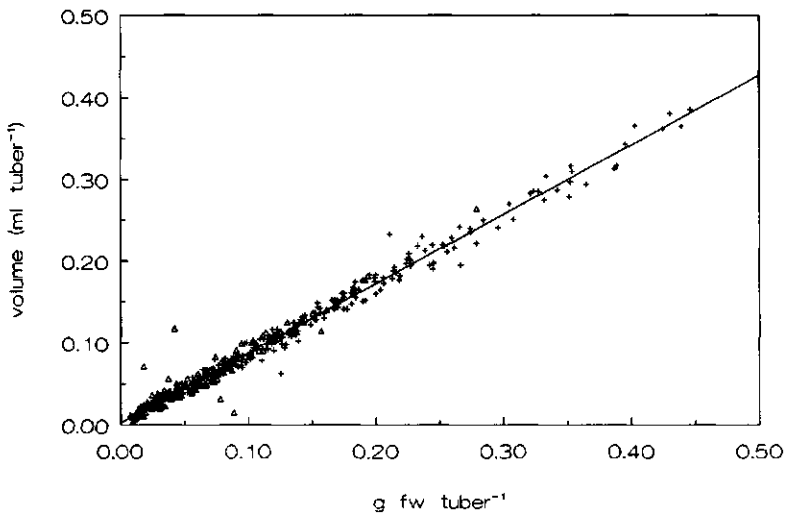


Fig. 2.3. Relation of tuber volume to fresh weight (fw) for the 'Veluwe' (triangles) and 'Texel' populations (crosses). The linear regression line for the two populations together is plotted: volume = 0.003 + 0.851 * fw, r^2=0.981, $p<0.001$.

fore consider the afdw/fw relation for the two populations also to be described best by the line based on the combined samples. The parameters of the linear regression lines of the two samples together are given in Table 2.1. Regressions with forced zero intercepts fitted the data equally well as those with non-zero intercepts.

3.2 Growth form

Most remarkably, the planted samples of tubers from the two populations differed strongly in the numbers that sprouted: for the 'Veluwe' population this was 82%, for 'Texel' 95%, which is a significant difference (χ^2, $p<0.005$). Also, we observed two forms of sprouted plants. One developed 'normal' tall stems, growing regularly to the water surface. The other remained close to the sediment, attained a

Table 2.1. Parameters for linear regression between fw, afdw and volume of tubers from 'Veluwe' and 'Texel' samples together. Regression line: y = a + b*x. Given are y and x, a, b, r^2 and n, the number of data pairs used. All four regressions were highly significant ($p \leq 0.001$). Regressions with forced zero intercept (a=0) described the data sets equally well (F tests with error sums of squares, $p>0.05$).

y	x	a	b	r^2	n
volume	afdw	0.014	1.975	0.976	131
		0	2.104	0.987	
volume	fw	0.003	0.851	0.981	398
		0	0.865	0.993	
afdw	fw	0.004	0.421	0.986	131
		0	0.405	0.992	
fw	afdw	0.012	2.339	0.986	131
		0	2.452	0.992	

Table 2.2. Number of not-sprouted tubers, dwarfed and 'normal' plants in samples of spring tuber banks from a brackish ditch on the island of Texel and from Lake Veluwe. Tuber size-classes: (1) 0 - 25, (2) 25 - 50, (3) 50 - 75, (4) 75 - 100 mg fw, tubers of exactly 25 mg are included in the first size-class etc.

tuber size-class:	1	2	3	4	total
'Veluwe'					
not sprouted	10	4	4	3	21
dwarf	6	7	8	6	27
tall	12	19	21	14	66
total	28	30	33	23	114
'Texel'					
not sprouted	1	2	2	1	6
dwarf	1	1	1	0	3
tall	14	30	30	32	106
total	16	33	33	33	115

Table 2.3. Multiple comparisons of fitted growth curves for the total number of leaves per plant (n leaves), the number of bundles (n bundles), plant length (cm), and sum of shoot bundle-lengths (Σ length, cm). The four size classes (cf. Table 2.2) are compared here per morphometric characteristic and per population ('Veluwe' and 'Texel' respectively). EER is held at p=0.05. Compare text for comparisons between populations and the determination of CER. Different letters indicate a significant difference between curves.

tuber size-class:	1	2	3	4
'Veluwe' dwarf				
n leaves	a	b	bc	bc
n bundles	a	b	b	b
plant length	a	ab	b	ab
Σ length	a	ab	b	b
'Veluwe' tall				
n leaves	a	a	b	c
n bundles	a	a	b	c
plant length	a	b	b	c
Σ length	a	a	b	c
'Texel'				
n leaves	a	b	b	c
n bundles	a	b	b	c
plant length	a	b	b	b
Σ length	a	b	b	c

dwarfed 'bushy' appearance producing numerous bundles of increasingly smaller size and apparently lacked the capability of stem elongation. The latter 'dwarfs' were virtually absent (3% of the sprouted plants) in the 'Texel' sample but markedly present in the 'Veluwe' sample (29%, Table 2.2, difference significant, χ^2, $p<0.001$).

For the 'Veluwe' sample, the number of not-sprouted tubers was significantly correlated with tuber size-class (Kendall's Tau B or C, $p=0.017$). Thus, in the 'Veluwe' sample the smallest size-class had a significantly higher proportion of not-sprouted tubers.

3.3 Growth during the experiment - morphometric characteristics

All fitted logistic curves were highly significant ($p<0.001$). Figs 2.4 and 2.5 give the curves for the number of leaves and plant length of the 'Veluwe' and 'Texel' populations respectively and separately for the four size-classes.

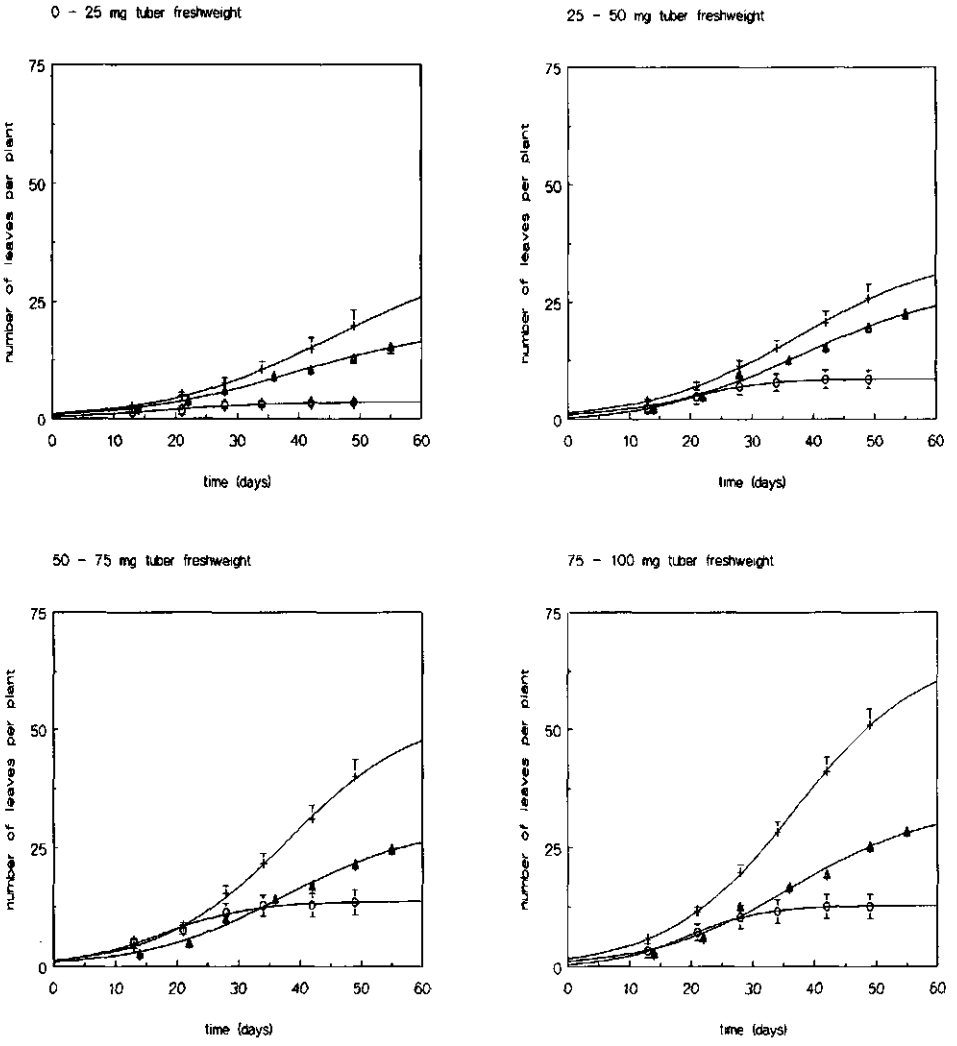


Fig. 2.4. Development of the number of leaves on a plant and the fitted logistic growth curve for four tuber weight classes of both populations. Crosses indicate the 'normal' plants, open circles the 'dwarfed' plants from the 'Veluwe' population. Filled triangles indicate the 'Texel' population. Bars represent standard errors, plotted only up- or downwards if this improved the clarity of the graph.

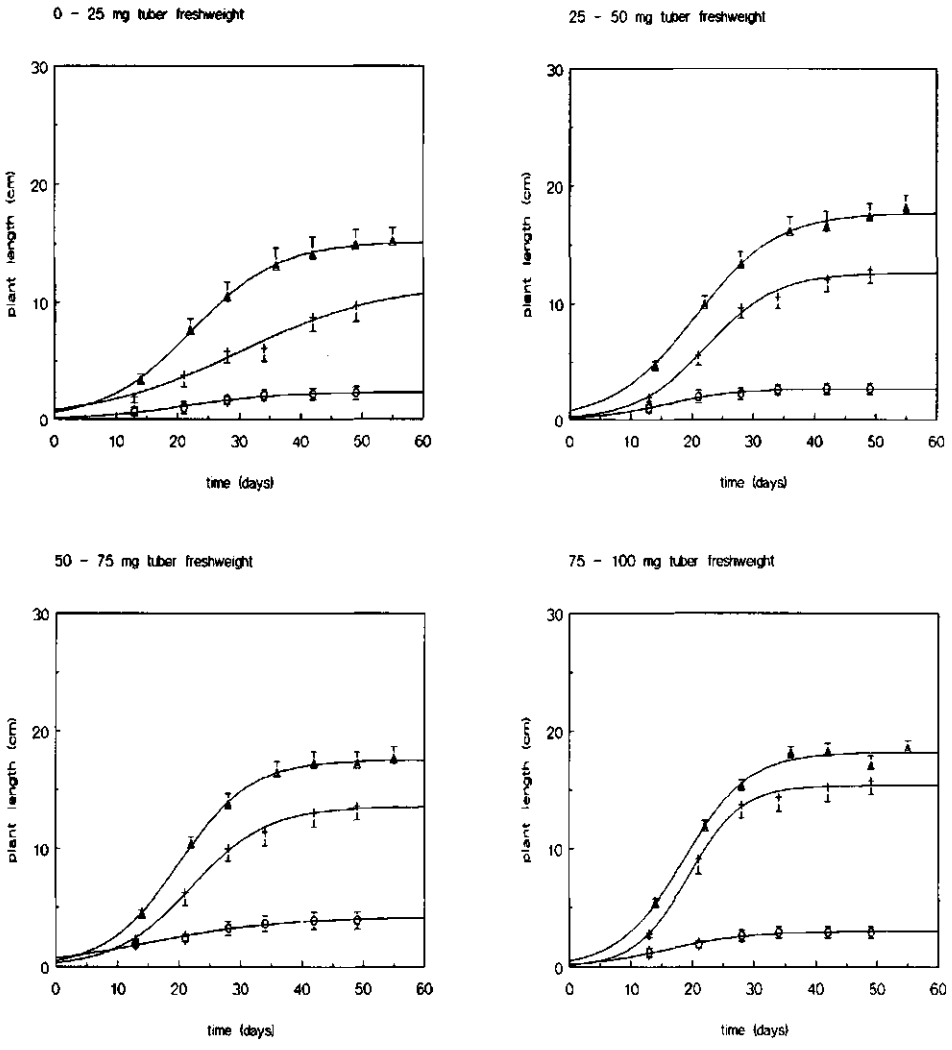


Fig. 2.5. Development of plant length and the fitted logistic growth curve (further as in Fig. 2.4).

Table 2.4. Parameter estimates for the logistic fits to several morphometric characteristics of the "tall" 'Veluwe' plants and the 'Texel' plants. Parameters are K (units: number or cm), q (initial scaling parameter, dimensionless) and r (day^{-1}) and given are mean and standard error (se). For an explanation of the morphometric characteristics, see Table 2.3.

	size-class:	1		2		3		4	
		mean	se	mean	se	mean	se	mean	se
n leaves	'Veluwe' tall								
	K	34.1	28.1	35.4	12.1	53.2	12.9	66.7	11.3
	q	30.2	15.5	23.7	11.3	40.0	19.1	36.8	16.7
	r	0.08	0.04	0.08	0.03	0.10	0.02	0.10	0.02
	'Texel'								
	K	19.5	4.2	28.2	3.3	29.5	2.5	33.4	2.4
	q	20.4	7.7	26.0	7.1	29.0	7.0	27.1	5.9
	r	0.08	0.02	0.08	0.01	0.09	0.01	0.09	0.01
n bundles	'Veluwe' tall								
	K	10.4	7.6	10.7	3.1	17.6	4.6	19.3	2.6
	q	23.3	12.3	21.2	10.6	36.8	16.7	40.8	19.2
	r	0.08	0.04	0.09	0.03	0.09	0.02	0.11	0.02
	'Texel'								
	K	4.6	0.6	6.2	0.3	6.3	0.3	6.9	0.2
	q	16.8	9.8	20.2	13.2	37.9	16.6	55.9	24.8
	r	0.10	0.03	0.12	0.02	0.13	0.02	0.16	0.02
plant length	'Veluwe' tall								
	K	11.6	3.6	12.6	0.8	13.5	0.9	15.3	0.7
	q	12.9	9.2	49.2	44.2	37.4	38.9	89.3	96.7
	r	0.09	0.04	0.17	0.04	0.16	0.04	0.23	0.06
	'Texel'								
	K	15.1	0.9	17.7	0.7	17.5	0.5	18.2	0.3
	q	23.7	19.9	21.2	13.8	31.0	18.4	33.6	14.2
	r	0.14	0.04	0.15	0.03	0.17	0.03	0.19	0.02
Σ length	'Veluwe' tall								
	K	84.2	82.6	79.1	21.8	131.6	29.6	162.0	19.7
	q	73.8	77.7	42.3	39.2	69.5	57.5	73.0	50.6
	r	0.10	0.06	0.11	0.04	0.12	0.04	0.13	0.03
	'Texel'								
	K	40.7	4.7	61.8	4.1	60.7	2.9	67.8	2.2
	q	49.8	45.1	49.4	27.9	60.1	30.6	66.6	29.9
	r	0.12	0.03	0.12	0.02	0.14	0.02	0.15	0.02

Table 2.5. Morphometric data of "tall" 'Veluwe' and 'Texel' plants after 49 days. Given are mean, standard error (se) and the level of significance (p) from a t'-test (variances were not homogeneous, Steel & Torrie, 1980) comparing the two samples. Replication was 46 for the 'Veluwe' sample and 109 for 'Texel'. For explanation of the morphometric characteristics, see Table 2.3.

	Veluwe		Texel		p
	mean	se	mean	se	
n leaves	41.9	2.3	21.1	0.7	0.001
n bundles	13.6	0.7	5.9	0.2	0.001
bundle length	8.3	0.2	9.5	0.2	0.001
leaves/bundle	3.1	0.4	3.7	0.9	0.001
plant length	14.7	0.7	16.9	0.5	0.014
Σ length	117.6	7.5	55.3	2.0	0.001

Per morphometric characteristic, the fitted growth curves were entered in a multiple comparisons' scheme comparing: (a) the "dwarfed" and the "tall" 'Veluwe' plants for each tuber weight class, (b) the "tall" 'Veluwe' and the 'Texel' plants for each weight class, and (c) the four weight classes for the 'Veluwe' and 'Texel' samples separately (Table 2.3). To maintain an experimental error rate (EER) at $p=0.05$, these 26 comparisons in total necessitated a comparisonwise error rate (CER) of 0.0019. The 'Veluwe' datasets were described significantly better ($p<0.001$, i.e. less than the CER) by two curves, i.e. one for the "dwarfed" and one for the "tall" plants. Estimated logistic parameters for all four morphometric characteristics are given in Table 2.4.

The growth curves of "tall" 'Veluwe' and 'Texel' plants were compared per size-class and characteristic: all curves were significantly different except the Σ length curves of classes 1 and 2. Figs 2.4 and 2.5 show that the "tall" 'Veluwe' plants produced more leaves than 'Texel' plants, but the 'Texel' plants grew taller. This was probably (individual logistic parameters were not tested, cf. section 2) not due to a difference in initial exponential growth rate (r, Table 2.4), but in the asymptotic maximum (K) to be attained. The 'Texel' plants already had closely approached this maximum at the end of the experiment, while the 'Veluwe' plants had not. The 'Veluwe' plants also produced more bundles and photosynthetical area, though individual bundles were shorter and had less leaves (Table 2.5).

For the tested range of tuber weights and for 'normal' plants from both populations, initial tuber weight had a significant effect on all morphometric characteristics: a plant that sprouted from a larger tuber grew more rapidly (Table 2.4, cf. values of r) and produced more leaves and bundles, taller plants and a larger photosynthetical area. This difference was less distinct for the dwarfed 'Veluwe' plants. Here, size-classes 2, 3, and 4 were not significantly different in morphometry and only tubers from the smallest weight class produced significantly less leaves, bundles and photosynthetical area than those from the three other classes. This smallest weight class also had the highest fraction of not-sprouted tubers (Table 2.2). None of the plants had formed new tubers at the termination of the experiment. Some flowering had occurred in the 'Veluwe' sample, but only by two plants. The 'Texel' sample had not produced any flowers yet at the termination of the experiment.

3.4 Final biomass

Biomass data are given in Table 2.6. All differences are significant, except for the L/S ratio (leaf biomass/shoot biomass)

Table 2.6. Final biomass data of "tall" 'Veluwe' and 'Texel' plants after 52 and 55 days respectively. Given are mean, standard error (se) and the level of significance from a t-test comparing the two samples (or from a t'-test when variances were not homogeneous, Steel & Torrie, 1980). Replication was 40 for the 'Veluwe' sample and 109 for 'Texel'. All values are in mg afdw plant^{-1}, unless stated otherwise.

	Veluwe		Texel		p
	mean	se	mean	se	
shoot	24.2	2.2	29.7	1.2	0.015
leaves	16.0	0.9	19.3	6.3	0.003
roots & rhiz.	17.1	1.9	24.7	0.9	0.001
final tuber	3.8	0.5	7.1	0.4	0.001
new	41.9	4.0	54.4	1.8	0.006
total	45.7	4.1	61.5	2.0	0.001
initial tuber	18.8	1.7	19.9	1.1	0.581
S/R	1.52	0.09	1.20	0.03	0.001
L/S	0.83	0.05	0.77	0.02	0.228
afdw/fw	0.104	0.003	0.143	0.002	0.000

Shoot = leaves + stem, total = final tuber + belowground + aboveground, new = belowground + aboveground, i.e. the truely newly formed biomass, initial tuber biomass in mg afdw is derived from initial tuber fw with the appropriate regression formula from Table 2.1. S/R = aboveground/belowground biomass, L/S = leaf biomass/shoot biomass (both dimensionless and calculated per individual plant). Leaf biomass was calculated from the number of leaves on a plant, the fw of an individual leaf and the aboveground afdw/fw ratio (replication was 32 for both samples).

and, as expected, initial tuber biomass. Apparently, 'Texel' plants produced more new biomass while the tubers decreased less in biomass. Because the S/R ratio was significantly higher for the 'Veluwe' plants, they invested relatively more in aboveground matter. Since the L/S ratio was not significantly different, plants from the two populations allocated aboveground biomass similarly over stems and leaves.

When initial tuber fw was used as a covariable in an ANOVA comparing the two samples, the covariable had a significant effect on shoot, roots and rhizomes, final tuber, new and total biomass ($p<0.001$) but not on the S/R ratio ($p=0.528$). This is illustrated in Figs 2.6 and 2.7. Fig. 2.6 gives the depletion of the tubers as a function of their initial biomass. This linear function is significant for both populations and the slopes of the two fitted lines are significantly different ($p<0.001$). So, with increasing initial tuber-biomass the 'Veluwe' population depleted its tubers more than the 'Texel' plants did.

In Fig. 2.7 the newly formed biomass is plotted against initial tuber biomass. A fair amount of scatter is present, but for both samples a significant ($p<0.001$) linear and hyperbolic ($y=a*x/(b+x)$) fit could be made. The hyperbolas did not fit the data significantly better or worse than the linear regressions, although they conceptually appear to fit better due to their inherent zero intercept. Still, we will restrict us to the linear fits. The 'Veluwe' and 'Texel' linear fits are significantly different ($p<0.001$). Up to about 30 mg initial tuber afdw (i.e. $\approx$ 75 mg fw) the 'Texel' line is above the 'Veluwe' line. Thus, for equal initial tuber biomass, 'Texel' tubers produced more new biomass than the 'Veluwe' tubers did, while they used less of their initial tuber in terms of afdw to achieve this.

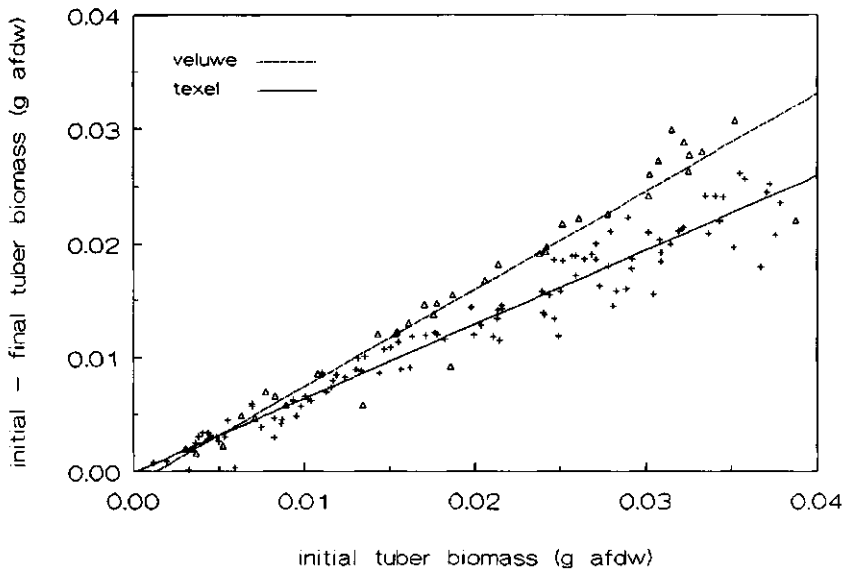


Fig. 2.6. Tuber depletion as a function of initial tuber biomass for the 'Veluwe' (triangles) and 'Texel' populations (crosses).

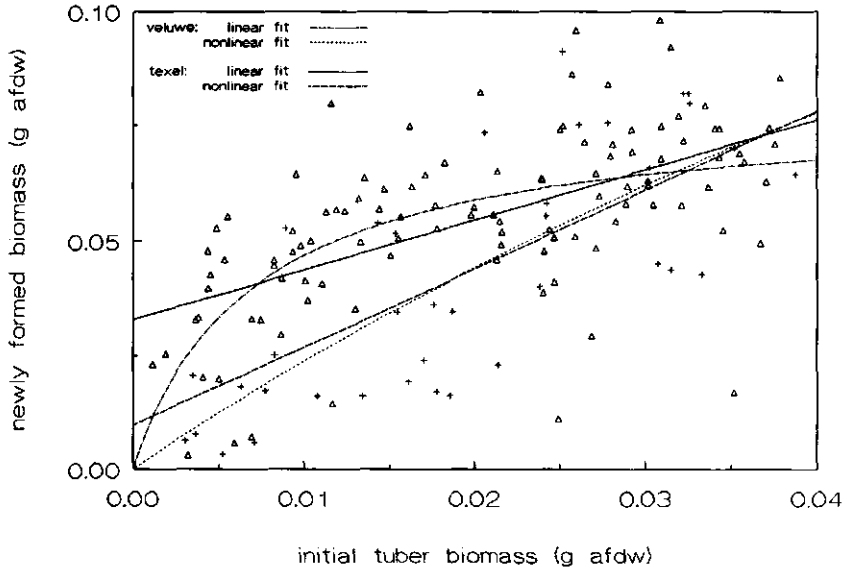


Fig. 2.7. Newly formed biomass as a function of initial tuber biomass for the 'Veluwe' (triangles) and 'Texel' populations (crosses).

3.5 Chlorophyll-content at harvesting

Total chlorophyll (i.e. chlorophyll a + b) and the fraction of chlorophyll b in this total are presented in Table 2.7. While phaeophytin a was not detectably present in the samples, some phaeophytin b was detected, though in small amounts only (8% of chlorophyll b at most, no significant difference between 'Veluwe' and 'Texel').
It can be concluded that the plants from the 'Veluwe' sample had a significantly higher chlorophyll content and a significantly higher fraction of chlorophyll b. Applying initial tuber fw as a covariable in an ANOVA comparing the two samples gave no significant effect of initial tuber fw on total chlorophyll content ($p=0.850$) but a significant effect on the fraction of chlorophyll b ($p=0.046$).

Table 2.7. Total chlorophyll (a+b) content of leaves (mg g afdw leaves^{-1}) and the fraction of chlorophyll b (frac-b = chl-b/chl(a+b)) in this total for the 'Veluwe' and 'Texel' samples. Given are mean, standard error (se) and the level of significance (p) from a 't' test comparing the two samples (cf. Table 2.5). Replication was 32 for both samples, each of the replicates consisted of 5 to 10 leaves.

	Veluwe		Texel		p
	mean	se	mean	se	
chl(a+b)	4.69	0.27	3.78	0.18	0.006
frac-b	0.25	0.01	0.21	0.01	0.001

4. Discussion and conclusions

4.1 Environmentally induced versus genotypic variation

We demonstrated significant differences between the two populations under controlled laboratory conditions and with consideration of interference by initial tuber size. Plants from tubers of similar weight from the 'Veluwe' population produced more leaves, bundles and photosynthetical area and had a higher chlorophyll content and fraction of chlorophyll b whilst aboveground biomass and plant length were less than that of the 'Texel' plants. From the above we can conclude that these differences have a genetic basis.

Furthermore, the number of not-sprouted tubers and dwarfed plants was higher in the 'Veluwe' sample and the amount of new biomass that could be produced from more heavily used tubers (final tuber biomass was less) was less than that in the 'Texel' sample. This may indicate that a qualitative factor like condition may also have played a role. Since *specific density (biomass/volume, cf.* Table 2.1) of the tubers from the two populations was not significantly different, other qualitative aspects may have been involved (like nutrient, sugar or protein content). The tubers from the 'Veluwe' sample then may have been in a worse condition. This difference in condition may have been genetically as well as environmentally based: adverse environmental conditions during the previous growing season may have affected the condition of the tubers. This may also have had consequences for the quantitative performance of the sprouting tubers that resulted in 'tall' plants.

Little information on qualitative aspects of *P. pectinatus* tubers from different populations or habitats is available, or, specifically, of the effect of tuber 'quality' on the plant that sprouts from it. Thus, no quantitative measurements as reported here can be excluded a priori from being influenced by tuber 'quality'.

To our knowledge only Van Wijk (1989b) reported on nutrient concentrations in *P. pectinatus* tubers from different field locations. His data show wide ranges of nutrient contents but no distinct differences between locations. In a consecutive paper, Van Wijk (1989c) reported on laboratory experiments concerning plant growth in nutrient gradients. Below minimal nutrient concentrations in the culture medium the plants failed to produce new tubers. Unfortunately, he did not report on the nutrient contents in the newly formed tubers, a possible measure of tuber 'quality'.

Summarizing we conclude that to date we have strong evidence of truly genotypic variation between different populations of *P. pectinatus*. However, the possibility of an environmental basis for the observed phenotypic variation via a difference in quality of the initial experimental material can never be excluded fully. In general, the latter seems to be inevitable when plant material collected from different locations is used without controlled culture of one or more pre-experimental generations.

4.2 Phenotypic plasticity

While the differences between the tested populations reported here may be more or less environmentally based, plasticity itself, as a genetically based trait, may have clear adaptive value (Bradshaw, 1965; Venable, 1984). More specifically, genetically based plasticity in S/R ratios, chlorophyll content, plant height, allocation ratios between different reproductive/propagative organs or rate of passing through different developmental stages may allow *P. pectinatus* populations to survive succesfully in a variety of environments.

The presently reported differences between the two populations, then, may be interpreted tentatively as of adaptive value in the specific habitats, whether caused by phenotypic plasticity or genotypic differences. The relatively faster transition through different developmental stages in the 'Veluwe' population (tubers were produced earlier than in other populations (Van Wijk et al., 1988), combined with a relatively higher investment in photosynthetic tissue (S/R ratio, chlorophyll content) and the relative compactness of the plants then may be of adaptive value in the wind-exposed Lake Veluwe with high turbidity, high dislodgement risk due to wave action and a fairly short growing season (Kautsky, 1987; Van Wijk, 1988; Van Dijk & Van Vierssen, 1991). Van Wijk et al. (1988) also found relatively short and compact plants for his sample from Lake Veluwe, their average shoot length is similar to the average of the pooled 'dwarf' and 'tall' plants in this study. Their average plant biomass was much higher than that reported here (0.75 vs. 0.05 g afdw plant^{-1}), probably due to differences in initial tuber size and irradiance.

Since the contrast 'brackish versus freshwater habitat' in the present study is coupled with the contrast 'lake versus ditch', interpretation of the reported differences with respect to salinity will not be endeavoured. When comparing two simultaneously cultured samples populations, one from a brackish (Camargue, France) and one from a freshwater habitat (Lake Veluwe), Van Wijk et al. (1988) concluded that the Camargue sample had produced more biomass and thus had a higher photosynthetic efficiency. We have demonstrated here that tuber size and tuber condition may also have been important.

It may be postulated that a certain degree

It may be postulated that a certain degree of plasticity is obligatory for a species depending as heavily on vegetative propagation as *P. pectinatus*, since generative propagation appears to be a rare event when measured as the number of successfully establishing new seedlings (Van Wijk, 1989a). Bradshaw (1965) stated that especially perennial species show marked plasticity and he tentatively explained the success of certain weed species by their remarkable plasticity. In a review, Abrahamson (1980) concluded that the optimal balance between the two modes of propagation depended on a variety of biotic and abiotic factors. Various widespread and abundant macrophyte species appear to combine considerable plasticity with a well-developed capacity for vegetative propagation. As examples can be named: *Elodea canadensis* Michx. (Silvertown, 1982), *Hydrilla verticillata* (L.f.) Royle (Pieterse, 1981; Verkleij et al., 1983; Van Vierssen et al., 1986), *Zostera marina* L. (Dennison & Alberte, 1986; Hootsmans et al., 1987), *Ulva lactuca* L. (Vermaat & Sand-Jensen, 1987), the presently studied species *P. pectinatus* and other *Potamogeton* species (Spence & Chrystal, 1970; Spence & Dale, 1978; Brux et al., 1987).

Acknowledgements

Dr. M.A.J. van Montfort (Wageningen Agricultural University, Department of Mathematics) is thanked for statistical advice on multiple comparisons of fitted curves, Prof. Dr. W. van Vierssen and Prof. Dr. W.J. Wolff critically read the manuscript. Dr. R.J. van Wijk is thanked for his suggestions on the storage of the collected tubers.

5. References

Abrahamson, W.G., 1980. Demography and vegetative reproduction. In: Solbrig, O.T., (ed.), Demography and evolution in plant populations, Blackwell, Oxford, pp. 89-106.

Bradshaw, A.D., 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. in Genetics 13: 115-155.

Brux, H., Todeskino, D. & Wiegleb, G., 1987. Growth and reproduction of *Potamogeton alpinus* Balbis growing in disturbed habitats. Arch. Hydrobiol. Beih. 27: 115-127.

Causton, D.R. & Venus, J.C., 1981. The biometry of plant growth. Arnold, London, 307 pp.

Chambers, P.A., Spence, D.H.N. & Weeks, D.C., 1985. Photocontrol of turion formation by *Potamogeton crispus* L. in the laboratory and natural water. New Phytol. 99: 183-194.

Conway, G.R., Glass, N.R. & Wilcox, J.C., 1970. Fitting nonlinear models to biological data by Marquardt's algorithm. Ecology 51: 503-507.

Dennison, W.C. & Alberte, R.S., 1986. Photoadaptation and growth of *Zostera marina* L. (eelgrass) transplants along a depth gradient. J. Exp. Mar. Biol. Ecol. 98: 265-282.

Hootsmans, M.J.M., Vermaat, J.E. & Van Vierssen, W., 1987. Seed-bank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L. and *Zostera noltii* Hornem. Aquat. Bot. 28: 275-285.

Jefferies, R.L., 1984. The phenotype: its development, physiological constraints and environmental signals. In Dirzo, R. & Sarukhan, J. (eds), Perspectives on plant population biology, Sinauer Associates Inc., Sunderland, MA, USA, pp. 345-358.

Kalkman, L. & Van Wijk, R.J., 1984. On the variation in chromosome number in *Potamogeton pectinatus* L. Aquat. Bot. 20: 343-349.

Kautsky, L., 1987. Life-cycles of three populations of *Potamogeton pectinatus* L. at different degrees of wave exposure in the Askö area, northern Baltic proper. Aquat. Bot. 27: 177-186.

Luther, H., 1950. Verbreitung und Oekologie der höheren Wasserpflanzen im Brackwasser der Ekenäs-gegend in Südfinnland II. Spezieller Teil. Acta Bot. Fenn. 50: 1-370.

Moed, J.R. & Hallegraef, G.M., 1978. Some problems in the estimation of chlorophyll-a and phaeopigments from pre- and post-acidification spectro-photometric measurements. Int. Revue ges. Hydrobiol. 63: 787-800.

Morgan, D.C. & Smith, H., 1981. Non-photosynthetic responses to light-quality. In Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (eds), Physiological Plant Ecology I, responses to the physical environment. Encyclopedia of Plant Physiology, new series, volume 12a, Springer Berlin, pp. 109-134.

Norusis, M.J., 1986. SPSS-PC$^+$ manual. SPSS Inc., Chicago, USA, 559 pp.

Ozimek, T., Prejs, K. & Prejs, A., 1986. Biomass and growth rate of *Potamogeton pectinatus* L. in lakes of different trophic state. Ekol. Polska 34: 125-131.

Pieterse, A.H., 1981. *Hydrilla verticillata* - a review. Abstr. Trop. Agric. 7(6): 9-34.

Rodriguez, M.A., 1987. Estimating periphyton growth parameters using simple models. Limnol. Oceanogr. 32: 458-464.

Salisbury, F.B., 1981. Responses to photoperiod. In Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (eds), Physiological Plant Ecology I, responses to the physical environment. Encyclopedia of Plant Physiology, new series, volume 12a, Springer Berlin, pp. 135-167.

Silvertown, J.W., 1982. Introduction to plant population ecology. Longman, London, 209 pp.

Spence, D.H.N. & Chrystal, J., 1970. Photosynthesis and zonation of freshwater macrophytes II. Adaptability of species of deep and shallow water. New Phytol. 69: 217-227.

Spence, D.H.N. & Dale, H.M., 1978. Variations in the shallow water form of *Potamogeton richardsonii* induced by some environmental factors. Freshwat. Biol. 8: 251-268.

Spencer, D.F., 1986. Early growth of *Potamogeton pectinatus* L. in response to temperature and irradiance: morphology and pigment composition. Aquat. Bot. 26: 1-8.

Spencer, D.F., 1988. Tuber size and planting depth influence growth of *Potamogeton pectinatus* L. Am. Midl. Nat. 118: 77-84.

Spencer, D.F. & Anderson, L.W.J., 1987. Influence of photoperiod on growth, pigment composition and vegetative propagule formation for *Potamogeton nodosus* Poir. and *Potamogeton pectinatus* L. Aquat. Bot. 28: 103-112.

Steel, R.G.D. & Torrie, J.H., 1980. Principles and procedures of statistics, a biometrical approach. Second edition. McGraw Hill Book Company, Singapore, 633 pp.

Van der Aart, P.J.M., 1985. Demographic, genetic and ecophysiological variation in *Plantago major* and *P. lanceolata* in relation to vegetation type. In White, J. (ed.), The population structure of vegetation, Handbook of vegetation science III. Dr Junk publishers, Dordrecht, The Netherlands, pp. 441-462.

Van Dijk, G.M. & Van Vierssen, W., 1991. Survival mechanisms of a *Potamogeton pectinatus* L. population under different light conditions in a shallow eutrophic lake (Lake Veluwe) in The Netherlands. Aquat. Bot. 39: 121-129.

Van Vierssen, W., Breukelaar, A.W. & Peppelenbos, H.W., 1986. A comparison of some morphological characteristics of four *Hydrilla* strains under different environmental conditions. Proc. EWRS/AAB 7th Symposium on Aquatic Weeds, pp. 369-374.

Van Wijk, R.J., 1983. Life-cycles and reproductive strategies of *Potamogeton pectinatus* L. in The Netherlands and in the Camargue (France). Proc. Int. Symp. Aquat. Macrophytes, Nijmegen, 18 - 23 September 1983, pp. 317-321.

Van Wijk, R.J., 1988. Ecological studies on *Potamogeton pectinatus* L. I. General characteristics, biomass production and life cycles under field conditions. Aquat. Bot. 31: 211-258.

Van Wijk, R.J., 1989a. Ecological studies on *Potamogeton pectinatus* L. III. Reproductive strategies and germination ecology. Aquat. Bot. 33: 271-299.

Van Wijk, R.J., 1989b. Ecological studies on *Potamogeton pectinatus* L. IV. Nutritional ecology, field studies. Aquat. Bot. 35: 301-318.

Van Wijk, R.J., 1989c. Ecological studies on *Potamogeton pectinatus* L. V. Nutritional ecology, in vitro uptake of nutrients and growth limitation. Aquat. Bot. 35: 319-335.

Van Wijk, R.J., Van Goor, E.M.J. & Verkley, J.A.C., 1988. Ecological studies on *Potamogeton pectinatus* L. II. Autecological characteristics, with emphasis on salt tolerance, intraspecific variation and isoenzyme patterns. Aquat. Bot. 32: 239-260.

Venable, D.L., 1984. Using intraspecific variation to study the ecological significance and evolution of plant life-histories. In Dirzo, R. & Sarukhan, J. (eds), Perspectives on plant population biology, Sinauer Associates Inc., Sunderland, MA, USA, pp. 166-187.

Verkleij, J.A.C., Pieterse, A.H., Horneman, G.J.T. & Torenbeek, M., 1983. A comparative study of the morphology and isoenzyme patterns of *Hydrilla verticillata* (L.f.) Royle. Aquat. Bot. 17: 43-59.

Vermaat, J.E. & Sand-Jensen, K., 1987. Survival, metabolism and growth of *Ulva lactuca* under winter conditions: a laboratory study of bottlenecks in the life cycle. Mar. Biol. 95: 55-61.

Vermaat, J.E. & Hootsmans, M.J.M., 1991. Growth of *Potamogeton pectinatus* L. in a temperature-light gradient. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understand changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vermaat, J.E., Hootsmans, M.J.M. & Van Dijk, G.M., 1991. Ecosystem development in different types of littoral enclosures. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understand changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vernon, L.P., 1960. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Analytic Chemistry 32: 1144-1150.

White, J., 1980. Demographic factors in populations of plants. In Solbrig, O.T. (ed.), Demography and evolution in plant populations. Blackwell, Oxford, pp. 21-48.

GROWTH OF *POTAMOGETON PECTINATUS* L. IN A TEMPERATURE-LIGHT GRADIENT

3

J.E. Vermaat & M.J.M. Hootsmans

Abstract

The effect of temperature and light on the growth of *Potamogeton pectinatus* L. was investigated in a laboratory experiment with three temperatures (13°, 15° and 22 °C) and four irradiance treatments (dark, 50, 100 and 200 μE m^{-2} s^{-1}). The experiment lasted two months. The plants were sprouted from a standard tuber size class (100 - 200 mg fresh weight) from one population, originating from a brackish ditch on the island of Texel, The Netherlands.

Both quantitatively and qualitatively, temperature and light affected growth in an interacting, nonlinear way. Logistic fits for the number of leaves, leaf bundles and secondary shoots resulted in higher asymptotic maxima and relative growth rates with both increasing irradiance and temperature. The qualitative effect of light was a typical sun/shade acclimation: in low light the main shoot elongated strongly and few or no secondary shoots were formed, whilst in high light elongation was less pronounced and more secondary shoots were formed. Also, chlorophyll (a+b) content was higher in low light. Elongation increased with increasing temperature up to a plateau at 15 °C. The difference in elongation for different irradiances was similar to that reported for other macrophyte species with erect stems. Temperature and irradiance influenced belowground biomass equally strong, but temperature had a stronger effect on aboveground biomass, while irradiance affected the ratio of aboveground to belowground biomass more. Irradiance had no significant effect on tuber depletion, whilst temperature had.

After up to three months in the dark at the three temperatures, the tubers were still able to form 'normal' green shoots when transferred to the light. High temperature during dark incubation resulted in lower subsequent aboveground biomass.

From detailed examination of sprouting tubers in the dark it was concluded that these tubers have the capacity to develop a secondary dormancy, when exposed to temperatures above a compensation point of 13°-15° immediately following (physiological) winter stratification. This mechanism may be of adaptive value in potentially summer-dry aquatic habitats. It may also indicate that tubers, once in a state of secondary dormancy, are capable to survive a second winter.

Contents

1. Introduction

Temperature and light interact nonlinearly (Berry & Raison, 1981; Björkmann, 1981; Spencer, 1986; Bulthuis, 1987) in their quantitative effect on plant photosynthesis and growth. Furthermore, plant growth is also affected qualitatively by light (a.o. photomorphogenesis, phototropism, photoperiodism; Morgan & Smith, 1981; Salisbury, 1981) and temperature (a.o. dormancy and flowering induction; Berry & Raison, 1981). Specific growth conditions have been shown to induce distinct morphological responses like sun- and shade-adaptations in a vast series of vascular plant species (Abrahamson, 1980; Björkmann, 1981; Barko & Smart, 1981; Barko et al., 1982) among which also *Potamogeton pectinatus* L. (Van Wijk et al., 1988), the species that is the subject of this study.

To understand the performance of *P. pectinatus* in an environment under eutrophication stress, it was felt that basic information would be useful concerning the effects of temperature and light on plants growth. The separate effect of these factors is hard to assess under field conditions, since large scale (diel, seasonal) fluctuations occur simultaneously and both factors may affect other environmental parameters simultaneously and interactingly (Berry & Raison, 1981). Therefore, though controlled laboratory experiments can only be approximate simplifications of the field conditions, they are necessary when the effects of such factors as temperature and light and their interaction are to be established separately.

Thus, the primary aim of this study was to provide detailed baseline information on the effect of temperature and light on the growth of *P. pectinatus*. A laboratory experiment (hereafter referred to as experiment 1) was done in which *P. pectinatus* plants were grown under a series of temperature-light combinations, to enable the assessment of the separate effects and their interaction.

Two more specific questions are also addressed in this study. Firstly, we investigated the capacity of *P. pectinatus* plants that have just sprouted from tubers to survive prolonged periods of extremely low light as might occur during intense phytoplankton blooms in strongly eutrophicated lakes, and the impact of such periods on subsequent growth. In the pertaining experiment (hereafter referred to as experiment 2) we moved replicate samples of plants after dark incubation of increasing duration under different temperatures to aquaria in normal light. Temperature during dark incubation was also incorporated as a factor, since it was hypothesized that increased temperatures might increase respiratory needs during the incubation and thus decrease the amount of tuber reserves available to sustain subsequent shoot growth.

In a third experiment (referred to as experiment 3), the sprouting of tubers under different temperatures in the dark was followed in detail. It was felt that such detailed observations might further elucidate the patterns of dormancy present in *P. pectinatus* tubers (Van Wijk, 1989).

2. Material and methods

2.1 Experiments 1 and 2

The tubers were from the same tuber stock of the 'Texel' population as used in Vermaat & Hootsmans (1991). Pre-planting individual fresh weight (fw) of the tubers was determined as in Vermaat & Hootsmans (1991) as were ash-free dry weight (afdw) and chlorophyll after harvesting. The plants were grown from tubers of a standard size-class (100 - 200 mg fw). This standard size class was chosen for two reasons: (a) Vermaat & Hootsmans (1991) demonstrated that tuber size affected plant growth and thus should be controlled for. (b) Differences in tuber size especially affected subsequent plant growth below an initial tuber weight of about 75 mg fw. Above this level initial tuber weight apparently had much less effect, at least for this population and plants of 8 weeks age. Spencer (1988) also found a significant effect of tuber size on subsequent plant growth.

Three temperatures (10°, 15° and 20 °C) and four irradiance levels (dark, 50, 100 and 200 μE m^{-2} s^{-1}) combined to 12 treatments. Both the terms light or irradiance are used in this chapter, they stand for the quantum flux of photosynthetically active radiation (PAR, μE m^{-2} s^{-1}, 400-700 nm wavelength section, as measured with intercalibrated cosine-corrected submersible Bottemanne or Licor LI 192-S quantum sensors). Every treatment consisted of one aquarium (L*B*H: 50*30*30 cm, 45 l, waterdepth above the sediment 20 cm) that contained 20 plants (29 in the dark treatments) and was filled with tap water. Per temperature, the aquaria of the different light treatments were placed in one temperature room and connected with pvc tubing. Water circulation was ensured with an Eheim 2030 pump (flow rate 6.5 l min^{-1}). Irradiance was held at the desired level (variation 5-10%) with 400 W Philips HPIT lamps and neutral density shading sheets. The dark aquaria were wrapped in a layer of black pvc sheeting. Daily temperatures in the aquarium water ranged from 12.1° - 14.2° (minimum in the dark to maximum in the light) for the 10 °C temperature room, from 14.1° - 16.1° for 15° and from 21.1° - 23.4 °C for the 20 °C temperature room, respectively. These temperature increases were largely due to heat production by the HPIT lamps. The desired temperatures 10°, 15° and 20° therefore were not actually maintained, average temperatures in the three temperature rooms were respectively 13.2°, 15.1° and 22.3 °C. The temperature treatments will be further referred to as 13°, 15° and 22 °C. Photoperiod in the illuminated treatments was 12 hours. The tubers were planted separately in coffee beakers filled with the same sediment as in Vermaat & Hootsmans (1991).

Data were collected on morphometric characteristics during the course of the experiment and on biomass (various plant fractions) and chlorophyll after termination of the experiment and harvesting of the plants, as in Vermaat & Hootsmans (1991). The experiment started on June 18th, 1986. The plants from the illuminated treatments of experiment 1 were harvested after 55 days.

In experiment 2, a batch of 5 plants from every darkness-temperature combination of experiment 1 was placed in an illuminated aquarium (16 h photoperiod, 200 μE m^{-2} s^{-1}, 18 °C during illumination and 15° ($\pm$1 °C) in the dark) at days 15, 22, 29, 42 (only 4 plants per batch), 70 and 98, counted from the day of planting (June 18th). All plants that went from dark to light were harvested at day 105, i.e. in September 1986.

2.2 Morphometric measurements

Morphometric measurements identical to those of Vermaat & Hootsmans (1991, cf. their Fig. 2.1) were made at weekly intervals. The following characteristics were extracted from the dataset:

1. Total plant length, i.e. stem length plus length of the final bundle of leaves on the tallest shoot.
2. Total number of leaves per plant, i.e. on all shoots (n leaves).
3. Total number of bundles on all shoots (n bundles, a bundle is a group of leaves enclosing a common meristem).
4. Sum of bundle lengths on all shoots (Σ length). A characteristic that may be interpreted as an index of photosynthetic area.
5. Number of secondary shoots on a plant (n sec. shoots).
6. Number of leaves per bundle on the main shoot.
7. Idem on the secondary shoots.
8. Fraction of all leaves that are not in a bundle.

When a shoot emerges, all leaves except the top two to four are solitarily standing on the main vertical stem. During further plant growth groups of new leaves (i.e. bundles) may develop from buds enclosed by the sheaths of these solitary leaves. The stem internodes between the lower leaves of these bundles may subsequently elongate and thereby form stems of second order. We observed stems of third order as well.

Logistic growth curves as described in Vermaat & Hootsmans (1991) were constructed for the morphometric characteristics 1 to 4 and for the biomass data of the plants that were moved from dark to light. In the latter case the period that the plants had been in the light was entered as time in the logistic equation.

To compare the logistic fits for all 12 treatment combinations, 66 multiple comparisons would have been necessary, reducing the comparisonwise error rate (CER) to 0.0008 when an experimentwise error rate (EER) of 0.05 is to be maintained. This reduces the power of the test considerably. Therefore, a reduction of the number of multiple comparisons was realized by comparing irradiance levels per temperature only and, likewise, temperatures per irradiance only (30 comparisons with a CER of 0.0017). As in Vermaat & Hootsmans (1991), we did not enter individual parameter estimates and their standard errors in significance tests but only compared the complete curves. This was done to maintain the power of the tests, and because measurements made at different times on the same plant cannot be considered independent. Therefore, statements comparing parameter estimates can only be made tentatively.

2.3 Experiment 3

In the detailed tuber sprouting experiment (experiment 3), a batch of 20 tubers from the standard size class (100 - 200 mg initial tuber fw) was incubated at 13°, 15° and 22 °C each, in the dark. The experiment was started in August 1986, directly following experiment 1. For 44 days, we measured the sprout length from the connection to the tuberous mass (0.5 mm precision, for bended sprouts measured by carefully moving the ruler along the bend) and the type and number of leafy organs almost daily. We distinguished the following stages in the sprouting process:

0. No visible sprouting.
1. The sprout elongates and three scaly yellowish-white sheaths become visible.
2. Then a leaflike organ with a short

(< 3 cm) and stiff blade appears with the still minute tip of the shoot enclosed in its sheath. This blade may turn green.

3. The shoot starts to elongate and 'normal' leaves (i.e. not discernably different in morphology from adult leaves) become visible. The first roots start to develop.

Sprouting of a tuber is thus described here from externally visible, morphological changes of the sproutlike organ that encompasses the new shoot. Observations on physiological changes occurring within the tuber have not been made.

3. Results

3.1 Growth during experiment 1 - morphometric characteristics

3.1.1 Logistic growth curves

The fitted logistic curves of the number of leaves (n leaves), plant length and the number of secondary shoots (n sec. shoots) are presented in Figs 3.1 - 3.3. The curves for the number of bundles per plant (n bundles) and the sum of bundle lengths (Σ length) were fairly similar and are not given here. Most fitted curves were highly significant ($p < 0.001$). At 50 $\mu E\ m^{-2}\ s^{-1}$ and 13° and 15°, the fits for the numbers of secondary shoots were significant but poor ($p < 0.005$, but r^2 respectively 0.096 and 0.143). The reason is obvious from Fig 3.3: in low light (50 $\mu E\ m^{-2}\ s^{-1}$) and 22 °C considerable numbers of secondary shoots were formed relatively early, but at the two lower temperatures only a few were formed and at a much later stage. In the dark no secondary shoots were formed (Fig. 3.3). The plants in the dark did not form any bundles along the elongating stem either (with the exception of the 'bundle' on top of the main shoot).

Multiple comparisons of the fitted growth curves are summarized in Table 3.1. The significance patterns are identical for n leaves, n bundles and Σ length: higher asymptotic maxima (K) and relative growth rates (r) with both increasing temperature and irradiance.

As outlined in section 2.2, statements concerning the individual parameters are only tentative. Instead of values of K, the finally attained maxima (at day 55) were tested. Though these maxima are strictly not similar to estimates of K, the logistic asymptote, K was not approached closely in only 8 out of 120 fitted curves within the experimental period (Table 3.1). Thus, these maxima were considered to represent values of K fairly well. In twoway ANOVAs for n leaves, n bundles, plant length, Σ length and n sec. shoots the effects of temperature, light and their interaction were highly significant ($p < 0.001$). Initial tuber fresh weight, entered as a covariable, had no significant effect on any of the morphometric characteristics, except plant length ($p = 0.026$). However, this significant effect of initial tuber weight was only slight as compared to the treatment effects. When the data were pooled per light treatment, regression of plant length to initial tuber fresh weight was only significant for 200 $\mu E\ m^{-2}\ s^{-1}$ ($r^2 = 0.09$, $p = 0.024$, Fig. 3.4). The results of the consecutive multiple comparison tests (Table 3.2) corroborate the previous tentative statement on the effect of temperature and light on K.

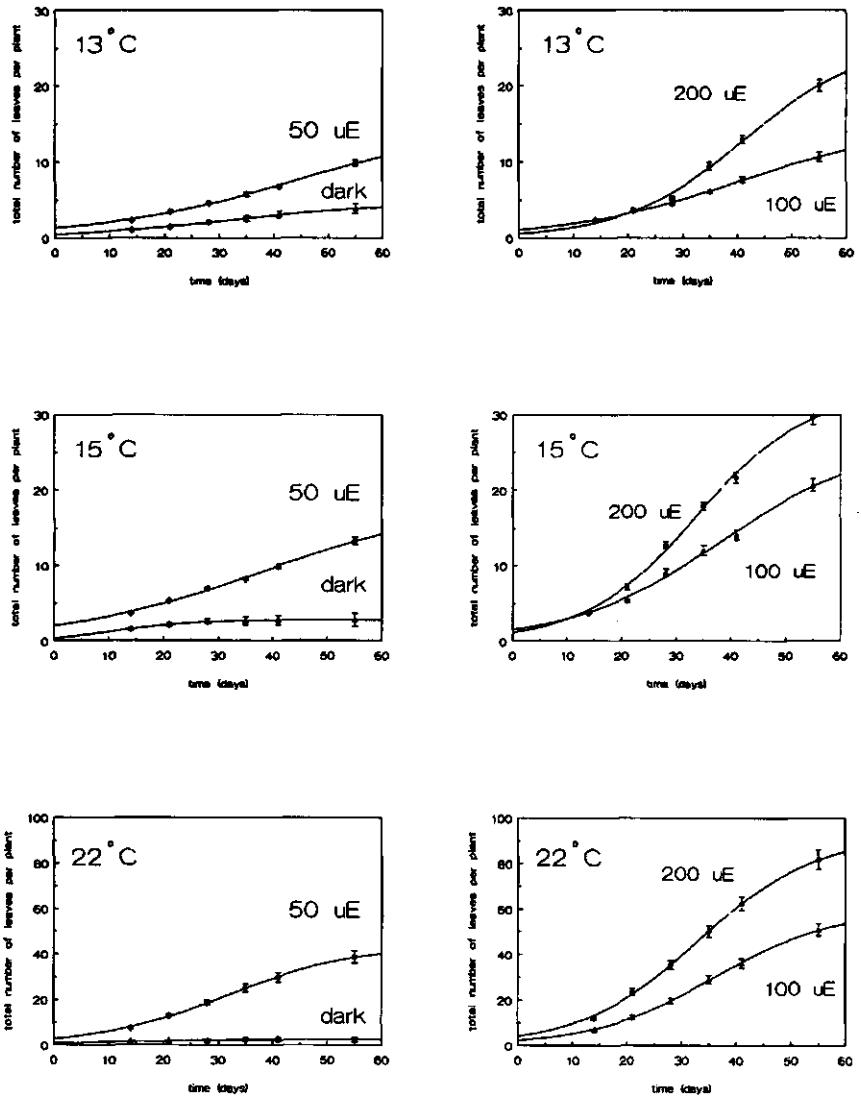


Fig. 3.1. Development of the total number of leaves on a plant and the fitted logistic growth curves for the twelve different treatments (temperature-light combinations) of experiment 1. Average values and standard errors are indicated (uE = μE m^{-2} s^{-1}).

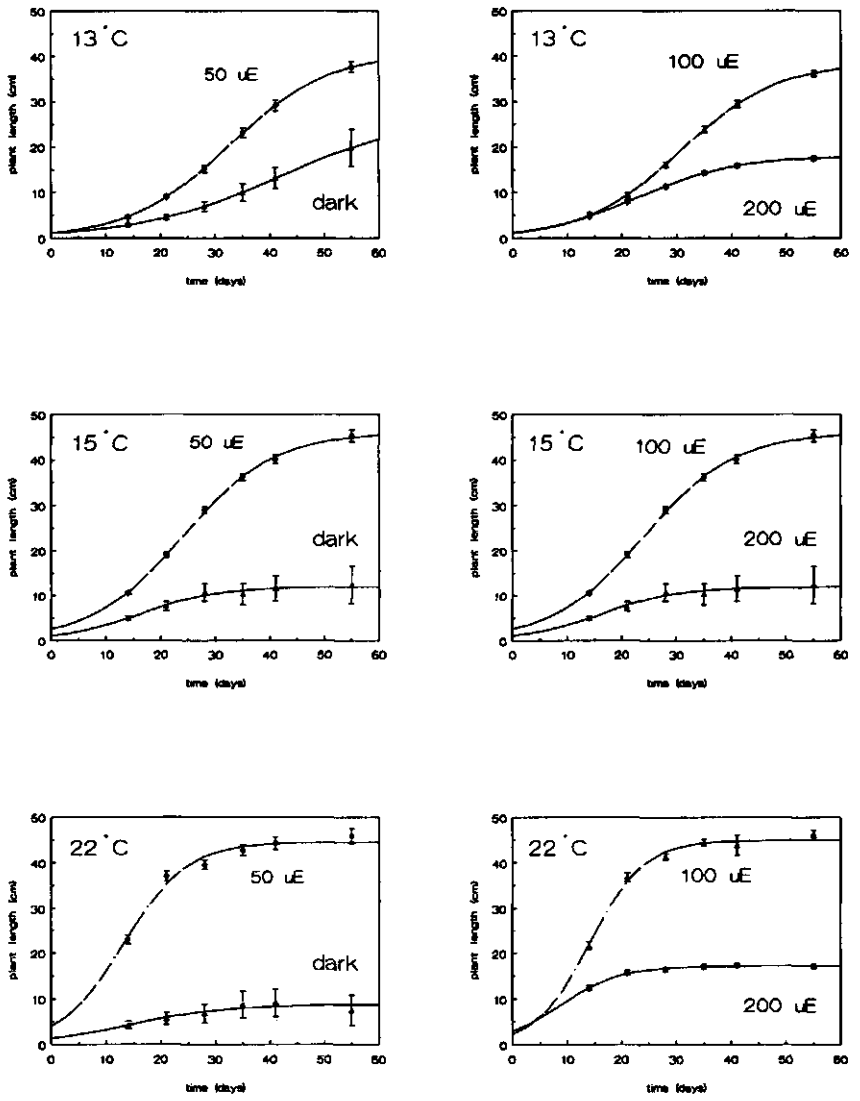


Fig. 3.2. Development of plant length and the fitted logistic growth curves for the twelve different treatments of experiment 1 (further as in Fig. 3.1).

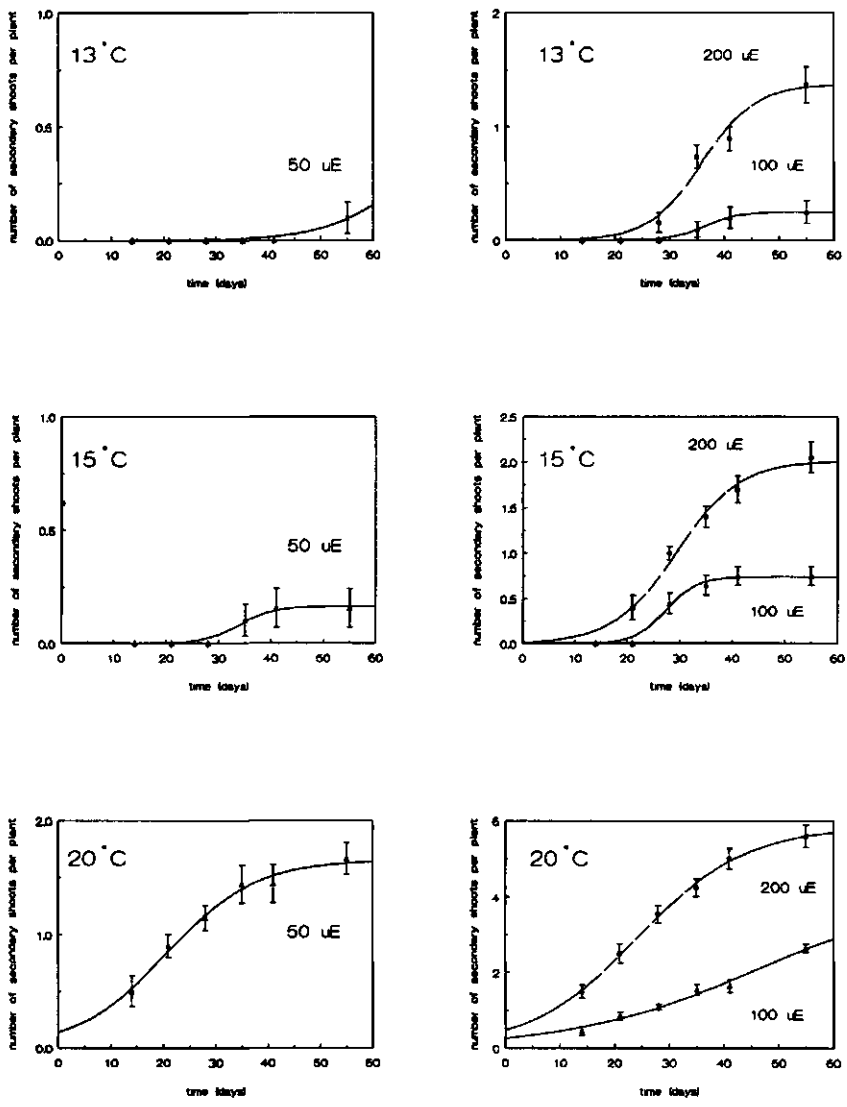


Fig. 3.3. Development of the number of secondary shoots on a plant and the fitted logistic growth curves for the twelve different treatments of experiment 1 (further as in Fig. 3.1).

Table 3.1. Multiple comparisons of fitted growth curves for the total number of leaves on a plant (n leaves), the number of bundles on a plant (n bundles), plant length (cm), the sum of bundle-lengths (Σ length, cm) and the number of secondary shoots (n secondary shoots). Experimental error rate was held at $p=0.05$. Multiple comparisons were made per irradiance level between temperatures (lower case, vertical comparisons in the table) and per temperature between irradiances (upper case, horizontal comparisons). Data presented are the K values from the logistic fits, i.e. the asymptotic maximum. An asterisc (*) is added to its value when 0.99*K was not reached within the experimental period of 55 days. Treatments that share the same letter are not significantly different. When letters are enclosed in brackets, this indicates that the individual logistic curves could not be fitted and a comparison between curves could actually not be made, the trends, however, were clear and are therefore indicated.

temperature (°C)	irradiance (μE m^{-2} s^{-1}) dark	50	100	200
n leaves				
13°	*4.8 aP	15.6 aQ	15.0 aQ	26.0 aR
15°	2.8 aP	18.6 bQ	26.6 bR	33.2 bS
22°	2.5 aP	44.2 cP	60.1 cR	92.4 cS
n bundles				
13°	1.0 (aP)	*2.2 aQ	*6.7 aQ	7.0 aR
15°	1.0 (aP)	*10.7 bQ	*5.1 bR	*7.4 bS
22°	1.0 (aP)	6.4 cQ	9.4 cR	15.2 cS
plant length				
13°	*27 bP	41 aR	39 aR	18 aQ
15°	12 abP	46 bS	37 bR	17 bQ
22°	9 aP	45 cR	45 cR	17 cQ
Σ length				
13°	6.9 aP	13.2 aQ	17.6 aQ	53.2 aR
15°	7.4 aP	14.8 bQ	43.5 bR	53.8 bS
22°	4.7 aP	51.3 cQ	76.7 cR	103.7 cS
n secondary shoots				
13°	0.0 (aP)	*0.5 aQ	0.3 aR	1.4 aS
15°	0.0 (aP)	0.2 aQ	0.7 bR	2.0 bS
22°	0.0 (aP)	1.7 bQ	4.2 cR	5.8 cS

Table 3.2. Multiple comparisons of final values of n leaves, n bundles, plant length, Σ length and n secondary shoots (as explained in Table 3.1). Given are mean, standard error (se) and results from lsd tests (experimental error rate held at p=0.05) with different lettering indicating significant difference. Replication was 18 to 20. To homogenize the variances, data were $\log_{10}(x+1)$-transformed before the lsd-tests were made.

temperature (°C)	13°			15°			22°		
irradiance ($\mu E\ m^{-2}\ s^{-1}$)	50	100	200	50	100	200	50	100	200
n leaves									
mean	9.9	10.7	20.1	13.3	20.7	29.8	38.8	51.1	82.0
se	0.3	0.6	0.8	0.5	0.8	1.0	2.6	2.7	4.3
lsd	a	ab	c	b	c	d	e	f	g
n bundles									
mean	1.4	1.8	6.2	1.9	4.9	7.7	6.2	9.3	14.7
se	0.1	0.2	0.2	0.2	0.3	0.3	0.6	0.5	0.7
lsd	a	a	c	a	b	cd	bc	d	e
plant length									
mean	37.6	36.2	17.5	45.2	35.3	16.7	45.9	46.4	17.2
se	1.2	0.7	0.4	1.3	1.0	0.7	1.6	0.1	0.5
lsd	b	b	a	c	b	a	c	c	a
Σ length									
mean	12.5	17.1	47.5	17.8	41.0	55.2	49.9	74.5	102
se	1.3	2.5	2.1	2.3	3.3	2.8	5.1	3.9	5.4
lsd	a	a	b	a	b	bc	b	cd	d
n secondary shoots									
mean	0.1	0.3	1.4	0.2	0.8	2.1	1.7	2.7	5.6
se	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.3
lsd	a	a	c	a	b	cd	c	d	d

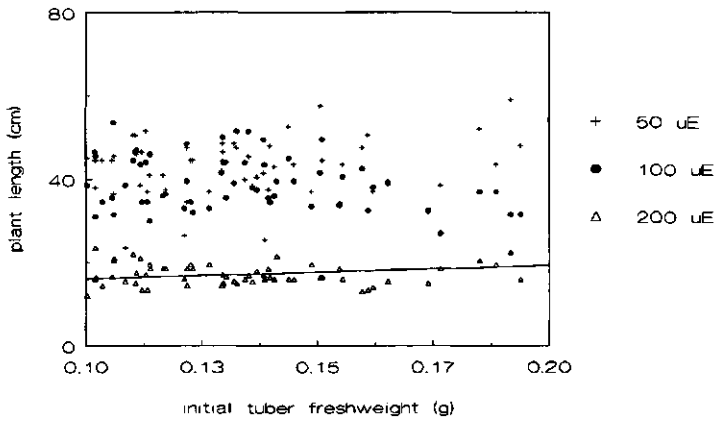


Fig. 3.4. Final plant length as a function of initial tuber fresh weight in experiment 1 (uE = μE m^{-2} s^{-1}). Linear regression was significant for 200 μE m^{-2} s^{-1} only (y = 12.9 + 32.4*x, r^2=0.09, p=0.024).

The significance pattern for the number of secondary shoots was not identical to that of n bundles, but the trends were similar: more secondary shoots were formed at higher irradiances and temperatures (Fig. 3.3, Tables 3.1 and 3.2).

The pattern for plant length was different and more complicated (Fig. 3.2, Tables 3.1 and 3.2). In the dark, plants grew longer at lower temperatures, while in the light they contrastingly grew longer at higher temperatures. Also, plant stems elongated significantly more in low than in high light.

We plotted the relative growth rates (r) from the logistic fits as a function of temperature for the separate light treatments (Fig. 3.5). To investigate the temperature effect, we applied the Q_{10}-concept in a logarithmic linearization with $Q_{10}/10$ as slope and c as intercept (Berry & Raison, 1981):

$$\log_{10}(r) = (\text{temperature} * Q_{10}) / 10 + c$$

Of the 60 (12 treatments * 5 morphometric characteristics) regressions only a few were significant (8 had a significance level $p<0.20$ and only 4 had $p<0.10$). This indicates that for the presently investigated temperature and irradiance ranges no loglinear relation existed between most growth rates and temperature. The few significant regressions that resulted in Q_{10} values that were in the range representative of biochemical processes (near 2, Berry & Raison, 1981) were for total number of leaves at 50 μE m^{-2} s^{-1} ($p=0.10$) and plant length at 50 and 200 μE m^{-2} s^{-1} ($p=0.03$ and 0.07 respectively). The other significant regressions had Q_{10} values that were in the range of 0.1 - 10.

3.1.2 Bundle characteristics

The similarity in significance patterns for n leaves, n bundles and Σ length suggests that they are strongly correlated. Thus, in other words, our 'bundle' concept indeed appears to depict a module with a considerable degree of constancy (cf. Vermaat & Hootsmans, 1991). This relative constancy of a bundle, then, should be

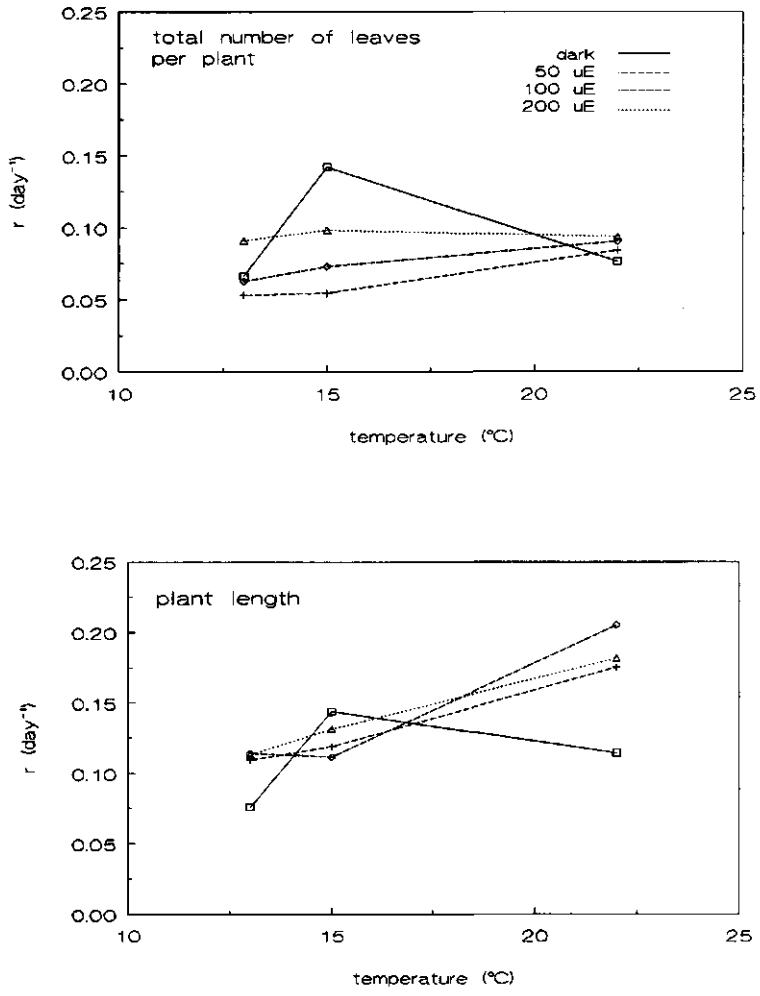


Fig. 3.5. Initial growth rate (r) of total number of leaves and plant length from the logistic fits of the different treatments in experiment 1 as a function of temperature and light (uE = μE m^{-2} s^{-1}).

reflected in (a) the number of leaves per bundle (Figs 3.6 and 3.7), and (b) the 'spectrum' of bundle lengths of a plant, in the sense that they must have been similar for the different treatments. Still, significant differences were present between treatments for both characteristics (Table 3.3). The combined effect of temperature and light however resulted in much larger differences in the number of bundles than in number of leaves per bundle or bundle length. The ratio of maximum/minimum number of bundles in any treatment was 10.9 (treatment 9/treatment 1, Table 3.2), while for number of leaves and bundle length on the main shoot this was only 1.7

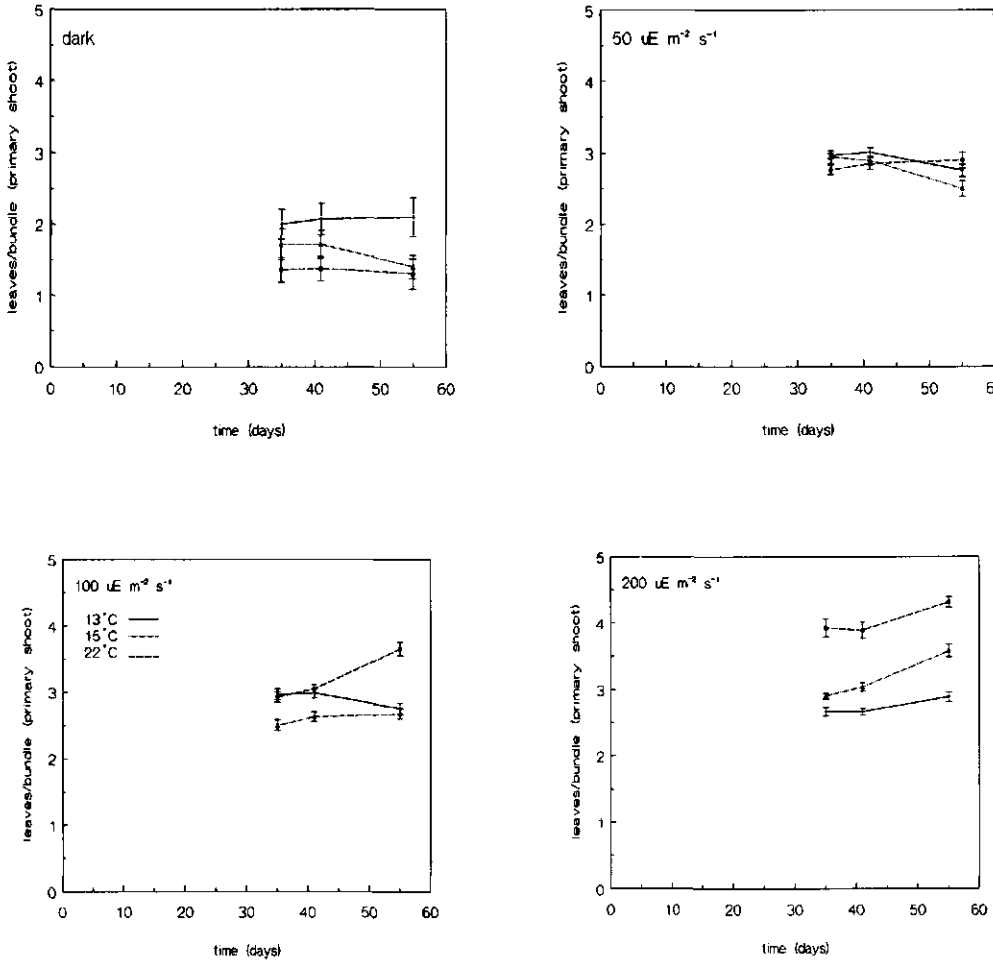


Fig. 3.6. Development of the number of leaves per bundle on the main shoot for the twelve different treatments of experiment 1 (further as in Fig. 3.1).

and 1.3 respectively. Thus, bundle dimensions were also significantly influenced by differences in temperature and light, but the effect was relatively small.

Both bundle length and number of leaves per bundle were significantly less on secondary shoots than on the main shoot ($p < 0.001$ in a threeway ANOVA incorporating the comparison secondary versus main shoots, temperature, light and the interactions).

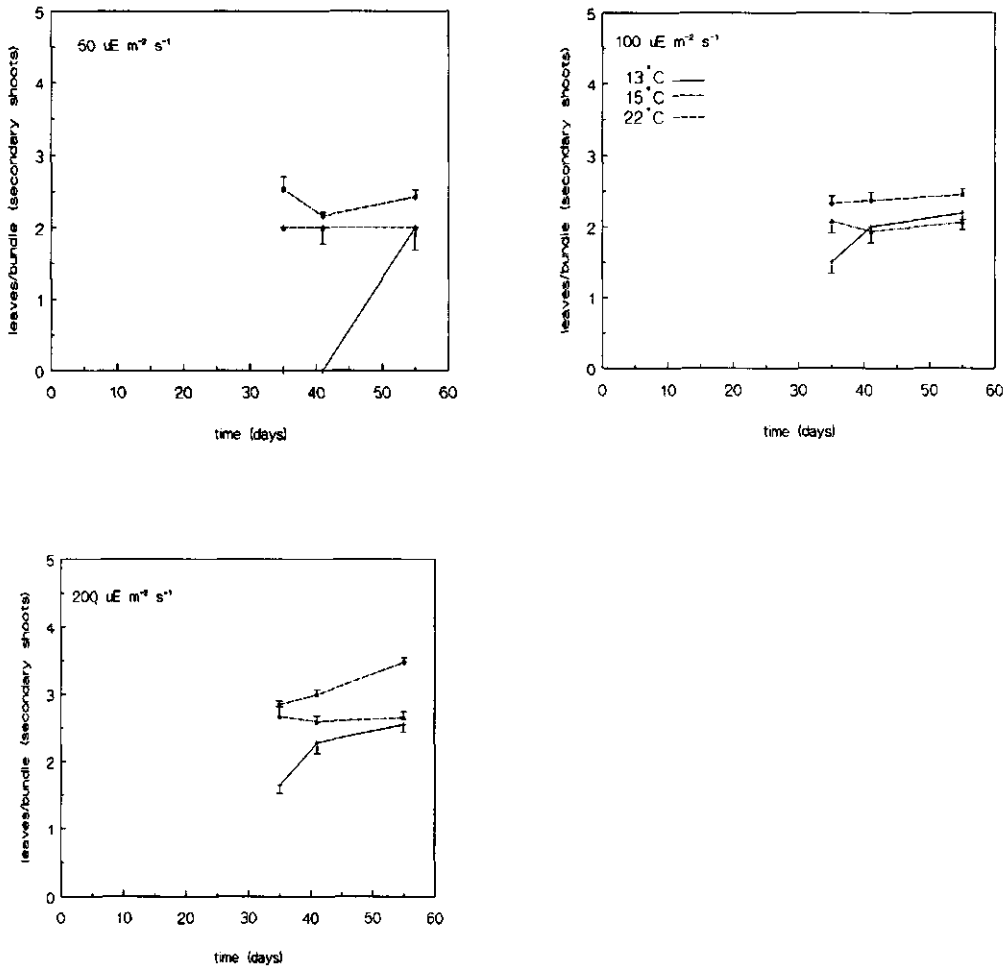


Fig. 3.7. Development of the number of leaves per bundle on secondary shoots for different treatments of experiment 1. No secondary shoots were formed in the dark (further as in Fig. 3.1).

3.2 Experiment 1 - final biomass and chlorophyll

Final biomass parameters were entered in analyses of covariance (ANCOVA) and in multiple regressions (Table 3.4). Final values and multiple comparisons are given in Table 3.5. In multiple regression analyses, deciding on the relative importance of different factors in the regression model is not straightforward and a number of indices have been developed (Norusis,

Table 3.3. Final values of number of leaves per bundle and bundle length (cm) for primary and secondary shoots separately. Given are mean, standard error (se), replication (n), significantly different groups of treatments if appropriate (presented as in Table 3.2) and, in the second half of the table, the significances of the different effects in twoway ANOVAs. Since no transformation satisfactorily homogenized the variances of the secondary shoot characteristics, multiple t' tests (Steel & Torrie, 1980) were performed maintaining an experimental error rate of 0.05. The few significant differences for secondary shoots could not be grouped interpretably and are not presented. Replication for the main shoot characteristics was 18 - 20.

temperature (°C)	13°			15°			22°		
irradiance (μE m^{-2} s^{-1})	50	100	200	50	100	200	50	100	200
(a) number of leaves per bundle, main shoot									
mean	2.8	2.8	2.9	2.5	2.7	3.6	2.9	3.7	4.3
se	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
lsd	a	a	a	a	a	b	a	b	c
(b) number of leaves per bundle, secondary shoots									
mean	2.0	2.2	2.5	2.0	2.1	2.7	2.4	2.5	3.5
se	0.3	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1
n	2	5	18	3	15	20	18	20	20
(c) bundle length, main shoot									
mean	9.6	9.8	8.1	9.2	8.9	7.9	8.4	8.9	7.6
se	0.3	0.2	0.1	0.3	0.2	0.2	0.3	0.2	0.1
lsd	e	e	abc	de	bcd	ab	bcd	cde	a
(d) bundle length, secondary shoots									
mean	4.5	6.3	6.1	7.5	5.5	5.3	7.0	6.0	6.4
se	1.0	0.9	0.2	0.8	0.3	0.2	0.2	0.2	0.1
n	2	5	18	3	15	20	18	20	20

twoway ANOVAs

	temperature	light	interaction	tuber fw (cov.)
(a)	0.001	0.001	0.001	0.584
(b)	0.001	0.001	0.068	0.726
(c)	0.001	0.001	0.179	0.877
(d)	0.013	0.561	0.003	0.074

Table 3.4. Significance levels from analyses of covariance of the effect of temperature (T), irradiance (I), initial tuber fresh weight (TW, covariable) and the temperature irradiance interaction (T*I) on selected biomass parameters: aboveground, belowground, remaining tuber and total biomass. New biomass = aboveground + belowground biomass and S/R = aboveground/belowground biomass. Also given are the overall explained variance (mult. r^2) from a multiple regression model incorporating temperature, irradiance and initial tuber fw, the sign of the effect (i.e. +: the biomass parameter increases with increasing factor level) and the partial correlation coefficient.

analysis of covariance

	T	I	TW	T*I
aboveground	0.0001	0.0001	0.0160	0.0010
belowground	0.0001	0.0001	0.0060	0.0001
final tuber	0.0010	0.8890	0.0000	0.2220
total	0.0001	0.0001	0.0001	0.0001
new	0.0001	0.0001	0.0070	0.0001
S/R	0.0001	0.0001	0.7090	0.8380

multiple regression

	T	I	TW	mult. r^2
aboveground	**+0.824**	+0.266	0.103	0.688
belowground	**+0.767**	**+0.802**	0.151	0.767
final tuber	−0.274	0.019	**+0.674**	0.502
total	**+0.816**	**+0.605**	+0.305	0.728
new	**+0.822**	**+0.596**	0.130	0.728
S/R	+0.270	**−0.818**	0.014	0.822

1986). We decided to use the partial correlation coefficient and interpret it cautiously, i.e. only when the differences were conspicuous.

Almost all factors had significant effects in the analyses of covariance, only irradiance had no significant effect on the final tuber weight and initial tuber weight had no effect on the shoot/root (S/R) ratio. The interaction between temperature and irradiance was significant for all biomass characteristics except for the final tuber weight and the S/R ratio.

According to the multiple r^2, the multiple regression model fitted the data fairly well, or, a good deal of the variation present in the data could be explained by temperature, light and initial tuber weight. In terms of partial correlation coefficients, aboveground biomass was more closely correlated with temperature than with light, while belowground biomass was equally correlated to both factors. This parallels the morphometric observations: the plants remained shorter but produced more secondary shoots under high irradiance. These secondary shoots sprouted from new rhizomes which explains the increasing belowground biomass with increasing irradiance. Accordingly, the S/R ratio was negatively correlated with irradiance, while temperature was far less important.

The relation between aboveground and belowground biomass and the number of secondary shoots pooled over all treatments is depicted in Fig. 3.8. Both linear regressions were significant ($p < 0.001$), but r^2 for aboveground biomass was significantly less. Thus, aboveground biomass correlated less with the number of secondary shoots than belowground biomass, which for the larger part consisted of rhizomes. The larger variation in aboveground biomass may be explained by relatively large variation in timing of the formation of secondary shoots and by variation in their size.

The emptying of the tubers was only governed by temperature with apparently higher emptying rates at higher temperatures (final tuber weight is nega-

Table 3.5. Biomass characteristics of *P. pectinatus* after 55 days growth in a temperature-light gradient. Except initial tuber fresh weight, all characteristics are explained in Table 3.4. Given are mean, standard error (se) and results from the multiple t'-tests (experimental error rate held at p=0.05) with different lettering indicating significant difference. $Log_{10}(x+1)$-transformation did not homogenize the variances, therefore t'tests were applied. Replication was 19 to 20. Biomass parameters are in mg plant^{-1} afdw unless otherwise indicated. The afdw/fw ratio for aboveground biomass was 0.14, i.e. similar to that for material from the same population in Vermaat & Hootsmans (1991). Ash fractions of dw were on average 0.11, 0.29 and 0.07 for aboveground, belowground and tuber biomass, respectively

temperature (°C)		13°			15°			22°		
irradiance (μE m^{-2} s^{-1})		50	100	200	50	100	200	50	100	200
initial tuber fw*	mean	132	134	125	143	138	134	124	127	135
	se	5	6	4	7	6	6	4	5	5
aboveground	mean	26	28	27	35	44	43	71	93	101
	se	1	1	1	2	2	2	4	5	4
	t'	a	ab	a	b	c	c	d	de	e
belowground	mean	13	16	33	13	25	43	29	44	86
	se	1	1	1	1	1	1	2	2	4
	t'	a	a	c	a	b	d	bc	d	e
final tuber	mean	20	22	18	22	19	19	14	15	19
	se	1	2	1	2	1	1	2	1	2
total	mean	60	66	78	70	88	105	114	153	207
	se	2	2	2	3	3	3	6	6	9
	t'	a	a	bc	ab	cd	b	d	e	f
new	mean	39	44	60	48	69	86	100	137	188
	se	2	2	2	2	2	2	6	7	10
	t'	a	a	b	a	b	c	c	d	e
S/R	mean	2.23	1.84	0.83	2.80	1.82	1.02	2.49	2.13	1.17
	se	0.19	0.06	0.03	0.15	0.07	0.03	0.09	0.06	0.04
	t'	cde	c	a	e	c	b	de	cd	b

* Initial tuber afdw was on average 52 mg, calculated with the overall regression formula from Vermaat & Hootsmans (1991).

Table 3.5. Continued. Chlorophyll (a+b) is in mg/g afdw leaves. For total chlorophyll, $\log_{10}(x+1)$-transformation homogenized the variances, replication was 4 - 5, Tukey's HSD test was used for the multiple comparisons.

temperature (°C)		13°			15°			22°		
irradiance (μE m^{-2} s^{-1})		50	100	200	50	100	200	50	100	200
chlorophyll (a+b)	mean	11.9	10.5	6.6	11.2	6.4	4.1	9.3	6.1	4.3
	se	3.3	0.7	1.1	2.0	1.2	0.7	1.2	1.3	0.2
	Tukey	e	e	cd	e	c	a	de	bc	ab
fraction chlorophyll b	mean	0.20	0.22	0.22	0.24	0.22	0.22	0.21	0.23	0.23
	se	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

tively affected by increasing temperature).

The significance patterns from the individual multiple comparisons mostly paralleled the patterns from the analyses of covariance (Table 3.4 and 3.5). The significant interactions are apparent: at 13 °C, for example, the aboveground biomasses were not significantly different for the three irradiance levels, while at higher temperatures significant differences did exist. No significant differences in initial tuber fresh weight nor in final tuber afdw existed. The former indicates that our randomization procedure was successful. The latter is probably due to the lower power of the multiple comparisons as compared to the ANCOVA due to the higher number of tests.

Phaeophytin a and b concentrations were not detectable in our plant samples. Phaeophytins are generally considered to be breakdown products of chlorophylls, their absence thus may be interpreted as a sign of good 'condition' of the plants. No significant effects of light and temperature were found on the fraction of chlorophyll b (frac-b). Total chlorophyll content (chl(a+b)) in general was less at higher irradiances, while temperature also had a 'diluting' effect (cf. Hootsmans & Vermaat, 1991), i.e. lower contents at higher temperatures, at least at the higher irradiances.

3.3 Experiment 2 - effect of prolonged dark exposure on subsequent plant growth in the light

The course of aboveground and belowground plant biomass with increasing time in the light is plotted in Fig. 3.9. For aboveground, belowground as well as total biomass, the data could be fitted equally well with a linear as with a logistic model, i.e. the F-ratios comparing the two residual sums of squares were not significant (cf. Vermaat & Hootsmans, 1991).

In a twoway ANOVA, temperature during dark incubation had a significant effect ($p < 0.05$) on aboveground biomass only, time had a significant effect on above-, belowground, final tuber as well as total biomass, while no interactions were significant. In other words, temperature during dark incubation growth,

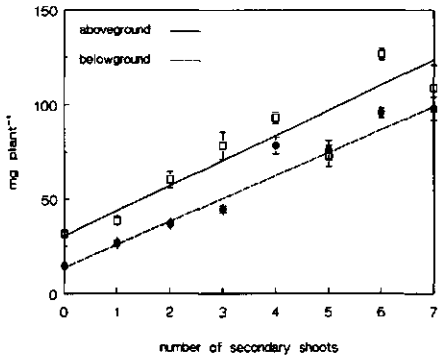


Fig. 3.8. Aboveground and belowground biomass as a function of the number of secondary shoots that a plant has formed at the end of experiment 1. Both linear regressions were significant ($p<0.001$). For aboveground biomass the formula was: $y = 30.5 + 13.3*x$, $r^2=0.586$, belowground: $y = 13.7 + 12.2*x$, $r^2=0.871$, the two r^2 values are significantly different ($p<0.001$). Biomass is in afdw in this and the following figure.

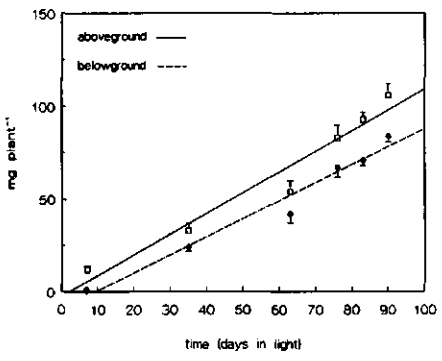


Fig. 3.9. Development of plant biomass in experiment 2 as a function of the time that the plants were in the light. The two linear regression lines were highly significant ($p<0.001$, $r^2>0.80$) and did not describe the data significantly worse than a logistic fit (F test, $p>0.30$). Aboveground: $y = -1.76 + 1.11*x$, belowground: $y = -9.06 + 0.98*x$, the two slopes were not significantly different (t test, $p>0.50$).

with higher 'dark' temperatures resulting in lower subsequent aboveground biomasses in the light.

When chlorophyll contents were analyzed, one group of plants, grown together in one aquarium in the light, appeared to contain significantly less chl(a+b) than the others. Though this difference could not be explained, this group has been excluded from further analysis of the chlorophyll data (Table 3.6). This difference between aquaria could not be detected in the biomass data, where no data had to be excluded. In a twoway ANOVA (time, temperature and their interaction) only time had a significant effect on the frac-b, all other effects on chl(a+b) or frac-b were not significant.

Table 3.6. Chlorophyll contents of plants that were moved to the light after variable periods of dark exposure. All samples taken after 105 days since the start of the experiment. Given are mean, standard error (se), replication (n) and significantly different groups for the fraction of chlorophyll b. Different letters indicate significantly different means. Chlorophyll (a+b) is in mg g^{-1} afdw of leaves.

days in dark	chlorophyll (a+b)			fraction chlor. b		
(in light)	mean	se	n	mean	se	Tukey
15 (90)	5.23	0.33	15	0.22	0.01	b
22 (83)	5.62	0.19	15	0.22	0.01	b
29 (76)	5.79	0.47	10	0.17	0.01	a
70 (35)	6.02	0.46	14	0.15	0.01	a

3.4 Development of sprouting tubers

We will report here on the sprouting stage and shoot-length of germinating tubers at the termination of experiment 3 (i.e. after 44 days), and make comparisons with data from the dark treatments of experiment 1 of similar age (measured at 42 days).

Table 3.7 gives the shoot lengths of the sprouting tubers after approximately 44 days in the dark. An overall oneway ANOVA comparing final lengths was highly significant ($p<0.0005$), but consequent non-orthogonal contrast tests (EER maintained at 0.05) demonstrated only significant differences between the 13° and 15° treatments of experiment 3 and between the 15° treatments of experiments 1 and 3 (this last difference was only significant at an EER of 0.10).

The high standard errors of the shoot lengths after 44 days (Table 3.7) reflect the fact that different individual tubers had arrived in different sprouting stages. Apparently, individual tubers of comparable size differ in the rate with which the sprouting process is passed through. This is illustrated in Table 3.8 for experiment 3. Only tubers in stage 3 have strongly elongating stems and developing leaves (definition in section 2.3). All three temperature treatments were significantly different with respect to the distribution over sprouting stages. At 13°, the bulk of the tubers has arrived in stage 3, whilst most are in stage 1 at 15° and 22°. Remarkably, no tubers reached stage 3 at 15 °C, while some tubers did at 20°.

Though no detailed observations on sprouting stage were made during experiment 1, we have noted the presence of the first and further leaves. Thus, we were able to distinguish whether a tuber had arrived in stage 3 or not. This enables a restricted comparison between experiments 1 and 3 (Table 3.9). The distribution of

Table 3.7. Shoot-lengths (mm) of sprouting tubers before sprouting (initial length) and after 44 days (final length) at three different temperatures in the dark. Given are mean and standard error (se), replication was 14 in experiment 1 and 20 in experiment 3 for every temperature. Initial length was measured as the length of the sproutlike organ that is connected to the tuber. The sprouting shoot emerges from this organ, as outlined in section 2.3.

temperature	initial length		final length	
	mean	se	mean	se
experiment 1				
13°	-	-	131.0	35.0
15°	-	-	115.5	43.5
22°	-	-	91.5	47.5
experiment 3				
13°	15.0	0.5	67.0	9.0
15°	15.5	0.5	25.0	1.5
22°	16.0	0.0	55.5	12.5

Table 3.8. Distribution of tubers over the four different sprouting stages after 44 days at three different temperatures in experiment 3. Levels of significance (p) of the overall χ^2-test and of the three separate additive tests are given below. For a definition of the sprouting stages see section 2.3.

sprouting stage:	0	1	2	3	total
13°	2	4	4	10	20
15°	3	15	2	0	20
22°	1	11	4	4	20
total	6	30	10	14	60

χ^2-tests	
p (overall)	0.005
p (13° - 15°)	0.005
p (13° - 22°)	0.050
p (15° - 22°)	0.050

Table 3.9. Comparison of the sprouting process of tubers in experiment 1 and 3. Sprouting stages 0, 1 and 2 were pooled in stage 'rest', stage 3 could also be discerned for tubers in experiment 1. The significance of χ^2 tests comparing tuber distributions of the two experiments per temperature are given (p).

	experiment 1		experiment 3		p
sprouting stage:	rest	3	rest	3	
13°	4	10	10	10	0.21
15°	9	5	20	0	0.04
22°	10	4	16	4	0.56

tubers over sprouting stages of the two experiments was significantly different at 15°.

4. Discussion and conclusions

4.1 The interactive effect of temperature and light on growth

Temperature and light affected the growth of *P. pectinatus* interactively, both in a quantitative and a qualitative way. A priori, interaction in the quantitative effects should have been the expected case, since in general both light and temperature affect growth nonlinearly and a basic assumption of analysis of variance is additivity, i.e. linearity of factor effects. Photosynthesis as well as growth of individual plants generally follow a saturation curve with increasing irradiance (Björkman, 1981; Hootsmans & Vermaat, 1991) and an optimum curve with increasing temperature (Eppley, 1972; Berry & Raison, 1981; Bulthuis, 1987). Indeed, the quantitative effect of temperature and light on growth of *P. pectinatus* was nonlinear (Figs 3.1 - 3.3).

Eppley (1972) clearly demonstrated that growth rates of marine planktonic microalgae increased exponentially with increasing temperature up to some maximum temperature. Growth rates decreased rapidly above this maximum. Optimum temperatures for macrophyte growth may be derived tentatively from studies that investigated a wider range of temperatures than was done in the present study, i.e. Barko & Smart (1981) and Barko et al. (1982, cf. Table 3.10). They reported optima of 24° to 32 °C at saturating irradiance for aboveground biomass development of five freshwater macrophyte species. Temperature optima for root growth were lower than for shoot growth in two species (*Elodea canadensis* Michx. and *Vallisneria americana* Michx.). According to Berry & Raison (1981), root biomass necessary to fulfil nutrient requirements may be less at higher temperatures, since nutrient diffusion rates generally are enhanced by increased temperature.

Since the reported temperature optima were above the temperature range we investigated here, we hypothesized that the growth rates from the logistic fits to our morphometric data would increase exponentially with increasing temperature and thus would obey to a Q_{10}-relation. This, however, was only the case in a few of the 60 loglinear regression fits (section 3.1, Fig. 3.5). Apparently, the rate at which the various morphometric characteristics were formed or grew was not influenced by temperature in a simple, loglinear way. The dark treatments had a clear optimum at 15 °C.

If a partial correlation coefficient indicates the relative weight of a factor in a multiple regression analysis, both temperature and irradiance were equally important in determining belowground

Table 3.10. Shoot length elongation ratios (SLR, cm cm^{-1}) of various aquatic macrophytes at different temperatures (T, °C), if appropriate. The SLR is the ratio of shoot length under low light divided by that under high light. Also given are the ratios of low and high irradiance (IRR, $\mu E\ m^{-2}\ s^{-1}$), photoperiod (P, hours) and plant age (in weeks) at determination. If not given by the authors, photoperiod was derived from the approximate latitude, the month of the year and a curve given in Salisbury (1981), when natural daylight was used.

species	age	T	IRR, P	SLR	reference
P. pectinatus	8	13°	50/200,	2.1	expt 1, this study
		15°	12 h	2.7	
		22°		2.6	
P. pectinatus	4	17-20°	50/200,	1.8	Hootsmans & Vermaat, 1991
	10		16 h	1.5	
	17			1.5	
P. pectinatus	8	20-23°	?, 16 h	2.1	Van Wijk et al., 1988
P. crispus	9	5°	20/93,	1.4	Tobiessen & Snow, 1984
		10°	? h	1.9	
		15°		2.0	
P. richardsonii	4	?	200/1500,	3.0	Spence & Dale, 1978
			15 h	1.5	
E. densa	6	24°	100/1500,	4.5	Barko & Smart, 1981
H. verticillata			14 h	2.7	
M. spicatum				2.5	
E. canadensis	8	12°	100/1500,	1.4	Barko et al., 1982
		16°	14 h	1.7	
		20°		2.3	
		24°		1.8	
P. nodosus	6	12°		1.7	
		16°		1.7	
		20°		2.1	
		24°		2.1	

species: *Potamogeton crispus* L., *Potamogeton richardsonii* (Benn.) Rydb., *Egeria densa* Planch., *Hydrilla verticillata* (L.f.) Royle, *Myriophyllum spicatum* L., *Elodea canadensis* Michx., *Potamogeton nodosus* Poir.

biomass, but temperature was more important for aboveground biomass. In general, though, the relative importance of an environmental factor to a species should be considered in terms of reproductive succes or survival (Pianka, 1979) and relative to the amplitude of the species and the total amplitude of that factor available in the habitat. Within the limited scope of the present experiment, no direct assessment of differences in reproductive succes or survival was feasible. Still, we can safely assume that the number of vegetative propagules or seeds produced per plant are also a function of plant size (Silvertown, 1982). Hence we can conclude that, within the tested ranges, both high temperature and irradiance had a positive effect on plant size, which may be of significance to survival.

Madsen & Adams (1988a) concluded from field studies that a temperature threshold of 15 °C may exist for *P. pectinatus* 'to thrive', but stressed the interrelatedness of temperature and daylength fluctuations. We demonstrated that *P. pectinatus* was able to grow well at lower temperatures (i.e. 13 °C) at 200 μE m^{-2} s^{-1}, i.e. at an irradiance sufficiently high for positive net photosynthesis and growth (Hootsmans & Vermaat, 1991). The relatively small difference in temperature regimes between the 13° and 15° treatments resulted in many significant differences, both in fitted growth curves and in biomass. This may indeed indicate the presence of a not-too-absolute threshold mechanism at about 15 °C for the presently studied population. Such a mechanism may be important for the correct seasonal timing of the presence of aboveground vegetation as a life cycle phase (Vegis, 1973).

Several authors measured growth of *P. pectinatus* in laboratory studies (Hodgson, 1966; Spencer, 1986; Spencer & Anderson, 1987; Van Wijk et al., 1988). Here, we will dwell upon a few aspects of comparative importance only.

Hodgson (1966) applied a loglinear model of growth thus assuming exponential growth for his entire experimental period. He used tubers of 400 - 1000 mg fw at 22 °C and 400 ft candela ($\approx$ 75 μE m^{-2} s^{-1}, conversion factor 0.19 from Harris (1978)). Hodgson (1966) found that of the initial tuber fw only 3% remained after 30 days, whilst we found 25% or more even after two months (Table 3.5). Explanations for this remarkable difference may be sought in differences in tuber characteristics (relatively less remobilizable material present in the presently studied tubers) or in differences in the need to exploit tuber resources in the newly developing plant under different experimental conditions.

Spencer & Anderson (1987) reported a total plant dry weight (dw) of 200 mg for plants of 56 days old at a photoperiod of 12 h, 80 μE m^{-2} s^{-1} and 24 °C, which is fairly comparable to our 175 mg dw at 22° and 100 μE m^{-2} s^{-1} (153 mg afdw).

For populations originating from brackish habitats (Yerseke, Vlake, both The Netherlands), Van Wijk et al. (1988) reported plant lengths similar to the values found here, after 2 months' culturing at 20° - 23 °C in a greenhouse. Their final biomasses, however, were much higher (400 mg $plant^{-1}$ aboveground afdw for the Yerseke population, versus maximally 200 mg afdw in Table 3.5). This may be due to a longer photoperiod (reportedly 16h), higher irradiances (greenhouse) or differences in initial tuber weight (cf. Vermaat & Hootsmans, 1991).

4.2 Acclimation to different irradiance regimes

The qualitative effect of light can be described as a typical sun/shade acclimation in the growth form. In low light the main shoot elongated strongly and few or no secondary shoots were formed, while in high light stem elongation was less pronounced and more secondary shoots were formed as described in section 3.1. Also the chl(a+b) concentration of the individual leaves increased in low light.

Similar acclimations from various other studies of aquatic macrophytes have been summarized in Table 3.10. For reasons of comparison, SLR, a relative measure of shoot elongation capacity was used, which was calculated as the ratio of shoot length in low and in high light.

SLR values calculated from Hootsmans & Vermaat (1991) were distinctly lower than those from the present experiment 1. This was probably due to differences in experimental set-up. Correction of the SLR values of the plants from experiment 1 (22°) for the difference in photoperiod (12 vs. 16 h, i.e. multiply with 0.75) and planting density (plants in Hootsmans & Vermaat (1991) had only 0.8 times as much space as experiment 1 plants), reduces the SLR for 22 °C to 1.6, which is clearly within the range derived from Hootsmans & Vermaat (1991). We therefore hypothesize that (1) shoot elongation is more pronounced at higher plant densities, and (2) stimulation of elongation may not be effectuated by the instantaneous irradiance, i.e. the flux at any moment, but by the integral diel irradiance received by the plant. The first hypothesis is conform with observations from agricultural practice (Silvertown, 1982), hence, for example, plants from 200 μE m^{-2} s^{-1} in Hootsmans & Vermaat (1991) elongated relatively more than in experiment 1 which resulted in a lower SLR. According to the second hypothesis, elongation was less stimulated in the plants grown at 50 μE m^{-2} s^{-1} with a 16h photoperiod of Hootsmans & Vermaat (1991), resulting in a lower SLR.

Temperature appeared to affect the elongation rate: though not linearly, SLR values generally increased with increasing temperature. Thus, light and temperature also interact in their qualitative effects.

Over the range of species and experimental conditions listed in Table 3.10, most SLR values remained within the range 1.5 - 3.0. Only *E. densa* elongated more vigorously. Where shoot elongation is of decisive importance relative to other traits, only *E. densa* must be considered a potentially strong competitor. Apparently, for other species not much 'room' is available for differentiation in competitive ability with respect to this trait.

Björkman (1981) reviewed different growth parameters of terrestrial sun and shade species or ecotypes. At irradiances similar to our 50, 100 and 200 μE m^{-2} s^{-1} he reported relative growth rates of 0.06, 0.09 and 0.10 day^{-1} for the shade-tolerant *Impatiens parviflora* Dc. and 0.01, 0.05 and 0.09 for the shade-intolerant *Helianthus annuus* L. The shade-tolerant species had a higher capacity to maintain its growth rate with decreasing irradiance. We can conclude from Fig. 3.5 that, in terms of maintenance of relative growth rates under low irradiance, *P. pectinatus* can be seen as a comparatively shade-tolerant species. Then, it may be postulated that most aquatic macrophytes are comparatively shade-tolerant, if we consider the similarity in SLR values in Table 3.10 and if we take the relatively low irradiances in aquatic environments as compared to terrestrial environments into account. A possible exception with respect to shade-

tolerance may be, for example, *Potamogeton polygonifolius* Pourr., generally being restricted to open, shallow water habitats (Spence & Chrystal, 1970a, 1970b).

An extensive literature exists on acclimation and/or adaptation of chlorophyll contents to changing light climates (see for example Björkman (1981) and Jeffrey (1981) for a review on terrestrial and aquatic environments respectively). The significant differences in chl(a+b) under different irradiances that were found here are similar to differences reported by other authors (Barko & Filbin, 1983; Spencer & Anderson, 1987; Hootsmans & Vermaat, 1991). Spencer (1986) reported remarkably lower chl(a) contents (0.2 mg g^{-1} fw as a grand mean) for *P. pectinatus* than was found in this study and by Spencer & Anderson (1987, respectively 0.8 and 0.9 mg g^{-1} fw).

Barko & Filbin (1983) demonstrated significantly higher frac-b values at intermediate temperatures (i.e. at 24 °C in a range of 12° - 32 °C) for *E. canadensis*, but not for *P. nodosus* and *V. americana*. They applied one of the least conservative multiple comparisons tests, Duncan's multiple range test (Steel & Torrie, 1980). Spencer & Anderson (1987) found no significant differences between treatments for *P. pectinatus*, whilst Spencer (1986) reported significantly increasing frac-b with increasing temperature (experimental range 10° - 37 °C) but did not test differences between different irradiances. We only found significant differences in the frac-b between two populations of *P. pectinatus* (Vermaat & Hootsmans, 1991), and at different age (cf. section 3.3 and Hootsmans & Vermaat, 1991). Overall however, the fractions of chlorophyll b were very similar between treatments (cf. Tables 3.5 and 3.6 above and Figs 4.7, 4.8 and 4.16 in Hootsmans & Vermaat, 1991) and to those of the referred literature. Therefore, we doubt whether changes in chl(b) content are a widespread means of short-term acclimation to changes in light climate in angiosperm freshwater macrophyte species, contrary to what among others Björkman (1981) reported for terrestrial plants.

4.3 Experiment 2 - effect of prolonged dark exposure on subsequent plant growth in the light

We demonstrated that temperature during dark exposure significantly affected subsequent plant growth in the light. This can be extrapolated to temperature regimes in the initial part of the growing season, i.e. during tuber sprouting in spring. High temperatures and low light in this phase may thus adversely influence subsequent plant growth.

The increase in the frac-b with time was remarkable. Similarly, Hootsmans & Vermaat (1991), found changes in the fraction of chlorophyll b with age: from 0.16 via 0.23 to 0.17 (averaged over irradiances) with 30, 70 and 120 days' age respectively. This increase may be correlated with the development of a self-shading concentration of leaves close to the water surface. The fraction of chlorophyll b indeed was correlated significantly (linear regression, $p<0.001$) with aboveground biomass in experiment 2. The lower values at 120 days in Hootsmans & Vermaat (1991) may be a diluting effect with increasing biomass or an effect of senescence (as discussed in Hootsmans & Vermaat, 1991). Thus, whilst we did not find significant differences in the fraction of chlorophyll b between treatments in experiment 1, *P. pectinatus* may change its fraction of

chlorophyll b and thus its relative amount of antenna chlorophyll (Björkmann, 1981) during its developmental course (long-term acclimation).

4.4 Development of sprouting tubers

Vegis (1973) discussed various types of dormancy and the widening effect of (physiological) stratification on the temperature range within which germination of seeds or bud break may occur. He described the onset of a secondary dormancy when high temperatures (above a certain compensation point around 14 °C) interrupt or follow a stratification period of low temperature (below this point). After secondary dormancy is established, stratification must start anew. Vegis (1973) also mentioned still higher temperatures as a means to break dormancy. The significant difference in the numbers of sprouting tubers in stage 3 that was found between experiments 1 and 3 may well be explained by the capacity of *P. pectinatus* tubers to develop such a secondary dormancy.

Based on these findings of Vegis (1973) we formulate the following hypothesis: (a) After physiological winter-stratification, the tubers germinate readily at the relatively low temperatures of the sediment in spring (probably less than 13°), higher temperatures (i.e. around 15°) inhibit further development of the sprouting tuber. (b) Still higher temperatures (22°) overrule the previous inhibition. (c) The inhibiting effect of temperatures around 15° increases with prolonged exposure of tubers to darkness and low, stratifying temperatures (i.e. about 5 °C).

Experiment 3 was done after termination of experiment 1, i.e. the tubers of experiment 3 had been stored at 4 °C for 2 months longer. Statements (b) and (c) explain the presence of 'stage 3' plants at 22° and the absence at 15° in experiment 3, while 'stage 3' plants were present in experiment 1.

Thus it appears that sprouting tubers of *P. pectinatus* that are exposed to temperatures above a compensation point between 13° and 15 °C immediately following winter stratification have the capacity to develop a secondary dormancy. This can be of adaptive value in habitats that may dry out during summer. Tubers that become secondary dormant may also survive another winter.

Madsen & Adams (1988b) also studied the sprouting of *P. pectinatus* tubers as a function of temperature and light. They collected tubers in March, stored them also at 4 °C prior to use and found a temperature range for germination of 17° - 26 °C, while the tubers grew hardly at 8 °C. Their results in the dark are not distinctly different from ours at comparable temperatures (i.e. their 17° and our 22 °C with shoot lengths of 6.0 and 8 cm, after 28 days, compare Fig. 3.2). Their 8 °C dark incubation had significantly shorter lengths (0.5 cm) than our 13 °C treatment. A shoot length of 0.5 cm may indicate that the tubers had not yet sprouted, since unsprouted tubers of 100 - 200 mg fw had shoot lengths of about 1.5 cm in the present study (following our definition of a shoot). We accidentally placed a batch of tubers at 8° in a dark temperature room in February: they germinated readily and many tubers had reached stage 3. Therefore we tentatively assume that germination rates at 8° are not very different from those at 13°, at least for the presently studied population.

The difference in germination in the 8° dark incubation of Madsen & Adams (1988b) as compared to our results can be explained in various ways: geographical

differences in environmental conditions, variation between populations as well as differences in collecting time (March versus January) may be held responsible.

Plant growth in the light, however, was far less in the experiments of Madsen & Adams (1988b) than in our experiment 1 (they found shoot lengths of 0.5, 9.5 and 8 cm respectively at 8°, 17° and 22°, and 60 $\mu E\ m^{-2}\ s^{-1}$, compare with Fig. 3.2). This difference may be attributed to crowding (they used 10 tubers in a 50 ml Erlenmeyer with 25 ml medium), a difference in initial tuber weight, the absence of sediment or low irradiances (self-shading) in the Erlenmeyer flasks.

All in all, a fairly complex picture emerges of the regulatory role of temperature being different in different phases of the life cycle. While the growth of adult plants appears to be enhanced at 15° and higher, the sprouting of tubers in spring seems to be stimulated most at temperatures below 15°. An inhibiting effect of temperatures around 15° apparently gains force with prolonged exposure to darkness and stratifying temperatures.

Acknowledgements

Dr. M.A.J. van Montfort (Wageningen Agricultural University, Department of Mathematics) is thanked for statistical advice on multiple comparisons of fitted curves, Prof. Dr. W. van Vierssen and Prof. Dr. W.J. Wolff critically read the manuscript.

5. References

Abrahamson, W.G., 1980. Demography and vegetative reproduction. In Solbrig, O.T. (ed.), Demography and evolution in plant populations. Blackwell, Oxford, pp. 89-106.

Barko, J.W. & Filbin, G.J., 1983. Influences of light and temperature on chlorophyll composition in submersed aquatic macrophytes. Aquat. Bot. 15: 249-255.

Barko, J.W. & Smart, R.M., 1981. Comparative influences of light and temperature on the growth and metabolism of selected submersed freshwater macrophytes. Ecol. Monogr. 51: 219-235.

Barko J.W., Hardin, D.G. & Matthews, M.S., 1982. Growth and morphology of submersed freshwater macrophytes in relation to light and temperature. Can. J. Bot. 60: 877-887.

Berry, J.A. & Raison, J.K., 1981. Responses of macrophytes to temperature. In Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (eds), Physiological Plant Ecology I, responses to the physical environment. Encyclopedia of Plant Physiology, new series, volume 12a, Springer Berlin, pp. 277-338.

Björkman, O., 1981. Responses to different quantum flux densities. In Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (eds), Physiological Plant Ecology I, responses to the physical environment. Encyclopedia of Plant Physiology, new series, volume 12a, Springer Berlin, pp. 57-108.

Bulthuis, D.A., 1987. Effects of temperature on photosynthesis and growth of seagrasses. Aquat. Bot. 27: 27-40.

Eppley, R.W., 1972. Temperature and phytoplankton growth in the sea. Fishery Bull. 70: 1063-1085.

Harris, G.P., 1978. Photosynthesis, productivity and growth: the physiological ecology of phytoplankton. Erg. Limnol. 10: 1-171.

Hodgson, R.E., 1966. Growth and carbohydrate status of Sago Pondweed. Weeds 14: 263-268.

Hootsmans, M.J.M. & Vermaat, J.E., 1991. Light-response curves of *Potamogeton pectinatus* L. as a function of plant age and irradiance level during growth. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Jeffrey, S.W., 1981. Responses to light in aquatic plants. In Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (eds), Physiological Plant Ecology I, responses to the physical environment. Encyclopedia of Plant Physiology, new series, volume 12a, Springer Berlin, pp. 249-276.

Madsen, J.D. & Adams, M.S., 1988a. The seasonal biomass and productivity of the submerged macrophytes in a polluted Wisconsin stream. Freshwat. Biol. 20: 41-50.

Madsen, J.D. & Adams, M.S., 1988b. The germination of *Potamogeton pectinatus* L. tubers: environmental control by temperature and light. Can. J. Bot. 66: 2523-2526.

Morgan, D.C. & Smith, H., 1981. Non-photosynthetic responses to light-quality. In Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (eds), Physiological Plant Ecology I, responses to the physical environment. Encyclopedia of Plant Physiology, new series, volume 12a, Springer Berlin, pp. 109-134.

Norusis, M.J., 1986. SPSS-PC$^+$ manual. SPSS Inc., Chicago, USA, 559 pp.

Pianka, E.R., 1976. Competition and niche theory. In May, R.M. (ed.), Theoretical ecology, principles and applications. Blackwell, Oxford, pp. 114-141.

Salisbury, F.B., 1981. Responses to photoperiod. In Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (eds), Physiological Plant Ecology I, responses to the physical environment. Encyclopedia of Plant Physiology, new series, volume 12a, Springer Berlin, pp. 135-167.

Silvertown, J.W., 1982. Introduction to plant population ecology. Longman, London, 209 pp.

Spence, D.H.N. & Dale, H.M., 1978. Variations in the shallow water form of *Potamogeton richardsonii* induced by some environmental factors. Freshwat. Biol. 8: 251-268.

Spence, D.H.N. & Chrystal, J., 1970a. Photosynthesis and zonation of freshwater macrophytes I. Depth distribution and shade tolerance. New Phytol. 69: 205-215.

Spence, D.H.N. & Chrystal, J., 1970b. Photosynthesis and zonation of freshwater macrophytes II. Adaptability of species of deep and shallow water. New Phytol. 69: 217-227.

Spencer, D.F., 1986. Early growth of *Potamogeton pectinatus* L. in response to temperature and irradiance: morphology and pigment composition. Aquat. Bot. 26: 1-8.

Spencer, D.F., 1988. Tuber size and planting depth influence growth of *Potamogeton pectinatus* L. Am. Midl. Nat. 118: 77-84.

Spencer, D.F. & Anderson, L.W.J., 1987. Influence of photoperiod on growth, pigment composition and vegetative propagule formation for *Potamogeton nodosus* Poir. and *Potamogeton pectinatus* L. Aquat. Bot. 28: 103-112.

Steel, R.G.D. & Torrie, J.H., 1980. Principles and procedures of statistics, a biometrical approach. Second edition, McGraw-Hill Book Company, Singapore, 633 pp.

Tobiessen, P. & Snow, P.D., 1984. Temperature and light effects on the growth of *Potamogeton crispus* in Collins Lake, New York State. Can. J. Bot. 62: 2822-2826.

Van Wijk, R.J., 1989. Ecological studies on *Potamogeton pectinatus* L. III. Reproductive strategies and germination ecology. Aquat. Bot. 33: 271-299.

Van Wijk, R.J., Van Goor, E.M.J. & Verkley, J.A.C., 1988. Ecological studies on *Potamogeton pectinatus* L. II. Autecological characteristics, with emphasis on salt tolerance, intraspecific variation and isoenzyme patterns. Aquat. Bot. 32: 239-260.

Vegis, A., 1973. Dependence of the growth processes on temperature. In Precht, H., Christophersen, J., Heusel, H. & Larcher, W. (eds), Temperature and life. Springer, Berlin, pp. 145-171.

Vermaat, J.E. & Hootsmans, M.J.M., 1991. Intraspecific variation in *Potamogeton pectinatus* L., a controlled laboratory experiment. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

LIGHT-RESPONSE CURVES OF *POTAMOGETON PECTINATUS* L. AS A FUNCTION OF PLANT AGE AND IRRADIANCE LEVEL DURING GROWTH

4

M.J.M. Hootsmans & J.E. Vermaat

Abstract

Macrophytes usually play a dominant role in a shallow aquatic ecosystem. Thus, among others, knowledge of plant photosynthesis in relation to light conditions and plant age is important to understand the functioning of this system. The relation between the rate of net photosynthesis P and light intensity I, the P-I curve or light-response curve, can be described with numerical equations incorporating the maximum rate of gross photosynthesis Pm, the rate of respiration R and a parameter determining the initial slope of the curve (e.g. the initial slope α or the light level Km at which the rate of gross photosynthesis equals half Pm).

In the first part of this chapter, attention is paid to curve-fitting methods, stressing the importance of unbiased, i.e. simultaneous estimation of all parameters needed in the P-I model. The results of various authors comparing the relative performance of different P-I models are discussed, leading to the conclusion that at the moment, no definitely 'best-fitting' model can be discerned. We decided to use both the hyperbolic tangent model (tanh-model), which is often considered the 'best' model, and the often used rectangular hyperbola or Michaelis-Menten model (MM-model). The latter model has the advantage that it can be integrated but it is sometimes regarded as 'poor' with regard to curve-fitting capabilities. A method for statistical comparison of parameter estimates from different curves is presented.

Accuracy of the three basic methods for photosynthesis measurements currently available (^{14}C, dissolved inorganic carbon, oxygen) is briefly discussed. In this study, the oxygen method was used with continuous registration of electrode signals. This enables the distinction of lag phases in plant response to changing conditions. In our case, the possible measurement error caused by storage of gases in internal lacunae was estimated to be less than 10%.

In the second part, the results from various photosynthesis experiments with *Potamogeton pectinatus* L. plants grown in the laboratory and in the field are presented. With regard to curve-fitting capabilities, the two models used were not significantly different. However, some datasets had to be described with a vertical asymptote by the MM model. In those cases, the tanh model still gave the familiar curve shape. Thus, for this subjective reason, the tanh model appeared 'better'.

In the laboratory cultures, plants were grown at 50, 100, 150 and 200 $\mu E\ m^{-2}\ s^{-1}$ for 30, 70 and 120 days. Effects of age and light were found on nutrient (i.e. N and P) content of aboveground and belowground plant material. N and P levels were lower for plants of higher age. Higher light levels during growth (referred to as light history level) resulted in a reduced P content. Both nutrients showed higher levels in aboveground tissue. The N/P molar ratio was reduced in aboveground tissue

with higher age. Both tissue compartments had a lower N/P ratio for lower light history levels.

Various morphological characteristics were affected. Higher age coincided with increased plant length and number of leaves per plant, decreased total leaf biomass per total aboveground biomass (leaf ratio) and decreased mean biomass of a leaf. Higher light history level resulted in more leaves per plant, reduced plant length, more secondary shoots and increased leaf ratio.

Light and age interacted in their effect on total chlorophyll (chl(a+b)) content of leaves and of total aboveground biomass. For lower light history levels, leaves had a lower chl(a+b) content with increased age. The two highest light history levels showed a decrease up till 70 days, but this tendency was reversed at 120 days. In total aboveground biomass, chl(a+b) content at 30 days became lower when light history levels decreased but increased with lower light history levels at age 70 days. At age 120 days this light effect was more or less absent. In general, chl(a+b) in total aboveground biomass was lower for higher age. Fraction chl(b) of total chlorophyll showed a slight increase only for age 70 days.

Effects of light history and age on photosynthesis of *P. pectinatus* were studied both by means of modelparameters of fitted P-I curves and with four derived parameters: gross and net rate of photosynthesis at 200 μE m^{-2} s^{-1}, referred to as GP200 and NP200, respectively, light compensation point LCP and the ratio Pm/R.

Laboratory grown plants from low light history levels had lower Pm, Km, Pm/R, GP200 and NP200 while LCP, R and α were not affected. Increasing age resulted in decreased Pm, R, Pm/R, GP200 and NP200 while LCP increased. Km and α were unaffected. Corrected for differences in chl(a+b) content of plant tissue, R slightly increased both with higher light history level and increased age.

P. pectinatus plants from the field showed a decrease in chl(a+b) content both of leaves and of total aboveground biomass with higher light history levels in June, but an increase with higher light history levels in July. Higher age coincided with lower chl(a+b) content. Fraction chl-b slightly decreased with higher light history levels and higher age.

A lower light history level correlated with increased Km and LCP. Higher age resulted in lower Pm, α, GP200 and NP200, while Km and LCP increased. Differences in response between field and laboratory material are attributed to differences in light history level during growth (higher in the field), and the actual age of plant tissue, which in field material is rather difficult to establish.

We conclude that *P. pectinatus* did not respond to low light intensities by a rigorous acclimation of its photosynthetic capabilities. In its normally shallow habitats, morphological adaptations, e.g. rapid shoot elongation and concentration of biomass in the upper water layer, possibly limit the necessity for such physiological changes.

Contents

1. Introduction

In the littoral zone of an aquatic ecosystem, energy flow and nutrient cycling usually are determined to a large extent by macrophytes. With respect to primary production the epiphytic algal community can be of even greater importance, especially in eutrophic lakes. Still, for their substratum these algae are almost totally dependent on the macrophytes. Examples are given in Wetzel (1983).

Knowledge of photosynthesis in aquatic macrophytes is crucial when insight in the functioning of a shallow macrophyte-dominated system is desired. More specifically, the relationship between photosynthesis and light, and the influence of various factors on this relationship, have to be known. In this chapter, light is defined as photosynthetically active radiation (PAR; McCree, 1972a; 1972b), meaning the incident quantum flux for which photosynthetic pigments are mainly sensitive (band 400-700 nm).

Many studies on photosynthesis in the aquatic environment have been published. Examples of the so-called photosynthesis versus light (P-I) curves or light-response curves can be found in Wetzel (1983). Most studies are concerned with phytoplankton. Usually, when P-I curves of macrophytes are determined, the plant is reduced to parts (i.e. leaves or parts of leaves; Drew, 1979; Kerr & Strother, 1985; Orr et al., 1988). Although such studies give insight in the process of photosynthesis, it is difficult to reconstruct the possible results for a whole plant from these data. An intact plant is an intricate combination of photosynthesizing and respiring tissues of different age and condition.

A further complication is that results are often expressed per unit of chlorophyll instead of (aboveground) biomass. Of course, the rate of photosynthesis is dependent on the chlorophyll content of the tissues (Gabrielsen, 1948), but the available amount of enzymes for the dark reaction also plays an important role (Wareing et al., 1968). However, as with data based on plant parts, extrapolation of results based on chlorophyll content to results for the whole plant is difficult without knowledge of the amount of chlorophyll per unit plant weight and the distribution of chlorophyll over the plant. A rapid change (within an hour) in the amount of chlorophyll-a and -b in response to light level was observed by Jiménez et al. (1987) for the seagrass species *Zostera noltii* Hornem. and *Zostera marina* L. If chlorophyll content is indeed this variable, then biomass might be a better reference basis for a P-I curve made by using the same plant material for all light levels.

When the light-response curve of an intact plant is known, the problem of extrapolation of the results to the whole vegetation is reduced. A vegetation consists of plants of different ages that are and have been exposed to different light levels. When the effect of light and age on the light-response curve of a single plant is known, the photosynthesis of a vegetation with a known age structure and light history can be calculated. We define light history as the mean amount of light to which a vegetation unit (plant, amount of biomass) has been exposed throughout its existence. Both age structure and light history are not easily measured in the field but can be calculated in a simulation model along with the light profile in the vegetation (Hootsmans, 1991).

Conclusions on photosynthesis-light relationships are based on a study of parameters derived from the light-response

curve. In other words, conclusions also depend on the technique by which experimental data are summarized in a number of parameters, and on the subsequent statistical analysis of the results. With respect to fitting techniques and model choice, several studies are available. However, up till now, not much attention has been paid to the use of statistical methods when comparing light-response curves. Only few studies exist in which parameters from P-I relations are mathematically derived and statistically tested to draw conclusions. Examples are Platt & Jassby (1976) and Madsen & Adams (1989).

This chapter will focus on three different subjects. We will start with a discussion of curve-fitting and the use of statistical methods in the study of light-response curves. Subsequently, attention is paid to the oxygen method for measuring photosynthesis in macrophytes. Finally, we present the results from the application of these techniques in a laboratory study of photosynthesis-light relationships in two macrophyte species: *Potamogeton pectinatus* L. and *Potamogeton perfoliatus* L., with emphasis on the former. Both species are present in considerable densities in Lake Veluwe.

2. Curve-fitting and the analysis of resulting parameter estimates

2.1 Introduction

When measurements of photosynthesis at different light intensities have been made, the usual result is an initially linear increase in the rate of photosynthesis with increasing light levels, followed by a leveling off to a maximum. Sometimes, at very high light intensities, a decrease in photosynthesis due to destruction or reorientation of photosynthetic organelles and/or pigments, photoinhibition, is observed (King & Schramm, 1976; Drew, 1979). Megard et al. (1984) have proposed a simple kinetic model in which photoinhibition is the result of a reversible inactivation of photosynthetic pigments because of the absorption of extra quanta. In the following we assume that photoinhibition does not occur. However, the discussion for situations in which photoinhibition occurs is only different with respect to the mathematical model that is needed to describe the curve.

Usually, in a graph with this kind of data a curve that more or less fits the data is added. Curves are often fitted by eye or are simplified to a linear regression of the light-limited part of the curve and a subjectively chosen maximum rate of photosynthesis (e.g. Drew, 1979). Nothing is wrong with this, but such subjective fits and the derived parameters do not satisfy basic statistical requirements. Hence, a statistically sound comparison of curves is not possible. A statistical test is necessary as an objective criterium with which the magnitude of observed differences can be judged; without it, we can only discuss subjective 'tendencies'. Thus, first of all it is necessary to use an objective curve-fitting technique to arrive at estimates of those parameters that are of interest to the experimenter.

The above is true for all studies of light-response curves, both with phytoplankton and with macrophytes. However, data for the different light levels are often obtained quite differently for these two

groups. In macrophyte studies the necessary measurements usually are done with the same plant material for all different light levels. As a consequence, data points for one light-response curve are not statistically independent. In phytoplankton studies, true replication of measurements for each light level is easily accomplished. One or more samples are used for each light level, and each sample is exposed to only one light intensity. In this way, all data points in the curve are independent from each other. Sometimes the same sample is exposed to all light intensities, giving the same dependency as in macrophyte studies (Iwakuma & Yasuno, 1983). An extensive discussion of the consequences of this so-called pseudoreplication is given by Hurlbert (1984).

In the following we will give an overview of several studies concerning curve-fitting and the validity of models that can be used. The consequences of the dependency of data in curves for macrophytes will be discussed along with a presentation of our own method for comparing different light-response curves.

2.2 Methods for fitting non-linear models

What is an objective curve-fitting technique? The above mentioned approach of distinguishing a light-limited linear part and a horizontal light-saturated part certainly is simple and straightforward. However, the experimenter must make the subjective decision which datapoints to include in which part. Despite the argument that such a decision when made by several people results in almost the same division (Jassby & Platt, 1976), the fact remains that the resulting parameter estimates are biased and thus cannot be subjected to a statistical test. Besides this fundamental objection, the model of course is not a very good representation of the non-linear nature of a photosynthesis-light relation. However, it is not always necessary to reject a simple linear relation. In some cases, depending on the light levels used in the experiment, light saturation does not occur within the range of the data set. An example is the study of Spence & Chrystal (1970).

When light saturation does occur, a non-linear model must be used. Many different models are available; examples are given in Jassby & Platt (1976), among others. In our analysis, we restricted ourselves to two different functions: the often used rectangular hyperbola (equation 1), also known as the Michaelis-Menten model, and the hyperbolic tangent (equation 2), introduced by Jassby & Platt (1976). In both functions, three parameters are used to fit a curve describing the relation between the independent variable light (I) and the dependent variable net productivity (P). Pm is the maximum rate of gross productivity; α is the slope of the curve at low (not-saturating) light levels; Km (the Michaelis-Menten constant in enzyme kinetics) is the light level where gross productivity is half the maximum gross productivity Pm; R is respiration. When only gross productivity is modelled, the parameter R is left out.

$$P = Pm * I / (Km + I) - R \qquad (1)$$

$$P = Pm * \tanh (\alpha * I / Pm) - R \qquad (2)$$

The first derivative of (1) is

$$dP/dI = Pm * Km / (Km + I)^2 \qquad (3)$$

For I=0, dP/dI is equal to α, the initial slope of the light response curve.

This results in:

$$\alpha = Pm / Km \qquad (4)$$

The light level at which the line described by $P = \alpha * I$ is equal to Pm is defined as the light level I_k (Talling, 1957). From (4) it can be seen that this parameter, used to indicate the onset of light saturation, is equal to Km.

Parameter estimates for non-linear models are not easily obtained. Sometimes, as in the case of a rectangular hyperbola without respiration, the function can be transformed into a linear equivalent by inversion. Using linear regression, parameters can then be calculated that minimize the sum of squared differences between measured and predicted values of the dependent variable. In the following, this so-called residual sum of squares of errors is referred to as RSS. However, inversion of the original data values means that the relative contribution of the different data pairs to the RSS is changed. Depending on the variation in the dataset, the resulting parameter estimates can be seriously biased (Dowd & Riggs, 1965). When the estimated parameter values are backtransformed, the resulting RSS usually is not the minimum RSS for the non-linear model. The same holds for other transformations.

