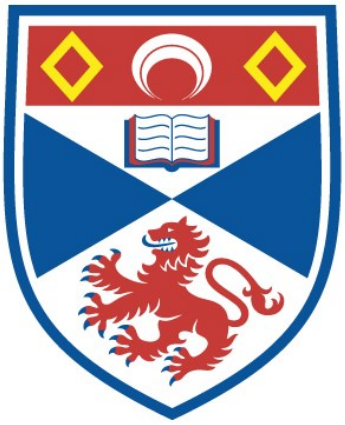
LOBELIA DORTMANNA - LIGHT, GROWTH STRATEGY AND ZONATION

Andrew M. Farmer

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1986

LOBELIA DORTMANNA LIGHT, GROWTH STRATEGY AND ZONATION

by

Andrew M. Farmer

A thesis submitted to the
University of St. Andrews
for the Degree of Doctor
of Philosophy

Department of Plant Biology and Ecology,
St. Salvator's College,
University of St. Andrews.

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Abstract

This thesis seeks to distinguish the main environmental parameters controlling the growth of *Lobelia dortmanna* and, by consideration of field conditions, describe which of these controls is most important in determining the zonation of the species, both horizontal and vertical, in Scottish lochs.

A detailed analysis of growth across one season was undertaken in a Sutherland lochan, studying populations on sites of different nutrient status. Continuous recordings of temperature and light and monthly measurements of photosynthesis, soluble carbohydrate content and leaf production show continuous growth across the year. However, major increases in growth are strongly correlated to light increases, and only weakly with temperature. No differences occur between sites, so nutrient limitation is not thought to be important in growth.

Germination studies reveal that seeds are absolute requirers of light for germination, are red-light promoted and require a cold stratification period before germination. Seeds germinate in low light, under conditions that seedlings subsequently cannot survive in. Examination of the light regime in Scottish lochs reveals that there is sufficient light for germination below the depth limit of zonation. Thus zonation is not controlled by a light requirement for germination.

Studies of photosynthesis, pigment and carboxylase variations with depth in *L. dortmanna* reveal some ability to respond to shading, particularly increasing chlorophyll levels. However, chlorophyll/carboxylase ratios do not change, so indicating the plant is not typical of shade-adapting species. It is concluded that light control of photosynthetic production is the most important factor in controlling growth and zonation.

Certificate

I hereby certify that the candidate has fulfilled the conditions of Resolution and Regulations appropriate to the degree of Doctor of Philosophy of the University of St. Andrews and that he is qualified to submit this thesis in application for that degree.

Dr. D.C. Weeks,
(Supervisor).

September 1985

DECLARATION

I hereby declare that this thesis is of my own composition, that it is based on an accurate record of work carried out by me, and that it has not been previously presented in application for a higher degree.

Andrew Farmer,
St. Andrews.

September 1985.

Declaration

I was admitted as a research student under Ordinance 12 in October 1982 and as a candidate for the degree of Ph.D. in October 1983; the higher study for which this is a record was carried out in the University of St. Andrews between 1982 and 1985.

Andrew M. Farmer.

September 1985

Acknowledgements

The greatest debt that I owe in the production of this thesis is to the late David Spence. His assistance and insight were invaluable in undertaking this study, and in particular, his continuing stimulation of my interest in freshwater ecology. His friendship I will greatly miss.

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I am also grateful to my parents for their continued support through all my studies.

I thank the Factor of the Westminster Estates, Sutherland, for permission to work on Lochan-na-Thuill and to Mr. & Mrs. D. Munro for their cheerful hospitality in Durness.

This study was undertaken whilst in the receipt of a N.E.R.C. Research Studentship.

Dundee and St. Andrews Universities Guild of Change Ringers.

Alloa, Central. St. John's Episcopal Church.

A quarter-peal of 1320 changes of *Lobelia dortmanna* Surprise Minor on 27th April 1985.

Treble	Paul Graupmer	4	Robin Beal
2	Katrina Rook	5	Andrew Farmer
3	Carol Lock	Tenor	James Aldridge

First quarter in the method.

New method: x 56 x 1456 x 56 x 1236 x 14 x 36.

CONTENTS

Abbreviations

Chemicals:

BSA	=	Bovine serum albumin
DNPH	=	2,4-Dinitrophenylhydrazine
DTT	=	Dithiothreitol
EDTA	=	Ethylene diamine tetraacetic acid
PVP	=	Polyvinylpyrrolidone

Other:

CAM	=	Crassulacean acid metabolism
F_r	=	Far red light at 730 nm
K_d	=	Vertical diffuse attenuation coefficient
K_G, K_R	=	K_d of light through broad band green and red filters
K_{min}	=	Least attenuated of the K 's for different parts of the visible spectrum
PAR	=	Photosynthetically active radiation (400-700 nm)
R	=	Red light at 660 nm
SCUBA	=	Self contained underwater breathing apparatus
SLA	=	Specific Leaf Area, unit leaf area per unit leaf dry weight
Z_c	=	Depth of zone colonised by macrophytes
Z_{eu}	=	Euphotic zone, having about 1% subsurface PAR

Note on light quantification:

In this thesis light is quantified in terms of Einsteins (E) which are taken to be equivalent to moles of light quanta. i.e.:

$$1\ \mu E\ m^{-2}\ s^{-1} \equiv 1\ \mu mol\ m^{-2}\ s^{-1}$$

CHAPTER ONE

INTRODUCTION

Chapter 1. Introduction

The most fundamental questions of aquatic plant ecology and physiology are those concerning the zonation of aquatic plants. Initially studies of lake vegetation were generally descriptions of lakes and their plant communities (e.g. West, 1905). However, in later descriptions (e.g. Pearsall, 1920) attempts were made to try to explain the observed distributions with reference to the environment of the lakes, i.e. their zonation.

Spence (1982) reviewed the current state of knowledge of plant zonation in freshwater lakes. He distinguished a number of environmental variables that can affect zonation. Zonation was also considered in two directions. Horizontal zonation concerns the changes in plant communities as, for example, one travels around the littoral shore of a lake. Vertical zonation is concerned with the changes in plant communities with increasing water depth.

The environment can be described in terms of a number of variables. Sediment characters include particle size, nutrient status and redox status. Water characters include nutrient status, colour, light attenuation, temperature and turbulence. This list is not exhaustive, and these characters are inter-related. They also have compounding effects on aquatic plants. It is, for instance, important in studies of light limitation of photosynthesis and productivity to account for any nutrient limitation. It is worthwhile considering these characters in more detail.

Horizontal zonation is generally controlled by sediment characters and water turbulence. Sheltered areas of a lake will allow the deposition of silt and organic particles. In oligotrophic lakes, which form the majority of Scottish lochs (Spence, 1964), such areas can have increased nutrient levels. Vegetation in these

regions is generally of emergent and floating species, with 100% cover. In exposed areas the sediment is of hard sand/gravel and, in oligotrophic lakes, low in nutrients. There is often little plant cover, and that is of small, submerged plants.

Vertical zonation has been attributed predominantly to changes in water turbulence and the light regime with increasing water depth. The latter effect can operate in two ways. First there is a reduction in the light available for photosynthesis. Secondly the spectral composition of the light changes with depth, the precise changes depending on the dissolved and colloidal substances in the water. This can cause alterations in photomorphogenic effects (Spence, 1976). The actual physical descriptions of the light environment of many lakes has been well studied (Spence, 1981), but the study of these effects on the aquatic plants has been little investigated. Spence and Chrystal (1970a and b) studied the effect of varying light levels in *Potamogeton* species by showing that the zonation of these plants is controlled by an ability of these species to photosynthesise under low light levels. In contrast, Spence (1982) has also suggested that zonation may be controlled in other species by photomorphogenic processes which depend on light quality, such as seed germination. These factors will be considered in greater detail in later chapters. This study will seek to distinguish a number of factors affecting zonation in one particular species - *Lobelia dortmanna* L.

L. dortmanna is one of a number of species belonging to a group of aquatic macrophytes known as the isoetids. Den Hartog and Segal (1964), based on Du Reitz (1921, 1930) defined aquatic macrophytes according to their growth forms. One of these forms is the isoetids, defined as "rhizophytes with a short stem, a rosette of stiff radial leaves, and with or without stolons". This type of growth habit is

spread across a taxonomically wide range of plants. In Britain the following species are considered as isoetids - *Isoetes lacustris* L. and *I. echinospora* Durieu (Isoetaceae), *Subularia aquatica* L. (Cruciferae), *Littorella uniflora* (L.) Aschers (Plantaginaceae), *Eriocaulon septangulare* With. (Eriocaulaceae) and *Lobelia dortmanna* (Lobeliaceae) (Haslam et al., 1975).

The isoetids are characteristic of the oligotrophic lakes of North-West Europe (Clapham et al., 1962). In Scotland the majority of the lochs and lochans are oligotrophic, particularly in the Highlands and Southern Uplands (Spence, 1964). In these lochs at least one isoetid species can usually be found. The distribution of *I. echinospora* and *S. aquatica* is widespread (Spence, 1964; Woodhead, 1951b), though these species are often overlooked (e.g. Ballantyne, 1977). *E. septangulare* is an historically recent arrival in Britain and is confined to parts of the West coast of Scotland, though it is spreading (personal communication to Spence). The remaining three species are widespread and are common in many oligotrophic Scottish lochs (Spence, 1964; Spence and Allen, 1979; Spence et al., 1979). *I. lacustris* is often a deep water species, certainly having a greater depth penetration than other genera of isoetids (Spence, 1964). It is less common to find large littoral populations of *I. lacustris* than *L. dortmanna* or *L. uniflora*. The two species of isoetids that are both relatively accessible in large numbers and of reasonably well known distribution are *L. dortmanna* and *L. uniflora*. In studying zonation and growth strategy this thesis will analyse growth in the field. *L. dortmanna* has a simple growth process, merely adding leaves to the rosette. *L. uniflora* also grows similarly.but, in addition, produces stolons and plantlets. This makes growth difficult to quantify. Such a factor is important in choosing *L. dortmanna* as a representative isoetid.

The zonation characteristics of the three commonest isoetids are well known. The depth limits of the species vary. *I. lacustris* can penetrate to 4-6 m, depending on the loch, *L. uniflora* to 3-4 m and *L. dortmanna* to 1.7-2.5 m (Spence, 1964; Pearsall, 1920). These depth limits are often well defined. A dive in a loch will reveal a population of large, healthy-looking plants of *L. dortmanna* that suddenly ends as the depth increases. Rørslett (1985) found that at the depth limit of *I. lacustris* there occur a number of dead individuals, so making an accurate determination of the limit of zonation difficult. This has not been observed for *L. dortmanna*. It is this short depth-range and sharp "cut-off" that make *L. dortmanna* ideal for studying the factors affecting vertical zonation.

The horizontal zonation of *L. dortmanna* is wide-ranging. In oligotrophic lochs the plants occur over a wide range of sediment types (Spence, 1964; Campbell, 1971). Only in the most exposed regions of lochs is this plant lacking. It is also limited in its ability to grow in dense vegetation. It is hoped that an analysis of the growth strategy of *L. dortmanna* will provide an indication of the factors limiting the distribution of *L. dortmanna* in its horizontal zonation.

This study will attempt to distinguish the most important environmental parameters that may control the growth and, hence, zonation of *L. dortmanna*. It is necessary to initially consider the seasonal changes in growth of the plant. This study will follow growth in the field, while monitoring a number of environmental variables. In particular this will seek to establish whether light, temperature, nutrients or CO_2 supply may limit growth. Such a study will generate a hypothesis concerning growth control that can be tested experimentally under controlled growth conditions.

The study will then go on to consider the reproductive strategy

of *L. dortmanna* and the environmental control of seed production and germination. In particular, the effect of temperature, light quantity and quality and anoxia on seed germination and seedling growth will be studied and compared to a physical description of the field environment. This will seek to generate a hypothesis on the control of vertical zonation.

Finally the metabolic physiology of *L. dortmanna* will be considered in greater detail. It will be necessary in this section to expand the study to include other macrophytes, particularly isoetids, as published data for comparisons are limited. The photosynthetic adaptability of *L. dortmanna* will be studied, in particular the ability of the plant to vary its light and carbon harvesting systems with increasing depth. This will be discussed in relation to the vertical zonation of the plant.

In concluding, the study will attempt to define the environmental factors that most limit growth in *L. dortmanna*. This will be used to define the growth strategy of the plant and so seek, at least in part, to explain the observed zonation, both vertical and horizontal.

CHAPTER TWO

THE ENVIRONMENTAL CONTROL OF THE SEASONAL GROWTH OF *LOBELIA DORTMANNA*

Chapter 2. The Environmental Control of the Seasonal Growth of *Lobelia dortmanna*

2.1. Introduction

Only one study (Moeller, 1978) has been carried out to investigate the seasonal variations in the growth of *L. dortmanna*. However, no account was made of any environmental parameters that may, in some way, control this growth. This chapter considers a number of growth and environmental parameters, seeking to determine which of the latter are more important in controlling growth.

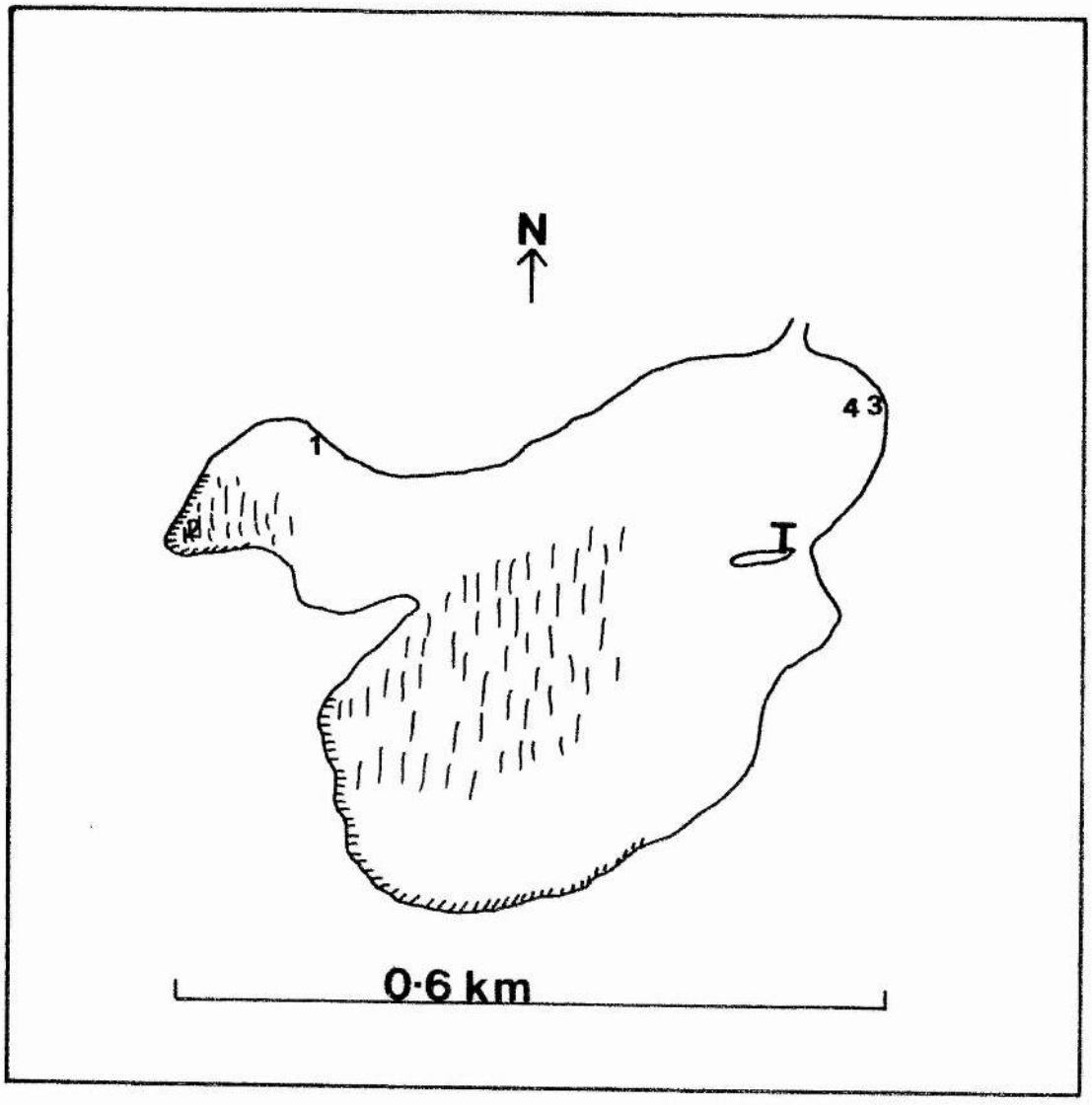
Initially an account is made of the study lochan, and of the study sites within that lochan. This is done so as to distinguish any variations in sediment characters and in plants of *L. dortmanna* in the lochan so that these can, if necessary, be taken into account in the subsequent analysis. Growth and environment were monitored throughout one season. A separate consideration of each growth and environmental character was carried out, and then the relationships between them were investigated. This allowed certain tentative conclusions to be drawn about the effects of the environment on growth. Finally, an account is given of the experimental growth of plants under controlled conditions which is designed to test the preliminary findings of the seasonal growth analysis.

2.2. The Study Lochan

The work on seasonal growth in *L. dortmanna* was carried out in a small feeder lochan to Loch-na-Thuill, Sutherland (5°00'W 58°24'N). Henceforth it is referred to as Lochan-na-Thuill. A map of the lochan is shown in Fig. 2.1. It is small and the water depth does not descend below 1.5 m. However, it contains very good populations of *L. dortmanna* over a range of substrate types.

Figure 2.1.

A sketch map of Lochan-na-Thuill. It shows the positions of the study sites (1-4) and of the permanent thermometer (T). It also shows those shores that are silty (small hatching) and areas of emergent vegetation (large hatching).


N
1
2
3
4
0·6 km

Following the classification of Spence (1964) a number of plant communities can be distinguished in the lochan. The more exposed, coarse sediment areas bear the *Littorella-Lobelia* open sociation. More sheltered areas and parts of the deep water sediments are covered with the *Lobelia-Littorella* sociation. One sheltered bay contains an example of the *Equisetum fluviatile-Littorella* sociation and in the deeper water may be found the *Nymphaea alba* subsp. *alba* sociation.

Most of the lochan, however, consists of the former two sociations. These communities have been noted to be very common in the West of Scotland (Spence, 1964; Spence and Allen, 1978; Spence, Allen and Fraser, 1979). Schoof-van Pelt (1973) studied the plant communities of the lochs of Sutherland and, using the classifications of Tuxen (1937), noted impoverished forms of the Isoeto-Lobelietum, which would correspond to much of the vegetation in lochan-na-Thuill. Similar communities are common in other parts of Britain, e.g. North Wales (Woodhead, 1951)[a], and Northern and Western Europe, e.g. Poland (Dambska, 1965 and 1966) and Finland (Eloranta and Marja-aho, 1982).

2.2.1. The Study Sites

Four sites were chosen around the littoral shores of the lochan which it was hoped would cover the full range in variation evident in the *L. dortmanna* populations and yet were easily accessible. These sites, which are located on the map (Fig. 2.1) were:

Site 1. An exposed, eroded area, with a sediment composed almost entirely of coarse gravel. Associated with *L. dortmanna* are *Littorella uniflora* and *Ranunculus flammula*.

Site 2. A sheltered, depositional area, with a fine-particled sediment. Present with *L. dortmanna* are *L. uniflora*, *R. flammula*, *Carex rostrata*, *Potamogeton polygonifolius* and *Utricularia minor*.

Site 3. Another exposed area, also with a sediment of coarse gravel. Associated with *L. dortmanna* are *L. uniflora*, *R. flammula* and *Isoetes lacustris*.

Site 4. This site is at the same point in the lochan as site 3, but at 45 cm depth. It has a coarse sediment and is mostly populated with *L. dortmanna*, with a little *L. uniflora*.

2.2.2. Analysis of the Study Sites

A full analysis of both the plants and their physical environment was undertaken at each of the sites in order to observe any differences that may be important in understanding a seasonal growth analysis. All analyses were done in September 1983. Unless otherwise stated the analyses were carried out the day the samples were collected in a small field laboratory in Durness, 27 kilometers from the lochan.

2.2.3. Physical Analysis

The following measurements were made.

1. Water Alkalinity.

The alkalinity of the lochan water was determined according to the methods of Mackereth, Heron and Talling (1978) using Gran Titration. There is some preliminary evidence that alkalinity may affect the distribution of isoetids, particularly *L. dortmanna* and *L. lacustris* (Spence, 1964; Spence, Allen and Fraser, 1978).

2. pH.

The pH of the lochan water was measured using a Pye-Unicam pH electrode. Like alkalinity, pH may also affect the distribution of isoetids (Spence, 1964).

3. Soluble Inorganic Phosphate.

Nutrient levels, and particularly phosphate levels, in both water and sediment are important in controlling the growth and distribution of a number of aquatic macrophytes (Gerloff and

Krombholz, 1966). The soluble inorganic phosphate levels of the lochan water were determined using the method of Mackereth, Heron and Talling (1978). At each study site interstitial water samples were taken as by Spence, Barclay and Allen (1984) and determined for inorganic phosphate as with the lochan water.

4. Sediment Organic Matter Content.

The sediment organic matter levels depend on the amount of silt in the sediment. This figure, therefore, bears a crude relationship to whether a site is sheltered and depositional or exposed. Samples of sediment from each study site were oven-dried at 80°C for 24 hours and then ignited in a furnace at 500°C for 8 hours. The difference between dry weight and ash weight is taken to be the organic content. This was determined in St. Andrews.

5. Plant Density.

The number of plants per m^2 was determined using 1 m^2 quadrats for each site. Care was taken that only the actual area that would be used for the seasonal growth analysis was counted.

2.2.4. Plant Analysis

1. Physical Description.

Ten plants were taken randomly from each site and the leaf length and length/breadth ratios were measured. The "forma" of the plants (see Discussion, section 2.2.5.) was also observed.

2. Chlorophyll Content.

Six plants were taken randomly from each site and the chlorophyll content was determined using the Dimethyl-sulphoxide extraction method of Hiscox and Israelstrom (1979). Spectrophotometric measurements were made at 663 nm for chlorophyll a and 645 nm for chlorophyll b and corrected by a 750 nm reading to account for any scattering.

Chlorophyll a and b levels were determined according to the

following formula:

$$\text{concentration (g litre}^{-1}) = \frac{\text{absorption}}{\text{pathlength} \times \text{specific absorption coefficient}}$$

The specific absorption coefficient for chlorophyll a is 78 and for chlorophyll b is 44. Results are expressed on a fresh weight basis.

3. Root Phosphatase Levels.

To complement the measurement of interstitial phosphate levels measurements were made of the root phosphatase levels. Studies on microalgae (Fitzgerald and Nelson, 1966) and macrophytes (Campbell, 1971) show that plants tend to increase the levels of alkaline phosphatases when under low phosphate conditions. Two plants were taken randomly from each site and studied for alkaline phosphatase levels. Campbell (1971) made a preliminary study of alkaline phosphatase levels in *L. dortmanna*, concluding that leaf levels were very much lower than root levels, and thus confirming autoradiographic studies that phosphate uptake occurs via the roots and not the leaves. In this study, therefore, alkaline phosphatase levels were determined on the leaves of plants from only two sites, in order to check Campbell's (1971) findings, but on the roots from plants from all sites.

Determinations were made in St. Andrews. Plants were, therefore, dug-up from each site using a trowel and potted, leaving as much of the sediment undisturbed as possible. The pots were kept in water from the lochan and used within two days. It was hoped that this would retain the in-situ phosphate environment.

The whole plant was washed to remove silt and some of the epiphytes. After wiping, the leaves were rubbed vigorously with GF-C filter-paper until no more "green" appeared on the paper. It was assumed that this removed most of the epiphytes. The thick cuticle prevents the leaves, themselves, from being damaged.

The basis of the method depends on the fact that the compound p-nitrophenol phosphate is colourless but if the phosphate group is hydrolysed yellow p-nitrophenol is liberated. This has an absorption maximum at 410 nm. One unit of alkaline phosphatase is defined as the amount of enzyme which liberates one micromole of p-nitrophenol per hour under the specified conditions.

The phosphatase levels were studied by suspending a piece of prepared tissue in 32 ml of Gorham's medium (Table 2.1) minus the phosphate component in a 100 ml conical flask. 4 ml of TRIS buffer were added (1M TRIS in 0.01M Magnesium chloride adjusted to pH 8.5 with acetic acid) followed by 4 ml of p-nitrophenol phosphate solution (30 mg in 100 ml distilled water). The assay mixture was incubated for 1½ hours at 36°C. After this time 10 ml were removed and added to a test-tube containing 0.5 ml of distilled water with 10 mg of orthophosphate. This stops the reaction. The absorbance at 410 was measured in a 1 cm cell using a SP600 spectrophotometer. The tissue was removed, blotted, dried at 90°C overnight in an oven and weighed. Controls to account for changes in the assay mixture during incubation and for the loss of material from the plants were also made. The results are calibrated against a standard solution of p-nitrophenol made up in 0.02N sodium hydroxide. Results are expressed on a dry weight basis.

2.2.5. Results and Discussion

The results for the site and plant analyses are given in Tables 2.2 and 2.3. The results show a number of variations between the sites, with site 2, in particular, being exceptional. The lochan itself is typical of many brown-water lochs in Western Scotland with a low soluble inorganic phosphate level, low pH and low alkalinity (Spence, Allen and Fraser, 1978). The interstitial

Table 2.1.

The Composition of Gorham's Medium (g/litre).

$NaNO_3$	0.496
K_2HPO_4	0.039
$MgSO_4.7H_2O$	0.075
$CaCl_2.2H_2O$	0.036
Na_2SiO_3	0.058
Na_2CO_3	0.020
Ferric citrate	0.006
Citric acid	0.006
EDTA	0.001
pH	8.5

For phosphatase analysis Gorham's medium is made up without the phosphate component.

Table 2.2.

A series of physical descriptions of the four study sites of Lochan-na-Thuill.

The sites are located in Figure 2.1.

	Site number			
	One	Two	Three	Four
Mean monthly water depth (cm) (1982-3)	15	15	15	45
Substrate description	coarse gravel	fine silt	coarse gravel	coarse gravel
% sediment organic matter Mean ± S.E. n = 3	0.98 ± 0.07	13.3 ± 1.2	1.13 ± 0.14	1.89 ± 0.44
Loch water soluble phosphorus Mean n = 2 (µg/litre)	2.0	2.0	2.0	2.0
Sediment interstitial soluble phosphorus Mean n = 2 (µg/litre)	22.8	249.	36.00	19.9
Loch water alkalinity Mean n = 2 (µeq/litre)	19.1	19.1	19.1	19.1
Mean montly plant density (plants/m^2)	65.1	46.5	52.4	35.0

Table 2.3.

A series of descriptions of plants of *L. dortmanna* from the four study sites in Lochan-na-Thuill. The four sites are located in Figure 2.1.

	Site number			
	One	Two	Three	Four
Plant form after Glück (1924)	"terrestris"	"ramosa"	"terrestris"	"terrestris"
Leaf length (mm) Mean ± S.E. n = 10 plants	23.5 ± 1.8	61.3 ± 2.9	24.4 ± 1.8	28.2 ± 0.7
Leaf length/breadth ratio Mean ± S.E. n = 10 plants	11.3 ± 0.5	22.8 ± 1.1	10.1 ± 0.7	10.0 ± 0.2
Leaf chlorophyll content (mg/g) Mean ± S.E. n = 5	0.616 ± 0.078	0.420 ± 0.034	0.635 ± 0.015	0.590 ± 0.050
Leaf chlorophyll a/b ratio Mean ± S.E. n = 5	1.42 ± 0.07	2.02 ± 0.73	1.28 ± 0.03	1.61 ± 0.06
Root phosphatase level (μM nitrophenol/hr/g) Mean ± S.E. n = 3	46.6 ± 12.3	18.5 ± 0.95	30.4 ± 5.1	38.3 ± 0.9
Leaf phosphatase level (μM nitrophenol/hr/g) Mean n = 2	3.93	2.14	-	-

phosphate levels are much higher than the water concentration, but that in site 2 is approximately ten times the level in the other three sites. This site is also distinguished by its fine sediment and higher sediment organic content.

Plant attributes also vary between the sites. Site 2 is distinguished by much larger plants. Glück (1924) described two "forma" of *L. dortmanna*. These were a large, submerged form - "ramosa", and a small terrestrial form - "terrestris". Terrestrial plants observed around the shores of other lochs in the Highlands and Western Isles are almost identical to the small plants found in sites 1, 3 and 4. It is probable that these forms represent differences between exposed and sheltered/silty habitats. It is well known that aquatic macrophytes exposed to wave action can be smaller, or more "compact" (Haslam, 1978; Lobban and Wynne, 1981; Dommasnes, 1978). Aberg (1943) also found, using plants transplanted in uniform sediment, that large plants of *L. dortmanna* can be formed due to the reduction in light levels caused by the increasing depth of water in a lake. It is possible that sedimentation in shallow water may shade the apex, which is situated close to the sediment surface. In this way it may act by shading, so causing the morphogenic effect. It should also be mentioned that whereas plants in exposed areas only have a rudimentary stem or corm, plants from silty areas have a large stem with leaf scars and obvious internodes. This ability to change growth form due to sedimentation has been previously noted for *L. uniflora* (Webster, 1975; Spence, 1982) but has been found to be lacking in *I. lacustris* (Pearsall, 1921).

There appears to be some relationship between the root phosphatase levels and sediment interstitial inorganic soluble phosphate levels. Thus in site 2 there is a much higher phosphate level, and a reduction in root phosphatase. This negative relationship

is well known in microalgae and other macrophytes (Fitzgerald and Nelson, 1966; Fitzgerald, 1969; Campbell, 1971).

The study sites, therefore, show a wide range in physical and plant attributes. They are probably representative of the littoral shores of lochan-na-Thuill, though they do not include any deep water plants. These sites are, therefore, able to be used to investigate the effects of inorganic phosphate levels on growth through the season in addition to the other environmental parameters that will be investigated.

2.3. The Seasonal Analysis of Growth and Environment

Growth is a complex physiological process and so any estimation of growth by analysis of one particular aspect may be subject to error or over-simplification. Three aspects of production and growth in *L. dortmanna* were, therefore, studied. These were photosynthetic rates, soluble carbohydrate content and leaf production. As about 50% of the biomass of *L. dortmanna* is leaf (Luther, 1983), a measure of leaf production is a good indicator of growth. The first two aspects studied are measures of the basic production process, while leaf production is a measure of the final outcome of production-growth.

The physical environment was monitored for changes in total daily irradiance, temperature and water level fluctuations. All growth and physical measurements were made monthly from November 1982 to September 1983 (excluding January 1983).

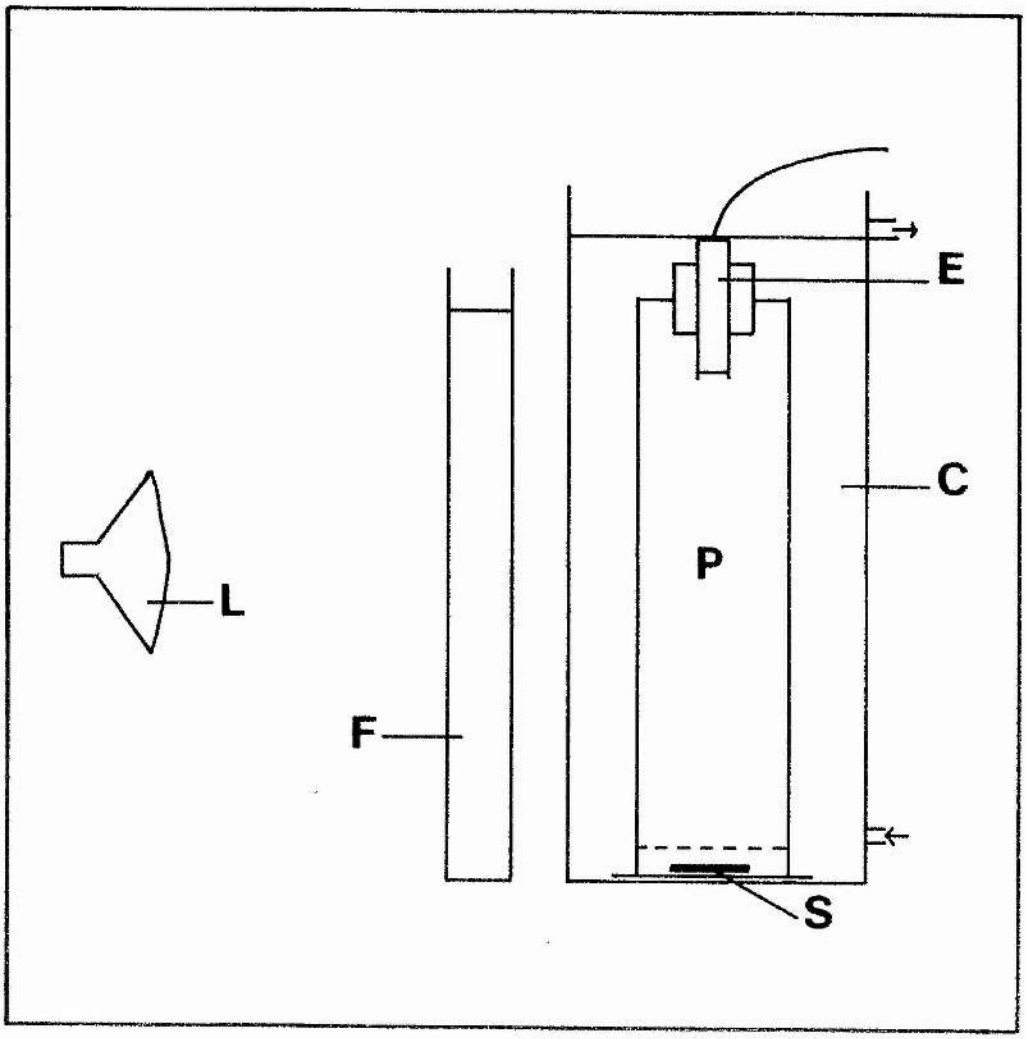
2.3.1. Methods

1. Photosynthetic Rate.

Two plants were taken each month from site 2 and transported to St. Andrews. Their photosynthetic rate was determined using a Beckmann oxygen electrode in the apparatus shown in Figure 2.2.

