Structural and functional aspects of epiphytic and benthic algae in the acidified Lake Gårdsjön, Sweden

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STRUCTURAL AND FUNCTIONAL ASPECTS OF EPIPHYTIC AND BENTHIC ALGAE IN THE ACIDIFIED LAKE GÅRDSJÖN, SWEDEN

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Structural and functional aspects of epiphytic and benthic algae in the acidified Lake Gårdsjön, Sweden

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The microstructure, distribution, taxonomic composition and primary production capacity of epiphytic and benthic algae were studied in an acidified lake.

The epiphytic cover on *Lobelia dortmanna* was made up of two distinct layers of epiphytes. Diatoms (*Eunotia*), bacteria and blue-green algae formed a firmly attached layer, which showed few seasonal changes in species composition. The loosely attached layer consisted of green algal filaments (*Mougeotia*, *Binuclearia*). This layer showed clear seasonal changes in species representation.

The benthic mat was built up of blue-green algal filaments (*Lyngbya*). Some species present in the filamentous matrix were also common in the epiphyton (*Merismopedia*).

Both algal assemblages contained mucilage. Most metals were found in the mucilaginous structures of epiphyton. Liming of the lake caused changes in the algal structures, favouring diatoms (*Achnanthes*) and desmids. The primary productivity rates of epiphyton were highest at 1.0 m. Photosynthesis in the benthic mat was restricted to the uppermost 2-3 mm.

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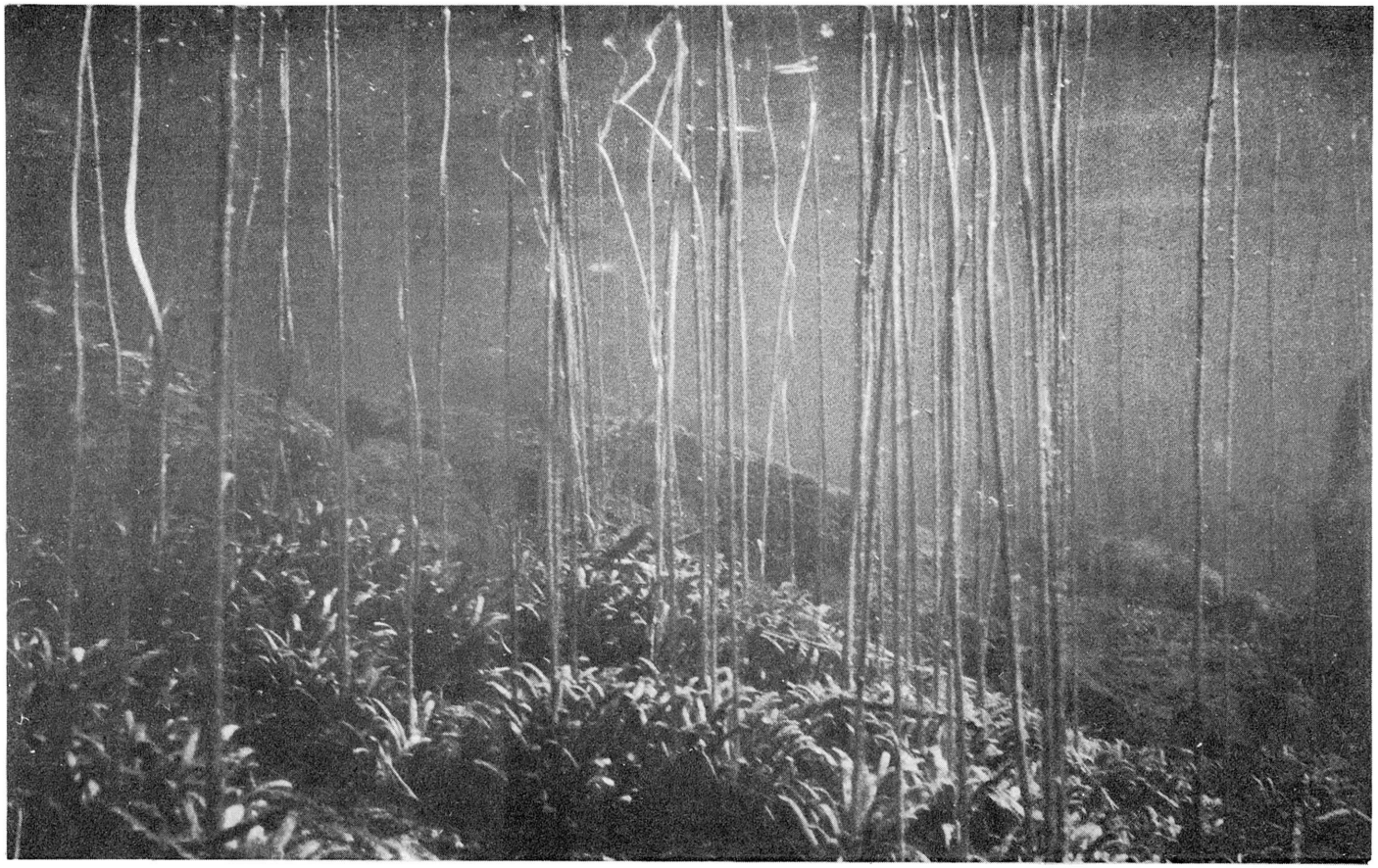
STANISLAW LAZAREK

DISSERTATION

Lund 1983

I do not know what I may appear to the world, but to myself I seem to have been only like a boy, playing on the seashore and diverting myself, in now and then finding a smoother pebble or a prettier shell than ordinary, while the great ocean of truth lay all undiscovered before me. SIR ISAAC NEWTON



View of the littoral in Lake Gårdsjön showing *Lobelia dortmanna* L. at 1.0 m. Note the elongated stalks which bear the flowers above the water level.

PREFACE

Life may be unpredictable but events are not as unconnected as they seem. It is not hard to see how the first idyllic impressions of the ponds and meadows of my childhood gradually gave way to more inquiring, analytical observations. Yet, it is these memories which kindle the hope that the harmony between man and nature is not beyond restoration.

I am deeply indebted to the many people who befriended me, inspired me and helped me to see with new eyes. I would like to mention them all individually, but on this short page there is space only to name a few.

Maggan and Lasse Jonsson supported me loyally through thick and thin and opened their home and hearts to me without reservations. Dr Gunnar Andersson, never short of patience, was always ready with sound advice and open to discussion. Without him I may never have found may way out of the many bureaucratic labyrinths. Dr Wilhelm Granéli offered me help and friendship when it was most needed. I am grateful to Professor Sven Björk and my colleagues at the Institute of Limnology for accepting me and giving me the opportunity of proving myself.