The only proper way to fit a non-linear model is a direct estimation of the parameters using an iterative method that minimizes the RSS through small changes in initial parameter estimates. Even the best parameter values for non-linear models, in terms of minimum RSS, are estimates: up to now there is no method that can directly calculate the parameter set as in linear regression analysis. A commonly used method is Marquardt's algorithm, described in Conway et al. (1970). Final estimates are reached when a certain criterion is fulfilled. Usually, this is a minimum change in RSS resulting from a change in the parameter values. An illustrative example of the difference in the results from a non-linear fit and a backtransformed linear fit is given in Fig. 4.1. It is based on one of our datasets. The rectangular hyperbola can only be inverted when respiration is added as a known third parameter. Therefore, the respiration R measured during this particular experiment was added to all datapoints. The curves were fitted to the resulting dataset without values for the zero light level. The rectangular hyperbola

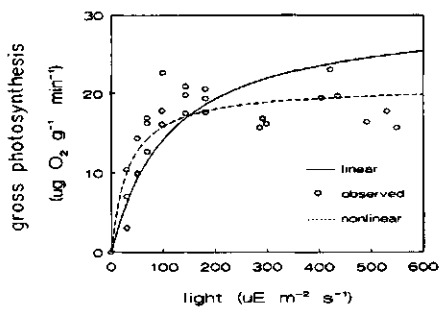


Fig. 4.1. Comparison of two fitting techniques for a rectangular hyperbola (see the text).
(a) Inverting the data followed by linear regression results in the curve indicated with linear.
(b) Direct parameter estimation with a non-linear fitting method gives the curve indicated with non-linear. Data values are indicated with observed. All weights in this and following figures are in g ash-free dry weigth, unless specified otherwise.

fitted by inversion and backtransformation has a RSS of 572.15, while the non-linear fitting method resulted in a RSS of 237.25, both with 25 degrees of freedom. These RSS's differ significantly ($F=2.412$, $p<0.025$).

An important aspect of fitting a model is that parameters must be determined simultaneously. Only in this way,

parameter estimates are independent and can be statistically analysed. Jassby & Platt (1976) did use a non-linear fitting method for a comparison of eight different models. However, they were not satisfied with the fact that the resulting estimates for parameter α differed for the different models and from subjective estimates of α based on the low-light part of the curve. Even worse, the estimates were 'far outside the range of published values'. Therefore they reverted to a linear regression of the low-light part of the curve to estimate α, and subsequently used this estimate as a constant in the non-linear curve-fitting process. Clearly, as has been explained in the beginning of this paragraph, this method is questionable.

Lederman & Tett (1981) pointed out that Jassby & Platt (1976) choose this fitting method because of their strict biological interpretation of model parameters: i.e., all models using the same parameter are estimating the same biological property. Thus, estimates of the same parameter in different models should be interchangeable. Our two models also use the same parameters (as shown in eq. 4, α and Km are related via Pm). However, when both models are fitted to the same dataset, the resulting parameter estimates are those that minimize the RSS for each model. There is no reason why two mathematically different models should have to use the same parameter values to describe the same curvature. This may result in differing parameter estimates, while there is no reason to reject one of the two models on the basis of the RSS. We agree with Lederman & Tett (1981) that the parameter estimates can be seen as sample statistics estimating population parameters that are dependent upon the underlying biochemical processes. When models are used that describe these processes in much more detail, estimates of the same parameter in different models are more likely to be interchangeable. In the light of our current level of understanding and especially because of the error in measurements, the development of such intricate models seems not yet rewarding. Therefore, parameter estimates can be seen as the best estimates of the biological parameters which they represent, but only in the model for which they were estimated.

2.3 Choosing the right model

Many different models have been used to describe P-I relationships. For choosing the 'best' model, some criterion must be found. Several authors have studied this problem. In the following, we will discuss the results of some of them. Often, the conclusion is drawn that the rectangular hyperbola is one of the poorest models to fit a P-I curve and should be rejected. We will try to show that this verdict probably depends on the use of fitting techniques which were incorrect (as outlined in the previous section).

In the study of Jassby & Platt (1976) eight different models were compared. Using the approach outlined in 2.2, they fitted an extensive data set of 188 light-response curves obtained for marine phytoplankton to each of these models. Every curve was based on 12 to 20 independent data pairs. As criterion for goodness-of-fit for each model i they used the mean squared error, defined as the sum of RSS's found with this model for all experiments, devided by the number of experiments. Apart from this criterion, Jassby & Platt also used N_i, defined as the number of times model i gave the lowest RSS for an experiment. With both methods, they found that the rectangular hyperbola in most cases gave bad fits, and

the hyperbolic tangent often the best.

Lederman & Tett (1981) repeated the work of Jassby & Platt (1976) using the same data set, but they estimated all modelparameters independently. As test criterion they used the sum of all RSS's found for a model. Models were compared by dividing these overall RSS's by the smallest overall RSS and comparing the result with an F-test. Number of degrees of freedom was calculated as: (number of observations for the curve - number of modelparameters) * (number of curves). They found that they could not distinguish between five of the eight models: the rectangular hyperbola and a straight line resulted in a significantly poorer fit, but it was not particularly bad. They concluded that a rejection of the rectangular hyperbola seemed not justified, also regarding other aspects such as the possibility to integrate the equation (this is not possible with the hyperbolic tangent).

Chalker (1980) found the hyperbolic tangent as the only biologically realistic solution for a quadratic expansion of dP/dI in P. In his introduction, he rejects the rectangular hyperbola because 'it usually yields a poor fit to experimental data. The predicted rates of photosynthesis at relatively low irradiances are usually greater than the observed data.'. As support, light-response curve data were fitted with the inverted version of this model. However, we cannot accept his conclusions as objections can be raised against this fitting method (see section 2.2), and no results of statistical tests were provided.

Iwakuma & Yasuno (1983) also compared several equations. Their criterion for goodness-of-fit was the mean RSS per measurement, calculated as the sum of RSS's for all experiments divided by the total number of measurements in all these experiments. Iwakuma & Yasuno (1983) also employed N_i. They concluded that the rectangular hyperbola and the hyperbolic tangent, both described above, 'did not always fit well our empirical data'. As with RSS's, these mean RSS's can be compared with an F-test, using the same formula for the calculation of the degrees of freedom as Lederman & Tett (1981) did. We applied the F-test using the mean RSS values from Table 2 in Iwakuma & Yasuno (1983) and 170 degrees of freedom. Significant differences existed only between the linear model and all other models, and between an exponential saturation model and the hyperbolic tangent. Critical values for F at these large degrees of freedom were calculated with the SAS statistical package (SAS Institute Inc., 1985).

Cossby et al. (1984) found no significant difference in goodness of fit between the models tested, including the rectangular hyperbola and the hyperbolic tangent. They used a different discrimination method, the so-called extended Kalman filter, described in Cossby & Hornberger (1984).

Pokorný et al. (1984) were not able to fit the rectangular hyperbola to their data on net photosynthesis. However, they did not include a parameter for respiration in the equation, making it impossible for the model to generate the necessary negative values at low light intensities. Thus, the rectangular hyperbola cannot be rejected on the basis of their results.

Orr et al. (1988) fitted both the rectangular hyperbola and the hyperbolic tangent model to light-response curve data. They used subjective estimates of net Pm, which were combined with dark respiration measurements to obtain gross Pm. Subsequently, α was fitted with a non-linear curve fitting technique. They arrived at the same conclusion as Jassby

& Platt (1976) who also estimated one of the modelparameters separately: 'it was visually evident and statistically shown that the model proposed by Jassby & Platt (1976) (i.e. the hyperbolic tangent model; MH & JV) provided the closest approximation to the data in all cases'. Regrettably, no statistical information was given, and again, objections can be raised against the fitting method used.

For fitting our own data sets we have used both the rectangular hyperbola and the hyperbolic tangent. Curves were fitted as described in section 2.2. The resulting curves were tested for a statistically significant difference in the goodness of fit between the two equations. The results, presented in section 5.3.2, did not lead to the rejection of one of the two models.

The conclusion is, that given the inherent error in even the best data sets available, it is impossible to make an objective choice between several non-linear models that are often used in fitting light-response curves. Therefore, there is no objective reason to reject the relatively simple rectangular hyperbola.

2.4 Comparing parameter estimates for different curves based on the same model

After choosing a model and fitting it with a correct technique, the problem remains of comparing the estimated parameter values for different data sets. An estimation of parameter variances is needed. Normally, in linear regression, these are obtained by calculation of the so-called variance-covariance matrix. Conway et al. (1970) have described a technique in which the non-linear model is approximated by a linearized Taylor series expansion, in the vicinity of the least-squares parameter estimates. After this, the variance-covariance matrix can be calculated. This method is valid as long as the estimated confidence intervals do not extend beyond the region for which the expansion is valid.

Another, much more time-consuming method, is the Monte Carlo approach (Silvert, 1979; Gallegos & Platt, 1981). In this method, the best parameter estimates are taken to simulate a data set for the light levels that were used. To each estimated value a random error term with mean zero and variance equal to that of the original data set is added. This variance can be estimated by dividing the RSS of the fitted model by n-k degrees of freedom (n=number of datapoints, k=number of model parameters). The new data set is fitted again, giving a new set of parameter estimates. When this procedure is repeated often enough, a reasonable estimate of parameter variance can be calculated from the replicate parameter estimates.

Zimmerman et al. (1987) compared the 'linearization method' with the 'Monte Carlo method' and found close agreement of the results.

Several software packages provide a non-linear fitting technique based on the Marquardt algorithm and give parameter estimates together with their standard errors (se). For comparisons, however, these se's can only be used when the estimation of the parameters is based on a set of independent data points. As was described in section 2.1, especially in macrophyte studies but sometimes also in phytoplankton work, the condition of independency of datapoints in the curve is not met. In this case, none of the methods described can be used to estimate parameter variance.

We solved this problem in the same way as Madsen & Adams (1989) by using

the parameter estimates from separate, replicate light-response curves as a sample from which mean and variance could be calculated. The number of replicate curves and the number of light levels per curve that can be measured with macrophytes are usually rather limited for practical reasons. Therefore, the calculated confidence limits may become rather wide. Some cold comfort may be the remark of Silvert (1979) that 'this is a frequent and painful conclusion that often arises out of proper analysis of the confidence limits'.

One final comment should be made on outliers. Results of experiments are subject to all kinds of variation, due to the method used and inherent in the material under inspection. The occurrence of outliers is therefore not uncommon. Sometimes the reason for their occurrence is clear, so that they can be nicely disposed of. Often, their cause is unknown. No definite method exists to make an objective decision whether to reject such values or not. Excluding such data inevitably introduces a subjectiveness in the composition of the data set that is to be studied, a fact against which we have objected in the previous sections. However, simply including outliers for objectiveness' sake also affects the results. Variation is increased and the chances for determining existing effects of experimental variation are reduced. In choosing between these two evils, we have used the following policy: if on visual inspection a case appeared to lie more than 100% above or below the value that was predicted on the basis of the rest of the data set, it was rejected.

3. Photosynthesis measurements in macrophytes with the oxygen method

3.1 Introduction

Up to now, three main methods for the measurement of photosynthesis exist. The ^{14}C method measures the incorporation of this carbon isotope into plant tissue. The oxygen method measures O_2 changes in the medium, and the DIC method measures changes of dissolved inorganic carbon (DIC) in the medium. All have their particular problems and advantages. Especially the first two methods are discussed in Vollenweider (1969). The three methods have not frequently been applied simultaneously to compare them. Examples are given by Kemp et al. (1986) and Lipkin et al. (1986).

We will discuss the performance of the oxygen method as compared with the other two methods, especially regarding the problem of lacunar storage and internal cycling of oxygen. Subsequently, the technical aspects of measurements with an oxygen electrode will be treated.

3.2 Comparison of methods and the consequences of lacunar space for the oxygen method

Lindeboom & De Bree (1982) used the oxygen method and the ^{14}C method for measuring benthic primary production of a *Z. marina* dominated community. When determining an annual mass balance, the oxygen method gave much better results: O_2 production and O_2 consumption were balanced, while the O_2 consumption estimate was twice as high as the ^{14}C production estimate. However, O_2

measurements were done with an intact community of *Z. marina*, while ^{14}C uptake was measured with isolated young shoots, making a direct comparison difficult (cf. section 1).

Lipkin et al. (1986) compared growth rates based on biomass measurements with estimates based on photosynthesis measurements. They found that the three different techniques could predict these growth rates with varying success for two different plant species (the angiosperm *Ceratophyllum demersum* L. and a red alga, *Gracilaria* sp.). For the alga, the O_2 and DIC method were comparable, but ^{14}C seriously underestimated the growth rate. For *C. demersum* the results were much less in agreement with each other. Kemp et al. (1986) measured biomass increase and O_2 production of *P. perfoliatus* for 6 weeks and found the total biomass increase to be almost identical to predictions based on oxygen production. Both studies found no reason to favour one of the three methods for measuring productivity.

An important aspect when applying any method is that the response of a plant to a change in light intensity is not always instantaneous. One explanation for this phenomenon is the internal storage and/or cycling of O_2 and CO_2. Usually, this problem is regarded as being only associated with the O_2 method. Wetzel (1969) mentions it as 'an important limitation and source of error in the application of the oxygen techniques' and Zieman & Wetzel (1980) therefore discouraged the use of this method. However, as Zieman & Wetzel (1980) also point out, incorporated carbon can also be respired and released as CO_2 in the lacunar space of a macrophyte and thus the ^{14}C and DIC method in principle suffer from the same problem.

Westlake (1978) presented data on the duration of the lag phase of oxygen production in a stirred situation before a constant rate was reached. This time was on average 9 minutes for various experiments with *Myriophyllum spicatum* L. and *Vallisneria americana* Michx. For the first species, it could be less than one minute. For the bryophyte *Fontinalis antipyretica* L. and the alga *Cladophora* sp. the time was on average 2 minutes. Kelly et al. (1981) present a graph of net community productivity measured with oxygen electrodes in a Danish river, related to solar insolation. The system showed only short time lags (10 minutes) following changes in insolation. In the laboratory they studied the duration of the lag phase for 9 species of aquatic macrophytes, in an experiment in which plants were exposed to subsequent short periods of light (22 min) and dark (38 min). When going from dark to light, duration of the lag phase was 5-13 min. When going from light to dark, this was 9-24 min. In contrast with Westlake (1978), they found no differences between vascular plants, the alga *Cladophora glomerata* (L.) Kütz. and the bryophyte *F. antipyretica*. The latter two have no aerenchyma and cannot store gases. Kelly et al. (1981) therefore concluded that the cause of the lag phase is not so much storage in aerenchyma, but the rate of exchange of gases between plant tissue and the surrounding water. Indeed, Madsen & Søndergaard (1983) found a stimulation of the oxygen exchange rate of *Callitriche stagnalis* Scop. by stirring the medium.

However, Sorrell & Dromgoole (1986) point out that although the lag phase may be short, internal storage of oxygen can still be important due to its low solubility in water compared to air. They measured oxygen exchange rates of *Egeria densa* Planch. with intact plants and plants in which all lacunar space was filled with water. They found that O_2

production was underestimated with 7-17%, and respiration with 23-53%, when internal storage was neglected. Lag periods were short (7-10 min) and not different for the two treatments. This indicates that a short lag period does not mean that internal storage is negligible. The error in O_2 production was in close agreement with the expected distribution of the produced amount of oxygen over the available air and water space, based on the different solubilities of oxygen in air and water. Respiration error was 2-3 times larger than expected. As CO_2 has a much higher solubility in water than O_2, the expected error in production measurements based on DIC is 0.5%. Sorrell & Dromgoole (1986) therefore recommended the DIC method. Still, a lag phase has to be taken into account.

When an estimate of gross photosynthesis is needed, a problem is the quantification of respiration in the light. Usually, this is taken to be equal to dark respiration. Hough (1974) pointed out that this is not the case, partly due to refixation of respired carbon in the light (Søndergaard, 1979) and to photorespiration. Zelitch (1966) found for tobacco that photorespiration increased strongly above 30 °C (amounting up to 60% of gross photosynthesis) but that it was negligible at 25 °C. This seems to be confirmed by the results of Jana & Choudhuri (1979) who measured very high rates of photorespiration (even higher than dark respiration) in three aquatic macrophytes at 35 °C, while both Hough (1974) and Søndergaard (1979), among others, found light-dark ratios of respiration normally to be less than unity at 20-25 °C. The latter two authors found light respiration rates usually to be less than 10% of net photosynthesis. Thus, photorespiration below 30 °C may not be very important (as long as oxygen concentrations are not high), but refixation in aquatic macrophytes can be very efficient at these temperatures. Due to refixation, precise measurements of light respiration are difficult. Therefore, light-response curves and concomitant estimates of gross photosynthesis and dark respiration may be erroneous when used to predict 'real' gross photosynthesis and 'real' light respiration, but are correct when used to predict 'real' net photosynthesis and growth. And for the plant, net photosynthesis determines possibilities for growth.

A consequence of the occurrence of a lag phase in the response to a change in light level is that a more or less continuous registration of the concentration may be needed. This necessitates repetitive sampling which is much more tedious and can never be done as continuous with the ^{14}C or DIC method as with an oxygen electrode.

Besides, the changes in oxygen or DIC concentration that are measured in short-term photosynthesis measurements are usually small. A reasonably accurate estimate of the rate of photosynthesis is difficult to be obtained when only a few samples are taken and analyzed with the Winkler method for oxygen, or with an infrared gas analyzer for DIC. Although it has a lower accuracy for each single sample than the Winkler method, an electrode allows for very frequent sampling, i.e. it can give a continuous picture of the process. The rate of change in the oxygen concentration is estimated by the regression coefficient in a linear regression. The variance of this parameter is dependent not only on the error within each measurement, but it is also inversely related to the number of measurements. This means that on average, the rate estimate based on many electrode measurements will be more reliable than

the result from a few Winkler data, as long as the error in the electrode measurements is not excessive. The continuous registration furthermore enables the detection of lag phases which subsequently can be excluded in the analysis, thereby improving the accuracy.

3.3 Oxygen electrodes

Several types of electrodes for the measurement of oxygen exist. The main difference is between galvanic cells and polarographic cells. In the first type, reduction of oxygen is a spontaneous process generating a current that can be measured directly or via a resistance as a potential difference. In the second type, the reaction must be driven by an externally generated potential difference over cathode and anode. Almost all commercially available electrodes are of the polarographic type. In our laboratory we had several electrodes of the galvanic type to our disposal. The type is very much comparable to the one described by Johnson et al. (1964) and Borkowski & Johnson (1967). As no external power source for polarisation is needed, this electrode is easy to use for long-term measurements in the field (which was the main reason for their acquisition by our laboratory).

The main problem with all oxygen electrodes is calibration. Membrane thickness, condition of the electrolyte, temperature and of course oxygen concentration influence the reading. Mancy et al. (1962) defined the steady-state current I at constant temperature as:

$$I = \frac{n * F * a * P * [O_2]}{b}$$

n = moles of electrons exchanged per mole of O_2 reduced
F = Faraday's constant
a = indicating electrode area (shown to be the membrane area by Briggs & Viney, 1964)
P = membrane permeability coefficient
b = membrane thickness
$[O_2]$ = oxygen concentration in sample

Apart from P, all constants in this formula are independent of temperature. Mancy et al. (1962) also provided a formula to compensate for this temperature dependence:

$$\ln(I/[O_2]) = k - m/T$$

in which m is the temperature coefficient for this specific membrane, and k is a combination of the other constants. This equation is used to calibrate an electrode when it is new; when subsequent drift occurs, the electrode is recalibrated by a correction of parameter k. Parameter m is considered constant as long as the membrane is not changed. Possible causes for drift can be fouling of the membrane and changes in membrane thickness due to changes in membrane tension.

Reference values for oxygen concentration can be found in oxygen saturation tables (Mortimer, 1981) or are directly measured with the Winkler method for determining dissolved oxygen (accuracy 0.1 %, Carpenter, 1965). Temperature compensation is usually done with a built-in thermistor and the temperature coefficient of the average membrane. This means that compensation is reasonable as long as membrane characteristics do not change much when membranes are changed. Thomsen & Thyssen (1979) found measurement errors of 10% for commercially available systems used at temperatures 5 - 10 °C above or below

the calibration temperature. When this error is to be avoided, it is advisable to perform calibration measurements at different temperature-oxygen combinations. In this way, an individually tailored oxygen-temperature calibration is reached for each electrode used (an approach we followed). Only a few commercially available systems allow for an automatic, electrode specific, temperature calibration.

4. Effects of light history and age on light-response curves and some other characteristics in *Potamogeton pectinatus* L. from laboratory cultures

4.1 Introduction

A monospecific vegetation of macrophytes may be seen as a rather simple community, easily described in terms of structure and function. However, this is not the case. Even when the epiphytic and benthic microalgal and faunal communities are omitted from consideration, such a vegetation still is a complex structure of plants that vary in age and in their perception of the environment. Young plants are short and thus more shaded than older plants, and grow but do not reproduce yet. Older plants have relatively more non-photosynthesizing tissue and may be in the process of vegetative and/or generative reproduction. It is very likely that these differences between plants of different ages have consequences for the resulting light-response curves. Effects of light history on chlorophyll-content and morphology are well known. Shaded plants have more chlorophyll-a and -b per unit biomass and a higher chl-b/chl(a+b) ratio (Boardman, 1977). They also show a stronger elongation of the shoots (Barko & Smart, 1981).

Description of a natural vegetation in terms of age structure and light history is tedious, if not impossible. Besides, such information will only be valid for a particular season and area. However, in a model vegetation age structure and light history can be calculated relatively easily. We hypothesize that when the effect of age and light history on light-response curves of individual plants is known, this will be the basis for any reasonably accurate simulation model of the growth and development of a vegetation.

In the following, photosynthesis results are presented for laboratory cultures of *P. pectinatus*. Data were gathered concerning the effects of light history and age on photosynthesis of intact plants. Information on these effects is almost absent for this species, but necessary if we want to predict the performance of a whole vegetation during the growing season in a model. Carbon availability and temperature were kept constant, since they too can influence the light-response curve (Barko & Smart, 1981; Sand-Jensen, 1983; Orr et al., 1988; Madsen & Adams, 1989). First, attention is given to effects of light history and age on nutrient content (nitrogen and phosphorus), plant morphology and chlorophyll content. These effects are then taken into account when the light-reponse curves for plants of different light history-age combinations are analyzed.

4.2 Material and methods

4.2.1 Plant material: collection and cultivation

In January 1986, tubers of *P. pectinatus* were collected in an experimental ditch at the Research Institute for Nature Management, Texel, The Netherlands. This locality was chosen for its high tuber density, enough to provide material for all experiments that were to be done as part of the whole research project on eutrophicated systems. This is the 'Texel' population discussed in Vermaat & Hootsmans (1991a). Water in this ditch was oligohaline (<3 ‰ Cl^-); tubers were stored at 4 °C and at the same chlorinity.

Before use, chlorinity was lowered to 0 ‰ by dilution with fresh tapwater in 24 hours. Tubers were planted in separate plastic coffee cups filled with 135 ml of a 1:3 clay-sand mixture. The cups were placed in four 100 l aquaria filled with tapwater and situated in a large flow-through waterbath. Water temperature was kept between 17 and 20 °C (night-day difference). To prevent algal growth, no nutrients were added. As light source 400 W Philips HPIT metal halide lamps were used, giving a light intensity of 200 μE m^{-2} s^{-1} at 1 cm below the water surface.

Four different light levels were created by covering three aquaria per set of four with neutral density netting of different mesh width: 200, 150, 100 and 50 μE m^{-2} s^{-1}. Light levels were measured with intercalibrated Licor 192s and Bottemanne underwater sensors. These cosine-corrected quantum sensors measure PAR.

In the period July - November 1986, 4 cultures were set up. The photoperiod was 16 h light - 8 h dark. Two sets started in July, which were used in September (age 70 days) and October (120 days). Another set was started in October and was used in November (30 days). The last culture was set up in November and was used in January (70 days). This last set replicated the culture used in September. In this way, we increased the amount of data on the effect of light history on photosynthesis. During the growing period, none of the plants formed tubers.

4.2.2 Experimental set-up for photosynthesis measurements

All photosynthesis measurements were performed in a 100 l perspex aquarium in a large flow-through bath which kept the temperature in the aquarium during the experiment between 18-20 °C. The aquarium was filled with tapwater and 20 g $NaHCO_3$ was added to arrive at saturating inorganic carbon levels (Sand-Jensen, 1983) and a pH comparable to that in Lake Veluwe.

Three independent, replicate closed systems were used. Each consisted of an Eheim circulation pump, perspex electrode chamber and a perspex tube of 40 cm length and 5 cm diameter, interconnected with pvc tubing. The tubes were submerged horizontally in the aquarium. Tube surface was kept 1 cm beneath the water surface.

Each tube had a thermotransducer for temperature registration during each photosynthesis measurement. Every 30 seconds electrode and transducer potentials were registrated with a Hewlett Packard datalogging set.

Light was provided by a Philips 400 W HPIT metal halide lamp. Different light levels were created by changing the distance between lamp and aquarium and using a neutral density filter. Illumination caused a temperature increase of 2 °C in

the September and October experiments. From November onwards, a shallow (5 cm) flow-through waterbath made of perspex was suspended beneath the lamp to absorb most of the infrared radiation. This restricted the temperature increase during the measurements to 1 °C.
Flow rate was 1.5 l min^{-1}, or about 12 mm s^{-1}, comparable to that in the set-up described by Sand-Jensen (1983). In a similar incubation chamber, Westlake (1967) found that rates of photosynthesis of *P. pectinatus* did not increase further above flow rates of 0.4 mm s^{-1}. Madsen & Søndergaard (1983) measured an optimum at 8-12 mm s^{-1} for *C. stagnalis*. When their shoot number was higher, photosynthesis at 20 mm s^{-1} was 10% higher than at 8 mm s^{-1}. In the light of these findings, we expect that our flow rate had no limiting effect on photosynthesis.

Four to six plants were used per tube. To reduce the amount of respiring material and thus to increase the net amount of oxygen produced, all rhizomes, roots and tubers were removed prior to the measurements. After at least thirty minutes in the dark (to deplete lacunar oxygen reserves), dark respiration of the plant material was measured for another 30 minutes. Subsequently, the tubes were exposed to 7-9 different light levels (15 minutes each), starting with the lowest intensity and ending with the highest. Between each measurement the tubes were opened and the medium inside was completely replenished with the surrounding water. Light intensities varied between 30 and 550 $\mu E\ m^{-2}\ s^{-1}$ at 1 cm below the water surface outside the tubes. As the light field could not be held homogeneous for the highest light levels, we measured light on three points along each tube and used the average intensity for further calculations.

Based on estimates of lacunar volume for our plant material and the volume of a measuring system we expect that the error in O_2 exchange rate on the basis of internal storage (Sorrell & Dromgoole, 1986) is less than 10% in our set-up.

4.2.3 Characterization of plant material

Per plant, six characteristics were analysed: number of leaves, number of secondary shoots, plant length, average leaf weight, leaf ratio (calculated as leaf biomass per total aboveground biomass), and the number of leaves per gram aboveground biomass.

A sample of 6-10 leaves per plant, covering the range of age and vitality, was taken. Fresh weight of this leaf sample and of the remaining plant was measured after blotting with tissue paper, and the leaf sample was frozen until chlorophyll determinations could be made. Subsequently, the remaining plantmaterial was divided in three fractions (aboveground, tuber and rhizomes with adhering roots), dried for at least 24 hours at 70 °C and weighed. A number of dried plants was chosen for nutrient determinations and stored at room temperature until further analysis. The rest of the plants was dried (105 °C) and ashed (520 °C) to obtain dry weight (dw) and ash-free dry weight (afdw). Using the appropriate ratios, the original amount of plant material for each replicate measurement (see section 4.2.2) in terms of afdw was calculated.

Chlorophyll-a and -b were determined with the method described in Vermaat & Hootsmans (1991a). Using the data on mean fresh weight of a leaf, aboveground plant fresh weight and the number of leaves per plant, the leaf-stem ratio was calculated. Together with the fresh weight to ash-free dry weight ratio

of aboveground material from the same experiment, the chl(a+b) content of the aboveground parts in terms of mg chl(a+b) g^{-1} afdw could then be estimated.

Total N and P content of aboveground and belowground material was determined spectrophotometrically with a Technicon autoanalyzer after digestion of about 300 mg dry weight with a mixture of sulfuric acid, salicylic acid, H_2O_2 and Se (Novozamsky et al., 1983). Material from several plants had to be combined to obtain two samples of aboveground and belowground material per experiment. In the September experiments with plants from 100 and 50 $\mu E\ m^{-2}\ s^{-1}$, only one sample was available due to the limited amount of plant material.

4.2.4 Calculations and statistical analysis

Each data set relating oxygen concentration with time for a particular light level was checked for measurement errors. Peaks or dips due to environmental noise and lag phases (cf. section 2.4) were excluded. O_2 exchange rates were calculated by linear regression and expressed in μg O_2 per g afdw of aboveground plant tissue per minute.

The resulting data set for each treatment replica consisted of the experimental light levels and the corresponding O_2 exchange rates. If outliers (as defined in section 2.4) occurred, they were removed. The corrected data set was fitted with the Marquardt algorithm. Both the hyperbolic tangent and the rectangular hyperbola, two models described in section 2.2, were used in order to compare their goodness of fit. The resulting model parameter estimates were used for comparisons, together with some derived parameters, e.g. the light compensation point (light level at which respiration and gross photosynthesis are balanced). Model parameter estimates may occur well beyond the measured light level range. As confidence intervals consequently may be wide, the use of derived parameters that are within the experimental light level range appears worthwhile.

The estimated respiration R may be seen as the biologically meaningful parameter dark respiration. Although evidence exists that dark respiration is different from respiration in the light, this has no consequences for the comparison of light-response curves. When the curves are used to predict gross production by adding the estimated dark respiration, this may result in an overestimation (see section 3.2).

Statistical analyses were done with the SAS statistical package (SAS Institute Inc., 1985). Analysis of variance (ANOVA), analysis of covariance (ANCOVA) and linear regression were performed with the general linear models (GLM) procedure, after $\log_{10}$ transformation if residuals were not normally distributed. Homogeneity of residual variances was checked with a plot of predicted versus residual values. For multiple comparisons, we used the LSMEANS option in SAS, giving the least squares means that are the basis for comparisons in unbalanced designs (SAS Institute Inc., 1986).

When variance distribution is not homogeneous and log transformation gives no improvement, the results from subsequent multiple comparisons must be watched with caution. When multiple comparisons are made, a mean variance is used, based on the RSS of the particular ANOVA. This means, that a treatment with only one replicate can be compared with other means, using the variance estimate. However, when variances are

not homogeneous, the estimated variance may be very different from the actual variance, resulting in unpredictable errors and perhaps erroneous conclusions. Statistical solutions for this problem are scarce. Snedecor & Cochran (1967) give the following solution. When sample sizes are equal, a t-test with n-1 instead of 2(n-1) degrees of freedom solves the problem. When sample sizes are not equal, the significance level of t is based on the t-values for n_1-1 and n_2-1, and the variances and sample sizes of the two means. However, in SAS there is no possibility to use this calculation in the LSMEANS option. This means that all comparisons of interest must be made separately, which is very time consuming. This problem occurred in the analysis of morphological characteristics. Although the consequences of inhomogeneity of variances can be severe, adequate statistical tests for its detection do not exist. Bartlett's test, for instance, is very sensitive to nonnormality, especially kurtosis, and can give many erroneous verdicts of inhomogeneity (Snedecor & Cochran, 1967; Glaser, 1982). We have used the results from the multiple comparisons with caution when inhomogeneity was suspected. The ANOVA itself is rather insensitive to these problems (Glaser, 1982).

In all ANOVA tables where significances for factor effects are given, these are the significances for the so-called Type III sum of squares (SAS Institute Inc., 1986). They represent the contribution of a factor corrected for all other factors and interactions.

Regression equations were compared by means of a regression model with dummy variables. When two equations are compared, one dummy variable Z is added, equal to 1 for equation 1 and 0 for equation 2. In a full model, stating that both lines have different intercepts and slopes, the dependent Y is related to Z, the independent X and the interaction Z*X. A reduced model can be that both lines do not differ at all (Z and its interaction Z*X are left out). The difference between the two models in terms of RSS which has been standardized on the difference in degrees of freedom f1, is divided by the RSS of the full model, standardized on its degrees of freedom f2. The outcome is distributed as F with f1,f2 degrees of freedom. When F is significant, the two data sets are best described by two different equations.

When making multiple comparisons with the LSMEANS option, a comparisonwise error rate (CER) was calculated for each comparison by dividing the experimentwise error rate (EER) by the number of comparisons. This approach is necessary to keep the EER within reasonable limits. As a consequence, the number of significant differences decreases when EER decreases and/or the number of comparisons per experiment increases.

In the present experiment, multiple comparisons were made between the four light history levels within each of the three age levels, i.e. 4*3/2=6 comparisons per age level and thus 18 comparisons in total. Comparisons were also made between the three age levels within each of the four light history levels (4*(3*2/2)=12 comparisons). CER was kept at 0.0017 to ensure an EER of 0.05 with this grand total of 30 comparisons. All other comparisons were excluded because they were regarded as less important. If all possible comparisons were made, the CER would have been 0.0007; this would have reduced the power of the test strongly.

It was always checked whether the two data sets for plants of 70 days (September and January experiments) differed. First, an ANOVA was done in

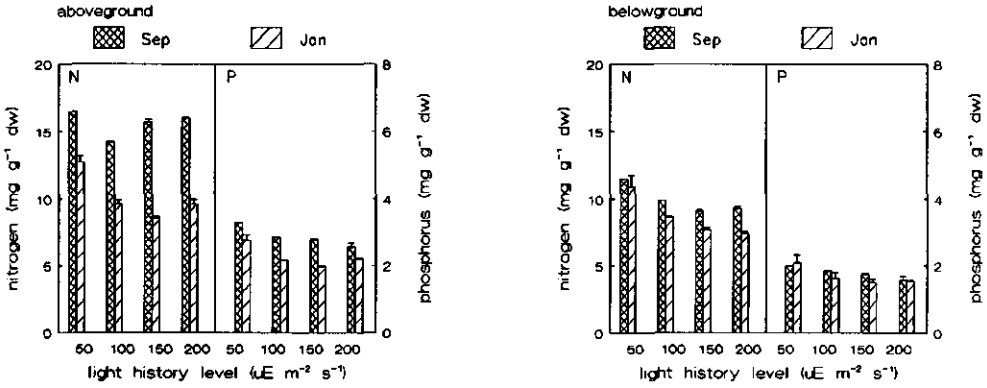


Fig. 4.2. Aboveground and belowground N and P content in plants from the September and January experiments (age 70 days).

which the two sets were entered as independent levels of the factor age, together with the other two data sets (October and November). This ANOVA is referred to as the 'separate' ANOVA. Subsequently, pairwise comparisons were done per light history level for these two data sets for 70 days. In this way the estimated variance, used for the calculation of the significance of differences encountered in the pairwise comparisons, is based on the information from all available data. The CER was kept at 0.01 to ensure an EER of 0.05 for the 4 comparisons between the two sets. In most cases, the two sets for 70 days were subsequently combined as one level of factor age together with the other two age groups in the so-called 'combined' ANOVA.

In the following, the different light history levels are indicated with their light level during the culture period: 200, 150, 100 and 50 $\mu E\ m^{-2}\ s^{-1}$. For factor age, this becomes 30, 70 and 120 days; in the case of the two replicate experiments for 70 days, the name of the month of the two experiments is used. All figures show standard error bars when appropriate.

4.3 Results of plant analysis

4.3.1 Nitrogen and phosphorus content

In Fig. 4.2, results for the two sets of 70 days are shown. Nutrient levels in the January experiment seemed to be lower than those in September for aboveground parts (stems and leaves). For belowground parts (roots, rhizomes and tubers), the differences were much less pronounced. In the following, aboveground and belowground parts are referred to as 'plant parts'. First, the significance of the difference in nutrient content between the two levels of factor 'plant parts' was checked. This was done in a three way ANOVA with the factors light history, age and plant parts, and their twoway interactions. Data for nitrogen were $\log_{10}$-transformed. Table 4.1 gives the results.

Table 4.1. Significances of the effects of the factors light history, age and plant parts (aboveground and belowground tissue), together with their interactions, on nitrogen (N) and phosphorus (P) content (mg g^{-1} dw) of *P. pectinatus* in a threeway ANOVA. In the separate ANOVA, the two sets for 70 days were entered as two independent levels of factor age. In the combined ANOVA, these two sets were taken together as one level.

	separate		combined	
	N	P	N	P
age	0.0001	0.0001	0.0001	0.0001
light history	0.0921	0.0001	0.4981	0.0001
plant parts	0.0001	0.0001	0.0001	0.0001
age*light	0.0020	0.0001	0.2127	0.0067
age*plant	0.0001	0.0001	0.0001	0.0001
light*plant	0.7338	0.2780	0.8220	0.7682

Table 4.2. Significances of the effects of the factors light history, age and their interaction age*light on nitrogen (N) and phosphorus (P) content (mg g^{-1} dw) of aboveground and belowground parts of *P. pectinatus* in a twoway ANOVA. Data from September and January were entered as independent levels of factor age. Probabilities for pairwise comparisons of the two groups of plants of 70 days are given together with their significance: * = significant, CER = 0.01, EER = 0.05.

twoway ANOVA				
	aboveground		belowground	
	N	P	N	P
age	0.0001	0.0001	0.0120	0.0001
light history	0.1548	0.0001	0.3904	0.0036
age*light	0.0049	0.0005	0.1199	0.3682

pairwise comparisons of the two 70 day groups				
	aboveground		belowground	
light history	N	P	N	P
200	0.0001*	0.0136	0.2001	0.9528
150	0.0001*	0.0001*	0.3320	0.2093
100	0.0001*	0.0011*	0.4817	0.3163
50	0.0123	0.0048*	0.7514	0.6699

There was a significant difference between aboveground and belowground parts, both when the two 70 day data sets were combined as one level of age and when they were entered as independent levels in this factor. To check for differences between these two data sets for 70 days, the results were reanalyzed with a twoway ANOVA (factors light history and age) for both levels of factor plant parts separately, followed by pairwise comparisons between the two 70 day data sets. Again, data for aboveground nitrogen content were $\log_{10}$-transformed. The results are shown in Table 4.2.

It can be concluded that the two data sets for 70 days differed for aboveground results, but not for belowground results. In the final twoway ANOVAs for above and belowground data, we reanalyzed the results from all experiments with the two sets for 70 days combined. For aboveground data, we also did an ANOVA without the January results as

Table 4.3. Significances of the effects of the factors light history, age and their interaction age*light on nitrogen (N) and phosphorus (P) content (mg g^{-1} dw) of aboveground and belowground parts of *P. pectinatus*. In this final analysis, the two data sets for 70 days were combined as one level in factor age. The effect of an analysis of aboveground nutrient content without January data for age 70 days is also shown.

	aboveground		belowground	
	N	P	N	P
age	0.0001	0.0001	0.0067	0.0001
light history	0.8514	0.0424	0.4078	0.0024
age*light	0.8157	0.0933	0.0401	0.2274

	aboveground (January data left out)	
	N	P
age	0.0001	0.0001
light history	0.2000	0.0008
age*light	0.1496	0.0020

Table 4.4. Multiple comparisons (mc) for nutrient content (mg g^{-1} dw). Letters a-d indicate differences within each age group (column mca). Letters e-g indicate differences within each light history level group (mcl column). For aboveground data, both results with (+) and without (−) the January set are given. CER = 0.0017, EER = 0.05. Number of replicates is 1-4, but 1-2 for aboveground data when January is left out.

aboveground plant parts: nitrogen content

	(−)						(+)					
age	30	70	120				30	70	120			
light	mca	mca	mca	mcl			mca	mca	mca	mcl		
200	a	a	a	f	e	e	a	a	a	f	e	fe
150	a	a	a	f	e	e	a	a	a	e	e	e
100	a	a	a	f	e	e	a	a	a	f	e	fe
50	a	a	a	f	fe	e	a	a	a	e	e	e

aboveground plant parts: phosphorus content

	(−)						(+)					
age	30	70	120				30	70	120			
light	mca	mca	mca	mcl			mca	mca	mca	mcl		
200	a	a	a	e	e	e	a	a	a	e	e	e
150	ab	a	a	f	e	e	ab	a	a	e	e	e
100	b	a	a	f	e	e	b	a	a	f	e	fe
50	b	a	a	f	e	e	b	a	a	f	e	e

belowground plant parts, both 70 day sets combined

	nitrogen						phosphorus					
age	30	70	120				30	70	120			
light	mca	mca	mca	mcl			mca	mca	mca	mcl		
200	a	a	a	f	e	fe	a	a	a	e	e	e
150	a	a	a	e	e	e	ab	ab	a	e	e	e
100	a	a	a	e	e	e	ab	ab	a	e	e	e
50	a	a	a	e	e	e	b	b	a	f	fe	e

these were lower than the other three data sets. The results are given in Table 4.3. Multiple comparisons within age groups and light history groups are given in Table 4.4. In Fig. 4.3, the data from the two 70 day sets are combined.

The only differences between the ANOVAs for aboveground data with and without January were found in the number of significantly different comparisons and in the occurrence of the age*light history interaction for phosphorus. Aboveground levels for both nutrients were higher than belowground levels. The levels of both nutrients decreased with increasing age, irrespective of plant parts. Light history only influenced phosphorus content, both above and belowground: increasing light history level led to a reduced phosphorus content, especially for age 30 days.

While the levels of both nutrients were influenced by the three factors studied, the behaviour of their ratio could have been different. The molar ratio of nitrogen and phosphorus might have remained constant. In a linear regression analysis the relation between phosphorus as independent and nitrogen as dependent variable (both in μmol g^{-1} dw) appeared highly significant, both for aboveground and belowground tissue. In this analysis, the data from all four experiments were included. The resulting equations with intercepts were compared with each other and with the results from regressions forced through the origin. The equations and tests are given in Table 4.5. The four regression lines are shown in Fig. 4.4.

The best fit in terms of minimum RSS was reached with two different equations for above and belowground plant parts having a non-zero intercept. When the regression was forced through the origin, the difference between the equations for above and belowground tissue disappeared. This suggests that the N/P molar ratio was not significantly different for above and belowground tissue. However, as the two equations with intercepts did differ from each other, there may be other factors (like light history and age) that also influence the relation between the two nutrients. The intercept represents the effect of all these factors

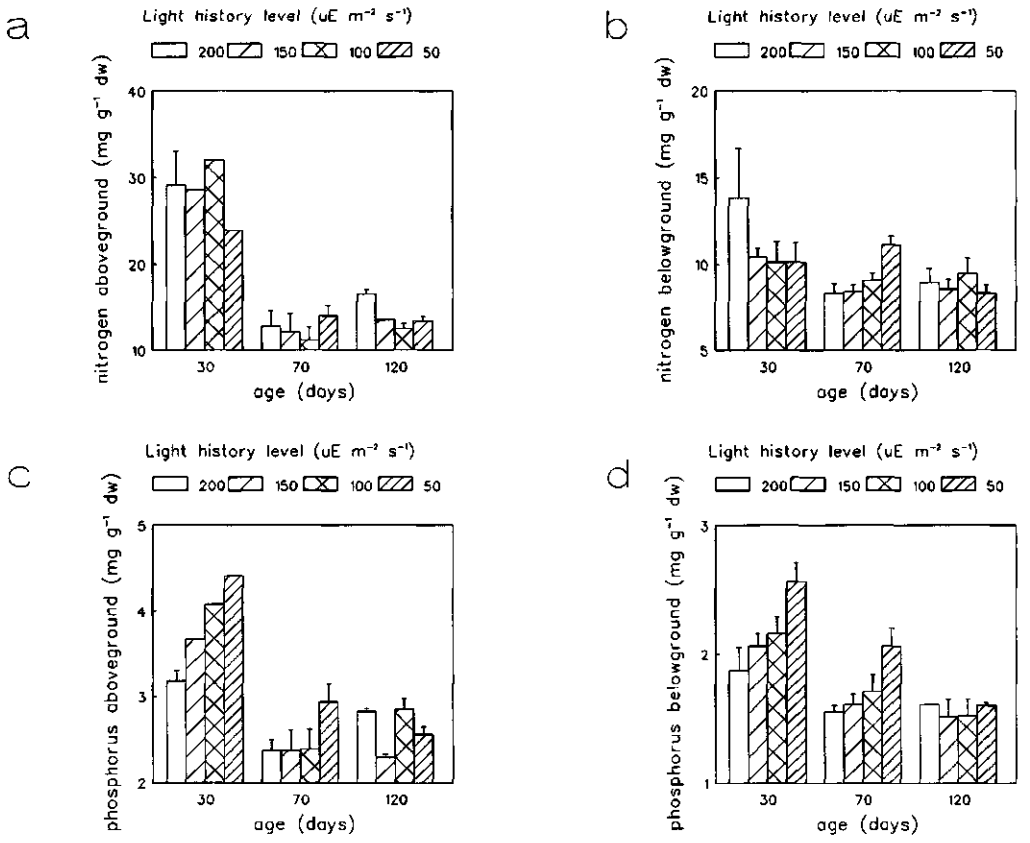


Fig. 4.3. Nutrient content of plant material from all four experiments (September and January data are combined). (a) Aboveground N content (b) Belowground N content (c) Aboveground P content (d) Belowground P content.

together.

Effects of light history and age on the N/P molar ratio can be studied most easily in a threeway ANOVA with factors light history, age and plant parts, followed by twoway ANOVAs per level of the factor plant parts. In this analysis, each value of the N/P ratio can be regarded as a 'regression' forced through the origin. Based on the results from such a regression (Table 4.5), it can be expected that the effect of the factor plant parts will be low. Again, first the two data sets for 70 days were compared. The two sets did not differ and were therefore combined in the following analyses. The results of the ANOVAs are shown in Table 4.6, mean values for individual treatments in Fig. 4.5.

Besides a small but significant effect of plant parts, there was a strong effect of age and light history on the N/P molar ratio. Belowground plant parts, higher age and lower light history levels had a lower ratio. For belowground tissue, the age effect was not significant, but the age*light history interaction existed, in contrast with aboveground tissue. As a consequence, age*light history and age*plant parts interactions were

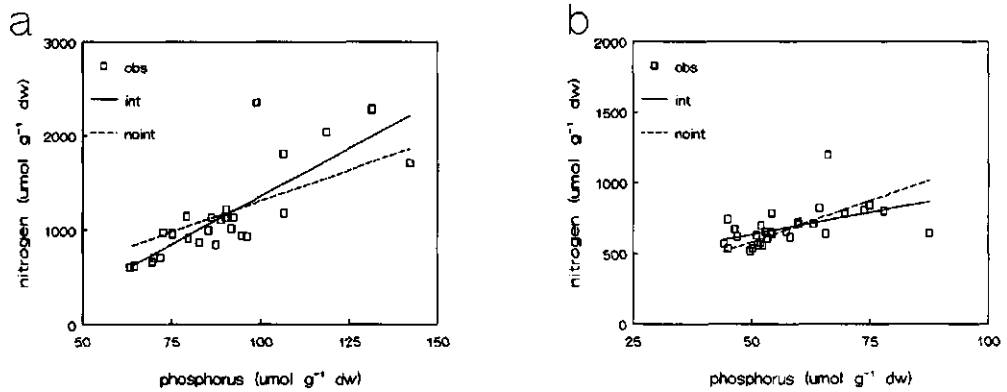


Fig. 4.4. Relation between N and P content (in μmol g^{-1} dw) in plant material from all four experiments. Obs = observed data, int = regression with intercept, noint = regression with no intercept.
(a) Aboveground (b) Belowground.

Table 4.5. Linear regression of nitrogen on phosphorus content (both in μmol g^{-1} dw) for aboveground and belowground tissue of *P. pectinatus*. The assumption that the two equations are different is tested with a regression with dummy variables. The equations are also compared with equations forced through the origin. ns =not significant.

(a) regression with intercept
aboveground: $N = -672.4 + 20.3*P$; $r^2 = 0.65$, $p < 0.0001$, $RSS = 2126*10^3$, $df = 25$
belowground: $N = 323.4 + 6.2*P$; $r^2 = 0.25$, $p < 0.0044$, $RSS = 378*10^3$, $df = 28$

model with different slopes and intercepts: $RSS = 2504*10^3$, $df = 53$
model assuming no differences: $RSS = 3028*10^3$, $df = 55$

$F_{2,55} = 5.52$, $p < 0.01$

(b) regression forced through the origin:
aboveground: $N = 13.1*P$; $r^2 = 0.57$, $p < 0.0001$, $RSS = 2655*10^3$, $df = 26$
belowground: $N = 11.6*P$; $r^2 = 0.06$, $p < 0.0001$, $RSS = 479*10^3$, $df = 29$

model with different slopes: $RSS = 3134*10^3$, $df = 55$
model assuming no differences: $RSS = 3282*10^3$, $df = 56$

$F_{1,56} = 2.59$, ns

(c) comparison of the equations with and without intercept for above and belowground tissue:
aboveground: $F_{1,25} = 6.22$, $p < 0.025$
belowground: $F_{1,28} = 7.48$, $p < 0.025$

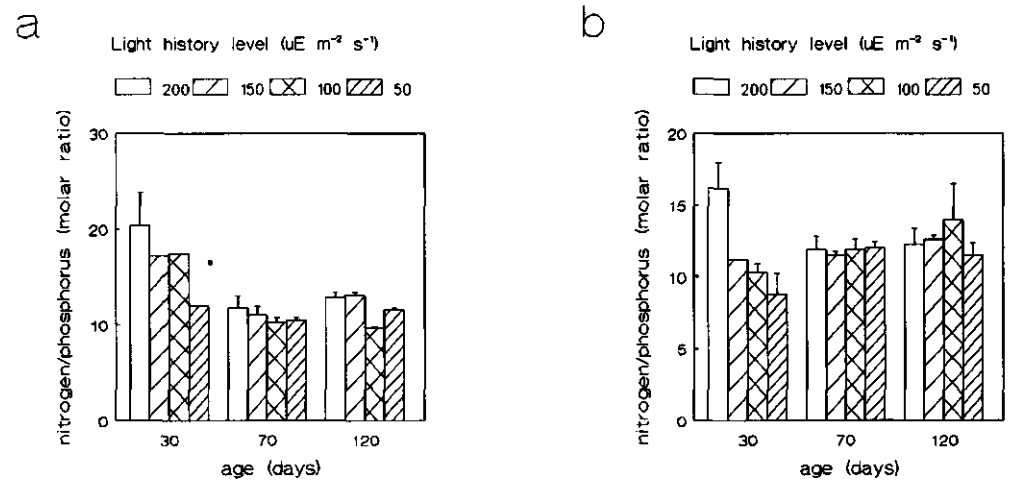


Fig. 4.5. N/P ratio (mol mol^{-1}) in plant material from all four experiments (September and January data are combined). (a) Aboveground (b) Belowground.

Table 4.6. Three- and twoway ANOVA: significances of the effects of factors light history, age, plant parts and their interactions on the N/P molar ratio's. Data sets for 70 days combined. Number of replicates per light history-age combination: 1-4.

overall		aboveground	
age	0.0002	age	0.0001
light history	0.0004	light history	0.0244
plant parts	0.0332	age*light	0.2201
age*plant	0.0001		
light*plant	0.5185	belowground	
age*light	0.0052		
		age	0.4465
		light history	0.0332
		age*light	0.0182

significant in the threeway ANOVA. Significant differences between individual treatments are limited to the aboveground 200 μE m^{-2} s^{-1} group (70 and 120 days were less than 30 days) and the belowground 30 days group (50 and 100 μE m^{-2} s^{-1} were less than 200 μE m^{-2} s^{-1}).

4.3.2 Plant morphology

Plant photosynthesis may also depend on plant morphology. For instance, plant elongation and the relative biomass allocation to leaves and stems may vary for plants from different light history levels and/or different ages. This can cause a different photosynthetic performance under the same light conditions of the whole

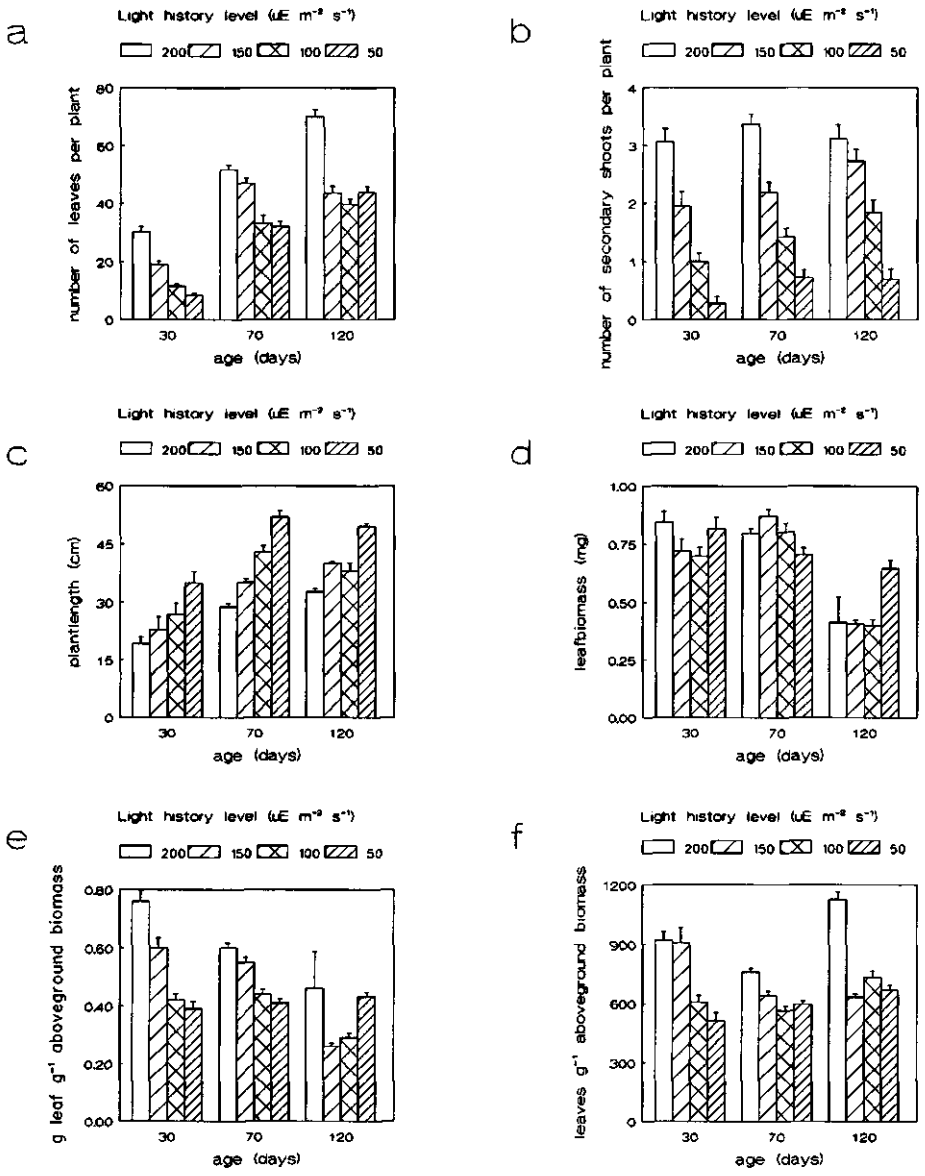


Fig. 4.6. Effects of light history and age on various morphological characteristics. In all cases, data from September and January are combined.

(a) Number of leaves per plant.

(b) Number of secondary shoots per plant.

(c) Plant length.

(d) Biomass of one leaf.

(e) Leaf ratio (total leaf biomass per total aboveground biomass).

(f) Number of leaves per g aboveground biomass.

Table 4.7. Significances of the effects of factors light history, age and their interaction on several morphological characteristics of *P. pectinatus*. For number of leaves and plant length, both ANOVAs with (+) and without January data are shown. All quantities expressed per plant.

	light history	age	age*light
number of leaves (+)	0.0001	0.0001	0.0001
number of leaves	0.0001	0.0001	0.0001
number of secondary shoots	0.0001	0.0014	0.2857
plant length (+)	0.0001	0.0001	0.0284
plant length	0.0001	0.0001	0.7739
biomass of leaf (afdw)	0.1237	0.0001	0.0001
leaf ratio	0.0001	0.0001	0.0001
number of leaves per aboveground biomass	0.0001	0.0001	0.0001

plant, even when photosynthesis of individual leaves may not be that different (although it probably is affected also). Thus, effects of light history and age on morphology may help to explain the effects of the two factors on parameters in the light-response curve models.

When the two replicate data sets for plants of 70 days were compared, they showed only a few significant differences: for light history level 100 μE m^{-2} s^{-1}, plants from September were shorter, with less leaves than the January material. When cultured at 50 μE m^{-2} s^{-1}, plants from September had less leaves. Thus, for all six morphological characteristics, a twoway ANOVA was done with the two sets for 70 days combined. For number of leaves and plant length, an ANOVA was performed also without the results from January. The choice for the September data was based on the results from the nutrient analysis (see section 4.3.1).

The results from these ANOVAs showed highly significant effects for both factors and their interaction. Table 4.7 gives the significances of the factor effects for the morphological characteristics that were measured. In Fig. 4.6, these characteristics are plotted. Multiple comparisons are made in Table 4.8. Apart from numbers of leaves and secondary shoots, data sets had no normal distribution. Residual plots showed no clear inhomogeneity of variances. Still, the results from the multiple comparisons should be used with caution (see section 4.2.4).

Levels of significance in the two ANOVAs for the number of leaves with and without January did not differ. The number of leaves continued to increase up till day 120, except for 150 μE m^{-2} s^{-1}. Lower light history levels resulted in fewer leaves per plant. The amount of secondary shoots was only weakly affected by age: no significant differences occurred within any light history level group. The light history effect is strong: more secondary shoots were found for higher light history levels. For plant length, the ANOVA without the January results showed no interaction between age and light history; the ANOVA with January did. Plant length increased with age up till day 70. Plants were longer when light history level was lower, especially for the lowest light history level.

Mean leaf biomass was not affected directly by light history. However, light history did influence the age effect: leaf biomass decreased between age 70 and age 120 days for all light history levels, except for 50 μE m^{-2} s^{-1}. Leaf ratio decreased with lower light history levels and increasing age. The interaction of the two factors is clear for 50 μE m^{-2} s^{-1}, where no age effect was apparent. The number of leaves per aboveground biomass showed a rather intricate behaviour for the different light history levels. For the lowest light history levels, increasing age led to a small increase in the number of leaves per

Table 4.8. Multiple comparisons (mc) for morphological characteristics. Letters a-d indicate differences within each age group (column mca). Letters e-g indicate differences within each light history level group (mcl column). For number of leaves and plant length, both results with (+) and without (−) the January data are shown. CER = 0.0017, EER = 0.05. Number of replicates per treatment is 13-18, but 31-33 for age 70 when the two data sets could be combined.

number of leaves (−)							number of leaves (+)						
age	30	70	120				30	70	120				
light	mca	mca	mca	mcl			mca	mca	mca	mcl			
200	a	a	a	e	f	g	a	a	a	e	f	g	
150	b	a	b	e	f	f	b	a	b	e	f	f	
100	bc	b	b	e	e	f	bc	b	b	e	f	f	
50	c	b	b	e	f	g	c	b	b	e	f	g	

plant length (−)							plant length (+)						
age	30	70	120				30	70	120				
light	mca	mca	mca	mcl			mca	mca	mca	mcl			
200	a	a	a	e	f	f	a	a	a	e	f	f	
150	a	a	a	e	f	f	a	a	a	e	f	f	
100	ab	a	a	e	f	f	ab	b	a	e	f	f	
50	b	b	b	e	f	f	b	c	b	e	f	f	

number of secondary shoots							biomass of one leaf						
age	30	70	120				30	70	120				
light	mca	mca	mca	mcl			mca	mca	mca	mcl			
200	a	a	a	e	e	e	a	ab	a	f	f	e	
150	b	b	ab	e	e	e	a	a	a	f	f	e	
100	c	c	b	e	e	e	a	ab	a	f	f	e	
50	c	d	c	e	e	e	a	b	b	e	e	e	

leaf ratio							number of leaves per aboveground biomass						
age	30	70	120				30	70	120				
light	mca	mca	mca	mcl			mca	mca	mca	mcl			
200	a	a	a	f	fe	e	a	a	a	f	e	g	
150	a	ab	b	f	f	e	a	b	b	f	e	e	
100	b	bc	ab	e	e	e	b	b	b	fe	e	f	
50	b	c	ab	e	e	e	b	b	b	e	e	e	

gram. Multiple comparisons per light history level, however, did not show differences. For 150 μE m^{-2} s^{-1}, the number of leaves per gram decreased between 30 and 70 days, and remained constant till 120 days. For 200 μE m^{-2} s^{-1}, there also was a decrease between 30 and 70 days, but this effect reversed between 70 and 120 days. For the youngest plants, the lower light history levels had fewer leaves per gram; for age 120 days, only 200 μE m^{-2} s^{-1} was significantly higher than the other three.

4.3.3 Chlorophyll content

As nitrogen is an essential component of chlorophylls, it can be expected that the differences that were encountered in the aboveground nitrogen levels between the two data sets for 70 days will be reflected in differences between these data sets for amount of chl(a+b) and fraction chl-b of total chlorophyll in leaves. Thus, January chl(a+b) content and fraction chl-b should be much lower compared to September. Fraction chl-b was not normally distributed, but no inhomogenity of variances was apparent. The results are shown in Table 4.9 and Fig. 4.7.

The two data sets for 70 days differed strongly, January indeed having a very low chl(a+b) content, also considering the other data sets (October and November). The deviation of January was particularly clear for fraction chl-b, January being 2-5 times lower (except for 50 μE m^{-2} s^{-1}: no difference) than the other three data sets.

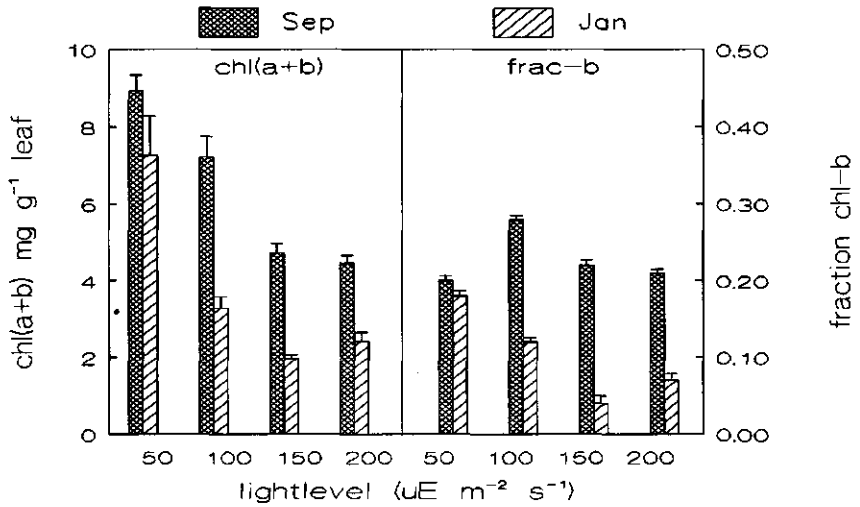


Fig. 4.7. Chl(a+b) content and fraction chl-b of chl(a+b) of leaves from plants of September and January.

Table 4.9. (a) Significances of the effects of factors light history, age and their interaction age*light on chl(a+b) content of leaves (mg g^{-1} afdw) and fraction chl-b of total chl(a+b) of *P. pectinatus*. Chl(a+b) was $\log_{10}$-transformed. Pairwise comparisons (pc) are done for each light history level in the two sets for age 70 days. Probabilities are shown together with their significance: * = significant, CER = 0.01, EER = 0.05, Number of replicates is 8-18. (b) Final ANOVAs. Data from January were included (+) or left out (−). Number of replicates is 8-18.

(a) Separate ANOVAs and comparison of the two 70 day sets

	chl(a+b)	frac-b	light	pc chl	pc frac-b
age	0.0001	0.0001	200	0.0001*	0.0001*
light history	0.0001	0.0001	150	0.0001*	0.0001*
age*light	0.0001	0.0001	100	0.0001*	0.0001*
			50	0.0069*	0.0054*

(b) Final ANOVAs

	(+)		(−)	
	chl(a+b)	frac-b	chl(a+b)	frac-b
age	0.0001	0.1021	0.0001	0.0001
light history	0.0001	0.0668	0.0001	0.0001
age*light	0.0001	0.0567	0.0001	0.0001

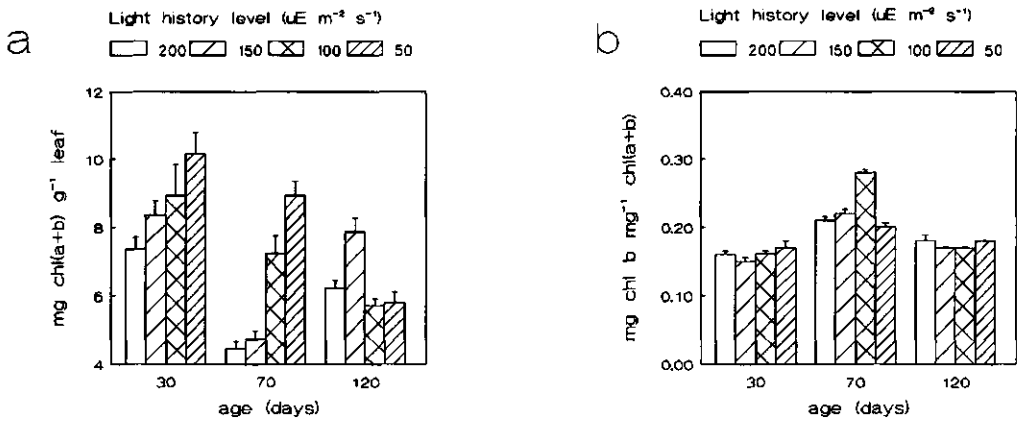


Fig. 4.8. (a) Effect of light history and age on chl(a+b) content of leaves from plants of all experiments (January data left out; see text). (b) Effect of light history and age on fraction chl-b in the same material.

Table 4.10. Multiple comparisons (mc) for chl (a+b) content (mg g^{-1} afdw) and fraction chl-b of leaves of *P. pectinatus*. Letters a-d indicate differences within each age group (column mca). Letters e-g indicate differences within each light history level group (mcl column). Both results with (+) and without (−) January are shown. CER = 0.0017, EER = 0.05. Number of replicates is 8-18.

chl(a+b) in leaves

	(−)						(+)					
age	30	70	120				30	70	120			
light	mca	mca	mca	mcl			mca	mca	mca	mcl		
200	a	a	ab	f	e	f	a	a	a	f	e	f
150	ab	a	a	f	e	f	a	a	a	f	e	f
100	ab	b	b	f	ef	e	a	b	a	f	e	ef
50	b	b	b	f	f	e	a	c	a	f	ef	e

fraction chl(b) in leaves

	(−)						(+)					
age	30	70	120				30	70	120			
light	mca	mca	mca	mcl			mca	mca	mca	mcl		
200	a	a	a	e	f	e	a	a	a	e	e	e
150	a	a	a	e	f	e	a	a	a	e	e	e
100	a	b	a	e	f	e	a	b	a	e	e	e
50	a	a	a	e	f	e	a	ab	a	e	e	e

Subsequently, to test the effects of light history and age on chl(a+b) and fraction chl-b in leaves, two ANOVAs were done for each characteristic: one in which the two sets for 70 days were combined and one without the data from January. These two ANOVAs differed only for fraction chl-b. The results are given in Table 4.9. The subsequent multiple comparisons are given in Table 4.10. Effects of light history, age and their interaction were significant for chl(a+b), both with and without the January data. For fraction chl-b, this was so only without the January data. The inclusion of the very low fraction chl-b values from January caused a strong increase in variance, leading to the disappearance of any effect.

In Fig. 4.8 the results are given without the January data. The interaction between light history and age was clear for chl(a+b). The lowest light history levels had a steady decrease in chl(a+b) content with increasing age, whereas at 150 and 200 μE m^{-2} s^{-1}, chl(a+b) decreased from 30 to 70 days, and increased again up till 120 days. Chl(a+b) content

was lower when light history levels were higher, but this picture was not clear for 120 days, when 150 μE m^{-2} s^{-1} had the highest chl(a+b) content (not significant when both sets for 70 days are combined). Fraction chl-b showed a slight increase between 30 and 70 days, and a decrease again between 70 and 120 days. Effect of light history was minimal, and mostly due to a peak for 100 μE m^{-2} s^{-1} from the September 70 days data.

When data on leaf biomass per total aboveground biomass and on chl(a+b) content of leaves are combined, an estimation of the amount of chl(a+b) per gram aboveground biomass is possible. This figure can be used to act as covariable in ANCOVAs of parameters from the light-response curve models. In that way, it is possible to detect direct effects of light history and age on these parameters apart from their indirect effects via changes in morphology, nutrient and chlorophyll content. For this purpose, we combined the mean chl(a+b) content of leaves and the mean leaf ratio per tube (see section 4.3.2), resulting in three independent estimates of chl(a+b) content per gram aboveground biomass per factor combination (thus, one for each replicate light-response curve). Again, differences in chl(a+b) content between the two sets for 70 days were checked. Remarkably, the differences were minor compared to the results for nutrient content per aboveground biomass and chl(a+b) content per leaf biomass. The two sets were thus pooled together in the ANOVA. The data are shown in Fig. 4.9. ANOVA results and multiple comparisons can be found in Table 4.11.

Chl(a+b) content of aboveground biomass was lower when age was higher, although this tendency reversed for 200 μE m^{-2} s^{-1} between 70 and 120 days, and 150 μE m^{-2} s^{-1} did not change after age 70

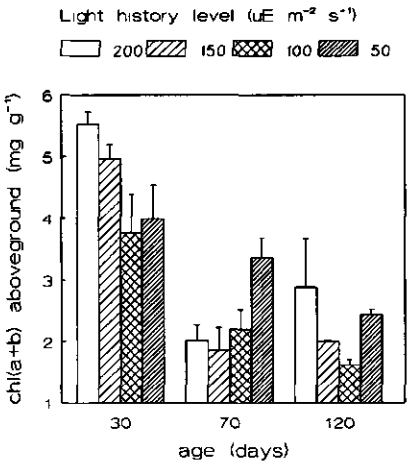


Fig. 4.9. Chl(a+b) content of aboveground biomass from all experiments (September and January data are combined).

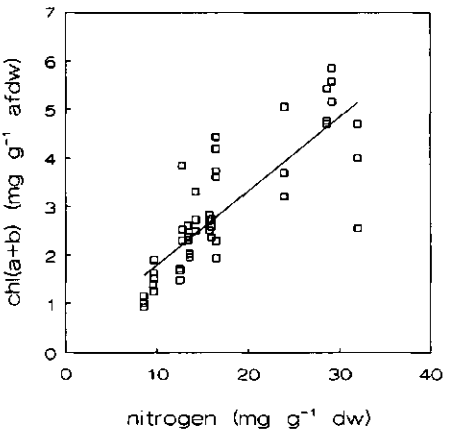


Fig. 4.10. Relation between nitrogen and chl(a+b) content of aboveground biomass from all experiments.

days. The effect of light history was intricate. On age 30, chl(a+b) content of lower light history levels was lower than for higher light history levels; on day 70, 50 μE m^{-2} s^{-1} was highest. Comparisons per age group only led to significant differences for age 70 days.

The relation between chlorophyll

Table 4.11. (a) Significances of the effects of light history, age and their interaction on chl(a+b) content of aboveground biomass (mg g^{-1} afdw) of *P. pectinatus*. Column 1: results from ANOVA in which the two sets for 70 days were entered as independent levels of factor age. Column 2: results from ANOVA in which these two sets were combined. (b) Pairwise comparisons (pc) are done for each light history level in the two sets for age 70 days. Probabilities are shown together with their significance: * = significant, CER = 0.01, EER = 0.05, number of replicates is 3. (c) Multiple comparisons (mc) for chl(a+b) content of aboveground biomass (mg g^{-1} afdw). Results for age 70 days were combined. Letters a-d indicate differences within each age group (column mca). Letters e-g indicate differences within each light history level group (mcl column). CER = 0.0017, EER = 0.05. Number of replicates per treatment is 3, but 6 for age 70 days.

(a) ANOVA

	1	2
age	0.0001	0.0001
light history	0.0043	0.0339
age*light	0.0015	0.0080

(b) pairwise comparisons

light history	pc chl
200	0.0281
150	0.0015*
100	0.0108
50	0.0535

(c) multiple comparisons

age	30	70	120	
light	mca	mca	mca	mcl
200	a	ab	a	f e e
150	a	a	a	f e e
100	a	ab	a	f ef e
50	a	b	a	e e e

content and nutrient content was analysed in a multiple linear regression with N and P content of aboveground tissue as independent and chl(a+b) content of aboveground biomass as dependent. The regression showed no significant effect of phosphorus, and a highly significant effect of nitrogen (r^2=0.68, $p<0.0001$, Fig. 4.10). The resulting equation was: chl(a+b) (mg g^{-1} afdw)=0.287 + 0.152 * nitrogen (mg g^{-1} dw).

4.4 Discussion: plant analysis

4.4.1 Nutrient content

Data on nitrogen and phosphorus content of *P. pectinatus* tissue from various places and authors are presented in Table 4.12 for comparison. With respect to nitrogen, our values for plants of 70 and 120 days (Fig. 4.3) are well within the ranges found by other authors (our data are expressed per g dw (70 °C) and should be multiplied with 1.02 to arrive at dw (105 °C) basis). For 30 days, our aboveground nitrogen levels are rather high compared with other material from The Netherlands. Phosphorus content of our plants is somewhat low, but still within the ranges found for Dutch material. Clearly, as was stressed by Van Vierssen (1982), it is worthwhile to analyze aboveground and belowground material separately: the two can differ strongly.

Our main reason for analyzing nutrient content was to find out whether plant photosynthesis and growth might have been reduced due to nutrient limitation. Our sediment was completely inorganic, and especially nitrogen supply might have been limiting. The question is: were the nutrient levels in our plants after 70 and 120 days too low? Not much is known about the effect of nutrient content on photosynthesis. Gerloff & Krombholz (1966) studied growth of several macrophytes with alga-free cultures in nutrient medium without sediment. They varied levels of N and P in the culture medium to determine the so-called 'critical' nutrient level, defined as 'the minimum tissue concentration necessary for maxi-

Table 4.12. Average nitrogen and phosphorus contents of *P. pectinatus* tissue from various field locations, in mg g^{-1} dw. Ranges between brackets. Total = total plant, above = aboveground biomass, below = belowground biomass. Our own data represent overall means over all experimental treatments.

origin	plant part	nitrogen	phosphorus	reference
Poland	above	16.1	0.4	1
Scotland	total	50.1 (40.4-60.1)	6.5 (4.6- 8.1)	2
South Africa	leaves	16.4	1.0	3
South Africa	stems	12.3	0.7	3
South Africa	roots	13.7	0.7	3
South Africa	rhizomes	10.3	0.1	3
The Netherlands	above	21.2 (12.4-41.2)	6.3 (1.4-11.1)	4
The Netherlands	below	14.7 (6.6-25.1)	5.3 (1.7- 9.1)	4
The Netherlands	above	16.2 (10.6-30.5)	4.4 (1.5- 9.8)	5
The Netherlands	below	13.7 (8.2-22.7)	4.4 (1.9- 7.1)	5
The Netherlands	above	15.9 (8.5-33.0)	2.8 (2.0- 4.4)	6
The Netherlands	below	9.6 (7.2-16.7)	1.8 (1.4- 2.7)	6

References: (1) Bernatowicz (1969), (2) Ho (1979), (3) Howard-Williams (1981), (4) Van Vierssen (1982), (5) Van Wijk (1989a), (6) This study.

mum growth'. Plants were not divided into above and belowground parts. For six species (*P. pectinatus* was not studied), the critical N level was about 13 mg g^{-1} dw (70 °C) and the critical P level 1.3 mg g^{-1}. Above these levels, nutrient content did not influence plant biomass; below these levels, a positive linear correlation between the two was found. Van Wijk (1989b) found a critical nutrient level of 1.5 mg P g^{-1} dw for *P. pectinatus*. However, in a nutrient enrichment study, Howard-Williams (1981) did not find any effect of the amount of nutrients added on biomass levels. In his experiments, tissue concentrations of P reached the critical 1.3 mg g^{-1} level only at the highest enrichment level. In our experiments, aboveground nitrogen levels were close to the supposedly critical 13 mg g^{-1} in plants of 70 and 120 days. In all our treatments, belowground nitrogen values were even lower. Aboveground and belowground phosphorus always remained above the critical level.

Our N/P molar ratios for aboveground tissue of plants of 30 days (Fig. 4.5a) are close to the value 17.1 which we calculated from the data in Ho (1979), and to the value 22.1 calculated from the critical N and P values of Gerloff & Krombholz (1966). Data in van Vierssen (1982) suggest ratios of about 5-7. Data in Van Wijk (1989a) result in values of 6-12 for Dutch populations, comparable to our data for plants of 70 and 120 days. These rather low values might be due to a sediment relatively rich in phosphorus and consequent luxury uptake.

We conclude that photosynthesis and growth of plants of 70 and 120 days may have been influenced by a rather low nitrogen content. This influence is expected to be minor as the aboveground nitrogen levels were still close to the critical levels mentioned by Gerloff & Krombholz (1966). The decrease in nutrient content with age, as shown in

Fig. 4.3, can be attributed to a dilution of the available nutrients over an increasing plant biomass. We did observe an effect of light history on phosphorus content, but not on nitrogen. Barko & Smart (1981) who worked with *Hydrilla verticillata* (L.f.) Royle, *M. spicatum* and *E. densa* found the highest biomass and the lowest nutrient levels for plants grown under the highest light levels.

4.4.2 Morphology

Looking at the data on plant morphology (Fig. 4.6) the (very obvious) conclusion is that when plants are older, they are longer and have more leaves. At the same time, leaves become smaller and the ratio of leaf biomass to total aboveground biomass decreases, except at light history level 50 μE m^{-2} s^{-1}. As the process of photosynthesis is located in the leaves, a decreasing leaf ratio must lead to a moment at which supply of photosynthate is lower than demand. This moment may have been reached for light history levels 100 and 150 μE m^{-2} s^{-1} between 70 and 120 days, because plant length and leaf number at these two ages are not significantly different. However, at the remaining light history levels, leaf number still increases between 70 and 120 days. If decreasing leaf biomass and leaf ratio are a consequence of 'senescence', it can be concluded that when plants are grown at 50 μE m^{-2} s^{-1}, they remain physiologically younger.

Apart from our own work (Vermaat & Hootsmans, 1991b), there are very few other literature data on long-term growth experiments with *P. pectinatus* under different temperature-light conditions. Van Wijk et al. (1988) studied growth of *P. pectinatus* in a greenhouse for two months. Plants from brackish habitats, grown in freshwater, formed longer shoots than plants from freshwater habitats (30-40 cm vs. 10-17 cm). Plants from Lake Veluwe showed shoot elongation when grown under 'reduced' light conditions. Mean shoot length increased from 9.4 to 19.3 cm.

The increase in plant length and reduction in number of secondary shoots of our plants in reaction to lower light history levels is comparable with our own findings (Vermaat & Hootsmans, 1991b) and with the results of Barko & Smart (1981) for other aquatic macrophytes.

Tuber formation was never observed in our experiments. Such a long period before tuber formation starts appears to be a characteristic of the 'brackish' forms of *P. pectinatus* according to Van Wijk et al. (1988). They cultured four brackish populations in the laboratory in different salinities. After 3 months of culture, only some tubers were found. The amount of tubers eventually formed by these plants remained rather low under freshwater conditions.

4.4.3 Chlorophyll

Chl(a+b) levels in leaf tissue from plants of 150 and 200 μE m^{-2} s^{-1} behave differently from those in plants grown at lower light history levels (Fig. 4.8). It seems as if for the higher light history level chlorophyll synthesis cannot keep up with the increase in total leaf biomass, and chl(a+b) content decreases strongly between 30 and 70 days. From day 70, when the number of leaves remains constant and the total leaf biomass decreases, chl(a+b) levels increase again (cf. Vermaat & Hootsmans, 1991b). For lower light levels during the first two months, chl(a+b) content of leaves is higher, and total leaf biomass is lower than for higher

light history levels. In this case, chlorophyll synthesis apparently is high enough to prevent a 'dip', but there is a steady decrease in chl(a+b) per gram leaf biomass with age. For chl(a+b) per total aboveground biomass (Fig. 4.9), the concentrations for low light history levels are lower (30 days) or higher (70 days) than those for high light history levels. Low light history levels lead to a strong elongation of the plant, and a lower leaf ratio. Consequently, no strong increase in chlorophyll content per total aboveground biomass as adaptation to low light was apparent, consistent with the findings of Spence & Chrystal (1970) for *Potamogeton polygonifolius* Pourr. and *Potamogeton obtusifolius* Mert. & Koch.

Peñuelas et al. (1988) found 4.7 mg chl(a+b) g^{-1} dw in leaves of *P. pectinatus*, comparable to our results for plants of 70 and 120 days. For stems, they found 2.5 mg chl(a+b) g^{-1} dw. We did not find a measurable amount of chlorophyll in stems. Much higher values were given by Best & Dassen (1987) for top sections of *C. demersum*: up to 16.5 mg chl(a+b) g^{-1} afdw. These chl(a+b) levels decreased to 30-60% of this maximum value in deeper layers of the vegetation. Gabrielsen (1948) studied several terrestrial species and found that the maximum energy yield (the energy equivalent of the apparent quantum yield α in the hyperbolic tangent model) did not increase further when chl(a+b) content per leaf area reached 4-5 mg dm^{-2}. Björkman (1981) found a 'normal' chl(a+b) level for leaves of terrestrial plants to be 4-6 mg dm^{-2}. Average light intensity under water is usually much lower than on land; therefore, disadvantageous selfshading of chlorophyll might occur at lower chl(a+b) concentrations than in terrestrial plant species. However, Drew (1979) found a linear relation between chl(a+b) content and photosynthesis (presumably a Pm estimate) up to 6 mg chl(a+b) dm^{-2} in leaf fragments of the seagrasses *Phyllospadix torreyi* S. Watson and *Posidonia oceanica* (L.) Delile. Specific leaf area for our plants when grown at 100 μE m^{-2} s^{-1} was 6-8 dm^2 g^{-1} afdw (single-sided, data from plants used in grazing experiments grown at 100 μE m^{-2} s^{-1}, described in Vermaat, 1991). Combined with the chl(a+b) content of leaves of plants from 70 days and 100 μE m^{-2} s^{-1} (Fig. 4.8a) this leads to a rather low chl(a+b) content of 1 mg dm^{-2}. This means that chl(a+b) content of our plants probably remained well within the range where chl(a+b) content can influence photosynthesis at low light levels. Indeed, our estimated α values (see Fig. 4.13c) showed a significant linear increase with chl(a+b) content of aboveground biomass (see Fig. 4.9), although r^2 was low: 0.19 ($p < 0.003$). Though the relation between α and chl(a+b) can be expected to level off above a certain chl(a+b) value, a rectangular hyperbola was not significantly different (in terms of RSS) from the linear equation.

The fraction chl(b) of total chlorophyll (Fig. 4.8) shows practically no change with age or light history, contrasting with the data of Boardman (1977) who mentioned an increase as adaptation to lower light levels. Our average figure of 0.18 is somewhat low compared to the fraction 0.25 based on the data of Peñuelas et al. (1988) for *P. pectinatus* leaves. Wiginton & McMillan (1979) found 0.3-0.4 for different species of tropical seagrasses. In their study, the fraction did increase with decreasing light levels for some species, but remained constant for other. No effect of light quality was observed. Jiménez et al. (1987) gave a summer value of 0.16 and a winter value of 0.23 for the intertidal seagrass *Z. noltii*, while a subtidal popu-

lation of *Z. marina* had a constant value of 0.28. Pokorný et al. (1984) gave a rather constant value of 0.26 for *Elodea canadensis* Michx. In August, this fraction showed a slight increase from 0.28 to 0.32 when going from top of the vegetation to the bottom. Best & Dassen (1987) presented chl(b)/chl(a) data of *C. demersum*. We have converted these into our fraction chl-b ratios. Their data showed an increase from 0.20 to 0.28 when going down for about 1 m from the apex of a vegetation at 2 m water depth. In a study of the marine macro-alga *Ulva lactuca* L. Vermaat & Sand-Jensen (1987) found no change in the fraction chl-b in response to low light levels: it remained 0.4. Compared to these data, *P. pectinatus* in our experiments maintained a rather low fraction chl-b under all light history conditions and did not increase this ratio in response to lower light history levels.

We have to make a comment on the observed differences in nutrient and chlorophyll contents between the experiments with plants of 70 days in September and January. There is no evidence for any difference in experimental conditions apart from a 4 months longer storage time of tubers for the January experiment under the 4 °C conditions. It is unclear how this difference could have had such a strong effect, especially regarding the results with the plants harvested in November: tubers for this experiment were stored 3 months longer than tubers used in September and October, but had the highest nutrient and chl(a+b) levels of all experiments.

For the moment, it cannot be ruled out that the observed variation between two apparently equal treatments is 'natural', and should be taken into account when making comparisons. Thus, in the following analysis of light-response curves, data from the two 70 day sets were compared with each other, but finally they were always pooled.

4.5 Results of curve fitting

In total, 48 light-response curves were obtained. One of the three replicates in the September experiment with plants of 150 μE m^{-2} s^{-1} showed a strongly erratic behaviour and was left out.

The remaining data sets were fitted with the two mathematical models described in 2.2. For the rectangular hyperbola (or Michaelis-Menten equation; MM-model in the following) 38 curves had an r^2 of 0.85 or higher. For the hyperbolic tangent (tanh-model in the following) this was achieved for 40 data sets. In the non-linear fits, r^2 is equal to the ratio SS(model)/SS(total). These sums of squares are calculated without subtracting the mean observed value from each of the estimated and observed values (i.e., they are 'not-corrected'). For the calculation of r^2 in linear regression, SS(model) and SS(total) are corrected in this way.

Only in two cases, in which only 5 and 6 datapoints were available, the F-test for the model was not significant. Table 4.13 gives an overview of the data sets including the number of datapoints per set that were used for curve fitting, occurrence of cases in a set for which measurements failed and cases that were left out of a set (outliers). In the following, treatments and replicates are indicated with an abbreviation: e.g. Sep100-1 is replicate 1 in the September experiment with light history level 100 μE m^{-2} s^{-1}. All curves are shown in Figs. 11a-d.

The non-linear fitting procedure estimated negative Km values for the MM-model for a number of replicate

Table 4.13. Overview of the data sets from photosynthesis-light measurements. Sep, Oct, Nov and Jan indicate the respective months of the four experiments. Light history level during growth is indicated with 50, 100, 150 and 200. Replicate number is 1, 2 or 3. For each experiment are given: the number of photosynthesis measurements that was used for the calculation of the light response curve, the number of results that was left out of the analysis (outliers) and the number of measurements that failed due to technical problems.

experiment	number of datapoints	datapoints left out	measurements failed
Sep200-1	9	0	0
Sep200-2	7	2	0
Sep200-3	5	1	3
Sep150-1	9	0	0
Sep150-2	9	0	0
Sep100-1	8	0	1
Sep100-2	8	0	1
Sep100-3	6	1	2
Sep50 -1	8	0	1
Sep50 -2	8	1	0
Sep50 -3	9	0	0
Oct200-1	9	0	0
Oct200-2	8	0	1
Oct200-3	9	0	0
Oct150-1 to 3	9	0	0
Oct100-1 to 3	9	0	0
Oct50 -1 to 3	9	0	0
Nov200-1 to 3	10	0	0
Nov150-1 to 3	10	0	0
Nov100-1	9	1	0
Nov100-2 to 3	10	0	0
Nov50 -1	9	0	1
Nov50 -2 to 3	9	1	0
Jan200-1 to 3	10	0	0
Jan150-1 to 3	10	0	0
Jan100-1 to 3	10	0	0
Jan50 -1 to 3	10	0	0

curves (Sep100-1 and -2, Sep50-1 and -2, Oct100-1 and -3, Oct50-1). In these cases, curves for net photosynthesis showed hardly any increase with light level and remained below zero. Extremely high Pm and Km values were estimated for the MM-curve of Sep100-3. The tanh-model gave an α estimate for Sep100-1 which was more than 3 times higher than any other α estimate.

These 9 curves, more or less 'aberrant' when their model parameter estimates are compared with those of the other curves, still gave the best fit of the data sets according to the least squares criterion. Therefore, estimates of gross and net productivity at 200 μE m^{-2} s^{-1} (GP200 and NP200) based on these curves still can be used. However, the very high parameter values of Sep100-3 (MM-model) and Sep100-1 (tanh-model) have a strong influence on the ANOVA results. Thus, Sep100-3 for the MM-model and Sep100-1 for the tanh-model were left out from the analysis of model parameters and light compensation point (LCP). Curves with negative Km values were included in the analysis as our goal was to arrive at a description of the influence of light history and age on the form of the light-response curve. The biological meaning of the parameter estimates is only secondary (see section 2.2). LCP did not always exist (curves of net photosynthesis remained below zero because respiration was larger than the estimated Pm). Consequently, the analysis in the following sections is based on 46 data sets when model parameters are concerned, on 47 data sets for GP200 and NP200, and on 37 data sets for LCP.

When looking at the curves (Figs. 4.11a-d), two features are very clear: although each replicate curve is a reasonably good fit of its datapoints, replicate curves more often than not are rather

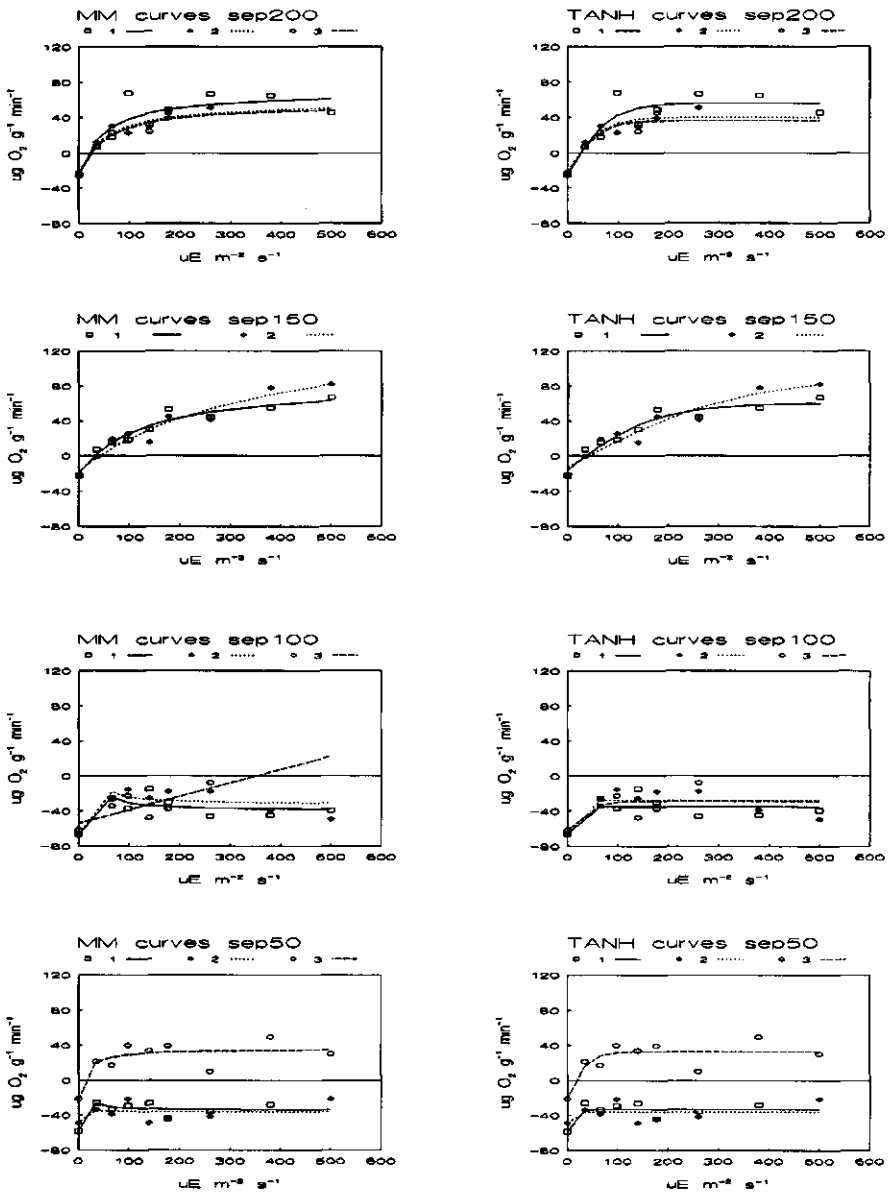


Fig. 4.11. Light response curves from all experiments with *P. pectinatus*. MM curve = rectangular hyperbola (Michaelis-Menten formula); tanh curve = hyperbolic tangent. Treatments are indicated with the abbreviated name of the respective month and the light history level (50, 100, 150 and 200 μE m^{-2} s^{-1}). Replicate curves are indicated with 1, 2 and 3.

(a) September data (age 70 days).

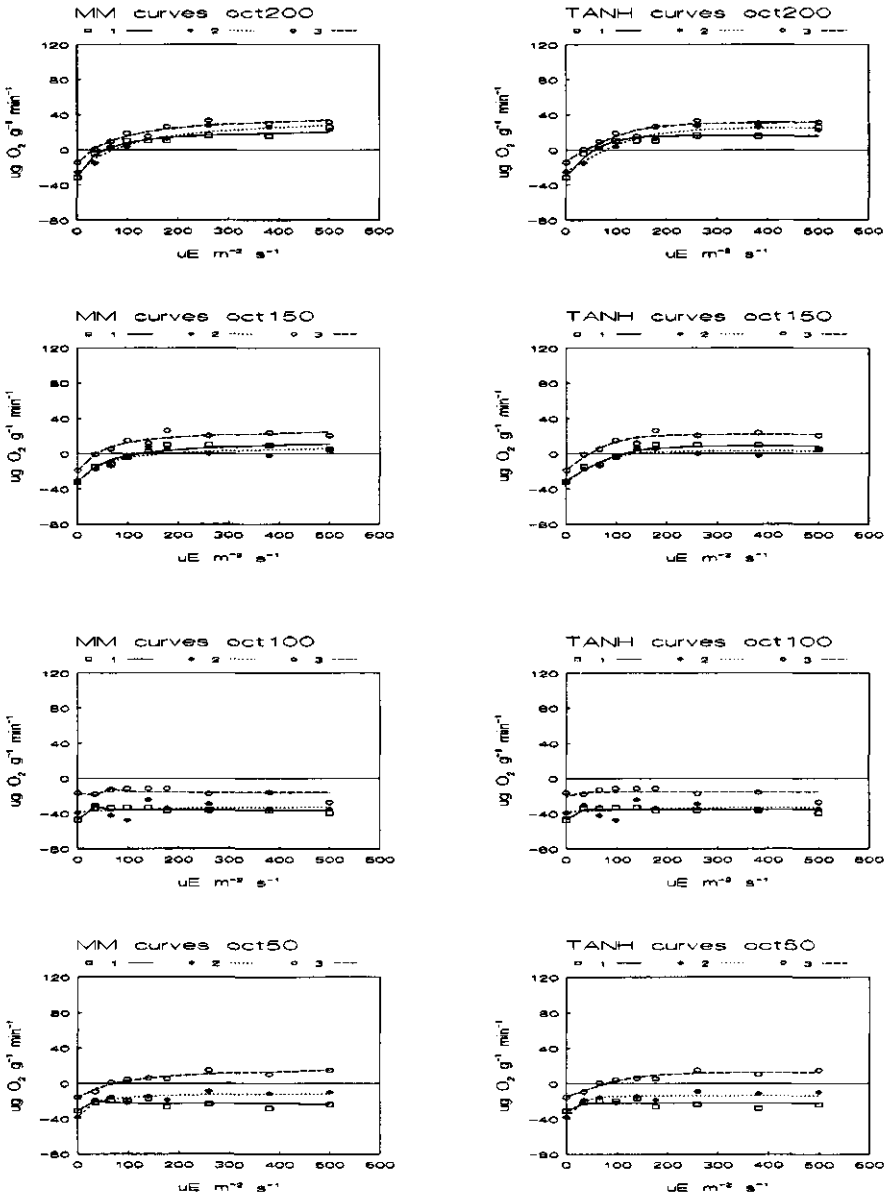


Fig. 4.11. (b) October data (age 120 days).

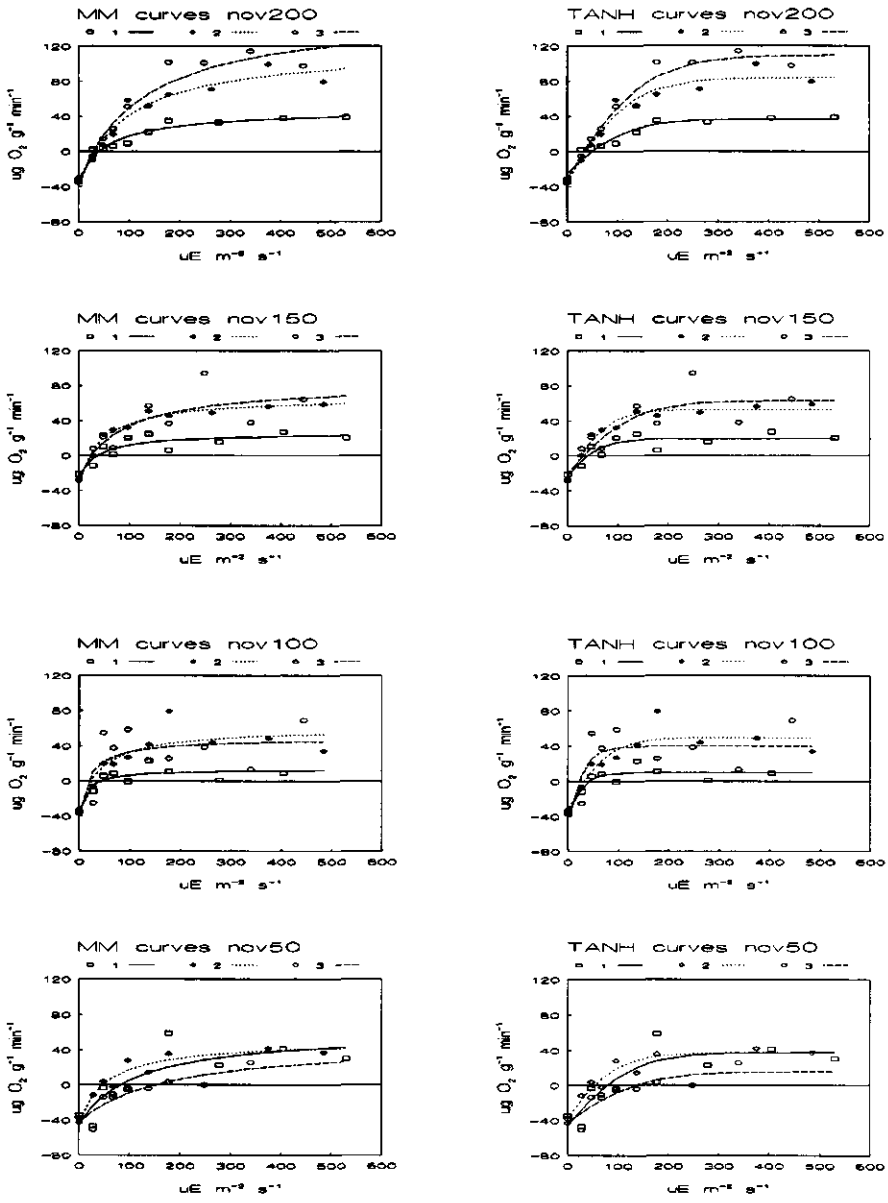


Fig. 4.11. (c) November data (age 30 days).

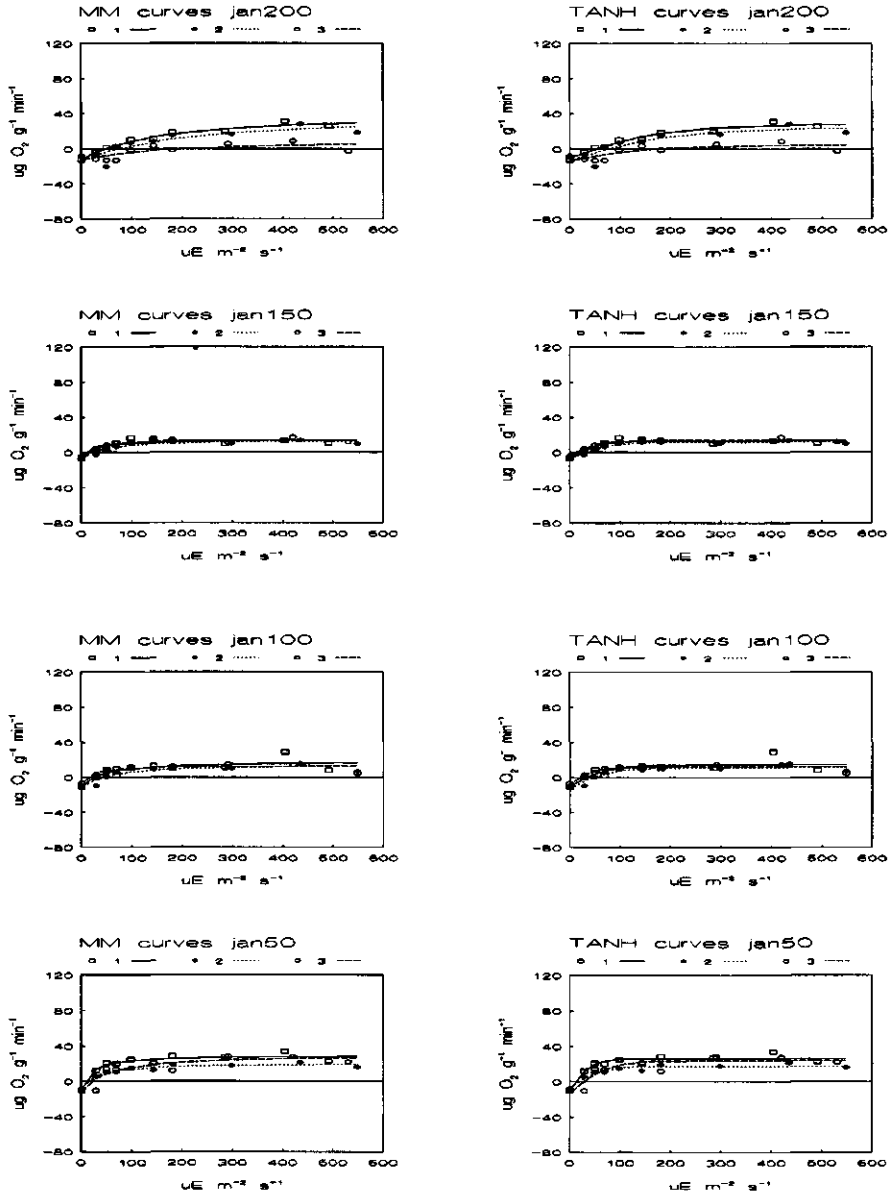


Fig. 4.11. (d) January data (age 70 days).

different, at least to the eye. Furthermore, the MM-model and tanh-model in most cases give almost the same curvature. Problems occur for the MM-model when photosynthesis shows almost no reaction to changes in light level, and even has a tendency to decrease with increasing light levels (Figs. 4.11a-b, 100 and 50 μE m^{-2} s^{-1}). In these cases, the model generates negative Km values, with a vertical asymptote for light level I = −Km as a consequence. The tanh-model clearly is more 'robust' for such situations, and still fits a 'normal' curve through the data. This might be a (subjective) reason to prefer the tanh-model above the MM-model.

4.5.1 The MM model

Data for Pm, Km and respiration R were $\log_{10}$-transformed to achieve normality and homogeneity of residual variance. For R, residual variance remained rather inhomogeneous.

Comparisons between the two sets for 70 days were made as in section 4.3. Pairwise comparisons showed a difference between the sets for light history level 150 μE m^{-2} s^{-1} for all three parameters. For R, dif-ferences also occurred for 100 and 50 μE m^{-2} s^{-1}. When differences occurred, the January estimates were lower than those for September. Table 4.14 gives the results of these 'separate' ANOVAs and the pairwise comparisons between the two sets for 70 days for the three parameters Pm, Km and R. Subsequently, the two sets for 70 days were pooled. Results from these 'combined' ANOVAs are also given in Table 4.14. Means (with the two 70 day sets combined) are shown in Figs. 4.12a-f.

The 'combined' ANOVA for Pm showed a significant effect of light history and age, and of the interaction age*light history. The significance level of the latter

Table 4.14. (a) ANOVA results for parameters Pm, Km and R from the MM-model. Number of replicates is 46, all data were $\log_{10}$-transformed. Separate = data from September and January entered in the analysis as independent levels of factor age, combined = these two sets combined. (b) Pairwise comparisons between September and January data with CER = 0.01, * = significant. (c) ANCOVA results with the same parameters. Data from September and January were combined.

(a) twoway ANOVA

	separate			combined		
	Pm	Km	R	Pm	Km	R
age	0.0001	0.2612	0.0001	0.0001	0.4145	0.0027
light history	0.0001	0.0002	0.0012	0.0002	0.0177	0.3568
age*light	0.0015	0.0055	0.0303	0.0276	0.5298	0.8842

(b) pairwise comparisons of the two 70 day groups

light history	Pm	Km	R
200	0.0940	0.0147	0.0183
150	0.0005*	0.0019*	0.0003*
100	0.7107	0.0388	0.0001*
50	0.3858	0.2196	0.0001*

(c) ANCOVA

	Pm	Km	R
age	0.0244	0.3869	0.0085
light history	0.0022	0.0190	0.0349
age*light	0.0089	0.8974	0.1883
chlor	0.0332	0.4722	0.0002

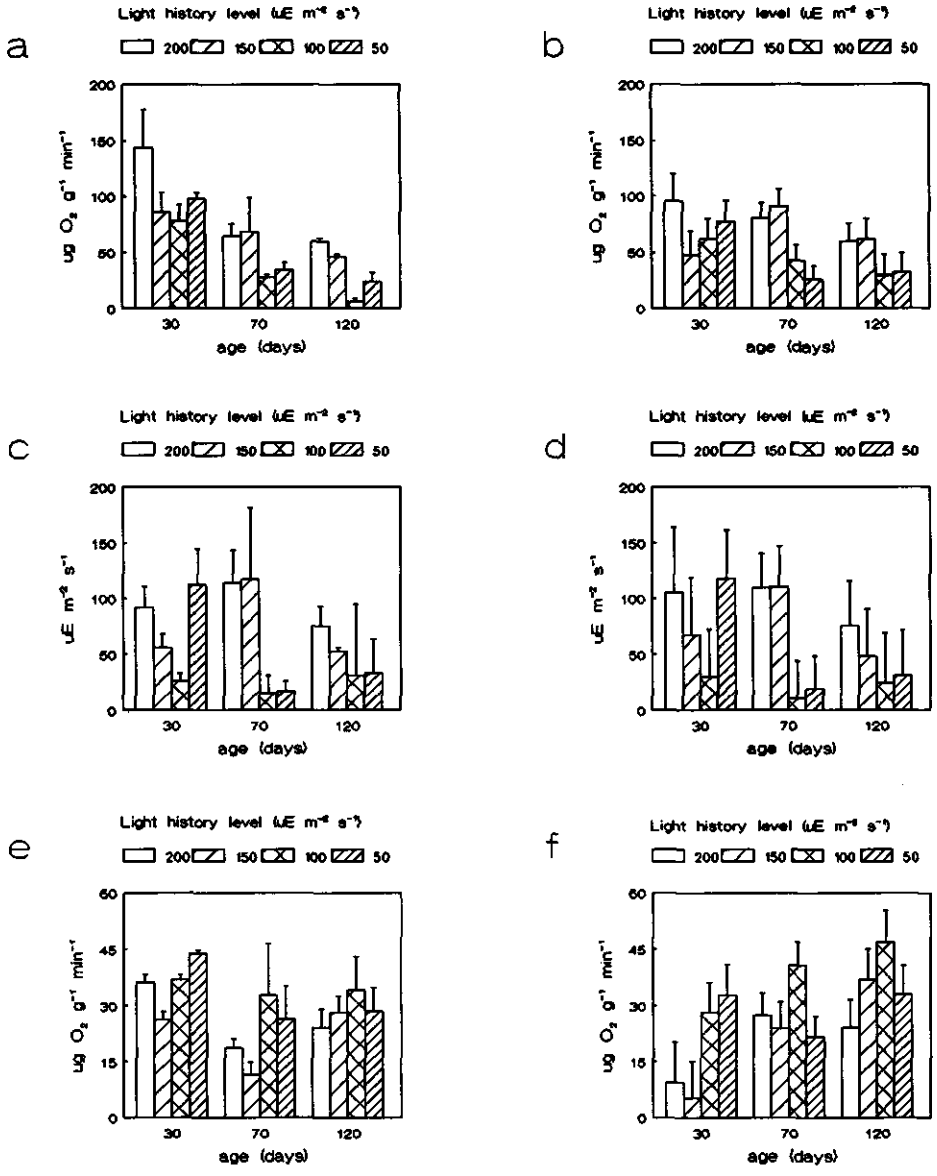


Fig. 4.12. Effects of light history and age on the model parameters in the MM model (data from September and January combined).

(a) Pm (b) Pm, LSMEANS (=adjusted means after ANCOVA; see text).

(c) Km (d) Km, LSMEANS.

(e) R (f) R, LSMEANS.

was much higher in the 'separate' ANOVA, because the effect of light history in January was much less than that in September, October and November. Pm was lower for lower light history levels and when plants were older.

The 'combined' ANOVA for Km gave an overall F-value only significant at $p < 0.0656$. Light history was significant: Km was lower for lower light history levels. In the 'separate' ANOVA, the age*light history interaction was also significant, due to the high Km at 50 μE m^{-2} s^{-1} for plants of 30 days. This effect disappeared in the 'combined' ANOVA due to the increased RSS.

The 'combined' ANOVA for R had an overall F that was significant only at $p < 0.0774$. The factor age was significant: R decreased between 30 and 70 days, and slightly increased between 70 and 120 days. In the 'separate' ANOVA, both factors and their interaction were significant: lower light history levels had a higher R value for September 70 days and for 30 days, but no effect for January 70 days and for 120 days.

Multiple comparisons have been made between the treatments, again with pooled results for age 70 days. Significant differences only existed for Pm: at light history level 100 μE m^{-2} s^{-1}, 120 days was lower than 30 and 70 days; for age 120, 100 μE m^{-2} s^{-1} was lower than 150 and 200 μE m^{-2} s^{-1}.

The effects of light history and age on the three model parameters may be partly due to chl(a+b) content, as this was also dependent on these factors (section 4.4.3). A way to filter out these indirect effects of light history and age on Pm, Km and R is an ANCOVA with chl(a+b) as covariate. The model parameter means, 'adjusted' for the covariate, are given in Fig. 4.12, together with the unadjusted means (both sets for 70 days combined). The results from the 'combined' ANCOVAs are given in Table 4.14. Multiple comparisons were not different from those after the ANOVA. For Pm, the covariate was significant, showing an effect of chlorophyll content on Pm. Still, age, light history and their interaction were also significant. For Km, no effect of chl(a+b) was apparent. The ANCOVA for Km was significant at $p < 0.0866$. The adjusted means were hardly different from the unadjusted means (Fig. 4.12). In contrast with the ANOVA for R, the ANCOVA for R was highly significant ($p < 0.0007$). Chlorophyll was a significant covariate for R, together with age and light history. The covariate filtered out a lot of the 'noise' that was due to the combination of effects of age and light history via chl(a+b) content on R. Thus, adjusted means for R were quite different from the unadjusted means, especially for the highest light history levels. The increase in respiration when age is higher or light history level is lower was very clear. We can conclude that, apparently, R increased also with increasing chl(a+b) levels.

4.5.2 The tanh-model

As with the parameters for the MM-model, parameter estimates for the tanh-model were $\log_{10}$-transformed. Data for R remained non-normally distributed. The 'separate' and 'combined' ANOVAs, together with the pairwise comparisons between the two data sets for 70 days, are given in Table 4.15. Means (data from September and January combined) are shown in Fig. 4.13. Comparing the two sets for 70 days, differences occurred for Pm at 200 and 150 μE m^{-2} s^{-1} (higher in September). For parameter α, at the light history level 200 μE m^{-2} s^{-1}, January

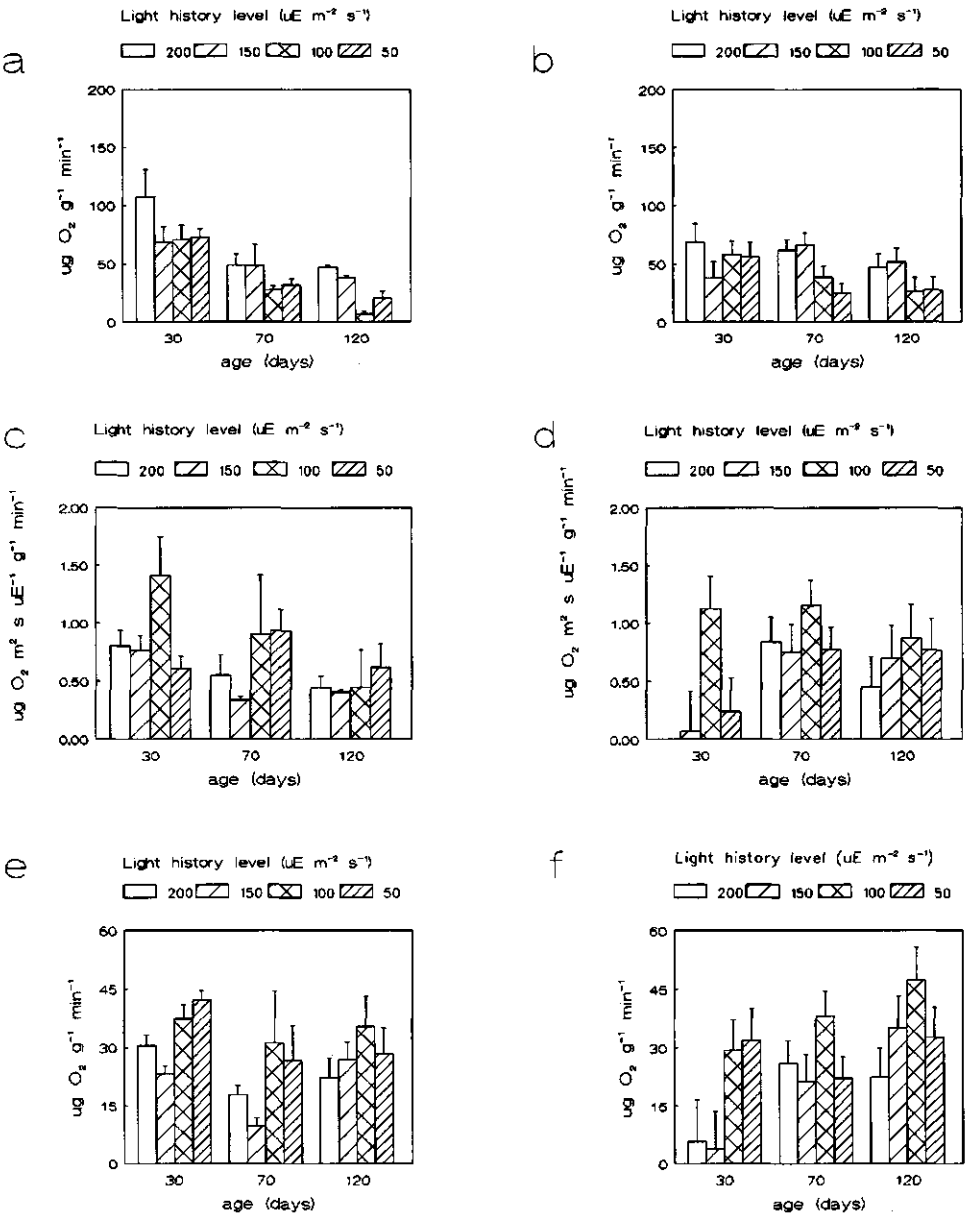


Fig. 4.13. Effects of light history and age on the model parameters in the tanh model (data from September and January combined). (a) Pm (b) Pm, LSMEANS (c) α (d) α, LSMEANS (e) R (f) R, LSMEANS.

Table 4.15. (a) ANOVA results of parameters Pm, α and R from the tanh-model. Number of replicates is 46, all data were $\log_{10}$-transformed. Separate = data from September and January entered in the analysis as independent levels of factor age, combined = these two sets combined. For α, the overall F for the 'combined' ANOVA was not significant ($p<0.1476$). (b) Pairwise comparisons between September and January data with CER = 0.01, * = significant. (c) ANCOVA results with the same parameters. Data from September and January were combined.

(a) twoway ANOVA

	separate			combined		
	Pm	α	R	Pm	α	R
age	0.0001	0.0002	0.0001	0.0001	0.0373	0.0029
light history	0.0001	0.2852	0.0002	0.0006	0.8010	0.1754
age*light	0.0003	0.0851	0.0384	0.0367	0.2789	0.8340

(b) pairwise comparisons of the two 70 day groups

light history	Pm	α	R
200	0.0072*	0.0010*	0.0230
150	0.0001*	0.7338	0.0069*
100	0.1584	0.0141	0.0001*
50	0.5210	0.1027	0.0001*

(c) ANCOVA

	Pm	α	R
age	0.0272	0.3160	0.0063
light history	0.0051	0.5047	0.0121
age*light	0.0057	0.3873	0.1641
chlor	0.0035	0.0072	0.0004

was lower than September; for R, at all light history levels except 200 μE m^{-2} s^{-1} the September values were higher.

Both factors and their interaction were significant for Pm in the 'separate' and 'combined' ANOVAs. Pm was lower with lower light history levels and with increasing age. For α, the combined ANOVA was not significant ($p<0.1476$). The separate ANOVA showed an effect of age, caused by the much lower values in January compared with September. The combined ANOVA for R was significant ($p<0.0491$). An increase in age coincided with lower respiration from 30 to 70 days, and with no change or an increase again from 70 to 120 days. The separate ANOVA also showed a significant effect of light history and the interaction age*light history: a slight increase in respiration with decreasing light history level, which was not clear for January and October. Multiple comparisons (with data for 70 days combined) were not made for α, as its ANOVA was not significant. Like in the MM model, differences were only significant for Pm: at age 120, 100 μE m^{-2} s^{-1} was lower than 150 and 200 μE m^{-2} s^{-1}; for 100 μE m^{-2} s^{-1}, 120 days was lower than 30 and 70 days, and for 50 μE m^{-2} s^{-1}, 120 days was lower than 30 days.

As with the analysis of the MM model parameters, it is interesting to perform an ANCOVA with chl(a+b) in aboveground biomass as covariate. Results from these ANCOVAs for Pm, α and R are presented in Table 4.15. In Fig. 4.13, graphs of means and adjusted means are given. Chlorophyll as covariate was significant for all three parameters. For Pm, both factors and their interaction remained significant together with the covariate. For α, the overall F test was significant ($p<0.0209$) but this was only due to the covariate (see section 4.4.3); age and light history were not significant. Apart from

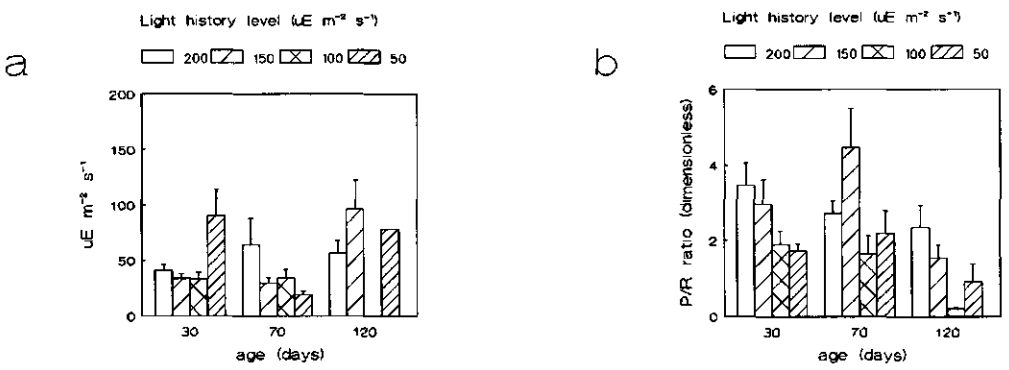


Fig. 4.14. Effects of light history and age on parameters LCP and Pm/R calculated with the tanh model (see text; data from September and January combined). (a) LCP (b) Pm/R.

age, light history also became significant for R. Again, as for the MM-model, the covariate 'cleared-up' the picture at the highest light history level for this parameter. R became higher for lower light history levels and increasing age. Multiple comparisons were not different from those after the ANOVA, except for Pm at 50 μE m^{-2} s^{-1}: no differences between the three ages were found.

4.5.3 Light compensation point and Pm/R ratio

When the net photosynthesis-light curve remains negative, no light compensation point (LCP) exists. This happens when Pm is less than R. An analysis of light history and age effects on LCP is therefore hampered, as such a 'non-existent' LCP is very meaningful, but cannot influence the ANOVA results. An alternative is to analyze the ratio Pm/R, which exists for every of the 46 curves that were used in the analysis of model parameters. But when Pm/R remains constant, LCP may change. Therefore, both derived parameters, LCP and Pm/R, have their value and are analyzed in this section.
Data were $\log_{10}$-transformed for the MM-model and for the tanh-model. Results for the two models were very similar. In the following, only the tanh-model results are discussed.

The results of 'separate' ANOVAs and comparisons between the two sets (September and January) are given in Table 4.16. Differences between the two 70 day sets occurred for LCP (at light history level 200 μE m^{-2} s^{-1}) and for Pm/R (light history level 100 and 50 μE m^{-2} s^{-1}).

Results from the 'combined' ANOVAs (Table 4.16) did not differ from their respective ANCOVAs: the covariate chlorophyll was not significant. Means for LCP and Pm/R are depicted in Fig. 4.14. The effect of age was significant for both parameters. Higher age correlated with higher LCP and lower Pm/R. Light history was significant for Pm/R only: lower light history levels had a lower Pm/R ratio. For LCP, the age*light history interaction was significant both in the

Table 4.16. ANOVA results of derived parameters LCP, Pm/R, GP200 and NP200 (tanh-model). ANCOVA only showed a significant effect of chl(a+b) content as covariate in GP200. Number of replicates is 37 for LCP, 46 for Pm/R, 47 for GP200 and NP200. Data for LCP and Pm/R were $\log_{10}$-transformed. Separate = data from September and January entered in the analysis as independent levels of factor age, combined = these two sets combined. Pairwise comparisons between September and January data with CER = 0.01, * = significant, n.e. = LCP not existent.

(a) twoway ANOVA with LCP and Pm/R

	separate		combined	
	LCP	Pm/R	LCP	Pm/R
age	0.0016	0.0001	0.0050	0.0003
light history	0.3828	0.0001	0.8528	0.0001
age*light	0.0004	0.0012	0.0179	0.1538

(b) twoway ANOVA and ANCOVA with GP200 and NP200

	separate		combined		ANCOVA	
	GP200	NP200	GP200	NP200	GP200	NP200
age	0.0001	0.0001	0.0001	0.0001	0.1640	0.0394
light history	0.0009	0.0001	0.0066	0.0004	0.0307	0.0015
age*light	0.0846	0.0046	0.6334	0.8227	0.2162	0.8359
chlor	-	-	-	-	0.0006	0.5454

(c) pairwise comparisons of the two 70 day groups

light history	LCP	Pm/R	GP200	NP200
200	0.0007*	0.5939	0.0007*	0.0231
150	0.1026	0.1222	0.0036*	0.0408
100	n.e.	0.0031*	0.3015	0.0032*
50	0.8694	0.0005*	0.8888	0.0151

separate and in the combined ANOVA: probably caused by the relatively high LCP of plants of 30 days and light history level 50 μE m^{-2} s^{-1}. This interaction also existed in the separate ANOVA for Pm/R, but disappeared in the combined ANOVA: it is caused by a decrease in Pm/R with lower light history levels for September while January remained relatively constant with changing light history level. Multiple comparisons for LCP and Pm/R (with both 70 day sets combined) showed a few significant differences. For light history level 50 μE m^{-2} s^{-1}, LCP of 70 days was lower than LCP of 30 days. For 100 μE m^{-2} s^{-1}, Pm/R of 120 days was lower than 30 and 70 days. For 120 days, Pm/R at light history level 100 μE m^{-2} s^{-1} was lower than at 150 and 200 μE m^{-2} s^{-1}.

4.5.4 Net and gross photosynthesis at 200 μE m^{-2} s^{-1}

An estimate of net and/or gross photosynthesis at a certain light level within the range used in the experiment is very worthwhile when estimated model parameter values such as Pm and Km are large. As extrapolations the latter two are usually subject to more variation then such a derived parameter. The fitted curve

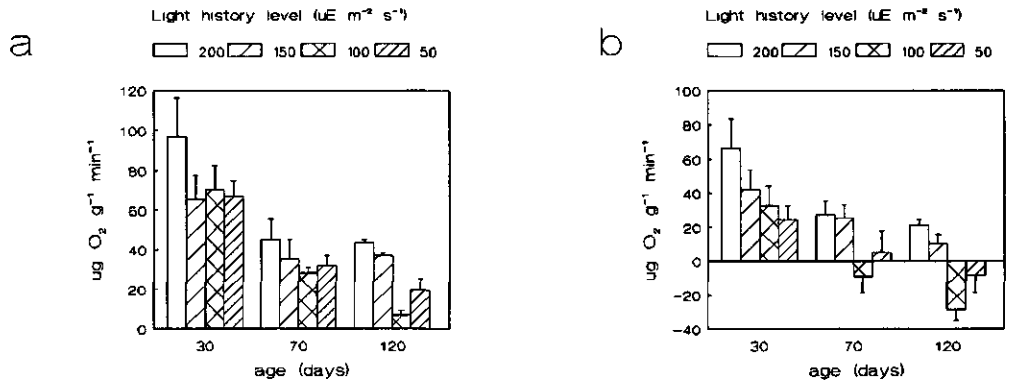


Fig. 4.15. Effects of light history and age on parameters GP200 and NP200 calculated with the tanh model (see text; data from September and January combined). (a) GP200 (b) NP200.

gives the 'best' approximation (in terms of minimized RSS) of the 'real' relation between light and photosynthesis within the range of light levels used during the experiment. In this section, the effects of light history and age on net (NP200) and gross (GP200) photosynthesis at 200 μE m^{-2} s^{-1} are studied. The data used were estimated with the tanh-model. The MM-model estimates gave comparable results (see Fig. 4.11).

No $\log_{10}$-transformation was necessary. The results of the ANOVAs and ANCOVAs are given in Table 4.16. In Fig. 4.15, GP200 and NP200 for the tanh-model are shown. For both parameters differences existed between the data sets for 70 days: for GP200 at light history levels 200 and 150 μE m^{-2} s^{-1}, for NP200 at 100 μE m^{-2} s^{-1}. Separate ANOVAs showed significant effects for age, light history and their interaction on GP200 and NP200. When the 70 day data were combined, the significance of the interaction effect was lost. Lower light history levels and higher age resulted in lower GP200 and NP200. Chlorophyll as covariate was significant only for GP200, leading to the disappearance of an age effect. Multiple comparisons for the tanh-model parameters showed significant differences between plants from 30 and 120 days, grown at 100 μE m^{-2} s^{-1} for GP200 and NP200, and between plants of 30 days and 70-120 days, 200 μE m^{-2} s^{-1} for GP200. The few significant differences among the multiple comparisons disappeared in the means adjusted for the covariate chlorophyll (not shown).

4.6 Discussion: photosynthesis curves

4.6.1 Variation between replicate measurements

As mentioned in section 4.5.1, we sometimes found rather large differences between replicate curves. Westlake (1967), working with *Ranunculus peltatus* ssp. *pseudofluitans* (Syme) C. Cook and *P. pectinatus*, also mentioned significant differences in rates of photosynthesis between plants, ranging up to 20-30%.

Sometimes, but certainly not always, this coincided with a different origin of the material and/or measurements in different times of the year. Orr et al. (1988) gave parameter values on a chl(a+b) basis for a tanh curve fit of light-response data from *Myriophyllum salsugineum* A.E. Orchard. When we recalculated their parameter values on a dw basis with their data on chl(a+b) content, it appeared that they also had a strong variation among replicate curves. Due to a remarkably strong variation in chl(a) content (4-9.25 mg g^{-1} dw) of plant material apparently collected at the same moment and at the same location, net Pm varied with 25%, α with 50-100%, while respiration was rather constant.

Reasons for such strong variation within treatments are unclear; variation of this kind is not often mentioned in studies on P-I relationships. However, as a source of variation, normal measurement error almost becomes negligible in the light of such differences between seemingly comparable plant material.

4.6.2 Comparison of photosynthesis of *P. pectinatus* with literature data

In Table 4.17, we present a short overview of data on photosynthesis parameters from various plant species. Although comparisons within and between species may have a limited value due to differing experimental conditions and calculations, they are necessary when photosynthetic capacities of a species, i.e. *P. pectinatus*, are evaluated. For easy interpretation, we have converted data from other authors into our own units. This may have introduced bias, as sometimes not all data necessary for proper conversion were available. Conversion factors used are given also in Table 4.17. The results from Van der Bijl et al. (1989) were averaged by us over the season using their Fig. 2; their results showed distinct peak values in June, declining during the rest of the season.

The first impression from Table 4.17 is a rather strong variation between and within species. Our net photosynthesis data are within the range of those from other authors working with *P. pectinatus*. The rather low value found by Jana & Choudhuri (1979) can be explained by the very low light intensity used, and a rather high temperature with high light respiration. The results found by Hough & Fornwall (1988) appear extremely low. Net rates of photosynthesis of *P. pectinatus* are of the same magnitude as results found for *E. canadensis*. Data for *H. verticillata* and *E. densa* at 24 °C are also in this range. Much higher values were reported for *M. salsugineum* and *Z. noltii* at comparable temperatures.

Our dark respiration values appear somewhat high compared to results from Peñuelas et al. (1988) and Madsen & Adams (1989), but are comparable with those of Van der Bijl et al. (1989). *P. pectinatus* respiration is not very different from that of other species, only respiration of *E. densa* appears rather high. A remarkable difference exists between the two *H. verticillata* values: dark respiration seems much lower at higher temperatures.