Figure 2.2.

The apparatus used to measure seasonal changes in photosynthetic rate. It shows the lamp (L), a water filter to filter excess infra-red (F), the oxygen electrode (E), the constant temperature cooling jacket (C), a magnetic stirrer (S), and chamber for the plant (P). The photosynthetic chamber contains filtered loch water.


L
F
E
C
P
S

Determinations were made at a standard irradiance (150 $\mu Em^{-2}s^{-1}$) and at the ambient/lochan temperature. Until used the plants were kept at 5°C. CO_2 was not found to be limiting in these determinations.

2. Soluble Carbohydrate Content.

Ten plants were taken each month from sites 1 and 2 and used for determining the soluble carbohydrate content. The plants were washed and wiped free of epiphytes. The leaves and roots of each plant were determined separately. After washing, the leaves or roots were weighed, chopped-up and placed in McCartney bottles containing 10 ml of 80% ethanol. This was done as soon as possible after collection in the field laboratory in Durness. The samples were then stored until convenient for analysis.

The soluble carbohydrates were extracted by boiling in ethanol. Each sample was boiled in four changes of 80% ethanol and three changes of 60% ethanol. The latter is thought to remove any more water-soluble carbohydrates (Crawford and Huxter, 1977). Each extraction took 20 minutes, and the extracts were then filtered through Whatman No. 4 filter paper. All flasks, filters, etc., were finally washed with distilled water to ensure complete collection of the carbohydrate. 0.5M sulphuric acid was then added to the extract (to make a 0.05M solution) and then boiled for one hour. After cooling the extract was neutralised with 0.5M sodium hydroxide. This ensures the hydrolysis of non-reducing sugars (Smith, 1969), particularly sucrose, to reducing sugars. The final extract was again filtered and the volume measured.

2 mls of each sample were used for the soluble carbohydrate determination based on the method of Somogi (1952). The principle of the analysis is the reduction, by the reducing sugars, of a Copper (II) reagent to Copper (I). This is then reacted with an arsenomolybdate reagent to form a coloured complex, the intensity

of which can be read spectrophotometrically. The composition of the reagents is given in Table 2.4.

The assay procedure involved adding 1 ml of the copper reagent to the 2 mls of extract and boiling for 1 hour. After cooling, 2 ml of the arsenomolybdate reagent was added, mixed and allowed to stand for 10 minutes. The absorption was read at 510 nm, 1 cm pathlength, in a Beckman D8-GD spectrophotometer. The results were compared with glucose standards and a distilled water blank. They are expressed on a fresh weight basis.

3. Leaf Production.

At each of the study sites a permanent line quadrat was set up parallel to the water level. Along the line plants were chosen and the leaf numbers recorded for each plant each month. As *L. dortmanna* is slow growing, it was quite possible to observe the growth of the same individual leaf repeatedly over 2-3 months, and also the slow degeneration of individual leaves. It was, therefore, possible to distinguish both production and loss in plants that maintained the same leaf number from one month to the next. The number of plants studied in each site is shown in Table 2.5.

4. Temperature.

A permanent record of the temperature in the lochan was made using a Casella chart recording thermometer. This was sealed and placed at 50 cm depth in the position shown in Figure 2.1.

5. Irradiance.

A Kipp and Zonen solarimeter was set up in an open area of ground in Durness, 27 kilometers to the north of the lochan. It is unlikely that there are any differences in average weather patterns between the two sites. The lochan is, however, surrounded by low hills so that the total irradiance reaching the water may be less than in Durness. This would be a proportionally similar effect

Table 2.4.

The composition of the copper reagent and arsenomolybdate reagent used for soluble carbohydrate analysis.

Copper reagent:-

Three solutions were made up:

1) 24 g of anhydrous sodium carbonate and 12 g of sodium potassium tartrate were dissolved in 250 mls of distilled water.
2) 4 g of hydrated copper sulphate were dissolved in 40 ml of distilled water.
3) 180 g of anhydrous sodium sulphate were dissolved in 300 mls of hot distilled water. This was boiled for 5 minutes to expel any air.

Solutions 1 and 2 were mixed together, before adding solution 3. The reagent was made up to 1 litre, stoppered and stored in the dark and cold.

Arsenomolybdate reagent:-

Two solutions were made up:

1) 25 g of ammonium molybdate was dissolved in 450 mls of distilled water. To this is added, slowly, 21 mls of concentrated sulphuric acid.
2) 3 g of sodium hydrogen arsenate was dissolved in 25 mls of distilled water.

Thw two solutions were mised and made up to 1 litre. This solution must be stored warm (35-40°C) overnight before use, but is otherwise stored dark and stoppered.

throughout the year, so any discussion of the relationship between irradiance and growth remains valid.

6. Water Level.

A permanent mark was made on a rock by the side of the lochan and each month the level of the water below this mark was measured. Fluctuations in water level could, therefore, be observed.

2.3.2. Results and Preliminary Discussion

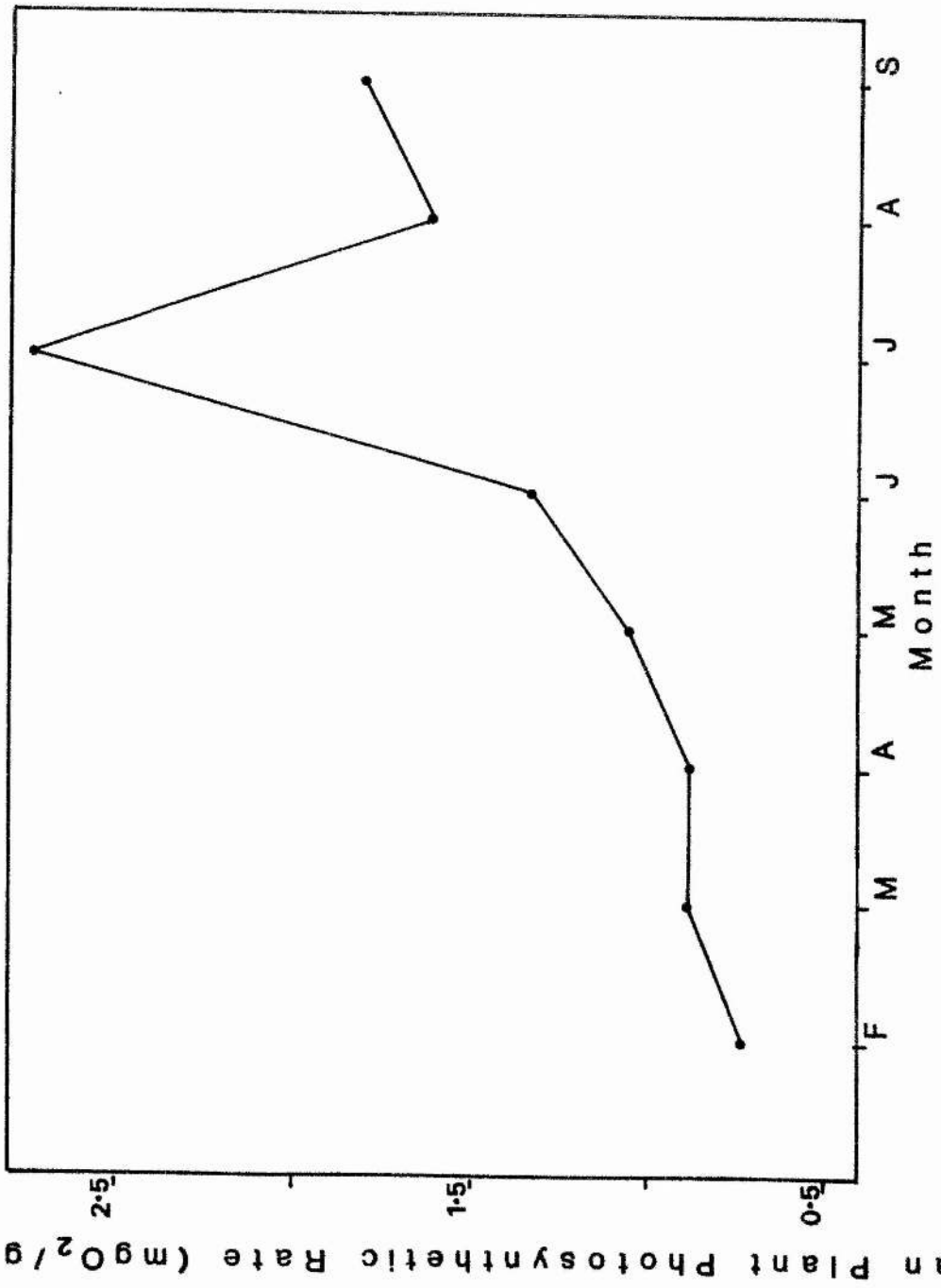
1. Photosynthetic Rate.

The results are presented in Figure 2.3. It can be seen that *L. dortmanna*, in this lochan, continues to photosynthesise net throughout the winter. Direct measurements of photosynthesis across the winter season in macrophytes are rarely made. In 1976 Boylen and Sheldon noted that ten species of macrophytes that maintained high winter population densities were able to photosynthesise under ice cover. Rates were 10-20% of the summer maxima. In this study *L. dortmanna* shows winter rates of about 20% of the summer level. This higher level is probably due to the mild nature of the winters in North-West Scotland, where ice-cover is intermittent. In severe winters plants are known to survive freezing in North Wales (Woodhead, 1951), Grampian (Mathews, 1946) and Germany (Glück, 1924). Kunii (1984) noted that *Elodea nuttallii* was able to photosynthesise during the Japanese winters when temperatures rose above 4°C.

It should be noted that there is a sharp rise in photosynthetic rate in May-July. In their studies on *L. uniflora* and *Isoetes macrospora* Boston and Adams (1985) made measurements of both C_3 assimilation and CAM across the growing season. They found a general, steady rise from February to August, and then a slow decline to December.

Figure 2.3.

Seasonal changes in the gross photosynthetic rate of whole plants of *L. dortmanna*. Each point is a mean of two measurements.


Mean Plant Photosynthetic Rate (mgO2/gdwt/hr)
2·5
1·5
0·5
F
M
A
M
J
J
A
S
Month

2. Soluble Carbohydrate Content.

The results are presented in Figures 2.4 and 2.5. The leaf soluble carbohydrate levels are always greater than the root levels, which is probably due to the fact that the leaves are the site of production. The level remains high over the winter and reaches a peak in early spring, prior to both the peak in photosynthesis (see above) and leaf production (see below). The only studies on carbohydrates in freshwater aquatic vascular plants have been carried out on emergent species. Roseff and Bernard (1979) studied seasonal variations in the non-structural carbohydrates of *Carex lacustris*. These reach a peak in the summer in the shoots, with a minimum level in December. The rhizomes show a reverse trend. Such a transfer of carbohydrates from growing to storage tissue does not occur in *L. dortmanna*.

3. Leaf Production.

Leaf production was monitored in all four quadrats. In order to consider its relationship with other growth parameters and environmental variables it is necessary to estimate any significant differences between the quadrats. This was achieved using the Mann-Whitney test (Snedecor and Cochran, 1971). In each test two quadrats were compared for significant differences in production each month. Thus for nine months there are 54 tests. In all only one proved significant (at $P = 0.05$), and that was between quadrats on sites 3 and 4 in June. It is, therefore, thought reasonable to treat all the data as a whole, and this is presented in Figure 2.6. The most important feature of this is the apparent lack of the influence of the substrate on leaf production, there being no significant differences between sites 1 and 2.

An initial consideration of the production and loss of leaves in *L. dortmanna* shows two important points. Firstly production

Figure 2.4.

Seasonal changes in the soluble carbohydrate content of leaves (L) and roots (R) of *L. dortmanna* from site 1. Each point is a mean of ten determinations ± standard error.

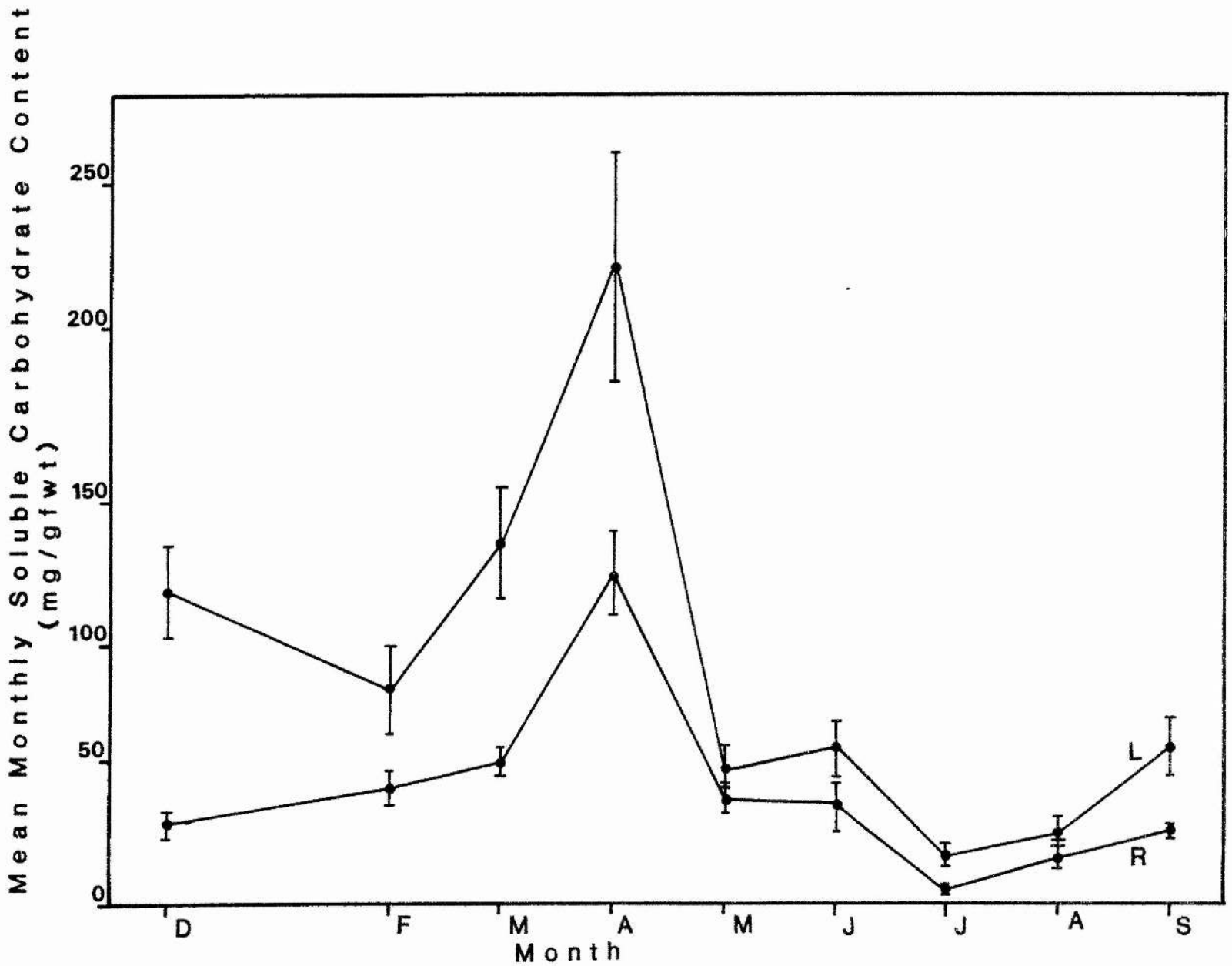

Mean Monthly Soluble Carbohydrate Content
(mg/gfwt)
250
200
150
100
50
0
D
F
M
A
M
J
J
A
S
Month
L
R

Figure 2.5.

Seasonal changes in the soluble carbohydrate content of leaves (L) and roots (R) of *L. dortmanna* from site 2. Each point is a mean of ten determinations ± standard error.

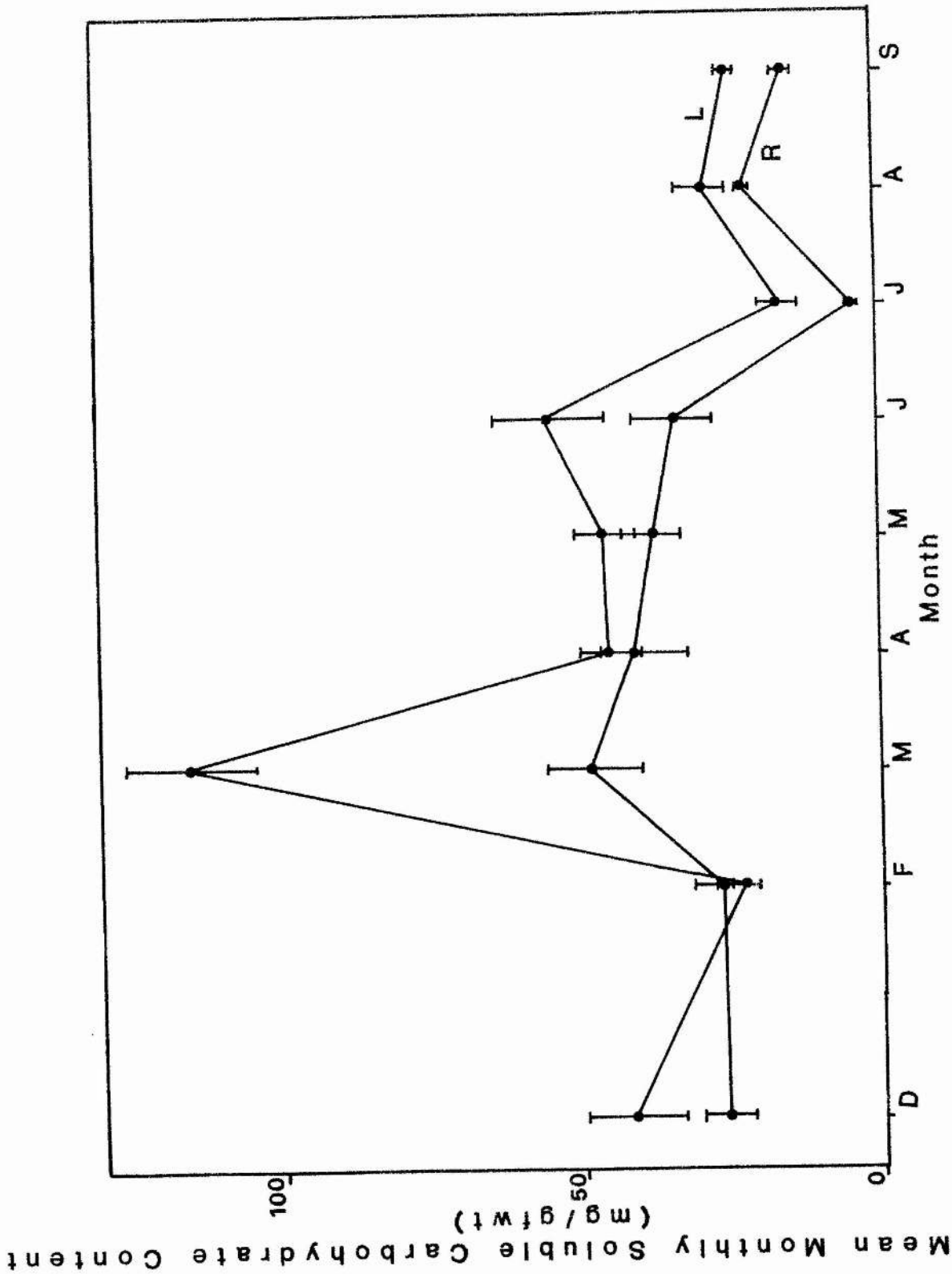
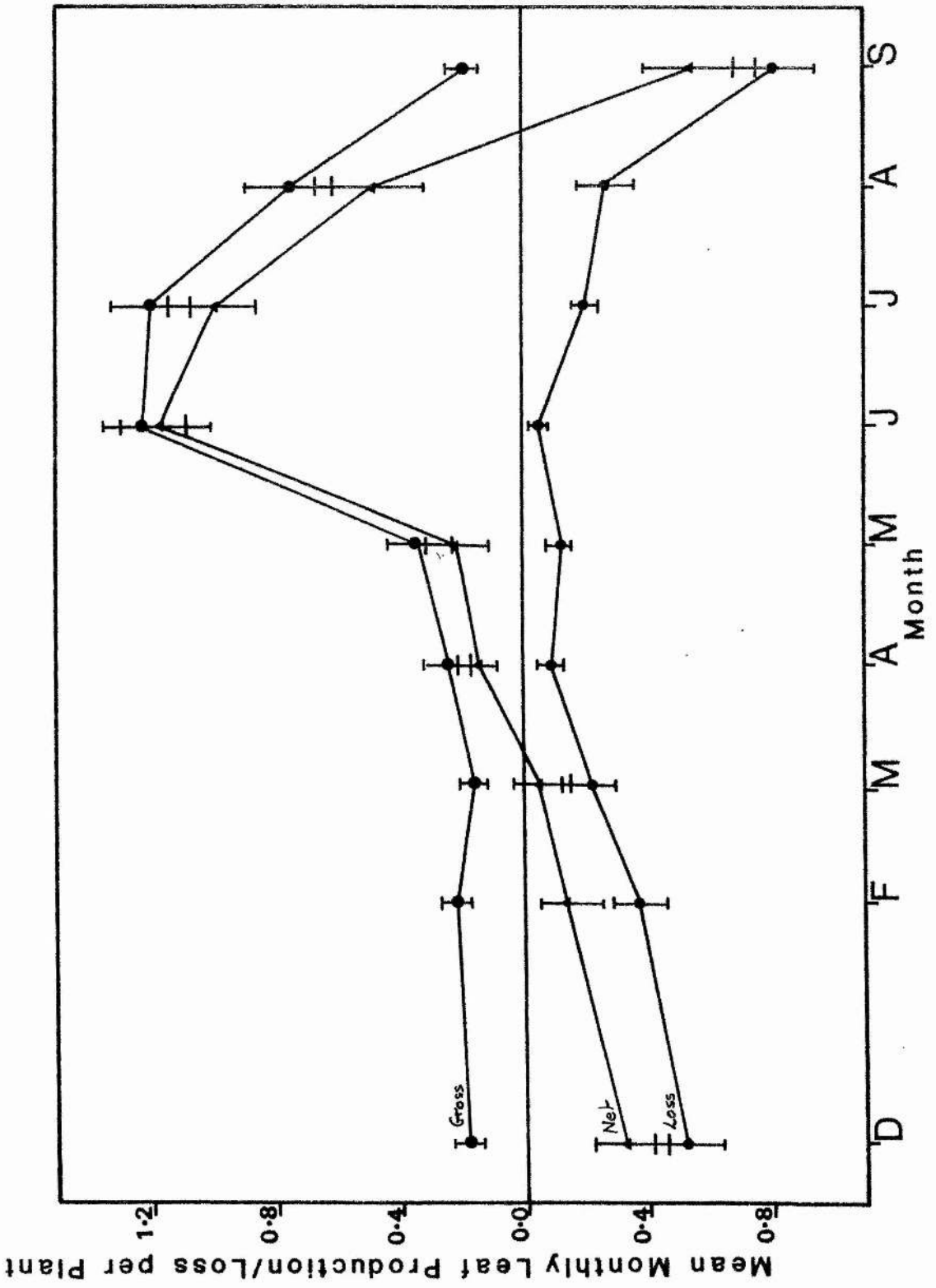

Mean Monthly Soluble Carbohydrate Content
(mg/gtwt)
100
50
0
D
F
M
A
M
J
J
A
S
Month
L
R

Figure 2.6.

Seasonal changes in gross and net leaf production and leaf loss. Each point is a mean of all study sites combined (n=45) ± standard error.


Mean Monthly Leaf Production/Loss per Plant
1·2
0·8
0·4
0·0
0·4
0·8
Gross
Net
Loss
D
F
M
A
M
J
J
A
S
Month

takes place for the whole year, and secondly loss occurs continually as well. This is in contrast to the results of Moeller (1978) and the observations of Aberg (1943). Moeller found no growth over the winter, and Aberg found an abrupt decrease in relative leaf length from the last autumnal leaves to the first spring leaves, so suggesting a cessation of growth over the winter. These studies were carried out, respectively, in New Hampshire and Sweden, which have much more severe winters than North-West Sutherland. Other Isoetids have also been found to cease growth over the winter. These are *I. lacustris* (Eriksson et al., 1974; Kansanen and Niem, 1974) and *L. uniflora* (Sand-Jensen and Søndergaard, 1978). Both of these results are from Scandinavia. Other submerged macrophytes, e.g. *Zostera marina* (Sand-Jensen and Borum, 1980), have been shown to grow continuously through the winter.

The main rise in leaf production does not take place till late spring and early summer. Sand-Jensen and Borum (1984) suggest that this may be due to a spring bloom in epiphytes reducing the amount of light available for growth, so that as the bloom disappears in the early summer, growth can resume. In Lochan-na-Thuill there are almost no epiphytes visible, so this is probably unimportant in this lochan. However, this can be tested by a consideration of the effects of irradiance on growth (see section 2.3.4). It should also be remembered that, e.g. in site 2, *L. dortmanna* can be shaded by other macrophytes that have grown in the spring. In this case the early summer growth would not appear to be an advantage. The peak in production and biomass is in July. This is similar to Moeller's (1978) and for other macrophytes, e.g. *L. uniflora* (Sand-Jensen and Søndergaard, 1979) and *Elodea canadensis* (Pokorny et al., 1984).

Moeller (1978) uses an expression for leaf turnover:

$$t \text{ (turnover)} = \frac{\text{number of new leaves produced per year}}{\text{number of leaves at the time of maximum biomass}}$$

This is also known as the Production/Biomass or P/B ratio, as is commonly used in macrophyte studies. Moeller's figure for *L. dortmanna* is 0.69 and Westlake (1982) in his review quotes this as the lowest for a macrophyte, with 3.8 being the highest for *Myriophyllum spicatum*. In this study the P/B ratio (all quadrats ± S.E.) is 0.58 ± 0.03.

This expression, though convenient for comparing many diverse species, has certain restrictions. It depends on the timing of leaf gains and losses, not on the quantity lost and gained. Therefore, if during one year:

Plant A has 5 leaves, gains 5 and then loses 5 P/B = 0.50

Plant B has 5 leaves, gains 1 and then loses 1 for a total of 5 gains and losses P/B = 0.83

Plant C has 5 leaves, loses 1 and then gains 1 for a total of 5 losses and gains P/B = 1.00.

In all of these cases the effect on the plant is the same, yet the P/B is very different. Turnover is a consideration of the time taken to replace the original biomass. Production is involved, but this also contributes to new biomass besides replacing old biomass. We can, therefore, distinguish a number of indices:

Turnover index for one year $t = \frac{n_L}{n_i} =$ Number of leaves lost / Initial number of leaves

Production index $P = \frac{n_p}{n_i} =$ Number of leaves produced

Growth index $G = \frac{n_p - n_i}{n_i}$ - a net figure

Table 2.5 gives the indices for each of the quadrats. It should be noted that the two sites which differ most are sites 1 and 3, which look ecologically similar and have morphologically similar plants.

The most useful expression of growth, however, is some measure of relative growth rate. This compares the number of leaves produced each month with the number at the beginning of the month. In this study the following expression is used:

$$R_n = \frac{\text{Log}N_m - \text{Log}N_o}{\text{Log}N_o}$$

N_m = Mean leaf number per plant at the end of the month

N_o = Mean leaf number per plant at the start of the month

In some instances it is usual to use this expression:

$$R_n = \frac{\text{Log}N_m - \text{Log}N_o}{\frac{1}{2}(\text{Log}N_o + \text{Log}N_m)}$$

R_n is, therefore, a measure of relative leaf growth rate (RALGR).

This takes account of the contribution of biomass produced during a period to the contribution of further biomass in that period, i.e. it is an "instantaneous" figure. *L. dortmanna* produces very few leaves each month, and, except in June and July, they take more than one month to expand and mature. As growth is steady (not proceeding in discontinuous "spurts") such a figure is irrelevant. Table 2.6 contains the values for R_n for each site and for all the sites combined.

4. Environmental Parameters.

The results are presented in Figures 2.7 to 2.9. Water level changes were never very great during the year, and at no time were any of the shallow-water plants exposed. Temperatures remained mild throughout the winter and rose to a maximum of 24°C in the summer. It must be remembered that the thermometer was positioned at 50 cm depth. A site such as site 2 which is shallow (<40 cm) has a dark sediment and is sheltered would almost certainly have recorded higher

Table 2.5.

The Turnover (t), Production (P) and Growth (G) indices for each of the study sites and all sites combined.

± Standard Error.

Site	n	t	P	G
1	11	0.40 ± 0.14	1.34 ± 0.30	0.94 ± 0.42
2	12	0.77 ± 0.13	1.33 ± 0.12	0.42 ± 0.14
3	12	0.56 ± 0.13	0.86 ± 0.09	0.25 ± 0.16
4	15	0.78 ± 0.15	1.34 ± 0.29	0.39 ± 0.17
Total	50	0.63 ± 0.16	1.22 ± 0.20	0.50 ± 0.22

Table 2.6.

The Relative Growth Rate (Rn) and the mean leaf number per plant (N) for each of the study sites and for all of the sites combined.

Month	Site 1		Site 2		Site 3		Site 4		Site 5	
	N	Rn	N	Rn	N	Rn	N	Rn	N	Rn
N	4.44	-	4.57	-	3.90	-	5.25	-	4.64	-
D	4.22	-0.046	4.43	-0.015	3.80	-0.017	4.63	-0.069	4.31	-0.060
F	4.22	0.016	4.38	-0.015	3.60	-0.034	4.25	-0.060	4.12	-0.016
M	4.00	-0.048	4.75	-0.063	3.50	-0.036	4.06	-0.032	4.04	-0.016
A	4.27	0.050	4.88	0.015	3.70	0.056	4.06	0.000	4.18	0.016
M	4.18	-0.016	5.13	0.029	4.00	0.053	4.25	0.030	4.33	0.032
J	5.36	0.177	6.50	0.141	4.90	0.150	5.69	0.206	5.58	0.172
J	6.55	0.123	7.00	0.037	5.90	0.116	6.75	0.092	6.56	0.093
A	6.09	-0.049	7.00	0.000	6.70	0.078	7.63	0.060	6.93	0.024
S	5.82	-0.026	6.13	-0.000	5.60	-0.096	7.25	-0.023	6.33	-0.048

Figure 2.7.

Seasonal changes in mean total daily irradiance for each week, starting at the beginning of February 1983. Each point is the mean for the week (n=7) with the maximum and minimum.

Mean Daily Total Solar Irradiance (/MJ/m²)÷3·6
0·0
2·0
4·0
6·0
0
10
20
30
40
50
Time (Weeks)

Figure 2.8.

Seasonal changes in mean daily temperatures for each week, starting in the last week of November 1982. Each point is the mean of the mean daily temperatures for the week (n=7) with the maximum and minimum temperatures reached at any point in that week.