I acknowledge Professor Brian Allanson for introducing me to the abstracts of the epiphytic microcosm and for emphasizing the stringent verification of results. I think warmly of my friend Dr Clive Howard-Williams. His generous and enthusiastic assistance extended beyond scientific necessities. Dr Jack Talling encouraged and supported me in my work. I thank my colleagues at Brookhaven National Laboratory for receiving me so warmly and for introducing me to the acidification problems in the Adirondacks. I am grateful for the kind assistance of the staff at the Section of Structural Zoology at the University of Lund.

Far from thinking that this work has exhausted the subject, I consider this as merely a good opportunity to critically revise and analyze my progresses and failures. I may therefore be permitted to hope that the ideas presented here provide the reader with some inspiration despite the obvious gaps in information and the sometimes faultering evidence.

This dissertation is dedicated to Thora.

LIST OF PAPERS

This thesis is based on the following published and unpublished papers:

I. Structure and productivity of epiphytic algal communities on *Lobelia dortmanna* L. in acidified and limed lakes. *Water, Air, and Soil Pollution*, 18:333-342, 1982.

II. Structure and function of a cyanophytan mat community in an acidified lake. *Canadian Journal of Botany*, 60:2235-2240, 1982.

III. Microstructure and metal content of the *Lobelia*-epiphyte complex in acidified lakes. *Freshwater Biology*, accepted for publication, 1983.

IV. Epiphytic algal production in the acidified Lake Gårdsjön, southwestern Sweden. *Ecological Bulletins (Stockholm)*, in press, 1983.

V. Epiphytic colonization of *Lobelia dortmanna* L. in the acidified and limed Lake Gårdsjön, southwestern Sweden. Manuscript.

In the summary, the above Roman numerals will be used when referring to these papers.

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ABSTRACT

The microstructure, distribution, taxonomic composition and primary production capacity of epiphytic and benthic algae were studied in an acidified lake.

The epiphytic cover on *Lobelia dortmanna* was made up of two distinct layers of epiphytes. Diatoms (*Eunotia*), bacteria and blue-green algae formed a firmly attached layer, which showed few seasonal changes in species composition. The loosely attached layer consisted of green algal filaments (*Mougeotia*, *Binuclearia*). This layer showed clear seasonal changes in species representation.

The benthic mat was built up of blue-green algal filaments (*Lyngbya*). Some species present in the filamentous matrix were also common in the epiphyton (*Merismopedia*).

Both algal assemblages contained mucilage excreted by algae, which was important in the process of epiphytic attachment and in the consolidation of the benthic mat. Most metals found in the epiphytic cover were localized in the mucilaginous structures. Liming of the lake caused changes in the epiphytic structures and species composition, favouring diatoms (*Achnanthes*) and desmids. No such changes were observed in the benthic mat.

The productivity rates of epiphyton were highest at 1.0 m. The optimum conditions (light, availability of substrate and CO_2-content) for epiphytic production were found at 1.0 m. 14-C activity was higher in the firmly attached layer than in the loosely attached layer of epiphytes. Photosynthesis in the benthic mat was restricted to the uppermost 2-3 mm.

Use of the collodion technique and epiphyte-free *Lobelia* plants suggested their applicability to studies in acidified lakes.

SUMMARY OF RESULTS

INTRODUCTION

Wetzel & Allen (1972)*, illustrated the complex interactions between the various biotic and abiotic components of the littoral zone. The rôle of the littoral and its communities has been well-documented (Howard-Williams & Lenton, 1975; Mickle & Wetzel, 1978) as an inseparable part of the lake ecosystem.

Earlier descriptive studies of periphytic communities (e.g. Cholnoky, 1927; Godward, 1937) established a basis for today's more comprehensive approach.

Benthic algal microcommunities are more stable than are free-living communities (Moss, 1980). Their distribution is related to the availability and topography of adequately illuminated substrate (Talling, 1975). Benthic and epiphytic algae are less exposed to frequent light fluctuations than phytoplankton. The dead cells are not as easily sedimented as are those of free-living algae. This implies that nutrients which are released from the attached algae are recycled within the algal microcosm, providing an effective strategy of adaptation and survival in nutrient-poor conditions. Carignan & Kalff (1982) calculated that of the total amount of phosphorus recycled diurnally within the epiphyton, 90% originated from epiphytes.

A striking feature of benthic algae in acidified lakes in Scandinavia is the mat-like structural appearance.

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* Most references have been presented in the papers. Here I refer to the most relevant or to those which are not mentioned in the papers.

Environmental perturbations caused by acidification are reflected by structural and functional changes in the existing communities. The species diversity is low and the energy transfer between the various components of the lake ecosystem is less efficient than in undisturbed ecosystems.

A decline in the growth of macrophytes in lakes, specifically *Lobelia dortmanna* L. (van Dam & Kooyman-van Blokland, 1978), has been attributed to acidification. However, a gradual disappearance of *L. dortmanna* was reported earlier in Germany (Roll, 1939) - in connection with increasing eutrophication.

I allotted four years of study to illuminate some aspects of benthic and epiphytic algal structures and the production capacity of these algae in relation to environmental conditions. I concentrated on two representative and interacting algal assemblages present in the same microhabitat in the acidified Lake Gårdsjön*:

1) the epiphyton associated with *Lobelia dortmanna*.

2) the cyanophytan (blue-green algal) benthic mat.

I used *L. dortmanna* as a natural host-plant, to overcome problems resulting from the heterogeneity of the substrata. The plant is common in the littoral of the temperate oligotrophic lakes of northern Europe and northern America. Its morphological and physiological features made it suitable for my studies. Moon (1935) was the first to use trays with another isoetid, *Littorella*, for sampling the

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* A map of Lake Gårdsjön showing the distribution of *Lobelia* and the sampling sites is presented in Appendix A. The environmental characteristics of the lake are presented in Appendix B and in the papers.

diverse communities in the littoral zones of the Lake District, England. I extended and elaborated his idea.

Recent studies of the ecology and physiology of *L. dortmanna* have been undertaken in the Netherlands (H. van Dam, personal communication) and in the United States at the University of Wisconsin-Madison (H. Boston, personal communication).