Literature data on α and Km values for macrophytes are scarce. Titus & Adams (1979) estimated Km values for *V. americana* and *M. spicatum* coming from various light history levels. They concluded that the former species is a much more efficient carbon fixer at low light levels than the latter. It is important to realize that this conclusion, based on Km values, is only valid when Pm values of the two species do not differ very much (which apparently was the case). Species

Table 4.17. Comparison of various photosynthesis parameters. Light intensity (μE m^{-2} s^{-1}) used is indicated by a number. Sat = saturating light intensity. Apex, basis: apical and basal plant material. 30-200 = results from this study, age 30 days, light history level 200 μE m^{-2} s^{-1}. Other = results from this study, range of data from other conditions than 30-200. All parameter estimates from this study are based on the tanh model, except Km values.

Conversion factors used: afdw=10% fw, afdw=80% dw, average chl(a+b) content=5 mg g^{-1} afdw, PQ=1, molar gas volume = 22.4 l. Light intensity units were converted to μE m^{-2} s^{-1} following McCree (1972b).

net rate of photosynthesis P (μg O_2 g^{-1} afdw min^{-1})

conditions	P	species	reference
15 °C, sat	53	Pp	1
35 °C, 40	17	Pp	5
20 °C, sat	80 to 120	Pp	10
20 °C, 288	1	Pp	15
20 °C, 500	4-6	Pp	15
20 °C, sat	61	Pp	18
varying temp, apex	100	Pp	19
varying temp, basis	0	Pp	19
20 °C, 200, 30-200	66	Pp	20
20 °C, 200, other	-29 to 42	Pp	20
20 °C, 30-200, sat	77	Pp	20
20 °C, other, sat	-28 to 45	Pp	20
20 °C, sat	294	Ms	16
24 °C, 1050	66	Ed	7
24 °C, 1050	44	Hv	7
30 °C, sat	145	Hv	4
30 °C, sat	135	Cd	4
30 °C, sat	155	Msp	4
20 °C, sat	73	Ec	11
15 °C, sat	166 to 333	Zn	13
20 °C, 200	62 to 151	Cd	12

dark respiration R (μg O_2 g^{-1} afdw min^{-1})

conditions	R	species	reference
15 °C	10	Pp	1
35 °C	46	Pp	5
18 °C	12	Pp	18
varying temp, apex	60	Pp	19
varying temp, basis	40	Pp	19
18 °C, 30-200	31	Pp	20
18 °C, other	12 to 44	Pp	20
25 °C; leaves	26	Pp	17
25 °C; stems	9	Pp	17
20 °C	26	Ms	16
24 °C	78 to 100	Ed	7

dark respiration R (μg O_2 g^{-1} afdw min^{-1})

conditions	R	species	reference
24 °C	44 to 55	Hv	7
30 °C	7	Hv,Cd,Msp	4
20 °C	21	Ec	11

apparent quantum yield α (μg O_2 m^2 s μE^{-1} min^{-1} g^{-1} afdw)

conditions	α	species	reference
varying temp, apex	3.1	Pp	19
varying temp, basis	1.0	Pp	19
20 °C,30-200	0.8	Pp	20
20 °C, other	0.3 to 1.4	Pp	20
20 °C	6.5	Ms	16
varying temp	0.7 to 4.3	Cd	9
varying temp	8.6 to 11.5	P	3
varying temp	6.7	P	8

Michaelis Menten constant Km (μE m^{-2} s^{-1})

conditions	Km	species	reference
20 °C	249	Pp	18
20 °C, 30-200	92	Pp	20
20 °C, other	15 to 117	Pp	20
25 °C	60 to 197	Va	6
25 °C	164 to 365	Msp	6
20 °C	50	Ms	16
varying temp	50 to 350	Cd	9
varying temp	114 to 120	P	3
varying temp	177	P	8

light compensation point LCP (μE m^{-2} s^{-1})

conditions	LCP	species	reference
15 °C	35	Pp	1
varying temp, apex	20	Pp	19
varying temp, basis	100	Pp	19
20 °C, 30-200	41	Pp	20
20 °C, other	19 to 96	Pp	20

Table 4.17. Continued.

light compensation point LCP (μE m^{-2} s^{-1})				light compensation point LCP (μE m^{-2} s^{-1})			
conditions	LCP	species	reference	conditions	LCP	species	reference
30 °C	15	Hv	4	20 °C	6 to 8	Ppo	2
30 °C	35	Cd	4	20 °C	0 to 3	Po	2
30 °C	35	Msp	4	10 °C	1 to 6	Ul	14
5 to 30 °C	23 to 37	Ec	11	varying temp	3.5 to 4.6	P	3
15 °C	30 to 35	Zn	13	varying temp	4.7	P	8
20 °C	4	Ms	16				

Species: Cd = *C. demersum*, Ec = *E. canadensis*, Ed = *E. densa*, Hv = *H. verticillata*, Ms = *M. salsugineum*, Msp = *M. spicatum*, P = marine phytoplankton, Po = *P. obtusifolius*, Pp = *P. pectinatus*, Ppo = *P. polygonifolius*, Ul = *U. lactuca*, Va = *V. americana*, Zn = *Z. noltii*.

References: (1) Westlake (1967), (2) Spence & Chrystal (1970), (3) Platt & Jassby (1976), (4) Van et al. (1976), (5) Jana & Choudhuri (1979), (6) Titus & Adams (1979), (7) Barko & Smart (1981), (8) Côté & Platt (1983), (9) Fair & Meeke (1983), (10) Sand-Jensen (1983), (11) Pokorný et al. (1984), (12) Best & Dassen (1987), (13) Jiménez et al. (1987), (14) Vermaat & Sand-Jensen (1987), (15) Hough & Fornwall (1988), (16) Orr et al. (1988), (17) Peñuelas et al. (1988), (18) Madsen & Adams (1989), (19) Van der Bijl et al. (1989), (20) this study.

with high Km values can overcome problems at low light intensities when they also have high Pm values. Fair & Meeke (1983) presented I_k (=Km) and α values for *C. demersum* based on field measurements during a year. The strong variation found during the season was attributed to some sort of light adaptation. Our Km values are much lower than those found by Madsen & Adams (1989). They worked with *P. pectinatus* shoots collected in the field, which were cultured for two weeks in the lab at 400 μE m^{-2} s^{-1} prior to the experiments. Their results are in line with the increase in Km that we found with increasing light history level. Van der Bijl et al. (1989) found much higher α values for *P. pectinatus* than we did: up to 8.3 in spring for apical parts. Again, this might be attributed to higher light intensities received by their field material. In their experiments, basal parts (more shaded, older) had lower α values, comparable to our results. From Table 4.17 it appears that *P. pectinatus* and *C. demersum* have more or less comparable α values, although the latter has a higher maximum. Our α values are very low in comparison with marine phytoplankton and *M. salsugineum*. Roughly, Km of *P. pectinatus* is about equal to Km of *C. demersum*, *M. salsugineum* and *V. americana*. The latter three have higher upper limits. Results from *M. spicatum* and the marine phytoplankton results from Côté & Platt (1983) are higher than the values found for *P. pectinatus*.

Literature data on LCP can be divided into two groups. LCPs of 15-41 μE m^{-2} s^{-1} are found for several species of macrophytes: *C. demersum*, *E. canadensis*, *H. verticillata*, *M. spicatum*, *P. pectinatus* and *Z. noltii*. LCPs of 1-6 μE m^{-2} s^{-1} are found for marine phytoplankton, for the macro-alga *U. lactuca* and for the macrophytes *M. salsugineum*,

P. obtusifolius and *P. polygonifolius*. Spence & Chrystal (1970) used leaf material, which might explain the rather low LCP of their plants (i.e. presumably not very much non-photosynthesizing supporting tissue present). However, Jiménez et al. (1987) also used young leaf material for *Z. noltii*. LCP values for our older *P. pectinatus* plants are rather high. LCP and dark respiration appear not correlated: Orr et al. (1988) found an LCP of 4 μE m^{-2} s^{-1} and an R of 26 μg O_2 g^{-1} afdw min^{-1} while Pokorný et al. (1984) with an R of 21 μg O_2 g^{-1} afdw min^{-1} found an LCP of 23 μE m^{-2} s^{-1}. Van et al. (1976) measured an LCP of 15-35 and a respiration of 7 μg O_2 g^{-1} afdw min^{-1}.

Summarizing, we conclude that compared to other species, *P. pectinatus* does not show remarkably deviant photosynthetic parameter values. Net photosynthesis and dark respiration are more or less 'average'. Parameter α, determining the slope of the light response curve, is at the lower end of the range of values found for other species. This may indicate that physiologically, *P. pectinatus* is not very well adapted for photosynthesis under low light intensities. However, physiological shortcomings may be overcome through an adaptive morphology, i.e. strong elongation under low light intensities (see Fig. 4.6c and Vermaat & Hootsmans, 1991b).

4.6.3 Effects of light history and age

In general, our plants from low light history levels had lower Pm, Pm/R, GP200, NP200 and Km, while α, LCP and R were not affected. LCP and α behaved rather erratic. The decrease in Pm occurred despite the increase in chl(a+b) in the leaves. This may be a result of the reduced amount of leaf biomass per aboveground biomass. Acclimation to low light levels can be achieved by an increased capability to absorb quanta. This can be reached by increasing chlorophyll content, or more specifically, the number and/or size of photosynthetic units (PSU). A PSU can be defined as the number of chlorophyll molecules per molecule of oxygen produced under saturating light conditions (Falkowski, 1981). The latter author, working with marine phytoplankton species, mentioned an increase in the time between successive oxygen molecules produced per PSU when chlorophyll content increases. Alternatively, Perry et al. (1981) defined PSU as the molar ratio of total chlorophyll to reaction centre chlorophyll, P700. They found decreasing α, increasing chl(a) content and increasing PSU size with decreasing light level for several marine algae. These results can be related to self-shading of the photosynthetic pigments and/or increased 'path-length' of the electron transport chain in a large PSU. In our case, as the fraction chl(b) of total chlorophyll is not much influenced by light history, increase in PSU size presumably did not occur.

The presently found decrease in Pm with lower light history levels is in accordance with Boardman (1977). He also mentioned a lowering of R and LCP as adaptation to low light, but this was not observed in our experiments. When we corrected R for chl(a+b) content, R even slightly increased when the light history level was lower. According to Spence & Chrystal (1970), *P. polygonifolius* and *P. obtusifolius* showed a reduction in initial slope of the light response curve (comparable to α), reduced respiration and LCP in response to shade conditions. King & Schramm (1976) found a lowered R and LCP for marine macro-algae collected in winter compared to summer material,

which can be due to lower light level but also to lower temperatures in winter (see 4.6.3). Vermaat & Sand-Jensen (1987) found a change in LCP from 6 to 1 μE m^{-2} s^{-1} in *U. lactuca* when light level in the culture was lowered from 60 to 2 μE m^{-2} s^{-1}. Barko & Smart (1981) measured P-I curves in freshwater macrophytes grown under different experimental light-temperature conditions in a greenhouse. Remarkably, regarding the results from Boardman (1977), they found an increasing net rate of photosynthesis when light level during growth was lower for *H. verticillata* and *E. densa*. Respiration of *H. verticillata* was hardly affected, while *E. densa* showed a maximum for 50% shade.

Effect of age on photosynthesis is scarcely mentioned. In our experiments, Pm decreased strongly with increasing age, especially in the first two months. GP200, NP200, R and Pm/R decreased and LCP increased with increasing age. Km and α were unaffected (possibly due to large variation in the data on these parameters). Remarkably, when R was corrected for chl(a+b) content, R increased with age. A rectangular hyperbola gave a reasonable fit of the 47 R and chl(a+b) datapairs ($p<0.001$) but its RSS was not significantly lower than the RSS of a simple linear regression ($r^2=0.18$, $p<0.003$). However, a saturating curve can be expected for such a relation. In the hyperbolic fit, R increased with chlorophyll content up to 5 mg chl(a+b) g^{-1} afdw. Apparently, the increase in R due to age is counteracted by the decrease in R due to lowering chl(a+b) content when plants get older. Why R and chl(a+b) are thus related is unclear; perhaps chl(a+b) content reflects synthetic and consequently respiratory activity of plant tissue.

Van der Bijl et al. (1989) worked with whole shoots of *P. pectinatus* from the field during a growing season. Pm, R, and chl(a+b) levels decreased while LCP increased during the season. The results were attributed to increased mean age and increased self-shading. The same trend was found comparing basal (older) parts and apical (younger) plant parts. Jana & Choudhuri (1979) compared young, mature and old leaves of *Vallisneria spiralis* L. and found a decrease in gross photosynthesis and respiration for leaves of increasing age. Drew (1979) found decreasing chlorophyll content and photosynthesis in the seagrass *P. oceanica* in summer as compared to spring. He mentioned 'leaf senescence, probably caused by daylength changes rather than by irradiance or temperature changes' as the cause for these phenomena. Kemp et al. (1986) measured net photosynthesis at 120-140 μE m^{-2} s^{-1} of shoots of *P. perfoliatus* from a laboratory culture on 6 occasions in a 5 month period but did not find any differences. It is not clear, however, whether the mean age of the material that was used was really increasing during the experimental period. The indicated length of the intact plants was between 20 and 30 cm, and thus the plants in subsequent experiments may have been fresh tips of about the same, low, age. Urbanc-Berčič & Gaberščik (1989) presented light-response curves of *Eichhornia crassipes* (Mart.) Solms plants collected in August, September and October. They found a clear depression of the rate of photosynthesis which correlated with decreasing ambient temperature and insolation. It is tempting to suggest an age effect also, but again, the real age of the material is unknown. In general, the problem of establishing the age of material increases with the complexity of the organism. A growing plant consists of many organs of varying age. Thus, it is difficult to link the response of the plant

as a whole to a precise age. In fact, we are using the time period since the beginning of the culture to indicate the age of the plant material. The mean age of the plant tissue will probably be lower, depending a.o. on the rate of leaf turnover.

It should be realized that senescence and ageing are only descriptive terms for a complex process that is linked to age and may be influenced by other factors like light history and temperature. We can see its effect in reduction of photosynthesis and growth, changes in cell content and finally in the occurrence of death, but the real nature of the process remains unclear.

4.6.4 Temperature effects

Temperature was kept constant in our experiments. It is almost certain that temperature can influence the results that we have presented. Therefore, some attention is given here to possible effects of temperature on photosynthesis. Bulthuis (1987), in a review of temperature effects on photosynthesis in seagrasses, found a doubling of Pm, R and LCP, but no effect on α, when temperature increased from 5 to 30 °C. Gross photosynthesis at saturating light levels increased linearly with temperature in several tropical seagrasses, while respiration was less influenced, according to Drew (1979). Based on his Table 4, we calculated that when temperature increased from 10 to 30 °C, gross photosynthesis at least doubled. Orr et al. (1988) studied temperature effects on *M. salsugineum*. For comparison, their data are presented in units used in this study. Net Pm, which they estimated subjectively from graphs, increased from 32 to 72 $\mu g\ O_2\ mg^{-1}$ chl(a) min^{-1} when temperature increased from 15 to 30 °C. On a dry weight basis, calculated with their data on chl(a) content, the increase in net Pm is less dramatic: 237 to 292 $\mu g\ O_2\ g^{-1}$ dw min^{-1}. Respiration increased from 14 to 60 $\mu g\ O_2\ g^{-1}$ dw min^{-1}. No effect on α was found; this parameter was rather variable. Km and LCP increased exponentially with increasing temperature. Kerr & Strother (1985) worked with the seagrass *Zostera muelleri* Irmisch ex Aschers. They found at 47 $\mu E\ m^{-2}\ s^{-1}$ an increase of net photosynthesis from 5 to 60 $\mu g\ O_2\ cm^{-2}$ leaf surface h^{-1} when temperature was increased from 3 to 30 °C, while respiration increased from 5 to 79 $\mu g\ O_2\ cm^{-2}\ h^{-1}$. *E. canadensis* reacted to increasing temperatures (5 to 20 °C) with increasing respiration (8 to 20 $\mu g\ O_2\ g^{-1}$ dw min^{-1}) and net Pm (20 to 60 $\mu g\ O_2\ g^{-1}$ dw min^{-1}) and rather constant LCP (23 $\mu E\ m^{-2}\ s^{-1}$; Pokorný et al., 1984). From 20 to 30 °C, net Pm decreased to 30 $\mu g\ O_2\ g^{-1}\ min^{-1}$ while respiration and LCP increased further, up to 24 $\mu g\ O_2\ g^{-1}$ dw min^{-1} and 37 $\mu E\ m^{-2}\ s^{-1}$ (figures derived by us from Fig. 11 in Pokorný et al., 1984). Madsen & Adams (1989) studied temperature effects on photosynthesis in *P. pectinatus*. From their linear equations, we estimate that Pm increased with 17 $\mu g\ O_2\ g^{-1}$ afdw min^{-1} and Km increased with 50 $\mu E\ m^{-2}\ s^{-1}$ for a 10 °C increase in temperature. These relations were linear up to 30-35 °C, when photosynthesis decreased sharply. R increased exponentially with temperature, again up to 35 °C. We calculated also that within the temperature range of 15-25 °C (normal for the growing season in temperate regions), the net photosynthetic rate at 200 $\mu E\ m^{-2}\ s^{-1}$ varied not more than 15% around the mean rate for this range.

It is clear, that the quantitative effect of temperature is rather variable. This may be related to the fact that temperature effects usually can be described

with optimum curves, different for different species. Qualitatively, we can expect for *P. pectinatus* that up till a certain level (30 °C), increasing temperatures will lead to increased Pm, Km, R and LCP, while α will remain rather constant (or erratic). Changes in these parameters up to 50% with 5-10 °C temperature difference may occur, but this seems less likely for *P. pectinatus* regarding the results of Madsen & Adams (1989).

4.7 Conclusions

P. pectinatus plants from our laboratory cultures did not show an effective shade acclimation of their photosynthetic capabilities. Chl(a+b) content of leaves did increase when light levels decreased, but expressed per total aboveground biomass, this increase was rather small. The fraction chl(b) did not change at all. Respiration and LCP were not influenced, while Pm was even reduced under low light intensities. The initial slope of the curve, α, did not increase. The only parameter showing some acclimation might be Km, which decreased with lower light history levels, thereby making it possible that Pm was reached at lower light intensities. However, the general impression is that the laboratory cultures showed decreased photosynthetic rates when grown at lower light intensities.

'Ageing' correlated with reduction of chl(a+b) content, Pm and R while LCP increased. Corrected for chl(a+b), R increased with increased age. These age effects can be expected to cause a deterioration of tissue condition and an increased probability of sloughing through wave action during the growing season.

5. Light-response curves of *Potamogeton pectinatus* L. and *P. perfoliatus* L. from Lake Veluwe

5.1 Introduction

In 1987 plant material was obtained from an experimental area in Lake Veluwe for comparison of photosynthetic characteristics of *P. pectinatus* from the field with those of the laboratory cultures of this species (section 4). In the field, growth and development of this species was studied under four different shading levels (see Van Dijk & Van Vierssen, in press). We collected material for photosynthesis measurements at three times during the growing season. In June, *P. pectinatus* was used from all four light treatments (100%, 74%, 55% and 27% of daylight, indicated with light history level 1, 2, 3 and 4 in the following). In July, no plants were found under the light history level 4; for comparison, *P. perfoliatus* from level 1, referred to as P, was used in addition to the other three *P. pectinatus* treatments. In August, only at level 1 vegetation was left. We therefore used *P. pectinatus* and *P. perfoliatus* only from this light history level. Both species are common in Lake Veluwe.

5.2 Material and methods

Plants with underground material were dug out and adhering sediment was gently washed off. In the laboratory, about 20 g of fresh plant weight was combined for a replicate measurement. These plants were

kept overnight in the dark in the experimental set-up prior to photosynthesis measurements during the following day. Photosynthesis measurements were done as described in section 4.2.2. The morphometric description of the plants from Lake Veluwe (referred to as 'field') was kept simple as the architecture of these plants was much more complex than laboratory-cultured plants from Texel (referred to as 'laboratory'). Individual plants (i.e. material originating from one tuber) could not be discerned. In June we counted the number of leaf-bundles per shoot (sensu Vermaat & Hootsmans, 1991a) for a subsample of shoots, and we measured shoot length. Chlorophyll samples of leaves were taken and analyzed in the same way as for the laboratory cultures (section 4.2.3). No nutrient analyses were done. Shoots from each replicate measurement were divided in leaves, stems, and belowground parts, and 105 °C dw and 520 °C afdw were determined. In July and August, only chlorophyll content and biomass were measured, in the same way as for the June samples.

Treatments and replicates are indicated by the abbreviated name of the month of collection, the light history level and the replicate number, e.g. Jun1-2 is replicate 2 from light history level 1 in June. In the case of *P. perfoliatus*, P is used in stead of the light history level (which was 1 in all cases). The three different collection dates are referred to as age although it is possible that the actual age of the plant material did not increase with the same amount as the time difference between collection dates (see discussion of results from Kemp et al. (1986) in section 4.6.3).

In the Jul1 experiment the six lowest light level measurements failed due to electrode malfunction in two of the three replicates. The normal procedure was continued for the remaining three light levels with new electrodes, and subsequently the missing light levels were measured again for all three replicates.

For information on statistical analysis, we refer to section 4.2.4. For fitting of the light-response curves we used the MM and tanh models as in section 4. The inclusion of a second species made it necessary to perform two ANOVAs. One, including both species, was used to make multiple comparisons within light history and age groups of *P. pectinatus*, between both species and within the age group of *P. perfoliatus*. In total, 17 multiple comparisons were made. A second ANOVA was performed to evaluate effects of light history and age on photosynthetic characteristics of *P. pectinatus* alone.

5.3 Results

5.3.1 Morphology and chlorophyll content

In Fig. 4.16a the number of bundles per shoot length for *P. pectinatus* in June is given. The one-way ANOVA with $\log_{10}$-transformed data was significant ($p < 0.0001$). The ratio was lower for lower light history levels.

Data on leaf ratio (leaf biomass per total aboveground biomass) remained not-normally distributed after $\log_{10}$-transformation. Thus, multiple comparison results may be biased (section 4.2.4). The ANOVA with untransformed data showed a significant effect of light history and the interaction age*light history (Table 4.18). Data are shown in Fig. 4.16b. The ratio was constant in June but in July light-2 was significantly lower than the other light history levels. In August, the ratio for *P. pectinatus* was higher than for *P. perfoliatus*.

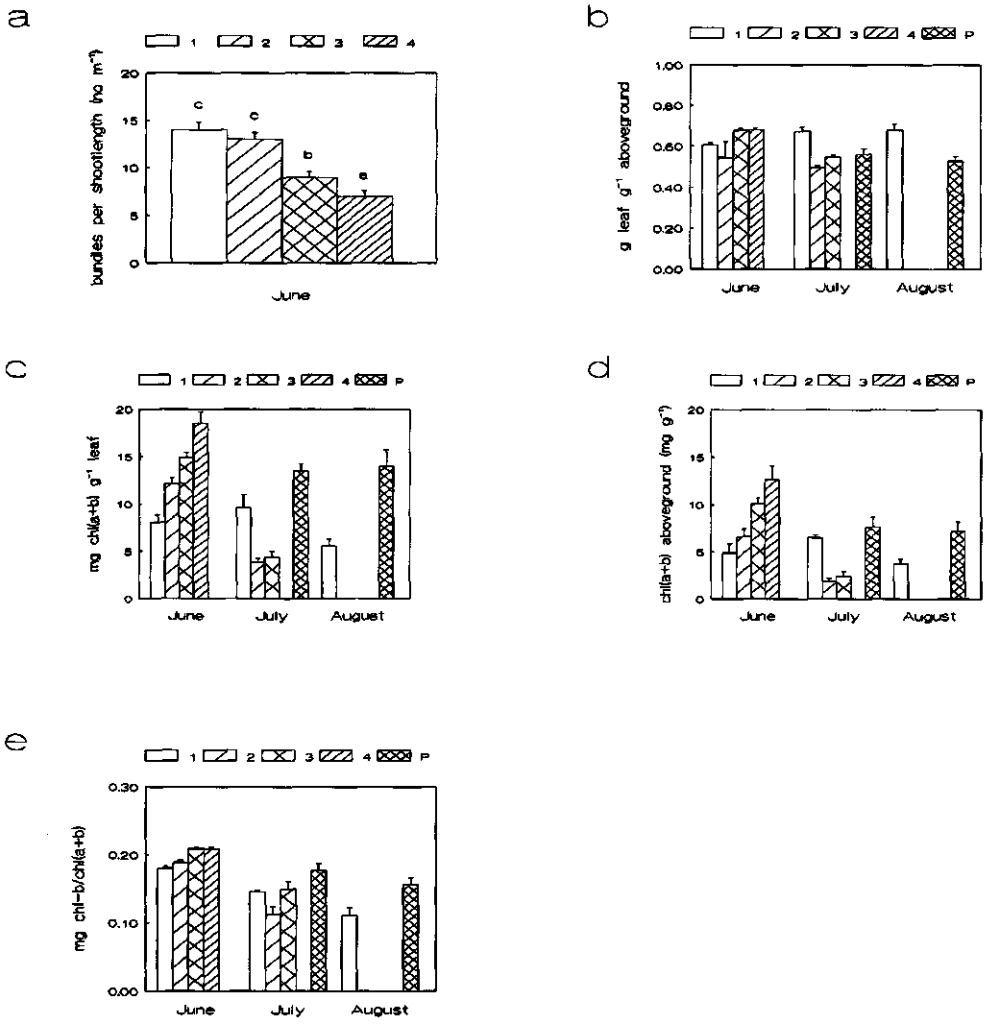


Fig. 4.16. Effects of light history and age on various characteristics of plant material from Lake Veluwe. Light history level for *P. pectinatus* material indicated with 1 to 4 (increased shading; see text). *P. perfoliatus* material from light history level 1 indicated with P.

(a) Number of leaf bundles per meter shoot length of *P. pectinatus* plants collected in June. Columns with the same letter are not significantly different ($p < 0.05$).

(b) Leaf ratio (total leaf biomass per aboveground biomass).

(c) Chl(a+b) content of leaves.

(d) Chl(a+b) content of aboveground biomass.

(e) Fraction chl-b of chl(a+b).

Table 4.18. (a) ANOVA results for chl(a+b) content of leaves (chl) and aboveground biomass (chlor), fraction chl-b and leaf ratio of *P. pectinatus* from light history levels 1-4. Frac-b was $\log_{10}$-transformed.
(b) Multiple comparisons (mc) for chl (a+b) content (mg g^{-1} afdw) of leaves and aboveground biomass, fraction chl-b and leaf ratio (see 5.3.1) of *P. pectinatus* from light history levels 1-4 and *P. perfoliatus* from light history level 1 (P). Pairwise comparisons between the two species (only for light history level 1) are indicated with letters p-q. CER = 0.0029, EER = 0.05. Number of replicates is 6-12. Not available is indicated with -.

(a) twoway ANOVA

	chl	chlor	frac-b	leaf ratio
age	0.0001	0.0001	0.0001	0.2967
light history	0.0001	0.0001	0.0497	0.0072
age*light	0.0001	0.0002	0.1004	0.0363

(b) multiple comparisons

chl(a+b) in leaves							chl(a+b) in aboveground biomass					
	Jun	Jul	Aug				Jun	Jul	Aug			
light	mca	mca	mca	mcl			mca	mca	mca	mcl		
1	a	ap	p	ef	f	e	a	ap	p	e	e	e
2	ab	b	-	e	f	-	ab	a	-	e	f	-
3	b	b	-	e	f	-	bc	a	-	e	f	-
4	b	-	-	-	-	-	c	-	-	-	-	-
P	-	p	q	-	e	e	-	p	p	-	e	e

frac chl-b in leaves							leaf ratio					
	Jun	Jul	Aug				Jun	Jul	Aug			
light	mca	mca	mca	mcl			mca	mca	mca	mcl		
1	a	ap	p	f	ef	e	a	ap	p	e	e	e
2	a	a	-	f	e	-	a	b	-	e	e	-
3	a	a	-	f	e	-	a	ab	-	e	e	-
4	a	-	-	-	-	-	a	-	-	-	-	-
P	-	p	q	-	e	e	-	p	q	-	e	e

After $\log_{10}$-transformation, the data on the fraction chl-b were homogeneous but still not-normally distributed. Trans-formation was not necessary for chl(a+b) in leaves and in total aboveground biomass. The results of ANOVAs for chlorophyll content and fraction chl-b are found in Table 4.18. Both per leaf biomass and per aboveground biomass, chl(a+b) showed a significant effect of light history, age and their interaction. Results are shown in Figs. 4.16 c-e. In June, chl(a+b) increased with lower light history level, but in July a decrease was found. Increasing age led to lowered chl(a+b) levels. Fraction chl-b was affected by light history and age: a slight increase with lower light history level, and a decrease with higher age. Multiple comparisons are given in Table 4.18. *P. perfoliatus* showed no age effect for these three characteristics; in August, chl(a+b) per leaf biomass and fraction chl-b were higher in *P. perfoliatus* than in *P. pectinatus*.

5.3.2 Results of curve fitting

We measured 30 P-I curves, 24 for *P. pectinatus* and 6 for *P. perfoliatus*. Table 4.19 gives an overview of the data sets used. The F-test for the fitted model was not significant for Jul1-2 (both models), Jul1-3 (MM model), Jul2-1 (both models) and JulP-1 (tanh model). R^2 was 0.85 or higher for the other 26 curves. Jul1-2 gave very high estimates for Pm, Km and α in the MM and tanh model. Because we had had technical problems in measuring this replicate, the curve was left out of all analyses. In Fig. 4.17, all 30 curves are shown. As was found in section 4, again much variation between replicates existed, while both models yielded very comparable curves.

Using the 47 curves from section 4 and the 29 from this section, we calculated the overall RSS's for the MM and the tanh model like Lederman & Tett (1981). An F-test revealed no significant

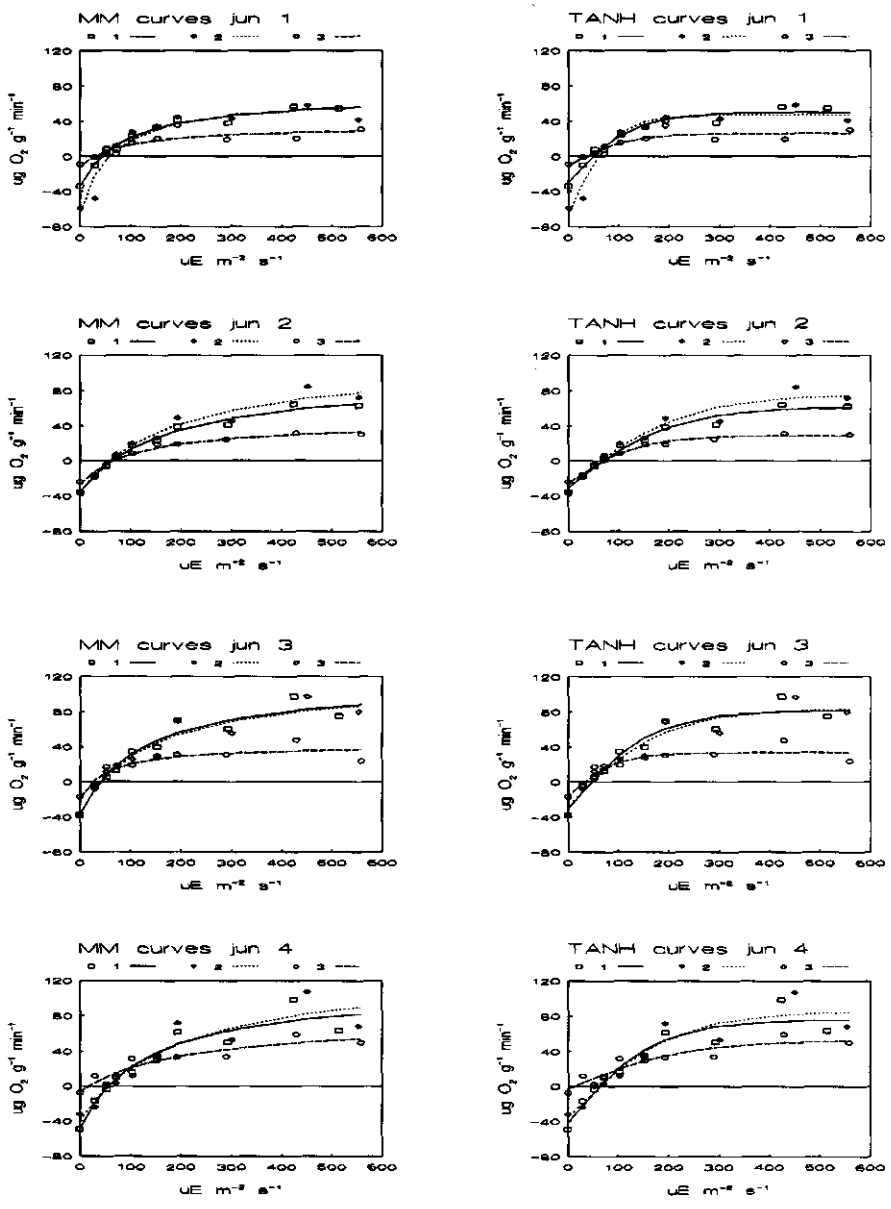


Fig. 4.17. Light response curves from all experiments with *P. pectinatus* and *P. perfoliatus* from Lake Veluwe. MM curve = rectangular hyperbola; tanh curve = hyperbolic tangent. Treatments are indicated with the abbreviated name of the respective month and the light history level (1, 2, 3 and 4 for *P. pectinatus*). *P. perfoliatus* is indicated with P (light history level 1). Replicate curves are indicated with 1, 2 and 3.
(a) June data.

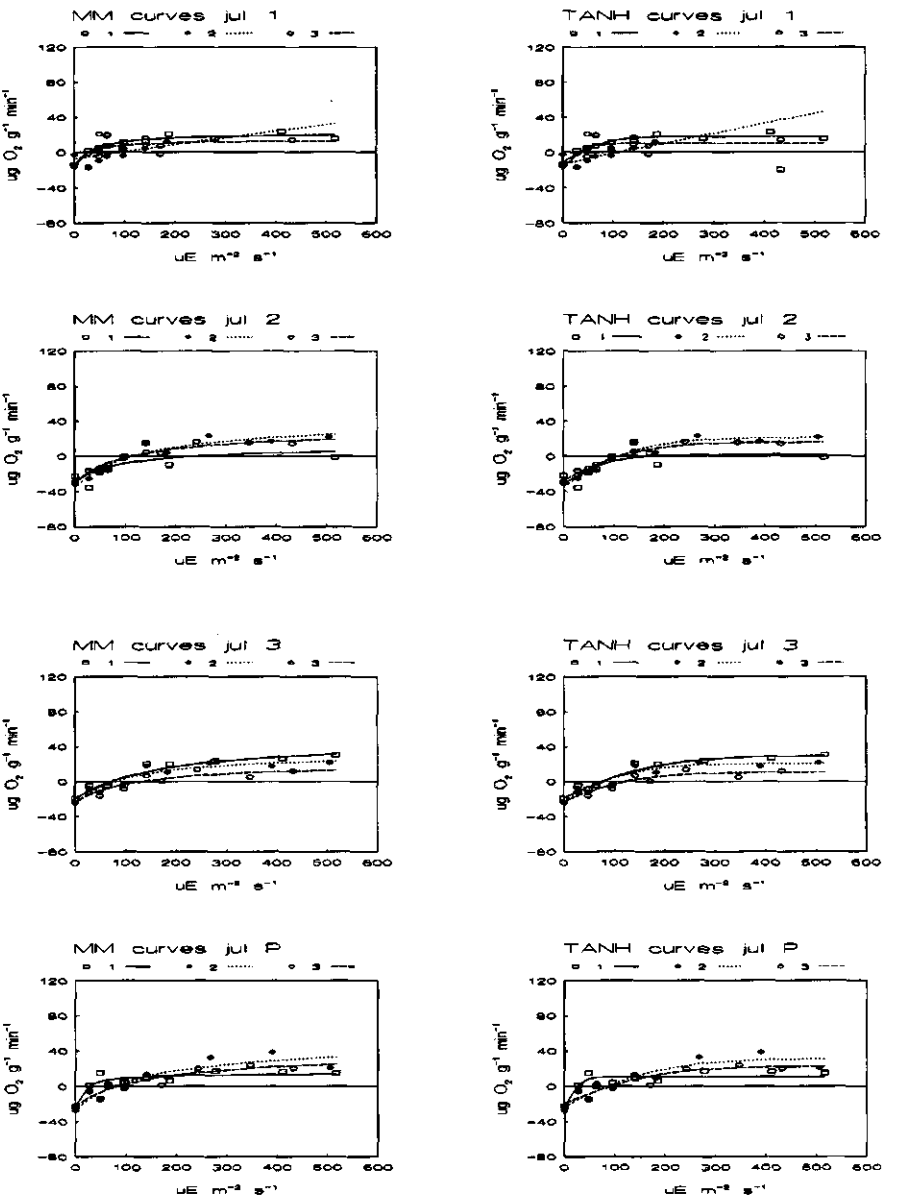


Fig. 4.17. (b) July data.

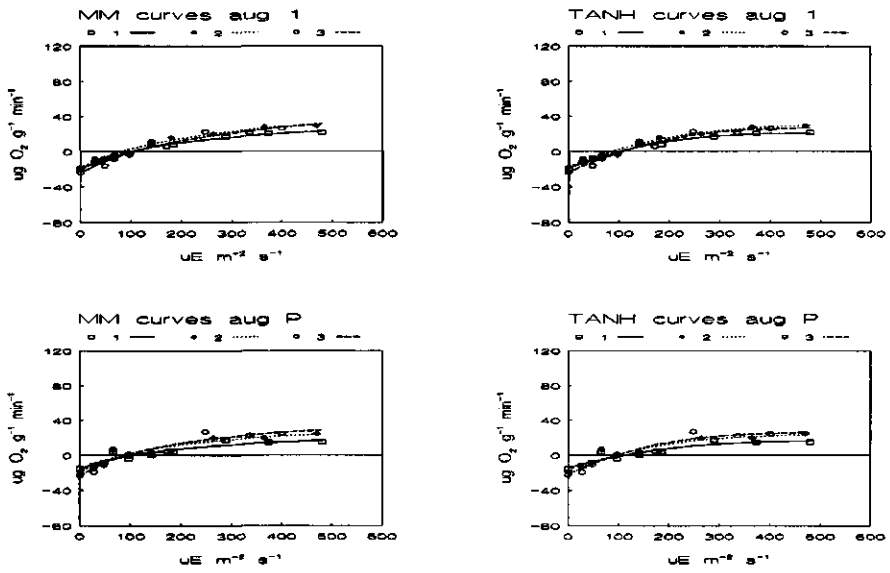


Fig. 4.17. (c) August data.

Table 4.19. Overview of the data sets from photosynthesis-light measurements with plants from Lake Veluwe. Jun, Jul and Aug indicate the respective months of the experiments. Light history level during growth is indicated with 1,2,3,4 for *P. pectinatus* and with P (=light history level 1) for *P. perfoliatus*. Replicate number is 1, 2 or 3. For each experiment are given: the number of photosynthesis measurements that was used for the calculation of the light response curve, the number of results that was left out of the analysis (outliers) and the number of measurements that failed due to technical problems.

experiment	Number of Datapoints Measurements		
	datapoints	left out	failed
Jun	10	0	0
Jul1-1	10	0	6
Jul1-2	7	0	9
Jul1-3	14	2	0
Jul2-1	8	2	0
Jul2-2 and 3	10	0	0
Jul3	10	0	0
JulP	10	0	0
Aug	10	0	0

differences between the overall RSS's of the two models. Thus, their goodness-of-fit was not significantly different, in accordance with our conclusions in section 2.3.

5.3.3 Effects of light history and age on photosynthetic parameters

Data for Pm (MM-model) were homogeneous but still not-normally distributed after $\log_{10}$-transformation. For the tanh model, data on Pm were not-normal and also not-homogeneous. Both parameters were analyzed without transformation. For R, transformation improved homogeneity but data remained not-normally distributed, while α had a normal distribution with homogeneous variances after $\log_{10}$-transformation. Km data were homogeneous and normally distributed.

The ANOVA results for model parameters are found in Table 4.20. Data are shown in Fig. 4.18. Both models

Table 4.20. ANOVA results for model parameters from the MM and tanh model of *P.pectinatus*. The overall F for the ANOVA of parameter R was not significant. Parameter α was $\log_{10}$-transformed.

MM:	age	light history	age*light
Pm	0.0105	0.4375	0.9908
Km	0.0001	0.0028	0.1703
tanh:			
Pm	0.0082	0.6892	0.9505
α	0.0016	0.2811	0.3214

Table 4.21. ANOVA results for derived parameters LCP, Pm/R, GP200 and NP200 in the tanh model of *P. pectinatus*. Overall F for Pm/R was only significant at $p<0.0518$. All parameters except LCP were $\log_{10}$-transformed.

	age	light history	age*light
LCP	0.0004	0.0024	0.0177
Pm/R	0.0824	0.1668	0.6802
GP200	0.0026	0.9020	0.7359
NP200	0.0001	0.2778	0.5831

showed a decrease in Pm with age and no effect on R. Km was affected by both factors, increasing with lower light history level and with age. The age effect is mainly due to the increase for light history level 1 in August. Parameter α showed a decrease with age. Significant differences only existed for Km at light history level 1: Km in August was higher than in June and July. In July, Km for *P. perfoliatus* was higher than for *P. pectinatus*.

The analysis of derived parameters showed comparable results for both models; only the tanh results are given in Fig. 4.19. GP200 and Pm/R data became normal and homogeneous after $\log_{10}$-transformation, but data on NP200 remained not-normally distributed.

The results from the ANOVAs are given in Table 4.21. LCP was affected by age and light history. LCP increased with lower light history level in July, but was not influenced in June. There was an increase in LCP with age, which occurred earlier for lower light history levels (indeed, the interaction age*light history was also significant for LCP). GP200 and NP200 were lower with increasing age. Pm/R was hardly affected: the overall-F was significant at $p<0.0518$. The only weak effect was caused by age, because of the rather high value for light history level 4 in June. Significant differences existed for LCP in July (light history level 2 higher than light history level 1) and for NP200 at light history level 2 and 3: higher in June than in July.

5.4 Discussion

5.4.1 Morphology and chlorophyll

The decrease in bundles per shoot length with lower light history levels in June (Fig. 4.16a) clearly reflects elongation in response to low light history level. This elongation was also found in *P. pectinatus* from our laboratory cultures (see section 4.3.2, and Vermaat & Hootsmans, 1991b). Leaf ratio in field material was not affected by age, in contrast to our findings in 4.3.2. However, it is very well possible that part of the material from July and August was not so much older as material collected in June. Determination of the age of plants from the field is almost impossible. Thus, it is to be expected that a true age effect will be rather difficult to measure. The effect of light history on leaf ratio was rather small and variable. While our laboratory cultures showed a clear decrease with lower light history level, the ratio in the field remained rather constant in June and de-

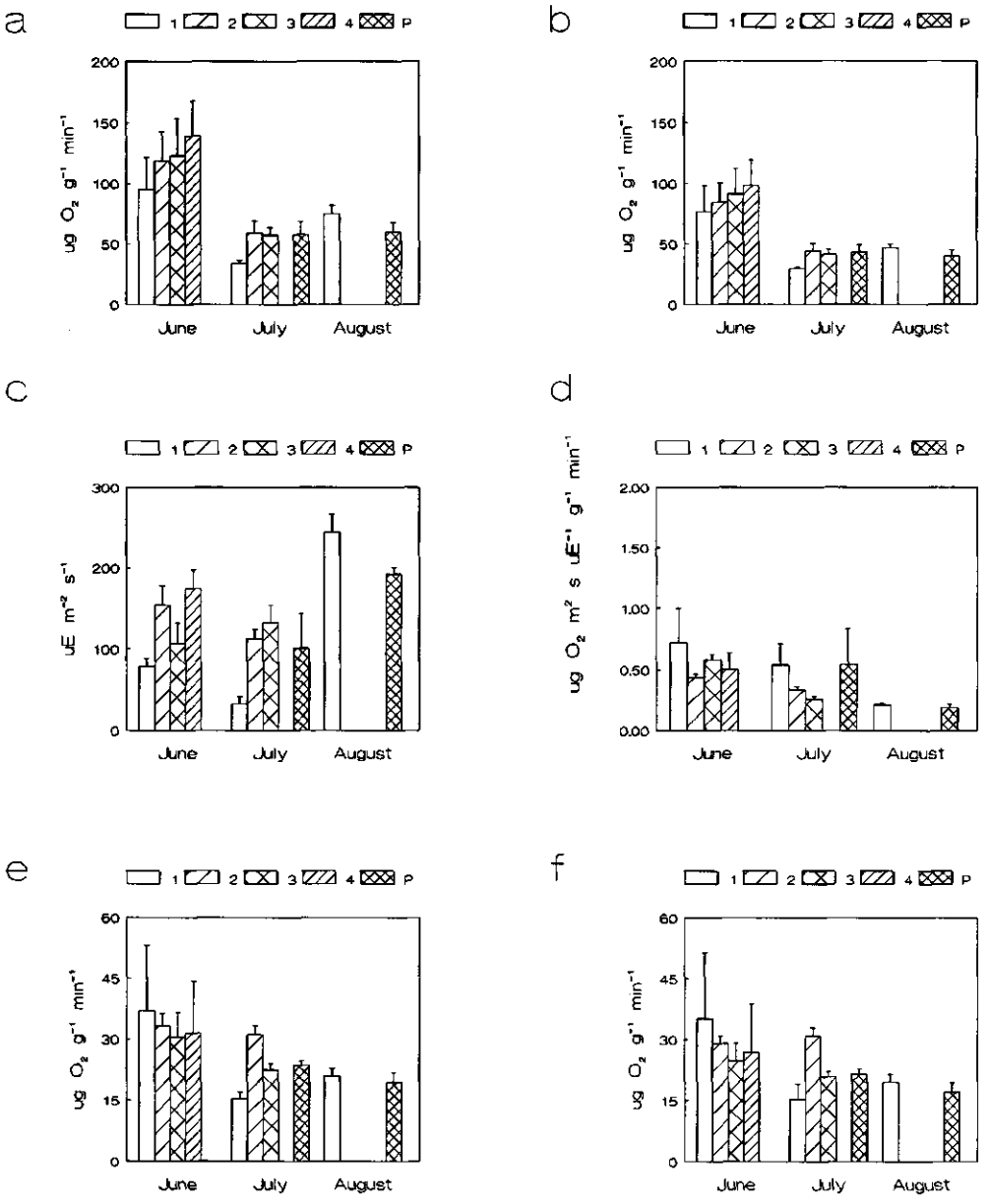


Fig. 4.18. Effects of light history and age on the model parameters from the MM and tanh model. (a) Pm, MM model (b) Pm, tanh model (c) Km (d) α (e) R, MM model (f) R, tanh model.

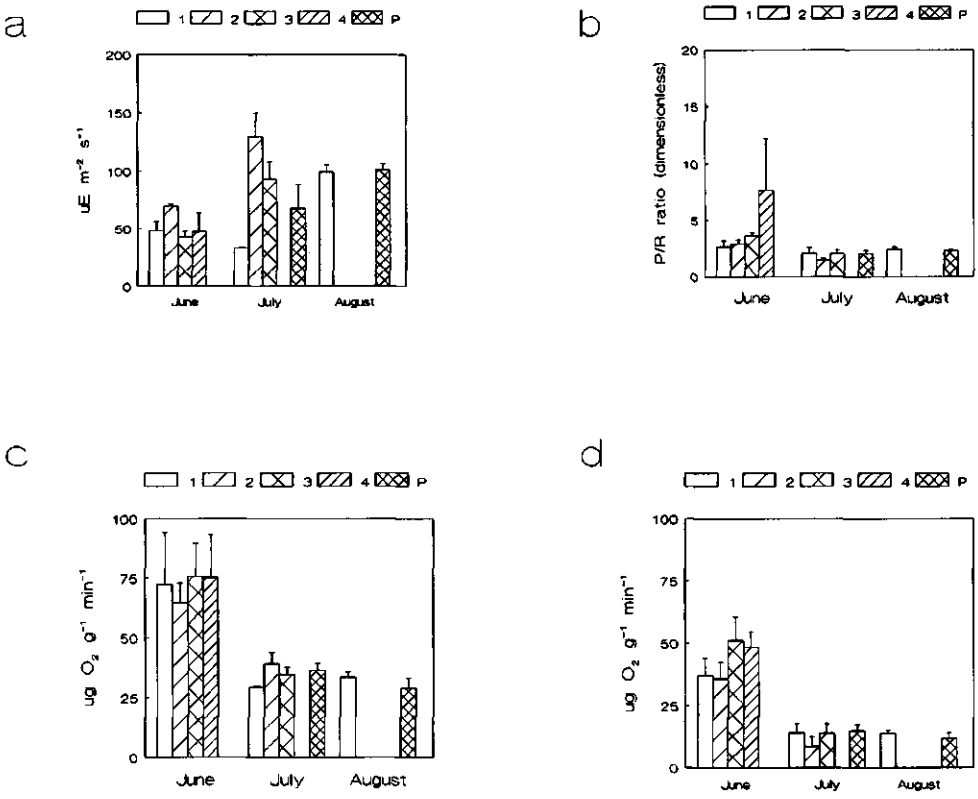


Fig. 4.19. Effects of light history and age on parameters calculated with the tanh model.
(a) LCP (b) Pm/R (c) GP200 (d) NP200.

creased in July. Data from the field for all three months were comparable to the values found for laboratory plants of 30-70 days, which supports our comment on the interpretation of the factor age in the field.

Chl(a+b) content in leaves and in aboveground biomass showed the same factor effects as in 4.3.3. In contrast with the laboratory cultures, field material showed a strong increase of chl(a+b) content in aboveground biomass with lower light history level in June. However, the July results showed a decrease in chl(a+b). Levels in leaves and in aboveground biomass were higher for field plants than for the laboratory cultures in June, but July and August values were comparable to laboratory results. *P. perfoliatus* appeared to have a higher chlorophyll content than *P. pectinatus* from the same light history level.

The fraction chl-b showed a small effect of light history and age; for laboratory cultures, these effects were not significant. Fractions in June were comparable to, in July and August lower than those found in the lab.

As in the laboratory, acclimation of chlorophyll content to light conditions in the field is only found in the amount of chl(a+b). The significant decrease in fraction chl-b in the field with age for lower light history levels can hardly be seen as adaptive. It is probably due to deterioration of plant condition with age under these low light conditions. In July, plants had already disappeared under light history level 4, and the same happened with light history level 2 and 3 in August. Under the very 'sheltered' laboratory conditions, this deterioration followed by sloughing of plant material is much less expressed. A 'premature senescence' of leaves is mentioned for the terrestrial species *Helianthus annuus* L. in Björkman (1981), as a consequence of heavy shading and concomitantly reduced allocation of carbohydrates to new leaf growth. This may also explain the strongly reduced chl(a+b) content of the light history levels 2 and 3 in July.

5.4.2 Photosynthetic parameters

Values of the model parameters were comparable for the two species and also comparable to those of the laboratory cultures. No effect of light history and the interaction age*light history on Pm as found in the laboratory was found in the field material. Km in the laboratory showed no age effect. Also, lower light history level in the laboratory gave lower instead of higher Km values. Parameter α for field material behaved the same as in the lab. R in the laboratory was influenced by age, but no effect at all was found in the field. In the lab, all parameters except Km had significant ANCOVA results with chl(a+b) content as covariate. Remarkably, this was not so in the field. For example: a separate linear regression for each month of Pm of *P. pectinatus* with chl(a+b) in aboveground biomass was never significant. With all data for *P. pectinatus* from the field combined, a significant positive relation between the two variables was found. However, this was mostly due to the overall higher chl(a+b) and Pm values of June. Thus, no strong reduction in variation by chl(a+b) as covariate could occur.

Clearly, field and laboratory material differed in some respects. For the age effect, differences may partly be due to the supposed variation in age within field material. In the laboratory, Pm is much more affected by age than R, and thus age may remain significant for Pm also in field material, but not for R. The average light intensity experienced by the plants in the field (factor light history) is also different from the laboratory. At light history level 4, light levels at the water surface are still 27% of daylight, i.e. still somewhat higher or about equal to the highest light history level in the laboratory cultures (200 μE m^{-2} s^{-1}). When field light history levels are seen at the higher end of a range with the laboratory light history levels at the lower end, the reaction of Km to light history level resembles an optimum curve, while Pm first increases and then remains constant.

The increase in Km with lower light history level in the field might be due to the concomitant increase in the amount of newly formed tubers (tuberization). In the field, tuberization was maximal and length of the life-span of aboveground biomass shortest under light history level 4 (Van Dijk & Van Vierssen, in press). Some evidence exists that the two are linked through changes in photosynthetic characteristics. Two replicate P-I curves of tuberized plants of 2 months old grown in the laboratory from Lake Veluwe tubers (photoperiod 16 h, 200 μE

m^{-2} s^{-1}) showed a lower α and higher Km than our non-tuberized laboratory plants (Van Vierssen et al., a, in prep.). However, the difference was not significant. In general, the results with field and laboratory material indicate that Pm and Km of *P. pectinatus* do change in response to changing light conditions, but not in a way that can be seen as a successful acclimation.

Derived parameters LCP, Pm/R, GP200 and NP200 for the field plants are comparable in magnitude to the laboratory results. Our NP200 of *P. perfoliatus* (12-15 μg O_2 g^{-1} afdw min^{-1}) was rather low compared to values of Kemp et al. (1986): 30-40 μg O_2 g^{-1} dw min^{-1} at 120-140 μE m^{-2} s^{-1}. Field plants of *P. pectinatus* differed from laboratory plants with respect to significances of factor effects. A main difference existed for the factor light history: it had significant effects on all derived parameters except LCP in the laboratory, but in the field it was only significant for LCP. The light history effect on LCP in the field was due to the rather high LCP values for light history level 2 and 3 in July. The trend in LCP in the field was caused by an increase of Km with lower light history level (in June Km was already high for light history level 2-4, while light history level 1 reached the same Km level not before August).

As an increased LCP means a reduced rate of photosynthesis (net and gross), the same relation with light history level can be expected for GP200 and NP200. However, this was not the case, probably due to the value of Pm which was somewhat higher for lower light history levels (though not significantly). A light intensity of 200 μE m^{-2} s^{-1} is not yet fully saturating, but to check for light history effects at lower light levels, we also calculated gross and net photosynthesis at 100 μE m^{-2} s^{-1}. In an ANOVA, NP100 indeed showed a significant effect not only of age (like NP200) but also of light history level and their interaction. The light effect was probably mainly due to the July results, in which decreased light history level correlated with decreased NP100 values. However, GP100 (log_{10}-transformed) behaved like GP200 and did not decrease with decreasing light history level. Thus, the light history effect on NP100 cannot be attributed to light history effects on Km and/or Pm but must be due to changes in parameter R which itself did not show any significant factor effects.

When an increase in Pm with decreasing light history level cannot keep up with the increasing Km, the result will be that plants disappear earlier in the growing season when light history level is relatively low. This phenomenon was indeed observed in the field (Van Dijk & Van Vierssen, in press). It can be hypothesized that light history may have an indirect effect on the rate of ageing of the plant, when the above described possible effect on Km through tuberization really exists. Decreased light history levels will lead to an increased tuberization and thus cause an increased demand for photosynthate, while at the same time photosynthetic rates are decreased because of an increase in Km. Thus, at the same time after germination (i.e. at the same age), plants from lower light history levels can be regarded as physiologically older than plants from higher light history levels. The increase in Km with decreased light history levels was not observed in our non-tuberized laboratory material, which is consistent with the above hypothesis. On the contrary, Km of the laboratory cultures showed a decrease with decreased light history levels, which can be seen as an acclimation to these circumstances (Pm can be reached at lower light intensities).

This decrease in Km is presumably overshadowed when tuberization causes an increase in Km.

Apart from the possible causes mentioned above, differences between laboratory and field material also point to differences between two populations of *P. pectinatus*: the 'brackish' and 'freshwater' forms differ in several aspects (Van Wijk et al., 1988; Vermaat & Hootsmans, 1991a).

6. Conclusions

We used two different models to describe a light-response curve: the rectangular hyperbola and the hyperbolic tangent. The two models did not differ significantly in their average goodness-of-fit based on all light-response curves from our experiments. Thus, we conclude that on the basis of these results, there is no reason to reject the rectangular hyperbola as inferior compared to the hyperbolic tangent.

Regarding the photosynthetic characteristics of plants grown in the laboratory and in the field, we can conclude that *P. pectinatus* apparently is not very well able to acclimate its photosynthetic system to low light levels. Changes in photosynthetic parameter values did occur but this only reduced the photosynthetic performance under turbid conditions. Increases in chl(a+b) were found with decreasing light history level, but these were not enough to keep photosynthetic rates at low light intensities unaffected. In the field in June, an increase in chl(a+b) with decreased light conditions was found, but this did not coincide with a change in Pm. In July, the opposite was the case: chl(a+b) content even decreased, although Pm again did not change.

A clear difference existed between field and laboratory grown material with respect to the relation between Km and light history level. These were positively correlated for laboratory material, but negatively for field plants. Evidence exists that tuberization of plant material coincides with an increase in Km. As low light levels stimulate tuberization, this may explain the observed difference. Thus, for a better understanding of the effects of light on photosynthesis, information on the effects of tuber formation on photosynthesis appears necessary also.

Despite the apparent lack of photosynthetic acclimation to low light levels, this species is one of the few aquatic macrophytes that have maintained considerable biomass levels in the highly eutrophicated and turbid, shallow lakes in The Netherlands. The solution to this apparent contradiction might be found in morphological adaptations like elongation and the availablity of carbohydrate reserves in the tuber. Early growth of *P. pectinatus* is mainly supported by the tuber, at least up to 16 days after sprouting (Hodgson, 1966; Van Vierssen et al., b, in prep.). These reserves can be used to reach the water surface by stem elongation as soon as possible. When the water surface is reached, turbidity of the water layer can only have a minor influence on plant photosynthesis. Thus, for growth in shallow habitats, acclimation of photosynthetic capabilities to low light levels might not be very important for *P. pectinatus*.

Besides low light history levels, ageing also had a strongly negative effect on photosynthesis. It can be expected that a decrease in the photosynthetic rate during

the growing season leads to assimilate shortage and tissue damage. Knowledge of the effects of both factors on the shape of the light-response curve can thus provide a physiologically based explanation for the decline and disappearance of the vegetation at the end of the growing season.

Acknowledgements

Thanks are due to A.W. Breukelaar and R. Gijlstra for technical assistance during the design and construction of the experimental set-up for the photosynthesis measurements. Plant material from Lake Veluwe was supplied by G.M. van Dijk. Prof. Dr. W. van Vierssen and Prof. Dr. W.J. Wolff critically read the manuscript.

7. References

Barko, J.W. & Smart, R.M., 1981. Comparative influences of light and temperature on the growth and metabolism of selected submersed freshwater macrophytes. Ecol. Monogr. 51: 219-235.

Bernatowicz, S., 1969. Macrophytes in the Lake Warniak and their chemical composition. Ekol. Polska, Ser. A 17: 447-467.

Best, E.P.H. & Dassen, J.H.A., 1987. Biomass, stand area, primary production characteristics and oxygen regime of the *Ceratophyllum demersum* L. population in Lake Vechten, The Netherlands. Arch. Hydrobiol., Suppl. 76: 347-367.

Björkman, O., 1981. Responses to different quantum flux densities. In Lange, O.L., Nobel, P.S., Osmond, C.B., & Ziegler, H. (eds), Physiological plant ecology I. Responses to the physical environment. Encyclopedia of plant physiology, new series, vol. 12a. Springer, Berlin, pp. 57-108.

Boardman, N.K., 1977. Comparative photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol. 28: 355-377.

Borkowski, J.D. & Johnson, M.J., 1967. Long-lived steam-sterilizable membrane probes for dissolved oxygen measurement. Biotechnol. Bioeng. 9: 635-639.

Briggs, R. & Viney, M., 1964. The design and performance of temperature compensated electrodes for oxygen measurements. J. Sci. Instrum. 41: 78-83.

Bulthuis, D.A., 1987. Effects of temperature on photosynthesis and growth of seagrasses. Aquat. Bot. 27: 27-40.

Carpenter, J.H., 1965. The accuracy of the Winkler method for dissolved oxygen analysis. Limnol. Oceanogr. 10: 135-140.

Chalker, B.E., 1980. Modelling light saturation curves for photosynthesis: an exponential function. J. theor. Biol. 84: 205-215.

Conway, G.R., Glass, N.R. & Wilcox, J.C., 1970. Fitting nonlinear models to biological data by Marquardt's algorithm. Ecology 51: 503-507.

Cossby, B.J. & Hornberger, G.M., 1984. Identification of photosynthesis-light models for aquatic systems. I. Theory and simulations. Ecol. Modelling 23: 1-24.

Cossby, B.J., Hornberger, G.M. & Kelly, M.G., 1984. Identification of photosynthesis-light models for aquatic systems. II. Application to a macrophyte dominated stream. Ecol. Modelling 23: 25-51.

Côté, B. & Platt, T., 1983. Day-to-day variations in spring-summer photosynthetic parameters of coastal marine phytoplankton. Limnol. Oceanogr. 28: 320-344.

Dowd, J.E. & Riggs, D.S., 1965. A comparison of Michaelis-Menten kinetic constants from various linear transformations. J. Biol. Chem. 240: 863-869.

Drew, E.A., 1979. Physiological aspects of primary production in seagrasses. Aquat. Bot. 7: 139-150.

Fair, P. & Meeke, L., 1983. Seasonal variations in the pattern of photosynthesis and possible adaptive response to varying light flux regimes in *Ceratophyllum demersum* L. Aquat. Bot. 15: 81-90.

Falkowski, P.G., 1981. Light-shade adaptation and assimilation numbers. J. Plankton Res. 3: 203-216.

Gabrielsen, E.K., 1948. Effects of different chlorophyll concentrations on photosynthesis in foliage leaves. Physiol. Plant. 1: 5-37.

Gallegos, C.L. & Platt, T., 1981. Photosynthesis measurements on natural populations of phytoplankton: numerical analysis. Can. Bull. Fish. Aquat. Sciences 210: 103-112.

Gerloff, G.C. & Krombholz, P.H., 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. Limnol. Oceanogr. 11: 529-537.

Glaser, R.E., 1982. Bartlett's test of homogeneity of variances. In Kotz, S., & Johnson, N.L., (eds), Encyclopedia of statistical sciences, vol.1. Wiley & Sons, New York, pp 189-191.

Ho, Y.B., 1979. Inorganic mineral nutrient level studies on *Potamogeton pectinatus* L. and *Enteromorpha prolifera* in Forfar Loch, Scotland. Hydrobiologia 62: 7-15.

Hodgson, R.H., 1966. Growth and carbohydrate status of sago pondweed. Weeds 14: 263-268.

Hootsmans, M.J.M., 1991. A growth analysis model for *Potamogeton pectinatus* L. In Hootsmans, M.J.M. & Vermaat, J.E. Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Hough, R.A., 1974. Photorespiration and productivity in submersed aquatic vascular plants. Limnol. Oceanogr. 19: 912-927.

Hough, R.A. & Fornwall, M.D., 1988. Interactions of inorganic carbon and light availibility as controlling factors in aquatic macrophyte distribution and productivity. Limnol. Oceanogr. 33: 1202-1208.

Howard-Williams, C., 1981. Studies on the ability of a *Potamogeton pectinatus* L. community to remove dissolved nitrogen and phosphorus compounds from lake water. J. Appl. Ecol. 18: 619-637.

Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187-211.

Iwakuma, T. & Yasuno, M., 1983. A comparison of several mathematical equations describing photosynthesis-light curve for natural phytoplankton populations. Arch. Hydrobiol. 97: 208-226.

Jana, S. & Choudhuri, M.A., 1979. Photosynthetic, photorespiratory and respiratory behaviour of three submersed aquatic angiosperms. Aquat. Bot. 7: 13-19.

Jassby, A.D. & Platt, T., 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol. Oceanogr. 21: 540-547.

Jiménez, C., Niell, F.X. & Algarra, P., 1987. Photosynthetic adaptation of *Zostera noltii* Hornem. Aquat. Bot. 29: 217-226.

Johnson, M.J., Borkowski, J. & Engblom, C., 1964. Steam sterilizable probes for dissolved oxygen measurement. Biotechnol. Bioeng. 6: 457-468.

Kelly, M.G., Moeslund, B. & Thyssen, N., 1981. Productivity measurement and the storage of oxygen in the aerenchyma of aquatic macrophytes. Arch. Hydrobiol. 92: 1-10.

Kemp, W.M., Lewis, M.R. & Jones, T.W., 1986. Comparison of methods for measuring production by the submersed macrophyte, *Potamogeton perfoliatus* L. Limnol. Oceanogr. 31: 1322-1334.

Kerr, E.A. & Strother, S., 1985. Effects of irradiance, temperature and salinity on photosynthesis of *Zostera muelleri*. Aquat. Bot. 23: 177-183.

King, R.J. & Schramm, W., 1976. Photosynthetic rates of benthic marine algae in relation to light intensity and seasonal variations. Mar. Biol. 37: 215-222.

Lederman, T.C. & Tett,.P., 1981. Problems in modelling the photosynthesis-light relationship for phytoplankton. Bot. Mar. 24: 125-134.

Lindeboom, H.J. & de Bree, B.H.H., 1982. Daily production and consumption in an eelgrass (*Zostera marina*) community in saline lake Grevelingen: discrepancies between O_2 and ^{14}C method. Neth. J. Sea Res. 16: 362-379.

Lipkin, Y., Beer, S., Best, E.P.H., Kairesalo, T. & Salonen, K., 1986. Primary production of macrophytes: terminology, approaches and a comparison of methods. Aquat. Bot. 26: 129-142.

Madsen, J.D. & Adams, M.S., 1989. The light and temperature dependence of photosynthesis and respiration in *Potamogeton pectinatus* L. Aquat. Bot. 36: 23-31.

Madsen, T.V. & Søndergaard, M., 1983. The effects of current velocity on the photosynthesis of *Callitriche stagnalis* Scop. Aquat. Bot. 15: 187-193.

Mancy, K.H., Okun, D.A. & Reilley, C.N., 1962. A galvanic cell oxygen analyzer. J. Electroanal. Chem. 4: 65-92.

McCree, K.J., 1972a. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9: 191-216.

McCree, K.J., 1972b. Test of current definitions of photosynthetically active radiation against leaf photosynthesis data. Agric. Meteorol. 10: 443-453.

Megard, R.O., Tonkyn, D.W. & Senft, W.H., 1984. Kinetics of oxygenic photosynthesis in planktonic algae. J. Plankton Res. 6: 325-337.

Mortimer, C.H., 1981. The oxygen concentration of air-saturated fresh waters over ranges of temperature and atmospheric pressure of limnological interest. Mitt. int. Verein. Limnol. no. 22, 23 pp.

Novozamsky, I., Houba, V.J.G., van Eck, R. & Van Vark, W., 1983. A novel digestion technique for multi-element plant analysis. Comm. Soil Sci. Plant Anal. 14: 239-248.

Orr, P.T., Pokorný, J., Denny, P. & Sale, P.J.M., 1988. Photosynthetic response of *Myriophyllum salsugineum* A.E. Orchard to photon irradiance, temperature and external free CO_2. Aquat. Bot. 30: 363-378.

Peñuelas, J., Murillo, J. & Azcón-Bieto, J., 1988. Actual and potential respiration rates and different electron transport pathways in freshwater aquatic plants. Aquat. Bot. 30: 353-362.

Perry, M.J., Talbot, M.C. & Alberte, R.S., 1981. Photoadaptation in marine phytoplankton: response of the photosynthetic unit. Mar. Biol. 62: 91-101.

Platt, T. & Jassby, A.D., 1976. The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton. J. Phycol. 12: 421-430.

Pokorný, J., Květ, J., Ondok, J.P., Toul, Z. & Ostrý, I., 1984. Production-ecological analysis of a plant community dominated by *Elodea canadensis* Michx. Aquat. Bot. 19: 263-292.

Sand-Jensen, K., 1983. Photosynthetic carbon sources of stream macrophytes. J. Exp. Bot. 34: 198-210.

SAS Institute Inc., 1985. SAS/STAT Guide for personal computers, version 6 edition. Cary, N.C., pp. 378.

SAS Institute Inc., 1986. SAS for linear models, a guide to the ANOVA and GLM procedures. Cary, N.C., pp. 231.

Silvert, W., 1979. Practical curve fitting. Limnol. Oceanogr. 24: 767-773.

Snedecor, G.W. & Cochran, W.G., 1967. Statistical methods. Iowa State University Press, Ames, pp. 593.

Søndergaard, M., 1979. Light and dark respiration and the effect of the lacunal system on refixation of CO_2 in submerged aquatic plants. Aquat. Bot. 6: 269-283.

Sorrell, B.K. & Dromgoole, F.I., 1986. Errors in measurements of aquatic macrophyte gas exchange due to oxygen storage in internal airspaces. Aquat. Bot. 24: 103-114.

Spence, D.H.N. & Chrystal, J., 1970. Photosynthesis and zonation of freshwater macrophytes II. Adaptability of species of deep and shallow water. New Phytol. 69: 217-227.

Talling, J.F., 1957. Photosynthetic characteristics of some freshwater plankton diatoms in relation to underwater radiation. New Phytol. 56: 29-50.

Thomsen, E. & Thyssen, N., 1979. Måling og regristrering af vigtige tilstandsvariabler i vandløb (Measurement and registration of important parameters in streams, in Danish). Vand 2: 2-7.

Titus, J.E. & Adams, M.S., 1979. Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria americana* Michx. Oecologia 40: 273-286.

Urbanc-Berčič, O. & Gaberščik, A., 1989. The influence of water temperature and light intensity on activity of water hyacinth (*Eichhornia crassipes* (Mart.) Solms). Aquat. Bot. 35: 403-408.

Van, T.K., Haller, W.T. & Bowes, G., 1976. Comparison of the photosynthetic characteristics of three submersed aquatic plants. Plant Physiol. 58: 761-768.

Van der Bijl, L., Sand-Jensen, K. & Hjermind, A.L., 1989. Photosynthesis and canopy structure of a submerged plant, *Potamogeton pectinatus*, in a Danish lowland stream. J. Ecol. 77: 947-962.

Van Dijk, G.M. & Van Vierssen, W., 1991. Survival of a *Potamogeton pectinatus* L. population under various light conditions in a shallow eutrophic lake (Lake Veluwe) in The Netherlands. Aquat. Bot. 39:121-129.

Van Vierssen, W., 1982. The ecology of communities dominated by *Zannichellia* taxa in western Europe. III. Chemical ecology. Aquat. Bot. 14: 259-294.

Van Vierssen, W., Hootsmans, M.J.M. & Van Dijk, G.M., a. Tuber induction in *Potamogeton pectinatus*. In Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E. (eds), Dynamics of a macrophyte dominated system under eutrophication stress: an integrated approach. Geobotany, Junk, in prep.

Van Vierssen, W., Vermaat, J.E. & Mathies, A., b. Early growth characteristics of *Potamogeton pectinatus*. In Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E. (eds), Dynamics of a macrophyte dominated system under eutrophication stress: an integrated approach. Geobotany, Junk, in prep.

Van Wijk, R.J., 1989a. Ecological studies on *Potamogeton pectinatus* L. IV. Nutritional ecology, field observations. Aquat. Bot. 35: 301-318.

Van Wijk, R.J., 1989b. Ecological studies on *Potamogeton pectinatus* L. V. Nutritional ecology, in vitro uptake of nutrients and growth limitation. Aquat. Bot. 35: 319-335.

Van Wijk, R.J., van Goor, E.M.J. & Verkley, J.A.C., 1988. Ecological studies on *Potamogeton pectinatus* L. II. Autecological characteristics, with emphasis on salt tolerance, intraspecific variation and isoenzyme patterns. Aquat. Bot. 32: 239-260.

Vermaat, J.E., 1991. Periphyton removal by freshwater micrograzers. In Hootsmans, M.J.M. & Vermaat, J.E. Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vermaat, J.E. & Hootsmans, M.J.M. 1991a. Intraspecific variation in *Potamogeton pectinatus*, a controlled laboratory experiment. In Hootsmans, M.J.M. & Vermaat, J.E. Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vermaat, J.E. & Hootsmans, M.J.M., 1991b. Growth of *Potamogeton pectinatus* in a temperature-light gradient. In Hootsmans, M.J.M. & Vermaat, J.E. Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vermaat, J.E. & Sand-Jensen, K., 1987. Survival, metabolism and growth of *Ulva lactuca* under winter conditions: a laboratory study of bottlenecks in the life cycle. Mar. Biol. 95: 55-61.

Vollenweider, R.A., 1969. A manual on methods for measuring primary production in aquatic environments. IBP handbook no. 12, Blackwell, Oxford, pp. 225.

Wareing, P.F., Khalifa, M.M. & Treharne, K.J., 1968. Rate-limiting processes in photosynthesis at saturating light intensities. Nature 220: 453-457.

Westlake, D.F., 1967. Some effects of low-velocity currents on the metabolism of aquatic macrophytes. J. Exp. Bot. 18: 187-205.

Westlake, D.F., 1978. Rapid exchange of oxygen between plant and water. Verh. int. Verein. Limnol. 20: 2363-2367.

Wetzel, R.G., 1969. The enclosure of macrophyte communities. In R.A. Vollenweider (ed.). A manual on methods for measuring primary production in aquatic environments. IBP handbook no. 12, Blackwell, pp. 81-88.

Wetzel, R.G., 1983. Limnology, 2nd edition, Saunders College Publishing, New York, pp. 767.

Wiginton, J.R. & McMillan, C., 1979. Chlorophyll composition under controlled light conditions as related to the distribution of seagrasses in Texas and the U.S. Virgin Islands. Aquat. Bot. 6: 171-184.

Zelitch, I., 1966. Increased rate of net photosynthetic carbon dioxyde uptake caused by the inhibition of glycolate oxydase. Plant Physiol. 41: 1623-1631.

Zieman, J.C. & Wetzel, R.G., 1980. Productivity in seagrasses: methods and rates. In Phillips, R.C. & McRoy, C.P., (eds), Handbook of seagrass biology: an ecosystem perspective. Garland STPM Press, New York, pp. 87-119.

Zimmerman, R.C., Beeler SooHoo, J., Kremer, J.N. & D'Argenio, D.Z., 1987. Evaluation of variance approximation techniques for non-linear photosynthesis-irradiance models. Mar. Biol. 95: 209-215.

ALLELOPATHIC LIMITATION OF ALGAL GROWTH BY MACROPHYTES

5

M.J.M. Hootsmans

Abstract

Growth inhibition of phytoplanktonic algae by macrophyte secretions is an important aspect of the eutrophication model that serves as the working hypothesis for the present study. However, evidence for allelopathic effects in aquatic ecosystems is scarce. Often, the phenomena found can also be attributed to various other causes like competition for light and/or nutrients. Although the active secretion of substances like nutrients by macrophytes is well-documented, not much unequivocal evidence exists for algal growth suppression by exudates from intact plant populations.

Based on a literature review, it was concluded that although other explanations than allelopathy are often possible, the occurrence of allelopathy cannot simply be excluded. The effect may be obscured by interaction with nutrient competition and can be dependent on the age of the organisms involved.

The question remained whether intact macrophytes release substances that can inhibit algal growth. Therefore, several laboratory experiments were performed, in which medium from cultures of different *Chara* spp. was added to cultures of the phytoplanktonic algae *Ankistrodesmus bibraianus* (Reinsch) Korš. and *Scenedesmus communis* Hegew. Nutrient levels of control and test cultures were kept identical.

Growth of *Ankistrodesmus* was stimulated or not significantly affected by all *Chara* cultures tested. *Scenedesmus* growth was stimulated or not affected by *Chara tomentosa* L., *C. hispida* L. and *C. delicatula* Agardh. Medium from *C. globularis* Thuill. often reduced growth of *Scenedesmus* significantly (mean biomass change during the experiment was about 10% lower than in control cultures). Within this *Chara* species, various strains differed in their effect on algal growth.

It was concluded that these macrophytes indeed excreted substances that influenced the growth of the test algae either directly or via interaction with bacteria. The effect was dependent on the *Chara* species, probably even on the *Chara* ecotype and on the species of alga used. It could change over time.

Subsequently, evidence had to be found for allelopathic growth limitation of algae by macrophyte secretions in the field. Therefore, algal cell-free filtrates from various stations in Lake Veluwe were collected on five occasions in the period June-August 1988. The stations differed in amount and kind of macrophyte vegetation. The filtrates were inoculated with *Scenedesmus*.

Allelopathic effects occurred in the water samples, differing both with regard to place and time. Final algal biomass could be reduced with 10-15% relative to the mean final algal biomass for all stations on a specific date. The effects could not readily be attributed to macrophyte biomass, so other factors, like the relative age composition of the macrophyte vegetation and its photosynthetic activity, may have played a role.

In recent years, a demand has risen for restoration of highly turbid, eutrophicated waters in The Netherlands. Till sofar, attention in restoration projects has focused mainly on manipulation of fish communities. However, it seems likely that the intricate balance between aquatic macrophytes, periphyton and phytoplankton should also be taken into consideration. Some evidence for allelopathic algal growth limitation in two biomanipulation projects, characterized by increased *Chara* development after restoration measures, are presented.

Contents

1. Introduction

The observation that some plants seem to limit the growth possibilities of other plants by the excretion of substances is certainly not recent. In his extensive review on the subject, Rice (1984) mentions several authors back to 300 B.C. who described this phenomenon. Molisch (1937) used the term allelopathy to describe all inhibitory and stimulatory biochemical interactions between plants, including microorganisms. Rice (1974) defined allelopathy more restrictedly as 'any direct or indirect harmful effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment'. However, in his second edition, Rice (1984) reverted to the original definition of Molisch (1937) because he was convinced by additional experiments and literature review that 'elimination of stimulatory effects from the definition is artificial'. Rice (1984) also stressed that allelopathy and competition are different: the first interaction depends on adding a chemical substance to the environment, whereas competition involves the reduction of the availability of some factor in the environment required by all competing organisms, like water, nutrients and light. Allelopathic and competitive interaction are combined in the term interference, suggested by Muller (1969) for the overall influence of one plant on another. In this chapter we will follow the definition of allelopathy by Molisch (1937) and Rice (1984).

The very existence of allelopathy, at least in terrestrial systems, seems not much challenged anymore. However, clear unequivocal evidence is scarce. Harper (1977) mentioned several examples of experimental results that were interpreted as caused by allelopathy, but which could also be explained in terms of nutrient competition. He doubted the importance of allelopathic interaction for higher plants with the argument that adaptation to heavy metals and herbicides can occur quite rapidly, while secreted complex organic molecules, supposedly allelopathic, are quickly decomposed in the soil.

The discrimination in the field between results from allelopathy and competition is inherently difficult. We can expect that the two act in concert to form an intricate web of stimulatory and inhibitory relations in the ecosystem. Although firm evidence therefore is hard to get, we still should credit allelopathy '..with its due significance - neither greater nor less than is justified by the present state of our understanding.' (Muller, 1969).

In the following sections, first a review is given of current literature on allelopathy in the aquatic environment. Then, results are presented from laboratory experiments on allelopathic effects on the growth of phytoplanktonic algae in nutrient-enriched water from cultures of various *Chara* species. Subsequently, algal growth data in nutrient-enriched water samples from various places within Lake Veluwe are presented. Finally, the possible presence of allelopathic interaction in three lake restoration projects in The Netherlands is discussed.

2. Review of existing evidence for allelopathy in the aquatic environment

2.1 Introduction

During the last decades a major shift from macrophyte dominance towards phytoplankton blooms has become apparent in many eutrophicated lakes. In The Netherlands, virtually none of the many shallow lakes still has a luxurious macrophyte vegetation and clear water. This dramatic change is only occasionally documented (Best, 1987; De Nie, 1987), but eyewitness reports are abundant. The often mentioned explanation for the phenomenon is increased phytoplankton growth because of the increased availability of nutrients. In these changed nutrient conditions, macrophyte growth supposedly cannot increase as strongly as the algal growth. Thus, the submerged vegetation has to cope with increased shading by the algae which finally leads to macrophyte extinction.

In Phillips et al. (1978) a more intricate mechanism was proposed in which shading by epiphytes and subsequent reduction in allelopathic growth inhibitor production by the macrophytes finally leads to planktonic algal blooming and macrophyte disappearance (see chapter 1). The hypothesized allelopathic interaction between phytoplankton and macrophytes is of crucial importance. It offers an explanation for the palaeolimnological data presented in Phillips et al. (1978). They found that an increase of phytoplanktonic algal remains in lake sediments in the Norfolk Broads, England, occurred much later in this century than the increase of typical epiphytic species. The latter coincided with documented increases in nutrient loading of the systems studied and with subsequent loss of macrophytes.

Evidence for the occurrence of allelopathy in an aquatic ecosystem is scarce but increasing. Rice (1974, 1979, 1984) in his reviews on predominantly terrestrial ecosystems mentioned a few studies concerning allelopathic interactions between algal species. Aquatic macrophytes were not discussed at all, although he suspected members of the phylum Charophyta to produce such substances.

2.2 Effects among algae

Toxic secretions by algae are often mentioned for blooms of Cyanobacteria. Gorham (1964) gave several examples of livestock and waterfowl kills caused by drinking lake water containing *Microcystis aeruginosa* Kütz and/or *Anabaena flos-aquae* (Lyngb.) de Bréb. Berg et al. (1987a, b) studied the toxicity of *M. aeruginosa* in mice. The strong toxin, a cyclic polypeptide, could not be detected in lakewater during the bloom. However, in a laboratory study, Berg et al. (1987a) found that it was released when decomposition of the algae began. Wolfe & Rice (1979) performed several tests in which sterile filtrates from algal cultures were inoculated with another algal species after nutrient enrichment to prevent effects due to nutrient shortage. They found growth limitation and stimulation, as well as no effect. Different but statistically significant results also occurred for repeated experiments with the same algal combination. This suggests changes over time in concentration and/or composition of the allelopathic substances excreted by the algae. Indeed, the stimulatory effect on the growth of *Pandorina morum* Bory by

filtrates from a *Scenedesmus incrassulatus* G.M. Smith culture decreased with the age of the *Scenedesmus* culture (Wolfe & Rice, 1979).

Fitzgerald (1964) suggested that growth-limiting substances excreted by algae might influence or even determine algal bloom sequences. Keating (1977) showed that allelopatic growth inhibition and stimulation between several species of Cyanobacteria were closely correlated with a bloom sequence recurring during 3 years in a particular lake. A sterile filtrate from a dominant algal species inhibited growth of its predecessor, but stimulated in turn the growth of its successor. Also, the spring diatom bloom was negatively correlated with the density of the preceding bloom of Cyanobacteria: cell-free filtrates from the Cyanobacteria inhibited diatom growth (Keating, 1978). The presence of bacteria appeared to reduce the allelopathic effect, suggesting bacterial decomposition of the active compound(s). Jüttner (1981) isolated various substances from lake water during algal blooms, which he could also detect in axenic cultures of various algae. Some of these substances inhibited growth of the alga *Synechococcus* sp.. Rijstenbil (1989) found allelopathic interaction in laboratory cultures of the two marine diatom species *Ditylum brightwellii* (West) Grunow and *Skeletonema costatum* (Grev.) Cleve. Algal extracts were more toxic than cell-free filtrates of algal cultures. The latter were more effective when coming from algal cultures in their exponential growth phase than from cultures in their stationary growth phase.

2.3 Effects of algae on macrophytes

To our knowledge, two examples exist of negative effects of algal excretions on the growth of macrophytes. Sharma (1985) reports growth inhibition of *Eichhornia crassipes* (Mart.) Solms in a large tank with an algal bloom. Inorganic phosphorus concentration in the control (without algae) was low (0.01 mg P l^{-1}) but only traces could be detected in the treatment. Total nitrogen was 0.32 mg l^{-1} in the control and 0.58 mg l^{-1} in the treatment. Thus, competition for P is not completely excluded.

Van Vierssen & Prins (1985) studied the effect of various amounts of cell-free filtrates of a predominantly *Anabaena* sp. culture on growth and photosynthesis of the macrophyte *Zannichellia peltata* Bertol. In this case, the varying amounts of filtrate added were made up to 2 l with nutrient solution. The macrophyte showed a decreased biomass and photosynthesis when the 'dose' was increased. As the algal culture can be expected to have used part of the nutrients in the filtrate, again nutrient effects may be an alternative explanation for the results. However, regarding the instantaneous effect on photosynthesis, this seems unlikely.

2.4 Effects of macrophytes on algae

The ability of macrophytes to excrete organic substances in considerable amounts is documented by Allen (1971), Wetzel & Manny (1972), Hough & Wetzel (1975) and Søndergaard (1981). Anthoni et al. (1980) isolated sulphur containing compounds from aquatic macrophytes (Characeans). Wium-Andersen et al. (1983) found sulphur in *Ceratophyllum demersum* L. These substances were shown to have an inhibitory effect on algal photosynthesis (Wium-Andersen et al., 1982).

Photosynthesis of the epiphytic

diatom *Nitzschia palea* (Kütz) W. Smith was reduced less than that of phytoplankton samples from two lakes (Wium-Andersen et al., 1982). This is in accordance with the hypothesized role of epiphytes in the early stages of the eutrophication process (Phillips et al. 1978): epiphytic algae should not be inhibited, or at least not as much, as phytoplankton algae by excretions from macrophytes.

Planas et al. (1981) isolated various phenolic compounds from tissue of *Myriophyllum spicatum* L. which appeared to limit growth of several (presumably axenic) monospecific algal cultures and lake phytoplankton. Harrison & Chan (1980) and Harrison (1982) studied the effect of water extracts of leaves of *Zostera marina* L. in various stages of decomposition on the growth of algae and bacteria. A growth-limiting effect of fresh and somewhat decomposed material was found, which decreased upon ageing of leaf material. Three phenolic acids, also present in the leaf extracts, had comparable effects.

Thus, various substances that can limit algal growth have been found in macrophyte tissues. However, the occurrence of allelopathic substances within plants is no proof of their release and activity in the environment (Wium-Andersen, 1987).

Hasler & Jones (1949) found a strong decrease in phytoplankton and rotifer biomass in artificial ponds with *Elodea canadensis* Michx. compared to ponds without plants. Crustacean zooplankton was not affected. This case again suffers from a problem that occurs in many papers favouring allelopathy: nutrient competition cannot be excluded.

Fitzgerald (1969) found a growth-limiting effect of macrophytes (*Ceratophyllum* sp., *Myriophyllum* sp. and *Lemna minor* L.) and filamentous algae (*Cladophora* sp. and *Pitophora oedogonium* (Mont.) Withrock) on epiphytes and phytoplankton under N-limiting conditions. No effect was found under P-limiting or not nutrient limited conditions: plants were overgrown by epiphytes and phytoplankton developed strongly. In experiments with *P. oedogonium*, it appeared that no differences in nitrogen concentration occurred between aquaria with and without the filamentous alga. Water from the cultures with *P. oedogonium*, filtered through glassfibre filters and enriched with nutrients, appeared to limit growth of *Chlorella pyrenoidosa* (strain Wis. 2005) but not of an *Ankistrodesmus* sp. The growth-limiting effect disappeared after sterilization through membrane filtering or autoclaving. The effect was attributed to a bacteria-sized organism, associated with the filamentous alga. Allelopathic substances excreted by the filamentous alga were not considered as the active agents, because the effect disappeared under non-limiting N levels. However, there seems to be no reason why an allelopathic growth inhibition could not be counteracted by high nutrient levels. Fitzgerald (1969) found a comparable effect for the effluent of a wastewater treatment plant: growth of *M. aeruginosa* (strain Wis. 1036) was inhibited by it, while *C. pyrenoidosa* growth was not. After membrane filter sterilization or autoclaving, *M. aeruginosa* grew well. Field data on *Cladophora* sp. showed that heavy epiphytizing took place only when nitrogen was not limiting.

Kogan & Chinnova (1972) and Kogan et al. (1972) tried to grow various species of phytoplanktonic algae together with *C. demersum*. It appeared that two species of *Anabaena* and *Anabaenopsis intermedia* Kog. were inhibited by the macrophyte, while *Chlorella* sp. and

Scenedesmus sp. showed no effect. It is not clear whether light competition played a role. They state that 'trophic competition....is impossible: a lot of nitrogen and phosphorus have remained in the cultural solution after algal extinction'. Surprisingly, a water extract of the macrophyte tissue stimulated algal growth (Kogan & Chinnova, 1972).

Brammer (1979) found in lakes in Sweden and Poland that phytoplankton apparently was growth-limited in stands of *Stratiotes aloides* L. However, although he did not exclude the possibility of an allelopathic effect, nutrient competition could explain the phenomenon. Crawford (1979) stated that farm ponds with a dense *Chara vulgaris* L. vegetation had stable sediments and low phytoplankton densities. Subsequent epiphyte growth under the usually eutrophic conditions in these systems presumably caused the decline of the macrophytes and a shift to phytoplankton dominance. Although allelopathy was not mentioned, she did stress the 'beneficial effects' of *C. vulgaris* on water quality, which are lost when the species is replaced in the succession by other macrophytes.

Godmaire & Planas (1983) did an enclosure experiment in which phytoplankton densities and rates of photosynthesis appeared to be enhanced especially in enclosures with *M. spicatum* but, to a lesser extent, also in those with a mixture of *Sparganium* sp. and *Nuphar microphyllum* (Pers.) Fern. compared to enclosures with plastic plants. The effect occurred in the first 8 weeks of the experiments and disappeared during the following 6 week period. Laboratory experiments were done with glassfibre-filtered water from cultures of *M. spicatum* and *E. canadensis*, enriched with N and P and inoculated with concentrated lake phytoplankton. Phytoplankton primary production was measured after 60 hours. The only effect found was a stimulation by water from cultures of young (2 weeks) *M. spicatum*. As the authors found that excretion of organic substances by *M. spicatum* is minimal during the first weeks, the quality of these substances seems of main importance. The growth stimulation by water from undisturbed *M. spicatum* cultures contrasts with the growth-limiting effect of extracts of the same species (Planas et al., 1981). This stresses the fact that results based on plant extracts cannot easily be used to predict the allelopathic effects that can be expected for intact plant populations. Wium-Andersen et al. (1987) isolated 2 organic compounds from *Sium erectum* Huds. that reduced the photosynthetic rate of the diatom *N. palea*. However, release of the substances from the macrophyte was not found. Wium-Andersen et al. (1987) suggested that these substances might be attached to the cuticle and in this way limit epiphyte colonization.

Weaks (1988) found allelopathic inhibition of growth of the alga *Selenastrum capricornutum* Printz in laboratory experiments with water from a beaver marsh. The effect disappeared after autoclaving, suggesting a heat-labile compound to be responsible. Addition of micronutrients reduced the effect also to some extent.

2.5 Effects among macrophytes

Some papers report allelopathic growth limitation between different macrophyte species. Frank & Dechoretz (1980) did various laboratory experiments to investigate the growth-limiting effect of *Eleocharis coloradoensis* (Britt.) Gilly on *Potamogeton pectinatus* L. and *Potamogeton nodosus* Poir. Water was

lead through sods of *E. coloradoensis* to each of the two *Potamogeton* species. Both in combined cultures and in monocultures *P. pectinatus* and to a lesser extent *P. nodosus* showed a lower amount of newly formed shoots and a lower shoot weight, compared to controls. Phosphorus concentration in water coming from *E. coloradoensis* cultures was 60-70% of that in the water coming from controls (aquaria with sediment, but no vegetation). A nutrient effect on plant growth seems to be unlikely as Peltier & Welch (1969) found no effect on *P. pectinatus* growth within a phosphorus concentration range including much lower P concentrations than the one used by Frank & Dechoretz (1980).

Kulshreshtha & Gopal (1983) found a growth-limiting effect of *Hydrilla verticillata* (L.F.) Royle on *C. demersum* and *Ceratophyllum muricatum* Chamisso when grown together in a tank, but separated by wire netting. No nutrient data were given, and nutrient competition seems to be an alternative explanation for the phenomena. Szczepańska (1987) reviewed results from earlier work by her and A. Szczepańsky on allelopathy among helophytes. *Phragmites australis* (Cav.) Trin ex Steud. appeared to be inhibited when grown together with *Typha latifolia* L. The latter then was stimulated. However, competition for nutrients and space was not excluded. Kleiven & Szczepańska (1988) found a limiting effect of water extracts from *Chara tomentosa* L. on early growth of germinated *Lepidium sativum* L. (a terrestrial species). Extracts from *C. demersum* had this effect to a lesser extent. No effect of water extracts from this and other *Chara* spp. was found on algal growth (unpublished data, mentioned in their paper).

2.6 Conclusions

From the results presented here, it seems clear that although sometimes nutrient competition may be a reasonable alternative explanation, in several cases allelopathic interactions cannot be excluded. Still, the actual excretion of algal growth-limiting substances by macrophytes which is part of the model of Phillips et al. (1983) is not yet firmly based on experimental evidence. Fitzgerald (1969), Kogan & Chinnova (1972) and Kogan et al. (1972) found algal growth inhibition while nutrient competition seemed unlikely, but in the case of Fitzgerald (1969), bacteria-sized organisms were suggested to be the actual agents, while in the latter two papers light competition is not clearly excluded. Godmaire & Planas (1983) could exclude competition for light and nutrients, but found stimulation of algal growth by macrophytes. Their results and those of Fitzgerald (1969) point to the fact that allelopathic growth limitation of algae by macrophytes may be rather complex: some algal species are growth-limited, whereas others are not or may even be stimulated.

Allelopathic effects may interact with nutrient competition and can be dependent on the age of the macrophyte. It is important to keep in mind that the role of allelopathic algal growth inhibition by macrophytes in the eutrophication model is not dependent on the actual origin of the secretions: macrophytes or a bacterial complex associated with them. Thus, results from non-axenic experiments (which are much easier to carry out) can be used to demonstrate whether allelopathic effects on algal growth, caused directly or indirectly by macrophytes, indeed occur.

3. Allelopathic effects from *Chara* spp. on two species of unicellular green algae

I. Blindow & M.J.M. Hootsmans

3.1 Introduction

In the extracts of several species of submerged plants, both of angiosperms (Su et al., 1973; Planas et al., 1981; Wium-Andersen et al., 1983), filamentous algae (Pankow, 1961) and *Chara* spp. (Anthoni et al., 1980, 1987) substances have been found that appeared to be toxic for unicellular algae.

Allelopathic reduction of microalgal growth by macrophytes has early been suggested to be responsible for the low phytoplankton and periphyton densities often observed in beds of submerged macrophytes (Willer, 1923; Hasler & Jones, 1949). Phillips et al. (1978) incorporated this relationship in their eutrophication model.

Compounds extracted from *Chara* spp. reduced the photosynthesis of unicellular algae (Wium-Andersen et al., 1982). Low periphyton densities were often observed on *Chara* spp. and have been explained by the production of allelopathic substances by these plants (Wium-Andersen et al., 1982).

However, the actual excretion of growth-limiting substances by healthy, intact submerged plants has not been demonstrated up till now. Therefore, the first aim of this study was to find out whether *Chara* spp. excrete toxic substances that significantly reduce the growth of unicellular algae.

Periphyton densities on *Chara* spp. vary considerably. Periphyton densities were high on *Potamogeton pectinatus* L. in Swartvlei, South Africa, but low on *Chara globularis* Thuill. growing close to each other (Howard-Williams, 1978). In Lake Tåkern (southern Sweden), however, periphyton densities were higher on *C. tomentosa* L. and *Nitellopsis obtusa* (Desv.) J. Groves than on *P. pectinatus* (Blindow, 1987). Also Zaneveld (1940) observed high periphyton densities on Charophyta in Malaysia.

To elucidate this apparent controversy, our second aim was to examine the occurrence of differences in allelopathic growth reduction when different taxa of *Chara* are tested on unicellular algae.

3.2 Material and methods

Plants were collected at different sites in southern Sweden and The Netherlands between August and October 1987. They were cultivated in aquaria containing 30 l tap water without sediment plus additional Na_2CO_3 (36 mg l^{-1}), $CaCl_2$ (23 mg l^{-1}) and $MgSO_4$ (50 mg l^{-1}). Light intensity was 100 $\mu E\ m^{-2}\ s^{-1}$ in a 12L:12D light regime (Philips HPIT lamp). Six cultures of *Chara* were used for the experiments. Five of them came from Sweden: *C. tomentosa*, *C. hispida* L., *C. delicatula* Agardh and *C. globularis* (two localities on Öland, named 1 and 2 in the following). One culture of *C. globularis* came from the Netherlands (Lake Veluwe, subsequently referred to as 3). As test algae, *Scenedesmus communis* Hegew. (strain 276-4b) and *Ankistrodesmus bibraianus* (Reinsch) Korš. (strain 6181, both from the Pflanzenphysiologisches Institut der Universität Göttingen) were used. Stock cultures of these algae were cultivated axenically in a nutrient medium

Table 5.1. Composition of Dutch Standard nutrient medium (NPR 6505) for the cultivation of algae (in mg l^{-1}).

		trace elements	
$NaNO_3$	500	H_3BO_3	2.9
K_2HPO_4	52	$MnCl_2$	1.81.
$MgSO_4$	75	$ZnCl_2$	0.11
$CaCl_2$	35	$CuSO_4$	0.08
Na_2CO_3	54	$(NH_4)_6Mo_7O_{24}$	0.018
Fe(III) citrate	6		
Citric acid	6		
NH_4NO_3	330		

(Dutch standard, NPR 6505; see Table 5.1). Experiments were carried out in a two-month period from October till December 1987. Each experiment consisted of two *Chara*-treatments and one control. For the control, six Erlenmeyer flasks (0.5 l) were filled with 300 ml nutrient solution in tap water. A treatment consisted of six test flasks filled with 150 ml of double-concentrated nutrient solution in tap water plus 150 ml of filtered water (Whatman GF/C glassfibre filter) from a *Chara* culture. The 18 flasks were inoculated with the same test alga (mean initial concentration in all experiments 8.7 mg l^{-1} ash-free dry weight (afdw), se 3.5 mg l^{-1}). They were placed in a temperature room at 15 °C, with a light regime of 15L:9D and a light intensity of 155-210 μE m^{-2} s^{-1} (Philips HPIT). The flasks were placed so that variation in light intensity was equally distributed among treatments. After 3-8 days, depending on the initial density of the algal culture, the water in the flasks was GF/C-filtered and the afdw of the algae was determined after drying (105 °C) and ashing (530 °C).

Each combination of *Chara* and alga was tested twice. When a significant growth reduction occurred in one or both of these two experiments, a treatment was repeated. As this extra information was conditional, we only used the first two experiments of each treatment in the overall statistical analysis.

To examine whether the effect of allelopathic substances was concentration dependent we did one additional experiment. GF/C-filtered water from the culture of *C. globularis*-3 was added in different concentrations to *Scenedesmus* cultures: 'standard concentration' as described above and 'half standard concentration'. The latter flasks contained 150 ml double concentrated nutrient solution in tap water, 75 ml GF/C-filtered water from the *Chara* culture and 75 ml tap water.

The results from all experiments were examined as follows. For each experiment we compared the mean biomass change of the two treatments per experiment with the control biomass change using Student's t-test with a comparisonwise error rate (CER) of 0.025 to ensure an experimentwise error rate (EER) of 0.05.

To compensate for differences in duration of the experiment, inoculation density and differences in light level between experiments, we first made a linear regression with treatment final biomass as dependent variable and control final biomass, inoculation biomass and duration as independent variables. Subsequently, the residuals from this regression were analysed in a twoway analysis of variance (ANOVA) for each alga with *Chara* (the different *Chara* treatments) and 'time' (replicate experiments per *Chara* treatment) as independent factors. As the datasets for the two test algae were rather unbalanced and separated in time, we could not test the interactions between *Chara* and test alga and between *Chara* and time although they may have existed. However, as the

effects other than those from *Chara* are eliminated when using the residuals from the regression, a significant time-effect can be regarded as an indication of a change in *Chara*-effect over time.

The regressions and ANOVAs were performed with the GLM procedure in the SAS statistical package (SAS Institute Inc., 1985). Multiple comparisons were done with least square means, obtained with the LSMEANS option in SAS. CER was kept at 0.003 to maintain an EER of 0.05 with the 15 comparisons that could be made between the 6 *Chara* treatments per test alga.

The mean difference between each treatment biomass change and control biomass change was calculated and expressed as percentage of the change in control biomass, together with its standard error. These 'standardized biomass change' data (which can be positive or negative) were used in the figures.

3.3 Results

In Figs. 5.1a-b standardized biomass changes from all experiments are shown. Both significant growth stimulations and growth reductions did occur. The latter were restricted to *Scenedesmus*. Differences between replicate experiments with the same combination of *Chara* and test alga were also apparent.

The results of the different ANOVAs are given in Table 5.2. In the ANOVA for *Ankistrodesmus*, no significant factor effects were found. *Scenedesmus* showed a highly significant *Chara* effect and a time effect ($p < 0.0001$). In Table 5.3, the 6 *Chara* treatments for *Scenedesmus* are compared with each other. Significant growth reductions occurred only when *Scenedesmus* was grown in filtrate from *C. globularis*-2 and 3. All other combinations resulted in no effect or a significant stimulation of algal growth. *C. globularis*-2 and 3 formed a distinct group without any differences in between but differing from all other combinations except *C. globularis*-1. The latter was much less pronounced than the other two and together with *C. delicatula* stood

Table 5.2. ANOVA results showing the significances of the effects of factors *Chara* and time on algal biomass development. SS = sum of squares, df = degrees of freedom.

a. *Ankistrodesmus* data

factor	df	type III SS	F	p
CHARA	5	0.00003306	0.49	0.7803
TIME	3	0.00001171	0.29	0.8318

b. *Scenedesmus* data

factor	df	type III SS	F	p
CHARA	5	0.00282500	23.64	0.0001
TIME	3	0.00067257	9.38	0.0001

Table 5.3. Multiple comparisons (mc) of the 6 *Chara* treatments with *Scenedesmus*. Data shown are the residual values (res; mg l^{-1}) from the regression needed to standardize the biomass data from the various experiments. Residuals that differ significantly from 0 point to a significant overall treatment effect averaged over time. These are indicated with * (t-test, $p < 0.05$). Treatments with the same letters are not significantly different. CER = 0.003, EER = 0.05. Replication is 12 for each treatment.

	res	mc	
C. tomentosa	11.8	*	A
C. hispida	9.0	*	A
C. delicatula	2.3		B
C. globularis-1	1.5		BC
C. globularis-2	−8.2	*	C
C. globularis-3	−8.5	*	C

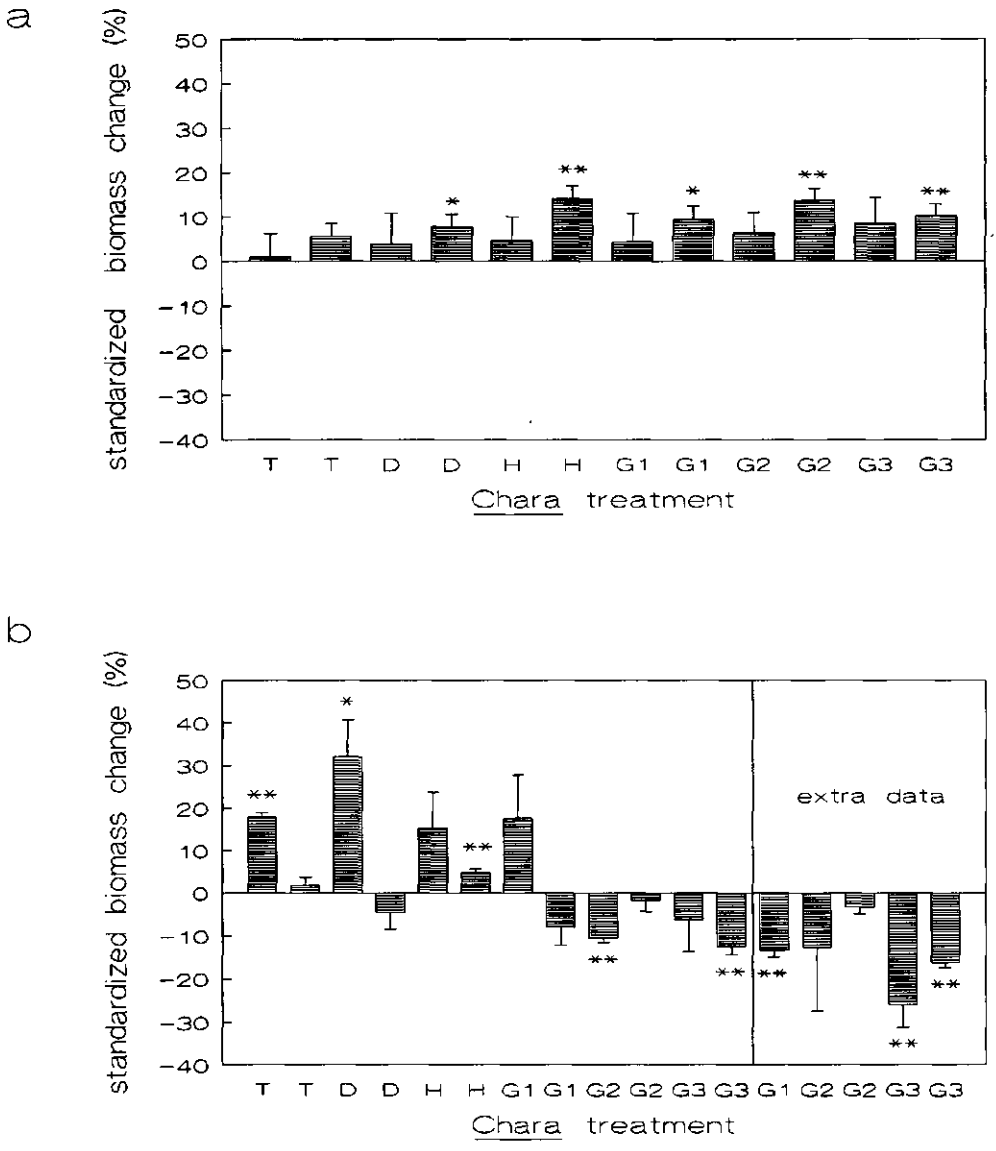


Fig. 5.1. The standardized biomass change for the various combinations of *Chara* and test alga. Given are means and standard errors. Asteriscs indicate significance of the difference between treatment and control biomass change: * = $p<0.05$, ** = $p<0.01$. For comparisons between treatments, see text and Table 5.3. T = *C. tomentosa*, D = *C. delicatula*, H = *C. hispida*, G1, G2, G3 = *C. globularis* strain 1, 2 and 3 (see text). Results from extra experiments performed for *C. globularis* cultures are presented as 'extra data'.

a. All experiments with *Ankistrodesmus*.

b. All experiments with *Scenedesmus*.

more or less in between this group and the other three species. *C. tomentosa* and *C. hispida* differed from the other species and stimulated algal growth.

In the additional experiment, 'standard' concentation of water from cultures of *C. globularis*-3 reduced the mean biomass change of *Scenedesmus* by 16% compared to the control ($p < 0.01$). 'Half standard' concentration of the same medium gave a mean biomass change 8% lower than the control ($p < 0.01$). The difference in mean biomass change between 'standard' and 'half standard' concentration was also significant ($p < 0.01$).

3.4 Discussion

Harper (1977) criticized most experiments that 'show' allelopathy, because alternative possibilities to explain the results are often not excluded. Thus, nutrient concentrations added to test and control cultures are different in many experiments. In our study, the control cultures received a slightly lower nutrient concentration than the test culture so that effects from differences in nutrient addition can be excluded (strictly, we suppose a positive relationship between nutrient concentration and growth). As we added medium from cultures of healthy macrophytes instead of medium from decaying macrophytes or extracts, any significant effect points to an excretion (active or passive) of substances by intact macrophytes.

Growth of *Ankistrodesmus* was stimulated or not affected by water from all *Chara* cultures. It is suggested that this stimulation was not caused by additional nutrients in the water from the *Chara* cultures as the nutrient content in this medium (tap water) was negligible compared to the amounts added with the nutrient medium to both test and control algal cultures. Instead, we assume the excretion of some stimulating organic substances by the macrophytes, as suggested by Allen (1971). We conclude on the basis of our results that chemical interaction exists between various *Chara* taxa and unicellular algae.

The reactions of *Scenedesmus* to different cultures of *Chara* agree well with the low periphyton density observed on *C. globularis* (Howard-Williams, 1978) and the high periphyton density described for *C. tomentosa* (Blindow, 1987). Growth of *Scenedesmus* was significantly reduced by cultures of *C. globularis* with about 10%. Based on the results from 'standard' and 'half standard' medium, the allelopathic effect appeared to be related to the concentration of the excreted substances (perhaps even linearly). Since medium from the macrophyte cultures was added just once and the substances identified from *Chara* are volatile (Anthoni et al., 1980), we suggest that the growth reduction may be higher in the field where phytoplankton is continuously exposed to these substances because of their steady production.

Even if the excretion of these compounds does not have a significant influence on the total biomass of phytoplankton in eutrophic water, an effect on the phytoplankton species composition may be suggested when some species are inhibited and others are stimulated by the same *Chara*. Effects of allelopathic substances excreted by planktonic algae on the succession of phytoplankton were already suggested by Keating (1977). Further studies could have practical importance when specific macrophytes can be found that lead to a substitution of Cyanobacteria by other taxa (e.g., green algae).

In our experiments different strains

of the same *Chara* 'species' (*C. globularis*) had different effects on algae. This is not surprising as widespread genetic isolation was found between different populations of this plant (Proctor, 1971) so that differences in their physiological behaviour can be expected. Furthermore, our results suggest that the excretion of allelopathic substances by the same macrophyte culture may change with time. Wium-Andersen (pers. comm.) observed that the content of allelopathic substances in extracts of Charophyta was lower in autumn than in summer.

We can not exclude that the observed growth reduction of *Scenedesmus* was caused by bacterial interaction. We assume that this point does not have ecological importance as the algal growth reduction should also occur in the field, independent of whether it is caused by a toxic substance from *Chara* and/or by bacterial interaction.

4. Seasonal changes in allelopathic properties of water samples from various localities in Lake Veluwe: a laboratory study

4.1 Introduction

In the preceding section it was shown that healthy, growing macrophytes in laboratory culture do excrete allelopathic substances that can influence the growth of phytoplanktonic algae. As the experiments were not sterile, bacterial interaction was possible. However, this is regarded as of minor interest for an evaluation of the role of allelopathy in the field: conditions in nature of course are rarely sterile.

Far more important is the question whether the effects that were found in the laboratory also occur in the field. Perhaps the concentration of growth mediators in the cultures was much higher than is normal in an aquatic system with macrophyte vegetation. To our knowledge, only Godmaire & Planas (1983) have investigated allelopathic activity in the field. In their enclosures with and without macrophytes in a lake, nutrient differences could not explain the stimulation of phytoplankton production in the enclosures with macrophytes (especially those with *Myriophyllum spicatum* L.).

Clear evidence for inhibition of algal growth by allelopathic interaction with macrophytes under field conditions is still not available. Of course, several studies exist that describe phytoplankton suppression in the neighbourhood of macrophytes (e.g. Hasler & Jones, 1949; Brammer, 1979; Crawford, 1979; cf. section 2 in this chapter) but in all cases, nutrient competition was not excluded. Such evidence is important to support the model proposed by Phillips et al. (1978) to explain the phenomena occurring in an ecosystem during eutrophication.

Stimulated by the laboratory results (see previous section 3) a research project was started to study the occurrence of allelopathic algal growth inhibition and its seasonal variation in the field. As light and nutrient effects are very difficult to eliminate in the field, we did algal growth experiments in the laboratory using nutrient-enriched water samples from various places in Lake Veluwe.

4.2 Material and methods

4.2.1 Experimental set-up

In the period June-August 1988, water samples were collected from five locations in Lake Veluwe. At each location, two samples were taken at 50-100 m from each other. Station 1 had a very low aboveground macrophyte biomass (mainly *Potamogeton perfoliatus* L.). Station 2, 3 and 5 had a *Potamogeton pectinatus* L. vegetation. Station 4 had a mixed vegetation of *P. pectinatus*, *Potamogeton pusillus* L. and *Chara globularis* Thuill. Very sparsely, the latter species also occurred at station 5. Water samples were collected in polythene jerrycans and stored overnight in the dark at 15 °C. The following day, samples were filtered over Whatman GF/C glassfibre filters. Nutrients were added according to the Dutch standard NPR 6505 (see section 3). The water samples were not sterilized, as bacterial interaction was regarded as a normal phenomenon in the field. Besides, as allelopathic substances may be volatile and/or heat labile (Keating, 1977, Wium-Andersen et al., 1982), autoclaving or sterile filtering with membrane filters might have eliminated the allelopathic agents.

The test organism was the green alga *Scenedesmus communis* Hegew., also used in the previous experiments (section 3). It was grown axenically in batch cultures, which were restarted weekly, in the same nutrient medium as mentioned above. Prior to each experiment, culture density was measured with a spectrophotometer at 750 nm. Algal biomass and optical density were linearly related up to 0.6 g l^{-1} (all weights are ash-free dryweight, afdw). When culture density was higher, a diluted sample (less than 0.6 g l^{-1}) was measured. Using this biomass estimate, the amount to be inoculated was calculated to arrive at an initial biomass of 0.1 mg l^{-1} in each experimental flask. The initial biomass was also measured directly by filtering an amount of algal culture through pre-combusted, pre-weighed glassfibre filters (Whatman GF/C). Filters with residu were dried (105 °C) and ashed (530 °C). Initial biomass appeared to be on average 0.11 mg l^{-1} (se 0.011 mg l^{-1}) with a relatively low value for the first experiment (0.08 mg l^{-1}) and a relatively high value for the last experiment (0.14 mg l^{-1}).

In total, 5 experiments were done. Experiment 1 lasted from June 17-27, 2 from July 1-11, 3 from July 14-21, 4 from July 28-August 4, 5 from August 12-19. Due to lack of laboratory space during the first two experiments, station 2 was not used in experiment 1 and 2, station 3 was not used in experiment 2 and station 4 was not used in experiment 1. A treatment consisted of 5 erlenmeyer flasks (0.5 l) filled with 300 ml nutrient enriched water for each of the two replicate samples from a station. To compensate for possible changes in experimental conditions over time, 10 control flasks (nutrient-enriched destilled water) were used in each experiment. The flasks were incubated in a waterbath (17 $\pm$ 1 °C) at 135 $\pm$ 5 μE m^{-2} s^{-1} provided by 400 W Philips HPIT lamps, at long day conditions (16 h photoperiod). Care was taken that each treatment had the same average light level. After 10 (exp. 1 and 2) or 7 days (exp. 3-5), all flasks were harvested by filtering over pre-combusted, pre-weighed glassfibre filters and final algal biomass (mg l^{-1}) was determined. In experiment 3 and 4, a subsample of each treatment was checked for the occurrence of other algal species originating from the lake or the laboratory. These algae might have infected the treatment because of

their small size (effective pore size of GF/C filter is 1.2 μm) or by leakage around the filter.

4.2.2 Statistical analysis

Comparison of the treatment effects on final algal biomass was done per experiment by analysis of variance (ANOVA) with factors 'station' and 'place' (i.e. the two replicate samples per station). Subsequent multiple comparisons of the final algal biomass were done with the Tukey test at $p<0.05$ (Steel & Torrie, 1980).

Analysis of the effect of time, i.e. comparisons of treatment effects between experiments, made it necessary to compensate for changes in experimental circumstances, indicated by differences in light level, duration of the experiment, final biomass of the various controls and the initial biomass levels. These 4 effects were used as independent variables in a multiple linear regression with final treatment biomass as dependent variable. The residuals from this regression can be considered as corrected for variation due to the 4 regressors. Any remaining significant trends in the residuals are attributed to treatment effects (i.e. effect of time). This can be tested in an ANOVA of the residuals with factors station, place and time. Significant stimulation, inhibition or no effect of each treatment was tested by comparing the mean residual value of each treatment with zero (Student t-test, $p<0.05$ for each comparison). Finally, multiple comparisons over time per station were made by t-tests with comparisonwise error rates (CER) low enough to ensure an experimentwise error rate (EER) of 0.05 for each station. All statistical calculations were done with the SAS statistical package (SAS Institute Inc., 1985).

4.3 Results

The analysis of subsamples from all treatments in experiment 3 and 4 revealed that virtually no other algae besides *Scenedesmus* occurred. In three cases, some diatoms (Pennales) were found. In one case, a filament of *Oscillatoria aghardii* Gomont was present.

In all experiments, final biomass of the treatments was about twice as high as the final biomass of the control. Final biomasses of the treatments are shown in Fig. 5.2a. The results of the ANOVAs for each experiment are given in Table 5.4. In all experiments, place was not significant. Apparently, on a scale of 50-100 m, the lake can be considered well-mixed. The interaction place*station was only significant for experiment 3. For experiment 1, 2, 4 and 5 a significant station effect was found.

Table 5.4. Significances from a twoway ANOVA per experiment with water from various localities in Lake Veluwe. Given are the significances of the effects of factors station, place and their interaction on the final biomass of *Scenedesmus*.

	experiment				
factor	1	2	3	4	5
station	0.000	0.002	0.129	0.024	0.000
place	0.514	0.580	0.790	0.377	0.895
place*station	0.275	0.275	0.011	0.415	0.249

Multiple comparisons between stations per experiment were performed with the results of the two 'places' per station combined except in the case of experiment 3 in which places were kept separately. The results are shown in Fig. 5.2a.

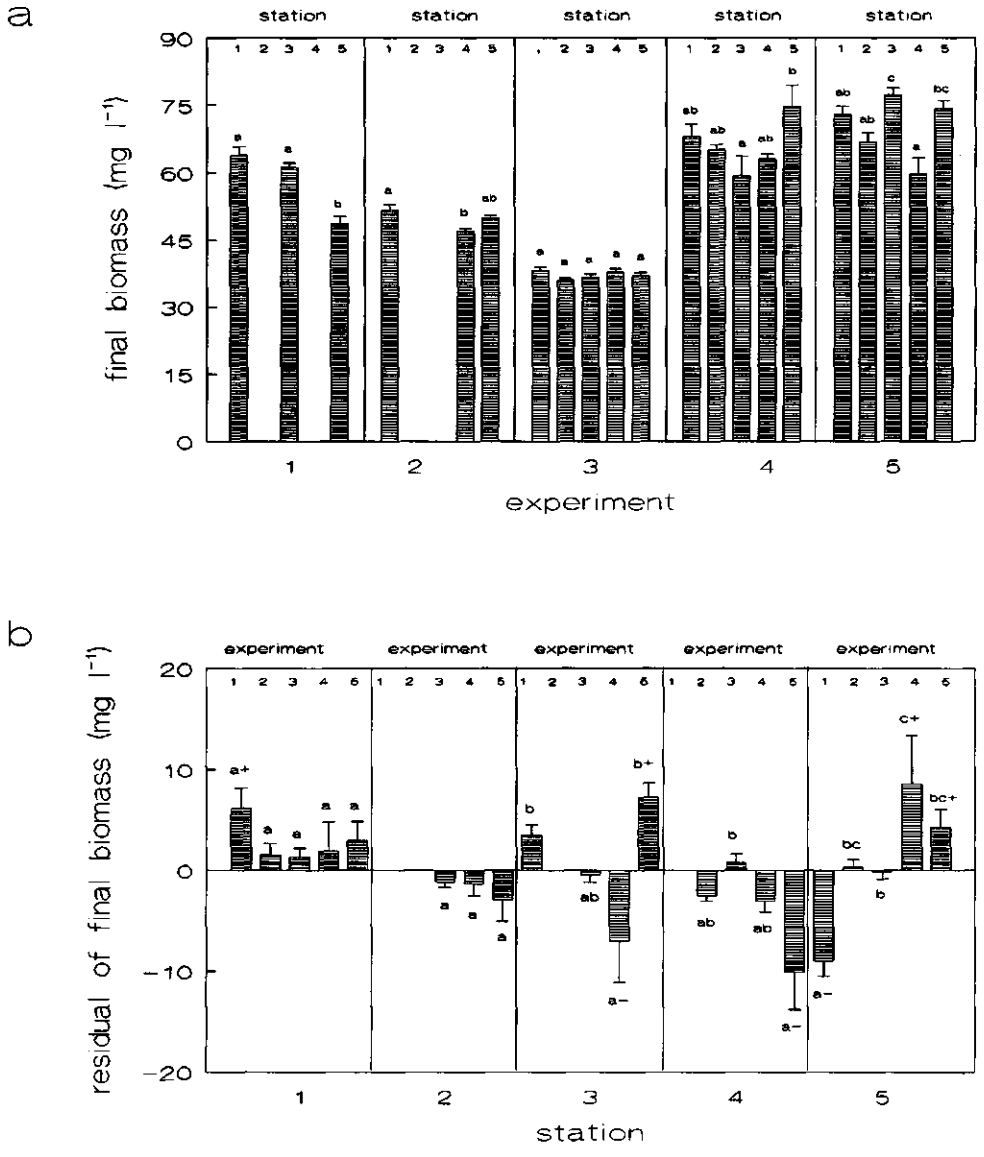


Fig. 5.2. Comparison of *Scenedesmus* biomass (mg l^{-1}) grown in water from various stations in Lake Veluwe. Lines on top of bars indicate standard errors.

(a) Comparison between stations per experiment. Per experiment, means with the same letter are not significantly different (Tukey test, $p < 0.05$).

(b) Comparison per station over time. Shown are mean residuals from a regression to standardize the data (see the text). Per station, bars with the same letter are not significantly different (t-test, EER = 0.05). Significant stimulation (+) or inhibition (−) is indicated besides the multiple comparison letter for each station.

Table 5.5. Parameter estimates and their significance in the multiple regression of final treatment biomass (g l^{-1}) of *Scenedesmus* with factors light (light level during experiment, $\mu E\ m^{-2}\ s^{-1}$), control (final control biomass of the experiment, g l^{-1}), begin (initial biomass of the experiment, g l^{-1}) and duration (duration of the experiment, days).

Parameter	estimate	p
intercept	0.01204	0.4596
light	0.00008	0.3474
control	1.80847	0.0001
begin	−0.16504	0.0045
duration	−0.00118	0.0425

In experiment 1, station 5 gave a significantly lower final biomass than station 1 and 3. Significant differences in experiment 2 existed between station 4 and station 1, but not anymore between 1 and 5. In experiment 3, no differences were found. In experiment 4, the situation reversed for station 5, which gave a rather high final biomass, differing from station 3 (the other stations were intermediate). In experiment 5, the effect of station 3 had changed. It gave the highest final biomass, differing from stations 1, 2 and 4. Station 4 was significantly lower than station 5.

In experiment 1, 4 and 5, the difference between lowest and highest final biomass (relative to highest biomass) was 15-20%. For experiment 2 and 3 this was 10% and 5%, respectively. It is clear that significant effects on algal growth occurred, and that stations differed in their effect. However, it is not possible to decide on the basis of these data whether the effects were also inhibitory: as all controls were much lower than the treatments, apparently some unknown factor in all treatments had a positive influence on algal growth.

Comparisons between the experiments were made with the residual values of the original data obtained by a multiple linear regression (see methods section). The results from this regression of the final treatment biomass data from all experiments are given in Table 5.5. The regression was highly significant (r^2 = 0.73, $p<0.0001$). Apart from light, all factors contributed significantly to the explanation of the variation in final biomass. As light intensity and distribution were kept as equal as possible for all experiments and treatments, the non-significance of the factor light is not surprising.

The resulting mean residual values for all experiments are shown in Fig. 5.2b. They show the remaining variation in the data. The residuals were analyzed for treatment effects with ANOVA, but were also used to draw conclusions on growth inhibition or stimulation by testing the mean residuals per station and experiment against zero (i.e. no treatment effect).

Table 5.6. Analysis of effects on residual values of final treatment biomass (g l^{-1}) by factors station, time and place.

factor	df	type III SS	F	p
time	4	0.00006960	0.40	0.8054
station	4	0.00115738	6.72	0.0001
place	1	0.00003799	0.88	0.3486
time*station	12	0.00347783	6.74	0.0001
error	187	0.00804636		

The ANOVA of the residuals is shown in Table 5.6. There was no effect of place and time, but station and its interaction with time were significant ($p<0.0001$). The other interactions were considered of no interest and were not tested. Again, as was expected after the ANOVA results for

each experiment, stations differed from each other in their effect on algal growth. The significant interaction indicates that in general the effect of a station changed over time, but not in the same manner for each station.

Significant differences between the mean residual values for each station and zero are indicated in Fig. 5.2b, together with multiple comparisons over time for each station. The number of significant differences in this analysis is less than in the separate analysis of the experiments. This is mainly caused by the use of different variance estimates, due to the different amount of data in the respective datasets. For example, in the test of experiment 2 (30 data), final biomasses of station 1 and 4 differed. In the residual analysis (209 data), none of the mean residuals from experiment 2 was significantly different from zero. The apparently negative effect on algal growth in experiment 3 (see the mean residuals in Fig. 5.2b) was not strong enough to be significant regarding the variation in the total dataset with all experiments combined. The number of significantly stimulating or inhibiting effects found with the residual analysis thus can be seen as a rather conservative estimate.

4.4 Discussion

Based on the results from the inspection of subsamples for contamination of the experimental cultures with other algae than *Scenedesmus*, it is concluded that the effects found reflect the reaction of this test alga only: filtration effectively removed all algae from the lakewater samples.

It must be stressed that no inorganic nutrient limitation or difference in inorganic nutrient concentration can

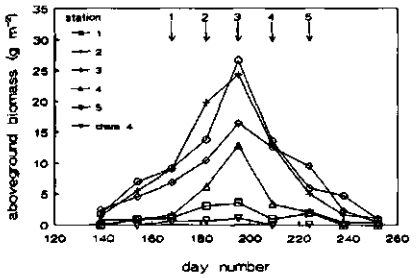


Fig. 5.3. Total aboveground biomass (g m^{-2}) of macrophyte vegetation at the different stations used in the experiments. Sampling dates of water for the five experiments are indicated with arrows.

explain the present results, as these were available in the same non-limiting quantities in all experimental cultures. The much higher final biomass in all treatments compared to their controls may be attributed to the occurrence of some important organic compound(s), like amino acids, vitamins and hormones in the lakewater from all stations.

Data on total aboveground macrophyte biomass for the various stations during the season are shown in Fig. 5.3. These results were taken from Doef (1990) who collected them together with the water samples used in the present study.

Station 1, which all the time had a low macrophyte biomass, showed no effect except for a stimulation in experiment 1. This may indicate that there was a release of stimulatory organic compounds from the sediment by microbial degradation of material at the time of this experiment. The significant growth inhibition of station 5 in experiment 1 may be attributed to a rather high production and excretion of allelopathic substances. At the same time, station 2 and 3 had a comparable biomass, while they did not show any significant effect.

Biomass development at station 2 almost paralleled the vegetation at station 5, while the vegetation peak biomass at station 3 was about 30% lower. Remarkably, station 2 never differed significantly from low biomass station 1 when both were used in the same experiment.

In the course of the season, activity of *P. pectinatus* apparently diminished (station 5 had no effect in experiments 2 and 3, station 2 and 3 had no effect in experiment 3). In experiment 4, station 3 strongly inhibited and station 5 strongly stimulated algal growth, while station 2 showed no effect. Despite this difference, vegetation biomass of these three stations on this date was about equal. One of the 10 replicate *Scenedesmus* biomass samples for station 3 was very low in this experiment (30% of the mean algal biomass value found for this station) but there was no reason to reject it as a valid observation. The difference between station 3 and 5 is paralleled by a slower biomass decrease at station 3, compared with station 2 and 5. In experiment 5, both station 3 and 5 strongly stimulated algal growth. At this time in the season the vegetation was senescing, which introduces the possibility of a release of essential organic substances that can stimulate algal growth.

For station 4, with a mixed vegetation with *C. globularis*, no effect was found until experiment 5. The strong inhibition in that experiment may be attributed to the release of inhibitors from the decaying *Chara* vegetation. If any stimulatory effect of the decaying *P. pectinatus* vegetation occurred also at this time, it was completely obscured. The allelopathic activity of at least two compounds from *C. globularis* extracts has been shown by Wium-Andersen et al. (1982). The expected growth inhibition of *Scenedesmus* by actively growing *Chara* (station 4 in experiment 2 and 3) was not found, in contrast with results from laboratory cultures (see section 3 in this chapter).

The demonstrated effects indicate that also in the field, substances occur in the water that can influence algal growth, both positively and negatively. The magnitude of the effects, within the time period of these experiments, was small. Still, when algal species are differentially affected by these allelopathic compounds, a 20% difference in biomass after 7 days can have a distinct influence on the outcome of competition between these algae.

The comparison of the results with vegetation data shows that a clear-cut explanation, i.e. a correlation of the allelopathic effects with vegetation biomass, is not possible. Differences in wind exposure and concomitant water exchange between the macrophyte stands and surrounding open water devoid of vegetation cannot explain the results either. Station 5 can be regarded as the most exposed, while at the same time, most effects were found for this vegetation. The stations may have differed with respect to macrophyte activity (i.e. photosynthesis, excretion) and age composition. The somewhat different vegetation development at station 3 may point in this direction. Unfortunately, information on such differences is not available.

Although the quantitative importance of the interaction found in this study may be minor, the results presented here provide to our knowledge for the first time evidence for the supposed allelopathic limitation of algal growth in situ. The rather strong inhibition of *Scenedesmus* growth by *P. pectinatus* as found for station 5 in experiment 1 is

especially interesting. It more or less coincides with both the spring algal bloom and the critical period of growth of young plants from the bottom to the water surface. Especially in this period, a suppression of algal growth seems advantageous. It is speculated that this is part of the reason why *P. pectinatus* is one of the few species that can survive under highly eutrophic conditions with potentially dense algal blooms.

Assuming that an increased rate of photosynthesis correlates with an increased production and excretion of (allelopathic) substances, it can be hypothesized that macrophytes and/or the bacterial complex associated with them will be more active in producing and excreting allelopathic substances under less turbid conditions than in Lake Veluwe in its present condition. The effects found in this study thus may be a minimum estimate of the potential allelopathic growth limitation of phytoplanktonic algae by macrophytes under clear water conditions.

5. Allelopathic effects in three lake restoration projects

5.1 Introduction

Regarding the results in the two previous sections, it appears that indeed, macrophytes are able to secrete substances that can influence algal growth. Allelopathic effects as hypothesized in our working hypothesis (the model based on Phillips et al., 1978; see chapter 1) thus can occur in the field. The question remains how strongly this interaction can determine the overall outcome of the various interactions occurring in the system. We will not address this problem here. Still, it seems that macrophytes cannot simply be considered as 'sitting ducks' that have to endure shading effects due to phytoplankton blooms.

Recently, much interest and activity has been deployed in the field of biomanipulation as a means for restoring heavily eutrophicated lakes. An overview of the various activities in this field in The Netherlands is given in Van Donk & Gulati (1989). During eutrophication, fish communities in many lakes have become dominated by bream (*Abramis brama* L.; Hosper, 1989, Lammens, 1989). Within the frame of the model 1 approach (see chapter 1) this can be seen as the major cause for macrophyte disappearance and algal bloom occurrence. Therefore, emphasis in the restoration efforts is usually placed on manipulating the fish stocks.

