MACROALGAL MATS IN A EUTROPHIC LAGOON: DYNAMICS AND CONTROL MECHANISMS

een wetenschappelijke proeve op het gebied van de
Natuurwetenschappen, Wiskunde en Informatica

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Katholieke Universiteit Nijmegen, volgens beslui
van het College van Decanen in het openbaar te
verdedigen op donderdag 3 februari 2000, des
namiddags om 3.30 uur precies

door

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geboren op 1 augustus 1969 te Wormerveer

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NIOO thesis 5 from the Netherlands Institute of Ecology, NIOO-CEMO, Yerseke, the Netherlands.

ISBN 90 – 9013449 – 2

Cover: *Ulva* spp. mats in the Veerse Meer, SW Netherlands (photography: Erik-jan Malta).

Printed by Ponsen & Looijen bv, Wageningen.

‘t Veerse gat

Zeeland, recreatieland
Het kleinste dorp, de grootste stad, zij hebben allemaal wel wa
Een kanovijver, echoput, een oude man, een oude hu
En aan het strand verdomd veel zand
Zeeland recreatieland

Veere, recreatiestad
Een toren met een restaurant, een walle en een waterkan
Een havenkroeg één havenmeid en veel antiek uit d’oude tijd
Het meterslange wandelpad en tot besluit het Veerse….
Hee, wat is dat?
Waar is het Veerse gat?

Het gat is dicht, de haven lich
Voor jo-ho-ker, voor jo-ho-ho-ho-ker
Als alle havens zonder gaten
Voor jo-ho-ker, voor jo-ho-ho-ho-ker
Und Fritsch und Wilhelm komen nu well schnell
Want Veere vreet hun bitte wel, zoals wij vroeger vraten
Voor jo-ho-ker, voor jo-ho-ker, voor jo-ho-ker

’t was nacht, ’t was nacht, ’t was midden in de nach
Toen heeft een man uit Delft dit bedach
Dat was een hele knappe, voor Delft een hele knappe
Hij had daar thuis wel 17 caissons, en daarbij nog een heleboel pontons
Die had ‘ie van zijn ome, om hogerop te komen
Hij dacht voor Delft heel diep en zei toen, wat heb ik aan die dingen zonder gat
Om kort te gaan dames en heren
Dit werd het gat van Veere

Veere, recreatiestad
Een toren met een kabelbaan, een frietkraam met een vlag er aan
De havenkroeg wordt uitgebreid, er komt een tweede havenmeid
Aus Bremen
De vissers op het wandelpad, zij krabben stug hun Veerse
Ach nee…

Jaap Fisher/Joop Visser, 1962

The research presented in this thesis was carried out at the Department of Marine Microbiology (former Dept. of Estuarine Ecophysiology) of the Netherlands Institute for Ecology – Centre for Estuarine and Coastal Ecology.

Parts of the research presented in this thesis were carried out in the framework of the EUMAC project. EUMAC was funded by the Environment and Climate programme of the European Commission (no. EV5V-CT93-0290).

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CHAPTER I: GENERAL INTRODUCTION AND OUTLINE

EUTROPHICATION: DEFINITION AND CAUSES

Eutrophication of coastal waters has become a major nuisance over the last four to five decades. The loading of the water with nutrients from diffuse and point sources has given rise to blooms of planktonic and benthic macro- and microalgae and bacteria. This has drasticall changed the structure and composition of entire ecosystems (Rosenberg, 1985; Nienhuis, 1992b; Nixon, 1995). Many definitions of the term "eutrophication" exist, but none of them is entirely valid, at least where it applies to the estuarine and marine environment (Nixon, 1995). In this thesis I use a definition which is derived from those given by Rosenberg (1985) and Nienhuis (1989):

> *Eutrophication is the process of increasing concentrations and load of nutrients, inducing changes in the structure and species composition of the aquati community.*

This definition involves ecological implications, which enables us to classify ecosystems in various trophic stages using biological parameters such as the presence or absence of certain macrophytes and other biological parameters.

The nutrients giving rise to the eutrophication processes are of anthropogenic origin (Rosenberg, 1985). Nutrient loads come from several sources. A few decades ago, the discharge of wastewater was the most important source, but nowadays, agricultural run-of enriched with fertilisers is more important. However, wastewater, together with atmospheric deposition ("acid rain"), can still be an important nutrient source (Nixon & Pilson, 1983; Rosenberg, 1985; Valiela *et al.*, 1992; Jeffrey *et al.*, 1995). Evidently, nitrogen is the key element limiting primary production in most shallow coastal systems (Ryther & Dunstan, 1971; Rosenberg, 1985; Nienhuis, 1989), although phosphorous (Lapointe, 1987; Peckol *et al.*, 1994; de Casabianca *et al.*, 1997a) or simultaneous N and P (Gordon *et al.*, 1981; Lapointe, 1987) limitation of primary production have also been observed.

IMPACT OF EUTROPHICATION ON MARINE MACROPHYTOBENTHIC COMMUNITIES

Eutrophication is often accompanied with other types of pollution, e.g. heavy metals, herbicides, antifouling compounds, etc. It is therefore not always possible to attribute changes in the macrophyte community to eutrophication alone (Fletcher, 1996). The large body o literature, however, consisting of both monitoring data and laboratory and field experiments (including some on mesocosm scale, e.g. Isaksson *et al.*, 1994; Short *et al.*, 1995; Taylor *et al.*, 1995), reveals several general trends. In general, eutrophication leads to a decrease in species diversity of the benthic flora and associated fauna (see a.o. Thorne-Miller *et al.*, 1983; Breuer & Schramm, 1988; Tewari & Joshi, 1988; Nienhuis, 1992a; Villano & Warwick, 1995). Phytoplankton blooms increase both in intensity and duration and microphytic and macrophytic primary production increases (see however Borum & Sand-Jensen, 1996). The

attenuation coefficient in the water increases. This causes changes in the depth distribution of benthic algae and seagrasses, often leading to the disappearance of characteristic zonation patterns (reviews by Duarte, 1995; Fletcher, 1996; Morand & Briand, 1996).

Probably the most conspicuous floristic change associated with eutrophication in macrophyte communities is the decline of perennial macroalgae, being part of the so-called climax vegetation of intertidal and subtidal regions throughout the world. For hard substrates, decreases have been reported in various fucoids such as *Fucus* L. spp., *Ascophyllum nodosum* (L.) Le Jol. and *Cystoseira* C. Agardh. spp., laminarians such as *Laminaria digitata* (Huds.) Lamour. and other species such as *Sargassum* C. Agardh. spp. and the rhodophytes *Chondracanthus canaliculatus* (Harvey) Guiry (former *Gigartina canaliculata* Harvey), *Gelidium robustum* (N.L. Gardner) Hollenberg & I.A. Abbott and *Lithophyllum subantarcticum* (Foslie) Foslie (review by Fletcher, 1996). As to the soft bottom macrophytes, several reports mention a decline of seagrass communities and their associated macroalgae due to eutrophication (Guist & Humm, 1976; Verheij & Erftemeijer, 1993; Den Hartog, 1994; Short *et al.*, 1995).

The perennial macrophytes are replaced by phytoplankton blooms and fast-growing macroalgae, such as members of the chlorophyte genera *Ulva* L., *Enteromorpha* Link, *Cladophora* Kütz. and *Chaetomorpha* Kütz., members of the rhodophyte genera *Ceramium* Roth and *Gracilaria* Greville and the phaeophytes *Ectocarpus siliculosus* (Dillw.) Lyngb. and *Pilayella littoralis* (L.) Kjellm. (reviewed by Fletcher 1996; Morand & Briand, 1996; Valiela *et al.*, 1997). In forming large deposits on the shoreline, some of the green algae blooms are commonly known as "the green tides" or "les marées vertes des ulves" (Fletcher, 1996; Morand & Briand, 1996). The high surface-area to volume ratio of these mass-forming macroalgae favours rapid nutrient uptake, high production and high growth rates, which enable them to overgrow the original vegetation (Littler & Littler, 1980; Hein *et al.*, 1995). In many areas, these bloom-forming algae occur as free-floating thalli that are frequentl arranged in thick canopies. In areas subjected to tidal movement and strong waves, the canop structure of these detached algae may be temporary (on the time scale of one tidal cycle, Dion & le Bozec, 1996). In lagoons, étangs and other areas only marginally subjected to tidal and wave exposure, the canopy structure may persist for weeks or even for an entire season (Vergara *et al.*, 1998).

The changes in biomass and species composition of the vegetation because of eutrophication have a strong impact on the fauna. The decomposing macroalgal organic matter and the phytoplankton blooms may lead to periods of complete anaerobic circumstances (dystrophic crises) that can persist for several weeks or even longer. Most benthic species are not able t survive these long anaerobic periods and will eventually disappear (Nicholls *et al.*, 1981; Reise, 1983; Warwick & Clarke, 1994; Pih *et al.*, 1995). Clearly, this also affects fish species, especially juvenile stage and the (often econom cally important) demersal species such as young cod, *Gadus morhua* L. and plaice, *Pleuronectus platessa* L. (e.g. Lenanton *et al.*, 1985; Isaksson *et al.*, 1994; Pihl *et al.*, 1995). However, for some herbivorous species, especially birds, positive effects can be seen when eutrophication is not too severe, viz. when no dystrophic crises occur (Coosen *et al.*, 1990; Seys *et al.*, 1991).

The phenomenon of large floristic changes in relation to pollution is certainly not new. One of the first reports dates back to 1905 and deals with the proliferation o *Ulva* in the Belfast Lough (Letts & Richards, 1911). Although the assumption of a correlation between eutrophication and the occurrence of macroalgal blooms is generally accepted (Duarte, 1995;

Schories *et al.*, 1997; Valiela *et al.*, 1997), fundamental knowledge is lacking for an understanding of the control mechanisms of the blooms (Nixon, 1995). Most studies on macroalgal blooms provide descriptions of biomass accumulation and production rates, however, studies on the coupling of spatial and seasonal biomass patterns to environmental variables, and on the bottlenecks in the life-cycle, are still rare (cf. Sfriso *et al.*, 1987; Laver *et al.*, 1991; de Casabianca & Posada, 1998; Lotze *et al.*, 1999). Macroalgal mats are increasingly considered as separate subcompartments within the ecosystem with differen physical and chemical characteristics (Sundbäck *et al.*, 1990; Thybo-Christensen *et al.*, 1993; Vergara *et al.*, 1997). Experimental evidence that these mats are spatially heterogeneous in environmental and physiological characteristics is accumulating (e.g. Krause-Jensen *et al.*, 1996; McGlather *et al.*, 1997; Vergara *et al.*, 1998) but this evidence needs to be backed-up by field results.

Outline of this Thesis

This thesis aims to describe the regulation of seasonal and spatial dynamics of macroalgal growth and biomass distribution by environmental and biological variables. Studies on different levels of organisation (ecosystem, community and organism) are integrated to come to an understanding of the processes controlling macroalgal blooms. The eutrophic coasta lagoon the "Veerse Meer" (Southwest Netherlands) has been serving as a model ecosystem, with *Ulva* cf. *scandinavica* Bliding as the main study organism.

Chapter II describes the dynamics of abiotic and biotic variables in the macroalgal growing season during the years 1992 and 1994 at one study site in the Veerse Meer. Biomass development, growth rate, tissue composition and habitat characteristics of macroalgal blooms are monitored in order to determine the seasonal and the between-year variability o macroalgal biomass and growth as a function of the variability in the environmental variables.

Chapter III compares macroalgal biomass and species distribution at two sites in the Veerse Meer during the growth season of '94/'95. Measurements of growth rates, biomass and environmental variables are compared in order to elucidate the mechanism responsible for the spatial distribution of macroalgal biomass. Four phases are distinguished in the seasonal dynamics of growth and biomass. The dynamics in environmental variables are related to the phase transitions.

Chapter IV focuses on the life cycle of *Ulva* spp. and its role in the initiation of the bloom The viability of *Ulva* spp. fragments, buried in Veerse Meer sediment is investigated. Conditions for resistance to freezing are tested experimentally in the laboratory. A field experiment is described in which the role of bioturbation by the lugwor *Arenicola marina* L. for the bloom initiation is tested. The role of cold resistance and burial with respect to the spring bloom initiation is discussed.

In Chapter V, the taxonomy, morphological variation and autoecology of the dominan *Ulva* species in the bloom are examined. Rooted phylogenetical trees are constructed, based on the sequences of the spacer regions of parts of the ribosomal DNA and compared with morphological data. The optimal temperature and salinity conditions for growth of the differen *Ulva* species and morphologies are determined and discussed with respect to their temporal and spatial distribution patterns.

Chapter VI investigates the existence of multiple growth-limiting gradients in *Ulva* spp. canopies. Nitrogen concentrations in the water and in the algae, quantum efficiency,

glutathione content and redox ratios and light absorbance of various layers in an *Ulva* canopy are measured during three phases of the bloom. The results are discussed in relation to *Ulva* physiology and macroalgal bloom dynamics.

Finally, Chapter VII presents a general discussion of the main results presented in this thesis in the form of a conceptual model.

The EUMAC Project

The work presented in this thesis has largely been carried out in the framework of the EUMAC (EUtrophication and MACrophytes) project (no. EV5V-CT93-0290). EUMA lasted from 1994 to 1996 and was financed by the European Community. It was a research co-operation between partners in 6 European countries, set-up with the following objectives: 1) to carry out comparative field and laboratory studies; 2) to carry out experimental studies in macroalgal taxonomy and ecophysiology; 3) to standardise and unify the kinetic parameters for a number of properly identified dominant nuisance algae along the European coast; 4) to construct a validated and balanced modelling concept for the "eutrophication-macrophyte" problem in marine coastal areas, to be implemented by the leading European institutions in this field (EUMAC, 1993a; EUMAC, 1993b). In the Netherlands, the research has been concentrated on the Veerse Meer (presented in this thesis). The other EUMAC study sites were Langstone Harbour, UK, the Schlei fjord, Germany (Lotze, 1998; Lotze *et al.*, 1999), the Bay de Lannion, France (Dion *et al.*, 1998), the Étang de Thau, France (de Casabianca *et al.*, 1997a, b; de Casabianca & Posada, 1998), Venice lagoon, Italy (Sfriso & Marcomini, 1996; Sfriso & Marcomini, 1997), S' Ena Arrubia lagoon, Italy (Bondavalli *et al.*, 1998) and Thermaikos Gulf, Greece (Orfanidis *et al.*, 1998). Rijstenbi *et al.* (1996) present a synthesis of the results gained in the EUMAC project.

The Study Area

All fieldwork presented in this thesis is carried out in the brackish lake the "Veerse Meer", a former part of the Rhine-Scheldt estuary, situated in the Southwest Netherlands. Hydrodynamically, this lake should be considered as a coastal lagoon. It was constructed after the flood of 1953 by the closing of the Veerse Gat from the North Sea and the Oosterschelde. Construction works were finished in 1961. The resulting non-tidal lagoon is connected to the Oosterschelde by the Zandkreek sluice (Fig. 1.1). During winter, the lagoon mainly serves as a catchment area for fresh, nutrient-rich polderwater. To facilitate this function, the water level is maintained at MSSL (Mean Standard Sea Level) - 0.70 m in winter and at MSSL – 0 m in summer. The annual residence time of the water is about 180 days. 60 % of the lagoon has a depth of 5 m or less, a few deep gullies (max. 25 m) run from east to west. The sedimen consists of sand, silty sand or silt, depending on location. The main morphometric and hydrographic data are summarised in Table 1.1. The salinity of the Veerse Meer shows large temporal and spatial variation and varies between 12.5 – 15 psu in winter and early spring to 17 – 22 psu in summer (van der Meulen & Havermans, 1981a, b; Coosen *et al.*, 1990). The large freshwater load, mainly from the polders, causes a high nitrogen load: 2.4 – 2.9 mo $N \cdot m^{-2} \cdot yr^{-1}$ (Nienhuis, 1993); total phosphorus load is moderate, 5 - 6 g $P \cdot m^{-2} \cdot yr^{-1}$ (Nienhuis, 1993). The P concentration in the water is mainly determined by the delivery from the sediment (de Vries *et al.*, 1990). Dissolved oxygen in the water and oxygen content in the bottom of the lagoon are strongly fluctuating, depending on depth, time of the year and water motion. In most years, low oxygen values (less than 30 % saturation) occur on 10 % of the area of the lagoon bottom; during warm summers, this area can increase to 40 %. The anoxic area is then about 25 % of the area of the lagoon bottom Coosen *et al.*, 1990; van de Kamer *et al.*, 1990.

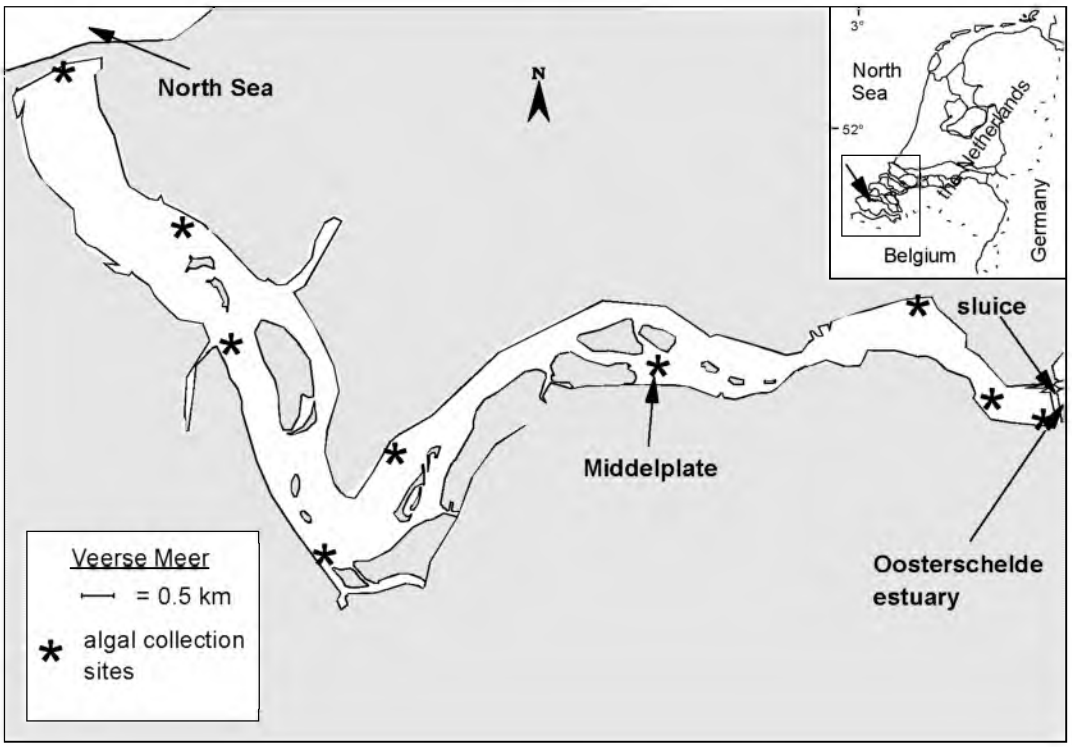

Fig. 1.1: Location of the study site, the Veerse Meer lagoon (SW Netherlands). Algal collection sites are marked with a *. Most of the fieldwork is carried out at the site "the Middelplaten".

The isolation from the sea and the Oosterschelde, the high freshwater and nutrient load and the artificial, semi-annual, tidal regime has had strong consequences for the macrophyte vegetation of the Veerse Meer. Before the closure, the hard-substrate vegetation on the dikes was dominated by perennial seaweeds (mainly fucoids), while on the soft-bottom areas the phanerogams *Zostera marina* L. and *Z. noltii* Hornem. were abundant. After the closure, both the fucoids and the seagrasses rapidly declined and were replaced by annual seaweeds. The number of seaweed species decreased from 64 before the closure (Den Hartog, 1959) to 48 in 1964 (Munda, 1967). At present, approximately 30 seaweed species occur in the Veerse Meer (E. Malta, S.G.A. Draisma, J.M. Verschuure and H. Stegenga, unpubl. results). Almost 90 % of the total July macrophyte biomass consists of species of the genus *Ulva* L. (Hannewijk, 1988; Apon, 1990). *Ulva* spp. dominate the western and central areas of the lagoon, where they occur free-floating in dense canopies. In the eastern part of the lagoon, free-floating *Ulva* spp. co-occur with dense *Chaetomorpha linum* (O.F. Müll.) Kütz. canopies (approximately 5 % of the total seaweed biomass o the lagoon). Other common macroalgae include various *Cladophora* spp. and *Enteromorpha* spp. (chlorophytes) and the rhodophytes *Callithamnion roseum* (Roth) Lyngb., *Ceramium diaphanum* (Lightf.) Roth, *C. rubrum* (Huds.) C. Ag., *Dasya baillouviania* (Gmel.) Mont. and cf *Gracilaria gracilis* (Huds.) Papenf. Eelgrass (*Zostera marina*) covers less than 3 % of the area and stil decreases slowly (Verschuure, 1994,

Table 1.1: Main morphometric and hydrographic data of the Veerse Meer lagoon (SW Netherlands).

surface area (April – October) (k ^{2})	22
surface area (October – April) (k ^{2})	18
volume (April – October) (^{3})	$102.1 \cdot 10^{6}$
volume (October – April) (^{3})	$88.6 \cdot 10^{6}$
average depth (m)	5
maximum depth (m)	25
length (km)	23
width (km)	0.5 - 2.0
residence time (d)	180
freshwater load ($m^3 \cdot y^{-1}$)	$83.0 \cdot 10^{6}$

1996, 1998). 74 % of the macrophyte biomass is concentrated on 16 % of the surface area o the lagoon. The sites used for algal collection and experiments in this thesis are marked in Fig. 1.1. Most field data are collected at a site near the islands called the "Middelplaten" (Fig. 1.1), which is considered representative for most of the shallow areas (0.50 – 1.50 m) of the lagoon. Detailed site descriptions are presented in the materials and methods sections of the subsequent chapters.

The Veerse Meer lagoon plays an important role in the tourism and recreation developments of the province of Zeeland. Related to the total recreational capacity of the province o Zeeland, about 15 % of the tourists spending their holidays in Zeeland and the related employment is localised in and around the Veerse Meer (Thomaes, 1991). The manipulations of the water level, the dense biomass and the stench caused by decomposing macroalgae all shorten the tourist season and reduce the number of tourists visiting the lagoon. The *Ulva* blooms thus limit the potential for economical growth in the tourist sector. Other important functions of the lagoon concern fishery (mainly for eel) and the nature reserve. These functions also suffer from the problems caused by the macroalgal blooms.

CHAPTER II: EFFECTS OF ENVIRONMENTAL VARIABLES ON BETWEEN-YEAR VARIATION OF *ULVA* GROWTH AND BIOMASS IN A EUTROPHIC BRACKISH LAKE

Abstract

Biomass development, growth rate, tissue composition and habitat characteristics o macroalgal blooms were monitored in the eutrophic Veerse Meer (the Netherlands) in 1992 and in 1994 to determine seasonal and between-year variation in relation to environmental factors. In both years, the blooms were dominated b *Ulva* species (more than 95 % of total macroalgal biomass). In 1992, the maximum biomass was 602 g DW·m^{-2}, in 1994 the maximum biomass was only 282 g DW·m^{-2}. Growth rates (μ), measured in cages, were high at the beginning of May 1992, but quickly dropped to values between 0.05 - 0.10 d^{-1}. In 1994, high growth rates were observed for 1 week in May only. Water nitrogen concentrations (DIN) and tissue nitrogen levels in *Ulva* spp. were higher in 1994 than in 1992. No overall difference was found in irradiance between 1992 and 1994, but at the beginning of the growing season irradiance levels were much higher in 1992. The results of a stepwise multiple regression analysis indicate that in 1992 the part of variation in growth rate that could be explained by regression was due to water DIN. In 1994, water phosphorus concentration and light were the variables explaining this part of the variation in growth rate. It is concluded that macroalgal biomass development in the Veerse Meer shows high variability, both within one season and between years. Although positive correlations were shown between tissue nitrogen levels and DIN, differences in DIN could not explain between-year variability. In a eutrophic lagoon, incident irradiance levels are probably more importan in regulating maximum macroalgal yield than DIN.

Erik-jan Malta and Jacobus M. Verschuure (1997) - J. of Sea Res. 38: 71 – 84

Introduction

Coastal zones, estuaries and lagoons comprise about 1 to 2 % of the surface of the ocean. Despite their relatively small area, the primary production represents about 20 % of the global oceanic production (Smith, 1981; Charpy-Robaud & Sournia, 1990). The high production per area has been attributed to the shallowness of most of these coastal waters, which allows the presence of benthic microphytes and macrophytes in addition to phytoplankton. In the las decades, a tendency towards increased loadings with nutrients originating from industrial and domestic waste-water, agricultural run-off and many other sources has been observed in many coastal waters the world over. This process of eutrophication is increasingly regarded as a nuisance. Eutrophication is defined here as the process of increasing the input of inorganic nutrients, inducing changes in aquatic communities (Nienhuis, 1989).

A recent overview concerning the effects of eutrophication on the benthic vegetation of European coastal waters is given by Schramm & Nienhuis (1996) and references therein. Similar effects have been described for other areas in the world (Thorne-Miller *et al.*, 1983; Tewari & Joshi, 1988; Brown *et al.*, 1990). In general, the process of eutrophication leads to a shift in the macrophytobenthic community from slow-growing seagrasses and large macroalgae towards dominance by fast-growing macroalgae and phytoplankton. The fast-growing macroalgae often form dense, free-floating mats. Characteristic species o eutrophicated macroalgal communities are mostly chloroph tes of the genera *Ulva*, *Enteromorpha* and *Cladophora* (e.g. Ho, 1981; Laver *et al.*, 1991; den Hartog, 1994), bu also other genera can be involved, including several phaeophyte and rhodophyte genera.

Duarte (1995) states that the causal factors and the main processes of eutrophication have been intensively studied, in the field as well as in experimental situations, but that these studies have failed to provide a basis for quantitative predictions on macroalgal biomass. Most studies on macroalgal blooms provide descriptions of biomass accumulation and production rates, but studies on the coupling of seasonal biomass patterns to environmental variables are rare (cf. Sfriso *et al.*, 1987, 1993; Laver *et al.*, 1991). Several mat ematical models have been developed to describe the eutrophication process (Ménesguen & Salomon, 1988; Piriou & Ménesguen, 1992; Bendoricchio *et al.*, 1994; Fong *et al.*, 1994). These models often provide an accurate description of seasonal patterns of variation of biotic and abiotic parameters, but cannot determine causal relations. Besides, application of most models is limited to the small geograph cal area for which they are developed.

The aims of the present study are: (1) to describe the between-year and seasonal variability of dominant macrophytobenthic communities in the eutrophic lake the "Veerse Meer" (the Netherlands) and: (2) to analyse the correlations between environmental variables and biomass and stoichiometry of the dominant macroalgae in order to detect the main regula ing factors for the occurrence and maintenance of these blooms.

Materials and Methods

Study area

The study was conducted in the Veerse Meer, a shallow man-made brackish lake, situated in the southwestern part of the Netherlands (51° 32' N 3° 46' E, see Fig. 2.1). Sluices connect its eastern part with the Oosterschelde estuary (S = 28 psu on average). The lake is non-tidal, but from October to April an artificial low water level (0.70 m below Mean Standard Sea Level) is maintained to facilitate its function as a receiving water body for agricultural run-off. In summer, the water level is raised again to Mean Standard Sea Level. Detailed information on hydrography and hydrochemistry can be found in Nienhuis (1989, 1992), Coosen *et al.* (1990)

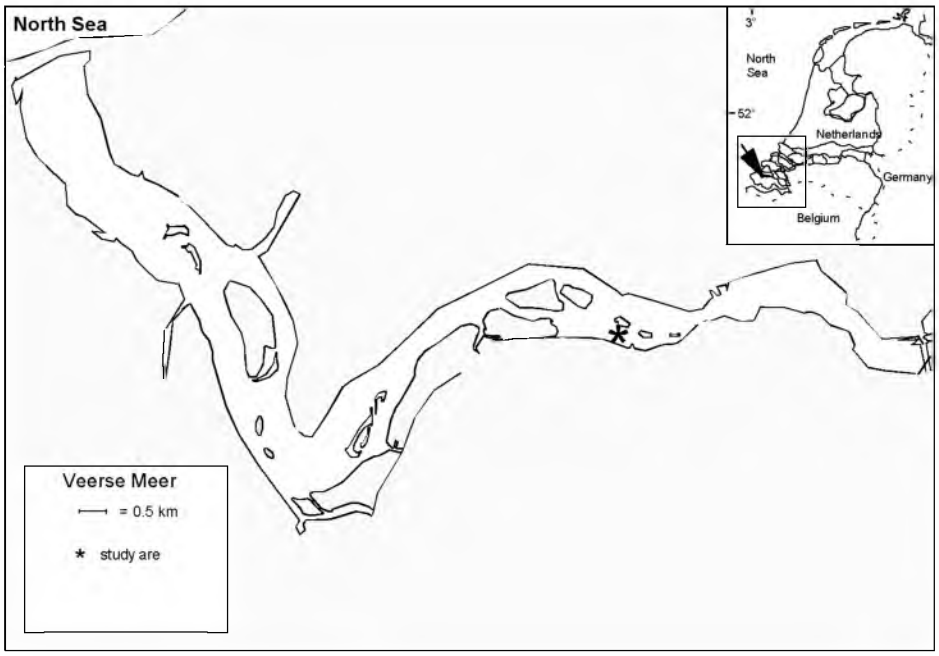

Fig. 2.1: Location of the study area and sampling site.

and van de Kamer *et al.* (1990). A range of variables has been studied at a site close to the islands called the Middelplaten (Fig. 2.1). Earlier observations (Hannewijk, 1988; Apon, 1990) showed that this site can be considered representative of most shallow areas in the lake with respect to macroalgal biomass. The site has a depth ranging from 0.70 to 0.90 m in summer. Data were collected from March to August 1992 and from March 1994 to March 1995. From the last period, only the data from March to August 1994 are used; other data will be presented elsewhere.

Water column variables, light and wind

In 1992, surface water temperature was determined fortnightly in March and weekly from April to the end of August. Each time, two water samples were taken in 1 d ^{3} PVC bottles at mid-depth (0.4 m), transported to the laboratory in ice, then filtered through Whatman GF/C glass fibre filters. Filters had been weighed prior to use. After filtration, filters were dried for 48 h at 60 °C and weighed after staying in an exsiccator for 20 minutes, in order to determine seston content. The water was analysed for nitrate + nitrite, ammonium and phosphate with a Skalar 5100 autoanalyzer and chlorinity was determined by titration with $AgNO_3$, using a Radiometer Copenhagen titrator with $_2CrO_4$ as indicator, according to Strickland & Parsons (1972). pH values of the water were determined using a Radiometer Copenhagen PHM 92 pH meter.

In 1994 sampling was continued. Samples were taken fortnightly in March and April and weekly from May to the end of August. Salinity was measured using a WTW microprocessor salinity meter mounted with a WTW KLE 1/T electrode. The salinity meter was calibrated using the titration method mentioned above.

Data on hourly incident irradiance (I_0, 300-2200 nm) in 1992 were obtained from a local station (Wilhelminadorp) of the Royal Dutch Meteorological Institute (KNMI). In 1994 I_0 was measured at the institute in Yerseke, using a LI-190SA quantum meter fitted with a PAR (400-700 nm) sensor. For comparison, the data from 1992 were transformed applying a conversion factor of 1.94 according to Kromkamp & Peene (1995). The hourly means were

summed to weekly totals. Light measurements *in situ* were performed during sampling days above and just below the water surface and at 0.20, 0.40, 0.60 and 0.80 m depths with a LiCor light meter, connected to a 2π PAR (400 - 700 nm) sensor. Reflection was calculated as the difference between above and just below surface irradiance. Because this method is quite sensitive to wave action, an overall mean reflection percentage of 11.7 (± 3.3) was used for the calculations of formula 2.1. A weekly attenuation coefficient was calculated by averaging the attenuation coefficients for the four depths. Weekly incident irradiance (I_z) at depth z was then calculated using the Lambert-Beer equation (Jerlov 1970):

$$I_z = I_{-0} \cdot e^{-kz} \quad (2.1)$$

where I_{-0} is weekly incident irradiance just below the water surface (I_0 minus reflection) and k is the weekly attenuation coefficient. Data on monthly average wind speed and wind direction at the station Wilhelminadorp were taken from the monthly weather reports of the KNMI (KNMI, 1992, 1994).

Macroalgal variables

Data on macroalgal variables were always collected on the same day as the collection of the water samples. Parts of algal material or, if possible, whole plants were transported to the laboratory for species identification with a light microscope, using the identification characteristics of Koeman & van den Hoek (1981) and Stegenga & Mol (1983). Representative samples were stored in a 4 % formaldehyde / Veerse Meer water solution and identified by Dr. H. Stegenga of the Rijksherbarium, Leiden. Algal biomass samples were collected fro the central area of the study site (determined every time by visual orientation on landmarks) by three times throwing a PVC cylinder (area = 0.16 ^{2}) into the water in a random direction. The biomass enclosed in the cylinder was collected and weighed wet after spinning for one minute in a domestic laundry centrifuge. Dry weight (DW) was determined after 48 h at 60 °C. A mean biomass value was calculated from three samples.

Growth rates of the dominant species (*Ulva* spp.) were determined from May to the end o August using cages. The cages were made of green plastic-coated steel mesh (0.01 x 0.01 mesh size) and were 0.75 m high and 0.25 x 0.25 m square and were vertically divided in three compartments (height 0.25 m) to determine growth rates at different depths. The cages were fixed to the sediment with steel poles. *Ulva* discs (42 mm in diameter) were punched from free-floating specimens with a sharpened stainless steel tube and incubated in these cages for 1 week. In order to rule out the possibility that variation was caused by variability in tissue condition and to be able to make corrections afterwards for tissue that had disappeared from the cages, five discs originating from different pieces o *Ulva* thalli were incubated per compartment. Growth was measured as the increase of biomass per compartment, because after incubation the discs could not be distinguished from each other. Before incubation, the total wet weight per compartment was determined. Fifteen other discs, sampled at the same time, were weighed wet, dried for 48 h at 60 °C and weighed again. From these discs a wet weight to dry weight conversion factor was determined (coefficient of variation was ranging from 1.5 to 3 %), which was applied to calculate the dry weight of the incubated discs. After incubation, total dry weight per compartment was determined and growth rate (μ) was calculated as the increase in dry weight per compartment as:

$$\mu = (\ln W_t - \ln W_0)/t \quad (2.2)$$

where W_0 is the initial and W_t the final dry weight after t days of incubation. Five cages were

used each week, the compartments at equal depth were considered as five replicates. When discs had disappeared from the cages (2.5 and 3.9 % of all incubated discs in 1992 and 1994, respectively), the calculation was corrected. Discs that were heavily grazed upon (0.4 and 0.5 %) were treated as missing discs. Thallus parts removed by minor grazing was very small (mostly less than 1 m^{2}) and considered to be insignificant.

To determine chemical tissue composition, pieces (1 - 2 g dry weight) of the dominan macroalgae were stored in ice and transported to the laboratory. The material was dried for 48 h at 60 ºC and ground to powder using a bullet mixer. The carbon and nitrogen contents of the algae were determined on a Carlo-Erba NA 1500 CHN-analyzer. Phosphorus contents were determined using the colorimetric procedure of Chen (1956), after tissue destruction with HNO_3-HCl in a microwave oven (Nieuwenhuize & Poley-Vos, 1989).

Data analysis

Data were analysed statistically according to Sokal & Rohlf (1995). Significance o differences in variable values between years was tested using a t-test, data obtained from the samplings taken at different intervals in April 1992 were not included in the test. Weekly differences in growth rate between years at equal depths were tested with a t-test. The effec of depth on growth (differences between compartments) was tested with an analysis of variance (ANOVA), followed by the Tukey-Kramer procedure for multiple comparisons in case of a significant ANOVA result. A significance level of 5 % was used in all tests. Data were tested for heteroscedasticity prior to t-tests or ANOVA by a Bartlett test for homogeneity. Heteroscedastic data (seston, nutrients, tissue nutrient concentrations) were log transformed. In the case of the nutrient data, logarithms of (concentration + 1) were calculated to avoid zeros. Pearson correlation coefficients were calculated between growth rate and weekl biomass increase (Δbiomass) and between nutrient concentrations in the water and in the plant tissue. The relation between growth rates and environmental variables was studied using stepwise multiple regression analysis. For correlati and for regression, nutrient concentrations and nutrient tissue concentrations were transformed in the same way as for the t-test.

Table 2.1: Ranges and means (± SD) of water column data for the Veerse Meer in 1992 and 1994. Temperature is water surface temperature, k is attenuation coefficient, n is the number of measurements; significant differences between means are marked: *** $p < 0.001$.

		1992	1994
temperature (°C)	range	6.0 - 26.5	7.0 - 26.2
	mean	18.4 ± 4.9	18.4 ± 5.2
	n	35	33
salinity (psu)	range	16.3 - 21.3	13.0 - 16.1
	mean	19.9*** ± 1.2	13.8 ± 1.0
	n	19	20
pH	range	7.7 - 9.0	8.3 - 8.9
	mean	8.6 ± 0.4	8.5 ± 0.1
	n	17	19
seston ($mg \cdot l^{-1}$)	range	7.6 - 34.9	6.2 - 16.5
	mean	14.9*** ± 6.5	9.6 ± 2.6
	n	15	15
k (m^{-1})	range	0.52 - 1.47	0.56 - 2.27
	mean	0.92 ± 0.33	1.03 ± 0.41
	n	13	13

RESULTS

Water column variables, light and wind

Data on water column variables are shown in Table 2.1. The mean water temperature for the observation period was equal for 1992 and 1994. The mean salinity was significantly lower in 1994 than in 1992 (t-test, $p < 0.001$) and week to week variation was much

higher in 1994 than in 1992. In both years, salinity increased from the onset of Mean Standard Sea Level in April until the lowering of the water level in November and decreased again from November to April of the next year. Water pH appeared to be constant with very little variability between years. Large weekly variations in seston levels were observed. The mean seston level of the water was significantly higher in 1992 than in 1994 (t-test, $p < 0.001$). However, the higher seston values in 1992 did not result in a significantly lower attenuation coefficient for the water column in that year (t-test, $p > 0.05$).