The benthic mat in L. Gårdsjön is a low diversity system made up of filamentous cyanophytes of the order Oscillatoriaceae. This highly controversial observation is interesting from a physiological and evolutionary point of view. Similar findings to mine from other parts of the world (e.g. Leupold, 1982; M. Leupold, personal communication), hint at an unknown pattern of biological adaptation to acid conditions.

It has been pointed out (e.g. Wetzel, 1975) that we too often attempt to describe the function of biological systems in terms of sophisticated mathematical formulas, neglecting the importance of contact with the living components of the system. I have tried to keep this in mind while providing detailed information on the structural properties of epiphytic and benthic algae and their production capacity.

I hope that the results presented here may be useful as a reference when we attempt to reverse the effects of acidification by liming acidified lakes.

STRUCTURAL ASPECTS

Species composition, *Microstructure*, *Microdistribution*, *Content of elements* (I,II,III,V).

Structural studies were performed in the spring, summer and autumn 1980, 1981 and 1982, at approximate monthly intervals. Scanning electron microscope (SEM) was used to determine the structural features of the epiphytic complex (I), the construction of the benthic cyanophytan mat (II) and a detailed survey of the *Lobelia* leaf surface with the patterns of epiphytic attachment (III). SEM was used in combination with an X-ray spectrometer to localize and check the relative content of some metals and phosphorus in the epiphytic complex (III). Transmission electron microscopy (TEM) was used to study bacterial colloidal products excreted during the attachment process (III). Microdistribution of the epiphytic cells was studied using a collodion impression technique (I,IV). The technique was elaborated (V) so that the firm epiphytic layer could be photographed at a magnification > X1000 and simultaneously, the cells could be counted under a phase-contrast microscope. The method proved particularly effective on *Lobelia* leaves but was also used successfully on *Nuphar* petioles, *Menyanthes* stalks and internodes of *Equisetum*. The collodion impression technique was used to study the epiphytic colonization of initially epiphyte-free surfaces. With this method, colonization rates, succession of species and the microdistribution of species could be investigated.

There are two types of benthic algae in L. Gårdsjön: the epiphytic algae associated with submerged vegetation, and the cyanophytan mat covering the sediment proper. A distinct flocculent mat composed of *Mougeotia* and

Binuclearia, forms an intermediate assemblage (metaphyton) in the warmer periods of the year (IV).

The epiphytic complex in L. Gårdsjön is a low diversity system. The values of the Shannon's index of diversity ranged from 0.31 to 0.76 in July–August, 1981, and in 1982 increased after liming in April, to between 0.64 and 1.20 in July–August. Some of the benthic species in L. Gårdsjön are presented in Appendix C.

The epiphytic complex associated with *Lobelia* consists of two layers of organisms which differ in their taxonomic composition, means of attachment, seasonal abundance and primary production capacity (IV).

The first layer is made up of the firmly attached diatom *Eunotia* with associated bacteria and colonial cyanophytes (I,III,V). After the liming of L. Gårdsjön, *Eunotia* gave way to the diatoms, *Achnanthes* and *Synedra* (V). This is consistent with an earlier observation from the limed reference lake, L. Högsjön, where these two diatom species dominated (I). There is evidence that *Achnanthes* and *Synedra* were abundant in L. Gårdsjön before it became acidified. The firmly attached layer contributed the highest percent to the total number of cells in the spring and winter (I,IV).

The second layer is built up of loosely attached green algae interwoven with bacteria, blue-green algae and diatoms. This layer could be removed by agitation. The seasonal changes in the species composition of the epiphyton were most apparent within the loosely attached fraction. The composition of the firm layer remained almost unchanged until 1982, when liming was performed.

Filamentous green algae (*Mougeotia*, *Binuclearia*) and blue-green algae were abundant in the summer (I,IV). The only abundant epiphytic desmid in L. Gårdsjön was *Penium*. After liming, the abundance and diversity of desmids increased (V).

The perennial benthic mat in L. Gårdsjön is composed of the motile filaments of two cyanophytan genera, *Lyngbya* and *Oscillatoria*. The occurrence of these two genera seems to have a local character, as the filamentous matrix of the benthic mat in a nearby lake, L. Hästevatten (pH 5.1-5.7) is composed of *Hapalosiphon* (II). *Lyngbya* and *Oscillatoria* were not present in the epiphyton. Several algal genera were represented in both the benthic mat and the epiphyton, e.g. *Merismopedia*, *Anomoeoneis*. Limited representation of invertebrates (nematods, chironomids) was encountered in both communities. Similar to the firm layer of epiphyton, the benthic mat did not show seasonal changes in the composition of the dominating species (II). After liming, the taxonomic composition of the epiphyton changed considerably, but so far no such changes have been observed in the benthic mat.

The epiphytic complex is built up on the epidermis of the *Lobelia* leaf, which is covered by a mucilaginous film (< 2 µm thick) of plant origin. The initial active settlement of rod-shaped bacteria and colonial cyanophytes is a rapid process, evident within one day (V). A passive attachment (mainly filamentous green algae and chain-forming diatoms) occurs on the barren leaves after a few hours of exposure (V). The development and function of the epiphytic complex and benthic mat is associated with the release of polysaccharideous material. The material is

important in the active attachment of cells and for the consolidation and protection of the algal assemblages against unfavourable environmental conditions (II,III). The epiphytic complex and benthic mat include sedimented detrital particles and entangled filaments of metaphytic algae with their associated bacteria.

The relative content of some metals in the epiphytic cover was estimated and they were localized in view of:

1) the high content of some metals in acid lakes and the possibility that a high metal content in the surviving algae limits grazing.
2) the possible connection to the high amounts of mucilage produced by algae in metal-contaminated, acid waters.
3) the impact of metals on algal metabolic processes, particularly photosynthesis.

The mucilage formations contained the greatest variety of metals and had the highest content of calcium, sodium and aluminium. The content of aluminium in the leaves of *Lobelia* with epiphytic cover was twice that in the leaves without cover. Biological uptake of aluminium by the firm layer of diatoms would explain this. Acid and metal resistant algal species appeared in L. Gårdsjön due to their structural and metabolic adaptation to a low pH environment (III). A chemical and physical modification of the existing microenvironment leads to the presence of low diversity, stable algal assemblages (II,III).

The successful experiments with epiphyte-free *Lobelia*-plants (V) suggest their applicability for monitoring the effects of acidification. The same set of plants may be exposed to different conditions in lakes and variations in the epiphytic structures may be compared. The collodion

impression technique offers a simple method of assessing the epiphytic cover without needing to remove the attached cells.