In this section, three of these projects in The Netherlands are given some attention from an allelopathic point of view. One of the results found after the fish stock manipulations was a rapid increase of *Chara* vegetations in two of the three projects. As was shown before, these macrophytes can supress algal growth by allelopathic exudates. Thus, these projects offered an interesting additional possibility to test the allelopathic effect of a macrophyte vegetation on phytoplankton growth. In the following, the results are presented of experiments comparing the algal growth in nutrient-enriched water from these restoration projects.

5.2 Study area, material and methods

The first location is called Bleiswijk. It was described in Meijer et al. (1989). Here, a small lake was divided into two compartments in 1987, using a small wooden dam. This dam was provided with a small opening (10*20 cm) covered with gauze. This allowed water exchange but was impossible for fish to pass through. In one part, Galgje, the fish community was changed by removing the major part of bream and carp (*Cyprinus carpio* L.). Pikeperch (*Stizostedion lucioperca* L.) was added to increase predation pressure on juvenile bream during the initial, still turbid, period. This predator is much less dependent on sight for prey capture than pike (*Esox lucius* L.). The other compartment, Zeeltje, acted as the control.

Location Wolderwijd is a large pond (1 ha, constructed in 1987) isolated from Lake Wolderwijd, a lake comparable and adjacent to Lake Veluwe. In this pond, no fish was present, while Lake Wolderwijd, the control, has a dense bream population.

Location Noorddiep is an isolated branch of the river IJssel, which is the northern branch of the river Rhine in The Netherlands. This location was divided into an untreated control (referred to as +fish) and a section from which bream and carp were removed (−fish).

In all three locations, fish stock changes resulted in increased Secchi depth, decreased chlorophyll-a concentration and decreased Cyanobacteria presence. In Galgje and in the Wolderwijd pond, a *Chara* vegetation developed (Meijer et al., 1989; Hootsmans & Breukelaar, 1990).

At the end of July, 1988, water samples were collected in the three locations. The water was filtered over GF/C filters. Erlenmeyer flasks (0.5 l) were filled with 300 ml water sample which was nutrient-enriched according to the Dutch standards for algal cultures (NPR 6505; see section 3). After inoculation with *Scenedesmus communis* Hegew., the water samples were incubated for 6 days at 17 °C and 140 μE m^{-2} s^{-1}. Five replicates were used for each treatment and ten for the control (nutrient-enriched distilled water). Final biomasses of treatments were compared with each other and with the control. Multiple comparisons between locations were done with a Tukey test ($p<0.05$).

5.3 Results and discussion

In Fig. 5.4, the resulting final biomass of *Scenedesmus* in nutrient-enriched water samples from the six treatments after 6 days is shown, together with the results

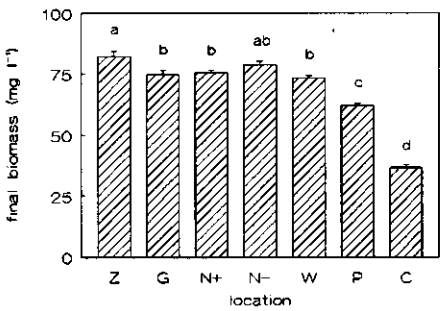


Fig. 5.4. Final biomass (mg l^{-1} ash-free dryweight) of *Scenedesmus* in water samples from three locations in The Netherlands where the fish population was manipulated. Per location, the left column represents the control situation. For comparison, the laboratory control is shown also. Lines on top of the columns are standard error bars. Columns with the same letter are not significantly different (Tukey test, $p<0.05$). Z = Zeeltje (control), G = Galgje (manipulated), N+ = Noorddiep control, N− = Noorddiep manipulated, W = Wolderwijd (control), P = pond (manipulated), C = laboratory control.

from multiple comparisons. The final biomass of the control is given also.

Again, as was found in section 4, final biomass in the treatments was twice as high as the control. This probably indicates the presence in the treatments of organic substances that enhanced algal growth compared to growth in a completely inorganic medium. Both Bleiswijk and Wolderwijd showed a reduced algal growth in the water sample from the manipulated compartment compared to their untreated situation. Noorddiep +fish and −fish did not differ from each other.

These results were correlated with the development of a *Chara* vegetation in Bleiswijk (Galgje) and Wolderwijd (pond). This supports the evidence from other studies that *Chara* spp. can inhibit algal growth to a certain extent (Wium-Andersen et al., 1982; section 3 in this chapter). The difference in final biomass between treated and untreated stations is small. Still, minor changes in growth rate may have serious consequences for the success of a species during competition and succession.

Despite the presence of an opening in the dam in location Bleiswijk, differences between the treated Galgje and untreated control Zeeltje existed. Apparently, water exchange was not very intense, or the lake compartments were not thoroughly mixed.

The size of the effects measured may be a consequence of the high inorganic nutrient levels in the experiments: perhaps allelopathic inhibition can partly be overcome when nutrient levels are high. This means that when nutrient conditions are reduced also during the restoration process, the effect of allelopathy might even be enhanced.

It is concluded that the observed positive effects of the restoration measures can at least partly be due to allelopathic effects from the macrophytes. Thus, it may be possible that active measures to restore the macrophyte vegetation, especially of *Chara* spp., can reduce the need for intensive manipulations of the fish community.

Acknowledgements

Thanks are due to I. Blindow (University of Lund, Sweden), R. Gijlstra, K.P. Raap, N.W. van den Brink and J.E. Vermaat for their support and advice during the experiments. Valuable statistical assistance was given by Dr. A.G. Pols from the Department of Mathematics (Wageningen Agricultural University). Prof. Dr. W. van Vierssen, Prof. Dr. W.J. Wolff and J.E. Vermaat critically read the manuscript.

6. References

Allen, H.L., 1971. Primary productivity, chemo-organotrophy, and nutritional interactions of epiphytic algae and bacteria on macrophytes in the littoral of a lake. Ecol. Monogr. 41: 97-127.

Anthoni, U., Christophersen, C., Øgård Madsen, J., Wium-Andersen, S. & Jacobsen, N., 1980. Biologically active sulphur compounds from the green alga *Chara globularis*. Phytochemistry 19: 1228-1229.

Anthoni, U., Nielsen, P.H., Smith-Hansen, L., Wium-Andersen, S. & Christophersen, C., 1987. Charamin, a quaternary ammonium ion antibiotic from the green alga *Chara globularis*. J. Org. Chem. 52: 694-695.

Berg, K., Skulberg, O.M. & Skulberg, R., 1987a. Effects of decaying toxic blue-green algae on water quality - a laboratory study. Arch. Hydrobiol. 108: 549-563.

Berg, K., Carmichael, W.W., Skulberg, O.M., Benestad, C. & Underdal, B., 1987b. Investigation of a toxic water-bloom of *Microcystis aeruginosa* (Cyanophyceae) in Lake Akersvatn, Norway. Hydrobiologia 144: 97-103.

Best, E.P.H., 1987. The submerged macrophytes of Lake Maarsseveen I: changes in species composition and biomass over a six year period. Hydrobiol. Bull. 21: 55-60.

Blindow, I., 1987. The composition and density of epiphyton on several species of submerged macrophytes - the neutral substrate hypothesis tested. Aquat. Bot. 29: 157-168.

Brammer, E.S., 1979. Exclusion of phytoplankton in the proximity of dominant water-soldier (*Stratiotes aloides*). Freshwat. Biol. 9: 233-249.

Crawford, S.A., 1979. Farm pond restoration using *Chara vulgaris* vegetation. Hydrobiologia 62: 17-31.

De Nie, H.W., 1987. The decrease in aquatic vegetation in Europe and its consequences for fish populations. EIFAC/CECPI occasional paper no. 19, 52pp.

Doef, R.W., 1990. Inventarisatie van water- en oeverplanten in de Randmeren van 1987-1989 (Inventory of aquatic vascular plants in the Dutch Border Lakes, in Dutch). Institute for Inland Water Management and Waste Water Treatment, Report 90.015.

Fitzgerald, G.P., 1964. The biotic relationships within waterblooms. In Jackson, D.F., (ed.), Algae and Man. Plenum Press, New York, pp. 300-306.

Fitzgerald, G.P., 1969. Some factors in the competition or antagonism among bacteria, algae, and aquatic weeds. J. Phycol. 5: 351-359.

Frank, P.A. & Dechoretz, N., 1980. Allelopathy in dwarf spikerush (*Eleocharis coloradoensis*). Weed Sci. 28: 499-505.

Godmaire, H. & Planas, D., 1983. Potential effect of *Myriophyllum spicatum* on the primary production of phytoplankton. In Wetzel, R.G., (ed.). Periphyton of freshwater ecosystems. Junk, The Hague, pp. 227-233.

Gorham, P.R., 1964. Toxic algae. In Jackson, D.F., (ed.), Algae and Man. Plenum Press, New York, pp. 307-336.

Harper, J.L., 1977. Population biology of plants. Academic Press, London, pp. 369-381.

Harrison, P.G., 1982. Control of microbial growth and of amphipod grazing by water-soluble compounds from leaves of *Zostera marina*. Mar. Biol. 67: 225-230.

Harrison, P.G. & Chan, A.T., 1980. Inhibition of the growth of micro-algae and bacteria by extracts of eelgrass (*Zostera marina*) leaves. Mar. Biol. 61: 21-26.

Hasler, A.D. & Jones, E., 1949. Demonstration of the antagonistic action of large aquatic plants on algae and rotifers. Ecology 30: 359-364.

Hootsmans, M.J.M. & Breukelaar, A.W., 1990. De invloed van waterplanten op de groei van algen (The influence of aquatic macrophytes on algal growth, in Dutch). H_2O 23: 264-266.

Hosper, S.H., 1989. Biomanipulation, new perspectives for restoring shallow, eutrophic lakes in The Netherlands. Hydrobiol. Bull. 23: 5-10.

Hough, R.A. & Wetzel, R.G., 1975. The release of dissolved organic carbon from submersed aquatic macrophytes: diel, seasonal and community relationships. Verh. int. Verein. Limnol. 19: 939-948.

Howard-Williams, C., 1978. Growth and production of aquatic macrophytes in a south temperate saline lake. Verh. int. Verein. Limnol. 20: 1153-1158.

Jüttner, F., 1981. Biologically active compounds released during algal blooms. Verh. int. Verein. Limnol. 21: 227-230.

Keating, K.I., 1977. Allelopathic influence on blue-green bloom sequence in a eutrophic lake. Science 196: 885-887.

Keating, K.I., 1978. Blue-green algal inhibition of diatom growth: transition from mesotrophic to eutrophic community structure. Science 199: 971-973.

Kleiven, S. & Szczepańska, W., 1988. The effects of extracts from *Chara tomentosa* and two other aquatic macrophytes on seed germination. Aquat. Bot. 32: 193-198.

Kogan, S.I. & Chinnova, G.A., 1972. On the relations between *Ceratophyllum demersum* L. and some blue-green algae. Gidrobiol. Zh. 8: 21-27.

Kogan, S.I., Chinnova, G.A. & Kravchenko, M.E., 1972. The effect of macrophytes on certain algae in joint cultivation. Izv. Akad. Nauk Turkm. SSR Ser. Biol. Nauk 3: 3-8.

Kulshreshtha, M. & Gopal, B., 1983. Allelopathic influence of *Hydrilla verticillata* (L.f.) Royle on the distribution of *Ceratophyllum* species. Aquat. Bot. 16: 207-209.

Lammens, E.H.R.R., 1989. Causes and consequences of the success of bream in Dutch eutrophic lakes. Hydrobiol. Bull. 23: 11-18.

Meijer, M.-L., Raat, A.J.P. & Doef, R.W., 1989. Restoration by biomanipulation of the Dutch shallow, eutrophic Lake Bleiswijkse Zoom: first results. Hydrobiol. Bull. 23: 49-58.

Molisch, H., 1937. Der Einfluss einer Pflanze auf die andere - Allelopathie. G. Fischer Verlag, Jena, pp. 106.

Muller, C.H., 1969. Allelopathy as a factor in ecological process. Vegetatio 18: 348-357.

Pankow, H., 1961. Uber die Ursachen des Fehlens von Epiphyten auf Zygnemales. Arch. Protistenkunde 105: 417-444.

Peltier, W.H. & Welch, E.B., 1969. Factors affecting growth of rooted aquatics in a river. Weed Sci. 17: 412-416.

Phillips, G.L., Eminson, D. & Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4: 103-126.

Planas, D., Sarhan, F., Dube, L., Godmaire, H. & Cadieux, C., 1981. Ecological significance of phenolic compounds of *Myriophyllum spicatum*. Verh. int. Verein. Limnol. 21: 1492-1496.

Proctor, V.W., 1971. *Chara globularis* Thuillier (=*C. fragilis* Desvaux): Breeding patterns within a cosmopolitan complex. Limnol. Oceanogr. 16: 422-436.

Rice, E.L., 1974. Allelopathy. Academic Press, New York, 353 pp.

Rice, E.L., 1979. Allelopathy - an update. Bot. Rev. 45: 15-109.

Rice, E.L., 1984. Allelopathy. 2nd ed., Academic Press, Orlando, 422 pp.

Rijstenbil, J.W., 1989. Competitive interaction between *Ditylum brightwellii* and *Skeletonema costatum* by toxic metabolites. Neth. J. Sea Res. 23: 23-27.

SAS Institute Inc., 1985. SAS/STAT Guide for Personal Computers, Version 6 Edition. SAS Institute Inc., Cary, NC, 378 pp.

Sharma, K.P., 1985. Allelopathic influence of algae on the growth of *Eichhornia crassipes* (Mart.) Solms. Aquat. Bot. 22: 71-78.

Søndergaard, M., 1981. Kinetics of extracellular release of ^{14}C-labelled organic carbon by submerged macrophytes. Oikos 36: 331-347.

Steel, R.G.B. & Torrie, J.H., 1980. Principles and procedures of statistics, a biometrical approach. McGraw-Hill, London, 2nd ed., pp. 185-186.

Su, K.L., Staba, E.J. & Abul-Hajj, Y., 1973. Preliminary chemical studies of aquatic plants from Minnesota. Lloydia 36: 72- 79.

Szczepańska, W., 1987. Allelopathy in helophytes. Arch. Hydrobiol. Beih. 27:173-179.

Van Donk, E. & Gulati, R.D., (eds), 1989. Biomanipulation in The Netherlands: applications in fresh-water ecosystems and estuarine waters. Hydrobiol. Bull. 23: 1-99.

Van Vierssen, W. & Prins, Th.C., 1985. On the relationship between the growth of algae and aquatic macrophytes in brackish water. Aquat. Bot. 21: 165-179.

Weaks, T., 1988. Allelopathic interference as a factor influencing the periphyton community of a freshwater marsh. Arch. Hydrobiol. 111: 369-382.

Wetzel, R.G. & Manny, B.A., 1972. Secretion of dissolved organic carbon and nitrogen by aquatic macrophytes. Verh. int. Verein. Limnol. 18: 162-170.

Willer, A., 1923. Der Aufwuchs der Unterwasserpflanzen. Verh. int. Verein. Limnol. 1: 37-57.

Wium-Andersen, S., 1987. Allelopathy among aquatic plants. Arch. Hydrobiol. Beih. 27: 167-172.

Wium-Andersen, S., Anthoni, U., Christophersen, C. & Houen, G., 1982. Allelopathic effects on phytoplankton by substances isolated from aquatic macrophytes (Charales). Oikos 39: 187-190.

Wium-Andersen, S., Anthoni, U. & Houen, G., 1983. Elemental sulphur, a possible allelopathic compound from *Ceratophyllum demersum*. Phytochemistry 22: 2613.

Wium-Andersen, S., Jørgensen, K.H., Christophersen, C. & Anthoni, U., 1987. Algal growth inhibitors in *Sium erectum* Huds. Arch. Hydrobiol. 111: 317-320.

Wolfe, J.M. & Rice, E.L., 1979. Allelopathic interactions among algae. J. Chem. Ecol. 5: 533-542.

Zaneveld, J.S., 1940. The Charophyta of Malaysia and adjacent countries. Blumea 4: 1-222.

PERIPHYTON DYNAMICS IN A TEMPERATURE-LIGHT GRADIENT

6

J.E. Vermaat & M.J.M. Hootsmans

Abstract

Periphyton was cultured on glass slides in a laboratory set-up combining three temperatures (10°, 15° and 20 °C) and three irradiances (50, 100 and 200 μE m^{-2} s^{-1}), with nutrient concentrations simulating eutrophic conditions. Replicate biomass (mg cm^{-2}, ash-free dry weight = afdw) and attenuance (irradiance transmittance reduction relative to blanks) samples were taken every second or third day for a period of 49 days.

Differences in temperature caused more significant differences in logistic growth curves of biomass and algal taxonomic composition than did differences in irradiance. *Scenedesmus* became the dominant genus at high temperature, while *Navicula* dominated at low temperature and low irradiance. Maximum periphyton biomass (1.5 - 3.0 mg cm^{-2} afdw) and instantaneous growth rates that were reached are within the range reported for periphytic mats. Only periphyton dominated by filamentous green algae reached biomasses that were one order of magnitude higher.

Most significant differences between attenuance-biomass curves existed between different irradiances. This pattern is different from that of the growth curves. The different periphyton communities apparently were able to acclimate efficiently to the different irradiance regimes: under low irradiance the initial slope of the curves was steeper, i.e. attenuance per unit biomass increased more, than under high irradiance.

Suspensions of periphytic communities systematically underestimated attenuance of the intact communities by about 40%. Application of Lambert-Beers' law for exponential extinction appeared invalid since intact periphyton communities did not behave as dilute homogeneous suspensions. A rectangular hyperbola was shown to describe the relation between proportional attenuance and periphyton biomass satisfactorily.

Contents

1. Introduction

Wetzel (1983a) gave a definition of 'periphyton': "a complex community of microbiota (algae, bacteria, fungi, animals, inorganic and organic detritus) that is attached to substrata. The substrata are inorganic or organic, living or dead." He concluded that though the word probably "is something of a misnomer in that a literal translation is too restrictive", and that "it is perhaps best to accept the word periphyton as it is widely understood". We follow this rationale and Wetzels' definition.

Differences in view exist on the specificity of periphyton communities on specific macrophyte species and on the intensity of mutual relations between periphyton and the host plant (Cattaneo & Kalff, 1978, 1979; Eminson & Moss, 1980; Gough & Gough, 1981; O'Neill-Morin & Kimball, 1983; Jenkerson & Hickman, 1986; Blindow, 1987). On the basis of their data, Eminson & Moss (1980) proposed an elegant hypothesis as a solution for the specificity controversy: with increasing eutrophication specificity disappears, or, for periphyton communities that develop under eutrophic conditions, all substrata are similar. From this viewpoint, the controversies on specificity and intensity of mutual relations appear to be closely related. Generally, it may be hypothesized that development of recycling mechanisms in the macrophyte-periphyton complex has distinct adaptive value for both 'sides' under nutrient-limiting, i.e. non-eutrophic conditions (Wetzel, 1983a).

Moeller et al. (1988) demonstrated in the laboratory that only 2% of cumulative phosphorus uptake by periphyton originated from the macrophyte and Carignan & Kalff (1982) reported 3 - 9% from incubations in mesotrophic Lake Memphremagog (Canada). The latter authors therefore concluded that "previously suggested macrophyte-epiphyte nutrient interactions are of relatively minor importance".

To date no clear evidence exists of any beneficiary effects of a dense periphyton cover to a macrophyte (Wetzel, 1983c). Protection from grazers (Hutchinson, 1975) and shading from too intense irradiation have been suggested (Van Vierssen, 1983). The latter phenomenon seems to play no significant role for temperate climate regions, since to our knowledge no photo-inhibition has been reported for submerged freshwater macrophyte species. The former remains questionable to date, since most grazers on macrophytes are considered to be true microherbivores (Cummins, 1973; Calow, 1970, 1973; Soszka, 1975; Reavell, 1980; Van Montfrans et al., 1982; Haynes & Taylor, 1984; Vermaat, 1991). Rogers & Breen (1983) demonstrated that periphyton grazing by snails even prolonged tissue-lifetime of the macrophyte by reducing invasion of necrotrophic bacteria. Therefore, it seems reasonable to assume that any nutrients or organic compounds coming available to periphyton from the macrophyte are not excreted to stimulate periphyton growth and that the significance of this 'leaking' to periphyton must be considered relative to the amount available in the surrounding water. Allelopathic effects of excreted organic compounds are treated by Hootsmans (1991).

Dense periphyton covers that reportedly develop under eutrophic conditions may adversely affect photosynthesis and growth of the macrophyte through shading or competition for inorganic carbon and/or nutrients (Sand-Jensen, 1977; Phillips et

al., 1978; Sand-Jensen & Søndergaard, 1981; Bulthuis & Woelkerling, 1983; Sand-Jensen & Borum, 1984; Silberstein et al., 1986).

Dynamics of periphyton communities have been studied mainly under field- or semi-field conditions (Allen, 1971; Allanson, 1973; McMahon et al., 1974; Herder-Brouwer, 1975; Cattaneo & Kalff, 1978, 1979; Eminson & Moss, 1980; Gons, 1982; O'Neill-Morin & Kimball, 1983; Kairesalo, 1984; Meulemans & Roos, 1985; Jenkerson & Hickman, 1986; Blindow, 1987; Fairchild & Everett, 1988; among others) on a fairly diverse range of substrata (submerged or emergent macrophytes, natural rock substrata and a series of artificial substrata).

Laboratory studies are less frequent. Eminson & Phillips (1978) reported higher periphyton densities on macrophytes in nutrient-enriched experimental units, but did not quantify the density or effect of a developing mat of filamentous *Oedogonium* spec. on the macrophytes and periphyton. Wilhm & Long (1969) did long-term batch experiments (109 days) on artificial substratum with three different initial nutrient levels and found higher photosynthesis, respiration and maximum biomass of the developed periphyton for higher initial nutrient levels.

In this study we investigated growth, light attenuance and chlorophyll concentration of periphyton communities under eutrophic conditions, i.e. with nutrients in high or approximately uptake-rate-saturating concentrations, as a function of temperature and light in a semi-continuous culturing system. Under field conditions, the separate effects of temperature and light are hard to assess since large scale (diel, seasonal) fluctuations occur simultaneously and interactingly (Berry & Raison, 1981). Therefore, a laboratory set-up was considered necessary to study the separate effects of these factors and their interaction.

We used microscopic glass slides as substrate to facilitate manipulation and replication and to be able to measure light attenuance by the intact periphyton community. For a discussion on the use of artificial substrata the reader is referred to e.g. Mason & Bryant (1975) and Robinson (1983). The reported similarity of periphyton communities on different substrata under eutrophic conditions as discussed above should permit extrapolation of results from glass slides to macrophyte surfaces for these conditions.

In the field, periphyton accumulation always is a function of growth and colonization (Herder-Brouwer, 1975; Rodriguez, 1987). Since colonization is restricted to inoculation by the experimenter in laboratory experiments, biomass accumulation thus can be considered a reliable measure of growth.

Though its significance was postulated repeatedly (e.g. Sand-Jensen, 1977; Phillips et al., 1978), light transmittance by intact periphyton communities has not been measured often and many authors used suspensions (Sand-Jensen & Søndergaard, 1981; Losee & Wetzel, 1983; Sand-Jensen & Borum, 1983; Borum et al., 1984). Bulthuis & Woelkerling (1983), Silberstein et al. (1986) and Meulemans (1987) measured transmittance by intact periphyton layers. From the 'point of view' of the macrophyte covered by an intact periphyton layer, total attenuance, i.e. absorbance as well as back-scattering (cf. Kirk, 1983) should be considered. Until now it remains unclear whether the attenuance of a suspension is similar to that of an intact periphyton layer. Back-scattering probably is not similar due to the damaging of the

structure of the layer. To answer this question, we compared transmittance reductions (attenuances) of intact periphyton communities with readings from subsequently made suspensions. We also compared several methods to describe attenuance-biomass curves of periphyton. No attempt was made to measure Lambert-Beer extinction coefficients since a true depth of an intact, undisturbed periphyton community is hard to measure due to its variability. Meulemans (1987), however, developed a promising method to measure attenuation profiles in intact periphyton communities using a micromanipulatable glass fibre of 1 mm width.

2. Material and methods

Periphyton was cultured on microscopic glass slides that were held horizontally in racks of plastified iron wire suspended 3 cm below the water surface in 33 l. aquaria. The 9 treatments consisted of combinations of three temperatures (10, 15 and 20 °C) and three irradiance levels (50, 100 and 200 μE m^{-2} s^{-1}, measured at 1 cm depth). Temperature was maintained within 10% of the average value in three temperature rooms. Each temperature room held three irradiated aquaria, connected in series via three dark 50 l containers and a pump (Eheim 2013, flow rate 390 l h^{-1}) to prevent eventual phytoplankton blooms. Irradiances were maintained within 5 - 10% of the average, with 400 W Philips HPIT metal halide lamps suspended above the aquaria. Light was reduced to the desired intensity with neutral density filter-sheets). Each aquarium held 52 slides from which 3 were selected randomly for attenuance measurements and biomass determination about three times a week. The experiment lasted 49 days. Periphyton was inoculated from a carefully homogenized mixture of periphyton communities present in our laboratory.

Except for nitrogen, nutrients were added at 12, 21 and 36 days according to Dutch Standards (Nederlandse Praktijkrichtlijn NPR 6505, 1984; cf. Hootsmans, 1991). The aquaria were filled with tap water. Nitrogen added as nitrate was brought to 140 μmol l^{-1} three times a week following analysis (Technicon AA2 autoanalyser). Phosphorus was added with the other nutrients, but in supersaturating concentration (140 μmol l^{-1}) thus having only nitrogen as a limiting factor and thereby restricting the number of analyses to keep the experiment 'manageable'. Nitrogen concentrations agree with 'fairly' eutrophic field concentrations (Wilhm & Long, 1969; Eminson & Moss, 1980; Wetzel, 1983b). This 'semi-continuous' experimental set-up was our closest practicable alternative of a continuous periphyton culture. No significant differences (ANOVA, $p > 0.05$) in nitrogen fluctuations existed between temperature rooms.

Taxonomic composition of the periphyton communities was determined to the genus level according to Streble & Krauter (1985) and Belcher & Swale (1976). Abundance was scored using a five points' scale (i.e. very abundant, abundant, frequent, occasional and sparse). Samples were taken at day 34.

Biomass (as ash-free dry weight (afdw), which is dry weight (dw, 105 °C, 24 h) minus ash weight (aw, combustion at 520-540 °C)) was determined on preweighed and pre-combusted Whatman GF/C filters. The filters contained all material that was scraped off the upper side of a slide with a razor blade and filtered with a small quantity of demineralized water. Due to the horizontal position of the slides in the racks, no periphyton had accumulated on the under-

sides of the slides.

Chlorophyll was determined at day 34 and 53 (2 slides per treatment) after extraction of GF/C filtered material in 80% acetone with the method of Vernon (1960), modified according to Moed & Hallegraeff (1978) to control post-acidification pH in the sample.

Attenuance was measured as proportional transmittance reduction relative to transmittance through a clean slide with a HPIT lamp as light source and intercalibrated Licor 192s or Bottemanne submersible quantum flux sensors as irradiance sensors (measuring photosynthetically active radiation). Thus we assume that absorbance nor back-scattering by the periphyton layer are affected by light intensity, i.e. in the presently used ranges the chance of a photon to be transmitted through the layer remains the same.

Rectangular hyperbolae were fitted to attenuance (E, proportion) versus biomass (B, mg cm^{-2} afdw) datasets:

$$E = (a * B)/(c + B)$$

where a and c are the constants of the rectangular hyperbola.

For modelling biomass development, we applied the following logistic formula:

$$B_t = K / (1 + q * \exp^{(-r * t)})$$

where B_t is periphyton biomass at time t (mg cm^{-2} afdw), K is the asymptotic maximum, q is an integration constant determining biomass at time zero ($q=K/(B_t-1)$) and r is the instantaneous, 'unrestricted' growth rate (Rodriguez, 1987; Causton & Venus, 1981). Logistic growth curves and attenuance-biomass hyperbolae were fitted nonlinearly with the Marquardt algorithm (Conway et al., 1970).

Multiple comparisons among fitted curves were performed applying an experimentwise error rate of 0.05 (comparisonwise error rate of 0.0014 with all 36 comparisons of 9 treatments) and the F statistic described in Vermaat & Hootsmans (1991a). Statistical analyses were performed with the SPSS/PC$^+$ statistical package (Norusis, 1986).

For a comparison of our method to measure attenuance of intact communities with that of Sand-Jensen & Søndergaard (1981) who measured attenuance of suspensions (ES), we followed their methodological description including depth of the suspension (2 mm) and area of the petridish (19.6 vs. 19.3 cm^2 area of the slides) using a series of periphyton-covered slides previously measured with our routine method.

Furthermore, we compared three methods to describe the curvilinear attenuance-biomass relationship in terms of the statistically best fit: the method of Losee & Wetzel (1983), the one of Sand-Jensen & Borum (1983) and the one derived in this paper. Also, the basic assumption (Lambert-Beer's law is valid) of the method of Losee & Wetzel was tested for the present data set from intact periphyton communities.

Losee & Wetzel (1983) used suspensions of periphyton and applied a semi-Lambert-Beer formula. We applied their formulae to our complete set of attenuance/biomass data pairs (i.e. all temperature-light combinations were pooled, attenuances of intact periphyton) and calculated their attenuance, EL:

$$EL = \log_{10} \frac{\text{(transmittance control)}}{\text{(transmittance sample)}}$$

giving an almost linear relation between EL and periphyton biomass, apart from

scatter. To answer the question as to which method will give the best description of attenuance as a function of biomass, we compared both methods using the residual sums of squares (RSS) from our nonlinear hyperbolic fit and the linear EL-biomass regression in a two-sided F-test. Losee & Wetzel (1983) stated that "When attenuance (EL) measurements follow the Lambert-Beer relationship the extinction coefficient (i.e. EXT = EL/B) ... will be constant for all densities (B)". This was also tested for our complete dataset. Thus, we did not investigate whether this Lambert-Beer extinction coefficient based on biomass instead of depth is appropriate for suspensions of periphyton, but only whether it is appropriate for intact communities.

Sand-Jensen & Borum (1983), applying the method of Sand-Jensen & Søndergaard (1981) to produce suspensions, fitted a negative exponential curve through an attenuance/biomass dataset from Borum & Wium-Andersen (1980):

$$ESB = 1 - a * \exp^{(-c * B)}$$

This formula was also fitted through our complete dataset and compared with the hyperbolic fit. The same formula was used by Silberstein et al. (1986) to fit attenuance-biomass data.

Table 6.1. Parameter estimates of the nonlinear logistic fit for the 9 different treatments. Given are mean and standard error (se) for K (mg cm^{-2} afdw), q (dimensionless) and r (day^{-1}). Also are presented the number of data-pairs (n) and the time to reach 0.99*K (t, in days), calculated with the appropriate K, q and r.

irradiance (µE m-2 s-1)		50		100		200	
temperature (°C)		mean	se	mean	se	mean	se
10	K	4.6	3.2	3.8	0.3	3.0	0.2
	q	296	120	438	70	1172	505
	r	0.11	0.03	0.13	0.01	0.17	0.01
	n	27		30		32	
	t	93		80		70	
15	K	3.7	2.3	2.6	0.2	1.2	0.1
	q	147	50	320	192	773	1065
	r	0.10	0.02	0.16	0.02	0.26	0.06
	n	32		34		24	
	t	97		65		43	
20	K	2.8	2.8	2.5	1.1	3.6	4.2
	q	115	61	55	53	107	89
	r	0.17	0.06	0.19	0.08	0.17	0.10
	n	18		18		17	
	t	56		46		53	

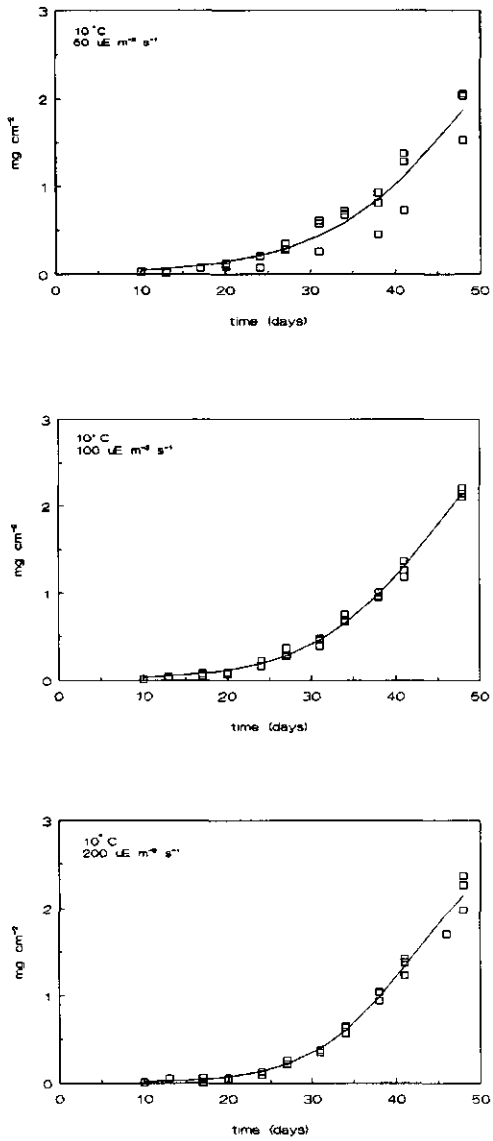


Fig. 6.1. Development of periphyton biomass and fitted logistic growth curves in 9 experimental treatments.

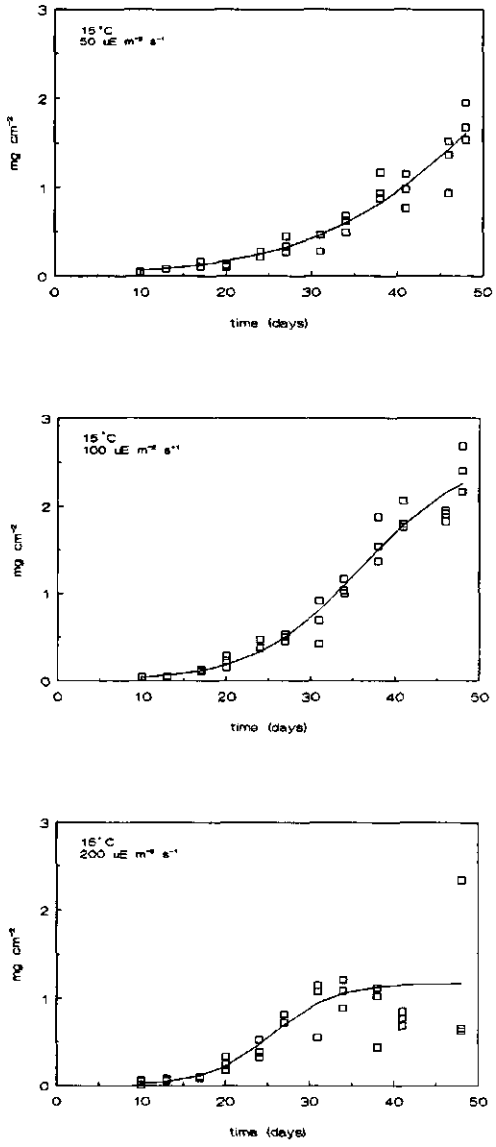


Fig. 6.1. Continued.

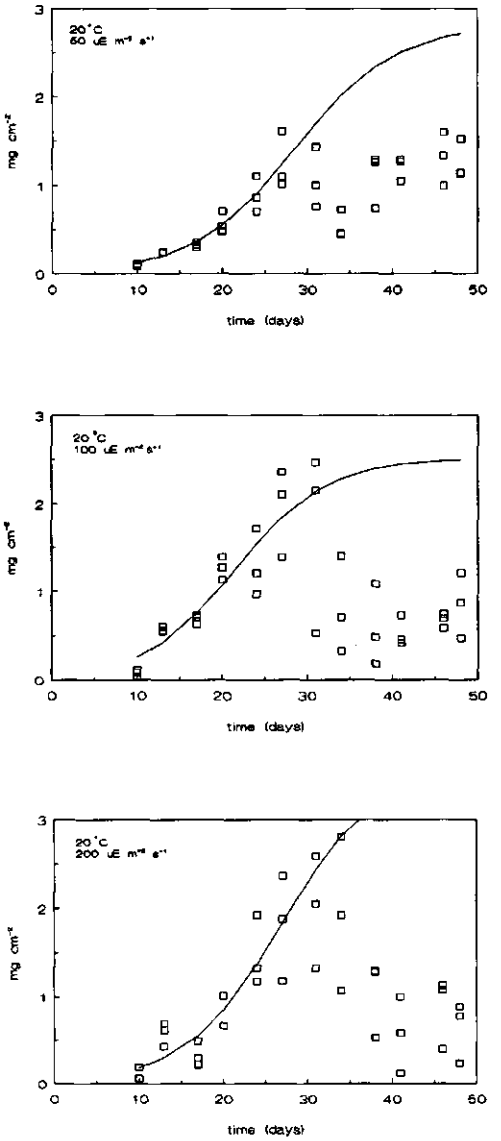


Fig. 6.1. Continued.

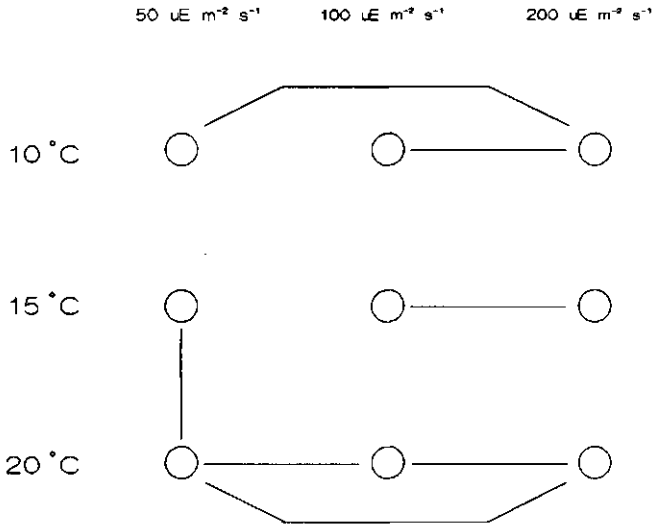


Fig. 6.2. Multiple comparisons between different logistic growth curves in 9 treatments (connected with a line: not significantly different).

3. Results

3.1 Periphyton growth

The growth curves for the nine treatments are presented in Fig. 6.1. In all 20 °C and in 15 °C/200 μE m^{-2} s^{-1} spontaneous dislodgements of parts of the periphyton layer were observed after 27 and 34 days respectively. For these treatments the logistic fitting was restricted to the period before dislodgement took place. All non-linear regressions are highly significant ($p < 0.001$, $r^2 = 0.92$ for 200 μE m^{-2} s^{-1}, 20 °C, all others 0.95 or more). Parameter estimates are given in Table 6.1. Most of the 36 multiple comparisons resulted in significant differences, so for the sake of clarity it was decided to present similarities, i.e. pairs of curves that were not significantly different (Fig. 6.2). Most similarities were observed between curves of equal temperature, suggesting that the effect of temperature was more divergent, i.e. resulted in more significantly different growth curves, than that of irradiance. The fitted curves suggest that the asymptotic biomass (K) could have been approached within the experimental period only for the three 20 °C treatments and for 15 °C, 200 μE m^{-2} s^{-1} (cf. Table 6.1). Because standard errors of individual parameter estimates generally are fairly high, significance tests were restricted to complete curves and conclusions on individual K and r values remain tentative. Table 6.1 suggests that most significant differences between fitted curves were caused by differences in r.

The dominant algal taxa in the periphyton at day 34 are given in Table 6.2. Distinct differences existed between treat-

Table 6.2. Dominant (= very abundant, abundant) algal taxa for the 9 different treatments at day 34.

irradiance (μE m^{-2} s^{-1})	50	100	200
temperature (°C)			
10	*Navicula* *Scenedesmus* *Lyngbya*	*Navicula* *Scenedesmus* *Lyngbya*	*Scenedesmus* *Chlamydomonas* *Lyngbya*
15	*Chlamydomonas* *Lyngbya*	*Chlamydomonas* *Lyngbya*	*Scenedesmus* *Chlamydomonas* *Lyngbya*
20	*Scenedesmus*	*Scenedesmus*	*Scenedesmus*

ments, and, though up to 12 taxa were recorded at this 'genus'-level, only a few gained dominance. While at 10 °C and the lower two irradiances the diatom *Navicula* was codominant, Cyanobacteria and green algae dominated at 10 °C, 200 μE m^{-2} s^{-1} and all 15 °C treatments. The green alga *Scenedesmus* was dominant at 20 °C. As for the growth curves, most dissimilarities occurred between temperatures: for 10° and 15 °C the 50 and 100 μE m^{-2} s^{-1} treatments had similar dominant taxa.

The fitted attenuance-biomass hyperbolae for the 9 treatments are given in Fig. 6.3. Apart from two extreme outliers (15 °C, 200 μE m^{-2} s^{-1}, day 48, not shown in Fig. 6.3), all data pairs were used. All fits were highly significant ($p<0.001$, $r^2=0.96$ at least). Contrary to the growth curves, most multiple comparisons produced no significant differences (30 out of 36), for clarity's sake the significant differences are therefore given in Fig. 6.4. Also contrary to the growth curves, most significant differences occurred among irradiances for a similar temperature. A fit using the pooled data from all treatments ($n=279$, $r^2=0.98$) resulted in the following constants: $a=1.23$ ($se=0.03$), $c=0.68$ ($se=0.04$).

Few distinct patterns can be discerned in the chlorophyll data. In three-way ANOVAs for chlorophyll content (chl(a+b), μg chlorophyll (a+b) mg^{-1} afdw), percentage of chlorophyll b (% chl-b, μg chl-b/chl(a+b) * 100%) and chlorophyll density (D_{chl}, μg chl(a+b) cm^{-2}), all factor effects (time, temperature and irradiance) and their interactions were significant ($p<0.05$, after $\log_{10}(x+1)$ transformation for chl(a+b) to cope with inhomogeneity of variances), except for the effect of irradiance on chl(a+b). The significant interactions are clearly illustrated in Table 6.3: for almost every temperature, the pattern of the different irradiances is different on the two harvesting days. Tukey multiple comparisons revealed no significant differences in chl(a+b) in week 5 and 4 of the 9

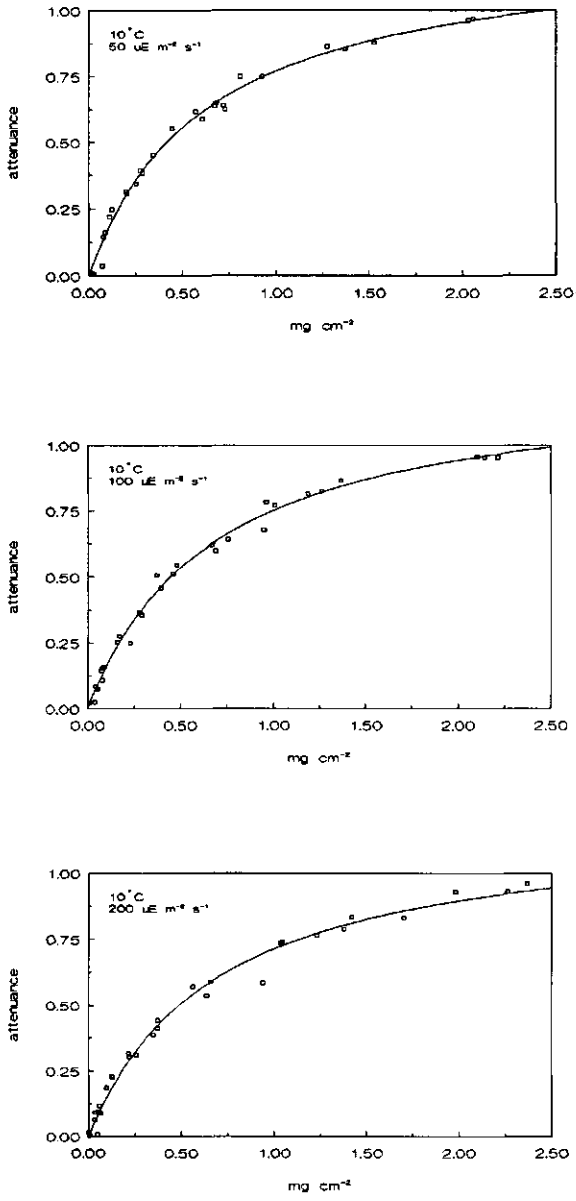


Fig. 6.3. Attenuance-biomass data pairs and fitted rectangular hyperbolae in 9 experimental treatments.

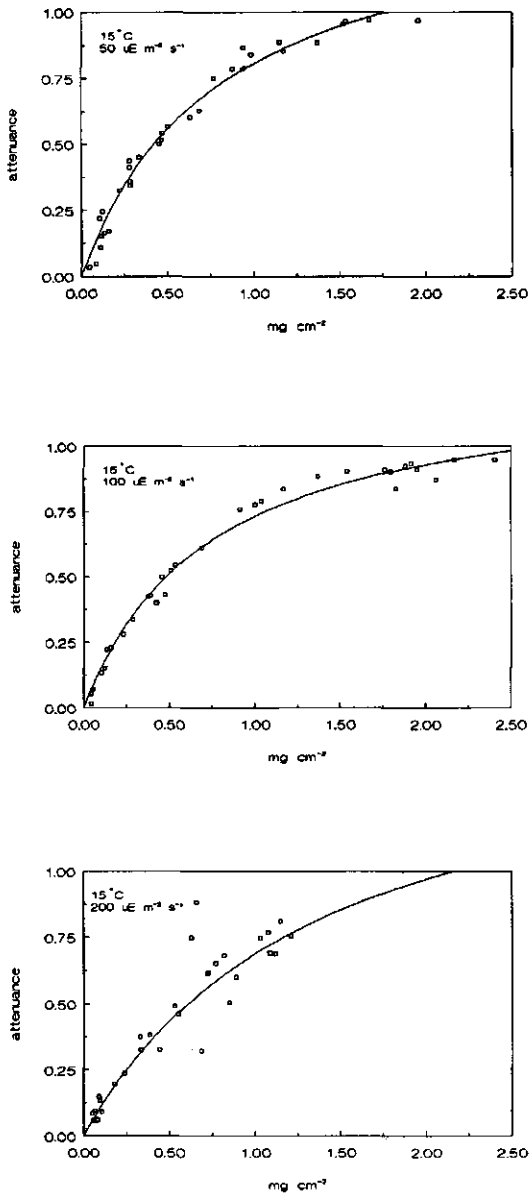


Fig. 6.3. Continued.

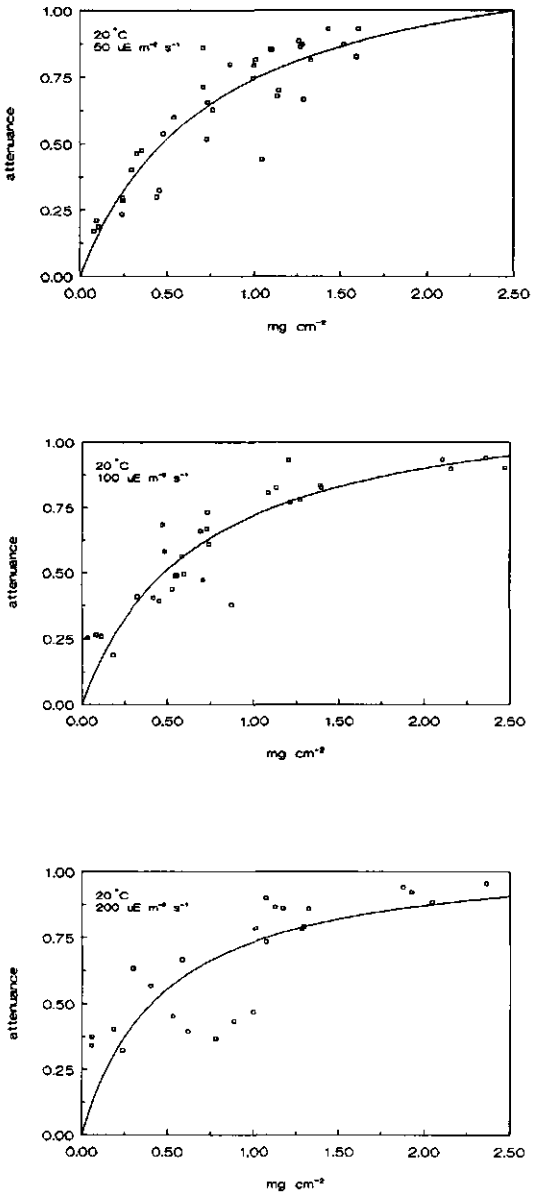


Fig. 6.3. Continued.

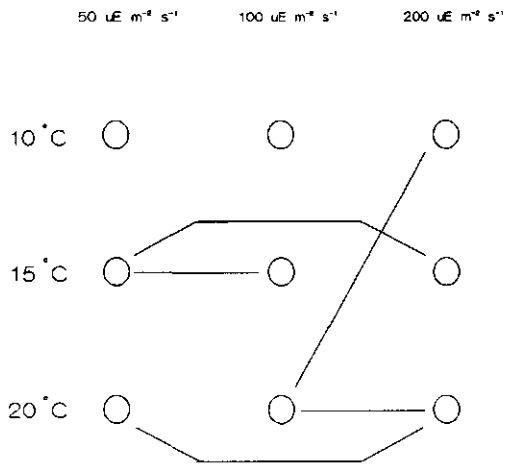


Fig. 6.4. Multiple comparisons of attenuance-biomass curves. Contrary to Fig. 6.2, but for the sake of clarity, the differences are indicated here: when two treatments are connected with a line, their fitted curves are significantly different.

Table 6.3. Periphyton chlorophyll content in weeks 5 and 8 (day 34 and 53) for a combination of three temperatures (°C) and irradiances (μE m^{-2} s^{-1}). Given are mean and standard error (se) for chlorophyll content (chl(a+b), μg chlorophyll (a+b) mg^{-1} afdw), percentage of chlorophyll b (% chl-b, μg chl-b/chl(a+b) * 100%) and chlorophyll density (D_{chl}, μg chl(a+b) dm^{-2}). Replication was 3.

temperature:			10°			15°			20°		
irradiance:			50	100	200	50	100	200	50	100	200
	week										
chl(a+b)	5	mean	7.9	5.8	3.7	4.7	6.9	11.2	1.4	10.7	3.2
		se	0.5	2.1	2.1	2.7	2.6	6.3	0.1	5.0	1.1
	8	mean	20.9	15.8	6.1	10.1	0.5	3.2	18.6	2.0	13.7
		se	1.2	1.0	0.7	0.6	0.1	0.2	1.9	0.1	1.2
% chl-b	5	mean	6.2	11.6	12.6	19.1	17.4	28.2	16.4	19.3	23.0
		se	1.2	1.5	2.6	1.7	0.5	1.2	0.3	1.9	2.0
	8	mean	10.3	19.6	50.7	22.7	76.6	32.8	22.8	26.0	22.6
		se	0.2	1.7	2.0	1.2	4.3	0.8	1.8	1.7	0.4
D_{chl}	5	mean	574	389	145	328	583	553	71	70	237
		se	37	140	74	223	184	207	15	27	51
	8	mean	3833	3156	1089	2002	91	218	2279	268	1003
		se	454	217	117	145	19	114	171	42	217

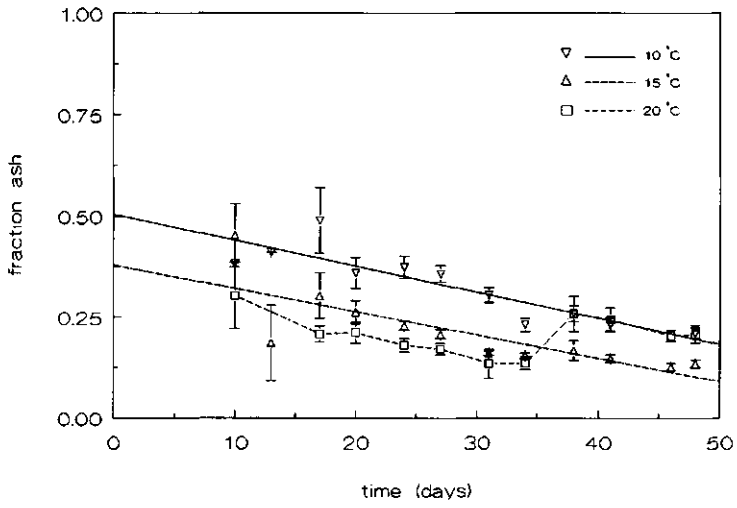


Fig. 6.5. Development of the ash content of the experimental periphyton communities. Data for the different irradiances are pooled per temperature. Given are means ± 1 standard error and the significant linear regression lines for 10° (hanging triangles) and 15 °C (standing triangles). The 20° treatment is indicated with open squares.

treatments in week 8 were not significantly different from this group either. For % chl-b and D_{chl} no interpretable patterns were apparent in the multiple comparisons. Thus, though threeway ANOVAs indicated significant effects of the factors, the significant interactions and relatively high variation in the samples prevented any conclusions on patterns in chlorophyll content.

Ash content of the periphyton cultures declined gradually in the course of growth (Fig. 6.5). A threeway ANOVA showed a significant effect of time and temperature, but not of light and interactions. The different irradiance treatments were therefore pooled per temperature in Fig. 6.5. The slopes of the linear regression lines (significant, $p<0.001$) fitted to the 10° and 15 °C data were not significantly different ($p>0.20$). The rate at which the ash fraction declined thus was not different. For the 20 °C data, however, no significant linear regression could be fitted. This is probably due to the switch to higher fractions at day 38, which, in turn, is probably correlated with the large-scale dislodgement that had taken place by that time (Fig. 6.1).

3.2 Comparison of attenuance measurements

Attenuances of suspensions measured according to Sand-Jensen & Søndergaard (1981, ES) and attenuances as measured according to our method (E) were compared for the same slides. A paired t-test revealed a significant underestimation by ES (on average 41% in this case, Table 6.4). The linear regression of ES against E was significant (Fig. 6.6). The slope (0.85) of this line was not significantly different from 1 ($p>0.40$), indicating a constant, systematic deviation.

A linear regression was fitted through EL (attenuance calculated according to Losee & Wetzel (1983)) and

Table 6.4. Comparison of attenuance of a periphyton suspension measured according to Sand-Jensen & Søndergaard (1981, ES) and attenuance of an intact periphyton layer measured according to the method used in this study (E). Given are mean and standard error (se) for both methods and the level of significance (p, paired t-test) of the observed difference, replication was 5.

method	mean	se	p
ES	0.41	0.08	0.001
E	0.69	0.09	

Table 6.5. Comparison of attenuance calculation according to Losee & Wetzel (1983, EL) with subsequent linear regression and the nonlinearly fitted rectangular hyperbola. Given are r^2 and the residual sums of squares (RSS) for both methods and an F statistic calculated as F = RSS{EL, linear}/RSS{E, nonlinear} (with 276, 276 degrees of freedom) and its level of significance.

method	r^2	RSS	F	p
EL, linear regr.	0.86	5.863	3.022	0.0000
E, nonlinear regr.	0.98	1.941		

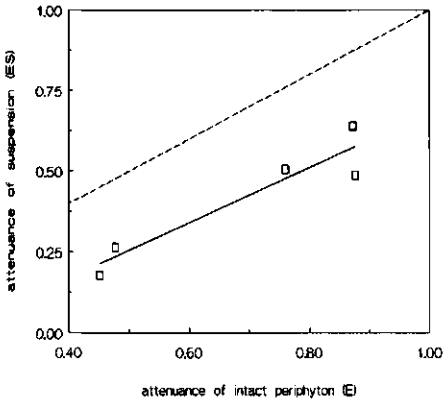


Fig. 6.6. Attenuance of suspensions plotted against attenuance of the intact community. The broken line indicates ES = E, i.e. if both were equal. The drawn line represents the significant linear regression: ES = 0.17 + 0.85*E, r^2=0.89, p=0.016.

periphyton biomass (mg cm^{-2} afdw) from our pooled dataset from all 9 treatments (Fig. 6.7). The RSS of this regression is compared with that of the nonlinearly fitted hyperbola of E against biomass for the same data in Table 6.5. The RSS of the nonlinear fit of E is significantly lower than that of the linear fit of EL, i.e. the former attenuance-biomass relation leaves less residual variation and thus results in a better fit.

The validity of the Lambert-Beer's extinction coefficient as used by Losee & Wetzel (1983, EXT = EL/B), i.e. its constancy for all densities, was tested with a linear regression of EXT against B for the same dataset (Fig. 6.7). Constancy should result in a non-significant slope. The slope, however, was significant (EXT = 0.796 - 0.140 * B, p{slope}=0.0005, r^2=0.05). Thus it can be concluded that, at least for the undisturbed periphyton communities in the present dataset, Lambert-Beer's relationship as used by Losee & Wetzel (1983) does not hold.

The negative exponential formula as used by Sand-Jensen & Borum (1983, attenaunce parameter ESB) is compared with a rectangular hyperbola for the complete dataset in Fig. 6.8. Parameter estimates of the negative exponential were: a=0.95 (se=0.01), c=-1.32 (se=0.03). Residual sums of squares are not significantly different (p=0.51) and r^2 is identical (0.98). Therefore, statistically speaking, both formulae fit the data equally well. A reason to choose the hyperbola might be that the negative

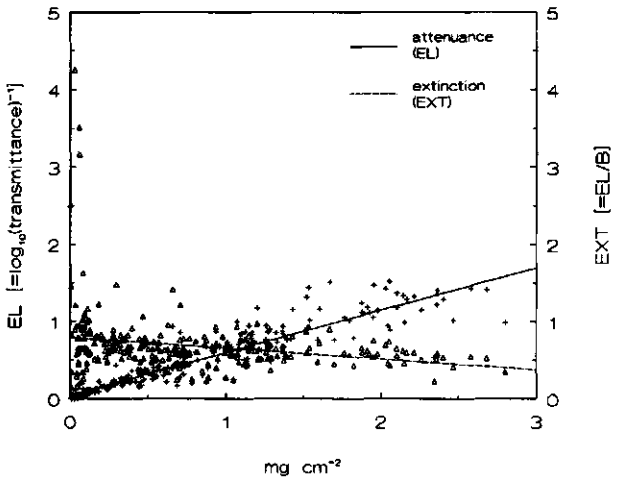


Fig. 6.7. Attenuance (EL, crosses) and extinction (EXT, triangles) of undisturbed periphyton as calculated according to Losee & Wetzel (1983).

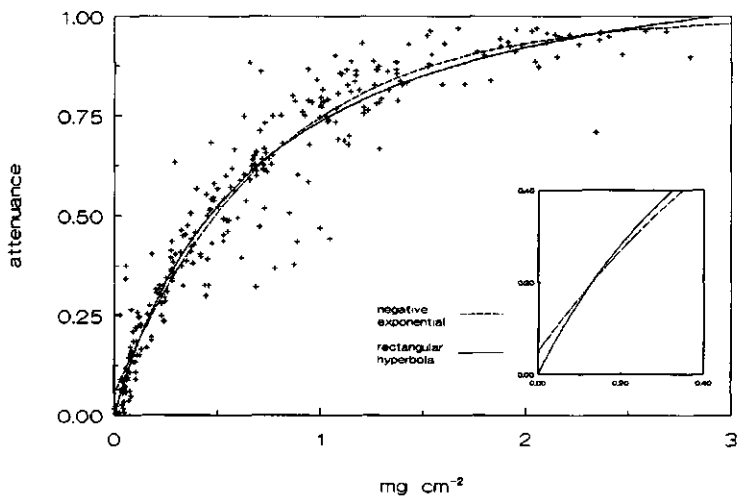


Fig. 6.8. Fits of the negative exponential and rectangular hyperbola to the complete dataset, with all treatments pooled. The inset shows the non-zero intercept of the negative exponential.

exponential gives a non-zero intercept, i.e. 1-a, contrary to the hyperbola used in this study, which has a zero intercept (Fig. 6.8 inset). Thus, at densities less than 0.08 mg cm^{-2} afdw, use of the negative exponential may result in significant overestimations of attenuance (a two-sided 5% confidence interval of the coefficient 'a' is $\pm$ 0.02).

4. Discussion

4.1 Periphyton growth

Temperature caused more significant differences in growth curves than irradiance. This also held for the differences in taxonomic composition. The presently used lowest irradiance (50 μE m^{-2} s^{-1}) must be considered a relatively low irradiance, since it is within the linear range of photosynthesis-irradiance curves of most primary producers (cf. Hootsmans & Vermaat, 1991). Lüning (1981) reported compensation points ranging from 5 - 20 μE m^{-2} s^{-1} for a range of marine macroalgae, and Vermaat & Sand-Jensen (1987) reported similarly low compensation points for low light and low temperature adapted *Ulva lactuca* L. (compensation points of 1 and 6 μE m^{-2} s^{-1} for algae grown at 10 °C and 2 and 56 μE m^{-2} s^{-1} respectively). Richardson et al. (1983), extracted a comparable range from the literature for microalgae: 1 - 21 μE m^{-2} s^{-1}. However, the growth curves at this irradiance do not differ distinctly from those of the higher irradiances. Apparently the light-harvesting part of the photosynthetic apparatus of the experimental periphyton communities acclimated efficiently to the irradiance range of this experiment.

In general, temperature affects enzymatic processes. Higher temperatures thus result in higher dark reaction rates (Richter, 1978) enabling higher growth rates, but also in higher rates of respiration. The net effect of these counteracting processes and the relative efficiency of nutrient uptake mechanisms will largely determine the succes of the different taxa in the periphyton community at the different temperatures. Apparently, high temperatures favoured the green alga *Scenedesmus*, low temperatures and low irradiances favoured the diatom *Navicula*. This is in general agreement with the findings of e.g. Eppley (1972) and Tilman et al. (1986). Also, it is not in contradiction with seasonal succession patterns (diatoms dominant in spring, green algae and/or Cyanobacteria increasingly dominant through summer) observed in periphyton communities (e.g. Cattaneo & Kalff, 1978; Meulemans & Roos, 1985) and in phytoplankton (Sommer et al., 1986) in freshwater lakes. Other factors (e.g. nutrient and substrate availability, turbulence, sedimentation from seston, grazing) evidently may also be important in determining seasonal succession patterns in periphyton communities in the field, but temperature and light seem to play a similar key role as for phytoplankton.

Periphyton biomass maxima as reported in the literature are given in Table 6.6. Despite pronounced differences in taxonomic composition, nutrient conditions and experimental set-up, most maximum densities from laboratory studies are within one order of magnitude and the maxima from the present study are within this range (cf. Table 6.1). Only the high irradiance treatment of McIntire & Phinney (1965) had a distinctly higher maximum. This may have been caused by continuous colonization from the natural stream water, a substantial secondary epiphytism sensu Rodriguez (1987) on the filamentous green algae (*Oedogonium*, *Ulothrix*) and a high biomass build-up by the filamentous greens themselves. Most maxima from field studies remain within the same range (Table 6.6, cont.). Only Fairchild & Everett (1988) reported a lower maximum for their unenriched treatment in an oligotrophic lake and Hunter (1980) did so for a 'shallow pond' of unreported trophic status. High field densities mostly coincided with high

Table 6.6. Reported maximum biomass (B_m) and time (t) to reach that maximum biomass for periphyton communities from (a) laboratory and (b) field studies. For the laboratory studies, experimental irradiance (I, $\mu E\ m^{-2}\ s^{-1}$), temperature (T, °C), taxonomic composition and a note on the experimental set-up are given. For the field studies taxonomic composition, substratum type and the trophic status of the studied water body are given. All densities are in mg cm^{-2} afdw, except those marked with dw, which are in mg dw cm^{-2}. Time is in days, sometimes approximately read from the authors' figures.

(a) laboratory studies

I	T	B_m	t	tax. comp	comments (reference)
40	13-16	6.7	85	diatoms	lab. streams with natural stream water (1)
100	13-16	14.0	71	diatoms, filamentous green algae	idem (1)
40	23	1.2dw	30	green algae Cyanobacteria	no clear maximum, exp. lasted 109 d., batches in battery jars, 190 μmol l^{-1} N, 20 μmol l^{-1} P, their level "N2" only (2)
		4.5dw	109		
50	30-34	3.8	36	*Scenedesmus*, *Lyngbya*	lab. streams with recycled natural water, initially enriched to 1900 μmol l^{-1} N, 210 μmol l^{-1} P, ungrazed only (3)
full sun	7-12	3-4.5	50	diatoms, Cyanobacteria	open air, artificial streams (4)
28	10-25	1-1.5	72	filamentous diatoms	lab. streams, 4 μmol l^{-1} N, 1 μmol l^{-1} P, the 113+N treatment received extra N to 14 μmol l^{-1} (5)
113	10-25	4	72		
113+N	10-25	5	72		

references: (1) McIntire & Phinney, 1965; (2) Wilhm & Long, 1969; (3) Kehde & Wilhm, 1972; (4) Eichenberger & Wuhrmann, 1975; (5) Sumner & McIntire, 1982.

Table 6.6. continued.

(b) field studies

status	B_n	t	tax. comp.	comments (reference)
eutrophic	4dw	34	?	slides, July-Aug. incubated in ditch (6)
eutrophic	1.8dw	120	diatoms	glass rods (7)
	1.8dw	?	*Cladophora*	submerged *Typha*-stems (7)
?	0.3dw	45	filamentous & non-filamentous diatoms	ungrazed slides in cages in shallow lake (8)
eutrophic	2	56	diatoms, filamentous green algae	perspex plates, April-June (9)
eutrophic	2-6	?	diatoms, green algae	reed stems from two locations and different seasons (10)
eutrophic	2dw	?	?	*Potamogeton perfoliatus* leaves, lake, Aug. (11)
	6dw	?	?	old reed stems, Sept. (11)
oligo-trophic	0.3-0.5	32-38	*Scenedesmus*, cryptophytes	controls (12)
	2-4	32-38		various enrichments (12)
eutrophic	4-5	45-60	?	clay tiles in stream (13)
eutrophic	0.4	?	diatoms	*Potamogeton pectinatus* in stream spring maximum (14)

references: (6) Herder-Brouwer, 1975; (7) Mason & Bryant, 1975; (8) Hunter, 1980; (9) Gons, 1982; (10) Meulemans & Heinis, 1983; (11) Riber et al., 1983; (12) Fairchild & Everett, 1988; (13) Feminella et al., 1989; (14) Sand-Jensen et al., 1989.

colonization rates.

No attempt was made to convert dry weights to ash-free dry weights in Table 6.6 since ash-free fractions of dry weight reportedly vary from 0.2 (McIntire & Phinney, 1965; Gons, 1982; Osenberg, 1989; Sand-Jensen et al., 1989) to 0.6 (Kehde & Wilhm, 1972, who reported a range of 0.4 - 0.6 from different laboratory stream studies). In the course of development of the presently observed communities, ash-free fractions increased from 0.55 to 0.87 (ash fractions decreased, Fig. 6.5). The relatively high organic fractions of the present laboratory cultures must have been caused by the relatively low accumulation of inorganic material as compared to field conditions, where the macrophyte-periphyton complex may act as a sieve for seston with high ash contents (Vermaat et al., 1991) and a substrate for $CaCO_3$ deposition due to high photosynthetic activity (Cattaneo & Kalff, 1978; Gons, 1982). In the latter phenomenon may lay a mechanistic explanation for the observation of Eminson & Moss (1980) that substrate specificity of periphyton communities disappeared with increasing eutrophication. Under eutrophic conditions $CaCO_3$ deposition may be similar on all substrata since the contribution of phytoplankton to total photosynthetic activity is proportionally large. Under oligotrophic conditions this may be much less so, i.e. to the extent that differences between substrate types become evident in periphyton composition. $CaCO_3$ deposition may be an important structuring 'external factor' (Eminson & Moss, 1980) to periphyton communities. Van Vierssen & Bij De Vaate (1990) found that periphyton on *Potamogeton perfoliatus* L. contained more inorganic material than periphyton on *Potamogeton pectinatus* L. in Lake Veluwe, but had a similar algal taxonomic composition.This may be explained by a difference in $CaCO_3$ deposition due to differences in photosynthetic activity.

Rodriguez (1987) fitted the logistic model to field data from (among others) Herder-Brouwer (1975) and Gons (1982). He estimated growth rates r of 0.28 and 0.41 (day^{-1}) respectively. Since the artificial substrates in these two studies were submerged close to the water surface and in summer, a comparison with 15° or 20 °C and 200 $\mu E\ m^{-2}\ s^{-1}$ from the present experiment is probably allowed (r respectively 0.27 and 0.17 day^{-1}). These rate estimates appear relatively close. However, since Rodriguez (1987) gave no standard errors no conclusion on significance of differences can be made. We fitted a logistic curve to data from the ungrazed treatment of Kehde & Wilhm (1972, cf. Table 6.6). This gave a growth rate r of 0.10 $\pm$ 0.06 (average $\pm$ standard error, $r^2=0.95$) which is not different from the rate observed here for 20 °C, 50 $\mu E\ m^{-2}\ s^{-1}$. Thus, as far as comparisons can be made, the presently found growth rates and maximum densities seem comparable to those found elsewhere.

Dislodgement, as observed in 4 of the 9 treatments of this study, has also been reported in a number of studies in the field and laboratory. McIntire & Phinney (1965) observed correlations between dislodgement peaks and high silt load in their natural stream water and concluded that especially filamentous greens were sensitive to the scouring effect of the silt. In our aquaria no scouring silt was present and other authors (Eichenberger & Wuhrmann, 1975; Herder-Brouwer, 1975; Gons, 1982) also observed 'spontaneous' dislodgement. Meulemans & Roos (1985) presented a hypothesis for the process after detailed observations. They concluded that the condition of the basal layer deteriorated

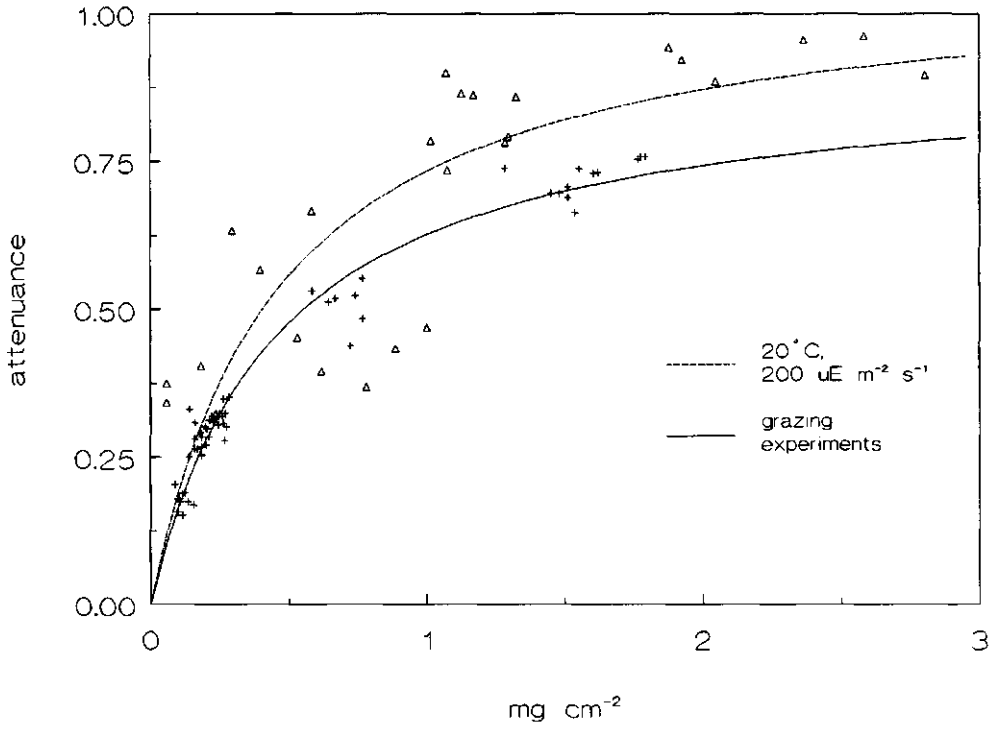


Fig. 6.9. Attenuance-biomass curves from the 20 °C,200 μE m^{-2} s^{-1} treatment and the ungrazed controls from Vermaat (1991).

after development of intermediate and top layers in the periphyton due to shading, which was concluded from the high frequency of chlorotic cells in the basal layer, and due to a heavy infection by fungi and bacteria. This deteriorating condition of the basal layer consequently facilitated dislodgement from the substratum. As in the present experiment significant dislodgement mainly occurred in the 20 °C treatments where high densities were reached early, both chlorosis of basal layer cells through shading by top layers (cf. attenuance/biomass curves in Fig. 6.3) and enhanced development of heterotrophic bacteria and fungi may have occurred.

It can be concluded that most periphyton communities reach similar maximum densities provided that the development of filamentous greens is restricted. Temperature and probably also nutrient conditions (cf. Table 6.6, field studies; Wilhm & Long, 1969) influence the rate of increase stronger than irradiance in the investigated range of irradiances. In the field, colonization and sedimentation from seston (Gons, 1982; Vermaat et al., 1991), dislodgement and grazing (Mason & Bryant, 1975; Cattaneo, 1983;

Meulemans & Roos, 1985; Vermaat, 1991) may greatly influence this pattern.

Most significant differences in attenuance/biomass curves occurred between treatments with different irradiance and equal temperature. This agrees well with the conclusion that the periphyton communities apparently were able to acclimate efficiently to the different irradiance regimes: under low irradiance the curves ascended faster, i.e. attenuance per unit biomass increased more, than under high irradiance.

The attenuance/biomass curve of ungrazed periphyton on slides in Vermaat (1991, used as controls in grazing experiments) was significantly different from the curve for 20 °C, 200 μE m^{-2} s^{-1} (F test, $p<0.0001$). The datasets from Vermaat (1991) and this periphyton growth experiment were thus best described by two different curves. Fig. 6.9 gives the two fitted curves. The difference may be attributed to a difference in nutrients (70 μmol l^{-1} N in the grazing experiments and 140 μmol l^{-1} N in the growth experiment) or in taxonomic composition (most periphyton used in the grazing experiments had codominant Cyanobacteria). The latter explanation seems to be the most probable explanation, possibly due to a different succession caused by differences in nitrogen availability.

The almost complete absence of interpretable patterns in the chlorophyll data may be caused by interaction between the experimental factors light and temperature, age of and succession in communities of different treatments, different taxonomical composition and probably by a relatively high variation of chlorophyll content per unit area. It illustrates that periphyton densities expressed in units of chlorophyll per unit area or substrate biomass (Cattaneo & Kalff, 1978, 1979; Sand-Jensen & Søndergaard, 1981; Antoine & Benson-Evans, 1983; Cattaneo, 1983; Sand-Jensen & Borum, 1984; Meulemans & Roos, 1985) should be interpreted with caution since (a) single algal species are reportedly able to vary their chlorophyll content with changing irradiance (Richardson et al., 1983; Vermaat & Sand-Jensen, 1987) and (b) succession in multi-species periphyton communities may distinctly alter chlorophyll contents per unit biomass.

Chlorophyll (a+b) contents varied from 1 - 21 μg mg^{-1} afdw in the present study (Table 6.3), which agrees well with reported chlorophyll a concentrations from the literature (0.8 - 15 μg mg^{-1} afdw; McIntire & Phinney, 1965; Kehde & Wilhm, 1972; Sumner & McIntire, 1982; Gons & Van Keulen, 1983; Fairchild & Everett, 1988). The % chl-b found in this study (10 - 30% with the exception of two extreme outliers) agrees well with values reported for phytoplankton dominated by green algae (Jeffrey, 1981).

4.2 Comparison of attenuance measurements

The measurement of light attenuance (i.e. transmittance reduction) in suspensions of periphyton communities systematically underestimated attenuance as measured in intact periphyton communities. The systematic character may indicate that backscattering by a suspension is systematically less than that of an intact periphyton layer, but it cannot be decided whether true absorbance is also different. It can be concluded that if true transmittance reduction (i.e. attenuance as defined in this chapter) by periphyton is to be measured, the use of suspensions will lead to a serious underestimation of attenuance: 41% for the community tested here. Though the correlation between atte-

nuation by the undisturbed layer and that of the suspension was high, this relation is not necessarily similar for different communities. Thus, it remains questionable whether the use of suspensions is valid for relative comparisons between communities from different sites.

Application of the version of Lambert-Beer's law according to Losee & Wetzel (1983) to undisturbed periphyton communities resulted in inconstancy of the extinction coefficient. Therefore, the use of this law for the presently investigated communities is not permitted. Losee & Wetzel (1983) state that applicability of Lambert-Beer's law is restricted to density ranges where absorbing particles form a 'uniform layer' and these ranges appeared to be different for suspensions of different species with a maximum of 0.9 mg cm^{-2} dw for *Anabaena flos-aquae*. Apart from our criticism on the use of suspensions, two comments must be made here: (1) 0.9 mg cm^{-2} dw ($\pm$ 0.4 mg cm^{-2} afdw) as a tentative maximum restricts the use of this method to periphyton communities in early phases of growth (cf. Fig. 6.1 & 6.7, Table 6.6). (2) Originally, Lambert-Beer's law was defined for attenuance by dilute solutions or suspensions. Chang (1977) stated that "At higher concentrations ($\geq$ 0.5 M), deviations occur and A (absorbance sensu Lambert-Beer) is no longer a linear function of c (concentration)", i.e., the extinction coefficient is no longer constant. Most likely, intact periphyton communities do not behave like homogeneous, dilute suspensions at all. This, together with the variation in depth of a well-developed multi-layered periphyton community in our opinion invalidates the use of Lambert-Beer's law.

Application of the negative exponential formula from Sand-Jensen & Borum (1983) resulted in an equally good fit to the data as the Michaelis-Menten hyperbola when compared with statistical criteria (minimization of residual sums of squares and maximization of r^2). Since the former results in a non-zero intercept, a Michaelis-Menten or alike hyperbola seems a better alternative for a formal description of attenuance-biomass relations of periphyton.

Table 6.7. Error in the calculation of attenuance (%) of a single layer of periphyton as half the attenuance of the periphyton on the upper and lower side of a leaf together (E½), as a function of true attenuance (ET) of one layer. Irradiance at the top of the first periphyton layer was 100% and attenuance by the leaf was set to zero. Transmittance = 100% - attenuance.

ET	20	40	60
transmittance after layer 1	80	60	40
transmittance after layer 2	64	36	16
E½	18	32	42
deviation (ET - E½)	2	8	18

Whilst the above cited authors all found a curvilinear attenuance-biomass relationship, Bulthuis & Woelkerling (1983) found a linear relation for a biomass range of 0.4 - 2.1 mg cm^{-2} dw ($\pm$ 0.3 - 1.5 mg cm^{-2} afdw, 13 data pairs). Incorporation of more data probably would have revealed the curvilinear nature of the relation. Also, their erroneous calculation of attenuance of a single layer of periphyton as half the attenuance of the periphyton on the upper and lower side of a leaf together, may have played a significant role (cf. Table 6.7). The assumption that both periphyton layers on the upper and lower side of a leaf transmit equal proportions of light is feebly based,

since at least irradiance reaching the lower layer must be significantly less. However if it is held valid, the transmittance of a single layer can be estimated as the square root of the proportionate transmittance of both layers together.

Finally, we conclude that, although the importance of irradiance reduction by periphyton as a major agent in the process of declining macrophyte vegetations in the course of eutrophication has been stressed regularly (Sand-Jensen, 1977; Phillips et al., 1978; Wetzel, 1983c; Borum, 1985), a generally accepted method to directly measure this irradiance reduction to our knowledge has not been agreed upon till now. We feel to have demonstrated that a combination of direct measurements through intact periphyton layers and a formal description of the attenuance-biomass relation with a rectangular hyperbola, at present is the best alternative for periphyton with biomass values up to 3 mg cm^{-2} afdw.

Acknowledgements

J. Smits and H. de Vries did most of the practical work and analyses, we gratefully acknowledge their motivated and active cooperation to fulfil the laborious sampling scheme. Identifications of periphytic algal taxa were checked by R. Suykerbuyk and Dr. R.M.M. Roijackers (Agricultural University, Dept. of Nature Conservation). Dr. M.A.J. van Montfort (Agricultural University, Dept. of Mathematics) gave statistical advice on multiple comparisons of fitted curves. Prof. Dr. W. van Vierssen and Prof. Dr. W.J. Wolff critically read the manuscript.

5. References

Allanson, B.R., 1973. The fine structure of the periphyton of *Chara* sp. and *Potamogeton natans* from Wytham Pond, Oxford, and its significance to the macrophyte-periphyton metabolic model of R.G. Wetzel and H.L. Allen. Freshwat. Biol. 3: 535-542.

Allen, H.L., 1971. Primary productivity, chemo-organotrophy, and nutritional interactions of epiphytic algae and bacteria on macrophytes in the littoral of a lake. Ecol. Monogr. 41: 97-127.

Antoine, S.E. & Benson-Evans, K., 1983a. The effect of light intensity and quality on the growth of benthic algae 1. Phytopigment variations. Arch. Hydrobiol. 98: 299-306.

Belcher, H. & Swale, E., 1976. A beginner's guide to freshwater algae. Culture Centre of Algae and Protozoa, Cambridge, UK, 47 pp.

Berry, J.A. & Raison, J.K., 1981. Responses of macrophytes to temperature. In Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (eds), Physiological Plant Ecology I. Responses to the physical environment. Encyclopedia of Plant Physiology, new series, vol 12a, Springer, Berlin, pp. 277-338.

Blindow, I., 1987. The composition and density of epiphyton on several species of submerged macrophytes - the neutral substrate hypothesis tested. Aquat. Bot. 29: 157-168.

Borum, J., 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. Mar. Biol. 87: 211-218.

Borum, J. & Wium-Andersen, S., 1980. Biomass and production of epiphytes on Eelgrass (*Zostera marina* L.) in the Øresund, Denmark. Ophelia, Suppl. 1: 57-64.

Bulthuis, D.A. & Woelkerling, W.J., 1983. Biomass accumulation and shading effects of epiphytes on the leaves of *Heterozostera tasmanica*, in Victoria, Australia. Aquat. Bot. 16: 137-148.

Calow, P., 1970. Studies on the natural diet of *Lymnaea pereger obtusa* (Kobelt) and its possible ecological implications. Proc. malac. Soc. Lond. 39: 203-215.

Calow, P., 1973. Field observations and laboratory experiments on the general food requirements of two species of freshwater snail, *Planorbis contortus* (Linn.) and *Ancylus fluviatilis* Müll. Proc. malac. Soc. Lond. 40: 483-489.

Cattaneo, A., 1983. Grazing on epiphytes. Limnol. Oceanogr. 28: 124-132.

Cattaneo, A. & Kalff, J., 1978. Seasonal changes in the epiphytic community of natural and artificial macrophytes in Lake Memphremagog (Que & Vt). Hydrobiologia 60: 135-144.

Cattaneo, A. & Kalff, J., 1979. Primary production of algae growing on natural and artificial aquatic plants: a study of interactions between epiphytes and their substrate. Limnol. Oceanogr. 24: 1031-1037.

Causton, D.R. & Venus, J.C., 1981. The biometry of plant growth. Arnold, London, 307 pp.

Chang, R., 1977. Physical chemistry with application to biological systems. Macmillan, New York, 538 pp.

Conway, G.R., Glass, N.R. & Wilcox, J.C., 1970. Fitting nonlinear models to biological data by Marquardt's algorithm. Ecology 51: 503-507.

Cummins, K.W., 1973. Trophic relations of aquatic insects. Ann. Rev. Ent. 18: 183-206.

Eichenberger, E. & Wuhrmann, K., 1975. Growth and photosynthesis during the formation of a benthic algal community. Verh. int. Verein. Limnol. 19: 2035-2042.

Eminson, D. & Phillips, G., 1978. A laboratory experiment to examine the effects of nutrient enrichment on macrophyte and epiphyte growth. Verh. int. Verein. Limnol. 20: 82-87.

Eminson, D. & Moss, B., 1980. The composition and ecology of periphyton communities in freshwaters 1. The influence of host type and external environment on community composition. Br. Phycol. J. 15: 429-446.

Eppley, R.W., 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. 70: 1063-1085.

Fairchild, G.W. & Everett, A.C., 1988. Effects of nutrient (N P C) enrichment upon periphyton standing crop, species composition and primary production in an oligotrophic softwater lake. Freshwat. Biol. 19: 57-70.

Feminella, J.W., Power, M.E. & Resh, V.H., 1989. Periphyton responses to invertebrate grazing and riparian canopy in three northern Californian coastal streams. Freshwat. Biol. 22: 445-457.

Gons, H.J., 1982. Structural and functional characteristics of epiphyton and epipelon in relation to their distribution in Lake Vechten. Hydrobiologia 95: 79-114.

Gons, H.J. & Van Keulen, R., 1983. Seasonal dynamics in organic matter and dark oxygen uptake of epiphyton and epipelon in relation to seston deposition in Lake Vechten. In Wetzel, R.G. (ed.), Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 175-183.

Gough, S. B. & Gough, L.P., 1981. Comment on "Primary production of algae growing on natural and artificial plants: a study of interactions between epiphytes and their substrate (Cattaneo and Kalff). Limnol. Oceanogr. 26: 987-988.

Haynes, A. & Taylor, B.J.R., 1984. Food finding and food preference in *Potamopyrgus jenkinsi* (E.A. Smith) (Gastropoda Prosobranchia). Arch. Hydrobiol. 100: 479-491.

Herder-Brouwer, S.J., 1975. The development of periphyton on artificial substrates. Hydrobiol. Bull. 9: 81-86.

Hootsmans, M.J.M., 1991. Allelopathic limitation of algal growth by macrophytes. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Hootsmans, M.J.M. & Vermaat, J.E., 1991. Light-response curves of *Potamogeton pectinatus* L. as a function of plant age and irradiance level during growth. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Hunter, D.R., 1980. Effects of grazing on the quantity and quality of freshwater aufwuchs. Hydrobiologia 69: 251-259.

Hutchinson, G.E., 1975. A Treatise on Limnology III, Limnological Botany. Wiley, New York, 660 pp.

Jeffrey, S.W., 1981. Responses to light in aquatic plants. In Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (eds), Physiological Plant Ecology I, responses to the physical environment. Encyclopedia of Plant Physiology, new series, volume 12a, Springer Berlin, pp. 249 - 276.

Jenkerson, C.G. & Hickman, M., 1986. Interrelationships among the epipelon, epiphyton and phytoplankton in a eutrophic lake. Int. Revue ges. Hydrobiol. 71: 557-579.

Kairesalo, T., 1984. The seasonal succesion of epiphytic communities within an *Equisetum fluviatile* L. stand in Lake Pääjärvi, Southern Finland. Int. Revue ges. Hydrobiol. 69: 475-505.

Kehde, P.M. & Wilhm, J.L., 1972. The effects of grazing by snails on community structure of periphyton in laboratory streams. Am. Midl. Nat. 87: 8-24.

Kirk, J.T.O., 1983. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge, UK, 401 pp.

Losee, R.F. & Wetzel, R.G., 1983. Selective light attenuation by the periphyton complex. In Wetzel, R.G. (ed.), Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 89-96.

Lüning, K., 1981. Light. In Lobban, C.S. and Wynne M.J. (eds), The biology of seaweeds, Botanical Monographs vol. 17, Blackwell, Oxford, pp. 326-355.

Mason, C.F. & Bryant, R.J., 1975. Periphyton production and grazing by chironomids in Alderfen Broad, Norfolk. Freshwat. Biol. 5: 271-277.

McIntire, C.D. & Phinney, H.K., 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecol. Monogr. 35: 237-258.

McMahon, R.F., Hunter, R.D. & Russel-Hunter, W.D., 1974. Variation in aufwuchs at six freshwater habitats in terms of carbon biomass and of carbon:nitrogen ration. Hydrobiologia 45: 391-404.

Meulemans, J.T., 1987. A method or measuring selective light attenuation within a periphytic community. Arch. Hydrobiol. 109: 139-145.

Meulemans, J.T. & Heinis, F., 1983. Biomass and production of periphyton attached to dead red stems in Lake Maarsseveen. In Wetzel, R.G. (ed.), Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 169-173.

Meulemans, J.T. & Roos, P.J., 1985. Structure and architecture of the periphytic community on dead reed stems in Lake Maarsseveen. Arch. Hydrobiol. 102: 487-502.

Moed, J.R. & Hallegraef, G.M., 1978. Some problems in the estimation of chlorophyll-a and phaeopigments from pre- and post-acidification spectrophotometric measurements. Int. Revue ges. Hydrobiol. 63: 787-800.

Moeller, R.E., Burkholder, J.M. & Wetzel, R.G., 1988. Significance of sedimentary phosphorus to rooted submerged macrophytes (*Najas flexilis* (Willd.) Rostk. and Schmidt) and its algal epiphytes. Aquat. Bot. 32: 261-281.

Nederlandse Praktijkrichtlijn, 1984. NPR 6505, benodigdheden, werkwijze en medium voor het kweken van algen, (Dutch Standards, materials and methods for the cultivation of alga, in Dutch). Nederlands Normalisatie Instituut, Delft, The Netherlands, 4 pp.

Norusis, M.J., 1986. SPSS-PC$^+$ manual. SPSS Inc., Chicago, USA, 559 pp.

O'Neill-Morin, J. & Kimball, K.D., 1983. Relationship of macrophyte-mediated changes in the water column to periphyton composition and abundance. Freshwat. Biol. 13: 403-414.

Osenberg, C.W.,1989. Resource limitation, competition and the influence of life history in a freshwater snail community. Oecologia 79: 512-519.

Phillips, G.L., Eminson, D.F. & Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated waters. Aquat. Bot. 4: 103-125.

Reavell, P.E., 1980. A study of the diets of some British freshwater gastropods. J. Conch. 30: 253-271.

Reynolds, C.S., 1988. The concept of ecological succession applied to seasonal periodicity of freshwater phytoplankton. Verh. int. Verein. Limnol. 23: 683-691.

Riber, H.H., Sørensen, J.P. & Kowalczewski, A., 1983. Exchange of phosphorus between water, macrophytes and epiphytic periphyton in the littoral of Mikolajskie Lake, Poland. In Wetzel, R.G. (ed.), Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 235-243.

Richardson, K., Beardall, J. & Raven, J.A., 1983. Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phytol. 93: 157-191.

Richter, G., 1978. Plant metabolism, physiology and biochemistry of primary metabolism. Thieme, Stuttgart, 475 pp.

Robinson, G.G.C., 1983. Methodology: the key to understanding periphyton. In Wetzel, R.G. (ed.), Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 245-251.

Rodriguez, M.A., 1987. Estimating periphyton growth parameters using simple models. Limnol. Oceanogr. 32: 458-464.

Rogers, K.H. & Breen, C.M., 1983. An investigation of macrophyte, epiphyte and grazer interactions. In Wetzel, R.G. (ed.), Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 217-226.

Sand-Jensen, K., 1977. Effect of epiphytes on eelgrass photosynthesis. Aquat. Bot. 3: 55-63.

Sand-Jensen, K. & Borum, J., 1983. Regulation of growth of eelgrass (*Zostera marina* L.) in Danish coastal waters. Mar. Techn. Soc. J. 17: 15-21.

Sand-Jensen, K. & Borum, J., 1984. Epiphyte shading and its effect on photosynthesis and diel metabolism of *Lobelia dortmanna* L. during the spring bloom in a Danish lake. Aquat. Bot. 20: 109-119.

Sand-Jensen, K. & Søndergaard, M., 1981. Phytoplankton and epiphytic development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Revue ges. Hydrobiol. 66: 529-552.

Sand-Jensen, K., Borg, D. & Jeppesen, E., 1989. Biomass and oxygen dynamics of the epiphytic community in a Danish lowland stream. Freshwat. Biol. 22: 431-443.

Silberstein, K., Chiffings, A.W. & McComb, A.J., 1986. The loss of seagrass in Cockburn Sound, Western Australia III. The effect of epiphytes on the productivity of *Posidonia australis* Hook. F. Aquat. Bot. 24: 355-371.

Sommer, U., Gliwicz, Z.M., Lampert, W. & Duncan, A., 1986. The PEG-model of seasonal succesion of planktonic events in freshwaters. Arch. Hydrobiol. 106: 433-471.

Soszka, G.J., 1975. Ecological relations between invertebrates and submerged macrophytes in the lake littoral. Ekol. Polska 23: 393-415.

Streble, H. & Krauter, D., 1985. Das Leben im Wassertropfen: Mikroflora und Mikrofauna des Süsswassers; ein Bestimmungsbuch. 7. Auflage, Kosmos - Franckh'sche Verlagshandlung, W. Keller & Co, Stuttgart, 369 pp.

Sumner, W.T. & McIntire, C.D., 1982. Grazer-periphyton interactions in laboratory streams. Arch. Hydrobiol. 93: 135-157.

Tilman, D., Kiesling, R., Sterner, R., Kilham, S.S. & Johnson, F.A., 1986. Green, bluegreen and diatom algae: taxonomix differences in competitive ability for phosphorus, silicon and nitrogen. Arch. Hydrobiol. 106: 473-485.

Van Montfrans, J., Orth, R.J. & Vay, S.A., 1982. Preliminary studies of grazing by *Bittium varium* on eelgrass periphyton. Aquat. Bot. 14: 75-89.

Van Vierssen, W., 1983. The influence of human activities on the functioning of macrophyte-dominated aquatic ecosystems in the coastal area of Western Europe. Int. Symp. Aquat. Macrophytes, Nijmegen, The Netherlands, 1983, pp. 273-281.

Van Vierssen, W. & Bij De Vaate, A., 1990. Licht en waterplanten, oorzaken van biomassafluctuaties van onderwatervegetaties in het Veluwemeer (Light and submerged macrophytes, causes for macrophyte biomass fluctuations in Lake Veluwe, in Dutch). Report of the Institute for Inland Water Management and Waste Water Treatment, Wageningen Agricultural University, Dept. of Nature Conservation, and the International Institute for Hydraulic and Environmental Engineering, 233 pp.

Vermaat, J.E., 1991. Periphyton removal by freshwater micrograzers. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vermaat, J.E. & K. Sand-Jensen, 1987. Survival, metabolism and growth of *Ulva lactuca* under winter conditions: a laboratory study of bottlenecks in the life cycle. Mar. Biol. 95: 55-61.

Vermaat, J.E., Hootsmans, M.J.M. & Van Dijk, G.M., 1991. Ecosystem development in different types of littoral enclosures. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vernon, L.P., 1960. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal. Chem. 32: 1144-1150.

Wetzel, R.G., 1983a. Opening remarks. In Wetzel, R.G. (ed.), Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 3-4.

Wetzel, R.G., 1983b. Limnology, 2nd edition. Saunders College Publishing, Philadelphia, 860 pp.

Wetzel, R.G., 1983c. Attached algal-substrata interactions: fact or myth, and when and how. In Wetzel, R.G. (ed.), Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 207-215.

Wilhm, J.L. & Long, J., 1969. Succession in algal mat communities at three different nutrient levels. Ecology 50: 645-652.

PERIPHYTON REMOVAL BY FRESHWATER MICROGRAZERS

7

J.E. Vermaat

Abstract

In various experiments, the freshwater snail species *Lymnaea peregra* (Müll.), *Physa fontinalis* (L.), *Valvata piscinalis* (Müll.) and *Bithynia tentaculata* (L.) significantly removed periphyton from glass slides, while the two tested crustacean species, *Asellus aquaticus* (L.) and *Gammarus pulex* (L.), did not. *B. tentaculata* removed similar amounts of periphyton accumulated in the field as it did of laboratory-cultured periphyton.

Removal rates ranged from 0.1 - 2.2 mg animal^{-1} day^{-1} ash-free dry weight (afdw) for different species, different temperatures and differing amounts of initially available periphyton. Differences in removal rate per individual snail between and within species could largely be accounted for by differences in snail biomass and activity. A significant effect of temperature on periphyton removal and activity was observed in only one out of four experiments. Apparently, an efficient temperature compensation mechanism is present in the tested species. The four species differed in grazing trail width, linear velocity, and also in the density and irradiance transmittance of the periphyton that is left in the grazing trail. Thus, differences in periphyton removal capacities of field populations of the different species are to be expected.

With similar periphyton density and taxonomic composition, velocity nor activity of *L. peregra* was influenced by the type of substratum (glass slides versus plant surface of *Potamogeton pectinatus* L.). Taxonomic composition of the periphyton did influence velocity and activity. Higher periphyton density resulted in increased periphyton removal in *L. peregra* (up to a plateau at 0.2 mg cm^{-2} afdw) and *P. fontinalis* but not in *B. tentaculata* and *V. piscinalis*.

In a growth experiment lasting 9 weeks, the density of periphyton on *P. pectinatus* was reduced in the presence of *L. peregra*. Taxonomic composition of periphyton on grazed plants differed from that on control plants: dominance by tightly adhering unicellular green algae in the presence of snails versus filamentous Cyanobacteria in their absence. Ungrazed control plants with a higher periphyton cover produced more leaf material than plants with snails, at the expense of the tubers. In a second growth experiment with *P. pectinatus* in the presence of the grazers *B. tentaculata*, *V. piscinalis* and juvenile *L. peregra*, all snail species significantly reduced periphyton density. Contrary to the previous experiment, however, no differences in plant growth were apparent. This was due to a lower periphyton and phytoplankton density in the controls as compared to the previous experiment. Irradiance reaching the plants in the first growth experiment was close to the compensation point for *P. pectinatus* of this age and acclimized to this irradiance, while in the second experiment net photosynthetic rates were estimated to be about twice as high. This resulted in a fivefold difference in newly formed biomass after 9 weeks.

The three tested species clearly showed different activity patterns. *L. peregra* remained active throughout the experiment and was present on the plants and aquarium walls in considerable numbers. *V. piscinalis* was present on the macrophytes for about one month, during which oviposition took place. Subsequent post-breeding mortality rapidly removed all adults of this semelparous species. *B. tentaculata* showed a somewhat intermediate pattern. In the first part of the experiment, densities on plants were similar to those of *L. peregra*, but after oviposition a large proportion of the animals moved to the sediment and burrowed themselves, whilst large-scale mortality did not take place.

Two methods were developed to estimate irradiance transmittance improvement due to periphyton removal. These methods were evaluated for different species and conditions. On glass slides, transmittance of the remaining periphyton after a period of exposure to grazing animals can be estimated without significant error from its biomass and a hyperbola relating irradiance attenuance (1-transmittance) to biomass (method A), provided that at least 60% of the biomass is removed by the grazers. Else, more detailed measurements of trail width, linear velocity and attenuance in grazing trails of individual snails are to be preferred (method B). When the two methods were evaluated on the basis of periphyton growth and removal rate on *P. pectinatus* in the first growth experiment, method B appeared to estimate the removed amounts more accurately.

Contents

1. Introduction

Periphyton communities reportedly respond strongly to eutrophication (Phillips et al., 1978; Borum, 1985; Cambridge et al., 1986). Dense periphyton covers that develop under eutrophic conditions may significantly reduce photosynthesis and growth of the host plant through shading or competition for inorganic carbon (Sand-Jensen, 1977; Sand-Jensen & Borum, 1984; Silberstein et al., 1986). Vermaat & Hootsmans (1991c) demonstrated high growth rates of periphyton communities under eutrophic laboratory conditions, with the periphyton cover absorbing 60% of incoming irradiance within about 14 days at 20 °C. Periphyton grazing by gastropod or arthropod microherbivores, then, may alleviate adverse effects for the macrophyte caused by a dense periphyton cover (Orth & Van Montfrans, 1984; Brönmark, 1985).

Hootsmans & Vermaat (1985) indeed demonstrated a positive effect of periphyton removal by different species of microherbivores in field densities on the growth of *Zostera marina* L., a marine macrophyte. Similar results were reported by Howard & Short (1986) from an experiment with subtropical *Halodule wrightii* Aschers. Therefore, consideration of the potential effect of microherbivores on periphyton seems worthwile when hypotheses are formulated to explain the decline of macrophytes in the course of eutrophication (cf. Phillips et al., 1978 and chapter 1). The present study reports on several aspects of the removal of periphyton communities present in eutrophic freshwaters by different species of freshwater microherbivores.

The term periphyton 'removal' is purposely used here since the process of periphyton grazing by snails and probably also arthropods involves both ingestion and dislodgement of parts of the structurally complex mass of algae, bacteria and organic and inorganic debris that forms a periphyton mat (cf. photographs in Van Montfrans et al., 1982). The term periphyton is used as defined in Wetzel (1983, cf. citation in Vermaat & Hootsmans, 1991c).

The primary aim of the present study was to quantify the removal of periphyton on macrophytes under eutrophic conditions by freshwater microherbivores. This is treated in section 2 of this chapter, which pertains to experiments that were all done using microscopic glass slides, i.e. artificial substrata, as used by Vermaat & Hootsmans (1991c). The use of glass slides enabled replication and direct measurement of irradiance reduction by an intact periphyton layer, as outlined by Silberstein et al. (1986) and Vermaat & Hootsmans (1991c). The removal of periphyton then may result in increased irradiance transmittance, which will be considered in section 3 including some methodological aspects. Throughout this chapter the term 'attenuance' is used (sensu Kirk, 1983), designating the fraction of light that is not transmitted by the periphyton layer, i.e. incorporating the absorbed as well as the backscattered fraction.

The use of rectangular glass slides instead of the fine linear leaves of the macrophyte model species of this study, *Potamogeton pectinatus* L., though, raises the question whether this might affect the behaviour of the microherbivore. In section 4 this subject is dealt with as well as the effect of taxonomically different periphyton communities inhabiting *P. pectinatus* on the grazing behaviour of *Lymnaea peregra* (Müll.). Section 5 reports on two experiments on the effect of

periphyton removal by snails on growth and biomass allocation of *P. pectinatus*. Also, the two methods to estimate attenuance reduction are tested that were developed in section 3.

2. Periphyton removal from glass slides

2.1 Introduction

Different grazer species have different dietary preferences (Calow, 1973a; Calow & Calow, 1975; Bovbjerg, 1968; Lodge, 1986), different feeding apparatus morphologies (Calow, 1970) and probably different temperature-feeding relations (Hylleberg, 1975; Calow, 1975). This may well be reflected in different removal capacities on specific periphyton communities and justifies the screening of several species of microherbivores to obtain a broader picture.

Evidence from marine environments (Van Montfrans et al., 1982; Hootsmans & Vermaat, 1985; Howard & Short, 1986) pointed to both snails and crustaceans as potential periphyton grazers. Furthermore, Marcus et. al. (1978) showed that *Asellus aquaticus* (L.) grew well on pelletized periphyton from *Elodea canadensis* Michaux, and Moore (1975) demonstrated that both *A. aquaticus* and *Gammarus pulex* (L.) fed on the filamentous green alga *Cladophora glomerata* (L.) Kz. in English streams. The following freshwater snails were selected as microherbivorous grazer species: *Lymnaea peregra* (Müll.), *Physa fontinalis* (L.), *Valvata piscinalis* (Müll.) and *Bithynia tentaculata* (L.). *A. aquaticus* and *G. pulex* were selected as crustaceans.

No planorbid snails were tested here because they reportedly feed on detritus and associated bacteria (Calow, 1975; Calow & Calow, 1975; Lodge, 1986), whereas periphyton on macrophytes in the euphotic zone of lakes in general is not a detritus-bacteria association but dominated by algae and precipitated or sedimented inorganic matter (Gons, 1982; Meulemans & Heinis, 1983; Van Vierssen & Bij De Vaate, 1990). Though chironomids and oligochaetes reportedly feed on periphyton (Mason & Bryant, 1975; Kairesalo, 1984; Kairesalo & Koskimies, 1987), they have not been included in the present study. For practical reasons, it was decided to concentrate on snails.

Since gastropods are poikilotherm animals, their energy requirements will increase with rising temperatures. For *Ancylus fluviatilis* Müll. and *Planorbis contortus* L., Calow (1975) reported increasing food absorption rates while absorption efficiencies (absorption/ingestion) remained constant. Ingestion thus increased. The slope of the absorption-temperature curve being less for *A. fluviatilis* than for *P. contortus*, he concluded that the former species is more effective in compensating temperature effects. Thus, though poikilothermic, freshwater gastropods are able to compensate for a temperature effect reducing the differences in enzymatic rates or activity in general. Furthermore, different species apparently differ in compensating capacity.

However, periphyton removal by the moving snail and food ingestion or absorption through the gut wall probably will not be related directly. We thus measured periphyton removal as a function of

temperature. Since it is the periphyton removal that is of importance to the macrophyte, ingestion and absorption efficiencies were not estimated here.

The five different experiments that are dealt with in this section are shortly described in Table 7.1. In all but one experiment laboratory-cultured periphyton was used. Natural periphyton that had accumulated on glass slides incubated in Lake Veluwe was tested in experiment 5.

Table 7.1. Short overview of periphyton removal experiments with glass slides.

no	description
1	Periphyton removal by various species of freshwater snails. April 1988.
2	Similar to experiment 1, with partly different snail species. May 1988.
3	Periphyton removal by two crustacean species. May 1988.
4	Temperature effect on periphyton removal by various species of snails. January to March of 1987 and 1988.
5	Removal by *B. tentaculata* of periphyton that accumulated on glass slides in eutrophic Lake Veluwe. July and August 1987.

2.2 Materials and methods

2.2.1 Periphyton culture

For experiments 1, 2 and 4 (cf. Table 7.1), periphyton was cultured on microscopic glass slides (19.3 cm^2 area) in 600 l tanks containing algal culture medium according to Dutch Standards (Nederlandse Praktijkrichtlijn NPR 6505, 1984; cf. Hootsmans, 1991) but with nitrogen and phosporus concentrations reduced to 70 and 12 μmol l^{-1} respectively, with an average irradiance of 180 $\pm$ 5 $\mu E\ m^{-2}\ s^{-1}$ PAR (mean $\pm$ standard deviation, sd), provided by 4 Philips 400 W HPIT lamps per culture aquarium and an average water temperature of 23 °C (sd: 2.5 °C). Four Eheim aquarium pumps provided water circulation to prevent the build-up of any nutrient or temperature gradients. The slides were positioned horizontally in plastic trays (8 slides each) on a plastified metal gauze frame and were used after 5 to 8 days. Periphyton for experiment 3, the crustacean experiment, was cultured slightly differently, as outlined below in section 2.2.3.

Three weeks before the start of experiment 5, racks holding vertically positioned slides were suspended at 10 cm waterdepth in Lake Veluwe, to allow for sufficient accumulation of periphyton under summer conditions in the field.

Taxonomic composition of the periphyton communities was determined according to Streble & Krauter (1985) and Belcher & Swale (1976) to the genus level. Abundance was scored using a five points' scale.

For biomass determinations, the upper sides of the slides were scraped clean with a razor blade into a small quantity of demineralized water. This water was filtered over a precombusted, preweighed Whatman GF/C filter. Biomass was determined as ash-free dry weight (afdw), which is dry weight (dw, 105 °C, 24 hours) minus ash weight (aw, combustion at 520-540 °C, 3 hours).

Since the true thickness (or depth) of a periphyton community is not easily determined, it was not endeavoured to estimate extinction coefficients sensu Lambert Beer (cf. Vermaat & Hootsmans,

1991c). Instead, attenuance was measured, being the total irradiance reduction by an intact periphyton layer relative to clean control slides, as defined in Vermaat & Hootsmans (1991c).

2.2.2 Animals

All animals used in the laboratory experiments were collected in autumn, winter and early spring of 1987 and 1988 in a few ditches in the vicinity of Wageningen. Prior to the experiments they were kept in aquaria with copperfree tapwater in controlled temperature rooms at the ditch water temperature at collection. For all temperature experiments the animals were acclimized to the desired temperature and light/dark cycle for at least seven days. The animals were also accustomed to periphyton as food for at least a week by adding periphyton-covered glass slides to the stock aquaria. All species clearly foraged on the slides during this acclimation period.

2.2.3 Experimental set-up

All experiments with snails (experiments 1, 2, 4 and 5) were done in perspex grazing chambers: containers designed to have the inner walls completely covered with periphyton-bearing glass slides (Fig. 7.1). The construction allowed for an easy insertion and removal of the slides from the chamber walls. In experiments 1, 2 and 4, the grazing chambers were placed in controlled temperature rooms. The chambers were placed in trays with the waterlevel just above the rim of the vertical slides. The trays were filled with copperfree tap water that was allowed to equilibrate to temperature room conditions for at least 24 hours prior to the experiment.

Table 7.2. Characteristics of snail species and dominant periphyton taxa used in experiments 1 and 2. *L. peregra*-L were adults collected in the field, *L. peregra*-S were small, juvenile animals from a laboratory-raised stock. Size is the maximal shell length, density is number of snails per chamber.

exp.	species	size (mm)	density	dominant periphyton taxa
1	*B. tentaculata*	7-10	10	*Scenedesmus*
	L. peregra-L	10-15	4	*Gloeocystis*
	P. fontinalis	4-7	8	*Coelastrum*
	V. piscinalis	3-5	20	
2	*B. tentaculata*	7-10	10	*Scenedesmus*
	L. peregra-L	10-15	4	*Gloeocystis*
	L. peregra-S	5-8	10	
	P. fontinalis	6-8	8	
	V. piscinalis	3-5	20	

All laboratory experiments were done with a light/dark cycle of 12/12 hours. To facilitate behavioural observations, light/dark cycles in the temperature rooms were 12h out of phase with the normal outdoor cycle. Illumination was provided for by 4 fluorescent white tubes per temperature room producing an irradiance at the water surface in the trays of $\pm$ 50 μE m^{-2} s^{-1}.

Experiments 1 and 2 differed only in the moment that the snails were collected in the field, respectively the first week of April and the last week of May of 1988 (a difference of 6 weeks) and in an additional grazer type in May: juvenile *L. peregra* from a laboratory population. Characteristics of the snail species used and dominant periphyton taxa are listed in Table 7.2. Water temperature was held at 15 °C, the experiments lasted 2 days.

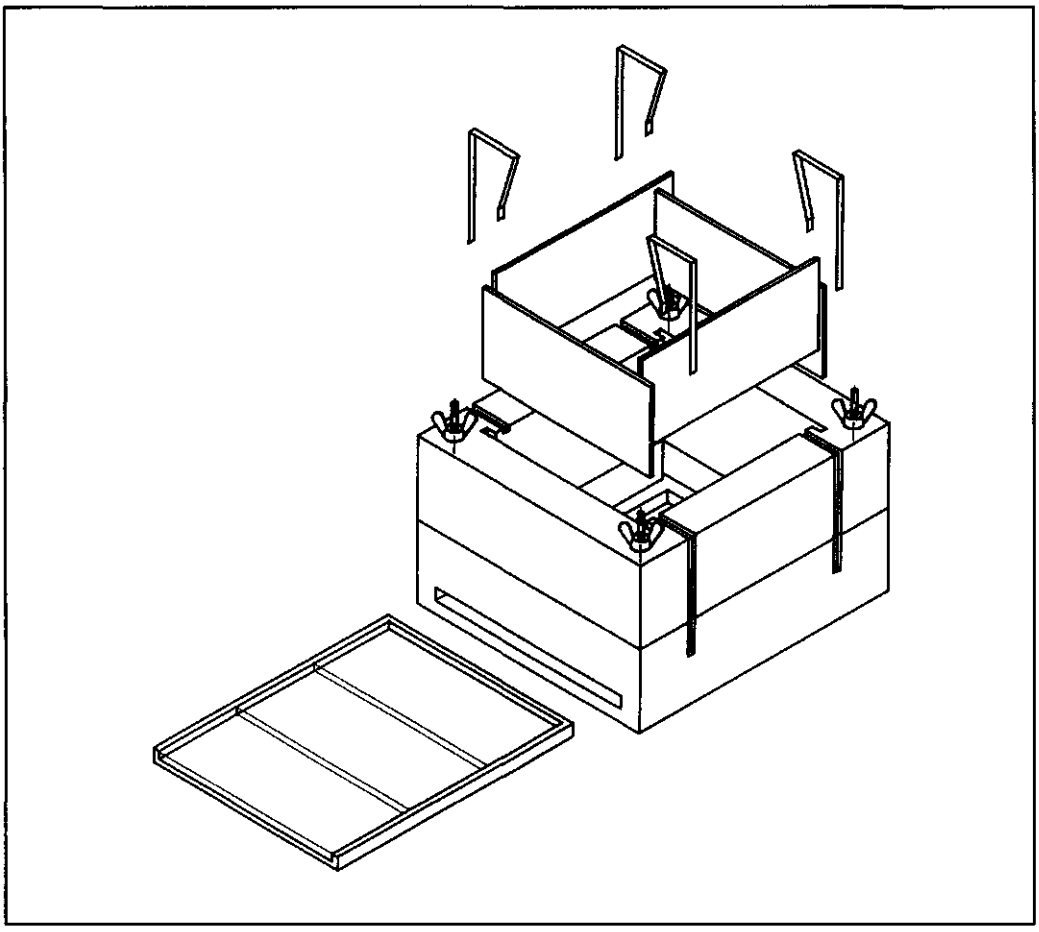


Fig. 7.1. Grazing chamber.

Periphyton removal, snail activities during day and night and snail biomasses were measured. Especially for the smaller individuals, accurate distinction between actually grazing or moving over the periphyton was hardly possible. Therefore all animals moving actively over the periphyton were considered as being actively grazing. For comparisons between treatments, activities were averaged over whole experimental periods or day and night separately, and expressed as a fraction of the total number of snails present in a grazing chamber.

In preliminary experiments both *G. pulex* and *A. aquaticus* showed cannibalism in the grazing chambers. Therefore, small plastic petri dishes (5 cm diameter) were used in experiment 3 as grazing chambers for individual animals (7-13 mm length). Prior to the experiment, the petri dishes were placed in the periphyton culture aquarium to be colonized by periphyton along with the slides for experiment 2. Periphyton communities on the glass slides and the petri dishes

Table 7.3. Characterization of the temperature experiment (no 4). Given are the different snail species, the size class used (shell length), the numbers of snails per grazing chamber, duration of the experiment and dominant periphyton taxa.

species	size (mm)	density	length (days)	dominant periphyton taxa
L. peregra-1 (Dec. 1986)	4-10	10	1	*Scenedesmus* *Chlamydomonas* *Lyngbya*
L. peregra-2 (Feb. 1987)	10-11	3	4	*Scenedesmus* *Gloeocystis* *Anabaena* *Oscillatoria*
B. tentaculata (March 1988)	7-10	10	7	*Gloeocystis* *Kirchneriella* *Anabaena*
V. piscinalis (March 1988)	3-5	22	2	*Gloeocystis* *Chlamydomonas* *Anabaena*

proved similar. Every petri dish containing one animal was allotted a control petri dish. The amount of periphyton initially available to an animal in a petri dish was comparable to that available to the snails in experiment 1 and 2. The experiment lasted 2 days and the temperature was held at 15 °C in a temperature room. Water depth was held at 7 mm. After removal of the animals, periphyton was carefully removed from the walls and floor of the petri dish and processed similarly as periphyton from glass slides.

For experiment 4, three temperatures were selected: 10, 15 and 20 °C, a compromise between replication and cover of a realistic temperature range. The species tested and some other experimental characteristics are summarized in Table 7.3. The different species could not be tested simultaneously due to the limited availability of temperature rooms and grazing chambers. *L. peregra* was tested twice: once in December 1986 and once in February 1987, with different initial periphyton densities. The periphyton for the '*L. peregra*-1' test was cultured in copperfree tapwater, i.e. without added nutrients. Duration of every test was adjusted to allow for sufficient periphyton to be removed.

For experiment 5, stocks of adult *B. tentaculata* (8-10 mm), sampled from our 'source' ditches in the vicinity of Wageningen in June 1987, were transferred to containers with partially gauze walls placed in the experimental *P. pectinatus* bed in Lake Veluwe (Van Dijk & Van Vierssen, in press) for acclimation. Experiment 5A was done in July 1987, with three densities of snails: 6, 12 and 24 per chamber. Lake water temperature was 22 $\pm$ 2 °C (mean $\pm$ sd). Experiment 5B was done in August, with densities 4 and 8, water temperature was 15 $\pm$ 1 °C. The trays containing the grazing chambers were placed under a shading gauze to simulate light conditions in the submerged vegetation. Lake water in the trays was continuously renewed from a 100 l buffer vessel at a rate of about 0.5 l h^{-1} and the waterlevel in the trays was held constant with an overflow pipe. *Scenedesmus* was the dominant algal genus in the periphyton. The experiments lasted 22 h.

2.2.4 Calculations

Periphyton removal was always measured relative to control chambers without animals. To establish whether periphyton was significantly removed in a specific experiment, the appropriate contrast tests comparing treatment with control were

done following analysis of variance (ANOVA) and tests for homogeneity of variance and normality (Steel & Torrie, 1980).

Consequently, every average difference between treatment and control and its standard deviation was divided by the number or average total biomass of animals in a chamber and the length of an experiment to arrive at the periphyton removal rate per individual animal per day (PRI) or per unit biomass per day (PRB), respectively (units are mg animal^{-1} day^{-1} and g g^{-1} day^{-1}, where weights are in afdw). These could be entered in ANOVAs comparing different experiments. Thus PRI allows for comparisons between species on an individual basis, while PRB enables comparisons on a biomass or unit tissue basis.

Whilst dividing the standard deviation of the difference between treatment and control by the average total biomass of snails per container to estimate PRB, the between container variation in this snail biomass was neglected. This was considered correct since the coefficient of variation (sd/mean * 100%) of snail biomass per container was always at least one order of magnitude less than that of the difference in periphyton density between control and treatment.

For multiple comparisons of means with equal replication, Tukey's Honestly Significant Difference test was used. With unequal replication least significant difference (lsd) tests were performed with the experimental error rate (EER) held at 0.05 (unless stated otherwise), and comparisonwise error rates (CER) reduced accordingly to maintain a similar overall conservativity as in Tukey's test (Steel & Torrie, 1980).

Inhomogeneity of variances and/or nonnormality in tested data sets were coped with by log- or square root transformation ($\log_{10}(x+1)$ and $\sqrt{(x+½)}$) if appropriate or applying t'-tests with EER held at 0.05 (Steel and Torrie, 1980). Statistical analyses were performed with the SPSS/PC$^+$ (Norusis, 1986) statistical package.

2.3 Results

2.3.1 Periphyton removal by different species of snails and crustaceans (experiments 1, 2 and 3)

Periphyton densities in grazed and control chambers are given in Table 7.4. Except *B. tentaculata*, all species significantly reduced the periphyton density at an EER of 0.10, and only the small *L. peregra* did not so at an EER of 0.05.

Periphyton removal is expressed as a rate per individual per day (PRI) and per unit biomass per day (PRB, Figs 7.2 and 7.3 respectively), calculated as described in section 2.2.4. Clearly, most of the differences between species in PRI are removed when we consider PRBs. Only *P. fontinalis* had a significantly higher PRB in the first experiment than in the second experiment. Most of the variation in PRI therefore might possibly be explained by differences in snail individual biomass (Fig. 7.4). This, however, was not the case. *B. tentaculata* had an individual biomass equal to that of 'large', adult *L. peregra* in both experiments, but removed only ± 10% of the amount removed by *L. peregra*. The difference can be largely accounted for, however, when we consider the activity of the different species: *B. tentaculata*'s fraction of actively grazing animals was only 20% of that of *L. peregra* (Fig. 7.5). For the prosobranchs *B. tentaculata* and *V. piscinalis* no significant differences in

Table 7.4. Periphyton densities (mg chamber^{-1} afdw) in control and grazed chambers, given are mean, standard error (se), the reduction as a percentage of the control density (%) and the probability of the observed difference between grazed and control (p). Replication was 4 in experiment 1 and 3 in experiment 2, control densities in mg cm^{-2} afdw were 0.17 and 0.08 and ash amounted to 40 and 75% of dry weight. Significant probabilities at an EER of 0.05 are indicated with an asterisc.

exp.	treatment	density mean	se	%	p
1	control	13.3	0.5	-	-
	B. tentaculata	11.7	0.9	12	0.179
	L. peregra-L	2.0	0.5	85	0.000*
	P. fontinalis	3.3	0.8	75	0.000*
	V. piscinalis	8.4	1.3	37	0.001*
2	control	6.4	0.8	-	-
	B. tentaculata	4.4	0.6	31	0.044
	L. peregra-L	1.0	0.1	84	0.000*
	L. peregra-S	3.8	0.2	41	0.012
	P. fontinalis	0.9	0.7	86	0.000*
	V. piscinalis	2.7	1.0	58	0.001*

PRI, PRB or activities existed between the two experiments.

Activities split up over day and night are presented in Fig. 7.6. Only the pulmonate snails, *L. peregra* and *P. fontinalis*, differed significantly in day and night activity. Per species, no significant differences existed between the two experiments.

Adult and juvenile *L. peregra* removed equal amounts of periphyton per unit of snail biomass (Fig. 7.3). Thus, it can be concluded that, apart from size differences, any difference in need of food between juvenile and adult snails is not reflected in the amount of periphyton removed.

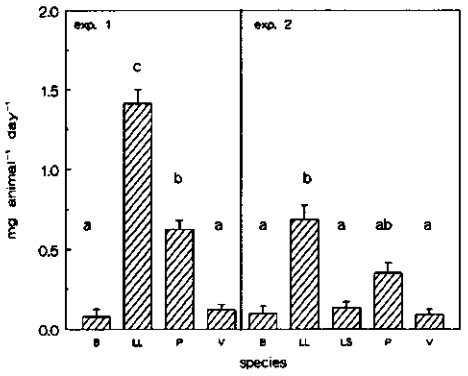


Fig. 7.2. Periphyton removal per animal (PRI) by different snail species in experiment 1 and 2; B = *B. tentaculata*, LL = adult *L. peregra*, LS = juvenile *L. peregra*, P = *P. fontinalis*, V = *V. piscinalis*. Different letters indicate significantly different means. Unless stated otherwise, in this and other figures all weights are in ash-free dry weight (afdw).

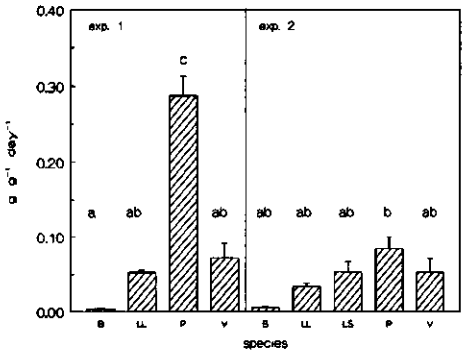


Fig. 7.3. Periphyton removal per unit snail biomass (PRB) by different snail species in experiment 1 and 2. Species and significant differences between means are indicated as in Fig. 7.2.

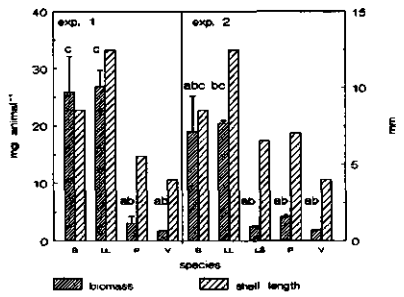


Fig. 7.4. Individual biomass and median shell length of the snail species used in experiments 1 and 2. Species and significant differences indicated as in Fig. 7.2.

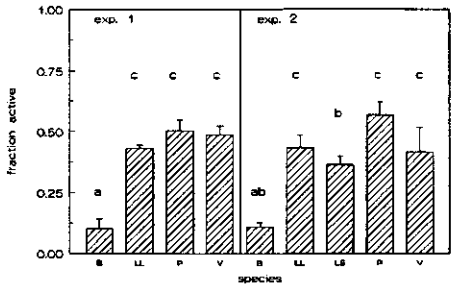


Fig. 7.5. Activities of different snail species during experiment 1 and 2. Activities are averaged over day and night. Further as in Fig. 7.2.

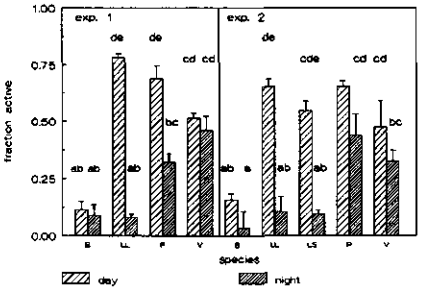


Fig. 7.6. Activities of different snail species during experiment 1 and 2, during day and night separately. Further as in Fig. 7.2.

Table 7.5. Periphyton removal and activity of *B. tentaculata* with periphyton culture medium instead of copperfree water as medium in the grazing chambers. The difference between control and grazed was significant ($p < 0.016$). As in experiment 2, 3 control and 3 grazed chambers were used.

quantity	mean	se
fraction active	0.355	0.050
PRI (mg animal^{-1} day^{-1})	0.350	0.055
PRB (g g^{-1} day^{-1})	0.016	0.003

The remarkably low activity of *B. tentaculata* raised curiosity since the snails were fairly active in our stock aquaria. Therefore an extra experiment with this species was undertaken directly following experiment 2, with periphyton from the same culture and snails from the same stock. In contrast to the previous experiments, no equilibrated copperfree tap water was used but a quantity of the periphyton culture medium, also being allowed to equilibrate to climate room conditions for 24 h. Table 7.5 gives the results of this experiment, showing a much higher activity and periphyton removal, both within the range of the other species in the previous experiments.

Since the two experiments were done with different initial periphyton densities, a possible periphyton density effect on PRB (a functional response; Hassel, 1978) could be evaluated. Here, also the data from the extra *B. tentaculata* experiment and the temperature experiment data with similar temperature were incorporated (15 °C, section 2.3.3). A significant density effect could only be demonstrated for *L. peregra* and *P. fontinalis* (Fig. 7.7), but not for *B. tentaculata* or *V. piscinalis* (ANOVA: $p > 0.20$, *B. tentaculata* was tested up

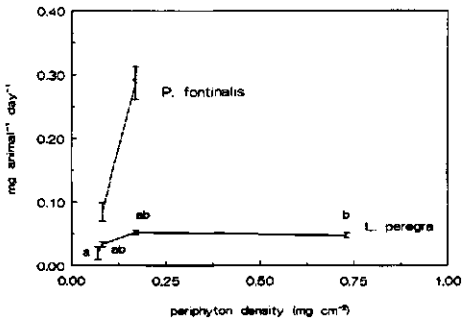


Fig. 7.7. The effect of periphyton density on periphyton removal by *L. peregra* and *P. fontinalis*. Significant differences between means are indicated as in Fig. 7.2.

Table 7.6. Periphyton densities (mg dish^{-1} afdw) in petri dishes with and without *A. aquaticus* or *G. pulex* (experiment 3): mean, standard error (se), replication (n) and significance of the paired t-test (p).

species	treatment	mean	se	n	p
A. aquaticus	with	3.4	0.2	10	0.208
	without	3.0	0.1	10	
G. pulex	with	2.4	0.1	10	0.153
	without	2.8	0.1	10	

to a density of 1.09 mg cm^{-2} afdw, *V. piscinalis* up to 0.17). The data points for *P. fontinalis* were already tested in Fig. 7.3: they were significantly different.

Results from the periphyton removal experiment with the two crustacean species (experiment 3) are given in Table 7.6. Clearly, neither *A. aquaticus* nor *G. pulex* did significantly remove any periphyton under these experimental conditions. Some freshly green faecal pellets were observed in the chambers, though, and excluded from periphyton density determinations. This suggests that the animals had fed on the periphyton to some extent.

2.3.3 The effect of temperature on periphyton removal by snails (experiment 4)

Periphyton densities in the control and grazed chambers of experiment 4 are given in Table 7.7. Six contrasts were tested: the grazed treatments versus their control at each temperature (contrasts 1 to 3), the difference between control minus grazed of 10 °C and 15 °C (contrast 4), the difference between 10 °C and 20 °C (contrast 5) and the difference between 15 °C and 20 °C (contrast 6). The first three contrasts test whether any significant periphyton removal did occur, the second three test for temperature effects on periphyton removal.

Comparing the four tests (Table 7.7), we can conclude that periphyton removal was not significant (EER = 0.05) in the *B. tentaculata* test and for the lowest two temperatures in the *L. peregra*-1 test. If an EER of 0.10 is applied, periphyton removal in the 10° *B. tentaculata* and the 15° *L. peregra* treatments were also significant. For *B. tentaculata*, this agrees with the data from experiments 1 and 2. Only the *L. peregra*-2 test showed a significant temperature effect: periphyton removal was significantly higher at 20° than at 10° and 15 °C.

Periphyton removal per individual per day and per unit snail biomass per day (PRI and PRB, respectively) are shown in Figs 7.8 and 7.9. The temperature effect of the *L. peregra*-2 test is still significant when comparing PRIs, but not when

Table 7.7. Periphyton densities (mg chamber^{-1} afdw) in control and grazed chambers and probabilities of six contrast tests in the temperature experiment (experiment 4). Given are mean and standard error (se), replication was 2-7, but generally 3. The set of contrasts is not orthogonal, resulting in a CER of 0.009 for an EER of 0.05.

species	temp.	periphyton densities			
		control		grazed	
		mean	se	mean	se
L. peregra-1	10°	6.1	3.6	3.8	0.2
	15°	9.4	1.4	6.5	0.7
	20°	6.6	1.5	3.1	0.1
L. peregra-2	10°	104.9	0.2	94.8	2.7
	15°	98.6	0.5	83.2	4.0
	20°	100.2	1.6	73.7	3.8
B. tentaculata	10°	108.7	7.5	81.2	1.8
	15°	83.9	5.0	68.0	11.4
	20°	60.2	6.7	35.5	5.0
V. piscinalis	10°	6.5	0.7	2.2	0.1
	15°	10.4	0.4	5.0	1.5
	20°	9.4	0.9	3.3	0.4

contrasts		control/ grazed	10°/15°	10°/20°	15°/20°
L. peregra-1	10°	0.051	0.661	0.743	0.446
	15°	0.013			
	20°	0.004*			
L. peregra-2	10°	0.002*	0.108	0.001*	0.006*
	15°	0.001*			
	20°	0.001*			
B. tentaculata	10°	0.015	0.415	0.842	0.534
	15°	0.128			
	20°	0.026			
V. piscinalis	10°	0.003*	0.521	0.287	0.643
	15°	0.001*			
	20°	0.001*			

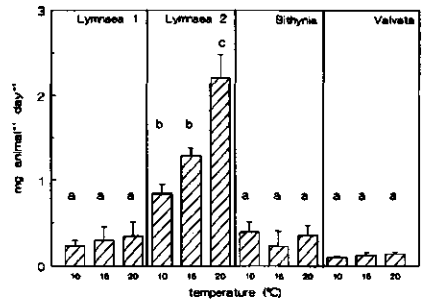


Fig. 7.8. The effect of temperature on periphyton removal per animal (PRI). Lymnaea 1 and 2 indicate two different tests (cf. Table 3). Significant differences between means are indicated as in Fig. 7.2.