Nutrient concentrations in the water at 0.40 m depth showed clear, but contrasting, seasonal patterns (Fig. 2.2). These patterns were largely similar between years. Phosphorus levels increased from March to August. By contrast, total dissolved inorganic nitrogen levels (DIN) were high at the beginning of March, followed by a sharp decline in spring leading to low values (sometimes below detection limits) in summer. This contrast between nitrogen and phosphorus concentrations is clearly expressed in their strong negative correlation coefficient in both years (Table 2.2). From May to the beginning of September, DIN consisted mainly of ammonium (70 – 100 %) in both years, whereas in March, April and the end of August, nitrate + nitrite were the main constituents of the DIN. Initial DIN concentrations were almost

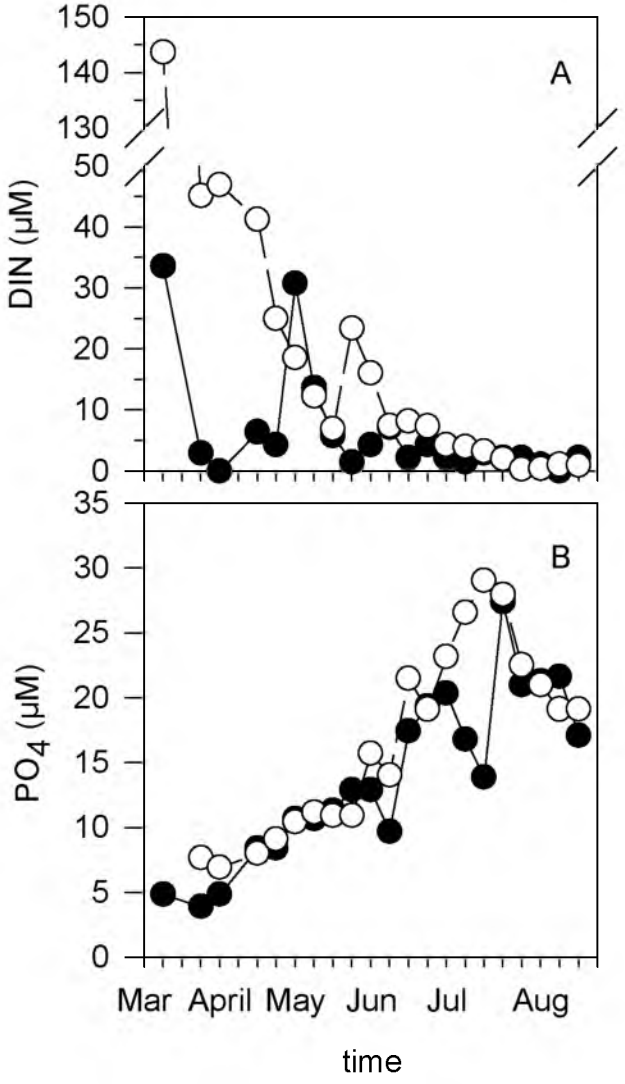

Fig. 2.2: Nutrient concentrations in surface water at the study site in 1992 (dots, full line) and in 1994 (circles, dotted line). (A) Dissolved inorganic nitrogen (DIN, µM). (B) total inorganic phosphorus (µM). Ticks on x-axis represent weeks.

four times higher in 1994 than in 1992, suggesting a higher winter nitrogen load. Mean DIN concentrations were significantly higher in 1994 compared to 1992 (t-test, $p < 0.01$). No significant difference was found for phosphorus.

Table 2.2: Pearson correlation coefficients (r) between growth rates (μ), weekly biomass increase (Δbiomass) of *Ulva* spp. and between nutrient concentrations in the water and in the *Ulva* spp. tissue. n=15 for all correlations, except fo Δbiomass (n=14). Significant correlations are marked: * $p < 0.05$; ** $p < 0.01$; n not significant.

		1992 r	1994 r
Δbiomass	μ	0.33 ns	0.05 ns
DIN	Phosphate	-0.70 **	-0.68 **
tissue N	DIN	0.38 ns	0.14 ns
tissue P	Phosphate	-0.29 ns	-0.74 **
tissue N	Tissue P	0.69 **	0.60 *
tissue N	tissue C	0.14 ns	-0.06 ns

In Fig. 2.3 the weekly incident irradiance (I_z) at mid-depth (0.4 m) in 1992 and in 1994 is plotted. I_z in 1992 was similar to I_z in 1994 (154 mol photons $m^{-2} wk^{-1}$ and 152 mol photons $m^{-2} wk^{-1}$ respectively). However, large differences were observed in the distribution over the season. In 1992 (dots in Fig. 2.3), the majority of the total I_z (between the 3rd week of March and the 2nd week of August) was recorded in the first 11 weeks of the observation period (1808 mol photons m^{-2}, 62 % of total). In 1994, in the first 11 weeks only 52 % of the total I_z was measured (1551 mol photons m^{-2}). From then on, I_z was higher in 1992 than in 1994.

The average wind speed in the period from March to August was slightly lower in 1992 than in 1994 (4.9 and 5.1 m s^{-1} respectively). The average over the growing season (May t August) was 4.4 m s^{-1} in both years. During the growing season, no storms or heavy wind (≥ 8 Beaufort) occurred. Wind direction was predominantly southwest to west-southwest in al month in both years except for July 1994 when north-eastern winds were prevailing.

Macroalgae variables

Biomass distribution was always patchy in both years, leading to (sometimes) high standard deviations. In 1992 the first measurable biomass was found in mid-May (Fig. 2.4). The vegetation was dominated by several unattached *Ulva* species (*U. curvata* [Kütz.] De Toni *U. scandinavica* Bliding and *U. lactuca* L., Ulvales, Chlorophyta). Identificati problems, resulting from taxonomic uncertainties, made a quantitative comparison of the relative share of each species to the total biomass impossible, so only tota *Ulva* spp. biomass will be considered further. Algal biomass increased rapidly in 1992, reaching its maximum value in the last week of June (602 g DW m^{-2}). This was followed by an almost equally rapid decrease to approximately 390 g DW m^{-2} by the

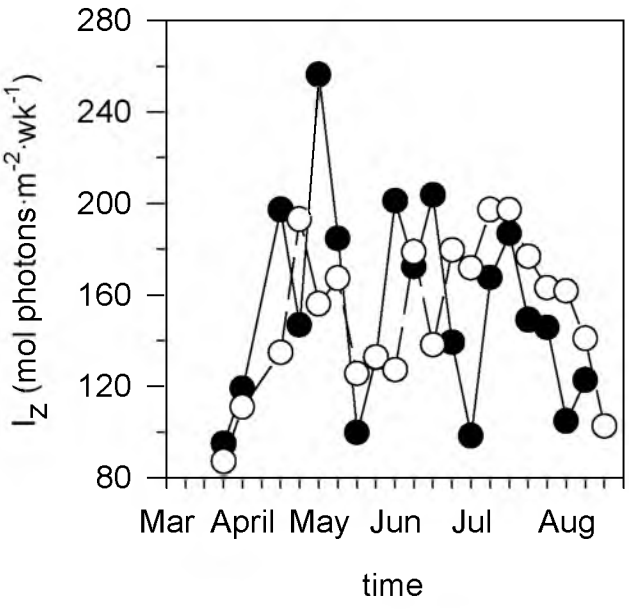

Fig. 2.3: Irradiance (I_z, mol photons $m^{-2} wk^{-1}$) at 0.40 m depth at the study site in 1992 (filled circles, straight line) and in 1994 (open circles, dotted line). Ticks on x-axis represent weeks.

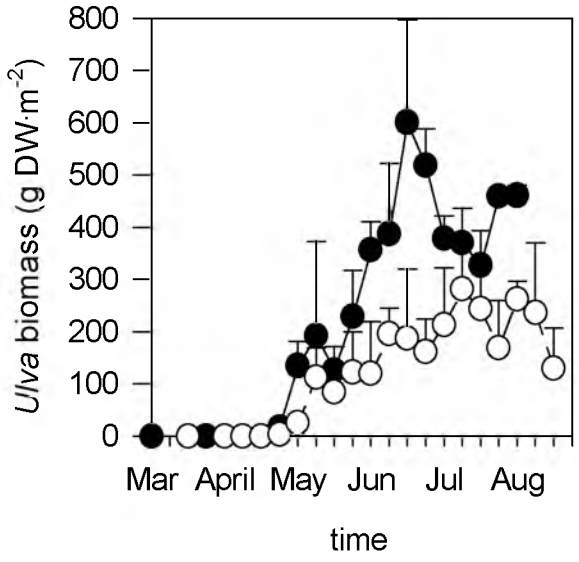

Fig. 2.4: Biomass values (g DW·m^{-2}) of *Ulva* spp. at the study site in 1992 (dots, full line) and in 1994 (circles, dotted line). The figures are mean values + 1 SD (n=3). Ticks on x-axis represent weeks.

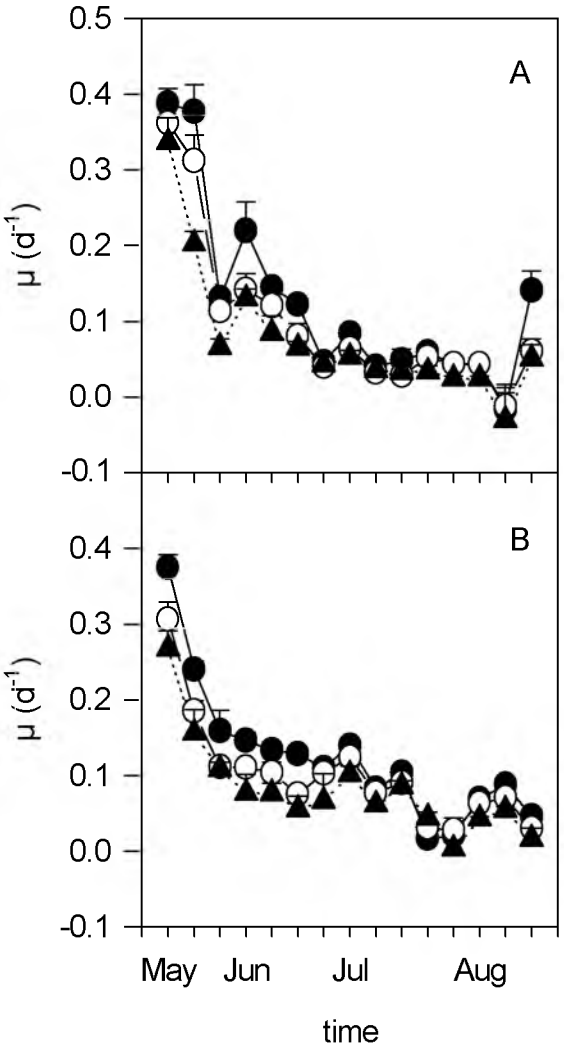

Fig. 2.5: Specific growth rates (μ, d^{-1}) of *Ulva* sp. in cages in 1992 (A) and 1994 (B). Growth rates from upper compartment (dots, full lines), middle compartment (circles, dotted line) and lower compartment (triangles, dotted line). The figures are mean values and + 1 SD is indicated (n=5). Ticks on x-axis represent weeks.

beginning of August, after which high values were found again. Apart from *Ulva* spp., the macroalgal vegetation consisted of floating thalli of the rhodophytes *Ceramium diaphanum* [Lightf.] Roth. and *C. rubrum* [Huds.] C. Ag. and the chlorophyte *Chaetomorpha linum* [O.F. Müll.] Kütz. However, the biomass of these species was negligible (less than 1 % o *Ulva* biomass, data not shown). Several individuals of *Enteromorpha linza* [L.] J. Ag. (*Ulva*les, Chlorophyta) were observed growing as epiphytes on *Ulva* spp.

In 1994, macroalgal biomass developmen followed largely the same pattern as in 1992 (Fig. 2.4). Biomass build-up started in the same week as in 1992. The rate of biomass increase was lower (especially in June), resulting in a considerably lower maximum value (282 g DW·m^{-2}), which was reached in mid-July. Four weeks later biomass levels decreased to around 150 g DW·m^{-2}. A qualitative observation of the *Ulva* bloom revealed that *U scandinavica* appeared first, followed b *U curvata*. At the end of August, *U lactuca* also appeared in small amounts and stayed until the end of the observation period. In the summer of 1994 some unattached *Gracilaria verrucosa* [Huds.] Papenf. (Graciariales, Rhodophyta) thalli were found and at the end of August the chlorophyte *Cladophora vagabunda* [L.] van den Hoek grew as an epiphyte on *Ulva* spp., accompanied by floating thalli of the phaeophytes *Pilayella littoralis* [L.] Kjellm. and *Ectocarpus siliculosus* [Dillw.] Lyngb. However, the biomass of these algae was always less than 1 % of the *Ulva* biomass (data not shown).

Ulva growth rates in the upper compartment in 1992 were high at the beginning of May (μ =

0.39 d^{-1}), but quickly decreased to values around $\mu = 0.07\ d^{-1}$ in July and even became negative (decomposition) in the 2nd week of August (Fig. 2.5a). A slight recovery was observed in the last week of the observation period. In 1994, growth rates in the upper compartment were high for one week only ($\mu = 0.38\ d^{1}$) and decreased thereafter (Fig. 2.5b). Decrease was much more gradual compared to 1992. The lowest values were observed at the end of July, after which a short recovery was observed, followed by another decrease. Mean growth rates of the upper compartments were higher in May, the beginning of June and in the 3rd week of August of 1992 than in the same periods in 1994. However, in the remaining period, growth rates were higher in 1994 than in 1992. All weekly differences between years were significant (t-test, $p < 0.05$ - 0.001), except for the last week of June and the 1st week o July. Testing between years for the middle and bottom compartments gave similar results as testing between the upper compartments. The mean annual (mean of all weeks) growth rate was equal for 1992 and 1994 ($\mu = 0.10\ d^{-1}$).

In most weeks in both 1992 and 1994, growth rates were highest in the top compartment and lowest in the bottom compartment, with the middle compartment having intermediate values (Fig. 2.5). In most weeks in 1992, differences between the top and middle compartments and between the middle and bottom compartments were not significant, except for a few weeks when differences were large. Differences between the top and bottom compartments were almost always significant (ANOVA, $p < 0.05$ - 0.01) except for the last week of June, the 2nd and 3rd week of July and the 2nd week of August. In 1994, differences between the top and middle compartments were significant from May to the 3rd week of June (ANOVA, $p < 0.01$ - 0.001), but not after that period. Differences between the middle and bottom compartments were usually not significant. Differences between the top and bottom compartments were significant in all weeks (ANOVA, $p < 0.01$ - 0.001) except for the 2nd and the last week o July. In both years, no significant correlation was found between the weekly biomass increase and growth rate (Table 2.2).

Trends in the seasonal cycles of tissue nitrogen, carbon and phosphorus were comparable for *Ulva* spp. in 1992 and 1994 (Fig. 2.6). Tissue N levels were high in early spring, decreased with the onset of biomass build-up in May and became high again in late summer and autumn (Fig. 2.6b). Tissue C and P levels roughly followed this pattern in both years (Fig. 2.6a,c). Tissue P showed a significant positive correlation with tissue N in both years (Table 2.2). Maximum tissue N levels for *Ulva* spp. were 5.55 in 1992 and 5.01 in 1994 (% DW). Minimum values were 0.89 for 1992 and 1.49 for 1994 (% DW). Mean tissue N levels in *Ulva* spp. were significantly higher in 1994 than in 1992 (t-test, $p < 0.05$). Differences for tissue C and P were not significant between years. Annual mean atomic C:N ratios were 17.2 for 1992 and 13.2 for 1994. Atomic C:N ratios from May to September were always > 20 in 1992 (maximum 31.9) and always > 15 in 1994 (maximum 25.6). Tissue nitrogen concentrations were not correlated to DIN in both years (Table 2.2). Tissue P and phosphorus concentrations were significantly negatively correlated in 1994; the negative correlation was not significan in 1992. Tissue N and P showed significant positive correlations in both years. No correlations were found between tissue C and tissue N (Table 2.2).

Growth rates and environmental variables

To examine the relation of growth rates with nutrients, temperature, salinity and ligh together, stepwise multiple regression analysis was used (Table 2.3). In 1992, 48 % of the variation observed in growth rates was explained by only one variable, DIN. When tissue N was used in the analysis instead of DIN, growth rates was only related to I_z. However, in this model R^2 was lower than in the first model. In 1994, coefficients of determination were much

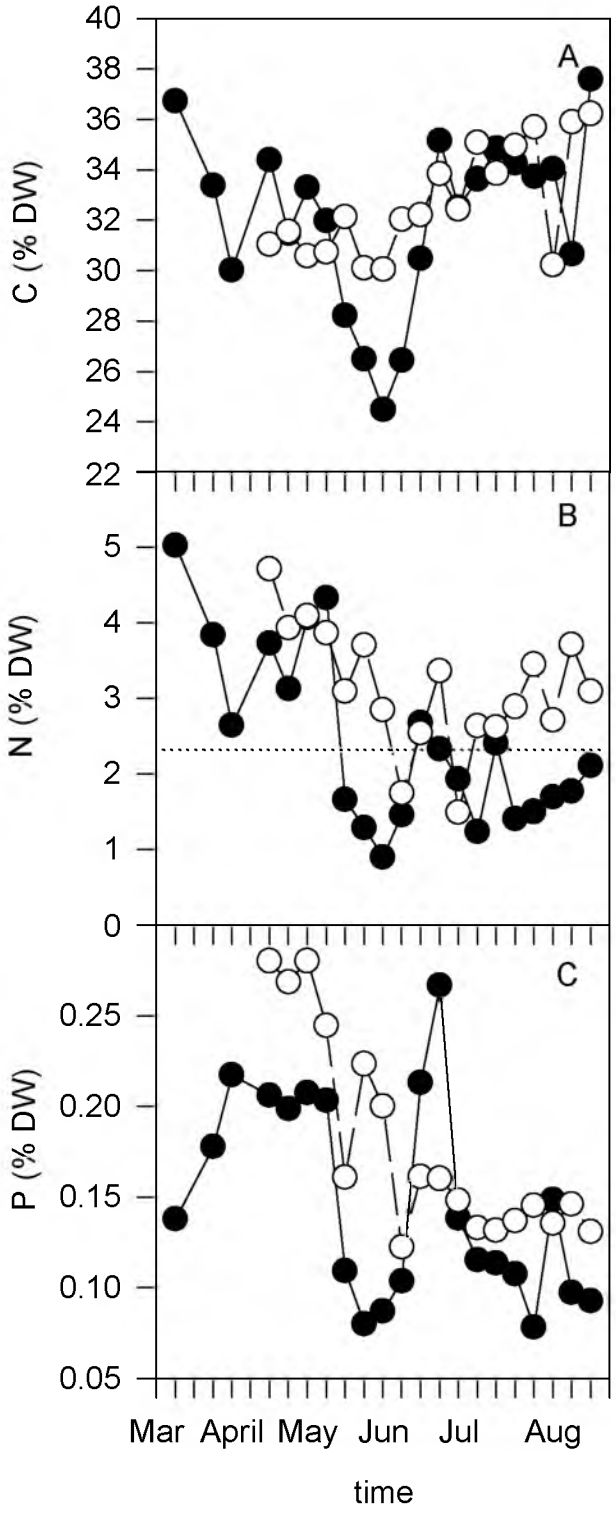

Fig. 2.6: Tissue carbon (A), nitrogen (B) and phosphorus (C) content (% DW) of *Ulva* sp. at the study site in 1992 (dots, full line) and in 1994 (circles, dotted line). Ticks on x-axis represent weeks. Horizontal line in (B) indicates critical nitrogen values, see text in discussion for explanation.

higher. Phosphorus concentration showed a negative partial regression coefficient, light was positively regressed in growth rates. When nutrient concentrations in the water were replaced by tissue nutrient concentrations in the analysis, only tissue P showed a positive significant regression coefficient. R^2 was similar for both regression models in 1994.

Discussion

Several distinct differences were found between the 2 years studied. *Ulva* spp. biomass was much higher in 1992 than in 1994, but, average annual growth rates were equal. Chemical composition of *Ulva* spp. tissue showed strong seasonal variability as was observed before in various systems (Duke *et al.*, 1987; Lavery & McComb, 1991). In addition, the existence o large between-year variation in tissue composition is shown in the present study.

Biomass and growth rates

In 1992 and 1994, algal mass development in the Veerse Meer started in May (Fig. 2.4). In 1992, biomass increased rapidly within two weeks. In 1994, peak biomass values were reached much later than in 1992 and they were much lower. In June 1992, biomass collapsed, followed by a recovery in the las 2 weeks of the observation period. In 1994 the decrease was less pronounced. Maximu biomass values found (602 and 282 g DW m^{-2} for 1992 and 1994, respectively) were in the same order of magnitude as the values found for mats of *Ulva* and other macroalgal species in other eutrophic areas. Niel *et al.* (1996), and references therein, report biomass values for *Ulva*-dominated algal mats on the Spanish Atlantic coast ranging from 250 to 827 g DW m^{-2}. In the hypertrophic Venice lagoon, *Ulva* biomass values between 700 and 1400 g DW m^{-2} were recorded (Sfriso *et al.*, 1992). Reports for *Enteromorpha* spp. mats, a closely related green alga, include values of 425 - 625 g DW m^{-2} (Pihl *et al.*, 1996) for the Swedish west coast and 750 g DW m^{-2} for a mudflat in Oregon (Pregnall Rudy, 1985).

High spring growth rates (up to 0.39 d^{-1} in the upper cage compartments) were also observed in both years (Fig. 2.5).

Table 2.3: Partial regression coefficients and ANOVA test results of stepwise multiple regression analysis relating specific growth rate (μ) of *Ulva* spp. in 1992 and 1994 in the Veerse Meer to nutrient concentrations, salinity, water temperature and light at 0.40 m depth (upper part of table). In the lower part of the table, tissue nitrogen and phosphorus values were used instead of nutrient concentrations in the water. Constant is the constant value returned by the multiple regression model. Variables, marked a ns (non-significant) did not show significant partial correlations and were not included in the multiple model. Significant partial regression coefficients and F values are marked: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

independent	dependent variables	
variables	μ 1992	μ 1994
constant	-0.04 ns	0.52 ***
DIN	0.11 **	ns
phosphate	ns	-0.22 ***
salinity	ns	ns
temperature	ns	ns
light	ns	0.001 *
F	13.69 ***	16.02 ***
R^2	0.48	0.68

	μ 1992	μ 1994
constant	-0.11 ns	0.55 ***
tissue N	ns	ns
tissue P	ns	0.25 ***
salinity	ns	ns
temperature	ns	ns
light	0.001 *	ns
F	6.82 ***	28.33 ***
R^2	0.30	0.66

Cage-average growth rate corresponded with growth values found for *U lactuca* in Denmark (μ values between 0.04 and 0.19 d^{-1}; Geertz-Hansen *et al.*, 1993) and for subtropical *U fasciata* in outdoor flow-through tanks (μ values between 0.10 and 0.35 d^{-1}; Lapointe & Tenore, 1981). Biomass fluctuations observed in June, July and the first half of August could not be explained by variations in growth rate found in the cages. This is in line with the lack of correlation between growth rates and weekly biomass variation (Table 2.2). The possible causes of discrepancies between cage-growth results and biomass variations have been pointed out earlier by Sfriso (1995). Algae grown in cages cannot model all the processes tha occur in dense algal masses. Processes which are not, or to a lesser extent, accounted for when measuring growth rates of macroalgae in cages or bottles, can include self-shading (Pregnall & Rudy, 1985), transport by waves and wind (Lowthi *et al.*, 1985; Soulsby *et al.*, 1985), grazing (Geertz-Hansen *et al.*, 1993), decomposition (Sfriso, 1995) and desiccation (Beer & Eshel, 1983), which are mainly loss processes. However, cages are helpful to determine the rate of the gain process, growth, and how it is related to the environmenta variables in the natural environment. Pihl *et al.* (1996) present a model in which cage-growth rates of various green algae agree with variable fluorescence measurements.

Tissue composition and nutrient limitation

Nitrogen is often considered to be the main limiting factor for macroalgal growth in mos coastal waters of temperate regions (Hanisak, 1983; Rosenberg, 1985; Fong *et al.*, 1993). Our results support this view, as the higher nitrogen load and average annual nitrogen concentration in 1994 was reflected in the higher tissue nitrogen levels of *Ulva* spp. in that year. Nitrogen mainly occurred in the Veerse Meer in the form of nitrate and ammonium. During the growing period, ammonium was the main component of DIN. Although the use of ammonium as the main nitrogen source is usually preferred by macroalgae (Hanisak, 1983), *Ulva* spp. have been shown to be able to take up both ammonium and nitrate at high rates (Pedersen, 1994; Hein *et al.*, 1995), therefore we only considered the total nitrogen concentration. It is commonly suggested that the chemical composition of algae can be used to predict nutrien limitation (Atkinson & Smith, 1983; Neori *et al.*, 1991; Lobban & Harrison, 1994). According to Atkinson & Smith (1983) the optimum atomic C:N ratio for *Ulva* growth is 9.6. In this concept, a higher C:N ratio would be an indication of nitrogen limitation. In *Ulva* from the Veerse Meer, C:N ratio was always higher from May to September, both in 1992 (≥ 20) and in 1994 (≥ 15). This suggests that growth o *Ulva* spp. was always nitrogen limited, in 1992 even more than in 1994.

Another, and probably better, way to draw conclusions on possible nitrogen limitation is not to look at the C:N atomic ratio, but the tissue nitrogen content per se. This can be compared with published experimental data on critical nitrogen values, i.e. the nitrogen content below which growth becomes nitrogen-limited. These critical values were determined for *U rigida* (2.0 % DW, Lavery & McComb, 1991 or ≤ 2.4 % DW, Fujita *et al.*, 1989) and for *U lactuca* (2.1 % DW, Pedersen, 1994) and show a remarkable similarity. Applying these values to the *Ulva* species in the Veerse Meer, it can be seen from Fig. 2.6b that tissue nitrogen levels were lower than the experimental critical levels at most sampling dates in 1992. In 1994 this was the case only at two moments. We conclude tha *Ulva* spp. growth in 1992 was mainly nitrogen limited, while in 1994 critical nitrogen levels were rarely reached. This conclusion is supported by the results of the stepwise multiple regression analysis. In the best model in 1992 (model with DIN, highest ^{2}), DIN appeared to be the only variable which could explain some of the variation in μ, while in 1994, incident irradiance, phosphorus and tissue P seemed to be the explaining variables in models with a similar R^2.

Phosphate limitation of algal growth rarely occurs in the marine environment (Ryther & Dunstan, 1971; Lobban & Harrison, 1994). To our knowledge no critical phosphate levels have been determined for *Ulva* spp. However, considering the high and increasing phosphorus concentration in the water during the growing season in both years (Fig. 2.6) it is very unlikely that phosphorus was limiting *Ulva* growth in the Veerse Meer in 1992 and 1994. The negative correlations found between tissue P and phosphorus (Table 2.2) support this conclusion. The increase in phosphorus concentration in the water is caused by ground water seepage and temperature-mediated release from (anoxic) sediments (Coosen *et al.*, 1990; Nienhuis, 1992) and these processes occur at the same time and on the same time scale as macroalgal growth. This explains why phosphate and tissue P were included in the multiple regression model of 1994 (Table 2.3), which in this case represents a merely coincidental relationship.

Between-year variation, relation with environmental variables

Data on biomass variation between different years are very scarce, and on growth rate variation no data at all are available in the literature. Sfriso *et al.* (1992) showed large biomass variation between two consecutive years for two sites in the Venice lagoon, while for two other sites, variation was very small. Pihl *et al.* (1996) found large differences in biomass between 1993 and 1994 in one of the nine Swedish bays they studied. This variation was accompanied by a large environmental variation (the study site was emerged for weeks in April and May 1994 due to extreme meteorological conditions). In Veerse Meer, the weather in 1992 was very similar to that in 1994, with hot summers in both years (KNMI, 1993, 1995), resulting in equal mean surface water temperatures (Table 6.1). Average wind speed was also very similar in both years and the dominant wind direction was the same in all months, except for July 1994 when northeastern winds were prevailing. As the Veerse Meer is non-tidal, macroalgal transport is mainly driven by wind action. However, the wind was not very strong in July and with a northeastern wind one would expect a piling up of biomass at the site in 1994, which was not the case. From this it can safely be assumed that wind-driven transport processes were not much different between 1992 and 1994.

Decomposition and grazing were not included in this study. Decomposition mainly occurs a the bottom of the algal mats, where anoxic conditions occur (de Casabianca-Chassany, 1989; Sfriso, 1995) and is enhanced at high temperatures (Mohsen *et al.*, 1974; Viaroli *et al.*, 1992; Israel *et al.*, 1995). Consequently, decomposition is important mainly during summer, when maximum biomass values have already been reached. Thus, in years with similar summer temperatures, no large between-year difference can be expected from decomposition processes. Invertebrate grazing can be an important cause of algal biomass reduction (Geertz-Hansen *et al.*, 1993; Sfriso & Pavoni, 1994). In contrast to these observations, the *Ulva* thalli used in our cages (not excluding herbivore grazers) usually showed no signs of grazing. Results from cage experiments in the Veerse Meer in 1995 also showed that no significan biomass reduction can be expected by invertebrate grazing (Kamermans *et al.*, unpubl. data). Large amounts of biomass can be consumed by birds such as the coot, *Fulica atra* and mute swan, *Cygnus olor* (Coosen *et al.*, 1990; Seys *et al.*, 1991; Horne *et al.*, 1994). However, thi only takes place during late summer and autumn: coots are most numerous in November (Meire *et al.*, 1991).

Variables that differed between 1992 and 1994 were salinity, nitrogen levels and the seasona distribution of incident irradiance. Salinity, which is of paramount importance for the distribution and growth of seaweeds (Lüning, 1990), was much higher and fluctuated less in 1992 than in 1994. No optimum salinity values are known in the literature for *Ulva* growth, but several authors have reported the presence of various members of the genus at a wide

range of salinity levels (Munda, 1978; Josselyn & West, 1985). Preliminary results fro growth experiments at various salinity levels with *Ulva* spp. from the Veerse Meer (Chapter 5), suggest that for *U. scandinavica* (probably the main species in the Veerse Meer) μis higher at high salinity levels. As the rate of biomass increase in the Veerse Meer was highest when salinity was still at a low level (spring) and as no significant correlations were found between μ and salinity in the 2 years, it can be concluded that salinity was not, or only to minor extent, the factor responsible for the lower biomass in 1994.

From the comparisons of the tissue N levels with published data on critical nitrogen levels, we conclude that in 1992 μ was probably nitrogen-limited for the major part of the growing season, while in 1994, this was much less important. The main delay in growth and biomass development in 1994, compared to 1992, occurred at the beginning of the season (May and early June). In this period, irradiance was higher in 1992 than in 1994 (Fig. 2.3). This suggests that light may have been the main regulating factor responsible for the between-year difference in biomass. It also indicates that a relatively short period (20 days or less) of the growing season can be crucial in controlling total macroalgal yield. The suggestion for light limitation is supported by the growth data. At the beginning of the season, μ was higher in 1992 compared to 1994. Differences between the vertical compartments, especially between the top and the middle compartment, were more pronounced in 1994 and mostly significant. This indicates a stronger response to low irradiance levels, while in 1992 irradiance values were probably near saturation for growth. Coutinho and Zingmark (1993), demonstrated the importance of light over nitrogen as a regulating factor in outdoor growth experiments with *U. curvata*. It remains unclear, however, why in 1994 biomass build-up is not continued later in the season, when there was more than enough light available in both years. High temperatures may stimulate respiration, causing temporal anoxia, resulting in die-off and decomposition. This phenomenon was observed during dystrophic crises in the Po River Delta (Viaroli *et al.*, 1992), the Venice lagoon (Sfriso *et al.*, 1987, 1992) and in the tank experiments of Israel *et al.* (1995). High temperatures might also have an inhibitory effect on the growth of the *Ulva* spp. Experiments on how growth responds to temperature are needed to draw further conclusions.

CHAPTER III: SPATIAL VARIATION OF MACROALGAL BIOMASS IN A BRACKISH EUTROPHIC LAKE IS CAUSED BY LOSS PROCESSES

Abstract

The seasonal regulation of *Ulva* growth and biomass production and the spatial distribution of the algae were investigated at two contrasting sites in the eutrophic Veerse Meer (the Netherlands). At the sheltered site Middelplaten, macroalgal biomass was dominated (more than 95 % of macroalgal biomass) by *Ulva* species, while at the more exposed site Kwistenburg, a mixture of *Ulva* spp. and *Chaetomorpha linum* dominated. Total summer macroalgal biomass was higher at Middelplaten than at Kwistenburg (282 and 79 g $DW \cdot ^{-2}$ respectively). Growth rates o *Ulva* spp., measured in cages at both sites, were high in the beginning of May 1992 (0.28 - 0.30 d^{-1} cage mean), but quickly dropped to values between 0.05 - 0.10 d^{-1}. During the biomass build-up phase, growth rates were significantly highest at Kwistenburg, while during the rest of the season; growth rates were similar for both sites or slightly higher at Middelplaten. Temperature, pH, dissolved oxygen, salinity, light attenuation, phytoplankton and nutrient concentrations did not display between-sit differences. It is concluded that the difference between sites in *Ulva* spp. biomass in the Veerse Meer is determined by loss processes and not by growth. The exact cause for this remains unclear, however it is hypothesised that transport of macroalgae by wind and waves is a very important factor. Four phases were distinguished in the seasonal biomass variation of *Ulva* spp. at Middelplaten: a dormant phase, a build-up phase, a stationary phase and a decomposition phase. At Kwistenburg, this pattern was less pronounced. The relation between variation in *Ulva* sp. growth rates and environmental parameters was analysed using stepwise multiple regression. The analysis showed that during the build-up phase, light and temperature were the main variables regulating *Ulva* spp. growth rates. At the end of the build-up phase, the algae became nitrogen limited. Temperature probably plays an importan role in the transition from the stationary phase to the decomposition phase. Field observations on the nitrogen and chlorophyll contents and the chlorophyll *a*:*b* ratio of the algae suppor these conclusions.

Erik-jan Malta, Jacobus M. Verschuure & Pieter H. Nienhuis – Bot. Mar. (accepted)

Introduction

One of the most conspicuous results of eutrophication in shallow coastal waters is the mass development of macroalgae. Typical species of eutrophicated macroalgal communities are Chlorophytes of the genera *Ulva*, *Enteromorpha* and *Cladophora* (e.g. Ho, 1981; Lavery *et al.*, 1991; den Hartog, 1994). Eutrophication effects on the benthic vegetation of coastal waters is reviewed by Schramm and Nienhuis (1996) for Europe and by Thorne-Miller at al (1983), Tewari and Joshi (1988) and Brown *et al.* (1990) in other parts of the world. In general, the process of eutrophication leads to a shift in the macrophytobenthic com munity from slow-growing seagrasses and large macroalgae (for instance *Fucus* spp. and *Pelvetia canaliculata*) to phytoplankton and fast-growing macroalgae (Sand-Jensen & Borum, 1991; Duarte, 1995). The latter often form dense, free-floating mats. The high surface-area to volume ratio of these massforming macroalgae favours rapid nutrient uptake, high production and rapid growth rates, which enable them to outcompete the original vegetation (Littler & Littler, 1980; Hein *et al.*, 1995).

The causal factors and main processes of eutrophication have been intensely studied, in the field as well as in the laboratory. Conditions that determine the vulnerability of water bodies to eutrophication and the subsequent development of mass blooms are high nutrient loadings, a long residence time and reduced mixing with nutrient poor (oceanic) waters (Morand & Briand, 1996). However, the seasonal regulation of algal growth and biomass production and the spatial distribution of the algae is still poorly understood (Duarte, 1995). Especially the mechanisms that lead to variation between sites within one estuary or lagoon are only faintly described.

Within the framework of the European EUMAC project (EUMAC, 1994), a field study was started. The aim of the EUMAC project was to detect correlations between the temporal, seasonal and spatial variation in macrophyte biomass and species distribution and environmental variables in eight European lagoons. In a previous paper, Malta & Verschuure (1997) stressed the importance of light as the main variable responsible for the between-year variation in macroalgal biomass at the Dutch site (the Veerse Meer). Nitrogen was more important for the seasonal regulation of growth and biomass development. In this study, we compared two sites in the Veerse Meer, which are contrasting with respect to macroalga biomass and species distribution. It is hypothesised that differences in growth rates o dominant macroalgae (*Ulva* spp.) are responsible for the observed differences in alga biomass and species distribution and that these differences are caused by between-site differences in environmental variables. These hypotheses are tested using growth experiments and by the calculation of Pearson correlations and multiple regression coefficients between the seasonal variation of growth rates and the environmental parameters.

Materials and Methods

Study area

The study was conducted between March 1994 and March 1995 in the Veerse Meer, a shallow man-made, brackish (salinity = 15 - 20 psu average) lake situated in the southwestern part of the Netherlands (Fig. 3.1). The lake is non-tidal, but from October to April, an artificial low water level (Mean Standard Sea Level -0.70 m) is maintained. See Nienhuis (1989, 1992) and Coosen *et al.* (1990) for details on hydrography and hydrochemistry. We selected two sites for this study (Fig. 3.1) which were contrasting with respect to macroalga species and biomass distribution (Hannewijk, 1988; van Lent & Verschuure, 1994). The first site, Middelplaten, is representative for most of the shallow sites of the lake and is situated close to the islands called the Middelplaten, about 50 - 75 m from the gully. The second site i

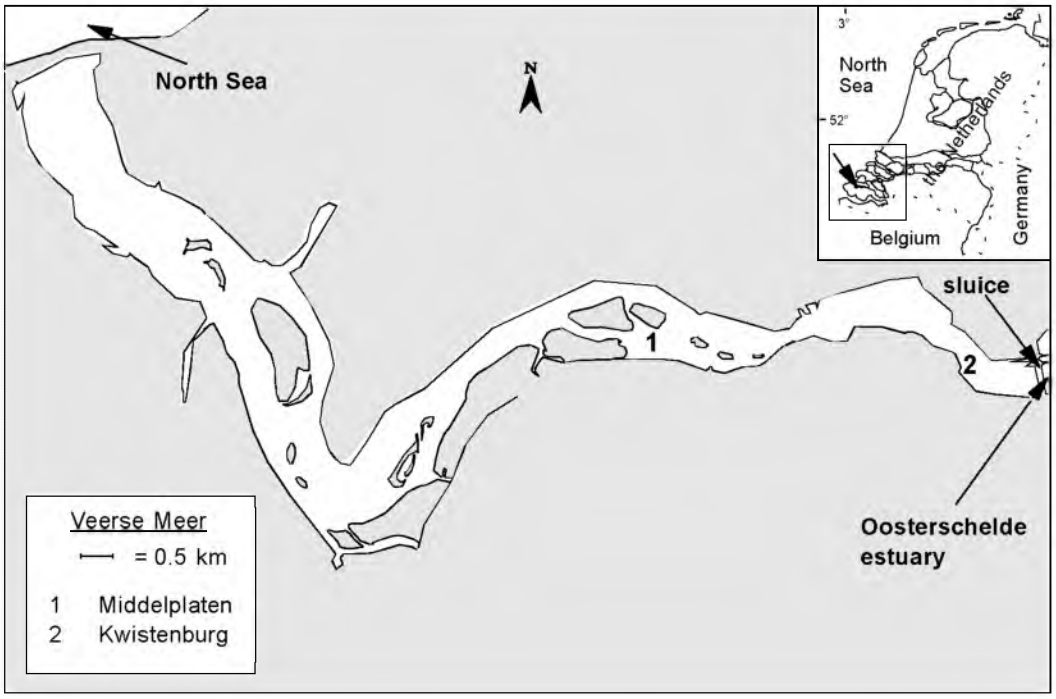

Fig. 3.1: Location of the study area and the study sites.

called Kwistenburg (4.5 km east of Middelplaten). It is bordering the main gully opposite to the sluices called the Zandkreeksluis. Water depth in summer in both sites is between 0.70 and 0.90 m.