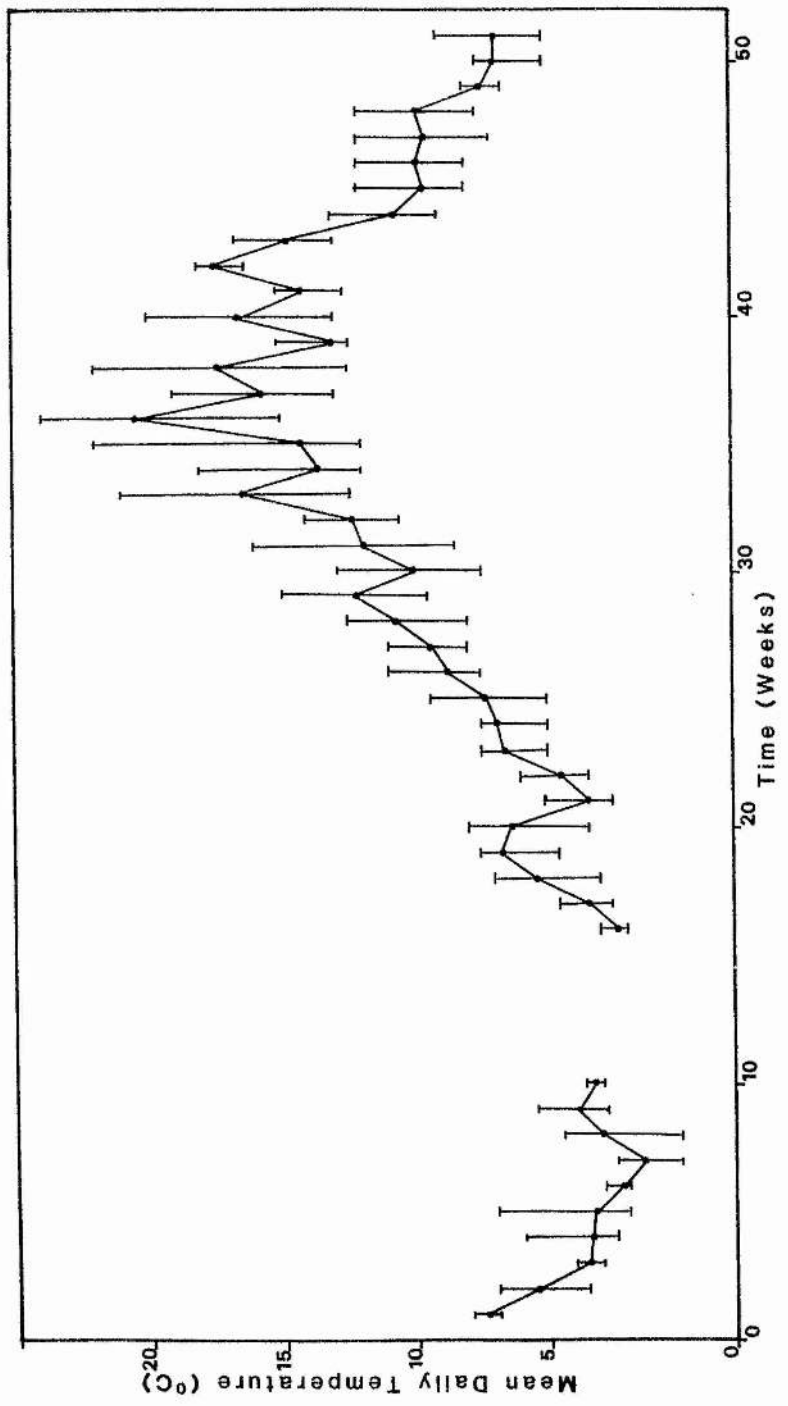
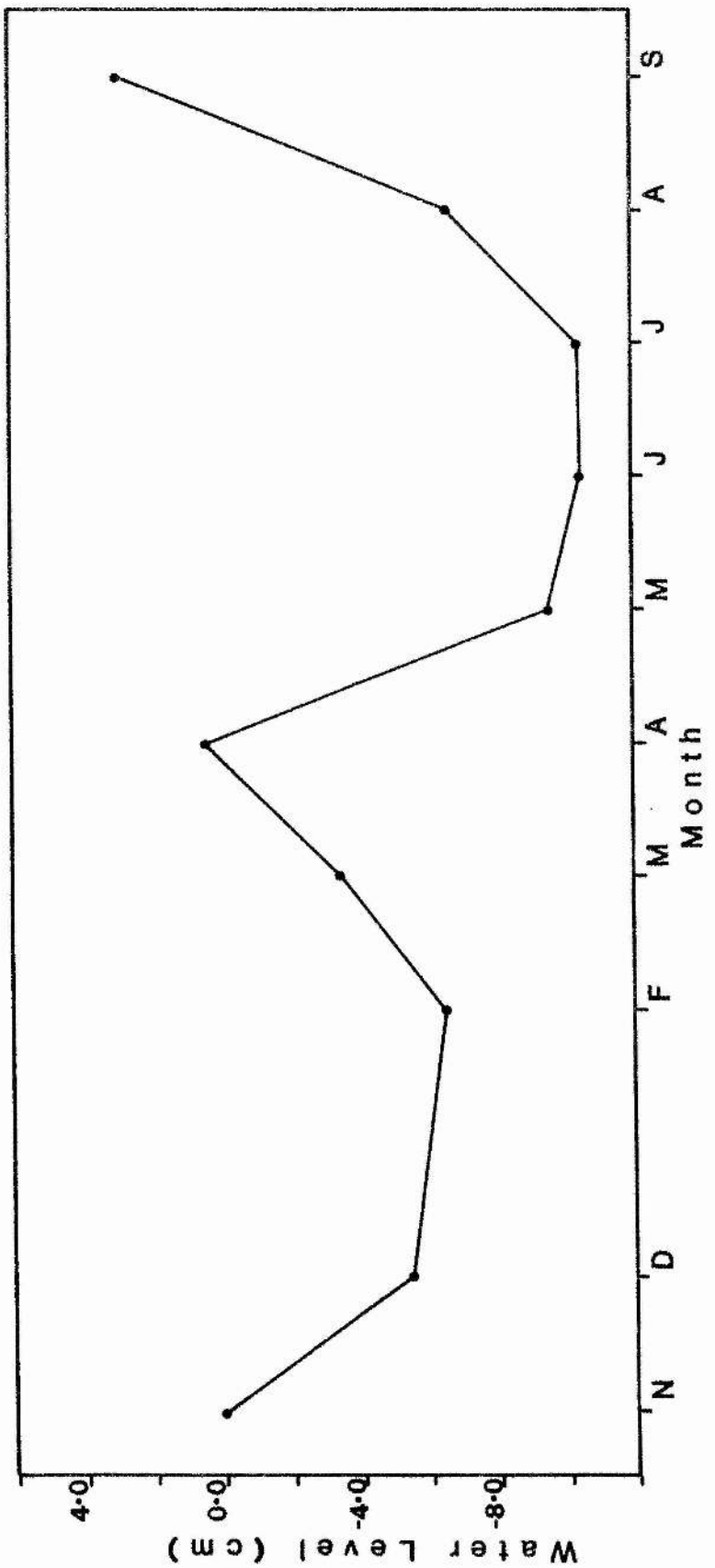

Mean Daily Temperature (°C)
0
5
10
15
20
Time (Weeks)
0
10
20
30
40
50

Figure 2.9.

Seasonal changes in the water level of Lochan-na-Thuill. Each point is a spot reading for that month and is compared to an arbitrary zero for November 1982.


Water level (cm)
4·0
0·0
-4·0
-8·0
N
D
F
M
A
M
J
J
A
S
Month

temperatures.

2.3.3. Further Analysis and Discussion - Inter-relation of Growth and Environmental Parameters.

1. The Growth Parameters.

A consideration of the seasonal changes in soluble carbohydrate content, photosynthesis and leaf production reveal how soluble carbohydrate content produces an early spring peak, which is subsequently followed by a peak in photosynthesis and leaf production (Figure 2.10 and 2.11). Figure 2.12 describes the relationship between leaf soluble carbohydrate and leaf production. This is a typical reverse-J curve, such a relationship being characteristic of a number of macroalgae (Dring, 1982). The decline in soluble carbohydrate during growth could be due merely to a dilution of a relatively constant amount of carbohydrate in a plant as it grows. As the content is expressed as a per fresh weight basis, a dilution will register as a reduction. However, the relative decline in soluble carbohydrate is many times greater than the relative increase in leaf number. It is thus probable that growth also uses the carbohydrate present in high levels in early spring.

2. The Environmental Parameters.

Figure 2.13 shows the relationship between the mean daily solar irradiance each month and the mean temperature for that month. The correlation between the two parameters is significant at $p = 0.05$ ($r=0.711$), and a regression line can be fitted ($Temp = 1.39 \times 2.88I$). However, the points of graph have been plotted as a time sequence. This reveals more information, as it shows the thermal lag that exists in the lochan. The lochan warms up slowly during the spring, and retains heat during the autumn. Any consideration of plant growth must, therefore, recognise the fact that the changes in irradiance

Figure 2.10.

Seasonal changes in soluble leaf carbohydrate content (●) for site 2 and mean monthly gross leaf production (▲). This illustrates how the peak in soluble carbohydrate precedes that in leaf production.

Mean Monthly Leaf Soluble Carbohydrate Content (mg/g)
150
100
50
0
Mean Monthly Gross Leaf Production per Plant
1·5
1·0
0·5
0·0
D
J
F
M
A
M
J
J
A
S
Month

Figure 2.11.

Seasonal changes in soluble leaf carbohydrate content (●) for site 2 and mean monthly gross photosynthetic rate (▲). This illustrates how the peak in soluble carbohydrate precedes that in photosynthesis.

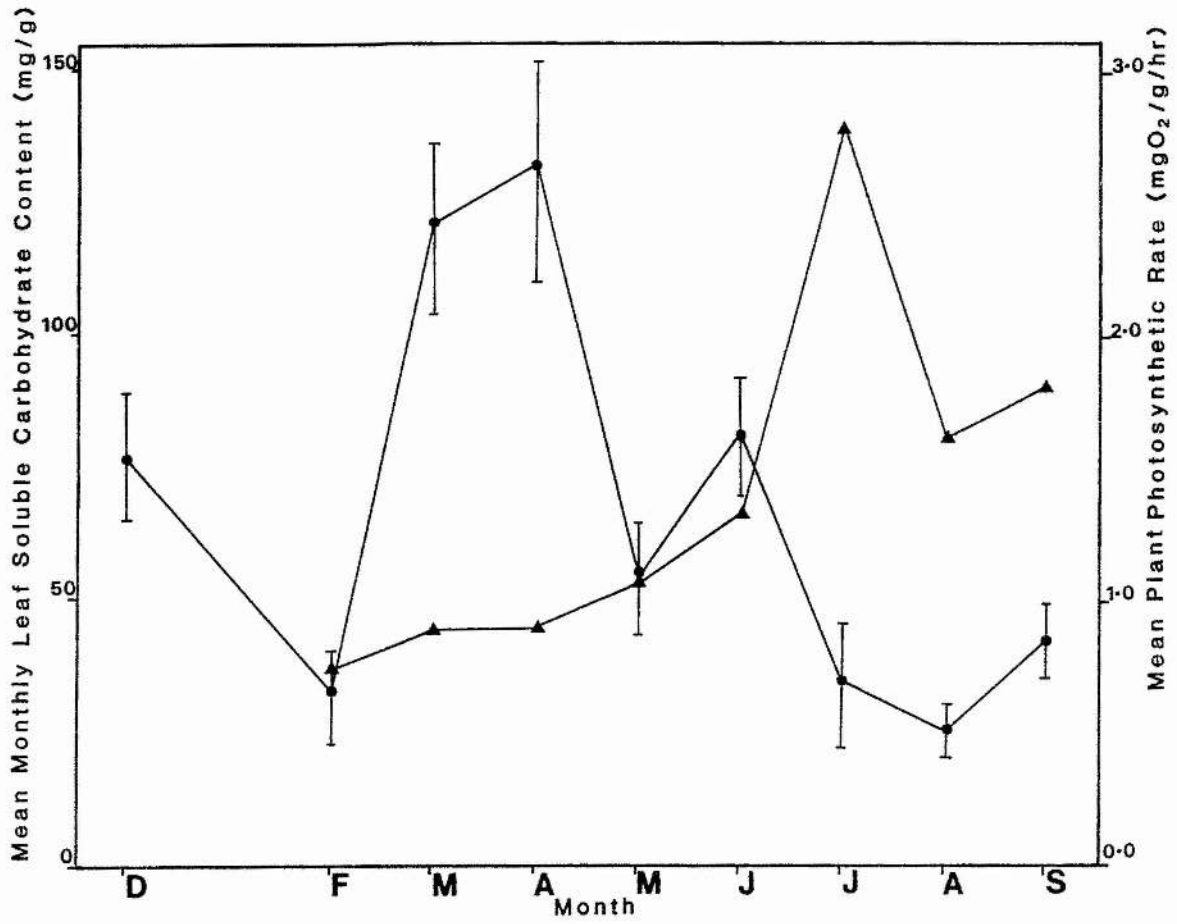

Mean Monthly Leaf Soluble Carbohydrate Content (mg/g)
150
100
50
0
Mean Plant Photosynthetic Rate ($mgO_2/g/hr$)
3·0
2·0
1·0
0·0
D
F
M
A
M
J
J
A
S
Month

Figure 2.12.

This figure shows the relationship between mean monthly leaf soluble carbohydrate content (both sites 1 and 2) and gross leaf production for all sites.

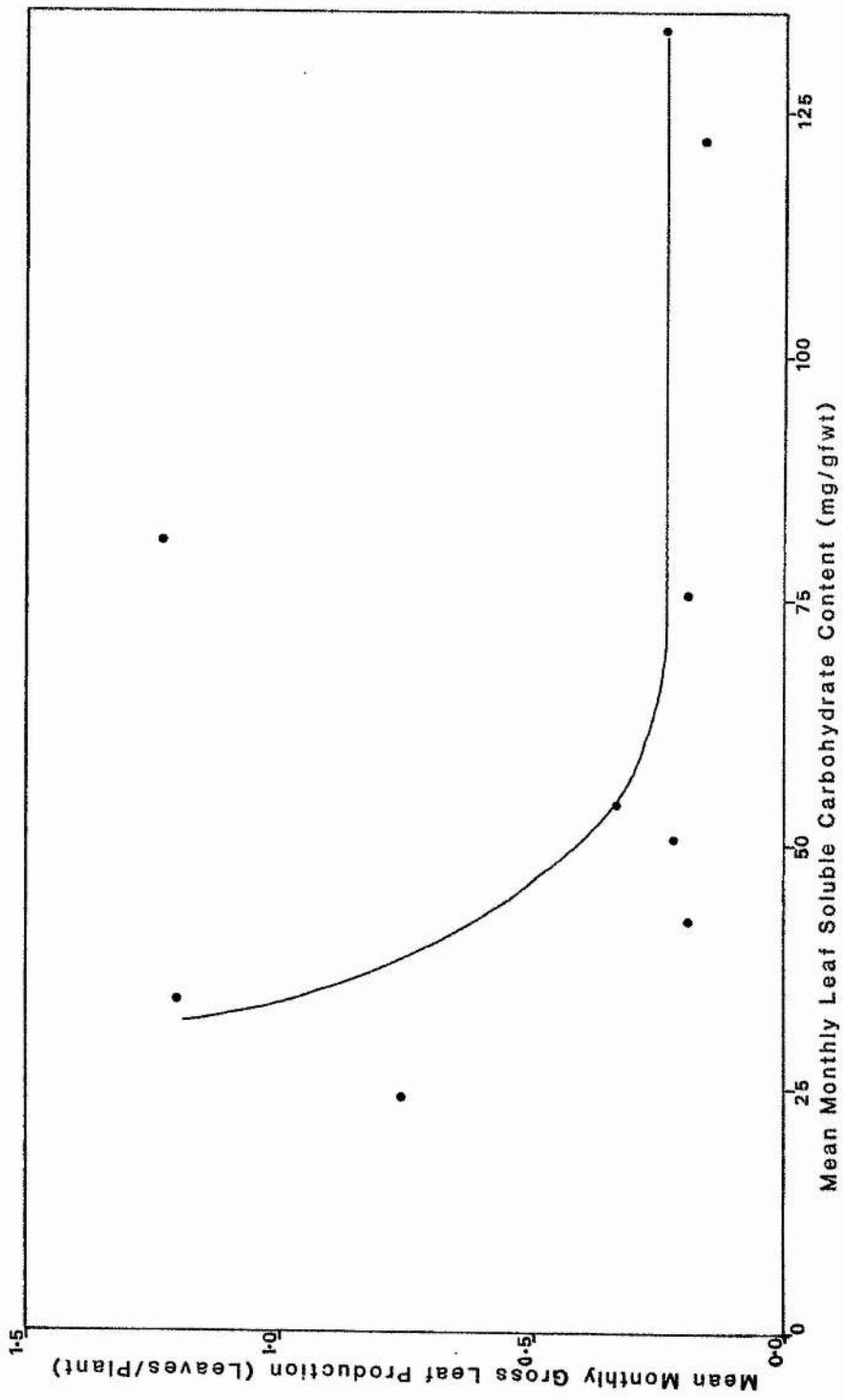
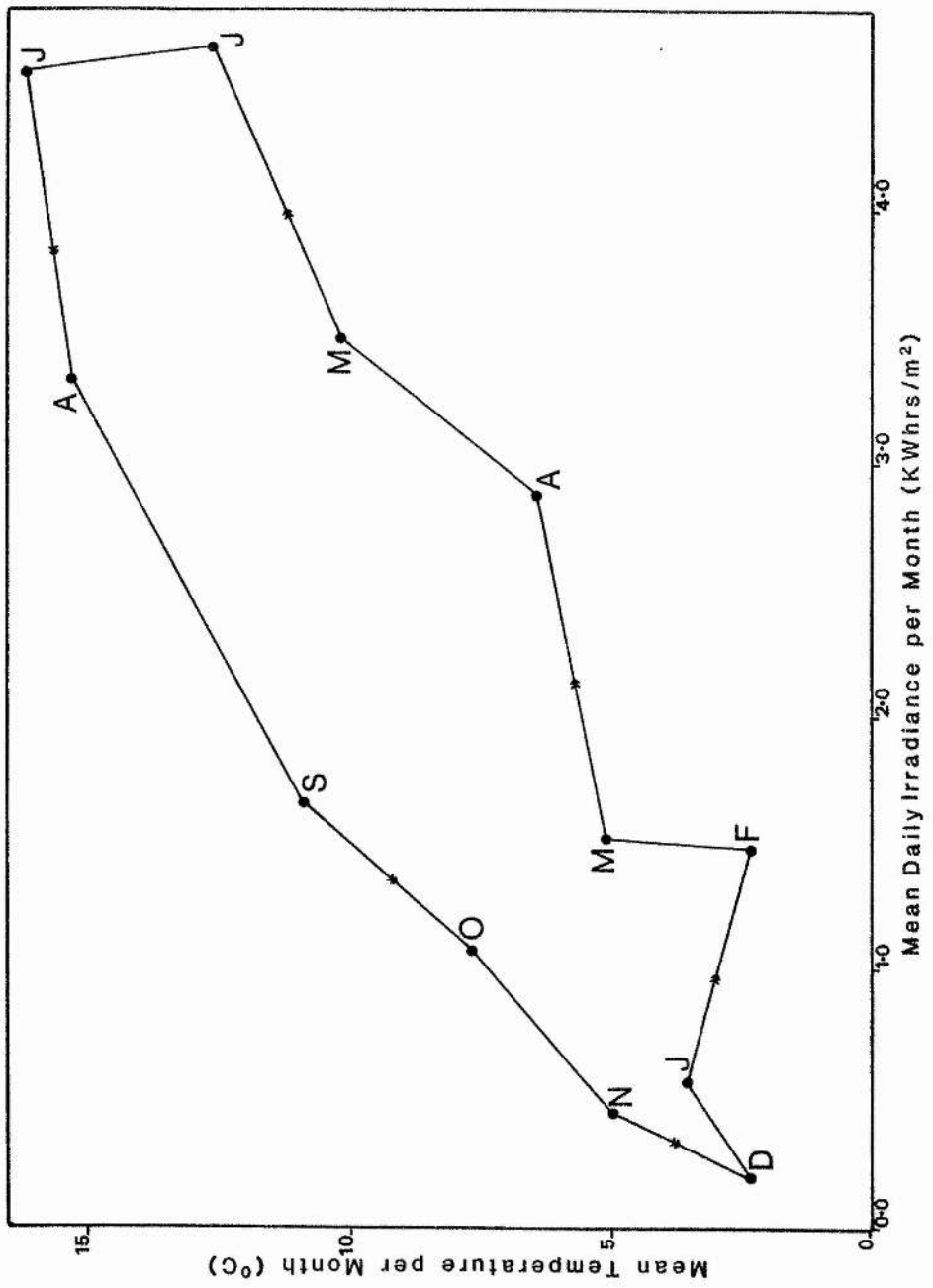

Mean Monthly Gross Leaf Production (Leaves/Plant)
0·0
0·5
1·0
1·5
0
25
50
75
100
125
Mean Monthly Leaf Soluble Carbohydrate Content (mg/gfwt)

Figure 2.13.

The relationship between mean monthly temperature and the mean total daily irradiance for that month. The figure illustrates the relationship by drawing out the temporal sequence.


Mean Temperature per Month (°C)
15
10
5
0
0·0
1·0
2·0
3·0
4·0
Mean Daily Irradiance per Month (KWhrs/m²)
J
J
A
M
A
S
M
F
O
J
N
D

and temperature are not in phase with each other.

3. Growth and the Environment.

Relative growth rate (R_n) is positively correlated with both temperature and irradiance. The correlation coefficients and regression equations are shown in Table 2.7 and Figures 2.14 and 2.15. It can be seen that only the correlation between R_n and irradiance is statistically significant. Table 2.7 also contains a linear multiple regression analysis of R_n and temperature and irradiance. This seeks to determine the relative contribution that the two environmental parameters make to R_n, recognising that temperature is dependent on irradiance, and not the reverse. From this 76.1% of the variation in R_n can be explained by changes in irradiance and only 5.2% by changes in temperature. Wium-Andersen and Borum (1984) performed a similar analysis with *Zostera marina*. They found that 75% of the variation in leaf growth could be explained by changes in irradiance, and 6% by changes in temperature. They did not take continual temperature recordings, but used "spot" readings while sampling growth. They consider this supports the suggestions of Harrison and Mann (1975), Sand-Jensen (1975) and Jacobs (1979) that light is more important than temperature in controlling growth, and thus refuting the opposite assertion of Setchell (1929). This is thought to be reasonable in that leaf area index is important in forming production, and this has been shown to be controlled by light (Spence and Chrystal, 1970b). Solander (1982) studied the production of four species of macrophytes on the edge of their ranges in sub-arctic Northern Sweden (*Carex rostrata*, *Equisetum fluviatile*, *Isoetes echinospora*, and *Sparganium* sp.). They find a rough parallel between the variations in summer temperature and biomass production for a five-year period. They, therefore, consider temperature to be important in controlling growth. As temperature and light are inter-related and no measurement of

Table 2.7.

The Results of the regression analysis for Rn and Irradiance and Temperature.

1. Rn against Temperature

The Regression Equation is:

$$Rn = -0.0428 + 0.00759T \ (°C)$$

Standard Deviations

Coefficient	Standard Deviation of Coefficient
-0.0428	0.05379
0.00759T	0.004933

R^2 = 0.163 adjusted for degree of freedom (D of F).

Analysis of Variance

Due to:	D of F	Sum of Squares
Regression	1	0.009796
Residual	6	0.024853
Total	7	0.034649

2. Rn against Irradiance

The Regression Equation is:

$$Rn = -0.1118 + 0.0488I \ (KWhrm^{-2})$$

Standard Deviations

Coefficient	Standard Deviation of Coefficient
-0.1118	0.0304
0.0488I	0.0095

R^2 = 0.782 adjusted for degree of freedom (D of F).

Analysis of Variance

Due to:	D of F	Sum of Squares
Regression	1	0.028174
Residual	6	0.006475
Total	7	0.034649

Table 2.7 (continued)

3. Temperature against Irradiance

The Regression Equation is:

$$T = 1.39 + 2.877I$$

Standard Deviations

Coefficient	Standard Deviation of Coefficient
1.394	3.220
2.877	1.009

R^2 = 0.505 adjusted for degree of freedom (D of F).

Analysis of Variance

Due to:	D of F	Sum of Squares
Regression	1	97.92
Residual	6	72.29
Total	7	170.21

4. Rn against Temperature and Irradiance

The Regression Equation is:

$$Rn = -0.105 + 0.0635I - 0.00511T$$

Standard Deviations

Coefficient	Standard Deviation of Coefficient
-0.105	0.0285
0.0635I	0.0135
-0.00511	0.00356

R^2 = 0.815 adjusted for degree of freedom (D of F).

Analysis of Variance

Due to:	D of F	Sum of Squares
Regression	2	0.030065
Residual	5	0.004584
Total	7	0.034649

Table 2.7 (continued)

Further analysis of variance, sum of squares explained by each variable.

Due to:	D of F	Sum of Squares
Regression	2	0.030065
Irradiance	1	0.028174
Temperature	1	0.001891

Figure 2.14.

The relationship between the relative growth rate (Rn) for each month and the mean total daily irradiance for that month.

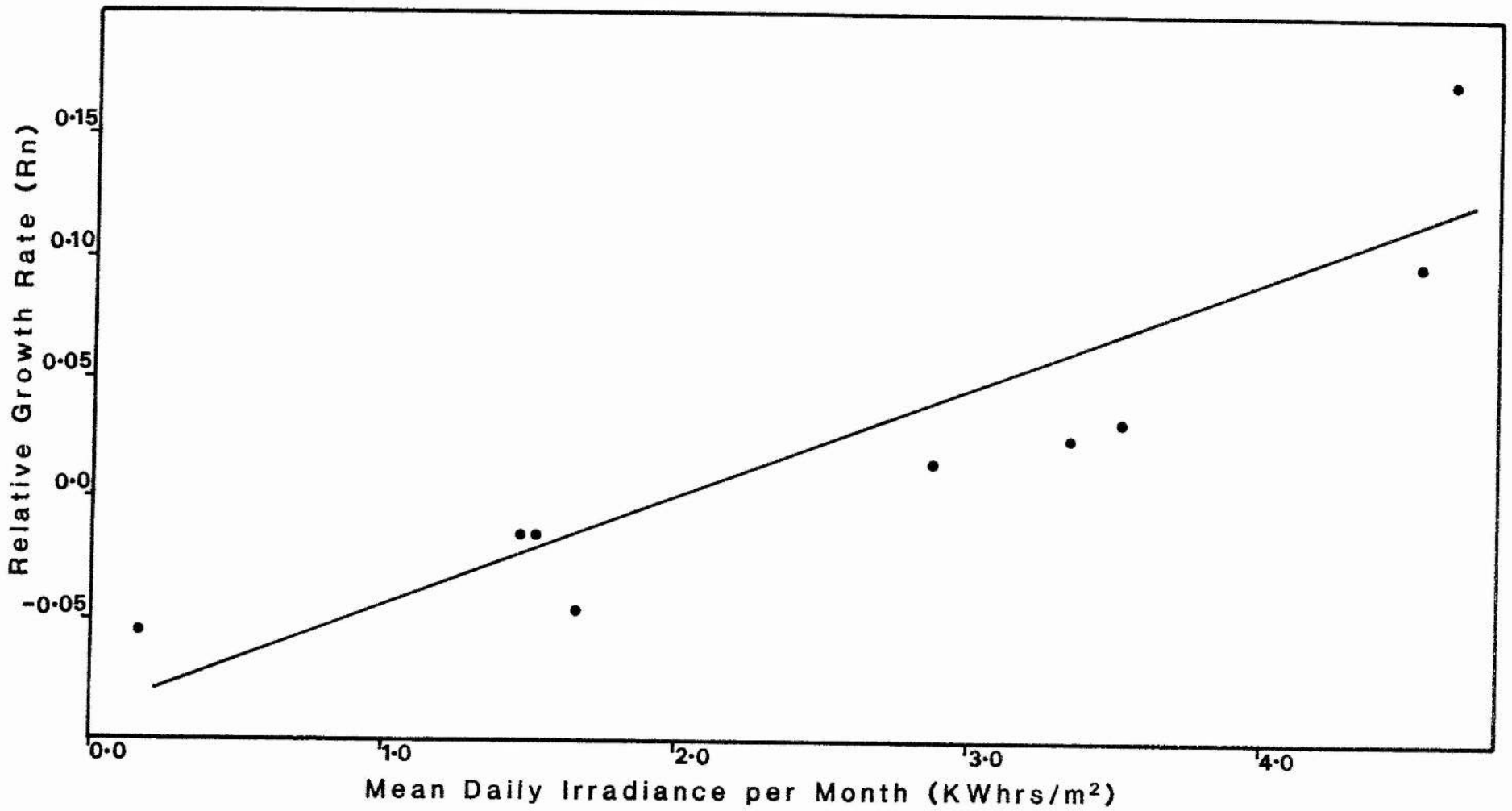
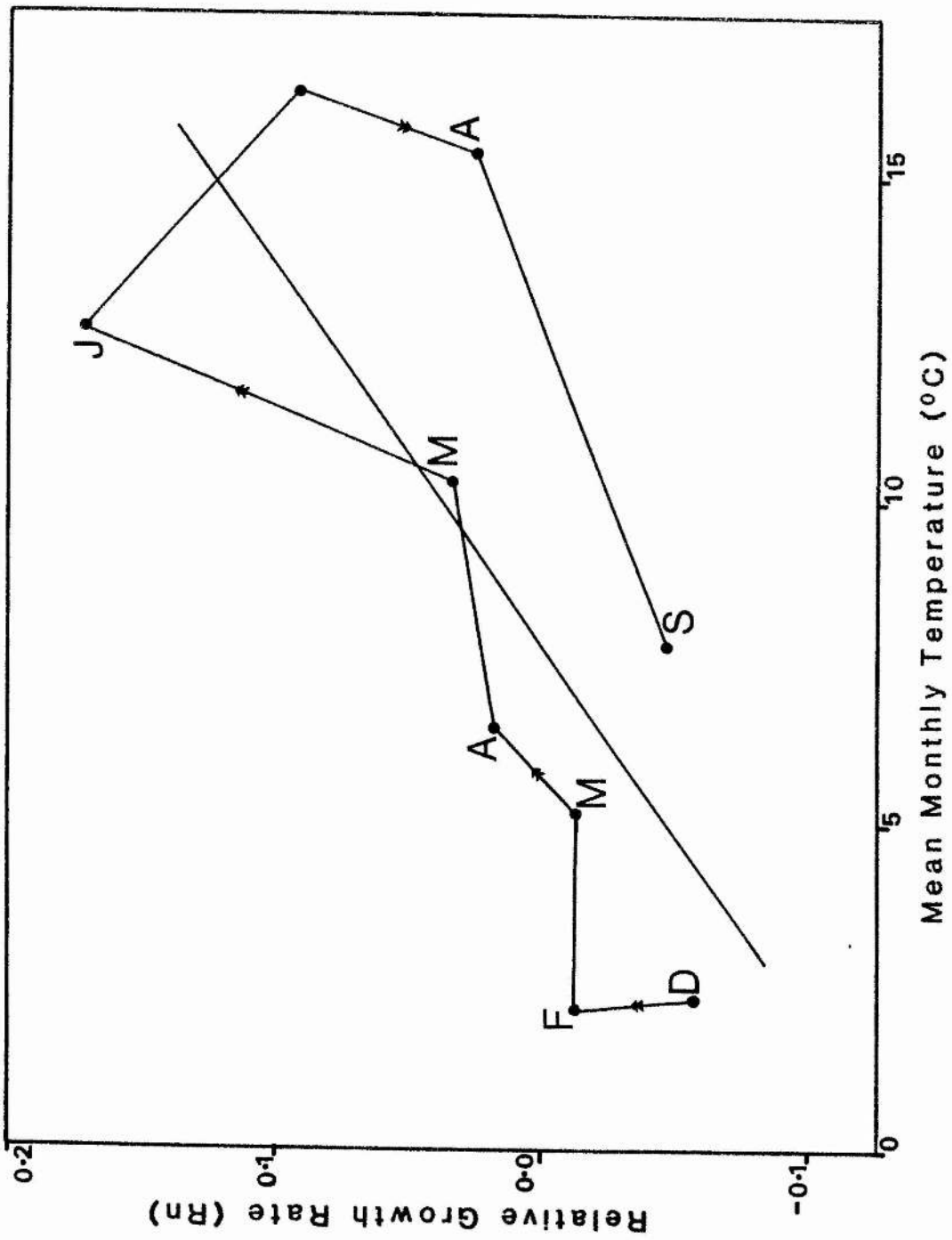

Relative Growth Rate (Rn)
0·15
0·10
0·05
0·0
-0·05
0·0
1·0
2·0
3·0
4·0
Mean Daily Irradiance per Month (KWhrs/m²)

Figure 2.15.