FUNCTIONAL ASPECTS

Biomass, chlorophyll a, primary productivity rates, primary production (I,II,IV).

The epiphytic and benthic algal production in L. Gårdsjön was estimated on the basis of *in situ* 14-C uptake experiments. The experiments were performed in 1980 and 1981 at 0.5, 1.0 and 2.0 m with the help of SCUBA diving. Several comparative measurements were carried out in two other acidified lakes in southwestern Sweden (I,IV). I built three types of plexiglas chambers (details are given in Appendix D), which were adjusted to the particular host-plant, type of lake bottom and community under investigation.

The daily light intensity was measured automatically at the side of the lake. In addition, the light intensity and light transmission were measured in the water during the 14-C incubation periods (Appendix E). 1 ml of $NaH^{14}CO_3$ (4 µCi, 1Ci= 37 GBq) was injected via a rubber septum. The period of incubation was either two or four hours. 14-C activity was determined on a liquid scintillation counter, and the concentration of dissolved inorganic carbon (DIC) in the water was determined in an IR Carbon Analyzer. To distinguish between bacterial and algal photosynthesis in the benthic mat, a photosystem II inhibitor (DCMU) was used (II).

Epiphytic biomass reached a maximum in the summer (1.0 mg

ash-free dry wt cm^{-2}). *Eunotia* had a stable biomass throughout the study period, while distinct seasonal changes in the biomass of the dominating loosely attached algae were noted (IV).

The highest chlorophyll *a* content was found in autumn (3.4 µg cm^{-2}). The mean chlorophyll *a* content in the benthic mat (II) was 81.3 µg cm^{-2}. At a 1.0 m depth the epiphytic chlorophyll *a* values were not significantly correlated with the epiphytic productivity rates (I).

Productivity rates for the epiphyton associated with *Lobelia* compared favourably with reported values from other soft-water, nutrient-poor waters. I encountered the highest productivity rates in June 1980 (1.0 mgC m^{-2} hr^{-1}). Maximum daily productivity rates for the epiphyton on *Lobelia* were calculated in July 1981, as 69.6 mgC m^{-2} leaf surface area at a 1.0 m depth (IV). The daily productivity rates correlated best with the stable biomass of the tightly attached fraction of algae (IV). Higher daily productivity rates were found for the epiphyton associated with *Isoetes* and *Nuphar*. However, due to the fact that *Lobelia* is the dominating macrophyte in the lake (Appendix B), it provides the largest biotic substrate area available to epiphytic colonization. The mean daily productivity rate in the benthic mat was equal to 32.8 mgC m^{-2}. The photic zone in the mat is restricted to the uppermost 2–3 mm.

The annual production of epiphyton on *Lobelia* was 1.8 gC m^{-2} leaf surface area. This value corresponds to the extremely low values reported for epiphytic production in soft-water lakes.

It was not possible to establish the total annual primary

production of the lake, as the annual production of the cyanophytan mat and the metaphyton in the lake has not been estimated. This requires detailed knowledge of the seasonal and daily changes in the CO_2 turnover and the light conditions at the bottom of the lake, as well as information on the distribution of these communities.

The DIC content in the water was highest at 0.5 m, declining with depth. Liming caused an eightfold increase in the DIC content in the water. This should have a positive long-term effect on primary production in the lake.

PERSPECTIVES

The results I have presented suggest several questions which would be rewarding to tackle in future research on benthic algae. Some of the following problems are of immediate practical importance for acidified waters, others are of value in theoretical limnology. Here are some aspects I would particularly like to elaborate on in future investigations:

1) The experimental manipulation of epiphyte-free *Lobelia* plants has afforded promising possibilities for continued studies on the epiphytic colonization of *Lobelia* and other macrophytes. Experiments may be performed in lakes with different physico-chemical conditions. Epiphytic communities could be subjected to certain grazers in a given period and the effect of grazing on the epiphytic structures could be compared in the laboratory and under natural conditions. The energy value (protein content) of the algal material could be determined.

2) Sediments from various acidified lakes could be inoculated with blue-green algae and the pattern of colonization, the physical structure of the developing cover, the succession of species and the physico-chemical modifications of the sediment-water interface could be followed.
3) 14-C track autoradiography could be used to determine the production capacity of individual acid-resistant algae under controlled light conditions. Studies of this kind will be initiated later this year during my stay at the University of Toronto, Canada.
4) The mechanisms regulating the formation of green algal clusters in the littoral of acidified lakes should be established, and the impact of these formations on the rates of nutrient diffusion from the sediment to the water should be determined.
5) Studies on the physiology of species such as *Mougeotia*, *Binuclearia*, *Batrachospermum* and *Zygnema* should be intensified.

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STRUCTURE AND PRODUCTIVITY OF EPIPHYTIC ALGAL COMMUNITIES ON *LOBELIA DORTMANNA* L. IN ACIDIFIED AND LIMED LAKES

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Abstract. The epiphytic algal community growing on *Lobelia dortmanna* L. was studied in two acidified lakes in southwestern Sweden from May through October 1980; Lake Gårdsjön (pH 4.3 to 4.7) and L. Högsjön (pH 6.3 to 6.7 after liming in 1978/79).

In both lakes a layer of firmly attached diatoms, *Eunotia veneris* and *E. rhomboidea*, covered the *Lobelia* leaves. Scanning electron microscope study revealed a mucoid matrix, and in L. Gårdsjön, heavy cover of detritus. The spring period in L. Gårdsjön was characterized by red alga *Batrachospermum sp.*, which was followed by the appearance of *Binuclearia sp.*, and *Mougeotia spp.* Blue-green algae appeared during the warmer period of the year. The spring period in L. Högsjön was characterized by the development of desmids. Diatoms dominated the community in the summer while green algae appeared in autumn.

The biomass in L. Gårdsjön showed spring and late summer maxima, while in L. Högsjön it increased gradually reaching a maximum in autumn. Chlorophyll *a* concentration was highest in L. Gårdsjön in late summer.

Primary productivity rates calculated per unit substrate surface area at 1.0 m depth were highest in early summer and decreased in late summer.

The results suggest that the liming of L. Högsjön caused significant structural changes in the epiphytic community, favoring diatoms and green algae, especially desmids. Blue-green algae were characteristic for L. Gårdsjön despite that lake's low pH. Productivity rates at 1.0 m depth show similar trends in both lakes.