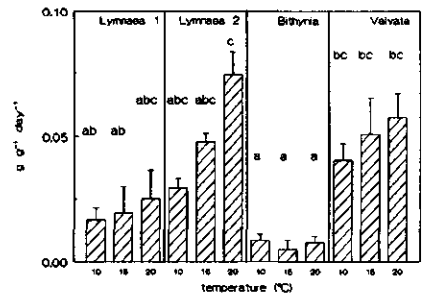


Fig. 7.9. The effect of temperature on periphyton removal per unit snail biomass (PRB). Further as Fig. 7.8.

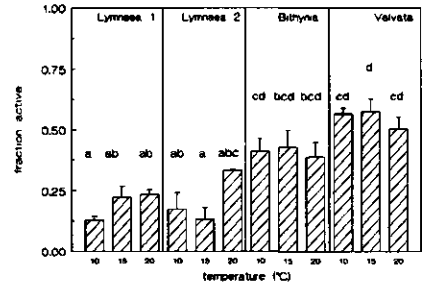


Fig. 7.10 The effect of temperature on the activities of different snail species. Activity is averaged over day and night. Further as Fig. 7.8.

comparing PRBs. Due to the increased amount of comparisons (all 12 means are compared) these tests are more conservative. The difference in significance comparing PRI and PRB indicates that the 20° grazing chambers in the *L. peregra*-2 test on average contained more snail biomass. No significant differences in snail biomass per grazing chamber, however, existed (ANOVA, $p=0.558$).

All PRIs and PRBs in experiment 4 were of the same order of magnitude as those in experiments 1 and 2 (Figs 7.2 and 7.3, observe differences in abscissa scales).

The fraction of active animals did not show any significant temperature effect (Fig. 7.10). Only the *L. peregra*-1 experiment, when considered separately, had a significantly lower activity at 10 °C (Tukey, $p<0.05$). It is remarkable that differences in activities between this temperature experiment and experiments 1 and 2 (*L. peregra* significantly less and *B. tentaculata* significantly more active in the temperature experiment, EER=0.05) were not reflected in clear differences in PRI or PRB (cf. Fig. 7.7).

2.3.3 Removal by *Bithynia tentaculata* of periphyton that accumulated in the field (experiment 5)

Removal by *B. tentaculata* of *Scenedesmus*-dominated periphyton that had accumulated in Lake Veluwe was significant for all three applied snail densities in experiment 5A but not for the lowest density in experiment 5B (Table 7.8). Furthermore, a significant effect of snail density was apparent when PRIs were compared: in experiment 5A individual snails had removed less periphyton at higher snail densities (Table 7.9). This significant density effect was also apparent in PRBs, but no significant differences existed in activities. Ash fractions were relatively high as compared to lab-grown periphyton (Table 7.4). In experiment 5A the ash content was significantly higher at the highest snail density than in the controls: the snails appeared to have selected for the organic fraction of the periphyton.

The difference between experiment 5A and 5B in PRI and PRB at comparable snail density (average PRI or PRB of density 4 and 8 in experiment 5B is compared with density 6 of experiment 5A) is considerable: they are five- to sixfold higher in experiment 5A. This may be due to a difference in temperature (22° versus 15 °C), initial periphyton density or periphyton composition (fraction ash, Table 7.8).

Table 7.8. Periphyton densities (mg chamber^{-1} afdw) after periphyton removal by different densities of *B. tentaculata* (experiment 5). Periphyton that had accumulated on glass slides suspended in Lake Veluwe was used. Control periphyton density was 0.24 and 0.13 mg cm^{-2} afdw in experiment 5A and 5B respectively. Given are mean, standard error (se), replication (n) and the result of lsd tests for afdw and fraction ash of dry weight. Inhomogeneity of variances was coped with by $\log_{10}(x+1)$ transformation prior to comparison of means with lsd (EER maintained at 0.05, different letters indicate significantly different means).

exp.	D	afdw		fraction ash		
		mean	se	mean	se	n
5A	0	18.7c	1.8	0.68a	0.02	8
	6	9.0b	0.3	0.70ab	0.01	4
	12	4.6a	0.5	0.74ab	0.03	4
	24	3.4a	0.1	0.76b	0.01	4
5B	0	10.4b	0.3	0.88a	0.00	8
	4	8.9ab	0.4	0.87a	0.01	6
	8	8.5a	0.6	0.88a	0.02	6

2.4 Discussion

All tested snail species were able to remove significant amounts of periphyton. Only *B. tentaculata* did not readily do so under all experimental conditions. The tested crustaceans, in contrast, did not remove significant amounts of periphyton in the present experimental set-up, though they reportedly are able to feed on periphytic microalgae (Moore,1975; Marcus et al., 1978). The present findings on the two tested crustacean species are in agreement with the conclusion of Moore (1975), who estimated that grazing by both crustacean species had little effect on the algal cover present in the investigated rivers.

Differences in PRI between snail species from the laboratory experiments could largely be explained when individual snail biomass and differences in activity were taken into account. Only *P. fontinalis* had a significantly higher PRB in experiment 1 than in experiment 2, while snail biomass and activity were not significantly different from those in experiment 2. This may have been due to a difference in available periphyton, which was less in experiment 2 (Table 7.4). The other tested pulmonate species, *L. peregra*, also showed a significant effect of periphyton density (Fig. 7.7). Above a periphyton density of about 0.2 mg cm^{-2} afdw, *L. peregra* did not increase its PRB (at a snail density of 4 animals per chamber, which is about 520 m^{-2} periphyton-covered substrate). In general, high snail densities may counteract the positive effect of periphyton density on PRB. However, for *B. tentaculata* on periphyton of similar density, this did not occur until a snail density of 12 per chamber (experiment 5A, about 1550 m^{-2} periphyton-covered substrate), which must be considered a very high density. Adult snail densities of species like *L. peregra* and *B. tentaculata* rarely exceed 300 m^{-2} bottom substrate (Dussart, 1979; Soszka, 1975; Young, 1975; Lodge, 1985), numbers that may be one order of magnitude less in macrophyte beds when expressed per unit periphyton-covered substrate. Newly hatched juveniles and smaller species (e.g. *Potamopyrgus jenkinsi* (Smith); Van Vierssen, 1982) however may easily reach densities of 1000 m^{-2} periphyton-covered substrate or higher.

Absence of distinct temperature effects may be due to a relatively low precision of the applied experimental set-up. This at least indicates that the absolute effect, if any, was rather small. However, effective temperature compensation mechanisms have been demonstrated for food absorption (=ingestion*absorption efficiency; Calow, 1975). Though absor-

Table 7.9. Periphyton removal rates per individual (PRI, mg $animal^{-1}$ day^{-1}) and per unit biomass (PRB, g g^{-1} day^{-1}) and snail activity on the periphyton (fraction active of total number of animals present in a grazing chamber) for different densities (n $chamber^{-1}$) of *B. tentaculata* on *Scenedesmus*-dominated periphyton accumulated in Lake Veluwe. Given are mean and standard error (se), replication was 4 (experiment 5A) or 6 (experiment 5B). Significant differences between means are indicated with different letters (lsd test comparing all 5 treatments). D = snail density.

exp.	D	PRI		PRB		activity	
		mean	se	mean	se	mean	se
5A	6	1.81c	0.34	0.028c	0.011	0.56a	0.06
	12	1.31bc	0.17	0.018bc	0.005	0.59a	0.06
	24	0.71ab	0.08	0.011ab	0.003	0.61a	0.02
5B	4	0.38a	0.14	0.006a	0.003	0.70a	0.03
	8	0.25a	0.10	0.003a	0.002	0.59a	0.02

ption and ingestion did increase with increasing temperature, Calow (1975) observed a Q_{10} of only 1.4 for *A. fluviatilis* and 1.8 for *P. contortus*, where a value of 2 would have indicated no compensation. Thus, the increase in ingestion with increasing temperature was not very high in *A. fluviatilis*. Since periphyton removal is probably not related very tightly to ingestion, relatively small differences in ingestion rate due to differences in temperature will not be reflected in clear differences in periphyton removal. This may explain the absence of a temperature effect in three out of our four tests: only in the second experiment with *L. peregra* the combination of snail density and activity, periphyton density and experimental duration was sufficiently favourable to show at least some temperature effect. For the presently tested temperature range, however, we can conclude that temperature effects need not be incorporated in estimations of periphyton removal capacities of field populations.

It may be hypothesized that well-developed temperature compensation mechanisms are of adaptive benefit to semelparous freshwater snail species that overwinter as adults and breed in spring or early summer (*L. peregra*, *P. fontinalis*, *V. piscinalis* among others; Calow, 1978). Adults and early hatched juveniles thus may profit from commonly occurring spring blooms of periphytic algae (McMahon et al., 1974; Cattaneo & Kalff, 1978; Mason & Bryant, 1975; Gons, 1982; Meulemans & Heinis, 1983; Meulemans & Roos, 1985) while temperature is still low. Also significant in this respect are the findings of McMahon et al. (1974) and Mason & Bryant (1975) indicating the nutritive superiority of periphyton over microbenthic communities.

Differences in PRI between juvenile and adult *L. peregra* could be explained by a difference in biomass alone, since PRBs were not significantly different.

Some words must be spent on the remarkable behaviour of *B. tentaculata* in the laboratory experiments. The low activity during experiment 1 and 2 suggested lethargy due to senescence or starvation, or inattractiveness of the offered periphyton as food. Both possibilities were refuted by the subsequent experiment with different medium: the animals were fairly active and removed significant quantities of periphyton. *B. tentaculata* apparently was sensitive to an unknown qualitative aspect of the water. Periphyton removal rates from the latter experiment were compared with those of a comparable field experiment (5A, periphyton density and taxonomic composition similar, snail density 8): PRIs were not significantly different, but PRBs were ($p=0.002$) due to a significant difference in individual snail biomass. Though of comparable size (7-10 mm versus 8-10 mm), the animals from the lab stocks had an almost four times lower biomass: 19 ± 10 (afdw, mean $\pm$ sd) versus 67 ± 5. Since the animals were collected from different ditches, and those from the laboratory stocks in early spring, both between-population variation (Osenberg, 1989) and tissue degrowth (Russell-Hunter & Eversole, 1976) during winter and stocking in the laboratory may have caused this difference. Vitality of the labstocks, however, apparently was not affected, since PRIs were not different.

A general conclusion from the above is that knowledge of snail biomass per area of periphyton-covered substrate, snail activities and periphyton density appear to be sufficient to estimate periphyton removal in the field, provided that individual snail biomass from the laboratory experiments does not deviate much

from field values. If such deviations are apparent, PRIs can be used and size- and biomass-distributions of the studied field populations need to be established.

3. Estimation of attenuance reduction due to periphyton removal by snails

3.1 Introduction

In the previous section we concluded that periphyton removal in the field can be estimated from a restricted number of variables. For a macrophyte, however, it is not the amount of periphyton present on its leaves that is of ultimate importance, but the effects of this cover on the performance of the macrophyte. Though a developing periphyton layer may have several effects like increased drag and competition for nutrients, in this section only the effect of removal on irradiance attenuance will be treated.

Vermaat & Hootsmans (1991c) demonstrated that the relation between irradiance attenuance by periphyton and density (whether in terms of afdw or dry weight per unit area) could well be described with a (Michaelis-Menten) hyperbola. A straightforward approach to estimate attenuance then would be to estimate the density of the remaining periphyton and apply this hyperbolic function with appropriate parameters to arrive at an estimate of attenuance.

However, two aspects of this method may be a source of error. Firstly, all remaining periphyton is treated as a homogeneous layer covering the total substrate area, while in reality the snails' grazing trails cause spatial variation with high transmittances in the grazing trail and low ones in untouched areas. Secondly, removal rates were estimated in grazing chambers containing several animals. The choice of the number of animals and the duration of the experiment was a compromise to allow for sufficient behavioural observations, significant changes between control and treatments and comparability of the different treatments. Thus, for highly active species like *L. peregra* and *P. fontinalis*, that may have covered the chamber's area several times during the experimental period, removal capacities may have been underestimated.

Therefore a series of observations on individual animals and their grazing trails was conducted parallel with experiments 1 and 2. Attenuance inside the grazing trail, the width of this trail and the linear velocity were measured for *L. peregra*, *P. fontinalis*, *B. tentaculata* and *V. piscinalis*. These measurements enable an estimation of the total area covered by an animal per unit time. Together with attenuances in- and outside the trail, an estimation of irradiance improvement over the total area may be made and compared with estimates from the first method described above.

Prior to a comparison of the two methods, the measurements on the individual grazing trails will be discussed. Attenuance estimates derived from the grazing chambers will be referred to as being derived with 'method A', those from the individual trails as 'method B'. The two methods are compared using data from experiments 1 and 2.

3.2 Materials and methods

Attenuance was measured as transmittance reduction by a periphyton layer relative to transmittance by a clean glass slide as in Vermaat & Hootsmans (1991c). A Philips 400 W HPIT metal halide lamp was used as a light source. Attenuances within and outside grazing trails were measured on glass slides with a Licor 192S quantum irradiance sensor where the measuring area of the sensor was reduced to 2*3 mm with 100% opaque pvc. The thus reduced sensor area could be fitted to the dimensions of most grazing trails. Measurements were done parallel with experiment 1 and 2 on periphyton-bearing slides from the same cultures and snails from the same stocks and sizes. Animals that appeared actively engaged in grazing were selected for observation.

Along with experiment 1 only attenuances were measured, while during experiment 2 also trail width and linear velocity were measured. Glass slides were laid on the bottom of small aquaria (15*25*7 cm), on a mm grid. Trail width was estimated with 0.1 mm precision.

Movement patterns of the snails in the chambers were mapped on mm-paper using the mid-point between the tentacles on the head as a reference point. Every 10th second a mark was noted on the mapped track. The distance moved can be calculated from the number of crossed lines on the paper with the formula by Reddingius et al. (1983):

$$E(R) = \tfrac{1}{4}\pi * m * E(N)$$

where E(R) is the distance, m the mesh width of the mm-paper (1 mm) and E(N) is the number of crossed lines on the mm-screen. The 10 seconds marks enabled the calculation of linear velocities. Areal velocity (V) was calculated from linear velocity (L) and trail width (W): V = L * W.

Irradiance attenuance by periphyton remaining in the grazing chambers at termination of experiments 1 and 2 was estimated with a rectangular hyperbola (method A). The hyperbola was fitted iteratively (as in Vermaat & Hootsmans, 1991c) to attenuance-density data pairs of undisturbed control slides from experiments 1, 2 and 4, and has the form:

$$\text{attenuance} = (a * \text{density})/(b + \text{density})$$

where attenuance is in relative units (0.0 - 1.0) and periphyton density in mg cm^{-2} afdw. Values of a and b were 0.913 and 0.457, respectively (se: 0.023 and 0.027, $r^2=0.99$, n=58).

The estimation of attenuance of the remaining periphyton in the chambers according to method B was derived as follows. The total area that could have been 'cleaned' in a grazing chamber during the grazing experiments (A, cm^2) was derived from areal velocities (V) and average activities in the chamber:

$$A = V * \text{activity} * N * 2 * 1440$$

where V is in $cm^2\ min^{-1}$, activity is a fraction of total time, N is the number of snails in a chamber, 2 is the duration of an experiment in days and 1440 is the number of minutes in a day. Average attenuance (ME) of the remaining periphyton was calculated according to:

$$ME = (A * ET + (TA-A) * EC) / TA$$

where ET is attenuance in a trail, EC is attenuance of an untouched, control area and TA is the total area of periphyton-covered substrate available in a grazing chamber (4 slides, 77.2 cm^2). If A was larger than TA, ME was simply set to

ET. Thus, it was assumed that the snails grazed a surface only once, i.e. no overlap in grazing trails occurred.

3.3 Results and discussion

3.3.1 Measurements on individual grazing trails

Attenuances in individual grazing trails as measured during experiments 1 and 2 are given in Fig. 7.11. Significant differences in path clearance existed between species: *B. tentaculata* and *P. fontinalis* in general cleared their path most thoroughly in terms of attenuance, while *L. peregra* and *V. piscinalis* left more material in their trail. Significant differences existed between the two experiments for *L. peregra* and *V. piscinalis*. Both species left paths that were less transmittant during the second experiment. Compared to experiment 1, the untouched control areas transmitted significantly more during experiment 2 (t test: $p<0.001$) and had a lower initial density (Table 7.4) and more abundant gelatinous *Gloeocystis* (Table 7.2). For none of the species attenuance in the trail was related significantly to attenuance in the control areas (linear regression). Taxonomic differences in the periphyton thus probably were responsible for the difference in the attenuance of the trail between experiments.

Significant differences in trail width (Table 7.10) paralleled differences in snail size (Fig. 7.12). Linear regression of path width to individual snail biomass was significant ($p=0.0115$, path width = 1.49 + 0.11 * individual afdw, $r^2=0.91$, $n=5$). Variation in path width was explained equally well by individual biomass as by median shell length (path width = 0.16 + 0.31 * median shell

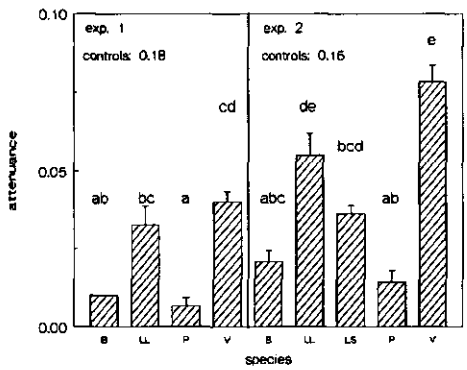


Fig. 7.11. Attenuance in the grazing trail of different snail species. Attenuance is defined here as the fraction of light that is not transmitted by the periphyton layer. Species and significant differences between means are indicated as in Fig. 7.2.

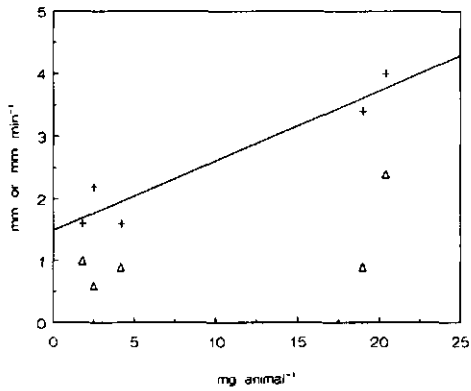


Fig. 7.12. Path width (crosses) and linear velocity (open triangles) of different snail species as a function of individual snail biomass. See text for further explanation.

Table 7.10. Mean attenuance (ET, % of irradiance not transmitted) in a grazing trail, path width (W, mm), linear velocity (L, mm min^{-1}) and the resultant areal velocity (V, mm^2 min^{-1}). Attenuances are averaged over experiment 1 and 2, mean attenuance of an ungrazed control (EC) was 17%. Given are mean, standard error (se) and replication (n). Different letters indicate significant differences (lsd, EER=0.05). Significance tests on linear and areal velocity were done after log_{10}-transformation to homogenize variances, mean and se in the table are untransformed.

species	attenuance (ET)			path width (W)		velocity linear (L)		areal (V)		
	mean	se	n	mean	se	mean	se	mean	se	n
B. tentaculata	1.8ab	0.3	17	3.4b	0.4	0.9a	0.1	3.1b	0.4	12
L. peregra-L	4.1c	0.5	26	4.0b	0.3	2.4b	0.3	9.6c	1.3	10
L. peregra-S	3.6bc	0.3	8	2.2a	0.3	0.6a	0.1	1.4a	0.2	10
P. fontinalis	1.0a	0.2	19	1.6a	0.2	0.9a	0.1	1.4a	0.2	7
V. piscinalis	5.1c	0.4	22	1.6a	0.2	1.0a	0.1	1.5a	0.2	10

length, $r^2=0.80$; difference between the two regression lines: $F = RSS_2/RSS_1 = 2.23$, df = 3, 3, $p>0.10$). Thus, differences in width of a grazing trail between snail species could be explained well by differences in snail size alone and specific differences between the tested species were not apparent.

Patterns in linear velocity (Table 7.10) were not explained well by individual snail biomass (Fig. 7.12). Linear velocity was entered in linear regressions with snail biomass and median shell length as independent variables. Neither variation in biomass ($p=0.219$) nor in median shell length ($p=0.098$) significantly explained variation in linear velocity. Still, the small probability ($p<0.10$) of the regression to median shell length indicates that also for the presently tested species, animal size in general does affect attainable velocity.

Areal velocities of the different species were calculated from path width and linear velocity. Significant differences in areal velocity between species thus reflect differences in both constituent variables, which indeed is apparent from the significance patterns (Table 7.10). Large *L. peregra* had the highest areal velocity whilst grazing on periphyton-covered glass slides, *B. tentaculata* was intermediate and the three remaining species had equal, relatively low linear velocities.

Kairesalo & Koskimies (1987) estimated from phosphorus budgets that adult *L. peregra* "scraped an area of 20 - 25 cm^2 a day" of periphyton-covered stems of *Equisetum fluviatile* L., with temperature ranging from 10 - 25 °C. With an average activity of 0.4 for day and night together (experiment 1 and 2) and the above areal velocity (Table 7.10), adult *L. peregra* covered 55 cm^2 in 24 h in the present experiment. Despite completely different methodology, the

Table 7.11. Irradiance attenuances (%) by periphyton remaining in the grazing chambers at termination of experiments 1 and 2 as estimated with methods A and B (cf. section 3.2). Given are mean, standard error (se), replication (n) and probability of the difference between method A and B (p, t-test). For the estimations of experiment 1 according to method B, the mean observed areal velocities were used that were observed during experiment 2.

exp.	species	method A			method B			p
		mean	se	n	mean	se	n	
1	*B. tentaculata*	22.7	1.2	4	1.0	0.0	5	0.001*
	L. peregra-L	4.9	1.0	4	3.3	0.6	16	0.251
	P. fontinalis	7.7	1.8	4	0.7	0.3	12	0.001*
	V. piscinalis	17.4	2.1	4	4.0	0.3	16	0.001*
2	*B. tentaculata*	10.1	1.3	3	3.4	0.7	12	0.001*
	L. peregra-L	2.4	0.3	3	5.5	0.7	10	0.377
	L. peregra-S	8.8	0.3	3	3.6	0.3	8	0.001*
	P. fontinalis	4.3	2.1	3	1.4	0.4	7	0.072
	V. piscinalis	6.5	2.3	3	7.8	0.5	6	0.439

difference between the two estimates remains satisfyingly well within one order of magnitude.

It can be concluded that especially adult *L. peregra* had a high areal velocity, almost 10 mm² were cleaned within a minute and the remaining material in the trail transmitted 96% of the incident light where ungrazed control areas transmitted 83%. The other tested species have distinctly lower areal velocities but increased transmittance in the grazing trails to values of similar magnitude.

3.3.2 Comparison of two methods to estimate irradiance improvement

Irradiance attenuances by periphyton remaining in the grazing chambers at termination of experiments 1 and 2, as estimated with methods A and B, are given in Table 7.11. The difference between the two estimations were significant for three of the four species in experiment 1 and only for two of the five in experiment 2. Also, the differences were numerically larger in experiment 1: up to 21.7% for *B. tentaculata*. This may be partly due to the fact that areal velocity estimates from experiment 2 had to be used. In the following section 4 it is demonstrated that differences in taxonomic composition of the periphyton may influence the velocity of *L. peregra*. The higher abundance of gelatinous *Gloeocystis* in experiment 2 may have caused a similar effect and thus may have been responsible for differences in areal velocities between the two experiments. Extrapolations from experiment 2 to experiment 1 therefore should be considered with some caution.

For those species that could have covered the total periphyton-covered area more than about twice during experiment 2, the two methods were not significantly different (large *L. peregra*, *P. fontinalis* and *V. piscinalis*, Table 7.11). *B.*

tentaculata and small *L. peregra*, however, did not remove sufficient material to prevent an overestimation with method A.

A linear regression of the difference between method A and B against the ratio of the total area potentially cleaned during the experiment to the area in the chamber covered with periphyton (i.e. A/TA, section 3.2), gave a highly significant negative slope for the data from experiment 2 (Fig. 7.13, $p<0.005$, $r^2=0.97$). Inclusion of all data from experiment 1 resulted in a nonsignificant regression, while exclusion of only *B. tentaculata* and *V. piscinalis* of experiment 1 produced a significant regression ($p<0.025$, $r^2=0.75$). For experiment 1, the two methods deviated most strongly for *B. tentaculata* and *V. piscinalis*, but not for *L. peregra* (Table 7.11). As argued above, the reason for the stronger deviations of the estimates for experiment 1 must probably be sought in differences in linear velocity. Apparently, our approach to estimate average attenuance in a chamber from trail attenuances and areal velocities obtained in another experiment was still sufficiently robust to produce satisfying results for the highly active *L. peregra*. Or, in short, probably the areal velocity of this species was not very different in the two experiments.

Two possible reasons for a deviation from true values of attenuance estimations from periphyton density in grazing chambers were formulated a priori. The first, neglection of spatial inhomogeneity in the estimation of attenuance from density, indeed appeared to be a source for significant deviations. The second, overlap of grazing trails, however, did not appear to have much effect. Apparently, the potentially strong overlap in grazing trails as for *L. peregra* (A/TA = 6.2) did not result in further

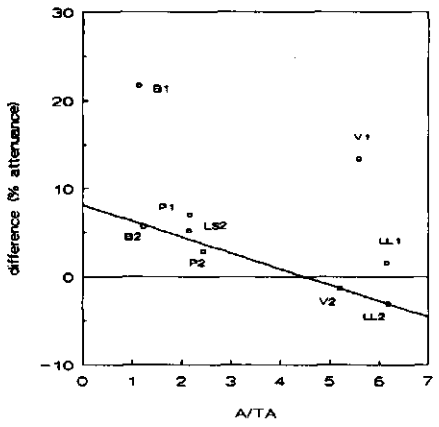


Fig. 7.13. Proportional difference in attenuance estimates between methods A and B as a function of the ratio grazed area/total available area (A/TA). Capitals indicate the different snail species as in Fig. 7.2, numerals 1, 2 indicate the two experiments. See text for further explanation.

reduction of attenuance in the trails. This may indicate that either the remaining material is not 'removable' by the snails or not worth the effort.

It can be concluded that the estimation of irradiance attenuance from the remaining periphyton density (mg cm^{-2} afdw) in a grazing chamber does not result in a significant overestimation provided that sufficient material has been removed. This amount can be roughly estimated at 60% for experiment 2 (i.e. *V. piscinalis*, Table 7.4). Though significant differences were present in the data of experiment 2, the maximum difference between the two methods was not very high: about 6% attenuance for *B. tentaculata*. Grazing chambers thus form a sensitive tool to evaluate the periphyton removal capacities of a species in terms of density, and, if the above provision (i.e.

60% removal) is taken into consideration, can produce reliable estimates of irradiance attenuance of the remaining periphyton.

4. The effect of periphyton type and substratum on snail behaviour (experiment 6)

4.1 Introduction

As outlined in section 1, extrapolation of grazer behaviour from glass slides to macrophytes under field conditions supposes that the behaviour of grazers is not different on these two types of substrate. This was studied by observing adults (9 - 11 mm shell length) of a relatively large snail species, *L. peregra*, on glass slides in grazing chambers and on *P. pectinatus* in aquaria with similar periphyton communities. *P. pectinatus* was chosen for two reasons: (a) its linear leaves obviously differ in form from the rectangular glass slides, and (b) *P. pectinatus* has been used throughout the present research project as model macrophyte species.

A second aim of this section was to establish whether a difference in taxonomic composition of the periphyton could elicit differences in snail behaviour. Therefore, periphyton communities on *P. pectinatus* with a different taxonomic composition were tested also. Linear moving rates as well as behavioural patterns were recorded.

4.2 Material and methods

The data from the grazing chambers used in this section are from a simultaneously performed experiment on the temperature effect on periphyton removal (experiment 4, *L. peregra*-2, cf. section 2). *P. pectinatus* plants were grown from standard size class tubers (0.1-0.2 g fresh weight) in aquaria (four plants per 25*25*50 cm aquarium) with a standard periphyton nutrient medium (section 2.2.1), at 200 $\pm$ 25 μE m^{-2} sec^{-1} (mean $\pm$ sd, dark/light cycle 12h/12h) and 19 °C (sd: 2.5 °C). Periphyton was inoculated from a 1 liter Erlenmeyer containing a homogenized sample of a periphyton batch culture. The plants were 6 to 10 weeks old when the snails were added (10 per aquarium). Observations were made during the consecutive 24 to 48 h. Periphyton afdw and dry weight and aboveground plant biomass and area were determined prior to (1 plant per aquarium) and following the observational period (remaining 3 plants per aquarium). To determine the amount of periphyton available per unit area, plant area was measured with the assumption that leaves are flat and rectangular and stem cross-section is circular. Two-sided leaf area thus was measured as 2 * length * width and stem area as 2π * radius * length with 1 mm precision. The area is taken double since both sides of a leaf can be colonized by periphyton and both sides were stripped clean at harvesting.

Four experiments were done with plants: (1) with all periphyton carefully removed (experiment 6a), (2) with a Cyanobacteria-dominated periphyton (experiment 6b), (3) with periphyton dominated by both Cyanobacteria and green algae (experiment 6c), and (4) with a green algae dominated periphyton (ex-

Table 7.12. Effect of substrate type and periphyton taxonomic composition on the behaviour of *L. peregra*. Shown are dominant periphyton taxa, linear moving velocities of *L. peregra* (mean, standard error, se, and number of observations, n), percentage of animals actively grazing during day and night respectively on plants (experiments 6a-6d) and glass slides (experiment 4). Significant differences are indicated with different letters (lsd, EER kept at 0.05).

exp	periphyton taxa	velocity (mm min^{-1})			percentage grazing day			night		
		mean	se	n	mean	se	n	mean	se	n
6a	-	13.5b	2.4	13	18.9a	4.5	9	0.0a	0.0	4
6b	*Anabaena* *Calothrix* *Oscillatoria*	13.2b	1.6	24	16.2a	1.3	19	8.3a	2.6	12
6c	*Scenedesmus* *Chlamydomonas* *Anabaena* *Calothrix*	10.0b	1.6	22	34.4b	3.2	40	20.6a	5.8	9
6d	*Scenedesmus* *Chlamydomonas*	4.5a	1.1	15	14.0a	2.4	40	3.7a	2.6	9
4	*Scenedesmus* *Gloeocystis* *Anabaena* *Oscillatoria*	15.5b	2.1	26	34.5b	3.7	29	16.8a	4.6	19

periment 6d). Taxonomic composition of periphyton in experiment 6c was similar to that on the glass slides.

Movement patterns of the snails in the chambers were measured according to Reddingius et al. (1983), as described in section 3.2. The approach differed from the one adopted in section 3 in the selection of the animal that was to be observed. Here, a moving animal was randomly selected and followed for 5 minutes or until it stopped longer than 30 seconds. Thus both actively grazing animals that moved at a relatively slow pace and more swiftly moving snails were incorporated in the sample. The moved distances on the plants were measured with help of a wire-thin ruler carefully held along the linear leaves or stems of the plant. Disturbance of the snails by this procedure was not observed.

From the behavioural protocols the proportion of animals grazing on periphyton (activity of the mouth parts could be observed in *L. peregra*) was analyzed.

4.3 Results and discussion

The results from the velocity and activity measurements are shown in Table 7.12. The multiple comparison tests indicate that

the velocity was significantly lower on green algae dominated periphyton then on all other substrata. Consideration of the amount of time spent grazing during daytime groups the 'green algae + Cyanobacteria-dominated' plants together with the slides. Grazing activities during the night were not significantly different.

It can be concluded that the snails did not show different velocities or grazing activities on slides as compared with plants with similar periphyton (experiment 6c). A comparable result is reached when experiment 6d is compared with the linear velocities as measured during experiment 2, where periphyton of comparable taxonomical composition was used (Table 7.2): linear velocities were not significantly different (t test, $p=0.128$). Thus, the somewhat different way in which moving snails were selected for observation did not result in significant differences in linear velocities.

With respect to the second question that is addressed with this series of experiments, it can be concluded that the taxonomic composition of a periphyton community had distinct effects on both linear velocity and activity patterns.

The snails had no measurable effect on average periphyton density on the plants during the present experiments. Thus, our behavioural observations can be considered as not being influenced by changes in periphyton density during the course of the experiment. Accordingly, only pooled densities are given in Table 7.13. Periphyton densities did not differ significantly between the three different experiments. Furthermore, the tested densities were above the range where *L. peregra* showed a functional response (above 0.2 mg cm^{-2} afdw, section 2.3.2, Fig. 7.7).

It is recognized that the experiments were performed in still water.

Table 7.13. Periphyton densities (mg cm^{-2} afdw) from three experiments where behaviour of *L. peregra* on periphyton-covered *P. pectinatus* was observed (experiments 6b, 6c, 6d). Because the difference in density between samples before and after addition of the snails was not significant, these data are pooled. Given are mean, standard error (se) and replication (n, number of plants). No significant differences existed between the three experiments (ANOVA, $p=0.0811$).

exp	mean	se	n
6b	1.25	0.09	16
6c	0.87	0.50	4
6d	0.65	0.21	4

Whether high turbulence or flow velocities affect the animals' performance on different substrata remains to be established. Calow (1981) demonstrated clear differences in growth and reproduction patterns between *L. peregra* populations from 'exposed' and 'sheltered' habitats when kept under identical conditions. Growth halted earlier in 'exposed' populations while reproduction started earlier. This may indicate that populations from 'exposed' habitats spend less time grazing on a seasonal basis. It cannot be decided whether this may also have effect on periphyton removal, though it seems unlikely.

To explain the overall pattern of velocities and activities the following rationale can be forwarded: A 'lawn of food' may consist of preferred and less preferred and easily and less easily ingestable food items or patches. To fulfil the animals' daily requirements it will spend more time on less easily ingestable food when it has no choice. Calow (1970) and Calow & Calow (1975) concluded a preference of *L. peregra* for green algae. In

terms of the above, the periphyton of experiment 6d may be considered an easily ingestable food type. Therefore, when grazing, the animals dropped their velocity and needed only a relative short time to fulfil their requirements. The periphyton on slides and in experiment 6c still offered enough of the preferred food, but with the periphyton at one particular site not being very profitable, a higher velocity was adopted and more time was spent on grazing. Finally, the least attractive substrata (clean plants (experiment 6a) and experiment 6b with complete blue-green dominance) were inspected at a relatively high velocity and apparently not paid fairly long visits. Thus an interacting pattern of velocity and time spent grazing emerges, a behavioural pattern that may fit well into general theories on food intake optimization through reactions to food patch profitability (Krebs & Davies, 1984).

5. The effect of periphyton removal by snails on the growth and biomass allocation of *Potamogeton pectinatus* L., a laboratory study

5.1 Introduction

In previous sections it was demonstrated that all tested snail species were able to remove significant amounts of periphyton. In the present section the macrophyte component is incorporated. In two laboratory experiments the effects of periphyton and its removal by snails on plant growth were investigated. The two experiments 7 and 8 had different aims with accordingly different experimental set-ups.

In experiment 7 biomass allocation in the macrophyte *P. pectinatus* was followed over time by harvesting replicate aquaria at different moments in the course of the experiment. Two densities of the snail *L. peregra* were applied. The aim of this experiment was to elucidate whether any difference in growth pattern is recognizable over time under different periphyton removal regimes. Experiment 7 is dealt with in section 5.2.

Using data from experiment 7, in section 5.3 a comparison is made between the two methods that were derived in section 3 to estimate irradiance improvement due to periphyton removal. From the results for *L. peregra* reported in section 3 it can be hypothesized that both methods should arrive at similar estimates, since on glass slides no significant differences existed.

In experiment 8 the impact of different grazer species on *P. pectinatus* growth was investigated. Two densities of *L. peregra*, *B. tentaculata* and *V. piscinalis* were applied and all harvesting was performed at the termination of the experiment. Care was taken to simulate late spring and summer conditions (May, June, July) with respect to grazer-density, -size class and -condition, macrophyte stage of development and water temperature. Experiment 8 is treated in section 5.4.

Periphyton grazing experiments in aquaria have one distinct 'unrealistic' but inevitable feature: an aquarium has walls. This increases the area that is colonizable for periphyton and thereby available for grazers. Grazing pressure on the macrophyte area in the aquaria therefore should pertain to snail numbers actually present

on the macrophytes. Activities on the different types of substrate have therefore been scored separately.

5.2 The effect of *Lymnaea peregra* L., repeated measurements (experiment 7)

5.2.1 Materials and methods

In each of 25 aquaria (heigth 25 cm, basis 15 * 15 cm) four tubers (standard size class: 0.1 - 0.2 g fresh weight) were planted in a 4 cm deep sediment layer (clay/sand mixture: 1/3). The aquaria were placed in a 5 * 5 array in a cooling basin maintaining a temperature of 18 °C in the aquaria. Homogeneous irradiance was provided by 4 400W Philips HPIT metal halide lamps: 100 $\pm$ 4 μE m^{-2} s^{-1} (mean $\pm$ sd) at 1 cm below the water surface, with a 12h light/12 h dark diurnal cycle. The experiment was done in spring 1987.

Nutrient medium composition was identical to that in the periphyton cultures (section 2.2). New medium was added weekly by flushing the aquaria with 10 liter each (approximately twice its volume). Periphyton was inoculated from a homogenized sample of a periphyton culture (used for the *L. peregra*-2 of experiment 4) in the second and third week of the experiment. To prevent infestation with periphyton consuming chironomid larvae the adults of which were present in the experimental rooms, all aquaria were covered with a mosquito gauze netting. Irradiance measurements were made below this netting. Chironomids have been reported to reduce periphyton densities (Mason & Bryant, 1975; Cattaneo, 1983).

Adult snails were collected from the standard '*L. peregra* source' ditches (water temperature $\pm$ 5 °C) in the vicinity of Wageningen. Prior to the experiment the animals were acclimized to the experimental temperature for a week. Snail densities were chosen to span a range of reported field densities for adult *L. peregra*: about 100 - 300 per unit of bottom area in well-developed populations (Soszka, 1975; Dussart, 1979; Young, 1975; Lodge, 1985): a low density (2 snails per aquarium $\approx$ 90 animals m^{-2}, shell length 11.8 $\pm$ 1.1 (mean $\pm$ sd)) and a high density (9 per aquarium $\approx$ 400 animals m^{-2}, shell length 11.6 $\pm$ 2.3). Snail behaviour was observed in the week prior to the second harvest (week 5). Activities were scored during 10 periods of 10 minutes length in a night- and a day-session.

The treatments and moments of harvest were allocated randomly over 24 aquaria, 1 aquarium remained unused. Harvesting in the aquaria took place after 3 (6 initial controls), 6 and 9 weeks (3 control aquaria, 3 with a low density and 3 with a high density of *L. peregra*) after planting of the tubers.

At harvesting periphyton was carefully removed manually from the macrophytes and processed as described in section 2.2. Microscopical inspection of a thus cleaned sample of leaves proved manual removal to be sufficiently efficient (99% removed). From every plant a sample (1 cm leaf) was fixed in Lugols solution for taxonomic characterisation.

After periphyton removal the number of leaves per plant were counted, and leaf length and width and stem diameter were measured (1 mm precision) to allow leaf and stem area calculation as in section 4.2. A sample of 2 leaves per plant (8 leaves per aquarium) was deep frozen for subsequent chlorophyll determination and biomass of the different plant parts was determined as in Vermaat

& Hootsmans (1991a). Fresh weight (blotted dry with tissue paper) of the chlorophyll subsample and the remaining leaves was determined to allow calculation of chlorophyll per unit afdw.

Chlorophyll was determined with the method of Vernon (1960) modified according to Moed & Hallegraeff (1978) to control post-acidification pH in the sample. The method enables determination of chlorophyll a, b and their phaeopigments in one sample.

Especially in the high snail densities blooms of planktonic *Kirchneriella* spec. developed. At the weekly medium renewal they were washed out but had developed again at the end of the week. To account for this interaction, exponential extinction was measured in the appropriate aquaria and the contribution of this alga to total irradiance extinction could be calculated.

5.2.2 Results

Periphyton density and taxonomic composition

The results are summarized in Table 7.14. One control aquarium of the 9 weeks harvest was excluded from analysis because it was infested with chironomids that also reduced periphyton density distinctly (to 5 mg dm^{-2} afdw on average on the plants). As is clear from Table 7.14, presence of snails had a distinct effect on periphyton. Both on walls and plants the density was significantly reduced after 9 weeks as compared to the controls. Mainly due to less variation in periphyton density on plants than on walls, at six weeks ungrazed plants already had significantly more periphyton than grazed plants while walls had not. Snail density had no significant effect on periphyton density, both snail densities maintained periphyton density at the 3 weeks' control level. No significant differences existed in periphyton density per unit area between walls and plants.

Taxonomic composition of the periphyton on both walls and plants changed markedly under grazing pressure. In the controls the periphyton developed into a mat of filamentous Cyanobacteria while a layer of closely adhering unicellular greens developed under grazing pressure.

Plant biomass and morphology

All plant data are presented in Table 7.15. Total biomass had not increased significantly during the last 6 weeks of the experiment. Aboveground biomass, however, did increase: significant differences existed both over time in the controls as well as between the control and high snail density in the third harvest. The increase in aboveground biomass in the controls was paralleled by a significant decrease in tuber biomass. Relative biomass distributions are shown in Fig. 7.14.

The differences in aboveground biomass were reflected in similarly significant differences in numbers of leaf and leaf area. With respect to stem area, stem length and specific aboveground area, no effect of snail density was present, while time had a significant effect (twoway ANOVA). Time nor snail density had any significant effect on chlorophyll (a+b) content, percentage chlorophyll b or phaeophytins.

Snail activity

The proportional distribution of active snails over different substrates is shown in Fig. 7.15. Day and night activities are pooled here. For all three separate activity categories: 'active' on plants, walls or sediment, no significant effect of snail density (number per aquarium) was present (t-tests, $p > 0.3$). When activity on the different substrates was normalized to

Table 7.14. Periphyton density (mg dm^{-2} afdw) and taxonomic composition on plant surfaces and aquarium walls. For the density data, mean and standard error (se) are given. Replication was 12 per treatment for periphyton on plants and 3 for periphyton on aquarium walls. Periphyton density on aquarium walls was determined only for the 6 and 9 weeks harvest. One control aquarium of the 9 weeks harvest was excluded from analysis because it was infested with chironomids. Different letters indicate significant differences (lsd, BER=0.05). Taxonomic composition and taxon abundance was determined as described in section 2.2.1, nd = not determined, va = very abundant, a = abundant, f = frequent, o = occasional and r = rare.

harvest:	3 weeks	6 weeks			9 weeks		
snails $aquarium^{-1}$:	0	0	2	9	0	2	9
(a) on plant surface							
density							
mean	10a	26bc	15a	9a	33c	17ab	13a
se	1	3	2	2	3	4	0
abundance							
Scenedesmus	a	va	a	va	f	a	a
Kirchneriella	-	-	s	a	o	a	a
cf. *Chlamydomonas*	o	f	a	a	f	-	-
Anabaena	va	va	va	a	va	a	a
Oscillatoria	-	a	a	f	o	-	-
Calothrix	o	f	-	-	a	-	-
(b) on aquarium walls							
density							
mean	nd	20ab	9a	0a	35b	4a	3a
se		10	3	0	7	2	1
abundance							
Scenedesmus	nd	a	a	-	a	f	-
Kirchneriella		-	-	f	-	-	-
cf. *Chlamydomonas*		a	a	-	f	-	-
Palmella		f	f	-	a	-	-
cf. *Pleurococcus*		-	-	a	f	a	a
Anabaena		-	a	f	a	-	-
Oscillatoria		a	a	-	-	-	-
Calothrix		-	-	-	o	-	-

Table 7.15. Plant biomass, morphology and chlorophyll content as affected by periphyton grazing in experiment 7. Given are mean and standard error (se) of: (a) total, tuber, root + rhizome and aboveground biomass (mg plant^{-1} afdw); (b) number of leaves, leaf area (cm^2, two-sided), stem area, specific aboveground plant area (two-sided, cm^2 g^{-1} afdw) and stem length (cm) per plant; (c) chlorophyll (a+b) content (chlor (a+b), μg mg^{-1} afdw), the percentage chlorophyll b in chlorophyl (a+b) and the percentage phaeopigments of total chlorophylls (= chlor (a+b) + phaeopigments). Initial afdw of the tubers was calculated with the linear relation between afdw and freshweight of Vermaat & Hootsmans (1991b). Replication was 24 for the 3 weeks' control and 12 for the other treatments for (a) and (b) and 6 and 3, respectively, for (c). Different letters indicate significant difference between treatments (lsd, EER=0.05).

harvest in week:		3	6			9		
snails aquarium^{-1}:		0	0	2	9	0	2	9
(a) biomass								
total	mean	43a	42a	45a	37a	39a	41a	37a
	se	1	3	2	3	2	3	2
tuber	mean	28d	20bc	24cd	21bc	14a	18ab	18 ab
	se	1	2	1	3	1	2	2
% of initial weight		42	30	36	31	21	27	27
roots + rhiz.	mean	1.9ab	1.9ab	1.5a	1.4a	2.2b	1.7ab	1.8ab
	se	0.1	0.1	0.2	0.1	0.2	0.2	0.1
aboveground	mean	13a	19bcd	19bcd	16ab	23d	22cd	18abc
	se	0	2	2	1	1	1	1
(b) morphometry								
n leaves	mean	8.0a	14.1b	13.6b	11.8ab	19.7c	18.8c	15.0b
	se	0.4	1.3	1.1	1.0	1.3	1.5	1.1
leaf area	mean	5.8a	11.1b	10.5b	9.6b	15.5c	12.4bc	11.0b
	se	0.3	1.1	1.0	0.8	1.1	0.9	1.2
stem area	mean	3.6a	6.9a	5.6a	5.4a	8.3a	6.3a	6.3a
	se	0.2	0.8	0.5	0.5	0.6	0.4	0.5
specific area	mean	717a	930b	846ab	933b	1028b	863ab	998b
	se	42	47	40	38	39	55	25
stem length	mean	11a	19bc	18b	16b	24c	19bc	21bc
	se	1	2	1	1	1	1	1
(c) chlorophyll								
chl(a+b)	mean	7.5a	4.3a	7.4a	6.2a	3.8a	3.3a	7.3a
	se	0.7	0.7	2.2	0.8	1.6	0.8	2.0
% chl-b	mean	13a	13a	20a	12a	7a	15a	13a
	se	2	4	1	3	2	4	3
% phaeopigm.	mean	11a	15a	8a	8a	26a	20a	24a
	se	2	9	0	1	9	9	10

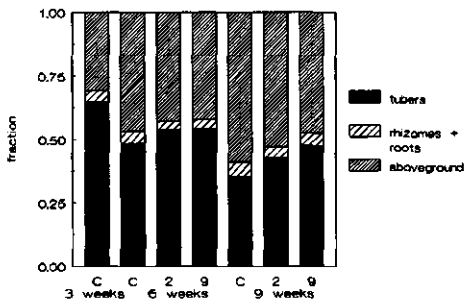


Fig. 7.14. Relative biomass distribution of *P. pectinatus* in experiment 7, C = control, 2 = 2 snails aquarium^{-1}, 9 = 9 snails aquarium^{-1}.

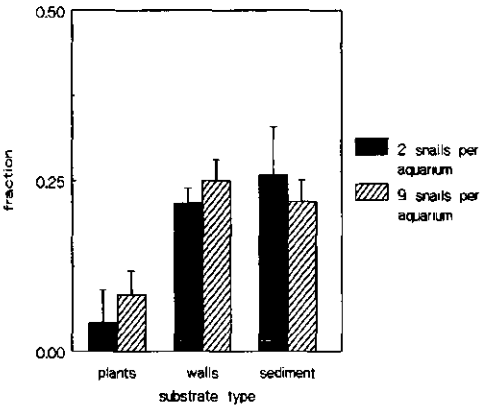


Fig. 7.15. Distribution of active *L. peregra* over different types of substrate in experiment 7. Together with the category 'non active', the different proportional activity types sum up to unity for each snail density. Per substrate type, no differences between density existed ($p > 0.3$). The fraction of animals active on walls and sediment were not significantly different but both significantly higher than on plants.

Table 7.16. Activity of *L. peregra* on three types of substrate normalized to unit area (% active cm^{-2}). Given are mean and standard error (se). Since $\log_{10}$-transformation did not homogenize the variances, t'-tests were used (EER=0.05). Replication was 6, i.e. all observations on one aquarium were pooled into one replicate, and since no significant differences existed between snail densities, the six grazed aquaria were taken as replicates. Plant area was taken as 4 * 15 cm^2 (4 plants times an estimate of plant area at the moment of observation), sediment area was 225 and wall area 1200 cm^2.

substrate	mean	se	t'-test
plants	0.10	0.04	ab
sediment	0.11	0.01	b
walls	0.02	0.00	a

unit area of each substrate (i.e. expressed in % cm^{-2}), one significant difference existed (Table 7.16): the animals preferred the sediment above the walls.

When activities on the different types of substrate were pooled to 'overall' activity, both snail density and the day/night effect as well as their interaction were significant (twoway ANOVA, $p < 0.05$). During night time, the animals of the high density were more active than the low density ones, while during day time no difference was observed (Table 7.17).

On the aquarium walls it was possible to discern between active but not grazing animals and actively grazing animals, since the movements of the mouth parts were visible to the naked eye. Deeper into the aquaria, on the plants and the sediment, such observations were not possible. Snail density had no significant effect on the proportion of active animals

Table 7.17. Total activities (%) of *L. peregra* on all substrata during day and night for two snail densities. Observations were made in the week prior to the second harvest. Given are mean and standard error (se), replication was 10. Different letters indicate significantly different means (Tukey, EER=0.05).

snails aquarium^{-1}		mean	se	Tukey
2	day	71	5	c
	night	30	6	a
9	day	73	3	c
	night	53	4	b

that were grazing on the aquarium wall (t-test, p=0.229). On average 31% of the active snails were not grazing, but simply moving over the substrate without touching the periphyton cover with their mouth parts.

5.2.3 Discussion

Periphyton removal by grazing or moving snails had distinct effects on the periphyton community on both the aquarium walls and plants. Similar to the findings of Nicotry (1977), Sumner and McIntyre (1982) and Cuker (1983) for non-living substrates, periphyton grazing resulted in dominance of unicellular, closely adhering forms, while filamentous algae developed in absence of grazers. Sumner & McIntyre (1982) hypothesize that the filamentous form is at an advantage with respect to nutrient uptake from the surrounding water in a well-developed periphyton community but at disadvantage with respect to susceptibility to dislodging. They postulate that filamentous 'overstory' algae, though possibly qualitatively 'poorer' food (Nicotry, 1977) are easier accessible to grazers. It remains questionable, however, to which extent filamentous algae can develop at all under a relatively high, more or less constant grazing pressure as in the present experiment. Evidence exists (Table 7.14) that especially the protruding filamentous forms like *Calothrix* do not develop at all, while chain-like, mat forming species like *Anabaena* do.

It has been demonstrated here that the freshwater pulmonate *L. peregra* has the capacity to reduce periphyton density significantly at a relatively low snail density (90 adults m^{-2} bottom area). Though not for the present species combination (*L. peregra* on *P. pectinatus*), significant periphyton density reductions by grazers have been reported before in the literature (Hunter, 1980; Cattaneo, 1983; Hootsmans & Vermaat, 1985; Howard & Short, 1986).

Hootsmans & Vermaat (1985) demonstrated that *Z. marina* growth was enhanced in the presence of grazers. In the present experiment, however, no apparent growth enhancement occurred because total biomass of the plants remained the same after 3 weeks. When inspected in more detail though, differences in growth did occur: ungrazed plants with a higher periphyton density produced relatively more aboveground biomass, i.e. more leaves. This happened at the account of the tubers, so increased aboveground 'growth' probably was not so much growth from new photosynthetic products as well as increased or prolonged translocation from tubers in the ungrazed plants. Apparently a mechanism similar to that postulated by Sand-Jensen (1977) for *Z. marina* is present: under increased periphyton cover the macrophyte allocates relatively more effort to the production of leaves. Because new leaves are devoid of periphyton, a higher leaf production rate

Table 7.18. Estimation of the contribution of periphyton and phytoplankton to total irradiance reduction and the irradiance finally reaching the macrophyte. Data from the 6 weeks' harvest. Irradiance at 1 cm depth was 100 μE m^{-2} s^{-1}. The extinction of the water of the '0 snails' treatment was 1.0, that of the high density 1.8 m^{-1}, the value for the low density was interpolated. Periphyton attenuance was estimated using the Michaelis-Menten hyperbola of section 3. To facilitate calculations, the 10 cm depth was taken to represent the median position of all photosynthesizing macrophyte material.

snails $aquarium^{-1}$	0	2	9
irradiance at 10 cm depth (μE m^{-2} s^{-1})	90	87	84
periphyton density (mg cm^{-2})	0.26	0.15	0.09
periphyton attenuance (fraction)	0.33	0.23	0.15
irradiance reaching macrophyte at 10 cm depth	60	67	71

may compensate for high periphyton colonization rates.

The surprising absence of any increase in total biomass after 3 weeks and the relatively high contribution of the tubers to total plant biomass may have been caused by high light attenuances of periphyton and planktonic *Kirchneriella*. Extinction by the phytoplankton has been measured and attenuance by the periphyton community was estimated with the Michaelis Menten curve from section 3.2 (Table 7.18).

Apparently, growth of planktonic *Kirchneriella* was stimulated in the presence of grazing snails, either by direct detachment from the macrophyte (not the aquarium walls, cf. Table 7.14) whilst moving over the plant or by indirect stimulation of growth through nutrient release from snail faeces or by enhancing the planktonic state of viable algae from snail faeces (Cuker, 1983). Whichever the process, phytoplanktonic extinction counteracted the reduction of periphyton attenuance due to grazing, (a) leaving only a small difference in irradiance between treatments, and (b) bringing irradiance levels close to the compensation point for *P. pectinatus* of this age (30 - 40 μE m^{-2} s^{-1}, Hootsmans & Vermaat, 1991), where seemingly small differences in irradiance may have significant effects on photosynthesis due to the slope of the photosynthesis-irradiance curve. Indeed, when representative constants from Hootsmans & Vermaat (1991) were used to estimate net photosynthesis at respectiviely 60 and 70 μE m^{-2} s^{-1}, the rate at 70 μE m^{-2} s^{-1} was twice as high (interpolated from calculations with Michaelis Menten light-response curves from plants that had been grown at 50 and 100 μE m^{-2} s^{-1} for 30 days). Competition for inorganic carbon by periphytic and planktonic algae may also have contributed (Sand-Jensen, 1977).

Since phaeopigment concentrations did not increase significantly in the course of the experiment, the plants apparently did not senesce under the unfavourable irradiance conditions. Thus it can be assumed that respiratory needs of the plants still were covered by photosynthesis and allocation from the tuber together. The fact that an increase in leaf biomass in the ungrazed controls was paralleled by a decrease in tuber biomass, stresses the significance of the tuber to plants of this size that experience adverse conditions for photosynthesis.

Similar to the findings in section 2 of this chapter, the snails were less active during night time. This may well indicate

that the assumption of Calow (1973b), that snails feed continuously during day and night when food is continuously available, is not correct. In the present experiment, *L. peregra* did not significantly prefer plants over aquarium walls, which is in agreement with the conclusion from section 4, where no significant effect of substratum on linear velocities and activities was detected for slides compared to *P. pectinatus* covered with similar periphyton.

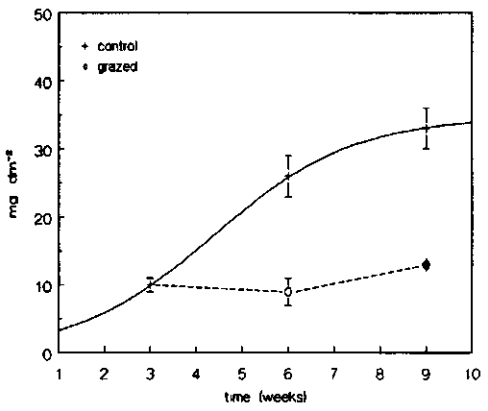


Fig. 7.16. Periphyton density on *P. pectinatus* in the ungrazed and heavily grazed treatments of experiment 7. A logistic curve is fitted through the ungrazed data.

5.3 Estimation of irradiance attenuance by periphyton on *P. pectinatus*, a methodological test

Two methods to estimate transmittance of periphyton remaining after grazing were developed in section 3 and tested for glass slides. In this section a comparison is made on the basis of the high snail density data from experiment 7, to evaluate the methods on true macrophytes. Terminology is similar as in section 3: method A estimates attenuance of remaining periphyton from its biomass and a hyperbola relating biomass to irradiance attenuance. Method B estimates attenuance from direct measurements in a grazing trail, trail widths and linear velocities. Since attenuances of the periphyton on the plants could not be measured directly, the two methods are compared in terms of biomass.

Periphyton growth was estimated with a nonlinear logistic fit of the controls as in Vermaat & Hootsmans (1991c). The fit was highly significant ($r^2=0.88$, $F[3,41]=102.7$, $p<0.001$, Fig. 7.16). Growth on the three sampling days was estimated as the increase in biomass over the sampling day (i.e. a period of one day) as given by the fitted logistic curve. This value was integrated over the total plant area present at that moment. Since periphyton density on the grazed plants did not change significantly during the experiment, it can be assumed that all growth was removed by the snails. Thus, the estimated total removal should be equal to or larger than the integrated growth.

Periphyton removal as estimated by method A (RA) was calculated from the rate of periphyton removal during activity from experiment 2 times the number of active snails present on the plants (n) in experiment 7, i.e. as follows:

$$RA = PRI/activity_{(exp\ 2)} * n_{(exp7)}$$

PRI is a measure of removal averaged over a whole experiment, including both active and inactive periods of an individual snail. Therefore removal during activity was derived from PRI divided by an estimate of the fraction of time that the animals were active during that experiment. RA was estimated at 1.14 mg aquarium^{-1} day^{-1}.

To estimate periphyton removal according to method B, several approaches are possible. It cannot be decided a priori whether linear or areal velocities (trail width * linear velocity) of the snails should be used. If a snail is able to adjust its path width according to the width of the substrate, i.e. the macrophyte leaves, and change its linear speed accordingly, areal velocities are probably more appropriate. However, if changes in the width of a trail have no effect on linear velocity, linear velocities can be used. A linear estimate of total plant area available (i.e. the total sum of all leaf and stem lengths, ΣL) then is necessary. Furthermore, a direct evaluation of the area covered daily by the snails does not necessitate any transformations and may equally well allow conclusions to be drawn on the removal capacity of the snails. Removal according to method B (RB) was estimated as follows:

RB = DB * Fraction of Area Cleaned * Total Plant Area

where DB is the difference in biomass between grazed trails and periphyton before grazing. The biomass in grazed trails was estimated at 3 mg dm^{-2} afdw, from the attenuance in the measured trails of experiment 2 and the appropriately reworked Michaelis-Menten hyperbola. Biomass before grazing was taken from the non-grazed controls (Table 7.14). The fraction of the area cleaned (FAC) was calculated on an areal and a linear basis, by dividing the total area or distance covered by actively grazing animals on the plants in a day by total plant area (TPA). If FAC was higher than 1, it was set to 1.

The average number of actively grazing snails on the plants was estimated at 0.55 from the ratio grazing/active as observed on the aquarium walls and the number of active animals on the plants. Linear velocity was estimated at 190 cm day^{-1} $aquarium^{-1}$, Areal velocity at 76 cm^2 day^{-1} $aquarium^{-1}$. Further results are summarized in Table 7.19. Activity observations were done only in week 5. Thus, if significant variation over time has occurred in activities, accuracy of the removal estimates is less for weeks 3 and 9.

Table 7.19. Estimation of periphyton growth and removal in experiment 7 by two methods. Calculations are explained in the text. Periphyton growth and removal is in mg $aquarium^{-1}$ day^{-1} afdw.

week	3	6	9
plant area (cm^2, two-sided)	37.4	60.3	69.4
ΣL (cm)	274	451	525
FAC (linear)	0.69	0.42	0.36
FAC (areal)	1.0	1.0	1.0
periphyton growth	0.26	0.39	0.54
periphyton removal			
- method A	1.14	1.14	1.14
- method B (linear)	0.77	0.76	0.75
- method B (areal)	1.12	1.81	2.08

As is apparent from Table 7.19, all estimates of removal are higher than those for growth, or, whichever method used to estimate removal, the snails must have been able to suppress periphyton growth. Clearly, method B, using linear velocities comes closest (removal maximally three times higher than growth), while the application of areal velocities produces the largest deviations (removal maximally 5 times higher). Still, all removal estimates remain within one order of magnitude of

growth, which must be considered fairly satisfying when it is taken into account that various values are extrapolated from other experiments.

A general conclusion can be that method B, using the linear basis, provides the closest estimates and thus probably is the best alternative when removal on macrophytes is to be estimated. For fine-leaved macrophytes the approach using linear velocities and an estimation of ΣL, 'total plant length', seems to be the most promising. For more broad-leaved plants the approach using areal velocities may be equally suited, since the snails will probably be able to exploit the full width of their grazing trail.

5.4 The effect of different snail species (experiment 8)

5.4.1 Materials and methods

Three different species were tested, each in an 'intermediate' and a 'high' density: *B. tentaculata*, *L. peregra* and *V. piscinalis*. The intermediate density (45 snails aquarium^{-1} ≈ 500 snails m^{-2} bottom area) was comparable to the high density of experiment 7, the high density amounted to 90 snails aquarium^{-1}, (≈ 1000 snails m^{-2}). To simulate late spring and summer conditions (May, June, July), snail size (shell-length) classes were adjusted accordingly (*B. tentaculata*: 7 - 10 mm, *L. peregra*: 4 - 8 mm, *V. piscinalis*: 3 - 5 mm). These size classes were similar to those used in experiment 2 (section 2). For *L. peregra* relatively small, juvenile animals from a laboratory-reared stock were used, since oviposition and consequent mortality of most of the adult snails of this annual and semelparous species generally has taken place in May in the shallow macrophyte-dominated waters of our latitude (Russel-Hunter, 1961; Young, 1975; Calow, 1978; Dussart, 1979; Reynoldson & Piearce, 1979). *B. tentaculata* reaches an age of 2 to 3 years and reproduces more than once (Calow, 1978) and in *V. piscinalis*, though annual and semelparous, the adults reportedly survive to October (Cleland, 1954). No effort was made to adjust snail densities to initial densities after apparent mortality in the course of the experiment, since this was considered a 'natural' process.

To decrease the 'wall-effect', aquarium dimensions were increased as compared to experiment 7. Nine aquaria of 30 * 30 * 30 cm were placed in a container of 118 * 118 * 50 cm. The walls of the aquaria consisted partly (70%) of gauze (mesh width 1 mm). Together with two Eheim aquarium pumps circulating the container water, this allowed for complete homogenization with respect to nutrient concentrations, temperature and periphyton colonization. The container was filled with 400 liter tap-water to fill the aquaria up to 29 cm. Nutrient concentrations were the same as described in section 2.2.1. The whole system was flushed weekly with approximately 2000 liter tap water and new nutrients were added.

The aquaria were filled with 3 cm of sediment (clay/sand mixture: 1/3) and 25 *P. pectinatus* tubers of a standard size class (0.1 - 0.2 g fresh weight) were planted. The experiment was started in April 1988 and tubers were left to sprout and develop roots. After one week, the snails were added and periphyton was inoculated after homogenization from the periphyton culture of experiment 1 (section 2.2.1). To simulate natural colonization rates, a new periphyton inoculation was performed weekly after every medium

Table 7.20. Summary of χ^2 tests between activity type of live snails and time for the three snail species and two densities. Given are the significance of χ^2 ($p(\chi^2)$), Pearsons correlation coefficient (r) and its significance (p(r)). Thus 5 activity types and 7 moments were cross-tabulated. A significantly positive r indicates that snails spent increasingly more time on the sediment in the course of time and a significantly negative r indicates that increasingly more time was spent on plants and walls.

species	density	$p(\chi^2)$	r	p(r)
B. tentaculata	high	0.000	0.39	0.000
	low	0.000	0.30	0.000
L. peregra	high	0.000	-0.02	0.341
	low	0.002	-0.20	0.001
V. piscinalis	high	0.000	0.38	0.000
	low	0.000	0.49	0.000

flushing. The whole container was covered with mosquito gauze netting to prevent infestation with periphyton consuming chironomid larvae. Irradiance (93 $\pm$ 7 μE m^{-2} s^{-1}, mean $\pm$ sd of five readings at 1 cm depth in every aquarium) was realized with four Philips HPIT 400W lamps, in a diurnal cycle of 12 h dark and 12 h light. Water temperature in the aquaria was maintained at 18 °C with a plastic tube spiral of 20 m length positioned in the container between the aquaria and connected with a chiller-heater at 15 °C. The weekly flushing reduced the water temperature to 16°C and the desired 18 °C was reached again within 6 hours.

The 9 aquaria were assigned randomly to the 3 snail species (each 2 densities) and control treatments (3 aquaria). Observations on snail behaviour were made weekly prior to flushing, except for week 8 and 9. The following activity types were discerned: (1) active on plants, (2) active on aquarium walls, (3) active on sediment, (4) inactive on sediment and (5) rest. Apparently dead animals were also scored (empty shells).

The experiment was terminated in June, 9 weeks after the snails had been added. Five plants per aquarium were selected randomly to be analyzed for plant length and biomass. Previously, periphyton was removed with a razor blade. Single-sided aboveground plant area from 3 plants out of these 5 per aquarium was determined with a Licor LI 3000 leaf area meter and periphyton density was determined as described in section 2.2.1.

5.4.2 Results

Snail activity patterns

The activity patterns that are given in Fig. 7.17 (a-f) for each species at its two densities were tested with χ^2 for interaction between activity type and time. Thus, changes over time in distribution of live snails over activity types were tested. The results of these tests are summarized in Table 7.20. All six χ^2 tests are significant, indicating that indeed the activity patterns changed in the course of the experiment for all three species and at both densities.

Both *B. tentaculata* and *V. piscinalis* showed increasing numbers of animals on or in the sediment in weeks 5 to 7. The snails of these species moved from the plants and aquarium walls to the sediment. The *B. tentaculata* that were classified as inactive in the sediment had clearly burrowed themselves in a hole, the sediment reaching halfway up the shell. Contrary, low density *L. peregra* spent relatively more time on the plants in the second part of the experiment. In weeks 1, 2 and 3 numerous egg capsules had been deposited on the aquarium walls and

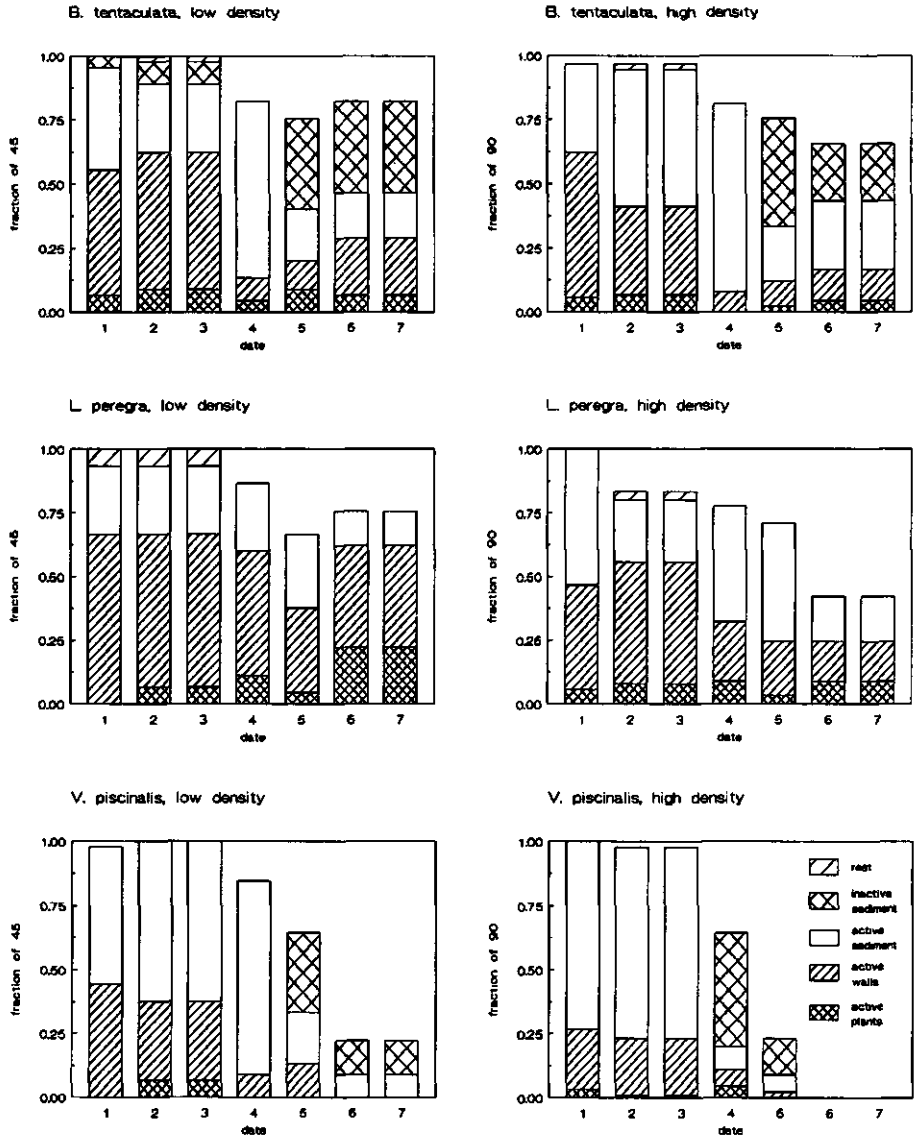


Fig. 7.17. Activity patterns of the three snail species (each two densities) in experiment 8.

Table 7.21. Significances (p) of various factors in two- and threeway ANOVAs relating fractions of animals active on plants and aquarium walls to 'species', 'density' and 'time', and significances of pairwise contrasts after averaging over time or time and density. For the fraction of animals active on plants these are t' contrasts due to inhomogeneity of variances caused by the zero's at time 6 and 7 for *V. piscinalis*. For an EER of 0.05, the CER is held at 0.009 in these sets of 6 non-orthogonal contrasts. Interaction had to be suppressed in the threeway ANOVAs since only one observation per cell remained. Significant p's are indicated with an asterisc.

two- and threeway ANOVAs

	active plants			active walls		
	2way	2way	3way	2way	2way	3way
species	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
density	0.09	-	0.09	0.03*	-	0.00*
time	-	0.36	0.47	-	0.00*	0.00*
species*density	0.63	-	-	0.52	-	-
species*time	-	0.11	-	-	0.93	-

pairwise contrasts	plants	walls
B. tentaculata high vs low	0.021	0.388
L. peregra high vs low	0.379	0.028
V. piscinalis high vs low	0.742	0.388
B. tentaculata vs *L. peregra*	0.119	0.047
B. tentaculata vs *V. piscinalis*	0.000*	0.038
L. peregra vs *V. piscinalis*	0.003*	0.000*

Table 7.22. Linear regression of numbers of alive snails present in the different aquaria against time. Given are the squared correlation coefficient (r^2), the significance of the regression ANOVA (p[AOV]), the slope (b) and the significance of the t test comparing the slopes of the high and low density of each species (p[slope]). Intercepts are not presented since they represent the initial stocking densities (90 and 45, respectively). Also presented are the significances of between-species comparisons of slopes. To maintain an EER of 0.05, the CER of every slope test was held at 0.006; significant p's are indicated with an asterisc. D is snail density.

linear regressions

species	D	r^2	p[AOV]	b	p[slope]
B. tentaculata	high	0.91	0.001*	-5.68	0.0009*
	low	0.67	0.024*	-1.82	
L. peregra	high	0.90	0.001*	-8.61	0.0005*
	low	0.73	0.014*	-2.50	
V. piscinalis	high	0.91	0.001*	-18.32	0.0009*
	low	0.85	0.003*	-6.71	

between-species slope tests

density	high	low
B. tentaculata vs *L. peregra*	0.0316	0.1570
B. tentaculata vs *V. piscinalis*	0.0003*	0.0019*
L. peregra vs *V. piscinalis*	0.0260	0.0053*

the macrophytes by *B. tentaculata* and *V. piscinalis*. No effort was made to quantify this repro-ductive output. Hatching was observed to take place after termination of the experiment, i.e. 10 weeks after the adults had been added to the aquaria and about 8 weeks after deposition of the egg capsules.

Analysis of variance was performed on fractions of animals active on plants and active on aquarium walls (Table 7.21), to establish whether significant differences existed (a) between species, (b) over time, and (c) per species betweendensities. Due to the presence of only one observation per cell, not all factors and their interactions could be tested simultaneously in one threeway ANOVA. To allow evaluation of some interaction terms, the data were entered

into twoway ANOVAs, one with densities pooled and one with the data from different moments pooled. The relevant twoway interactions were not significant, thus a threeway ANOVA with suppressed interactions could be performed. Only the factor 'species' had a significant ($p < 0.05$) 'overall' effect on the fraction of animals active on plants. For active animals on walls all three factors were significant. Subsequently, a set of 6 non-orthogonal contrasts (6 pairwise comparisons: CER=0.009 for an EER of 0.05) was tested, addressing differences between species, and, per species, between densities. Here, only 'species' showed significant effects in the individual contrast tests: *L. peregra* was more active than *V. piscinalis* on both walls and plants, *B. tentaculata* was more active than *V. piscinalis* on plants.

The fractions in Fig. 7.17 sum up to the fraction of animals still alive and present in the aquaria. Numbers of animals alive were entered in a linear regression against time (Table 7.22). For all three species and two densities the regression was significant ($p < 0.05$) and for all three species the slope is significantly steeper for the high density than for the low density. Thus a distinct density effect on the number of surviving animals in the aquaria was present. Also, the slopes of *V. piscinalis* and *B. tentaculata* and those of low density *V. piscinalis* and *L. peregra* were significant. *V. piscinalis* thus died at a significantly higher rate than *B. tentaculata*. It must be mentioned that for *L. peregra* the decline in numbers alive in an aquarium cannot be attributed solely to survival since considerable numbers (10-20) were observed outside the aquaria, apparently having been able to pass the aquarium walls above the water. Therefore, the actual number of surviving animals has been higher and the density dependent reduction in numbers present in the aquaria was the result of both mortality and dispersal. For the two other species the number of remaining animals in the aquaria were true survivors.

Table 7.23. Numbers of animals active on walls and plants in two periods. Period 1 was chosen to represent the time of oviposition for *B. tentaculata* and *V. piscinalis* (weeks 1, 2, 3), period 2 was chosen as the post-breeding period for these two species (weeks 6, 7). Given are mean, standard error (se) and the percentage of live animals that was present on walls or plants, replication in period 1 was 6, in period 2 it was 4. Data were averaged over density, since no significant density effect was present in the two periods (t-tests). Different letters indicate significant differences between means (lsd, EER=0.05, CER=0.003).

species	period 1			period 2		
	mean	se	%	mean	se	%
B. tentaculata	35 c	5	55	14 ab	1	30
L. peregra	39 c	4	65	25 bc	2	70
V. piscinalis	20 b	3	30	0 a	0	-

In section 5.2 it was concluded that aquarium walls and macrophytes can be considered equivalent with respect to snail activity. Thus the fractions active on walls can be added to fractions active on plants for a tentative estimation of the active fraction of a population present on submerged vegetation under field conditions. For this purpose, two distinct periods have been discerned: period 1, the oviposition period for *B. tentaculata* and *V. piscinalis* (weeks 1, 2 and 3 of Fig. 7.17), and period 2, the post-breeding period of these two species (weeks 6 and 7). Period 2 can be considered as a period in which densities of *L. peregra* had stabilized (Fig. 7.17). For each separate period and each species, no significant differences in numbers of snails present on both walls and plants could be detected between the two

Table 7.24. Periphyton density (mg dm^{-2} leaf surface, afdw and ash weight, ash also expressed as % of dry weight) and plant characteristics of experiment 8. Given are mean and standard error (se), replication was 3 (9 for the controls) for periphyton and plant area (cm^2 $plant^{-1}$, two-sided) and 5 (15 for the controls) for biomass (mg $plant^{-1}$ afdw) and length (cm). For every characteristic the significance (p) of a oneway ANOVA is also given. Periphyton densities (afdw and ash) pooled over snail density (i.e. high + low) were significantly lower than controls except for *V. piscinalis* (contrast tests, EER=0.05).

		control	*B. tentaculata*		*L. peregra*		*V. piscinalis*		p
			high	low	high	low	high	low	
periphyton									
afdw	mean	13.0	7.5	3.9	3.8	2.6	10.4	4.0	0.009
	se	2.3	0.8	1.2	1.2	0.4	1.1	0.3	
ash	mean	16.2	6.1	2.2	4.1	7.0	8.7	3.7	0.001
	se	2.3	0.8	0.3	0.5	1.9	0.9	1.1	
% ash of dw		56	45	38	53	71	46	45	
plant biomass									
tuber	mean	8	10	7	10	11	8	7	0.504
	se	1	3	1	2	1	1	2	
roots +	mean	35	31	47	33	33	47	41	0.394
rhizomes	se	3	4	11	4	8	4	3	
aboveground	mean	95	95	109	114	119	101	107	0.797
	se	6	10	19	18	11	8	10	
plant morphometry									
aboveground	mean	130	136	166	160	154	104	134	0.756
area	se	24	18	42	36	22	6	16	
length	mean	70	75	72	66	68	69	64	0.368
	se	3	3	3	2	5	2	3	

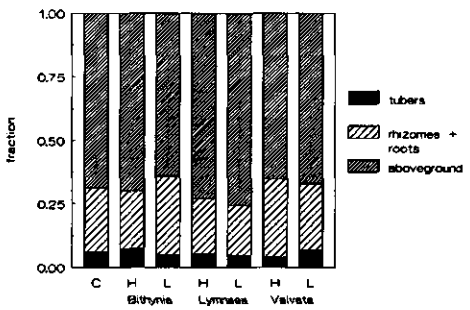


Fig. 7.18. Relative biomass distribution of *P. pectinatus* in experiment 8. C = control, H = high density, L = low density.

densities (t-tests). This is similar to the results of experiment 7. Densities were therefore pooled to test for differences in numbers present on walls and plants between species and periods (Table 7.23). It can be concluded that slightly more than half of the population of *B. tentaculata* was present on plants and walls during the oviposition period. This was significantly less in the second period. Although migration had obviously occurred, the numbers of *L. peregra* were not significantly different in the two periods. For *V. piscinalis*, post-breeding mortality had been very high, with obvious consequences for the number of animals present on plants or walls. When comparing species, *L. peregra* and *B. tentaculata* did not differ significantly in numbers present in the first period, while *V. piscinalis* was significantly lower in the first period. In the second period, *B. tentaculata* was intermediate.

Periphyton density and macrophyte characteristics

All data are gathered in Table 7.24 and Fig. 7.18. Presence of the different grazers apparently had no effect on any plant characteristic, though periphyton density, in terms of dry or ash weight, was significantly reduced relative to controls by all three species. In terms of afdw, *V. piscinalis* did not reduce periphyton density significantly. No phytoplankton blooms were observed during the experiment. Initial tuber weight was estimated from the regression line relating tuber freshweight to afdw in Vermaat and Hootsmans (1991a) at 67 mg afdw. Final tuber weight after 9 weeks thus was 12% of initial weight (8 mg overall mean). Specific aboveground area was 1300 cm^2 g^{-1} afdw (two-sided) on average.

5.4.3 Discussion

Snail activity patterns

V. piscinalis spent a relatively short time on macrophytes (and aquarium walls). Contrary to the findings of Cleland (1954), who found survival of adults into October, most post-breeding mortality had taken place within three weeks after the animals left the plants. Presence of adult *V. piscinalis* on macrophytes and consequent impact on periphyton thus probably is restricted to a relatively short period of one month at most. With respect to this, the present findings are in agreement with those of Cleland (1954) and Young (1975). Whether juvenile, recently hatched *V. piscinalis* had any effect cannot be concluded from the present data. Cleland (1954) reported that their presence on macrophytes was restricted to a few weeks. A tentative estimate of the proportion of adults that is present on macrophytes during the oviposition period may be 30% for a population of relatively high density (assuming that walls can be considered similar to plants, Table 7.23).

From the macrophytes' point of view, the activity pattern of *B. tentaculata* was more similar to that of juvenile *L. peregra*. During oviposition 50% of the animals or more may be present on the macrophytes, in densities similar to those of *L. peregra*. After the eggs had been deposited, a considerable fraction of the adult animals returned to the sediment and burrowed themselves, apparently turning into some state of low activity. Still, some 30% were observed on macrophytes and walls, which is also in agreement with the conclusions of Cleland (1954) and Young (1975).

In contrast to the other two species, *L. peregra* remained fairly active during the whole experiment and present on the plants and walls in high proportions, which is in agreement with the results of Young (1975). Though quite some snails were able to leave the aquaria to the main container, this did not significantly influence the total number of snails present on walls and plants (Table 7.23). The proportion of a high density population of *L. peregra* present on macrophytes in the field then may tentatively be estimated at about 70% (week 6 and 7). Total plant and wall area amounted to 88% of the total available area after 9 weeks. If this figure is corrected for assumed growth of the plants, these two percentages do not differ much. *L. peregra* apparently did not prefer any of the three substrates. Similarly, Lodge (1985) did not find any substrate preference (surface of different species of macrophytes) in *L. peregra* while Lodge (1986) did find preferences for specific types of periphyton.

In general it can be concluded that the present findings on overall activity distribution patterns are in good agreement with field observations. Hence, the relative distribution estimates over plants and sediments as given above can probably be extrapolated to field populations.

Various factors may be of significance to the timing of presence and oviposition on macrophytes. Two of these will be discussed in the following. For a more general discussion of life cycle strategies in freshwater gastropods, the reader is referred to Calow (1978, 1981) or Lodge & Kelly (1985).

A first factor is the generally observed periphyton spring bloom of temperate latitudes (McMahon et al., 1974; Mason & Bryant, 1975; Cattaneo & Kalff, 1978; Gons, 1982; Cattaneo, 1983; Meulemans & Heinis, 1983; Kairesalo, 1984; Meulemans & Roos, 1985; Cattaneo, 1987). Also, periphyton is reportedly superior in nutritive quality to benthic communities (McMahon et al., 1974; Mason & Bryant, 1975). Thus presence on macrophytes during the spring bloom of periphyton may have clear nutritive advantages. Apparently, the three species tested here have different timing in their annual cycles with respect to this spring bloom: in *V. piscinalis* and *B. tentaculata* adults and in *L. peregra* juveniles are present during the spring bloom (Cleland, 1954; Young, 1975; Calow, 1978; Dussart, 1979).

The second factor is the seasonal fluctuation of predation pressure. Various fish species are known molluscivores (Covich & Knezevich, 1978; Brown & DeVries, 1985; Lodge, 1986; Lammens, 1989; Osenberg, 1989), but their food-intake is generally low in winter and early spring (Gilinsky, 1984). Oviposition early in the season then may be of adaptive value to the soft-shelled, relatively vulnerable *L. peregra* in reducing mortality among the relatively costly adults that survived through winter.

Differences between waterbodies in seasonal availability of a suitable habitat may well determine the presence of spec-

ific snail species. Aquatic vegetation must be considered an important refuge for soft-shelled pulmonates against decimation through predation (Covich & Knezevich, 1978; Gilinsky, 1984) in a similar vein as suggested for zooplankton in a.o. Irvine et al. (1989). In an exclosure experiment, Brönmark (1988) found that snail density increased with increasing macrophyte biomass, both with and without fish, but in the latter case the slope was steeper. Lodge et al. (1987) state that 'among and within waterbodies available habitats and food determine distribution and abundance' of freshwater snails, 'if levels of disturbance, competition and predation are low', while in permanent lakes especially 'predation reduces species richness'. Since in permanent lakes the presence of aboveground vegetation is often restricted (e.g. June - August in Lake Veluwe, The Netherlands; Van Dijk & Van Vierssen, 1991), it is probably the interaction between absence of refuge and predation that reduces species richness and abundance. This is of especial significance to species like *L. peregra*, for which the refuge also forms the substratum that provides food, i.e. periphyton. Though contradicted by Cleland (1954), Lodge & Kelly (1985) suggested that *V. piscinalis* and *B. tentaculata* are 'capable of a form of filter feeding in which suspended particles are extracted by the gills'. A similar statement can be found in Fretter & Graham (1962). Together with their reportedly lower vulnerability to predation (Lodge & Kelly, 1985; Lodge et al., 1987) this may explain why these prosobranchs persist in permanent lakes where macrophyte abundance is reduced.

Periphyton density and macrophyte characteristics

Though *L. peregra* and *B. tentaculata* had significantly reduced periphyton density in terms of afdw, no differences were found in any of the measured plant characteristics. This is contrary to the results of experiment 7. Another striking difference between the two experiments is apparent in plant biomass: newly formed biomass was 5 times higher in experiment 8 for plants of equal age. Two hypotheses may explain this difference:

(1) The differences in plant biomass were caused by differences in available experimental 'space', i.e. water- or sediment-volume or area per plant.

(2) The differences can be explained by differences in light climate and/or competition for carbon and nutrients due to differences in periphyton and phytoplankton density.

The differences in plant biomass are listed in Table 7.25, together with the results from a growth experiment with plants of similar age grown under a similar light and temperature regime from Vermaat & Hootsmans (1991b). Total newly formed biomass (new biomass = aboveground + roots & rhizomes) of experiment 8 and the temp./light experiment was not significantly different, even though large differences existed between the two experiments in aquarium depth, area per plant, water volume per plant and sediment volume. The difference in biomass between experiment 7 and 8 cannot be explained by differences in available 'space' since it is similar (depth, available volume per plant) or higher for experiment 7 (area, sediment volume per plant). The first hypothesis thus can be rejected.

Turning to the second hypothesis, the following points should be mentioned: (a) Though covered with periphyton, the plants from experiment 8 reached a similar biomass as the 'clean' plants of the temp./light experiment. (b) The periphyton cover of the controls in experiment 8 after 9 weeks was similar to that of the

Table 7.25. Final plant biomass (mg plant^{-1} afdw) and experimental dimensions in three different experiments: experiment 7 (ungrazed controls of the harvest at 9 weeks, replication is 11), experiment 8 (ungrazed controls, replication is 15) and the 100 μE m^{-2} s^{-1}/22 °C treatment of Vermaat & Hootsmans (1991b, 'temp./light', harvested after 8 weeks, replication is 20). Given are mean, standard error (se) and the result of an lsd test per biomass characteristic (EER = 0.05), different letters indicate significant differences between means.

experiment	7		8		temp./light	
experimental dimensions per plant						
water volume (ml)	1025		856		1505	
sediment volume (ml)	210		101		135	
area (cm^2)	53		34		72	
depth (cm)	20		26		21	
plant biomass	mean	se	mean	se	mean	se
aboveground	23 a	2	93 b	6	94 b	5
roots & rhizomes	2 a	1	35 b	3	44 c	2
total new	25 a	2	128 b	8	137 b	7
tuber	14 b	1	8 a	1	15 b	1

highly grazed plants in experiment 7 (cf. Tables 7.14 and 7.23), estimatedly transmitting 80% of incoming irradiance (Michaelis-Menten hyperbola, section 3.2), while no phytoplankton bloom occurred, (c) Net photosynthetic rates at 74 μE m^{-2} s^{-1} (i.e. the light reaching controlplants in experiment 8) have probably been about twice as high as the rates at 60 μE m^{-2} s^{-1} (the light reaching control plants of experiment 7, compare section 5.2). Therefore the second hypothesis is not rejected, and the cumulative effect of a seemingly small difference in irradiance reaching the macrophyte ($\pm$ 74 versus 60 μE m^{-2} s^{-1}) together with possible competition for carbon and nutrients is held responsible for the large differences in plant biomass after 9 weeks.

The absence of an effect of periphyton removal now can easily be explained from the above: periphyton cover of the control plants was simply not high enough. In this respect, the applied inoculation procedure apparently failed to simulate accumulation rates as observed in the field during the spring bloom in June (up to 2.1 mg dm^{-2} day^{-1} afdw in Lake Veluwe, Van Vierssen & Bij De Vaate, 1990). Still, it may be speculated that the significantly lower remaining biomass of the tuber in experiment 8 as compared to the temp./light experiment (Table 7.25) is the result of a higher reallocation of tuber material, possibly as a response to the higher periphyton cover reducing photosynthesis. The remaining tuber mass in experiment 7 was not significantly different from that of the temp./light experiment (Table 7.24). This may be explained by the fact that growth in experiment 7 was low after the third week and the assumption that reallocation from the tuber is directly coupled to aboveground growth in a source-sink relation (Watson & Cooper, 1984).

6. Conclusions

All tested freshwater snail species (*B. tentaculata*, *L. peregra*, *P. fontinalis* and *V. piscinalis*) proved able to significantly remove periphyton from glass slides, while the two crustacean species (*A. aquaticus* and *G. pulex*) did not. For the snails, most between-species differences in removal rate per individual could be explained by differences in snail biomass and activity. The four snail species differed also in width of the grazing trail, linear velocity and in density and attenuance of the remaining periphyton in the trail.

A significant temperature effect on periphyton removal was found only once within the tested temperature range (10° - 20 °C). A fairly efficient temperature compensation mechanism appears to be present.

For similar taxonomic composition and density of periphyton, *L. peregra* behaviour on glass slides was not different from that on the macrophyte *P. pectinatus*. Different taxonomic composition of periphyton on *P. pectinatus*, however, did influence both linear velocity and activity of the snails.

In both experiments on the effect of periphyton grazing on macrophyte growth (nos 7 & 8), periphyton density was significantly reduced in the presence of grazers. Plant growth was affected only in the first experiment due to more favourable light conditions in the second experiment. In the first experiment the plants reacted to a denser periphyton cover by reallocating more tuber material and investing this in leaves.

The activity patterns of *L. peregra*, *B. tentaculata* and *V. piscinalis* showed distinct differences in the course of experiment 8. *L. peregra* remained active throughout the whole period and was present on plants and aquarium walls in considerable numbers. *V. piscinalis*, in contrast, only spent a month on the macrophytes during oviposition, after which all adults rapidly died. *B. tentaculata* had a somewhat intermediate pattern: after oviposition the larger part of the animals burrowed themselves in the sediment, but remained alive.

Attenuance reduction as a consequence of periphyton removal can be estimated from the remaining material on slides and an attenuance-density hyperbola, provided that 60% of the biomass is removed by the grazers. Else, more detailed measurements are to be preferred, involving estimates of trail width, linear velocity and attenuance in the grazing trail. In an evaluation on the basis of periphyton growth and removal in a *P. pectinatus* vegetation (experiment 7), the latter method produced the more accurate estimates.

Acknowledgements

The students H. de Vries and J. Smits are thanked for their contribution to experiment 4, A. Fentener van Vlissingen for part of experiment 4 and for experiment 6, J. Veth and A. Smit for experiment 5 and H. Bakker for experiment 7. Dr. M.A.J. van Montfort and Dr. A. Pols (Agricultural University, Dept. of Mathematics) for statistical advice and M.J.M. Hootsmans for valuable discussions and practical assistence. M.J.M. Hootsmans, Prof. Dr. W. van Vierssen and Prof. Dr. W.J. Wolff critically read the manuscript.

7. References

Belcher, H. & Swale, E., 1976. A beginner's guide to freshwater algae. Culture Centre of Algae and Protozoa, Cambridge, UK, 47 pp.

Borum, J., 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. Mar. Biol. 87: 211-218.

Bovbjerg, R.V., 1968. Responses to food in Lymnaeid snails. Physiol. Zool. 41: 412-423.

Brönmark, C., 1985. Interactions between macrophytes, epiphytes and herbivores: an experimental approach. Oikos 45: 26-30.

Brönmark, C., 1988. Effects of vertebrate predation on freshwater gastropods: an exclosure experiment. Hydrobiologia 169: 363-370.

Brown, K.M. & DeVries D.R., 1985. Predation and the distribution and abundance of a pulmonate snail. Oecologia 66: 93-99.

Calow, P., 1970. Studies on the natural diet of *Lymnaea pereger obtusa* (Kobelt) and its possible ecological implications. Proc. malac. Soc. Lond. 39: 203-215.

Calow, P., 1973a. Field observations and laboratory experiments on the general food requirements of two species of freshwater snail, *Planorbis contortus* (Linn.) and *Ancylus fluviatilis* Müll. Proc. malac. Soc. Lond. 40: 483-489.

Calow, P., 1973b. On the regulatory nature of individual growth: some observations from freshwater snails. J. Zool. Lond. 170: 415-428.

Calow, P., 1975. The feeding strategies of two freshwater gastropods, *Ancylus fluviatilis* Müll. and *Planorbis contortus* Linn. (Pulmonata), in terms of ingestion rates and absorption efficiencies. Oecologia 20: 33-49.

Calow, P., 1978. The evolution of life-cycle strategies in freshwater gastropods. Malacologia 17: 351-364.

Calow, P., 1981. Adaptational aspects of growth and reproduction in *Lymnaea peregra* (Gastropoda: Pulmonata) from exposed and sheltered habitats. Malacologia 21: 5-13.

Calow, P. & Calow L.J., 1975. Cellulase activity and niche separation in freshwater gastropods. Nature 255: 478-480.

Cambridge, M.C., Chiffings, A.W., Brittan, C., Moore, L., & McCombe A.J., 1986. The loss of seagrass in Cockburn Sound, Western Australia II. Possible causes of seagrass decline. Aquat. Bot. 24: 269-285.

Cattaneo, A., 1983. Grazing on epiphytes. Limnol. Oceanogr. 28: 124-132.

Cattaneo, A., 1987. Periphyton in lakes of different trophy. Can. J. Fish. Aquat. Sci. 44: 296-303.

Cattaneo, A. & Kalff, J., 1978. Seasonal changes in the epiphytic community of natural and artificial macrophytes in Lake Memphremagog (Que & Vt). Hydrobiologia 60: 135-144.

Cleland, D.M., 1954. A study of the habits of *Valvata piscinalis* (Müller) and the structure and function of the alimentary canal and reproductive system. Proc. malac. Soc. Lond. 30: 167-203.

Covich, A.P. & Knezevich, B., 1978. Size-selective predation by fish on thin-shelled gastropods (*Lymnaea*); the significance of floating vegetation (*Trapa*) as a physical refuge. Verh. internat. Verein. Limnol. 20: 2172-2177.

Cuker, B.E., 1983. Grazing and nutrient interactions in controlling the activity and composition of the epilithic algal community of an arctic lake. Limnol. Oceanogr. 28: 133-141.

Dussart, G.B.J., 1979. Life cycles and distribution of the aquatic gastropod molluscs *Bithynia tentaculata* (L.), *Gyraulus albus* (Muller), *Planorbis planorbis* (L.) and *Lymnaea peregra* (Muller) in relation to water chemistry. Hydrobiologia 67: 223-239.

Fretter, V. & Graham, A., 1962. British prosobranch molluscs. Ray Society, London, UK, 755 pp.

Gilinsky, E., 1984. The role of fish predation and spatial heterogeneity in determining benthic community structure. Ecology 65: 455-468.

Gons, H.J., 1982. Structural and functional characteristics of epiphyton and epipelon in relation to their distribution in Lake Vechten. Hydrobiologia 95: 79-114.

Hassel, M.P., 1978. The dynamics of arthropod predator prey systems. Monographs in Population Biology 13. Princeton University Press, Princeton, New Jersey, USA, 237 pp.

Hootsmans, M.J.M. & Vermaat, J.E., 1985. The effect of periphyton grazing by three epifaunal species on the growth of *Zostera marina* L. under experimental conditions. Aquat. Bot. 22: 83-88.

Hootsmans, M.J.M. & Vermaat, J.E., 1991. Light response curves of *Potamogeton pectinatus* L. as a function of plant age and irradiance level during growth. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Howard, R.K. & Short, F.T., 1986. Seagrass growth and survivorship under the influence of epiphyte grazers. Aquat. Bot. 24: 287-302.

Hunter, D.R., 1980. Effects of grazing on the quantity and quality of freshwater aufwuchs. Hydrobiologia 69: 251-259.

Hylleberg, J., 1975. The effect of salinity and temperature on egestion in mud snails (Gastropoda: Hydrobiidae), a study on niche overlap. Oecologia 21: 279-289.

Irvine, K., Moss, B. & Balls, H., 1989. The loss of submerged plants with eutrophication. II. Relationships between fish and zooplankton in a set of experimental ponds, and conclusions. Freshwat. Biol. 22: 89-107.

Kairesalo, T., 1984. The seasonal succession of epiphytic communities within an *Equisetum fluviatile* L. stand in Lake Pääjärvi, southern Finland. Int. Rev. ges. Hydrobiol. 69: 475-505.

Kairesalo, T. & Koskimies, I., 1987. Grazing by oligochaetes and snails on epiphytes. Freshwat. Biol. 17: 317-324.

Kirk, J.T.O., 1983. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge, UK, 401 pp.

Krebs, J.R. & Davies, N.B., 1984. Behavioural ecology: an evolutionary approach. Second edition, Blackwell, Oxford, UK, 514 pp.

Lammens, E.H.R.R., 1989. Causes and consequences of the succes of Bream in Dutch eutrophic lakes. Hydrobiol. Bull. 23: 11-18.

Lodge, D.M., 1985. Macrophyte gastropod associations: observations and experiments on macrophyte choice by gastropods. Freshwat. Biol. 15: 695-708.

Lodge, D.M., 1986. Selective grazing on periphyton: a determinant of freshwater gastropod microdistributions. Freshwat. Biol. 16: 831-841.

Lodge, D.M. & Kelly, P., 1985. Habitat disturbance and the stability of freshwater gastropod populations. Oecologia 68: 111-117.

Lodge, D.M., Brown, K.M., Klosiewski, S.P., Stein, R.A., Covich, A.P., Leathers, B.K. & Brönmark, C., 1987. Distribution of freshwater snails: spatial scale and the relative importance of physicochemical and biotic factors. Amer. Malac. Bull. 5: 73-84.

Marcus, J.H., Sutcliffe, D.W. & Willoughby, L.G., 1978. Feeding and growth of *Asellus aquaticus* (Isopoda) on food items from the littoral of Windermere, including green leaves of *Elodea canadensis*. Freshwat. Biol. 8: 505-519.

Mason, C.F. & Bryant, R.J., 1975. Periphyton production and grazing by chironomids in Alderfen Broad, Norfolk. Freshwat. Biol. 5: 271-277.

McMahon, R.F., Hunter, R.D. & Russel-Hunter, W.D., 1974. Variation in aufwuchs at six freshwater habitats in terms of carbon biomass and of carbon:nitrogen ration. Hydrobiologia 45: 391-404.

Meulemans, J.T. & Heinis, F., 1983. Biomass and production of periphyton attached to dead reed stems in Lake Maarsseveen. In Wetzel, R. (ed.), Periphyton of freshwater ecosystems. Junk, The Hague, The Netherlands, pp. 169-173.

Meulemans, J.T. & Roos, P.J., 1985. Structure and architecture of the periphytic community on dead reed stems in Lake Maarsseveen. Arch. Hydrobiol. 102: 487 - 502.

Moed, J.R. & Hallegraef, G.M., 1978. Some problems in the estimation of chlorophyll-a and phaeopigments from pre- and post-acidification spectrophotometric measurements. Int. Revue ges. Hydrobiol. 63: 787-800.

Moore, J.W., 1975. The role of algae in the diet of *Asellus aquaticus* L. and *Gammarus pulex* L. J. Anim. Ecol. 44: 719-730.

Nederlandse Praktijkrichtlijn, 1984. NPR 6505, benodigdheden, werkwijze en medium voor het kweken van algen (Dutch Standards, Materials and methods for the cultivation of algae, in Dutch). Nederlands Normalisatie Instituut, Delft, 4 pp.

Nicotri, M.E., 1977. Grazing effects of four marine intertidal herbivores on the microflora. Ecology 58: 1020-1032.

Norusis, M.J., 1986. SPSS-PC$^+$ manual. SPSS Inc., Chicago, USA, 559 pp.

Orth, R.J. & Van Montfrans, J., 1984. Epiphyte seagrass relationships with an emphasis on the role of micrograzing: a review. Aquat. Bot. 18: 43-69.

Osenberg, C.W., 1989. Resource limitation, competition and the influence of life history in a freshwater snail community. Oecologia 79: 512-519.

Phillips, G.L., Eminson, D.F. & Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated waters. Aquat. Bot. 4: 103-125.

Reddingius, J., Schilstra, A.J. & Thomas, G., 1983. The grid method in estimating path length of a moving animal. J. Anim. Ecol. 52: 199-209.

Reynoldson, T.B. & Piearce, B., 1979. Predation on snails by two species of triclad and its bearing on the distribution of *Planaria torva* in Britain. J. Zool. London 189: 459-484.

Russel Hunter, W., 1961. Life cycles of four freshwater snails in limited populations in Loch Lomond, with a discussion of infraspecific variation. Proc. Zool. Soc. London 137: 135-171.

Russel-Hunter, W.D. & Eversole, A.G., 1976. Evidence for tissue degrowth in starved freshwater pulmonate snails (*Helisoma trivolvis*) from tissue, carbon, and nitrogen analyses. Com. Biochem. Physiol. 54A: 447-453.

Sand-Jensen, K., 1977. Effect of epiphytes on eelgrass photosynthesis. Aquat. Bot. 3: 55-63.

Sand-Jensen, K. & Borum, J., 1984. Epiphyte shading and its effect on photosynthesis and diel metabolism of *Lobelia dortmanna* L. during the spring bloom in a Danish lake. Aquat. Bot. 20: 109-119.

Silberstein, K., Chiffings, A.W. & McComb, A.J., 1986. The loss of seagrass in Cockburn Sound, Western Australia III. The effect of epiphytes on the productivity of *Posidonia australis* Hook.F. Aquat. Bot. 24: 355-371.

Soszka, G.J., 1975. The invertebrates on submerged macrophytes in three Masurian lakes. Ekol. Pol. 23: 371-391.

Steel, R.G.D. & Torrie, J.H., 1980. Principles and procedures of statistics, a biometrical approach. Second edition. Mc Graw Hill Book Company, Singapore, 633 pp.

Streble, H. & Krauter, D., 1985. Das Leben im Wassertropfen: Mikroflora und Mikrofauna des Süsswassers; ein Bestimmungsbuch. 7. Auflage, Kosmos - Franckh'sche Verlagshandlung, W. Keller & Co, Stuttgart, BRD, 369 pp.

Sumner, W.T. & McIntire, C.D., 1982. Grazer-peripyton interactions in laboratory streams. Arch. Hydrobiol. 93: 135-157.

Van Dijk, G.M. & Van Vierssen, W., 1991. Survival mechanisms of a *Potamogeton pectinatus* L. population under different light conditions in a shallow eutrophic lake (Lake Veluwe) in The Netherlands. Aquat. Bot. 39: 121- 129.

Van Montfrans, J., Orth, R.J. & Vay, S.A., 1982. Preliminary studies of grazing by *Bittium varium* on eelgrass periphyton. Aquat. Bot. 14: 75-89.

Van Vierssen, W., 1982. The ecology of communities dominated by *Zannichellia* taxa in western Europe. II. Distribution, synecology and productivity aspects in relation to environmental factors. Aquat. Bot. 13: 385-483.

Van Vierssen, W. & Bij De Vaate, A., 1990. Licht en waterplanten, oorzaken van biomassafluctuaties van onderwatervegetaties in het Veluwemeer (Light and submerged macrophytes, causes for macrophyte biomass fluctuations in Lake Veluwe, in Dutch). Report of the Institute for Inland Water Management and Waste Water Treatment, Wageningen Agricultural University, Dept. of Nature Conservation and the International Institute for Hydraulic and Environmental Engineering, 233 pp.

Vermaat, J.E. & Hootsmans, M.J.M., 1991a. Intraspecific variation in *Potamogeton pectinatus* L., a controlled laboratory experiment. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vermaat, J.E. & Hootsmans, M.J.M., 1991b. Growth of *Potamogeton pectinatus* L. in a temperature-light gradient. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vermaat, J.E. & Hootsmans, M.J.M., 1991c. Periphyton dynamics in a temperature-light gradient. In Hootsmans, M.J.M. & Vermaat, J.E., Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Vernon, L.P., 1960. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Analytic Chemistry 32: 1144-1150.

Watson, M.A. & Cooper, B.B., 1984. Morphogenetic constraints on patterns of carbon distribution in plants. Ann. Rev. Ecol. Syst. 15: 233-258.

Wetzel, R.G., 1983. Opening remarks. In Wetzel, R.G. (ed.), Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 3-4.

Young, M.R., 1975. The life cycles of six species of freshwater molluscs in the Worcester-Birmingham canal. Proc. malac. Soc. London 41: 533-548.

ENCLOSURE EXPERIMENTS IN *POTAMOGETON PECTINATUS* L. DOMINATED FRESHWATER ECOSYSTEMS

8

J.E. Vermaat & M.J.M. Hootsmans

Contents

1. Introduction

Enclosures are considered a useful experimental tool since they combine the separability and replicability of treatment effects of laboratory experiments with a higher degree of natural reality (environmental variation, interactive complexity of a natural ecosystem; Kuiper, 1977; Bloesch et al., 1988). Thus, the use of enclosures enables one to test conclusions from indoor laboratory experiments and, in general, field hypotheses under controlled field conditions.

Since Lund (1972) presented the first results from two large enclosing tubes, the use of enclosures of various dimensions and fabric has become increasingly popular among aquatic ecologists. Most freshwater studies concerned pelagic trophic interactions and relatively deep (> 2 m) enclosures were used with (cf. Lack & Lund, 1974; Andersson et al., 1978) or without sediment contact (McCauley & Briand, 1979; Shapiro & Wright, 1984; Riemann & Søndergaard, 1986; Brabrand et al., 1987; Post & McQueen, 1987; Bloesch et al., 1988).

Relatively few investigators (Jupp & Spence, 1977; Leah et al., 1978; Moss & Leah, 1982; Godmaire & Planas, 1983) applied enclosures in a shallow littoral ecosystem including aquatic macrophytes. Leah et al. (1978) and Moss & Leah (1982) attempted to elucidate the mechanisms responsible for the large scale reduction of aquatic vegetation in the lake system of the Norfolk Broads (England). Here, we will try to make a connection between the mainly littoral-oriented model of Phillips et al. (1978) that we used as a general working hypothesis for our understanding of macrophyte-dominated ecosystems, and the pelagic-oriented hypothesis on the so-called cascading effect of piscivorous fish via planktivorous fish and zooplankton on phytoplankton (cf. Carpenter et al., 1985; McQueen & Post, 1988).

Our laboratory experiments suggested that (a) *Potamogeton pectinatus* L. beds can produce allelopathic substances that significantly reduce phytoplankton growth (Hootsmans, 1991) and (b) freshwater snails can significantly reduce periphyton densities and consequently enhance the growth of *P. pectinatus* (Vermaat, 1991). The aim of our enclosure experiments was to assess the significance of these two mechanisms for macrophyte performance relative to effects of pelagic (zoo-)planktivorous and benthivorous fish. Also, we wanted to elucidate the zooplanktivorous potential of small littoral fish that inhabit aquatic vegetation. Planktivory by littoral fish may be significant for pelagic zooplankton and phytoplankton dynamics and may thereby influence environmental conditions for macrophytes.

In 1987 and 1988 four efforts were made to install an experimental enclosure set-up in a *P. pectinatus* vegetation in Lake Veluwe (The Netherlands), but only the last two experiments were successful. The 1987 experiments were done with pvc as the flexible material of the enclosing column, and had to be pre-treated to reduce the diffusion of highly toxic pvc-flexibilizing phtalates. The pre-treatment consisted of a spreading out of the pvc columns in tanks with running tap water for three weeks. The first experiment was started in early June 1987 but the enclosures were torn to pieces by a storm in the second week. Consequently, a second effort was made in July, but the pre-treatment was reduced to a 10 days' washing with warm water, to save time and keep the experimental period within the growing season of the macrophyte. In the

third week, however, we observed high mortality of the added snails and a significant deterioration of the vegetation in the enclosures as compared to surrounding vegetation, probably due to still too high phtalate concentrations.

We used late summer of 1987 to investigate the effects of small littoral fish (three-spined sticklebacks, *Gasterosteus aculeatus* L.) on zooplankton and phytoplankton stocks in an enclosure experiment that lasted four weeks. This will be dealt with in section 2 of this chapter. The third section deals with the enclosure experiment done in 1988 using non-toxic polythene and gauze as the flexible material.

2. Zooplankton predation by three-spined sticklebacks (*Gasterosteus aculeatus* L.)

J.E. Vermaat & M.J.M. Hootsmans

Abstract

The three-spined stickleback (*Gasterosteus aculeatus* L.) is an abundant polyphagous fish in shallow waters and the littoral zones of lakes of North-Western Europe. In enclosures, three-spined sticklebacks stocked in field densities significantly reduced daphnid and bosminid numbers relative to enclosures without fish, while phytoplankton density was higher in the presence of the sticklebacks. This resulted in zooplankton and phytoplankton densities similar to those in the surrounding eutrophic Lake Veluwe, suggesting that the potential planktivorous effect of three-spined sticklebacks in field densities is similar to that of a pelagic fish community dominated by bream (*Abramis brama* L.).

2.1 Introduction

Three-spined sticklebacks (*Gasterosteus aculeatus* L.) occur in relatively high numbers in shallow waters and the littoral zones of lakes (2-17 m^{-2}, Wootton, 1976) of North-Western Europe. They are polyphagous: they consume copepods, cladocerans, other crustaceans, oligochaetes, larvae and pupae of chironomids, juvenile lymnaeids and a variety of laboratory feeds (Beukema, 1968; Wootton, 1976; personal observations). A short life-span (generally about one year) and annual breeding may enable strong numerical responses to favourable food conditions. Increased densities of periphyton-feeding invertebrates, as a result of increasing periphyton growth in the course of eutrophication (Brown & DeVries, 1985; Lodge et al., 1987; Osenberg, 1989), may constitute such favourable food conditions. Three-spined sticklebacks are known to feed also on various types of zooplankton (Wootton, 1976) and in general littoral fish may use the pelagic as a foraging area (Mittelbach, 1981; Butler, 1989). Thus, a numerical response of sticklebacks to an increase in periphytic invertebrates may have consequences for pelagic zooplankton food stocks. Reduced zooplankton density then may result in increased phytoplanton density and shading of macrophytes.

In general, the pelagic 'cascading effect' (or 'top-down') studies (Carpenter et al., 1985; McQueen & Post, 1988) have concentrated on pelagic fishes. Especially in shallow lakes with well-developed littoral zones, littoral fish stocks may also be of importance for the dynamics of zooplankton and, eventually, of phytoplankton.

We hypothesized that three-spined sticklebacks in densities within the published range have a similar effect on zooplankton and phytoplankton densities as pelagic planktivorous fish. The experiment was done in pvc enclosures in shallow eutrophic Lake Veluwe, just after the late summer die-back of aboveground *Potamogeton pectinatus* L. vegetation. The fish fauna of this lake is dominated by bream (*Abramis brama* L.; Hosper & Jagtman, 1990), a species known to feed efficiently on pelagic zooplankton (Lammens, 1989).

2.2 Materials and methods

We used the pvc enclosures from the second 1987 effort, i.e. they were amply pre-treated at the start of the present experiment.

The enclosures consisted of a flexible pvc column (0.3 mm thickness, clear) of 1 m diameter and ± 1.20 m height (Fig. 8.1). The column was enforced on the top and bottom end with an inner and outer pvc ring (both 7 mm thickness) that closely fitted each other to allow the flexible column to be slipped through, clamped tightly and retained in position. The bottom ring was locked into a pvc collar that rested on the sediment and thus enclosed 0.785 m^2 of bottom area. The collar was kept in position on the sediment with 8 iron hooks of 40 cm length. The top ring was held ± 50 cm

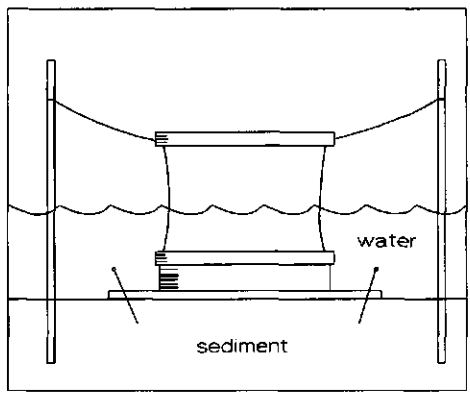


Fig. 8.1. Schematic side-view of an enclosure.

above the water surface with 4 steel chains connected to the top ring and an equal number of gas-tube type poles (steel, 2 m length, 3 cm diameter) that had been driven into the sediment. This allowed for considerable flexibility of the column and a fixed position of the bottom of the enclosure. Water depth inside the enclosures was ± 70 cm.

We used three densities in the enclosures: 0, 5 and 10 sticklebacks per enclosure (0, 6.4 and 12.7 m^{-2}). The lake was considered a fourth treatment, representing the effect of a bream-dominated fish stock. Thus we were able to test our hypothesis and get further information on density effects within a realistic density range of three-spined sticklebacks. The treatments were replicated five times and were distributed randomly over the enclosures. In the fourth and last week of the experiment five enclosures were severely damaged by heavy weather.

Three-spined sticklebacks (3-4 cm length) were collected from the outflow ditches of a trout farm in the vicinity of Vaassen (The Netherlands). Prior to the addition of the fish, the top ring was lowered to the bottom and raised again to

fill all enclosures with new and similar lake water. The experiment was started at August 11th and zooplankton and seston samples were taken after two and four weeks.

Seston was sampled by filling a 1 liter flask whilst moving it through the whole column. The samples were fixed immediately with formalin to a final concentration of 4% to prevent any further zooplankton grazing, stored cool and dark and a known subsample was filtered over precombusted and preweighed Whatman GF/C filters within 12 h. Seston dry weight (dw) was determined after 24 h at 105 °C and ash weight (aw) after 4 h at 520° C. Ash-free dry weight (afdw) was determined by subtraction (afdw = dw - aw).

Zooplankton sampling was similar to seston sampling. The sample was stored cool and dark until further processing within 12 h. A known volume of 500 - 1000 ml was filtered over a 55 μm mesh-width zooplankton net, the remaining concentrate was carefully pippetted onto pre-weighed and pre-combusted GF/C filters and fixed with formalin to a concentration of 4%. Whole samples were counted at 20x magnification in a 5 cm diameter petri-dish with a 2 mm grid. Finally, zooplankton afdw was determined as for seston.

Results were analyzed statistically with the SPSS/PC$^+$ package (Norusis, 1986). Differences between treatments (0, 5, and 10 sticklebacks and the lake) were tested with a modified lsd test. This lsd test had to be used because of unequal replication, but was held similarly conservative as Tukey's HSD test by maintaining an experimentwise error rate (EER) of 0.05 (and, consequently, a comparisonwise error rate (CER) of 0.009 for the six possible comparisons of the treatments; Steel & Torrie, 1980). Data were tested for normality and homogeneity of variances and $\log_{10}$-transformed if necessary (this was the case only for the *Bosmina* densities). Zooplankton data were not analysed with a multiple comparisons test but with a set of two orthogonal contrasts, comparing fish versus no fish and lake versus sticklebacks (both densities combined) respectively. This increases the power of the test since the CERs of a set of orthogonal contrasts can be set at 0.05 (Steel & Torrie, 1980).

Table 8.1. Levels of significance for the factors time, treatment and their interaction (int.) from twoway ANOVAs for zooplankton numbers, seston afdw and ash and non-zooplanktonic seston afdw (NZ-seston). Probabilities indicated with an asterisc (*) are significant at an experimental error rate of 0.05. All four treatments are incorporated, levels for the factor time are two and four weeks.

	time	treatment	int.
zooplankton numbers			
daphnids	0.667	0.001*	0.777
bosminids	0.013*	0.008*	0.057
copepods	0.227	0.309	0.625
rotifers	0.277	0.538	0.666
seston weight fractions			
seston afdw	0.185	0.001*	0.102
seston ash	0.020*	0.047*	0.071
NZ-seston afdw	0.237	0.000*	0.139

2.3 Results

The initial zooplankton community had low numbers overall. Most numerous were copepods (11 individuals l^{-1} on average), while daphnids, bosminids and rotifers were present in lower densities (< 5 individuals l^{-1}, Fig. 8.2). Twoway ANOVAs demonstrated a significant

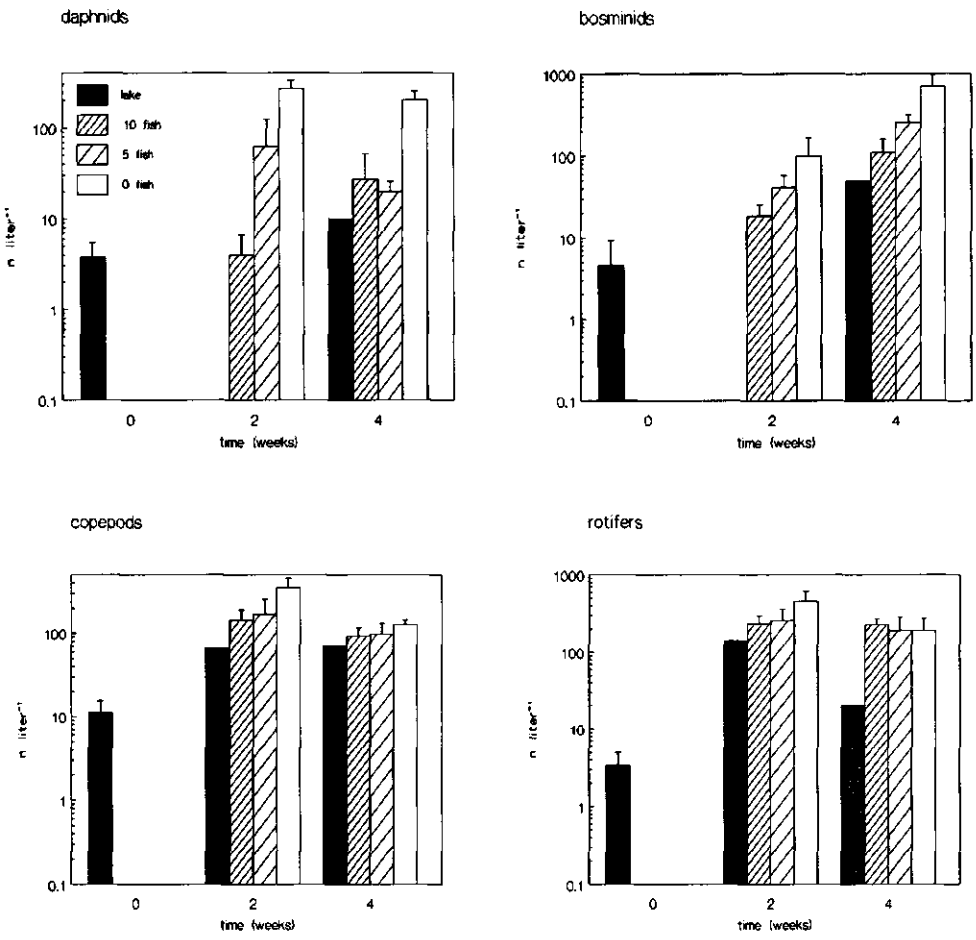


Fig. 8.2. Development of densities of four categories of zooplankton in enclosures with different densities of three-spined sticklebacks and in Lake Veluwe. Indicated are means and standard errors. Observe the log scale of the vertical.

treatment effect for daphnids and bosminids and a significant time effect for bosminids (Table 8.1). Thus, during this experiment the sticklebacks and the fish community in the lake only affected the numbers of daphnids and bosminids. For daphnids, the contrast tests revealed a significant difference between treatments with fish and the enclosures without fish, both for two and four weeks (Table 8.2). For bosminids, this difference was only significant after four weeks. No significant differences existed between the lake and enclosures with sticklebacks (Table 8.2). Sticklebacks thus had kept zooplankton stocks at similar levels as present in the lake.

Linear regressions with the number of zooplankters as dependent and the number of sticklebacks as independent

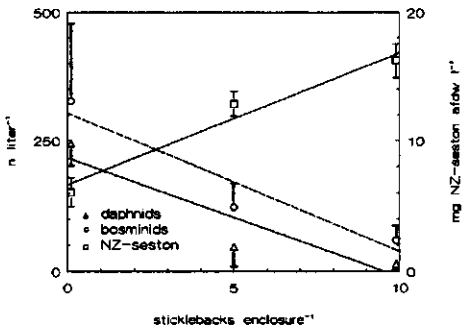


Fig. 8.3. Zooplankton density and Non-Zooplanktonic seston afdw as a linear function of stickleback density in the enclosures. Daphnids: slope=-22.7, r^2=0.493, p=0.0001; bosminids: slope=-26.7, r^2=0.168, p=0.0419; NZ-seston afdw: slope=1.018, r^2=0.663, p=0.001. Data from two and four weeks were pooled: n=25.

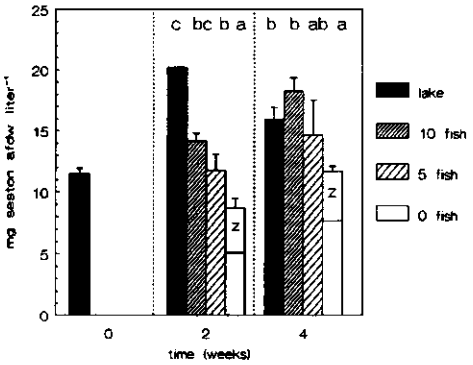


Fig. 8.4. Seston ash-free dry weight development in enclosures with different densities of three-spined sticklebacks and in Lake Veluwe. The upper part of the bar of the no fish treatment (marked with a 'z') is due to zooplankton, the remainder is phytoplankton and dead organic matter. Differences in non-zooplanktonic seston afdw between treatments were tested with a modified lsd test for the two periods separately. Differences in letters indicate significant differences between means (EER=0.05).

Table 8.2. Levels of significance for a set of two orthogonal contrasts. The treatments are compared separately for 14 and 28 days. Contrast 1 compares density in the lake with that in the enclosures with sticklebacks (both densities combined). Contrast 2 compares treatments with fish (lake, enclosures with sticklebacks) with the enclosures without sticklebacks. Only daphnids and bosminids are used because they showed a significant treatment effect in the twoway ANOVAs. Bosminid data were $\log_{10}$-transformed.

zooplankton category	time (days)	contrasts 1	2
daphnids	14	0.780	0.003*
	28	0.833	0.003*
bosminids	14	0.154	0.093
	28	0.323	0.024*

variable had significant negative slopes for both daphnids and bosminids (Fig. 8.3). Thus, for these two groups of zooplankters, stickleback density and zooplankton density showed an inverse relation.

In the enclosures without sticklebacks, a large proportion of the seston afdw was made up by the zooplankton: 44% and 35% after two and four weeks, respectively (Fig. 8.4). The biomass of the zooplankton in the other treatments was below the detection limit. This difference was mainly due to the significantly higher numbers of large daphnids in the enclosures without fish (> 200 individuals l^{-1}, Fig. 8.2).

We subtracted zooplankton afdw from seston afdw to get non-zooplanktonic seston (NZ-seston) afdw, being the summed weight of dead organic matter and phytoplankton. Any effect of zooplankton on phytoplankton biomass would be reflected more clearly in NZ-seston afdw than in total seston afdw.

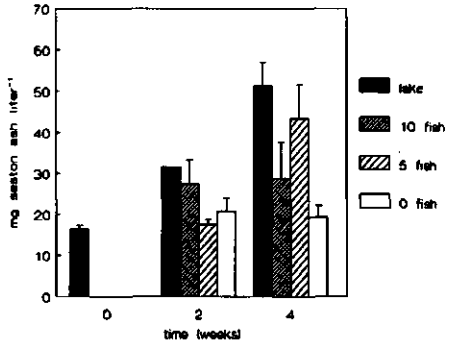


Fig. 8.5. Seston ash development in enclosures with different densities of three-spined sticklebacks and in Lake Veluwe. The contrast enclosures versus lake was significant at four weeks.

In a twoway ANOVA with time and treatments as factors, only treatments had a significant effect on NZ-seston afdw (NZ-seston, $p<0.001$, Table 8.1). The differences after two weeks were more pronounced than after four weeks, but the general trend was similar. NZ-seston afdw in the enclosures without sticklebacks was significantly lower than that in the treatments with fish in all cases but one: after four weeks it was not significantly different from the enclosures with five sticklebacks. The presence of the sticklebacks resulted in seston densities of similar magnitude as in the lake, and a positive relation between stickleback and NZ-seston density is suggested. This is corroborated by a linear regression of NZ-seston afdw against stickleback density (Fig. 8.3).

Seston ash was not affected as much as seston afdw and the variation between samples was higher (Fig. 8.5). In a twoway ANOVA time and treatment had a significant effect ($p=0.020$ and 0.047 respectively) but the consequent lsd tests for the separate sampling times produced no significant differences. Only the contrast lake versus enclosures was significant after four weeks ($p=0.025$): the enclosures had significantly less seston ash than the lake, which is probably caused by lower resuspension rates in the enclosures.

2.4 Discussion and conclusions

We demonstrated that three-spined sticklebacks in field densities can strongly reduce daphnid and bosminid numbers. Furthermore, our hypothesis that the effect of stickleback predation on zooplankton and phytoplankton densities is of similar magnitude as that of predation by pelagic planktivores, is not contradicted by the present results. Daphnid and bosminid densities in the lake were not significantly different from those in the stickleback enclosures, and phytoplankton afdw in the lake was not significantly different from that in the enclosures with 10 sticklebacks. Thus, in the enclosures a clear 'top-down' effect (Fig. 8.3, cf. McQueen & Post, 1988) was apparent in the presence of the sticklebacks. The potential interfering effect of this abundant small fish therefore should be taken into consideration in restoration schemes for lakes with extensive littoral zones that include the removal of pelagic planktivorous fish. Though similar in their 'top-down' effect, three-spined sticklebacks probably have a less disturbing impact on the sediment than larger pelagic fish species like bream. Bream reportedly feeds strongly on benthic chironomids with concomitant frequent disturbance of the sediment (Lammens, 1989) which may be detrimental to aquatic vegetation (Ten Winkel & Meulemans, 1984; section 3 of this chapter).

Absence of predation pressure in the enclosures without fish resulted in a rapid increase in daphnid numbers in the present late summer experiment. Enclosures without fish can be viewed as absolute refuges for zooplankters (Hassel, 1978; Irvine et al., 1989; Shapiro, 1990). Irvine et al. (1989) suggested that aquatic vegetation may also provide an efficient refuge for large zooplankters, 'from which they may move out to graze in the open water'. Aquatic vegetation, however, can only be a relative refuge, the effectiveness of which may be reduced by the presence of small fish like three-spined sticklebacks in the vegetation.

The vulnerability of zooplankters to predation by the polyphagous sticklebacks is relative to the presence of other readily eaten prey. In the enclosures, periphytic invertebrates will not have been very abundant since most vegetation had died back. Benthic invertebrate densities were low and thus zooplankters probably were the main food of the sticklebacks in the present experiment. In earlier summer months, however, alternative food items certainly will be available in aquatic vegetation. This may reduce the predation pressure by sticklebacks on zooplankton, but, as outlined in section 2.1 above, may also result in numerical responses followed by switching with possible contrary effects. It is unknown whether numerical responses of sticklebacks to periphytic invertebrates exist. Evidence is present of a response of the invertebrates to increasing availability of their periphytic food as in the course of eutrophication (Moss, 1976; Brown & DeVries, 1985; Lodge et al., 1987; Osenberg, 1989). Thus, interactions in the 'periphytic' (sensu lato) foodweb remain open for further research.

Whether sticklebacks prey on pelagic zooplankton and exert a 'top-down' stimulating effect on phytoplankton stocks, or prey on littoral periphytic macro-invertebrates that possibly influence periphyton densities, the effect on the environmental conditions of aquatic macrophytes may be similar: enhanced shading. If the above mentioned periphyton numerical response chain ending with the sticklebacks exists, it may have played an accelerating role in the change from a macrophyte-dominated to a phytoplankton-dominated ecosystem, which reportedly has been a sudden one in many cases (Phillips et al., 1978; Moss & Leah, 1982; De Nie, 1987).

3. Ecosystem development in different types of littoral enclosures

J.E. Vermaat, M.J.M. Hootsmans & G.M. van Dijk

Abstract

Macrophyte growth was studied in two enclosure types (gauze and polythene) in a homogeneous bed of *Potamogeton pectinatus* L. in Lake Veluwe (The Netherlands). The gauze was expected to allow for sufficient exchange with the lake to maintain similar seston densities, the polythene was expected to exclude fish activity and most water exchange.

Polythene enclosures held higher total *P. pectinatus* biomass (ash-free dry weight, afdw) than the lake, gauze enclosures were intermediate. The enclosures had a higher abundance of other macrophyte species (*Chara* sp., *Potamogeton pusillus* L.) than the lake. Seston ash content was not but seston afdw, periphyton ash content and afdw were lower in polythene than in gauze enclosures. Rotifer densities were higher in polythene enclosures than in the lake, gauze was intermediate. Daphnids occurred earlier in the polythene enclosures than in gauze, and reached higher densities, whilst they were absent in the lake.

Light attenuation by seston and periphyton and the consequent irradiance reaching the macrophytes was estimated: the plants in the polythene enclosures received more light than those in gauze and the lake, that received an almost similar amount. We explain the difference in macrophyte biomass by improved light conditions in polythene and absence of sediment-disturbing fish (e.g. bream) in both types of enclosures, and conclude that both factors are of similar importance.

3.1 Introduction

The present study was set up to investigate the simultaneous effect of (a) water exchange between adjacent lake areas with and without macrophytes and consequent dilution of possibly produced allelopathic substances, and (b) periphyton removal by snails, both in semi-field conditions in enclosures. Apart from experimental manipulations, physical conditions inside enclosures will rarely be completely similar to those outside. We therefore explicitly incorporated the most obvious ones, differences in wave exposure and water exchange, in our hypotheses and analysis. An earlier form of this paper has been published elsewhere (Vermaat et al., 1990).

Though the experiment was also designed to study the effect of snail activity and half of the enclosures had been stocked with 500 adult *Bithynia tentaculata* (L.) each, less than 10% of these animals were still present at termination of the experiment. Presence of snails had no significant effect on any measured parameter. We thus pooled the 'plus snail' replicates with the appropriate other treatments. *B. tentaculata* reportedly burrows in the sediment (Lilly, 1953; Young, 1975; Vermaat, 1991) thus the construction of the enclosures with the bottom collar laying flat on the sediment probably enabled large numbers of snails to migrate out of the enclosures.