The relationship between the relative growth rate (Rn) for each month and the mean temperature for that month. The figure shows both the temporal sequence and the regression line.


Relative Growth Rate (Rn)
0·2
0·1
0·0
-0·1
0
5
10
15
Mean Monthly Temperature (°C)
J
A
M
A
M
F
D
S

irradiance was made, such a direct relationship may not be caused by temperature.

4. Soluble Carbohydrate and the Environment.

The mean leaf soluble carbohydrate content for sites 1 and 2 for each month has a small negative correlation for both irradiance ($r=-0.20$) and temperature ($r=-0.53$) for those months. However, in both cases these are not significant. The relationship is probably best explained by the reverse -J type relationship of soluble carbohydrate with leaf production (section 2.3.2) and the strong positive correlation of the latter with both environmental variables (see above).

5. Photosynthesis and Temperature.

Figure 2.16 shows the relationship between mean monthly photosynthetic rate and the temperature at which it was taken. There is a strong, significant correlation ($r=0.83$). It is, of course, not possible to correlate photosynthesis and irradiance, as all photosynthetic measurements were made at saturating irradiance.

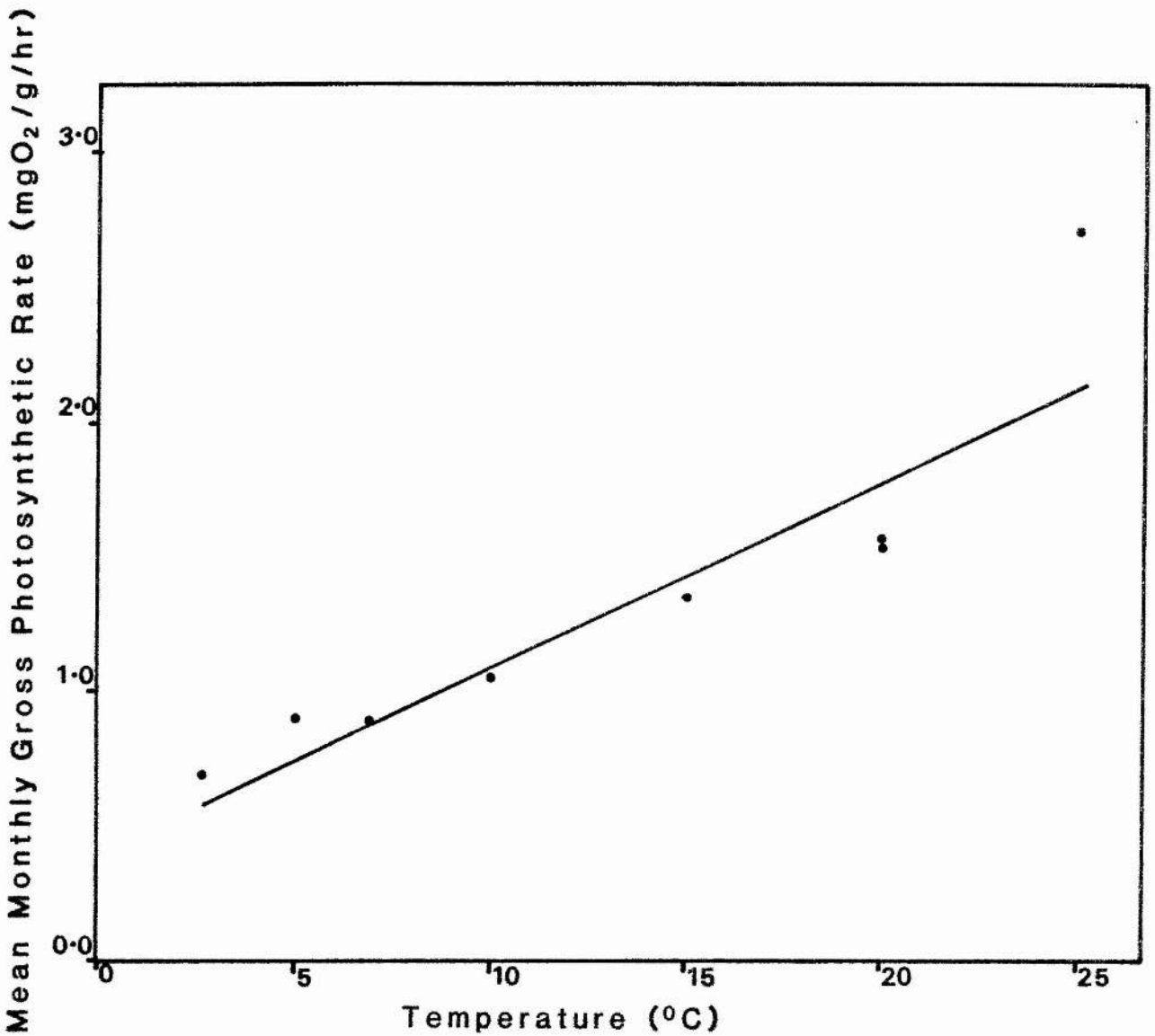
2.4. Growth under Controlled Conditions

2.4.1. Introduction

In section 2.3.3 it was calculated that 76.1% of the seasonal variation in the relative growth rate (R_n) could be explained by changes in measured irradiance, whereas only 5.2% of the variation could be explained by changes in measured temperature. As temperature is also dependent upon irradiance the exact contribution each makes to the growth of *L. dortmanna* in the field is necessarily complex in the data collected. This section separates the two environmental variables, by growing plants in controlled conditions such that one environmental factor is kept constant and the other is varied. This will distinguish experimentally the relative contribution of each to

Figure 2.16.

The relationship between the mean monthly gross photosynthetic rate and the temperature at which the measurements were made.


Mean Monthly Gross Photosynthetic Rate ($mgO_2/g/hr$)
3·0
2·0
1·0
0·0
0
5
10
15
20
25
Temperature (°C)

growth.

2.4.2. Methods

Plants were collected in March 1985 from Loch na Craige, Tayside. In order to ensure uniform substrate conditions, all plants were washed free of soil and epiphytes and potted in 5 cm diameter pots in a 50:50 sand/peat mixture with a small constant amount of added fertilizer (composition is in Table 2.8). The plants were allowed to "settle" for two weeks in dim light in a greenhouse before being transferred to the growth room. Plants were grown at two temperatures (10°C and 20°C) and at three different light levels at each temperature (70, 35 and 17.5 $\mu Em^{-2}sec^{-1}$ PAR). The plants were grown in large tanks, in which the water was continually stirred and aerated. Light was provided by white fluorescent tubes and measured using a Macam quantum photometer. All reductions in light were achieved by placing a neutral density filter, muslin, over the tank. The number of leaves on each plant was counted before and after the experiment, which lasted 60 days. Ten plants were used in each treatment. The leaves of all the plants were of approximately equal size. The period for growth was not long enough to cause any changes in growth form that may have been brought about by shading.

2.4.3. Results and Discussion

The results presented in Table 2.9 with the relative growth rate (R_n) calculated on leaf production as previously (section 2.3.2). The study provides four results of the effect of increasing temperature from 10 to 20°C and three of the effect of doubling PAR irradiance. This increase in temperature causes an increase of 1.94 ± 0.29 (± S.E.) in R_n, and for irradiance, doubling causes an increase of 1.28 ± 0.13 in R_n. It thus seems that this increase in temperature has a greater effect on R_n. However, under natural conditions the mean monthly

Table 2.8

The composition of the general fertilizer used for study of the growth of *Lobelia dortmanna* under controlled growth conditions.

Nutrient	g nutrient/100 g fertilizer
Nitrogen	5.300
Phosphorus total	3.300
" soluble	3.000
" insoluble	0.300
Potassium	0.300
Boron	0.020
Copper	0.040
Iron	0.250
Manganese	0.115
Magnesium	0.300
Molybdenum	0.001

Table 2.9.

The relative growth rate (Rn) of *L. dortmanna* under controlled conditions.

Irradiance (PAR) ($\mu Em^{-2}s^{-1}$)	Rn 10°C ± S.E. n = 10	Rn 20°C ± S.E. n = 10
70	0.213 ± 0.067	0.379 ± 0.064
35	0.173 ± 0.069	0.266 ± 0.084
17.5	0.113 ± 0.026	0.282 ± 0.050

temperature extremes are 2.2°C and 21.5°C, whereas the highest mean monthly irradiance is 26.13 times the lowest. This increase in temperature would increase R_n by 668% and the rise in irradiance would increase R_n by 33.45%. The relative contribution of temperature is, therefore, 12.96% and irradiance 72.07%. This would suggest that temperature may be more important than predicted in the field analysis of the seasonal growth measurements. It should be remembered, however, that the theoretical increases in R_n are not realised in the field. This is probably due in part to adverse environmental factors, such as wave action, that do not occur under controlled conditions, and the fact that fertilizer was added in the experiment. Such an extrapolation of laboratory Q_{10} values to the field conditions over wider ranges of conditions is open to considerable error.

2.5. General Discussion

The growth of aquatic macrophytes can be affected by many environmental parameters. The four most notable are light, temperature, nutrient levels and exposure (Spence, 1982). This study has concentrated on the first two factors. The study sites in Lochan-na-Thuill do represent a range of nutrient levels (at least with respect to sediment interstitial soluble phosphate) and exposure. Little difference is found in growth between the quadrats. Phosphorus is known to be probably the most important limiting nutrient in oligotrophic lakes (Goldman and Horne, 1983). Gerloff and Krombholz (1966) studied thirteen species of macrophytes and in eight out of nine lakes found the phosphorus was more likely to limit growth than nitrogen. Moeller (1978) studied seasonal variation in tissue nutrient levels in *L. dortmanna*, suggesting that summer growth and flowering severely depletes both phosphorus and nitrogen, but that winter losses of biomass affect phosphorus levels more than nitrogen. *L. dortmanna* possesses mycorrhizae that, in the

absence of root hairs, probably aid nutrient uptake (Søndergaard and Laegaard, 1977 and Farmer, 1985).

The classic growth pattern of microalgae consists of a spring bloom in production. This is not continued into the summer, not because of lack of light, but of nutrients. Their growth is, therefore, not correlated to light conditions. *L. dortmanna* does not show this. As the light increases, so does its growth. This suggests that neither nutrients nor carbon supply are limiting. This would confirm the finding of no greater increase in growth rate for site 2, which has a higher inorganic soluble phosphate level in the sediment.

The overall importance of light in influencing the seasonal growth of *L. dortmanna* must be stressed. This control is a direct consequence of the production of photosynthate. In the next chapter the effect of light on a classical photomorphogenic response (seed germination) will be considered. In order to flower and produce seeds any plant will require adequate levels of photosynthate. In this way light, via chlorophyll, is controlling some of the fundamental growth processes in *L. dortmanna*.

CHAPTER THREE

REPRODUCTION, GERMINATION AND ZONATION

Chapter 3. Reproduction, Germination and Zonation

3.1. Introduction

In many aquatic plants growth and vegetative reproduction are difficult to distinguish. Most macrophytes reproduce predominantly by vegetative means (Sculthorpe, 1967). In many cases this occurs by the production of rhizomes or stolons that can be considered as part of a continuum of growth of the plant's organs. In other cases macrophytes may vegetatively reproduce by means of turions. This has been considered to play an ecologically similar role to seeds, especially in their dormancy and ability to disperse (Bartley and Spence, 1985).

Few macrophytes reproduce by seeds. In some instances, e.g. the Nymphaceae, plants may exhibit both extensive seed and vegetative reproduction. Aquatic annuals, e.g. *Elatine* spp. and *Subularia aquatica*, rely heavily on seed production. However, few perennials rely so extensively on this. An exception appears to be *Lobelia dortmanna*. This species produces many seeds and shows little vegetative reproduction.

Little work has been done on the ecological significance of seed germination for aquatic macrophytes. The germination of some species' seeds has been studied in the laboratory, but few attempts have been made to interpret these results in relation to the natural environment. The most important hypothesis that has been made is that the requirements for seed germination of a species may control the zonation of that species. In particular, Stross (1981) has suggested that the lower depth limits of the macroalga *Nitella flexilis* may be set by the amount of red light available for red-light requiring processes such as spore germination or sporeling differentiation. Spence (1982) suggested that *L. dortmanna* may be

limited to shallow water because its seeds may have a high light requirement for germination.

This chapter seeks to test this hypothesis. It will consider the entire reproductive process in *L. dortmanna* from flower production to seedling establishment. The importance of seed production and dispersal as a reproductive strategy for the distribution of *L. dortmanna* is discussed. Seed germination is controlled by many environmental influences (Bewley and Black, 1982). In this experimental study the following are considered - cold stratification, light quantity, light quality, temperature and anoxia. Finally, the natural environment is considered in detail in order to predict from laboratory findings where in any loch the seeds of *L. dortmanna* could, in theory, germinate. This enables a conclusion to be reached as to whether seed germination may, in this species, control zonation.

3.2. Flowering and Seed Production

3.2.1. The Seasonal Production of Flowers

Flowering in *L. dortmanna* begins with the initiation of a flowering stalk from the apex at the centre of the leaf rosette. The internodes expand, producing a stem, usually, 25 cm-1 m in length. It has small leaves, the flowers being borne in their axils. Examination reveals that the stems and leaves both contain chlorophyll and have stomata.

The standard floras (Clapham et al., 1962; Haslam et al., 1975) describe the flowering season of *L. dortmanna* as July and August. In effect the season is considerably greater than this. The first flowering stalks can commonly be seen in May in North-West Scotland. In 1983, a particularly warm year, open flowers were seen in Lochan-na-Thuill in May. The latest open flowers personally observed were in mid-October 1982, also in Lochan-na-Thuill. If a plant is damaged

a second flowering stalk can be produced. The flowers remain open for 1-2 weeks and then form a capsule, which is green and, presumably, photosynthetic.

The timing of flowering may vary throughout Scotland. Southern latitudes, which are generally warmer, have been seen to have an earlier flowering period. In 1983, for example, flowering stalks were degenerate in Lochs Dow and Lurg in Fife (3°27'W 56°9'N) in late September, though flowering was still extensive in Lochan-na-Thuill, Sutherland (5°0'W 58°24'N). In 1984 in July it was noticed that while most of Scotland had lochs with extensive stands of open *L. dortmanna* flowers, the lochs of Shetland were limited to plants that had only just begun the initiation of flowering stems.

3.2.2. The Distribution of Flowering

1. Which plants flower?

a) Introduction.

A casual glance at a sheltered bay of a Highland lochan in August will often reveal a dense bed of flowering plants of *L. dortmanna*. However, closer inspection reveals that only a small proportion of these plants are actually flowering. During the seasonal growth analysis (Chapter 2) it was noted that relatively few of the plants studied ever flowered. This section presents a short study to determine what proportion of any population may flower and give some guide as to the factors that may affect flowering.

b) Methods.

During the flowering period a number of lochs from the Highlands, Lowlands, Western and Northern Isles were visited. Sites were chosen in these lochs to illustrate ranges in substrate type and exposure. At each site 25 cm^2 quadrats were randomly cast and the leaf number and flowering were counted for every plant in the

quadrat. In all cases over 100 plants were studied at each site. In Lochan-na-Thuill a site at 1 m depth was also examined in exactly the same manner using SCUBA. Also in Fetlar, Shetland, a particularly fine flowering (terrestrial) population of *Litorella uniflora* was similarly studied.

c) Results.

Table 3.1 presents a brief description of the sites studied, giving details of sediment characters and co-existing species. Table 3.2 presents the percentage of flowering plants and the number of flowers per individual shoot in relation to the type of site in which the population is found. Part of this data are presented graphically in Figure 3.1. Figure 3.2 shows the actual population structure at each of the sites.

d) Discussion.

The most obvious conclusion to be drawn from Figure 3.2 is that flowering is not evenly distributed thoughout the population. Larger plants are more likely to flower, and the smallest plants do not flower. The size at which flowering becomes likely varies between populations, generally being around 5-7 leaves. In most populations the very largest plants always flower. The same trend is observed over all of the sites, regardless of substrate type, and also at 1 m deep in the water. Young (1984) noted that the "giant" E. African *Lobelias*, *L. telekii* and *L. keniensis*, both have a minimum rosette size before they produce an inflorescence. This is a common feature in herbaceous species, e.g. *Dipsacus sylvestris* (Werner, 1975), *Frasera speciosa* (Inouye and Taylor, 1980) and others (Gross, 1981). Young (1984) suggests that a minimum size for flowering is due to a plant slowly building up the reserves necessary for flowering, something that only the larger plants can do. Moeller (1979) noted that flowering in *L. dortmanna* uses up inorganic nutrients.

Table 3.1.

The sediment characters and co-existing species for the sites studied in Figure 3.2.

Site	Name and Locality	Description
A	Lochan-na-Thuill, Sutherland	1 m depth. Fine silt with *L. uniflora* and *J. bulbosus*.
B	Sligachan, Skye	Exposed, gravel with *E. septangulare*, *R. flammula* and *L. uniflora*.
C	Loch Marulaig, South Uist	Sheltered, silty with *Nymphaea alba*, *Phragmites communis*, *Eleocharis palustris*.
D	Loch Kearsinish, South Uist	Exposed, gravel with *L. uniflora*, *J. bulbosus* and *Carex nigra*.
E	Loch Druidibeg, South Uist	Sheltered. Fine sand and silt with *L. uniflora*, *I. lacustris*, *E. palustris*.
F	Loch Druidibeg, South Uist	Exposed. Gravel with *L. uniflora*.
G	Loch Airigh na Saorach, Skye	Sheltered. Sand/silt with *L. uniflora*, *R. flammula*, *Potamogeton polygonifolius*.
H	Loch Airigh na Saorach, Skye	Exposed, hard gravel with *R. flammula*.
I	Loch Dow, Fife	Sheltered, gravel/sand with *L. uniflora* and *I. lacustris*.
J	Unnamed lochan, West Mainland, Shetland	Sheltered. Very silty. Pure stand.
K	Many Crooks, West Mainland, Shetland	Exposed, hard gravel with *L. uniflora* and *J. bulbosus*.
L	Skutes Water, Fetlar, Shetland. *L. uniflora* population	Terrestrial. Pure stand.
M	Sandy Water, West Mainland, Shetland	Exposed, gravel with *L. uniflora* and *J. bulbosus*.

Figure 3.1.

The number of plants with different flower numbers for four populations. Two exposed sites, Loch Druidibeg (A) and Loch Kearsinish (B) and two sheltered sites Loch Marulaig (C) and Loch Druidibeg (D).

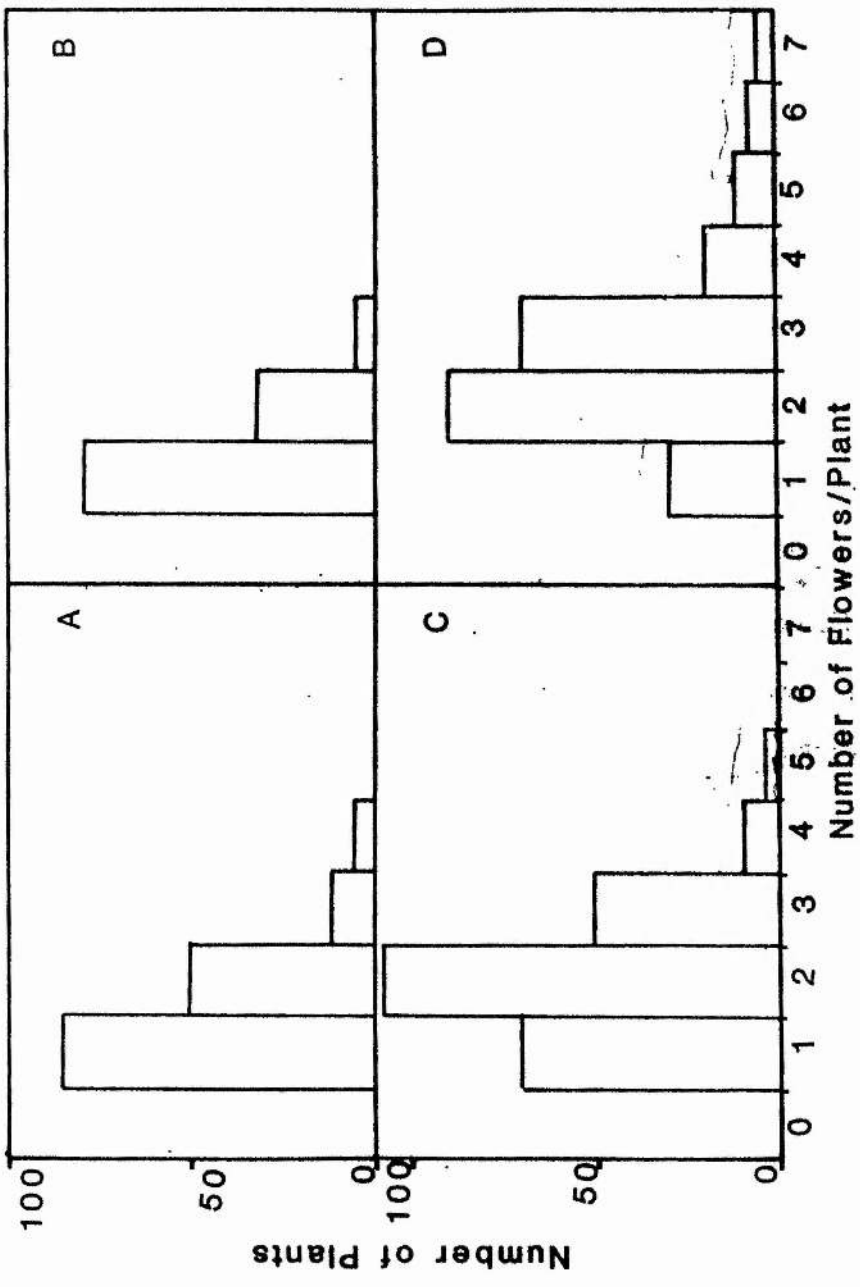

A
B
C
D
100
50
0
0 1 2 3 4 5 6 7
Number of Plants
Number of Flowers/Plant

Figure 3.2.

The population structure of 13 populations of *L. dortmanna* and one of *L. uniflora*. Each shows the percentage of plants of each size class (by leaf number) and the part of the population which is flowering. The site numbers (A-M) correspond to those in Table 3.1.

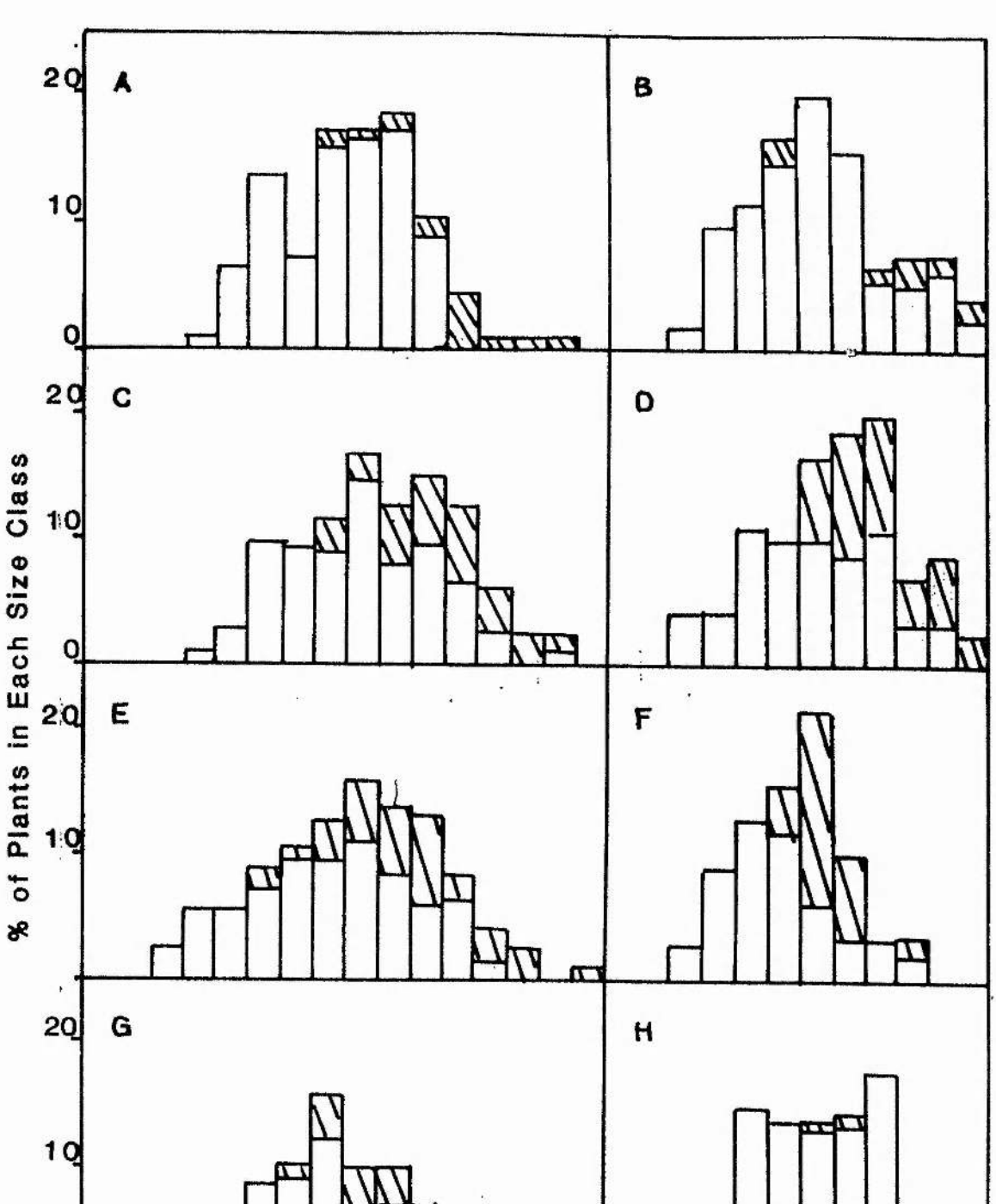
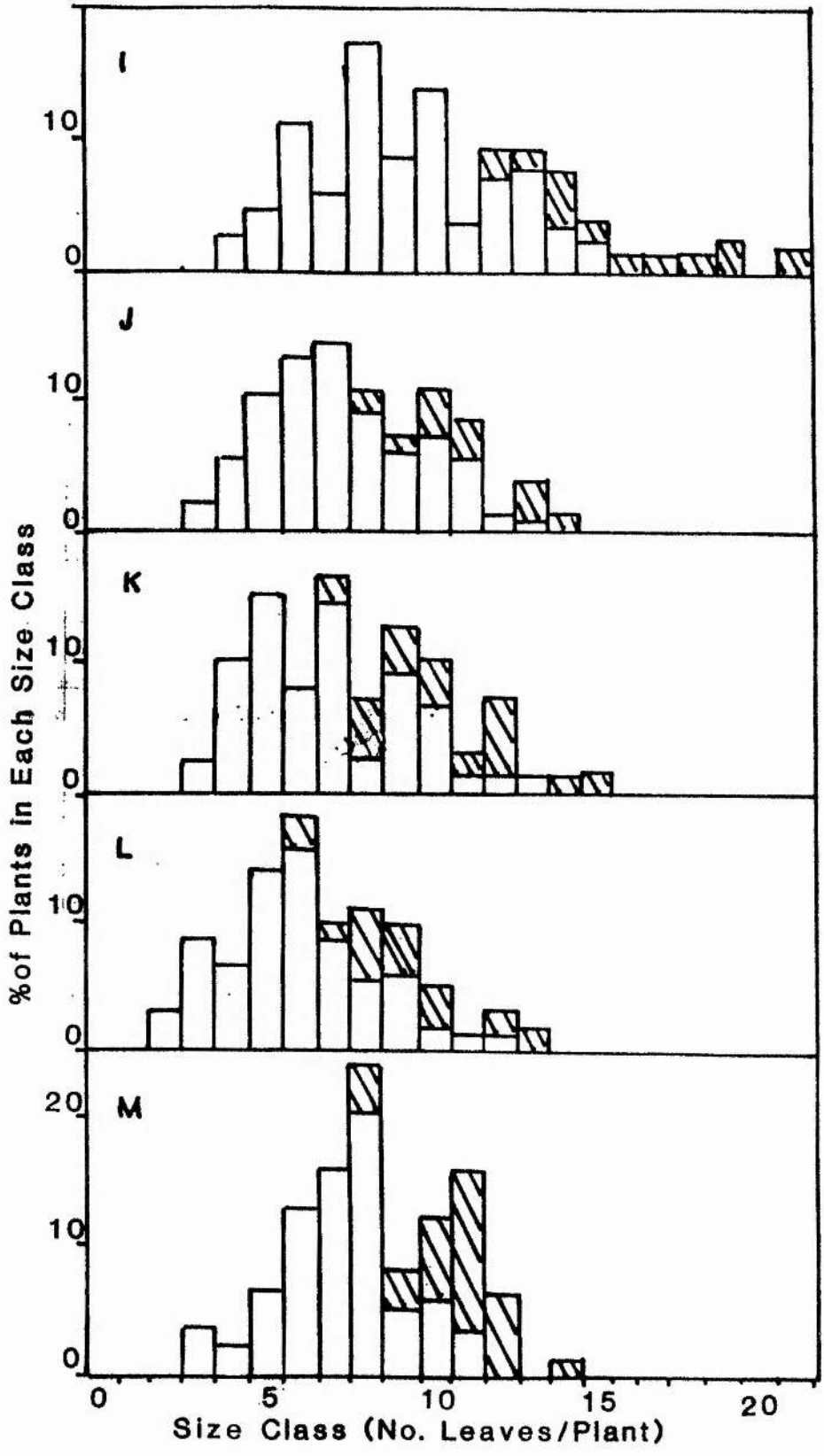

A
B
C
D
E
F
G
H
20
10
0
% of Plants in Each Size Class
0
5
10
15
0
5
10
Size Class(No. Leaves/Plant)

Figure 3.2.

(continued).


I
J
K
L
M
10
0
20
%of Plants in Each Size Class
0
5
10
15
20
Size Class (No. Leaves/Plant)

L. dortmanna seems, therefore, to behave as a typical herbaceous species, as does *L. uniflora* (Figure 3.2).

It is, of course, not possible to age the plants in a population. Chapter 2 showed that leaf production rates vary between plants to that two plants of a comparable size may be of very different ages. Figure 3.2 illustrates the effect of exposure on the plant sizes in a population. This may either reflect a younger population due to higher mortality, or smaller plants adapted to exposure. In either case, Table 3.2 shows that exposure has no discernible effect on the percentage of the plants that flower. In only one case, Lochan-na-Thuill, does shelter increase the number of flowering plants. The two sites studied here are sites 1 and 2 of Table 2.1. This table shows that site 2 is considerably richer in sediment interstitial soluble inorganic phosphate. Moeller (1978) showed that tissue phosphorus is depleted in particular during flowering, so that this nutrient may be a limiting factor. No studies of the nutrient levels in the other lochs were undertaken, however. Table 3.2 and Figure 3.1 does show, however, that exposure reduces the number of flowers on any shoot. Exposed plants cannot produce long shoots with many flowers as these are particularly susceptible to wave action.

2. Flowering and water depth.

Moeller (1978) found that flowering in *L. dortmanna* declined exponentially with depth to 1.7 m, below which no further plants flowered. The previous section showed that flowering was common for for plants at 1 m depth in Lochan-na-Thuill. However, this loch does not descend below 1.25 m. Nearby is Loch Fiacail, which is much deeper (unknown depth, but below 5 m). In this loch *L. dortmanna* does not grow below 1.8 m, when pure stands of *Isoetes lacustris* become the only vegetation. Using SCUBA this population was examined

Table 3.2.

The extent of flowering of *Lobelia dortmanna* for a number of lochs in Sutherland, Skye and South Uist.

Loch	Plant Density m^{2} $^{-1}$	% Flowering	Flowers Per Flowering Shoot
Airigh na Saorach, Skye			
a) Silty, sheltered	403	19.4	1.81
b) Gravel, exposed	88	14.5	1.14
Sligachan lochan, Skye			
Gravel, exposed	227	4.9	1.36
Druidibeg, South Uist			
a) Silty, sheltered	210.4	24.7	2.49
b) Gravel, exposed	190.4	32.7	1.63
Kearsinish, South Uist			
Gravel, exposed	132.0	33.0	1.23
Marulaig, South Uist			
Silty, sheltered	358.4	25.4	1.90
Loch na Thuill, Sutherland			
a) Gravel, exposed	60.5	3.0	-
b) Silty, sheltered	51.0	19.5	

September 1984. 5-10% of the *L. dortmanna* plants were flowering at the depth limit. The stems were tall and unbranched. Jensen (1977) also noted that plants may flower at the depth limit. West (1905) observed stems reaching the water surface from 2 m depth. In Loch Fiacail stems from plants at the depth limit remain submerged. Flowering at depth is reduced (Moeller, 1978) and this is probably due to the reduction in light and thus photosynthate here. Photosynthetic adaptation to deep water is considered in greater detail in Chapter 4.