1. Introduction

An increase in biomass of benthic and epiphytic algae has been observed in many lakes undergoing acidification. Reduced microbial decomposition at low pH (Bick and Drews, 1973) and reduced invertebrate grazing (Hendrey and Wright, 1975) have been proposed as possible mechanisms responsible for this increase. Hendrey (1976) and Müller (1980) both observed low diversity of periphytic communities in their experiments with artificial acidification, but the mechanisms remain unexplained. Acidification apparently changes the structure of algal communities and may, as was observed in phytoplankton (Yan, 1979), reduce their biomass, at least at the beginning of the acidification process. However, this reduction is a transient phenomenon, while the changes in community structure may remain permanent as acidification progresses.

Lime ($CaCO_3$) has been applied to acidified lakes to restore their buffering capacity. The rapid transition from acid to neutral conditions may be harmful to algal communities, however knowledge of their response is poor.

The littoral zone forms a link between terrestrial and aquatic ecosystems. These shallow parts of lakes have a potential to influence the whole lake metabolism by altering the chemical composition of water entering a lake. Organic compounds and metabolities

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released by the macrophyte-epiphyte associations in the littoral zone are added to inflowing water. The role of the littoral zone and its communities was documented by Allen (1971) and reviewed by Wetzel (1975). As was pointed out by Wetzel (1975), a large ratio of the littoral to the pelagial zone in many lakes coincides with a high contribution of attached algae to the total autochthonous carbon production. This should be particularly true of shallow, acidified lakes in southwestern Sweden. In addition, the increase of light penetration and accumulation of organic material on bottoms of acidified lakes may favor the growth of benthic and epiphytic algae.

In situ studies of the ecology of epiphytic algae growing on natural substrate in acidified lakes have not been performed. This study draws attention to the seasonal changes in the structure, biomass and rate of photosynthetic activity of epiphytic algae growing on *Lobelia dortmanna* L. (isoetides) in acidified and limed lakes in southwestern Sweden.

2. Study Area

Studies were performed in southwestern Sweden on the acidified Lake Gårdsjön and on Lake Högsjön which was limed in 1978 and 1979. Some limnological data on these lakes are given in Table I. The lakes are situated 40 km north and 70 km southeast of Gothenburg. Granite rocks form the bedrock of L. Gårdsjön. L. Högsjön region is dominated by gneiss. In general, southwestern Sweden is characterized by soils with weak buffering capacity, and lakes whose watersheds contain mixed deciduous and coniferous forests and a high percent of peat bogs. The littoral zones are dominated by *Sphagnum spp.* and the isoetides *Isoetes lacustris*, and *Lobelia dortmanna*. *Lobelia* often dominates the submerged macrophyte communities in the oligotrophic, ion-poor lakes of northern Europe and America. In the studied lakes, *Lobelia* is found in the littoral zone from 0.0 to 4.0 m, with a maximum development at approximately 0.5 to 1.0 m depth. Vast areas of the bottom of L. Gårdsjön are covered with felt-like cyanophytan mats (Lazarek, 1980). *Littorella uniflora* is common in L. Högsjön.

3. Material and Methods

Quantitative and qualitative sampling of epiphytic algae on *Lobelia* was conducted at approximately monthly intervals during the period May to October 1980. Productivity rates of epiphytic algae were studied between June and October 1980. A structural study of epiphytes was made using a scanning electron microscope (Cambridge Mark 11). Epiphytes on *Lobelia* leaves were fixed in 10% glutaraldehyde fixative with a phosphate buffer. The material was dehydrated with methanol and freon before critical point drying.

Species composition was determined and percent contribution of each species to the total epiphyte biomass was estimated on the basis of cell number. The Sedgewick-Rafter counting cell was used for loosely attached algae. Collodium 'peel-off' technique (Lazarek, in prep.) in combination with a square-marked occular and phase-contrast microscopy was used for counting firmly attached algae. Epiphytes for biomass, chlorophyll *a* and 14-C determinations were removed by agitation and with a soft brush.

TABLE I

Some limnological data on the studied lakes

	Gårdsjön 1980	Högsjön[a]	
		1978	1980
Lake area (km^2)	0.3		2.5
Mean depth (m)	7.9		9.0
Transparency (m)	9.5	5.5 –7.5	3.0 –5.8
Colour (mgPt $\times l^{-1}$)	2 –6	10 –40	25 –50
pH	4.3–4.7	4.2 –5.3	6.3 –6.7
Alkalinity (meq $\times l^{-1}$)	0.00	0.00	0.13–0.19
Conductivity (mS $\times m^{-1}$)	6.2–8.1	5.4 –6.9	6.1 –7.2
Ca (mg $\times l^{-1}$)	1.86	2.2	5.6
Mg (mg $\times l^{-1}$)	1.21	2.0	4.8
Al (mg $\times l^{-1}$)	0.26	0.08–0.11	0.01–0.09
SO_4 (mg $\times l^{-1}$)	10.94	9.6	9.6
SiO_2 (mg $\times l^{-1}$)	0.25	0.17–0.32	0.20–0.30
PO_4–P ($\mu g \times l^{-1}$)	3.7	3.0	—
Total–P ($\mu g \times l^{-1}$)	6.1	3 –13	6 –11
Total–N ($\mu g \times l^{-1}$)	380	360 –500	380 –870

[a] Unpublished data provided by Länsstyrelsen, Halmstad. L. Högsjön was limed in 1978 and 1979.

The total biomass of epiphytes was determined as ash-free dry weight (110 and 550 °C) per unit substrate surface area. Chlorophyll *a* per square cm substrate surface area, corrected for pheophytin *a*, was determined spectrophotometrically according to Lorenzen (1967) using reagent grade 90% methanol. Calculations were made in accordance to Marker *et al.* (1980), with the absorption coefficient of chlorophyll *a* in methanol equal to 77.0. The extraction period was 30 min. in darkness at room temperature, with a final brief boiling. All readings were corrected for turbidity at 750 nm.