Macroalgal parameters

The sites were visited at two-week intervals in April and October 1994, weekly from May to October 1994 and monthly from November 1994 to April 1995. Macroalgal thalli were transported to the laboratory for identification using a light microscope. Biomass was determined by randomly taking three samples with a PVC cylinder (area = 0.16 ^{2}). The *Ulva* spp. coverage in each cylinder was estimated and the biomass enclosed was collected, dried for 48 h at 60 °C and weighed. To account for the patchy distribution of the algae, a permanent quadrate (PQ) of 10 x 10 m, which was considered representative for the site, was laid out at both sites. Macroalgal coverage in the PQ was estimated with an underwater viewer. Biomass per cylinder was corrected for PQ coverage as:

$$\text{biomass}_{PQ}\ [m^{-2}] = \text{biomass}_{cylinder}\ [m^{-2}] \times (\text{coverage}_{PQ} / \text{coverage}_{cylinder})$$

Growth rates of the dominant species (*Ulva* spp.) were determined from May until the second week of October (last week of high water level) using cages. The cages, covering the whole water column, were vertically divided in three compartments (see Malta & Verschuure, 1997 for details). Each compartment contained five *Ulva* discs (42 mm diameter), punched from free-floating specimens with a sharpened stainless steel tube. Growth rate per compartmen was calculated as:

$$\text{growth rate} = \ln (W_t - W_0)/t$$

where W_0 is the initial and W_t the final dry weight after t days of incubation. Five cages per site were used, the cages were cleaned every week. A correction was made for missing discs and discs that were heavily grazed upon (3.9 and 0.5 % of all incubated discs respectively) by

multiplying the initial weight with the number of discs found after one week divided by five, assuming that discs have a similar weight. Thallus removed by minor grazing was very smal (less than 1 mm^2) and considered being insignificant. It can be argued that net growth rates (including grazing) may be more valuable than potential growth, however, considering the rare occurrence of heavy grazing and the point that flushing out of the cages is probably the most important loss factor we felt that the correction is needed to prevent an underestimation of growth. The discussion further reviews the role of grazing.

We attempted to measure the growth rate of the other dominant alga at Kwistenburg, *Chaetomorpa linum*. The algae were loosely wrapped in nylon nets, which were weighed we before and after one week incubation in the cages. However, only negative growth rates were found, probably due to biomass losses during the procedure. As we doubted the reliability o the method, we decided not to use these data any further.

Small samples of algal material were dried for 48 h at 60 °C and ground using a bullet mixer. The carbon and nitrogen content was determined on a Carlo-Erba NA 1500 CHN-analyser. Phosphorus content was determined using the colorimetric procedure of Chen (1956), after tissue destruction with HNO_3-HCl in a microwave oven (Nieuwenhuize & Poley-Vos, 1989). Additionally , pieces of macroalgae were stored at -80 °C in tin foil. The samples were freeze-dried and ground in a bullet mixer and tissue chlorophyll *a* and *b* were determined using reverse phased HPLC (Millipore Waters) according to Brown *et al.* (1981) after overnight extraction in the dark in a 90% acetone solution.

Water column parameters and light

During each visit, air and surface water temperature and weather conditions were recorded. Water samples were taken at mid-depth at each site with 1 L PVC bottles and filtered over Schleicher & Schuell no. 6 glass fibre filters that were weighed before filtration. Two filters were dried for 48 h at 60 °C and weighed after 20 min of cooling in an exsiccator to determine the seston content. The other filters were stored in tin foil at -80 °C and later extracted overnight in the dark in 90% acetone. The extract was analysed for chlorophyl *a* and *b* using reverse phased HPLC (Millipore Waters) according to Brown *et al.* (1981).The water was analysed for nitrate + nitrite, ammonium and phosphate on a Skalar 5100 autoanalyzer and salinity was measured using a WTW microprocessor salinitymeter mounted with a WTW KLE 1/T electrode.

Hourly incident irradiance (I_0) was measured with a LI-190SA quantummeter fitted with a PAR (400-700 nm) sensor, which was situated on top of the NIOO-CEMO building in Yerseke (approximately 30 km from the field sites). Light attenuation was measured at four depths in the Veerse Meer with a LiCor light meter, connected to a 2π PAR (400 - 700 nm) photon sensor. Reflection was calculated as the difference between above and just below surface irradiance. Because this method is sensitive to wave action, an overall average reflection of 11.7 % ($\pm$ 3.3) was used in the irradiance calculations. A weekly attenuation coefficient (k) was calculated by averaging the attenuation coefficients for the four depths. Weekly incident irradiance (I_z) at depth z was then calculated using the Lambert-Beer equation (Jerlov, 1970).

Data analysis

Differences between sites in water column parameters, light, weekly algal growth rates and chemical composition of the algae were examined for significance using a t-test. Water chlorophyll, seston, oxygen and nutrient concentrations and tissue nutrient concentrations were log transformed in order to avoid heteroscedasticity (tested with a Bartlett test for homogeneity, Sokal & Rohlf, 1995). Differences in growth rates between cage compartments were tested for significance using a one-way analysis of variance followed by a Tukey-Kramer a posteriori test in case of a significant ANOVA result. To detect relations between growth rate and weekly biomass change (dbiomass) and between nutrient concentrations in the water and in the plant tissue, Pearson correlation coefficients were calculated. The degree of variation in weekly mean growth rate that can possibly be explained by the simultaneous effect of the environmental parameters (dissolved inorganic nitrogen, phosphate, salinity, temperature, light and tissue nutrient concentrations) was estimated using a multiple stepwise regression analysis (Sokal & Rohlf, 1995). For the regression equation and further explanations, see the results and discussion sections. The data for the correlations and the multiple regression were transformed in the same way as for the ANOVA and t-tests.

Results

Macroalgae parameters

At Middelplaten, macroalgal biomass consisted almost entirely of two free-floating *Ulva* L. (Ulvales, Chlorophyta) species: *U curvata* (Kütz.) De Toni and *U scandinavica* Bliding. Identification problems, resulting from taxonomic uncertainties (Malta *et al.*, 1997) made a quantitative comparison of the relative contribution of each species to the total biomass impossible, therefore only tota *Ulva* spp. biomass is be taken into account. At Kwistenburg, *Ulva* spp. dominated together with *Chaetomorpha linum* (O.F. Müll) Kütz. The *Ulva* biomass consisted of the same species as were found at Middelplaten. The biomass of other algae found at both sites was negligible (less than 1 % of *Ulva* spp. and *Ulva* spp. and *C. linu* biomass at Middelplaten and Kwistenburg respectively, data not shown).

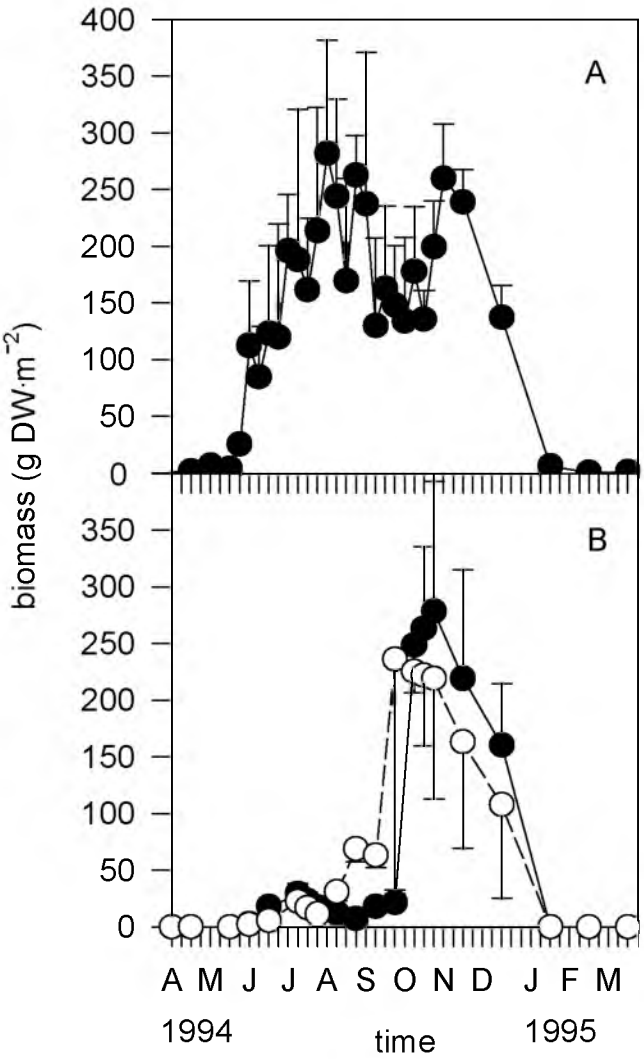

Fig. 3.2: Biomass values (gDW·m^{-2}) of macroalgae in the Veerse Meer (the Netherlands) from April 1994 until march 1995. (A) Biomass of *Ulva* spp. at the site Middelplaten. (B) Biomass of *Ulva* spp. (closed circles, full line) and *Chaetomorpha linum* (open circles, dotted line) at the site Kwistenburg. Ticks on x-axis represent weeks. Error bars represent + 1 SD for *Ulva* spp. and - 1 SD for *C. linum.*

Roughly, four different periods can be distinguished in biomass build-up at Middelplaten (Fig. 3.2a). From January to April, a dormant phase can be distinguished, followed by a build-up phase that started in the second week of May and lasted for about ten weeks

when the maximum biomass was reached (282 g DW· $^{-2}$). Four weeks later, biomass levels decreased to around 150 g DW·m^{-2} and a stationary phase sets in, with little biomass fluctuations. At the end of October, a short increase was observed followed by a decomposition phase in which the biomass decreased and eventually completely disappeared. At Kwistenburg, the dormant phase lasted longer and a stationary phase could not clearly be recognised here (Fig. 3.2b). Compared to Middelplaten, total macroalgal biomass levels were low during summer (maximum in August 79 g DW·m^{-2}). At the end of September, biomass increased sixfold within two weeks time. Biomass distribution became very patchy in this period, explaining the high variation in the observations. At the end of 1994 almost all algae had disappeared from the site.

Growth rates of *Ulva* spp. at Middelplaten were high during the build-up phase (beginning of May, 0.28 - 0.30 d^{-1} cage mean) but decreased quickly after two weeks to a cage mean of 0.10 d^{-1} in June (Fig. 3.3a). During the stationary phase, growth rates were more or less constant (around 0.05 d^{-1}). At Kwistenburg *Ulva* growth rates were also high at the beginning of May and decreased after two weeks (Fig. 3.3b). However, the decline was less steep than at Middelplaten. Cage mean growth rates were significantly higher at Kwistenburg than at Middelplaten during the last week of May, the firs three weeks of June and in the third and fourth week of July (t-test, $p < 0.05$ - 0.001). During the first week of July and the first, third and last week o August growth rates were slightly, but significantly higher at Middelplaten (t-test, $p < 0.05$ - 0.001). For all other weeks, the differences were insignificant. The mean annual growth rate was not significantly different between Middelplaten (0.086 d^{-1}) and Kwistenburg (0.096 d^{-1}). Testing between corresponding compartment gave the same results as the testing between the cage means.

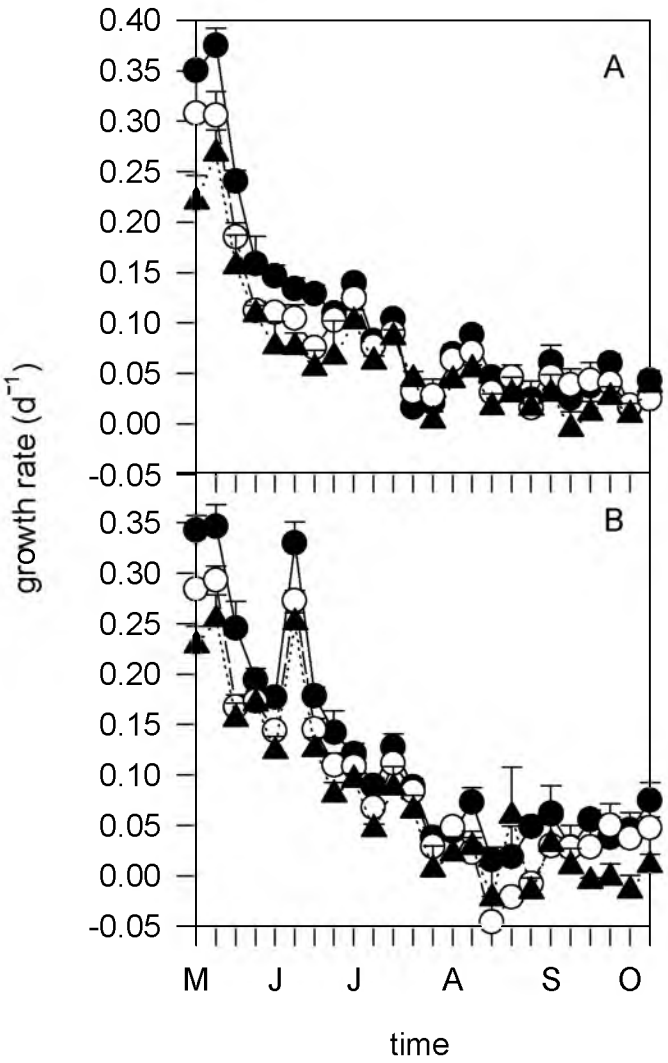

Fig. 3.3: Growth rates (d^{-1}) of *Ulva* spp. in 1994 in compartimented cages in the Veerse Meer (the Netherlands) at the sites Middelplaten (A) and Kwistenburg (B). Growth rates of top compartment (dots, full line), middle compartment (circles, dotted line) and lower compartments (triangles, dotted lines). Each value represents the average of five measurements, error bars represent + 1 SD. Ticks on x-axis represent weeks.

As expected, growth rates were highest in the upper cage compartment (a) and lowest in the lower compartment (c) with the middle compartment (b) having intermediate values at both sites (Fig. 3.3). Differences between a and c were always significant for both sites (ANOVA, $p < 0.05$ - 0.001), except for the third and fourth week of Augus (both sites) and the last two weeks of

the observation period (Middelplaten). Differences between (a) and (b) were significant fro May until the fourth week of June at both sites and in the second, third and fifth week of August at Kwistenburg only (ANOVA, $p < 0.01 - 0.001$). Differences between (b) and (c) were significant in the first two weeks of May, all weeks of June and the second week of July at both sites (ANOVA, $p < 0.05 - 0.001$). At Middelplaten, a significant difference was also found in the last week of July and the first two weeks of August, while at Kwistenburg during the last five weeks of the observation period a significant difference was found (ANOVA, $p < 0.01$). At both sites, no significant correlation was found between growth rates and weekly biomass change (Table 3.1).

Trends in the seasonal cycles of tissue nitrogen (N), carbon (C) and phosphorus (P) were more or less comparable for *Ulva* spp. (Middelplaten) and *C. linum* (Kwistenburg) (Fig. 3.4a - c). Tissue N and P levels were high at the end of the dormant phase, decreased during the build-up phase and increased again during the stationary phase, eventually reaching their initial high levels during the decomposition phase. Tissue C in both species pronounced a less obvious pattern. Annual average tissue C was lower and tissue P was higher for *C. linum* than for *Ulva* sp. (t-test, $p < 0.001$ for both C and P), while annual average tissue N levels were similar. A significant correlation was found between dissolved inorganic nitrogen (DIN) and tissue N for *Ulva* sp., but not for *C. linum* (Table 3.1). Tissue P in *Ulva* sp. showed a significant negative correlation with dissolved inorganic phosphorus (DIP), the correlation for *C. linum* was insignificant. Tissue N and P and tissue N and C were positively correlated in both species.

In *Ulva* spp. total chlorophyll content was high at the end of the dormant phase, decreased to less than 1.5 mg·g DW^{-1} during the build-up phase, increased again in August and remained stable during the stationary phase (Fig. 3.5a). The chlorophyll *a*:*b* ratio increased from 1.1 - 1.3 in the dormant phase to 1.8 - 2.3 during the remaining period (Fig. 3.5b). For *C. linum*, total chlorophyll levels were around 1.5 to 2.0 mg·g D^{-1} in the beginning of the build-up

Table 3.1: Pearson correlation coefficients (r) between growth rate and weekly biomass increase (dbiomass) of *Ulva* spp., between nutrient concentrations in the water and between these and tissue nutrient concentrations and tissue pigment concentrations in *Ulva* sp. at Middelplaten and *C. linum* at Kwistenburg in the Veerse Meer (data from Fig. 2 - 6). For dbiomass n=24, otherwise n=30 fo Middelplaten and n=20 for Kwistenburg. Significant correlations are marked: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns not significant ($p > 0.05$).

		r	r
		Middelplaten	Kwistenburg
dbiomass *Ulva*	growth *Ulva*	0.05 ns	0.17 ns
		Ulva spp. (Middelplaten)	*C. linum* (Kwistenburg)
DIN	DIP	-0.64 ***	-0.47 *
tissue N	DIN	0.56 **	0.34 ns
tissue P	DIP	-0.50 **	-0.05 ns
tissue N	tissue P	0.57 **	0.65 **
tissue N	tissue C	0.35 *	0.67 **
tissue N	total pigments	0.57 **	0.73 ***

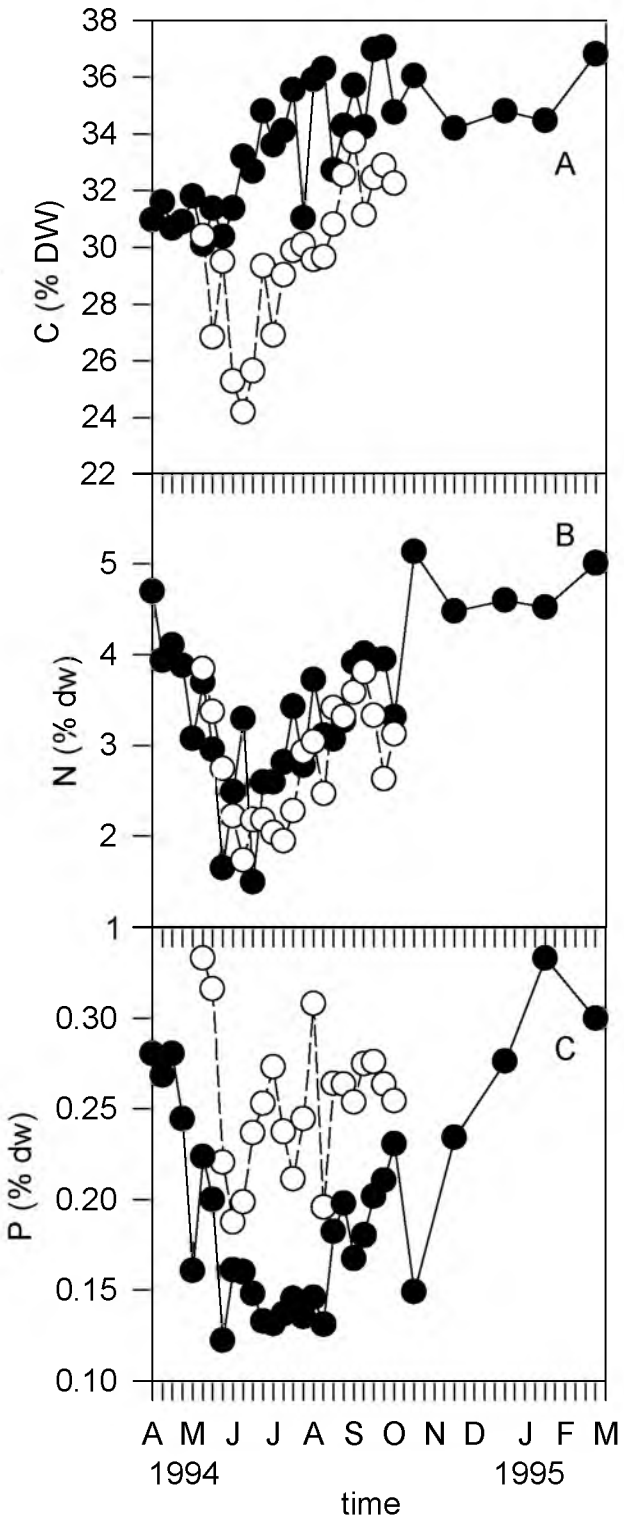

Fig. 3.4: Tissue carbon (A), nitrogen (B) and phosphorus (C) content (% dw) of *Ulva* spp. at the site Middelplaten (closed circles, full line) and *Chaetomorpha linum* (open circles, dotted line) at the site Kwistenburg in the Veerse Meer (the Netherlands) from April 1994 until march 1995. Ticks on x-axis represent weeks.

phase, increased six-fold in July and August, followed by a period of high values (Fig. 3.5a). During the decomposition phase, total chlorophyll content decreased again. The chlorophyll *a*:*b* ratio showed large fluctuations, reached a maximum value of 4.0 in spring and decreased again to approximately 1.8 - 2.0 in autumn and winter (Fig. 3.5b). In both species, total chlorophyll content was significantly, positively correlated to tissue N content (Table 3.1).

Environmental parameters

Annual average water temperature, salinity, pH, attenuation coefficient (k), chlorophyll levels and oxygen did not differ significantly between sites (Table 3.2). Chlorophyll *a* levels in the water in '94/'95 were always below 10 $mg \cdot m^{-3}$ for both sites, apart for two peak values in Apri and the beginning of May (values up to 50.5 $mg \cdot m^{-3}$ for Middelplaten and 100 $mg \cdot m^{-3}$ for Kwistenburg). Dissolved oxygen levels showed minimal fluctuations, except for peaks in April and October '94 and one in March '95, occurring at both sites at the same time. The April peak coincided with a phytoplankton bloom, the other two were observed right after the change of the water level. At both sites, the raising of the water level had a significant effect on salinity (t-test, $p < 0.001$) and seston levels (t-test, $p < 0.001$).

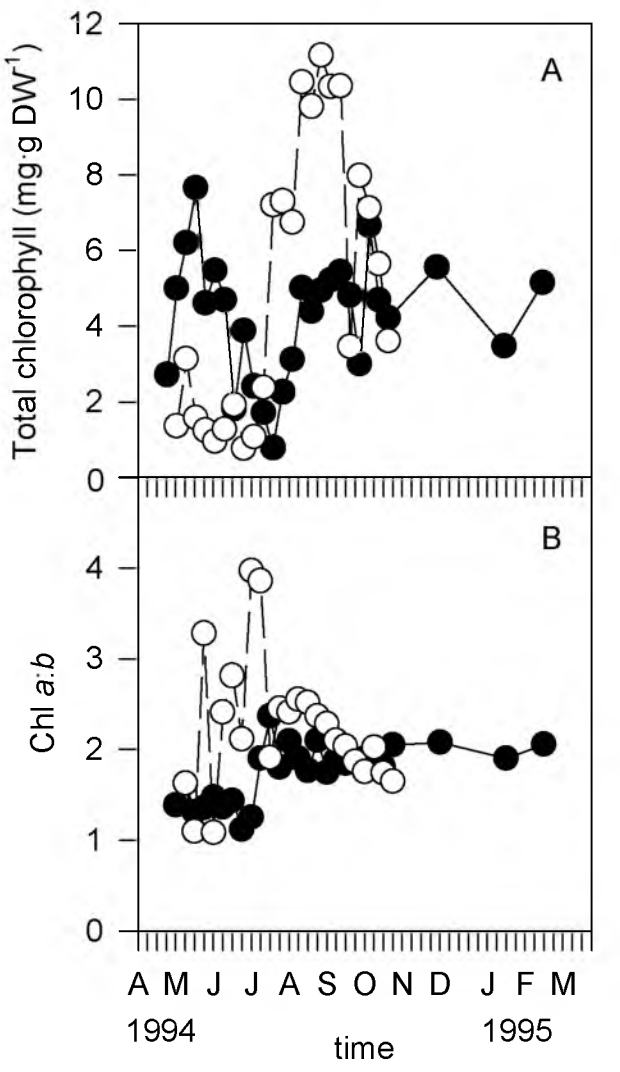

Fig. 3.5: Total tissue chlorophyll content (A) and chlorophyll *a*:*b* (B) of *Ulva* spp. at the site Middelplaten (closed circles, full line) and *Chaetomorpha linum* (open circles, dotted line) at the site Kwistenburg in the Veerse Meer (the Netherlands) from April 1994 until march 1995. Ticks on x-axis represent weeks.

Dissolved inorganic phosphate (DIP) levels increased during the build-up phase and decreased again during the biomass decomposition phase (Fig. 3.6a). Concentrated agricultural run-off after heavy rainfall, probably caused the peak value at both sites in September 1994. Total dissolved inorganic nitrogen levels (DIN) however, increased during the decomposition and dormant phase, followed by a sharp decline during the build-up phase leading to low values (sometimes below detection limits) during the stationary phase (Fig. 3.6 b). The significant negative correlation coefficients between DIN and DIP at both sites clearly express these contrasting patterns (Table 3.1). A peak value for DIN was observed in October. High DIN values (over 200 µM) were reached in winter 1995 at both sites. During the build-up phase, DIN consisted mainly of ammonium (70 - 100% of DIN) at both sites, while during the rest of the year nitrate + nitrite were the main constituents of the DIN (80 - 95 %). Mean annual DIN and DIP concentrations were not significantly different between sites.

Table 3.2: Ranges and means (± SD) of water column parameters at two sites in the Veerse Meer (the Netherlands). From April to October 1994, the water level was at Mean Sea Level, between November 1994 and April 1995 the water level was at MSL - 0.70 m. Temperature is water surface temperature, k is attenuation coefficient, n is the number of measurements, n.m. is no measurements. Significant differences (t-test) of means between seasons are marked: *** = $p < 0.001$.

		Middelplaten		Kwistenburg	
		MSL	MSL - 0.70	MSL	MSL - 0.70
temperature (°C)	range	8.0 - 24.1	5.9 - 12.0	8.0 - 24.1	5.9 - 12.0
	mean	16.7 ± 4.5	8.5 ± 2.4	16.7 ± 4.5	8.5 ±2.4
	n	27	5	27	5
salinity (psu)	range	13.0 - 17.4	9.6 - 15.0	13.5 - 17.5	10.9 - 15.4
	mean	15.0*** ± 1.3	12.2 ± 2.1	15.3*** ± 1.2	13.3 ± 2.1
	n	27	5	27	5
pH	range	8.3 - 8.9	7.9 - 8.7	8.3 - 8.8	8.1 - 8.8
	mean	8.6 ± 0.1	8.4 ± 0.3	8.5 ± 0.1	8.5 ± 0.3
	n	22	5	24	5
seston (mgl^{-1})	range	5.6 - 16.5	13.6 - 45.0	6.6 - 20.8	8.8 - 27.1
	mean	9.4*** ± 2.4	30.0 ± 14.3	10.4*** ± 2.9	18.7 ± 6.9
	n	27	5	27	5
k (m^{-1})	range	0.5 - 2.2	n.m.	0.4 - 1.6	n.m.
	mean	0.9 ± 0.3		1.0 ± 0.3	
	n	27		27	
planktonic chlorophyll (mgm^{-3})	range	0.9 - 30.4	1.7 - 10.1	1.1 - 59.6	1.5 - 3.3
	mean	7.3 ± 7.3	5.5 ± 3.1	9.4 ± 11.8	2.1 ± 0.8
	n	27	5	27	5
oxygen (mgl^{-1})	range	6.6 - 23.6	7.5 -23.5	6.6 - 22.3	9.8 - 22.3
	mean	10.4 ± 0.1	15.0 ± 0.2	8.9 ± 0.1	13.2 ± 0.4
	n	24	5	24	5

Relations of environmental parameters and tissue nutrient levels with growth rates
Testing the simultaneous effects of all environmental parameters on weekly average growth of *Ulva* sp. with a multiple regression analysis resulted in a negative partial correlation of growth with salinity and DIP, explaining 59 % of the variation (Table 3.3). Leaving out salinity and DIP (explained in discussion) resulted in a negative partial correlation of growth with temperature and a positive correlation with light, explaining 36 % of the variation. Replacement of the water nutrient concentrations (DIN and DIP) by tissue N and tissue P (explained in discussion) returned a positive partial correlation with tissue P and a negative correlation with salinity, explaining 65 % of the variation. When tissue P and salinity were excluded, 44 % of the variation was explained by tissue N (positive), temperature (negative) and light (positive).

At Kwistenburg, a negative partial correlation with salinity and temperature explained 75 %

of the variation. When salinity and DIP were excluded, 37 % of the variation was explained by DIN (positive) and light (positive). Testing of the different cage compartments gave similar results for both sites.

Discussion

Algal biomass distribution

The occurrence of large differences between sites in species distribution and biomass has been reported earlier and could in some cases be attributed to environmental parameters, such as salinity, nutrient loading, turbidity, etc. (Josselyn & West, 1985; Thom & Albright, 1990; Brouwer *et al.*, 1995). These studies rarely include data on algal growth rates and/or daily biomass production (see however Laver *et al.*, 1991; Geertz-Hansen *et al.*, 1993; Sfriso, 1995). Analysing growth rates however, gives an indication of the growth potential of a certain species at a specific location and can thus reveal some of the underlying mechanisms that contribut to the observed variation in biomass (Sfriso, 1995; Hernández *et al.*, 1997). We hypothesised, that in the Veerse Meer, the lower biomass at Kwistenburg is due to lower growth rates of *Ulva* spp. at this site. Growth rates at Kwistenburg, however, were equal to those a Middelplaten during the largest part of the season and even higher in May and June (Fig. 3.3), thus we have to reject this hypothesis. From the growth measurements, we can conclude that the difference between sites is not caused by the gain process, but by loss processes. Similar conclusions were drawn for *Ulva* spp. biomass at sites in the lagoon of Venice, Italy (Sfriso, 1995) and the Palmones estuary, Spain (Hernández *et al.*, 1997).

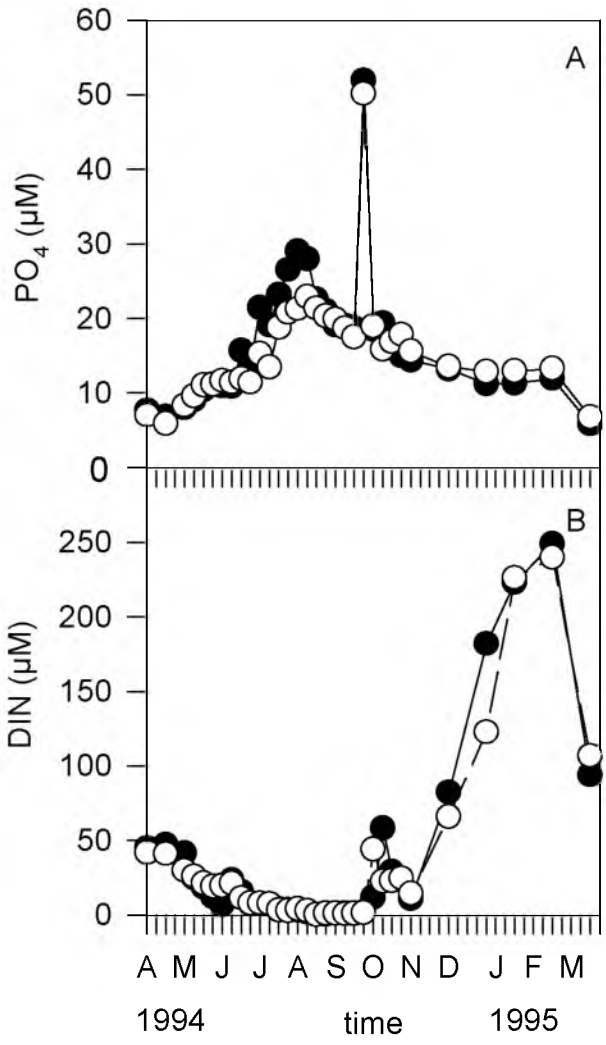

Fig. 3.6: Nutrient concentrations in the surface water of the Veerse Meer (the Netherlands) at the sites Middelplaten (closed circles, full line) and Kwistenburg (open circles, dotted line) from April 1994 until march 1995. (A) Total inorganic phosphorus (DIP, µM). (B) Dissolved inorganic nitrogen (DIN, µM). Ticks on x-axis represent weeks.

The most important loss processes for macroalgal biomass are decomposition (Sfriso, 1995), grazing (Geertz-Hansen *et al.*, 1993) and transport by waves and wind (Lowthion *et al.*, 1985; Soulsby *et al.*, 1985). Anoxic conditions, which often occur in dense mats, enhance decomposition rates (de Casabianca-Chassany, 1989). One would thus expect that the biomass at Middelplaten would be more sensitive to these processes, which makes decomposition an unlikely cause of the difference between the sites. It has been shown tha grazing by invertebrates and birds can reduce macroalgal biomass considerably (Coosen *et al.*, 1990; Sey *et al.*, 1991; Geertz-Hansen *et al.*, 1993; Horne *et al.*, 1994). Experiments carried out by Kamermans *et al.* (unpubl.data), show however that there is a low grazing pressure of invertebrates in the Veerse Meer, which does not differ between sites. Bird

Table 3.3: Partial regression coefficients and ANOVA test results of stepwise multiple regression analysis relating growth rate of *Ulva* spp. in the Veerse Meer (the Netherlands) in 1994 to:
- water column parameters (dissolved inorganic nitrogen [DIN], dissolved inorganic phosphate [DIP], salinity, temperature and light at mid-depth) at the sites Middelplaten (nr. 1) and Kwistenburg (nr. 5)
- water column parameters excluding phosphate and salinity at the sites Middelplaten (nr. 2) and Kwistenburg (nr. 6)
- salinity, light at mid-depth, water temperature and plant tissue nitrogen and phosphorus (nr. 3) and temperature, light and plant tissue N (nr. 4) at Middelplaten.
See table 3.1 and figs. 2 - 6 for data. The regression equations are of the form $Y = a + bX_1 + cX_2 \ldots$ etc., wher Y is *Ulva* spp. growth, a is the regression constant, X are the dependent variables and b, c, etc. are the partial coefficients. Significant partial regression coefficients and F values are marked: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant.

nr. 1	dependent variable	nr. 2	dependent variable
independent variables	*Ulva* spp. growth Middelplaten	independent variables	*Ulva* spp. growth Middelplaten
constant	0.74 ***	constant	0.17 *
DIN	ns	DIN	ns
DIP	-0.22 **	temperature	-0.02 **
salinity	-0.03 *	light	0.001 **
temperature	ns		
light	ns		
F	17.42 ***	F	7.45 **
R^2	0.59	R^2	0.36
nr. 3	dependent variable	nr. 4	dependent variable
independent variables	*Ulva* spp. growth Middelplaten	independent variables	*Ulva* spp. growth Middelplaten
constant	0.86 ***	constant	ns
tissue N	ns	tissue N	0.28 *
tissue P	0.36 **	temperature	-0.01 *
salinity	-0.03 **	light	0.002 ***
temperature	ns		
light	ns		
F	22.24 ***	F	7.08 **
R^2	0.65	R^2	0.44
nr. 5	dependent variable	nr. 6	dependent variable
independent variables	*Ulva* spp. growth Kwistenburg	independent variables	*Ulva* spp. growth Kwistenburg
constant	1.39 ***	constant	ns
DIN	ns	DIN	0.08 **
DIP	ns	temperature	ns
salinity	-0.07 ***	light	0.001 *
temperature	-0.01 **		
light	ns		
F	35.76 ***	F	7.76 **
R^2	0.75	R^2	0.37

grazing takes place mainly during the late summer and autumn when the difference between sites is already evident, thus we assume that grazing was also not responsible for the observed difference. Recently, several authors demonstrated the importance of wind and currents as important factors for transport of free-floating macroalgae (Coffaro & Sfriso, 1997; Flind *et al.*, 1997; Hernández *et al.*, 1997; Salomonsen *et al.*, 1997). In the Veerse Meer, transport from the more exposed site Kwistenburg may be more important as a loss factor than a Middelplaten. Future studies should examine and quantify the role of transport, to understand both the spatial and seasonal dynamics of macroalgal biomass.

Seasonal variation, relation with environmental parameters
Roughly, three to four phases in the *Ulva* spp. biomass dynamics could be distinguished in the Veerse Meer. The dormant phase is the period from December to May when there is no above-ground biomass at all. Kamermans *et al.* (1998) have shown that during this phase *Ulva* spp. fragments survive buried in the sediment. Viable remainders of these fragments can initiate the spring biomass build-up. They suggested that temperature, wind to uncover the buried thalli and probably also light (both intensity and daylength) are the variables tha determine the onset of growth.

Multiple regression explained a limited, but significant part of the variation in cage growth rates during the other three phases (Table 3.3). Tissue nutrient concentrations proved to be better in accounting for the variation in *Ulva* growth than nutrients in the water. The capacity of *Ulva* spp. of luxury uptake and storage of nutrients when other factors are limiting growth explains this. When nutrient concentrations in the water become very low, the algae can grow on their internal reserves (Fujita, 1985; Viaroli *et al.*, 1996; Pedersen & Borum, 1997). However, the results should be interpreted with care, as intercorrelation of the independen variables can greatly influence the outcome of a multiple regression analysis (Robertson *et al.*, 1993; Sokal & Rohlf, 1995). It is very likely that the correlations with phosphate/tissue P and salinity are the result of these intercorrelations and have no causal meaning. Both DIP and salinity increased during the year. Considering the concentrations and the negative correlation of phosphorus with tissue P for *Ulva* at Middelplaten, neither phosphorus limitation nor phosphorus toxification seem a likely cause of variation in *Ulva* spp. growth in the Veerse Meer. A negative effect of increasing salinity is very unlikely, as the optimum salinity for growth of *Ulva* spp. is still higher than that observed in the Veerse Meer (Bliding, 1968; Koeman & van den Hoek, 1981; Dickson *et al.*, 1982).

For the reasons explained above, we excluded phosphorus and salinity from the analysis, which resulted in a positive relation of growth with tissue N and light and a negative relation with temperature. The degree to which an alga is nitrogen limited can be determined by comparing the observed tissue N levels of the algae with the so-called critical tissue N levels determined in experimental set-ups (i.e. the nitrogen level below which growth rates decrease, Morand & Briand, 1996). Critical values determined for *Ulva* spp. range from 2.0 to 2.4 % DW (Fujita *et al.*, 1989; Lavery & McComb, 1991; Pedersen, 1994). As tissue N levels of *Ulva* spp. at Middelplaten were close to or below the critical values at the end of the build-up phase, it is concluded that nitrogen limitation may have occurred during this period, causing the transition from the build-up period to the stationary period. The increase in tissue nitrogen during the stationary and the decomposing phase is probably mainly due to the leakage of nitrogen-free compounds from decomposing thalli (Hanisak, 1993; Viarol *et al.*, 1996). However, also self-shadowing, leading to strong light limitation and luxury uptake of nitrogen may increase the average tissue N of a dense algal mat, as suggested by the data of Vergara *et al.* (1998). Studies on such vertical gradients in macroalgal mats are very rare, however

certainly deserve more attention as they can be helpful in explaining parts of the complex dynamics of macroalgal biomass. Although tissue N data for *Ulva* at Kwistenburg were not available, the results of the multiple regression analysis and the observed similar pattern in seasonal variation in growth suggests that here also nitrogen has been limiting for some part of the season.

Light and temperature also showed a significant correlation with growth, supporting the earlier conclusion (Malta & Verschuure, 1997) that light was the main growth-limiting variable during the build-up phase. Both the higher growth rates in the upper cage compartments during the first weeks of the season and the observed low chlorophyll *a*:*b* ratio and the high total chlorophyll content of *Ulva* spp. in spring support their conclusion (Ramus *et al.*, 1976; Henley & Ramus, 1989). Temperature may have affected growth rates in two ways. High water temperatures (over 20 °C) have been shown to inhibit growth in *U lactuca* from the North Sea (Fortes & Lüning, 1980) although Malta *et al.* (1997) still found hi growth rates for *U scandinavica* from the Veerse Meer at 25 °C. Another way in which temperatures can affect the biomass variation is by stimulating respiration, causing tempora anoxia, resulting in die-off and decomposition (Sfriso *et al.*, 1987). We observed indications of anoxia (black sediment and sulphuric smell, E. Malta and J.M. Verschuure, pers. obs.) at both sites in the Veerse Meer. In this way, temperature enhanced decomposition, possibly together with bird grazing, can be the variables that determine the transition from the stationary phase to the decomposition phase.