Moeller (1978) suggested that the lack of flowering at the depth limit may explain his observation that very few seedlings are observed at this point. Seedlings were observed at 1.8 m in Loch Fiacail, and Jensen (1977) also reported seedlings at the depth limit. Moeller's suggestion ignores the possibility of seed dispersal from elsewhere. In Loch Fiacail the shallow population, producing thousands of seeds, is only 4 m away. Some of these seeds are likely to settle at 1.8 m.

3. Submerged flowers.

The presence of submerged flowers has been noted in a number of aquatic macrophytes (Hutchinson, 1975). Seed production is by autogamy or pseudocleistogamy, i.e. by self-pollination in an air-filled bud. Hooker (1847) noted this in *Limosella aquatica*, and Ernst-Schwarzenbach (1956) noted both normal and cleistogamous flowers in *Ottelia ovalifolia*. Sculthorpe (1967) lists twelve genera that have shown sumberged flowers. In particular the isoetid *Subularia aquatica* (Thunmark, 1931) produces autogamous submerged flowers.

Faegri and Van der Pijl (1979) suggested that the flowers of *L. dortmanna* are formed underwater and are already self-fertilized by the time they are opened above the water. I have not been able to

confirm this. Many flowers are initiated above the water surface. Confusion may result from the presence of underwater flowers. Moeller (1978) noted plants flowering at 1.7 m and these had underwater flowers (personal communication in Spence, 1982). Spence (1982) noted them in Lochan-na-Thuill.

During three flowering seasons a close observation was made of submerged flowers. Plants growing below 0.75 m rarely produce flowering stems that rise above the water. All of the flowers are formed and set seed under water. The flowers do not open, so seed set is probably by cleistogamy. Woodhead (1951a) notes that this may be a possibility for *L. dortmanna*. In addition, shallow water plants may have submerged flowers. Although the stems rise above the water the lowest flowers may be submerged. This has been observed in a number of lochs, including Lochan-na-Thuill where water level changes were monitored (Chapter 2), showing that these flowers are not aerial ones that have been subsequently submerged.

It is obvious, therefore, that the production of flower buds is not due to exposure to the air. It is more likely to be a light effect, though there is no evidence for this. The process may be analogous to the induction of aerial-type leaves on shoots of *Hippuris vulgaris*, which has been shown to be due to light changes near the water surface rather than exposure (Bodkin, Spence and Weeks, 1980).

The production of submerged flowers, which set seed, is naturally important to a species like *L. dortmanna* that has little vegetative reproduction and yet grows down to 2 m in depth. Aerial flowering stems from deep plants would be liable to damage. Shorter, submerged stems are less easily broken. In shallow water populations aerial flowers may allow for cross pollination. Faegri and van der Pijl (1979) and Woodhead (1951a) think that insect visitors are unlikely

though Sculthorpe (1967) suggests it is entomophilous. Although many "showy" flowers are formed, I have never seen any insect visitors.

Conversely to the production of submerged flowers, plants from terrestrial populations, e.g. those at Loch Freuchie, Tayside, also readily flower and set seed. The stems are, however, rather short (15-20 cm) and there are rarely more than three flowers per shoot (often only one). Sylven (1903) noted multiple, axillary stalks in emersed plants. I have never seen this, except for one plant grown in culture.

3.3. Seed Dispersal

3.3.1. Seed Number

The mean number of flowers produced per plant for a number of sites is given in Table 3.2. The full range of flower numbers stretches from one to seven, though the higher numbers are rare. Almost every flower forms a capsule with seeds. Occasionally I have noted a dead flower and no capsule production. The capsules contain widely differing seed numbers. Woodhead (1951a) quotes previous studies giving a range of 41-175 seeds/capsule, but he found an average of 250 in North Wales. I have noted a range from 76 to 302. An individual plant could, theoretically, produce over 2000 seeds, though the average found is about 350. No attempt was made to count the seeds of the underwater capsules, as the capsules degenerate easily, making an accurate assessment difficult. A casual observation suggests the seed numbers are of a similar order.

3.3.2. The Mode of Dispersal

Seeds have been observed to have been liberated from capsules in a number of ways. Submerged capsules tend to slowly degenerate

and the seeds are dispersed in the water currents. Aerial capsules become quite dry and the seeds are slowly liberated via pores in the capsule. Although small and not wetted the seeds are still quite heavy and will drop immediately onto the water surface. In some lochs the water level may rise quite quickly in the autumn and inundate capsules. In others, rough water may break the stems and again cause the capsules to be flooded. The seeds are then liberated as with the submerged capsules. The old flowering stems may persist for many months, in some instances until the initiation of the next flowering stem.

The seeds float only for a few seconds and then sink (Sculthorpe, 1967). In some macrophytes floating seeds are an important means of dispersal. In others the seeds sink but the seedlings are buoyant and may float and disperse, e.g. *Baldellia ranunculoides* (Hutchinson, 1975). The seedlings of *L. dortmanna* are certainly buoyant, though whether they aid dispersal is unknown.

Seeds may be dispersed over short distances by water currents. In the North-West of Scotland the many small lochans are linked by streams that probably facilitate some dispersal. The mechanism of long range dispersal is unknown. *L. dortmanna* has dispersed in post-glacial Europe and North America. It is, however, limited to one island in the Faeroes and has not reached Iceland (Ostenfeld and Grӧntued, 1924), unlike *I. lacustris*, *L. uniflora* and *S. aquatica*. The major means of long-distance dispersal in aquatic plants is by birds (Hutchinson, 1975), and it is probably also so for the spores/seeds of the isoetids. This seems to be either less efficient for *L. dortmanna* or its seeds have narrower germination requirements.

3.3.3. Dispersal in the Loch Sediment

1. Introduction.

Once the seeds have settled on the sediment surface wave action

would be likely to cause their burial by the deposition of further sediment over the seeds. In some areas of a loch deposition may be slow, and the seeds may sink into the sediment, especially if they are more dense than the sediment. In this study seeds were tested for "self-burial" in undisturbed conditions. As sediment burial will affect the environment of the seeds, particularly the light climate, any self-burial may be important.

2. Methods.

Fresh seeds were collected from aerial capsules on the shores of Loch Fiacail. Sediment was collected using a corer (10 cm diam., 25 cm long) from 1.5 m depth in the middle of a bed of *L. dortmanna*, using SCUBA. The sediment was placed in an upright polythene tube (2.5 cm diam., 25 cm long), sealed at the base. It was allowed to settle, retaining a 5 cm column of water above the surface.

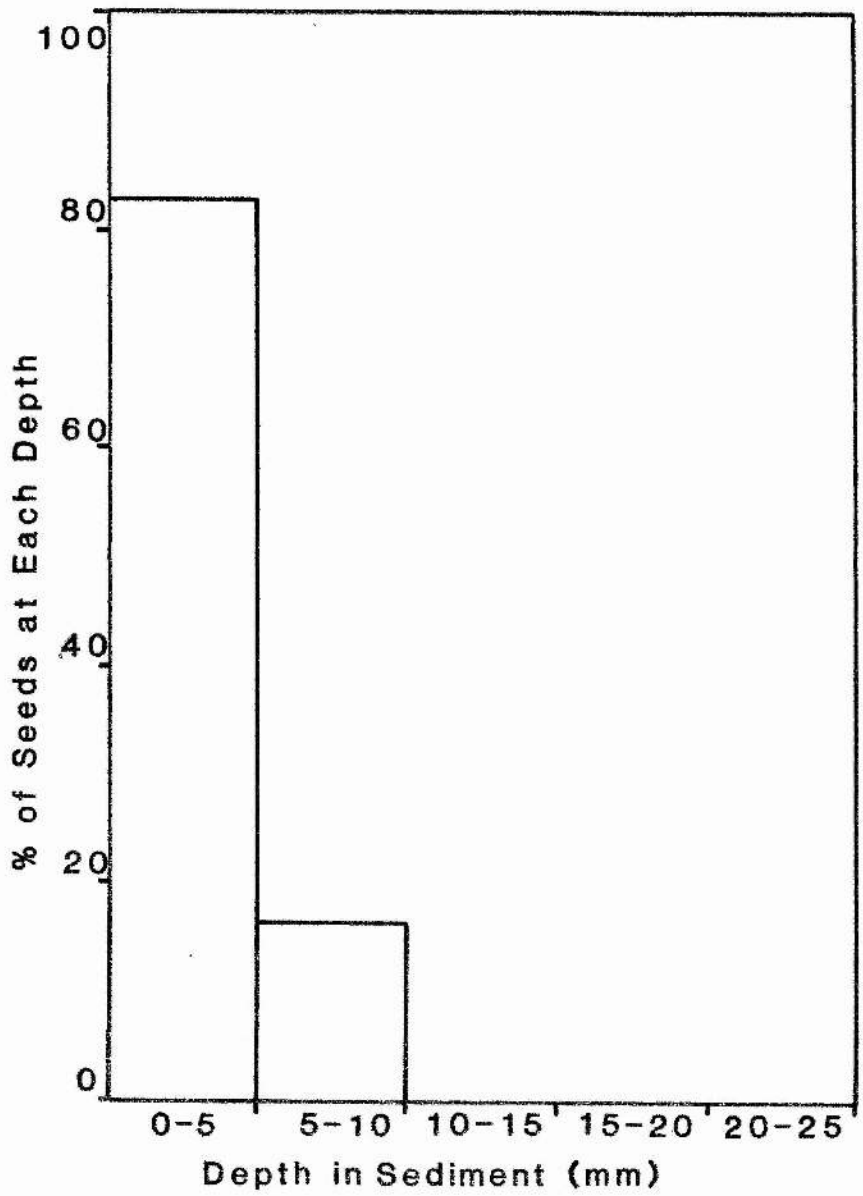
The seeds were st.ained with safranin in order to make their later detection more easy. The seeds (over 300) were floated on the surface of the water column. The tube was incubated at 20°C in the light for five weeks. It was then frozen at -5°C and cut into 5 mm segments. The number of seeds in each segment was counted, upon thawing, using a dissecting microscope.

3. Results and Discussion.

The results are presented in Figure 3.3. It can be seen that the seeds do not sink below 10 mm in the sediment due to their difference in density between sediment and seed. A significant proportion, 16.1%, have sunk below 5 mm. The light conditions in loch sediments are discussed later. Nicholson and Keddy (1983) noted that some lakes have a very shallow seed-bank. Certainly with *L. dortmanna* burial by wave action would be necessary to explain more than a superficial covering of sediment.

Figure 3.3.

The distribution of seeds of *L. dortmanna* allowed to settle in a core of sediment from Loch Fiacail for one month.


100
80
60
40
20
0
% of Seeds at Each Depth
0-5
5-10
10-15
15-20
20-25
Depth in Sediment (mm)

3.4. Seed Germination

3.4.1. Seed Storage

1. Introduction.

The storage conditions of any seeds are important in determining the subsequent requirements for germination. Seeds stored hydrated are able to respond to stratification and any exposure to light will affect phytochrome conversion in the seed. This happens even though the seeds may be stored at a temperature below that required for germination. Seeds stored dry will not stratify (Bewley and Black, 1982), though they can still respond to light changes (Bartley and Frankland, 1984). The best state in which to store seeds for experimental purposes is, therefore, dry and dark. However, some aquatic plant seeds will not survive drying so this study attempts to discover the conditions under which *L. dortmanna* seeds can be stored.

2. Methods.

Seeds from both aerial and submerged capsules were stored under two conditions. In the first, seeds were placed in a tube in a glass chamber containing water and nitrogen gas. This was stored dark (wrapped in black plastic) at 1-3°C. In the second, seeds were dried over silica gel at 15°C for 5 days until the water content dropped to about 0.06 g/g. The seeds were then transferred in the dark to a small box and frozen at -5°C.

After storage for a month the seed viability was tested by treating the seeds with Giberellic Acid (GA_3) (1 g/litre). In this, and all subsequent experiments, all treatments consist of four 5 cm petri-dishes with three layers of Whatman No. 1 filter paper, each dish with 50 seeds. 10 ml of GA_3 was added. The petri-dishes were placed in the dark at 25°C until no further germination occurred.

All transfers and preparations of the treatments were carried out in a dark room with a green safe-light box. This minimises even the green light in the room.

3. Results and Discussion.

The results of the germination tests are as follows (± S.E.).

% Germination Aerial Frozen dry	96.8 ± 0.9
% Germination Aerial Anaerobic wet	96.6 ± 0.3
% Germination Submerged Frozen dry	98.5 ± 0.6
% Germination Submerged Anaerobic wet	97.8 ± 0.5

From this it can be seen that submerged flowers produce viable seed. The seeds also survive drying to conditions more extreme than that at which would be experienced in the field. Muenscher (1936a) failed to find this for *L. dortmanna*, though Guppy (1897) found many aquatic plant species have seeds that survive drying. *Eichornia* (Crocker, 1907) even loses its dormancy by drying. The ability to survive drying may be important to seeds in capsules exposed to strong sunlight and especially to those an terrestrial plants.

As the stored seeds in anaerobic, wet conditions are kept for a few months they become infected with fungi. This causes further infection of experimental dishes and probably reduces viability. In all the subsequent studies, therefore, the seeds used were those stored frozen and dry.

3.4.2. Seed Stratification

1. Introduction.

The seeds of many plant species require a period of cold stratification before they will germinate (Bewley and Black, 1982). This usually ensures that seeds do not germinate in warm autumn

conditions when they may not grow to an adequate size to survive the winter. Guppy (1897) noted that few seeds, therefore, germinate in the year that they are shed, and Muenscher (1936a) showed that a cold period was necessary in some species, including *L. dortmanna*. Conversely, Churchill (1983) found, in field tests, that *Zostera marina* will germinate before the onset of winter, and McNaughton (1966) showed that three species of *Typha* do not require cold stratification. This section seeks to determine whether the seeds of *L. dortmanna* require a period of cold stratification and, if so, of what duration.

2. Methods.

Seeds were placed in the dark in petri-dishes and flooded with 10 ml of distilled water. The dishes were placed in dark boxes and put in cold storage at 1-3°C. At regular intervals four dishes were placed in a conviron cabinet with white fluorescent light (continuous light, PAR = 90 $\mu Em^{-2}s^{-1}$) at 25°C. Germination was allowed to proceed until it was complete. Fresh seeds were used in one treatment to test for immediate germination upon shedding. Another treatment using seeds stored in ice for 1 month was also undertaken.

3. Results and Discussion.

The results are presented in Table 3.3. It can be seen that *L. dortmanna* seeds will not germinate immediately, but require a cold period. They survive imbedding in ice, as do a number of other aquatic species (Guppy, 1897). The optimum cold period seems to be of 1-2 months. It can also be seen that longer periods of storage cause a reduction in viability. Some petri-dishes become infected by fungi and this may explain some of the reduction in viability.

Sylven (1903) observed that *L. dortmanna* seeds germinated in the late summer and overwintered in the early stages of germination.

Table 3.3.

The % germination of seeds of *L. dortmanna* at 25°C in continuous light (PAR = 90 $\mu Em^{-2}s^{-1}$) for fresh seeds, seed stored in ice and seeds cold stratified at 1-3°C for varying periods of time. Germination allowed to go to completion.

Pre-treatment	% germination of 50 seeds ± S.E. n = 4
Fresh seeds	0.0
Ice (1 month)	83.0
1 month cold	96.0 ± 0.8
2 months cold	97.0 ± 0.9
3 months cold	83.0 ± 2.4
4 months cold	56.7 ± 5.1

Field observations reveal that seedlings become visible in August, but as they are small they may have germinated some time before this. As seed production is not well advanced in July, it would suggest these seeds are produced in the previous year. The only other British isoetid studied adequately in its germination is *Isoetes echinospora*. This species requires a minimum of twelve weeks cold stratification (Kott and Britton, 1982).

3.4.3. Light, Temperature and Germination

1. Introduction.

The interaction of light and temperature in seed germination is complex. This study attempts to evaluate the effects of light quantity, quality and temperature. Aquatic plant seeds vary considerably in their response to these factors. Guppy (1897) found that light promoted germination in *Nuphar lutea*, but prevented it in *Iris pseudacorus*. Light is also inhibitory in *Najas marina* (Forsberg, 1965) and *N. flexilis* (Wetzel and McGregor, 1968). It promotes germination in some *Potamogeton* species (Spence et al., 1971) and *Typha latifolia* (Sifton, 1959). The requirement for light is a common feature of ruderal species, invaders of open spaces.

The requirement of light for germination is often mediated via phytochrome (Bewley and Black, 1982). This is testable by subjecting seeds to red and far-red light, so causing phytochrome conversions. This has been rarely done in aquatic species. Spence et al. (1971) showed red promotion and far-red inhibition in *Potamogeton thunbergii* and *P. schweinfurthii*. *T. angustata* is promoted by red light (Gopal and Sharma, 1983) but no tests have been made with far-red light.

The actual quantity of light may be important. Spence (1982) suggested that some aquatic species may have a minimum light requirement that limits the depth to which they can germinate in a loch. Spence

et al. (1971) did show that the deeper water *P. schweinfurthii* required about a quarter of light quantity of *P. thunbergii*.

The effects of temperature on germination in aquatic species are poorly understood. Most species increase the germination rate with increasing temperature, though *Zostera marina* has a low optimum temperature (Churchill, 1983). A few species may require a fluctuating temperature to induce germination, e.g. *Neptunia oleracea* (Sharma et al., 1984). This may limit species to shallow water where diurnal fluctuations in temperature occur, as deep water exhibits a strong thermal stability and lacks diurnal variation in temperature (Hutchinson, 1975).

2. Methods.

Previously stored and stratified seeds were given the following treatments:

1) Some were placed under constant white fluorescent light over a range of temperatures from 5°C to 27°C. Constant temperatures were maintained either in growth cabinets or by floating petri-dishes on water baths.

2) Some petri-dishes were given daily flashes of 5 min red light (PAR = 10 $\mu Em^{-2}s^{-1}$) or far-red light (PAR = 5 $\mu Em^{-2}s^{-1}$) until germination was complete. Another treatment given was red followed by far-red or far-red followed by red. Light sources were constructed as recommended in Kendrick and Frankland (1983) for seed germination. Spectroradiometric scans of the two light sources are presented in Figures 3.4 and 3.5. Between exposures the seeds were incubated at 25°C in dark boxes.

3) Seeds were also placed, at 25°C and constant white fluorescent light, in a range of light levels. The reduction in fluence rate was achieved using muslin, a neutral density filter.

In all cases after one month GA_3 (1 g/litre) was added to the

Figure 3.4.

A spectral scan of the red-light source (after Kendrick and Frankland, 1983) used in seed germination. The scan was taken using a Macam Spectroradiometer SR 3010 from 360 to 800 nm. It shows a peak in the red region of the spectrum (660 nm) and very little far-red light (730 nm).

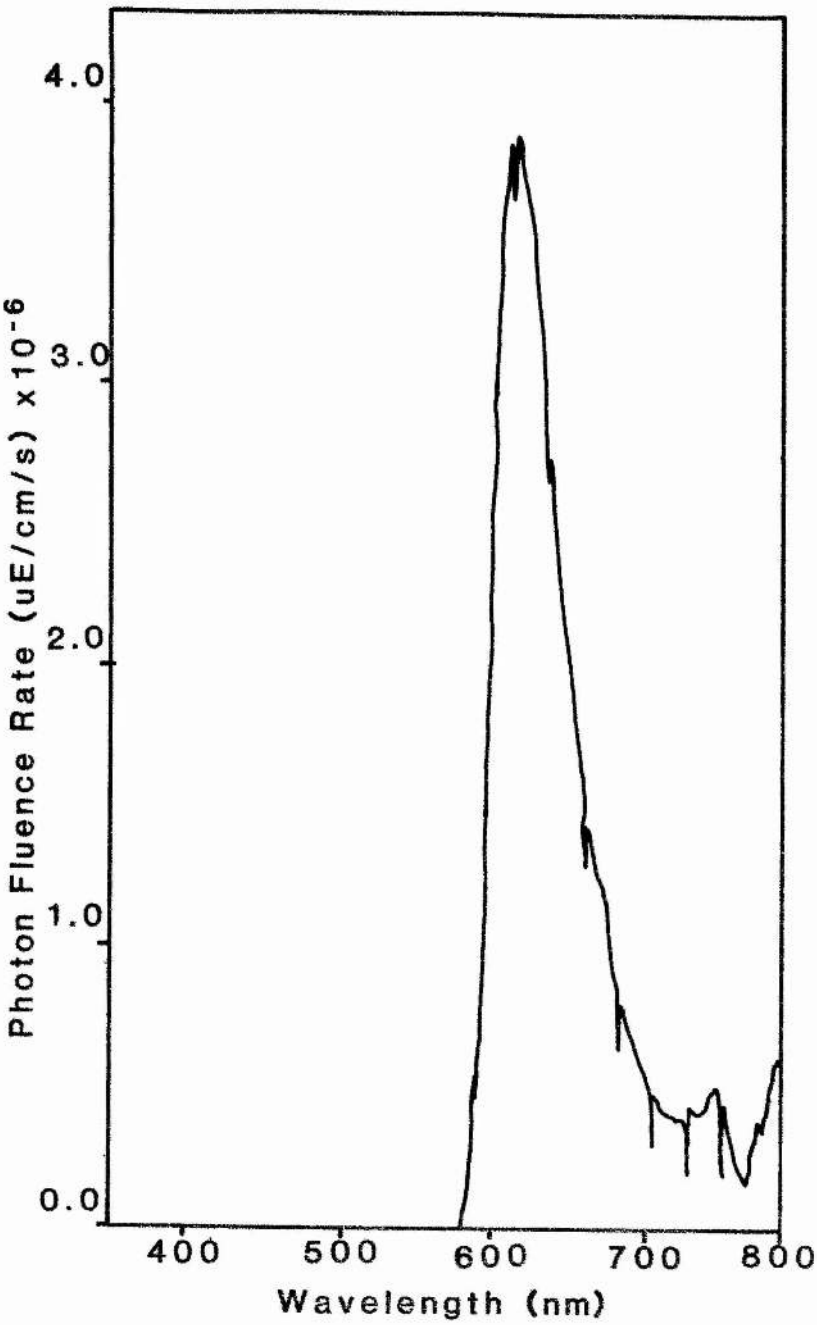
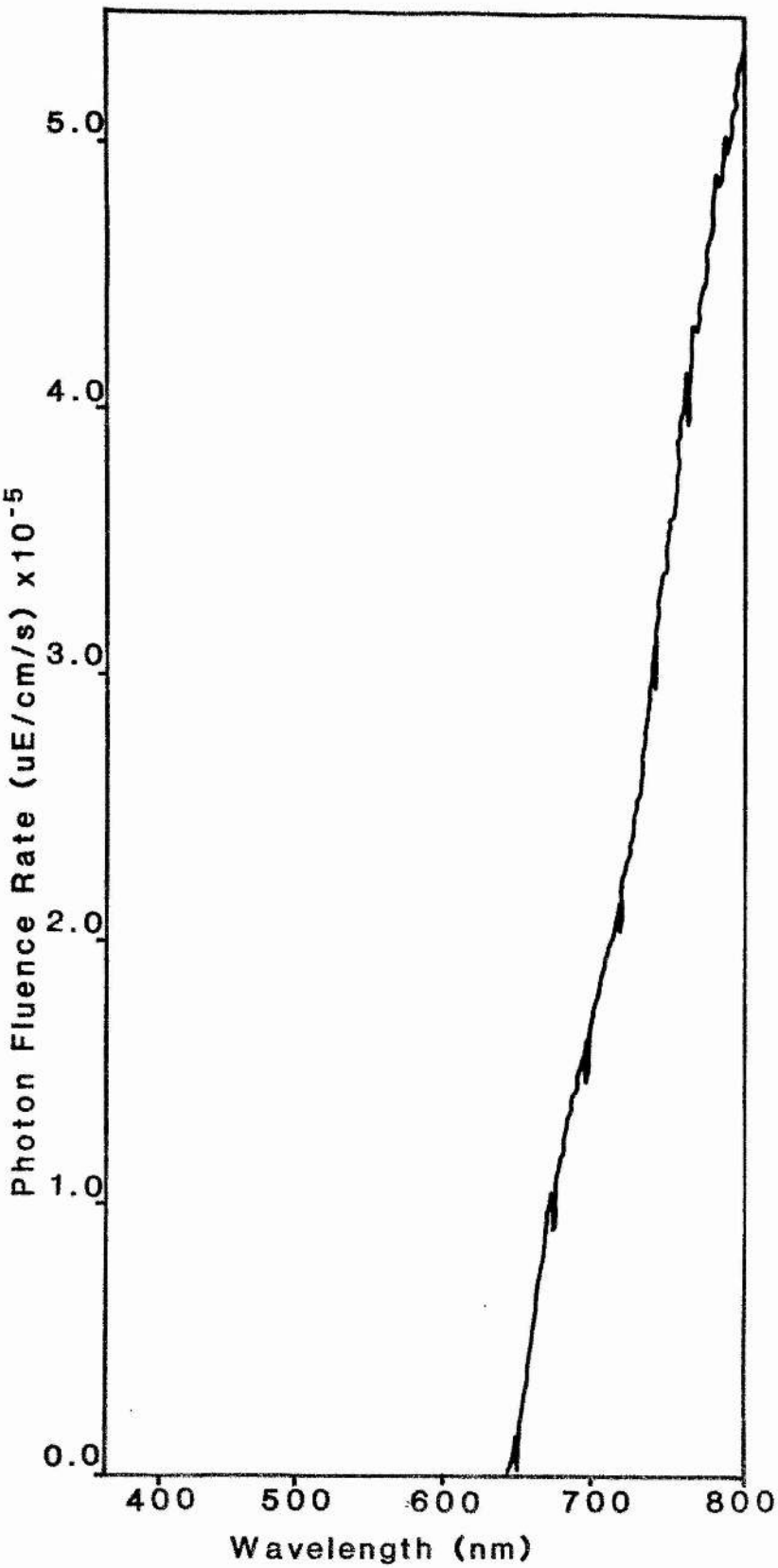

4.0
3.0
2.0
1.0
0.0
Photon Fluence Rate (uE/cm/s) x10-6
400
500
600
700
800
Wavelength (nm)

Figure 3.5.

A spectral scan of the far-red source (after Kendrick and Frankland, 1983) used in seed germination. The scan was taken using a Macam Spectroradiometer SR 3010 from 360 to 800 nm. It shows very little red light and a large peak in the far-red and infra-red regions of the spectrum.


5.0
4.0
3.0
2.0
1.0
0.0
Photon Fluence Rate (uE/cm/s) x10-5
400
500
600
700
800
Wavelength (nm)

ungerminated seeds to test viability so that results can be expressed accurately as percentage germination of the seeds available for germination.

3. Results and Discussion.

The results are presented in Figure 3.6 and Table 3.4. No dark germination occurs at any temperature. This is ecologically extremely important. Seeds buried too deeply in sediment will not germinate and, as they tend to lose their viability over a few months, deeply buried seeds will tend to die unless exposed after a relatively short time.

Germination will proceed over a range of temperatures, though after 1 month none was observed at 10°C or 5°C. It is most rapid at 27°C and 25°C, being markedly reduced at 20°C. This slow rate of germination has also been noticed in the spores of a number of *Isoetes* species (Kott and Britton, 1982). As temperatures rise in Lochan-na-Thuill (Chapter 2), 15°C is only reached in mid-May, 20°C in June. The maximum temperature recorded was 24°C and the mean weekly temperature was at 20°C or above for only one week. This would suggest that germination in the field is not very rapid.

Germination takes place at constant temperature. No diurnal fluctuations are necessary. Obviously the shallow-water distribution of *L. dortmanna* cannot be explained by a requirement for such fluctuations.

The results of the red/far-red treatments are not conclusive. Red light is definitely better at promoting germination than far-red. Its promotive effect can also be reversed by exposure to far-red. The reversal of far-red by red is clear. One possible mechanism of seedling zonation underwater would be for seeds to be far-red promoted. As far-red light attenuates more rapidly in a loch than red light (Spence, 1976), germination would be greater in shallow

Figure 3.6.

The time courses of the germination of seeds of *L. dortmanna* under constant white fluorescent light at different temperatures (PAR = 90 $\mu Em^{-2}s^{-1}$).

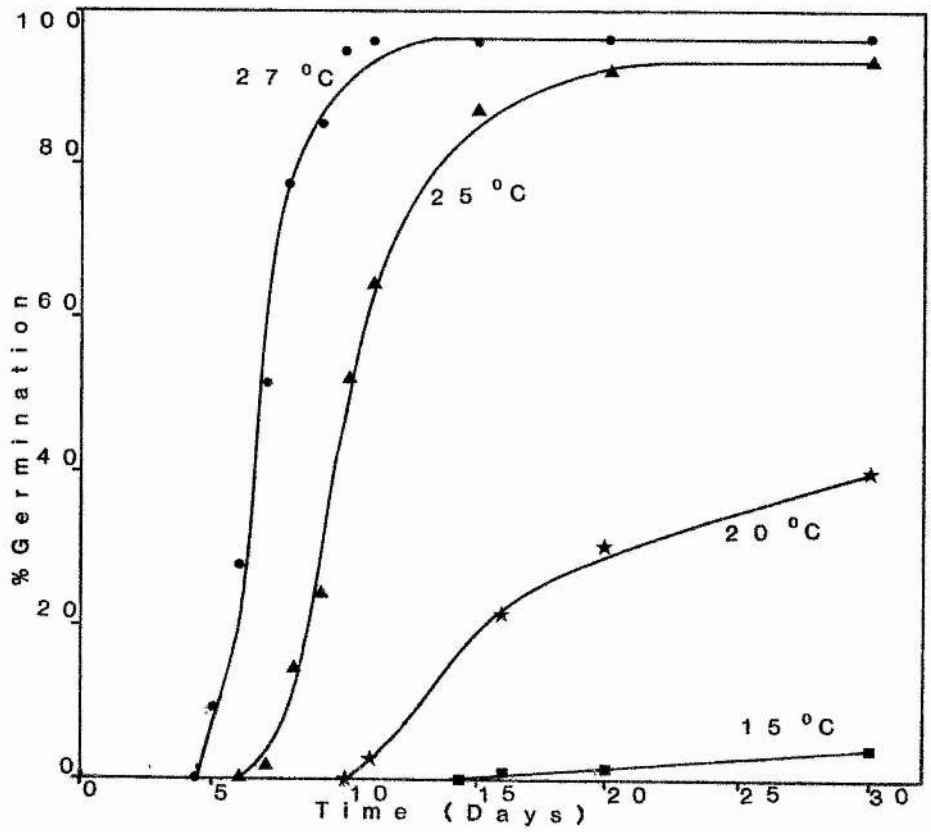

100
80
60
40
20
0
% Germination
27 °C
25 °C
20 °C
15 °C
0
5
10
15
20
25
30
Time (Days)

Table 3.4.

Germination of seeds of *L. dortmanna* under different white fluorescent light fluence rates and different light qualities. Germination allowed to go to completion at 25°C. The spectral composition of the red and far-red light sources are given in Figures 3.4 and 3.5.

Fluence rate PAR $\mu Em^{-2}s^{-1}$ constant	% Germination of 50 seeds ± S.E. n = 4
87	100 ± 0.0
34	71 ± 16.0
12	87 ± 7.0
4	100 ± 0.0
0	0.0 ± 0.0
Light quality	**% Germination of 50 seeds ± S.E. n = 4**
Red	34.0 ± 6.0
Red/Far-red	14.8 ± 4.5
Far-red	14.0 ± 2.1
Far-red/red	22.5 ± 4.9

water. Such a germination response has only been noted in one terrestrial species - *Bromus sterilis* (Hilton, 1982). As *L. dortmanna* is red-promoted such a mechanism of zonation cannot be operating.

L. dortmanna, although absolutely requiring light, will germinate at the very low light levels for 4 $\mu Em^{-2}s^{-1}$ PAR). A lower limit was not determined in these treatments. Clearly this will allow the seeds to germinate at low light levels either in deep water or buried sediment. At what depths these may occur is considered in section 3.5.

3.4.4. The Effect of Light Quantity on Seedling Growth

1. Introduction and Methods.

Although the seeds may germinate at very low light levels, the seedlings may subsequently not be able to grow. In this way the depth zonation of the plant may be controlled not by an effect on germination but on seedling survival.

Seedlings germinated in other experiments were placed in sterile conical flasks containing 50 mls of sterilized Gorham's medium (see Table 2.1). These flasks were wrapped in muslin to provide three different fluence rates. They were placed under a lamp (constant light) on an orbital shaker at 20°C. Three flasks, each with about 20 seedlings were used in each treatment. At the start and after one month, the number of seedlings and the number of leaves on each were counted. The seedlings were then given fresh sterile flasks and medium. The flasks at the two higher fluence rates subsequently became infected with algae, but those at the lowest rate were grown for a further four months.