We used two different types of enclosure, polythene and gauze, to create a gradient in physical and biological conditions. The enclosures were situated in a

Table 8.3. Physical and biological conditions in polythene (pe) and gauze enclosures and the lake, and a set of hypotheses on the development of the different studied components. The last column gives the references on which the hypothesis is based. Numerals in brackets indicate the conditions in the upper part of the table that are the basis of the postulated hypothesis.

	pe	gauze	lake	reference
conditions				
1. wave action	less	intermediary	high	
2. water exchange	minimal	intermediary	high	
3. fish activity	no	no	normal	
hypotheses				
zooplankton density	high	intermediary	low	b, c, g (2, 3)
seston density	low	high	high	b, d, f (2, 3)
periphyton density	?	?	?	
macrophytes	high	intermediary	low	a, e (1, 3)

references: a) Jupp & Spence, 1977; b) Andersson et al., 1978; c) Lynch, 1979; d) Lynch & Shapiro, 1981; e) Ten Winkel & Meulemans, 1984; f) Riemann & Søndergaard, 1986; g) Post & McQueen, 1987.

homogeneous vegetation of *Potamogeton pectinatus* L. in shallow, wind-exposed Lake Veluwe (about 70% of its area is shallower than 0.9 m). We studied the development of macrophytes, periphyton, seston and zooplankton in the two types of enclosure and the lake. Both enclosure types were expected to exclude all fish activity. From its physical properties (flexibility and porosity), the gauze was considered to be intermediate between polythene and lake with respect to water exchange and wave action. This has not been verified experimentally, since we were not aware of a simple and straightforward method. Based on the conditions in the enclosures and the literature we derived a set of hypotheses on the development of the different biotic components of the ecosystem on which we collected data. Conditions and hypotheses are summarized in Table 8.3 and outlined below.

Wave action may affect macrophyte performance in different ways. Sedimented particulate matter may be resuspended thus influencing turbidity. Also, wave action may influence sediment stability and rooting possibilities or have direct adverse mechanic effects on the macrophyte tissue. The significance of exposure to wave action for the distribution of macrophyte communities in general (Keddy, 1982; Duarte & Kalff, 1988) and, specifically, of *P. pectinatus* (Anderson, 1978) has been stressed. Jupp & Spence (1977) found an increased biomass of *Potamogeton filiformis* Pers. in unrepli-

cated perspex containers (165 cm^2 area) as compared to unenclosed vegetation. They however stated that 'initial plant densities were not the same in each treatment', without explicitly mentioning initial densities. Kautsky (1987) found significant differences in *P. pectinatus* biomass over a natural exposure gradient in the Askö skerries. However, other environmental variables like sediment type and organic matter content covaried with wave exposure in the work of Kautsky (1987). We hypothesize that both direct effects of a reduction of wave action and indirect effects (increased sedimentation of suspended matter) will be beneficiary to macrophyte growth (Table 8.3).

A priori, we cannot decide whether seston densities in the gauze enclosures will be intermediate or equally high as in the lake since we have no estimates of water exchange rates (nutrients, algal inocula) or reduced turbulence and consequently influenced resuspension rates. Still, for the sake of an unequivocal hypothesis, we hypothesize that seston density in the gauze enclosures will be similar to that in the lake.

Finally, we will refrain from hypothesizing about periphyton development, since too many factors may affect it interactingly. Consider for example the interactive effect of increased growth of the macrophytes providing an increasing area available for colonization, reduced nutrient diffusion or increased sedimentation rates into the periphyton community under less turbulent conditions.

3.2 Materials and methods

The enclosures were of similar construction as described in section 2.2. The flexible column however was made of polythene (pe, 0.3 mm thickness, 'clear') or gauze (extra flexible Monyl plankton gauze type 1050 HC, mesh width 1 mm).

The 16 enclosures were laid out in a randomized block design of 4 blocks and an adjacent area of the lake was designated as control area. Each block had two polythene and two gauze enclosures, one of each type with and one without added snails. The whole experimental area was clearly marked with gas-tube poles and a rope with red and white floats, to alert recreating visitors and minimize disturbance. The experiment was started on May 9, 1988, and lasted 9 weeks, until July 12. In the first week, cracks appeared in the polythene, after which a second layer of polythene was enveloped around the first.

Seston was sampled weekly, zooplankton samples were taken at the start, halfway and at termination of the experiment. Sample processing is described in section 2.2. No zooplankton biomass was determined and the remaining concentrate on the 55 μm zooplankton net was carefully pippetted into 10 ml glass vials and fixed with formalin to a concentration of 4% for consequent counting within a week.

At termination of the experiment, macrophyte shoot samples of at least 20 leaves with stems were taken from the top (upper 10 cm) and bottom water layer to determine periphyton density. In the laboratory, periphyton was scraped off with a razor blade and biomass was determined as for seston. One-sided surface area of these scraped plant parts was determined with a conveyor-belt-type Licor LI 3000 area-meter and biomass (afdw) as for seston. All material within the enclosures were finally dug out to a depth of about 20 cm to sieve and collect macrophytes and snails. The weight-proportion of other macrophyte species in the sample was estimated and the *P. pectinatus* material

was separated into tubers, roots + rhizomes and aboveground material of which the biomass was determined as above.

Results were analyzed statistically with ANOVA facilities of the SPSS/PC+ statistical package (Norusis, 1986). Data were tested for normality and homogeneity of variances. They were $\log_{10}$-transformed if these requirements were not met. This transformation proved to satisfy the requirements in all cases. Differences between treatments (pe, gauze, lake) were tested with Tukey's HSD test or, if replication was not equal, with a modified lsd test. This lsd test was held similarly conservative as Tukey's HSD by maintaining an experimentwise error rate of 0.05 (and consequently a comparisonwise error rate of 0.017 for the comparison of three treatments, Steel & Torrie, 1980).

3.3 Results

The initial zooplankton community was dominated by copepods, with an average density of 187 individuals l^{-1} (standard error (se) 10, n=5). No bosminids or daphnids and only a few rotifers were present (average 4 l^{-1}, se=1). During the experiment, differences in densities of daphnids and rotifers developed between treatments (Fig. 8.6). For the daphnids, the pattern is clear, but was difficult to test due to the occurrence of zero values for all replicates of some of the treatments. Daphnids occurred later in our samples from gauze than from polythene enclosures and were not found in the lake. This fits our hypothesis (Table 8.3). The rotifer patterns were testable and also fitted our hypothesis.

Seston afdw and ash patterns were clearly influenced by wind force (Fig. 8.7): week 2 had strong winds. For this period, differences in seston ash were all significant, while afdw in the enclosures was significantly less than in the lake. This was the only occasion that seston ash in enclosures and lake were significantly different. In week 5 to 8, seston afdw was

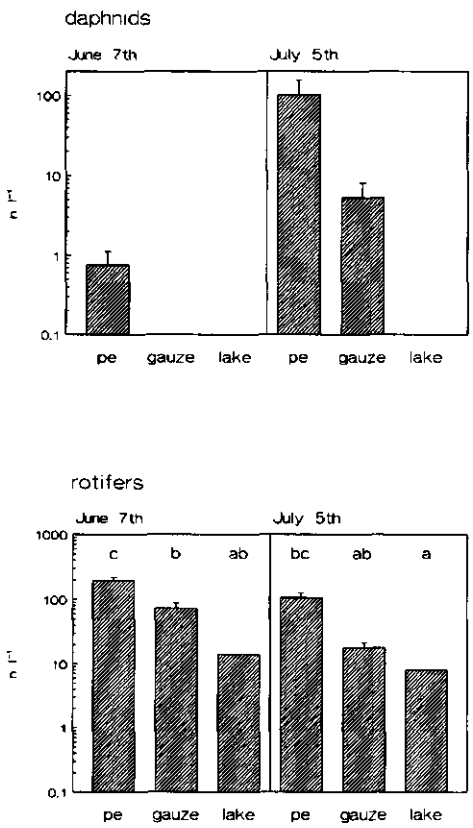
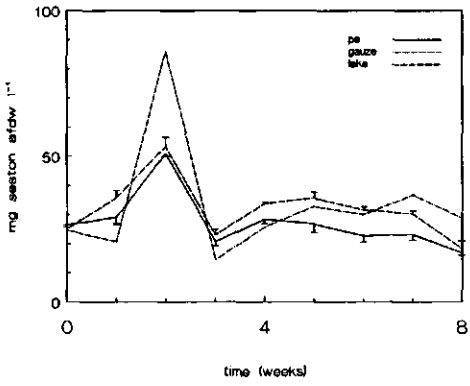


Fig. 8.6. Development of daphnids and rotifers in polythene (pe) and gauze enclosures and in Lake Veluwe. Average values and standard errors are indicated. The vertical axis has a log scale. If two treatment means share the same letter they are not significantly different. June 7th was halfways the experiment, after 4 weeks, July 5th was at the end of the experiment.



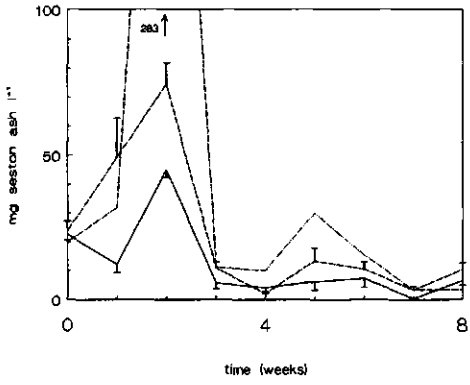


Fig. 8.7. Development of seston ash-free dry weight (afdw) and ash (for explanation cf. Fig. 8.6).

significantly less in polythene than in gauze enclosures. For seston afdw these results do not contradict our tentative hypothesis: polythene held significantly less afdw than gauze. Seston afdw and ash were analysed separately for every week, because in a twoway ANOVA the factors time, treatment and their interaction were highly significant for both parameters. The significant time effect stresses the importance of temporal variation in seston density, caused by, e.g., temporal variation in wind-induced turbulence.

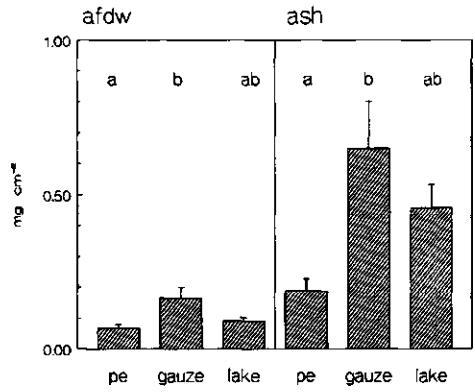


Fig. 8.8. Periphyton density at termination of the experiment. Pooled values for top and bottom samples (for explanation cf. Fig. 8.6).

Top and bottom periphyton densities were significantly different ($p < 0.05$) both with respect to ash and afdw. Still, they are pooled in Fig. 8.8. The ratio top/bottom periphyton density was about 0.4 in enclosures and 0.8 in the lake for both ash and afdw. Thus periphyton on the part of the plant in the top 10 cm of the water column was less dense than in the lower part, and this difference was more pronounced in the enclosures than in the lake. Periphyton density in the gauze enclosures (Fig. 8.8) was highest, that in the lake intermediate, again both for ash and afdw.

Total macrophyte biomass (at least 95% *P. pectinatus*) was significantly higher in polythene enclosures than in the lake, the gauze enclosures were intermediate, i.e. neither significantly different from polythene nor from the lake (Fig. 8.9). Relative biomass distribution and plant length were not significantly different (overall averages: tubers 2%, roots + rhizomes 9%, aboveground matter 89%, plant length 69 cm), but specific leaf area and numbers of inflorescences

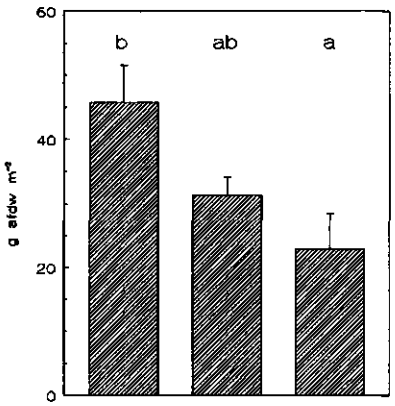


Fig. 8.9. Total macrophyte biomass at termination of the experiment (for explanation cf. Fig. 8.6).

per unit biomass were (Fig. 8.10). Furthermore, the proportions of *Potamogeton pusillus* L. and *Chara* spec. in the biomass sample were significantly higher in the polythene enclosures than in the lake (Fig. 8.11). With respect to *P. pusillus* the gauze was similar to the lake, for *Chara* it was intermediate.

3.4 Discussion

In the present study, seston concentration appeared to be greatly influenced by wind action while enclosure type did not influence sedimentation much since seston ash was not distinctly less in the enclosures as compared to the lake. Seston afdw, however, was significantly lower in polythene than in gauze enclosures while daphnid densities were consistently higher in pe. This suggests that the daphnids reduced phytoplankton stocks and thus seston afdw, which is in agreement with the conclusions of a.o. Andersson et al. (1978), Shapiro & Wright (1984), Riemann & Søndergaard (1986) and those from the stickleback experiment (section 2.4 of this chapter). A variation on the caveat of Post & McQueen (1987) on possible nutrient interactions however must be stressed here: the macrophyte-periphyton complex may have competitively reduced nutrient concentrations in the polythene enclosures. Furthermore, inhibition of phytoplankton growth by allelopathic excretions from the macrophytes may also have played a role. Hootsmans (1991) demonstrated allelopathic activity of *P. pectinatus* beds in Lake Veluwe in spring. In a laboratory experiment, he found biomass reductions in the range of 10-15% for inhibited phytoplankton, a difference of similar magnitude as found between polythene enclosures and the lake (whole-experiment averages respectively 27 and 33 mg seston l^{-1} afdw).

Though daphnid numbers did not reach densities > 200 individuals l^{-1} as in the stickleback experiment, daphnids will have contributed to seston afdw in the polythene enclosures at the end of the experiment (July 5th, Fig. 8.6). Based on an average individual biomass of 16.8 μg afdw for daphnids from the stickleback experiment, we estimate daphnid afdw at July 5th to be 1.7 mg l^{-1}, which is only 7% of total seston afdw. We explain the difference in maximum daphnid densities between the two experiments with between-year and seasonal variation in initial inocula at the filling of the enclosures (Brinkman & Van Raaphorst, 1986).

Periphyton densities appeared to be maximal in the gauze enclosures, equally with respect to ash and afdw. This may indicate that the periphyton in the gauze enclosures acted as a seston trap. A constant inflow of lake water through the gauze then maintained high seston ash and afdw concentrations, while the macrophyte-periphyton complex acted as a

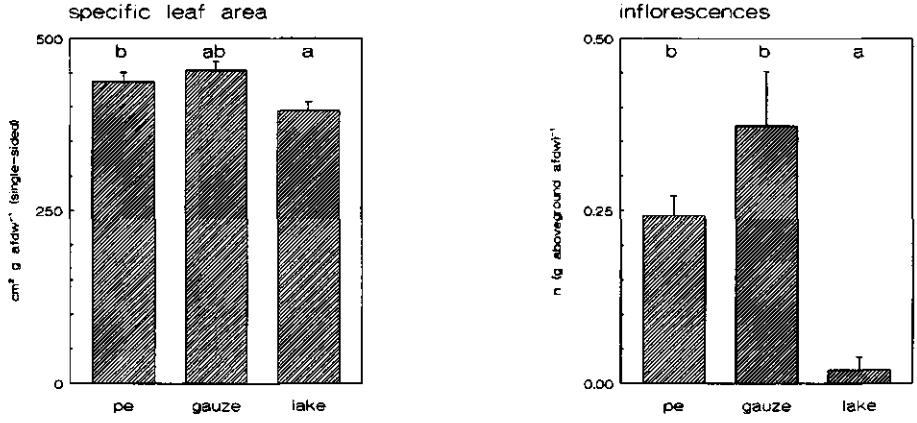


Fig. 8.10. Specific leaf area and number of inflorescences per unit aboveground biomass of *Potamogeton pectinatus* (for explanation cf. Fig. 8.6).

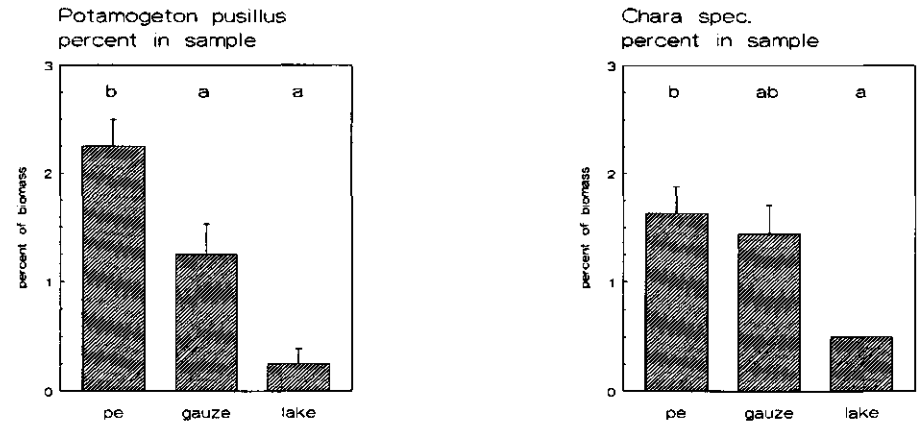


Fig. 8.11. proportional contribution of *Potamogeton pusillus* and *Chara* spec. to the total enclosure biomass (for explanation cf. Fig. 8.6).

sieve. Meanwhile, sedimentation rates were not altered as much as to result in significant differences in seston ash contents between enclosure types.

Based on an absorbance-biomass hyperbola for Lake Veluwe periphyton (Van Vierssen & Bij De Vaate, 1990) we estimate average irradiance absorbance (PAR) by the periphyton in the gauze enclosures to be approximately 40% while this is about 20% for the polythene enclosures. Absorbance by the periphyton in

Table 8.4. Estimation of the irradiance reaching the plants surface after the summed attenuation by phytoplankton and periphyton in polythene and gauze enclosures and the lake. An average phytoplankton extinction coefficient (k) of 2.8 m^{-1} is assumed for gauze and lake (Van Vierssen & Bij De Vaate, 1990). For polythene (pe), k is reduced by multiplication with the ratio A = BP/BL (BP is average seston afdw in pe, and BL is average seston afdw in the lake). Periphyton absorbance is derived from a biomass-absorbance hyperbola (Van Vierssen & Bij De Vaate, 1990). Total waterdepth is assumed to be 70 cm, 'effective' (i.e. a weighted average waterdepth above the photosynthetic tissue of the vegetation) phytoplankton depth to be 40 cm and average irradiance during a diel photoperiod in summer in The Netherlands is set at 720 $\mu E\ m^{-2}\ s^{-1}$ (calculated from De Wit, 1965). No correction was made for surface reflection.

	pe	gauze	lake
seston afdw (average over experiment, mg l^{-1})	27.2	31.8	33.3
seston extinction coefficient (m-1)	2.3	2.8	2.8
periphyton attenuance (%)	20	40	30
irradiance ($\mu E\ m^{-2}\ s^{-1}$ and as % of 720 $\mu E\ m^{-2}\ s^{-1}$)			
after seston	287 (40%)	235 (33%)	235 (33%)
after periphyton	230 (32%)	141 (20%)	165 (23%)
relative to lake	1.4	0.9	1.0
macrophyte biomass (g m-2 afdw)	45.7	31.3	22.9
relative to lake	2.0	1.4	1.0

the top layer, where the largest part of plant photosynthesis presumably occurs (Van Wijk, 1988; Van der Bijl et al., 1989), must have been considerably less.

Both phytoplankton and periphyton influence the amount of light that reaches the photosynthetically active tissue of the macrophyte. Based on average values and a few assumptions, we can estimate the summed effect of both on the irradiance reaching the plant in the three different treatments. We calculated the change in estimated irradiance that reached the plants in polythene and gauze enclosures relative to the conditions in the lake (as a ratio, Table 8.4). The irradiance conditions in the polythene enclosures appear to have improved (ratio 1.4 > 1) due to the combined effect of decreased periphyton and phytoplankton, whilst they were slightly worse in the gauze enclosures (ratio 0.9 < 1). When similar ratios relative to the lake values are calculated for total macrophyte biomass, however,

the picture was different (Table 8.4). Differences between irradiance ratios and biomass ratios for both gauze and polythene enclosures thus indicate that the response in macrophyte biomass in the present experiment cannot be explained by differences in irradiance conditions alone. For the gauze enclosures, an increase in macrophyte biomass has not been due to irradiance improvements, whilst the biomass increase in the polythene enclosures can only be partly attributed to this factor.

Apart from differences in the light climate, other factors thus must also have played a role. Any effects of zooplankton grazing and allelopathic growth inhibition on light availability have already been incorporated in the above estimations (Table 8.4). We consider the impact of zooplankton and allelopathy via other factors such as increased nutrient availability to be negligibly small as compared to that via light availability. Since seston ash was not distinctly less in the enclosures than in the lake, we assume that differences in seston resuspension were absent. Other factors may have been water exchange, fish activity and the mechanic effect of wave action.

Differences in water exchange between polythene and gauze enclosures may affect availability of nutrients and inorganic carbon (CO_2 and HCO_3^-) to the macrophytes through competition by phytoplankton. It seems unlikely that macrophyte growth in the gauze enclosures was reduced much through nutrient or carbon competition since the difference in seston afdw was not very large (difference was 22% of average seston afdw in polythene). Furthermore, Peltier & Welch (1969) found that growth of *P. pectinatus* was only 5.4% less in tapwater than in a nutrient-rich medium. This indicates that even at very low nutrient concentrations in the water, growth of this species is not affected very much.

Sediment disturbance by benthivorous fish like bream (Ten Winkel & Meulemans, 1984; Lammens, 1989) was equally absent in both types of enclosures. This thus can be a probable explanation for the higher macrophyte biomass in the gauze enclosures as compared to the lake. Improved light conditions then explain the even higher biomass in the polythene enclosures. Furthermore, since the increase in ratios due to these two factors is of similar magnitude (Table 8.4), we conclude that they are of similar importance. Finally, it cannot be excluded that the decreasing mechanical disturbance with decreasing exposure to wave action also has played a role.

Acknowledgements

In 1987, many people from the Dept. of Nature Conservation have assisted during the installation and sampling of the first two unfortunate experimental trials: we thank R. Gijlstra, J.A.J. Beijer, L.J. Langelaan, M. Kuipers, R.A.E. Knoben, B.A. Koops, K.P. Raap, H. Kieft, A. Smit, J.J.R.M. Vet, I.J.J. Barten and R.D. Koeze. We gratefully acknowledge the assistance of R. Gijlstra, R.G.J. Lobbes and D. Ramondt during the installation of the enclosures in 1988. R. Gijlstra and R. Lobbes assisted with the sampling. Prof. Dr. W. van Vierssen and Prof. Dr. W.J. Wolff critically read the manuscript.

4. References

Anderson, M.G., 1978. Distribution and production of Sago Pondweed (*Potamogeton pectinatus* L.) on a northern prairie marsh. Ecology 59: 154-160.

Andersson, G., Berggren, H., Cronberg, G. & Gelin, C., 1978. Effects of planktivorous and benthivorous fish on organisms and water chemistry in eutrophic lakes. Hydrobiologia 59: 9-15.

Beukema, J.J., 1968. Predation by the three-spined stickleback (*Gasterosteus aculeatus* L.): the influence of hunger and experience. Brill, Leiden, 126 pp.

Bloesch, J., Bossard, P., Bührer, H., Bürgi, H.R. & Uehlinger, U., 1988. Can results from limnocorral experiments be transferred to in situ conditions? (Biomanipulation in limnocorrals VI). Verh. int. Ver. Limnol. 23: 762-763.

Brabrand, Å., Faafeng, B. & Nilssen, J.P.M., 1987. Pelagic predators and interfering algae: stabilizing factors in temperate eutrophic lakes. Arch. Hydrobiol. 110: 533-552.

Brinkman, A.G. & Van Raaphorst, W., 1986. De fosfaathuishouding in het Veluwemeer (Phosphorus dynamics in Lake Veluwe, in Dutch). PhD Thesis, Twente Technical University, The Netherlands, 481 pp.

Brown, K.M. & DeVries, D.R., 1985. Predation and the distribution and abundance of a pulmonate snail. Oecologia 66: 93-99.

Butler IV, M.J., 1989. Community responses to variable predation: field studies with sunfish and freshwater macroinvertebrates. Ecol. Monogr. 59: 311-328.

Carpenter, S.R., Kitchell J.F. & Hodgson, J.R., 1985. Cascading trophic interactions and lake productivity. BioScience 35: 634-639.

De Nie, H.W., 1987. The decrease in aquatic vegetation in Europe and its consequences for fish populations. EIFAC/CECPI Occasional Paper no. 19, 52 pp.

De Wit, C.T., 1965. Photosynthesis of leaf canopies. Agr. Res. Rep. 663: 1-57.

Duarte, C.M. & Kalff, J., 1988. Influence of lake morphometry on the response of submerged macrophytes to sediment fertilization. Can. J. Fish. Aquat. Sci. 45: 216-221.

Godmaire, H. & Planas, D., 1983. Potential effect of *Myriophyllum spicatum* on the primary production of phytoplankton. In R.G. Wetzel (ed.), Periphyton of freshwater ecosystems. Junk, The Hague, pp. 227-233.

Hassel, M.P., 1978. The dynamics of arthropod predator-prey systems. Monographs in Population Biology 13, Princeton University Press, New Jersey, 237 pp.

Hootsmans, M.J.M., 1991. Allelopathic limitation of algal growth by macrophytes. In Hootsmans, M.J.M. & Vermaat, J.E., Aquatic macrophytes: a key to understanding changes in shallow ecosystems resulting from eutrophication, PhD Thesis, Wageningen Agricultural University.

Hosper, S.H., 1989. Biomanipulation, new perspectives for restoration of shallow eutrophic lakes in The Netherlands. Hydrobiol. Bull. 23: 5-10.

Hosper, S.H. & Jagtman, E., 1990. Biomanipulation additional to nutrient control for restoration of shallow lakes in The Netherlands. Hydrobiologia 200/201: 523-534.

Irvine, K., Moss, B. & Balls, H., 1989. The loss of submerged plants with eutrophication II. Relationships between fish and zooplankton in a set of experimental ponds, and conclusions. Freshwat. Biol. 22: 89-107.

Jupp, B.P. & Spence, D.H.N., 1977. Limitations of macrophytes in a eutrophic lake, Loch Leven II. Wave action, sediments and waterfowl grazing. J. Ecol. 65: 431-446.

Kautsky, L., 1987. Life-cycles of three populations of *Potamogeton pectinatus* L. at different degrees of wave exposure in the Askö area, northern Baltic proper. Aquat. Bot. 27: 177-186.

Keddy, P.A., 1982. Quantifying within-lake gradients of wave energy: interrelationships of wave energy, substrate particle size and shoreline plants in Axe Lake, Ontario. Aquat. Bot. 14: 41-58.

Kuiper, J., 1977. Development of North Sea coastal plankton communities in separate plastic bags under identical conditions. Mar. Biol. 44: 97-107.

Lack, T.W. & Lund, J.W.G., 1974. Observations on the phytoplankton of Blelham Tarn, English Lake District. I. The experimental tubes. Freshwat. Biol. 4: 399-415.

Lammens, E.H.R.R., 1989. Causes and consequences of the succes of bream in Dutch eutrophic lakes. Hydrobiol. Bull. 23: 11-18.

Leah, R.T., Moss, B. & Forrest, D.E., 1978. Experiments with large enclosures in a fertile, shallow, brackish lake, Hickling Broad, Norfolk, United Kingdom. Int. Revue ges. Hydrobiol. 63: 291-310.

Lilly, M., 1953. The mode of life and the structure and functioning of the reproductive ducts of *Bithynia tentaculata* (L.). Proc. Malac. Soc. Lond. 30: 87-110.

Lodge, D.M., Brown, K.M., Klosiewski, S.P., Stein, R.A., Covich, A.P., Leathers, B.K. & Brönmark, C., 1987. Distribution of freshwater snails: spatial scale and the relative importance of physicochemical and biotic factors. Amer. Malac. Bull. 5: 73-84.

Lund, J.W.G., 1972. Preliminary observations on the use of large experimental tubes in lakes. Verh. int. Ver. Limnol. 18: 71-77.

Lynch, M., 1979. Predation, competition, and zooplankton community structure: an experimental study. Limnol. Oceanogr. 24: 253-272.

Lynch, M. & Shapiro, J., 1981. Predation, enrichment, and phytoplankton community structure. Limnol. Oceanogr. 26: 86-102.

McCauley, E. & Briand, F., 1979. Zooplankton grazing and phytoplankton species richness: field tests of the predation hypothesis. Limnol. Oceanogr. 24: 243-252.

McQueen, D.J. & Post, J.R., 1988. Limnocorral studies of cascading trophic events. Verh. int. Ver. Limnol. 23: 729-747.

Mittelbach, G.G., 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. Ecology 62: 1370-1386.

Moss, B., 1976. The effect of fertilization and fish on community structure and biomass of aquatic macrophytes and epiphytic algal populations: an ecosystem experiment. J. Ecol. 64: 313-342.

Moss, B. & Leah, R.T., 1982. Changes in the ecosystem of a guanotrophic and brackish shallow lake in Eastern England: potential problems in its restoration. Int. Revue ges. Hydrobiol. 67: 625-659.

Norusis, M.J., 1986. SPSS-PC$^+$ manual. SPSS Inc., Chicago, USA, 559 pp.

Osenberg, C.W., 1989. Resource limitation, competition and the influence of life history in a freshwater snail community. Oecologia 79: 512-519.

Peltier, W.H. & Welch, E.B., 1969. Factors affecting growth of rooted aquatics in a river. Weed Sci. 17: 412-416.

Phillips, G.L., Eminson, D. & Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4: 103-126.

Post, J.R. & McQueen, D.J., 1987. The impact of planktivorous fish on the structure of a plankton community. Freshwat. Biol. 17: 79-89.

Riemann, B. & Søndergaard, M., 1986. Regulation of bacterial secondary production in two eutrophic lakes and in experimental enclosures. J. Plankton Res. 8: 519-536.

Shapiro, J., 1990. Biomanipulation: the next phase - making it last. Hydrobiologia 200/201: 13-27.

Shapiro, J., & Wright, D.W., 1984. Lake restoration by biomanipulation: Round Lake, Minnesota, the first two years. Freshwat. Biol. 14: 371-383.

Steel, R.G.D. & Torrie, J.H., 1980. Principles and procedures of statistics, a biometrical approach. 2nd edition. McGraw Hill, New York, 633 pp.

Ten Winkel, E.H. & Meulemans, J.T., 1984. Effects of fish upon submerged vegetation. Hydrobiol. Bull. 18: 157-158.

Van der Bijl, L., Sand-Jensen, K. & Hjermind, L., 1989. Photosynthesis and canopy structure of a submerged plant, *Potamogeton pectinatus*, in a Danish lowland stream. J. Ecol. 77: 947-962.

Van Wijk, R.J., 1988. Ecological studies on *Potamogeton pectinatus* L. I. General characteristics, biomass production and life cycles under field conditions. Aquat. Bot. 12: 103-155.

Vermaat, J.E., 1991. Periphyton removal by freshwater micrograzers. In Hootsmans, M.J.M. & Vermaat, J.E., Aquatic macrophytes: a key to understanding changes in shallow ecosystems resulting from eutrophication, PhD Thesis, Wageningen Agricultural University.

Vermaat, J.E., Hootsmans, M.J.M. & Van Dijk, G.M., 1990. Ecosystem development in different types of enclosures. Hydrobiologia 200/201: 391-398.

Van Vierssen, W. & Bij De Vaate A., 1990. Licht en waterplanten, oorzaken van biomassafluctuaties van onderwatervegetaties in het Veluwemeer (Light and submerged macrophytes, causes for macrophyte biomass fluctuations in Lake Veluwe, in Dutch). Report of the Institute for Inland Water Management and Waste Water Treatment, Wageningen Agricultural University, Dept. of Nature Conservation and the International Institute for Hydraulic and Environmental Engineering, 233 pp.

Wetzel, R.G., 1983. Limnology, second edition. Saunders college publishing, Philadelphia, 860 pp.

Wootton, R.C., 1976. The biology of sticklebacks. Acad. Press, London, 387 pp.

Young, M.R., 1975. The life cycles of six species of freshwater molluscs in the Worcester-Birmingham canal. Proc. Malac. Soc. Lond. 41: 533-548.

A GROWTH ANALYSIS MODEL FOR *POTAMOGETON PECTINATUS* L.

9

M.J.M. Hootsmans

Abstract

A description is given of the macrophyte growth model SAGA1, developed for *Potamogeton pectinatus* L. Based on various laboratory and field experiments, the model incorporates effects of light and age on plant photosynthesis, growth and development and on tuber bank dynamics. The vegetation is described in layers and consists of three subvegetations, each with its own tuber bank with a fixed tuber size. Self-shading between and within subvegetations is incorporated. Light and age effects on photosynthetic parameters generate a decrease in production. This leads to an increased chance for wave damage. In most cases, this results in the disappearance of the vegetation at the end of the growing season.

A sensitivity analysis of most model parameters has been carried out. Apart from parameters connected with photosynthesis and respiration, the model output variables that were used showed a low sensitivity to changes in most other parameters. Model validation resulted in a reasonable agreement with field data from a *P. pectinatus* vegetation in Lake Veluwe (The Netherlands). Temperature effects were not incorporated in the model and appeared not necessary to reach this agreement. This may be due to the limited width of the actual temperature range during the rather short period of plant growth in this lake. Furthermore, deterioration and loss of biomass at the end of the growing season already occur in Lake Veluwe when water temperature has not decreased at all.

Model results for various light conditions are presented. They suggest among others that in shallow water, both periphyton light regime and water layer extinction coefficient influence biomass development. In deeper water (depth 1.0 m and more), periphyton shading becomes less important relative to the light extinction by the water layer. In deeper, turbid water (extinction 2.0 m^{-1}), rather small changes in the extinction coefficient can cause large changes in total vegetation biomass.

Tuber induction and tuber bank development play an important role in vegetation survival when periphyton shading shows a peak in spring. Combined with an increased water clarity this strongly stimulates biomass development, mainly through increased tuber growth. The resulting total biomass of the vegetation is comparable to that reached in clear water with a constant low periphyton shading level.

Contents

1. General introduction

Clearly, dealing with the enormous amount of data, variables and relations that are obtained from a system ecology study is tedious. It is often difficult or impossible to estimate the relative importance of all the discerned interactions. The number of laboratory and field experiments that can be done is usually limited. Therefore, the construction and use of mathematical models is increasing. Such models can give insight in the concerted result of many factors acting together, and in their relative effect on the main processes in the system.

It is important to realize that a model is a simplified image of reality. Simplification is necessary to get insight into a system. However, the decision which factors are to be included and which interactions between factors should be incorporated in a model is usually rather tentative: knowledge of the system grows along with the growing model. This inevitable process can easily lead to erroneous conclusions, especially when the understanding of system functioning is still rather limited. In that stage, a model can only give a rough description of the system, and broad ranges are usually accepted when the outcome of a model simulation is judged. Seemingly good results may however still be based on completely wrong assumptions regarding the underlying mechanisms. A model with much more detail probably will show such discrepancies more clearly. The necessity to fill in more and more parameter values quickly points out gaps in understanding.

Nevertheless, models are useful tools when the consequences of (usually complex) impacts on an ecosystem have to be predicted, and when questions concerning management are raised. Ideally, a model is a comprehensive integration of all available knowledge and insights concerning the system under study. Thus, an answer based on such a model can be seen as the best that can be obtained. The disappointing fact that results from model simulations more often than not differ from the real world only stresses how little we really know about ecosystem functioning.

The main reason for the development of the current macrophyte growth model was the existing and growing need for predictions of macrophyte development in the management of shallow eutrophicated lakes. As outlined above, a model can be a good tool for this purpose.

Up till now only a few mathematical models exist that simulate aquatic macrophyte growth in more or less detail. Examples are a model for the freshwater macrophyte *Myriophyllum spicatum* L. (Titus et al., 1975) and models for the seagrass *Zostera marina* L. (Short, 1980; Verhagen & Nienhuis, 1983; Wetzel & Neckles, 1986). Collins & Wlosinsky (1989) presented a general model for macrophyte biomass development in a freshwater reservoir. Of these examples, the freshwater models describe the vegetation in several layers. In this way, changes with depth in environmental and physiological characteristics, and especially changes in photosynthetic parameters, can be taken into account (Ikusima, 1970). Ondok et al. (1984) modelled changes in oxygen, carbon dioxide and bicarbonate in an *Elodea canadensis* Michx. vegetation. They also used this layer approach to calculate the photosynthesis of the whole vegetation.

The incorporation of several layers in a model can lead to a strong increase in complexity and in the amount of equations that have to be solved. However, for a

macrophyte vegetation which can achieve a considerable height, like a vegetation of *Potamogeton pectinatus* L., such an approach appears absolutely necessary to accomodate physiological acclimation to light changes with depth.

The model of Collins & Wlosinsky (1989) did not distinguish between macrophyte parts. The aboveground biomass was just distributed over depth using a maximum volumetric density, creating a biomass column. Our research project aimed at a more detailed description of specific growth characteristics of *P. pectinatus* including tuber initiation and growth, secondary shoot formation and canopy development. Therefore, it appeared necessary to construct a growth model in which light intensity could influence photosynthesis, phenology as well as plant morphology.

In the following, a description is given of the generalized life cycle of a vegetation of *P. pectinatus*. This aquatic macrophyte is one of the few species that are still common in most Dutch shallow, eutrophicated lakes. The description is the result of a compilation of our current knowledge of the physiology and ecology of the species in a temperate region (a.o. Van Wijk, 1988, 1989; Madsen & Adams, 1989; Hootsmans & Vermaat, 1991; Van Dijk & Van Vierssen, 1991; Vermaat & Hootsmans, 1991a, b; Van Vierssen & Hootsmans, in press). This life cycle has functioned as the basis for the development of the growth model SAGA1, which is subsequently described. The behaviour of a preliminary version of the model was already discussed shortly in Hootsmans & Van Vierssen (in press). The present model deviates mainly regarding the parameter values used.

2. Generalized life cycle of *P. pectinatus*

2.1 Wintering and sprouting of the tuber bank

During winter, a vegetation of *P. pectinatus* usually survives by means of specialized rhizome organs, the tubers. They form a tuber bank with a distinct size frequency distribution. This distribution can be rather different for different habitats (Vermaat & Hootsmans, 1991a). The hibernating tuber bank steadily looses biomass. This is caused by maintenance metabolism and mortality due to diseases and grazing (e.g. wintering Bewick's Swan, *Cygnus bewickii* Yarr.; see chapter 10).

The evidence for a possible dormancy and its nature is somewhat confusing. For various European populations, Van Wijk (1989) demonstrated a positive effect of stratification on the subsequent sprouting percentage when temperatures were 15 °C and lower. However, at higher temperatures, the effect of stratification appeared less important. Van Vierssen et al. (b, in prep.) even showed that recently formed tubers from a Californian population could sprout immediately, provided that they were removed from the rhizome system. Tuber size also seemed to play a role in the sprouting process. The time period up till sprouting was longer for smaller tubers. This was attributed to the physiological condition of the tuber: small tubers were supposed to have stronger sink characteristics (Van Vierssen et al., b, in prep.). Thus, the presence of an innate dormancy in the tuber seems to depend on tuber size

and stratification. The temperature of sediment and water can (partly?) overrule it, but when too low (below 10 °C), it can also impose an enforced dormancy. In temperate regions, when the rhizome system has desintegrated in late autumn, tubers do not sprout until the following spring. Then, most tubers sprout synchronously when temperature rises again above 10 °C.

Part of the tuber bank does not sprout in spring. These tubers are able to sprout, but for unknown reasons remain dormant (Van Vierssen & Bij de Vaate, 1990). This opens the possibility that some tubers can survive more than one winter. Thus, the survival potential of the vegetation can be increased.

2.2 Initiation of plant growth

The juvenile plant can rely on the reserves in its mother tuber for a certain period (Hodgson, 1966; Van Vierssen et al., c, in prep.). First of all, energy is invested in the development of aboveground parts. This ensures the future energy supply by photosynthesis. Growth of belowground biomass is initiated about one week after sprouting (pers. obs.). The importance of the reserves in the mother tuber relative to photosynthesis decreases steadily. Still, an effect of the initial tuber size on plant development can be evident even two months after sprouting, especially in plants from small tubers (<0.1 g fresh weight; Vermaat & Hootsmans, 1991a, b; Van Vierssen et al., c, in prep.).

2.3 Light and photosynthesis

The light intensity experienced by the growing plant is determining photosynthesis and thus plant survival. Photosynthesis is directly influenced because light supplies the energy for the process. Indirect effects of light also occur. Photosynthetic characteristics of plant tissue are dependent on the average light intensity experienced by this biomass during its existence (light history level; Hootsmans & Vermaat, 1991). Plants with a low light history level show decreased gross and net rates of photosynthesis. Comparable changes occur when plants become older. It appears that temperature does not strongly affect the net rate of photosynthesis in *P. pectinatus*. Within the normal temperature range during the growing season (15-25 °C) net photosynthesis varied $\pm$ 15% around the mean rate for this temperature range (Madsen & Adams, 1989).

2.4 Plant growth and development

The demands of the growing plant in terms of energy have to be fulfilled by the available resources. These comprise the reserves that can be mobilized from the mother tuber and the photosynthetic production. The way in which these resources are allocated in the aboveground biomass is strongly influenced by the light intensity reaching the plant. Elongation is stimulated by low light levels, while secondary shoot formation is increased under high light intensities (Vermaat & Hootsmans, 1991b).

The growing vegetation can be described as a heterogeneous community of young and old plants, developing from different tuber sizes. Plants from small tubers remain smaller and are increasingly subjected to shading by larger neighbours.

Clearly, light plays an important role in determining growth and development. The amount of light reaching the water surface changes both daily and

seasonally. Seasonal fluctuations in biotic factors as phytoplankton, zooplankton, periphyton and epifaunal grazers may also affect the light climate for the plant. Physical and stochastic factors like sediment composition and stability, wind fetch, depth, storm and wave formation influence sediment resuspension (Van Vierssen & Bij de Vaate, 1990).

In turbid waters, *P. pectinatus* exhibits rapid growth to the water surface, concentrating its biomass in the upper water layers (canopy formation; Van Wijk, 1988; Van der Bijl et al., 1989). By growing to the water surface, the importance of light extinction by the water layer for plant photosynthesis is reduced. Periphyton shading cannot be evaded in this way, but its effect is limited also as light intensity near the water surface is much higher.

2.5 Tuber formation

The formation of new tubers can already start within a few weeks from the beginning of the growing season (Van Dijk & Van Vierssen, 1991). Tubers are initiated at the end of rhizomes. Thus, the amount of tubers formed will partly be determined by the available rhizome biomass (or 'space').

Tuber initiation and growth are strongly influenced by light. Apart from the obvious direct effect of light quantity and its consequences for the available resources, photoperiodic effects play a role. Especially the so-called photosynthetic period is important. This is defined analogous to the photoperiod as the daylength during which the light intensity surmounts a certain minimum treshold value. The precise value of the treshold light intensity determining the photosynthetic period is not known. It seems to be rather high, i.e. at a level above which the rate of photosynthesis does not change very much (Van Vierssen, unpub.). Shading experiments in the field showed that the ratio of photosynthetic period to photoperiod strongly determined the tuber initiation rate (Van Dijk & Van Vierssen, 1991). Laboratory experiments with various combinations of photoperiods and photosynthetic periods confirmed these findings (Van Vierssen et al., a, in prep.). When this ratio decreases, the tuber initiation rate is increased. It is not clear how the photosynthetic period is measured by the plant. The obvious mechanism would be somehow via the photosynthetic process.

2.6 Decay and loss of biomass

Cells can divide with a rate that is limited. Thus, for instance at the start of the growing season, periods may occur during which the resources become available at a much higher rate than can be accomodated by growth. Then reserves can be formed in various plant parts.

During the growing season, ageing and shading negatively affect photosynthesis (Hootsmans & Vermaat, 1991). Resources decrease and from a certain moment onwards, the condition of plant tissue declines visibly. Increased wave action caused by storms leads to biomass loss and disappearance of the aboveground vegetation. Tubers remain in the sediment to survive to the following spring. Under more sheltered conditions (e.g. ponds, ditches), part of the aboveground biomass may survive during winter (Van Wijk, 1988). This biomass can contribute to the establishment of the new vegetation in the next season.

3. SAGA1: description of the model

3.1 Modelling concepts and implementation

The macrophyte growth model SAGA1, short for Sago pondweed Growth Analysis model version 1, was not designed for a particular situation. It should represent a generalized life cycle of *P. pectinatus* as described in section 2. It was supposed that by changing the relevant physiological parameter values, the model could give a useful description of the annual cycle of this species in many different situations.

The following conditions played an important role in the modelling process:

1. The model simulates the vegetation as biomass per m^2. The biomass is not necessarily homogeneously distributed over depth.
2. The vegetation originates from a tuber bank with a certain tuber size distribution.
3. Photosynthesis determines the possibilities for growth, together with material derived from the tuber bank. Photosynthesis is integrated over the height of the vegetation.
4. The rate of photosynthesis is related to light through a light response curve.
5. The parameters determining the shape of the light response curve are dependent on the age and average light level experienced by that particular part of the vegetation for which photosynthesis is determined.
6. Plant development (rate of elongation, secondary shoot formation, tuber initiation) is determined by light.
7. The decrease and disappearance of the vegetation at the end of the vegetation is a result of physiological and stochastic (wave action) events.
8. Light climate as experienced by the vegetation is determined by self-shading, periphyton shading, water layer turbidity and the seasonal and daily changes in the amount of light reaching the water surface.

The precise mathematical formulation of these constraints will be dealt with in subsequent sections. A short overview of the model structure is given in the following.

The vegetation is simulated as three subvegetations, each with its own tuber bank and a specific, fixed initial tuber size. In this way, a tuber size distribution with three classes is simulated. The subvegetations can influence each other through shading effects. Total aboveground biomass is arranged in layers stacked over plant height for each discerned vegetation. Within each layer, biomass is assumed to be distributed homogeneously. Each subvegetation consists of biomass sprouted from the tuber bank (referred to as mother vegetation) and secondary shoot biomass. Combining the biomass of mother vegetation and secondary shoots, average age and light history of each layer in a subvegetation are calculated. These variables determine the shape of the light response curve of each layer. Total production of a subvegetation is summed over depth.

The available resources (photosynthetic production, reserves from mother tubers and reallocated material from dead biomass) are used for growth of aboveground, belowground and new tuber biomass. Growth of aboveground biomass of a subvegetation is realized in three different ways. The length of mother vegetation and existing secondary shoots can increase (referred to as elongation), new secondary shoots can be formed, and the biomass in each layer of the mother

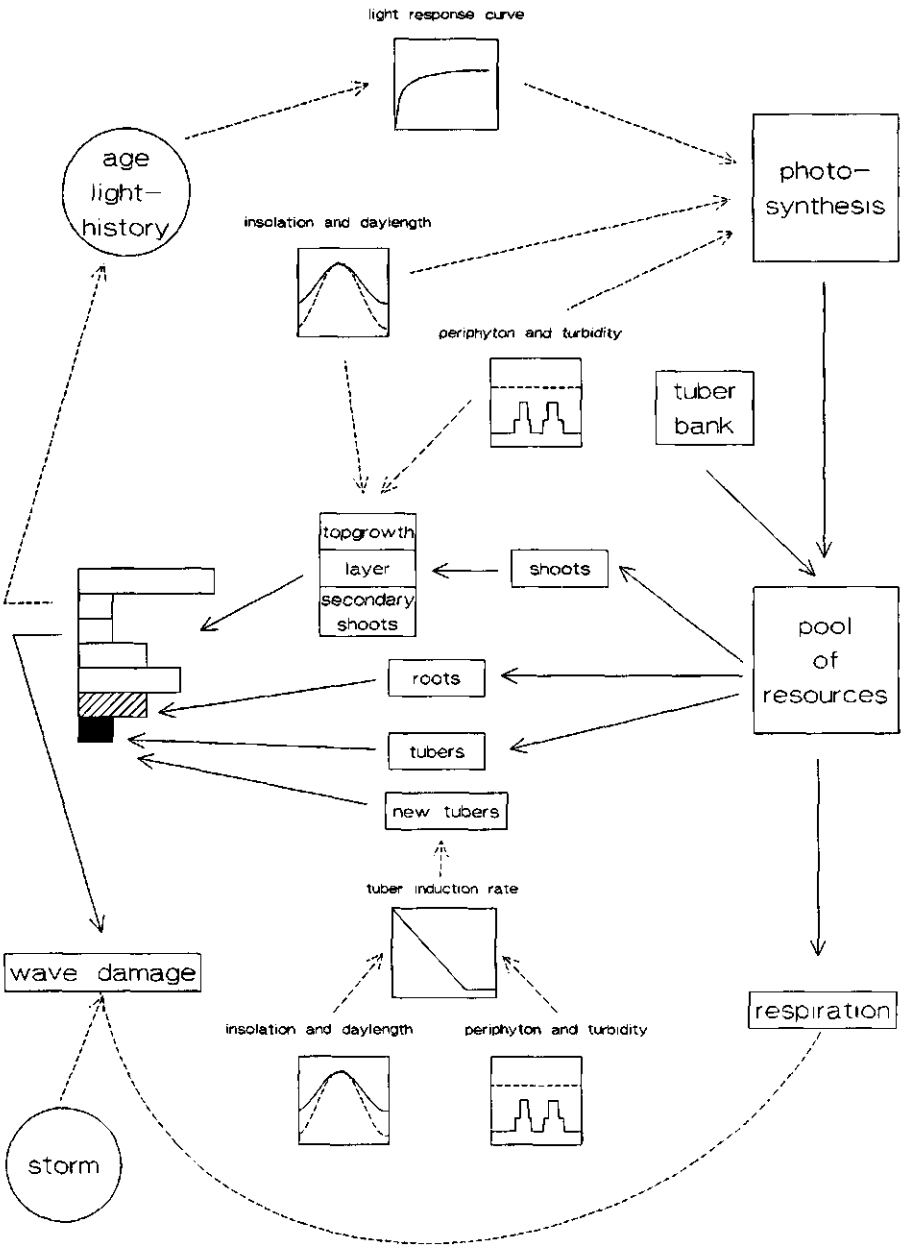


Fig. 9.1. An overview of the important relations and flow of information in the model SAGA1. Only one subvegetation is represented, showing its construction in layers. Mass flows are indicated with a solid line, flow of information with a broken line.

vegetation and the existing secondary shoots can be increased. The precise distribution of the resources available for aboveground growth among the three possibilities is decided upon by means of tables. These provide the fraction of the resources that can be used for growth of a specific component, which is dependent on light intensity.

When there are not enough resources to fulfil respiratory needs, the rate of photosynthesis is decreased and chances for damage due to wave action are increased. This leads to a decrease in vegetation standing stock with increasing age. Also, chances for storm events increase in autumn. A schematic representation of the relations in the model, for clarity with only one subvegetation, is given in Fig. 9.1.

Clearly, photosynthesis and changes in photosynthetic parameters due to light acclimation and ageing play a central role in the model. It was hoped that the occurrence of biomass losses through wave action could be based on changes in photosynthesis. In this way, the often observed rapid decrease in biomass at the end of the season, especially in wind-exposed habitats (Van Wijk, 1988, and pers. obs.), might be given a physiological basis other than e.g. increased mortality or respiration. The latter solutions are probably indirect results from changes in photosynthetic capacity.

Both through its influence on photosynthesis and its effects on plant elongation and secondary shoot and tuber formation, light is the most important environmental factor in SAGA1.

3.2 Overview of model structure

In this section, a description of the simulation model SAGA1 is given. The mathematical details of the actual implementation are presented in Appendix 1.

The model consists of a main program from which calls are made to various subroutines. Basically, three subroutines (VEG1, VEG2 and VEG3) are centered around the main program. They represent the three subvegetations. Each subroutine has the same structure, and uses the same set of assisting subroutines in calculating the seasonal biomass development. The results from the three subvegetations are combined within the main program. A scheme giving the position of the various subroutines in the model is given in Fig. 9.2. The relation between the model subroutines and the generalized life cycle described in section 2 is indicated also. The parameter values that are used in the model will be discussed in the next section.

At the start of a simulation, the main program calls the subroutine INFO which opens several files for input and output. Every day a call is made to the subroutine METEO for the actual day-length and daily insolation. Subsequently, the subroutine LIGHT is called. This subroutine uses the amount of shading due to periphyton, turbidity and self-shading in the vegetation to calculate the daily insolation halfway in each of the various layers of the vegetation. Also, a depth distribution of the instantaneous insolation halfway each of these layers at three times of the day is calculated. The latter information is used in the calculation of photosynthesis.

Each of the three subvegetation routines VEG1-3 has the same sequence of procedures, which will now be discussed. During the year, the tuber bank is steadily loosing biomass because of maintenance costs, diseases and predation. These effects are combined in a constant decay rate. Every day, in subroutine

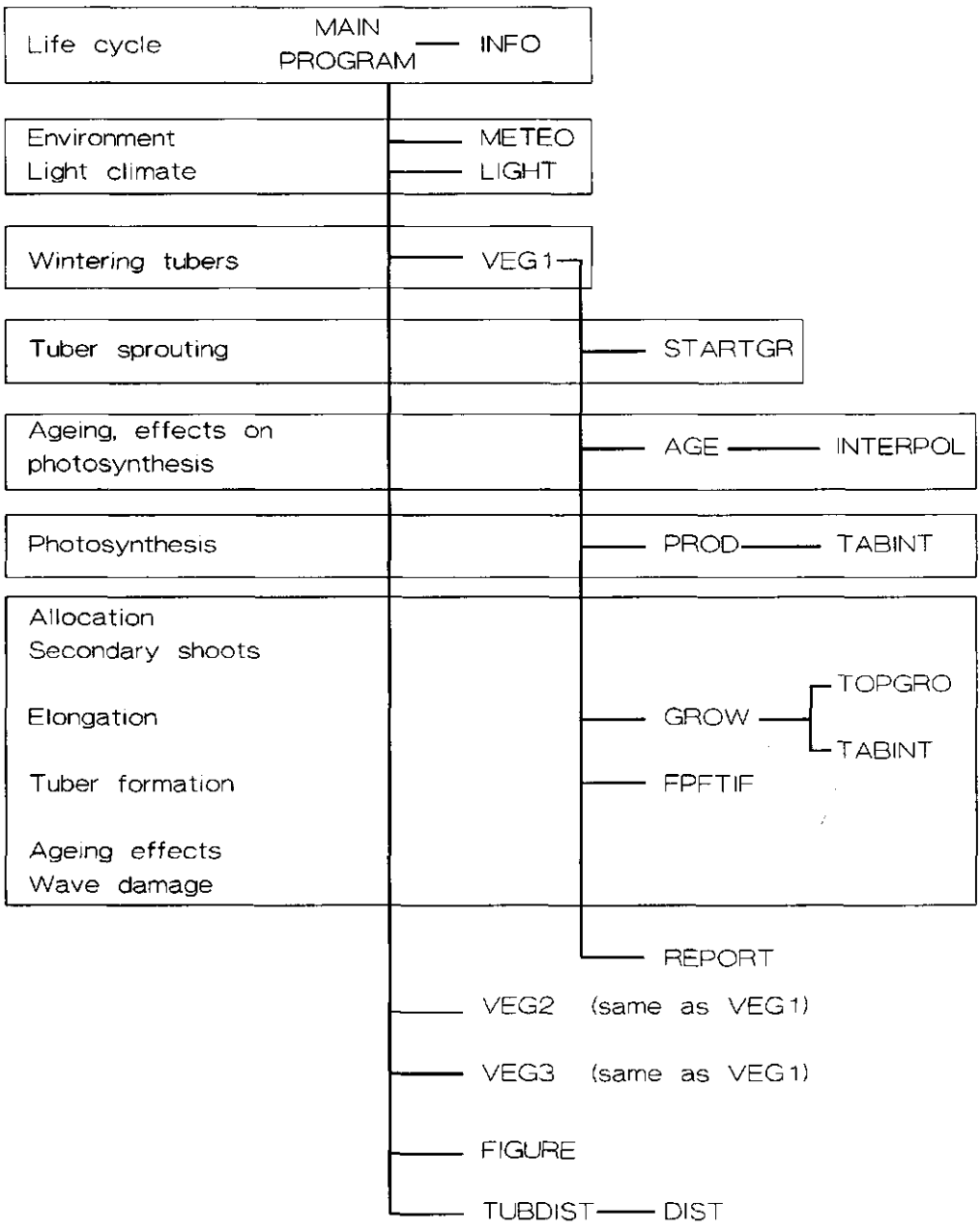


Fig. 9.2. Generalized life cycle of *P. pectinatus* in temperate regions, and the subroutine structure of the derived simulation model SAGA1.

STARTGR it is checked whether the growing season has begun or not. When it has, aboveground and belowground biomass are initiated and subsequently, other subroutines are called from VEG. In AGE, the average ages of the mother vegetation, secondary shoots and of the total biomass are calculated for each layer. Together with the daily light distribution obtained from LIGHT, the age distribution and the biomass of mother vegetation and secondary shoots, the average light level experienced by each layer during its existence (light history) is obtained. Age and light history of a layer determine the two necessary photosynthetic parameters PM (maximum rate of gross photosynthesis) and KM (light intensity at which the rate of gross photosynthesis is half the maximum rate). These are obtained in INTERPOL by interpolation from a table in which these photosynthesis constants are provided for various light history levels and ages.

In FPFTIF, the photosynthetic period factor FPF is determined from a tentative relation of FPF with the ratio of photosynthetic period to photoperiod (FPRATIO; a more detailed explanation is given in section 4.8). FPF influences the rate at which new tubers will be formed.

The photosynthetic production for each day by each subvegetation is calculated in PROD. Total photosynthesis is integrated over depth and time of the day. A call is made to subroutine TABINT for a linear interpolation of the effect of temperature on photosynthesis from a table. TABINT was taken from Ng & Loomis (1984). In SAGA1, the temperature effect is not operational.

The largest subroutine is GROW. Here, the daily production and the reserves coming from the mother tubers are combined in the pool of assimilates available for respiration and growth. Based on specific growth rates for roots, shoots and newly formed tubers, the potential growth of the various fractions is calculated. Modifications through factors derived with TABINT are possible. In SAGA1, only light efects on shoot growth are used. Effects of temperature, assimilate shortages and age on a.o growth rates are incorporated, but not operational. The potential growth then is compared with the available resources after respiratory demands have been subtracted. In principle, all potential growth is realized, but when the pool does not contain enough resources, the requested growth is curtailed with a certain percentage to be in balance with the available amount of resources. When shortages occur, tuber growth is favoured above shoot and root growth. The assigned shoot growth subsequently is divided between elongation, secondary shoot formation and horizontal growth within the existing layers. This allocation is made separately for shoot growth of the mother vegetation, and for shoot growth of each of the secondary shoots. The new length after elongation is determined in TOPGRO. Increase in length is strong when the daily insolation on the top layer is low.

Secondary shoot formation is increased when daily insolation reaching the vegetation is high. To determine secondary shoot formation, insolation on the bottom layer was taken instead of insolation on the top layer, as the latter would result in an increased secondary shoot formation under turbid conditions when the vegetation reaches the water surface. Such an effect seems rather unlikely.

Based on the calculated photosynthetic period factor FPF and the amount of root biomass, a number of new tubers is initiated daily from the second month in the growing season onwards. When growth of existing tubers is limited

due to shortage of resources, no new tubers are initiated. This reflects a strongly negative influence of growing tubers on young tubers in their neighbourhood.

Using a call to TABINT, the chance for storm damage (wave action) is determined with a random number. This chance is dependent on the time of the year. Chances for damage due to increased wave action are also increased when respiratory needs are not fulfilled completely. Losses occur first in the uppermost layers of the vegetation. In this way, the breaking of a few old main stems resulting in loss of part of the canopy is simulated.

The concomitant increase in mean age and a decrease in the average light intensity received by the remaining tissue leads to a further decrease in photosynthesis, increased shortages and increasing chances for wave loss.

Finally, in REPORT, results for each day per subvegetation are written to three output files (SAGA1-3).

When all subvegetation subroutines have been completed, the results for the total vegetation are summarized in the main program and written to the file SAGA. A graphic representation of the depth distribution of biomass is printed to the screen for each subvegetation by subroutine FIGURE.

At the end of each year, the main program calls TUBDIST. This subroutine makes calls to subroutine DIST to summarize the tuber bank data from each subvegetation. In this way, a new mother tuber bank for the whole vegetation, consisting of three size classes, is constructed. In the next year, the new tuber bank initiates the new vegetation.

4. Defining the system and its environment: the choice of parameter values

4.1 Introduction

A complex simulation model like SAGA1 includes many different parameters that can be defined with varying certainty. Full parametrization has to be based partly on comparing model results with reality. A limited number of parameters was directly taken from experimental results (e.g. effects of light and age on photosynthesis, Hootsmans & Vermaat, 1991). Other processes, like tuber induction and tuber growth, could be defined qualitatively using lab and field data (Van Dijk & van Vierssen, 1991; Van Vierssen et al., a, in prep.). The quantitative implementation was reached by experimental data and by model calibration. Likewise, effects of light on elongation and secondary shoot formation were based on experimental results (Vermaat & Hootsmans, 1991b) but their final numerical definition was based on the actual model response. Sometimes, the criteria for the choice of a specific parameter value could only be found in the response of the model system to this parameter together with all other parameters. When several parameters interact, it may be that the estimated value of a parameter strongly depends on the already chosen values for other parameters.

Model calibration was done by comparing total biomass development, total tuber biomass and average tuber size in the model system with data from field work in Lake Veluwe, The Netherlands (results from the unshaded control; Van

Table 9.1. Parameter values used in SAGA1, and their status. Field data = Lake Veluwe data (unshaded control).

parameter	model name	value	reference
light and temperature, storm effects			
- maximum daylength	DMAX	16.45 hour	5
- minimum daylength	DMIN	7.48 hour	5
- light reflection	REFLEX	0.10	5
- thickness of layer	THICKN	0.1 m	user defined
- effect of daynumber on storm chance	ESTORM	see Fig. 9.4	tentative
- fraction lost in case of wave damage	FRAC	0.1	tentative
tuber bank			
- tuber bank VEG1	WTUB(1)	2.4 g afdw	field data
- tuber bank VEG2	WTUB(2)	3 g afdw	field data
- tuber bank VEG3	WTUB(3)	0.6 g afdw	field data
- average tuber weight VEG1	ATUB(1)	0.04 g afdw	field data
- average tuber weight VEG2	ATUB(2)	0.025 g afdw	field data
- average tuber weight VEG3	ATUB(3)	0.01 g afdw	field data
- decay rate tuber bank	TUBDEC	0.001 day^{-1}	1
initiation of growth			
- start growing season	IDAYBEG	120 days	field data
- fraction of tuber bank for initiation of biomass	CITUBFL	0.15	tentative
- fraction of tuber bank coming available per day	CTUBFL	0.10 day^{-1}	3, 8
- weight of young biomass per unit length	WLINI	0.05 g afdw m^{-1}	4
- fraction of initiation biomass used for root initiation	ROOTIN	0.25	tentative
photosynthesis and respiration			
- maximum rate of gross photosynthesis	PM	see Fig. 9.8a	4, calibrated
- light intensity at which half PM is reached	KM	see Fig. 9.8b	4
- conversion O_2 - afdw	CCO2GR	0.82	2, 6, 10
- rate of dark respiration	RESPMAX	0.018 day^{-1}	4, calibrated

Table 9.1. Continued.

parameter	model name	value	reference
growth and morphology of the vegetation			
- maximum root growth rate	RGP	0.20 day^{-1}	9, calibrated
- maximum shoot growth rate	SGP	0.20 day^{-1}	9, calibrated
- maximum secondary shoot growth rate	SECGP	0.20 day^{-1}	9, calibrated
- death rate roots	DRTR	0.001 day^{-1}	tentative
- death rate aboveground	DRTS	0.001 day^{-1}	tentative
- conversion efficiency of dead material (reallocation)	CEDEAD	0.5	tentative
- self-shading extinction coefficient	KPLANT	0.02 $m^2 g^{-1}$	11
- number of shoots per secondary shoot biomass	INSEC	20	calibrated
- initial secondary shoot biomass	SECSTART	0.1 g m^{-2}	4
- maximum number of secondary shoot biomasses	SECMAX	25	user defined
- effect of light on elongation	ELTOP	see Fig. 9.9	9, calibrated
- effect of light on secondary shoot formation	ELSEC	see Fig. 9.9	9, calibrated
tuber initiation			
- light level determining tuber induction rate	BOUNDLIGHT	200 μE $m^{-2} s^{-1}$	7
- maximum ratio of photosynthetic period and photoperiod	FRMAX	0.70	calibrated
- minimum tuber induction factor	FPFMIN	0.10	calibrated
- place factor	PF	60	field data
- maximum number of tubers formed per day	TUBNUM	5 day^{-1}	calibrated
- initial tuber biomass	TUBIN	0.006 g afdw	field data
tuber growth			
- maximum tuber growth rate	TGP	0.08 day^{-1}	field data
- period of maximum tuber growth rate	ITUBDAY	21 days	calibrated
- minimum tuber growth rate	TGPMIN	0.01 day^{-1}	calibrated
- minimum fraction of potential tubergrowth realized per day	TUBMIN	0.5	calibrated
- maximum weight of newly formed tuber	TUBMAX	0.06 g afdw	user defined

References: (1) Anderson & Low (1976), (2) Best & Dassen (1987), (3) Hodgson (1966), (4) Hootsmans & Vermaat (1991), (5) Kirk (1983), (6) Van der Bijl et al. (1989), (7) Van Vierssen (pers. comm.), (8) Van Vierssen et al. (c, in prep.), (9) Vermaat & Hootsmans (1991b), (10) Westlake (1963), (11) Westlake (1964).

Dijk & Van Vierssen, 1991). Only a general order of magnitude agreement was aimed for. Various interactions and processes are not yet described with much detail at the moment. Besides, a generalized insolation curve and periphyton regime was used, together with a constant average cloud cover and water layer extinction coefficient. Thus, exact agreement was highly unlikely.

Most parameters are supplied to the model by the input datafile SIMPARAM.PRN. The use of such an inputfile facilitates changes in parameter values. Some parameters that are not often changed are incorporated in the model, e.g. the tables reflecting the effects of light history and age on photosynthetic parameters. Data on periphyton shading percentage PERIF and water layer light extinction EXTW are provided in the input file PERFEXTW.PRN. In Table 9.1, the values of the parameters in the file SIMPARAM.PRN as used in SAGA1 are shown. These will be discussed in the following paragraphs, together with parameters whose values are incorporated in the model.

4.2 Light and temperature, storm effects

The model generates a sinusoidal fluctuation of daylength between the average yearly maximum and minimum values (DMAX-DMIN). The actual values of DMAX and DMIN were based on a daylength formula given by Kirk (1983) using a latitude of 52° N for The Netherlands. A cosine function causes the maximum to occur on June 21st, the minimum on December 20th (the model year has 365 days).

In calculating daily insolation, a long yearly average of daily global insolation of 10^7 J m^{-2} day^{-1} in the Netherlands (1961-1970) is modified with a goniometric formula. In Fig. 9.3, the actual average annual cycle of global insolation is given together with the cycle generated by the model. For further use in the model, the global insolation values are multiplied with 4.66 μE J^{-1} to convert units to μE m^{-2} day^{-1} (based on McCree, 1972), and with 0.5 to restrict insolation to PAR (400-700 nm, based on Straškraba & Gnauck, 1985). Average cloud cover is already incorporated in the long yearly average. Reflection of light by the water surface, REFLEX, is kept at 10%. This value was chosen somewhat higher than mentioned by Kirk (1983) as Lake Veluwe is rather wind-exposed. This causes waves, and thus more reflection. The daily insolation value is fixed in subroutine METEO, together with the conversion factors 4.66 μE J^{-1} and 0.5. The thickness of a layer in the water column can be user-defined by parameter THICKN. It was kept at 0.1 m.

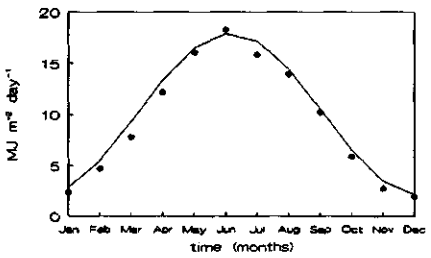


Fig. 9.3. Annual cycle of daily global insolation, as generated by the model (solid line), compared with the long yearly monthly averages for 1961-1970 in The Netherlands (dots).

The chance for a storm event is dependent on daynumber: decreasing from 0.25 in January to zero in the beginning of April (day 100), and increasing again from mid September (day 265) through December. The relation is partly fixed in

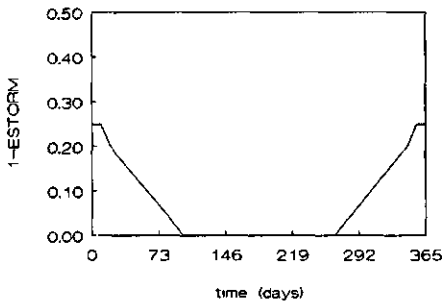


Fig. 9.4. Relation between daynumber and storm chance 1-ESTORM (see text). When ESTORM equals 1, storm chance is zero.

GROW by the daynumber on which storm chance becomes zero. The chance distribution was chosen qualitatively and is not based on actual long yearly data on storm occurrences. The relation between storm chance and daynumber is stored in the table ESTORM. The difference 1-ESTORM represents the storm chance (Fig. 9.4). When a storm occurs, a certain fraction FRAC of the total aboveground biomass disappears. The actual value of FRAC was tentatively kept at 10%.

4.3 Periphyton shading and water layer light extinction

In the present version of the model, dynamics of periphyton and phytoplankton/seston are not incorporated. Seasonal fluctuations in periphyton shading percentage PERIF and water layer light extinction EXTW are provided by daily values read from the input file PERFEXTW.PRN. No difference was made in periphyton cover between the various layers in the vegetation or between plant parts of different age. The shading percentages used in the model simulations were based on data from two-week incubations in the field in Lake Veluwe (Van Vierssen & Bij De Vaate, 1990). Long-term incubations indicated that after four weeks, a constant shading percentage of about 80-90% was reached. However, maximum periphyton shading percentage used in the simulations was 50%. Thus, effects of leaf age and leaf-sloughing on average periphyton cover are more or less compensated for.

Of course, the general shape of the periphyton shading curve and the timing of the peaks may vary between years, depending a.o. on weather conditions. In 1986, the spring peak in Lake Veluwe occurred in June, while a late summer peak was almost absent. In 1987, the spring peak was earlier, and a late summer peak occurred during the second half of August and the first half of September (Van Vierssen & Bij De Vaate, 1990). Especially a spring peak can be expected to influence plant growth. During the late summer bloom, the vegetation in the field has already reached or passed its peak biomass level. Fluctuations in periphyton biomass during the season are also known from other temperate lakes. Mason & Bryant (1975) report a spring peak from

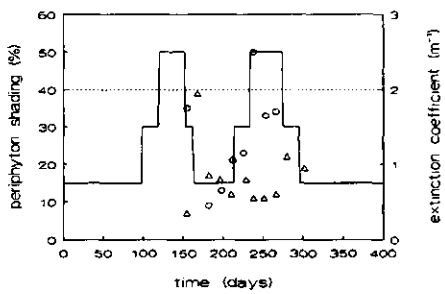


Fig. 9.5. Periphyton shading regime (solid line) and water layer light extinction (dotted line), as used during model calibration ('Lake Veluwe conditions'). The actually measured periphyton shading percentages during 1986 (triangles) and 1987 (circles) under unshaded conditions in Lake Veluwe are shown also.

March to the end of April. Cattaneo & Kalff (1978) found a spring peak in June and a second peak in September. Meulemans & Heinis (1983) found a spring peak in May on reed stems.

For model calibration, a periphyton and seston shading regime was used as shown in Fig. 9.5. Also shown are measured shading percentages in Lake Veluwe in 1986 and 1987 (Van Vierssen & Bij de Vaate, 1990). In line with the use of an average insolation curve, the model periphyton cycle is a generalized seasonal curve based on the information given above.

Timing of the first peak in the model was chosen to coincide with the first month of the growing season in the model, i.e. May. In this way, early plant growth can be most strongly affected by periphyton shading, both through photosynthesis and the determination of the tuber induction rate (cf. section 4.8). Compared to Lake Veluwe in 1986, the spring peak in the model was 4 weeks earlier. The late summer peak in the model lasted from the second half of August till the end of September, comparable to the situation in 1987 in Lake Veluwe. Actual water layer extinction coefficient data showed strong variation during the year. It was decided to use a constant value of 2.0 m^{-1} for model calibration, based on the average value of 2.2 m^{-1} in 1986 for Lake Veluwe.

4.4 Tuber bank

The model tuber bank (implemented with the arrays WTUB and ATUB) was derived from data on actual tuber size distribution as well as tuber bank biomass data, both from Lake Veluwe (unshaded control; see Van Dijk & Van Vierssen, 1991). Tuber size distribution in the model, with only three size groups, of course can only be a rough approximation of the field data. The size distribution used in SAGA1 is shown in Fig. 9.6, together with the two field size distributions on which it was based (one from January 1986, the other from April 1987). Size class boundaries in this figure were based on fresh-weight data and were subsequently converted into ash-free dry-weight (afdw).

To estimate the model tuber bank

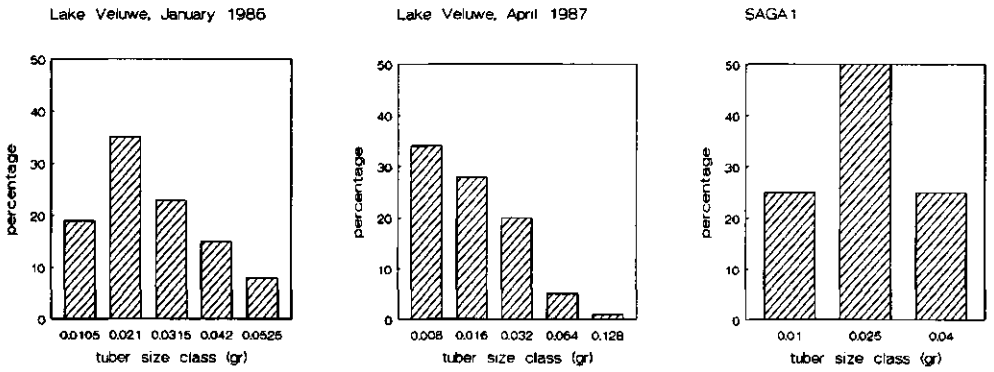


Fig. 9.6. Model tuber size distribution, together with two independent datasets representing tuber size distributions in Lake Veluwe. All weights in this and following figures are in afdw. Tuber sizes for the two field samples represent upper class limits; model tuber sizes are class averages.

Table 9.2. Tuber bank biomass changes during winter, using an average tuber decay rate of 0.003 day^{-1} in Lake Veluwe. Model values are based on 0.001 day^{-1}. Lake Veluwe tuber bank biomass values are based on data from October 2nd and November 3rd, 1986 and April 15th and May 13th, 1987. Model values are from the first year and year 10 (stabilized cycle) of the nominal simulation with the periphyton and seston regime shown in Fig. 9.5. All values in g afdw m^{-2}, except number of tubers (number m^{-2}).

	November 1	January 1	April 30		
	actual	estimate	actual	estimate	tuber no.
control	8.0	6.7	3.5	4.7	150
25% shaded	7.0	5.9	3.5	4.1	150
50% shaded	3.0	2.5	2.2	1.7	90
75% shaded	2.0	1.7	1.0	1.2	60
model year 1	-	6.0	-	5.3	212
model year 10	-	8.6	-	7.6	251

biomass on January 1st, a value for the tuber decay rate was also necessary. Using Lake Veluwe tuber bank biomass data from the beginning of November 1986 and the end of April 1987, the decay rate TUBDEC appeared to be 0.003 day^{-1} on average for the four light treatments, combined with a tuber bank biomass on January 1st of about 6-7 g afdw m^{-2} for the unshaded control (Table 9.2). However, during model calibration this value for TUBDEC appeared too high to reach a reasonable agreement in vegetation development between model and reality. Based on the Lake Veluwe tuber bank biomass at the start of the growing season, the model vegetation biomass remained much lower than the Lake Veluwe control.

Other data on tuber decay during winter were found in Anderson & Low (1976). Several *P. pectinatus* plots protected from grazing by wildfowl because of ice cover showed a tuber bank biomass decrease from the end of October to early June (i.e. about 220 days) varying from 15 to 30% of the initial biomass. This leads to an estimated tuber decay rate of 0.001 day^{-1}. Combined with a total tuber bank biomass on January 1st in year 1 in the model of 6 g afdw m^{-2}, this decay rate resulted in a reasonable biomass development in the model system compared to the Lake Veluwe situation. Despite the lower TUBDEC in the model, the model tuber bank in the stabilized situation (simulation year 10) still becomes rather large compared to the Lake Veluwe control situation (Table 9.2). Apparently, the model vegetation needs more support from the tuber bank during early growth than is necessary in reality. I will return to this in section 5 (model validation).

4.5 Initiation of growth

The start of the growing season (IDAYBEG=120 days) was taken from Lake Veluwe data. An initial fraction CITUBFL is taken from the tuber bank biomass on this day to initiate vegetation biomass. The model calibration suggested that CITUBFL should be higher than the daily fraction CTUBFL coming available from the tuber bank. It was fixed at 0.15 day^{-1}.

CTUBFL (0.1 day^{-1}) was based on

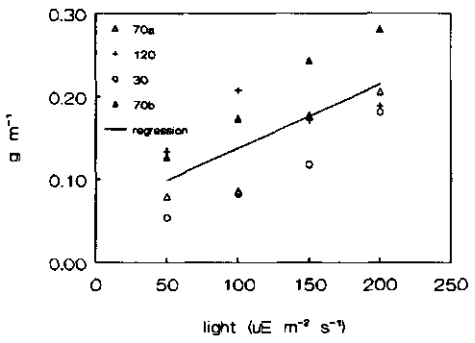


Fig. 9.7. Relation between biomass per unit length (WEIGHTL, g afdw m^{-1}) and light intensity during growth, for *P. pectinatus* plants of various ages. 70a, 70b = age 70 days, 120 = age 120 days, 30 = age 30 days. Regression data: WEIGHTL = 0.06 + 0.0008 * LIGHT, $r^2 = 0.50$, $p < 0.005$.

data for early growth of *P. pectinatus* in the laboratory (Hodgson, 1966; Van Vierssen et al., c, in prep.). The calibrated value had to be somewhat higher than the value 0.08 day^{-1} based on the laboratory data, again reflecting the strong dependence of the model vegetation on tuber bank material during early growth. Although a certain energy loss will be associated with the reallocation of tuber biomass to other plant parts, I have assumed this to be negligible.

Plant length associated with the initial amount of biomass on the first day in the growing season is dependent on the parameter WLINI. This parameter represents the amount of biomass per unit length of newly formed biomass. Its value was derived from length and biomass data of the plants used in the photosynthesis measurements (Hootsmans & Vermaat, 1991). The lowest value from these data appeared to be 0.05 g afdw m^{-1}, belonging to young plants growing under low light conditions, i.e. showing the strongest elongation (see Fig. 9.7). These conditions were supposed to be close to the situation in the field during the first period of growth. The value of WLINI was set at 0.05 g afdw m^{-1}.

Root/rhizome biomass is started on the 7th day in the growing season (based on personal observations in various laboratory experiments). An arbitrary fraction ROOTIN (0.25) of the total amount of biomass that is available for total biomass initiation (CITUBFL*WTUBER on IDAYBEG) is then transformed into root/rhizome biomass.

4.6 Photosynthesis and respiration

Plant photosynthesis parameters were taken from laboratory measurements on *P. pectinatus* plants from various ages grown at different light levels (Hootsmans & Vermaat, 1991). PM values were increased by 20% during model calibration. This was considered reasonable, as photosynthesis data were obtained with plants that were not forming new tubers. Results for potato plant photosynthesis suggested a strong increase in PM during tuber formation, up to 50% (Ng & Loomis, 1984). Although effects on KM may also occur, changes in KM values appeared not necessary to obtain a model behaviour comparable to the Lake Veluwe situation. The relation between age, light intensity during growth and these two photosynthesis parameters is shown in Fig. 9.8. The resulting two-dimensional tables were used for interpolation in the model (see Appendix 1).

Gross production was expressed in O_2 weight units and had to be converted to afdw units using the conversion coefficient CCO2GR. The amount of carbon fixed per molecule of oxygen produced varies depending on the material that is being synthesized by the plant. When glucose is

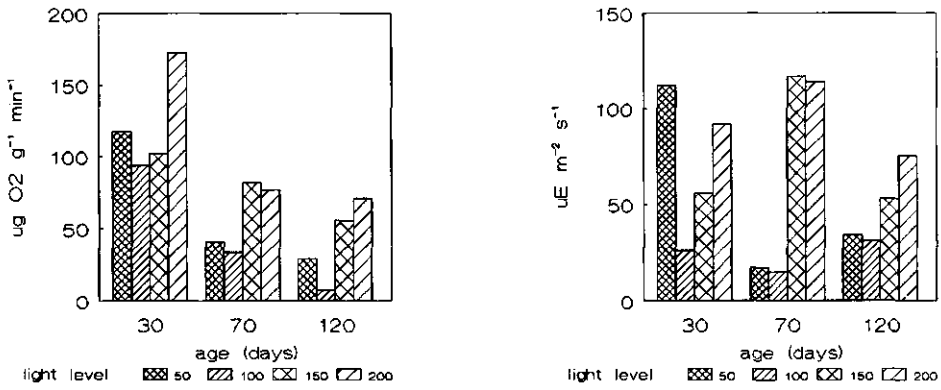


Fig. 9.8. The effect of age and average light level during growth on the photosynthesis parameters as used in the model. Left: Pm (maximum rate of gross photosynthesis, $\mu g\ O_2\ g^{-1}$ afdw min^{-1}); right: Km (light intensity at which half Pm is reached, $\mu E\ m^{-2}\ s^{-1}$).

synthesized, the photosynthetic quotient PQ (O_2/C, molar basis) is 1. Westlake (1963) gives a range of 1.0-1.25. Guterstam (1981) used a PQ of 1.2 for *Fucus vesiculosus* L. Sepers (1981) found a PQ of 1.29 for algae. Seasonal variation in PQ (0.56-1.76, average 1.12) was found by Best & Dassen (1987) for *Ceratophyllum demersum* L. For the moment, the present model uses an average PQ of 1.15.

Carbon content data of afdw vary between 39% (Nienhuis & de Bree, 1981) and 45-46% (Westlake, 1965). Van der Bijl et al. (1989) give a carbon content of dry weight of 35% for *P. pectinatus*. Combining this information, a carbon content of afdw of 40% was used. This led to a CCO2GR of 0.82.

Dark respiration RESPMAX was derived from laboratory data on plant photosynthesis (Hootsmans & Vermaat, 1991). The same value was used for all plant parts. The actually measured value of 0.025-0.035 day^{-1}, based on aboveground material, had to be lowered to 0.018 day^{-1} during model calibration.

4.7 Growth and morphology of the vegetation

Maximum relative growth rates of various plant parts (RGP, SGP, and SECGP for roots, mother vegetation and secondary shoots, respectively) were based on measured growth rates for plant length of Lake Veluwe plants in the laboratory (0.15 day^{-1}, Vermaat & Hootsmans, 1991b). RGP, SGP and SECGP were increased to 0.20 day^{-1} during model calibrations. It is stressed that these values are only reached for a short period early in the growing season, after which they are curtailed by the available amount of photosynthate. Still, they seem rather high.

Mortality rates of aboveground (DRTS) and belowground biomass (DRTR) were tentatively kept at 0.001 day^{-1}. A fraction CEDEAD of dead material was reallocated to the resources pool. Its value was arbitrarily set at 0.5, since no data are available on this parameter.

When shortage in the amount of

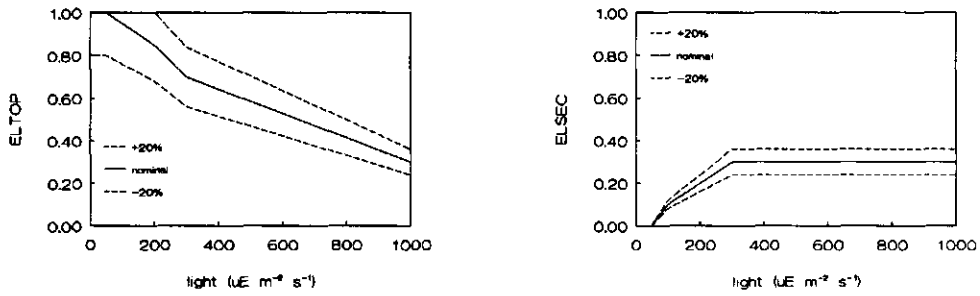


Fig. 9.9. Relation between light intensity and elongation parameter ELTOP (left) and secondary shoot formation parameter ELSEC (right). The relations used during sensitivity analysis are also shown (see text).

assimilates occurs and respiratory demands are no longer met, gross production is limited by the photosynthetic compensation parameter FC, which varies from 1 (no effect when respiratory demands are completely met) to 0 (no gross production when shortage equals 5% of total biomass). The 5% limit is incorporated in subroutine GROW.

Elongation and secondary shoot formation are determined by the light level on the top or bottom layers, respectively, of the vegetation. This light level is influenced by the periphyton shading percentage PERIF and the water layer extinction coefficient EXTW, but also by self-shading. The latter was modelled to be dependent on vegetation biomass. The accompanying extinction coefficient KPLANT was set to 0.02 m^2 g^{-1} afdw, based on data in Westlake (1964). Van der Bijl et al. (1989) found a somewhat higher value of 0.024 m^2 g^{-1} dw (all values for an e-power extinction formula).

Secondary shoots actually were modelled as biomass units, each unit representing a number of INSEC (20) secondary shoots. A new 'secondary shoot unit' is formed when the cumulative total amount of biomass that has been allocated to secondary shoot formation equals SECSTART (0.1 g afdw m^{-2}). These two parameters were chosen in such a way, that a new 'secondary shoot unit' has an initial length of 0.1 m, using WLINI=0.05 g afdw m^{-1}. A maximum number of SECMAX (25) 'secondary shoots', equivalent to 500 individual secondary shoots per m^2, can be formed by each subvegetation. This maximum was imposed to maintain a reasonable calculation speed. Secondary shoot formation starts on day 15 after the start of the growing season, based on data from laboratory experiments (Van Vierssen, unpublished; Vermaat & Hootsmans, 1991a, b). Model results stressed the importance of this delay. Immediate secondary shoot formation caused a strong decrease in elongation rate of the main vegetation. In turn, this reduced elongation increased the lowest light level under which the vegetation could survive.

The relation between light on top of the vegetation and the amount of biomass allocated to elongation is defined in a table, ELTOP (see Fig. 9.9a). The analogous relation between light level on the bottom vegetation layer and secondary shoot formation is given by ELSEC (Fig. 9.9b). The two relations were qualitatively based on laboratory results (Vermaat & Hootsmans, 1991b) but quantified by model calibration.

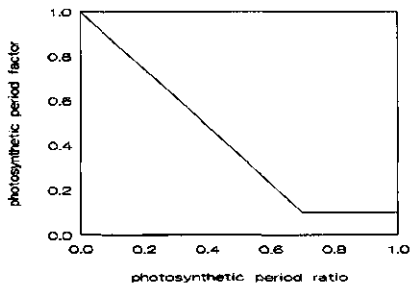


Fig. 9.10. Relation between FPRATIO and photosynthetic period factor FPF (see text).

4.8 Tuber initiation

Newly formed tubers were not found within one month after the growing season started (Van Dijk, pers. comm.). Thus, tuber growth begins at IDAYBEG+30 (incorporated in GROW). Results from shading experiments in Lake Veluwe (Van Dijk & Van Vierssen, 1991) indicated a strong effect of the so-called photosynthetic period. In the model, this parameter has been defined as: the fraction FPRATIO of the photoperiod during which at least 200 μE m^{-2} day^{-1} (BOUNDLIGHT) reaches the top of the vegetation. The lower this fraction, the higher the amount of tubers formed per unit of plant biomass (Van Dijk & Van Vierssen, 1991). The exact nature of the relation is unknown, and it was therefore kept linear. A tentative maximum FPRATIO (FRMAX=0.7) was chosen. Above this maximum, the photosynthetic period factor FPF that modifies the tuber induction rate is kept constant at 0.1 (FPFMIN). Below FRMAX, FPF increases to 1 when FPRATIO is zero. The value of BOUNDLIGHT was based on results from a shading experiment in a *P. pectinatus* vegetation in an experimental ditch on the island of Texel, The Netherlands (Van Vierssen, unpub.). Here it appeared that moderate shading resulted in a significant increase in tuber biomass production, while aboveground biomass was not much affected compared to the control (no shading). In these experiments, photoperiod was not different between treatments, while photosynthetic periods became shorter when shading increased. Apparently, tuber induction rate was influenced by a photosynthetic period determined for rather high light levels (e.g. 200 μE m^{-2} s^{-1}) which do not have much effect on photosynthesis. In Fig. 9.10, the assumed relation between FPRATIO and FPF is shown.

The amount of tubers that can be induced on one day is dependent on FPF but also on the number of tubers already present on the root/rhizome system (referred to as root biomass in the following). Lake Veluwe data indicated a maximum of 40-100 tubers per g of root biomass. The so-called place factor PF was chosen to be 60 tubers g^{-1} root biomass. Furthermore, a maximum number of 5 tubers that can be induced per g root biomass per day (TUBNUM) appeared necessary during calibration. Otherwise, under very turbid conditions, all tuber places would be filled within a few days, resulting in no tuber size differentiation.

Initial tuber weight was fixed at 0.006 g afdw (TUBIN), based on the weight of the smallest recognizable tubers found in Lake Veluwe.

4.9 Tuber growth

Results from Lake Veluwe indicated that full-grown new tubers (approximately 0.035 g afdw) already existed after one month of tuber development (Van Dijk, pers. comm.). This value could be achieved within one month in the model

by using a relative growth rate of new tubers (TGP) of 0.08 day^{-1}. Model results indicated that, after a period of 21 days of tuber growth (ITUBDAY), the relative growth rate of a tuber had to decrease to TGPMIN (0.01 day^{-1}) in order to keep maximum tuber weights within reasonable limits. A maximum tuber size TUBMAX (0.06 g afdw) was used in calculating the tuber size distribution at the end of the year.

To account for competition between growing tubers for the available carbon pool, a factor TUBMIN was incorporated. This factor indicates the fraction of the potential tuber growth rate that must at least be accomplished when the available carbon pool is limiting. When this fraction becomes less than TUBMIN, tuber induction rate becomes zero. TUBMIN was set to 0.5 by model calibration.

5. Behaviour of the calibrated model

5.1 Seasonal cycling

The simulated development of total vegetation biomass during 10 years is shown in Fig. 9.11. Total vegetation biomass includes newly formed tubers, but not the tuber bank biomass from which it sprouts. In this run, water depth was 0.75 m. Parameter values were kept at the values given in Table 9.1. Periphyton shading regime and water layer light extinction coefficient were kept the same as presented in Fig. 9.5. This set of conditions will be referred to as 'Lake Veluwe conditions' in the following. The results of this particular simulation are referred to as the 'nominal run' and will be used as a standard to compare with results from other light regimes and/or with runs using other parameter values.

Clearly, within a few years, a stable cyclic behaviour is established. The increase in biomass during the growing season is caused by reallocation of tuber bank biomass and subsequent onset of photosynthesis. An interesting question is: why does the vegetation decrease again at the end of the season? Apart from tissue death, which occurs at a much too low rate to explain the observed decrease, two possible causes have been incorporated in the model: storm damage and tissue damage due to unfulfilled respiratory demands, followed by biomass loss due to sloughing. The first cause is much more 'trivial' than the second, which represents an 'innate' trigger for biomass decrease at the end of the season.

In Fig. 9.12a, the simulated total biomass fluctuation in the 10th year of the nominal run shown in Fig. 9.11 is presented. Also, the changes in daily photosynthetic pro-

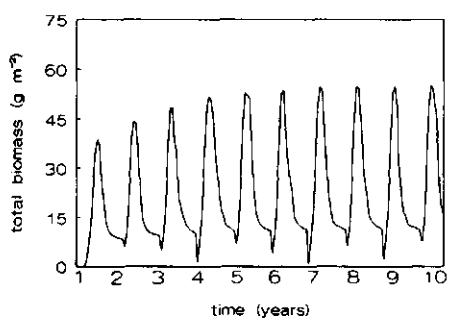


Fig. 9.11. Nominal long-yearly cycle: simulation of total vegetation biomass at 0.75 m waterdepth during ten years. Periphyton shading and water layer extinction regime were similar to Fig. 9.5 (Lake Veluwe conditions).

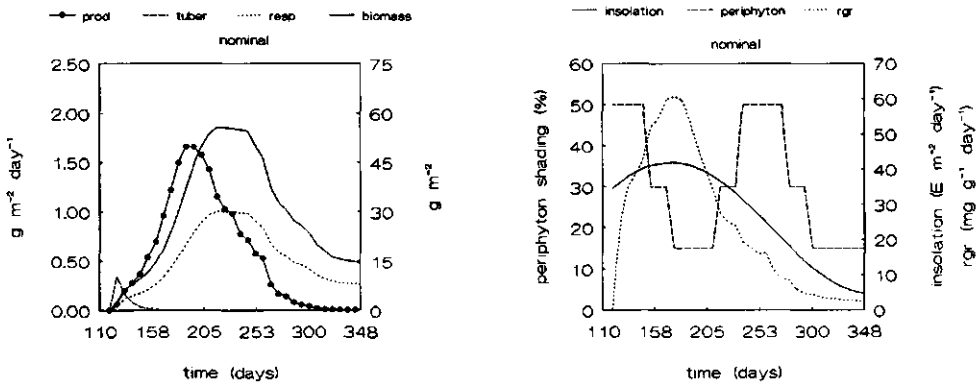


Fig. 9.12. Dynamics of the vegetation in the 10th year of the simulation shown in Fig. 9.11. Left: total biomass, gross photosynthetic production, respiration and rate of tuber depletion. Right: daily insolation, periphyton shading and gross production per total aboveground biomass (rgr).

duction, tuber depletion rate and vegetation respiration are shown. Material coming available from tuber depletion initiates development of the vegetation. The rate of photosynthesis increases and more than compensates for the decreasing input from the tuber. The subsequent decrease in production precedes the decline in total biomass, which occurs from day 224 (beginning of August) onwards. Storms cannot occur before day 265 (half September). Thus, the cause for the decrease in total biomass in the model vegetation under these conditions is physiologically based.

The position of the peak in photosynthetic production and its subsequent decrease is related (at least partly) to the seasonal change in daily insolation. This is more clearly seen when gross production is expressed relative to aboveground biomass (Fig. 9.12). Periphyton regime cannot be held directly responsible, as the decrease occurs during early summer when periphyton shading is lowest. Furthermore, other simulations with constant periphyton regimes showed comparable changes in gross production.

Apart from the seasonal insolation curve, three other factors can be suspected to cause the shortage in photosynthate. These are: new tuber formation, self-shading and the effect of light and age on photosynthetic parameters. To answer the question which of them is important, three simulations lasting only one year were performed using the tuber bank from the stabilized model vegetation at the end of year 9 from the nominal run. In one, no tubers were formed by setting TUBNUM to 0; in the second, KPLANT was set to 0. The third was performed with a fixed age BEGIN of each vegetation layer of 50 days and a fixed light history of 200 μE m^{-2} s^{-1}. This means: no effect of changing age and light history on photosynthesis. All other parameters were kept the same as in the nominal run. The results are presented in Fig. 9.13 a-c.

Clearly, tuber formation and self-shading do affect total biomass development. However, on a relative scale, the various curves do not differ much from the results in Fig. 9.12. The absence of light and age effects on photosynthetic parameters causes a completely different

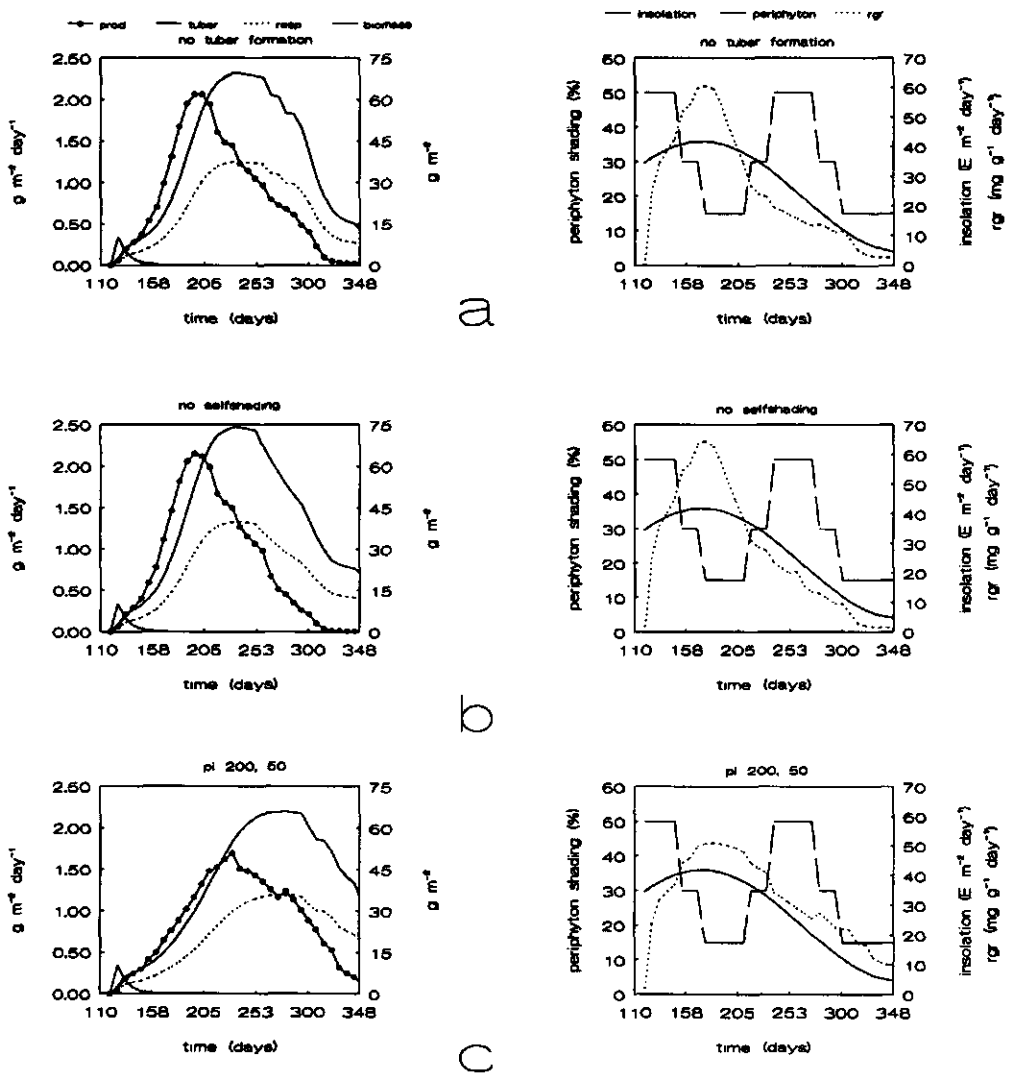


Fig. 9.13. Vegetation dynamics in the 10th year of the simulation in Fig. 9.11.
(a) no tuberformation (b) no self-shading (c) no effects of light and age on photosynthetic parameters (i.e. parameters used for plants of 50 days, grown at 200 μE m^{-2} s^{-1}).

picture (Fig. 9.13c). Gross production per aboveground biomass closely follows the daily insolation cycle. Total biomass now starts to decrease from day 265 onwards, i.e. solely caused by storm occurrence.

It can be concluded that the decrease in aboveground biomass at the end of the season in the model vegetation is mainly triggered by light history and age effects on photosynthesis of the vegetation. When gross production becomes less than respiration, chances for wave damage increase strongly.

5.2 Sensitivity of the model to changes in various parameter values

A sensitivity analysis of a simulation model is necessary to find out which parameters are likely to have a strong effect on model behaviour, and consequently should be assessed very precisely. In evaluating sensitivity, a definition of this term is necessary. The seasonal cycle of the model vegetation is the combined expression of all parameters together, and the effect of a change in one parameter is probably also determined by changes in other parameters. Thus, sensitivity could be defined as the average response to a change in one parameter value for all combinations of changes in the other parameter values. In the case of SAGA1, about 30 parameters were evaluated. This would lead to 2^{30} possible parameter combinations, when all parameters are both once increased and once decreased. To limit the amount of test simulations for this purpose, the present analysis was based on the effect of a change in a parameter when all other parameters are kept the same. As reference level, the nominal parameter values were chosen as given in Table 9.1, under Lake Veluwe conditions at 75 cm depth. In a one year simulation starting with the stabilized tuber bank from the end of year 9 of the nominal run, the value of the parameter under study was changed. The results were compared with the nominal 10th year.

Each parameter was once increased by 20% and once decreased by 20%. The results from both entries are not necessarily symmetrical: an opposite change in value may lead to other changes via various interactive processes that are certainly not always simple linear relations (i.e. light response curves, the determination of tuber induction rate). In the case of the tables for ELTOP and ELSEC, each table value was increased or decreased by 20%. The resulting relations are shown in Fig. 9.9.

Six different characteristics were taken to study the effect of parameter changes: biomass of the total mother vegetation, the biomass of all secondary shoots together and the total root biomass, all at the peak of total aboveground biomass; total tuber biomass and tuber number at the peak of total tuber biomass; and the maximum rate of gross production per m^2 of the total vegetation.

The change in each of these variables was calculated as a percentage relative to its nominal value, and expressed as percent change per percent change in the parameter value. The sensitivity of a parameter was then defined as the average relative change of all six variables for the same change in this parameter. Thus, each parameter had two sensitivity values: one for a 20% increase, one for a 20% decrease. The separate values on which they were based are given in Appendix 2. In this sensitivity definition and its calculation, I followed Ng & Loomis (1984).

The various parameters have been grouped according to the process or

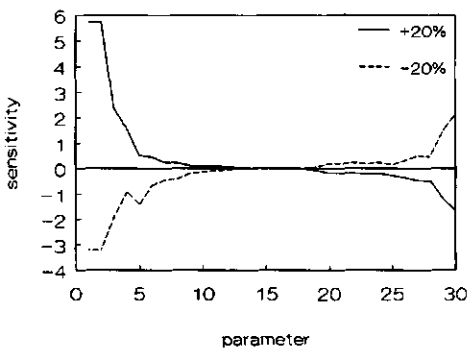


Fig. 9.14. Averaged sensitivity of six model variables (maximum shoot, root, secondary shoot and tuber biomass, maximum tuber number and maximum gross production, all per m^2) to deviations in parameter values from their nominal values as presented in Table 9.1. Results were obtained in a one year simulation under Lake Veluwe conditions, starting with the tuber bank from the end of year 9 of the long-yearly nominal cycle presented in Fig. 9.11. Parameters are ranked from high to low sensitivity with respect to the +20% change result. Parameter numbers correspond with the numbers in Appendix 2.

characteristic that was directly influenced by them. They are shown in Table 9.3. The groups are the same as those in Table 9.1, apart from the weather-related parameters. Besides daylength, these parameters were not analysed. All average sensitivities for the 30 parameters, both for + and −20% change in value, are shown in Fig. 9.14. The parameters were ranked according to their average sensitivity with a +20% change in value. It can be seen that the response to a positive change is not complementary to the effect of a negative change. High sensitivities (absolute value more than 0.5) are found for 8 parameters. The average sensitivity per group is also given in Table 9.3. Clearly, the photosynthesis and respiration group has most influence on model results, which is not really surprising. The other groups may be divided in three with an average sensitivity of 0.3-0.4 (tuber bank, growth initiation, growth and morphology), and two with an average sensitivity of 0.15 (tuber initiation and tuber growth). In the following the results for some parameters will be discussed.

The high sensitivity values of PM and CCO2GR, although not unexpected, are still rather unpleasant from the modeller's viewpoint. The variation in these parameters is inherently high, and thus their exact determination may be impossible. Still, when more data for these parameters will become available, especially of plants forming tubers, the present sensitivity may be decreased. The pronounced sensitivity value of DMAX compared to that of DMIN can be explained by the calculation of DPAR. The latter is very dependent on daylength. From the point of view of the vegetation, daylength is only important during the growing season, and thus especially DMAX will have a strong effect.

The potential relative growth rates RGP, SGP and SECGP were not based on many data. Fortunately, their sensitivity value is not very high. The same is true for the effect of light on secondary shoot formation ELSEC. However, it should be realized that ELSEC is only affecting the allocation of photosynthates after the effect of light on elongation ELTOP has had its influence. The latter is more strongly affecting model performance.

Despite the expected strong influence of tuber bank parameters on growth, their sensitivity values are not very high compared to those of photosynthesis and respiration. The low sensitivity value of TUBDEC is comforting regarding the big difference in the nominal TUBDEC value used (0.001 day^{-1}), and the most likely value in Lake Veluwe (0.003 day^{-1}).

Table 9.3. Results from the sensitivity analysis. Sensitivity was defined as the averaged percentual (%) change of 6 model variables (maximum shoot, root, secondary shoot and tuber biomass, maximum tuber number and maximum gross production, all per m^2) per percent deviation of the respective parameter from its nominal value (shown in Table 9.1) in a one year simulation under Lake Veluwe conditions, using the tuber bank at the end of year 9 from the long-yearly cycle shown in Fig. 9.11. In the analysis, each parameter was both increased and decreased with 20% relative to its nominal value, while all other parameters remained nominal. The average sensitivity per group of parameters was calculated using the absolute sensitivity values. For further details, see the text. + = 20% increase, − = 20% decrease in nominal parameter value.

parameter		sensitivity	parameter		sensitivity	parameter		sensitivity
tuber bank			**initiation of growth**			**tuber initiation**		
WTUB	−	−0.65	IDAYBEG	+	1.58	TUBIN	−	0.22
WTUB	+	0.46	IDAYBEG	−	−0.92	TUBIN	+	−0.21
ATUB	−	−0.41	ROOTIN	−	0.49	BOUNDLIGHT	+	−0.20
ATUB	+	0.23	ROOTIN	+	−0.48	BOUNDLIGHT	−	0.19
TUBDEC	+	−0.09	WLINI	+	−0.27	FRMAX	−	0.18
TUBDEC	−	0.07	WLINI	−	0.14	FRMAX	+	−0.17
			CTUBFL	+	0.09	TUBNUM	+	−0.18
			CTUBFL	−	−0.08	TUBNUM	−	0.18
			CITUBFL	−	0.03	PF	−	−0.13
			CITUBFL	+	0.00	PF	+	0.10
						FPFMIN	−	0.01
						FPFMIN	+	−0.01
mean		0.32	mean		0.41	mean		0.15
photosynthesis and respiration			**growth and morphology**			**tuber growth**		
PM	+	5.73	ELTOP	−	−1.41	TGP	+	−0.37
CCO2GR	+	5.73	ELTOP	+	0.52	TGP	−	0.32
PM	−	−3.19	KPLANT	+	−0.50	ITUBDAY	−	0.22
CCO2GR	−	−3.19	KPLANT	−	0.47	ITUBDAY	+	−0.19
DMAX	+	2.37	RGP-SECGP	−	−0.44	TGPMIN	−	0.03
RESPMAX	−	2.13	RGP-SECGP	+	0.25	TGPMIN	+	0.01
DMAX	−	−1.93	ELSEC	−	−0.17	TUBMIN	+	0.00
RESPMAX	+	−1.66	ELSEC	+	0.11	TUBMIN	−	0.00
KM	−	1.50	CEDEAD	+	0.07			
KM	+	−1.17	CEDEAD	−	−0.05			
DMIN	+	0.04	DRTR/DRTS	−	0.02			
DMIN	−	−0.02	DRTR/DRTS	+	0.00			
mean		2.39	mean		0.33	mean		0.14

The starting date of the growing season, IDAYBEG, shows an interesting asymmetry. A decrease in IDAYBEG means a prolonged growing season, and thus might be expected to result in higher biomass values. The opposite is true. This is probably related to the timing of tuber germination and the daily insolation: when the vegetation starts earlier in the year, average light level received during this crucial period of growth will be lower, and photosynthetic production per day is decreased. Tuber induction also will begin earlier in the season. Tuber demand for photosynthate will be the same or even higher (tuber induction rate may be increased due to lower light intensity) compared to their claim when the growing season starts later in the year. Thus the available amount of photosynthate for other plant parts will be decreased. This will affect the amount of aboveground tissue, and thus total biomass development will be limited.

The amount of tuber biomass used for growth initiation (CITUBFL) and for growth during the early season (CTUBFL) both have rather low sensitivities. This may be different for development under other, more light-limited conditions. Still, the nominal light conditions during early growth are also regarded as rather turbid. Therefore, although important for the first days during the growing season, these two parameters do not strongly affect the eventual peak biomass levels. The same appears true for tuber initiation and tuber growth parameters, apart from TGP. Apparently, when a certain minimum number of tubers necessary for survival can be developed, a further increase does not have much effect. TGP does have more influence. From the results it follows that the present value of TGP is limiting the peak biomass levels: thus, under nominal conditions, not so much an even more increased tuber bank size in spring but a decrease in the demand for photosynthate by the tuber bank during its development appears to have positive consequences for total biomass development of the vegetation.

The effects of changes in periphyton shading regime and extinction coefficient on biomass development will be dealt with in section 6.

5.3 Model validation: seasonal cycles in Lake Veluwe

After model calibration, it is customary to compare model performance with independent data. An evaluation of the agreement between model behaviour and reality is usually simply based on subjective criteria (Straškraba & Gnauck, 1985). With respect to the macrophyte models mentioned in section 1, a statistical analysis for this purpose was not given by the respective authors.

Twoway analysis of variance (ANOVA) with factor 'TIME' of the season and factor 'MODEL' (field results as one level, model result as the other) appeared to be a reasonable method to test for deviations between model and reality. A significant factor TIME indicates a seasonal trend (a more or less trivial result with this kind of data, although a not-significant effect of TIME is interesting); a significant factor MODEL indicates an overall difference between model and reality. A significant interaction between these factors indicates that the shape of the modelled seasonal development cycle differs from the real seasonal cycle.

Statistical tests like ANOVA for comparison of the shape of the model cycle with the field results do not show the exact moments on which significant deviations occur. For this, comparisons

per sampling occasion can be done with Student's t-tests at $p<0.05$. I have refrained from doing this for two reasons. One is that t-test results showed various significant differences while the overall F-test (ANOVA) was not significant. This is caused by the fact that multiple comparisons are made, necessitating the use of a much lower comparisonwise error rate (CER) than 0.05 to keep the experimentwise error rate (EER) at a reasonable level (0.05). However, in this case the interest is not in finding differences but in finding many agreements, i.e. not-significant differences, indicating a good model description of reality. This is facilitated by reducing the CER, which seems not justified. The second reason is that looking at the actual curve after an ANOVA also points out places were agreement between model and reality is poor.

For model validation, an extensive dataset was available on biomass development in a *P. pectinatus* vegetation in Lake Veluwe during 1986 and 1987. Apart from control areas, this vegetation was subjected to three different shading levels (see Van Dijk & Van Vierssen, 1991). The actual experimental shading levels of 26, 45 and 73% were modelled as 25, 50 and 75%.

During model parameterization, part of the 1986 dataset collected in the controls in Lake Veluwe was used. Thus, agreement between model and total biomass development in 1986 in this vegetation is no independent evidence for 'reasonable' model performance. Other data from this vegetation, like root biomass and tuber number, could still be used to validate the model results. Also, data from the three shaded treatments and all data from 1987 were available for this purpose.

In this and following sections, results presented are based on model simulations that used the parameter values given in Table 9.1, and that were performed under 'Lake Veluwe' conditions, unless specified otherwise. All simulations pertained to two years, starting from the tuber bank that existed at the end of year nine of the stabilized nominal run. Results from year one of each validation run were compared with 1986 data. The subsequent year two results from the model were compared with Lake Veluwe results from 1987.

When evaluating the results, the differences in seasonal insolation curve and periphyton regime between model and reality should be kept in mind. The major goal during calibration was to reach general agreement between model behaviour and field data from 'a' shallow eutrophic temperate lake, not an exact description of a particular system like Lake Veluwe.

Table 9.4 gives an overview of the ANOVA results for six characteristics. The interaction effect was never significant, indicating that, at least on a relative scale, the seasonal cycle as predicted by the model is not significantly different from the field. A TIME effect was often absent for all characteristics at 50% shading, and always absent at 75% shading. This was mainly due to strong variation in the field data.

In Fig. 9.15, the model results are compared with the unshaded total biomass fluctuation in Lake Veluwe in 1986 and 1987. The development in the first half of the growing season appears very similar for both years, and the model predicts the real biomass increase rather well. The model peak biomass is intermediate between the field levels of 1986 and 1987. The predicted rate of decrease in biomass at the end of the season is much lower than in the field. Probably the real losses

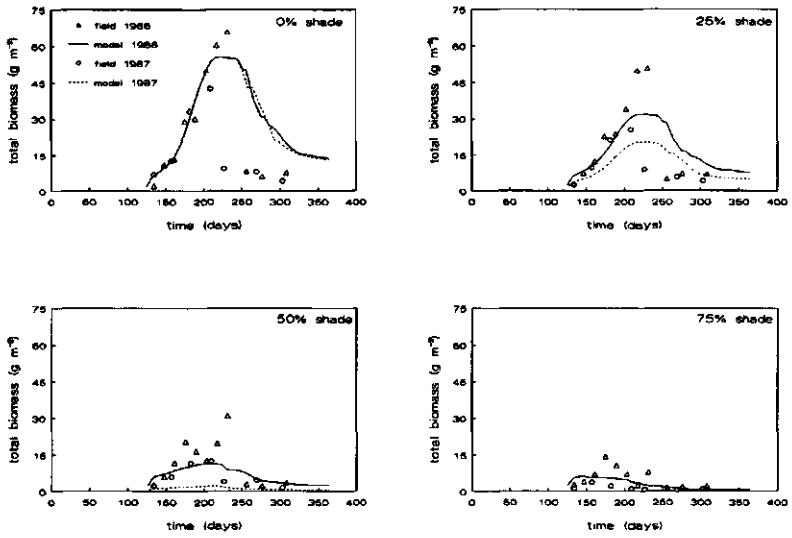


Fig. 9.15. Total biomass development in Lake Veluwe (1986 and 1987) compared with model results. The various shading levels are indicated also.

Table 9.4. Overview of the significances of the effects of factor MODEL (M) and TIME (T) in a twoway ANOVA (see text). The interaction MODEL*TIME was never significant. PF = place factor, i.e. number of tubers per g root biomass, + = $p<0.10$, * = $p<0.05$, ** = $P<0.01$, *** = $p<0.001$, ns = not significant, - = not available (model did not generate root and tuber biomass). To homogenize variances, all data were $\log_{10}(x+1)$-transformed.

shading %		0		25		50		75	
		M	T	M	T	M	T	M	T
total biomass	1986	ns	***	ns	***	ns	+	ns	ns
	1987	ns	***	ns	***	***	*	**	ns
aboveground	1986	ns	***	ns	***	ns	+	ns	ns
biomass	1987	ns	***	ns	***	*	+	*	ns
root biomass	1986	ns	***	ns	*	ns	ns	ns	ns
	1987	ns	+	ns	ns	+	ns	ns	ns
tuber biomass	1986	+	***	ns	**	**	ns	***	ns
	1987	ns	***	ns	**	**	ns	*	ns
tuber number	1986	**	***	ns	+	ns	ns	***	ns
	1987	ns	*	ns	ns	***	ns	***	ns
PF	1986	**	ns	ns	ns	ns	ns	**	ns
	1987	ns	ns	ns	ns	ns	ns	-	-

due to wave action are much larger than the 10% per occasion as supposed in the model. As a consequence, the data beyond the moment of peak biomass were always left out of the ANOVA. The ANOVA showed no significant MODEL effect for both years.

The differences between model results and field data in 1986 and 1987 can be attributed to differences in daily insolation. The average daily insolation (PAR) reaching the water surface during the growing season (May 1st-August 31st, 123 days) was 41 E m^{-2} day^{-1} in 1986 and 35 E m^{-2} day^{-1} in 1987 (Van Vierssen & Bij De Vaate, 1990). The daily insolation calculation as used in the model was on average 38 E m^{-2} day^{-1} for this period. Thus, especially regarding the good agreement with 1987, it seems that under nominal conditions the model predicts total biomass development rather well.

The nominal periphyton regime (Fig. 9.5) has a spring peak in May which is one month earlier than in Lake Veluwe in 1986 (see 4.3). To study the influence of different periphyton regimes, two other periphyton cycles were used. One had its spring peak moved to June (Fig. 9.16, left), the other was shifted also and had a narrowed periphyton peak (only the 50% part of the shifted regime, with 15% shading during the rest of the year; Fig. 9.16, right). The real periphyton shading percentages as measured in Lake Veluwe (Van Vierssen & Bij De Vaate, 1990) are also shown. In both cases, the model vegetation development was not very different compared with the results under the nominal periphyton regime (Fig. 9.17).

With the shifted periphyton regime of Fig. 9.16, periphyton shading during May is 30%. Apparently, this shading level creates light conditions for tuber induction and growth that are comparable to the nominal periphyton shading regime. The consequent shift of the 50% peak to June only had a minor effect on production since the major part of the vegetation by that time is already concentrated near the water surface. The regime with narrowed peaks resulted in a somewhat higher total biomass development, i.e. close to the 1986 peak biomass but deviating more from 1987 (Fig. 9.17). Thus, given the extinction coefficient used (EXTW=2.0 m^{-1}), the effect of such

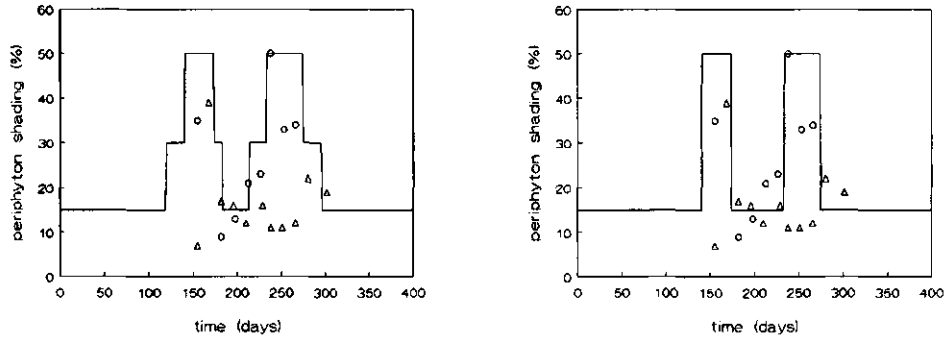


Fig. 9.16. Two periphyton regimes used to study the effect of changes in shape and timing of the peaks on biomass development. The actual values measured in Lake Veluwe are shown also (triangles: 1986, circles: 1987). Left: spring peak shifted to June. Right: also shifted, but peaks narrowed.

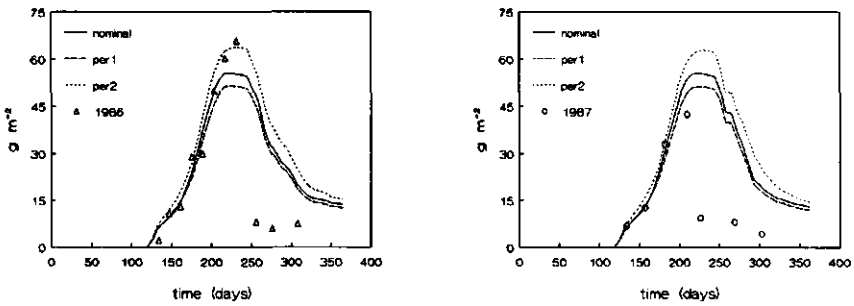


Fig. 9.17. The total biomass development simulated by the model for the different periphyton shading regimes shown in Fig. 9.16 left (per1) and Fig. 9.16 right (per2). The actual values measured in Lake Veluwe are shown also. (a) 1986 data (b) 1987 data.

changes in the shape of the periphyton shading curve on biomass development is not very large. Therefore, model results are always based on the nominal periphyton shading regime (Fig. 9.5) in the following.

In Fig. 9.15, total biomass data are given for the three shading levels used in the Lake Veluwe field work. For 25% shading the model still is reasonably close to the real total biomass development, although the increasing part of the curve generated by the model remains somewhat low in 1987. The ANOVA showed no significant effect of the factor MODEL for both years. For 50 and 75% shading, significant differences occur in 1987: the model vegetation suffers more from shading than is happening in reality.

With Figs. 9.18-21, a closer look into various biomass components is possible. This is worthwhile as a reasonable fit of model results with total biomass data might conceal a poor simulation of the different parts which comprise the vegetation, and vice versa. Looking at the data from Table 9.4, there appears to be a reasonable agreement between the model and the field data for the unshaded control and 25% shading. More differences exist for the two highest shading levels, especially in 1987. Most problems seem to be related to tuber formation, again most pronounced for 50 and 75% shading.

Aboveground biomass development is shown in Fig. 9.18. For the same reason as in the analysis of total biomass, the points in the decreasing part of the cycle were left out of the analysis. Significant MODEL effects were found in 1987 for 50 and 75% shading. Deviations tend to occur in the beginning of the growing season (model too high) and at the time of peak biomass (model too low). As aboveground biomass forms the major part of total biomass, the general impression is the same: the model shows more growth limitation by shading than the Lake Veluwe population did. However, in the case of 75% shading the model results seem to be not more than one year ahead of the real developments: the decrease in Lake Veluwe standing crop and tuber biomass over the two years

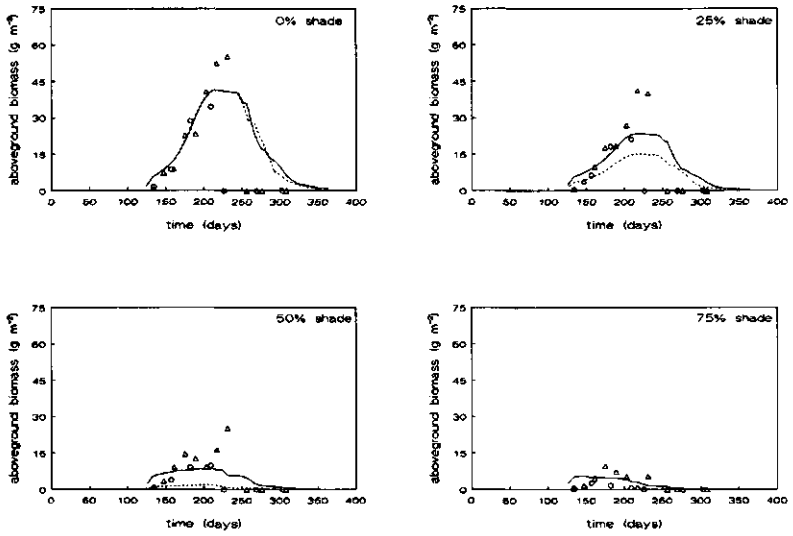


Fig. 9.18. Aboveground biomass development in Lake Veluwe (1986 and 1987) compared with model results. For legend see Fig. 9.15.

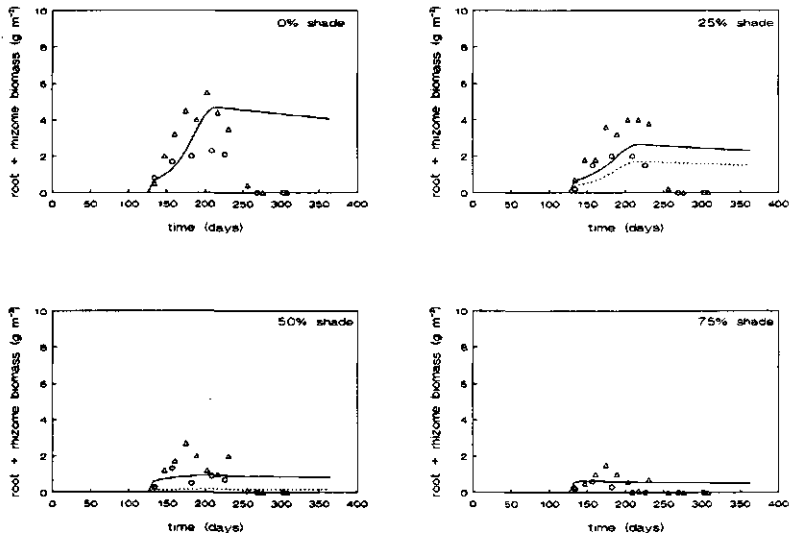


Fig. 9.19. Root biomass development in Lake Veluwe (1986 and 1987) compared with model results. For legend see Fig. 9.15.

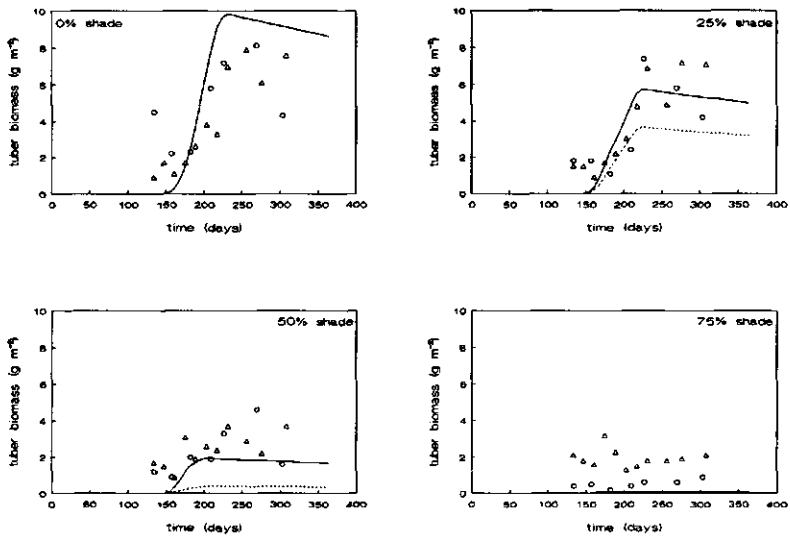


Fig. 9.20. Tuber biomass development in Lake Veluwe (1986 and 1987) compared with model results. For legend see Fig. 9.15.

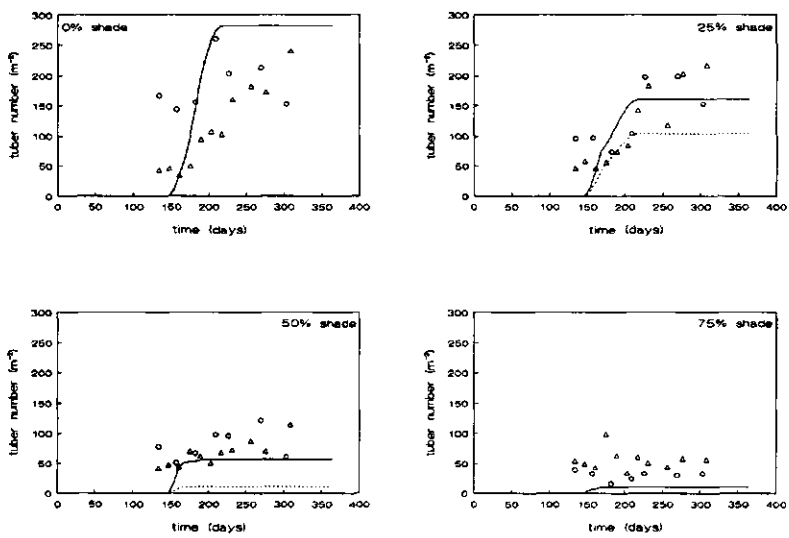


Fig. 9.21. Tuber number development in Lake Veluwe (1986 and 1987) compared with model results. For legend see Fig. 9.15.

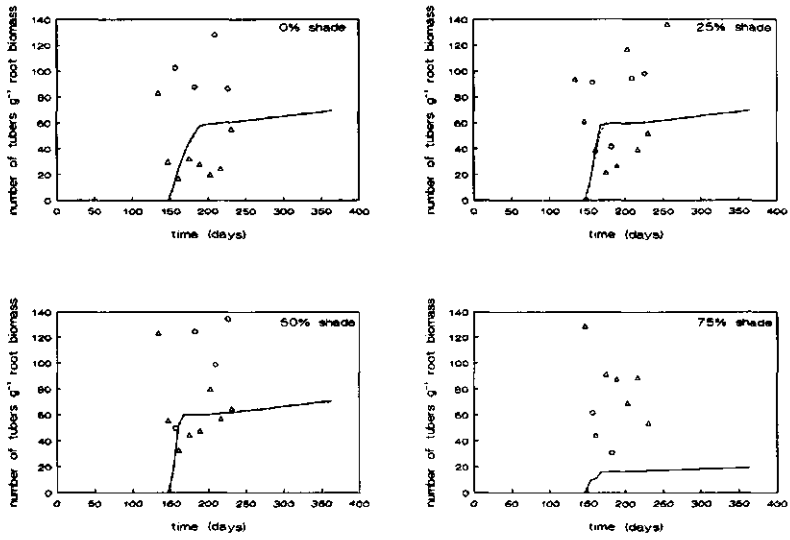


Fig. 9.22. Development of tuber number per gram root biomass in Lake Veluwe (1986 and 1987) compared with model results. For legend see Fig. 9.15.

for 75% shading is such that total extinction of the vegetation can be expected within one or two years.

Fig. 9.19 shows root biomass development. The model root biomass seems to grow at a lower rate and to reach lower levels than the Lake Veluwe vegetation under 25% and 50% shading levels in 1986 while the unshaded control in 1987 remains below the predicted levels. The other treatments are simulated reasonably well. Root biomass in the field decreased much stronger when aboveground biomass had disappeared than is simulated in the model. Therefore, these data were left out of the analysis. The ANOVA only showed a MODEL effect at $p < 0.10$ for 50% shading in 1987.

In the analysis of tuber data, field data for May were left out because these are a mixture of not-germinated tubers of the previous year and newly formed tubers. For 50 and 75% shading, tuber biomass, tuber number and the number of tubers per g root biomass (place factor, PF) showed no significant TIME effect in the ANOVA (Table 9.4). Under these circumstances, the moment at which the vegetation is not able to sustain the production and growth of tubers any longer is reached already early in the season.

Tuber biomass development (Fig. 9.20) occurs at a lower rate in the unshaded control than in the model. On the other hand, under the highest shading level, more tuber biomass is possible in reality than is simulated. The other field data on tuber biomass are simulated rather well. Significant MODEL effects are found for both years at the two highest shading levels, and weakly for the unshaded control in 1986.

Tuber number (Fig. 9.21), like tuber biomass and aboveground biomass, also reflects the tendency of the model to react faster to decreased light conditions than happens in the field: compared with the unshaded treatment, model tuber

number development occurs at a too high rate, while for the higher shading levels, the reverse is the case. Maximum number at the end of the season is too high for the unshaded control, and too low for the three shading levels.

The number of tubers per gram root biomass (Fig. 9.22) is limited to 60 in the model (PF). This seemed to be a reasonable amount, although the variation in this characteristic clearly is quite high. As PF increases strongly at the end of the season due to disappearance of root biomass, these PF values were left out of the analysis. Significant MODEL effects existed for the unshaded control and 75% shading in 1986. The model did not generate any root biomass or tubers in the second year under the 75% shading level, while these were produced in reality.