If and to what extent growth of *C. linum* is regulated by the same parameters o *Ulva* spp. remains unclear. The tissue N of *C. linum* in Veerse Meer was always well above the critica value of 1.15 % DW found by Pedersen and Borum (1996), so we conclude that growth of *C. linum* was not nitrogen limited. Light might have been limiting during the build-up phase, considering the low chlorophyll *a*:*b* ratios. The thick mats of *C. linum* started decomposing in October - November and were totally anoxic at certain locations, leading to the formation of H_2S and elemental sulphur in the water column (E. Malta, pers. obs.). This, most likely, has led to the final decline of the alga.

Conclusions

This study has demonstrated that loss processes are the main causes of large differences in macroalgal biomass and species distribution between sites only a few km apart. The measurements of in situ growth and/or production rates are of vital importance to get an idea of the mechanisms that are responsible for the observed seasonal and spatial variation of macroalgal biomass. Future studies should pay special attention to the complex set of loss processes in macroalgal populations.

CHAPTER IV: ROLE OF COLD RESISTANCE AND BURIAL FOR WINTER SURVIVAL AND SPRING INITIATION OF AN *ULVA* SPP. (CHLOROPHYTA) BLOOM IN A EUTROPHIC LAGOON (VEERSE MEER LAGOON, THE NETHERLANDS)

Abstract

In the eutrophic Veerse Meer lagoon (The Netherlands) large amounts of free-floating *Ulva* thalli are present from May to October. In winter however, no algae seem to occur in the lagoon. Sexual reproduction appears to be negligible as spore formation and germling growth are observed only sporadically. Results of a field survey showed that in winter, viable *Ulva* biomass is present buried in the sediment of the shallow parts of the lagoon. Freezing experiments demonstrated that the algae are able to survive temperatures of -5 °C for two weeks when kept in darkness. In spring, the buried *Ulva* thalli are liberated out of the sediment to initiate a bloom. A field experiment indicates that bioturbation by the lugwor *Arenicola marina* does not stimulate the release of the thalli. Burial and winter survival can explain the rapid increase in *Ulva* biomass in spring and suggests that the initial spring biomass is one of the major factors determining the maximal biomass in summer.

Pauline Kamermans, Erik-jan Malta, Jacobus M. Verschuure, L. Franca Lentz & Lonneke Schrijvers (1998) – Mar. Biol. 131: 45 - 51

INTRODUCTION

The mass development of macroalgae is one of the striking features of shallow eutrophic coastal waters. In most cases the blooms consist of green macroalgae of the genera *Ulva* and *Enteromorpha* (e.g. Ho, 1981; Lavery *et al.*, 1991; Fletcher, 1996). Several conditions have been put forward as being favourable for blooms of these macroalgae. For example, the waterbody must receive substantial nutrient supplies, and the residence time of the water mus be at least a few days (Morand & Briand, 1996). In addition, the morphology of most bloom-forming species ensures rapid nutrient uptake (Littler & Littler, 1980; Hein *et al*, 1995). Furthermore, macroalgae have an advantage over microalgae because their capacity to stor nutrients is much higher (Pedersen & Borum, 1996). Therefore, they can make better use of pulse supply of nutrients, which is often the case in eutrophicated areas. The life cycle of the species may also play an important role in the formation of a macroalgal bloom. For example, vegetative propagation and the ability to form free-floating or loose lying populations capable of almost unlimited vegetative growth have been related to the mass development o *Cladophora monteagneana* in Australia (Gordon *et al.*, 1985).

Ulva blooms regularly occur in the Veerse Meer lagoon in the Netherlands. This brackish lagoon is a former part of the river Rhine delta. In the early nineteensixties, two dams were constructed that separated this estuary from North Sea and river influence. The lagoon has deep gullies (up to 25 m) and shallow areas (depth of less than 5 m). The latter comprise 60 % of the area (Nienhuis, 1992). The bottom consists of sand or silt depending on the location. Dikes and some small loose stones and shells are the only hard substrates present. To increase the storage capacity for discharge of nutrient-rich polder water an artificial low water level (Mean Standard Sea Level - 0.70 m) is maintained during winter (1 November - 1 April). In summer, the water level is raised again to Mean Standard Sea Level.

In winter, the lagoon seems to be devoid of macroalgae. Nevertheless, in summer and fall large amounts of free-floating *Ulva* thalli (up to 350 gram ash-free dry weight (AFDW) m^{-2}) are present on the sediment surface of the shallow areas (Malta & Verschuure, 1997). Sexual reproduction of *Ulva* appears to be negligible in the Veerse Meer lagoon, spore formation is rarely observed. Only sporadically did we find germlings attached to hard substrates (P. Kamermans, E. Malta & J.M. Verschuure, personal observations). If sexual reproduction is not important, then the spring bloom must be based primarily on regeneration of old vegetative fragments.

Since the biomass initiating the bloom is not present on the surface of the sediment in the shallow areas it was unclear where the initiating material came from. In early spring of 1992, *Ulva* biomass (2 g AFDW m^{-2}) was found to be buried in the sediment of a shallow location in the Veerse Meer lagoon (Malta, 1993). If these thalli are able to resume growth in spring, overwintering in the sediment could be a survival strategy o *Ulva*. Santelices *et al.* (1984) reported survival of *Gracilaria* thalli buried in sand for up to six months. The winter that preceded the observation on buried *Ulva* biomass (1991/1992) had only 6 days with minimum temperatures below - 5 °C and can be considered mild (data from KNMI - the Royal Dutch Meteorological Institute). Cold periods of 10 to 20 days with minimum temperatures of - 5 ° or more are common during severe winters (KMNI, data from 1960-1995). In these cases the lagoon is partly, or completely, frozen. Vermaat & Sand-Jensen (1987) showed that *Ulva* was unable to survive freezing to -18 °C in the laboratory, but it did resume growth when collected in the field from fjord ice. It is thus unknown under which circumstances vegetative *Ulva* parts may be able to survive freezing. *Ulva* is capable of heterotrophic growth for at least 41 days (Markager & Sand-Jensen, 1990), and is thus able to survive prolonged periods of

darkness. However, we do not know if the anoxic and dark conditions produced by burial in the sediment may affect survival of *Ulva* under freezing conditions.

For initiation of the *Ulva* bloom in spring to occur the overwintering thalli have to get onto the sediment surface. A possible machanism can be digestion survival. Santelices & Ugarte (1987) found regeneration of new cells from *Ulva rigida* thalli fragments they collected from faecal pellets of herbivorous molluscs. The lugworm (*Arenicola marina*) is common at the shallow areas of the Veerse Meer lagoon. These animals live in an L-shaped burrow, where they ingest sediment with organic material at the lower end of the head-shaft and deposit faeces at the surface of the sediment through the tail-shaft (Cadée, 1976). It may be possible that the lugworm is feeding on the buried *Ulva* material and that algal fragments survive passage through the digestive tract. When temperature rises in spring, bioturbation by the lugworm increases. The feeding behaviour of the lugworm could bring undigested *Ulva* fragments to the surface of the sediment and thus initiate the bloom. On the other hand, the opposite effect is also possible, deposition of faeces mounts produced by the lugworms on the sediment surface may rebury already uncovered thalli.

In this paper, we present the results of experiments that were carried out to test the following hypotheses: 1. *Ulva* biomass that overwinters in the Veerse Meer lagoon buried in the sediment is viable. 2. *Ulva* is able to survive freezing in darkness and under anoxic conditions. 3. In spring, the bloom is initiated b *Ulva* fragments emerging at the sediment surface b bioturbation of the buried material

Materials and Methods

Viability of overwintering Ulva

In February 1995, 2 shallow sites (0.10 m water) in the Veerse Meer lagoon were sampled for the presence of *Ulva* (site 1: N 51° 32' 63" E 3° 46' 89"; site 2: N 51° 32' 61" E 3° 50' 79"). The locations were chosen because it was known from earlier work that an *Ulva* bloom developed at those sites (Malta & Verschuure, 1997). At each site, the upper 20 cm of the sediment o five 0.05 m^2 areas was sieved (2-mm mesh size). *Ulva* tissue collected was cultured to test the viability of the material. Discs of 2.2 cm diameter were cut out of the thalli. Five discs were used per incubation and four replicates were incubated for each location. Erlenmeyer flasks were filled with 1 l filtered (0.45 mm) water from the Veerse Meer lagoon and aerated continuously. All flasks were placed under the same natural light conditions in a NE window sill. The light cycle was 13 hours dark and 11 hours light and the average irradiation was 260 $\mu E \cdot m^{-2} \cdot s^{-1}$. The medium was renewed with fresh Veerse Meer water every two weeks. Mean salinity was 11 (psu) and the water temperature ranged from 12 to 29 °C. Every 3 - 13 days the total wet weight of the 5 discs in each replicate was determined. Before weighing, the discs were blotted between two paper sheets to remove adhering water. Growth rate (m) was assumed to be exponential and was calculated as:

$\mu = (\ln W_t - \ln W_0)/t$

in which W_0 is the initial and W_t the final wet weight after t days of incubation.

Freeze tolerance

To study the effect of darkness and anoxia on survival under freezing conditions, discs of 2.2 cm diameter were cut out of the *Ulva* thalli collected in February 1995 at site 1 in the Veerse Meer lagoon. Treatments involved placing 5 *Ulva* discs in 1 l of filtered (0.45 mm) Veerse Meer lagoon water in the dark or in a 12 h dark and 12 h light cycle (100 $\mu E \cdot {}^{-2} \cdot s^{-1}$). In addition, 5 discs were buried 5 cm in anoxic sediment (natural sediment collected in the Veerse Meer lagoon) or in oxic sediment (bird cage sand), both covered with a 2-cm layer of

filtered lagoon water. Two replicates were incubated for each of the 4 treatments. All treatments were acclimatised at 2 °C for 1 week, transfered to -5 °C for 2 weeks and after tha acclimatised again for 1 week at 2 °C. Viability after this 4-week period was tested b culturing the discs as described above. The light cycle during culturing was 11.5 hours dark and 12.5 hours light and the average irradiation was 490 $\mu E \cdot m^{-2} \cdot s^{-1}$. To check the presence or absence of oxygen in the different sediment types, oxygen concentration was measured for each 0.5 mm depth with a Clark oxygen microelectrode mounted on a micromanipulator. Measurements were done at 2 °C with an autoranging picoammeter.

Bioturbation effect

In March 1995, a total of 10 cages were placed at site 1 in the Veerse Meer lagoon (water depth of 0.10 m in winter and 0.80 m in summer). Iron frames were covered at all four sides with wire mesh (mesh size of 1 cm) leaving the top and bottom open. The cages were 1 high and the bottom covered 1 ^{2}. In 5 cages a 1-mm mesh screen was inserted horizontally into the sediment at approximately 10-cm depth to force lugworms out of experimental plots. This method has been used succesfully by Reise (1983) and by Philippart (1994). As a contro treatment the other 5 cages were left without the screen. To correct for the effect of sedimen disturbance when placing the screens, the sediment in the control cages was also removed and shovelled back into the holes. Twice a week, the number o *Arenicola* faeces mounts in each cage was counted with the aid of an underwater viewer. The number of faeces mounts shows good correlation with the actual number of lugworms present in the sediment ($r = 0.783$, $n = 9$, $p < 0.05$, Kamermans, unpublished data). At the same time as counting the faeces mounts, the *Ulva* coverage within the cages was visually estimated. The duration of the experimen was 63 days, until 15 May 1995. At the end of the experiment al *Ulva* biomass present in the cages was harvested and the wet weight was determined after 1 min rotation in a laundr centrifuge.

Statistical analyses

The effects of different pre-culture conditions and different culture times on the growth rate o *Ulva* was tested with two-way analyses of variance (ANOVA). The significance o differences between treatments was analysed with a Tukey-Kramer procedure as post-hoc test (Sokal & Rohlf, 1995). In the same manner the significance of differences in *Ulva* coverage and lugworm densities with or without a mesh screen buried in the sediment and differen sampling dates was tested. A student t-tests was used to test the effect of mesh screen cages on *Ulva* wet weight at the end of the experiment. Assumption of normal distribution of the dependent variables was examined using box plots. Data were tested for heteroscedacity with a Bartlett's test for homogeneity of variances. Data that scored significantly were *Ulva* coverage, *Ulva* wet weight and number of *Arenoicola* faeces mounts. These data were log-transformed ($\log x + 1$), which considerably improved the data: their distribution was normal and they did not show significant heteroscedacity anymore. Statistical tests were carried out with those log-tranformed data. A significance level of 5% was used in all tests. The statistical analyses were conducted using the STATISTICA programme.

RESULTS

Viability of overwintering Ulva

The viability test yielded growth rates of 0.03 - 0.08 d^{-1} (Fig. 4.1). Growth rates achieved with *Ulva* from the two sampling sites did not differ significantly (Table 4.1). These results show that in early spring 1995 *Ulva* fragments that had overwintered buried in the sediment of the Veerse Meer lagoon were able to resume growth.

Freeze tolerance

Results of the oxygen measurements show that in the natural sediment oxygen concentration is reduced to zero mM at a depth of 0.5 cm while the concentration in the bird cage sand stayed comparable to the concentration in the overlying water upto a depth of more than 5 cm (Fig. 4.2). The viability test showed that *Ulva* spp. can survive a period of 14 days at -5 °C (Fig. 4.3). The different treatments resulted in significantly different growth rates (Table 4.2). Growth was fastest after freezing in natural anoxic sediment. This was followed by freezing in oxic sediment and then freezing in water under dark conditions. No recovery was found after freezing in water exposed to a 12 hour light and 12 hour dark regime where the *Ulva* discs had turned completely white. The post-hoc tes showed significant differences in growth rates between the light-water treatmen and the other three treatments (Table 4.3). These results demonstrate that light conditions prevent the survival of *Ulva* during freezing. Burial of *Ulva* in the sediment produces dark conditions. Thus, in the field, buried *Ulva* may be able to survive freezing conditions during winter.

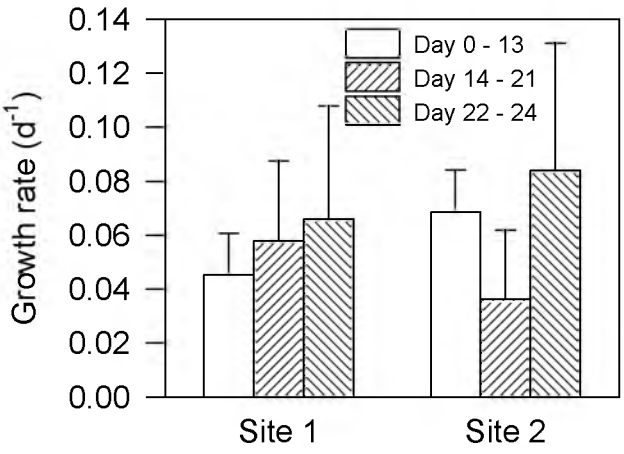

Fig. 4.1: Growth rates (d^{-1}) of *Ulva* during the viability test determined after the first 13 days, the middle 8 days and the last 3 days. Tissue was collected at two shallow locations (site 1 and 2). Bars represent mean value ± s.d. (n=4).

Table 4.1: Statistical evaluation of influences of location (buried in sediment of two shallow sites) and culture time on growth rates of Ulva in the viability test after winter survival. Values are degrees of freedom (df), mean square (MS) and probability (p) of ANOVA.

source of variation	df	MS	p
location	1	0.000	0.613
day	2	0.002	0.230
location x da	2	0.001	0.325
error	18	0.001	

The role of oxygen in survival is not clear. Growth rates after the oxic-sediment treatment did not differ significanty from those after the anoxic-sediment treatment (Table 4.3). However, growth rates after the dark-water treatment were significantly lower than those after the anoxic-sediment treatment (Table 4.3). Growth rates after the dark-water treatment did no differ significantly from the oxic-sediment treatment (Table 4.3), showing that, when frozen in water and in sediment, darkness and oxic conditions yield similar survival results.

Bioturbation effect

In the mesh-screen cages, faeces-mount density was significantly lower than in the contro treatment without mesh screen (Fig. 4.4a, Table 4.4). This shows that the horizontal insertion of the mesh screen effectively reduced the density of *Arenicola*. Treatment and time, as well as their interaction, had a significant effect on *Ulva* coverage (Table 4.5). Coverage was significantly higher in the cages with a mesh screen bottom (Fig. 4.4b, Table 4.5). This can not be caused by sediment pertubation at the beginning of the exper iment, since the sedimen was equally mixed in all cases. At the end of the experiment, *Ulva* biomass was also significantly higher in the screen-bottom cages (Fig. 4.4c, Student t-tests, $p < 0.01$). These results indicate that lugworms do not play a role in uncovering *Ulva* thalli from the sediment. In contrast, they seem to have a negative effect on *Ulva* biomass.

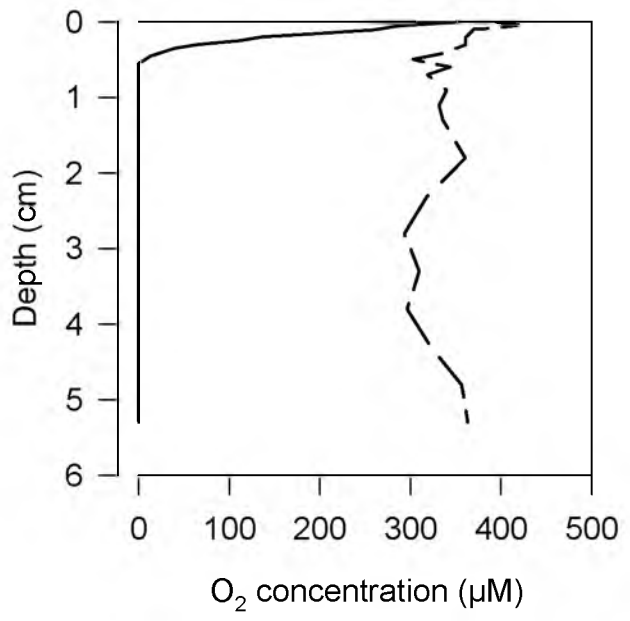

Fig. 4.2: Oxygen concentration (mM) at different depths in a beaker with submerged natural sediment (solid line) and a beaker with submerged bird-cage sand (dashed line). Depth 0 is the concentration in the overlaying water. Measurements were carried out at 2 °C.

Table 4.2: Statistical evaluation of influences of treatment (anoxic sediment, oxic sediment, frozen water without light and frozen water with a 12 hour light and 12 hour dark regime) and culture time on growth rates of Ulva in the viability test after freezing. Values are degrees of freedom (df), mean square (MS) and probability (P) of ANOVA.

source of variation	df	MS	p
treatment	3	0.019	0.000
day	1	0.002	0.125
treatment x day	3	0.001	0.518
error	8	0.001	

Table 4.3: Matrix of pairwise comparison probabilities of Tukey Kramer post hoc test.

treatment	anoxic-sediment	oxic-sediment	dark-water	light-water
anoxic-sediment	1.000			
oxic-sediment	0.208	1.000		
dark-water	0.012	0.233	1.000	
light-water	0.000	0.002	0.030	1.000

Discussion

Our results show that in winter, viable *Ulva* biomass is present buried in the sediment of the shallow parts of the Veerse Meer lagoon. We observed tha most fragments were found near holes left by foraging herbivorous birds such as coots and swans which are abundant in the lagoon (Coosen *et al.*, 1990). Thi suggests a possible explanation for burial of *Ulva*. Macroalgae accumulate in these depressions after which the holes may be filled with sediment by water motion. Overwintering of *Ulva* thalli is in contrast with the general view that *Ulva* is an annual plant starting with germlings attached to hard substrates (Bliding, 1968). The occurence of large loose lying thalli that do not seem to be related to the presence of nearby hard substrates certainly supports the idea of a perennial life style.

Our freeze experiments demonstrated that *Ulva* tissue is able to survive freezing when kept in darkness. Several other macroalgal species are able to withstand a certain amount of freezing. This freeze tolerance has been attributed to the presence of antifreeze substances in their cells (Lüning, 1990). Karsten *et al.* (1990) observed that Antarctic green algae contained high amounts of DMSP, a supposed antifreeze compound. DMSP formation has also been found in *Ulva rigida* (Karsten *et al.*, 1991). Karsten *et al.* (1991) also showed that the DMSP concentration in *U rigida* did not decrease under conditions of darkness. When frozen in light, the *Ulva* discs of our freeze experiment did not survive and turned white. This may be caused by photodamage as low temperatures reduce the ability of algae to use light. Under these circumstances excess ligh energy may damage the photosynthetic apparatus of the algae (Davison, 1991).

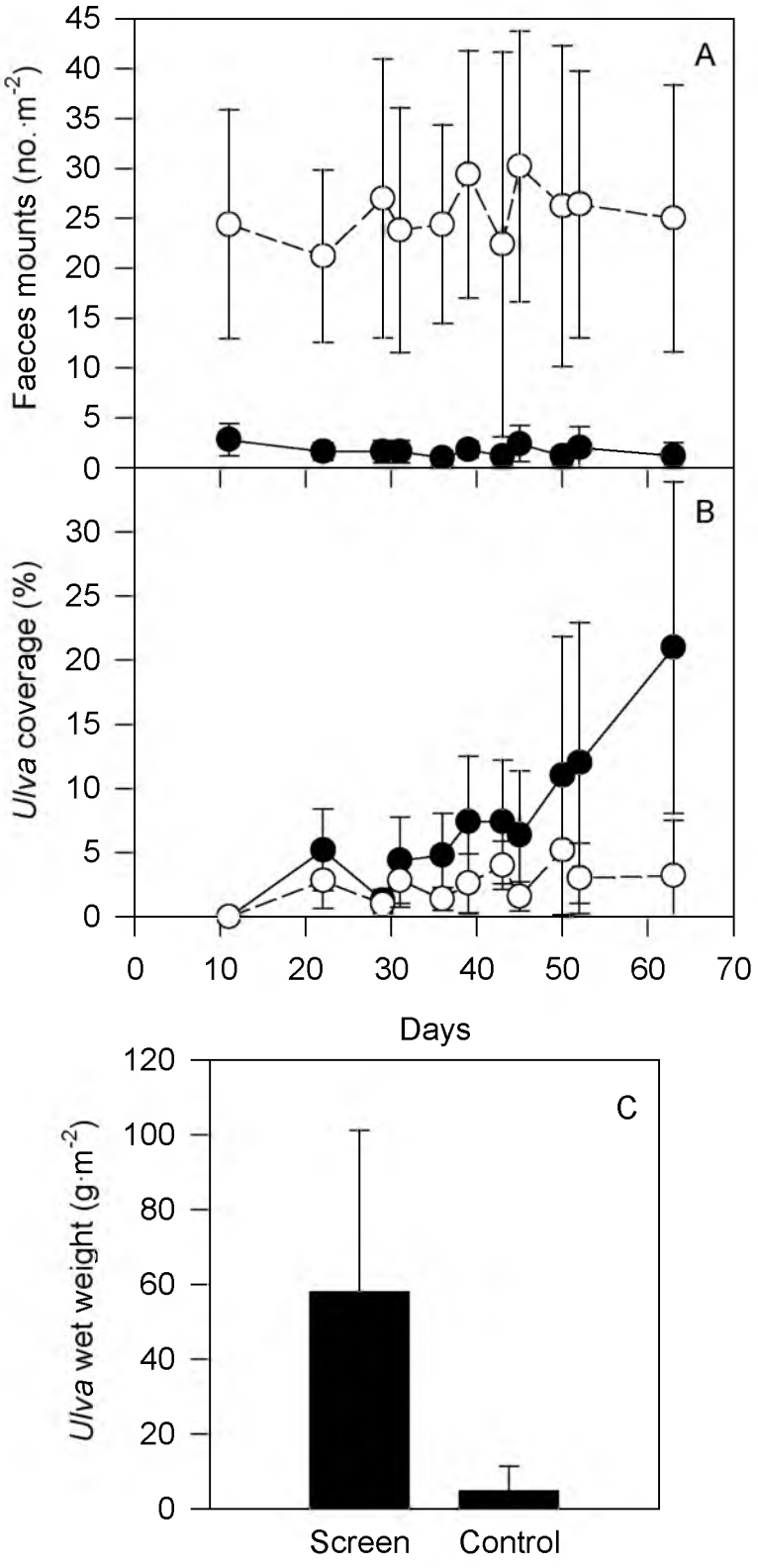

Fig. 4.4: (A) Density of *Arenicola* faeces mounts (number per m^2) and (B *Ulva* coverage (%) in cages with a 1-mm mesh screen inserted horizontally into the sediment at approximately 10 cm depth to force lugworms out of experimental plots (dashed lines and open symbols, mean value ± s.d., n=5) and in cages without a 1-mm mesh screen in the sediment as control treatment (solid lines and closed symbols, mean value ± s.d., n=5). (C) Wet weight of *Ulva* (grams) at the end of the cage experiment (mean value ± s.d., n=5).

Table 4.4: Statistical evaluation of influences of treatment (with or without mesh screen) and date on log-transformed number of *Arenicola faeces* mounts. Values are degrees of freedom (df), mean square (MS) and probability (p) of ANOVA.

source of variation	df	MS	p
treatment	1	143.5	0.000
day	10	0.2	0.724
treatment x day	10	0.1	0.955
error	88	0.4	

Table 4.5: Statistical evaluation of influences of treatment (with or without mesh screen) and date on log-transformed *Ulva* coverage data. Values are degrees of freedom (df), mean square (MS) and probability (p) of ANOVA.

source of variation	df	MS	p
treatment	1	15.13	0.000
day	10	3.32	0.000
treatment x day	10	0.76	0.038
error	88	0.37	

Vermaat & Sand-Jensen (1987) presented contradicting results on freeze tolerance o *Ulva lactuca*. On the one hand, they showed that 10 days of freezing at -18 °C reduced the viability of *Ulva lactuca* to virtually nil, but, on the other hand, when they collected *Ulva lactuca* frozen in ice from the the field it was able to resume growth. The authors suggest that, in the field, gradual freezing may allow the alga to acclimate to the lower temperatures. The freeze experiment of Vermaat & Sand-Jensen (1987) was carried out with *Ulva* thalli that were frozen in the dark under oxic conditions. In the Veerse Meer lagoon overwintering may take place buried in the sediment. In that case the *Ulva* thalli are subjected to darkness and anoxic conditions. Vermaat & Sand-Jensen (1987) demonstrated that at 4 °C, anoxia lead to an increase in respiration and a decline in growth capacity o *Ulva lactuca*. However, respiration seems to be more strongly inhibited by low temperatures than photosynthesis (Kirst & Wiencke, 1995). A small decrease in temperature may considerabl lower respiration rates and reduce the negative effect of anoxia. Therefore, we suggest tha anoxic conditions at temperatures lower than 4 °C (that are experienced during freezing) may not be detrimental to the survival of *Ulva* thalli. Freezing under anoxic conditions was not included as a treatment in the experiments of Vermaat & Sand-Jensen (1987).

Recovery of *Ulva* tissue was best in natural anoxic sediment. Therefore, burial in natura sediment seems to offer the a good overwintering location for *Ulva*. The winter of 1994/1995 that preceded our experiments had only 4 days below -5 °C (KNMI). The winter of 1995/1996 had 28 days with temperatures below -5 °C and was thus more severe (KNMI). A preliminary survey in March 1996 showed that *Ulva* tissue was still present buried in the sediment of the shallow areas of the Veerse Meer lagoon (Kamermans *et al.*, 1996). Apparently *Ulva* tissue is able to withstand freezing in sediment for such a long period.

In spring, buried *Ulva* thalli are not moved out of the sediment by bioturbation. Results of the cage-experiment indicate that lugworms had a negative effect on this process. The deposition of faeces mounts produced by the lugworms on the sediment surface may have reburied already uncovered macroalgae. Likewise, Philippart (1994) demonstrated a negative effect of lugworm density on seagrass survival and concluded that this effect was caused by sediment-reworking activities of lugworms resulting in burial of the seagrasses. It is more likely tha wind-induced water motion frees the *Ulva* thalli out of the sediment. Recent investigations carried out in the Veerse Meer lagoon by Kamermans *et al.* (1996) support this view. In March 1996 large amounts of *Ulva* thalli were found buried in the sediment of site 1. The amount was substantial (about 90 g AFDW $\cdot$ $^{-2}$ compared to 5 g AFDW m^{-2} in 1995). However, the spring of 1996 was very calm and, unlike in the spring of 1995, no thalli were uncovered from the sediment (Kamermans *et al.*, 1996). In March, April and May only 2 days

had wind velocities higher than 15 $m \cdot s^{-1}$, compared to 19 days during the same period in 1995 (KNMI). This observation suggests that wind may play a role in uncovering *Ulva* thalli from the sediment.

Ulva biomass (up to 660 g AFDW $\cdot m^{-2}$) has been observed in the gullies of the Veerse Meer lagoon in summer (Hannewijk, 1988). Sand-Jensen (1988) also reports large amounts of *Ulva lactuca* occurring at great depths in eutrophicated estuaries and relates this to the capacity o *Ulva* to maintain growth at very low light intensities. In the Veerse Meer lagoon, *Ulva* was also present on the bottom of the deeper parts in winter (Kamermans, unpublished data). The gullies may thus represent another winter-survival location. A trap experiment showed that healthy looking adult *Ulva* thalli were floating near the egde of the shallow areas before resident vegetative fragments had emerged on the sediment surface of those areas (Kamermans, unpublished data). Currents may carry the deposited *Ulva* from the gullies to the shallow areas. This is supported by the fact that the largest amounts o *Ulva* were captured in the traps during the period of water-level change (Kamermans, unpublished data). In addition, the first *Ulva* usually appears on the shallow parts directly after the period of water-level change. Rising of the water level in spring may induce current-driven transport o *Ulva* from the gullies to the shallow areas.

It can be concluded that vegetative *Ulva* spp. parts that survive the winter initiate next years bloom. This can explain the rapid increase in *Ulva* biomass in spring and suggests that the initial biomass present in the Veerse Meer lagoon in spring may determine to a large exten the maximal biomass in summer. When this is the case removal of overwintering *Ulva* could be considered as a strategy to control the development of the macroalgal bloom in the Veerse Meer lagoon.

CHAPTER V: FREE-FLOATING *ULVA* L. IN THE SOUTHWEST NETHERLANDS: SPECIES OR MORPHOTYPES? A MORPHOLOGICAL, MOLECULAR AND ECOLOGICAL COMPARISON

ABSTRACT

Free-floating *Ulva* L. biomass in the eutrophic brackish "Veerse Meer" lagoon (southwes Netherlands) consists of four morphologically identified species: *U. curvata* (Kützing) De Toni, *U. lactuca* L., *U. rigida* C. Agardh and *U. scandinavica* Bliding. *U. curvata* could be recognised easily because of the characteristic central cavity in the holdfast of the attached plants, the arrangement of cells in rows and the single pyrenoid in each cell. *U. rigida* was distinguished by the thick thallus and the large number of pyrenoids. The Veerse Meer isolate, however, was slightly different from the isolate from the Oosterschelde estuary (the Netherlands). *U. lactuca* and *U. scandinavica* showed a high degree of overlap in thallus thickness and cell size, bu *U. scandinavica* usually possessed more pyrenoids. However, doubts have frequently been expressed about the use of some morphological characters in *Ulva* taxonomy. To determine the validity of such characters in the identification of *Ulva* species, the morphological variation within and between morphological species was recorded and a molecular data set generated. To detect possible ecophysiological differences between species, optimum temperatures and salinities for growth were determined experimentally. The sequences of the nuclear ribosomal DNA internal transcribed spacer 2 (ITS2) and flanking regions of *U. lactuca*, *U. rigida* and *U. scandinavica* from the Veerse Meer were all identical, but differed from that of *U. rigida* from the Oosterschelde estuary. *Ulva* species from the Veerse Meer were most closely related to *U. armoricana* and *U. rigida* from Brittany (2.9 % and 3.5 % divergence respectively); the difference between *U. rigida* from the Veerse Meer and the Oosterschelde estuary was 7.5 %. Rooted trees, based on a comparison of these sequences with sequences of other *Ulva* and *Enteromorpha* species obtained from the literature, using *Monostroma arcticum* as outgroup suggested that *Ulva* is paraphyletic with respect to *Enteromorpha*. The optimum temperature for growth of *U. curvata* was 25 °C; for all other species it was 10 °C. The optimum salinity for growth was 30 °C for all isolates. It is concluded that *U. lactuca*, *U.rigida* and *U. scandinavica* from the Veerse Meer are al members of one highly polymorphic species.

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INTRODUCTION

In many shallow lagoons and estuaries, eutrophication processes have resulted in mass development of macroalgal biomass (Schramm & Nienhuis, 1996). The most characteristic species of these macroalgal communities include members of the closely related ulvophycean genera *Ulva* L. and *Enteromorpha* Link (e.g. Lowthion *et al.*, 1985; Brown *et al.*, 1990; Lavery *et al.*, 1991; Duarte, 1995). The distromatic, foliose morphology of *Ulva* species provides only a few features, which can be used as identification characters. In his genera revision of the European Ulvales, Bliding (1968) proposed a number of taxonomic criteria, by which he recognised eight species in Europe. Bliding used mainly microscopic characters: cell size and shape in the blade and basal area (both in surface view and in section), thallus thickness, cell arrangement, chloroplast position and number of pyrenoids. Koeman & van den Hoek (1981) largely confirmed the validity of these criteria in their study on Dutch *Ulva* species, although they stressed even more the importance of the morphology of the basal area and the holdfast of the thallus. Identification and revision o *Ulva* has been carried out along similar lines in other parts of the world (Coppejans & Gillis, 1983; Hoeksema & van den Hoek, 1983; Womersley, 1984).

Several authors, however, have questioned the validity of morphological characteristics for identification purposes, because of the large variation observed between individuals at different sites and between individuals at the same site in different seasons (Steffensen, 1976b; Mshigeni & Kajamulo, 1979; Phillips, 1984; Tanner, 1986). An additional problem is that under eutrophic conditions, *Ulva* spp. occur almost exclusively as free-floating thalli and lack basal parts, leaving only the morphology of the blade for identification. In the stagnan brackish lake the "Veerse Meer" (Southwest Netherlands), Malta & Verschuure (1997) observed dense blooms of free-floating *Ulva* thalli which were identified, using morphological characters, as a mixture of four species: *U curvata*, *U lactuca*, *U rigida* and *U scandinavica*. However, they questioned whether the observed morphological differences were actually indicative of different species or whether the differences instead represented morphotypes of a single species. Spatial and seasonal variability of morphological characteristics in the field has been described for *Ulva* species from Australia (Phillips, 1984, 1988), New Zealand (Steffensen, 1976b) and the USA (Tanner, 1986). For European species, the seasonal variability is almost unknown. Moreover, except for Steffensen (1976b), all studies have concentrated on attached populations. In our study, the variability of blade morphology of free-floating plants was described by comparing individuals within and between different sites in the Veerse Meer and the adjacent Oosterschelde estuary in the Netherlands.

The validity of the morphological characteristics was tested by comparing these results with phylogenetic analyses of DNA sequences. Analyses of the ribosomal (r)DNA sequences of the internal transcribed spacers (ITS) and flanking regions have been shown to provide good phylogenetic resolution at or below the species level in various red algae (Gof *et al.*, 1994; van Oppen *et al.*, 1995), brown algae (Peters *et al.*, 1997; Stache-Craine *et al.*, 1997; Leclerc *et al.*, 1998; Serrão *et al.*, 1999) and green algae (Bakker *et al.*, 1992; Kooistra *et al.*, 1993; Coleman *et al.*, 1994; Bakker *et al.*, 1995; Marks & Cummings, 1996; Pillman *et al.*, 1997). In the present study, nucleotide (nt) sequences of the 5.8S and ITS2 regions and parts of the flanking ITS1 and 26S regions of the nuclear-encoded ribosomal cistron for three putative *Ulva* species from the Veerse Meer and the Oosterschelde estuary were compared with each other and with *Ulva* sequences obtained from the literature. Sequences o *Enteromorpha linza* from the Veerse Meer and sequences obtained from van Oppen (1995) for *Enteromorpha* spp. from Greenland and *Monostroma arcticum* from Spitsbergen (Svalbard, Norway) served as

outgroups in the phylogenetic analyses. Furthermore, the optimum growth temperature and salinity of the differen *Ulva* species were studied experimentally to detect possible differences between morphologically recognised species.

The aim of this paper is to answer the following questions: (1) Are the morphologically recognised species separate monophyletic lineages on the basis of the ITS sequences or ar they members of a single polymorphic complex? (2) How well does the molecular phylogeny reflect what is known from morphological characters? (3) Do any morphological characters correlate with molecular data? (4) Do the morphologically recognised species differ in their optimum growth temperature and salinity and can these factors explain the distribution of the different species in the southwest Netherlands

Materials and Methods

Collection sites

Algal collection sites are listed in Table 5.1. We collected *Ulva* and *Enteromorpha* plants from the "Veerse Meer", a shallow, man-made, brackish (salinity = 15 - 20 psu average) lake, situated in the southwestern part of the Netherlands (lat. 51° 32' N, long. 3° 46' E). Additionally, two *Ulva* plants were collected from the Oosterschelde. The Oosterschelde is a former estuary. It is connected to the North Sea via a storm-surge barrier in the West. The Veerse Meer is connected to the saline Oosterschelde in the East by sluices. Seasona variation in thallus morphology o *Ulva* was monitored at two sites in the Veerse Meer. The first site, Middelplaten, is representative of most of the shallow sites of the lake and is situated close to the islands called the Middelplaten, about 50 - 75 m from a gully. The second site is called Kwistenburg (4.5 km east of Middelplaten). It borders the main gully opposite the sluices known as the Zandkreeksluis. Water depth in summer in both sites is between 0.70 and 0.90 m. More detailed information on the Veerse Meer can be found in Malta & Verschuure (1997) and references therein.