Primary productivity rates were based on *in situ* 14-C measurements. *Lobelia* has a relatively simple morphology and unlike other submerged phanerogams satisfies its CO_2 requirements by absorption from sediment (Steeman Nielsen, 1960; Wium Andersen, 1971). Therefore $^{14}CO_2$ injected into the water was assumed assimilated by the epiphytes and not by the host-plant. Incubation chambers were made from 3 mm plexiglas. Dark chambers were made from black plexiglas and were covered with aluminum foil. Chambers were placed over *Lobelia* plants and $NaH^{14}CO_3$ was injected via serum bottle stoppers using 2 ml syringes. It was assumed that pumping the syringe several times provided a homogenous distribution of 14-C in the chamber. After a 4 h incubation, the plant material was stored over formaldehyde in a desiccator for transport to the laboratory. Removed epiphytes were filtered onto a prewetted 0.45 µm Sartorius cellulose acetate membrane filter. *Lobelia* leaves were rinsed and dried on blotting paper. Leaf surface area was determined and the leaves were placed on prewetted membrane filters. All samples were fumed approx. 10 min above conc. HCl and placed in liquid scintillation vials after 10 min exposure to the air. Root cuts were sampled to determine if 14-C had

diffused below the sediment and entered the *Lobelia* leaves via the root system. The material was digested in scintillation vials approx. 5 h at 45 °C with 1.0 ml Soluene-350 (Packard Inst. Co. Inc.). Brown coloring was removed by the addition of 200 µl isopropanol and 200 µl 30% H_2O_2 and subsequent warming at 45 °C for 20 min. Dimilume-30 (Packard Inst. Co. Inc.) was added (9 ml), and counting done in an Intertechnique SL-31 liquid scintillation counter after a 48 h delay to allow for chemoluminescence decay. Dark uptake of 14-C was subtracted from the light values. Quench correction was calculated by external standard channels ratio using a quenched series prepared with blank membrane filters. The 14-C was added as hexadecane standard (Radiochemical Centre, Amersham, England) and acetone was used as the quenching agent. The efficiency of counting ranged from 75 to 95% in epiphyte samples.

Dissolved inorganic carbon (DIC) in the water was determined in an IR Beckman Carbon Analyzer. Water samples were injected into 15 ml Vacutainers (Becton-Dickinson Co., New Jersey) and kept at −5 °C in darkness until analyses were performed.

Photosynthetic rates in this study were calculated for one depth (1.0 m) as follows: milligrams of C fixed = (sample activity/added activity) × (milligrams of DIC available) × (1.06 correction for isotope discrimination).

Data on hourly and daily solar radiation measured at Landvetter airport in Gothenburg were utilized in the calculation of light conditions at both lakes. Underwater measurements of light intensity during the incubation period were made with a quantum meter (LI-185, Lambda Inst. Co.), combined with an underwater silicon photodiode quantum sensor. It was assumed that photosynthetically available radiation (PAR = 350 to 700 nm) equals 46% of the total solar radiation. A constant 10% loss of PAR due to surface reflection was assumed during the incubation.

4. Results and Discussion

4.1. Community structure

As *Lobelia* is a perennial hydrophyte, its leaves are available to epiphyte colonization throughout the year. Studies by Moeller (1978) showed that *Lobelia* is a slow-growing plant which results in a more even distribution of epiphytes on its leaves. In both lakes *Lobelia* leaves were covered by a layer of firmly attached acidophilic, morphologically similar diatoms; *Eunotia veneris* (Kütz.) O. Müller and *E. rhomboidea* Hust. (Figure 1a). A mucoid matrix with embedded epiphytes was observed on older leaves of *Lobelia* in both lakes (Figure 1b), and was similar to that proposed by Allen (1971) and described later by Allanson (1973), but due to the acid conditions in the lakes it was not impregnated by calcium carbonate. Nevertheless, it gives mechanical support to holdfasts of filamentous green algae. These in turn serve as substrate for other sessile organisms, particularly diatoms (Figure 1c) and epiphytic bacteria (Figure 1f). The epiphytic community in L. Gårdsjön contained a great amount of detrital material as reported earlier (Lazarek, 1979). The liming of L. Högsjön gradually brought new species to the firmly attached community (e.g. ubiquitous *Achnanthes minutissima* Kütz. and alkaliphilic