2. Results and Discussion.

The results are presented in Table 3.5. The seedings in the two highest fluence rates grew and remained healthy (until infected).

Table 3.5.

Growth and survival of seedlings of *L. dortmanna* at three different white fluorescent light fluence rates over five months. The seedlings at the two higher fluence rates were lost after 1 month.

% Increase in leaf number				
Fluence rate for growth $\mu Em^{-2}s^{-1}$ PAR	Initial mean leaf number	Time (months) 1	3	5
87	2.67	+7.5	-	-
34	2.84	+0.3	-	-
4	2.58	-0.4	-0.8	-1.2
% Survival of seedlings				
Fluence rate for growth $\mu Em^{-2}s^{-1}$ PAR	Initial number of seedlings	Time (months) 1	3	5
87	69	100	-	-
34	44	100	-	-
4	75	89.3	76.0	64.0

Those in the lowest fluence rate slowly died. Even after five months more than a half were still alive. The negative growth rate also shows that the largest seedlings were dying faster, though it is uncertain why this might be. The seedlings can, therefore, survive periods of low light, but dea th will eventually occur. It must be noted that no dark period was given and the imposition of dark respiration in the field would probably increase seedling mortality.

It seems, therefore, that seeds will germinate under light conditions in which the seedlings cannot subsequently grow. This could be either at too great a depth in a loch or too deep in the sediment. The seedlings are, however, buoyant, even though they have not developed the gas-filled lacunal system of the adult plant. They root well in sediment, but a seedling that has germinated too deep in a loch may become detached and float upwards. This seedling would have a chance, albeit slim, of being washed to a site where it might take root and grow.

3.4.5. Anoxia and Seed Germination

1. Introduction.

The effects of anaerobic conditions on seed germination can be varied. In aquatic species low oxygen tensions have been seen to promote germination in a number of species - *Nelumbe nucifera* (Ohga, 1926), *Euryale ferox* (Ohga, 1930), *Alisma triviale* (Crocker and Davis, 1914), *Trapa natans* (Teresawa, 1927), *Peltandra virginica* (Edwards, 1933), *Typha latifolia* (Morinaga, 1926 and Sifton, 1959) and *Najas marina* (Forsberg, 1965). In *Oryza sativa* germination some cultivars are promoted by low oxygen tensions, others are not. (Takahashi, 1985) In some terrestrial species, however, anaerobiosis induces a secondary dormancy, e.g. *Xanthium pennsylvanicum* (Esashi et al., 1978) and *Avena fatua* (Borthwick et al., 1954).

This study attempts to evaluate the effect of anoxia on the dormancy of the seeds of *L. dortmanna*.

2. Methods.

Petri-dishes of seeds stored and cold stratified for two months were placed in an anaerobic workbench (Forma Scientific Marietta Ohio anaerobic system model 1024) for 1-16 days at 24-26°C. Seeds were removed over this period and placed in a conviron growth cabinet, 25°C, constant white fluorescent light for three weeks. These conditions would normally cause germination if the seeds had been brought immediately from cold storage. Each treatment had 4 × 50 seeds.

Seeds treated with anoxia and allowed to germinate were either treated with GA_3 to test viability or given another 1 month cold period and again allowed to germinate in the same growth conditions.

3. Results and Discussion.

The results are presented in Table 3.6. Even one day in anoxia will induce a secondary dormancy in the seeds, but many remain viable, even after 16 days anoxia. This secondary dormancy is broken by one month in the cold. It is usual that a secondary dormancy is broken by the same conditions as a primary dormancy (Bewley and Black, 1982). Seeds in conditions of low oxygen tension in the field will not germinate. The actual degree of hypoxia necessary to induce the secondary dormancy has not been determined. This mechanism could act in conjunction with a light requirement for germination for seeds buried deep in sediment. However, once induced a secondary dormancy will prevent a seed germinating upon exposure to oxygen and light. As viability is probably not long it would, therefore, be unlikely that such a seed would survive until the following spring when this dormancy would have been broken by winter stratification.

Table 3.6.

Germination of seeds of *L. dortmanna* after pre-treatment under anoxia and subsequent treatment with GA_3 (1 g/litre) or 1 month cold storage at 1-3°C.

No. of days anoxia	% Germination	
	Without GA_3	With GA_3
1	2.0 ± 0.5	96.± 0.1
2	0.0 ± 0.0	91 ± 1.1
5	0.0 ± 0.0	81 ± 3.7
12	0.0 ± 0.0	63 ± 5.2
16	0.0 ± 0.0	51 ± 7.2
2 days followed by 1 month cold	73 ± 4.3	-

3.4.6. Seed Germination - Conclusions

The following points have been determined with respect to germination in *L. dortmanna*.

1. The seeds require a cold period before germination.
2. In cold storage the seed viability declines over a few months.
3. The seeds are absolutely requirers of light for germination.
4. They will germinate at very low light levels, though the seedlings are, subsequently, not able to grow or survive at these light levels.
5. There is tentative evidence for phytochrome action (red light promotion and far-red reversal) in germination.
6. A period of one day in anoxia will induce a secondary dormancy that is broken by a further cold period.

It is now necessary to examine the natural environment of lochs containing *L. dortmanna* to see how these conditions may control the germination, survival and zonation of the species.

3.5. The Natural Environment

3.5.1. Introduction

This section will attempt to describe the light and oxygen status of the loch water and sediment. The light environment of a number of lochs containing *L. dortmanna* is described in Spence (1982). The reduction in light with depth is described by a series of attenuation coefficients. In discussing photomorphogenesis the attenuation coefficients of red and far-red light (K_r and K_{fr}) are important. The photosynthetic environment is described in terms of the least attenuated wavelength (K_{min}), usually broad-band green (K_G). Talling (1971) described a relationship for waters in the Lake District and Lake Victoria:-

$$zeu \approx 3.7/K_{min}$$

Zeu (the euphotic zone) is defined as the lower limit of net photosynthesis in phytoplankton, usually the depth at which 1% subsurface PAR is found.

Using Talling's equation and the figures for K_G given in Spence (1982), it is possible to derive a rough estimate of the depths at which 1% PAR can be found in a number of lochs. These approximations are given in Table 3.8. It is necessary to describe the light environment in terms of PAR as the seed germination has been so defined.

The light environment of lake sediments has not been previously studied, though the redox status has. Allegeier et al. (1941) found that sediments became more anaerobic with depth, though oligotrophic lakes were less reducing.

This study will consider the light environment of a number of loch sediments and the light environment of the water and the redox status of the sediment of Loch Fiacail, from where the seeds were collected for the germination studies.

3.5.2. Methods

The light environment in Loch Fiacail was measured using a Macam Spectroradiometer SR3010. Scans of the spectrum from 360-800 nm were taken at 0.5 m intervals in the water down to 4.5 m. The sensors were lowered down the unshaded side of a boat from an outrigger projecting 75 cm over the water surface. In order to adjust for variations in daylight simultaneous measurements of PAR were made for each scan using a Macam quantum photometer placed in the boat. Vertical diffuse attenuation coefficients for R, FR and PAR were calculated by linear regression from the equation:

$$K = (\ln I_0 - \ln I_z)/z$$

where K is the vertical diffuse attenuation coefficient (in ln units m^{-1}), I_0 is the initial subsurface irradiance, and I_z is the irradiance at a depth of z_m.

The light environment in the sediment was determined by placing 1 mm thick layers of wetted sediment in a flat, perspex tube over the sensor of the Macam spectroradiometer SR3010. A scan from 360-800 nm was taken under each sediment when exposed to a mercury-vapour lamp light, which provides a more intense source than fluorescent light. The percentage transmission in PAR was calculated for each sediment sample.

A sediment core from Loch Fiacail was obtained using a 5 cm diameter corer. The redox potential was measured at a number of depths in the sediment using a number of platinum electrodes inserted to those depths. The potential is measured with reference to a calomel electrode (Figure 3.7) after Mansor (1981).

3.5.3. Results and Discussion

The results are presented in Tables 3.7 and 3.8 and Figure 3.8. *L. dortmanna* rarely grows below 2 m and yet the depth of 1% subsurface PAR is considerably below this in all the lochs considered. In absolute terms subsurface PAR will go well above 1000 $\mu Em^{-2}s^{-1}$, so that a seed at a depth where it receives 1% of this will have enough light to germinate. Even allowing for inaccuracies in calculating Table 3.7, it is clear that *L. dortmanna* can germinate in lochs well below the level at which plants are usually found to grow. Hence light does not control zonation by limiting germination. It more probably limits seedling growth (section 3.4).

Light attenuates rapidly in the sediment. Seeds below 3-4 mm will experience almost total darkness. Self-sedimentation of seeds (section 3.3), therefore, does allow seeds to sediment to a point where

Figure 3.7.

The apparatus used to record the redox potentials at a series of depths in a core of sediment from Loch Fiacail.

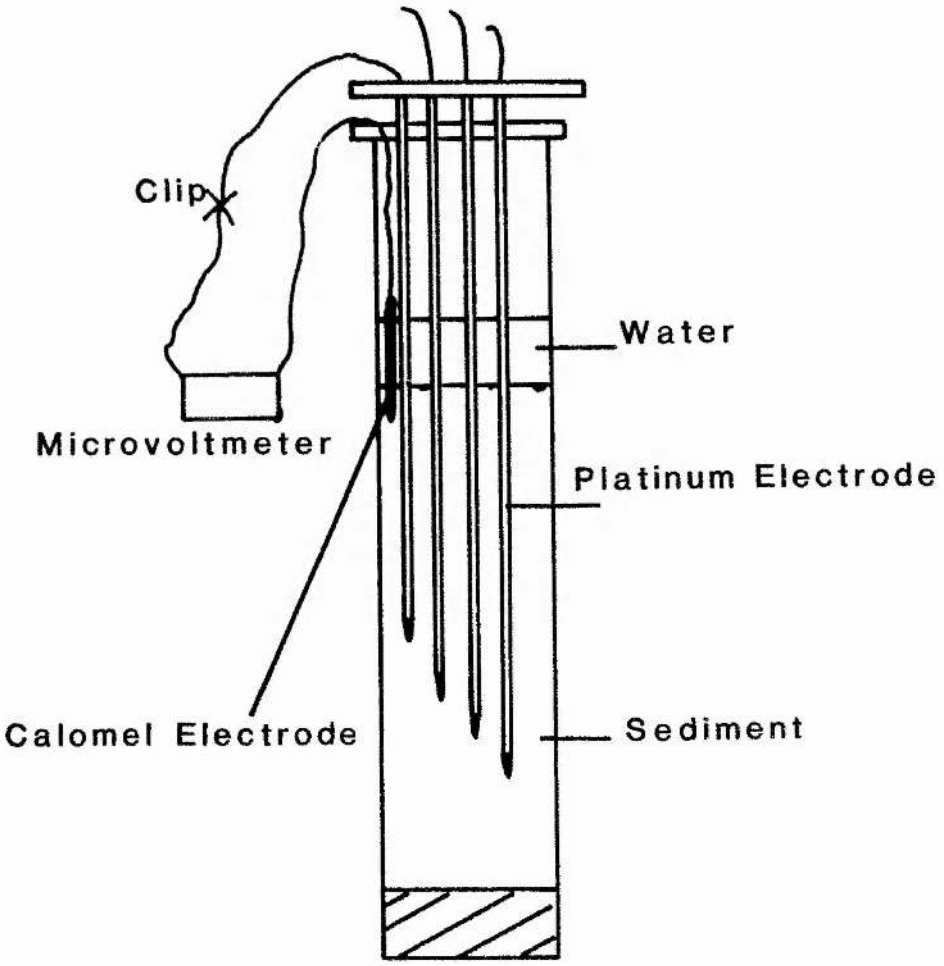

Clip
Water
Microvoltmeter
Platinum Electrode
Calomel Electrode
Sediment

Table 3.7.

The attenuation coefficients and calculated depth of 1% PAR for the waters for a number of British lakes containing *L. dortmanna*.

Lake or Loch	K_d ln units m^{-1}		1% PAR depth (m)
	K_G	K_R	
[1]Windermere	0.35	0.58	10.6
[1]Uanagan	0.37	0.43	10.0
[1]Esthwaite	0.48	0.52	7.7
[1]Clunie	0.46	0.47	8.0
[1]Lowes	0.60	0.54	6.2
[1]na Thuill	0.55	0.54	6.7

	K_{PAR}	K_R	K_{FR}	1% PAR depth
[2]Lomond	0.60	0.60	1.00	7.7
[3]Fiacail	1.16	0.94	2.65	4.0

[1]Spence (1982)

[2]Chambers and Spence (1984)

[3]This study

Table 3.8.

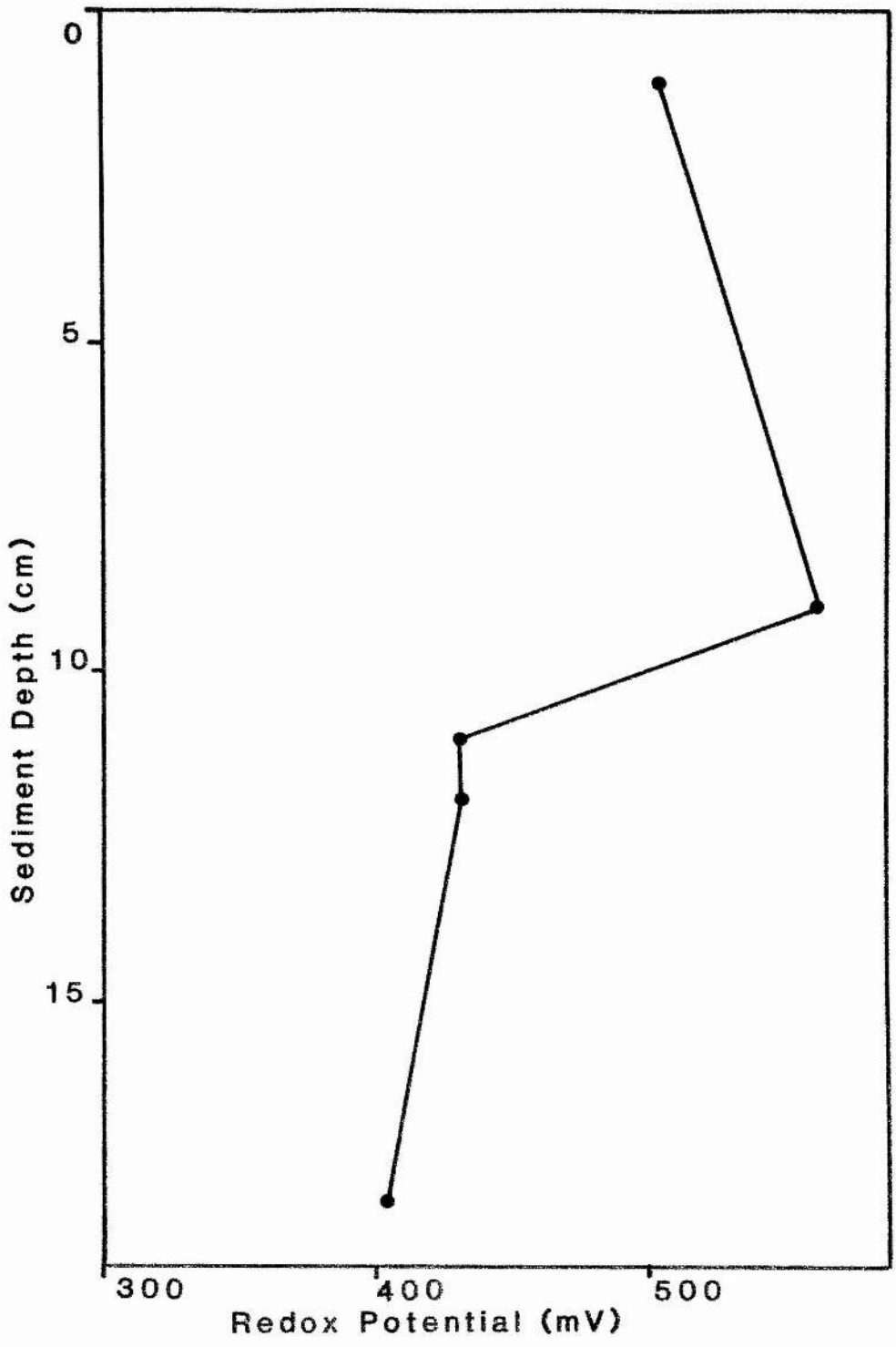
The percentage transmission of PAR by 1 mm depth of sediment from a number of lochs containing *L. dortmanna*.

Loch	Description	% transmission in PAR
Fiacail, Sutherland	Fine sand	18.2
Dow, Fife	Black silt	0.19
Craiglush, Tayside	Grey sediment	0.45
Lowes, Tayside	Sand and gravel	0.18
Lomond, Strathclyde	Gravel and sand	0.18
Dow, Fife	Gravel	0.11
Restil, Strathclyde	Silt and gravel	0.21
[1]Borralie, Sutherland	Fine sand, some silt	2.21

[1]This loch does not contain *L. dortmanna*.

Figure 3.8.

The change in the redox potential with depth through a core of sediment taken from Loch Fiacail.


0
5
10
15
Sediment Depth (cm)
300
400
500
Redox Potential (mV)

they will not experience conditions under which they can germinate. Any burial by wave action etc., which is probably much more important than self-sedimentation, will also prevent germination.

In lochs Fiacail and Lomond (Table 3.7) the water attenuates red-light less quickly than far-red. As red light promotes germination in *L. dortmanna* the change in R/FR ratio with water depth will not act so as to reduce the percentage germination. The zonation of *L. dortmanna* cannot, therefore, be explained by changes in the R/FR ratio in the water.

The redox profile of Loch Fiacail (Figure 3.8) reveals that this particular loch sediment is relatively oxidising compared to many other lake sediments (Allegeier et al., 1941). This study confirms others, e.g. Allegeier et al. (1941), that shows that the top two or three centimeters of sediment is well oxidised, and in many lochs the red colour of (oxidised) iron (III) hydroxide is associated with this upper layer (Hutchinson, 1975). It is probable, therefore, that seeds buried in the top few centimeters will not experience conditions that will induce a secondary dormancy. The control of germination in the sediment is, therefore, predominantly by light.

3.6. Vegetative Reproduction

In aquatic macrophytes vegetative reproduction takes the form of rhizomes, runners, tubers and turions (Hutchinson, 1975). Vegetative reproduction in *L. dortmanna* uses another means. Buchenan (1866) described that after flowering "side processes" were produced, new plants being formed at the base of the flower stalk. The process of flowering causes the loss of the apical bud, so that further vegetative growth involves the growth of an axillary bud. As the leaves are spirally arranged one bud grows in

dominance over the others so that it is usual to see only one growing at the close of the flowering season. Older plants, however, will often show the growth of a second bud, lower down and smaller than the first. Eventually the two plants will seem to be two individuals, though vestigial remains of a joined corm can be sometimes seen.

Aberg (1943) hypothesised that vegetative reproduction can only take place after flowering. This, of course, cannot be proven. However, all I have observed is in keeping with such a suggestion, having observed individual plants flower and subsequently undergo axillary bud expansion.

Vegetative reproduction generally incorporates some form of dispersal. The runners of *L. uniflora* can rapidly form dense mats over bare ground. Another isoetid, *E. septangulare*, also reproduces vegetatively by growth of short rhizomes. However, this can form dense mats by this method, unlike *L. dortmanna*. The other isoetids do not show vegetative reproduction, although *I. lacustris* and *I. echinospora* may exhibit vivipary in deep water (Goebel, 1879).

It seems that vegetative reproduction in *L. dortmanna* is of minor importance compared to seed production. In effect it is a means of continuing growth after flowering. Although two plants may result from one, over a number of years, there is no dispersal over more than a few centimeters.

Sculthorpe (1967) does suggest that *L. dortmanna* has runners. This is probably a mistake due to a confusion with *L. uniflora*.

4.7. Conclusions

L. dortmanna produces many seeds. This aids dispersal, fills all the available microsites and increases the chances of surviving unfavourable conditions (Cavers, 1983). A casual glance at a loch

may give the impression that there is a considerable number of "safe sites" (Harper, 1977) for the seeds. Yet in only a few sites does *L. dortmanna* from very dense swards.

A "safe site" for plant growth is not defined by the ability of the seeds to germinate. Certainly the seeds will not germinate when buried too deeply in sediment as they receive no light. It is doubtful if a small seedling would reach the surface if too deep in silt. However, seeds will germinate in water too deep to support seedling growth. It is probable that many seeds are lost this way. It may be that the effect of the seeds sinking rapidly is to keep more in the littoral zone of a loch, preventing seeds floating over deep water before sinking. Naturally, this makes long-distance dispersal more difficult.

Seed production seems to be quite costly. The inflorescence is large compared to the rest of the plant. Young plants do not flower and only a small proportion of the larger plants flower in any one year. *L. dortmanna* is, however, almost completely limited to sexual reproduction, particularly for dispersal.

In the conditions for seed germination it is seen again that light is very important in controlling growth in this species. The seeds require light and this may be mediated via phytochrome.

It can be concluded that the depth zonation of *L. dortmanna* is not controlled by seed germination. The seeds will readily germinate in light regimes that can be found at depths well below any known depth limit for *L. dortmanna*. Seedlings will not subsequently survive. It is also probable that seeds will germinate in areas of the littoral not suitable for further growth of the plant.

As the control of zonation is not by germination but by an effect on growth, it is therefore necessary to examine the ability

of *L. dortmanna* plants to vary their phsyiology with depth in a loch and test whether this can explain the zonation of the plant.

CHAPTER FOUR

METABOLIC ADAPTATION AND ZONATION

Chapter 4. Metabolic Adaptation and Zonation

4.1. Introduction

The physiology of the isoetids has been variously studied. However, most of the work has centered around a consideration of the supply of exogenous carbon for photosynthesis. Raven (1970) and Wium-Andersen and Andersen (1972) have shown that the carbon dioxide concentration in the sediments in the habitats normally colonised by isoetids is at least an order of magnitude greater than the water above. This is so in lochs with a low pH, which is the usual habitat of the isoetids (Spence, 1964). However, most species are capable of growing at higher pH (up to pH 9.0) and in lochs of high alkalinity (Spence et al., 1979; Seddon, 1965) where such a condition may not apply. Roeloffs et al. (1984) also showed that at a very low pH, associated with the acidification of lakes, the supply of sediment CO_2 may be considerably decreased.

Luther (1983) noted that isoetids have a root:shoot ratio of at least 0.5. This was considered large for aquatic macrophytes. This is consistent with the studies of Steemann-Nielsen (1960), Wium-Andersen (1971), Søndergaard and Sand-Jensen (1979), Richardson et al. (1984) and Keeley et al. (1984) who have shown that *Lobelia dortmanna*, *Littorella uniflora*, *Isoetes lacustris* and *Stylites andicola* absorb CO_2 from the sediment via the root system for photosynthesis. Sand-Jensen et al. (1982) also showed that these plants show a considerable oxygen efflux from the root systems. In *L. dortmanna* almost 100% of the gas exchange was found to occur via the root system. *L. uniflora* and *I. lacustris* show some gas exchange between the leaves and the lake water.

The isoetids have also been noted by their possession of submerged CAM, i.e. of dark CO_2 fixation and acidification and light

deacidification and photosynthetic fixation of the CO_2. *L. uniflora*, all submerged *Isoetes* species and *S. andicola* have been shown to possess such diurnal fluctuations in acidity (Keeley, 1982; Keeley and Morton, 1982 and Keeley et al., 1984). *L. dortmanna*, however, lacks such a feature (Boston and Adams, 1983; Richardson et al., 1984). Keeley (1981a, b) considered that such dark CO_2 fixation is a particular adaptive advantage to isoetids growing in lakes low in dissolved inorganic carbon. Boston and Adams (1985) have shown in a study of the relative contribution of daytime and nightime CO_2 fixation in *I. macrospora* and *L. uniflora* across the growth seasons that night fixation contributes approximately a half of the carbon fixed in these species.

A further adaptation of the isoetids to enhance carbon gain was shown by Søndergaard (1979). He found that the large lacunae in the leaves of *L. dortmanna* and *L. uniflora* are efficient at trapping CO_2 generated in the surrounding cells by photorespiration, instead of it being lost to the surrounding water.

The general morphology of the isoetids has been cited as being functionally extremely important in these physiological attributes. In particular the possession of large lacunae in the leaves and roots and a thick cuticle in *L. dortmanna* and *L. uniflora* have been suggested as being necessary for the diffusion of CO_2 and O_2 between the leaves and the sediment (Sand-Jensen et al., 1982). A detailed examination has not, however, been undertaken.

The physiology of aquatic macrophytes has been shown to be extremely important in determining zonation. In general these studies have concentrated on the effect of changing light regimes on photosynthesis. Spence, Campbell and Chrystal (1973) found that the specific leaf area (SLA) of five *Potamogeton* species increased linearly with depth. The degree of increase in SLA depended on the

spectral character of the lochs concerned, changes in SLA being correlated with the attenuation in PAR rather than any effect of the R:FR.ratios. The degree to which any species could alter its SLA determined the zonation of that species in a loch. For instance, *P. polygonifolius* was least able to increase its SLA and was found to be the species distributed in the most shallow water. Spence and Chrystal (1970a) also found that deep water *Potamogeton* exhibited a lower dark respiration rate than shallow water leaves of the same species. This is an obvious adaptation to reduce the loss of fixed carbon in deep water where carbon-fixation in the light is reduced. Sand-Jensen (1978) found that the SLA of *I. lacustris* (from 2 m depth) was greater (0.195 cm^2/mg dwt) than that of *L. uniflora* (0.240 cm^2/mg dwt) (from 0.5 m depth). Also that *I. lacustris* was able to photosynthesise at slightly higher rates in low light and exhibited much lower leaf and root dark respiration rates. Sand-Jensen suggested that this adaptation of *I. lacustris* enabled it to colonise to greater depths in a lake that *L. uniflora*. It is unfortunate, however, that the two species were not studied from the same depths where a direct comparison could be made.

Kirk (1983) also records further adaptations that aquatic plants may exhibit to allow for photosynthesis at depth. Some macrophytes exhibit changes in their photosynthetic pigments. Thus the total chlorophyll content may rise with depth, the a:b ratio may decline. Other antennal pigments such as carotenoids, apart from chlorophyll b, may also increase in concentration. Kirk (1983) states that this feature varies amongst macrophytes, and that further studies are necessary. Søndergaard (unpublished data) found plants of *L. uniflora* from 2.3 m depth had nearly twice the chlorophyll content of plants from 0.2 m. The chlorophyll a:b ratio also declined and the carotenoid content increased.

The photosynthetic consequences of these adaptations are revealed as changes in the variation of photosynthetic rate with changes in photon fluence rate. Thus high-light adapted plants require a higher light intensity to saturate photosynthesis than low-light adapted plants. Both types of plant have similar photosynthetic rates per unit chlorophyll in low light, but as low-light adapted plants have more chlorophyll per unit biomass they consequently have a high photosynthetic rate per unit biomass. This has been well demonstrated in a number of phytoplankta (Kirk, 1983).

Shade adaptation is further complicated by the fact that shading may cause the number of photosynthetic units to increase while causing the amount of carboxylase in a cell to decrease. In other words the plant is expanding more energy on the capture of light and less on the capture of carbon (Boardman, 1977). Again this has been demonstrated in phytoplankta (Kirk, 1983).

An understanding of carboxylases in freshwater aquatic macrophytes is, however, very limited. Two carboxylases have to be considered - Ribulose bis-phosphate carboxylase-oxygenase (RuBPcase) and Phosph oenol pyruvate carboxylase (PEPcase). These assimilate CO_2 into C_3 compounds and C_4 compounds respectively. Almost all angiosperms contain both enzymes but the ratio of the two has important physiological and ecological consequences. In macrophytes high PEPcase levels have been associated with both the re-fixation of photorespirationally lost CO_2 and low CO_2 compensation points. Terrestrial CAM plants also show high PEPcase levels.

This chapter will seek a wider understanding of the distribution and ratios of the two enzymes in aquatic macrophytes so that results from the isoetids can be set in context. A wide range of species will be considered, though the isoetids will be more closely studied. As CO_2 is absorbed via the root system the roots will also be

examined for the presence of carboxylases. *L. dortmanna* will also be studied for adaptations in its carboxylase:chlorophyll ratio with increasing depth. This latter study will be further complemented by an examination of the photosynthetic characters of plants from two depths.

The isoetids have not been completely examined with respect to diurnal acid fluctuations. This chapter will, therefore, extend this knowledge and place it in the context of the above enzymatic studies.

Finally detailed structural studies will be undertaken on *L. dortmanna* and *L. uniflora* to elucidate what morphological adaptations these plants have to allow for their physiological function.

This study will, therefore, provide a physiological comparison between *L. dortmanna* and other macrophytes, particularly the other isoetids. The adaptability of *L. dortmanna* to increasing aquatic shade will also be revealed. It is then hoped that this will provide a guide as to the zonation of the plant in freshwater lakes.

4.2. Methods

4.2.1. Photosynthetic measurements

In September 1984 plants of *L. dortmanna* were collected from 0.2 m and 1.0 m depth (using SCUBA) from Lochan-na-Thuill. These were transported to St. Andrews, and kept in low light at 5°C until use. Photosynthesis was measured using the apparatus in Chapter 2 (section 2.3.1.). Photosynthetic rates were measured at 10°C (the ambient temperature of the lochan) over a range of photon fluence rates. Two plants from each depth were studied. The changes in photon fluence rate were made by placing different thicknesses of a neutral density filter (white perspex) in front of the water jacket (Figure 2.1). After each alteration in fluence rate the recordings from the oxygen electrode were allowed to stabilise before any results were taken.

All determinations on any single plant were finished before a point where carbon supply was observed to become limiting.

4.2.2. Diurnal Acid Fluctuations

Two studies were made of diurnal acid fluctuations in a range of isoetids and similar plants. Specimens of the British isoetids (excluding *Isoetes echinospora*) and *Pilularia globulifera* were collected from a number of sites (see Table 4.2). *P. globulifera* is, like *I. lacustris*, a rooted, submerged pteridophyte. They were grown in a Conviron growth cabinet at 25°C under white fluorescent light, for 16 hr day each 24 hr day. Plants from a terrestrial population of *L. uniflora* were also included.

In November 1984 plants were collected from Lochan-na-Thuill and measured directly for titratable acidity in a nearby field laboratory in Durness. Plants were collected at two depths, *L. dortmanna*, *L. uniflora* and *I. lacustris* from 75 cm and *L. dortmanna*, *L. uniflora* and *R. flammula* from 10 cm.

In both experiments plants were harvested at dawn and dusk. The leaves were wiped and ground in 15 ml of cold CO_2-free de-ionized water. The extract was filtered through muslin and then centrifuged to remove remaining plant debris - 10 ml of the supernatant was titrated against 0.01 NaOH (made up with CO_2-free de-ionized water) to pH 6.4 (as in Keeley, 1982). In studies using plants grown in the Conviron growth cabinet, the extraction procedure was carried out in a 5°C cold room. This was not possible for the field studies.

4.2.3. Carboxylase Determinations

A wide range of aquatic macrophytes were collected from lochs or culture tanks from March to May 1985. Before assaying, all species were kept in illuminated, aerated water at 15°C for 24 hours to activate the carboxylase enzymes. For a range of isoetids the roots

were also examined for carboxylases. In order to prevent exposure of the roots to light, plants were potted by the lochside with as little disturbance to the sediment around the roots as possible.

Plants of *L. dortmanna* were collected from a range of depths (0.1-1.75 m) in Loch Fiacail using SCUBA. Deep water plants could not be collected with intact sediment around the root systems. However, they were immediately potted in fresh sediment upon being brought to the surface.

Healthy tissue was selected, washed and loosely attached epiphytes removed by wiping. The tough leaves of the isoetids were further rubbed with Whatman GF-C filter paper. About 0.5 g of tissue was used for each determination. One extraction was made for each species. However, with the plants of *L. dortmanna* collected in Loch Fiacail two determinations of leaf carboxylases were made for each depth. The tissue was ground in a chilled glass homogeniser in the extraction mixture, previously kept on ice, shown in Table 4.1. A sample from the extracted medium was removed for chlorophyll determination and the remainder was filtered through a 0.45 µm millipore filter. The carboxylase determinations were carried out in scintillation vials kept in a shaking water bath at 30°C. Each vial contained an assay mixture as in Table 4.1. Before assaying 1 ml of the extraction medium was added to 0.05 ml of $MgCl_2$ solution (4.0662 g/100 ml H_20) and 0.05 ml bicarbonate solution (200 µm) to activate the RuBPcase. This was used for RuBPcase determinations, whereas the original extract was used for PEPcase assays. 0.1 ml of extract was used for each RuBPcase or PEPcase assay. Three determinations of each enzyme per sample were taken. Results are expressed as a mean of these three determinations. Each RuBPcase assay reaction took 45 seconds to complete, at which point it was killed by a saturated solution of DNPH in 6N HCl. The PEPcase reaction took three minutes and was killed with 6N HCl.