Culture methods

The Veerse Meer algae were cultured in the laboratory using a 3:2 mixture of filtered (Schleicher & Schuell no. 6) natural seawater from the Oosterschelde estuary and demineralized water (final salinity = 16 psu, the annual average salinity of the Veerse Meer) to make up a modified f/2 enrichment (Guillard & Ryther, 1962) using NH_4Cl as the nitrogen source instead of $NaNO_3$. The Oosterschelde algae were cultured at 33 psu. During the firs week of cultivation, GeO_2 was added to a concentration of 47.8 µM to suppress diatom growth (Le Gall *et al.*, 1990). The *U curvata* cultures were treated with 100 $mg \cdot l^{-1}$ cefotaxime (ICN biomedicals, Aurora, OHIO, USA) for one week to eliminate cyanobacteria (Kooistra *et al.*, 1991). Stock cultures were maintained at 15 °C under a 14:10, light:dark cycle at 40 $\cdot$ $^{2} \cdot s^{-1}$. The four Veerse Meer morphotypes that most closely matched the species descriptions of Koeman & van den Hoek (1981), UcurVM, UlacVM, UrigVM and UscaVM94 (Table 5.1), were maintained in culture for a longer period and used in the temperature and salinity experiments. Parts of the algae collected in 1994 and June 1995 were grown as unialga cultures to avoid problems of contaminant ITS sequences. These cultures were obtained by cultivating small fragments of the algae in sterilised medium (light and temperature as above). After 2 weeks a visually clean piece of thallus was cut out and inoculated into fresh medium. This procedure was repeated three to five times until cultures were unialgal. The cultures were then transferred to aerated Erlenmeyer flasks at the same temperature, light and nutrien conditions. Medium was renewed every 2 weeks, until sufficient biomass (> 0.5 g fresh weight) was obtained for DNA extraction. Algae collected in September 1995 were not cultured, instead DNA was extracted from field material, because by then we had designed a

Table 5.1: Dutch *Enteromorpha* and *Ulva* isolates used in the phylogenetic analysis: names, codes, collection site and date, number of individuals sequenced and the accession numbers of the sequences in the Genbank database (upper panel). Names, codes, number of individuals sequenced and the accession numbers of the sequences in the Genbank database for the sequences of van Oppen (1995) and literature source for published ITS sequences used in the phylogenetic analyses (lower panel).

Species name	Code	Collection site	No. sequenced ind. (and acc. numbers)	Collection date or literature source
U. curvata (Kützing) De Toni [a]	UcurVM	Veerse Meer	0 [b]	Oct 1994
U. lactuca L. [a]	UlacVM	Veerse Meer	1 (AF153488)	Oct 1994
U. rigida C. Agardh [a]	UrigVM	Veerse Meer	1 (AF153489)	Sept1995
U. rigida C. Agardh	UrigOS	Oosterschelde	1 (AF153490)	Sept 1995
U. scandinavica sensu Koeman & van den Hoek (1981) [a]	UscaVM94	Veerse Meer	1 (AF153486)	Oct 1994
U. scandinavica sensu Koeman & van den Hoek (1981)	UscaVM10, VM11, VM25	Veerse Meer	3 (AF153483, AF153484, AF153485)	Jun 1995
U. scandinavica sensu Koeman & van den Hoek (1981)	UscaOS	Oosterschelde	1 (AF153490)	Sept 1995
E. linza (L.) J. Agardh	ElinVM	Veerse Meer	1 (AF153491)	Jun 1995
E. clathrata (Roth) Greville	EclaGL	Disko Island, Greenland	1 (AF153492)	van Oppen, 1995
E. intestinalis (L.) Link	EintGL	Disko Island, Greenland	1 (AF153493)	van Oppen, 1995
M. arcticum Wittrock	MarcSB	Spitsbergen, Norway	1 (AF153495)	van Oppen, 1995
U. armoricana Dion, de Reviers et Coat	UarmBri	Brittany, France		Coat *et al.*, 1998
U. rigida C. Agardh	UrigBri	Brittany, France		Coat *et al.*, 1998
U. rigida C. Agardh	UrigAus	Flinders, Australia	1 (AF153494)	van Oppen, 1995
U. rotundata Bliding	UrotBri	Brittany, France		Coat *et al.*, 1998

[a] Isolate used to determine optimum temperatures and salinities for growth
[b] Attempts to PCR-amplify *U. curvata* failed.

primer for the polymerase chain reaction (PCR) amplification specific for Ulvaceae.

Morphological observations
Morphological observations were made on the day of collection using a light microscope. Identification of morphotypes was based on the morphology of the blade following the criteria of Bliding (1968) and Koeman & van den Hoek (1981). Macroscopic observations on the algae included the thallus colour and texture and, if attached morphotypes were found, the holdfast was screened for a central cavity. The following microscopic characteristics were recorded: cell sizes (longest dimensions) of at least ten randomly chosen cells, thallus

thickness (observed in three cross-sections of each plant), shape and arrangement of the cells in surface view, shape and arrangement of the chloroplast in surface view (cap-like appearance or not) and number and distribution of pyrenoids. Several parts of the thallus were examined for each plant. Herbarium specimens were made of representative thalli and voucher samples of thallus material were stored in 4 % formalin-seawater.

Seasonal changes in morphology of the dominant *Ulva* forms were investigated *in situ*. To this end, cages made of plastic-coated steel mesh (0.01 x 0.01 m mesh width) were mounted on a frame of iron poles. One cage was placed at the Middelplaten site and inoculated with one large thallus of the locally dominan *U. scandinavica*; the other cage was placed a Kwistenburg and inoculated with *U. lactuca*. In the period from May to November 1995, pieces of algal thallus were sampled monthly and observations made on algal morphology (see above).

DNA extraction and ITS sequencing

DNA was extracted using the modified LiCl method of Hong *et al.* (1992), as described by van Oppen *et al.* (1995). DNA quality was checked on 0.8 % TBE (Tris borate EDTA) agarose gels (Sambrook *et al.*, 1989) and stained with ethidium bromide. Double-stranded (ds) - PCR amplifications were performed in a HYBAID OmniGene thermocycler with an initial denaturation step of 95 °C for 3 min followed by 5 cycles of 95 °C for 1 min, 55 °C for 2 min and 72 °C for 2 min. This was followed by 25 cycles with the following temperature profile: 1 min at 95 °C, 2 min at 52 °C and 2 min at 72 °C. The final step was at 72 °C for 2 min. The reaction volume was 50 µl, consisting of 20 - 100 ng genomic DNA, 200 µM each of dATP, dTTP, dCTP and dGTP, 0.5 µM of each primer, 5 µl of 10 x reaction buffer (500 mM KCl, 100 mM Tris/HCl pH 8.3, 0.1 % gelatine), 1 unit Taq DNA polymerase (Promega, Madison, WI, USA) and 1.5 mM $MgCl_2$. Reactions were overlaid with mineral oil. Two primers were used. Forward primer UR1 (5' – CGGTCTCGCATTGCTTTGTA – 3') was designed in our laboratory and anneals at position 217 in a partial 18S gene alignment by van Oppen (1995). Reverse primer ITS4 (5' – TCCTCCGCTTATTGATATGC – 3'), taken from White *et al.* (1990), anneals at position 57 (26S) in their alignment. Amplifications were examined for correct length, purity and yield on 0.8 % agarose TBE gels as described earlier. The desired DNA fragment was cut out of the agarose gel with a sterile scalpel and the DN purified using the QIAquick Gel Extraction Kit (Quiagen, Hilden, Germany) or the JETsorb Gel Extraction Kit (GENOMED, Bad Öyenhausen, Germany) according to the manufacturer's instructions. Clean DNA was finally dissolved in 40 µl of sterile milliQ water. The PCR product was cloned using the pGEM - T Vector System II Ligation Kit (Promega, Madison, WI, USA) and using JM109 High Efficiency Competent Cells (Promega) following the manufacturer's instructions. Plasmid DNA was isolated by means of alkali lysis and PEG precipitation, following the protocol of Birnboim & Doly (1979). Plasmids were sequenced using the Vistra Thermo Sequenase core sequencing kit with 7-deaza-dGTP from Amersha (Little Chalfont, Buckinghamshire, UK) following the manufacturer's instructions for dye-primer cycle sequencing (Murray, 1989). For each sequencing reaction, 1 - 5 µl of the redissolved isolated plasmid was used. Primers used were the Texas Red M13 (-21) forward 18-mer sequencing primer (Amersham) and the reverse 18-mer sequencing primer (Amersham). Samples were loaded on a Vistra Hydrolink Long Ranger gel (Amersham) and run on a Vistra DNA Sequencer 725 (Amersham).

Sequences were processed using the Sequencer 3.0 software package (Gene Codes Corporation, Ann Arbor, MI, USA) and format conversions were carried out in SeqApp 1.9a169 (Gilbert, 1993). Termini of rRNA coding regions and starting positions of the ITS

regions were determined by comparisons with published rDNA sequences of *Ulva* (Table 5.1). Final alignments were done manually using a text editor. Phylogenetic analyses were carried out using the aligned sequences from the isolates in this study, together with sequences found by van Oppen (1995) and Coat *et al.* (1998) of other Ulvales (Table 5.1). Phylogenetic analyses were performed with PAUP 4.0b2 (Swofford, 1998) using three different optimality criteria: maximum parsimony, maximum likelihood and neighbour-joining. In all cases, *M. arcticum* was used as outgroup and uninformative characters were ignored. Maximum-likelihood analysis was based on the Hasegawa-Kishino-Yano model. Neighbor-joining analyses were performed with two distance models: uncorrected distances and Jukes-Cantor. Confidence limits of clades were estimated by bootstrap analysis (Felsenstein, 1985) with 100000 replicates.

Determination of optimum temperature and salinity for growth
Specific growth rates at different temperatures were determined of the Veerse Meer *Ulva* morphotypes (see Table 5.1 for collection dates) using circulating flow cultures. Ten litres of modified f/2 medium (S = 16 psu, see above) was circulated through a flat perspex cylinder (diameter 20 cm, height 5 cm) at a speed of 18.9 l·h^{-1} (Cole-Parmer Masterflex pump). The cylinders were divided into four compartments using iron mesh (mesh width 2 x 2 mm) wit silicone tubing on the edges to prevent damage to the algae. The medium was constantly aerated to prevent oxygen and/or carbon limitation of the algae; light conditions were the same as those for the stock cultures. Algal material was taken from the stock cultures and discs (diameter 1.8 cm) were punched out from healthy thalli using a sharpened stainless stee cylinder. The discs were placed in the cylinders, one disc per compartment. The cylinders were then transferred to the experimental temperatures (5, 10, 15, 20, 25 and 30 °C) gradually, the algae never being subjected to temperature steps larger than 5 °C per week. Temperatures up to 20 °C were obtained in climate chambers; higher temperatures were reached by heating of the medium to the desired temperature using aquarium heater thermostats (Rena Cal). The temperature of the medium was monitored during the experimen using a Thermistor 10K sensor connected to a data logger (Omega RD-TEMP).

Specific growth rates (μ) were determined by measuring the wet weight of each disc (Sartorius basic balance) after removing excess water by gently blotting between four layers o tissue. The algae started to grow exponentially after 3-4 days and continued to do so until the end of the experiment. μ was defined as the slope of the line through the Napierian logarithms (ln) of the wet weight during the exponential growth phase. This slope was determined using linear regression (for all regressions $R^2 \geq 0.95$). All experiments lasted 2 weeks and were repeated three times so that 12 measurements of growth rates were available per morphotype per treatment. In two cases only 10 measurements were available because two discs died. During the first experiments, the four different morphotypes were put together in one cylinder. In the last two experiments, only one morphotype per cylinder was used. No significant differences within morphotypes were found between the experiments (ANOVA, $p > 0.05$), so we decided to pool all data per morphotype. If spore formation in the discs was suspected (little colourless or white spots) microscopic observations were made on the discs. The potential presence of spores or loose cells in the medium or on the cylinders was checked by analysing samples of the medium using a light microscope and an epifluorescence microscope.

The effect of salinity on μ was tested at 15 °C, using the same morphotypes and experimental set-up as in the temperature experiment. Eight different salinities were tested: 5, 10, 15, 20, 25, 30, 35 and 40 psu. Salinities lower than natural seawater (± 32 psu) were obtained by

diluting the filtered (Schleicher & Schuell no. 6) seawater with demineralized water. For the higher salinities, NaCl was added to the desired level. Medium salinity was checked with a WTW microprocessor conductivity-meter mounted with a WTW KLE 1/T electrode (reading psu). Modified f/2 enrichment (see above) was added to the medium. μ was determined as above. All experiments lasted 2 weeks and were repeated three times. One morphotype per cylinder was used in each experiment.

RESULTS

Morphology and distribution

Both free-floating and attached plants o *U. curvata* always had thin thalli, polygonal cells without rounded corners which were at least partly arranged in distinct rows and rarely more than one pyrenoid per cell (Table 5.2). Moreover, all attached plants possessed the characteristic central cavity in the holdfast. All other *Ulva* morphotypes were only found floating or lying on the sediment and never had a holdfast. The cells o *U. curvata* were usually slightly smaller than the cells of the other *Ulva* morphotypes.

U. rigida from the Oosterschelde had a thicker thallus than *U. rigida* from the Veerse Meer. Furthermore, many cells of the Oosterschelde specimens had more than two pyrenoids. *U. rigida* differed from *U. lactuca* and *U. scandinavica* by the thick thalli, the distinctly rounded cells, the relatively large number of cells with a cap-shaped chloroplast and the uniform distribution of pyrenoids. Large overlap in thallus thickness was found between *U. lactuca* and *U. scandinavica* and their cell shape was nearly identical. These morphotypes could be distinguished from each other by cell ordering and pyrenoid number and distribution. Much variation was observed in the number and distribution of pyrenoids in *U. scandinavica*, especially when plants were collected in late summer or autumn. *U. scandinavica* specimens from the Oosterschelde (which were only collected in September) had slightly thicker thalli compared with the Veerse Meer plants. The cells of most plants o *U. lactuca*, *U. rigida* and *U. scandinavica* contained many starch grains or unidentified vesicles surrounded by starch, obscuring the view on the chloroplast and the pyrenoid. All characteristics were maintained during cultivation except for thallus thickness, which slightly decreased in all morphotypes.

Marked seasonal variation was observed in the morphology of *U. lactuca* and *U. scandinavica* (Table 5.3). Thallus thickness increased during the growing season in both morphotypes, while cell size was more or less constant. Large variation was observed in the numbers and distribution of pyrenoids in the cells of *U. scandinavica*. At the onset of growt (May), at least 50 % of the cells had more than one pyrenoid, while at the end of the growing season the majority of the cells had only one pyrenoid and cells with more than two pyrenoids became very rare. In August, pyrenoids could not be observed in either morphotype because of the large amount of starch grains and unidentified vesicles present in the cells at that time. The thalli tended to become darker at the end of the year, except for *U. scandinavica* in October, when new tissue was formed on the remains of old thalli. The texture of the algae also changed, from smooth and slimy in spring to wrinkled and rough in autumn.

ITS sequence analysis

The alignment has a length of 482 nt positions. Complete sequences were obtained for the ITS2 region (213 nt positions) and the 5.8S gene (160 nt positions) and flanking regions (50 nt positions of the ITS1 region and 59 nt positions of the 26S gene) from eight *Ulva* individuals,

Table 5.2: Morphological and cytological characteristics of *Ulva* species from the Veerse Meer (VM) and the Oosterschelde (OS) (southwest Netherlands). *n* = number of individuals studied. For thallus thickness and cell diameter the most frequently observed range is listed, with extremes between brackets. Pyrenoid number and distribution are indicated as the approximate percentage of cells (values between brackets) which have a specified number of pyrenoids.

Characteristics:	*U. curvata* (VM)	*U. lactuca* (VM)	*U. rigida* (VM)	*U. rigida* (OS)	*U. scandinavica* (VM)	*U. scandinavica* (OS)
n	37	16	2	5	39	5
Thallus colour	Light green	Medium dark green	Dark green	Dark green	Light green	Light green
Texture	Membranous, slimy	Slimy, stronger thallus than *U. curvata* and *U. scandinavica*	Stiff strong thallus	Stiff strong thallus	Membranous, slimy	Membranous, slimy
Thallus thickness (µm)	(37.5-)40-42.5(-50)	(40-)52-64(-80)	85-90	95-110	(43-)48-56(-65-70)	(50-)52-58(-60)
Cell shape (surfac view)	Polygonal, corners not or slightly rounded	Polygonal, corners slightly rounded	Polygonal, distinctly rounded corners	Polygonal, distinctly rounded corners	Polygonal, corners slightly rounded	Polygonal, corners slightly rounded
Cell diameter (µm)	(10-)12-16(-22)	(12-)15-19(-23)	15-20	13-15(-17.5)	(10-)12-19(-26)	14-16
Number and distribution of pyrenoids	1 (100 %), 2 (very rare)	1 (80-95 %), 2 (5-20 %)	2	2-3 (100 %), 4 (very rare)	1 ([30-]40-60[-80] %), 2-5 ([20-]40-60[-70] %)	2-3 (70 %), 1(30 %)
Cell arrangement	Most of the cells in rows	Some cells in rows, mostly in indistinct groups	Some cells in rows, mostly in indistinct groups	Most of the cells in rows	Mostly without order	Mostly without order
Chloroplast cap-lik (surface view)	Mostl	Rare	Occasionally	Mostl	Extremely rare (1 observation)	No
Plants attached	In 3 of 7 cases	Never	Never	Never	Never	Never
Dentate thallus margin	No	No	No	No	No	No
Remarks	Attached plants with central cavity in holdfast	Many starch grains in most cells	Many starch grains in most cells	-	Many starch grains in most cells	Many starch grains in most cells

Table 5.3: Seasonal variation of morphological and cytological characteristics of *Ulva lactuca* from the site Kwistenburg and *U. scandinavica* from the site Middelplaten in the Veerse Meer (Southwest Netherlands) from May to October 1995. For thallus thickness and cell diameter the most commonly observed range is listed, with extremes between brackets. Pyrenoid number and distribution are indicated as the approximate percentage of cells (values between brackets) which have a specified number of pyrenoids. n.d. = not determined.

Month	Thallus thickness (µm)	Cell diameter (µm)	Distribution of pyrenoids in cells	Cell arrangemen	Remarks
	Ulva lactuca (Kwistenburg)				
May	45-50	(18)-19-(20)	1 (70 %), 2-3 (30 %)	Not ordered or indistinct groups	-
June	50-56	(16)-19-(23)	1 (75 %), 2 (25 %), 3 (rare)	Indistinct groups	-
July	52-58	(12)-15-(18)	1 (80 %), 2 (20 %), 3 (rare)	Not ordered	Many starch grains
August	50	(17)-19-(20)	Not visible	Not ordered	Filled with starch, some cap-like chloroplasts
September	60-66	n.d.	1 (80 %), 2-3 (20 %)	Not ordered	Thallus partly decomposing
October	57-64	n.d.	1 (70 %), 2 (30 %), 3-4 (rare)	Not ordered	Thallus looks young
	Ulva scandinavica (Middelplaten)				
May	48-55	16-18	1 (35 %), 2 (65 %), 3 (5 %)	Not ordered	-
June	44-48	(15)-19-(26)	1 (55 %), 2 (40 %), 3 (5 %)	Not ordered	-
July	48-54	(12)-17-(23)	1 (50 %), 2 (50 %), 3 (rare)	Not ordered	Many starch grains, pyrenoids difficult to see
August	50-53	n.d.	Not visible	Not ordered	Very dark green, filled with starch, thallus wrinkled
September	46-50	n.d.	1 (80 %), 2 (20 %)	Not ordered	Pyrenoids difficult to see
October	60-70	n.d.	1 (70 %), 2-3 (30 %)	Not ordered	Pyrenoids difficult to see

representing three morphologically identified species, and one individual of *Enteromorpha linza*. These sequences and those from van Oppen are available in the Genbank database (see Table 5.1 for accession numbers). Efforts to obtain PCR-amplifications of the ITS from three *U. curvata* isolates were unsuccessful. The complete alignment, including all sequences obtained from the literature, is also available in the Genbank database (accession number ds38662) The ITS alignment, including sequences from the literature, shows that UlacVM, UrigVM, UscaVM25, UscaVM94 and UscaOS were identical to UscaVM11. The alignmen of UscaVM10 differed by only one base substitution at position 247 (A instead of G). The partial ITS1, 5.8S, ITS2 and partial 26S sequences show respectively 10 (20 %), 4 (2.5 %), 69 (34 %) and 1 (1.7 %) indels/substitutions among the Ulvales (i.e. excluding *M. arcticum*). For

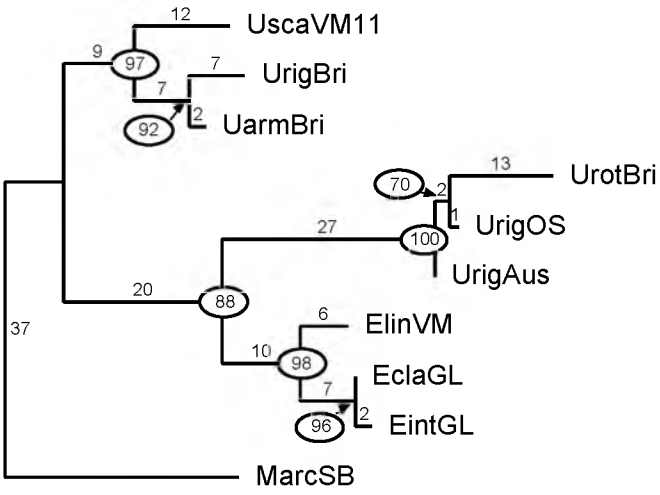

Fig. 5.1: Single most parsimonious tree (162 steps) of the Ulvales and the Ulotrichalean outgroup MarcSB based on rDNA 5.8S and ITS2 plus flanking regions. Unweighted parsimony under an exhaustive search option. See Table 5.1 for abbreviations. The isolates UlacVM, UrigVM, UscaVM94, UscaVM10, UscaVM25 and UscaOS are at the same position as UscaVM11. Total number of character state changes is given along each branch. Numbers at the nodes indicate bootstrap values.

the whole alignment of all isolates, 77 of the 482 sites are informative.

For the parsimony analysis, uninformative characters were ignored. Parsimony analyses of the alignment treating gaps as a fifth base or as missing data resulted in the same tree topologies. Excluding gaps or excluding a part of the gaps in order to treat two or more position gaps as one event, also resulted in the same tree topologies. Fig. 5.1 shows the most parsimonious tree (MPT) of 162 steps (consistency index = 0.79; retention index = 0.81; gaps included as fifth base). The data show low homoplasy. Besides the outgroup, *M. arcticum*, three main groups could be distinguished clearly. One group comprises the three *Enteromorpha* species. Another main group consists of UrotBri, UrigOS and UrigAus. A third main group consists of UrigBri UarmBri and all the Veerse Meer *Ulva* morphotypes plus *U. scandinavica* from the Oosterschelde. The high bootstrap values (Fig. 5.1) give strong support to these three groups. The neighbor-joining analyses also resulted in the same tree topology as with parsimony (not shown). The maximum likelihood tree also shows the two *Ulva* groups, but the three *Enteromorpha* species are paraphyletic with respect to the UrotBri/UrigAus/UrigOS group. ElinVM branches off first and EintGL last (not shown).

Temperature and salinity experiments

All isolates tested survived and grew over the tested temperature range of 5 - 30 °C during the 2 weeks of the experimentation period. At low temperatures, the specific growth rate (μ) o *U. curvata* was low and it increased almost linearly with temperature, reaching its maximum a 25 °C (Fig. 5.2a). At 30 °C, μ was still 67 % of the observed maximum. The other morphotypes exhibited a very different temperature response, reaching their maximum μ at 10 °C (Fig. 5.2a, b). The temperature responses of *U. lactuca* and *U. rigida* were not significantly different from one another (two-way ANOVA, $p > 0.05$). The temperature response of *U. scandinavica* was slightly different from those of *U. lactuca* and *U. rigida* in that at 15 °C a significant dip in μ was found and at 25 °C, μ was significantly higher, reaching 66 % of the maximum (two-way ANOVA, $p < 0.05$). At 15 °C, most discs o *U. scandinavica* and 20 - 25 % of the discs of the *U.lactuca* and *U. rigida* isolates showed littl white spots, indicating cell sloughing and/or sporulation. Growth rates of these discs were still exponential and not lower than in the other discs at 15 °C. In the medium only loose cells and debris were found, not spores. Extreme (90 % of the disc) cell sloughing occurred in two discs of *U. lactuca* at 15 °C, resulting in non-exponential, negative growth rates. These data were not used in the calculation of the average μ.

All morphotypes survived and grew over the tested salinity range of 10 - 40 psu (Fig. 5.3a, b). At 5 psu *U. curvata* died, while no growth and occasional cell sloughing was observed in the other morphotypes. The shape of the µ versus salinity curve was similar for al morphotypes. µ showed a linear increase with salinity up to a maximum at 30 psu, after which a decrease was observed. Thalli of *U. rigida* and *U. scandinavica* became more wrinkled when grown at a salinity of 30 psu or higher.

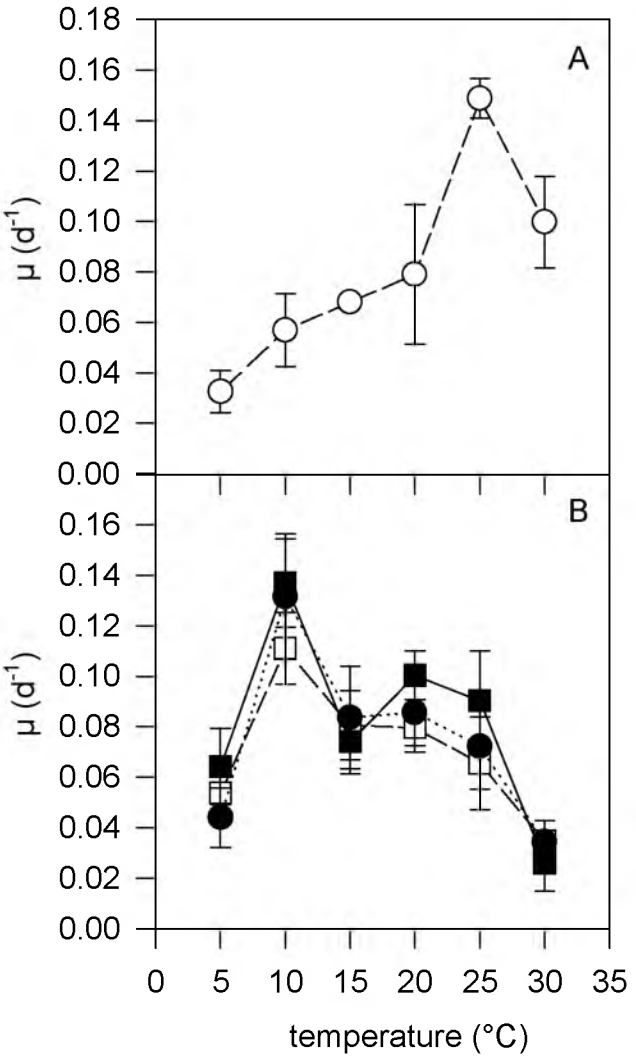

Fig. 5.2: Specific growth rates (µ, d^{-1}) of *Ulva* spp. as a function of temperature (°C). (A) *U. curvata* (open circles, dashed lines). (B) *U. lactuca* (filled circles, dotted line), *U rigida* (open squares, dashed lines) and *U scandinavica* (filled squares, continuous line). Error bars represent ± on standard deviation.

DISCUSSION

Congruence between morphological and molecular data

The results indicate that the analysis o ITS sequences of free-floating *Ulva* species can provide good phylogenetic resolution at the species level within the order Ulvales, since the sequences were relatively easy to align and contained a considerable number of informative sites (77). Recent studies on *Enteromorpha* spp. (Blomster *et al.*, 1998) and *Ulva* spp. (Coat *et al.*, 1998) have come to similar conclusions. In this study onl partial ITS1 sequences were obtained, whereas this type of phylogenetical study usually uses the entire ITS1. Considering the high bootstrap values that support the subgroups and the fac that the various methods used for tree construction gave highly similar results, we do not think that including the whole ITS1 region would have changed the results dramatically.

On the basis of morphological characteristics, Malta & Verschuure (1997) distinguished four species in the Veerse Meer lagoon: *U. curvata*, *U. lactuca*, *U. rigida* and *U. scandinavica*. The latter three could not, however, be distinguished from each other nor from *U. scandinavica* from the adjacent Oosterschelde estuary on the basis of the ITS sequences, bu they were different from *U. rigida* from the Oosterschelde. It is concluded that the Veerse Meer "species" (except for *U. curvata*) are in fact different morphotypes of one polymorphic species. From this, it follows that most of the morphological characteristics available in floating *Ulva* spp. in the Veerse Meer and the Oosterschelde vary too much to allow identification to the species level. A similar conclusion was drawn for floating *Ulva* species in Brittany, but there the identification of the isolates could be checked with attached plants (Coa *et al.*, 1998). Unfortunately, for unknown reasons we did not succeed in sequencing *U. curvata*. Morphologically, this isolate clearly appears to be a separate species. Due to the lack

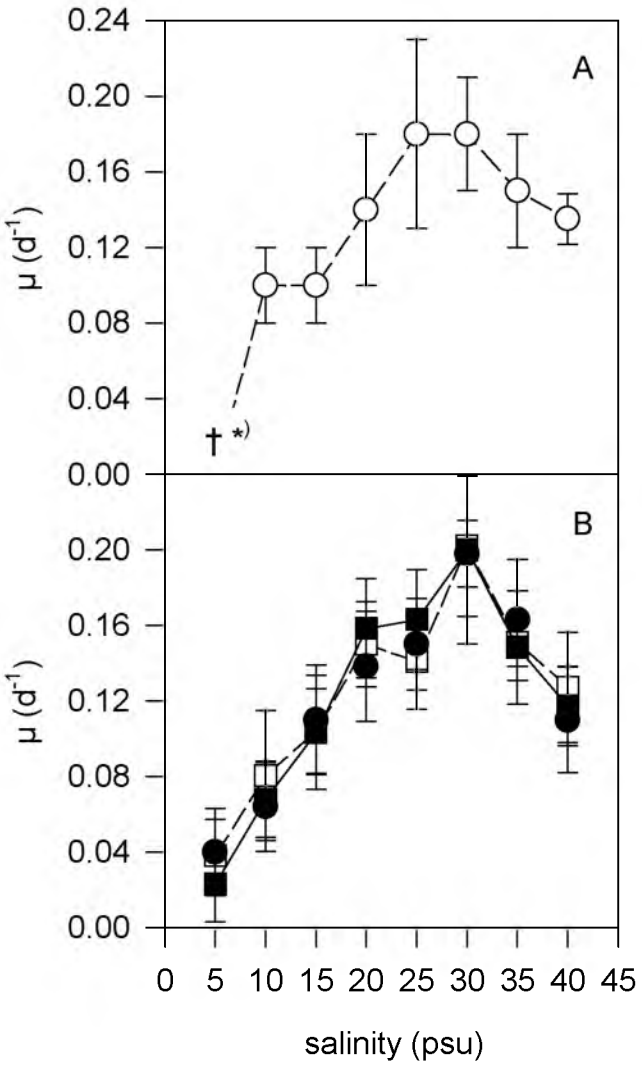

Fig. 5.3: Specific growth rates (μ, d^{-1}) of *Ulva* spp. as a function of salinity (psu). (A) *U. curvata* (open circles, dashed lines). (B) *U. lactuca* (filled circles, dotted line), *U rigida* (open squares, dotted lines) and *U. scandinavica* (filled squares, continuous line). Error bars represent ± on standard deviation. *) *U curvata* died at 5 psu.

of sequence data, however, we cannot be sure that this isolate indeed represents a different species.

The morphology o *U. curvata* was relatively constant and matches the escriptions of Koeman & van den Hoek (1981). It should be noted that Bliding (1968) does not mention a central cavit in the holdfast for *U. curvata*, although it seems to be present on one of his micrographs. The other morphotypes present more problems. Thallus thickness, cell diameter and pyrenoid number and distribution of *U. lactuca*, *U. rigida* and *U. scandinavica* in the Veerse Meer were all highly variable. Thallus thickness mainly varied through the season and is thus probably related to thallus age (Bliding, 1968; Phillips, 1988; Table 5.3). It seems that only the thallus characteristics of young (brigh and fresh-looking, fast growing) thalli match the species descriptions of Koeman & van den Hoek (1981). Cell size is known to vary under different environmental conditions such as salinity (Koeman & van den Hoek, 1981), temperature and light availability (Israel *et al.*, 1995) and for these reasons does not seem to be a good characteristic for identification. The seasonal variability of characteristics was observed within one genotype of the "species" *U. lactuca* and *U. scandinavica*, illustrating their phenotypic plasticity

The usefulness of pyrenoid number and distribution is still a matter of debate. Kapraun (1970), Tanner (1986) and Phillips (1988) all found this character to be too variable to draw conclusions, whereas Bliding (1968) and Koeman & van den Hoek (1981) found it useful for identification. In our study, *U. curvata* could clearly be identified on the basis of pyrenoi number and distribution, while variation between the other morphotypes was considerable. Considering pyrenoid numbers, two groups can be distinguished (Hoeksema & van den Hoek, 1983; Dion *et al.*, 1998): a group of species with one pyrenoid in most (90 - 100 %) of the cells (e.g. *U. curvata* and *U. lactuca*) and a group with varying pyrenoid numbers and distribution (e.g. *U. armoricana*, *U. rigida*), provided that observations are made on young thalli. It should be noted that pyrenoids can be very hard or even impossible to observe if the cells contain many starch grains, as was the case in mos *U. scandinavica* plants. Moreover,

the plants may be able to change pyrenoid numbers in the cells under changing environmental conditions. This may especially be the case when the algae are confronted with changes in the levels of dissolved inorganic carbon, as was observed in some planktonic chlorophyte species (Miyachi *et al.*, 1986; Ramazanov *et al.*, 1996). One can also question whether the starch grains can have taxonomic significance, considering their commonness in most individuals in all seasons. A possible physiological regulation of pyrenoid numbers and distribution and o starch grains certainly deserves attention in further studies.

Nomenclature

In the Veerse Meer, *U. scandinavica sensu* Koeman & van den Hoek (1981) was dominant, so we concluded that this should be the species name for all morphotypes except for *U. curvata*, for which there is enough evidence to maintain it under its present name. This name is chosen randomly until typification of the relevant entities can be carried out. Coppejans & Gillis (1983) have shown that this species can occur exclusively free-floating. Our *U. scandinavica* resembles closely the recently newly described species *U. armoricana* Dion, de Revier et Coat, but, the ITS sequence data are not identical (Coat *et al.*, 1998; Di *et al.*, 1998, Fig. 5.1). *U. rigida* from the Veerse Meer lagoon, the Oosterschelde estuary and Brittany al appear to represent different species based on ITS sequences. The *U. rigida* specimens from the Oosterschelde and Australia and *U. rotundata* from Brittany, however, are very similar. To solve this problem, herbarium material should be morphologically (re)described and, i possible, sequenced, resulting in a complete revision of the genus. The results of this stud may be helpful in investigating some of the morphological characteristics for their variability and suitability as identification characteristics, especially when free-floating populations are involved.

The position of the *Enteromorpha* species in our phylogenetic tree (Fig. 5.1) shows that the genus *Ulva* is paraphyletic. Species in the Ulvales are typically separated into genera based on flat, distromatic blades (*Ulva*) and tubular, monostromatic blades (*Enteromorpha*) (see Papenfuss, 1960). *E. linza*, until recently placed in the genus *Ulva* (Newton, 1931), often loses its tubular form and assumes a distromatic morphology. Conversely, *U. curvata* possesses a central cavity in the basal area and is thus partly tubular. On the basis of these overlaps in morphology and the similar developmental patterns (Bliding, 1963, 1968), Bonneau (1977) suggested that the genus *Enteromorpha* should be included in the genus *Ulva*. This view is supported by the molecular data presented in our study.

Temperature, salinity and distribution

The results of the temperature experiments show that the *U. scandinavica* morphotypes fro the Veerse Meer (*U. "lactuca"*, *U. "rigida"* and *U. "scandinavica"*) do not only differ in morphology, but also have a slightly but significantly different temperature - growth response. Similarly, Steffensen (1976a) found a different optimum growth temperature for free-floating and attached morphotypes o *U. lactuca* from New Zealand. The growth - temperature responses of the *U. scandinavica* isolates from the Veerse Meer are more or less typical for algae from a cold temperate region (Fortes & Lüning, 1980; Lüning, 1990), although the temperature range in which a high μ (> 60 % of maximum) could be maintained is quit broad. Kamermans *et al.* (1998) showed that *U. scandinavica* can survive at -5 °C for at leas 2 weeks. Considering data of other cold temperate algae, the lower survival limit is probabl close to -5 °C. Occasionally, cultures were subjected to temperatures higher than 30 °C. When this happened, the algae died within 2-3 days (E. Malta, pers. obs.), indicating that the upper survival temperature is in the region of 30 - 35 °C. The growth dip found at 15 °C in *U. "scandinavica"* and to a lesser extent also in *U. "lactuca"* and *U. "rigida"* is probably

caused by processes involved in preparing for gamete formation or vegetative reproduction (i.e. spore formation, cell sloughing or fragmentation; Bonneau, 1977, 1978; Beach *et al.*, 1995) as is indicated by the observed morphological changes (occurrence of white spots on the discs, fragmentation). A similar dip in the temperature - growth response curve caused b spore formation was observed in different North Atlanti *Cladophora* species by Cambridge *et al.* (1987).

The growth - temperature response of *U. curvata* differed markedly from those of the *U. scandinavica* morphotypes. The μ at 5 °C of *U. curvata* was very low (only 22 % of the maximum). Furthermore, the optimal growth temperature is 25 °C, which is quite high for an algal species from the cold to warm temperate region (Fortes & Lüning, 1980; Yarish *et al.*, 1986; Lüning, 1990). Duke *et al.* (1989) report a similar optimum value of 20 - 23 °C for *U. curvata* from North Carolina, USA. However, this is certainly not a unique phenomenon and other temperate species such as *Cladophora sericea* also show high optimum growth temperatures (Breeman & Pakker, 1994). Koeman & van den Hoek (1981) found that the distribution of *U. curvata* in the Netherlands was mainly restricted to the shallow parts of the stagnant man-made lagoons in the southwestern part. Temperatures in these lagoons can reach 25 °C or higher in summer. The high optimum growth temperature and the relatively poor performance at lower temperatures (less than 50 % of the maximum μ at temperatures below 20 °C) of *U. curvata* might be a good explanation for the restricted distribution pattern in the Netherlands.

The relationship between μ and salinity followed an optimum curve with a maximum μ observed at 30 psu in all isolates, which is typical for mos *Ulva* spp. (Bliding, 1968; Koeman & van den Hoek, 1981; Dickson *et al.*, 1982). Five psu seems to be the minimum tolerance limit for the *U. scandinavica* morphotypes, while *U. curvata* died at this salinity. These results are in good agreement with the results of Koeman & van den Hoek (1981) and Koeman (1985) on various Dutch *Ulva* species. They found that *U. scandinavica* germlings and young blades survived at 5 psu, while young *U. curvata* plants died and that both species grew best at higher salinities (17 - 34 psu). Both higher and lower optimal salinities have been reported for various *Ulva* species (e.g. 25 psu for *U. fasciata* (Morand & Briand, 1996) and *U. pertusa* (Floreto *et al.*, 1994), 35 - 40 psu for *U. lactuca* (Friedlander, 1992) and *U. rigida* (Zavodnik, 1975). Although species-specific differences and ecotypic variation in salinity tolerance can occur in *Ulva* (Zavodnik, 1975; Friedlander, 1992; Floreto *et al.*, 1994; Morand & Briand, 1996), this is not the case for the morphotypes in the brackish Veerse Meer.

Concluding remarks

We conclude that the morphologically recognised species *U. lactuca* and *U. rigida* from the Veerse Meer and *U. scandinavica* from the Veerse Meer and the Oosterschelde (Southwest Netherlands) in fact represent one polymorphic species, which is different from *U. curvata* (Veerse Meer) and *U. rigida* (Oosterschelde). We have shown that reliable taxonomic identification of *Ulva* species is unlikely to be obtained on the basis of morphology alone, especially when only free-floating individuals are present. ITS sequences are good markers but not suitable for a quick field identification. Thallus morphology can not only differ between different individuals of the same species, but can also vary considerably during the growing season. However, thallus thickness and pyrenoid number and distribution can be helpful for a distinction between groups of species, provided that observations are made on young thalli. Cell size shows too much overlap between species, and is thus not a good character. The relatively poor performance o *U. curvata* at temperatures below 20 °C might be an explanation for its restricted distribution pattern in the Netherlands.