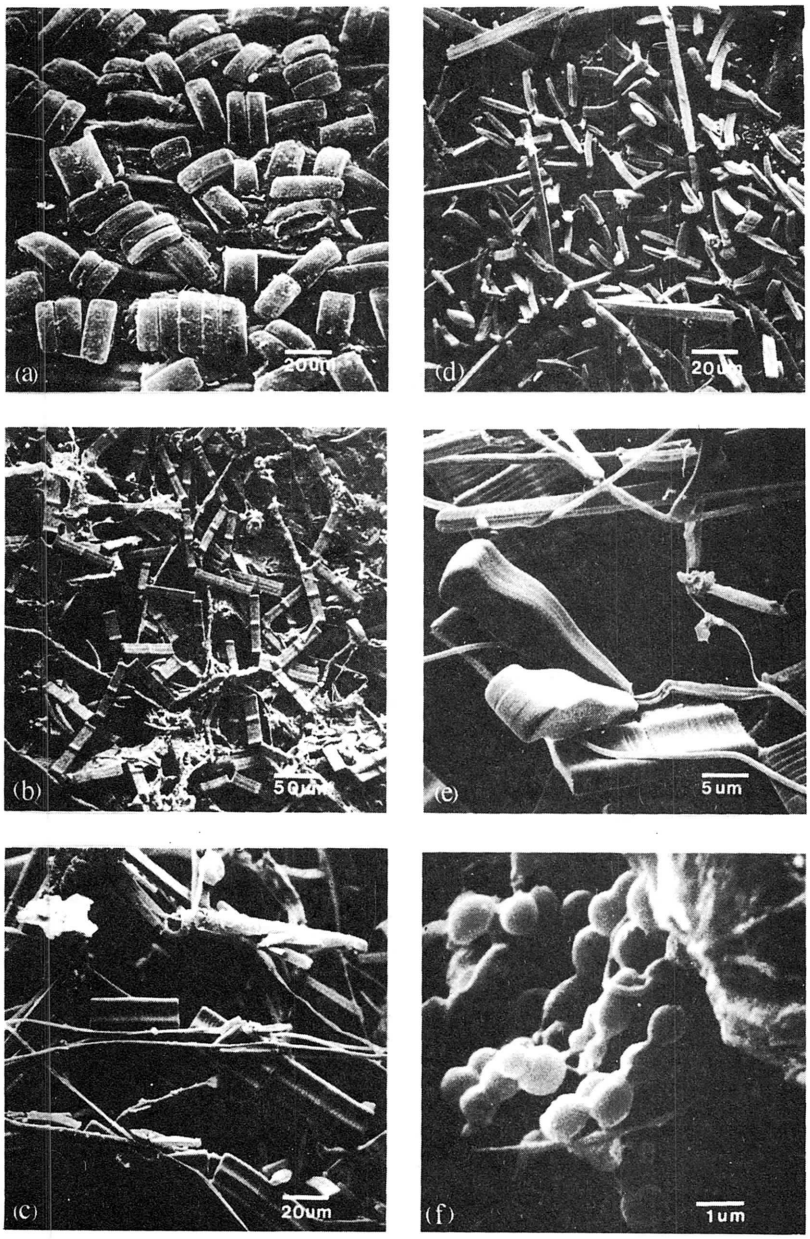

Fig. 1. Some characteristic epiphytes on *Lobelia* in the studied lakes. (a) a layer of firmly attached diatoms *Eunotia veneris* (Kütz.) O. Müller and *E. rhomboidea* Hust. in L. Gårdsjön; (b) cells of *Tabellaria fenestrata* (Lyngb.) Kütz. embedded in a mucoid matrix in L. Gårdsjön; (c) loosely attached cells of *T. flocculosa* (Roth) Kütz. in L. Gårdsjön; (d) a layer of firmly attached cells of *Achnanthes minutissima* Kütz. in L. Högsjön; (e) *Gomphonema constrictum* Ehrenb., *T. flocculosa* and *Synedra spp.* in L. Högsjön; (f) epiphytic bacteria in L. Högsjön. Scanning electron microscopy (SEM), 1980.

Gomphonema constrictum Ehrenb.) (Figure 1d, e). In contrast, studies in Sweden (Bengtsson *et al.*, 1980) show that two years after liming, planktonic communities were still dominated by the same species.

There was a marked seasonal pattern within the epiphytic communities in the studied lakes. In L. Gårdsjön, an initial dominance of the diatoms *Eunotia veneris* and *E. rhomboidea* in May–June (28% of the total cell number) was observed. Colonies of *Batrachospermum vagum* (Roth) Ag. and filaments of long pectin-rich cells (spring form) of *Binuclearia tectorum* (Kütz.) Berger in Wichm. were abundant (altogether 21% of the total cell number). Single filaments of blue-green alga *Homeothrix sp.* were characteristic. In summer, loosely attached *Mougeotia spp.* filaments, together with firmly attached colonies of *Bulbochaete spp.*, dominated the community (58% of the total cell number). As water temperature increased to 19 °C in July, colonial blue-green algae like *Aphanothece spp.* and *Microcystis spp.* became abundant. A high content of empty planktonic *Peridinium sp.* shells was found in summer samples. The diatom *Tabellaria fenestrata* (Lyngb.) Kütz. and filaments of *Binuclearia* (autumn form) became important components of the autumn community. The only important epiphytic desmid in L. Gårdsjön was *Penium sp.* forming from 2 to 7% of the total cell number throughout the study period.

In contrast to the statement of Brock (1973) that blue-green algae disappear below pH 5, they were abundant in the epiphytic community in L. Gårdsjön, composing 46% of the total cell number in July. The appearance of blue-green algae in such low pH conditions agrees with the observations of Kwiatkowski and Roff (1976), and Conroy *et al.* (1976) from Canadian lakes.

Spring time in L. Högsjön was characterized by a great number of desmids with dominating acidophilic *Hyalotheca dissiliens* J. E. Smith ex Bréb. (30% of the total cell number). Desmids remained an important component of the epiphytic community in L. Högsjön, throughout the study period. The diatom *Achnanthes minutissima*, which was rarely found in 1979 samples, dominated the community in summer, 1980 (42% of the total cell number). Stockner and Armstrong (1971) also reported the midsummer maxima of this diatom in ELA lakes of Ontario. Other diatoms like *Gomphonema constrictum, Synedra rumpens, Frustulia rhomboides* and *Tabellaria flocculosa* were common in the summer. Filamentous green algae (*Oedogonium spp.*) and desmids prevailed in the autumn. The planktonic blue-green alga *Anabaena lemmermannii* P. Richt. was found in samples during the warmer periods of the year.

The influence of environmental factors, such as the pH-CO_2-bicarbonate system, P and N, and toxicity of Al on the growth and distribution of epiphytes in acidified lakes is practically unknown. Experimental studies similar to that undertaken by Moss (reviewed in 1973) are necessary for the understanding of algal responses to acidification and liming.

4.2. Biomass

Both lakes showed a similar range for ash-free dry weight values (Figure 2). Spring and late summer maximum were noticed in L. Gårdsjön (1.0 mg cm^{-2}). The biomass of

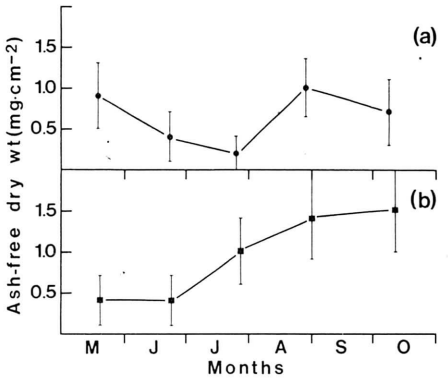

Fig. 2. Mean seasonal values of the dry weight biomass of epiphytes on *Lobelia* in L. Gårdsjön (a), and L. Högsjön (b), expressed as milligrams ash-free dry weight per square cm of the leaf surface area. Vertical bars equal two standard errors of the mean (n = 5).

epiphytes in L. Högsjön increased gradually to its maximum (1.5 mg cm^{-2}) in the autumn. The values presented are probably too high because of the accumulation of detrital material and dead algal cells within the epiphytic community. Obtained epiphyte biomass values are higher than reported from artificially acidified streams in Norway (Hendrey, 1976), and the periphyton biomass values reported for epiphytes in Lake George in the Adirondack Mountains, N.Y. (Sheldon and Boylen, 1976). Hargraves and Wood (1967) reported that in an oligotrophic pond (pH 4.0 to 4.9) attached algae peaked twice, in June due to the growth of green algae, and in September due to accumulation of blue-green algae. No clear relationship was found between changes in biomass and the occurrence of any of the main algal groups in these two lakes.