Table 4.1.

The composition of the extraction and assay media for carboxylase determinations.

Extraction medium

Constituents	Volume added per 6 ml in a glass homogenizer (ml)
Tris (6.07 g/100 ml H_2O) pH 8.0	0.6
$MgCl_2$ (4.0662 g/100 ml H_2O)	0.3
DTT (0.1542 g/10 ml)	0.6
Sodium Isoascorbate (0.0881 g/10 ml)	0.6
De-ionised water	3.3
EDTA (0.0372 g/100 ml)	0.6
PVP	250 mg } Added as
BSA	50 mg } solid

Assay medium

Constituents (made up as above)	Volume added to each vial (ml)
Tris	0.1
$MgCl_2$	0.05
EDTA	0.01
DTT	0.01
De-ionised water	0.35
PEP (0.1771 g/10 ml) or RuBP (5 mM)	0.1
$NaH^{14}CO_3$ (200 mM)	0.1 (0.05 + 0.5 H_2O for PEPcase)
Enzyme extract	0.1
Total assay	1.0

Blanks, without PEP or RuBP, were taken for both RuBPcase and PEPcase determinations.

After killing, the vials were dried-down overnight. For counting the samples were resuspended in 2-5 ml of de-ionized water and 5 ml of Phase Combining System (Amersham). Total counts of the stock $H^{14}CO_3^-$ solution were made up using 5 µl of the solution made up for counting as with the assay samples, but with the addition of a drop of NaOH solution to avoid loss of $^{14}CO_2$.

The samples were counted for 10 minutes (totals for 1 minute). This gives a figure of counts per minute. In order to convert this to destructions per minute counts are made using a standard solution of ^{14}C toluene (0.985×10^6 disintegrations/min. at 20°C). This method follows that of Salvucci and Bowes (1981, 1982). Although not optimised for these species, this procedure produces activities similar to other macrophytes.

Chlorophylls were determined as follows. 0.3 ml of the original extract was added to 0.3 ml of de-ionised water and to 2.6 ml of acetone. This was centrifuged and the absorbtion of the supernatant measured at 663 nm and 645 nm using a SP600 spectrophotometer. Corrections for general absorption were made using a reading taken at 720 nm. Three determinations were made for each extract. The estimation following that of Arnon (1949).

4.2.4. Plant Morphology

The structure of the leaves and roots of the different isoetids were variously studied. *L. dortmanna* and *L. uniflora* were the most intensively studied. Plants of both terrestrial and submerged forms were collected for both species. The gross morphology was examined

by dissection under a dissecting microscope. Sections were also made using a freezing microtome, and all studies were made using fresh material. The only stain used was Sudan IV in order to observe cuticle thickness and the Casparian strip in the endodermis. Measurements were made using an eyepiece graticule. Drawings were made using a drawing tube.

The general leaf and root morphology of *I. lacustris* and *I. hystrix* was also examined. *I. hystrix* was collected from a terrestrial location in Southern Spain and stored in 70% ethanol before examination.

4.3 Results and Discussion

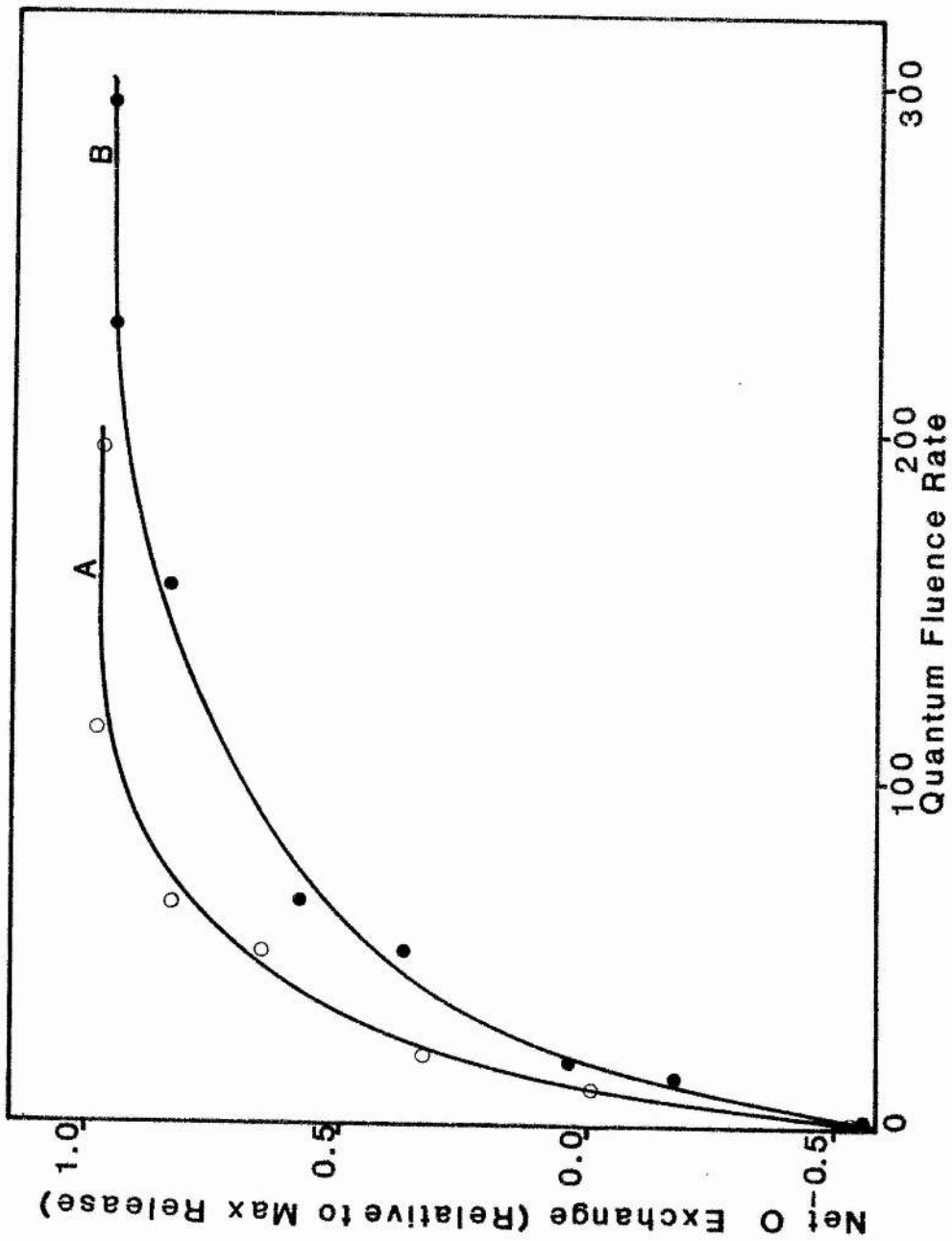
4.3.1 Photosynthetic Measurements

The effect of fluence rate on the rate of net O_2 exchange from plants of *L. dortmanna* from two depths is shown in Figure 4.1. There is a large difference between the two plant types. The plants from 1 m have a lower light compensation point (LCP) of about 10 $\mu E\ m^{-2}\ s^{-1}$ and saturate at 90-100 $\mu E\ m^{-2}\ s^{-1}$. The shallow water plants have a LCP of about 16 $\mu E\ m^{-2}\ s^{-1}$ and saturate at over 200 $\mu E\ m^{-2}\ s^{-1}$. The saturation level of the latter is similar to these found by Sand-Jensen and Borum (1984). Søndergaard (unpublished data) found that plants of *L. uniflora* from 2.3 m have a LCP of 10-14 $\mu E\ m^{-2}\ s^{-1}$ and from 0.2 m of 17-18 $\mu E\ m^{-2}\ s^{-1}$ from June to September. Later in the year, both types have LCPs of 5-6 $\mu E\ m^{-2}\ s^{-1}$.

This ability to alter photosynthetic responses to varying fluence rates is characteristic of many aquatic plants (Kirk, 1983). However, the differences in the LCPs are not great and Kirk (1983) suggests that such a feature is characteristic of plants not particularly able to adapt well to shade. Whether *L. dortmanna* can reduce its LCP further is unknown. *L. uniflora* (Sondergaard,

Figure 4.1.

Photosynthesis-light curves for two sets of plants of *Lobelia dortmanna* collected in September 1984 from two depths - 1.0m (A) and 0.2m (B). Determinations were made using the apparatus in Figure 2. Each point is a mean of two determinations made using two separate plants. The oxygen release is expressed as a relative figure compared to maximum release. Maximum release for plants from 1.0m was 1.51 mg O_2/g fwt/hr, and for plants from 0.2m was 1.92 mg O_2/g fwt/hr.


Net O Exchange (Relative to Max Release)
1.0
0.5
0.0
−0.5
0
100
200
300
Quantum Fluence Rate
A
B

unpublished data) can, and this may be due to changes in pigment levels (see Introduction). This may explain the ability of *L. uniflora* to penetrate to greater depths.

4.3.2. Diurnal acid fluctuations

The results are presented in Tables 4.2 and 4.3. The presence of diurnal acid fluctuations in *L. dortmanna* and *R. flammula* is confirmed (Keeley, 1982; Keeley and Morton, 1982; Boston and Adams, 1983). Keeley et al. (1983) found that as populations of *I. howellii* became emergent they lost any diurnal fluctuations in titratable acidity. In this study plants from terrestrial populations of *L. uniflora* have such fluctuations (Table 4.2). Keeley et al. (1984) did find such fluctuations in *S. andicola*, a terrestrial plant. Keeley et al. (1983) also found that shallow water plants of *I. howellii* exhibited greater absolute diurnal fluctuations than deep water populations. This study shows this to be the case also for *L. uniflora*. As it is unlikely that the carbon supply from the sediment is reduced at greater depth in the lochan, the reduction in accumulated acidity is probably due to lower enzyme levels in the plants. Such an adaptation has also been suggested as a reason for reduced respiration rates in deep water plants (Sand-Jensen, 1978).

The other isoetids and *P. globulifera* lack any diurnal acid fluctuations, and so, presumably, lack the CAM pathway. CAM is, therefore, only known from three isoetid genera - *Littorella*, *Isoetes* and *Stylites*. *Stylites* is closely related to *Isoetes* and some taxonomists would place the two genera together (Foster and Gifford, 1974).

The significance of submerged CAM has been heavily debated. Keeley et al. (1984) considered that the possession of CAM and root uptake of CO_2 by pteridophytes like *Isoetes* and *Stylites* may possibly

Table 4.2.

Leaves of titratable acidity (to pH 6.4) at start of day (06.00h) and end of day (22.00h) in leaves (and roots of *L. dortmanna*) of submerged plants of named species (and terrestrial plants of *L. uniflora*). Diurnal change in titratable acidity is expressed as the ratio of acidity at start of day to that at end of day. Plants were grown in a Conviron growth cabinet, 25°C, 16h day. Means ± S.E.

Species	Loch source in Scotland	Locality	n	Titratable Acidity µeq g^{-1} fwt		Diurnal change
				06.00	22.00	
Lobelia dortmanna						
Leaves	Loch Dow, Fife	3°27'W 56°9'N	3	14.0 ± 1.4	21.1 ± 1.7	0.7
Roots	Loch Dow, Fife	3°27'W 56°9'N	2	0.0 ± 0.0	0.0 ± 0.0	0.0
Littorella uniflora submerged	Loch na Thuill, Sutherland	5°0'W 58°24'N	3	65.7 ± 9.1	13.8 ± 0.4	4.8
L. uniflora terrestrial	Loch Fruechie, Tayside	3°50'W 56°31'N	3	139.1 ± 11.6	19.5 ± 1.6	7.1
Eriocaulon septangulare	Loch nan Eilean, Sligachan, Skye	6°12'W 57°17'N	3	4.5 ± 1.0	5.1 ± 0.8	0.9
Subularia aquatica	Loch Borrallen, Sutherland	4°56'W 58°03'N	2	12.5 ± 0.4	11.9 ± 0.7	1.1
Pilularia globulifera	Loch Borrallen, Sutherland	4°56'W 58°03'N	2	12.4 ± 5.9	25.0 ± 0.5	0.5
Isoetes lacustris	Loch na Thuill, Sutherland	5°0'W 58°24'N	2	82.8 ± 18.2	37.7 ± 11.7	2.2

Table 4.3.

Levels of titratable acidity (to pH 6.4) just after dawn (08.15h) and just before dusk (15.15h) in November 1984 from two depths in leaves of submerged species from Loch-na-Thuill, Sutherland. Diurnal change in titratable acidity is expressed as the ratio of acidity just after dawn to that just before dusk. Means ± S.E. n = 2.

Species	Titratable acidity $\mu eq\ g^{-1}$ fwt		Diurnal change
	8.15 hr	15.15 hr	
10 cm depth			
Lobelia dortmanna	21.8 ± 4.4	20.6 ± 10.8	1.1
Littorella uniflora	141.7 ± 26.7	57.4 ± 3.4	2.5
Ranunculus flammula	22.3 ± 7.1	24.0 ± 2.9	0.9
75 cm depth			
Lobelia dortmanna	15.4 ± 2.6	9.9 ± 1.1	1.5
Littorella uniflora	78.3 ± 13.0	29.5 ± 3.6	2.7
Isoetes lacustris	107.7 ± 14.5	20.3 ± 10.9	5.3

represent a primitive feature associated with the early colonization of land by vascular plants. However, CAM has arisen many times in evolution (Osmond, Winter and Ziegler, 1982) and *Isoetes* and *Stylites* are thought to be advanced genera (Thomas, 1985). These genera represent terrestrial families that have subsequently become aquatic, so it is inadvisable to make inferences about the evolution to the terrestrial habit from such physiological studies.

4.3.3. Carboxylases - species variation

The results are presented in Tables 4.4 and 4.5. They show that RuBPcase is the principal carboxylating enzyme in all the species studied, as has been found previously in a number of freshwater macrophytes (Beer and Wetzel, 1982; Salvucci and Bowes, 1981). The low RuBPcase/PEPcase quotient of *L. uniflora* is consistent with its possession of submerged CAM, although *I. lacustris* has a slightly higher quotient. *Fontinalis antipyretica*, *L. dortmanna*, *P. globulifera*, *Potamogeton crispus* all lack CAM (Keeley and Morton, 1982; Boston and Adams, 1983; and this study), which is consistent with the low levels of PEPcase activity found in these species. Two previously little-studied species, *Juncus bulbosus* var. *fluitans* and *P. praelongus* show low quotients. Both species deserve further study.

In the three amphibious species the aerial or floating leaves had higher total levels of carboxylating enzymes than the submerged leaves. This is probably due to photosynthesis being limited by rates of transport of CO_2 underwater (Black et al., 1981) rather than lower light levels. The RuBPcase/PEPcase quotient was lower in submerged, compared to aerial or floating leaves, which agrees with previous work (Salvucci and Bowes, 1981; Salvucci and Bowes, 1982), although there is only a small difference in the quotient in *Hippuris vulgaris*.

Table 4.4.

RUBPcase and PEPcase activities and RuBPcase/PEPcase quotients for freshwater macrophytes (non-isoetids).

Species	Collection site	Carboxylase activity µmol CO_2 mg $CH^{-1}h^{-1}$		RuPBcase/ PEPcase
		RuBPcase	PEPcase	
Fontinalis antipyretica	Loch Borrallen, Sutherland 4°56'W 58°03'N	64	0	-
Hippuris vulgaris (aerial)	Culture	1030	36	28.6
(submerged)	Culture	177	7	25.3
Juncus bulbosus var. *fluitans*	Loch na Thuill, Sutherland 5°0'W 58°24'N	177	54	3.3
Mentha aquatica (aerial)	Loch Lindores, Fife 3°11'W 56°20'N	431	13	33.2
(submerged)	Loch Lindores, Fife 3°11'W 56°20'N	161	17	9.5
Nitella flexilis	Lumley Den, Angus 2°58'W 3°33'N	53	5	10.6
Pilularia globulifera	Loch Borrallen, Sutherland 4°56'W 58°03'N	250	33	7.6
Potamogeton crispus	Lumley Den, Angus 2°58'W 3°33'N	262	38	6.9

Table 4.4 contd.

Species	Collection site	Carboxylase activity $\mu mol\ CO_2\ mg\ Ch^{-1}h^{-1}$		RuPBcase/ PEPcase
		RuBPcase	PEPcase	
P. polygonifolius (floating)	Loch na Thuill, Sutherland 5°9'W 58°24'N	417	12	34.8
(submerged)	Loch na Thuill, Sutherland 5°9'W 58°24'N	332	29	11.5
P. praelongus	Loch Caladail, Sutherland 4°46'W 58°33'N	115	35	3.3
P. schweinfurthii	Culture	158	18	8.8
Ranunculus flammula	Loch na Thuill, Sutherland 5°0'W 58°24'N	494	17	29.1
Riccardia pinguis	Loch na Thuill, Sutherland 5°0'W 58°24'N	36	3	12.0

Table 4.5.

RuBPcase and PEPcase activities and RuBPcase/PEPcase quotients for freshwater macrophytes (isoetids).

Species	Collection Site	Leaf or Root	Carboxylase activity				RuBPcase/ PEPcase
			µmol CO_2 mg fr wt^{-1}/h^{-1}		µmol CO_2 mg $Chl^{-1}h^{-1}$		
			RuBPcase	PEPcase	RuBPcase	PEPcase	
Lobelia dortmanna	Loch-na-Thuill, Sutherland 5°0'W 58°24'N	L	24.3	1.1	268	12	22.3
		R	-	4.5	-	-	
Littorella uniflora	Loch-na-Thuill, Sutherland 5°0'W 58°24'N	L	16.9	11.4	217	105	2.1
		R	-	-	-	-	
Isoetes lacustris	Loch Dow, Fife 3°27'W 56°9'N	L	44.2	12.9	75	22	3.4
		R	-	-	-	-	
Eriocaulon septangulare	Loch nan Eilean, Skye 6°12'W 57°17'N	L	30.2	6.0	48	10	4.8
		R	-	-	-	-	

Table 4.6.

RuBPcase and PEPcase activities, RuBPcase/PEPcase quotients and chlorophyll content for leaves and roots of *L. dortmanna* from a range of depths.

Depth (m)	Leaf or Root	n	Carboxylase activity				RuBPcase/ PEPcase	Leaf chlorophyll content mg/g fwt
			µmol CO_2 mg fr wt^{-1}/h^{-1}		µmol CO_2 mg $Chl^{-1}h^{-1}$			
			RuBPcase	PEPCase	RuBPcase	PEPcase		
0.1		2	21.9	1.6	188	14	13.4	130
		1	0.0	0.0	-	-		
0.25		2	48.3	7.1	299	38	7.9	160
		1	0.0	1.40	-	-		
0.75		2	39.6	1.7	222	10	22.2	160
		1	0.0	0.21	-	-		
1.25		2	29.6	1.3	165	8	27.6	180
		1	0.0	0.51	-	-		
1.75		2	55.8	3.6	291	18	16.2	210
		1	0.0	0.98	-	-		

An alternative approach to the study of the carboxylating enzymes of freshwater plants has been the use of carbon isotopes. This technique rests on the fact the RuBPcase selectively discriminates between two naturally occurring carbon isotopes, ^{12}C and ^{13}C, favouring $^{12}CO_2$. PEPcase, on the other hand, does not discriminate. For freshwater studies two values have to be established. These are the $^{12}C:^{13}C$ ratio for the sample and that of a recognised standard (a belnemnite limestone). The difference between these two ratios is referred to as $\delta^{13}C$. The $\delta^{13}C$ is also calculated for the carbon source of the plant. The difference between the $\delta^{13}C$ of the source and that of the plant is known as $\Delta\delta^{13}C$. Only with the latter figure can meaningful comparisons be made. Plants that fix carbon predominantly via RuBPcase have a more negative $\Delta\delta^{13}C$ value than those fixing carbon via PEPcase.

Published data suggests that CO_2 fixation is primarily via RuBPcase for *F. antipyretica*, emergent and submerged shoots of *H. vulgaris* (Osmond et al,, 1981), *P. crispus* (Lazerte and Szalados, 1982) and *L. dortmanna* (Richardson et al., 1984). This is in agreement with the data in Table 4. $\Delta\delta^{13}C$ values for *I. lacustris* (Richardson et al., 1984) suggests fixation of CO_2 via PEPcase, and this is consistent with the submerged CAM previously reported (Keeley, 1982) and the RuBPcase/PEPcase quotient found in this study.

It has been shown that PEPcase activity is higher in summer, compared to winter grown *Hydrilla verticillata* (Holaday et al, 1983). The material collected for this study was obtained in March. It is possible that higher PEPcase levels may be found in these species in the summer.

Table 4.5 also shows that for four isoetids only one has any carboxylase enzymes in its roots. Roots of *L. dortmanna* contain PEPcase, though the quantity is small compared to leaf levels.

I. lacustris and *L. uniflora* both exhibit root uptake of CO_2 (Richardson et al., 1984; Søndergaard and Sand-Jensen, 1979), but no fixation occurs in the roots. *E. septangulare* has not been studied for root uptake. The roots of *I. lacustris* and *L. uniflora* do not exhibit diurnal changes in acid fluctuations (Richardson et al., 1984; Boston and Adams, 1983) and this is consistent with their lack of carboxylating enzymes. *L. dortmanna* roots also have no diurnal fluctuations in acidity (Table 4.2). The PEPcase may play a role in absorbing CO_2 via the root system, both by taking CO_2 from the sediment during the day and by absorbing CO_2 lost by respiration at night. The level is, however, low and the physiological significance should not be over-stressed.

It should be noted, however, that PEPcase can occur in roots in a number of species. The PEPcase is postulated to be a means of generating malate instead of ethanol in flood-tolerant species (Crawford, 1978). As the roots of *L. dortmanna* are well oxygenated (from photosynthetic production in the leaves) the presence of the enzyme cannot be explained as a means of tolerating hypoxia.

4.3.4. Carboxylases - Depth Variation in *L. dortmanna*

Table 4.6 shows the leaf and root carboxylases for *L. dortmanna* from a range of depths from 0.1 m to 1.8 m. The low level of root PEPcase is consistent at most depths, though lacking in the most shallow plant. There is no observable trend in either carboxylase or the RuBPcase/PEPcase quotient. This would suggest that *L. dortmanna* is not able to vary the proportion of carboxylase compared to its chlorophyll content. This is characteristic of plants not able to adapt to shade conditions (Kirk, 1983).

Table 4.6 also reveals that the chlorophyll content in the leaves increases with depths. The plants at 1.75 m have about 1.6

times the concentration of chlorophyll of plants at 0.1 m. Similar increases have been reported in *L. uniflora* (Søndergaard, unpublished data). It thus seems that in *L. dortmanna* the total photosynthetic apparatus is increased with shading. This is not an adaptation to shading common to shade tolerant plants (Kirk, 1983), which increase the light capturing part of the photosynthetic apparatus without increasing the carbon accumulating part.

4.3.5. Plant Structure

The structural features and dimensions of *L. dortmanna* and *L. uniflora* are presented in Figures 4.2-4.6 and Table 4.7.

The leaves of *L. dortmanna* contain two longitudinally continuous lacunae. Surrounding these are highly vacuolated cortical cells, which do not contain chloroplasts. Chloroplasts are limited to the sub-epidermal layers, particularly concentrated on the upper surface of the leaf. This side also contains peripheral vascular bundles (Figure 4.3). The roots have a central stele surrounded by a number of lacunae. Dissection reveals that these are not continuous with the leaf lacunae, except in the oldest leaves. Usually a thin plate of tissue (250 µm thick) separates the two lacunal systems. The roots develop at the bases of the leaves, each root (two to four per leaf) being associated with a particular leaf lacuna.

In contrast, *L. uniflora* leaves have a series of discontinuous longitudinal lacunae. The cortical cells surrounding these contain chloroplasts, and peripheral vascular bundles are distributed around the whole leaf. The roots have a similar structure to those of *L. dortmanna*. However, they do not arise from the leaf bases, but from the stem, which is spongy, being filled with small lacunae. In the leaves of both species the vascular bundles are well-developed, in contrast to the reduced vascular systems of a number of macrophytes

Figure 4.2.

Diagrams of dissections of the leaves and roots of *Lobelia dortmanna* (L.d.) and the leaves, stem and roots of *Littorella uniflora* (L.u.). These show the lacunae (L) in the leaves (lf), stem (S) and roots (R).

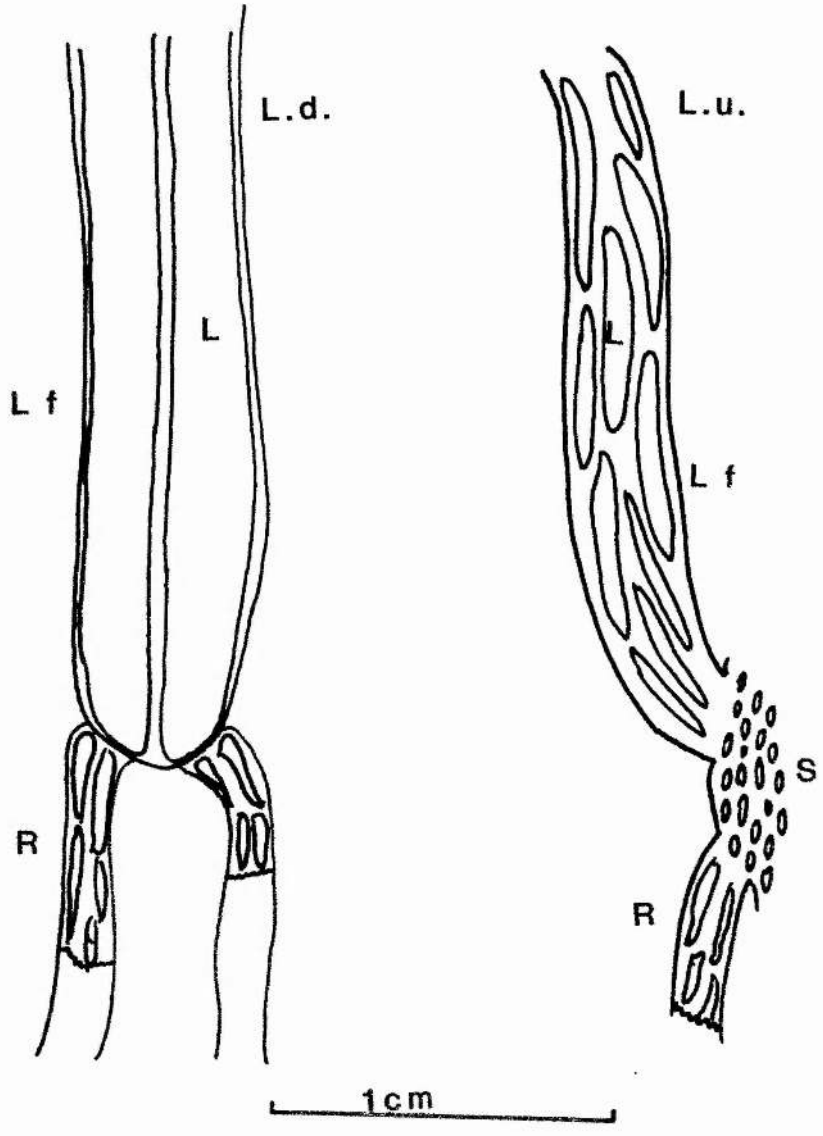
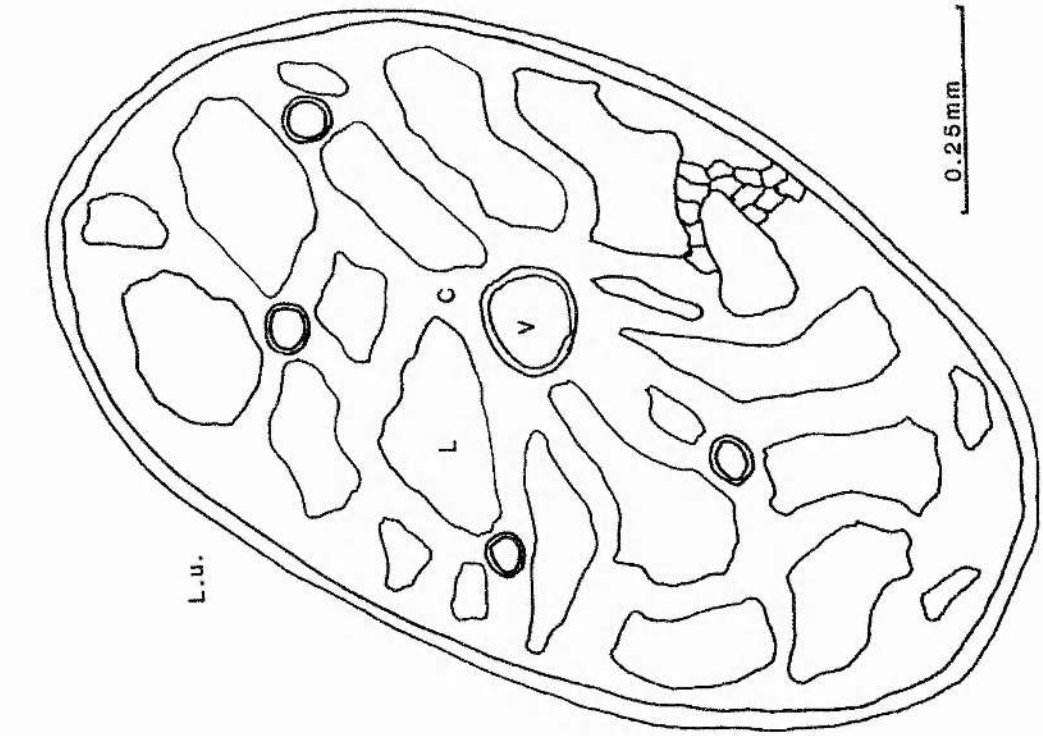

L.d.
L
L f
R
L.u.
L
L f
S
R
1 cm

Figure 4.3.

Transverse sections of leaves of *Lobelia dortmanna* (L.d.) and *Littorella uniflora* (L.u.) showing the position of the lacunae (L), cortex (C) and vascular traces (V), both central and peripheral.


0.25mm
L.u.
V
L
C

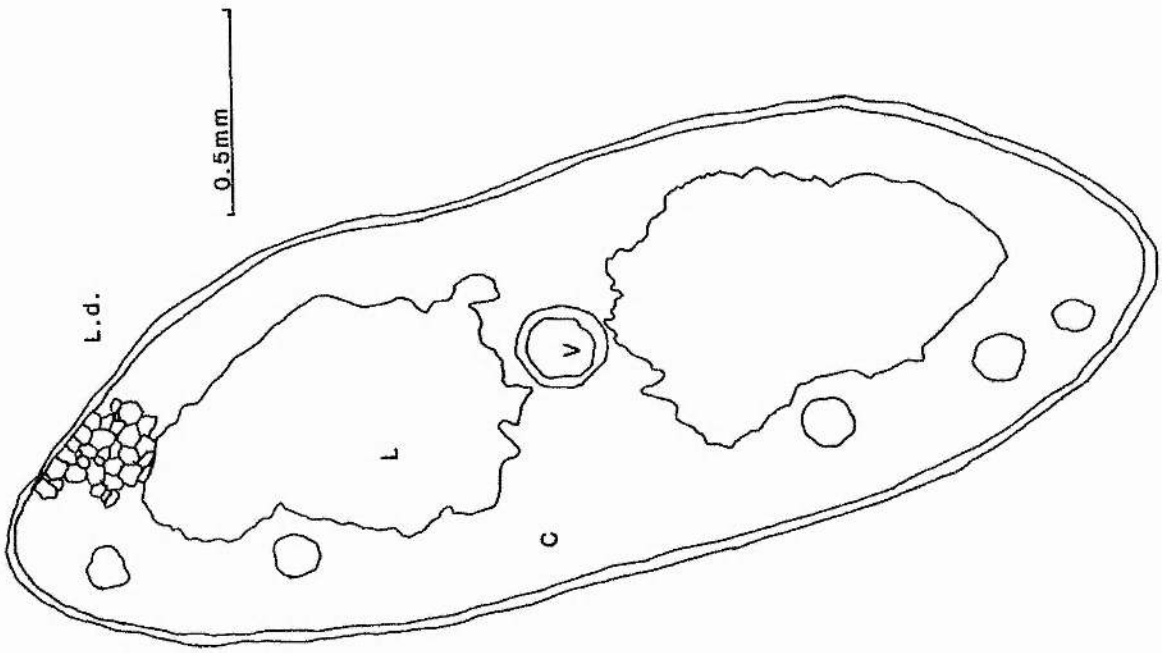
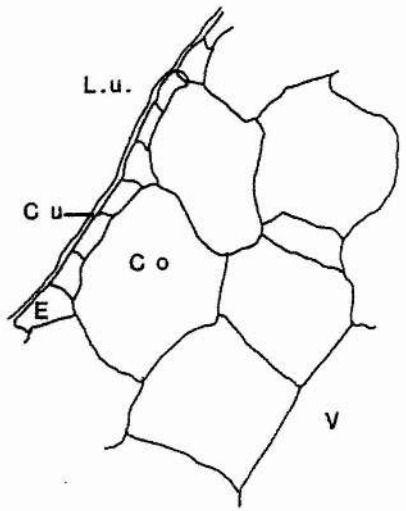
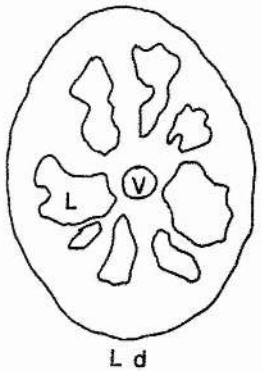

0.5mm
L.d.
V
L
C

Figure 4.4.