CHAPTER VI: GROWTH-LIMITING GRADIENTS IN MACROALGAL MATS AND CONSEQUENCES FOR *ULVA* L. PHYSIOLOGY

ABSTRACT

Eutrophic coastal waters are often characterised by dense mats of free-floating macroalgae. Experiments, described in the literature, predict the occurrence of at least two growth-limiting gradients in such mats: the light gradient will limit growth of bottom layers while upper layers become progressively nitrogen limited. This study was designed to test whether this prediction holds in a field situation in different seasons. Macroalgal mats were sampled in June, July and September in the eutrophic lagoon the Veerse Meer (SW Netherlands). At al three sampling dates, the mats were composed almost entirely of *Ulva* spp., in September the mats were in decay and covered with silt and epiphytes. In June and July, total dissolved inorganic nitrogen concentration (DIN) of the water in the mat was significantly higher than outside the mat. Tissue N levels in the algae indicated progressive nitrogen limitation towards the surface of the mat in June and July: tissue N in top layers was around 1 % DW, while tissue N in the bottom layers was always over 2.2 % DW. No vertical gradient in tissue C was found. Wide-band absorption increased with depth in the mat and throughout the season, due to higher chl *a* and *b* and lutein contents. The shape of the absorption spectrum was similar for all layers. The absorption of the silt/epiphyte film on the top *Ulva* layer was highest; it absorption spectrum (high absorption in the 500 – 560 nm) film indicates that film on the top layers of the macroalgal mats mainly consist of diatoms. In June, dark-adapted quantu efficiency (measured as chl fluorescence) and the glutathione redox ratio of the algae increased with depth in the layer. From this, it is concluded that upper layers suffer from oxidative stress leading to photoinhibition. September measurements of quantum efficienc indicate severe stress; the algae were in decay. Growth rates o *U scandinavica* were lower under green filters in an experiment, simulating self-shading. It is concluded that multiple growth-limiting gradients occur in macroalgal mats: upper layers suffer from nitrogen limitation and photoinhibition while bottom layers are light limited. The algae in the ma acclimatise to low light conditions by increasing heir absorption through increased pigment contents and by higher photosynthetic efficiency.

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INTRODUCTION

In shallow coastal areas (lagoons, bays, etc.) subjected to a high nutrient load, mass developments of green macroalgae can often be observed (Schramm & Nienhuis, 1996). The blooms mainly consist of members of the genera *Enteromorpha* Link and *Ulva* L., bu *Chaetomorpha* Kütz. and *Cladophora* Kütz. blooms are also common (Lowthion *et al.*, 1985; Brown *et al.*, 1990; Lavery *et al.*, 1991; Duarte, 1995). These algae often manifest themselves as free-floating thalli, which are frequently arranged in thick mats. In areas exposed to tida movement and strong waves, the mat structure may be temporary (in the time scale of one tidal cycle). In lagoons and other areas only marginally subjected to tidal and/or wave exposure, the mat structure may persist for weeks and sometimes even for an entire season (Vergara *et al.*, 1998). Consequently, the algae in the different layers experience very different light climates for a long time: upper layers may suffer from photoinhibition, while bottom layers have to cope with extremely low photon fluence rates (Vergara *et al.*, 1997). Moreover, as the algae are not optically neutral and preferably absorb blue and red light, consequently not only light quantity, but also light quality decreases in the layers (Salles *et al.*, 1996; Vergara *et al.*, 1997).

In laboratory experiments with mats consisting o *U rigida* and *U rotundata*, Vergara *et al.* (1998) showed that the thalli at the bottom of the mat acclimatise to the low photon fluence rates by increasing maximum photosynthesis rates, chlorophyll content and thereby absorption and by lowering the light compensation point and dark respiration. Furthermore, they found significantly higher C/N ratios in thalli from the upper layers, suggesting that these may suffer nitrogen limitation. Krause-Jensen *et al.* (1996) showed that in both natural and experimental mats of *C. linum* such a pattern of decreasing C/N ratios with depth can occur. Their experiments also showed that chlorophyll content of the algae increased with depth, suggesting acclimatisation to low light levels. The surface layers of the mat may experience negative effects of the high light and UV levels, which may lead to photoinhibition and oxidative stress (Hanelt, 1992, 1996; Herrmann *et al.*, 1995). However, thus far no studies have been performed on this topic in macroalgal mats.

Except for the C/N data by Krause-Jensen *et al.* (1996), experimental work on growth limiting gradients in algal mats and acclimatisation of the algae has not been confirmed with field data. This paper aims to fill this gap with field data on *Ulva* spp. mats. The study was carried out in the man-made, brackish lake the "Veerse Meer" (southwest Netherlands). In this eutrophic lake, each year large blooms consisting of various *Ulva* spp. develop, which are mainly controlled by light and nitrogen availability (Malta & Verschuure, 1997). Samples of the algae and the water were taken and analysed for the existence and physiologica consequences of a light and nutrient gradient. The maximum (dark-adapted) quantu efficiency (F_v/F_m) of thalli collected from different depths was measured as an estimate for the degree of photoinhibition of the algae (Baker & Bowyer, 1994). The level of oxidative stress experienced by the algae was determined by measuring the glutathione pools and their redox state (de Vos *et al.*, 1992).

A better understanding of the processes that occur in these mats and the physiologica responses o *Ulva* spp. can lead to a better understanding of the dynamics of macroalga blooms. The aim of this paper is to test the existence of multiple growth-limiting gradients in mats of *Ulva* spp. in the field. More specifically, the following hypotheses will be tested for an *Ulva* spp. mat: 1) nitrogen limitation will decrease with depth, 2) at the surface of the mat, high light and UV levels will lead to oxidative stress, indicated by high oxidised glutathione levels and to photoinhibition, expressed as a low maximum quantum efficiency, 3)

progressive light limitation with depth will cause an increase in pigment concentration and absorption capacity. Additionally we studied the time course of acclimatisation o *U scandinavica* to self-shading in a simulated spectrum of an algal mat in the laboratory.

Materials and Methods

Study site

The field data were collected in the "Veerse Meer", a shallow, man-made, brackish lake (salinity = 15 - 20 psu average), situated in the southwestern part of the Netherlands (lat. 51 ° 32' N, long. 3°46' E). The Veerse Meer receives a high nutrient load, mainly originating from agricultural run-off. The resulting eutrophic conditions give rise to a large macroalgal bio mass, mainly consisting of different *Ulva* species (Malta & Verschuure, 1997). Details on hydrography and hydrochemistry can be found in Nienhuis (1989, 1992) and Coosen *et al.* (1990). Observations were carried out at the site Middelplaten, which is considered representative with respect to macroalgal biomass for most shallow areas in the lake (Malta & Verschuure, 1997). Water samples and algal collections for pigment, carbon and nitrogen analysis and absorption measurements were carried out in 1997 during the build-up phase of the bloom (June, 10), during the stationary phase (July, 30) and during the degradation phase (September, 23) (Malta & Verschuure, 1997). Additionally, maximum quantum efficiency (F_v/F_m) of photosynthesis and the glutathione redox ratio of the algae were determined in June and September.

Water nutrient analyses

Water samples were collected by SCUBA divers between 12.00 and 14.00 h, using syringes mounted with perforated tubings. Samples were taken from the bottom (± 0.80 m depth, under 6 layers of *Ulva*), the middle (± 0.60 m depth, under 3 layers of *Ulva*), below and above the top layer (± 0.20 m depth) of algal mats and at four depths (0.20, 0.40, 0.60 and 0.80 m) in a water column overlying bare sediment. One sample per layer and depth was taken from each of three different algal mats and water columns. The water was filtered through 0.2 µm disposable filters (Whatman) and stored frozen until analysis. Nitrate, nitrite, ammonium and orthophosphate were analysed on a Skalar 5100 autoanalyzer.

Tissue nitrogen and pigment content

Ulva spp. were sampled from the top layer, the third (= middle layer) and from the sixth (= bottom layer) of each of three mats, approximately 500 g wet weight of algae per layer per mat was collected. Parts of the algae were transported on ice to the laboratory. Samples intended for pigment analysis were stored in tin foil at -80 °C. Later, samples were ground and extracts were made in 95 % methanol, buffered with 5 % ammonium acetate. Pigments were analysed by reversed phase HPLC after Wright et al. (1991). The samples were injected through a Waters 171 Plus autosampler into an Alltech column (Econosphere C18). The signal was detected at 436 and 658 nm with a Waters absorbance detector, see Barranguet et al. (1998) for details. The remaining material was dried for 48 h at 60 °C and ground using a bullet mixer. The carbon and nitrogen contents of the algae were determined on a Carlo-Erba NA 1500 CHN-analyser (Nieuwenhuize et al., 1994).

Absorbance and fluorescence

The *in vivo* absorption spectra and total wide-band (400 – 700 nm) absorbance of the algae were determined immediately after collection by placing the algae on a cosine sensor connected to a MACAM SR9910/PC spectroradiometer, using natural sunlight. A sunlight spectrum was determined just before each measurement, absorption was calculated with respect to that measurement. Algae were carefully wiped clean with tissue before the measurements. In September, the absorption of the epiphyte/silt layer on the *Ulva* thalli was

measured, by determining the absorption spectra before and after cleaning of the *Ulva* tissue.

Maximum (dark-adapted) quantum efficiency (F_v/F_m) can be used as an estimate for the degree of photoinhibition of the algae (Baker & Bowyer, 1994) , which happens when a plan is exposed to high light or UV-B radiation (Henley *et al.*, 1991a; Hanelt *et al.*, 1992). It has also been shown to be sensitive to other stresses such as nutrient limitation (including carbon) and high temperatures (Henley *et al.*, 1991b; Magnusson, 1997). Ch *a* fluorescence parameters F_0 and F_m were measured in June and September after 15 min dark acclimation, using a pulse amplitude modulated (PAM) fluorometer (PAM 100-103, Walz). From these parameters the variable fluorescence, $F_v = F_m - F_0$ and F_v/F_m were calculated. In June, mats were sampled at noon (12.30 h) and in the afternoon (15.00 h), to detect variation due to circadian patterns (Hanelt *et al.*, 1993). In September, mats were sampled only at noon.

Glutathione redox ratios

Glutathione, the tripeptide γ-glutamyl-cysteinyl-glycine, is one of the major antioxidant molecules in many organisms. One of its functions is therefore to protect plant cells against lipid peroxidation by active oxygen (de Vos *et al.*, 1992). Active oxygen species are formed due to electron leakage from the photosynthetic and respiratory electron transport chains. Reduced glutathione (GSH) is thereby oxidised to GSSG, both via a spontaneous chemical reaction and through enzymatic (peroxidase) reactions. Oxidative agents like the transient metal copper and ultraviolet radiation produce active oxygen (superoxide anion and hydroxyl radicals, hydrogen peroxide). Because such radicals oxidise part of the GSH pool, the redox state o this thiol expressed as GSH:(GSH + 0.5GSSG) can be a useful indicator of oxidative stress in algae living in a steep vertical gradient of sunlight. For this reason, we have measured, using a modified HPLC method (after Fahey & Newton, 1987; Rüegsegger & Brunold, 1992; Rijstenbil *et al.*, 1998), the glutathione pools and their redox state of *Ulva* spp. at different positions in the mat. *Ulva* thalli were stored in liquid nitrogen immediately after sampling. For each sample, run in triplicate, 200 mg wet weight of plant material was ground with pestle and mortar under addition of small portions of liquid nitrogen. After determining the initial weight of the cold powder, 1 ml of a mixture of 0.12 M HCl and 5 mM DTPA (diethylene triamine penta acetic acid) was added. In order to obtain a cell-free extract this homogenate was sonicated on ice (Soniprep MSA; 14-μm amplitude; 3 min; 0 °C). The suspension was centrifuged (Centrikon T-324 Kontron; 15,000 rpm; 20 min; 4 °C). The detailed procedures for this precolumn derivatisation and the subsequent reversed-phase HPLC runs are described in Rijstenbil & Wijnholds (1996). The supernatant was derivatised in an HEPPS-DPTA buffer with monobromobimane (MBrB, Molecular Probes). Separate HPLC runs without and with dithiothreitol (DTT) as reductor were performed to analyse reduced glutathione (GSH) and total glutathione (GSH + 0.5GSSG), respectively. From the peak areas, using GSH as a standard, the concentrations of GSH and its redox state were calculated.

Time course of acclimatisation to simulated self-shading

The time course and way of acclimation to self-shading was tested in a lightbox simulating natural sunlight. Light was provided from both the bottom and the top of the lightbox by two sets of 9 fluorescent lamps, each consisting of 6 "white" (Sylvania Activa 36W/172), 3 "red" (Sylvania F36W/GRO) and 1 UVA (Philips 40 W/TL05 Blacklight) lamp. The different lamp types produced a spectrum in the middle of the box that was similar in shape to the spectru produced by natural sunlight in the 350 – 750 nm range. The horizontal differences in strength and spectrum in the box were minimal and negligible (E. Malta and P. van Breugel, unpubl. data). The box was placed in a climate room at 15 °C. Lamp heat was removed by a ventilator. A sinusoidal light regime was applied to simulate the natural course of light. The length of the

photoperiod was 12 h, total light provided was 11.4 mol photons $\cdot m^{-2} \cdot d^{-1}$, with a maximum light intensity of 390 µmol photons $\cdot m^{-2} \cdot s^{-1}$ for two hours per day.

Cultivated *Ulva scandinavica* (clone VM94; Malta *et al.*, in press) were used in the laboratory experiments. Cultures were maintained at 15 °C under a 14:10, L:D light cycle (40 µmol $\cdot m^{-2} \cdot s^{-1}$), using a 3:2 mixture of filtered (Schleicher & Schuell no. 6) natural seawater (Oosterschelde estuary) and demineralised water. Final salinity was 16 psu, which is the annual average salinity of the Veerse Meer. A modified f/2 enrichment (Guillard & Ryther, 1962) using NH_4Cl as the nitrogen source instead of $NaNO_3$ was added. Discs of algal material (∅=1.8 cm) were punched out from the thalli using a sharpened stainless stee cylinder and transferred to flat perspex cylinders (∅ = 20 cm, height = 5 cm). The cylinders were divided in four compartments using stainless steel mesh (mesh width 2 x 2 mm) with silicone tubings on the edges. Six discs were placed in each cylinder; two compartments contained two discs and two others one. 10 L of modified f/2 medium (see above) was circulated through the cylinders at a rate of 26.9 L $\cdot h^{-1}$ (Cole-Parmer Masterflex pump). The medium was constantly aerated to prevent oxygen and/or carbon limitation in the algae.

Two different light conditions were assessed by covering two cylinders with 1 mm x 1 mm grey mesh and two with a combination of "pale green" (LEE, nr. 138) and "straw" (LEE, nr. 103) filters. The spectrum of the grey mesh was neutral in light transmission, while the transmission of the LEE filters was very similar to that of an *Ulva* layer (Fig. 6.1). Both filters absorbed 56 % of the initial irradiance. On days 0, 1, 2, 5, 6 and 12 small circular pieces (∅=0.4 cm) were cut out of two discs per treatment and chl *a* fluorescence parameters F_0 and F_m were measured with a PAM 2000. With these parameters, F_v/F_m was calculated (see above). The other discs were weighed wet every day (Sartorius basic balance) after removing excess water by gentle blotting between 4 layers of tissue. The algae started to grow exponentially after three to four days and continued to do so until day 12, which was the end of the experiment. Specific growth rate (µ) was defined as the slope of the regression line through the Napierian logarithms (ln) of the wet weight during the exponential growth phase. All linear regressions had a R^2 *in vivo* absorption spectra and total wide-band (400 – 700 nm) absorbance of the algae were determined using a spectroradiometer. The *Ulva* disc between two microscope object slides, which were mounted in fron of a light source (Hansatech LS2). Absorbance was measured with a MACAM SR9910/PC. At the end of the experiment, the algae were submersed in liquid nitrogen and dried in a freeze-drier in the dark for 72 h. Pigment content were determined in the same manner as the field collected algae.

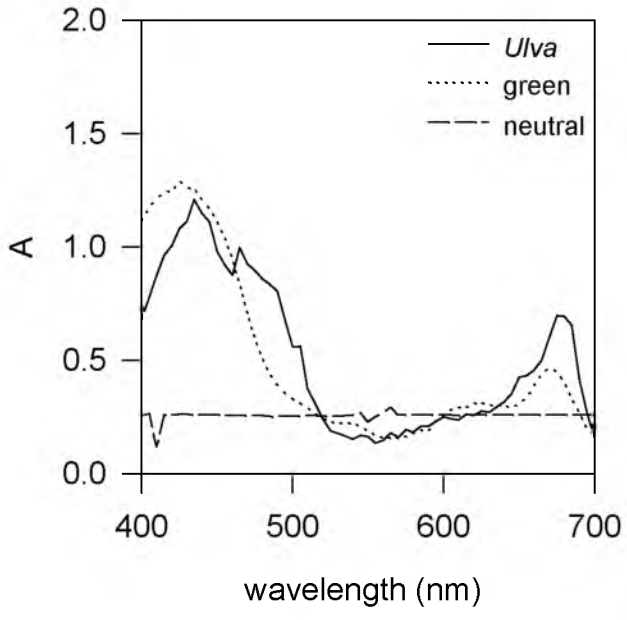


Fig. 6.1: Absorption spectra of a neutral and a green filter and of an *Ulva* spp. thallus.

Differences between sampling dates and the existence of a gradient in the algal ma of tissue C, tissue N, absorbance and pigment content were tested for significance by a two-way analysis of variance (two-way ANOVA, Sokal & Rohlf, 1995). Multiple *post hoc* comparisons were done by a Tukey honest significant difference test for unequa

sample sizes (Spjøtvoll & Stoline, 1973). DIN concentrations were tested for differences between samples taken from the inside and outside of a mat and for vertical differences using a two-way ANOVA, followed by a Tukey test for unequal sample sizes. Significance o seasonal differences in DIN and significance of differences between ammonia and nitrate concentrations were both tested using the nonparametric Kruskal-Wallis test (McGlathery 1995). A two-way ANOVA was used to detect significant differences in sampling time or layer in the mat of F_v/F_m data collected in June. The September measurements of F_v/F_m and the glutathione data were tested for significant differences between layers using a one-way ANOVA, followed by a Tukey test in case of a significant ANOVA result. Data were tested for heteroscedasticity with Bartlett's test for homogeneity (McGlathery, 1995). The variables that scored significant (DIN, F_v/F_m, Chl *a*/*b* and ß-carotene content) were transformed using the Box-Cox procedure ($x'=x^{\lambda} + 1$; Box & Cox, 1964), which removed the heterogeneity. The values for λ were estimated in an iterative procedure by the Statistica 5.1 software package (StatSoft, 1997). A significant difference of specific growth rates between treatments in the experiment was tested by means of a nested one-way ANOVA (McGlathery, 1995). Differences in time and between treatments of F_v/F_m and absorbance were tested for significance using two-way ANOVA's, following a Tukey test. Pigment contents of the tw treatments and the cultures were tested for significant differences using one-way ANOVA's followed by a Tukey test. Heteroscedastic data (Ch *a* and lutein content) were Box-Cox transformed prior to testing.

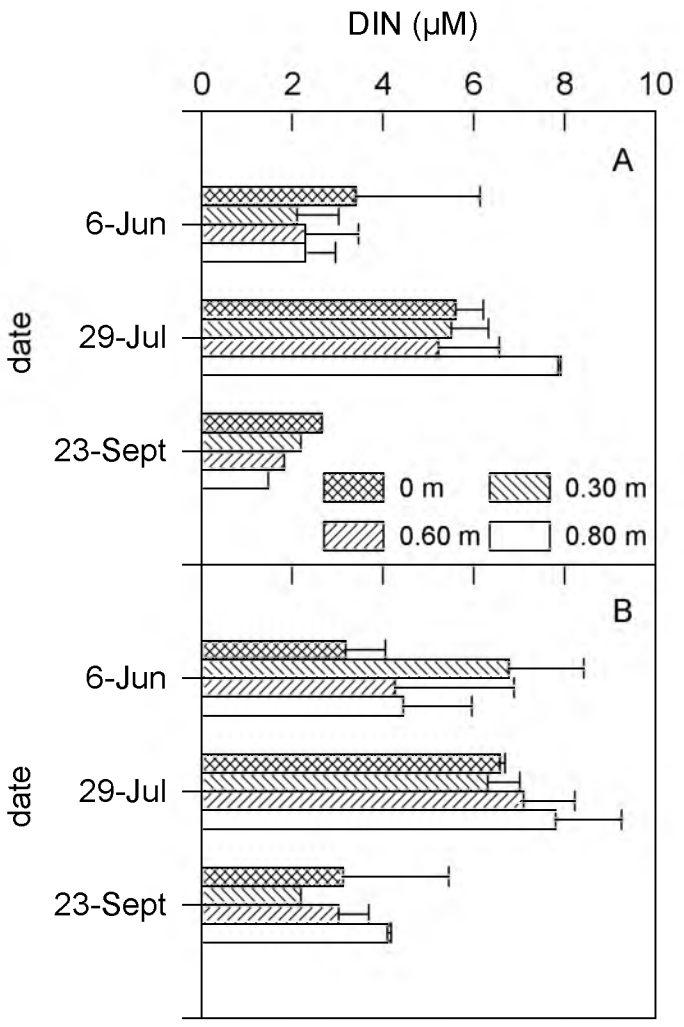


Fig. 6.2: Vertical profile of dissolved inorganic nitrogen (DIN) in the Veerse Meer (SW Netherlands) at three dates: (A) at different depths in a water column overlying bare sediment and (B) at different depths in the water in an *Ulva* spp. mat. Samples were taken from the surface water (just above the first layer of *Ulva* thallus), from 0.30 m depth (below the first *Ulva* layer), at 0.60 m (below the third layer) and at 0.80 m (below the bottom layer).

Results

Seasonal dynamics in mat appearance

At all three sampling dates, the mats were composed almost entirely o *Ulva* spp. (> 99 % of total macroalga biomass, data not shown) with a coverage of 90 – 100 %. In June and July, the upper and middle layers o the mat were floating in the water column. In this period, the upper *Ulva* layer was partly, and the middle and bottom layers were completel covered with a thin brown substance. Inspection of the thalli with a microscope revealed that a diatom film had settled on thalli from the top and, to a lesser extent, the middle layer. Bottom layers were with debris and silt. Cleaned thalli of the upper and middle layers looked brigh green; thalli from the bottom layer

usually had a dark brownish-green colour. In September, the mats were in decay and lying fla at the sediment. The algae were completely covered with a film of silt and epiphytes. Moreover, massive amounts of barnacles and tunicates had settled on the thalli of the bottom layers and, to a lesser extent, also the middle and top layers. The colour of the algae was dark green to brownish; some parts of the bottom layer were black.

Nutrients in the water and in algal tissue

In June and July, total dissolved inorganic nitrogen concentration (DIN) of the water in the mat was significantly higher than outside the mat (two-way ANOVA, $p < 0.05$, Fig. 6.2a-b). A significant vertical pattern was only found in July where DIN in the two upper layers was significantly lower than in the bottom layer. Average DIN was significantly highest in July, both inside and outside the mats (Kruskal-Wallis, $p < 0.01$, Fig. 6.2a-b). Both inside and outside the mats, NH_4^+ concentrations were 1.9 to 5.1 times higher than NO_3^- concentrations, without any significant vertical or seasonal pattern (Kruskal-Wallis, $p > 0.05$). Tissue C increased from June to September (two-way ANOVA, $p < 0.01$) and did not show a significant vertical pattern (two-way ANOVA, $p > 0.05$, Fig. 6.3a). Tissue N levels showed a clear and significant vertical pattern in June and July (Fig. 6.3b). A these two dates, nitrogen levels in the top layer were around 1 % of dry weight (DW) which was significantly lower than the 3.0 - 3.2 % DW measured in the bottom layers (two-way ANOVA, $p < 0.001$ for all dates). In June, tissue N in the middle layer was similar to that in the top layer and had an intermediate value in July, differing significantly from both the top and bottom layer (two-way ANOVA, $p < 0.001$). Values in September were highest for all three layers (two-way ANOVA, $p < 0.001$), without significant differences between layers.

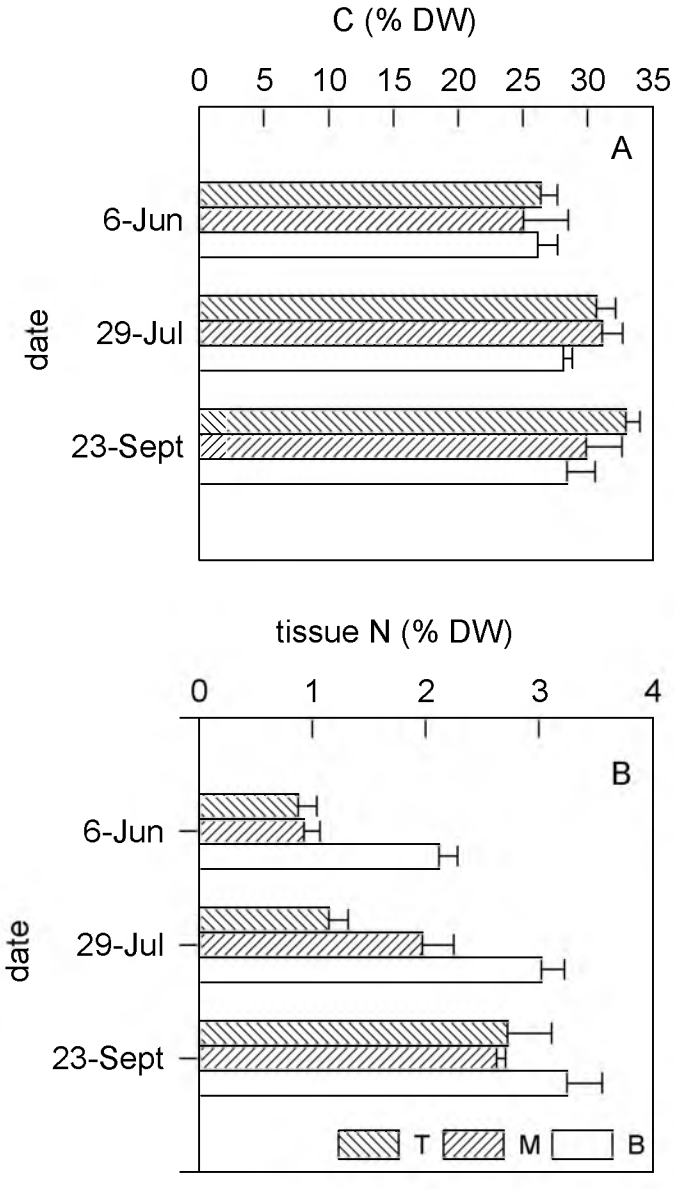


Fig. 6.3: Tissue carbon (A) and nitrogen (B) content in thalli of *Ulva* spp. from the top (T), middle (M) and bottom (B) layer of an algal mat at three dates in the Veerse Meer (S Netherlands).

Absorption, quantum efficiency and pigments

Wide-band (400-700 nm) absorbance in the bottom *Ulva* layers was significantly higher than in the top layers (two-way ANOVA, $p < 0.001$; Table 6.1); the middle *Ulva* layer showed intermediate values. Absorbance capacities of all layers increased significantly throughout the season (two-way ANOVA, $p < 0.001$). Calculated

Table 6.1: Absorbance of *Ulva* spp. at three depths (average ± standard deviation, n = 3) in mats collected from the Veerse Meer (SW Netherlands) at three sampling dates and calculated absorbance of the silt/epiphyte film on *Ulva* spp. thalli at three depths in September (see text for explanation).

date	top layer	middle layer	bottom layer
10 Jun	0.28 ± 0.01	0.38 ± 0.07	0.47 ± 0.02
30 Jul	0.36 ± 0.07	0.59 ± 0.02	0.68 ± 0.11
23 Sep	0.82 ± 0.02	0.90 ± 0.17	0.99 ± 0.03
23 Sep (silt/epiphytes)	0.39[1)]	0.25[1)]	0.32[1)]

[1)] no replicate measurements

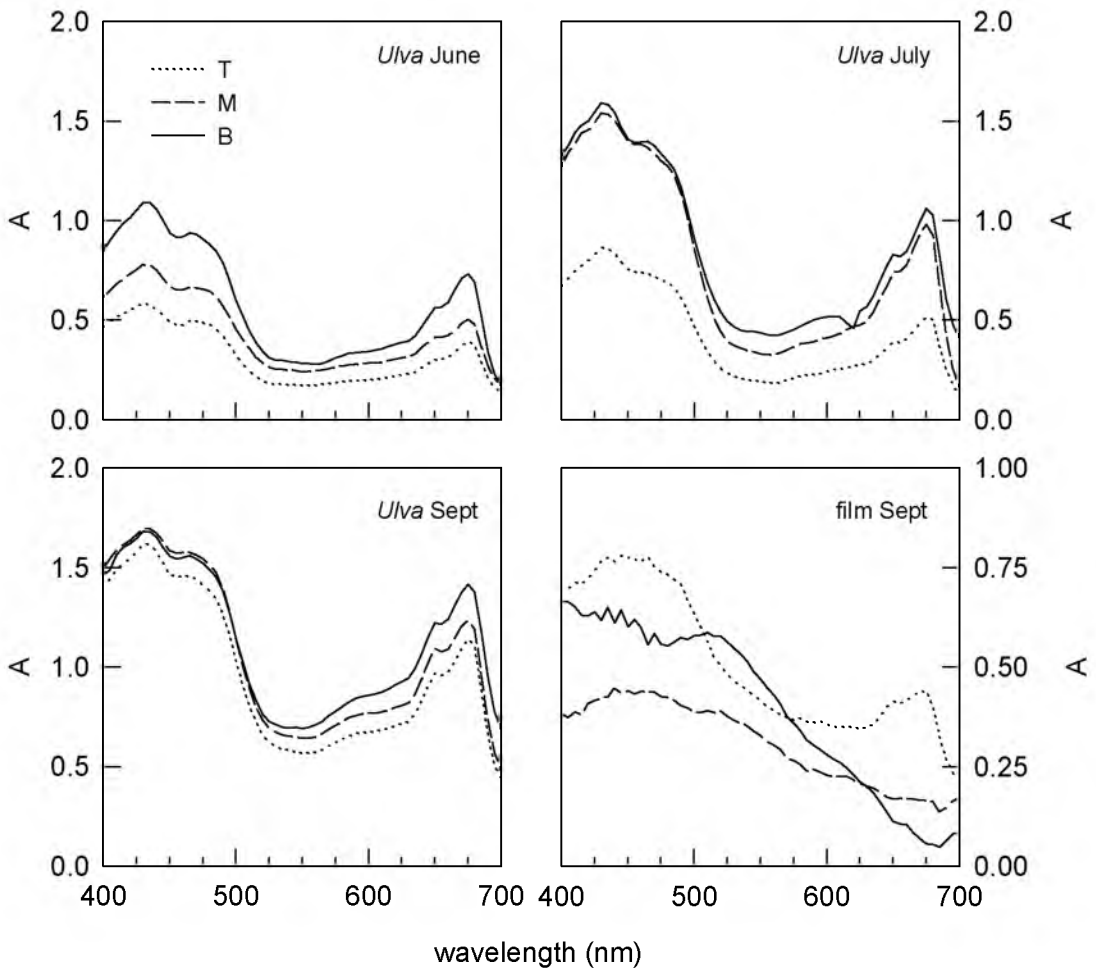


Fig. 6.4: Absorption spectra of thalli of *Ulva* spp. from the top (T), middle (M) and bottom (B) layer of an algal mat in the Veerse Meer (SW Netherlands), in June, July and September and the absorption of the silt/epiphyte film on top of *Ulva* thalli (note different scale), measured in September.

absorbance values (by comparing the absorbance of untreated and cleaned *Ulva* thalli) for the epiphyte/silt fil on the *Ulva* thalli in September were highest for the upper layer and lowest for the middle layer, but could not be tested for significance due to lack o replicates. The shape of the absorbance spectrum of the *Ulva* thalli was similar for all layers at all dates (Fig. 6.4a-c) and showed typical absorption peaks in the blue and red parts of the spectrum. The spectra for the epiphyte/silt film, however, did show considerable differences along the vertical gradient (Fig. 6.4d). The spectrum for the top layer was comparable with the *Ulva* spectrum, but showed more absorbance in the 500 – 625 nm region. No clear peaks were observed in the spectra from the epiphyte/silt film from bottom and middle layers.

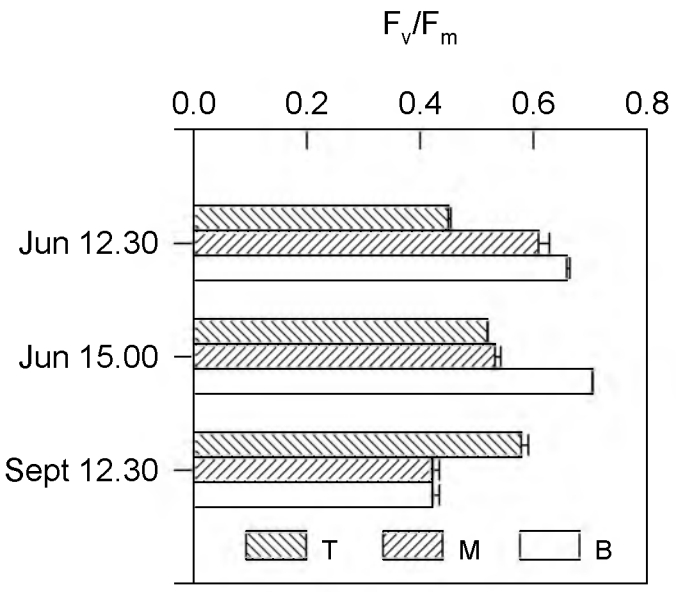


Fig. 6.5: Maximum quantum efficiency (F_v/F_m) of *Ulva* spp. thalli from the top (T), middle (M) and bottom (B) layer of an algal mat in the Veerse Meer (SW Netherlands), measured at noon and afternoon in June and at noon in September.

In June, maximum (dark-adapted) quantum efficiency ($_v/F_m$) measured at noon was signi ficantly highest in the bottom layer and lowest in the top layer (two-way ANOVA, $p < 0.001$, Fig. 6.5). The F_v/F_m values of afternoon samples of the top and bottom layers were significantly higher than those sampled at noon (two-way ANOVA, $p < 0.001$), while the F_v/F_m of the afternoon samples of the middle layer were significantly lower compared to the noon samples. Differences between the top and bottom layer were still significant for the afternoon samples (two-way ANOVA, $p < 0.001$). In September, F_v/F_m (measured at noon) were similar for the bottom and middle layer and significantly higher for the top layer (ANOVA, $p < 0.001$). Top layers in September had a higher F_v/F_m than in June; conversely, the September values of the bottom layers were lower than the June values.

All pigments showed a trend of increasing contents with depth (Table 6.2). A two-way analysis of variance with sampling date and depth as independent variables, revealed however, that only in July the top and middle *Ulva* layers had significantly lower chl *a* and *b* contents than the bottom layer ($p < 0.05$ for ch *a* and $p < 0.01$ for ch *b*). For lutein, a significant difference between top and bottom layers was found in July ($p < 0.01$) and September ($p < 0.05$) and between middle and bottom layers in July ($p < 0.05$). Ch *b* content of the middle layer was significantly higher in September than in June ($p < 0.05$). Lutein content of the bottom *Ulva* layer was significantly higher in September than in June ($p < 0.05$) and July ($p < 0.01$); for the middle layer the increase between June and September was significant ($p < 0.05$). No clear trend was observed in the ch *a*/*b* ratio (Table 6.2). Differences between layers were not significant, top layer values in June were higher than in July and September (two-wa ANOVA, $p < 0.05$).

Table 6.2: Pigment content of different pigments (µg pigment·g *Ulva* dry weight^{-1}) and chlorophyll *a*/*b* ratio of *Ulva* spp. at three depths (average ± standard deviation, n = 2 for June and n = 3 for July and September) in mats collected from the Veerse Meer (SW Netherlands) at three sampling dates. Chl *a*/*b* = chlorophyll *a* to *b* ratio; b.d. = below detection limit.

		Chl *a*	Chl *b*	ß-carotene	lutein	Chl *a*/*b*
10 Jun	top	125.4 ± 87.8	45.1 ± 24.9	b.d.	1.3 ± 1.8	2.65 ± 0.49
	middle	238.8 ± 195.3	99.3 ± 0.7	b.d.	1.3 ± 1.8	2.40 ± 1.95
	bottom	914.4 ± 528.9	717.1 ± 385.7	b.d.	13.9 ± 19.6	1.26 ± 0.06
30 Jul	top	94.5 ± 93.2	107.2 ± 59.2	0.8 ± 1.2	12.3 ± 11.3	0.76 ± 0.45
	middle	552.6 ± 399.4	444.5 ± 243.0	2.0 ± 2.4	40.7 ± 26.7	1.13 ± 0.34
	bottom	1621.5 ± 206.3	1308.8 ± 190.5	4.9 ± 4.3	133.0 ± 19.5	1.24 ± 0.12
23 Sep	top	455.4 ± 227.5	460.4 ± 135.0	0.6 ± 0.9	25.9 ± 10.7	0.96 ± 0.21
	middle	1190.6 ± 420.9	1073.1 ± 243.0	2.1 ± 3.0	94.7 ± 38.4	1.09 ± 0.14
	bottom	1427.4 ± 438.4	1224.4 ± 293.9	5.4 ± 2.1	127.3 ± 34.6	1.16 ± 0.08

Glutathione redox ratio

Glutathione redox ratios GSH:(GSH + 0.5GSSG), measured in June, were below 0.5 in all *Ulva* thalli (Fig. 6.6a). A vertical gradient was clearly present, with low values at the top and high values at the bottom. The GSH:(GSH + 0.5GSSG) redox ratio in *Ulva* from the top and middle layer was significantly lower than the redox ratio in the intermediate layer (ANOVA, $P<0.01$); the redox ratio of *Ulva* at the top was also significantly lower than that of the middle layer (ANOVA, $P<0.01$). Total glutathione content (GSH + 0.5GSSG, Fig. 6.6b) was significantly higher in the top layer than in the middle and bottom layer (ANOVA, $p < 0.05$; $p < 0.001$); the difference between the middle and bottom layer was also significant. The high concentration in the upper and middle layer could be attributed entirely to the high concentration of oxidised GSSG. No reliable glutathione data could be obtained from the algae collected in September, due to products of decaying algae interfering with the HPLC analysis.