5.4 Conclusions

It should be stressed that the agreement found in the various ANOVAs comparing model and reality is partly due to the rather high variation in field data. Looking at the curves, it is clear that the description of the field data could be better. Still, statistically there is no reason to reject the present model on the basis of its performance relative to reality.

Major deviations appeared to center around the tuber formation algorithm: although the model reaction to low light intensities is comparable to the behaviour of the field vegetation, possibilities for tuber formation and growth at these light intensities are estimated somewhat too pessimistic. This may not be surprising, as several parameters in this routine are still rather tentative.

The model is able to simulate the field situation quite well, despite the absence of any temperature effect. This is in agreement with the fact that the deterioration and loss of biomass in the Lake Veluwe vegetation already occurs when temperatures are still high. In other words, regarding temperature there seems to be no reason why the vegetation should disappear already. Thus other factors, like ageing and the concomitant decrease in the rate of photosynthesis, have to play a main role in the decline of the vegetation at the end of the growing season. The model results support this.

The stabilized model vegetation starts with a larger tuber bank than is found in Lake Veluwe. Modelled vegetation biomass tends to be too high early in the season, but especially under shaded conditions, it remains somewhat below the real peak biomass levels. This suggests that the rate of photosynthesis of plants forming tubers may still be underestimated.

Disappearance of large amounts of biomass through wave action is limited to aboveground parts. Clearly, the mortality rate DRTR used in the model for root biomass is not high enough to explain the decrease in root biomass after disappearance of aboveground biomass. This problem can be solved by a strong increase in DRTR when belowground respiration cannot be sustained by production of photosynthate. Perhaps grazing by waterfowl can also play a role.

6. Effects of changes in light climate on vegetation development

6.1 Introduction

One of the main reasons to develop the present model was to be able to predict the possibilities for macrophyte development under various light conditions. The latter are mainly dictated by periphyton development during the growing season and by water layer light extinction. After the previous sections, it now seems justified to study the effects of both factors on the model vegetation. As increasing depth basically has a similar effect on light climate as an increased extinction coefficient, the response of the model to different periphyton regimes and extinction coefficients was studied for various water depths.

In the following, the potential for biomass development under different light conditions is studied first. The specific light regime that was chosen remained constant during the simulation. Subsequently, the reaction is analyzed of a stabilized vegetation growing under nominal conditions when light conditions are changed.

6.2 Response of the model to different light regimes

To study the response of the model to different light regimes, results were taken from the 10th year of various simulations. In that year, stable cyclic behaviour (or extinction) usually is reached. All simulations used the initial tuber bank and the parameter values as shown in Table 9.1.

Regarding the effect of the periphyton regime, besides peak periphyton shading levels, the shape of the periphyton curve will also influence results. An indication in this direction was already given in section 5.3 (model validation). Therefore, in the present analysis, two types of simulations were done: one with a constant periphyton regime, and one with a fluctuating periphyton regime.

During a simulation, the selected periphyton regime was not changed. The shape of the fluctuating regime was identical to the nominal periphyton regime shown in Fig. 9.5. The summer value was always kept at 15%. Peak levels in spring and late summer-autumn were always equal to each other and were varied between 20% and 50% (10% increments). Constant periphyton regimes were varied between 0 and 50% shading (10% increments).

Water layer extinction coefficients were always kept constant during the simulation, and were varied from 0 to 4 m^{-1} (0.5 m^{-1} increments, but 0.5 and 3.5 m^{-1} were left out). The combination of no periphyton shading and zero extinction was not used.

6.2.1 Constant periphyton regime

Using the macrophyte peak biomass data from the various simulations, nomograms were made showing isobiomass lines. These connect points of equal peak biomass, and are called isoclines in the following. Fig. 9.23 gives the behaviour of peak total biomass in relation to the extinction coefficient and the periphyton shading regime for five different water depths.

A general trend can be seen. The isoclines are increasingly compressed and tilted vertically when the water becomes deeper. This shift to an almost vertical position indicates that with increasing

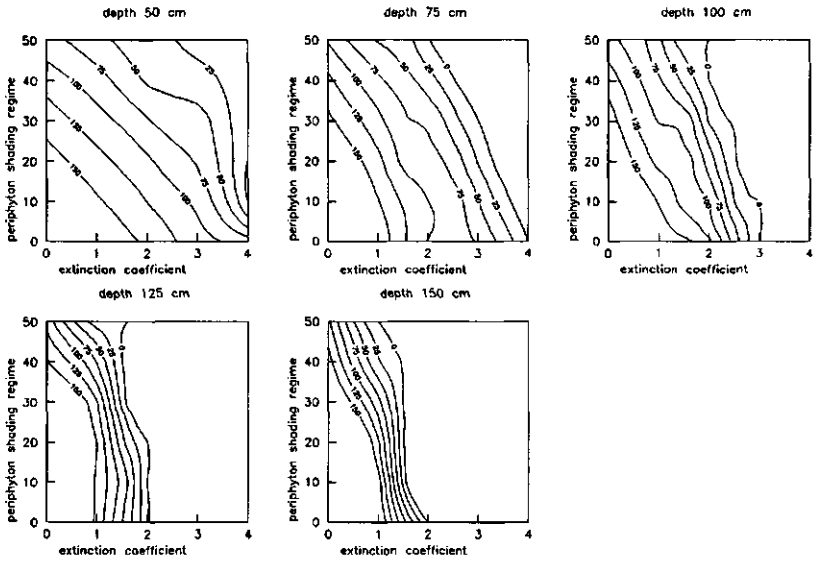


Fig. 9.23. Nomograms showing the effect of constant periphyton shading regime and constant water layer extinction coefficient on peak total biomass in the 10th simulation year for various water depths.

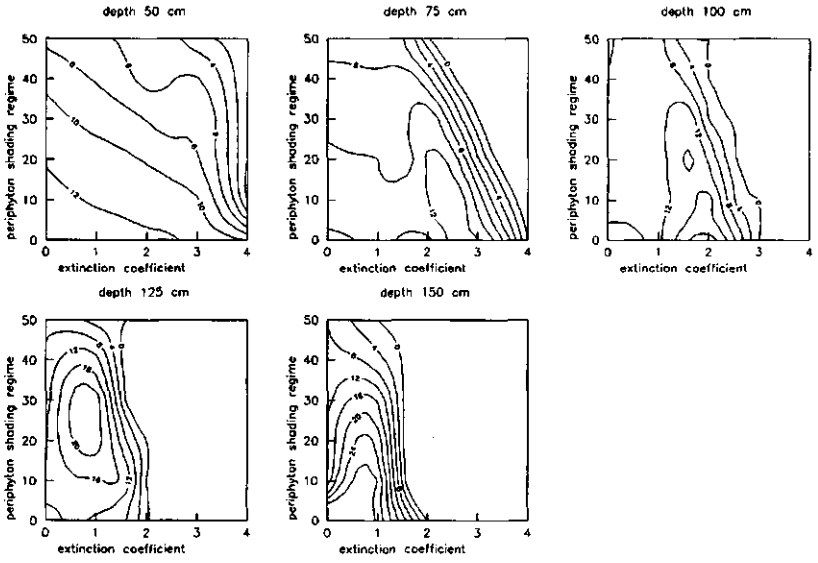


Fig. 9.24. Nomograms showing the effect of constant periphyton shading regime and constant water layer extinction coefficient on peak tuber biomass in the 10th simulation year for various water depths.

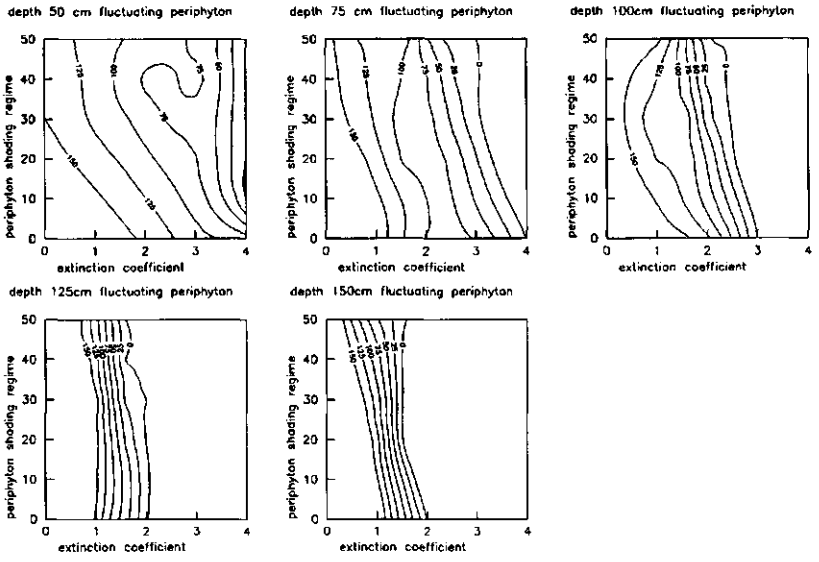


Fig. 9.25. Nomograms showing the effect of fluctuating periphyton regime and constant water layer extinction coefficient on peak total biomass in the 10th simulation year for various water depths.

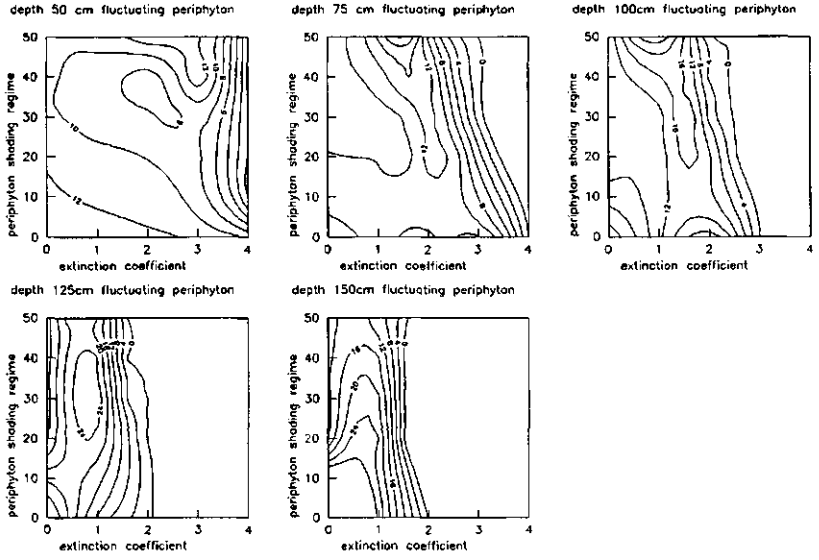


Fig. 9.26. Nomograms showing the effect of fluctuating periphyton regime and constant water layer extinction coefficient on peak tuber biomass in the 10th simulation year for various water depths.

water depth, the extinction coefficient of the waterlayer increasingly overshadows an effect of periphyton cover on the vegetation. In deep water (1.25-1.50 m) a periphyton effect is found only in clear water (i.e. extinction coefficient less than 1.0 m^{-1}). On the other hand, in shallow water (0.50 m) the isoclines approximately have a 45° inclination up to an extinction coefficient of 3.0 m^{-1}. This indicates that the effect of periphyton shading regime and extinction coefficient are of the same magnitude for this depth: an increase of 10% periphyton shading results in the same decrease in peak total biomass as an increase of 1.0 m^{-1} in the extinction coefficient. Under these conditions, peak biomass levels do not react very dramatically to relatively small changes in both factors.

In waters of 0.75-1.0 m, peak biomass levels and survival potential are strongly limited when the extinction coefficient increases above 2 m^{-1}. It can be seen from the nomogram that in the region of 2-3 m^{-1}, rather small changes in the extinction coefficient can cause large changes in peak biomass.

The situation becomes even more serious in deep water (1.25-1.50 m). No stable vegetation development is possible above an extinction coefficient of 2 m^{-1}. Below this value, very small changes in turbidity can make the difference between a dense vegetation or a very sparse one, regardless of periphyton cover.

Somewhat more intricate nomograms emerge for peak tuber biomass under the same periphyton and extinction regimes (Fig. 9.24). However, the general trends are similar to those found in total biomass. Increasing depth leads to a more and more vertical orientation of the isoclines, i.e. except for low extinctions, the effect of periphyton shading decreases.

In the model, tuber induction rate is increased with decreasing light intensity. Thus, it can be expected that the isoclines will curve back for low extinction coefficients and low periphyton shading levels, resulting in circular isoclines. For 0.50 m, the effect is not evident, because even for rather high extinction coefficients, the photosynthetic period ratio (see section 4.8) is still high, causing no change in the photosynthetic period factor FPF. Under these conditions, the peak tuber biomass is mainly dependent on the general growth potential for the total vegetation.

From depth 0.75 m onwards, the expected curvature can be seen. Circles disappear again in 1.50 m water. At this depth, for all extinction coefficients, light intensity near the bottom is already so low that decreased periphyton shading does not result in a decreased FPF anymore. Thus, tuber biomass will not decrease either when periphyton shading becomes less under these circumstances.

6.2.2 Fluctuating periphyton regime

At first sight, the nomogram for peak total biomass under fluctuating periphyton regimes (Fig. 9.25) shows an even less pronounced effect of periphyton shading compared with the nomogram for constant regimes. For all depths, isoclines run more vertically in Fig. 9.25 than in Fig. 9.23. However, periphyton regimes do play their role. When periphyton peaks in spring and autumn are increased, this correlates at first with a decrease in peak biomass. Above a peak shading of about 30%, the trend is reversed and peak biomass remains constant or even increases again. This phenomenon is caused by the increased FPF and concomitant tuber production when peak shading levels in spring are increased.

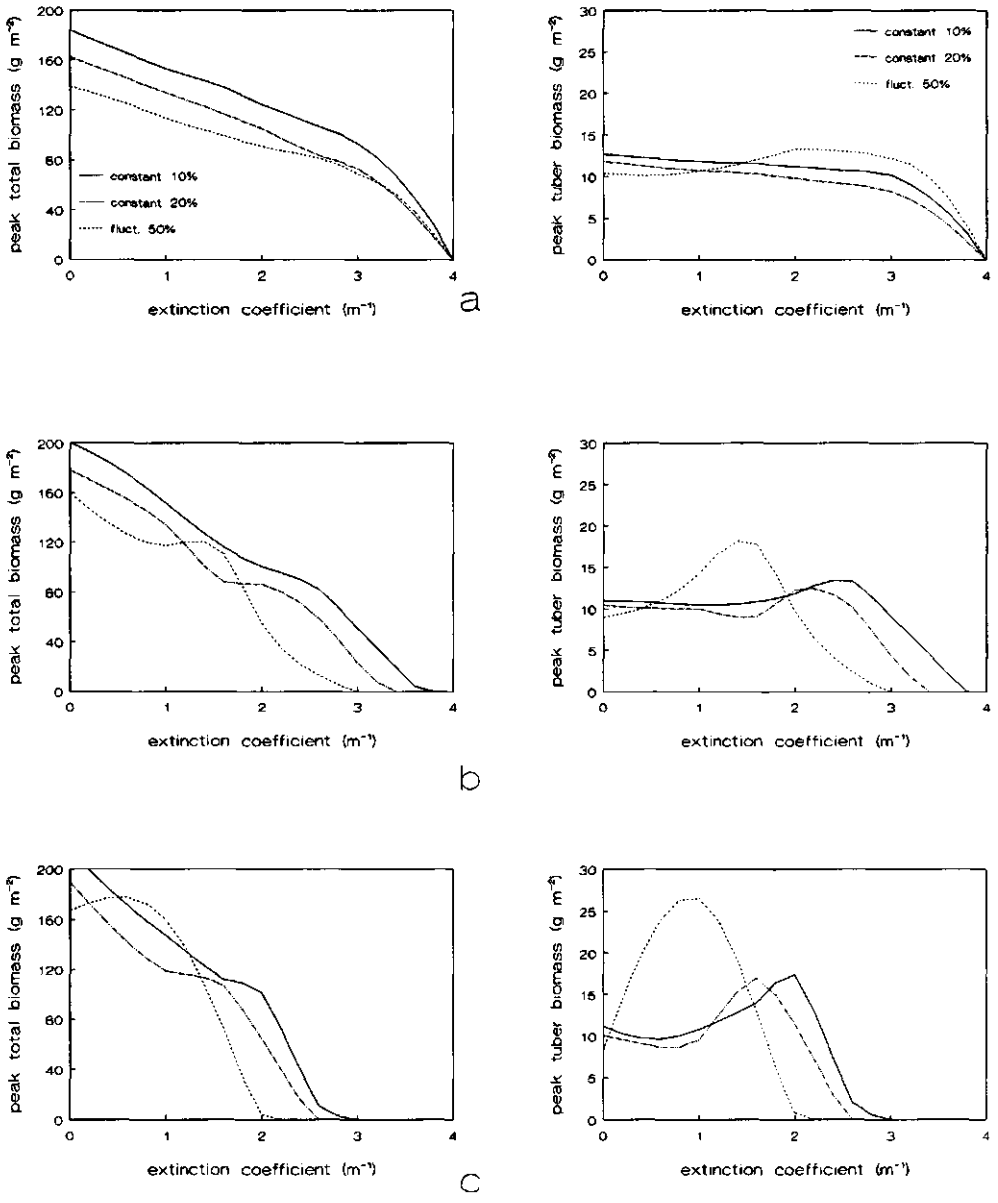


Fig. 9.27. Three cross sections along the extinction coefficient axis through the biomass planes as defined in the nomograms. Shown are peak total biomass and peak tuber biomass reached with a constant 10% or 20% periphyton shading regime and with a fluctuating periphyton regime with peak levels of 50% shading (see Fig. 9.5) (a) depth 0.50 m (b) depth 0.75 m (c) depth 1.00 m.

Fig. 9.26 shows the nomogram for peak tuber biomass. The strange shape of the 75 g afdw m^{-2} isocline in 0.50 m water in Fig. 9.25 can be explained by the similarly curved 10-12 g afdw m^{-2} isoclines in Fig. 9.26. The increased distance between the 100 and 125 g afdw m^{-2} isocline in 1.00 m water correlates with the plateau in peak tuber biomass for these conditions.

Because of the negative correlation between tuber induction and light intensity, the difference in peak total biomass levels between a fluctuating periphyton regime with peak levels of 50% (Fig. 9.25) and a constant periphyton shading regime of 10% (Fig. 9.23) can be very limited. This is illustrated in Fig. 9.27. Here, the peak total biomass and peak tuber biomass levels reached with three different periphyton regimes are shown for three depths. Apart from its peak levels of 50%, the fluctuating regime with a summer low of 15% can be seen as intermediate between the constant 10 and 20% periphyton shading regimes. The increased FPF and tuber production for the fluctuating regime result in a higher peak tuber biomass, especially with increasing depth. The increased tuber bank biomass compensates for the reduced photosynthesis in spring and thus enables the vegetation to reach the same or even higher peak biomass values as under the two constant periphyton regimes. Of course, this compensatory mechanism can only work up to a certain light intensity. Below this level, the demand for photosynthate by the developing tuber bank cannot be fulfilled any longer by the vegetation. As a consequence, the resulting tuber bank will be smaller than needed for full compensation of the decreased photosynthesis in spring. The peak total biomass levels than will remain increasingly below those of the two constant periphyton regimes (Fig. 9.27b and c, above an extinction coefficient of 1.5 m^{-1}).

6.3 Recovery of the model vegetation after reduced light conditions

When a vegetation shows a stable seasonal cycle, it can be regarded as in balance with the environmental conditions. In the present model, it can be studied how such a vegetation will react when light conditions change. This can be brought about by changing the water depth and/or the extinction coefficient of the water.

As a starting point, the stable vegetation developing under nominal conditions (parameter values from Table 9.1, Lake Veluwe light regime, 0.75 m water depth) was taken. The tuber bank at the end of the 10th year was used as the basis for

Table 9.5. Consequences of changing water layer extinction coefficient (EXTW, m^{-1}) and water depth (depth, m) for peak total biomass of a stable vegetation growing under nominal conditions (0.75 m depth). Given are the number of years (N) till a new stable situation is reached, and the consequent change in peak total biomass relative to the initial situation (% change). Extinction is indicated with ext. When stability is reached after more than 10 years, this is shown by >10.

	N			% change		
depth	0.50	0.75	1.00	0.50	0.75	1.00
EXTW						
2.2	4	9	ext	64	-33	-100
2.0	4	0	ext	66	0	-100
1.8	5	5	>10	73	40	-54
1.6	5	5	2	80	78	-2
1.4	4	4	7	86	101	65
1.2	4	4	6	96	112	128
1.0	3	3	6	105	115	189
0.5	2	3	3	132	175	188

subsequent 10 year simulations under different light conditions. Three depths (0.50, 0.75 and 1.00 m) were used to simulate decreased, constant or increased water depths. In all simulations, periphyton regime was maintained nominal (see Fig. 9.5). For each depth, eight different extinction coefficients were used, between 0.5 and 2.2 m^{-1}. The light conditions did not change during the simulation. The vegetation now had to change to reach a new stable situation. The number of years necessary to reach this new stable cycle is shown in Table 9.5. Also, the change in peak total biomass relative to that of the initial stable situation is given.

When water depth remains unaffected, i.e. at 0.75 m, a decreasing extinction coefficient results in an increasing biomass. The new stable situation is also reached faster when light extinction becomes less. A concomitant decrease in water depth does not have much effect on the time period needed to reach a new equilibrium. In water of 0.50 m, the new peak level first increases relative to 0.75 m depth, but when the extinction coefficient becomes less than 1.6 m^{-1}, it remains lower than the biomass peak in 0.75 m depth. This phenomenon is due to self-shading: the available 'room' for biomass development becomes limiting. This is also the case for 0.75 m, regarding the even higher new levels that can be reached under clear conditions in 1.00 m depth.

Increasing the extinction coefficient to 2.2 m^{-1} results in a strong decrease in biomass in 0.75 m; together with increasing water depth, the vegetation does not survive. Thus, the model predicts that a slight increase in water depth under the nominal conditions will result in a strong decrease in biomass development. A modest decrease in the extinction coefficient to 1.5 m^{-1} will result in an almost doubled peak biomass level within 4 to 5 years. As such rather small changes have big consequences, the stable equilibrium situation under nominal conditions (which were derived from the present situation in Lake Veluwe) can be seen as rather labile.

7. General conclusions and future model developments

In view of the main objectives, it can be concluded that the present model is certainly able to give a reasonable description of various characteristics of a field population of *P. pectinatus* growing under various light conditions. Especially the general agreement between the model and the behaviour of a real vegetation over more than one year is a promising fact.

When the inevitable uncertainty of model predictions is accepted, nomograms as the ones presented here can be used as a tool for deciding on management strategies. Influencing periphyton development seems much more difficult than reducing turbidity in a shallow, wind-exposed lake in which a large part of the water layer light extinction is caused by resuspended bottom material. Fortunately, according to the present model, when extinction coefficients are higher than 2.0 m^{-1}, management should not worry about periphyton but focus on reducing the extinction coefficient when an increased macrophyte development is desired.

In the near future, it is intended to include various other characteristics in the model. Many data are available on periphyton grazing by macrofauna, especially snails (Vermaat, 1991). This interaction is

likely to be of importance when a vegetation is exposed to increasing periphyton densities due to eutrophication, but also when nutrient levels are decreasing and the reverse process occurs. In the latter situation, data on the colonization rate of the still sparse vegetation by snails from refugia are also necessary. It may also be worthwhile to incorporate periphyton development in dependence of light. The probable effect of snail grazing can already be estimated when macrophyte development under different periphyton regimes is compared. An example of this approach is presented in chapter 10.

Resuspension of sediment particles and possible allelopathic limitation of algal growth by the macrophytes (Hootsmans, 1991) as well as the influence of the amount of vegetation biomass on these effects are not included in the model. Preliminary results from simulations in which a relation between vegetation biomass and the extinction coefficient was included pointed out that in the present version of SAGA1, its effect on vegetation biomass development is very limited. This may change when vegetation development characteristics are defined otherwise.

Nutrients do not play a direct role in vegetation development in the model, apart from their indirect consequences for light availability through effects on phytoplankton and periphyton growth. For eutrophic conditions, this simplification was considered justified. Within a broad range of nutrient concentrations, *P. pectinatus* biomass appeared uninfluenced by changes in major nutrient levels (Peltier & Welch, 1969).

The model vegetation is treated as annual: apart from tubers, no biomass survives to the next season. In reality, especially under more sheltered conditions (small ponds, ditches), *P.pectinatus* biomass can survive at least partly till the following year, and thus can contribute to the newly developing vegetation (Van Wijk, 1988, 1989). The absence of this phenomenon in the model may explain the present maximum total biomass of 150-200 g afdw m^{-2} generated by SAGA1 under the most favourable conditions. The incorporation of overwintering biomass may be necessary to simulate more sheltered situations correctly.

The production of seeds and seed germination does not seem to be of importance for *P. pectinatus* with regard to the short term goal of biomass development (Van Wijk, 1989). Genetically, a vegetation of this species can be considered as a rather homogeneous clone due to its predominantly vegetative reproduction by tubers. However, in the long run, genetic variation and selection within the population are important in determining survival. Prolific seed production, although not very important for short term success, then may be seen as of adaptive value for the long term goal of genetic survival. Such evolutionary aspects may not be very important for short-term simulations. Still, incorporating seed production and germination in the model will make it possible to simulate the life cycle of other species that do rely completely on seeds for reproduction and survival to the next season.

The present model has a weather routine (limited to insolation and daylength) for the temperate climate zone. For a successful application under different climatic conditions this routine of course must be adapted. Other changes may also be necessary. For instance, seasonal changes in photoperiod are very limited in tropical latitudes. Consequently, it may be that *P. pectinatus* stands in the tropics show a more or less continuous tuber production and sprouting. These stands might then be described as consis-

ting of a number of 'temperately' behaving vegetations that are out of phase. This means that the total vegetation could be modelled as several parallel SAGA1 models, somewhat out of phase. In this case, the basic principles do not change much and this approach will therefore be pursued in the near future.

Acknowledgements

The construction of a complex simulation model can never be claimed as the work of only one person. Dr. M. Scheffer suggested the use of the special integration method in the production subroutine. The cooperation and support provided by J.E. Vermaat and Prof. Dr. W. van Vierssen have been essential to arrive at SAGA1 in its present form. G.M. van Dijk and Prof. Dr. W. van Vierssen provided data for model calibration and validation. Prof. Dr. W. van Vierssen, Prof. Dr. W.J. Wolff and J.E. Vermaat critically read the manuscript.

8. References

Anderson, M.G. & Low, J.B., 1976. Use of sago pondweed by waterfowl on the Delta Marsh, Manitoba. J. Wildl. Manage. 40: 233-242.

Best, E.P.H. & Dassen, J.H.A., 1987. Biomass, stand area, primary production characteristics and oxygen regime of the *Ceratophyllum demersum* L. population in Lake Vechten, The Netherlands. Arch. Hydrobiol. Suppl. 76: 347-367.

Cattaneo, A. & Kalff, J., 1978. Seasonal changes in the epiphyte community of natural and artificial macrophytes in Lake Memphremagog (Que.&VT.). Hydrobiologia 60: 135-144.

Collins, C.D. & Wlosinsky, J.H., 1989. A macrophyte submodel for aquatic ecosystems. Aquat. Bot. 33: 191-206.

Goudriaan, J., 1986. A simple and fast numerical method for the computation of daily totals of crop photosynthesis. Agric. For. Meteorol. 38: 257-262.

Guterstam, B., 1981. In situ investigation on the energy flow in a Baltic *Fucus* community. In Levring, T. (ed.). Proc. Xth Int. Seaweed Symp., pp. 405-410.

Hodgson, R.H., 1966. Growth and carbohydrate status of sago pondweed. Weeds 14: 263-268.

Hootsmans, M.J.M., 1991. Allelopathic limitation of algal growth by macrophytes. In Hootsmans, M.J.M. & Vermaat, J.E. Macrophytes: a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University, in prep.

Hootsmans, M.J.M. & Van Vierssen, W. Computer simulations of macrophyte population dynamics during lake recovery after eutrophication. Verh. int. Verein. Limnol., in press.

Hootsmans, M.J.M. & Vermaat, J.E. 1991. Light-response curves of *Potamogeton pectinatus* L. as a function of plant age and irradiance level during growth. In Hootsmans, M.J.M. & Vermaat, J.E. Macrophytes: a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University, in prep.

Ikusima, I., 1970. Ecological studies on the productivity of aquatic plant communities IV. Light condition and community photosynthetic production. Bot. Mag. Tokyo 83: 330-341.

Kirk, J.T.O., 1983. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, 401 pp.

Mason, C.F. & Bryant, R.J., 1975. Periphyton production and grazing by chironomids in Alderfen Broad, Norfolk. Freshwat. Biol. 5: 271-277.

McCree, K.J., 1972. Test of current definitions of photosynthetically active radiation against leaf photosynthesis data. Agric. Meteorol. 10: 443-453.

Meulemans, J.T. & Heinis, F., 1983. Biomass and production of periphyton attached to dead reed stems in Lake Maarsseveen. In Wetzel, R.G.(ed.), Periphyton of freshwater ecosystems. Junk Publishers, The Hague, pp. 169-173.

Ng, E., & Loomis, R.S., 1984. Simulation of growth and yield of the potato crop. Pudoc, Wageningen, pp. 147.

Nienhuis, P.H. & de Bree, B.H.H., 1982. Production and growth dynamics of eelgrass (*Zostera marina*) in brackish Lake Grevelingen (The Netherlands). Neth. J. Sea Res. 14: 102-118.

Ondok, J.P., Pokorný, J. & Květ, J., 1984. Model of diurnal changes in oxygen, carbon dioxide and bicarbonate concentrations in a stand of *Elodea canadensis* Michx. Aquat. Bot. 19: 293-305.

Peltier, W.H. & Welch, E.B., 1969. Factors affecting growth of rooted aquatics in a river. Weed Sci. 17: 412-416.

Sepers, A.B.J., 1981. The aerobic mineralization of amino acids in the saline Lake Grevelingen and the freshwater Haringvliet basin (The Netherlands). Arch. Hydrobiol. 92: 114-129.

Short, F.T., 1980. A simulation model of the seagrass production system. In Phillips, R.C. & McRoy, C.P., (eds). Handbook of seagrass biology: an ecosystem perspective. Garland, New York, pp. 277-295.

Straškraba, M. & Gnauck, A.H., 1985. Freshwater ecosystems. Modelling and simulation. Developments in environmental modelling 8. Elsevier, Amsterdam, 309 pp.

Titus, J., Goldstein, R.A., Adams, M.S., Mankin, J.B., O'Neill, R.V., Weiler Jr., P.R., Shugart, H.H. & Booth, R.S., 1975. A production model for *Myriophyllum spicatum* L. Ecology 56: 1129-1138.

Van der Bijl, L., Sand-Jensen, K. & Hjermind, A.L., 1989. Photosynthesis and canopy structure of a submerged plant, *Potamogeton pectinatus*, in a Danish lowland stream. J. Ecol. 77: 947-962.

Van Dijk, G.M. & Van Vierssen, W., 1991. Survival of a *Potamogeton pectinatus* L. population under various light conditions in a shallow eutrophic lake (Lake Veluwe) in The Netherlands. Aquat. Bot. 39:121-129.

Van Vierssen, W. & Bij De Vaate, A., 1990. Licht en waterplanten, oorzaken van biomassafluctuaties van onderwatervegetaties in het Veluwemeer (Light and submerged macrophytes, causes for macrophyte biomass fluctuations in Lake Veluwe, in Dutch). Report of the Institute for Inland Water Management and Waste Water Treatment, Wageningen Agricultural University, Dept. of Nature Conservation and the International Institute for Hydraulic and Environmental Engineering, 233 pp.

Van Vierssen, W. & Hootsmans, M.J.M. On the origin of macrophyte population dynamics during lake recovery after eutrophication. Verh. int. Verein. Limnol., in press.

Van Vierssen, W., Hootsmans, M.J.M. & Van Dijk, G.M., a. Tuber induction in *Potamogeton pectinatus*. In Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E. (eds). Dynamics of a macrophyte dominated system under eutrophication stress: an integrated approach. Geobotany, Junk, in prep.

Van Vierssen, W., Spencer, D.F. & Vermaat, J.E., b. Source-sink relationships and their influence on tuber dormancy and germination. In Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E. (eds). Dynamics of a macrophyte dominated system under eutrophication stress: an integrated approach. Geobotany, Junk, in prep.

Van Vierssen, W., Vermaat, J.E. & Mathies, A., c. Early growth characteristics of *Potamogeton pectinatus*. In Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E. (eds). Dynamics of a macrophyte dominated system under eutrophication stress: an integrated approach. Geobotany, Junk, in prep.

Van Wijk, R.J., 1988. Ecological studies on *Potamogeton pectinatus* L. I. General characteristics, biomass production and life cycles under field conditions. Aquat. Bot. 31: 211-258.

Van Wijk, R.J., 1989. Ecological studies on *Potamogeton pectinatus* L. III. Reproductive strategies and germination ecology. Aquat. Bot. 33: 271-299.

Verhagen, J.H.G. & Nienhuis, P.H., 1983. A simulation model of production, seasonal changes in biomass and distribution of eelgrass (*Zostera marina*) in Lake Grevelingen. Mar. Ecol. Prog.Ser. 10: 187-195.

Vermaat, J.E., 1991. Periphyton removal by freshwater micrograzers. In Hootsmans, M.J.M. & Vermaat, J.E. Aquatic macrophytes: a key to understanding changes in shallow ecosystems resulting from eutrophication. PhD Thesis, Wageningen Agricultural University, in prep.

Vermaat, J.E. & Hootsmans, M.J.M., 1991a. Intraspecific variation in *Potamogeton pectinatus* L., a controlled laboratory experiment. In Hootsmans, M.J.M. & Vermaat, J.E. Aquatic macrophytes: a key to understanding changes in shallow ecosystems resulting from eutrophication. PhD Thesis, Wageningen Agricultural University, in prep.

Vermaat, J.E. & Hootsmans, M.J.M., 1991b. Growth of *Potamogeton pectinatus* L. in a temperature-light gradient. In Hootsmans, M.J.M. & Vermaat, J.E. Aquatic macrophytes: a key to understanding changes in shallow ecosystems resulting from eutrophication. PhD Thesis, Wageningen Agricultural University, in prep.

Westlake, D.F., 1963. Comparisons of plant productivity. Biol. Rev. 38: 385-425.

Westlake, D.F., 1964. Light extinction, standing crop and photosynthesis within weed beds. Verh. int. Verein. Limnol. 15: 415-425.

Westlake, D.F., 1965. Some basic data for investigations of the productivity of aquatic macrophytes. In: Goldman, C.R. Primary productivity in aquatic environments. Mem. Ist. ital. Idrobiol. 18 (Suppl.): 229-242.

Wetzel, R.L. & Neckles, H.A., 1986. A model of *Zostera marina* L. photosynthesis and growth: simulated effects of selected physical-chemical variables and biological interactions. Aquat. Bot. 26: 307-323.

GENERAL CONCLUSIONS AND IMPLICATIONS FOR LAKE MANAGEMENT

10

M.J.M. Hootsmans & J.E. Vermaat

Contents

1. Introduction

In this final chapter, we try to synthesize our results from the previous chapters. Part of this goal was already reached by the construction of the model SAGA1, presented in chapter 9. Still, several important results, e.g. from our grazing studies and the allelopathy experiments, are not incorporated in this model. Thus, a further, be it more qualitative synthesis seems worthwhile.

First, a summary is given of the various conclusions from the previous chapters, cross-linking where possible. Subsequently, we will reconsider the validity of our conceptual model, and discuss the possibilities of combining it with the fish interaction model (model 1 in the general introduction, chapter 1). Finally, implications of our results for lake management will be illustrated with some examples.

2. General conclusions from the previous chapters

2.1 The macrophyte

We found that phenotypic differences between the two populations of *Potamogeton pectinatus* L. which we studied appeared to have a genotypic component. This genetically based variation might have invalidated the extrapolation of our laboratory results from one population to the other. Apparently, regarding the results of our simulation model, this was not the case. Still, the existence of this kind of variation emphasizes that the combination of data from various sources may lead to large deviations between predicted and real behaviour of a system under study.

Tuber size played an important role in determining plant development and thus also influences the ultimate survival of the vegetation. Especially in small size classes (up to 0.1 g fresh weight), tuber size affected various morphological characteristics and effects on plant biomass remained significant and conspicuous up till two months after sprouting.

It was found that tubers that were incubated up to 3 months in the dark at temperatures between 13° and 22 °C could still produce healthy plants when transferred to the light. In the dark, however, the sprouting tubers produced stem and leaves at 13° and 22°, while they did not at 15°. We concluded that this indicated the induction of a secondary dormancy above a certain threshold limit at about 15°, as has been shown for other angiosperms. The still weak dormancy was easily overcome by the higher temperature treatment (i.e. 22°). Such a secondary dormancy might be induced in the field by a rapid temperature rise in spring, and may have adaptive value in potentially summer-dry habitats. It also implies that at least part of the tuber bank can survive more than one winter.

The effects of three factors (light, age and temperature) on plant growth and development were studied in detail in the laboratory. Plants grown under higher light intensities showed less elongation, produced more leaves and formed more secondary shoots. Chl(a+b) content of leaves decreased. Photosynthetic parameters Pm (maximum rate of gross photosynthesis) and Km (light intensity at which half Pm is reached) increased while α (initial slope of the light-response curve) and R (respiration) were not affected. Consequently, the rate of gross and net photosynthesis at 200 μE m^{-2} s^{-1} (GP200 and NP200) increased.

Increasing age resulted in increased length and more but smaller leaves. Pm and R decreased, Km and α were unaffected. GP200 and NP200 decreased. Photosynthetic performance of *P. pectinatus* can be regarded as 'average' when compared with various other species.

Plants from higher temperatures had higher relative growth rates and higher maxima for the number of leaves, leave bundles and secondary shoots. Interactions between the three factors existed also.

It can be concluded that in response to decreased light levels, *P. pectinatus* does not acclimate its photosynthetic capacities. Traits like shoot elongation and rapid canopy formation, besides the possibility to rely on its tuber reserves, apparently are sufficient to guarantee successful survival in turbid, shallow habitats. The elongation capacity of *P. pectinatus* is not extraordinary compared with that of various other species. Thus, canopy formation and perhaps especially its tuber characteristics may have been decisive for the fact that the species is still present in many eutrophicated systems while other species became extinct.

2.2 The periphytic subsystem

Periphyton development under eutrophic conditions in the lab was in the same range as reported from other lab and field studies (1.5 - 3.0 mg cm^{-2} ash-free dry weight (afdw), maximum densities). Differences in temperature caused more significant differences in the logistic density growth curves than light intensity. On the other hand, most significant differences between curves relating irradiance attenuance and periphyton density existed between different light intensities. It can be concluded that with respect to its density, the periphyton community had been able to acclimate efficiently to the different irradiance regimes. Differences in attenuation characteristics between communities of the same density could be related to differences in taxonomic composition at different irradiances. In general, within a temperature range of 10 - 20 °C and irradiances between 50 and 200 $\mu E\ m^{-2}\ s^{-1}$, the periphytic community reached a density of 0.5 mg afdw cm^{-2} after 3 to 4 weeks, attenuating about 50% of incident light. Regarding light-response curve characteristics of *P. pectinatus*, this means that its photosynthesis can rapidly become light-limited in turbid waters.

2.3 Snail grazing

In grazing experiments, four freshwater snail species significantly removed periphyton from glass slides. Removal rates varied from 0.1-2.2 mg afdw $individual^{-1}\ day^{-1}$. The two tested crustacean species did not remove significant amounts of periphyton. The removal rates of the snails appeared to be a function of activity, snail size and the taxonomic composition of the periphyton. Significant temperature effects were rarely found. In *Lymnaea peregra* (Müll.) no difference was observed in periphyton removal on *P. pectinatus* as compared to glass slides. Dominance of Cyanobacteria in the periphyton clearly limited the removal rate of this species. The performance of *Bithynia tentaculata* L. was similar on laboratory periphyton and on periphyton grown in Lake Veluwe.

Snail grazers could significantly limit periphyton accumulation on *P. pectinatus*. The effect of the consequent improvement of light climate on plant growth and development was clearly related to the ambient light intensity.

When light intensities received by the macrophyte tissue remained low (60 μE m^{-2} s^{-1}), and thus within the linear part of the light-response curve of *P. pectinatus*, differences in the reallocation of tuber reserves were found between grazed and ungrazed plants. The latter showed a relative increase in aboveground tissue. When light intensities were higher (74 μE m^{-2} s^{-1}), no effect on plant growth was found. Thus, seemingly small differences in irradiance caused by different combinations of periphyton shading and snail grazing can lead to pronounced differences in macrophyte growth.

Significant differences existed in the activity patterns of the snail species used during a simulated spring and early summer period. *L. peregra* remained active during the whole period. *Valvata piscinalis* (Müll.) was only present during the first month; after oviposition, post-breeding mortality removed all adults. *B. tentaculata* was active during the first half of the experiment, and subsequently burrowed in the sediment where they remained inactive.

From the plant's point of view, it seems that a population of *L. peregra* is the best grazing solution against periphyton accumulation. This species has one of the highest removal rates and a high and constant activity and presence on macrophytes during the season.

2.4 Allelopathy

Results from various experiments on allelopathy clearly demonstrated the existence of allelopathic effects of macrophyte exudates on phytoplankton growth. Nutrients and light were never limiting. The effect appeared to be rather unpredictable, and could be different for different times of the season, for different algae and for different macrophyte species. The average reduction in algal biomass production in a one week period when allelopathic growth limitation occurred was found to be 10-15%. Thus, this effect may be seen as minor, but might have consequences for algal competition and succession.

2.5 Ecosystem enclosures

In an experiment with polythene and gauze enclosures in Lake Veluwe, the combined effects of fish activity, periphyton, turbidity and possible allelopathic interaction on macrophyte development were studied. Polythene enclosures held higher total *P. pectinatus* biomass than the lake, gauze enclosures were intermediate. The enclosures also had a higher abundance of other macrophyte species. We estimated light attenuation by seston and periphyton and calculated that the plants in the polythene enclosures received more light than those in gauze and the lake, that received an almost similar amount. The differences in plant biomass could not be caused by the gradient in light conditions alone. Differences in nutrients or inorganic carbon concentrations do not seem very likely. We therefore explained the difference in macrophyte biomass by improved light conditions in polythene and absence of sediment-disturbing fish (e.g. bream) in both types of enclosures, and concluded that both factors are of similar importance.

The effect of a small predatory fish, *Gasterosteus aculeatus* L., on zooplankton density in enclosures appeared comparable to the planktivorous effect of the bream-dominated (*Abramis brama* L.) fish community in Lake Veluwe.

2.6 The plant growth model

The simulation model SAGA1 can reasonably describe vegetation development in Lake Veluwe under various light conditions. The seasonal decrease and disappearance of aboveground biomass is satisfactorily triggered by effects of light and age on photosynthesis.

Simulations under various light conditions pointed out that in shallow water, both periphyton shading and water turbidity play an important role in determining plant development. In deeper water, the importance of periphyton decreases strongly relative to turbidity.

Down to a certain light intensity or depth, a *P. pectinatus* vegetation exposed to increased shading maintains a biomass equal to that under very clear conditions. This is caused by the stimulating effect of low light on tuber formation.

Model results pointed out that the current, seemingly 'stable' situation in Lake Veluwe actually might be fairly labile. Relatively small deviations from the present turbidity and water depth values derived from this lake cause a strong biomass increase or total disappearance of the model vegetation.

3. Consequences for our basic model hypothesis: an integrating attempt

The supposed role of allelopathic interaction in our conceptual model can be supported by the evidence from our experiments on this subject. However, as was mentioned in section 2.4, the effect was rather unpredictable. It appeared that not all macrophyte species are able to produce such growth limiters under all circumstances and at every moment.

It may be hypothesized that a diverse macrophyte community in clear water will always produce sufficient allelopathic substances to guarantee a limitation of phytoplankton growth during the period of macrophyte presence. Hence, when increased periphyton growth due to eutrophication starts to disrupt macrophyte community structure, this will lead to a periodic and finally permanent collapse in the production of allelopathic growth limiters by the macrophytes.

The incorporation in our model of periphyton grazing by snails seems necessary regarding the removal capacities measured. Grazing per se will not always lead to improved macrophyte development. Especially when periphyton shading is not very intense, the effects from snail grazing activity may be restricted.

Nevertheless, keeping in mind a diverse macrophyte community with many different growth characteristics, snail grazing can be regarded as a useful means to at least postpone the disappearance of many, more sensitive macrophyte species during eutrophication.

An alternative for the all-important role of periphyton in our conceptual model recently was suggested by one of its original designers (B. Moss, in Stansfield et al., 1989). In their paper, Stansfield et al. (1989) attribute the sudden disappearance of zooplankton in lake sediment records to the increased use of pesticides like DDT in the fifties. However, the evidence from sediment cores regarding the 'rise and fall' of epiphyte dominance, followed by the increase in phytoplankton remains, still stands (Phillips et al., 1978). We think that both effects can have played their role simultaneously.

We now would like to discuss the apparent controversy between the two models mentioned in our introduction (chapter 1). From a scientific point of view, there seems to be no reason why

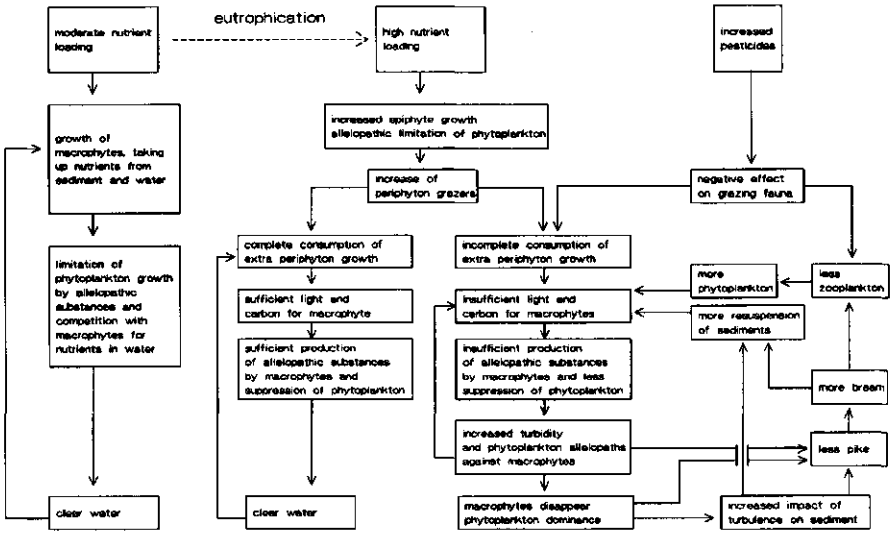


Fig. 10.1. An extended version of the conceptual model of the functioning of a shallow eutrophicated system.

the two models cannot be linked together. In our opinion, this is even necessary. Model 1, in which fish activities play a major role, cannot readily explain the initial changes occurring in a eutrophic community that lead to phytoplankton dominance. On the other hand, our conceptual model (model 2) simply disregards the presently overwhelming effect of fish activity on water turbidity.

Clearly, reality is much more complex than any model that we can develop to enhance our understanding. It is suggested to use model 2, possibly with the effects of pesticides on zooplankton added, to describe the processes that started the 'downfall of the macrophyte empire', while the present 'hegemony' of turbidity and especially its persistence despite decreased nutrient concentrations is more readily explained by the relations in model 1.

When a restoration scheme is discussed, it seems necessary to take both models into account. Although continuously adding more and more aspects to a conceptual model may not always result in an increased insight in the real system, at the present stage this fusion can be worthwhile. Explicit incorporation of the sediment-disturbing activities of benthivorous fish appears necessary. Another addition is the effect of fish predation on zooplankton. We found clear evidence for the ability of a littoral fish species to strongly limit zooplankton densities. Such predation pressure in the littoral may reduce the usefulness of macrophyte vegetation as a refuge for zooplankton from pelagic predation. An attempt to integrate both models is shown in Fig. 10.1.

Apart from the fusion of the two models, the negative effect of pesticides on zooplankton and grazing fauna is also incorporated. The 'fish' part of the model comes into action when the macrophyte vegetation is starting to deteriorate. This is caused by increased turbidity, which makes it more difficult for pike to catch its prey. In turn, the decrease in vege-

tation cover also leads to an increased turbidity because of enhanced sediment resuspension by wave action.

Basing ourselves on this enlarged conceptual model, some further research topics seem evident. Turbidity of the waterlayer in shallow lakes with only a sparse vegetation cover can be caused mainly by sediment resuspension (Gons et al., 1986, Meijer et al., 1990, Van Vierssen et al., in prep.). Resuspension in turn is the result of wind action and bottom-feeding fish like bream. The relative importance of both causes should be assessed. This probably differs depending on lake size and wind exposition. Furthermore, the direct effect of wind and fish action on possibilities for vegetation colonization and development through mechanical stress may be decisive too (Brewer & Parker, 1990).

Some information is available on the mechanisms behind tuber formation (Van Vierssen et al., in prep.). However, further quantification of various causal relations in this process seems necessary to improve our predictive capabilities for the development of a *P. pectinatus* vegetation. The same holds for allelopathic interactions.

Further information is especially necessary on the population dynamics and habitat use of snail populations. Are there still refugia where these animals exist? Could for instance the creation of reed belts in a eutrophicated lake provide the necessary habitat from where they will recolonize the macrophyte beds by themselves (Lodge et al., 1987) or do we have to introduce snail grazers?

In other words, future research should concentrate on interactions between fringes of emergent vegetation, the littoral zone and the pelagial, whilst stressing their role as refugia for various groups during various periods throughout the year.

4. Implications for lake management

Due to the inherently large variation in their subject, ecologists should take care when extrapolating their results to management schemes. However, at the same time they are faced with an overwhelming demand for advise. The ongoing man-caused destruction of our environment does not allow too much delay in taking measures. In the following, we will give some suggestions for the recovery of shallow, turbid lakes to their original, clear and macrophyte-dominated state.

An integration of part of our results is found in our simulation model. This model showed that rather small decreases in turbidity may lead to strongly increased vegetation biomass. This is the case when the extinction coefficient has a value of 2-3 m^{-1}, irrespective of the periphyton shading regime. Depending on the contribution of phytoplankton and resuspended sediments, measures could be taken to increase zooplankton grazing and to reduce sediment resuspension. When sediment resuspension by wind action is negligible, management should focus on fish stock manipulation. However, we suspect that especially in larger shallow lakes with only a sparse macrophyte vegetation, sediment resuspension will play an important, maybe even predominant role in determining the turbidity. In this case, at least part of the problem could be solved by reducing wind fetch on the sediments. By creating wind- and wave-sheltered areas, resuspension will be limited. In this manner, a compartmentation of the lake in areas with different uses is possible: i.e. clear water zones for macrophyte vegetation and more

turbid areas for recreation. Measures could comprise the stimulation of reed belts, the creation of small dikes or islands, and perhaps sediment stabilization by using a kind of netting material.

In the present situation, many lakes probably will have to undergo fish stock manipulation as well. However, the extent to which especially the bream population can be diminished is dependent on the relative isolation of the waterbody undergoing such measures. If there are several connections to other systems, restocking will occur easily (Van Donk et al., 1990). The removal of smaller bream might be accomplished by increased presence of young pike that find hiding places in patches of vegetation (planted or still existent) in sheltered areas.

After the vegetation has been reestablished or improved, sediment resuspension will be further limited, thereby improving possibilities for macrophyte growth: a positive feedback mechanism then has been reinstalled (Kemp et al., 1984).

To our opinion, the use of a relatively complex model incorporating the major interactions in the system can be very useful in determining a management strategy. We finish this chapter by presenting three very different cases in which the use of our macrophyte growth model is demonstrated.

The potential effect of a well-developed snail community on macrophyte growth through the reduction of periphyton accumulation

It has been established that various freshwater snail species have the capacity to remove significant amounts of periphyton from macrophytes (Vermaat, 1991). Here, we ask ourselves whether a well-developed community of common freshwater snail species has the capacity to compensate for increased periphyton accumulation rates due to eutrophication. We will estimate densities needed to cope with periphyton accumulation and compare these with figures available in the literature. Further, we will use output from SAGA1 (Hootsmans, 1991) to estimate the impact of periphyton removal on the biomass of *P. pectinatus*.

We have used periphyton accumulation rates as measured in 1986 (Van Vierssen & Bij de Vaate, 1990). Periphyton accumulation had two distinct maxima, one in spring and one in late summer, and a minimum in between. We accordingly distinguished three periods. Subsequently, we used periphyton removal rates (PRI) of *B. tentaculata* and *L. peregra*, to calculate the number of snails necessary to completely compensate for accumulation (Table 10.1). These two species are reasonably common in the freshwaters of North Western Europe and have adults of similar size. It appears that of both species densities of 120-150 m^{-2} of periphyton-covered area are needed to fully compensate for periphyton accumulation.

The figures from Table 10.1 need to be converted to numbers per unit sediment area for a comparison with literature data. This is done in Table 10.2, where we used two substrate preferences (i.e. sediment versus plants): (1) no preference, i.e. a distribution on the basis of available area, and (2) 100% preference for the plants. Data in Vermaat (1991) suggest the former for *L.peregra*, but we will use both here.

Reported densities for adults of *L. peregra* and *B. tentaculata* just before or during the ovipostion period range around 100 m^{-2}, but values up to 300 m^{-2} were found (Dussart, 1979; Soszka, 1975; Young, 1975; Lodge, 1985). If we assume that any substrate preference is absent, a

Table 10.1. Estimation of densities of *L. peregra* (Lp) and *B. tentaculata* (Bt) needed to compensate for periphyton accumulation in three periods. Periphyton accumulation on microscopic glass slides was taken from Van Vierssen & Bij De Vaate (1990). Periphyton removal rate (PRI) was taken from Vermaat (1991), taking periphyton density and functional reponses into account. Snail densities are expressed per unit area of periphyton-covered substrate.

period	accumulation (mg afdw dm^{-2} day^{-1})	PRI (mg afdw $snail^{-1}$ day^{-1})		snail density (n m^{-2})	
		Bt	Lp	Bt	Lp
spring bloom	2.1	1.8	1.4	120	150
summer low	0.6	0.4	0.5	150	120
late summer bloom	2.1	1.8	1.4	120	150

Table 10.2. Estimation of snail densities needed to compensate for periphyton accumulation that is integrated over plants and sediment. Densities of *L. peregra* (Lp) and *B. tentaculata* (Bt) were calculated on the basis of no and 100% preference for plants over sediment (cf. text). No calculations were made for the late summer bloom, since aboveground vegetation had already died back by that time. Two-sided plant area was calculated from aboveground biomass in 1986 (Van Dijk & Van Vierssen, 1991) and a specific area of 5 dm^2 g $afdw^{-1}$.

period	plant area (m^2 m^{-2})	snail density (n m^{-2}, integrated)			
		no preference for plants		100% preference for plants	
		Bt	Lp	Bt	Lp
spring bloom	0.5	360	450	60	80
summer low	1.1	290	230	170	130

total of about 400 adult snails m^{-2} is needed for a complete compensation of periphyton accumulation. This certainly is a high density for one species. However, snail communities in aquatic vegetation of freshwaters of Western Europe consist of several species and the combined effect of the whole community may well be equivalent to that of 400 adult *L. peregra* or *B. tentaculata*. We conclude that well-developed snail communities have the capacity to remove spring periphyton accumulations to quite some extent.

Output from SAGA1 on the effect of three periphyton regimes is given in Table 10.3. A partial reduction of periphyton to a maximal irradiance attenuance of 30% already results in a total peak biomass increase with a factor of 1.5 at a water depth of 75 cm. Complete absence of a periphyton spring bloom almost doubles peak biomass of the macrophytes. We can thus conclude that periphyton removal by a snail community may very well have significant effects on macrophyte biomass. The freshwater snail community of our model lake, Lake Veluwe, however, and probably also that

of similar lakes must be considered as poorly developed. *V. piscinalis* was the only species present in some numbers (< 25 adults m^{-2}), whilst *L. peregra* was rare (personal observations).

Table 10.3. Peak total biomass at three different periphyton regimes, 75 cm depth and a water extinction of 2 m^{-1}, as calculated by SAGA1. Total biomass = aboveground + belowground + new tubers. Partial compensation indicates that periphyton removal by snails partially compensates for accumulation during periphyton blooms.

periphyton regime	biomass	ratio to nominal
nominal (0.5-0.15-0.5)	55	-
partial compensation (0.3-0.15-0.3)	84	1.5
total compensation (0.1)	101	1.8

Several factors may be held responsible for the virtual absence of a snail community in Lake Veluwe. Osenberg (1989) and Lodge et al. (1987) showed that predation by fish may be an important decimating factor. Osenberg (1989) found that nutrient addition that increased the periphytic food stocks had a stronger effect on snail density than the prevention of predation by fish, but he worked in an oligotrophic lake. Food limitation does not seem to be that important in eutrophic Lake Veluwe, also because we calculated that fairly high numbers are necessary to compensate for periphyton accumulation. Food limitation may play an indirect role via the restricted time that submerged vegetation is available as a habitat. Bream stocks are probably high in Lake Veluwe, (cf. Hosper & Jagtman (1990) for the neighbouring and comparable Lake Wolderwijd) and predation pressure may be accordingly high.

We conclude that probable candidates to explain the lack of snails in Lake Veluwe are fish predation as well as the short availability of macrophytes as a habitat where high quality food and shelter can be found.

Possibilities for a wintering Bewick's swan population to feed on tubers in Lake Veluwe

Bewick's swan (*Cygnus bewickii* Yarr.) is an example of a bird species that is very dependent on the availability of submerged macrophyte beds for its survival during winter. During the last decades, the number of swans fluctuated strongly, paralleling the changes in macrophyte abundance. In Lake IJssel (The Netherlands), which came into existence after the closing of the Zuiderzee in 1932, beds of *P. pectinatus* expanded strongly. The number of wintering swans increased from several hundreds to 3000-5000 in the sixties (Timmerman, 1977). The decrease in macrophytes as a result of eutrophication resulted in a decline to about 1000 animals by the end of 1968. They also shifted for feeding to neighbouring grasslands. Recently, concurrent with the increase in *P. pectinatus* biomass, swan numbers in The Netherlands have increased again up to 3000-6500 animals (about 50% of the total western palearctic population of this species). Nowadays, they still often feed on grasslands, probably because of frequent disturbance by recreation on the lakes.

The model SAGA1 can be used to estimate the number of swans that could be sustained by a *P. pectinatus* vegetation during winter. The calculations are based on several assumptions, presented in Table 10.4. Although objections may be raised against these data, this does not influence the point we want to make: a simulation model of macrophyte growth could be

successfully applied for very different purposes.

In the calculations, swans arrive in autumn (October 15th) and are supposed to feed on tubers during the whole winter period, until they leave in early spring (March 15th). Starting from the tuber biomass of an 'ungrazed' vegetation on October 15th, a daily decrease in tuber biomass occurs, due to mortality and swan grazing. Swans stop foraging when the tuber biomass per m^2 drops below a certain level (6.5 g m^{-2}, according to Beekman et al., 1981). Because the swan population stays all winter, this minimum tuber biomass per m^2 has to be equal to the tuber biomass that is left per m^2 when the birds leave the area in early spring (March 15th). The tuber biomass on March 15th is used to calculate the new tuber biomass on October 15th that can be developed by the 'grazed' vegetation. This new tuber biomass is then used to calculate the number of swans that can be sustained during the next winter. Thus, two different swan numbers are obtained:

Table 10.4. Assumptions made during calculations of the number of wintering Bewick's swans feeding on a *P. pectinatus* tuber bank.

Lake surface that is vegetated	1000 ha
Lake depth	0.75 m
Decay rate of tuber biomass	0.001 g g^{-1}
Daily tuber consumption per swan	250 g afdw
Minimum tuber biomass at which swans stop grazing	6.5 g afdw m^{-2}
Arrival date of swans	October 15th
Departure date of swans	March 15th

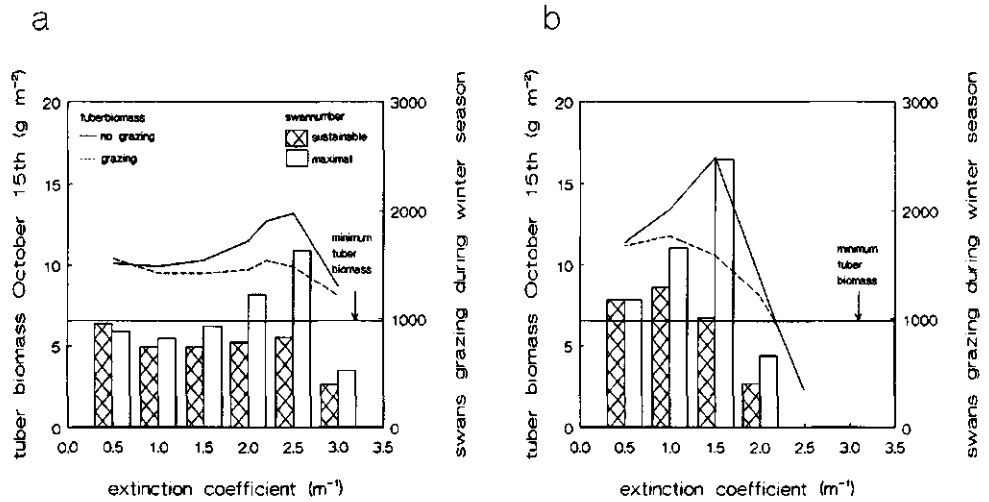


Fig. 10.2. Model predictions on tuberbank size and the possible number of swans feeding on tubers during winter. Starting in an ungrazed vegetation (solid line, open bars) and in a vegetation already grazed during the previous winter (dotted line, hatched bars). Depth 0.75 m; (a) periphyton regime constant 10% shading or (b) fluctuating between 50% in spring and autumn and 15% in summer (Lake Veluwe regime). See the text.

one, the maximal number, is based on the tuber biomass available in autumn in a previously ungrazed vegetation. The other, the sustainable number of swans, can return each year to the grazed vegetation.

Fig. 10.2 shows the effect of various light conditions on the tuber biomass in an ungrazed and in a grazed vegetation, together with the maximal and sustainable number of swans that could feed during the winter season. Clearly, the maximum number can seldom be maintained in the next winter as grazing decreases the tuber biomass available in spring and thus negatively affects vegetation development during the growing season. Only under very clear water conditions, the two numbers are about equal. Due to the stimulating effect of low light levels in spring on tuber formation (Van Dijk & Van Vierssen, 1991) tuber biomass in the ungrazed situation shows a maximum, which of course coincides with the highest maximal number of swans.

The sustainable number of swans remains more or less constant when the extinction coefficient is increased. The stimulating effect of decreased light levels on tuber production is just enough for a grazed vegetation to maintain an approximately constant tuber bank biomass in autumn. Because of the same effect, under a fluctuating periphyton regime the vegetation can support a higher sustainable number of swans than under a low, constant periphyton shading regime. However, when the water becomes rather turbid, the sustainable number of swans rapidly declines to zero. Rather small changes in the extinction coefficient in the region of 2.0 m^{-2} thus can have important consequences for swan wintering possibilities.

Light conditions in Lake Veluwe can be described as fluctuating like in Fig. 10.2b, with an extinction coefficient of 2.0 m^{-2}. Furthermore, the vegetated area and the average depth of this lake are more or less equal to the data shown in Table 10.4. The actual number of Bewick's swan wintering on this lake was between 300-700 in the winter of 1981-1982 (Brouwer et al., 1984). Our model calculations predict that about 400 swans could survive during winter under these conditions. Regarding the switching of the swans to terrestrial food sources during winter, the model results appear in rather good agreement with the field data.

As tuber peak biomasses are reached in August, timing of swan arrival only affects the development of the grazed vegetation (and, in turn, the sustainable number of swans) when the birds arrive before August. Up till now, this has never occurred. The time necessary to complete their breeding cycle in the summer habitat does not permit the birds to arrive so early. Thus, the timing of processes in the two populations (plant and bird) fits nicely.

Strategic mowing

Sometimes a macrophyte vegetation develops so profusely, that it is considered a nuisance. The 'weeds' then have to be 'controlled'. Several methods exist for this purpose. However, all of them need some strategic planning: when is the best moment to apply them? In this case, 'best' can be defined as: reaching the desired effect with the least effort, both in terms of labour and financial cost. Thus, it may be worthwhile to study the effects of various strategies with a simulation model. As an example, we will study the effect of mowing on different dates during the growing season on the development of *P. pectinatus*. These results are described in more detail in Van Vierssen & Hootsmans (1990).

To simplify things, mowing was

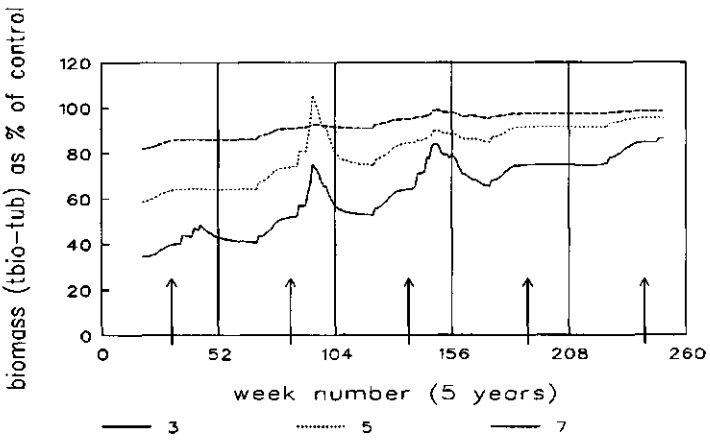


Fig. 10.3. Impact of the timing of mowing in the growing season on subsequent vegetation development. Three different initial tuber banks (3, 5 and 7 g m^{-2}) are the result of mowing a previously unaffected, stabilized vegetation on July 4th, 14th and 24th, respectively. Biomass of the total vegetation minus tuber biomass (tbio-tubbio) is presented relative to the untreated control. Simulation under Lake Veluwe conditions (see text), water depth 0.75 m. Arrows indicate the end of August.

defined as the complete removal of above-ground biomass. The tuber biomass that has developed on the date of mowing was taken as the new tuber bank for the next year. All simulations started with a stabilized model vegetation under conditions comparable to those in Lake Veluwe (see 7.4.2). Three different mowing dates were used: July 4th (when 3 g m^{-2} new tubers had been produced), July 14th (at 5 g m^{-2} new tubers) and July 24th (7 g m^{-2}). The resulting biomass development in the years following the mowing treatment is shown in Fig. 10.3, relative to the biomass of the untreated control vegetation. Clearly, rather small differences in timing can lead to large differences in the resulting vegetation biomass. The effects can last up to 4 years after the treatment.

5. References

Beekman, J., Van Dijk, K. & Dirksen, S., 1981. De begrazing van fonteinkruidvelden in de Lauwersmeer door de Kleine Zwaan (*Cygnus bewickii*) (Grazing of Pondweedbeds by Bewick's Swan, in Dutch). Student report Zoological Laboratory. Groningen University. 30 pp.

Brewer, C.A. & Parker, M., 1990. Adaptations of macrophytes to life in moving water: upslope limits and mechanical properties of stems. Hydrobiologia 194: 133-142.

Brouwer,H., Daalder, R. & Nieboer, E., 1984. Kleine zwanen en plankzeilers in het randmerengebied. Bewick's Swans and windsurfers in the 'Randmeren' area, in Dutch). De Levende Natuur 85: 77-83.

Dussart, G.B.J., 1979. Life cycles and distribution of the aquatic gastropod molluscs *Bithynia tentaculata* (L.), *Gyraulus albus* (Muller), *Planorbis planorbis* (L.) and *Lymnaea peregra* (Muller) in relation to water chemistry. Hydrobiologia 67: 223-239.

Gons, H.J., Veeningen, R. & Van Keulen, R., 1986. Effects of wind on a shallow lake ecosystem: resuspension of particles in the Loosdrecht lakes. Hydrobiol. Bull. 20: 109-120.

Hootsmans, M.J.M., 1991. A growth analysis model for *Potamogeton pectinatus* L. In Hootsmans, M.J.M. & Vermaat, J.E. Macrophytes, a key to understand changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Hosper, S.H. & Jagtman, E., 1990. Biomanipulation additional to nutrient control for restoration of shallow lakes in The Netherlands. Hydrobiologia 200/201: 523-534.

Kemp, W.M., Boynton, W.R., Twilley, R.R., Stevenson, J.C. & Ward, L.G., 1984. Influences of submerged vascular plants on ecological processes in upper Chesapeake Bay. In Kennedy, V.S., (ed.), The estuary as a filter. Academic Press, New York, pp. 367-394.

Lodge, D.M., 1985. Macrophyte gastropod associations: observations and experiments on macrophyte choice by gastropods. Freshwat. Biol. 15: 695-708.

Lodge, D.M., Brown, K.M., Klosiewski, S.P., Stein, R.A., Covich, A.P., Leathers, B.K. & Brönmark, C., 1987. Distribution of freshwater snails: spatial scale and the relative importance of physicochemical and biotic factors. Amer. Malac. Bull. 5: 73-84.

Meijer, M.-L., De Haan, M.W., Breukelaar, A.W. & Buiteveld, H., 1990. Is reduction of the benthivorous fish an important cause of high transparency following biomanipulation in shallow lakes? Hydrobiologia 200/201: 303-315.

Osenberg, C.W., 1989. Resource limitation, competition and the influence of life history in a freshwater snail community. Oecologia 79: 512-519.

Phillips, G.L., Eminson, D.F & Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated waters. Aquat. Bot. 4: 103-125.

Soszka, G.J., 1975. The invertebrates on submerged macrophytes in three Masurian lakes. Ekol. Pol. 23: 371-391.

Stansfield, J., Moss, B. & Irvine, K., 1989. The loss of submerged plants with eutrophication III. Potential role of organochlorine pesticides: a palaeoecological study. Freshwat. Biol. 22: 109-132.

Timmerman, A., 1977. De Kleine Zwaan (Bewicks Swan, in Dutch). Vogeljaar 25: 113-123.

Van Dijk, G.M. & Van Vierssen, W., 1991. Survival of a *Potamogeton pectinatus* L. population under various light conditions in a shallow eutrophic lake (Lake Veluwe) in The Netherlands. Aquat. Bot. 39:121-129.

Van Donk, E., Grimm, M.P., Gulati, R.D., Heuts, P.G.M., De Kloet, W.A. & Van Liere, L., 1990. First attempt to apply whole-scale food-web manipulation on a large scale in The Netherlands. Hydrobiologia 200/201: 291-301.

Van Vierssen, W. & Bij De Vaate, A., 1990. Licht en waterplanten, oorzaken van biomassafluctuaties van onderwatervegetaties in het Veluwemeer (Light and submerged macrophytes, causes for macrophyte biomass fluctuations in Lake Veluwe, in Dutch). Report of the Institute for Inland Water Management and Waste Water Treatment, Wageningen Agricultural University, Dept. of Nature Conservation and the International Institute for Hydraulic and Environmental Engineering, 233 pp.

Van Vierssen, W. & Hootsmans, M.J.M., 1990. Weed control strategies for *Potamogeton pectinatus* L. based on computer simulations. Proc. EWRS 8th Symposium on Aquatic Weeds, pp. 231-236.

Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E., (eds). Dynamics of a macrophyte-dominated system under eutrophication stress: an integrated approach. Geobotany, Junk. Accepted.

Vermaat, J.E., 1991. Periphyton removal by freshwater micrograzers. In Hootsmans, M.J.M., & Vermaat, J.E. Macrophytes, a key to understand changes caused by eutrophication in shallow freshwater ecosystems. PhD Thesis, Wageningen Agricultural University.

Young, M.R., 1975. The life cycles of six species of freshwater molluscs in the Worcester-Birmingham canal. Proc. malac. Soc. London 41: 533-548.

APPENDICES

Contents

SAGA1: A MATHEMATICAL OUTLINE 1

M.J.M. Hootsmans

This Appendix has to be seen as a commentary to the source code of the model, presented in Appendix 4. SAGA1 was written in Fortran 77 for use both on personal computers and mainframes. Model complexity dictates a run time of approximately 3 minutes for a 10 year simulation on an HP-9000 mainframe. One year simulations on an IBM-PC-AT or compatible with mathematical coprocessor take about the same amount of time. For references, see chapter 9.

1.1 Main program

In the main program, through a call to INFO, a number of input and output files is opened. Then, the year and day loops start. Several variables are available in the main program to store information on the various biomass components and other characteristics of the total vegetation. These variables are initialized each year, one day before the day IDAYBEG. Each day, a call is made to METEO to provide the daylength and daily insolation. Furthermore, the periphyton shading percentage PERIF and the waterlayer extinction coefficient EXTW are read from the file PERFEXTW.

When the growing season has begun on IDAYBEG, each day a random number RND is read from the file RANDOM. The random number file is the same for each simulation run. Thus, whenever SAGA1 is used, the same sequence of random numbers is read from RANDOM. This enables a direct comparison of the results from simulation runs performed with different parameter values.

The watercolumn is divided into layers of constant thickness THICKN. The length distribution HTOP of the total vegetation over these layers is calculated from THICKN and the vegetation length HMAX. Within each layer I, biomass LAYWT(I) is homogeneously distributed, except in the top layer TOP. Here, the layer is filled up till the top of the vegetation. Thus, HTOP(I) always equals THICKN, except in the top layer TOP.

The daily light distribution with depth is obtained through subroutine LIGHT. Subsequently, the main program makes calls to the requested number of subvegetation subroutines (VEG1-3). The length and the top layer of the longest subvegetation are taken as the new HMAX and TOP of the total vegetation. The biomasses of the various components of the subvegetations are totalled in variables representing the corresponding biomass components of the total vegetation.

If the requested print interval has elapsed, subroutine FIGURE is called, and the results for the total vegetation are written to the output file SAGA.

At the end of a year loop, the new tuber bank is calculated by subroutine TUBDIST.

1.2 INFO

The structure of the INFO subroutine is rather straightforward. It is called once in a simulation. All input and output files are opened then. Input files are providing data on

periphyton shading percentage and water layer light extinction coefficient (file PERFEXTW.PRN) and various simulation parameters (SIMPARAM.PRN). Random numbers are taken from the file RANDOM. Output files store data on daylength and insolation (SIMMETEO.PRN), light and biomass profiles of the total vegetation (LIGHT.PRN), various data on total vegetation (SAGA.PRN) and each subvegetation (SAGA1.PRN, SAGA2.PRN, SAGA3.PRN). Tuber bank data are stored in TUBER.PRN. Several other variables, like photosynthesis parameters, are stored in HELPSIM.PRN. Input is asked from the user regarding the depth DEPTH of the watercolumn and the shade percentage SHADE caused by artificial shading above the water surface. Also, the number of subvegetations and the number of years in the simulation, together with the time interval to write results to output files and the screen, are requested.

Parameter values provided by the input file SIMPARAM.PRN can be used to influence the behaviour of various processes in the simulation. They are written to the screen for reference. Values of parameters representing ratios are checked. The ratio of DEPTH and THICKN is not allowed to be higher than 20, the maximum number of layers which can be accomodated within the present array dimensions. Subsequently, the parameter values are stored again in SIMPARAM.PRN, together with the information provided on DEPTH and SHADE.

1.3 METEO

Daily weather calculations are limited to daily insolation DPAR (μE m^{-2} day^{-1}, photosynthetically active radiation), instantaneous insolation at a certain time of day HPAR (μE m^{-2} s^{-1}) and photoperiod DAYL (h). Basically, these values are obtained by interpolation over time between minimum and maximum values, using goniometric equations. Daynumber DAI is expressed in degrees (DAT) by multiplying with 360/365. DAYL is derived from maximum (DMAX) and minimum (DMIN) daylength in The Netherlands:

```
DAYL=(DMAX+DMIN)/2-((DMAX-DMIN)/2*COS(2*PI*(DAT+10)/360))
```

Average daily global insolation in The Netherlands amounts to $1*10^7$ J m^{-2} day^{-1}. Using a conversion factor of 4.66 μE J^{-1}, this becomes $4.66*10^7$ μE m^{-2} day^{-1}. Average cloud cover is already incorporated in this figure. DPAR depends on daynumber DAT as follows:

```
DPAR=4.66*1E7*(0.5-0.4*COS(2*PI*(DAT+10)/360))
```

Instantaneous insolation on a certain time HOUR of the day, HPAR, is calculated according to Kirk (1983):

```
T=DAYL/2+HOUR-12  (time in hours since sunrise)
HPAR=(((PI*DPAR)/(2*DAYL))*SIN(PI*T/DAYL))/3600
```

DPAR and DAYL data are written to the output file SIMWEER.PRN in the main program.

1.4 LIGHT

Distribution of the average instantaneous insolation PARTOP(I) (μE m^{-2} s^{-1}) halfway in each layer I of the watercolumn is of course the same for all subvegetations. The same holds for the depth distribution of the actual instantaneous insolation on three times J of the day, PAR(I,J). Both variables are calculated from the watersurface (layer SURFACE) down to the bottomlayer.
The amount of light reaching the water surface is calculated as:

```
PARTOP(SURFACE+1)=DPAR/(3600*DAYL)
```

Reflection from the water surface, and artificial shading, result in the insolation directly below the water surface:

```
PARTOP(SURFACE)=PARTOP(SURFACE+1)*(1-REFLEX)*(1-SHADE)
```

Following Ikusima (1970), the extinction through turbidity and self-shading is taken into account with extinction coefficients EXTW (m^{-1}) and KPLANT (m^2 g^{-1}) when the amount of light halfway in the surface layer is calculated:

```
LL(SURFACE)=PARTOP(SURFACE)*EXP(-0.5*THICKN*EXTW-
0.5*LAYWT(SURFACE)*KPLANT)
```

Subsequently, the amount of light halfway in the deeper layers is calculated:

```
PARTOP(I)=PARTOP(I+1)*EXP(-0.5*THICKN*EXTW-0.5*LAYWT(I+1)*KPLANT)
LL(I)=PARTOP(I)*EXP(-0.5*THICKN*EXTW-0.5*LAYWT(I)*KPLANT)
```

Finally, the periphyton shading percentage PERIF is incorporated and PARTOP is made equal to LL:

```
LL(I)=LL(I)*(1-PERIF)
PARTOP(I)=LL(I)
```

PAR(I,J), the depth distribution of the instantaneous insolation (μE m^{-2} s^{-1}) on three moments during the day, is calculated in the same way. Which three moments during the photoperiod are used depends on daylength and on the integration method used in PROD, and will be discussed there.

1.5 VEG1, VEG2, VEG3

These three subroutines are almost identical. The only difference is the tuber bank biomass WTUBER and mother tuber size ATUBW. By this difference, a tuber size distribution with three classes is simulated. The part of the data from each subvegetation that is necessary to calculate the results for the total vegetation is passed back to the main

program.

All assisting subroutines are shared by VEG1-3. The variables in these assisting routines are so-called local variables: they are used by all three VEG routines. A variable value has to be send back from the assisting routine to the calling routine VEG in order to remain available for further calculations. Thus, all data pertaining to the subvegetations that are necessary for the next day, e.g. information on age and light history of the various fractions and new tuber development, are saved in variables in their respective subroutine VEG1-3.

On day 1 of the year, WTUBER and ATUBW are initialized. The other necessary variables are not initialized until the day before IDAYBEG. This allows the possibility of aboveground biomass from the previous year to survive winter. Such biomass, together with the mother tuber bank, can initiate the new vegetation (Van Wijk, 1989). However, in SAGA1, apart from the tuber bank, all remaining biomass from the previous year is arbitrarily set to 0 when the new growing season begins.

Every day, tuber biomass WTUBER decreases with a fraction TUBDEC, simulating the combined effect of maintenance metabolism, decay and predation. The growing season starts when on IDAYBEG the call to STARTGR results in the variable GSEASON becoming 1. When all aboveground vegetation has disappeared, GSEASON becomes 0 again.

In the growing season, calls are made to AGE (age and light history of all biomass components, photosynthetic parameter values), FPFTIF (photosynthetic period factor determination), PROD (daily production of the subvegetation) and GROW (growth and development). The maximum length and the top layer of the subvegetation are determined from the length and top layer of the mother vegetation and the various secondary shoots. The results are stored in the respective output files SAGA1-3 in REPORT when the requested printinterval has elapsed.

1.6 STARTGR

In this subroutine, aboveground biomass of the subvegetation is initialized on IDAYBEG. The number of plants is determined:

```
AMOUNT=WTUBER/ATUBW
```

This number remains constant throughout the growing season, and is important in determining the length increase of the vegetation resulting from the biomass allocated to elongation. When this biomass has to be divided over many plants, the resulting length increase will be small.

A certain fraction CITUBFL of the tuber bank biomass WTUBER is available for shoot and root biomass initiation. The tuber bank is depleted with the same amount, TUBFLOW.

```
TUBFLOW=WTUBER*CITUBFL
WTUBER=WTUBER-TUBFLOW
```

A fraction ROOTIN is taken from TUBFLOW to initiate root biomass. It is stored in ROOTST. Root biomass initiation occurs one week after IDAYBEG in subroutine GROW. The remaining TUBFLOW is allocated to the biomass in the bottom layer, LAYW(1). No secondary shoot biomass and new tuber biomass is initiated yet. Thus, biomass of the mother vegetation in the bottom layer, SUMSPROUT(1), is equal to LAYW(1). Together with the constant weight/length ratio of initiated shoot biomass, WLINI (equal for all subvegetations), and AMOUNT, the length HEIGHT and the length distribution of the mother vegetation HEIDIS(I) of the young vegetation is calculated:

```
HEIGHT=(SUMSPROUT(1)/AMOUNT)/WLINI
HEIDIS(1)=HEIGHT
```

It is checked that both are less than or equal to THICKN. Finally, the season variable GSEASON is set to 1.

1.7 AGE

With regard to age calculations, many separate groups are recognized. This has been done to facilitate a future incorporation of age effects on growth and development. Age is calculated for root biomass (AGER) and aboveground biomass. With respect to aboveground biomass, age is calculated per layer I of subvegetation (MEANAGE(I)), per layer I of the mother vegetation (SUMSPROUT(I)) and for each layer I of each secondary shoot biomass J (AGESEC(J,I)). Age of tubers is not registrated specifically, as it is easily derived from the first array counter in the array KNOL(I,J) which contains tuber number and biomass data (see GROW).

The calculation is similar for all components: age is increased with one day, unless the biomass has increased. As an example, the calculation of the age AGES(1) of the bottomlayer of the mother vegetation SUMSPROUT(1) is shown. The biomass of this layer on the previous day has been saved in SUMOLD(1). AMIN is a Fortran function taking the minimum of the two values between the brackets.

```
AGES(1)=AGES(1)*AMIN1(1.,SUMOLD(1)/SUMSPROUT(1))+1
```

When the biomass decreases, age is simply increased by one each day. Thus, an extra increase of the mean age of the layer when especially young biomass has disappeared due to wave damage is not taken into account.

To determine the values of the two photosynthesis parameters PM and KM, information is needed on the age of a layer and its light history. The parameter values are based on experiments in which age was simply the period between planting and the photosynthesis measurements, while light history was equal to the constant light level during the growth period. In the model I also use the period since the first amount of biomass appeared in the respective layers as the 'age' of a layer. This quantity BEGIN(I) is simply incremented daily with 1, when a subvegetation layer exists. Thus, BEGIN(I) differs from the 'real', i.e. average age of the layer, MEANAGE(I).
Light history MEANPAR(I) of a layer is calculated somewhat more complicated. The

light history of both yesterday's biomass LAYOLD(I) in a layer and today's biomass LAYW(I) are weighed with respect to age MEANAGE(I) and biomass.

```
MEANPAR(I)=MEANPAR(I)*LAYOLD(I)*MEANAGE(I)+PARTOP(I)*LAYW(I)*1
MEANPAR(I)=MEANPAR(I)/(LAYOLD(I)*MEANAGE(I)+LAYW(I))
```

1.8 INTERPOL

In this subroutine, two tables are available with values for the photosynthetic parameters PM and KM (maximum rate of photosynthesis and the light level at which half this rate is reached, respectively). These data are taken from laboratory experiments described in Hootsmans & Vermaat (1991) with plants of three ages (30, 70 and 120 days) grown under 4 different light levels (50, 100, 150 and 200 μE m^{-2} s^{-1}, 16 hour photoperiod). The specific parameter value for a biomass layer LAYW(I) is derived from this 3*4 table with light history MEANPAR(I) and the time period BEGIN(I) during which the layer I has existed as entry parameters. Linear interpolation is used between the four table values whose coordinates are closest to the coordinates of the requested parameter value. When one of the entry coordinate values is outside the table boundaries, this value is reset to the closest table entry value. In this case, linear interpolation is done between only two table values. In extreme cases, when both coordinates are outside the table boundaries, the closest table value is taken.

1.9 FPFTIF

It is supposed that the tuber induction rate is determined during the first month of the growing season. This rate is based upon the daily ratio FPRATIO of the daily photosynthetic period FP defined as the time period during which at least a certain amount of light BOUNDLIGHT (μE m^{-2} s^{-1}) reaches the top layer, and the daily photoperiod DAYL. FPRATIO is averaged over the first 30 days (this number of days is incorporated in the subroutines VEG and FPFTIF). I have taken the ratio FP/DAYL instead of FP itself because FP is affected not only by turbidity and periphyton development, but also simply by the changing photoperiod.

The effect of FPRATIO on the tuber induction rate is realized through FPF, the photosynthetic period factor. FPF is deduced from FPRATIO with a tentative relation. Above an FPRATIO of FRMAX, FPF is set to a low value FPFMIN, ensuring a certain low tuber induction rate under clear water conditions. Below FRMAX, FPF is calculated as:

```
FPF=1-((1-FPFMIN)/FRMAX)*FPRATIO
```

It is supposed that tuber induction is maximal when FPRATIO is zero.

1.10 PROD

Daily photosynthetic production is calculated per layer for each subvegetation. Based on the parameter values PM and KM estimated in INTERPOL, a light response curve is constructed. The light response curve is fitted by a rectangular hyperbola:

```
P=LAYW(I)*(PMAX(I)*PAR(I,J))/(KM(I)+PAR(I,J))
```

In a special time-saving numeric integration method (Goudriaan, 1986) the rate of photosynthesis (g O_2 m^{-2} s^{-1}) is calculated on only three moments of the day, assuming that production is symmetrical around noon. The resulting daily production GROSP, expressed in g afdw m^{-2} day^{-1}, is summed for all layers of the subvegetation. GROSP is multiplied with FC (photosynthetic compensation), a factor which normally is 1, but decreases when respiratory needs are not completely satisfied. FC is determined in subroutine GROW. A temperature effect on photosynthesis is possible through the parameter ETEMPF, determined by linear interpolation in a table in TABINT. In SAGA1, this effect is not active.

1.11 TABINT

TABINT is a one dimensional linear interpolation routine which was taken from the model of Ng & Loomis (1984). Based on a table that is passed on in the call to this subroutine together with an independent parameter value, the requested dependent parameter value is calculated.

1.12 GROW

GROW is the central routine that determines plant growth and development. The subroutine starts each day by calculating the amount of tuber biomass TUBFLOW that is available for growth when the growing season has begun. On the seventh day since aboveground biomass was initiated in STARTGR, root biomass is initiated with the amount ROOTST.

Subsequently, the total amount of resources RES available for growth is determined. A fraction CEDEAD of the biomass that died the day before (dead material DEAD) is added to RES together with TUBFLOW and GROSP. This simulates reallocation.

Following Ng & Loomis (1984), a so-called assimilate status ASSTAT, defined as the ratio of RES and total biomass TOTBIOM, is used to introduce effects of the relative amount of the resources on growth of tubers, roots, mother vegetation and secondary shoots. In this way, competition between these four components for resources can be simulated. For instance, when ASSTAT is low, tubers could be given a stronger sink function than the other biomass components. At the present moment, ASSTAT is not yet used for this purpose in SAGA1. Unfulfilled respiratory needs RSHORT are expressed relative to TOTBIOM in the parameter SHORT.

A series of calls is made to TABINT to determine the magnitude of parameters reflecting effects of temperature, age, ASSTAT and SHORT on growth, development and death rate of the four main components of the subvegetation. The effect of age on growth is calculated for each layer of both mother vegetation and each secondary shoot. All parameter values are between 0 and 1. The latter value stands for no effect, the former for complete inhibition. Temperature and ASSTAT are assumed to affect the potential maximum growth rate. Based on this 'corrected' potential maximal growth rate, age can further influence the actual growth rate. From the effects of temperature and ASSTAT on growth, the factor with the smallest parameter value is taken. The effect of age is multiplicated with this minimum value. In SAGA1 none of these effects is actually operational.

Respiration is calculated in the same way for all fractions. The rate of respiration per gram biomass RESPMAX can be influenced by an effect of temperature ETEMP (not operational). For example, respiration by new tubers is calculated as:

```
RESPTUB=RESPMAX*KNOLBIOM*ETEMP
```

Respiration by all fractions is summed to arrive at the total respiration of the subvegetation, RESPTOT. Respiration of the tuber bank WTUBER is not separately modelled but incorporated in its decay rate TUBDEC which is realized in VEG1-3.

Potential growth by the four biomass components is dependent on the potential maximum growth rate, the already existing biomass and the effects of temperature, ASSTAT and age. Calculations are identical for all fractions except for new tubers. For example, the growth GRSPR(2) of the second layer SUMSPROUT(2) of the mother vegetation:

```
GRSPR(2)=SGP*SUMSPROUT(2)*ESHOOTG*EAGESG(2)
```

Here, SGP is the potential maximum shoot growth rate, ESHOOTG is the minimum of the effects of temperature and ASSTAT on mother vegetation growth, and EAGESG(2) is the effect of the age of this second layer on its growth. Total potential growth of the mother vegetation is stored in GRSHTT.

Growth of new tubers is determined as follows. All tubers formed on a day are considered as a separate age group during the rest of the year. It is assumed that each group of tubers grows with a high potential maximum growth rate TGP during an initial period of ITUBDAY days, and with a lower potential maximum growth rate TGPMIN in the following days. Age is assumed to have no effect on tuber growth. Apart from these differences, calculations are equal to the one for the mother vegetation described above. In this way, a much more balanced tuber size distribution results than in simulations in which tuber growth rate is constant and tubers formed early in the season can become very large. Total potential growth of the subvegetation is summed in GRTOT.

When the needs for respiration and growth are known, these must be balanced against the available resources. First of all, respiratory needs have to be fulfilled. When resources are not sufficient, the remaining respiratory need becomes RSHORT and no growth is possible. After respiration has been taken care of, the ratio PERC of the remaining RES and GRTOT and is calculated. When PERC is larger than one, clearly all

growth can be accomplished. When PERC is less than one, tuber growth has priority. If GRTUB is less than RES, tuber growth is realized completely. The remaining RES is allocated to roots, mother vegetation and secondary shoots relative to their 'requested' amounts: these are multiplied with the ratio PERC of RES (the resources that remain after tuber growth) and the remaining total potential growth of the subvegetation (GRTOT-GRTUB). If GRTUB is larger than RES, only a fraction PERCTUB (=RES/GRTUB) of the potential tuber growth can be realized, and growth of the other components is not possible.

For each fraction the new biomass can now be calculated. As an example, the calculations for root biomass are given. Mortality is effectuated before growth. In this way, both growth and mortality are based on the same biomass. Death rate of roots DRTR can be affected by SHORT through ETEKDR (not operational). The total amount of biomass of the subvegetation lost through mortality is saved in DEAD till the next day.

```
DEAD=DEAD+ROOTW*DRTR*ETEKDR
ROOTW=ROOTW*(1-DRTR*ETEKDR)
ROOTW=ROOTW+GRROOT*PERC
```

Growth and mortality of a layer of the mother vegetation and of each layer of each secondary shoot cannot be calculated until the effect of light on growth allocation is estimated: how much of the total available growth of the aboveground biomass, GRABG, should be invested in elongation, in secondary shoot formation and in horizontal growth?

A fraction ELTOP is allocated to elongation of mother vegetation (GRTOP). ELTOP is determined in a call to TABINT, based on the light level PARTOP(TOP) halfway in the top layer of the mother vegetation and the tentative table ELTOPT based on laboratory results (Vermaat & Hootsmans, 1991):

```
GRABG=GRSHTT*PERC
GRTOP=GRABG*ELTOP
```

A fraction ELSEC of the remaining growth is allocated to the initiation of new secondary shoots (GRNSEC). Secondary shoots are not initiated during the first 15 days of the growing season. ELSEC is obtained by a call to TABINT with the amount of light PARTOP(1) halfway in the bottomlayer of the mother vegetation and the table ELSECT also based on Vermaat & Hootsmans (1991).

```
GRNSEC=(GRABG-GRTOP)*ELSEC
```

The remaining growth is used for horizontal growth of each layer according to the respective potential growth GRSPR(I) of a layer. GRSPR(I) is corrected for new growth already spent on elongation and secondary shoot initiation with the factor ENEWGR. The latter is calculated as (1-ELTOP)*(1-ELSEC).

```
DEAD=DEAD+SUMSPROUT(I)*DRTS*ETEKDR
SUMSPROUT(I)=SUMSPROUT(I)*(1-DRTS*ETEKDR)
SUMSPROUT(I)=SUMSPROUT(I)*GRSPR(I)*PERC*ENEWGR
```

Each secondary shoot is treated in the same way as the mother vegetation: its total potential growth is corrected with the fraction PERC, and subsequently, the amount of growth for elongation and new secondary shoots is determined by parameters ELTOP and ELSEC (calculated for the light level PARTOP halfway in the top layer of the secondary shoot and in its bottomlayer, respectively). Finally, mortality and horizontal growth of each layer are calculated.

A secondary shoot actually is considered as a biomass unit with a constant number of individuals, INSEC. Thus, a 'secondary shoot' is modelled in the same way as the mother vegetation. Secondary shoots also form new secondary shoots.

A new secondary shoot biomass is initiated when the total amount of growth allotted to secondary shoot initiation, SECNEW, becomes higher than the necessary initial secondary shoot biomass SECSTART. The initiation procedure is comparable to the subroutine STARTGR. The number of secondary shoots, in fact secondary shoot biomasses, NSEC is increased by 1. The biomass SECSHT(NSEC,1) of the first layer, which is also the top layer, becomes equal to SECNEW. The height of the first layer, HSEC(NSEC,1), is determined on the condition that it never exceeds the thickness THICKN of layer 1:

```
HSEC(NSEC,1)=AMIN1(THICKN,SECSHT(NSEC,1)/(INSEC*WLINI))
```

Following these growth calculations, new tuber formation is determined. The so-called place factor, defined as the ratio PFACCENT of the amount of tubers that already exists (KNOLYE) and the root biomass, ROOTW, determines whether there is still place for more tubers. When PFACCENT is less than the maximum PF, the number of tubers TUBER that can be formed per gram of root biomass is determined as:

```
TUBER=AMIN1(TUBNUM,(PF-PFACCENT))
```

TUBNUM is the maximum amount of tubers that can be initiated per gram root biomass per day. Further limits are imposed: when PERCTUB is less than a minimum value TUBMIN, meaning that tuber growth is limited, no new tubers are initiated. Also, in the first 30 days of the growing season, it is supposed that tuber initiation has not yet begun, while no initiation is taking place beyond day 231 to limit the size of the tuber array KNOL(I,J). In this array, counter I indicates the daynumber, starting from the first day on which tuber formation starts (i.e. I equals 1 on IDAYBEG+30). The tuber biomass formed on day I is stored in KNOL(I,2), the corresponding number of tubers in KNOL(I,1). The amount of tubers formed is influenced also by the parameter FPF determined in subroutine FPFTIF, and by the effect of ASSTAT, EASSTI (the latter is not operational).

```
KNOL(I,2)=TUBER*FPF*ROOTW*EASSTI
KNOLYE=KNOLYE+KNOL(I,2)
```

Each tuber in the newly formed tuber group KNOL(I,2) receives an initial biomass TUBIN. Thus, the total initial biomass of this group, KNOL(I,1), equals:

```
KNOL(I,1)=KNOL(I,2)*TUBIN
```

As was already mentioned in the discussion of subroutine AGE, the age of each tuber group can easily be derived as the first array counter represents the daynumber since IDAYBEG on which this tuber group was initiated.

The new length of the mother vegetation and each secondary shoot is calculated by means of calls to TOPGRO (see 1.13). Then, the occurrence of storm and wave damage and their consequences are determined.

Storm events can only occur between day 265 of this year and day 100 of the next year. Chances for storm events increase between day 265 and day 365, and decrease again between day 1 and day 100. A call is made to TABINT to interpolate the table ESTORM relating daynumber to parameter STORM. STORM can vary between 1 (no storm) and 0.75 (25% chance for a storm event on this day). To determine whether a storm event indeed occurs, the status of SHORT is also taken into consideration. The photosynthetic compensation factor FC decreases from 1 when SHORT equals zero, to 0 when SHORT equals 5% of the total biomass of the subvegetation. The minimum of the two parameters FC and STORM, which is named LOSS, is compared with a random number RND between 0 and 1. When it is less than the random number, wave damage occurs. Thus, a healthy vegetation (FC=1) still may suffer from storm damage, while a poorly photosynthesizing stand rapidly disappears because of increased chances for wave damage through a low FC.

The amount lost through wave action is a constant fraction FRAC of the total aboveground biomass. Starting with the top layer, biomass disappears and the length of secondary shoots and mother vegetation of the subvegetation is adjusted. The loss is partitioned between secondary shoots and mother vegetation relative to their respective share in the biomass in the layer that is losing material. The length of the remaining biomass in the new top layer of mother vegetation and secondary shoots is calculated using the amount of biomass per unit length, WEIGHTL, and the number of plants, AMOUNT for the mother vegetation and INSEC for a secondary shoot. WEIGHTL is dependent on the daily average amount of light received halfway in the new top layer, PARTOP(TOP). WEIGHTL is taken as the maximum of 0.1 g m^{-2} and PARTOP(TOP)/1000, but is never greater than 0.2 g m^{-2}. These limits are based on data from Hootsmans & Vermaat (1991).

Finally, the new values of various variables are calculated (biomass per layer, total biomass of vegetation, length of vegetation etc.).

1.13 TOPGRO

Based on the amount of biomass GRTOP available, elongation of the mothervegetation and of the secondary shoot is calculated. In the calculations the amount of biomass per unit length, WEIGHTL, is kept equal to WLINI as top growth pertains to young biomass. Together with the number of plants AMOUNT in the mother vegetation or secondary

shoot, the total elongation TOPLEN is determined:

```
TOPLEN=(GRTOP/AMOUNT)/WEIGHTL
```

Using TOPLEN, the new length distribution of the vegetation is calculated. If necessary, a new layer becomes the top layer. Length of the vegetation increases until the water-surface is reached.

1.14 REPORT

When a user-defined interval has elapsed, results from a subvegetation are stored in its output file and written to the screen. The interval times for screen and file may differ.

1.15 FIGURE

This subroutine creates a simple graphical representation of each of the three subvegetations. It shows the biomass distribution of each subvegetation (mother vegetation and all secondary shoots together) over the water column.

1.16 TUBDIST and DIST

At the end of the year, the new tuber size and tuber bank for the next season is determined in TUBDIST. The tuber bank information of each subvegetation, stored in the arrays KNOL1, KNOL2 and KNOL3, is passed on to subroutine DIST. This subroutine uses a maximum individual tuber size TUBMAX (g afdw) to create the tuber size distributions SET1, SET2 and SET3. Each SET is a twodimensional array with 9 equal distance tuber size classes (boundaries between 0 g and TUBMAX). For each size class the tuber biomass and the number of tubers is calculated.

In TUBDIST, the three arrays SET are combined in the array TOTAL for the whole vegetation, and subsequently the size distribution is simplified to a distribution NEW with three classes by combining the size classes 1-3, 4-6 and 7-9. By dividing the amount of biomass in each of the three size classes in NEW by their respective tuber number, the new average tuber size ATUB(1-3) for each of the three subvegetations is obtained. Both the simplified tuber distribution NEW and the three distributions SET are written to the outputfile TUBER.PRN.

SENSITIVITY VALUES FOR SIX MODEL OUTPUT VARIABLES

2

Sprout = maximum aboveground biomass of mother vegetation (g m^{-2}); roots = maximum root biomass (g m^{-2}); secsht = maximum secondary shoot biomass (g m^{-2}); tubbiom = maximum tuber biomass (g m^{-2}); ntub = maximum number of tubers (m^{-2}); grospmax = maximum rate of gross photosynthesis (g m^{-2} day^{-1}). Rank = rank number as used in Fig. 9.14. All data based on the total vegetation.

Sensitivity to a 20% increase in the respective parameter value.

rank	sprout	roots	secsht	tubbiom	ntub	grospmax
1 CCO2GR	6.2	6.2	2.7	6.6	6.1	6.6
2 PM	6.2	6.2	2.7	6.6	6.1	6.6
3 DMAX	3.3	3.2	-2.3	3.6	3.1	3.3
4 IDAYBEG	1.4	1.5	4.5	-0.7	1.2	1.5
5 ELTOP	0.9	1	-1.8	1.1	0.9	1
6 WTUB	0.8	0.8	-0.9	0.6	0.7	0.7
7 RGP-SECGP	0.8	-0.2	0.5	0	-0.2	0.6
8 ATUB	0.4	0.4	-0.5	0.4	0.3	0.4
9 ELSEC	-0.1	0	0.9	-0.1	0	0
10 PF	-0.1	-0.1	-0.5	0.5	0.8	0
11 CTUBFL	0.1	0.1	0	0.1	0.1	0.1
12 CEDEAD	0.1	0.1	0	0.1	0.1	0
13 DMIN	0	0.1	0	0.1	0	0
14 TGPMIN	0	0	0	0.1	-0.1	0
15 TUBMIN	0	0	0	0	0	0
16 DRTR/DRTS	0	0	0	0	0	0
17 CITUBFL	0	0	0	0	0	0
18 FPFMIN	0	0	0	0	0	0
19 TUBDEC	-0.1	-0.1	0	-0.1	-0.1	-0.1
20 FRMAX	-0.1	-0.1	-0.5	-0.1	-0.1	-0.1
21 TUBNUM	-0.1	-0.1	-0.5	-0.1	-0.1	-0.2
22 ITUBDAY	-0.4	-0.3	-0.5	0.6	-0.4	-0.1
23 BOUNDLIGHT	-0.2	-0.1	-0.5	-0.1	-0.1	-0.2
24 TUBIN	-0.3	-0.3	-0.5	0.4	-0.4	-0.2
25 WLINI	-0.3	-0.2	0	-0.2	-0.3	-0.6
26 TGP	-0.6	-0.5	-0.9	0.7	-0.6	-0.3
27 ROOTIN	-1.1	0.1	-0.9	-0.1	0	-0.9
28 KPLANT	-0.2	-0.2	-1.8	-0.3	-0.2	-0.2
29 KM	-1.1	-1.1	-1.4	-1.2	-1.1	-1.1
30 RESPMAX	-1.7	-1.6	-1.8	-1.8	-1.7	-1.4

Appendix 2. Continued.

Sensitivity to a 20% decrease in the respective parameter value.

rank	sprout	roots	secsht	tubbiom	ntub	grospmax
CCO2GR	-3.1	-3	-3.6	-3.1	-3.1	-3.3
PM	-3.1	-3	-3.6	-3.1	-3.1	-3.3
DMAX	-2.2	-2.1	-0.5	-2.3	-2.2	-2.3
IDAYBEG	-0.4	-0.4	-3.6	0.2	-0.5	-0.8
ELTOP	-1.6	-1.5	-0.5	-1.4	-1.6	-1.9
WTUB	-0.8	-0.8	0	-0.7	-0.8	-0.8
RGP-SECGP	-0.9	0	-0.9	-0.1	0	-0.8
ATUB	-0.4	-0.3	-0.5	-0.2	-0.4	-0.6
ELSEC	0.1	0.1	-1.4	0.1	0	0
PF	0.3	0.3	0	-0.6	-0.8	0
CTUBFL	-0.1	0	0	-0.1	-0.1	-0.2
CEDEAD	-0.1	0	0	-0.1	-0.1	-0.1
DMIN	0	0	0	-0.1	0	0
TGPMIN	0.1	0.1	0	-0.1	0.1	0
TUBMIN	0	0	0	0	0	0
DRTR/DRTS	0	0.1	0	0	0	0
CITUBFL	0	0.1	0	0.1	0	0
FPFMIN	0	0	0	0	0	0
TUBDEC	0	0.1	0	0.1	0.1	0.1
FRMAX	0.3	0.3	0	0	0.3	0.2
TUBNUM	0.3	0.3	0	0.1	0.2	0.2
ITUBDAY	0.5	0.5	0.5	-0.8	0.5	0.2
BOUNDLIGHT	0.3	0.3	0	0	0.3	0.2
TUBIN	0.4	0.4	0.5	-0.5	0.4	0.2
WLINI	0.4	0.3	-0.9	0.4	0.3	0.4
TGP	0.6	0.7	0.5	-0.7	0.6	0.2
ROOTIN	1.3	0.2	0.9	0	-0.3	0.9
KPLANT	0.2	0.3	1.4	0.4	0.3	0.2
KM	1.7	1.6	0.5	1.9	1.6	1.7
RESPMAX	2.5	2.5	0.9	3	2.3	1.6

LIST OF VARIABLES USED IN SAGA1

3

Name	Type	Explanation
A	REAL	dummy variable
ABC	INTEGER*2	contains numeric answer
ABOVE	REAL	total aboveground biomass (g m^{-2})
AGE	REAL	age of layer of subvegetation, equal to BEGIN (days)
AGER	REAL	age of root biomass (days)
AGES	REAL	age of shoot biomass of mother vegetation (days)
AGESEC	REAL	age of secondary shoot biomass (days)
AMAX1		takes maximum of two input values (intrinsic Fortran function)
AMIN1		takes minimum of two input values (intrinsic Fortran function)
AMOUNT	REAL	number of plants (m^{-2})
ANS	INTEGER*2	contains numeric answers
ASIN		arcsine (mathematical function)
ASSTAT	REAL	assimilate status
ATUB	REAL	average biomass of tuber bank of subvegetation (g)
ATUBW	REAL	average individual tuber biomass of subvegetation (g)
BEGIN	REAL	number of days since a layer of aboveground biomass started (days)
BIO	REAL	dummy variable for SECSHT (g m^{-2})
BOUND	REAL	Boundaries used in determining tuber size distributions (g)
BOUNDLIGHT	REAL	boundary light intensity used to determine FP (μE m^{-2} s^{-1})
CCO2GR	REAL	conversion of O_2 to afdw
CEDEAD	REAL	conversion of dead material to reserves
CITUBF	REAL	fraction of tuber bank biomass available for biomass initiation
CLASS	REAL	tuber size distribution
CORRECTION	REAL	variable used in interpolation routine INTERPOL
COS		cosine (mathematical function)
COUNT	INTEGER*2	age of tuber classes (days)
CTUBFL	REAL	daily fraction of tuber biomass available for growth
DAI	INTEGER*2	day of the year (days)
DAT	REAL	day of the year in degrees
DAY	REAL	day of the year (days)
DAYBEG	REAL	begin of growing season (days)
DAYL	REAL	daylength (hours)
DEAD	REAL	dead material (g m^{-2})
DEMAND	REAL	potential growth of tuber class (g m^{-2} day^{-1})
DEPTH	REAL	water depth (m)

DIST1	REAL	distance between points in interpolation routine INTERPOL
DIST2	REAL	distance between points in interpolation routine INTERPOL
DIST3	REAL	distance between points in interpolation routine INTERPOL
DIST4	REAL	distance between points in interpolation routine INTERPOL
DMAX	REAL	length of longest day (hours)
DMIN	REAL	length of shortest day (hours)
DPAR	REAL	daily insolation (PAR, $\mu E\ m^{-2}\ day^{-1}$)
DRTR	REAL	death rate root biomass
DRTS	REAL	death rate aboveground biomass
E	CHAR*40	variable used for presentation of biomass depth distribution
EAGERG	REAL	effect of age on root growth
EAGESG	REAL	effect of age on shoot growth of mother vegetation
EAGET	REAL	effect of age on tuber growth
EAGEUG	REAL	effect of age on secondary shoot growth
EASSIT	REAL	table with effect of assimilate status on tuber induction
EASSRG	REAL	effect of assimilate status on root growth
EASSRT	REAL	table with effect of assimilate status on root growth
EASSSG	REAL	effect of assimilate status on shoot growth of mother vegetation
EASSST	REAL	table with effect of assimilate status on shoot growth of mother vegetation
EASSTG	REAL	effect of assimilate status on tuber growth
EASSTI	REAL	effect of assimilate status on tuber induction
EASSTT	REAL	table with effect of assimilate status on tuber growth
EASSUG	REAL	effect of assimilate status on secondary shoot growth
EASSUT	REAL	table with effect of assimilate status on secondary shoot growth
EFFECT	REAL	daynumber, used for determining the occurrence of storm
ELSEC	REAL	effect of light on development of new secondary shoots
ELSECT	REAL	table with effect of light on development of new secondary shoots
ELTOP	REAL	effect of light on elongation
ELTOPT	REAL	table with effect of light on elongation
ENEWGR	REAL	effect of elongation and the development of new secondary shoots on growth of mother vegetation
EROOTG	REAL	largest of the effects of temperature and assimilate status on root growth
ESECG	REAL	largest of the effects of temperature and assimilate status on secondary shoot growth
ESHODR	REAL	effect of SHORT on death rates
ESHODT	REAL	table with effect of SHORT on death rates
ESHOOT	REAL	largest of the effects of temperature and assimilate status on aboveground biomass growth
ESTORM	REAL	table with effect of day number on storm chance
ETEMFT	REAL	table with effect of temperature on photosynthesis
ETEMP	REAL	effect of temperature on growth
ETEMPF	REAL	effect of temperature on photosynthesis

ETEMPT	REAL	table with effect of temperature on growth
ETUBG	REAL	largest of the effects of temperature and assimilate status on tuber growth
EXP		power e (mathematical function)
EXTFACTOR	REAL	fraction of light lost between surface and top of sub-vegetation
EXTW	REAL	extinction coefficient of the water layer (m^{-1})
F	CHAR*40	variable used for presentation of biomass depth distribution
FACT1	REAL	variable used in interpolation routine INTERPOL
FACT2	REAL	variable used in interpolation routine INTERPOL
FACT3	REAL	variable used in interpolation routine INTERPOL
FACT4	REAL	variable used in interpolation routine INTERPOL
FACTOR	REAL	dummy variable for calculation of FP
FC	REAL	photosynthetic compensation factor
FINAL	INTEGER*4	number of years in simulation (years)
FLOAT		converts INTEGER into REAL (intrinsic Fortran function)
FP	REAL	photosynthetic period (hours)
FPF	REAL	photosynthetic period factor
FPFMIN	REAL	minimum FPF value
FPRATIO	REAL	ratio of FP and DAYL, averaged over the first 30 days of the growing season
FPSUM	REAL	ratio of FP and DAYL, summed over the first 30 days of the growing season
FRAC	REAL	fraction of aboveground biomass of a subvegetation lost through wave action
FRMAX	REAL	value of FPRATIO above which FPF remains at the value of FPFMIN
G	CHAR*40	variable used for biomass depth distribution
GR	REAL	dummy variable = GRTOPSEC
GRABG	REAL	growth of aboveground biomass (g m^{-2} day^{-1})
GRNSEC	REAL	growth of new secondary shoot (g m^{-2} day^{-1})
GROSP	REAL	gross production (g m^{-2} day^{-1})
GRR	REAL	dummy variable = GRSEC
GRROOT	REAL	growth of root biomass (g m^{-2} day^{-1})
GRSEC	REAL	total growth of secondary shoot
GRSECL	REAL	growth of one layer of a secondary shoot (g m^{-2} day^{-1})
GRSECT	REAL	total growth of all secondary shoots (g m^{-2} day^{-1})
GRSHTT	REAL	total growth of aboveground mother vegetation (g m^{-2} day^{-1})
GRSPR	REAL	growth of one layer of aboveground mother vegetation (g m^{-2} day^{-1})
GRTOP	REAL	elongation growth of aboveground mother vegetation (g m^{-2} day^{-1})
GRTOPSEC	REAL	elongation growth of secondary shoot (g m^{-2} day^{-1})
GRTOT	REAL	total growth of vegetation (g m^{-2} day^{-1})
GRTUB	REAL	growth of tubers (g m^{-2} day^{-1})
GSEASON	INTEGER*2	indicator for growing season

GSHORT	REAL	fraction of carbohydrate shortage for growth of total vegetation
H	REAL	dummy variable = HSEC
HEIDIS	REAL	height distribution of mother vegetation
HEIGH1	REAL	height distribution of subvegetation 1
HEIGH2	REAL	height distribution of subvegetation 2
HEIGH3	REAL	height distribution of subvegetation 3
HEIGHT	REAL	height of mother vegetation (m)
HELP	INTEGER*4	dummy variable
HELP1	REAL	variable used for tuber size distribution
HELP2	REAL	variable used for tuber size distribution
HELP3	REAL	variable used for tuber size distribution
HMAX	REAL	maximum of the heights of the three subvegetations (m)
HMAX1	REAL	height of subvegetation 1 (m)
HMAX2	REAL	height of subvegetation 2 (m)
HMAX3	REAL	height of subvegetation 3 (m)
HOUR	REAL	time of the day (decimal hours)
HPAR	REAL	insolation on time HOUR (PAR, μE m^{-2} s^{-1})
HSEC	REAL	height distribution of secondary shoot
HTOP	REAL	height distribution of the longest subvegetation
I	INTEGER*2	DO-LOOP counter
I1	INTEGER*2	DO-LOOP counter
ICOUNT	INTEGER*2	counter for SCREENINT
IDAYBEG	INTEGER*2	begin of growing season (days)
IDAYNR	INTEGER*4	equal to DAI
IH1	INTEGER*4	variable used in interpolation routine INTERPOL
IH2	INTEGER*4	variable used in interpolation routine INTERPOL
IHELP	INTEGER*4	variable used for tuber size distribution
IHMAX	INTEGER*4	integer value of HMAX
ILCOUNT	INTEGER*4	counter for output interval of light and biomass depth distribution
ILENA	INTEGER*4	variable used for presentation of biomass depth distribution
ILENB	INTEGER*4	variable used for presentation of biomass depth distribution
ILENC	INTEGER*4	variable used for presentation of biomass depth distribution
ILEND	INTEGER*4	variable used for presentation of biomass depth distribution
ILENE	INTEGER*4	variable used for presentation of biomass depth distribution
IMAX	INTEGER*4	number of water layers passed through by total vegetation
INIT	INTEGER*4	day of reinitialization of various arrays; equal to IDAYBEG-1
INSEC	REAL	number of secondary shoots in a secondary shoot biomass (m^{-2})
INT		truncation of REAL to INTEGER value
IPCOUNT	INTEGER*2	counter for PRINTINT
IPH	INTEGER*4	variable used in interpolation routine INTERPOL
IPV	INTEGER*4	variable used in interpolation routine INTERPOL
IROOT	INTEGER*4	number of days since IDAYBEG
ITHICK	INTEGER*4	integer value of THICK (m)

ITUBDAY	INTEGER*4	number of days with maximum tuber growth rate for a tuber class (days)
IV1	INTEGER*4	variable used in interpolation routine INTERPOL
IV2	INTEGER*4	variable used in interpolation routine INTERPOL
J	INTEGER*2	DO-LOOP counter
K	INTEGER*2	DO-LOOP counter
KM	REAL	Michaelis Menten constant (μE m^{-2} s^{-1})
KMTABLE	REAL	table with KM values for various combinations of age and light
KNOL	REAL	biomass (j=1; g m^{-2}) and tuber number (j=2; m^{-2}) of tuber biomass(i)
KNOL1	REAL	tuber array of subvegetation 1
KNOL2	REAL	tuber array of subvegetation 2
KNOL3	REAL	tuber array of subvegetation 3
KNOLBIOM	REAL	total tuber biomass (g m^{-2})
KNOLT	REAL	total tuber biomass of total vegetation (g m^{-2})
KNOLT1	REAL	total tuber biomass of subvegetation 1 (g m^{-2})
KNOLT2	REAL	total tuber biomass of subvegetation 2 (g m^{-2})
KNOLT3	REAL	total tuber biomass of subvegetation 3 (g m^{-2})
KNOLYE	REAL	total number of tubers on previous day (m^{-2})
KPLANT	REAL	extinction coefficient of vegetation (m^2 g^{-1})
L	INTEGER*4	variable used for presentation of biomass depth distribution
LAYOLD	REAL	biomass distribution of subvegetation on previous day
LAYW	REAL	total aboveground biomass in layer(i)
LAYW1	REAL	biomass distribution of subvegetation 1
LAYW2	REAL	biomass distribution of subvegetation 2
LAYW3	REAL	biomass distribution of subvegetation 3
LAYWT	REAL	biomass distribution of total vegetation
LENG1	REAL	length of subvegetation 1 (m)
LENG2	REAL	length of subvegetation 2 (m)
LENG3	REAL	length of subvegetation 3 (m)
LENGTH	REAL	dummy variable = LENSEC (m)
LENSEC	REAL	height of secondary shoot (m)
LIGHT	REAL	average light intensity experienced by a layer during its existence, equal to MEANPAR (μE m^{-2} s^{-1})
LL	REAL	PAR averaged over the day, halfway in layer of sub-vegetation (μE m^{-2} s^{-1})
LOSS	REAL	minimum value of STORM and FC
M	INTEGER*4	variable used for presentation of biomass depth distribution
MAXLAY	REAL	upper most layer in the water column containing vegetation
MEANAGE	REAL	average age of layer of a subvegetation (days)
MEANPAR	REAL	average light intensity experienced by a layer during its existence (μE m^{-2} s^{-1})
N	INTEGER*2	counter in TABINT for determination of the position of X and Y in TABEL
N1	INTEGER*2	counter in TABINT for determination of the position of X and Y in TABEL

NAME	CHAR*4	dummy variable for exchange of text
NEW	REAL	part of elongation growth going to a new layer (g m^{-2})
NKNOL	REAL	total tuber number of total vegetation (m^{-2})
NKNOL1	REAL	total tuber number of subvegetation 1 (m^{-2})
NKNOL2	REAL	total tuber number of subvegetation 2 (m^{-2})
NKNOL3	REAL	total tuber number of subvegetation 3 (m^{-2})
NSEC	INTEGER*2	total number of secondary shoots (m^{-2})
OLD	REAL	part of elongation growth remaining in present layer (g m^{-2})
P1	REAL	sums production of all layers in a subvegetation on a specific moment (g O_2 m^{-2} s^{-1})
P1TOT	REAL	sums total production of all layers of a subvegetation for three specific moments (g O_2 m^{-2} s^{-1})
P2	REAL	production of a layer in a subvegetation on a specific moment (g O_2 m^{-2} s^{-1})
PAR	REAL	instantaneous amount of light (PAR) on top of layer of subvegetation (μE m^{-2} s^{-1})
PARTOP	REAL	insolation in PAR halfway in layer averaged over the day (μE m^{-2} s^{-1})
PERC	REAL	balance potential total growth/reserves
PERCTUB	REAL	fraction of potential tuber growth that can be realized
PERIF	REAL	extinction by periphyton
PF	REAL	place factor: maximum number of tubers per g root biomass
PFACCENT	REAL	realized number of tubers per g root biomass
PH	REAL	variable used in interpolation routine INTERPOL
PI	REAL	constant PI
PM	REAL	maximum rate of gross photosynthesis (μg O_2 g^{-1} min^{-1})
PMAX	REAL	maximum rate of gross photosynthesis of a layer of a subvegetation (g O_2 g^{-1} s^{-1})
PMTABLE	REAL	table with PM values for various combinations of age and light
PRINTINT	INTEGER*2	printinterval to output file (days)
PROD1	REAL	total gross production of subvegetation 1 (g m^{-2} day^{-1})
PROD2	REAL	total gross production of subvegetation 2 (g m^{-2} day^{-1})
PROD3	REAL	total gross production of subvegetation 3 (g m^{-2} day^{-1})
PRODT	REAL	total gross production of total vegetation (g m^{-2} day^{-1})
PV	REAL	variable used in interpolation routine INTERPOL
Q	REAL	dummy variable = INSEC
RATE	REAL	tuber growth rate
REAL		converts INTEGER into REAL value
REFLEX	REAL	reflection percentage of light from water surface
RES	REAL	amount of reserves (g m^{-2})
RESPMAX	REAL	rate of respiration (g g^{-1} day^{-1})
RESPROOT	REAL	respiration of root biomass (g m^{-2} day^{-1})
RESPSEC	REAL	respiration of secondary shoots (g m^{-2} day^{-1})
RESPSHOOT	REAL	respiration of aboveground mother vegetation (g m^{-2} day^{-1})
RESPTOTAL	REAL	total respiration (g m^{-2} day^{-1})

RESPTUB	REAL	respiration of tubers (g m^{-2} day^{-1})
REST	REAL	remaining length in upper most layer of total vegetation (m)
RESULT	REAL	variable used in interpolation routine INTERPOL
RGP	REAL	root growth percentage
RND	REAL	random number
ROOT1	REAL	root biomass of subvegetation 1 (g m^{-2})
ROOT2	REAL	root biomass of subvegetation 2 (g m^{-2})
ROOT3	REAL	root biomass of subvegetation 3 (g m^{-2})
ROOTIN	REAL	fraction of tuber bank biomass for total biomass initiation available for root biomass
ROOTST	REAL	amount of tuber biomass available for root biomass initiation (g m^{-2})
ROOTT	REAL	root biomass of total vegetation (g m^{-2})
ROOTW	REAL	root biomass (g m^{-2})
ROOTYE	REAL	biomass of roots of a subvegetation on the previous day (g m^{-2})
RSHORT	REAL	fraction of carbohydrate shortage for respiration of total vegetation
SCREENINT	INTEGER*2	printinterval to screen (days)
SECGP	REAL	secondary shoot growth percentage
SECMAX	INTEGER*4	maximum number of secondary shoots (m^{-2})
SECNEW	REAL	initial biomass of new secondary shoot (g m^{-2})
SECOLD	REAL	biomass distribution of a secondary shoot on the previous day
SECSHT	REAL	biomass of layer of secondary shoot (g m^{-2})
SECSTART	REAL	minimum biomass necessary for secondary shoot initiation (g m^{-2})
SECTOT	REAL	total secondary shoot biomass of total vegetation (g m^{-2})
SECW	REAL	total biomass of secondary shoots (g m^{-2})
SET1	REAL	tuber size distribution of subvegetation 1
SET2	REAL	tuber size distribution of subvegetation 2
SET3	REAL	tuber size distribution of subvegetation 3
SGP	REAL	shoot growth percentage
SHADE	REAL	shading percentage due to artificial shading
SHORT	REAL	fraction of total shortage of carbohydrates for respiration and growth
SIN		sine (mathematical function)
SLOUGH	REAL	biomass loss through wave action (g m^{-2})
SPROUT	REAL	biomass of mother vegetation (g m^{-2})
SQRT		square root (mathematical function)
STORM	REAL	chance that storm occurs
SUMOLD	REAL	aboveground biomass distribution of mother vegetation on the previous day

SUMSP1	REAL	aboveground mother vegetation biomass of subvegetation 1 ($g\ m^{-2}$)
SUMSP2	REAL	aboveground mother vegetation biomass of subvegetation 2 ($g\ m^{-2}$)
SUMSP3	REAL	aboveground mother vegetation biomass of subvegetation 3 ($g\ m^{-2}$)
SUMSPROUT	REAL	biomass of layer of aboveground mother vegetation ($g\ m^{-2}$)
SUMSPT	REAL	total aboveground mother vegetation biomass of total vegetation ($g\ m^{-2}$)
SURFACE	INTEGER*4	water layer containing water surface
T	REAL	time in hours since sunrise
TAB1	REAL	variable used in interpolation routine INTERPOL
TAB2	REAL	variable used in interpolation routine INTERPOL
TAB3	REAL	variable used in interpolation routine INTERPOL
TAB4	REAL	variable used in interpolation routine INTERPOL
TABEL	REAL	dummy variable for exchange of table values
TABLE	REAL	dummy variable for exchange of table values
TEMP	REAL	water temperature (°C)
TGP	REAL	tuber growth percentage
TGPMIN	REAL	minimum tuber growth rate ($g\ g^{-1}\ day^{-1}$)
THICKN	REAL	thickness of one layer of the water column
TMAX	REAL	maximum water temperature (°C)
TMIN	REAL	minimum water temperature (°C)
TOP	INTEGER*2	top layer of longest subvegetation
TOP1	INTEGER*4	top layer of subvegetation 1
TOP2	INTEGER*4	top layer of subvegetation 2
TOP3	INTEGER*4	top layer of subvegetation 3
TOPLAY	INTEGER*2	layer with top of mother vegetation
TOPLEN	REAL	length of biomass available for elongation (m)
TOPLSEC	INTEGER*2	layer with top of secondary shoot
TOPSPR	INTEGER*4	top layer of mother vegetation of a subvegetation
TOTABG	REAL	total aboveground biomass ($g\ m^{-2}$)
TOTAL	REAL	tuber size distribution of total vegetation
TOTBIO	REAL	total biomass ($g\ m^{-2}$)
TUBDAY	REAL	number of days with maximal tuber growth rate for a tuber class (days)
TUBDEC	REAL	decay rate of tuber bank ($g\ g^{-1}\ day^{-1}$)
TUBER	REAL	number of tubers that can be initiated per g root biomass
TUBFLOW	REAL	amount of reserves coming from tuber bank ($g\ m^{-2}\ day^{-1}$)
TUBIN	REAL	initial biomass of newly formed tuber (g)
TUBMAX	REAL	maximum tuber biomass assumed in tuber size distribution (g)
TUBMIN	REAL	minimum fraction of realized potential tuber growth below which tuber growth stops
TUBNUM	REAL	maximum number of tubers initiated per g root per day

VEGMAX	REAL	height of a subvegetation (m)
WEIGHTL	REAL	biomass-length ratio (g m^{-1})
WLINI	REAL	biomass-length ratio of young biomass (g m^{-1})
WTUB	REAL	tuber bank of subvegetation (g m^{-2})
WTUB1	REAL	tuber bank biomass of subvegetation 1 (g m^{-2})
WTUB2	REAL	tuber bank biomass of subvegetation 2 (g m^{-2})
WTUB3	REAL	tuber bank biomass of subvegetation 3 (g m^{-2})
WTUBER	REAL	average biomass of tuber in tuber bank (g)
WTUBT	REAL	tuber bank biomass of total vegetation (g m^{-2})
X	REAL	dummy variable in TABINT (independent)
Y	REAL	dummy variable in TABINT (dependent)