Transverse sections of: a. Leaves of *Littorella uniflora* (L.u.) and *Lobelia dortmanna* (L.d.) showing the cortical cells (Co), epidermis (E), cuticle (Cu) and vacuole (V); b. Roots of the two species showing the lacunae (L) and vascular traces (V).



10um



0.5mm

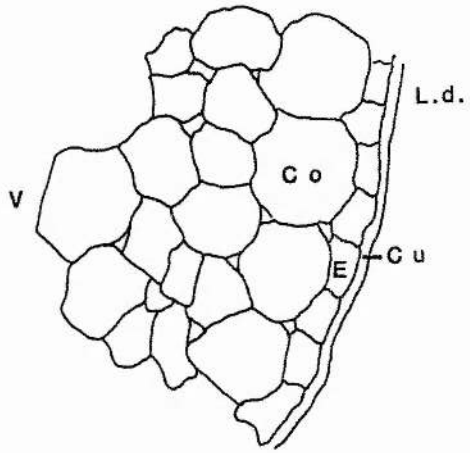
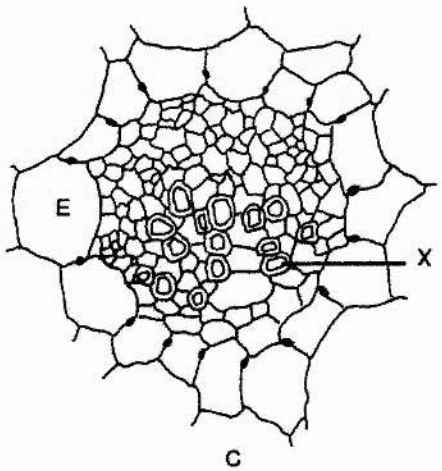


Figure 4.5.

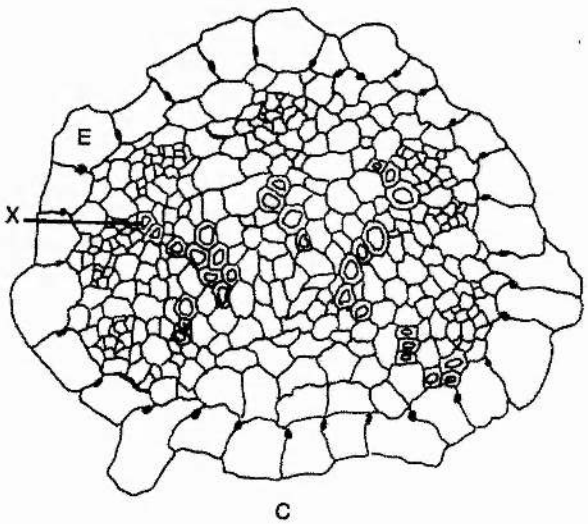
Transverse sections of the central (C) and peripheral (P) vascular traces of *Lobelia dortmanna* (L.d.) and *Littorella uniflora* (L.u.), showing the well developed vascular system with xylem cells (X) and Casparian strips in the endodermis (E).

L.u.

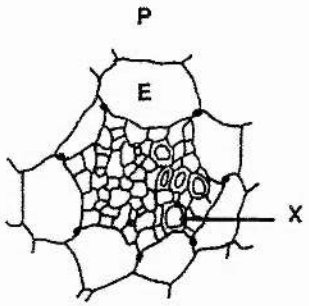


10um

L.d.



10um



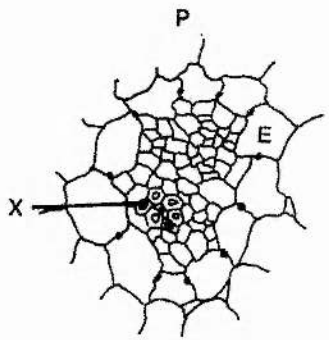


Figure 4.6.

Diagrams of transverse sections of leaves and roots of *Littorella uniflora* (L.u.) (terrestrial), *Isoetes lacustris* (I.l.) (submerged) and *I. hystrix* (I.l.) showing the changes in the lacunal systems (L) in these species caused by emergence.

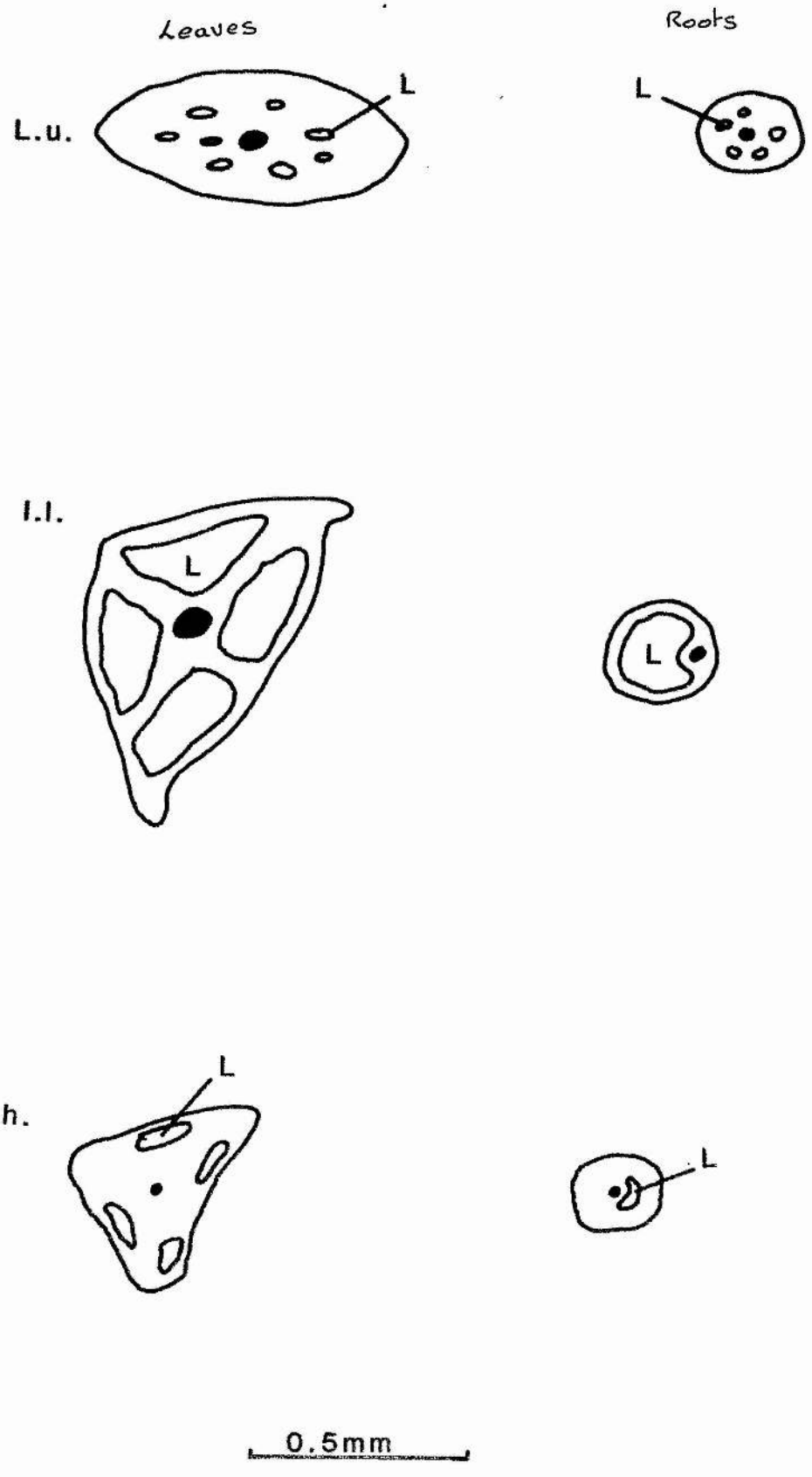

Leaves
Roots
L.u.
L
L
I.l.
L
L
I.h.
L
L
0.5mm

Table 4.7.

Cell dimensions, cuticle widths and stomata occurrence for the leaves of a number of isoetids, both submerged and terrestrial.

Species	Epidermal cells Area of T.S. (μm^2) ± S.E. n = 19	Cortical cell Area of T.S. (μm^2) ± S.E. n = 10	Cuticle width (μm) ± S.E. n = 10	Stomata present or absent
Lobelia dortmanna				
submerged	467 ± 17	2124 ± 416	4.1 ± 0.1	absent
terrestrial	389 ± 34	2472 ± 163	5.0 ± 0.3	few
Littorella uniflora				
submerged	346 ± 21	3985 ± 278	1.4 ± 0.1	absent
terrestrial	203 ± 20	2048 ± 150	2.7 ± 0.1	frequent
Isoetes lacustris	324 ± 14	2525 ± 193	1.7 ± 0.1	absent
Isoetes hystrix	292 ± 16	1315 ± 287	2.5 ± 0.2	frequent

(Sculthorpe, 1967).

Terrestrialisation causes a reduction of the lacunal system and cell sizes in *L. uniflora*, but this is barely noticeable in *L. dortmanna* (Table 4.7). This is consistent with the fact that *L. uniflora* commonly occurs as a terrestrial plant, but this is much less common for *L. dortmanna* (Haslam et al., 1975; Woodhead, 1951a). This reduction is also seen in the differences between the submerged *I. lacustris* and the terrestrial *I. hystrix* (Table 4.7). Terrestrialisation also causes the formation of stomata, though this is less pronounced in *L. dortmanna*. Whether any root uptake of CO_2 continues to take place under these conditions is unknown. The stomata of *L. uniflora* and *I. hystrix* open onto the lacunae (Scott and Hill, 1900), and it is unlikely that CO_2 limitation would occur, which is an assumed reason for root-uptake of CO_2 in submerged plants (Wium-Andersen, 1971). Similar changes were found in *Isoetes japonica* (West and Takeda, 1915) though on the same leaf. This species has large leaves, the apical regions of which are emergent.

L. dortmanna seems better adapted for root-uptake of CO_2 in submerged conditions than *L. uniflora*. There is a more intimate lacunal link between leaf and root, and in *L. uniflora* the discontinuous lacunae are probably a considerable resistance to CO_2 diffusion. Even with this reasonably open system *L. dortmanna* may be considered to have a considerable resistance to CO_2 diffusion. However, Richardson et al. (1984) found that the $\Delta\delta^{13}C$ value for *L. dortmanna* was typical of C_3 plants. A high diffusion resistance may effect the degree of isotope fractionation (O'Leary and Osmond, 1980). ^{12}C will diffuse down a concentration gradient faster than ^{13}C. Also it may be assumed that the discrimination of RuBPcase may cause a build-up of $^{13}CO_2$ in the lacunae. If either of these factors were to occur to a significant extent the $\Delta\delta^{13}C$ value would be altered. However, it is typically C_3

in its character.

The discontinuous lacunae of *L. uniflora* are more efficient at trapping CO_2 generated by photorespiration (Søndergaard, 1979). Gas exchange can occur from the leaves of *L. uniflora* to the water (Sand-Jensen et al., 1982), though not *L. dortmanna*. This is almost certainly a function of the differences in the thicknesses of the lacunae. The thick cuticle of *L. dortmanna* (Table 4.7) may prevent the loss of CO_2 generated in photorespiration which, as it is inefficiently trapped by the lacunae, could be rapidly lost to the surrounding water.

A further important distinction between the two species is that the cortical cells of *L. uniflora* are much larger than those of *L. dortmanna*. The former exhibits submerged CAM, the latter does not. The large cells would be similar to the large cells common in CAM plants, the vacuoles of which accumulate malic acid at night (Osmond et al., 1982). Similarly the cortical cells of *I. lacustris* (with CAM) are larger than those of *I. hystrix* (without CAM, Keeley, 1982). Terrestrial plants of *L. uniflora* have cortical cells, reduced in size, but not to the extent of *I. hystrix*. This will explain its ability to retain CAM upon terrestrialisation.

4.3.6. Conclusions

This chapter has sought to discuss how well adapted *L. dortmanna* is to its environment. The aquatic environment is often poor in CO_2, and the various carbon accumulating mechanisms discussed have been suggested as adaptations to this environment. Richardson et al. (1984) suggest that because *L. dortmanna* has carbon-accumulating strategies its growth is probably carbon-limited. A converse argument would suggest that the presence of such strategies may help free the plant from carbon-limitation. As can be seen in Chapter 2, seasonal growth is highly correlated with changes in irradiance, and so is not carbon-

limited. Table 4.8 illustrates the present state of knowledge of carbon accumulating mechanisms in isoetids. *L. uniflora* is seen to have the widest range of mechanisms and is also the most widely distributed (Haslam et al., 1975; Spence, 1964).

L. dortmanna has a seasonal growth pattern limited by light (Chapter 2). It has its lower depth limit in any loch set not by the responsivity of its seeds to light but by the ability of the seedlings to survive low light levels (Chapter 3). *L. dortmanna* is able to vary its physiology with changing light regimes - both with its photosynthetic/light curve characteristics and its chlorophyll content. It does not, however, seem to adapt to shade conditions by varying its carboxylase levels. This species is thus limited in its responsivity. It is this that is probably the most important factor in determining the zonation of the plant.

Table 4.8.

A summary of the present state of knowledge of the various carbon accumulation mechanisms and their presence or absence in British Isoetid species.

Species	Carbon Accumulating Mechanism		
	Submerged CAM	Root uptake of CO_2	Photorespiratory lacunal CO_2 trap
I. lacustris	Present	Present	-
I. echinospora	Present	-	-
L. uniflora	Present	Present	Present
L. dortmanna	Absent	Present	Present
S. aquatica	Absent	-	-
E. septangulare	Absent	-	-

CHAPTER FIVE

CONCLUSIONS

CHAPTER FIVE CONCLUSIONS

The previous chapters give an indication of the environmental parameters that most affect growth in *Lobelia dortmanna* and provide a background from which we can describe the growth strategy of the plant. Let us consider how we can define this strategy.

Grime (1979) presents one approach to growth strategies that has been followed by many workers. His approach is to define plants according to very broad categories, which seek to distinguish the different approaches of various plant species to the survival of environmental stress, competition and disturbance. This approach to plant strategies has been criticised for being generalised, though useful in its separation of growth and reproductive strategies (Verhoeven et al., 1982). These workers use an alternative approach of defining a strategy using precise environmental variables. Both approaches will be considered for *L. dortmanna*.

By considering the observed distribution of *L. dortmanna* it is obvious that it is one of the most successful macrophytes in colonising disturbed areas of loch shores (Spence, 1964). Such habitats are characteristic of ruderals (Grime, 1979), which are considered ephemeral plants, annuals with a high reproductive output. *L. dortmanna* does not fit this description. It is slow growing (Chapter 2) and does not flower frequently (Chapter 3). Similarly of the isoetids *Littorella uniflora*, which also colonises these habitats, is slow growing, but *Subularia aquatica* is a characteristic ruderal being an annual biennial with frequent seed production (Woodhead, 1951b). The perennial, slow growth of *L. dortmanna* and *L. uniflora* is more characteristic of a stress-tolerator (Grime, 1979).

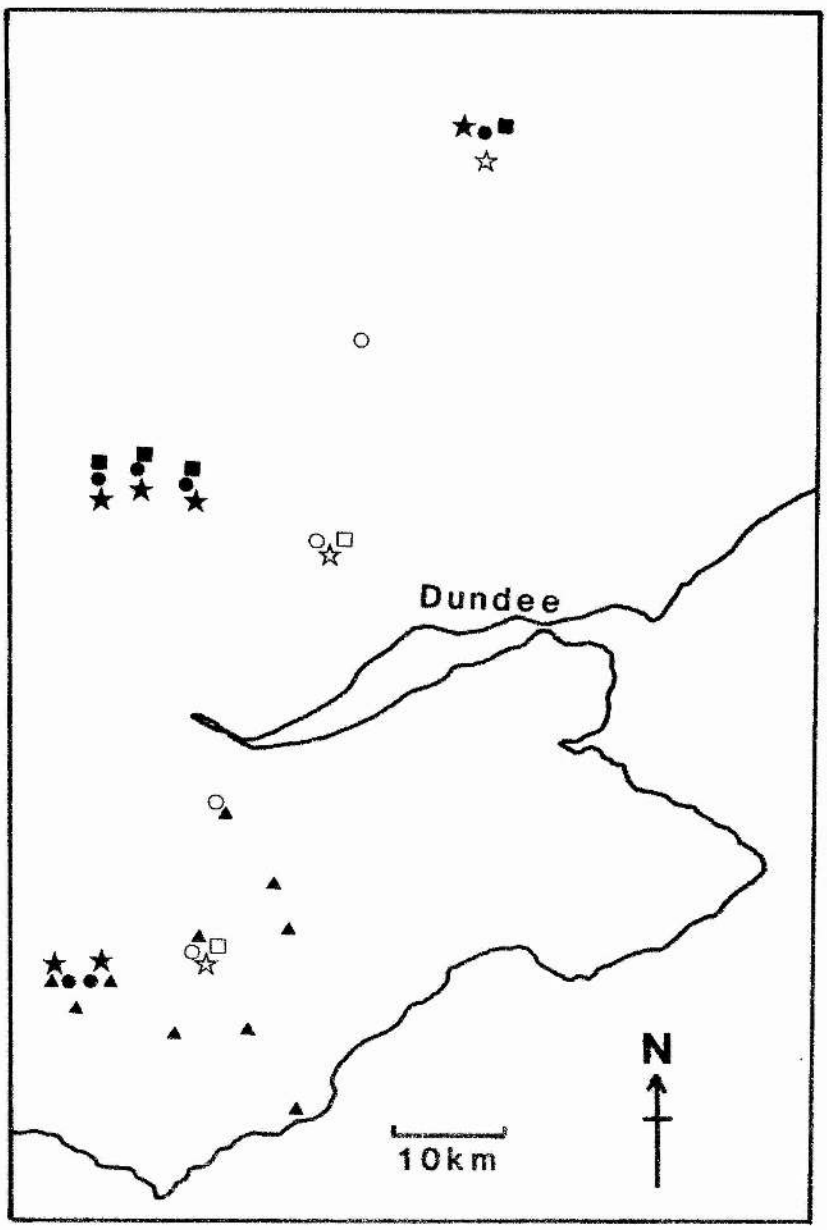
In considering stress tolerance, Grime (1979) is concerned particularly with light and nutrient stress. All of the isoetids are characteristic of very low nutrient lochs (see Introduction), so are evidently able to

tolerate this stress. The slow growth rate of *L. dortmanna* is not due to a limitation by the low nutrients of the loch sediment (Chapter 2), In aquatic habitats carbon may also be limiting (Black et al. 1981), but again this does not seem to be a cause of the slow growth of *L. dortmanna* (Chapter 2). This species does not, however, seem to be as well adapted to responding to light stress (shading) as other isoetids, being limited to more shallow water than *L. uniflora* or *Isoetes lacustris* (Spence, 1964). This study (Chapter 4) has shown that although *L. dortmanna* can vary its pigment composition with depth, it does not alter its carboxylation system in a way characteristic of shade-tolerant plant (Kirk, 1983). It is, therefore, tolerant of limited light stress only.

L. dortmanna can often be found growing in dense vegetation with other macrophytes. However, with increasing eutrophication, *L. dortmanna* tends to disappear from lakes (Jupp et al., 1974; Sand-Jensen and Borum, 1984). This has been found to be due to the epiphyte growth on the leaves severely limiting light penetration (Sand-Jensen and Borum, 1984). Figure 5.1 shows the decline in the distribution of isoetids in Fife and Angus. This is based on the information of Young (1936), Ballantyne (1977) and Ingram and Noltie (1981). The latter workers consider this decline to be due to eutrophication, caused by agricultural run-off. The last two remaining sites for *L. dortmanna* and *I. lacustris* in Fife are in the Cleish Hills, but in the past year the surrounding land has been ploughed. These lochs already contain much epiphytic growth, so an agricultural fertilization may cause the complete removal of these species from Fife. Thus *L. dortmanna* is not tolerant of shading. It seems able to compete with other macrophytes in some respects, as it tolerates very low nutrient levels and uses sedimentary CO_2 as its carbon source, so avoiding competition. However, it is unable to withstand severe competition for light, particularly by epiphytes. Such a cause of species decline has been suggested as a general feature

Figure 5.1.

A map of Fife and Angus in Eastern Scotland showing past (open symbols) and present (closed symbols) of *Lobelia dortmanna* (●), *Isoetes lacustris* (✶), and *Subularia aquatica* (■), and sites of *Littorella uniflora* in Fife (▲).


Dundee
10km
N

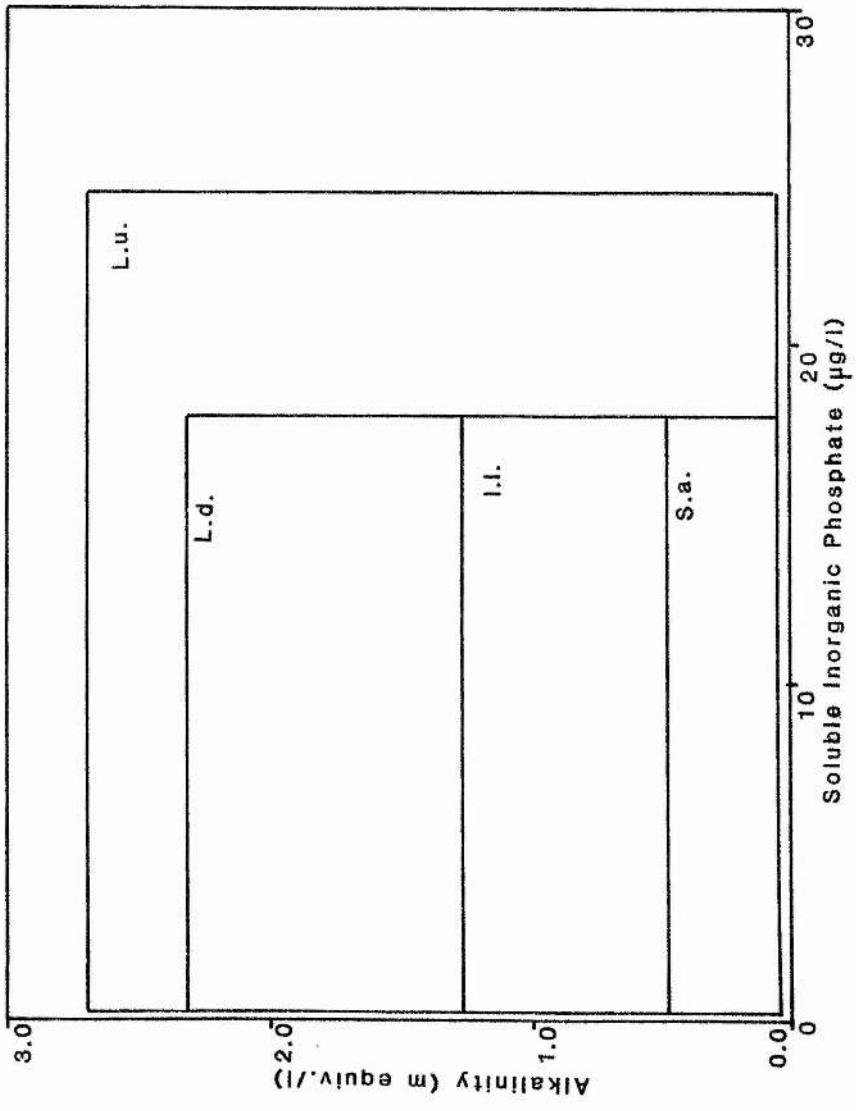
associated with eutrophication for many species (Phillips et al., 1978).

In following the type of analysis of Verhoeven et al. (1982), the distribution of *L. dortmanna* can be considered with reference to precise environmental parameters. Figure 5.2 shows the range of *L. dortmanna*, *L. uniflora*, *I. lacustris* and *S. aquatica* with reference to water alkalinity and water inorganic phosphorus level. This Figure is based on data from Spence and Allen, 1979; Spence et al., 1979; Spence et al., 1984 and Campbell, 1971, covering over seventy lochs and lochans. This reveals that *L. uniflora* is much broader in its range than other isoetids. It is particularly tolerant of mesotrophic lochs and lochs of high alkalinity. *L. dortmanna* is rarely found in mesotrophic lochs for reasons described above. However, it is also excluded from marl lochs, unlike *L. uniflora*. Neither plant uses bicarbonate as a carbon source (Spence and Maberly, 1985). In these lochs the leaves of *L. uniflora* become covered in a marl covering and it is possible that *L. dortmanna* may not be able to survive the light reduction caused by such a covering. It certainly can survive in lochs with a higher alkalinity, but with reduced marling, e.g. the Machair lochs of the Western Isles (Spence et al., 1979).

These definitions of growth strategy can, therefore, be used in helping to explain the observed zonation of *L. dortmanna*. Chapter 3 showed that the vertical zonation of the species cannot be explained by postulating a light requirement for germination that is only met in shallow water. The seeds can germinate well below the lower limits observed for *L. dortmanna* growth, but subsequent growth of the seedlings is impeded. This zonation is, therefore, attributable to the inability of the species to tolerate the stress of light reduction. It has been shown that this may be due to its limited ability to alter its photosynthetic apparatus to cope with shading.

Figure 5.2.

The "ecological amplitudes" of four isoetids after Verhoeven et al. (1982). The Figure shows the ranges of the species with respect to water alkalinity and water soluble inorganic phosphate for about seventy Scottish lochs. The species are: *Littorella uniflora* (L.u.), *Lobelia dortmanna* (L.d.), *Isoetes lacustris* (I.l.), and *Subularia aquatica* (S.a.).


L.u.
L.d.
I.l.
S.a.
Alkalinity (m equiv./l)
3.0
2.0
1.0
0.0
0
10
20
30
Soluble Inorganic Phosphate (µg/l)

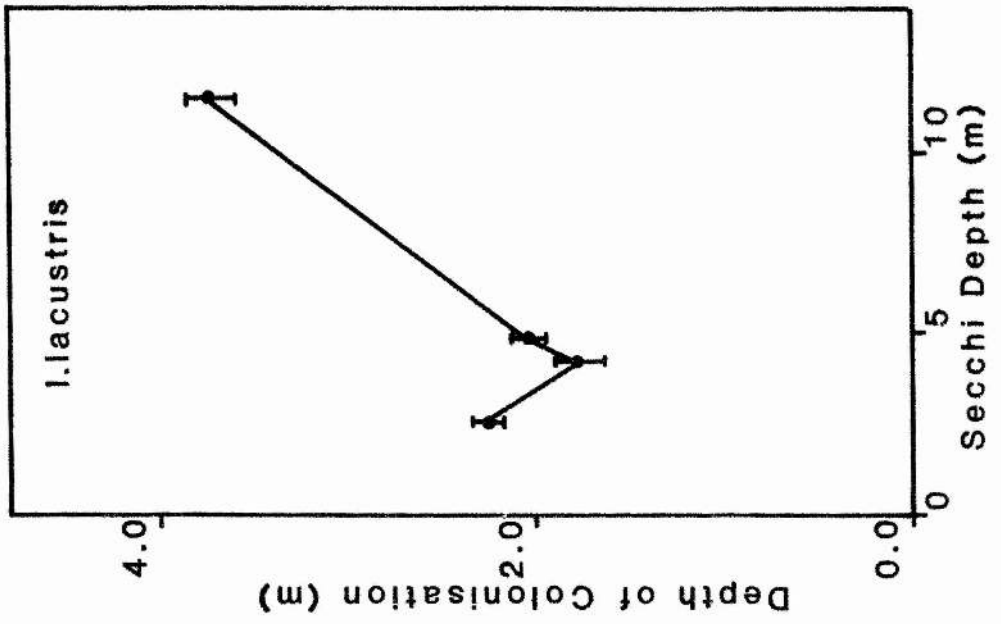
It is, therefore, possible to hypothesise that variations in the light attenuation of any lake would determine the lower depth limit of *L. dortmanna*. This can be tested by considering the data of Eloranta and Marja-aho (1982). They took transects around a large, oligotrophic Finnish lake. Many of these transects contained *L. dortmanna*. They also recorded the Secchi depth for these sites, which are a measure of water clarity. Analysis of their data is presented in Figure 5.3. It can be seen that increasing water clarity, i.e. decreasing light attenuation, allows deeper penetration of *L. dortmanna*. As a comparison the same analysis has been undertaken for *I. lacustris*. This shows a similar trend, though in all cases at greater depths. Chambers and Kalff (1985) have found a strong correlation between Secchi depth and Z_{eu} - the maximum depth of macrophyte colonisation. This analysis shows that the relationship holds for individual species as well as macrophyte populations as a whole. It also confirms the proposition of light quantity controlling the limits of vertical zonation in *L. dortmanna*.

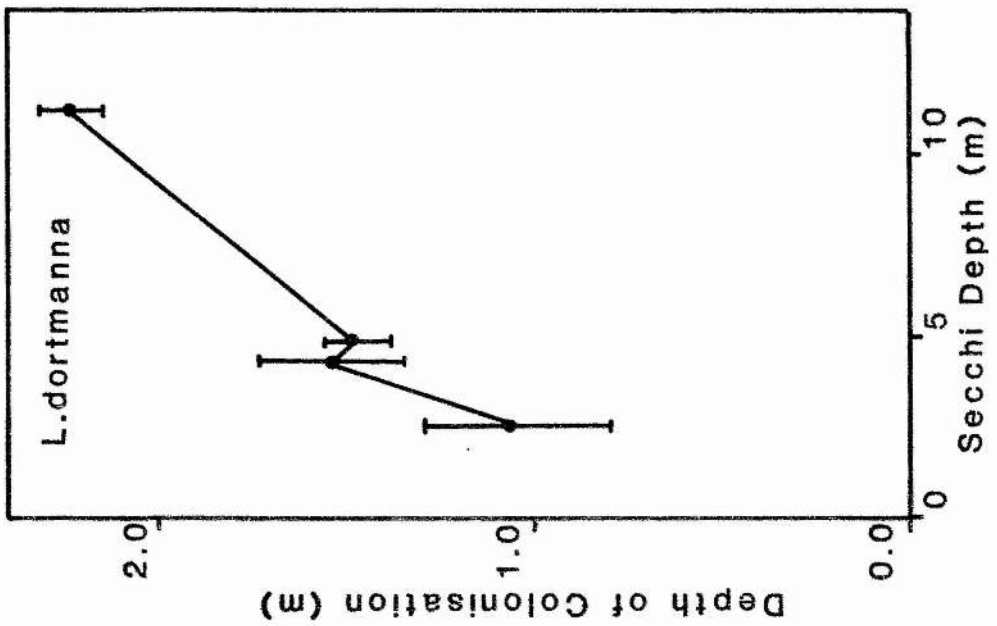
It also seems that light is important in controlling horizontal zonation. *L. dortmanna* is limited in its occurrence in dense vegetation, particularly being affected by epiphytes. This is attributable to light reduction rather than any other competitive effects (see above). The only other factor of importance noted to control horizontal zonation is water turbulence, as *L. dortmanna* is not found in areas of extreme exposure.

Light is, therefore, seen to be of overriding importance in controlling the zonation, both vertical and horizontal, of *L. dortmanna*. This is seen to be due to action via a reduction in photosynthetic production rather than any other photomorphogenic effect.

Figure 5.3.

The relationship between secchi depth and the maximum depth of colonisation for *Lobelia dortmanna* (A) and *Isoetes lacustris* (B). Each point is a mean of four colonisation records for each secchi depth (eight for the second point in each graph) ± S.E. Data are drawn from Eloranta and Marja-aho (1982).


l.lacustris
Depth of Colonisation (m)
4.0
2.0
0.0
0
5
10
Secchi Depth (m)


L.dortmanna
Depth of Colonisation (m)
2.0
1.0
0.0
0
5
10
Secchi Depth (m)

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