Time course of acclimatisation to simulated self-shading

Growth was significantly highest in the treatment with neutral filters (ANOVA, $p < 0.001$, Table 6.3). F_v/F_m was low (0.45) for both treatments at the start of the experiment, increased to values between 0.76 - 0.79 after five to six days and remained more or less constant for the rest of the experiment (Fig. 6.7). The increase was due to a simultaneous decrease in F_0 and an increase in F_m for both treatments (data not shown). F_v/F_m of *U scandinavica* under a neutral filter treatment was significantly higher than under a LEE filter at day 1 (two-way ANOVA, $p < 0.001$), values were similar between treatments thereafter. The time course o absorbance showed a pattern similar to that of F_v/F_m: low values in the beginning of the

experiment followed by a quick increase and more or less stable values after day 6-7 (Fig. 6.8a). Differences between treatments were not significant (two-way ANOVA, $p > 0.05$). No differences in the shape of the absorption spectra were found between treatments; also the shapes of spectra obtained from the experiments were similar to those obtained from field measurements (Fig. 6.8b) and remained constant during the course of the experiment (data not shown). Pigment contents and chl *a*/*b* ratio (Table 6.3) did not differ between treatments; initial pigment contents (of the stock cultures) were significantly lower than those of the algae used in the experiment for all pigments (ANOVA, $p < 0.01$) except for ß-carotene, which was below detection levels in all samples.

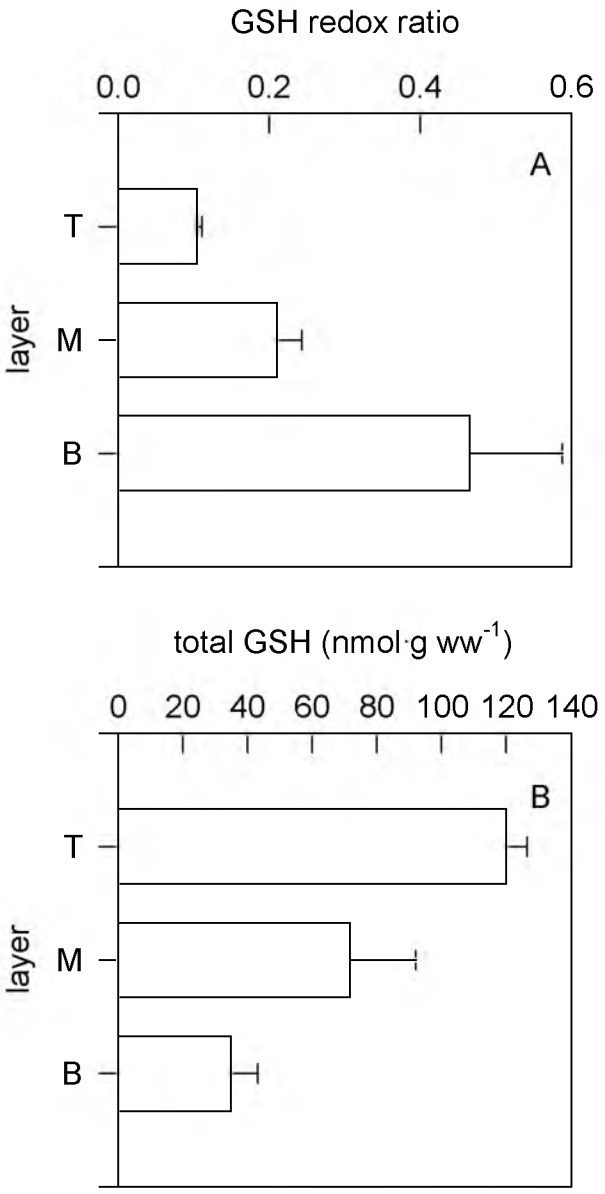


Fig. 6.6: Glutathione (GSH) redox ratio (A) and total reduced and oxidised glutathione pool (B) of *Ulva* spp. from the top (T), middle (M) and bottom (B) layer of an algal mat in the Veerse Meer (SW Netherlands) measured in June.

DISCUSSION

Nitrogen limitation

We observed a pronounced vertical gradient in tissue nitrogen in the *Ulva* mats (Fig. 6.3). Considering a critical tissue N level (i.e. the level at which growth becomes nitrogen limited) for *Ulva* spp. between 2.0 and 2.4 % DW, Fujita *et al.*, 1989; Laver & McComb, 1991b; Pedersen, 1994, this means there is a progressive N limitation towards the surface of the mat. The N content of the bottom *Ulva* layers was around the critical level in June and well above it in July and September. This is in good agreement with the predictions of Krause-Jensen *et al.* (1996) and Vergara *et al.* (1998), based on their experimental mats. Two independent processes can lead to the existence of such a vertical gradient: 1) Light intensities decreasing with depth, causing light limitation of growth in deeper layers. The internal nitrogen content of the thalli of the bottom layers is thus less diluted because growth rates are lower compared to top layers (Malta & Verschuure, 1997). This process established the vertical gradient observed by Vergara *et al.* (1998). 2) Decomposition and subsequent mineralisation of bottom layer and sediment organic material and short- or long-term anoxia at the bottom of the mat may cause an upward nutrient flux (Sfriso *et al.*, 1987; Lavery & McComb, 1991a; Bartoli *et al.*, 1996) that can be utilised by the viable algae in the mat. As these fluxes come from the sediment, the bottom layers are the first to benefit fro these nutrients (Thybo-Christensen *et al.*, 1993; Krause-Jensen *et al.*, 1996). The observed higher nutrient concentrations in ambient water from the mat compared to free water overlying bare sediment (Fig. 6.2) support this second hypothesis. In the field, most probably,

Table 6.3: Laboratory experiment with *U. scandinavica* subjected to green light (LEE filter), a neutral spectrum (neutral filter) and from stock cultures. Specific growth rates (SGR, d^{-1}), pigment contents (µg pigment · g *Ulva* dry weight^{-1}) and chlorophyll *a*/*b* ratio (average ± standard deviation, n = 2). Chl *a*/*b* = chlorophyll *a* to *b* ratio, n.m. = not measured, b.d. = below detection limit.

	SGR	Chl *a*	Chl *b*	ß-carotene	lutein	Chl *a*/*b*
LEE filter	0.08 ± 0.01	2451.4 ± 26.9	1512.0 ± 143.4	b.d.	81.5 ± 16.9	1.63 ± 0.14
neutral filter	0.11 ± 0.01	2617.6 ± 211.1	1694.9 ± 81.4	b.d.	103.7 ± 14.7	1.54 ± 0.05
culture	n.m.	531.3 ± 25.2	157.6 ± 20.1	b.d.	6.7 ± 0.1	3.41 ± 0.59

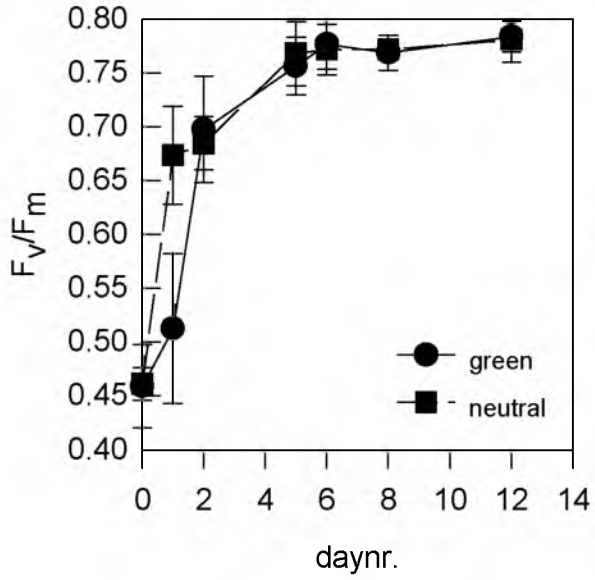
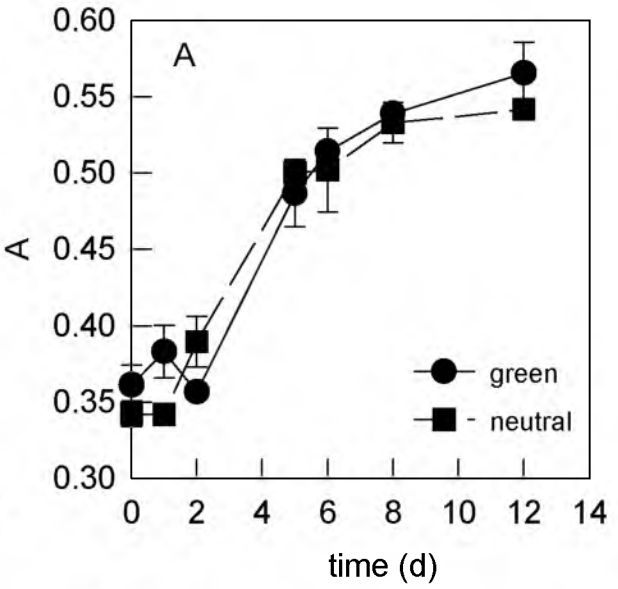


Fig. 6.7: Time course of acclimatisation of maximum quantum efficiency (F_v/F_m) of *Ulva scandinavica* in an experiment simulating self-shading using a green filter and a neutral filter.



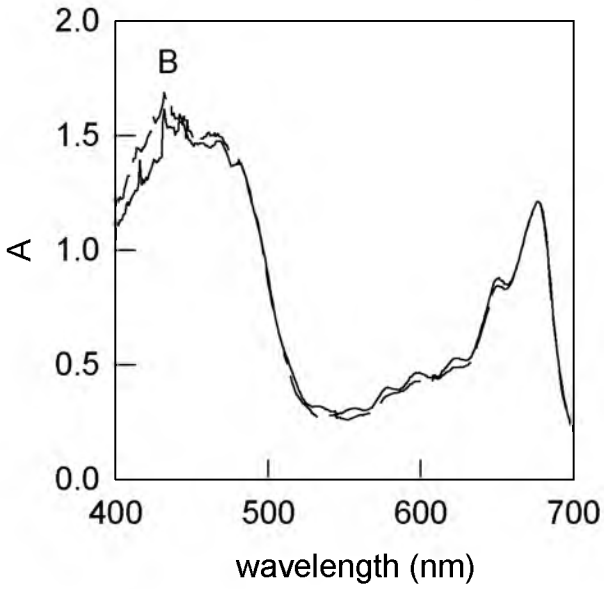


Fig. 6.8: Time course of acclimatisation of absorbance (A) of *Ulva scandinavica* (A) and absorption spectra (B) in an experiment simulating self-shading using a green filter and a neutral filter.

both processes occur simultaneously. It is obvious that nitrogen dilution resulting from growth is the most dominant process during the build-up phase while nutrient enrichment due to decomposition and anoxia become more important later in the season. However, further studies are required to be able to make a quantitative distinction between these two processes.

In September, no differences in *Ulva* tissue N were observed between layers. Wind-induced mixing of the mat in autumn cannot be excluded as a cause. It seems however more likely tha high tissue N levels during the degradation phase may be caused by recycling of nitrogen in the mat. Severely light-limited thalli on the bottom of the mat die off due to an imbalance between photosynthesis and respiration. Nitrogen can be released from these decomposing thalli and is taken up by viable algae in the mat (Sfriso *et al.*, 1987; Lavery & McComb, 1991a). Additionally, high tissue N values during the degradation phase may be caused by leaching and microbial growth on dissolved organic carbon and nitrogen, resulting in the attachment of nitrogen rich bacteria (Hanisak, 1993, Kristensen, 1994).

In most field studies, tissue N in algae is determined in order to be able to assess whether algae are nitrogen limited during certain parts of the season or that other factors are limiting growth (see for instance Soulsby *et al.*, 1985; Fujita *et al.*, 1989; Sfriso *et al.*, 1992; Wheeler & Bjørnsater, 1992; Malta & Verschuure, 1997). This study has shown that in an algal mat at a given point in time a gradient in tissue N exists which ranges between levels, saturating for maximum growth (bottom layers) to levels that are at the edge of the minimum levels required for survival (top layers). Conclusions about potential nitrogen limitations are thus very much dependent on the depth and position in the layer from which the sample is taken.

Photoinhibition and oxidative stress

Ulva spp. and other green algae typically show maximum F_v/F_m values ranging from 0.78 to 0.83 (Magnusson, 1997). The values found for the algae from the bottom layer in June are close to these maxima. In contrast, the much lower values of the algae from the middle and top layer indicate stress. This stress may be caused by photoinhibition, however, considering the low tissue nitrogen content it may also be caused by nitrogen limitation or a combination of both. A small recovery in F_v/F_m was found in the late afternoon measurements, indicating that the lower values were, at least partly, caused by reversible photoinhibition. In September (degradation phase), F_v/F_m values were low (< 0.4) for the middle and bottom layers, indicating severe stress. Most algae are dead or decomposing during this period. The values for the lop layer algae were higher (approximately 0.6), indicating that the algae in this layer still show some activity. Massive die-off of macroalgal blooms in autumn is a common phenomenon in eutrophic systems. Photoinhibition, high summer temperatures and severe nitrogen limitation in upper layers and prolonged light limitation and anoxia in deeper layers may all contribute to algal decomposition (Viaroli *et al.*, 1992; Rivers & Peckol, 1995b; Sfriso, 1995). However, the exact triggers for the die-off are scarcely studied and largely unknown.

All glutathione redox ratios were on average lower than 0.5, indicating mild (bottom layers) to considerable (middle and surface layers) oxidative stress in the *Ulva* thalli (Fig. 6.6). The almost four-fold higher total glutathione concentration in the top layers compared to the bottom layers further stresses the existence of severe oxidative stress in the top layers. Fro both higher plants and algae it is known that oxidative stress stimulates the production of reduced GSH (Ishikawa & Sies, 1988; Alscher, 1989; Agrawal, 1992), probably via induction through high GSSG concentrations (Agrawal, 1992). Oxidative stress in the bottom layers is most likely caused by growth limitations (especially carbon can be limiting in macroalga

mats; Frost-Christensen & Sand-Jensen, 1990; Rivers & Peckol, 1995a) or by formation of active oxygen in the respiratory pathways (Rijstenbil *et al.*, 1998). The low glutathione redox (0.10 ± 0.01) in the top layer should be attributed to UV and high light stress (Agrawal, 1992). When considering the glutathione levels in connection with the results of the fluorescence measurements, we can conclude that the photoinhibition in the top layer is largely due to oxidative stress, probably caused by high light intensities and UV radiation.

It is interesting to note that despite the nitrogen limitation this antioxidant mechanism is stil highly active. Our results indicate that it is a low energy investment mechanism in terms o nitrogen metabolism which can still be active when the algae suffer nitrogen shortage. The glutathione cycle is a very well studied antioxidative mechanism in organisms of almost all major taxonomical groups, such as mammals (including humans) (Ishikawa & Sies, 1988), higher plants, bacteria and diatoms (Rijstenbil & Wijnholds, 1996). For macroalgae glutathione data are rare (see Nakano *et al.*, 1995; Rijstenbi *et al.*, 1998). In their recen review, Davison and Pearson (1996) concluded that there is a strong need to develop markers to be able to determine specific environmental stresses or groups of stresses in seaweeds. This study has shown that glutathione can be a good candidate for such a stress marker for *Ulva* spp., especially in combination with fluorescence measurements.

Acclimatisation to self-shading

Considering the values for wide-band absorbance we measured in the field and the absorption spectra of the algae from the mats and the experiment, we conclude that the *Ulva* spp. from the Veerse Meer (probably mainl *U scandinavica*, Malta *et al.*, in press) mainly acclimatise to the spectral and intensity changes in a mat by increasing their absorption capacity. Some green algae, including at least one *Ulva* species, the deep-water alga *U japonica*, are able to absorb light in the so-called green window (roughly the 500 – 560 nm region), using the carotenoid siphonaxanthin (Kageyama *et al.*, 1977; Yokohama, 1981; Fork & Larkum, 1989). Neither siphonaxanthin, nor its esters siphonein A and B could be detected in the Veerse Meer *Ulva* spp. Although the light climate in the lower layers of a mat is very similar to that of deep-water (Mercado *et al.*, 1996; Salles *et al.*, 1996), apparently, mat-forming *Ulva* spp. do not possess the capacity to use siphonaxanthin for absorption in the 500 – 560 nm region. Absorbance did not differ between treatments in the shading experiment, over-all light levels might have been too low to induce such a difference.

The changes in pigment content and composition of *Ulva* spp. observed in the field, also indicate intensity acclimation rather than complementary chromatic acclimation (Table 6.2). Although the large variation in pigment content among thalli from the same layer makes it impossible to detect significant differences, the overall trend indicates an even increase of pigment concentration in all pigments with depth at all sampling dates. Probably, the increase in absorbance is, partly or fully, caused by an increase in pigment content. Additionally, pigment contents respond strongly to the nitrogen status of a macroalga (Lapointe & Tenore, 1981; Ramus, 1983; Krause-Jensen *et al.*, 1996). This may also contribute to the vertical trend and is most certainly the cause of the observed trend of the seasonal increase in pigmen contents. The significantly lower growth rates under green sheets (Table 6.3) confirms that *U scandinavica* cannot use the green light for growth, or at least not as efficient as blue and red light. The time course of acclimatisation in our experiment was similar for both treatments and took about 6 days. Considering the increase in chlorophyll content compared to the contents of the stock cultures, this time is mainly needed to produce photosynthetic pigments.

Vergara *et al.* (1997) showed that a mud film on the algae can also contribute significantly to

the light absorption by measuring absorption on different sediment dilutions. In our September measurements, the contribution of the epiphyte/silt film to total light absorption was calculated. Two important conclusions can be drawn from our measurements: 1) absorbance by a 'mud' film is considerable but less than that of an *Ulva* thallus (25 - 45 % o the absorbance of an *Ulva* layer) 2) the 'mud' film is not optically neutral. The latter conclusion is especially obvious in the absorption spectrum of the film obtained from the upper layers from the mat (Fig. 6.4d). This film shows an absorption spectrum which differs from the *Ulva* spp. by the higher absorption in the 500 – 560 nm region, typical for diatoms (see a.o. Huisman, 1997). Considering the spectra of the films on the middle and botto layers, combined with microscpical observations, it can be concluded that the diatoms are mainly present in the top layers of the macroalgal mat. The amount of silt and diatoms seems to increase throughout the season (E. Malta, pers. obs.), frequent measurements are needed to quantify the absorbance of the epiphyte/silt film throughout the season.

Concluding remarks

In this paper we tested the hypothesis that multiple growth-limiting gradients in mats o *Ulva* spp. occur in the field. We conclude that: 1) during the phase of fast growth, there is progressive nitrogen limitation towards the surface of the mat; 2) surface layers suffer from photoinhibition and oxidative stress caused by high sunlight intensities and UV-B radiation as is expressed by low quantum efficiencies and glutathione redox ratios; 3) progressive light limitation with depth leads to an increase in absorption and a higher quantum efficiency, furthermore there is a trend of increasing pigment content with depth. This information shoul be used to refine models estimating primary production and biomass development of systems dominated by free-floating macroalgae.

CHAPTER VII: GENERAL DISCUSSION AND MANAGEMENT PERSPECTIVES

INTRODUCTION

Eutrophication of shallow lagoons, bays and estuaries gives rise to large and rapid biomass development of foliose and filamentous macroalgae. *Ulva* spp. are among the most comm species in these blooms, which occur as dense, free-floating mats (Fletcher, 1996; Morand & Briand, 1996). This thesis aims to address the regulation of temporal and spatial dynamics of macroalgal biomass in eutrophic coastal areas. I will present a conceptual model, which synthesises the interactive effects of the different abiotic and biotic variables during differen phases of a macroalgal bloom.

In cold to warm temperate and subtropical areas, the dynamics of macroalgal biomass and growth follow a distinct annual cycle (Ch. 2 and 3; Sfriso, 1995; Hernández *et al.*, 1997; de Casabianca & Posada, 1998). In general, three to four phases can be distinguished (Fig. 7.1).

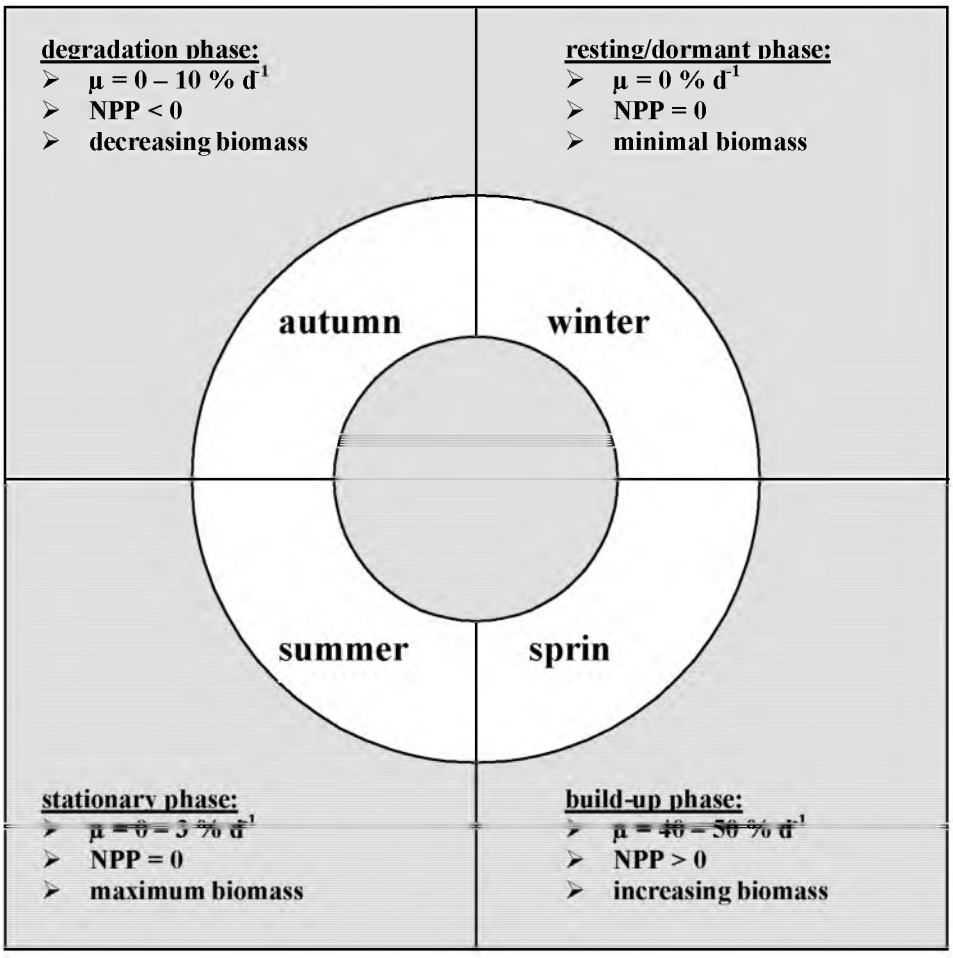


Fig. 7.1: The four phases and their properties in the seasonal dynamics of macroalgal blooms. μ = growth rate, NPP is net primary production.

During winter, above-ground biomass is minimal in warm-temperate to subtropical lagoons (e.g. Mediterranean lagoons, Sfriso, 1995; Romero *et al.* 1996) and absent in cold-temperate and cold areas such as the Veerse Meer (Ch. 3 and 4). In both cold to cold-temperate and warm-temperate to subtropical lagoons, algal growth rates and production are zero: the algae are in a resting or dormant phase. The build-up phase (spring) is characterised by high growth rates (40 – 50 % d^{-1} or even higher, Ch. 2 and 3, Lapointe & Tenore, 1981; Geertz-Hansen & Sand-Jensen, 1992; Geertz-Hansen *et al.*, 1993), rapid biomass increase and consequently positive net primary production (Krause-Jensen *et al.*, 1996). The build-up phase gradually shifts to a stationary phase (summer). During this period a thick macroalgal mat is present growth rates are generally low (0 - 3 % d^{-1}) and biomass is more or less constant. Growth rates match decomposition rates, resulting in zero net primary production. It should be noted that the phenomenon of a stationary phase mainly relates to areas which are only marginally subjected to tidal and/or wave exposure, since it is related to the persistence of the ma structure as will be discussed below. The results from the EUMAC project showed that dense mats do develop in the macrotidal sites along the Atlantic coasts in France and England (Rijstenbil *et al.*, 1996), however, these are constantly mixed and have different physiologica properties than the stable mats that persist in more sheltered areas (Ch. 6; Vergara *et al.*, 1998). The degradation phase (autumn) completes the annual cycle. This period i characterised by usually low, but varying growth rates (ranging from 0 – 10 % d^{-1}; Ch. 2; refs.), a rapidly decreasing biomass and hence a negative net primary production.

In this thesis I use the term mat for the dense masses formed by macroalgal blooms. This term is subject to some discussion. Although not defined, marine biologists (microbial ecologists in particular) usually refer to mats consisting of micro-organisms (e.g. bacteria, cyanobacteria, diatoms and other unicellular algae). Together, these organisms form communities that have a distinct oxygen and nutrient ecology and are in this respect more or less isolated from the res of the ecosystem (van Gemerden, 1993; Stal & Caumette, 1994). These microbial mats are often found on the sediments of intertidal areas. In the case of macroalgae, the term canopy, as adapted from terrestrial ecology, is more common. The term canopy as it is used in terrestrial systems, however, mainly relates to the light climate in a vegetation and remains close to the literal meaning of the word canopy = cover. For eutrophic marine systems the term macroalgal mat (including the dominant macroalgae and their epiphytes and associated bacteria and fauna) is increasingly used in literature (see a.o. Price & Hylleberg, 1982; Krause-Jensen *et al.*, 1996; Sundbäck *et al.*, 1996). As discussed below, these mats have a distinct nutrient, oxygen and carbon ecology and form their own subsystem, similar to microbial mats (Krause-Jensen *et al.*, 1996), therefore I consider the use of the term mat justified.

The Dormant or Resting Phase

However obvious the importance of the initiation of any bloom may seem, the dormant phase is probably the least studied period in the annual dynamics of macroalgal blooms in genera and of free-floating *Ulva* spp. in particular (Santelices, 1990; Lotze *et al.*, 1999). The result from the EUMAC project reveal two important modes of survival of the bloom-forming species (Rijstenbil *et al.*, 1996). 1) In warm-temperate areas, such as the Mediterranean, a low but viable biomass persists during winter and gives rise to a new bloom in spring. The transition to the biomass build-up phase is triggered by higher light intensities, longer daylengths and increasing water temperatures (Sfriso, 1995; Viarol *et al.*, 1996; de Casabianca & Posada, 1998). 2) In cold-temperate areas, such as the Veerse Meer and the Baltic Sea, no above-ground biomass is present during winter (Ch. 3 and 4, Lotze *et al.*, 1999). Survival of the Baltic Sea bloom species *Pilayella littoralis* and *Enteromorpha* spp.

occurs via settled propagules (Lotze 1998, Lotze *et al.*, 1999). A large difference between these species and most bloom-forming *Ulva* spp. is that the formers primarily develop as attached plants, while *Ulva* spp. can develop as exclusively free-floating plants. Furthermore, sexual reproduction of *Ulva* appears to be negligible in the Veerse Meer, whereas the formation of (asexual) zoospores has been observed only one or two times (Ch. 4). The experiments described in Ch. 4 clearly show that regeneration of old vegetative fragments initiate the new bloom. These fragments survive freezing during winter buried in the sedimen or on the bottom of deep gullies. The freezing experiments show that darkness is essential for the survival of the algae. The buried fragments thus form a fragment bank. This fragmen bank is functionally similar to the propagule bank observed in the Wadden Sea and the Baltic Sea for *Enteromorpha* spp. (Schories, 1995; Lotze *et al.*, 1999) and for *Pilayella littoralis* in the Baltic Sea (Lotze 1998, Lotz *et al.*, 1999), the "banks of microscopic forms" (Hoffmann & Santelices, 1991) and to the well known seed bank observed for many terrestrial plant species (e.g. Grime, 1979; Leck *et al.*, 1989).

The buried thalli need to be uncovered or transported from the gullies to the shallow areas. Apparently, this is not being accomplished by bioturbation (Ch. 3 and 4). Fragments, buried only superficially, may grow while still in the sediment, using stored reserves and a heterotrophic metabolism (Markager & Sand-Jensen, 1990), and thus emerge from the sediment. However, most fragments seem to be buried at a depth of at least 5 cm (E. Malta, pers. obs.). It seems that wind is most likely the trigger for both uncovering buried thalli and advective transport from the gully. Optimal growth of the dominan *Ulva* species in the Veerse Meer (*U scandinavica*) is attained at a temperature of 10 °C (Ch. 5). The Veerse Meer reaches this temperature usually in April, about a month prior to bloom initiation (Ch. 2 and 3; unpubl. data). Tissue and water nutrients are available in excess during the entire winter period. Obviously, other triggers are important for the initiation of the bloom. Endogenous, circa-annual growth rhythms and processes similar to dormancy in seed plants might be active in seaweeds as well. However, the importance of propagule banks and other below-ground overwintering forms in the life history of seaweeds is only slowly emerging (Santelices, 1990; Schories, 1995; Floresmoya *et al.*, 1996) and hardly anything is known about the mechanisms that play a role in these processes.

The Build-up Phase

The main variable regulating growth at the start of the build-up phase is light (Ch. 2; Geertz-Hansen & Sand-Jensen, 1992; Coutinho & Zingmark, 1993; Hernández *et al.*, 1997; de Casabianca & Posada, 1998). As in most coastal ecosystems, phosphorus was never limiting *Ulva* spp. growth in the Veerse Meer (Ch. 2 and 3; Ryther & Dunstan, 1971; Fujita *et al.*, 1989; Duarte, 1995) and the high tissue nitrogen values of the algae and the high concentrations in the water show that nitrogen is initially available in excess. From the result of the between-year comparison (Ch. 2), the following scenario emerges: if the weather in spring is clear, *Ulva* growth will continue until nitrogen becomes limiting as was the case in 1992. When the weather is cloudy and light intensities are relatively low, tissue nitrogen levels will not become limiting for growth, as was the case in 1994.

Decreasing growth rates during the build-up phase and the transition to a more stationary phase, or even directly to a degradation phase, are usually attributed to progressive nitrogen limitation as illustrated by decreasing tissue N levels (Rivers & Peckol, 1995; Fong *et al.*, 1996; Viaroli *et al.*, 1996). Additionally, negative effects of high summer temperatures are sometimes mentioned as a cause (Israe *et al.*, 1995; Rivers & Peckol, 1995). In Ch. 2 and 3 of this thesis, similar lines of logic were followed to explain the seasonal regulation o *Ulva*

blooms in the Veerse Meer. This traditional approach, however, cannot always satisfactorily explain a decrease in growth rates: often growth decreases when tissue N levels are still above the reported critical levels (Ch. 2 and 3; Hernández *et al.*, 1997). Moreover, the results presented in Ch. 6 and other recent data (Krause-Jensen *et al.*, 1996; Valiela *et al.*, 1997; Vergara *et al.*, 1998), show that as soon as a macroalgal mat is formed, a vertical gradient in tissue N sets in, which ranges from storage (> 2.4 % DW) to the lowest possible level for survival (0.8 – 1.0 % DW). Consequently, tissue N values taken from algal blooms mask the different processes and physiological states that occur in a mat if it is not indicated from which part of the mat they are obtained. In case of a mixed sample containing algae from different layers, tissue N levels may indicate a sort of average nitrogen status for the mat.

Measuring the physiological properties of the various layers of a mat, however, offers the opportunity to raise new hypotheses on the regulation of growth and biomass by environmental variables. The results in Ch. 6 show that there exist two principal, diametrically opposed growth-limiting gradients in an algal mat: light and nitrogen concentration. Within a month after the onset of growth, the mat has a thickness of at least six layers. The total amount of light (PAR) remaining for the bottom layer will be less than 3 % of the sunlight (Ch. 6.; Salles *et al.*, 1996; Vergara *et al.*, 1997, 1998). Moreover, only the green light remains at the bottom of a mat, as the algae preferentially absorb blue and red light. At low light levels, the total dose of green light required for growth of *U. pseudocurvata* germlings was at least three times higher then that of blue and red light (Leukart & Lüning, 1994). In the experiment presented in Ch. 6, adult thalli of *U. scandinavica* received either PAR or green light, such that total irradiance was equal for both treatments, resulting in 27 % lower growth rates under green light. Extrapolating these phenomena to the field, this means that at the bottom of a macroalgal mat the amount of light that can be used for photosynthesis will be less than 1 %, despite the fact that total PAR may be two to three times higher. In other words: algae living at the bottom of a mat are living at or below the euphotic zone, defined as the lower limit where primary production can occur (see a.o. Kirk, 1986). Self-shading is thus probably the main factor decreasing growth in these layers. The positive correlation of both chlorophyll content, absorption and maximum quantum efficiency versus depth in the mats supports this hypothesis (Ch. 6).

The vertical profile of tissue nutrient contents of *Ulva* clearly shows the importance o nitrogen as a growth-limiting factor for the upper layers of the mat (Ch. 6). It also shows that bottom layers are not nitrogen limited: here light limitation prevents nitrogen limitation. Other important conclusions that can be drawn from the existence of a tissue nitrogen gradien concern the stability of a mat. Results of growth experiments of Vergara *et al.* (1998) show that the gradients are reversible; as can be expected, frequently mixed mats (due to tidal and wind induced currents) do not show vertical differences. The shift from the build-up phase to the stationary phase can thus be explained by the heterogeneous environment within the mat. Additionally, evidence is available that growth rates of algae in mats tend to be lower than those of free-floating algae, because algal mats reduce the current velocity of the water, thereby increasing the boundary layer (Koch, 1993). This reduces the diffusive exchange o gas and nutrients and inhibits photosynthesis during parts of the day because of high oxygen concentrations and depletion of dissolved inorganic carbon (Parker, 1981; Gordon & Sand-Jensen, 1990; Krause-Jensen *et al.*, 1996).

The Stationary Phase

One of the major differences between a phytoplankton bloom and a macroalgal bloom is the persistence of the macroalgal bloom (Valiela *et al.*, 1997). The typical duration of a

phytoplankton bloom is within the order of days to weeks (Cloern, 1996) while a macroalga bloom lasts several months. Nutrient limitation and increased numbers and biomass of grazers (mainly zooplankton and filter-feeders) usually promote the end of a phytoplankton bloom (Howarth, 1988; Heip *et al.*, 1995). The mat structure of a macroalgal bloom is probably the reason of its capacity to maintain a high biomass during a relatively long period, while nutrient concentrations in the water are very low (often below detection limits). By intercepting the nutrient-flux from sediment to water and by the recycling of nutrients in the dense algal mats (Ch. 6; Valiela *et al.*, 1997; Vergara *et al.*, 1998), macroalgae are able to prolong the duration of the bloom. This process has been described and quantified for mats of *Chaetomorpha linum* (Krause-Jensen *et al.*, 1996, 1999; McGlathery *et al.*, 1997) and *Cladophora sericea* (Thybo-Christensen & Blackburn, 1993; Thybo-Christensen *et al.*, 1993) and is very likely to occur in *Ulva* mats as well. In this way the bloom can persist under low or even zero nitrogen concentrations in the water during summer.

The dependence on the mat structure makes the system inherently unstable (Krause-Jensen *et al.*, 1996). Autumn usually comes with strong winds, which have a strong impact on the biomass of free-floating algae (Flind *et al.*, 1997; Salomonsen *et al.*, 1997). Algal transport by wind-driven currents both causes biomass decrease by dispersal and temporal biomass increase in sheltered areas (Ch. 3). Additionally, strong winds disturb the mat structure and hence the nutrient recycling within the macroalgal mats and will permit the release o nutrients to the water column. Upper layers will become severely nitrogen limited and loose their viability, causing the shift from the stationary to the degradation phase. Phytoplankton cells, which are much better competitors with respect to nutrient uptake then macroalgae (Hein *et al.*, 1995; Pedersen & Borum, 1996), can then form a bloom, thereby reducing water transparency and consequently reduce growth conditions for the macroalgae. Actually, these processes were observed in Venice lagoon (Sfriso & Pavoni, 1994; McGlathery *et al.*, 1997), where blooms of phytoplankton and macroalgae (mostly *U rigida*) alternated. Alternatively, or additionally, bottom layers may suffer a prolonged carbon imbalance due to high respiration rates at high summer temperatures and very low to zero photosynthesis due to the severe light limitation in the mat or by phytoplankton blooms.

The Degradation Phase

This thesis pays relatively less attention to this phase, compared to the other phases. Massive decomposition of macroalgae occurs in hypertrophic systems, in which increased respiration at high summer temperatures leads to anoxia and H_2S production. In these systems the entire water column can become anoxic (dystrophic crisis). In the Veerse Meer, dystrophic crises do not occur, however, the bottom layers and sediment become partly anoxic (Coosen *et al.*, 1990; van de Kamer *et al.*, 1990). This may contribute to the decrease of the biomass of the bottom layers. Mats of *C. linum* become totally anoxic in the Veerse Meer (Ch. 3) as well as in other areas (Lavery & McComb, 1991; Krause-Jensen *et al.*, 1996), eventually leading to their decay.

A major quantitative biomass decrease is caused by birds, for which the Veerse Meer is an important overwintering area. Coots (*Fulica atra*) and mute swans (*Cygnus olor*) both consume considerable amounts of *Ulva* biomass (Coosen *et al.*, 1990; Meire *et al.*, 1991). Bird grazing in the Veerse Meer is facilitated by the lowering of the water level in October, giving the birds access to the sediment of the bloom areas. Alternative or additional causes of degradation may be diseases and viruses. Only recently, Delcampo *et al.* (1998) described a disease ("hole disease"), caused by infection with the endophytic filamentous green alga *Acrochaete geniculata* (Gardner) O'Kelly and subsequent bacterial "wound" infections. This

disease caused severe losses in cultures o *U rigida*, and was especially active when *Ulva* growth rates were low. However, it is yet unknown if and to what extent these infections play an important role in nature (Delcampo *et al.*, 1998).

Apart from degradation and decomposition, short periods of regrowth are observed during the degradation period (and sometimes also during the stationary phase). Mixing resaturates the water and parts of the sediment with oxygen and nutrients. Autumn rains cause run-off from agricultural land, which also increases the nutrient concentration. When temperatures are not too low (in October in particular), viable *Ulva* fragments can reach considerable growth rates, thereby temporarily reducing the rate of biomass decrease.