FORTRAN 77 SOURCE CODE OF SAGA1

4

```
C*************************************************************************
C******************************** SAGA1 **********************************
C*************************************************************************
C*   A simulation model for the life cycle of Potamogeton pectinatus L.   *
C*************************************************************************

        PROGRAM SAGA

C ************* COMMON BLOCK ***********************************

       INTEGER DAI,TOP,PRINTINT,SCREENINT,ANS,SURFACE,YEAR,FINAL,
      $SECMAX,TOP1,TOP2,TOP3

       REAL LAYW1(21),LAYW2(21),LAYW3(21),
      1LENG1,LENG2,LENG3,HEIGH1(21),HEIGH2(21),
      2HEIGH3(21),LL(21),LAYWT(21),
      3SUMSP1(21),SUMSP2(21),SUMSP3(21),
      4KNOLT1,KNOLT2,KNOLT3,NKNOL1,NKNOL2,NKNOL3,
      5KNOLT,NKNOL,KNOL1(200,2),KNOL2(200,2),KNOL3(200,2),INSEC,
      6KPLANT

       COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
      1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

       COMMON /PRODUC/ CCO2GR,KPLANT,PERIF,PAR(21,3),EXTW,SHADE

       COMMON /PRINT/ SCREENINT,PRINTINT

       COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
      1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
      2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

       COMMON /WEATHER/ DMAX,DMIN,REFLEX,TMAX,TMIN,HPAR,DPAR,
      1DAYL,TEMP,FPFMIN,FRMAX

C ************************ MAIN PROGRAM ***************************

       CALL INFO

       WRITE(*,*) ' One (1), two (2), or three (3) subvegetations?'
       READ(*,*) ANS
       WRITE(*,*) ' How many years to simulate? '
```

```
      READ(*,*) FINAL

C*********************BEGIN SIMULATION*****************************
      INIT=IDAYBEG-1

      DO 1 YEAR=1,FINAL
      write(*,*) ' YEAR',year
      DO 10 DAI=1,365
      READ(4,*) PERIF,EXTW

C ******************* begin of annual cycle **********************
      IF(DAI.EQ.INIT) THEN
C ** initialize arrays
      DO 3 I=1,21
      LAYW1(I)=0.
      LAYW2(I)=0.
      LAYW3(I)=0.
      LAYWT(I)=0.
      HEIGH1(I)=0.
      HEIGH2(I)=0.
      HEIGH3(I)=0.
      SUMSP1(I)=0.
      SUMSP2(I)=0.
      SUMSP3(I)=0.
3     CONTINUE
      LENG1=0.
      LENG2=0.
      LENG3=0.
      ENDIF
      CALL METEO(DAI,HOUR)
      ICOUNT=ICOUNT+1
      IF(ICOUNT.EQ.PRINTINT) THEN
      WRITE(15,100) DAI,TEMP,DPAR,DAYL
      ICOUNT=0
      ENDIF
100   FORMAT(I4,3(F14.3))

      IF((DAI.GE.IDAYBEG).OR.(YEAR.GT.1)) THEN
        READ(3,*) RND

C ** Calculation of length distribution of total vegetation over depth
C ** Variable TOP cannot become 0 when vegetation growth must still begin
      IF(TOP.EQ.0) TOP=1
      IHMAX=INT(10000*HMAX+.5)
      ITHICKN=INT(10000*THICKN+.5)
      IMAX=INT(IHMAX/ITHICKN)
      REST=HMAX-IMAX*THICKN
```

```
      DO 30 I=1,21
         HTOP(I)=0.
         IF(I.LE.IMAX) THEN
            HTOP(I)=THICKN
         ENDIF
         IF(I.EQ.IMAX+1) HTOP(I)=REST
30    CONTINUE

C ** calculation of light distribution over depth
      CALL LIGHT(DAI)
      ILCOUNT=ILCOUNT+1
      IF(DAI.EQ.1) ILCOUNT=1
      IF(ILCOUNT.EQ.PRINTINT) THEN
C ** output of light distribution of today and biomass depth distribution
C ** of yesterday
         DO 6 I=SURFACE,1,-1
           WRITE(25,'(I5,2F8.3)') DAI,PARTOP(I),LAYWT(I)
           ILCOUNT=0
6        CONTINUE
      ENDIF
      ENDIF
C ** calls to subvegetation routines VEG1-3
      N=11
      CALL VEG1 (DAI,N,LAYW1,LENG1,HEIGH1,ROOT1,SUMSP1,KNOLT1,NKNOL1,
     $WTUB1,PROD1,KNOL1,YEAR,TOP1,HMAX1)
      IF(ANS.EQ.1) GOTO 2
      N=12
      CALL VEG2 (DAI,N,LAYW2,LENG2,HEIGH2,ROOT2,SUMSP2,KNOLT2,NKNOL2,
     $WTUB2,PROD2,KNOL2,YEAR,TOP2,HMAX2)
      IF(ANS.EQ.2) GOTO 2
      N=13
      CALL VEG3 (DAI,N,LAYW3,LENG3,HEIGH3,ROOT3,SUMSP3,KNOLT3,NKNOL3,
     $WTUB3,PROD3,KNOL3,YEAR,TOP3,HMAX3)

C ** summary of data from subvegetations
2     WTUBT=WTUB1+WTUB2+WTUB3

      IF((HMAX1.GT.HMAX2).AND.(HMAX1.GT.HMAX3)) THEN
        HMAX=HMAX1
        TOP=TOP1
      ELSEIF(HMAX2.GT.HMAX3) THEN
        HMAX=HMAX2
        TOP=TOP2
      ELSE
        HMAX=HMAX3
        TOP=TOP3
      ENDIF
```

```
      ABOVE=0.
      SUMSPT=0.
      DO 5 I=1,21
      LAYWT(I)=LAYW1(I)+LAYW2(I)+LAYW3(I)
      ABOVE=ABOVE+LAYWT(I)
      SUMSPT=SUMSPT+SUMSP1(I)+SUMSP2(I)+SUMSP3(I)
5     CONTINUE
      SECTOT=ABOVE-SUMSPT
      ROOTT=ROOT1+ROOT2+ROOT3
      PRODT=PROD1+PROD2+PROD3
      KNOLT=KNOLT1+KNOLT2+KNOLT3
      NKNOL=NKNOL1+NKNOL2+NKNOL3

C ** output of biomass depth distribution for each subvegetation to
C ** the screen
      CALL FIGURE(LAYW1,LAYW2,LAYW3,DAI,HEIGH1)

C ** output of data from total vegetation
      IPCOUNT=IPCOUNT+1
      IF (IPCOUNT.EQ.PRINTINT) THEN
        WRITE(10,20) DAI,HMAX,WTUBT,SUMSPT,ROOTT,SECTOT,PRODT,KNOLT,
     $NKNOL
        IPCOUNT=0
      ENDIF
20    FORMAT(I5,8F8.3)
10    CONTINUE

C ** calculation and output of tuber size distribution
      CALL TUBDIST(KNOL1,KNOL2,KNOL3,TUB,WTUB,TUBMAX)
1     CONTINUE
      END
C ********************************************************************
      SUBROUTINE TUBDIST(KNOL1,KNOL2,KNOL3,TUB,WTUB,TUBMAX)

      REAL KNOL1(200,2),KNOL2(200,2),KNOL3(200,2),TUB(3),WTUB(3),
     $SET1(9,2),SET2(9,2),SET3(9,2),TOTAL(9,2),NEW(3,2)

      DO 10 I=1,9
        DO 20 J=1,2
          SET1(I,J)=0.
          SET2(I,J)=0.
          SET3(I,J)=0.
          TOTAL(I,J)=0.
20      CONTINUE
10    CONTINUE
      DO 30 I=1,3
```

```
        DO 40 J=1,2
           NEW(I,J)=0.
40      CONTINUE
30    CONTINUE

      CALL DIST(KNOL1,SET1,TUBMAX)
      CALL DIST(KNOL2,SET2,TUBMAX)
      CALL DIST(KNOL3,SET3,TUBMAX)

      DO 50 I=1,9
        DO 60 J=1,2
          TOTAL(I,J)=SET1(I,J)+SET2(I,J)+SET3(I,J)
60      CONTINUE
50    CONTINUE
      IHELP=0
      DO 70 I=1,3
        DO 80 J=1,2
          NEW(I,J)=TOTAL(1+IHELP,J)+TOTAL(2+IHELP,J)+TOTAL(3+IHELP,J)
80    CONTINUE
      IHELP=IHELP+3
70    CONTINUE
      DO 90 I=1,3
        IF(NEW(I,2).LE.0.) THEN
          TUB(4-I)=0.
          WTUB(4-I)=0.
          GOTO 90
        ENDIF
        TUB(4-I)=NEW(I,1)/NEW(I,2)
        WTUB(4-I)=NEW(I,1)
90    CONTINUE

      WRITE(40,200) '"class"','"ntub1"','"biom"','"mean"',
     $'"ntub2"','"biom"','"mean"','"ntub3"','"biom"','"mean"'
      DO 100 I=1,9
       IF(SET1(I,2).LE.0.) THEN
         HELP1=0.
       ELSE
         HELP1=SET1(I,1)/SET1(I,2)
       ENDIF
       IF(SET2(I,2).LE.0.) THEN
         HELP2=0.
       ELSE
         HELP2=SET2(I,1)/SET2(I,2)
       ENDIF
       IF(SET3(I,2).LE.0.) THEN
         HELP3=0.
       ELSE
```

```
         HELP3=SET3(I,1)/SET3(I,2)
       ENDIF
         WRITE(40,210) I,SET1(I,2),SET1(I,1),HELP1,SET2(I,2),
     $SET2(I,1),HELP2,SET3(I,2),SET3(I,1),HELP3
100    CONTINUE
       WRITE(40,220) '"class"','"ntub"','"biom"','"meantub"'
       DO 110 I=1,3
           WRITE(40,230) I,NEW(I,2),NEW(I,1),TUB(4-I)
110    CONTINUE

200    FORMAT(10A8)
210    FORMAT(I5,9F8.3)
220    FORMAT(4A9)
230    FORMAT(I5,3F8.3)

       RETURN
       END

C ******************************************************************

       SUBROUTINE DIST(KNOL,CLASS,TUBMAX)

       REAL KNOL(200,2),CLASS(9,2),BOUND(10)

       DO 10 I=1,9
         BOUND(I)=REAL(I)*TUBMAX/9.
10     CONTINUE
       BOUND(0)=0.
       DO 20 I=1,200
           DO 30 J=1,9
             IF(KNOL(I,2).LE.0.) GOTO 30
             IF(KNOL(I,1)/KNOL(I,2).LE.BOUND(J)) THEN
                  IF(KNOL(I,1)/KNOL(I,2).GT.BOUND(J-1)) THEN
                            CLASS(J,1)=CLASS(J,1)+KNOL(I,1)
                            CLASS(J,2)=CLASS(J,2)+KNOL(I,2)
                  ENDIF
             ELSEIF(J.EQ.9) THEN
                            CLASS(J,1)=CLASS(J,1)+KNOL(I,1)
                            CLASS(J,2)=CLASS(J,2)+KNOL(I,2)
             ENDIF
30         CONTINUE
20     CONTINUE

       RETURN
       END
C ******************************************************************
```

```
      SUBROUTINE FIGURE(LAYW1,LAYW2,LAYW3,DAI,HEIGH1)

      INTEGER SCREENINT,TOP,DAI,ILENA(21),ILENB(21),ILENC(21),
     $ILEND(21),ILENE(21),HELP,SURFACE,PRINTINT,SECMAX

      REAL LAYWT(21),LL(21),MAXLAY,KPLANT,HEIGH1(21),INSEC,
     $LAYW1(21),LAYW2(21),LAYW3(21)
      CHARACTER*40 E,F,G,H,I
      CHARACTER*80 J
      CHARACTER*1 K

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

      COMMON /PRODUC/ CCO2GR,KPLANT,PERIF,PAR(21,3),EXTW,SHADE

      COMMON /PRINT/ SCREENINT,PRINTINT

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

      ICOUNT=ICOUNT+1
      IF(DAI.EQ.1) ICOUNT=1

      IF(ICOUNT.LT.SCREENINT) RETURN
      ICOUNT=0
      E=' '
      F=' '
      G=' '
      H=' '
      I=' '
      J=' '
C ** A=veg1   B=veg2   C=veg3
      DO 10 L=SURFACE,1,-1
      IF(L.EQ.SURFACE) THEN
      WRITE(*,*) '------------------->:more than 15 g/m2---------------'
      ENDIF
      IF((LAYW1(L).GT.0.).AND.(LAYW1(L).LT..5)) THEN
         ILENA(L)=1
      ELSE
         ILENA(L)=INT(LAYW1(L)+.5001)
      ENDIF
      IF(ILENA(L).EQ.0) THEN
         K=' '
         ILENA(L)=1
      ELSE
```

```
          K='#'
        ENDIF
        IF(ILENA(L).GT.15) THEN
          ILENA(L)=14
          E((ILENA(L)+1):(ILENA(L)+1))='>'
        ENDIF
        DO 11 M=1,ILENA(L)
          E(M:M)=K
11      CONTINUE

        ILENB(L)=16-ILENA(L)
        DO 12 M=1,ILENB(L)
          F(M:M)=' '
12      CONTINUE
        IF((LAYW2(L).GT.0.).AND.(LAYW2(L).LT..5)) THEN
          ILENC(L)=1
        ELSE
          ILENC(1)=INT(LAYW2(L)+.5001)
        ENDIF
        IF(ILENC(L).EQ.0) THEN
          K=' '
          ILENC(L)=1
        ELSE
          K='@'
        ENDIF
        IF(ILENC(L).GT.15) THEN
          ILENC(L)=14
          G((ILENC(L)+1):(ILENC(L)+1))='>'
        ENDIF
        DO 13 M=1,ILENC(L)
          G(M:M)=K
13      CONTINUE

        ILEND(L)=16-ILENC(L)
        DO 14 M=1,ILEND(L)
          H(M:M)=' '
14      CONTINUE
        IF((LAYW3(L).GT.0.).AND.(LAYW3(L).LT..5)) THEN
          ILENE(L)=1
        ELSE
          ILENE(1)=INT(LAYW3(L)+.5001)
        ENDIF
        IF(ILENE(L).EQ.0) THEN
          K=' '
          ILENE(L)=1
        ELSE
          K='$'
```

```
      ENDIF
      IF(ILENE(L).GT.15) THEN
        ILENE(L)=14
        I((ILENE(L)+1):(ILENE(L)+1))='>'
      ENDIF
      DO 15 M=1,ILENE(L)
         I(M:M)=K
15    CONTINUE

      J(1:ILENA(L))=E(1:ILENA(L))
      J(ILENA(L)+1:ILENB(L)+ILENA(L))=F(1:ILENB(L))
      J(ILENA(L)+ILENB(L)+1:ILENA(L)+ILENB(L)+ILENC(L))=G(1:ILENC(L))
      HELP=ILENA(L)+ILENB(L)+ILENC(L)
      J(HELP+1:HELP+ILEND(L))=H(1:ILEND(L))
      J(HELP+ILEND(L)+1:HELP+ILEND(L)+ILENE(L))=I(1:ILENE(L))
      WRITE(*,*) J(1:HELP+ILEND(L)+ILENE(L))
10    CONTINUE
      WRITE(*,*)'++++++++++++++++++++++++++++++++++++'
      END

C ****************************************************************

      SUBROUTINE INFO

      INTEGER ABC,ANS,SCREENINT,PRINTINT,TOP,SECMAX,SURFACE
      REAL KPLANT,LL(21),LAYWT(21),INSEC

      COMMON /PRINT/ SCREENINT,PRINTINT

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

      COMMON /TABEL/ ETEMPT(15),EAGET(15),EASSTT(15),EASSRT(15),
     $EASSST(15),EASSUT(15),ESHODT(15),EASSIT(15),ETEMFT(15),
     $ELTOPT(15),ELSECT(15),ESTORM(15)

      COMMON /PRODUC/ CCO2GR,KPLANT,PERIF,PAR(21,3),EXTW,SHADE

      COMMON /WEATHER/ DMAX,DMIN,REFLEX,TMAX,TMIN,HPAR,DPAR,
     1DAYL,TEMP,FPFMIN,FRMAX

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

      WRITE (*,*)'************************************************'
      WRITE (*,*)'******************** SAGA1 *********************'
```

```
      WRITE (*,*)'************************************************'
      WRITE (*,*)' '
      WRITE(*,*)' '
      OPEN(3,FILE='RANDOM',STATUS='OLD')
      OPEN(4,FILE='PERFEXTW.PRN',STATUS='OLD')
      OPEN(10,FILE='SAGA.PRN',STATUS='NEW')
      OPEN(11,FILE='SAGA1.PRN',STATUS='NEW')
      OPEN(12,FILE='SAGA2.PRN',STATUS='NEW')
      OPEN(13,FILE='SAGA3.PRN',STATUS='NEW')
      OPEN(15,FILE='SIMMETEO.PRN',STATUS='NEW')
      OPEN(20,FILE='SIMPARAM.PRN',STATUS='OLD')
      OPEN(25,FILE='LIGHT.PRN',STATUS='NEW')
      OPEN(30,FILE='HELPSIM.PRN',STATUS='NEW')
      OPEN(40,FILE='TUBER.PRN',STATUS='NEW')


      WRITE(10,40) '"day"','"height"','"wtuber"','"sproutw"',
     $'"rootw"','"secw"','"grosp"','"tubw"','"ntub"'
      WRITE(11,39) '"day"','"height"','"wtuber"','"spruitw"',
     $'"rootw"','"secw"','"grosp"','"fp"','"n sec"','"tubw"',
     $'"ntub"','"perc"','"pertub"','"fprat"'
      WRITE(12,39) '"day"','"height"','"wtuber"','"sproutw"',
     $'"rootw"','"secw"','"grosp"','"fp"','"n sec"','"tubw"',
     $'"ntub"','"perc"','"pertub"','"fprat"'
      WRITE(13,39) '"day"','"height"','"wtuber"','"sproutw"',
     $'"rootw"','"secw"','"grosp"','"fp"','"n sec"','"tubw"',
     $'"ntub"','"perc"','"pertub"','"fprat"'
      WRITE(15,41) '"day"','"temp"','"dpar"','"dayl"'
      WRITE(*,*) ' The simulation uses these parameter values:'

C ** Input of parameter values from SIMPARAM.PRN

      READ(20,*)
      DO 55 I=1,15
      READ(20,*) ETEMPT(I),EAGET(I),EASSTT(I),EASSRT(I),EASSST(I),
     $EASSUT(I),ESHODT(I),EASSIT(I),ETEMFT(I),ELTOPT(I),ELSECT(I),
     $ESTORM(I)
55    CONTINUE
      READ(20,*)
      READ(20,*) CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP
      READ(20,*)
      READ(20,*) DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,
     $DAYBEG
      IDAYBEG=INT(DAYBEG)
      READ(20,*)
      READ(20,*) TUBDEC,CCO2GR,KPLANT,DMAX,DMIN,REFLEX,TMAX,TMIN
      READ(20,*)
```

```
      READ(20,*) WTUB(1),WTUB(2),WTUB(3),TUB(1),TUB(2),
     $TUB(3),WLINI,SHADE
      READ(20,*)
      READ(20,*) A,INSEC,TUBMAX,TGPMIN,TUBDAY,TUBMIN,TUBNUM,
     $FRAC
      SECMAX=INT(A)
      ITUBDAY=INT(TUBDAY)
      READ(20,*)
      READ(20,*) ROOTIN,TUBIN,FPFMIN,FRMAX
      CLOSE(20)
C *** output parameter values to screen

      GOTO 61
      WRITE(*,46) ' ETEMPT',' EAGET',' EASSTT',' EASSRT',
     $' EASSST',' EASSUT'
      DO 60 I=2,15
      WRITE(*,47) ETEMPT(I),EAGET(I),EASSTT(I),EASSRT(I),EASSST(I),
     $EASSUT(I)
60    CONTINUE
      WRITE(*,*) ' press ENTER'
      PAUSE
      WRITE(*,46) ' ESHODT',' EASSIT',' ETEMFT',' ELTOPT',
     $' ELSECT',' ESTORM'
      DO 70 I=2,15
      WRITE(*,47) ESHODT(I),EASSIT(I),ETEMFT(I),ELTOPT(I),ELSECT(I),
     $ESTORM(I)
70    CONTINUE
      WRITE(*,*) ' press ENTER'
      PAUSE
61    CONTINUE
      WRITE(*,42) ' CTUBFL',' CEDEAD',' CITUBFL',' RESPMAX',
     $' TGP',' RGP',' SGP',' SECGP'
      WRITE(*,43) CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP
      WRITE(*,42) ' DRTR',' DRTS',' SECSTRT',' PF',' THICKN',
     $' DEPTH',' BOUNDLI',' IDAYBEG'
      WRITE(*,43) DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,
     $DAYBEG
      WRITE(*,42) ' TUBDEC',' CCO2GR',' KPLANT',' DMAX',' DMIN',
     $' REFLEX',' TMAX',' TMIN'
      WRITE(*,43) TUBDEC,CCO2GR,KPLANT,DMAX,DMIN,REFLEX,
     $TMAX,TMIN
      WRITE(*,42) ' WTUB(1)',' WTUB(2)',' WTUB(3)',
     $' TUB(1)',' TUB(2)',' TUB(3)',' WLINI',' SHADE'
      WRITE(*,43) WTUB(1),WTUB(2),WTUB(3),TUB(1),TUB(2),
     $TUB(3),WLINI,SHADE
      WRITE(*,42) ' SECMAX',' INSEC',' TUBMAX',' TGPMIN',' ITUBDAY',
     $' TUBMIN',' TUBNUM',' FRAC'
```

```
      WRITE(*,43) A,INSEC,TUBMAX,TGPMIN,TUBDAY,TUBMIN,
     $TUBNUM,FRAC
      WRITE(*,44) ' ROOTIN',' TUBIN',' FPFMIN',' FRMAX'
      WRITE(*,45) ROOTIN,TUBIN,FPFMIN,FRMAX

C ** check SIMPARAM data: only values that lead to calculation errors
C ** are detected!
      IF(CTUBFL.GT.1.) WRITE(*,*)' CTUBFL >1! STOP with ctrl-C'
      IF(CEDEAD.GT.1.) WRITE(*,*)' CEDEAD >1! STOP with ctrl-C'
      IF(CITUBFL.GT.1.) WRITE(*,*)' CITUBFL>1! STOP with ctrl-C'
      IF(DRTR.GT.1.) WRITE(*,*)'    DRTR >1! STOP with ctrl-C'
      IF(DRTS.GT.1.) WRITE(*,*)'    DRTS >1! STOP with ctrl-C'
      IF(THICKN.GT.DEPTH) WRITE(*,*)' THICKN >DEPTH! STOP with ctrl-C'
      IF(TUBDEC.GT.1.) WRITE(*,*)' TUBDEC  >1! STOP with ctrl-C'
      IF(REFLEX.GT.1.) WRITE(*,*)' REFLEX  >1! STOP with ctrl-C'
      IF(TGPMIN.GT.1.) WRITE(*,*)' TGPMIN  >1! STOP with ctrl-C'
      IF(TUBNUM.GT.PF) WRITE(*,*)' TUBNUM  >PF! STOP with ctrl-C'
      IF(FRAC.GT.1.) WRITE(*,*)'  FRAC   >1! STOP with ctrl-C'
      IF(ROOTIN.GT.1.) WRITE(*,*)' ROOTIN  >1! STOP with ctrl-C'
      IF(FPFMIN.GT.1.) WRITE(*,*)' FPFMIN >1! STOP with ctrl-C'
      IF(FRMAX.GT.1.) WRITE(*,*)' FRMAX  >1! STOP with ctrl-C'

      WRITE(*,*) '  '
      WRITE(*,*) ' 1. Maximum number of layers is 20'
      WRITE(*,*) ' When layer thickness is 0.1 m, DEPTH is max. 2.0 m!'
      WRITE(*,*) ' 2. Weather routine for The Netehrlands'
20    WRITE(*,*) '  '

      WRITE (*,*)' Give: DEPTH (in m) and SHADE (between 0-1)'
      READ(*,*) DEPTH,SHADE
      IF(SHADE.GT.1.) THEN
         WRITE(*,*) ' SHADE cannot be more than 1!'
         GOTO 20
      ENDIF
      SURFACE=INT(DEPTH/THICKN+.5001)
      IF(SURFACE.GT.20) THEN
         WRITE(*,*) ' SURFACE layer higher than 20!'
         GOTO 20
      ENDIF
      WRITE (*,*)' Give print interval to screen in days'
      READ(*,*) SCREENINT
      WRITE (*,*)' Give print interval to files in days'
      READ(*,*) PRINTINT
      WRITE(*,*) ' The parameters used in this simulation'
      WRITE(*,*) ' are saved in SIMPARAM.PRN'
C ** output of simulation parameters
```

```
      OPEN(20,FILE='SIMPARAM.PRN',STATUS='NEW')

      WRITE(20,48) '"ETEMPT"','"EAGET"','"EASSTT"','"EASSRT"',
     $'"EASSST"','"EASSUT"','"ESHODT"','"EASSIT"','"ETEMFT"',
     $'"ELTOPT"','"ELSECT"','"ESTORM"'
      DO 80 I=1,15
      WRITE(20,49) ETEMPT(I),EAGET(I),EASSTT(I),EASSRT(I),EASSST(I),
     $EASSUT(I),ESHODT(I),EASSIT(I),ETEMFT(I),ELTOPT(I),ELSECT(I),
     $ESTORM(I)
80    CONTINUE
      WRITE(20,42) '"CTUBFL"','"CEDEAD"','"CITUBFL"','"RESPMAX"',
     $'"TGP"','"RGP"','"SGP"','"SECGP"'
      WRITE(20,43) CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP
      WRITE(20,42) '"DRTR"','"DRTS"','"SECSTRT"','"PF"','"THICKN"',
     $'"DEPTH"','"BOUNDLI"','"IDAYBEG"'
      WRITE(20,43) DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,
     $DAYBEG
      WRITE(20,42) '"TUBDEC"','"CCO2GR"','"KPLANT"','"DMAX"','"DMIN"',
     $'"REFLEX"','"TMAX"','"TMIN"'
      WRITE(20,43) TUBDEC,CCO2GR,KPLANT,DMAX,DMIN,REFLEX,
     $TMAX,TMIN
      WRITE(20,42) '"WTUB(1)"','"WTUB(2)"','"WTUB(3)"',
     $'"TUB(1)"','"TUB(2)"','"TUB(3)"','"WLINI"','"SHADE"'
      WRITE(20,43) WTUB(1),WTUB(2),WTUB(3),TUB(1),TUB(2),
     $TUB(3),WLINI,SHADE
      WRITE(20,42) '"SECMAX"','"INSEC"','"TUBMAX"','"TGPMIN"',
     $'"ITUBDAY"','"TUBMIN"','"TUBNUM"','"FRAC"'
      WRITE(20,43) A,INSEC,TUBMAX,TGPMIN,TUBDAY,TUBMIN,
     $TUBNUM,FRAC
      WRITE(20,44) '"ROOTIN"','"TUBIN"','"FPFMIN"','"FRMAX"'
      WRITE(20,45) ROOTIN,TUBIN,FPFMIN,FRMAX
      CLOSE(20)
39    FORMAT(14(A9))
40    FORMAT(9(A9))
41    FORMAT(4(A9))
42    FORMAT(8(A9))
43    FORMAT(8(F9.3))
44    FORMAT(4(A9))
45    FORMAT(4(F9.3))
46    FORMAT(6(A9))
47    FORMAT(6(F9.3))
48    FORMAT(12(A9))
49    FORMAT(12(F9.3))
      RETURN
      END
C *****************************************************************
```

```
      SUBROUTINE VEG1 (DAI,N,LAYW,HEIGHT,HEIDIS,ROOTW,SUMSPROUT,
     $KNOLBIOM,KNOLYE,WTUBER,GROSP,KNOL,YEAR,TOP1,HMAX1)

      INTEGER DAI,TOPLAY,TOP,COUNT,TOPLSEC(25),GSEASON,TOPSPR,
     $SECMAX,SURFACE,YEAR,TOP1

      REAL LAYW(21),HEIDIS(21),SUMOLD(21),SUMSPROUT(21),KNOL(200,2),
     1SECSHT(25,21),AGESEC(25,21),SECOLD(25,21),MEANAGE(21),
     2LAYOLD(21),MEANPAR(21),PMAX(21),KM(21),AGES(21),
     3LENSEC(25),HSEC(25,21),LAYWT(21),LL(21),KNOLBIOM,KNOLYE,
     4INSEC,BEGIN(21)

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

      IF(DAI.EQ.1) THEN
      WTUBER=WTUB(1)
      TUBW=TUB(1)
      ICOUNT=0
      IPCOUNT=0
      IHELP=0
      ENDIF
      INIT=IDAYBEG-1
      IF(DAI.EQ.INIT) THEN
      WRITE(*,*) ' Start of growing season. Initializing.....'
      FPSUM=0.
      GSEASON=0
      TOPLAY=1
      TOPSPR=1
      TOP1=1
      HMAX1=0.
      IROOT=0
      NSEC=0
      RES=0.
      KNOLYE=0.
      SECNEW=0.
      ROOTW=0.
      ROOTST=0.
      DEAD=0.
      GROSP=0.
      TOTBIOM=0.
      KNOLBIOM=0.
      TOTABG=0.
```

```
      SPROUTW=0.
      SECW=0.
      SHORT=0.
      RSHORT=0.
      GSHORT=0.
      FC=1.
      HEIGHT=0.
      DO 10 I=1,21
       LAYW(I)=0.
       HEIDIS(I)=0.
       SUMOLD(I)=0.
       SUMSPROUT(I)=0.
       MEANAGE(I)=0.
       BEGIN(I)=0.
       LAYOLD(I)=0.
       MEANPAR(I)=0.
       PMAX(I)=0.
       KM(I)=0.
       AGES(I)=0.
       LL(I)=0.
       HTOP(I)=0.
       PARTOP(I)=0.
        DO 20 J=1,25
          SECSHT(J,I)=0.
          AGESEC(J,I)=0.
          SECOLD(J,I)=0.
          HSEC(J,I)=0.
20      CONTINUE
10    CONTINUE
      DO 30 I=1,25
        LENSEC(I)=0.
        TOPLSEC(I)=0
30    CONTINUE

      DO 40 I=1,200
       DO 50 J=1,2
         KNOL(I,J)=0.
50     CONTINUE
40    CONTINUE

      ENDIF

      WTUBER=(1-TUBDEC)*WTUBER

      IF((YEAR.EQ.1).AND.(DAI.EQ.IDAYBEG)) THEN
        COUNT=DAI-IDAYBEG
      ELSEIF(GSEASON.EQ.0) THEN
```

```
        COUNT=0
       ELSE
        COUNT=COUNT+1
       ENDIF

       IF(GSEASON.EQ.1) THEN

       CALL AGE(COUNT,AGER,ROOTYE,ROOTW,TOPLAY,SUMOLD,SUMSPROUT,
      $NSEC,SECSHT,AGESEC,SECOLD,MEANAGE,LAYOLD,LAYW,MEANPAR,
      $PMAX,KM,AGES,BEGIN,IHELP)

       IF(COUNT.LE.30) THEN
       CALL FPFTIF(HEIGHT,FP,FPF,FPSUM,FPRATIO,TOPLAY,COUNT)
       ENDIF

       CALL PROD(COUNT,PMAX,KM,FC,LAYW,GROSP,TOPLAY)

       CALL GROW (WTUBER,DEAD,GROSP,RES,TOTBIOM,AGER,AGES,
      $NSEC,AGESEC,KNOLBIOM,ROOTW,TOPLAY,SUMSPROUT,SECSHT,COUNT,
      $KNOL,TOPLSEC,RSHORT,GSHORT,FC,SECNEW,KNOLYE,FPF,
      $HEIGHT,HEIDIS,LENSEC,HSEC,LAYW,TOTABG,SPROUTW,SECW,
      $GSEASON,SHORT,DAI,AMOUNT,IROOT,TOPSPR,PERC,PERCTUB,ROOTST)

       HMAX1=0.
       DO 60 I=1,NSEC
       IF(LENSEC(I).GT.HMAX1) THEN
          HMAX1=LENSEC(I)
          TOP1=TOPLSEC(I)
       ENDIF
60     CONTINUE
       IF(HEIGHT.GT.HMAX1) THEN
          HMAX1=HEIGHT
          TOP1=TOPSPR
       ENDIF
       ELSE
       IF(DAI.EQ.IDAYBEG) CALL STARTGR(GSEASON,
      $ROOTST,SUMSPROUT,HEIDIS,HEIGHT,LAYW,TUBW,AMOUNT,WTUBER,WLINI)
       SPROUTW=LAYW(1)
       ENDIF
C **   output data of VEG1

       CALL REPORT(ICOUNT,IPCOUNT,HMAX1,WTUBER,SPROUTW,ROOTW,
      $SECW,GROSP,FP,NSEC,KNOLBIOM,KNOLYE,SHORT,DAI,RES,DEAD,N,PERC,
      $PERCTUB,FPRATIO)

       RETURN
       END
```

```
C ***************************************************************

      SUBROUTINE VEG2 (DAI,N,LAYW,HEIGHT,HEIDIS,ROOTW,SUMSPROUT,
     $KNOLBIOM,KNOLYE,WTUBER,GROSP,KNOL,YEAR,TOP2,HMAX2)

      INTEGER DAI,TOPLAY,TOP,COUNT,TOPLSEC(25),GSEASON,TOPSPR,
     $SECMAX,SURFACE,YEAR,TOP2

      REAL LAYW(21),HEIDIS(21),SUMOLD(21),SUMSPROUT(21),KNOL(200,2),
     1SECSHT(25,21),AGESEC(25,21),SECOLD(25,21),MEANAGE(21),
     2LAYOLD(21),MEANPAR(21),PMAX(21),KM(21),AGES(21),
     3LENSEC(25),HSEC(25,21),LAYWT(21),LL(21),KNOLBIOM,KNOLYE,
     4INSEC,BEGIN(21)

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

      IF(DAI.EQ.1) THEN
      WTUBER=WTUB(2)
      ICOUNT=0
      IPCOUNT=0
      IHELP=0
      TUBW=TUB(2)
      ENDIF
      INIT=IDAYBEG-1
      IF(DAI.EQ.INIT) THEN
      WRITE(*,*) ' Start of growing season. Initializing.....'
      FPSUM=0.
      GSEASON=0
      TOPLAY=1
      TOPSPR=1
      TOP2=1
      HMAX2=0.
      IROOT=0
      NSEC=0
      RES=0.
      KNOLYE=0.
      SECNEW=0.
      ROOTW=0.
      ROOTST=0.
      DEAD=0.
      GROSP=0.
```

```
      TOTBIOM=0.
      KNOLBIOM=0.
      TOTABG=0.
      SPROUTW=0.
      SECW=0.
      SHORT=0.
      RSHORT=0.
      GSHORT=0.
      FC=1.
      HEIGHT=0.
      DO 10 I=1,21
       LAYW(I)=0.
       HEIDIS(I)=0.
       SUMOLD(I)=0.
       SUMSPROUT(I)=0.
       MEANAGE(I)=0.
       BEGIN(I)=0.
       LAYOLD(I)=0.
       MEANPAR(I)=0.
       PMAX(I)=0.
       KM(I)=0.
       AGES(I)=0.
       LL(I)=0.
       HTOP(I)=0.
       PARTOP(I)=0.
        DO 20 J=1,25
          SECSHT(J,I)=0.
          AGESEC(J,I)=0.
          SECOLD(J,I)=0.
          HSEC(J,I)=0.
20      CONTINUE
10    CONTINUE
      DO 30 I=1,25
        LENSEC(I)=0.
        TOPLSEC(I)=0
30    CONTINUE
      DO 40 I=1,200
       DO 50 J=1,2
         KNOL(I,J)=0.
50     CONTINUE
40    CONTINUE

      ENDIF

      WTUBER=(1-TUBDEC)*WTUBER

      IF((YEAR.EQ.1).AND.(DAI.EQ.IDAYBEG)) THEN
```

```
        COUNT=DAI-IDAYBEG
       ELSEIF(GSEASON.EQ.0) THEN
        COUNT=0
       ELSE
        COUNT=COUNT+1
       ENDIF

       IF(GSEASON.EQ.1) THEN

       CALL AGE(COUNT,AGER,ROOTYE,ROOTW,TOPLAY,SUMOLD,SUMSPROUT,
      $NSEC,SECSHT,AGESEC,SECOLD,MEANAGE,LAYOLD,LAYW,MEANPAR,
      $PMAX,KM,AGES,BEGIN,IHELP)

       IF(COUNT.LE.30) THEN
       CALL FPFTIF(HEIGHT,FP,FPF,FPSUM,FPRATIO,TOPLAY,COUNT)
       ENDIF

       CALL PROD(COUNT,PMAX,KM,FC,LAYW,GROSP,TOPLAY)


       CALL GROW (WTUBER,DEAD,GROSP,RES,TOTBIOM,AGER,AGES,
      $NSEC,AGESEC,KNOLBIOM,ROOTW,TOPLAY,SUMSPROUT,SECSHT,COUNT,
      $KNOL,TOPLSEC,RSHORT,GSHORT,FC,SECNEW,KNOLYE,FPF,
      $HEIGHT,HEIDIS,LENSEC,HSEC,LAYW,TOTABG,SPROUTW,SECW,
      $GSEASON,SHORT,DAI,AMOUNT,IROOT,TOPSPR,PERC,PERCTUB,ROOTST)

       HMAX2=0.
       DO 60 I=1,NSEC
       IF(LENSEC(I).GT.HMAX2) THEN
          HMAX2=LENSEC(I)
          TOP2=TOPLSEC(I)
       ENDIF
60     CONTINUE
       IF(HEIGHT.GT.HMAX2) THEN
          HMAX2=HEIGHT
          TOP2=TOPSPR
       ENDIF

       ELSE
       IF(DAI.EQ.IDAYBEG) CALL STARTGR(GSEASON,
      $ROOTST,SUMSPROUT,HEIDIS,HEIGHT,LAYW,TUBW,AMOUNT,WTUBER,WLINI)
       SPROUTW=LAYW(1)
       ENDIF
C **   output data of VEG2
       CALL REPORT(ICOUNT,IPCOUNT,HMAX2,WTUBER,SPROUTW,ROOTW,
      $SECW,GROSP,FP,NSEC,KNOLBIOM,KNOLYE,SHORT,DAI,RES,DEAD,N,PERC,
      $PERCTUB,FPRATIO)
```

```
      RETURN
      END

C *****************************************************************

      SUBROUTINE VEG3 (DAI,N,LAYW,HEIGHT,HEIDIS,ROOTW,SUMSPROUT,
     $KNOLBIOM,KNOLYE,WTUBER,GROSP,KNOL,YEAR,TOP3,HMAX3)

      INTEGER DAI,TOPLAY,TOP,COUNT,TOPLSEC(25),GSEASON,TOPSPR,
     $SECMAX,SURFACE,YEAR,TOP3

      REAL LAYW(21),HEIDIS(21),SUMOLD(21),SUMSPROUT(21),KNOL(200,2),
     1SECSHT(25,21),AGESEC(25,21),SECOLD(25,21),MEANAGE(21),
     2LAYOLD(21),MEANPAR(21),PMAX(21),KM(21),AGES(21),
     3LENSEC(25),HSEC(25,21),LAYWT(21),LL(21),KNOLBIOM,KNOLYE,
     4INSEC,BEGIN(21)

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

      IF(DAI.EQ.1) THEN
      WTUBER=WTUB(3)
      ICOUNT=0
      IPCOUNT=0
      IHELP=0
      TUBW=TUB(3)
      ENDIF
      INIT=IDAYBEG-1
      IF(DAI.EQ.INIT) THEN
      WRITE(*,*) ' Start of growing season; initializing...'
      FPSUM=0.
      GSEASON=0
      TOPLAY=1
      TOPSPR=1
      TOP3=1
      HMAX3=0.
      IROOT=0
      NSEC=0
      RES=0.
      KNOLYE=0.
      SECNEW=0.
      ROOTW=0.
```

```
      ROOTST=0.
      DEAD=0.
      GROSP=0.
      TOTBIOM=0.
      KNOLBIOM=0.
      TOTABG=0.
      SPROUTW=0.
      SECW=0.
      SHORT=0.
      RSHORT=0.
      GSHORT=0.
      FC=1.
      HEIGHT=0.
      DO 10 I=1,21
       LAYW(I)=0.
       HEIDIS(I)=0.
       SUMOLD(I)=0.
       SUMSPROUT(I)=0.
       MEANAGE(I)=0.
       BEGIN(I)=0.
       LAYOLD(I)=0.
       MEANPAR(I)=0.
       PMAX(I)=0.
       KM(I)=0.
       AGES(I)=0.
       LL(I)=0.
       HTOP(I)=0.
       PARTOP(I)=0.
        DO 20 J=1,25
          SECSHT(J,I)=0.
          AGESEC(J,I)=0.
          SECOLD(J,I)=0.
          HSEC(J,I)=0.
20      CONTINUE
10    CONTINUE
      DO 30 I=1,25
        LENSEC(I)=0.
        TOPLSEC(I)=0
30    CONTINUE
      DO 40 I=1,200
       DO 50 J=1,2
         KNOL(I,J)=0.
50     CONTINUE
40    CONTINUE

      ENDIF
```

```
      WTUBER=(1-TUBDEC)*WTUBER

      IF((YEAR.EQ.1).AND.(DAI.EQ.IDAYBEG)) THEN
        COUNT=DAI-IDAYBEG
      ELSEIF(GSEASON.EQ.0) THEN
        COUNT=0
      ELSE
        COUNT=COUNT+1
      ENDIF

      IF(GSEASON.EQ.1) THEN

      CALL AGE(COUNT,AGER,ROOTYE,ROOTW,TOPLAY,SUMOLD,SUMSPROUT,
     $NSEC,SECSHT,AGESEC,SECOLD,MEANAGE,LAYOLD,LAYW,MEANPAR,
     $PMAX,KM,AGES,BEGIN,IHELP)

      IF(COUNT.LE.30) THEN
      CALL FPFTIF(HEIGHT,FP,FPF,FPSUM,FPRATIO,TOPLAY,COUNT)
      ENDIF

      CALL PROD(COUNT,PMAX,KM,FC,LAYW,GROSP,TOPLAY)

      CALL GROW (WTUBER,DEAD,GROSP,RES,TOTBIOM,AGER,AGES,
     $NSEC,AGESEC,KNOLBIOM,ROOTW,TOPLAY,SUMSPROUT,SECSHT,COUNT,
     $KNOL,TOPLSEC,RSHORT,GSHORT,FC,SECNEW,KNOLYE,FPF,
     $HEIGHT,HEIDIS,LENSEC,HSEC,LAYW,TOTABG,SPROUTW,SECW,
     $GSEASON,SHORT,DAI,AMOUNT,IROOT,TOPSPR,PERC,PERCTUB,ROOTST)

      HMAX3=0.
      DO 60 I=1,NSEC
      IF(LENSEC(I).GT.HMAX3) THEN
         HMAX3=LENSEC(I)
         TOP3=TOPLSEC(I)
      ENDIF
60    CONTINUE
      IF(HEIGHT.GT.HMAX3) THEN
         HMAX3=HEIGHT
         TOP3=TOPSPR
      ENDIF

      ELSE
      IF(DAI.EQ.IDAYBEG) CALL STARTGR(GSEASON,
     $ROOTST,SUMSPROUT,HEIDIS,HEIGHT,LAYW,TUBW,AMOUNT,WTUBER,WLINI)
      SPROUTW=LAYW(1)
      ENDIF
C **  output data of VEG3
      CALL REPORT(ICOUNT,IPCOUNT,HMAX3,WTUBER,SPROUTW,ROOTW,
```

```
     $SECW,GROSP,FP,NSEC,KNOLBIOM,KNOLYE,SHORT,DAI,RES,DEAD,N,PERC,
     $PERCTUB,FPRATIO)

      RETURN
      END
C *****************************************************************

      SUBROUTINE METEO (DAI,HOUR)

      INTEGER DAI

      COMMON /WEATHER/ DMAX,DMIN,REFLEX,TMAX,TMIN,HPAR,DPAR,
     1DAYL,TEMP,FPFMIN,FRMAX

      PI=3.141592
      DAY=FLOAT(DAI)
      DAT=DAY*360.0/365.0

C **  calculation of DAYL
      DAYL=(DMAX+DMIN)/2.0-((DMAX-DMIN)/2.0*COS(2.0*PI*
     $(DAT+10.0)/360.0))

C **  calculation of DPAR
      DPAR=4.66*1E7*(.5-.4*COS(2.0*PI*(DAT+10.0)/360.0))

C **  calculation of HPAR
      T=DAYL/2.0+HOUR-12.0
      HPAR=((PI*DPAR)/(2.0*DAYL))*SIN(PI*T/DAYL)
      HPAR=HPAR/3600.0
      IF(HPAR.LT.0.0) HPAR=0.0

C **  calculation of water temperature
      TEMP=(TMAX+TMIN)/2.0-((TMAX-TMIN)/2.0*COS(2.0*PI*
     $(DAT-30.0)/360.0))
      RETURN
      END

      SUBROUTINE STARTGR (GSEASON,ROOTst,SUMSPROUT,HEIDIS,HEIGHT,
     $LAYW,TUBW,AMOUNT,WTUBER,WLINI)

      INTEGER GSEASON
      REAL SUMSPROUT(21),HEIDIS(21),LAYW(21)

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC
```

```
      IF(TUBW.LE.0.) GOTO 20
      AMOUNT=WTUBER/TUBW

C **  TUBFLOW=WTUBER*CTUBFL*CITUBFL
      TUBFLOW=WTUBER*CITUBFL
      WTUBER=WTUBER-TUBFLOW
      ROOTST=TUBFLOW*ROOTIN
      SUMSPROUT(1)=TUBFLOW*(1.-ROOTIN)
      LAYW(1)=SUMSPROUT(1)
      HEIGHT=(SUMSPROUT(1)/AMOUNT)/WLINI
      HEIDIS(1)=HEIGHT
      WRITE(*,10) ' initial number of plants= ',AMOUNT,
     $' biomass per plant= ',SUMSPROUT(1)/AMOUNT
10    FORMAT(A,F8.3,A,F8.3)
      IF(HEIDIS(1).GT.THICKN) THEN
      HEIGHT=THICKN
      HEIDIS(1)=THICKN
      WRITE(*,*) ' Initial biomass of aboveground vegetation too high!'
      ENDIF
      GSEASON=1
20    RETURN
      END

C ****************************************************************

      SUBROUTINE LIGHT(DAI)

      INTEGER TOP,DAI,SECMAX,SURFACE
      REAL KPLANT,LL(21),LAYWT(21),INSEC

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

      COMMON /PRODUC/ CCO2GR,KPLANT,PERIF,PAR(21,3),EXTW,SHADE

      COMMON /WEATHER/ DMAX,DMIN,REFLEX,TMAX,TMIN,HPAR,DPAR,
     1DAYL,TEMP,FPFMIN,FRMAX

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

C ****************************************************************************
C ** light incident on water surface, averaged over the day
      PARTOP(SURFACE+1)=DPAR/(3600*DAYL)
C ** correction for reflection and artificial shading
      PARTOP(SURFACE)=PARTOP(SURFACE+1)*(1-REFLEX)*(1-SHADE)
```

```
C ** correction for extinction by water and biomass in surface layer
      LL(SURFACE)=PARTOP(SURFACE)*EXP(-.5*THICKN*EXTW-.5*
     $LAYWT(SURFACE)*KPLANT)
C ** calculation of light profile: correction for extinction
      DO 50 I=SURFACE-1,1,-1
      PARTOP(I)=PARTOP(I+1)*EXP(-THICKN*EXTW-LAYWT(I+1)*KPLANT)
C ** calculation of light intensity halfway in a layer
      LL(I)=PARTOP(I)*EXP(-.5*THICKN*EXTW-.5*LAYWT(I)*KPLANT)
50    CONTINUE
C ** per layer with biomass: correction for periphyton
      DO 55 I=TOP,1,-1
      LL(I)=LL(I)*(1.-PERIF)
      PARTOP(I)=LL(I)
55    CONTINUE

C ** calculation of PAR incident on layer I on 3 moments of the days,
C ** necessary to calculate production

      DO 60 J=-1,1
        HOUR=12.+DAYL/2.*(.5+J*SQRT(.15))
        CALL METEO(DAI,HOUR)
        PAR(TOP,J+2)=HPAR*(1.-REFLEX)*(1.-SHADE)*
     $EXP(-(SURFACE-TOP)*THICKN*EXTW)
        DO 70 I=TOP-1,1,-1
          PAR(I,J+2)=PAR(I+1,J+2)*EXP(-THICKN*EXTW-LAYWT(I+1)*KPLANT)
70      CONTINUE

        DO 75 I=TOP,1,-1
          PAR(I,J+2)=PAR(I,J+2)*(1.-PERIF)
          PAR(I,J+2)=PAR(I,J+2)*EXP(-.5*THICKN*EXTW-.5*LAYWT(I)
     $*KPLANT)
75      CONTINUE
60    CONTINUE

      RETURN
      END

C ***************************************************************

      SUBROUTINE AGE(COUNT,AGER,ROOTYE,ROOTW,TOPLAY,SUMOLD,
     $SUMSPROUT,NSEC,SECSHT,AGESEC,SECOLD,MEANAGE,LAYOLD,LAYW,
     $MEANPAR,PMAX,KM,AGES,BEGIN,IHELP)

      INTEGER TOPLAY,COUNT,TOP,SECMAX,SURFACE
      REAL SUMOLD(21),SUMSPROUT(21),LL(21),LAYWT(21),KMM,
     1SECSHT(25,21),AGESEC(25,21),SECOLD(25,21),MEANAGE(21),
     2LAYOLD(21),LAYW(21),MEANPAR(21),PMAX(21),KM(21),AGES(21),
```

```
     3INSEC,BEGIN(21)

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

C ** root biomass
      IF(ROOTW.LE.0.) THEN
        AGER=0.
        GOTO 10
      ENDIF
      AGER=AGER*AMIN1(1.,ROOTYE/ROOTW)+1.
      ROOTYE=ROOTW
10    CONTINUE

C ** aboveground biomass
      DO 50 I=TOPLAY,1,-1
      IF(SUMSPROUT(I).LE.0.) THEN
         AGES(I)=0.
         GOTO 40
      ENDIF
      AGES(I)=AGES(I)*AMIN1(1.,SUMOLD(I)/SUMSPROUT(I))+1.

      SUMOLD(I)=SUMSPROUT(I)

40    DO 60 J=1,NSEC
        IF(SECSHT(J,I).LE.0.) THEN
         AGESEC(J,I)=0.
         GOTO 60
        ENDIF
        AGESEC(J,I)=AGESEC(J,I)*AMIN1(1.,SECOLD(J,I)/SECSHT(J,I))+1.
        SECOLD(J,I)=SECSHT(J,I)

60    CONTINUE
      IF(LAYW(I).LE.0.) THEN
        MEANAGE(I)=0.
        MEANPAR(I)=0.
        BEGIN(I)=0.
        GOTO 50
      ENDIF

      MEANPAR(I)=MEANPAR(I)*LAYOLD(I)*MEANAGE(I)+LL(I)*LAYW(I)
      MEANPAR(I)=MEANPAR(I)/(LAYOLD(I)*MEANAGE(I)+LAYW(I))
      MEANAGE(I)=MEANAGE(I)*AMIN1(1.,LAYOLD(I)/LAYW(I))+1.
      LAYOLD(I)=LAYW(I)

      BEGIN(I)=BEGIN(I)+1.
      HELP=MEANPAR(I)
```

```
      CALL INTERPOL(HELP, BEGIN(I),PMAX(I),KMM)

          PMAX(I)=PMAX(I)/(60.*1000000.)
          KM(I)=KMM
50    CONTINUE

C ** output data on layer biomass and photosynthesis parameters **
      IHELP=IHELP+1
      IDAYNR=COUNT+IDAYBEG
      IF(IHELP.EQ.14) THEN
         IHELP=0
         WRITE(30,90) '"LAY"','"LAYW"','"SPR"','"PMAX"','"KM"',
     $'"BEGIN"','"M PAR"','"day',IDAYNR
         DO 80 I=TOPLAY,1,-1
            HELP=60000000*PMAX(I)
            HELP2=KM(I)
            WRITE(30,91) I,LAYW(I),SUMSPROUT(I),HELP,
     $HELP2,BEGIN(I),MEANPAR(I)
80       CONTINUE
      ENDIF

90    FORMAT(8(A9),i4)
91    FORMAT(I4,6F8.3)

      RETURN
      END

C ***************************************************************

      SUBROUTINE INTERPOL(LIGHT,AGE,PM,KMM)

      REAL X(3),Y(4),KMM,LIGHT,AGE
      REAL PMTABLE(3,4),KMTABLE(3,4)

      DATA KMTABLE /112.,17.,34.,26.,15.,31.,56.,117.,53.,92.,114.,75./
      DATA PMTABLE /117.6,40.8,28.8,93.6,33.6,7.2,102.,81.6,55.2,172.8,
     $76.8,70.8/

      DATA X /30.,70.,120./
      DATA Y /50.,100.,150.,200./
      PV=LIGHT
      PH=AGE
      IH1=1
      IH2=1
      IV1=1
      IV2=1
```

```
      IPH=INT(PH+.5)
      IPV=INT(PV+.5)

      IF(IPH.LT.INT(X(1)+.5)) THEN
        PH=X(1)
        IH1=1
        IH2=1
      ENDIF
      DO 10 I=1,3
        IF(IPH.GE.INT(X(I)+.5)) THEN
           IH1=I
           IH2=I+1
        ENDIF
10    CONTINUE
      IF(IPH.GE.INT(X(3)+.5)) THEN
         PH=X(3)
         IH1=3
         IH2=3
      ENDIF

      IF(IPV.LT.INT(Y(1)+.5)) THEN
         PV=Y(1)
         IV1=1
         IV2=1
      ENDIF
      DO 20 I=1,4
      IF(IPV.GE.INT(Y(I)+.5)) THEN
         IV1=I
         IV2=I+1
      ENDIF
20    CONTINUE
      IF(IPV.GE.INT(Y(4)+.5)) THEN
      PV=Y(4)
      IV1=4
      IV2=4
      ENDIF

      TAB1=PMTABLE(IH1,IV1)
      TAB2=PMTABLE(IH2,IV1)
      TAB3=PMTABLE(IH1,IV2)
      TAB4=PMTABLE(IH2,IV2)

      DIST1=SQRT((PH-X(IH1))**2+(PV-Y(IV1))**2)
      DIST2=SQRT((PH-X(IH2))**2+(PV-Y(IV1))**2)
      DIST3=SQRT((PH-X(IH1))**2+(PV-Y(IV2))**2)
      DIST4=SQRT((PH-X(IH2))**2+(PV-Y(IV2))**2)
```

```
      FACT1=1/(DIST1+.0001)
      FACT2=1/(DIST2+.0001)
      FACT3=1/(DIST3+.0001)
      FACT4=1/(DIST4+.0001)

      CORRECTION=FACT1+FACT2+FACT3+FACT4
      RESULT=(FACT1*TAB1+FACT2*TAB2+FACT3*TAB3+FACT4*TAB4)
      PM=RESULT/CORRECTION

      TAB1=KMTABLE(IH1,IV1)
      TAB2=KMTABLE(IH2,IV1)
      TAB3=KMTABLE(IH1,IV2)
      TAB4=KMTABLE(IH2,IV2)

      RESULT=(FACT1*TAB1+FACT2*TAB2+FACT3*TAB3+FACT4*TAB4)
      KMM=RESULT/CORRECTION

      RETURN
      END

C *****************************************************************

      SUBROUTINE FPFTIF (HEIGHT,FP,FPF,FPSUM,FPRATIO,TOPLAY,COUNT)

      INTEGER TOP,HELFT,TOPLAY,COUNT,SECMAX,SURFACE
      REAL LL(21),LAYWT(21),INSEC,KPLANT

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

      COMMON /WEATHER/ DMAX,DMIN,REFLEX,TMAX,TMIN,HPAR,DPAR,
     1DAYL,TEMP,FPFMIN,FRMAX

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

      COMMON /PRODUC/ CCO2GR,KPLANT,PERIF,PAR(21,3),EXTW,SHADE

C*****************************************************************
      EXTFACTOR=(1-REFLEX)*(1-PERIF)*(1-SHADE)*EXP(-(SURFACE-TOP)*
     $THICKN*EXTW)
      EXTFACTOR=EXTFACTOR*PARTOP(TOPLAY)/PARTOP(TOP)

C **  EXTFACTOR and the HPAR curve over the day determine the time HOUR
C **  on which HPAR is equal to BOUNDLIGHT. The HPAR curve is
C **  symmetric around noon.
```

```
      PI=3.14159

      FACTOR=(BOUNDLIGHT*2.*DAYL*3600.)/(EXTFACTOR*(PI*DPAR))
      IF(FACTOR.GT.1.) FACTOR=1.
      HOUR=(ASIN(FACTOR)*DAYL/PI+12.-DAYL/2.)
      FP=2.*(12.-HOUR)
      FPRATIO=FP/DAYL
      FPSUM=FPSUM+FPRATIO

      IF(COUNT.EQ.30) THEN
         FPRATIO=FPSUM/30.
         IF(FPRATIO.LE.0.) THEN
                FPF=1.0
         ELSEIF(FPRATIO.GE.FRMAX) THEN
                FPF=FPFMIN
         ELSE
                FPF=1.-((1-FPFMIN)/FRMAX)*FPRATIO
         ENDIF
      ENDIF

      RETURN
      END

C *****************************************************************

      SUBROUTINE REPORT(ICOUNT,IPCOUNT,VEGMAX,WTUBER,SPROUTW,ROOTW,
     $SECW,GROSP,FP,NSEC,KNOLBIOM,KNOLYE,SHORT,DAI,RES,
     $DEAD,N,PERC,PERCTUB,FPRATIO)

      INTEGER SCREENINT,PRINTINT,DAI
      REAL KNOLBIOM,KNOLYE

      COMMON /PRINT/ SCREENINT,PRINTINT

      IPCOUNT=IPCOUNT+1
      IF(IPCOUNT.NE.PRINTINT) GOTO 4070
      WRITE(N,400) FLOAT(DAI),VEGMAX,WTUBER,SPROUTW,ROOTW,SECW,
     $GROSP,FP,NSEC,KNOLBIOM,KNOLYE,PERC,PERCTUB,FPRATIO
      IPCOUNT=0
4070  ICOUNT=ICOUNT+1
      IF(ICOUNT.NE.SCREENINT) RETURN
      WRITE(*,*) '-------------------------------------------------'
      WRITE(*,200) 'day','height','wtuber','sproutw','rootw',
     $'SECW','grosp','fp','n sec','tubbio'
      WRITE(*,410) FLOAT(DAI),VEGMAX,WTUBER,SPROUTW,ROOTW,SECW,
     $GROSP,FP,NSEC,KNOLBIOM
```

```
      WRITE (*,300)' SHORT =',SHORT,' n tubers =',KNOLYE
      WRITE(*,420) 'RES=',RES,'DEAD=',DEAD,'GROSP=',GROSP
      ICOUNT=0
200   FORMAT(10(A8))
300   FORMAT(A10,F8.3,A20,F8.3)
400   FORMAT(8F8.3,I8,5F8.3)
410   FORMAT(8F8.3,I8,F8.3)
420   FORMAT(3(A10,F8.3))
      RETURN
      END

C *****************************************************************

      SUBROUTINE  PROD(COUNT,PMAX,KM,FC,LAYW,GROSP,TOPLAY)

      INTEGER TOPLAY,COUNT,TOP,SECMAX,SURFACE
      REAL PMAX(21),KM(21),LAYW(21),KPLANT,LL(21),LAYWT(21),INSEC

      COMMON /WEATHER/ DMAX,DMIN,REFLEX,TMAX,TMIN,HPAR,DPAR,
     1DAYL,TEMP,FPFMIN,FRMAX

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

      COMMON /PRODUC/ CCO2GR,KPLANT,PERIF,PAR(21,3),EXTW,SHADE

      COMMON /TABEL/ ETEMPT(15),EAGET(15),EASSTT(15),EASSRT(15),
     $EASSST(15),EASSUT(15),ESHODT(15),EASSIT(15),ETEMFT(15),
     $ELTOPT(15),ELSECT(15),ESTORM(15)

      P1TOT=0.0
      P1=0.

      DO 80 J=1,3
          DO 70 I=TOPLAY,1,-1
             IF(PMAX(I).GT.0.) THEN
               P2=LAYW(I)*(PMAX(I)*PAR(I,J))
     $/(KM(I)+PAR(I,J))
               P1=P1+P2
             ENDIF
70        CONTINUE
      IF(I1.EQ.2) P1=1.6*P1
      P1TOT=P1TOT+P1
      P1=0.0
80    CONTINUE
      CALL TABINT(ETEMFT,TEMP,ETEMPF,'TEMF')
      GROSP=FC*CCO2GR*P1TOT*3600.0*DAYL/3.6*ETEMPF
```

```
1490  RETURN
      END

C ***************************************************************

      SUBROUTINE TABINT(TABLE,X,Y,NAME)

C ** TABLE INTERPOLATION ROUTINE
C ** from 'Simulation of growth and yield of the potato crop'
C ** E. Ng and R. S. Loomis, 1984. Pudoc Wageningen.
C ** Table is the vector of alternating independent and dependent
C **    variables. The independent variables must be monotonically
C **    increasing. The first element of Table is the total number of
C **    elements in the table
C ** X is the independent variable for which a corresponding dependent
C **    variable is sought.
C ** Y is the interpolated value which is returned.
C ** NAME is table name (max. 4 alphanumeric)
C ** If X is out of range, the smallest or largest Y in the table is
C ** returned.

      REAL TABLE(15)
      CHARACTER*4 NAME
C ** What is the number of elements in the table?
      N=TABLE(1)
      N1=N-1
      IF (X.LT.TABLE(2)) GOTO 300
      IF (X.GT.TABLE(N1)) GOTO 400
      DO 100 I=4,N1,2
      IF (X.LE.TABLE(I)) GOTO 200
100   CONTINUE
200   Y=TABLE(I-1)+(TABLE(I+1)-TABLE(I-1))
     $  *((X-TABLE(I-2))/(TABLE(I)-TABLE(I-2)))
      RETURN
300   Y=TABLE(3)
      RETURN
400   Y=TABLE(N)

      RETURN
      END

C ***************************************************************
```

```
      SUBROUTINE GROW (WTUBER,DEAD,GROSP,RES,TOTBIOM,AGER,AGES,
     $NSEC,AGESEC,KNOLBIOM,ROOTW,TOPLAY,SUMSPROUT,SECSHT,COUNT,
     $KNOL,TOPLSEC,RSHORT,GSHORT,FC,SECNEW,KNOLYE,FPF,
     $HEIGHT,HEIDIS,LENSEC,HSEC,LAYW,TOTABG,SPROUTW,SECW,
     $GSEASON,SHORT,DAI,AMOUNT,IROOT,TOPSPR,PERC,PERCTUB,rootst)

      INTEGER TOPLAY,COUNT,TOPLSEC(25),A,GSEASON,TOP,DAI,TOPSPR,
     $SECMAX,SURFACE

      REAL AGES(21),AGESEC(25,21),SUMSPROUT(21),LAYWT(21),
     $SECSHT(25,21),KNOL(200,2),HEIDIS(21),LENSEC(25),HSEC(25,21),
     $LAYW(21),GRSPR(21),DEMAND(200),GRSECL(25,21),GRSEC(25),
     $GRTOPSEC(25),EAGEUG(25,21),EAGESG(21),BIO(21),H(21),LL(21),
     $KNOLBIOM,KNOLYE,LENGTH,INSEC,LOSS

      COMMON /VEGINF/ TOP,LAYWT,HMAX,HTOP(21),WTUB(3),RND,
     1PARTOP(21),LL,TUB(3),WLINI,SECMAX,INSEC,TUBMAX,SURFACE

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

      COMMON /WEATHER/ DMAX,DMIN,REFLEX,TMAX,TMIN,HPAR,DPAR,
     1DAYL,TEMP,FPFMIN,FRMAX

      COMMON /TABEL/ ETEMPT(15),EAGET(15),EASSTT(15),EASSRT(15),
     $EASSST(15),EASSUT(15),ESHODT(15),EASSIT(15),ETEMFT(15),
     $ELTOPT(15),ELSECT(15),ESTORM(15)

C ** Maximum of 200 tuber classes
C ** Maximum of 25 secondary shoot biomasses

C **  reserves from tuber bank only to be used from IDAYBEG till end
C ** of the year. Otherwise, perennial biomass uses the new tuber bank

      IF(DAI.GT.IDAYBEG) THEN
         TUBFLOW=WTUBER*CTUBFL
         WTUBER=WTUBER-TUBFLOW
      ELSE
         TUBFLOW=0.
      ENDIF
      IROOT=IROOT+1

C ** On day 7 root biomass is initiated with ROOTST
      IF(IROOT.EQ.7) THEN
        ROOTW=ROOTST
      ENDIF
```

```
C **  reserves from dead material
      DEAD=DEAD*CEDEAD
C **  total resources
      RES=RES+GROSP+TUBFLOW+DEAD
      DEAD=0.
C **  status of the resources
      IF(COUNT.EQ.1) THEN
        ASSTAT=1.
        SHORT=0.
        FC=1.
      ELSE
        ASSTAT=RES/TOTBIOM
        SHORT=(RSHORT)/TOTBIOM

C ** when SHORT equals 5%, photosynthesis becomes 0
        FC=AMAX1(0.,1-SHORT*20)
        IF(ASSTAT.GE.1.) THEN
           ASSTAT=1.
        ENDIF
        IF(SHORT.GE.1.) THEN
           SHORT=1.
        ENDIF
      ENDIF

C **  Calculate growth regulation factors
      CALL TABINT(ETEMPT,TEMP,ETEMP,'ETEM')
      CALL TABINT(EASSTT,ASSTAT,EASSTG,'ASST')
      CALL TABINT(EASSRT,ASSTAT,EASSRG,'ASSR')
      CALL TABINT(EASSST,ASSTAT,EASSSG,'ASSS')
      CALL TABINT(EASSUT,ASSTAT,EASSUG,'ASSU')
      CALL TABINT(EAGET,AGER,EAGERG,'AGER')
      CALL TABINT(ESHODT,SHORT,ESHODR,'SHOD')
      CALL TABINT(EASSIT,ASSTAT,EASSTI,'ASTI')

      ETUBG=AMIN1(ETEMP,EASSTG)
      EROOTG=AMIN1(ETEMP,EASSRG)
      ESHOOTG=AMIN1(ETEMP,EASSSG)
      ESECG=AMIN1(ETEMP,EASSUG)
      DO 20 I=1,TOPSPR
      CALL TABINT(EAGET,AGES(I),EAGESG(I),'AGES')
20    CONTINUE
      DO 15 J=1,NSEC
      DO 16 I=1,TOPLSEC(J)
      CALL TABINT(EAGET,AGESEC(J,I),EAGEUG(J,I),'AGEU')
16    CONTINUE
15    CONTINUE
```

```
C **  Calculate respiration of the four fractions
C **  Tubers formed today do not respire yet

      RESPTUB=RESPMAX*KNOLBIOM*ETEMP
      RESPROOT=RESPMAX*ROOTW*ETEMP
      RESPSHOOT=0.
      RESPSEC=0.
      DO 30 I=1,TOPSPR
      RESPSHOOT=RESPSHOOT+RESPMAX*SUMSPROUT(I)*ETEMP
30    CONTINUE
      DO 25 J=1,NSEC
      DO 26 I=1,TOPLSEC(J)
      RESPSEC=RESPSEC+RESPMAX*SECSHT(J,I)*ETEMP
26    CONTINUE
25    CONTINUE

      RESPTOT=RESPTUB+RESPROOT+RESPSHOOT+RESPSEC

C **  calculate potential growth
C **      TUBERS
      GRTUB=0.0
      N=COUNT-31
      IF(N.GT.200) N=200
      DO 40 I=1,N
C **  N is number of tuber classes up till yesterday
      RATE=TGP
C **  maximal growth rate TGP is maintained during ITUBDAY days, followed
C **  by TGPMIN
      IF((COUNT-30-I).GT.ITUBDAY) RATE=TGPMIN
      DEMAND(I)=KNOL(I,1)*RATE*ETUBG
      GRTUB=GRTUB+DEMAND(I)
40    CONTINUE

C **      ROOTS
      GRROOT=ROOTW*RGP*EROOTG*EAGERG

C **      SHOOTS AND SECSHTS
      GRSHTT=0.
      GRSECT=0.
      DO 50 I=1,TOPSPR
      GRSPR(I)=SGP*SUMSPROUT(I)*ESHOOTG*EAGESG(I)
      GRSHTT=GRSHTT+GRSPR(I)
50    CONTINUE
      DO 45 I=1,NSEC
      DO 46 J=1,TOPLSEC(I)
      GRSECL(I,J)=SECGP*SECSHT(I,J)*ESECG*EAGEUG(I,J)
```

```
      GRRU=GRRU+GRSECL(I,J)
46    CONTINUE
      GRSEC(I)=GRRU
      GRSECT=GRSECT+GRSEC(I)
      GRRU=0.
45    CONTINUE

      GRTOT=GRTUB+GRROOT+GRSHTT+GRSECT

C **  Can the demand for respiration and growth be fulfilled?
C **  Respiratory demands have priority
      RES=RES-RESPTOT
      IF(RES.LT.0.) THEN
      RSHORT=-RES
      RES=0.
      ENDIF

C **  Make sure that growth does not exceed the available resources
      IF(GRTOT.LE.0.) THEN
      PERC=1.
      PERCTUB=1.
      GOTO 55
      ENDIF
      PERC=RES/GRTOT
55    IF (PERC.GE.1.) THEN
C ** there are more resources than the potential growth demands
         PERC=1.
         PERCTUB=1.
         RES=RES-GRTOT
      ELSE

C ** shortage: priority for tuber growth
         GSHORT=(1.-PERC)*GRTOT
         IF((RES-GRTUB).GT.0.) THEN
C ** demand by tubers is fulfilled; rest of the biomass receives
C ** its share partly
            RES=RES-GRTUB
            PERCTUB=1.
            PERC=RES/(GRTOT-GRTUB)
         ELSE
C ** everything available to tubers; rest of biomass does not grow
            IF(GRTUB.GT.0.) THEN
               PERCTUB=RES/GRTUB
            ELSE
C ** there are no tubers
               PERCTUB=0.
            ENDIF
```

```
            PERC=0.
          ENDIF
          RES=0.
        ENDIF

C **        TUBERS
        KNOLBIOM=0.
        DO 60 I=1,N
        DEAD=DEAD+KNOL(I,1)*DRTR*ESHODR
        KNOL(I,1)=KNOL(I,1)*(1-DRTR*ESHODR)
        KNOL(I,1)=KNOL(I,1)+DEMAND(I)*PERCTUB
C **      add todays tubers to KNOLBIOM
        KNOLBIOM=KNOLBIOM+KNOL(I,1)
60      CONTINUE

C **        ROOTS
C **    First mortality, then growth. In this way both are based on
C **    the same biomass
        DEAD=DEAD+ROOTW*DRTR*ESHODR
        ROOTW=ROOTW*(1-DRTR*ESHODR)
        ROOTW=ROOTW+GRROOT*PERC

C **        SHOOTS
C ** How much of the potential growth is allocated to elongation, and
C ** how much goes to new secondary shoots?
        CALL TABINT(ELTOPT,PARTOP(TOPSPR),ELTOP,'ELTO')
        ELSEC=0.
        IF(COUNT.LE.15) GOTO 61
        IF(NSEC.LT.SECMAX) CALL TABINT(ELSECT,PARTOP(1),ELSEC,
       $'ELRU')

61      ENEWGR=(1-ELTOP)*(1-ELSEC)
        GRABG=GRSHTT*PERC
C **    elongation has priority over secondary shoot formation
        GRTOP=GRABG*ELTOP
        GRNSEC=(GRABG-GRTOP)*ELSEC
C ** growth of each layer of the mother vegetation
        DO 70 I=1,TOPSPR
        DEAD=DEAD+SUMSPROUT(I)*DRTS*ESHODR
        SUMSPROUT(I)=SUMSPROUT(I)*(1-DRTS*ESHODR)
        SUMSPROUT(I)=SUMSPROUT(I)+GRSPR(I)*PERC*ENEWGR
70      CONTINUE

C ** Secondary shoots
C ** how much of the potential growth goes to elongation?
        DO 65 I=1,NSEC
        CALL TABINT(ELTOPT,PARTOP(TOPLSEC(I)),ELTOP,'ELTO')
```

```
      ELSEC=0.
      IF(NSEC.LT.SECMAX) CALL TABINT(ELSECT,PARTOP(1),
     $ELSEC,'ELUI')
      ENEWGR=(1-ELTOP)*(1-ELSEC)
C **  elongation has priority over new secondary shoot formation
      GRTOPSEC(I)=GRSEC(I)*PERC*ELTOP
      GRNSEC=GRNSEC+(GRSEC(I)*PERC-GRTOPSEC(I))*ELSEC

C ** growth of each layer of the secondary shoots
      DO 66 J=1,TOPLSEC(I)
      DEAD=DEAD+SECSHT(I,J)*DRTS*ESHODR
      SECSHT(I,J)=SECSHT(I,J)*(1-DRTS*ESHODR)
      SECSHT(I,J)=SECSHT(I,J)+GRSECL(I,J)*PERC*ENEWGR
66    CONTINUE
65    CONTINUE

C ** formation of new secondary shoots
      SECNEW=SECNEW+GRNSEC
      IF (SECNEW.GE.SECSTART) THEN
         NSEC=NSEC+1
         SECSHT(NSEC,1)=SECNEW
         TOPLSEC(NSEC)=1
         HSEC(NSEC,1)=AMIN1(THICKN,SECSHT(NSEC,1)/
     $(INSEC*WLINI))
         SECNEW=0.
      ENDIF
C **  *************** formation of new tubers

      IF(ROOTW.LE.0.0) THEN
         PFACCENT=0.0
      ELSE
         PFACCENT=KNOLYE/ROOTW
      ENDIF
      IF(PERCTUB.LT.TUBMIN) GOTO 74
      IF(COUNT.GE.231) GOTO 74
      IF(COUNT.LE.30) goto 74
      IF(PFACCENT.LT.PF) THEN
        TUBER=AMIN1(TUBNUM,(PF-PFACCENT))
      ELSE
        TUBER=0.
      ENDIF
      KNOL(COUNT-30,2)=TUBER*FPF*ROOTW*EASSTI
      IF(KNOL(COUNT-30,2).LT.0.0) KNOL(COUNT-30,2)=0.0
      KNOLYE=KNOLYE+KNOL(COUNT-30,2)
      KNOL(COUNT-30,1)=KNOL(COUNT-30,2)*TUBIN
74    CONTINUE
C ** elongation of mother vegetation
```

```
      IF(GRTOP.GT.0.) CALL TOPGRO(GRTOP,HEIGHT,
     $SUMSPROUT,HEIDIS,TOPSPR,AMOUNT,WLINI)
      TOPLAY=TOPSPR
C ** elongation of each secondary shoot
      DO 80, I=1,NSEC
      GR=GRTOPSEC(I)
      LENGTH=LENSEC(I)
      DO 75 J=1,21
      BIO(J)=SECSHT(I,J)
      H(J)=HSEC(I,J)
75    CONTINUE
      A=TOPLSEC(I)
      Q=INSEC
      IF(GR.GT.0.) CALL TOPGRO(GR,LENGTH,BIO,H,A,Q,WLINI)
      LENSEC(I)=LENGTH
      DO 76 J=1,21
      SECSHT(I,J)=BIO(J)
      HSEC(I,J)=H(J)
76    CONTINUE
      TOPLSEC(I)=A
      IF(LENSEC(I).GT.HEIGHT) THEN
         TOPLAY=TOPLSEC(I)
      ELSE
         TOPLAY=TOPSPR
      ENDIF
80    CONTINUE

C*********************************************************************
C ** Check for the occurrence of sloughing on the basis of SHORT/TOTBIOM
C ** and because of possible storm damage
      STORM=1.
      IF(DAI.LE.100) THEN
         EFFECT=FLOAT(DAI)
         CALL TABINT(ESTORM,EFFECT,STORM,'ESTO')
      ELSEIF(DAI.GT.265) THEN
         EFFECT=100.-FLOAT(DAI-265)
         CALL TABINT(ESTORM,EFFECT,STORM,'ESTO')
      ENDIF

      SLOUGH=0.
      LOSS=AMIN1(FC,STORM)
      IF (LOSS.LT.RND) THEN
         SLOUGH=FRAC*TOTABG
90       I=TOPLAY
         IF(LAYW(I).GT.SLOUGH) THEN
C ** distribute losses in this layer relative to the biomass of
C ** mother vegetation and secondary shoots in this layer
```

```
            IF(SUMSPROUT(I).LE.0.) GOTO 92
            SUMSPROUT(I)=SUMSPROUT(I)-SLOUGH*SUMSPROUT(I)/LAYW(I)
            WEIGHTL=AMAX1(.1,PARTOP(I)/1000.)
            IF(WEIGHTL.GT..2) WEIGHTL=.2
            HEIDIS(I)=AMIN1(THICKN,(SUMSPROUT(I)/AMOUNT)/WEIGHTL)
92          DO 95 J=1,NSEC
               IF (SECSHT(J,I).LE.0.) GOTO 95
               SECSHT(J,I)=SECSHT(J,I)-SLOUGH*SECSHT(J,I)/LAYW(I)
            WEIGHTL=AMAX1(.1,PARTOP(TOPLSEC(J))/1000.)
            IF(WEIGHTL.GT..2) WEIGHTL=.2
               HSEC(J,I)=AMIN1(THICKN,(SECSHT(J,I)/INSEC)/WEIGHTL)
95          CONTINUE
         ELSE
C **  a layer must disappear
            SLOUGH=SLOUGH-LAYW(I)
            TOPLAY=TOPLAY-1
            LAYW(I)=0.
            IF(SUMSPROUT(I).LE.0.) GOTO 93
            SUMSPROUT(I)=0.
            HEIDIS(I)=0.
            TOPSPR=TOPSPR-1
93          DO 96 J=1,NSEC
               IF(SECSHT(J,I).LE.0.) GOTO 96
               SECSHT(J,I)=0.
               HSEC(J,I)=0.
               TOPLSEC(J)=TOPLSEC(J)-1
96          CONTINUE
            GOTO 90
         ENDIF
      ENDIF
C*************************************************************************

C **      calculate biomass, height and totals per layer of
C **     mother vegetation and secondary shoots
      TOTABG=0.
      SPROUTW=0.0
      HEIGHT=0.0
      SECW=0.0
      DO 115 I=1,21
      LAYW(I)=0.
115   CONTINUE
      DO 120 I=1,NSEC
      LENSEC(I)=0.
120   CONTINUE
      DO 125 J=1,NSEC
      DO 127 K=1,TOPLSEC(J)
      SECW=SECW+SECSHT(J,K)
```

```
      LENSEC(J)=LENSEC(J)+HSEC(J,K)
      IF(LENSEC(J).GT.DEPTH) LENSEC(J)=DEPTH
      LAYW(K)=LAYW(K)+SECSHT(J,K)
127   CONTINUE
125   CONTINUE

      DO 130 I=1,21
      SPROUTW=SPROUTW+SUMSPROUT(I)
      HEIGHT=HEIGHT+HEIDIS(I)
      IF(HEIGHT.GT.DEPTH) HEIGHT=DEPTH
      LAYW(I)=LAYW(I)+SUMSPROUT(I)
      TOTABG=TOTABG+LAYW(I)
130   CONTINUE
      TOTBIOM=TOTABG+KNOLBIOM+ROOTW+WTUBER
      IF(SPROUTW.LE.0.01) THEN
          SPROUTW=0.0
          GSEASON=0
      ENDIF
      RETURN
      END

C *****************************************************************

      SUBROUTINE TOPGRO(GRTOP,HEIGHT,SUMSPROUT,HEIDIS,TOPLAY,
     $AMOUNT,WLINI)

      INTEGER TOPLAY
      REAL SUMSPROUT(21),HEIDIS(21),NEW

      COMMON /GROWTH/ CTUBFL,CEDEAD,CITUBFL,RESPMAX,TGP,RGP,SGP,SECGP,
     1DRTR,DRTS,SECSTART,PF,THICKN,DEPTH,BOUNDLIGHT,IDAYBEG,TUBDEC,
     2TGPMIN,ITUBDAY,TUBMIN,TUBNUM,ROOTIN,TUBIN,FRAC

      WEIGHTL=WLINI

80    TOPLEN=(GRTOP/AMOUNT)/WEIGHTL


C ** when the surface is reached, biomass in layer TOPLAY is
C ** added to GRTOP
      IF (HEIGHT.GE.DEPTH) THEN
        SUMSPROUT(TOPLAY)=SUMSPROUT(TOPLAY)+GRTOP
        GRTOP=0.
      ELSE

C ** which part of TOPLEN comes in the old top layer and which part goes
C ** to the new top layer?
```

```
      NEW=(TOPLEN-(TOPLAY*THICKN-HEIGHT))/TOPLEN

C ** does a new layer develop?
          IF ((NEW.LE.0.).OR.(TOPLAY*THICKN.GE.DEPTH)) THEN
             NEW=0.
             SUMSPROUT(TOPLAY)=SUMSPROUT(TOPLAY)+GRTOP
             HEIDIS(TOPLAY)=AMIN1(THICKN,HEIDIS(TOPLAY)+TOPLEN)
             GRTOP=0.
             HEIGHT=AMIN1(DEPTH,HEIGHT+TOPLEN)
          ELSE
          OLD=(1.-NEW)
          SUMSPROUT(TOPLAY)=SUMSPROUT(TOPLAY)+OLD*GRTOP
          HEIDIS(TOPLAY)=THICKN
          HEIGHT=HEIGHT+OLD*TOPLEN
          TOPLAY=TOPLAY+1
          GRTOP=NEW*GRTOP
          ENDIF
      ENDIF
C ** is top growth finished?
      IF (GRTOP.GT.0.) GOTO 80
      RETURN
      END
```

SAMENVATTING

1. Inleiding

In grote delen van de wereld zijn ondiepe zoete wateren beïnvloed door eutrofiëring (vgl. Parma, 1980). Vaak was hierbij sprake van een sterke achteruitgang in diversiteit en productie van ondergedoken waterplantenvegetaties. Het goeddeels verdwijnen van de waterplanten had meestal tot gevolg dat de diversiteit van de fauna sterk verminderde en dat voedselweb en stofkringlopen drastisch veranderden (Hall et al., 1970; Kemp et al., 1984; Carpenter & Lodge, 1986; De Nie, 1987). Ook de fytoplanktonsamenstelling veranderde, met als uiteindelijk resultaat een dominantie van blauwalgen zoals *Oscillatoria agardhii* Gom. gedurende het hele jaar.

Dit promotieonderzoek was deel van een groter onderzoeksproject onder leiding van Van Vierssen bij de vakgroep Natuurbeheer van de Landbouwuniversiteit. Hierin werden geëutrofieerde, potentieel door waterplanten gedomineerde zoetwaterecosystemen op verschillende schaal (laboratoriumproeven, veldwaarnemingen, veldexperimenten) en met een samenhangend conceptueel model als werkhypothese bestudeerd. Doel van het project was een bijdrage te leveren aan het ontwikkelen van een optimale en duurzame strategie voor de restauratie van dergelijke meren en het herstel van de potentiële biologische diversiteit. Het Veluwemeer was de veldlocatie van het onderzoeksproject. Volledige publicatie van het onderzoek dat binnen dit projectkader heeft plaatsgevonden zal gebeuren in Van Vierssen et al. (in prep.).

Voor het mechanisme dat ten grondslag ligt aan het op grote schaal verdwijnen van waterplanten zijn in de literatuur twee hoofdtheorieën te onderscheiden, hier noemen we ze model 1 en model 2.

In model 1 wordt het belang van fytoplankton en de veranderde samenstelling van de visfauna benadrukt. In deze theorie wordt verondersteld dat met de toenemende beschikbaarheid van nutriënten de fytoplanktonbiomassa sterk toeneemt. Dit heeft een sterke vermindering tot gevolg van de hoeveelheid licht die de waterplanten bereikt, die daardoor in biomassa afnemen. Zichtjagende roofvissen, zoals de snoek, zijn afhankelijk van waterplantenvegetaties, onder andere voor hun voortplanting (Grimm, 1989). Met de toenemende troebelheid en de afnemende hoeveelheid waterplanten neemt de predatie-efficiëntie van deze roofvis af en kunnen prooisoorten, zoals brasem, in aantal toenemen (Lammens, 1989). Brasem is zoöplanktivoor en benthivoor, en zowel door opwoeling van het sediment als door predatie op zoöplankton zal de troebelheid van het water toenemen en daarmee het licht dat waterplanten bereikt afnemen. Dit model is een combinatie van mechanismen gesuggereerd door Hrbaček et al. (1961), Andersson et al. (1978) en Andersson (1984) voor de zoöplankton-fytoplankton interactie, en van de suggestie van Jupp & Spence (1977) voor de relatie tussen fytoplankton en waterplanten.

Het tweede model is gepubliceerd door Phillips et al. (1978). Hierin wordt perifyton-ontwikkeling gezien als doorslaggevend voor de achteruitgang van de waterplanten. Fytoplanktonontwikkeling wordt volgens deze theorie in eerste instantie geremd door allelopatische stoffen die door de waterplanten uitgescheiden worden. Voor ons onderzoek is als werk-

hypothese een modificatie van dit model gebruikt zoals gepubliceerd door Van Vierssen et al. (1985; zie Fig. 1.1). De modificatie behelst een uitbreiding met de rol van perifytonbegrazende fauna (Hootsmans & Vermaat, 1985) en mogelijke allelopatische remming van waterplanten door fytoplankton (Van Vierssen & Prins, 1985).

De keuze voor model 2 als werkhypothese of conceptueel model voor dit onderzoek is gebaseerd op het feit dat model 1 ons inziens het plotselinge verdwijnen van waterplanten niet volledig verklaart. Vaak is een toename van de troebelheid van de waterlaag alleen niet voldoende om dat verdwijnen te verklaren (Phillips et al., 1978).

Aan de hand van het uitgebreide model 2 zijn een aantal onderzoeksthema's geïdentificeerd. Bovendien is gekozen voor één 'model'-soort waterplant, schedefonteinkruid (*Potamogeton pectinatus* L.), omdat deze soort ook onder de huidige omstandigheden nog vrij algemeen is. Hoewel *P. pectinatus* uitgebreid bestudeerd is (zie de literatuurlijsten van hoofdstukken 2 tot en met 4), was het onze overtuiging dat een aantal in dit kader belangrijke aspecten van de ecologie van deze soort nog niet voldoende duidelijk was. In de hoofdstukken 2 tot en met 4 wordt aandacht besteed aan plasticiteit, groei en fotosynthese. Allelopatie (hoofdstuk 5) en perifytonontwikkeling en -begrazing (hoofdstuk 6 en 7) zijn onderscheiden als belangrijke interacties in het model. Omdat in het veld verschillende processen en factoren tegelijkertijd inwerken, zijn gecontroleerde in-situ manipulaties van complete ecosystemen een uitstekende aanvulling van laboratoriumexperimenten. Hiertoe zijn een tweetal enclosure-experimenten uitgevoerd in het Veluwemeer (hoofdstuk 8). Een belangrijk deel van de resultaten is vervolgens geïntegreerd in een simulatiemodel van de seizoensontwikkeling van *P. pectinatus* (SAGA1, hoofdstuk 9). In hoofdstuk 10 wordt tenslotte samenvattend ingegaan op de conclusies uit de verschillende hoofdstukken van dit gezamenlijke proefschrift, met name in het kader van het beheer van waterplantenvegetaties en ondiepe meren.

2. Conclusies uit de deelonderzoeken

2.1 De waterplant, *Potamogeton pectinatus*

Fenotypische verschillen tussen twee populaties van *P. pectinatus*, één uit een brakke proefsloot op Texel en één uit het Veluwemeer, bleken een genotypische component te hebben. Dit betekent onder andere dat de combinatie van gegevens van verschillende populaties in bijvoorbeeld een simulatiemodel, zoals in hoofdstuk 9 is gebeurd, risico's met zich meebrengt. We hebben geen aanleiding te veronderstellen dat dit in het geval van SAGA1 ook werkelijk het geval is.

Het is niet onaannemelijk dat een aantal karakteristieken van de Veluwemeerpopulatie van adaptief voordeel is in dit sterk aan de wind geëxponeerde, troebele meer met relatief hoge kansen op afslag van materiaal. Deze eigenschappen zijn een snellere ontwikkeling gedurende het groeiseizoen, een relatief hogere investering in fotosynthetisch materiaal en een compactere groeivorm dan de populatie van Texel.

Tubergrootte bleek een belangrijke factor voor de zich ontwikkelende plant. Onder verder gunstige omstandigheden bleven verschillen in biomassa ook na twee maanden nog significant aanwezig. Uit een tuberkiemingsexperiment met

verschillende temperaturen concludeerden we dat zich in tubers van één winter oud een secundaire kiemrust ontwikkelde boven een drempeltemperatuur van rond de 15 °C. Deze zou in het veld geïnduceerd kunnen worden door een snelle temperatuurstijging in het voorjaar. Het verschijnsel zou voordelig kunnen zijn in aquatische habitats met een grote kans op uitdroging in de zomer.

Het effect van licht, temperatuur en leeftijd op de groei en de ontwikkeling is in detail bestudeerd. Voor veel eigenschappen waren de interacties tussen deze drie factoren significant. Planten die onder hoge lichtintensiteiten gekweekt werden elongeerden minder, produceerden meer bladeren en secundaire scheuten en hadden een lager chlorofylgehalte. Bij hogere temperaturen waren groeisnelheden en maxima voor een aantal morfometrische eigenschappen hoger. De fotosyntheseparameters Pm (maximum bruto fotosynthese) en Km (lichtintensiteit waarbij 0.5*Pm is bereikt) namen toe terwijl α (initiële helling van de curve) en R (respiratie) niet beïnvloed werden. Bijgevolg namen de bruto en netto fotosynthese bij 200 μE m^{-2} s^{-1} (GP200 en NP200) toe. Met toenemde leeftijd werden de planten langer en produceerden ze meer maar kleinere blaadjes. Pm en R namen af terwijl Km en α gelijk bleven, GP200 en NP200 namen daardoor af. Bij een vergelijking wat betreft fotosynthese-eigenschappen met andere soorten blijkt *P. pectinatus* zich 'gemiddeld' te gedragen.

We concluderen dat *P. pectinatus* zijn fotosynthese-eigenschappen niet aanpast aan lagere lichtniveau's. Andere eigenschappen zoals een snelle groei naar en een concentratie van het bladmateriaal aan het wateroppervlak, en de mogelijkheid daarbij te teren op energievoorraden in de tuber zijn blijkbaar voldoende om te overleven in ondiep, troebel water.

2.2 Perifytongroei en -verwijdering

De perifytonontwikkeling op objectglaasjes onder eutrofe omstandigheden in een laboratoriumproef was vergelijkbaar met literatuurgegevens (maximumdichtheden 1.5 - 3 mg cm^{-2} asvrijdrooggewicht, afdw). Temperatuurverschillen veroorzaakten meer significante verschillen in logistische groeicurves dan verschillen in lichtintensiteit. Het tegenovergestelde was het geval voor lichtextinctie-dichtheidscurven. Verschillen in lichtextinctie tussen perifytongemeenschappen van dezelfde dichtheid bleken gerelateerd aan de taxonomische samenstelling. In een temperatuurbereik van 10 - 20 °C en voor lichtintensiteiten tussen de 50 en 200 μE m^{-2} s^{-1} bereikten de perifytongemeenschappen een dichtheid van zo'n 0.5 mg cm^{-2} afdw na 3 tot 4 weken, een dichtheid waarbij nog slechts 50% van het ingestraalde licht werd doorgelaten.

De vier geteste soorten zoetwaterslakken verwijderden significante hoeveelheden perifyton van objectglaasjes (0.1 - 2.2 mg $slak^{-1}$ dag^{-1} afdw). Verwijdering door de twee geteste soorten zoetwatercrustaceeën was daarentegen niet significant. De verwijderingssnelheid van de slakken bleek een functie van activiteit, slakgrootte en taxonomische samenstelling van het aangeboden perifyton. Temperatuureffecten waren zelden significant. Voor *Lymnaea peregra* (Müll.) werd geen verschil in verwijdering gevonden van perifyton op *P. pectinatus* vergeleken met objectglaasjes.

De in twee laboratoriumproeven geteste soorten slakken bleken in staat de perifytonontwikkeling op *P. pectinatus* significant te onderdrukken. Het effect van de lichtklimaatsverbetering die hier het gevolg van was bleek sterk afhankelijk van de hoeveelheid licht die de planten uiteindelijk na extinctie door fytoplankton

en perifyton nog bereikte. In het tweede experiment ontvingen de planten iets meer licht (74 ten opzichte van 60 μE m^{-2} s^{-1} in het eerste experiment), maar deze intensiteiten lagen in het steile traject van de fotosynthese-instralingscurve (hoofdstuk 4) wat een duidelijk verschil in de groei van de plant tot gevolg had. In het eerste experiment spraken de planten waarop het perifyton zich 'ongestoord' kon ontwikkelen hun reservevoorraad in de tubers aan om daar relatief meer bladmateriaal mee te produceren. In het tweede experiment was er geen significant effect op de plantengroei waar te nemen. Vanuit het oogpunt van de plant lijkt een populatie *L. peregra* de beste 'begrazingsoplossing' tegen perifytonaangroei. Deze soort heeft een hoge verwijderingssnelheid en een constant hoge activiteit op waterplanten gedurende het gehele groeiseizoen.

2.3 Allelopatie

In experimenten waarin nutriënten noch licht beperkend waren is het voorkomen van een allelopatische effect van door intacte waterplanten uitgescheiden stoffen op de groei van fytoplankton duidelijk aangetoond. Het effect bleek echter relatief onvoorspelbaar: verschillen traden op op verschillende momenten in het seizoen, voor verschillende fytoplanktonalgen en voor verschillende soorten waterplanten. Voor de gevallen waarin allelopatische remming optrad, was de gemiddelde reductie in algengroei zo'n 10 - 15%. Dit lijkt een effect van relatief beperkt belang, maar het kan zeker consequenties hebben voor concurrentie en successie in het fytoplankton.

2.4 Enclosure experimenten

In een experiment met twee typen enclosures (wanden van respectievelijk polyetheen en gaas) in het Veluwemeer zijn de effecten van visactiviteit, perifyton en troebelheid van de waterlaag op de ontwikkeling van een natuurlijke waterplantenvegetatie bestudeerd. De *P. pectinatus* biomassa in de polyetheen-enclosures was twee keer zo hoog als in het meer, de biomassa in de enclosures van gaas lag er tussen in. Bovendien bevatten de enclosures meer andere soorten waterplanten. Lichtextinctie door perifyton en de waterlaag was gelijk in de gazen enclosures en het meer. In de polyetheen enclosures was de extinctie lager. Verschillen in beschikbare nutriënten of opgeloste anorganische koolstof lijken onwaarschijnlijk. We verklaren het verschil in waterplantenbiomassa door de verbeterde lichtomstandigheden in polyetheen en door de afwezigheid van sedimentomwoelende vis in beide typen enclosures. Beide factoren lijken even belangrijk.

Het effect van stekelbaars (*Gasterosteus aculeatus* L.), een soort die zich meestal tussen waterplanten ophoudt, op de dichtheid van zoöplankton en fytoplankton is in een ander enclosure-experiment onderzocht. Het bleek gelijk aan dat van de door brasem (*Abramis brama* L.) gedomineerde visgemeenschap in het Veluwemeer.

2.5 Het simulatiemodel, SAGA1

Het model SAGA1 bleek de vegetatieontwikkeling onder verschillende lichtomstandigheden in het Veluwemeer redelijk te kunnen beschrijven. Afname en verdwijnen van de waterplantenbiomassa aan het eind van het groeiseizoen konden

voldoende voorspeld worden door effecten van licht en leeftijd op de fotosynthese.

Simulaties onder verschillende lichtcondities lieten zien dat in ondiep water zowel extinctie door perifyton als door de waterlaag een belangrijke rol spelen. In dieper water wordt de waterlaag relatief belangrijker. Beneden een bepaalde lichtintensiteit of diepte is de *P. pectinatus* vegetatie in staat gelijke biomassa's te produceren als onder zeer heldere omstandigheden. Dit wordt veroorzaakt door het stimulerende effect van lage lichtniveau's op de vorming van tubers.

De modeluitkomsten geven aan dat de huidige, ogenschijnlijk stabiele situatie in het Veluwemeer in feite misschien wel tamelijk labiel is. Relatief kleine afwijkingen veroorzaken bij de huidige troebelheid en diepte van dit meer namelijk een sterke biomassatoename of het totale verdwijnen van de vegetatie.

3. Betekenis voor het conceptuele model

De veronderstelde rol van allelopatie wordt ondersteund door onze experimentele resultaten, hoewel het effect vrij onvoorspelbaar is. Blijkbaar produceert niet elke soort altijd en onder alle omstandigheden dergelijke stoffen. We kunnen echter veronderstellen dat een diverse waterplantengemeenschap in helder water altijd voldoende allelopatische stoffen zal produceren.

Het lijkt noodzakelijk perifytonbegrazing door slakken in ons model te incorporeren, gezien de waargenomen verwijderingscapaciteiten. Deze begrazing zal echter niet per definitie tot groeiverbetering bij de waterplant leiden. Met diezelfde gevariëerde waterplantengemeenschap in gedachten kunnen we dit effect van slakken als een middel zien om het verdwijnen van waterplanten tenminste uit te stellen.

In Stansfield et al. (1989) wordt een alternatief aangedragen voor de doorslaggevende rol van perifytonopbloei. Deze auteurs wijten het plotseling verdwijnen van zoöplankton uit de meersediment-profielen aan het toegenomen gebruik van pesticiden in de vijftiger jaren. De toe- en afname van perifyton is echter even goed gedocumenteerd in profielen uit dezelfde meren (en verzameld door gedeeltelijk dezelfde auteurs: B. Moss; Phillips et al., 1978). Ons inziens kunnen beide processen een rol gespeeld hebben.

We willen hier nog ingaan op de controverse die er ogenschijnlijk bestaat tussen de modellen '1 en 2' (zie de inleiding). Er is voor ons geen enkele reden waarom de twee modellen niet gekoppeld zouden kunnen worden. Model 2 beschrijft met de rol van perifyton de aanzet tot het verdwijnen van waterplanten beter, maar verwaarloost vervolgens het effect van vis op de troebelheid van het water volkomen.

We suggereren in hoofdstuk 10 (Fig. 10.1) een koppeling van beide modellen, waarbij we ook het eventuele effect van pesticiden op zoöplankton incorporeren. Op basis van dit uitgebreide conceptuele model doen we vervolgens een aantal aanbevelingen voor verder onderzoek.

4. Betekenis voor het beheer van ondiepe meren

Een belangrijk deel van onze resultaten is gecompileerd in het simulatiemodel. Dit model liet voor meren als het Veluwemeer zien dat relatief kleine veranderingen in de troebelheid grote veranderingen in planten-

biomassa tot gevolg kunnen hebben. Dit is het geval bij een extinctiecoëfficiënt van 2-3 m^{-1}, onafhankelijk van het perifytonregime. De maatregelen die in dit geval genomen moeten worden om een grotere helderheid te bereiken zijn afhankelijk van de bijdrage aan de troebelheid van fytoplankton enerzijds en door de wind geresuspendeerd bodemmateriaal anderzijds. Visstandmanipulatie moet de nadruk hebben als resuspensie door wind verwaarloosbaar is. We vermoeden echter dat in ondiepe meren met een spaarzame waterplantenbedekking resuspensie een belangrijke rol zal spelen. In dat geval zal tenminste een deel van de aandacht gericht moeten worden op de reductie van het windeffect. Door het creëren van voor wind en golven beschutte gebieden kan plaatselijk de resuspensie beperkt worden. Compartimentering van een meer in delen met verschillend gebruik is dan bovendien mogelijk: sommige delen met helder water en waterplanten met een 'natuurfunctie', andere delen met minder vegetatie voor recreatieve doeleinden.

Het is onze overtuiging dat het gebruik van een relatief complex model dat de belangrijkste interacties in een ecosysteem beschrijft van groot nut kan zijn voor het bepalen van een efficiënte beheersstrategie. We beëindigen hoofdstuk 10 met drie illustraties hiervan.

Perifytonbegrazing in ondiepe meren
In dit eerste voorbeeld schatten we het effect van een goed ontwikkelde slakkengemeenschap op de ontwikkeling van perifyton en waterplanten in bijvoorbeeld het Veluwemeer met behulp van veld- en labgegevens en SAGA1. We concluderen dat een niet al te hoge perifyton-begrazingsdruk al duidelijk positieve effecten op de plantengroei zou hebben. Afwezigheid van een redelijk ontwikkelde slakkengemeenschap in het huidige Veluwemeer moet vermoedelijk geweten worden aan vispredatie en de korte beschikbaarheid van waterplanten als habitat voor slakken.

Mogelijkheden voor overwinterende kleine zwanen
Met behulp van SAGA1 is geschat hoeveel kleine zwanen (*Cygnus bewickii* Yarr.) voor hun overwintering door een *P. pectinatus* vegetatie onderhouden kunnen worden onder verschillende troebelheids- en perifytonregimes. In de eerste plaats concluderen we dat model en veldwaarnemingen redelijk overeenstemmen, in de tweede plaats moeten we ook hier concluderen dat relatief kleine veranderingen in de extinctiecoëfficiënt grote gevolgen kunnen hebben.

Strategisch maaien
Voor een waterbeheerder kunnen waterplanten zowel gewenst als ongewenst zijn. In het laatste geval is 'controle' nodig. Met behulp van SAGA1 is geschat wat het effect van een verschillend maaitijdstip is op de ontwikkeling van een *P. pectinatus* vegetatie. Een verschil van 20 dagen (begin of eind juli) veroorzaakt een factor 2 verschil in biomassa in het volgende jaar en werkt nog minstens 4 jaar door.

5. Literatuur

Andersson, G., 1984. The role of fish in lake ecosystems - and in limnology. In Bosheim, S. & Nicholls, M., (eds), Nordic Limnology Symposium on interactions between trophic levels in freshwater. Norsk Limnologforening, Oslo, pp. 189-197.

Andersson, G., Berggren, H., Cronberg, G. & Gelin, C., 1978. Effects of planktivorous and benthivorous fish on organisms and water chemistry in eutrophic lakes. Hydrobiologia 59: 9-15.

Carpenter, S.R. & Lodge, D.M., 1986. Effects of submerged macrophytes on ecosystem processes. Aquat. Bot. 26: 341-370.

De Nie, H.W., 1987. The decrease in aquatic vegetation in Europe and its consequences for fish populations. EIFAC/CECPI occasional paper no. 19, 52 pp.

Grimm M.P., 1989. Northern pike (*Esox lucius* L.) and aquatic vegetation, tools in the management of fisheries and water quality in shallow waters. Hydrobiol. Bull. 23: 59-65.

Hall, D.J., Cooper, W.E. & Werner, E.E., 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol. Oceanogr. 15: 839-928.

Hootsmans, M.J.M. & Vermaat, J.E., 1985. The effect of periphyton grazing by three epifaunal species on the growth of *Zostera marina* L. under experimental conditions. Aquat. Bot. 22: 83-88.

Hrbaček, J., Dvořakova, M., Kořinek, V. & Procházkóva, L., 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton association. Verh. int. Verein. Limnol. 14: 192-195.

Jupp, B.P. & Spence, D.H.N., 1977. Limitation on macrophytes in a eutrophic lake, Loch Leven. I. Effects of phytoplankton. J. Ecol. 65: 175-186.

Kemp, W.M., Boynton, W.R., Twilley, R.R., Stevenson, J.C. & Ward, L.G., 1984. Influences of submerged vascular plants on ecological processes in upper Chesapeake Bay. In Kennedy, V.S., (ed.), The estuary as a filter. Academic Press, pp. 367-394.

Lammens, E.H.R.R., 1989. Causes and consequences of the success of bream in Dutch eutrophic lakes. Hydrobiol. Bull. 23: 11-18.

Parma, S., 1980. The history of the eutrophication concept and the eutrophication in The Netherlands. Hydrobiol. Bull. 14: 5-11.

Phillips, G.L., Eminson, D.F. & Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4: 103-126.

Stansfield, J., Moss, B. & Irvine, K., 1989. The loss of submerged plants with eutrophication III. Potential role of organochloride pesticides: a palaeoecological study. Freshwat. Biol. 22: 109-132.

Van Vierssen, W. & Prins, Th. C., 1985. On the relationship between the growth of algae and aquatic macrophytes in brackish water. Aquat. Bot. 21: 165-179.

Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E., 1985. Waterplanten: bondgenoten bij waterkwaliteitsbeheer? (The role of aquatic macrophytes in water quality management, in Dutch) H_2O 18: 122-126.

Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E. (eds), in prep. Dynamics of a macrophyte-dominated system under eutrophication stress: an integrated approach. Geobotany, Kluwer Academic Publishers, Dordrecht.

GENERAL ABSTRACT

Eutrophication of shallow waters has often been associated with the decline of submerged macrophytes. In this study, a conceptual model as a working hypothesis for the mechanism responsible for this decline was used to generate the research topics. Aims of the study were to better understand the mechanism by testing parts of the hypothesis, and to compilate the results in a simulation model, a.o. for management purposes.

The model hypothesis used suggests that periphyton development acted as a trigger for macrophyte decline, with a postponed phytoplankton reaction due to allelopathic growth limitation by substances excreted by the macrophytes.

We selected one abundant macrophyte species, *Potamogeton pectinatus* L., and studied growth, photosynthesis and plasticity. Tuber size appeared to be an important factor for the plant that developed from it. Temperature and light influenced growth nonlinearly and interactively. With increasing age, maximal photosynthetic rate and respiration decreased. Light level during growth also affected photosynthesis. With respect to photosynthetic performance and stem elongation capacity, *P. pectinatus* can be characterized as 'average' when compared to other macrophyte species.

Periphyton development on glass slides under eutrophic conditions in the laboratory was comparable to that in the field as found by other authors. Temperature distinctly affected the growth curves, whilst light did so for the attenuance-density curves of the experimental communities. In the studied light and temperature range, periphyton attenuated about 50% of the light within 3-4 weeks.

All four tested freshwater snail species significantly removed periphyton from glass slides, whilst the two tested crustaceans did not. Differences in removal between and within species could be explained largely by differences in snail activity, snail size and taxonomic composition of the periphyton. Temperature effects were rarely found. From the plant's point of view, a population of *Lymnaea peregra* (Müll.) appears to be the best solution against periphyton accumulation. It has one of the highest removal rates and a constant, high activity and presence on macrophytes during the season.

The existence of allelopathic effects of macrophyte exudates on phytoplankton growth was demonstrated clearly. When present, it was reflected in a growth limitation after one week of on average 10-15%. It could be different for different times of the season, different planktonic algae and different macrophyte species.

In enclosures with different types of wall material (gauze and polythene), we found that polythene had the highest macrophyte biomass, the lake had the lowest and gauze was intermediate. This could be explained by improved light conditions in the polythene enclosures due to reduced seston and periphyton density, and by the absence of sediment-disturbing fish in both enclosure types. In another enclosure experiment, sticklebacks (*Gasterosteus aculeatus* L.) in field densities had a similar effect on zoo- and phytoplankton as the bream-dominated (*Abramis brama* L.) fish community in Lake Veluwe.

The simulation model SAGA1 could reasonably well describe the seasonal vegetation development in Lake Veluwe under various light conditions. Simulations pointed out that in shallow water both periphyton shading and water turbidity are important in limiting plant development. In deeper waters the relative importance of periphyton is reduced. The present, seemingly stable situation in Lake Veluwe actually may well be very labile, since small deviations from the present conditions in the model resulted in large changes in simulated macrophyte biomass.

For our conceptual model, we may conclude that the supposed role of allelopathy is now experimentally supported. Periphyton removal by freshwater snails, as well as the role of benthivorous and piscivorous fish, and possible effects of pesticides on periphyton grazers and zooplankton appear components worthwhile to incorporate. Finally, some illustrations are given of the use of the present study for the management of shallow lakes and submerged macrophyte beds.

RÉSUMÉ GÉNÉRAL

Macrophytes, une clé pour comprendre les changements causés par l'eutrophisation dans les écosystèmes d'eau douce peu profonde

M.J.M Hootsmans & J.E. Vermaat

L'eutrophisation des eaux peu profondes a souvent été associée à la régression de macrophytes immergées. Dans cette étude, un modèle conceptuel a été utilisé en tant qu'hypothèse de travail pour les mécanismes gouvernant cette régression.
L'hypothèse de base était que le développement du périphyton fonctionne comme déclencheur de la régression de macrophytes. La réaction du phytoplancton est retardée à cause d'une limitation de croissance allélopathique par des substances sécrétées par les macrophytes.

Les buts de l'étude étaient de mieux comprendre les mécanismes en vérifiant des parties de l'hypothèse, et d'intégrer les résultats dans un modèle de simulation, entre autres pour des objectifs de gestion. Ainsi, outre l'information sur un certain nombre d'interactions dans l'écosystème, la connaissance du fonctionnement de la composante macrophyte était essentielle.

Nous avons choisi un lac eutrophique, le Lac Veluwe (Pays-Bas), comme location de recherche. Nous avons sélectionné une espèce de macrophyte abondante, le *Potamogeton pectinatus* L., et étudié sa croissance, sa photosynthèse et sa plasticité. La taille des tubercules parut être un facteur important pour la plante qui s'en développait. La température et la lumière influençaient la croissance de manière non-linéaire et interactive. Avec l'âge, la vitesse de photosynthèse maximale et la respiration diminuaient. Le niveau de la lumière pendant la croissance influençait également la photosynthèse. Quant à la performance photosynthétique et la capacité d'élongation de la tige, le *P. pectinatus* peut être caractérisé comme 'moyen', comparé à d'autres espèces de macrophytes.

Le développement de périphyton sur des lamelles de verre dans des conditions eutrophiques au laboratoire était comparable à celui sur le terrain, comme observé par d'autres chercheurs. La température influençait nettement les courbes de croissance, tandis que la lumière agissait ainsi pour les courbes atténuation-densité des communautés expérimentales. Dans la portée étudiée de la lumière et de la température, le périphyton atténuait environ 50% de la lumière en 3-4 semaines.

Toutes les quatre espèces de limnées enlevaient significativement le périphyton des lamelles de verre, tandis que les deux crustacés examinés ne le faisaient pas. Des différences dans le prélèvement entre individus et entre espèces purent être expliquées en grande partie par des différences d'activité des limnées, de taille des limnées et de composition taxonomique du périphyton. Des effets de température furent rarement observés. Du point de vue de la plante, une population de *Lymnea peregra* (Müll.) paraît être la meilleure solution contre l'accumulation de périphyton. Cette limnée a une des plus grandes vitesses de prélèvement et elle maintient une grande activité et une présence continue sur les macrophytes pendant la saison.

L'existence d'effets allélopathiques d'exsudats de macrophytes sur la croissance du phytoplancton fut clairement démontrée. Ils se manifestaient par une limitation de croissance de 10-15% en moyenne après une semaine. L'effet pouvait varier au cours de la saison, et selon les différentes espèces d'algues planctoniques et les différentes espèces de macrophytes.

Dans deux types d'enclos (gaze et polyéthylène) dans le Lac Veluwe, nous avons observé que le polyéthylène avait la biomasse de macrophytes la plus élevée, le lac avait la biomasse la plus basse et la gaze était intermédiaire. Ceci put être expliqué par des conditions de lumière améliorées dans les enclos en polyéthylène, à cause d'une densité réduite de seston et de périphyton, et par l'absence de poissons perturbant le sédiment dans les deux types d'enclos.

Le modèle de simulation SAGA1 peut décrire raisonnablement la dynamique saisonnière de la végétation du Lac Veluwe sous

différentes conditions de lumière. Les simulations indiquent qu'en eau peu profonde, à la fois l'ombrage causé par le périphyton et la turbidité de l'eau sont des facteurs importants limitant le développement des plantes. Dans des eaux plus profondes l'importance relative du périphyton est réduite. La situation actuelle dans le lac Veluwe, apparemment stable, pourrait en fait être très labile, vu que dans le modèle de petites déviations des conditions actuelles dans le modèle résultent en de grands changements de biomasse simulée.

Dans le cas de notre modèle conceptuel, nous pouvons conclure que le rôle présupposé de l'allélopathie est maintenant soutenu expérimentalement. Le prélèvement de périphyton par les limnées, comme par les poissons benthivores et piscivores semblent être des composantes valables à incorporer. Finalement, quelques illustrations ont été données concernant l'utilisation de cette étude pour la gestion des lacs peu profonds et des végétations de macrophytes immergées.

RESUMEN GENERAL

Los macrófitos acuáticos, un elemento clave para comprender los cambios causados por la eutrofización en ecosistemas de aguas someras

M.J.M. Hootsmans & J.E. Vermaat

Los procesos de eutrofización de humedales someros han estado asociados a menudo con el declive de sus poblaciones de macrofitos sumergidos. En este estudio, se ha utilizado un modelo conceptual como hipótesis de trabajo para investigar los mecanismos responsables de dicho declive.

La hipótesis básica es que el desarrollo del perifiton actua como un factor desencadenante del declive de los macrófitos acuáticos. La reacción del fitoplancton es postpuesta a consecuencia de la limitación alelopática de su crecimiento provocada por sustancias excretadas por los macrófitos.

El objetivo principal de este estudio era comprender mejor los mecanismos mediante el contraste de diversas partes de las hipótesis, e integrar los resultados en un modelo de simulación, p.ej. para su uso en problemas de gestión. Para ello, además de la información sobre un cierto número de interacciones presentes en este tipo de ecosistemas, era esencial obtener un adecuado conocimiento del funcionamiento de su componente macrofítico.

Como localidad de campo, elegimos el lago eutrófico Veluwe (Holanda). Seleccionamos una especie de macrófito sumergido abundante en dicho lago, *Potamogeton pectinatus* L., y estudiamos su crecimiento, fotosíntesis y plasticidad. El tamaño de los tubérculos resultó ser un importante factor para las plantas que se desarrollaban a partir de ellos. La temperatura y la iluminación influyeron el crecimiento de forma no linear e interactiva. Al aumentar la edad de las plantas, se producía una disminución en la tasa fotosintética máxima (maximal photosynthetic rate) y la respiración. El grado de iluminación durante el periodo de crecimiento también afectó a la fotosíntesis. Por lo que respecta al funcionamiento fotosintético y a la capacidad de elongación del tallo, *P. pectinatus* puede ser caracterizada como "media" en comparación con otras especies de macrófitos.

El desarrollo de perifiton sobre portaobjetos de vidrio bajo condiciones eutróficas en laboratorio fue comparable al encontrado en campo por otros autores. La temperatura afectó claramente a las curvas de crecimiento, mientras que la iluminación afectó a las curvas atenuación-densidad de las comunidades experimentales. Dentro de los rangos estudiados de iluminación y temperatura, el perifiton producía una atenuación de alrededor del 50% de la luz en 3-4 semanas.

Las cuatro especies de caracoles dulceacuícolas estudiadas retiraban significativamente el perifiton de los portaobjetos de vidrio, al contrario que las dos especies de crustáceos utilizadas. Las diferencias en la intensidad de consumo de perifiton entre y dentro de las diferentes especies podría ser explicada en gran parte por las diferencias en actividad y tamaño de los caracoles y en la composición taxonómica del perifiton. Encontramos raramente un efecto significativo de la temperatura. Desde el punto de vista de las plantas, una población de *Lymnaea peregra* (Müll.) parece ser la mejor solución contra la acumulación de perifiton. Dicha especie presenta una de las más altas tasa de remoción de perifiton y una constante y elevada actividad y presencia sobre los macrófitos durante el periodo de crecimiento de estos.

La existencia de efectos alelopáticos provocados por sustancias exudadas por los macrófitos que afectan al crecimiento del fitoplancton fue claramente demostrada. La presencia de macrófitos se reflejó en una limitación del crecimiento del fitoplancton que alcanzó un valor medio del 10-50% en una semana. Este efecto podría variar para diferentes momentos del periodo de crecimiento, diferentes algas planctónicas y diferentes especies de macrófitos.

En los dos tipos de cercados (exclosures) utilizados (de gasa y de polietileno, respectivamente) en el lago Veluwe, encontramos que los de polietileno presentaban la

maxima biomasa de macrófitos, el lago la menor y los de gasa un valor intermedio. Estos resultados podrían ser explicados por la mejora en las condiciones lumínicas que se produce en los cercados de polietileno debido a la reducción en la densidad de seston y perifiton, y por la ausencia de peces capaces de remover el sedimento en ambos tipos de cercados.

El modelo de simulación SAGA1 puede describir razonablemente bien la dinámica estacional de la vegetación sumergida en el lago Veluwe bajo diversas condiciones de iluminación. Las simulaciones señalan que en aguas someras tanto el sombreado provocado por el perifiton como la turbiedad del agua son factores importantes en la limitación del desarrollo de las plantas. En aguas más profundas, la importancia relativa del perifiton disminuye. La situación actual, aparentemente estable, en el lago Veluwe puede facilmente ser muy labil, puesto que pequeñas desviaciones a partir de las condiciones presentes resultan en el modelo en grandes cambios en las biomasas de macrófitos simuladas.

En lo referente a nuestro modelo conceptual, podríamos concluir que el papel que asignamos a la alelopatía en la limitación del crecimiento del fitoplancton ha sido confirmado por los datos experimentales. La remoción de perifiton por caracoles dulceacuícolas, así como el efecto de los peces bentívoros y piscívoros, parecen ser componentes a incorporar. Finalmente, se ilustran algunas posibilidades de uso del presente estudio para la gestión de lagos someros y praderas de macrófitos sumergidos.

大型水生植物，了解浅水水域淡水生态系统中由富营养化而引起的变化之关键

M. J. 米歇尔 霍沃曼斯
简 E. 弗梅特

摘要

浅水水域的富营养化常常伴随着大型沉水植物的消亡。我们从一个概念性模型出发，研究大型水生植物消亡之机制。

作为出发点，其基本假设为：水生着生植物的生长发育是引起大型水生植物消亡的关键，因为大型水生植物的分泌物质对浮游植物的生长有抑制作用（他感效应），所以浮游植物的作用有些滞后。

此项研究旨在通过检验部分假设，以求得对大型水生植物消亡机制有更深入的了解，并据研究结果建立一个为管理服务的模拟模型。因此，除了生态系统内部相互作用的信息之外，有关大型水生植物的功能方面的知识也是必需的。

野外试验基地设在富鲁夫湖（Veluwe），此乃荷兰境内的一个富营养型湖泊，我们选择了一种在湖内为数众多的大型水生植物篦齿眼子菜（Potamogeton pectinatus L.）作为研究对象，研究其生长、光合作用及适应性。观测结果表明，块茎的大小对于由它所发育的植株而言是一个重要的因子。温度和光照对植物的生长有着非线性的，相互协同的影响。最大光合作用率和呼吸作用随年龄的增长而衰减。在生长期光照强度对光合作用也有影响。就光合作用及茎杆伸长能力而言，篦齿眼子菜和其他大型水生植物相比属于中等水平。

正如其他作者所发现的那样，水生着生植物在实验室内置于富营养条件下的载玻片上的生长发育与野外的情形是可比的。通过对所选群落进行试验发现，温度对其生长曲线的影响是很明显的，而光照则对光衰减一密度曲线有显著影响。在试验所用光照，温度变化范围内，三至四周内着生植物即可衰减约50%之光照。

试验所用四种淡水螺类均能有效地从载玻片上去除着生植物，而所用的两种甲壳类动物则不能。种间及种内去除量的差异，主要地是由螺类的活性，个体大小以及着生植物组成成分的不同而引起的。在此，温度几乎没有影响。从植物的角度出发，一种螺类群体（Lymnaea peregra (Mull)）是控制着生植物富集的最佳选择。它具有最高的去除率，活性又总是很高，并且在生长期存在于大型水生植物之上。

大型水生植物分泌物对浮游植物的抑制作用的存在表现得很清楚。这种作用存在时，表现为一周之后浮游植物生长率平均下降10-15%。抑制作用可能因时间不同，浮游藻类不同，大型水生植物不同而不同。

在富鲁夫湖的两种围网（纱网，聚乙稀网）内，我们发现大型水生植物生物量在聚乙稀网内最高，湖内最低，纱网内居中。其原因可能是，在聚乙稀网内由于悬浮物及着生植物密度减小而改进了光照条件，同时在两种围网内均没有搅动底泥的鱼类存在。

模拟模型SAGA1能很好地描述富鲁夫湖内不同光照条件下的植物季节变化。模拟结果表明，在浅水水域着生植物的遮蔽作用和水的浊度对抑制大型水生植物的生长发育都很重要，而在较深的水域着生植物的相对重要性就降低了。目前富鲁夫湖貌似稳定的状况可能是极不稳定的，因为在模型中只要稍稍改变目前的条件，模拟所得的大型水生植物生物量即会产生很大变化。

对我们所用的概念性模型可以得出这样的结论，即假设的他感效应的作用已为实验所证实。看来值得综合利用淡水螺类，捕食底栖生物的鱼类和食鱼的鱼类，以去除着生植物。本文最后还给出了目前的研究工作在浅水湖泊及大型沉水植物植被管理方面的一些应用实例。

МАКРОФИТЫ, КЛЮЧ К ПОНИМАНИЮ ИЗМЕНЕНИЙ, ВЫЗЫВАЕМЫХ ЭУТРОФИКАЦИЕЙ В ПРЕСНОВОДНЫХ ЭКОСИСТЕМАХ НА МЕЛКОВОДЬЕ

М. Я. М. Хоотсманс, Я. Е. Вермаат

АННОТАЦИЯ

Эутрофикация мелководья всегда была связана со снижением количества подводных макрофитов. В данном исследовании в качестве рабочей гипотезы объяснения механизма такого снижения и генерирования изучаемых вопросов была использована концептуальная модель.

Целями исследования были лучшее понимание механизма путем проверки частей гипотезы и использование результатов в имитационной модели, в частности для целей управления.

Модельная гипотеза предполагает, что развитие перифитона послужило пусковым механизмом для снижения числа макрофитов, и задержки реакции фитопланктона в силу аллелопатического ограничения роста, вызываемого веществами, выделяемыми макрофитами.

Мы выбрали один из избыточных видов макрофитов, *Potamogeton pectinatus L*, и изучили рост, фотосинтез и пластичность. Оказалось, что размер клубня имел важное значение для растения, которое развилось из него. Температура и свет влияли на рост нелинейно и согласованно. С увеличением возраста возросли и максимальная скорость фотосинтеза и респирации. Влиял на фотосинтез также и уровень освещенности. По характеристикам способности к фотосинтезу и продолжению рода, *P. pectinatus* может быть охарактеризован как «средний», если сравнить его с другими видами макрофитов.

Развитие перифитона на стеклянных пластинках в лабораторных условиях эутрофикации было сравнимым с развитием в полевых условиях, описанным другими авторами. Температура явно влияла на кривые роста, в то время как свет влиял на кривые плотности в экспериментальных сообществах. В изученных диапазонах света и температуры перифитон рассеивал около 50% света в пределах 3-4 недель.

Все четыре изученных вида пресноводных улиток в существенной степени удаляли перифитон со стеклянных пластинок, в то время как два изученных ракообразных - нет. Различия в удалении между и внутри видов могут быть объяснены в значительной мере различиями в активности улиток, их размерах и таксономической композиции перифитонов. Влияние температуры обнаруживалось редко. С растительной точки зрения популяция *Lymnaea peregra* (Müll) оказалась лучшим решением против аккумуляции перифитона. Она имела одни из самых высоких скорость удаления, а также постоянную, высокую активность в течение сезона.

Было явно продемонстрировано существование аллелопатических эффектов, оказываемых выделениями макрофитов на рост фитопланктона. При его наличии, оно выражалось в ограничении роста через одну неделю в среднем на 10-15%. Оно было различным для разного времени в течение сезона, различных планктонных волн и различных видов макрофитов.

Мы обнаружили, что в огороженных местах при использовании различного материала для стен (марля и полиэтилен), полиэтилен имел самую высокую биомассу макрофитов, озеро - наинизшую, и марля - промежуточную. Это может быть объяснено лучшими световыми условиями в полиэтиленовых ограждениях из-за пониженного количество взвеси и плотности перифитона, а также отсутствием в обоих типах ограждений взбаламучивания рыбами. В другом эксперименте в ограждении, колюшки (*Gasterostius aculeatus L.*) в полевых плотностях оказывали похожий эффект на зоо- и фитопланктон в рыбном сообществе с доминированием леща (*Abramis brama L.*) в озере

Veluwe.

Имитационная модель SAGA1 могла достаточно хорошо объяснить сезонный рост в озере Veluwe при различных условиях освещения. Моделирование выявило, что на мелководье как затенение перифитоном, так и мутность воды имели важное значение для ограничения развития растений. На глубоководье относительное влияние перифитона ниже. Настоящая ситуация на озере Veluwe, кажущаяся устойчивой, может оказаться нестабильной, поскольку вводимые в модель малые отклонения от настоящих условий приводили к существенным изменениям в моделирумой биомассе макрофитов.

Для нашей концептуальной модели мы можем заключить, что предполагаемая роль аллелопатии теперь экспериментально подтверждена. Оказалось, что компонентами, которые требуют учета, являются удаление перифитона пресноводными улитками, роль травоядных и плотоядных рыб, а также возможные воздействия пестицидов на перифитон и зоопланктон. В заключение, проиллюстрировано использование настоящего исследования в задачах управлении мелководными озерами и поселениями макрофитов.

CURRICULA VITAE

Michael Joannes Maria (Michiel) Hootsmans werd geboren op 14 december 1959 in Voorschoten. In 1978 behaalde hij het diploma gymnasium-b aan het Christelijk Lyceum te Alphen aan den Rijn, waarna hij begon met zijn studie biologie aan de toenmalige Landbouwhogeschool in Wageningen.

In 1982 behaalde hij met lof het kandidaatsdiploma. Het doctoraal programma omvatte drie vakken. Het hoofdvak Aquatische Ecologie werd uitgevoerd samen met Jan Vermaat. Het onderwerp 'Functionele aspecten van zeegrasvelden in de Zandkreek, een subsysteem van de Oosterschelde' werd gedaan in 1983 onder de gezamenlijke begeleiding van Dr. P.H. Nienhuis (Delta Instituut voor Hydrobiologisch Onderzoek, Yerseke) en Dr. W. van Vierssen (Vakgroep Natuurbeheer, Landbouwhogeschool Wageningen). Het onderwerp 'Kiemingsecologie van de twee Nederlandse zeegrassoorten' werd aansluitend gedaan onder begeleiding van Dr. W. van Vierssen. Het bijvak Regionale Bodemkunde werd gedaan in 1984 samen met Michiel Wallis de Vries onder begeleiding van Dr. Ir. N. van Breemen, met als onderwerp 'Bodemkundige variatie in podzolgronden'. Het bijvak Dierecologie werd eveneens samen met Michiel Wallis de Vries uitgevoerd in 1984-1985 aan het Instituut voor Oecologisch Onderzoek in Heteren onder begeleiding van Dr. J. Tinbergen. Het onderwerp was 'Fourageergedrag van koolmezen'.

De praktijktijd werd gedeeltelijk doorgebracht in de Verenigde Staten bij het Virginia Institute of Marine Science. Gedurende vier maanden in 1982 werd samen met Jaap Graveland onder supervisie van Dr. R.J. Orth gewerkt aan de populatiedynamica van de blauwe krab en de restauratie van zeegrasvelden. De rest van de praktijktijd werd in 1985 gedaan bij het Instituut voor Oecologisch Onderzoek onder begeleiding van Dr. J. Tinbergen. Het onderwerp was 'Broedecologie van de koolmees in de Hoge Veluwe'.

Het doctoraalexamen werd met lof behaald in september 1985. In december 1985 werd samen met Jan Vermaat begonnen aan het promotieonderzoek dat leidde tot het voorliggende proefschrift.

Tijdens de aanstelling bij de vakgroep Natuurbeheer werkte hij mee aan opzet en uitvoering van het practicum Aquatische Ecologie op Terschelling. In oktober 1988 maakte hij deel uit van de Mauretanië-expeditie van de Stichting Onderzoek der Zee naar de Banc d'Arguin.

Sedert december 1988 is hij in dienst van het International Institute for Hydraulic and Environmental Engineering (IHE) te Delft, bij de afdeling Environmental Engineering. In oktober 1990 leverde hij een bijdrage aan de 'Joint Course on Ecology and the Sustainable Development of Watersheds' in Suzhou (Volksrepubliek China).

Jan Elbertus Vermaat werd op 31 januari 1959 geboren in Leidschendam. Hij behaalde in 1977 aan het Revius Lyceum te Doorn het diploma gymnasium-b, om vervolgens biologie te gaan studeren aan de toenmalige Landbouwhogeschool te Wageningen.

Het kandidaatsdiploma werd in 1983 met lof behaald. Het doctoraalpakket bestond uit drie vakken. Gedurende het hoofdvak Dierecologie werd onder begeleiding van Dr. Ir. M.W. Sabelis onderzoek gedaan naar de specificiteit van spintmijtkairomonen voor de roofmijt *Phytoseiulus persimilis* en de betekenis van deze stoffen voor het zoekgedrag van de roofmijt. Voor het verzwaarde hoofdvak Natuurbeheer zijn samen met Michiel Hootsmans de volgende twee aquatisch ecologische onderwerpen bewerkt: (a) 'Functionele aspecten van zeegrasvelden in de Zandkreek, een subsysteem van de Oosterschelde',

onder begeleiding van Dr. P.H. Nienhuis (Delta Instituut voor Hydrobiologisch Onderzoek) en Dr. W. van Vierssen (Vakgroep Natuurbeheer, Landbouwhogeschool Wageningen) en (b) 'Kiemingsecologie van groot en klein zeegras (*Zostera marina* en *Zostera noltii*)', onder begeleiding van Dr. W. van Vierssen. Vervolgens is in het extra vak Pedagogiek en Didactiek de onderwijskundige bevoegdheid in de biologie behaald. De praktijktijd is aan twee Europese instituten doorgebracht. Aan het Tvärminne Zoological Station in Finland is samen met Greta Rensenbrink een vegetatiekartering uitgevoerd op de scheren van het toekomstige nationale park Tammisaari Saaristo, onder begeleiding van Dr. P. Borg van het Finse bosbouwministerie (Metsähallitus). Het tweede deel is besteed op het Freshwater Biological Laboratory, University of Copenhagen (Hillerød, Denemarken), waar onderzoek is gedaan naar groei en fotosynthese van zeesla (*Ulva lactuca*), onder begeleiding van Dr. K. Sand-Jensen. Het doctoraalexamen werd in juli 1985 afgelegd.

In december 1988 werd hij samen met Michiel Hootsmans aangesteld als promotie-assistent op de vakgroep Natuurbeheer van de Landbouwuniversiteit, onder begeleiding van Dr. W. van Vierssen, met dit proefschrift als resultaat. Gedurende de aanstelling bij de vakgroep Natuurbeheer is meegewerkt aan de opzet en uitvoering van het practicum Aquatische Ecologie op Terschelling. Ook is in oktober 1988 deelgenomen aan de expeditie van de Stichting Onderzoek der Zee naar het waddengebied van de Banc d'Arguin in Mauretanië.

Vanaf april 1989 is hij werkzaam op de afdeling Environmental Engineering van het International Institute for Hydraulic and Environmental Engineering (IHE) te Delft. De aanstelling was eerst op basis van een projectcontract voor DBW/RIZA ('Rivieren, natuurlijk dynamisch, naar een geïntegreerd beheer van het Rijnstroomgebied, een verkenning vanuit hydraulisch-morfologisch en ecologisch gezichtspunt') en vanaf september 1989 is hij in dienst als onderzoeker in de aquatische ecologie.

Publicaties

Sabelis, M.W., Vermaat, J.E. & Groeneveld, A., 1984. Arrestment responses of the predatory mite, *Phytoseiulus persimilis*, to steep odour gradients of a kairomone. Physiol. Entomol. 9: 437-446.

Van Vierssen, W., Hootsmans, M.J.M. & Vermaat, J.E., 1985. Waterplanten: bondgenoten bij het waterkwaliteitsbeheer? Een visie op de toekomst van het beheer van waterplantenvegetaties. H_2O 18: 122-126.

Hootsmans, M.J.M. & Vermaat, J.E., 1985. The effect of periphyton-grazing by three epifaunal species on the growth of *Zostera marina* L. under experimental conditions. Aquat. Bot. 22: 83-88.

Hootsmans, M.J.M. & Wallis de Vries, M.F., 1986. A study of soil variation in podzols in The Netherlands. Neth. J. Agr. Sci. 34: 106-108.

Hootsmans, M.J.M., Vermaat, J.E. & van Vierssen, W., 1987. Seed-bank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L. and *Zostera noltii* Hornem. Aquat. Bot. 28: 275-285.

Vermaat, J.E. & Sand-Jensen, K., 1987. Survival, metabolism and growth of *Ulva lactuca* under winter conditions: a laboratory study of bottlenecks in the life cycle. Mar. Biol. 95: 55-61.

Vermaat, J.E., Hootsmans, M.J.M. & Nienhuis, P.H., 1987. Seasonal dynamics and leaf growth of *Zostera noltii* Hornem., a perennial intertidal seagrass. Aquat. Bot. 28: 287-299.

Hootsmans, M.J.M. & Breukelaar, A.W., 1990. De invloed van waterplanten op de groei van algen. H_2O 23: 264-266.

Van Vierssen, W. & Hootsmans, M.J.M., 1990. Weed control strategies for *Potamogeton pectinatus* L. based on computer simulations. Proc. EWRS 8th Symposium on Aquatic Weeds, 231-236.

Vermaat, J.E., Hootsmans, M.J.M. & Van Dijk, G.M., 1990. Ecosystem development in different types of littoral enclosures. Hydrobiologia 200/201: 391-398.

Vermaat, J.E. & Van Vierssen, W., 1990. Kansen voor waterplanten in de grote rivieren? H_2O 23: 534-536.

Hootsmans, M.J.M, & Van Vierssen, W., in press. Computer simulations of macrophyte population dynamics during lake recovery after eutrophication. Verh. int. Verein. Limnol.

Van Vierssen, W. & Hootsmans, M.J.M., in press. On the origin of macrophyte population dynamics during lake recovery after eutrophication. Verh. int. Verein. Limnol.

Hootsmans, M.J.M., J.A.J. Beijer & J.E. Vermaat. Periphyton density and shading as a function of tidal position and fiddler crab activity in intertidal seagrass beds of the Banc d'Arguin (Mauritania). In Van der Land, J., P.H. Nienhuis, De Wilde, P.A.W.J. & W.J. Wolff, Biological studies in the coastal waters of Mauritania. Hydrobiologia, submitted.

Vermaat, J.E., Beijer, J.A.J., Gijlstra, R.J., Hootsmans, M.J.M., Philippart, C.J.M., Van de Brink, N. & Van Vierssen, W. Leaf dynamics and standing stocks of intertidal *Zostera noltii* Hornem. and *Cymodocea nodosa* (Ucria) Ascherson in the Banc d'Arguin (Mauritania). In Van der Land, J., P.H. Nienhuis, De Wilde, P.A.W.J. & W.J. Wolff, Biological studies in the coastal waters of Mauritania. Hydrobiologia, submitted.