Regulation of Annual Dynamics: Conceptual Model and Recommendations for Future Research

Figure 7.2 summarises the regulation of the annual dynamics of the macroalgal bloom in a conceptual model. The hypothesis that wind-induced currents are the main factors uncovering algal fragments from the sediment, thereby triggering the transition from the dormant to the build-up phase, requires further testing, as well as do the possible role of other factors (e.g. daylength, annual rhythms). Light is the main factor regulating growth during the build-up phase. Only after an extended period of clear weather, nitrogen becomes growth limiting. Temperature (and in the case of the Veerse Meer also salinity) is suboptimal for *Ulva* growth. Progressive self-shading occurs in the mats during the build-up phase, slowing down biomass increase and eventually causing the shift of the build-up phase to a more or less stationary phase. During this phase high biomass levels can be maintained despite low nitrogen concentrations in the water, due to nutrient (mainly nitrogen) recycling in the mat. Growth in

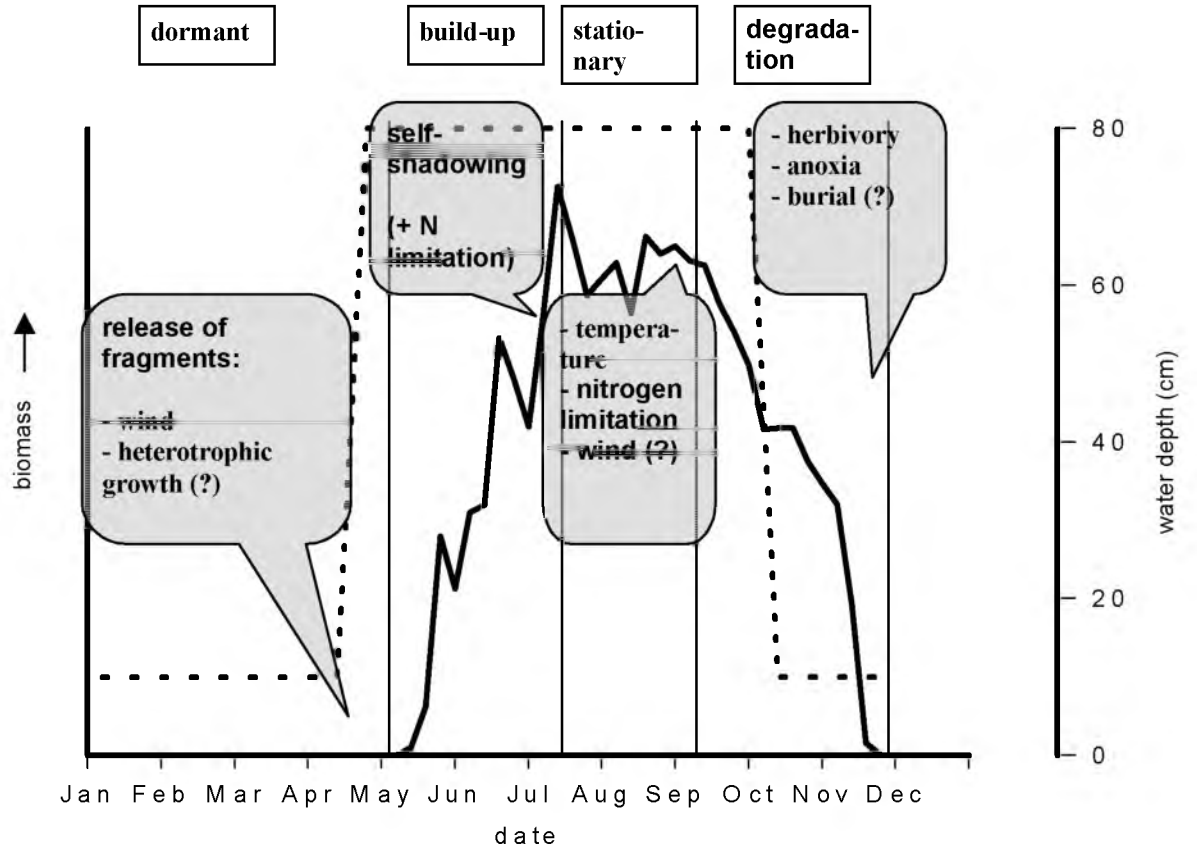


Fig. 7.2: The four phases in the annual cycle of *Ulva* biomass development in the Veerse Meer (SW Netherlands). Arrows indicate the causes for the shifts beteen phases. Solid line = biomass; broken line = depth of the water column.

the layers of the mat may, however, be hampered due to oxidative stress. Finally, the degradation phase starts, during which biomass again decreases to winter levels. This thesis does not pay much attention to this phase. Ch. 3 mentions increased respiration due to hi summer temperatures leading to anoxia and H_2S production as the main causes for the massive die-off of the algae. The role of herbivory of both invertebrates and birds, disturbance of the mats by autumn winds, virus attacks and diseases need further elucidation. Wind-induced currents therefore seem to be a crucial factor in the initiation and persistence o macroalgal mats. As can be expected, it also plays a major role in the spatial distribution o free-floating algae (Ch. 3). Recently developed mathematical models describing macroalga biomass budgets recognise the role of wind and algal transport (Flindt *et al.*, 1997; Salomonsen *et al.*, 1997; Solidoro *et al.*, 1997). This thesis shows that: 1) wind-induced currents are important in the spatial and seasonal biomass budget; 2) probably also play a major role in spring bloom initiation and as a potential factor in shortening the stationary phase by disturbing macroalgal mats. The role of currents and water motion therefore deserves major attention in further studies. Furthermore, it has become clear that macroalgal mats form an important entity in macroalgal blooms. Quantification of the recycling potential in these mats as well are more detailed studies to the functioning of these mats as a whole are necessary to gain insight in the dynamics of macroalgal blooms.

Management Perspectives

Mass development of macroalgae not only has an important impact on the ecosystem, it often also affects the local economy. The main ecological consequences include a decrease in biodiversity and a decrease in ecosystem stability. Clearly this has negative effects on fish and shellfish populations and thus also on fishery. Furthermore, algal blooms have a negative impact on tourism: the accessibility of the water and the beaches decreases and the decomposing algae cause noxious odour. In an attempt to minimise this impact, (local) authorities often harvest the algae, which is quite expensive: the annual costs of harvesting and cleaning of beaches range between about 20,000 Euro in Venice lagoon (Italy), to 325,000 Euro in Peel Inlet (Australia) and even as much as 500,000 Euro in Brittany (France) (review by Morand & Briand, 1996). Between 1978 and 1990 harvesting took place in the Veerse Meer, however only a minimal amount of the algae was removed (less than 3 %; Hannewijk, 1988; Apon, 1990), no data were found considering the costs. It is recognised however, that the decaying macroalgal blooms limit the length of the tourist season, withou blooms it is estimated that the number of tourists visiting the lagoon would grow with 20 – 30 % (Thomaes, 1991). Currently, the tourist industry concerning the Veerse Meer generates an annual income of about 60,000,000 Euro and provides work for approximately 1200 people (Thomaes, 1991). Both the ecological and the economical damage of these macroalgal blooms require a sustainable solution.

The main cause of the annual mass development of macroalgae (mainly *Ulva* spp.) is the high load of nitrogen-rich freshwater, combined with the long residence time of the water. Management plans aiming to prevent these blooms, should be primarily directed towards a decrease of the annual nutrient load, in combination with methods to reduce the residence time of the water in the lake. Modelling exercises indicate that the latter can, at least in part, be realised by increasing the exchange of the lake with the Oosterschelde and the return of a more natural (micro-)tide around the high summer water level. A permanently high water level will probably not affect the *Ulva* growth directly, considering that the entire growth season takes place during the period when the summer water level is maintained. However, a constant water level permits the regrowth of perennial macroalga, such as *Fucus* spp. on the hard substrates (dikes, boulders) and might stimulate the extension of the eelgrass meadows.

Under low to moderate nutrient loading rates these may act as nutrient sinks and thus help keeping *Ulva* and phytoplankton blooms within acceptable limits (see the conceptual mode of Nienhuis, 1989; Sand-Jensen & Borum, 1991).

Additionally, the amount o *Ulva* biomass can be reduced by removing the overwintering fragments in the gully and in the sediment during the winter (Ch. 4). The *Ulva* bloom period can probably be shortened by disturbing the mat structure, for example by harvesting. This will decrease the amount of nutrients available for recycling within the macroalgal system. However, the sediment-water nutrient flux will then be directed towards the water column, which will stimulate phytoplankton blooms. The effects of such blooms are yet unpredictable, however, they will most certainly not increase the water quality. Harvesting of overwintering fragments might, for that matter, have a similar effect. In this respect it is important to note the statement of Valiela *et al.* (1997) that the water quality in a macroalgae dominated syste can be notably better than in a phytoplankton dominated system.

In conclusion it can be said that the first step to prevent mass development o *Ulva* biomass in the Veerse Meer should be a drastic decrease in nitrogen load. For a further recovery of the ecosystem of the lake, a constant or microtidal water level and frequent exchange with water from the Oosterschelde estuary are indispensable. Additionally, harvesting of algal mat during summer and of fragments in the sediment during winter will bring some relief. Decreasing the level of eutrophication in the Veerse Meer will favour both the ecosystem as well as the local economy. Finally, the Veerse Meer is practically the only area in the Netherlands left where eelgrass, *Zostera marina*, exists. Restoration of the lake might contribute to the conservation of this species for the Netherlands.

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SUMMARY

Increased eutrophication of shallow coastal waters has become a major nuisance over the las five decades. One of the most striking features of this eutrophication is the mass accumulation of foliose or filamentous macroalgae, mostly chlorophytes belonging to the genera *Chaetomorpha*, *Cladophora*, *Enteromorpha* and *Ulva*. These species often occur free-floating or loose lying on the sediment, forming dense masses. These macroalgal blooms have a strong negative effect on the functioning of the ecosystem. Seagrasses and perennial macroalgae strongly decrease in abundance or disappear completely. The sediments underneath the dense masses become anoxic and suffocate the benthic fauna. Additionally, the blooms negatively affect fisheries activities and hinder tourism, thus threatening the economy of the region.

It has generally been accepted that these macroalgal blooms are caused by increased nutrien loads, mainly originating from agricultural run-off and (partially) untreated wastewater. In particular, high loads and concentrations of nitrogen have been correlated with changes in macrophytobenthic communities. Fundamental knowledge of the seasonal and spatial dynamics of these blooms is still lacking, however. Regulation of the bloom by environmenta and biological variables, bottlenecks in the life cycle of the dominant algae and the functioning of macroalgal mats are largely unknown. This thesis focuses on the regulation of seasonal and spatial dynamics of blooms of *Ulva* cf. *scandinavica* Bliding in the Veerse Meer, a eutrophic man-made lake in the SW Netherlands. Studies have been performed on ecosystem, community and organism level, to come to an understanding of the processes controlling macroalgal blooms.

Chapters 2 and 3 are field studies, concentrating on the mechanisms determining seasonal and spatial distribution of macroalgae in the Veerse Meer. Chapter 2 shows large variation within one season, but also between years. Positive correlations were shown between tissue nitrogen levels and dissolved inorganic nitrogen (DIN), however, differences in DIN could not explain the between-year variability. Irradiance levels are thought to be more important in regulating the maximum macroalgal yield than DIN. The importance of irradiance is again stressed in chapter 3. In this chapter it is concluded that the difference between sites in *Ulva* spp. biomass in the Veerse Meer is determined by loss processes and not by growth. The main loss process is probably transport by wind-induced currents. The seasonal biomass variation of *Ulva* spp. has been divided in four phases: a dormant phase, a build-up phase, a stationary phase and a degradation phase. During the build-up phase, light and temperature were the main variables regulating *Ulva* spp. growth rates. The algae became nitrogen limited at the end of the build-up phase. Temperature probably plays an important role in the transition from the stationar phase to the decomposition phase. These conclusions are supported by field observations on the nitrogen and chlorophyll contents and the chlorophyll a:b ratio of the algae.

Chapter 4 focuses on the life history of *Ulva* spp. in the Veerse Meer. It is hypothesised tha *Ulva* spp. fragments survive the winter while buried in the sediment or lying on the bottom of deep gullies. Viability of the algae and resistance to freezing was tested in the laboratory. Simultaneously, a field experiment was set up to determine if bioturbation could explain the uncovering of *Ulva* in spring. The results show that viable *Ulva* biomass is present buried in

the sediment and on the bottom of deep (anoxic) gullies. It is demonstrated that the algae are able to survive temperatures of –5 °C for 2 weeks, provided they are kept in darkness. Bioturbation by the lugwor *Arenicola marina* does not stimulate the release of the thalli; most likely wind-induced water motion frees the thalli out of the sediment. Additionally currents may carry the deposited *Ulva* from the gullies to the shallow areas. It is concluded that burial and winter survival can explain the rapid increase in *Ulva* biomass in spring and suggested that the initial spring biomass is one of the major factors determining the maximal biomass in summer.

Ulva is a genus notorious for its taxonomical difficulties. Initially, the bloom Veerse Meer was thought to consist of four species: *U curvata*, *U lactuca*, *U rigida* and *U scandinavica*. Chapter 5 presents a morphological, molecular and ecological comparison of these species. The spatial and seasonal variation in morphology of these species was compared, nuclear encoded ribosomal DNA was sequenced and optimum growth temperature and salinity were determined. It is concluded that the Veerse Meer species (except for *U curvata*) are in fac different morphotypes of one polymorphic species, *Ulva* cf. *scandinavica*. From this, it follows that most of the morphological characteristics available in floating *Ulva* spp. in the Veerse Meer and the Oosterschelde vary too much to allow identification to the species level. The rDNA o *U curvata* could not be sequenced, however, considering its very distinct and relatively constant morphology and different temperature – growth response, it is concluded that it is indeed a separate species. Phylogenetic trees were constructed, based on a comparison of sequences of internally transcribed spacer regions (ITS) rDNA. The trees were rooted using *Monostroma arcticum* as outgroup and suggested that *Ulva* is paraphyletic with respect to *Enteromorpha*. Optimum temperature for growth o *U curvata* was 25 °C, for the other species it was 10 °C. Optimum salinity for growth was 30 psu for all isolates.

Green tide algae often manifest themselves as free-floating thalli, which are frequently arranged in thick mats. In shallow areas, only marginally subjected to tidal and wave exposure, the mat structure may persist for weeks and sometimes even for an entire season. Consequently, the algae in the different layers in the mat are subjected to very different light climates for a long time: upper layers may suffer from photoinhibition, while bottom layers experience extremely low photon fluence rates. Chapter 6 tests the existence of multiple growth-limiting gradients in mats o *Ulva* spp. in the field. Tissue nitrogen concentrations reveal that, during the growth phase, there is a progressive nitrogen limitation towards the surface of the mat, while progressive light limitation with depth leads to an increase in absorption and higher quantum efficiency. At the surface of the mat, oxidative stress leads to high oxidised glutathione levels and photoinhibition, expressed as low maximum quantum efficiency.

The general discussion integrates the results of the different chapters in a conceptual model, which describes the annual dynamics of *Ulva* biomass development in the Veerse Meer. Four phases are distinguished in the bloom: a dormant phase, a build-up phase, a stationary phase and a degradation phase. Probably, wind (or better wind-induced currents) is the main factor causing the release of the algae from the sediment, thereby triggering the transition from the dormant to the build-up phase, however, further research is required. Light is the main factor regulating growth during the build-up phase. Only after a prolonged period of clear weather, nitrogen becomes growth limiting. Self-shading in the mats increases during the build-up phase, slowing down biomass increase and eventually causing the shift of the build-up phase to a more or less stationary phase. A high biomass can be maintained during this phase due to nutrient (mainly nitrogen) recycling in the mat. Finally, the degradation phase starts, during

which biomass again decreases to winter levels. This thesis does not pay much attention t this phase. Increased respiration due to high summer temperatures leading to anoxia and H_2S production and consumption by birds are probably the main causes for the massive decay of the algae.

Management measures, aiming to decrease the macroalgal biomass development in the Veerse Meer, should be primarily directed to a decrease in nitrogen load. For a further recovery of the ecosystem of the lake, a more natural tidal regime is requested. Additionally, harvesting of algal mats during summer and of fragments in the sediment during winter will bring some relief.

SAMENVATTING

Het watermilieu wordt in toenemende mate belast met voedingsstoffen. Deze toename leid tot grote veranderingen in de structuur en soortensamenstelling van het ecosysteem. He aantal soorten dat in het systeem voorkomt neemt sterk af. Voorheen dominante soorten worden vervangen door andere soorten, bovendien verandert het voedselweb sterk. Dit proces noemen we eutrofiëring. Eutrofiëring van kustwateren is gedurende de laatste vijf decennia een groot milieuprobleem geworden. Eén van de meest in het oog springende veranderingen van eutrofiëring in ondiepe baaien en lagunes langs de kust is het massale voorkomen van blad- of draadvormige zeewieren (ook wel macroalgen), meestal behorend tot de groep van de groenwieren. De meest kenmerkende soorten behoren bij de geslachten *Chaetomorpha* (borstelwieren), *Cladophora*, *Enteromorpha* (darmwieren) en *Ulva* (zeesla). Deze wieren vinden we vaak losdrijvend of op de bodem liggend. Hier kunnen ze dichte massa's vormen. We spreken dan wel van een macroalgenbloei. Deze macroalgenbloeien hebben een sterk negatief effect op het functioneren van het ecosysteem: kenmerkende soorten als zeegrassen en langlevende, vastzittende zeewieren nemen sterk af of verdwijnen. De bodem onder de wiermassa's worden zuurstofloos, waardoor de bodemfauna geheel kan afsterven. Daarnaas vormen de macroalgenbloeien een bedreiging voor de lokale economie: ze hebben een negatief effect op de vis- en schelpdierstand en dus op de visserij hierop en door de stank die ontstaat bij de rotting van deze wieren en de fysieke hinder die de dichte massa's geven, vormen de wieren een belemmering voor het toerisme.

De voedingsstoffen (nutriënten) die verantwoordelijk zijn voor het eutrofiëringsproces, worden door de mensen in het water gebracht. Deze nutriënten kunnen afkomstig zijn van verschillende bronnen. In het verleden was lozing van (ongezuiverd) rioolwater de voornaamste bron. Tegenwoordig is overbemesting van landbouwgronden, vaak met kunstmest, de belangrijkste oorzaak, hoewel rioolwater, samen met zogeheten atmosferische depositie ("zure regen") nog steeds een belangrijke bron van nutriënten kan zijn. Stikstofverbindingen als nitraat, nitriet en ammoniak zijn de belangrijkste veroorzakers van eutrofiëring in ondiepe kustwateren, hoewel in sommige gebieden ook fosfaten als oorzaak worden genoemd.

Vele studies hebben een verband gelegd tussen een toename van de nutriëntenbelasting en macroalgenbloeien. Het is dan ook algemeen geaccepteerd dat de macroalgenbloeien worden veroorzaakt door eutrofiëring. Echter, fundamentele kennis van de ruimtelijke verdeling en seizoensvariatie van deze bloeien is nog steeds schaars. De regulering van de bloeien door omgevings- en biologische variabelen, de kritieke momenten in de levenscyclus van de kenmerkende zeewieren en het processen die zich afspelen in de dichte massa's (matten) zijn nog grotendeels onbekend. Dit proefschrift concentreert zich op de regulering van de ruimtelijke- en seizoensvariatie van bloeien van zeesla (vermoedelijk *Ulva scandinavica*) in het Veerse Meer in Zeeland, ZW Nederland. Het Veerse Meer is een eutroof, brak meer, dat is gecreëerd in 1961 naar aanleiding van de watersnoodramp van 1953. Onderzoek is verrich naar het functioneren van het ecosysteem, de wierengemeenschap en de individuele zeewieren, met als doel inzicht te verkrijgen in de processen die het voorkomen van deze macroalgenbloeien controleren.

In de hoofdstukken 2 en 3 worden veldstudies beschreven die zijn uitgevoerd ten einde inzicht te krijgen in de mechanismen die de variatie in de hoeveelheid zeewieren gedurende he seizoen en op verschillende plekken in het Veerse Meer reguleren. De in hoofdstuk 2 gepresenteerde resultaten laten zien dat er zowel binnen het seizoen als tussen twee seizoenen grote variatie bestaat tussen de hoeveelheid zeewieren (voornamelijk zeesla) op een plek. Het totaal stikstofgehalte in de zeesla hangt samen met de totale hoeveelheid opgelost stikstof in het water. Verschillen in de stikstofconcentratie in het water kunnen echter niet de verschillen tussen twee seizoenen verklaren. De resultaten doen vermoeden dat de hoeveelheid ingestraald zonlicht bepalend is voor de hoeveelheid zeesla in een seizoen in plaats van de concentratie opgelost stikstof. Hoofdstuk 3 bevestigt dit vermoeden.

In hoofdstuk 3 wordt geconcludeerd dat de ruimtelijke verdeling van zeesla in het Veerse Meer vooral wordt bepaald door verliesprocessen en niet door verschillen in groeisnelheid. Het belangrijkste verliesproces is waarschijnlijk transport door stroming, hetgeen vooral door windsnelheid en windrichting bepaald wordt. Vier fasen kunnen worden onderscheiden in de ontwikkeling van de zeesla biomassa (= hoeveelheid zeewier per ^{2}) gedurende een groeiseizoen: een rustfase, een opbouwfase, een stationaire fase en een afbraakfase. Gedurende de opbouwfase wordt de groeisnelheid van zeesla vooral bepaald door de hoeveelheid zonlicht en de watertemperatuur. De overgang van de opbouwfase naar de stationaire fase komt waarschijnlijk door uitputting van de hoeveelheid opgelost stikstof in he water. Een hoge watertemperatuur in de nazomer zorgt daarna waarschijnlijk voor de overgang van de stationaire fase naar de afbraakfase.

Hoofdstuk 4 richt zich op de overwintering van zeesla in het Veerse Meer. In deze periode zijn er bovengronds geen wieren aanwezig. In dit hoofdstuk wordt de hypothese getest dat kleine zeewierfragmenten overwinteren terwijl ze in de bodem begraven zijn of op de bodem van de diepe geulen liggen. De levensvatbaarheid van de wieren is getest in het laboratorium. Daarnaast is er een veldexperiment uitgevoerd om te kijken of bioturbatie, het omwoelen van de bodem door bodemdieren als de wadpier, *Arenicola marina*, ervoor zorgt dat de wieren in het voorjaar weer uit de bodem komen. Uit de resultaten blijkt dat de fragmenten zeesla op de bodem van de diepe geulen en in de bodem, levensvatbaar zijn. In een bevriezingsexperiment wordt aangetoond dat de zeewieren een temperatuur van –5 °C gedurende tenminste 2 weken kunnen overleven, mits zij niet aan licht blootgesteld worden. Bioturbatie is nie verantwoordelijk voor het vrijkomen van de wieren; hoogstwaarschijnlijk worden de algen uit de bodem vrijgemaakt door waterstroming. Daarnaast kan de stroming er ook voor zorgen dat de zeesla die in de geulen overwintert in de ondiepe delen van het meer terechtkomt. Hieruit wordt geconcludeerd dat begraving in de bodem en de capaciteit van de wieren om lage temperaturen te overleven, de snelle groei in het voorjaar kan verklaren. Gesuggereerd word dat de biomassa die in het vroege voorjaar aanwezig is, bepalend is voor de maximale biomassa in de zomer.

In totaal zijn er vijf verschillende soorten zeesla gevonden in Nederland. Deze zijn echter zeer moeilijk van elkaar te onderscheiden, vooral als slechts los drijvende planten aanwezig zijn. Aanvankelijk werd er gedacht dat de zeesla biomassa in het Veerse Meer uit vier verschillende soorten bestond: *Ulva curvata*, *U. lactuca*, *U. rigida* en *U. scandinavica*. In hoofdstuk 5 worden de uiterlijke kenmerken (de morfologie), een gedeelte van het DN (erfelijk materiaal) en enkele ecologische eigenschappen van de soorten uit het Veerse Meer met elkaar vergeleken. Morfologische veranderingen gedurende het seizoen en van wieren van verschillende plekken in het Veerse Meer en de Oosterschelde, de basenvolgorde van een

stuk van het ribosomale DNA in de celkern (= een gedeelte van het erfelijk materiaal) werd bepaald en de optimale temperatuur en het optimale zoutgehalte voor de groei van de verschillende soorten werden bepaald. De conclusie uit deze vergelijking is dat, behalve *U. curvata*, alle andere onderscheiden soorten zeesla in het Veerse Meer in feite allen behoren to één soort die zeer veel verschijningsvormen kan aannemen. Hieruit volgt dat de beperkte morfologische informatie die van losdrijvende zeesla kan worden waargenomen te vee variatie vertoont om identificatie tot op soortniveau toe te staan. *U. curvata* had een optimale groeitemperatuur van 25 °C, voor de andere soorten was dit 10 °C. Het optimale zoutgehalte voor groei was 30 psu (30 g zouten per l water) voor alle soorten. De basenvolgorde van he DNA va *U. curvata* kon niet worden bepaald. Echter, uit diens karakteristieke en relatie constante morfologie en hoge optimale groeitemperatuur, kan geconcludeerd worden dat deze wel tot een andere soort behoort. Een stamboom, gebaseerd op de basenvolgorden van he erfelijk materiaal geeft een sterke aanwijzing da *Enteromorpha* (darmwieren) soorten tot hetzelfde geslacht behoren als *Ulva* (zeesla).

In een eutroof gebied manifesteren de zeewieren zich vaak als losdrijvende bladen, die zich vaak ophopen tot dikke matten. In ondiepe gebieden, die slechts beperkt onder de invloed staan van getijdenbewegingen en sterke stroming kan deze matstructuur weken en soms zelfs maanden standhouden. Dit heeft tot gevolg dat het lichtklimaat voor elke laag in zo'n mat verschillend is: de bovenste laag kan teveel licht krijgen en daardoor geremd worden in de groei, terwijl de onderste lagen zeer weinig licht krijgen. In hoofdstuk 6 wordt het effect van deze verschillende omstandigheden in een mat beschreven. Stikstofgehalten in de wieren laten zien dat de bovenste lagen gedurende de opbouwfase een stikstoftekort hebben. De onderste lagen krijgen te weinig licht, hetgeen ze gedeeltelijk kunnen opvangen door meer en efficiënter licht te absorberen dan de bovenste lagen. Metingen van de biomarker glutathion, een stof die een indicatie geeft over de stress waaraan de wieren zijn blootgesteld, laten zien dat de bovenste lagen zoveel licht krijgen dat ze hiervan stress ondervinden.

In de algemene discussie worden de resultaten van de voorafgaande hoofdstukken geïntegreerd in een conceptueel model dat de seizoensdynamiek van de zeesla in het Veerse Meer beschrijft. Het model richt zich vooral op de overgangen tussen de vier fasen waarin de zeeslabloei kan worden onderverdeeld. Wind, of, beter gezegd, door wind veroorzaakte waterstroming is waarschijnlijk de belangrijkste factor die de wieren in het voorjaar uit de bodem losmaakt, en zo de overgang van de rustfase naar de opbouwfase bewerkstelligt. De hoeveelheid zonlicht is de belangrijkste factor die de groeisnelheid reguleert gedurende de opbouwfase. Slechts na een relatief lange periode met veel licht (geen wolken), kunnen de wieren een tekort krijgen aan stikstof. De toenemende hoeveelheid biomassa hoopt zich op tot een algenmat. De zelfbeschaduwing die hierbij optreedt werkt remmend op de groeisnelheid op de onderliggende wieren, waardoor de toename van de biomassa steeds langzamer gaat en uiteindelijk stopt; de mat komt in een stationaire fase waarin de biomassa min of meer constant blijft doordat de nutriënten (in het bijzonder stikstof) in de mat hergebruikt kunnen worden. Tenslotte gaat de stationaire fase over in een afbraakfase, waarin de wierbiomassa snel afneemt en de wieren uiteindelijk geheel verdwijnen. Aan deze fase wordt weinig aandacht besteed in dit proefschrift. Hoge zomertemperaturen zorgen waarschijnlijk voor toenemend zuurstofgebruik door de wieren, hetgeen leidt tot anoxia (= zuurstofloosheid) en productie van het giftige sulfide. Daarnaast worden ook grote hoeveelheden zeesla door overwinterende vogels als zwanen en meerkoeten geconsumeerd.

Beheersmaatregelen die gericht zijn op een vermindering van de massale zeeslabiomassa in het Veerse Meer zullen zich in eerste instantie moeten richten op een vermindering van de

stikstofbelasting. Voor een verder herstel van het ecosysteem is een meer natuurlijk geti vereist. Aanvullende maatregelen kunnen bestaan uit het oogsten van de wiermatten gedurende de stationaire fase en het verwijderen van de overwinterende fragmenten in de bodem en op de bodem van de geulen gedurende de winter.

DANKWOORD

Bij het voltooien van een proefschrift ondervindt men zeer veel tegenwind. Op de fiets van Goes naar Yerseke en vice versa heb ik dit letterlijk aan den lijve kunnen ondervinden – in Zeeland heeft men om een of andere reden en in tegenstelling met hetgeen door alle natuurwetten wordt voorgeschreven vaker wind tegen dan mee. Meer figuurlijk gesproken heeft een aantal personen bij de voltooiing van dit proefschrift voor wat luwte gezorgd, hen wil ik op deze plaats daarvoor bedanken. In de eerste plaats gaat mijn dank uit naar Ko Verschuure, die mij zowel tijdens mijn studentenonderwerp in Yerseke als tijdens de eerste twee jaar van mijn promotieonderzoek heeft geassisteerd. Ko was zowel assistent als leermeester. Toen ik voor het eerst in Yerseke kwam wist ik weinig van de "natte" ecologie, mijn veldkennis en vaardigheden op dit gebied heb ik vooral van hem geleerd. Daarnaast weet ik nu ook hoe ik krabben en paling moet vangen in kooien die eigenlijk bedoeld waren om de groei van zeesla te meten. Ko beschikt over iets dat door Piet ecologische intuïtie is genoemd, hetgeen er op neer komt dat hij in staat bleek mijn niet altijd even concrete ideeën om te zetten in praktisch uitvoerbare veldexperimenten. De door Ko ontworpen kooien zijn legendarisch geworden in het Europese EUMAC project, daarnaast is hij gezegend met een bijna onverwoestbaar optimisme. De vele uren die wij samen in en op het Veerse Meer hebben doorgebracht behoren tot de mooiste herinneringen aan mijn AIO periode in Zeeland.

Piet Nienhuis, mijn promotor en Jan Rijstenbil, mijn begeleider en co-promotor hebben een grote rol gespeeld in de voltooiing van dit proefschrift. Piet stond als bedenker en projectleider aan de wieg van het EUMAC project, waar ik mijn aanstelling aan te danken had. Daarnaast trad hij op als "bewaker van de grote lijn", de theoretische achtergrond van dit proefschrift en als kritisch lezer van de manuscripten. Ik heb onze discussies, vooral gevoerd tijdens onze “EUMAC-Nederland” meetings, altijd als zeer plezierig en inspirerend ervaren. Jan stelde de middelen ter beschikking om het onderzoek in de praktijk uit te voeren en controleerde mijn manuscripten zeer nauwgezet. Ook hem ben ik daarvoor zeer erkentelijk.

Pauline, coördinator van het EUMAC project en in het “oude” instituut mijn kamergenoot. Pauline, ik heb vaak bewonderend toegekeken hoe je de niet altijd even leuke rol als projectcoördinator hebt vervuld. Onze gezamenlijke, spontane brainstormsessies, veldtochten en discussies hebben mij enorm gestimuleerd.

Identificatie van al die verschillende soorten zeesla was één van de moeilijkste problemen in dit onderzoek. Herre Stegenga bracht orde in de morfologische chaos die dreigde te ontstaan, veel dank hiervoor. Als lid van de laatste lichting dienstweigeraars ontrafelde Stefan Draisma gedeeltes van de DNA sequentie – hierdoor kwamen we er achter dat het eigenlijk maar om 1 soort ging (maar welke naam we hem nou moeten geven….?). Daarnaast hadden we veel lol tijdens onze veldtrips en in de Kêête. Stefan, ik ben blij dat je er bij wilde zijn.

Van het NIOO hebben velen bijgedragen aan dit proefschrift: Peter van Breugel, Johan van de Zande, wijlen Jaap van Liere, Joop Nieuwenhuize, Adri Merks, Jan Sinke en Yvonne Maas van de analyselaboratoria zorgden voor de analyses van de water- en zeeslamonsters. Peter van Breugel heeft tevens de speciale lichtbak ontworpen en gemaakt voor de groei

experimenten. De technische inzet van Bert Sinke, redder van menig NIOO onderzoeker, was onmisbaar in het programmeren van de lichtbak en uiteraard het laten functioneren van he gehele mesocosmosgebouw. Chris Almekinders, Ko Hoekman, Lowie Hazen en Arie van Vossen van de werkplaats verleenden diverse hand- en spandiensten. De bemanning van de "Luctor" zorgde voor een vlekkeloos verloop van onze twee vaartochten. Anita Wijnholds en Tanja Moerdijk verzorgden de glutathion analyses en hielpen bij de kweek. Met de leden van de werkgroep mariene microbiologie (voorheen estuariene oecofysiologie) en met vele anderen binnen het NIOO-CEMO heb ik vele, vaak inspirerende discussies mogen voeren. Nadat Pauline zich met de zeegrassen ging bezighouden, werd Patty Brouwer in het "nieuwe" instituut mijn kamergenoot. Patty, de korte tijd die wij mochten samenwerken en de vele discussies die wij hadden over het werk, de zeewierecologie en hoe het nou daarmee verder moest in Nederland en over nog heel veel andere dingen hebben mij enorm geholpen tijdens de laatste loodjes. Verder heb ik zeer veel steun mogen ondervinden van het roemruchte Yerseks AIO overleg, het zgn. YAIOO. Opgericht onder het motto "gedeelde smart is halve smart", zochten wij steun bij elkaar gedurende de YAIOO uitjes en de Antwerpse diners. Speciaal wil ik Monique de Bie bedanken voor de vriendschap, troost in zware tijden en lol in vrolijke tijden tijdens de vele jaren Yerseke die we, eerst als student en later als AIO, gehad hebben. Festina perenijsjes, Jack & John, Kinky Friedman, Gazi Bay – het zijn slechts een paar van de dingen die we gedeeld hebben en die voor mij onvergetelijk zijn. Verder wil ik iedereen van het NIOO-CEMO bedanken – hoe zou ik dit ooit hebben kunnen doen zonder systeembeheer, uitstekende bibliotheek, kantine….

Een groot aantal studenten heeft zich in de loop der tijd aan de zeesla gewaagd. Zelf heb ik Edwin Arens, Rian Rottier, Ghisêl Crielaart en Robert Trouwborst onder mijn hoede gehad, terwijl Franca Lentz, Lonneke Schrijvers en Angela Tjin A Lien door Pauline begeleid werden. Ik wil jullie nogmaals bedanken voor jullie inzet en de gezellige tijd die we hebben gehad. Ook de stagiaires van Jan Rijstenbil: Natasja Perk, Marjoke Muller en Nella Kleijweg wil ik bedanken voor hun bijdrage.

“De Harpoen” en later “Het Anker” hebben een belangrijke rol gespeeld in de relativering van het werk. Vele donderdagavonden heb ik hier doorgebracht, met zeer veel mensen. Twee waren er altijd bij: Johan Craeymeersch en Marco van der Land. Marco heeft dit proefschrift helaas niet meer mee mogen maken. Ik denk met dankbaarheid terug aan al die donderdagavonden met z’n drieën.

All people that participated in the EUMAC project and joined the EUMAC workshops: I hope you enjoyed our meetings and discussions as much as I did. I will never forget the legendar EUMAC lunches in Venice and Sète! Special thanks go to the EUMAC PhD's: Heike, Becky Karen and Eliane and to Serge who taught us all about nutrient uptake kinetics. We had a lot of fun (and discussion) during those days in Kiel, Yerseke and Pleubian! Ralph Kuhlenkamp, thanks for discussions, encouraging e-mails and the articles you gave me. At the beginning o my thesis, I visited several Danish groups to discuss my plans and to learn from their methods. Drs. Kaj Sand-Jensen and Ole Geertz-Hansen (Instite for Freshwater Ecology of the University of Copenhagen), Dr. Sören L. Nielsen (University of Roskilde) and Drs. Peter-Bondo Christensen and Sören Rysgaard and all others of the fjordengroup at Silkeborg are thanked for their hospitality and fruitful discussions.

Als het leven in het Zeeuwse mij teveel werd vluchtte ik een weekendje naar Leiden, waar altijd wel iemand bereid was om in deze of gene kroeg naar mijn geweeklaag te luisteren, muziek mee te maken (bedankt Be Lazy Lizzy! – Liesbeth, Roland, Ursula en Floris) en mij

daarna ook nog een bed of bank aan te bieden. Mijn dank is groot aan al mijn vrienden. Speciaal wil ik hier Arnoud en Majory bedanken voor al die weekends en hun kat Kiri die mij toch altijd weer op zijn bank moest tolereren. Inez was zo vriendelijk om tijdens de laatste periode haar huis ter beschikking te stellen als ik vanuit Amsterdam overkwam voor een paar daagjes Yerseke.

Stef, jij hebt me de energie gegeven die ik nodig had tijdens de laatste loodjes. Amsterda was de laatste twee jaar dankzij jou een veilig toevluchtsoord. Ik hoop dat ik voor jou net zo'n steun zal zijn als jij voor mij bent geweest. Tenslotte wil ik mijn oma, broer, overige familie en vooral mijn ouders bedanken voor alles wat ze voor mij hebben gedaan en het vertrouwen dat ze in mij gehad hebben. Uiteindelijk is dit proefschrift ook een beetje hun werk.

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CURRICULUM VITAE

Erik-jan Malta werd op 1 augustus 1969 geboren te Wormerveer. In 1987 behaalde hij he VWO diploma aan de gemeentelijke scholengemeenschap Noordendijk te Dordrecht. Aansluitend begon hij met een studie Biologie aan de Rijksuniversiteit Leiden. Een eerste doctoraalonderwerp werd uitgevoerd bij de vakgroep Theoretische Biologie, sectie Biologie en Samenleving van de RUL. Hier bestudeerde hij het gebruik van proefdieren in he onderzoek naar milieugevaarlijke stoffen en de ethische afwegingen hierbij. Een tweede onderwerp werd uitgevoerd bij het Centrum voor Estuariene en Mariene Oecologie van he Nederlands voor Oecologisch Onderzoek (NIOO-CEMO) te Yerseke. Hier deed hij onderzoek naar de effecten van eutrofiëring op de groei van zeesla soorten in het Veerse Meer. Vervolgens werd een doctoraalonderwerp uitgevoerd bij de vakgroep Botanische Oecologie, sectie Vegetatieoecologie bij de Universiteit van Utrecht. Het onderzoek richtte zich op de ro van de vorming van stamspruiten voor het behoud van de boomsoorten Greenheart en Clump Wallaba na de kap en omvatte een veldwerkperiode van 3½ maand in Guayana, Zuid-Amerika. In 1993 werd het doctoraalexamen behaald, met als hoofdvak plantenoecologie. Hierna werkte hij een korte periode als vrijwilliger bij de wetenschapswinkel van de RUL, alwaar hij een rapport maakte over de natuurwaarde van het Heerenschoolbos ten behoeve van de Groen Links fractie in de gemeente Katwijk. In maart 1994 werd hij aangesteld als assistent in opleiding bij het NIOO-CEMO bij de werkgroep Estuariene Oecofysiologie (tegenwoordig Mariene Microbiologie) in het kader van het Europese "Eutrophication and Macrophytes" (EUMAC) project. Hier werd onderzoek verricht naar de gevolgen van eutrofiëring op de massavorming van de zeewieren, resulterend in dit proefschrift. In maar 1998 volgde een korte aanstelling als onderzoeker bij het NIOO-CEMO. Hierin werd, in opdracht van het Rijksinstituut voor Kust en Zee van Rijkswaterstaat, onderzoek gedaan naar de effecten van het gedeeltelijk herstel van een zoet-zout gradiënt op de bodemfauna van de zachte substraten in de Oosterschelde. In 1999 werkte hij verder als gastmedewerker bij he NIOO-CEMO aan de voltooiing van zijn proefschrift. In januari 1999 begon hij als vrijwilliger bij de stichting Bureau Duin & Kust/EUCC Services te Leiden, alwaar hij de promotie van de verkoop van publicaties van de European Union for Coastal Conservation (EUCC) en de acquisitie voor het blad Coast*line* verzorgt. Sinds april 1999 is hij werkzaam bij de EUCC als coördinator van Coas *line*. Vanaf oktober 1999 is hij tevens werkzaam bij de Stichting de Noordzee te Utrecht. Hier werkt hij aan een project dat de juridische mogelijkheden van gebiedsbescherming in de Noordzee onderzoekt en geeft hij ecologisch advies bij diverse onderwerpen.