The highest chlorophyll *a* content was found in L. Gårdsjön in autumn (3.4 µg cm^{-2}), Figure 3. Chlorophyll *a* content followed the dry weight changes in this lake in summer

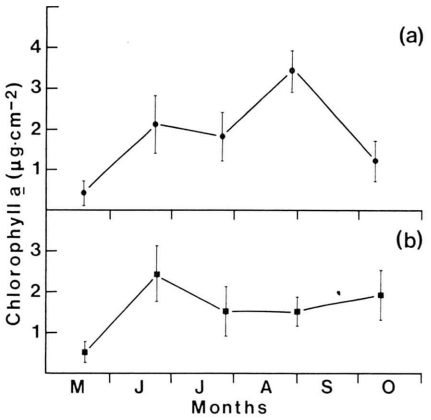

Fig. 3. Mean seasonal values of chlorophyll *a* content of epiphytes on *Lobelia* in L. Gårdsjön (a) and L. Högsjön (b), expressed as micrograms per square cm of the leaf surface area. Vertical bars equal two standard errors of the mean (n = 5).

and autumn. Low values observed in May suggest a high contribution of detrital material to the epiphyte biomass. In L. Högsjön chlorophyll *a* values are relatively low in summer and autumn, despite high dry weight values and abundance of green algae during these periods.

4.3. Primary productivity rates

Average epiphytic productivity rates, presented in Figure 4, indicate early summer and autumn maxima for both lakes. Comparative values for algae growing on glass slides,

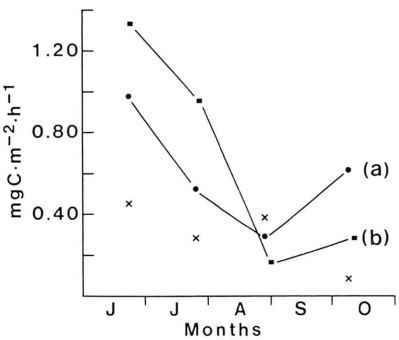

Fig. 4. Seasonal changes in the productivity rates of epiphytes on *Lobelia* at 1.0 m depth. (a) In L. Gårdsjön, (b) in L. Högsjön. (x) represents comparative values for the productivity rates of algae growing on glass slides suspended for one month in L. Gårdsjön. Values expressed in miligrams carbon-14 assimilated per square meter of leaf surface area, per hour. Duplicate estimations with a correction for dark uptake.

suspended for 1 mo in L. Gårdsjön show relatively low productivity rates. Uptake of 14-C in the dark was negligible, ranging from 1 to 3% of light fixation, thus indicating low heterotrophic metabolism within the epiphytic communities. No activity was detected in root-cuts.

Productivity was measured under light saturation conditions. Approximately 62% of (PAR) reaches 1.0 m depth in the littoral zone of L. Gårdsjön, and 33% in L. Högsjön. Water temperature during the incubation time varied between 18 °C in June and 12 °C in October. The observed drop in productivity in both lakes in late August coincided with reduced radiation during the incubation period and may indicate an ability of the epiphytes in these lakes to utilize the high intensity light. Other factors, like nutrient depletion (particularly P), may have been responsible for this drop in productivity but no chemical analysis of the littoral water during the incubation period was made.

Average DIC concentration at the sampling site in L. Gårdsjön was 1.2 mg C l^{-1}, and in L. Högsjön 4.9 mg C l^{-1}. These C concentrations were not considered a limiting factor in the photosynthesis of epiphytes in these lakes. Schindler *et al.* (1980) reported a concentration range from 1.4 to 0.8 mg C l^{-1} in the ELA lakes of Ontario, and found that phytoplankton photosynthesis was not C limited. Extremely low concentrations of DIC (less than 0.6 mg C l^{-1}) in the Canadian Shield lake were not C limiting for photosynthesis, when N and P were in sufficient supply (Schindler *et al.*, 1973).

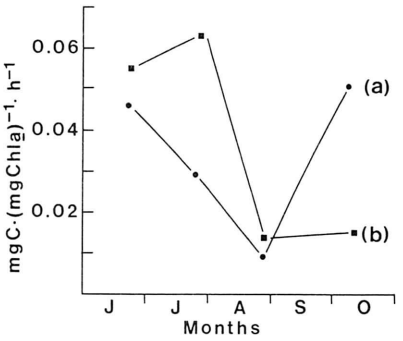

Fig. 5. Seasonal variation in specific productivity of epiphytes on *Lobelia* expressed as the ratio of assimilated carbon-14 to chlorophyll *a* content, per hour. (a) In L. Gårdsjön, (b) in L. Högsjön.

Specific productivity, calculated as a ratio of assimilated 14-C to chlorophyll *a* (Figure 5), follows the changes in productivity rates. There was no evidence of a close relationship between productivity and chlorophyll *a* content in the epiphytes at 1.0 m depth in L. Gårdsjön. Productivity in L. Högsjön was correlated with chlorophyll *a* values ($r = 0.58$).

Productivity rates of epiphytes in the limed lake, L. Högsjön, were not significantly higher (t-test on means at 95% confidence level) than in L. Gårdsjön. Presented productivity rates cannot, in any case, be representative for the studied lakes, due to the heterogeneity of the littoral zones and the limited number of replicas. The obtained results do show that the epiphytic productivity in the studied lakes at 1.0 m depth, is similar to that reported in oligotrophic lakes (Hooper and Robinson, 1976).

Arce and Boyd (1975) reported that the productivity of phytoplankton in limed ponds was generally higher than it was in untreated ponds. Bicarbonate could be a substantial source of CO_2. Also Sheldon and Boylen (1975) reported that in C limited conditions of Lake George, an addition of bicarbonate stimulated the growth of epiphytes by 30% in a 2 h period.

Significant productivity changes, both in acidified and limed lakes, may not be apparent for a long time due to the complexity of factors involved. However, the effect of acidification and/or liming on the structure of epiphytic communities cannot be ignored. As most algae have their origin in attached habitats (Wetzel, 1975), changes in the epiphytic community structure must also cause changes in phytoplankton. This study shows that epiphytes in the limed lake, L. Högsjön, are part of a more dynamic system of significant structural changes during the post-liming period.

There have been few attempts to measure the *in situ* rates of photosynthesis in epiphytic algae on their natural substrate. Technical problems and the uneven distribution of algae on host-plants make such attempts often difficult. This study demonstrates that some of these problems can be overcome. The choice of the host-plant is an important factor considering the existence of the intimate metabolic interactions between the plant and its associated epiphytes (Allen, 1971). The extent to which epiphytes utilize inorganic

compounds (O_2, PO_4, CO_2) and organic micronutrients released by macrophytes, is unknown. Further study of epiphytic communities in acidified and limed lakes requires an experimental design which includes the influence of P and Al on the growth of epiphytes.

5. Summary

The main purpose of this study was to provide some characteristics of the seasonal changes in the structure and productivity of epiphytes in acidified and limed lakes. The study was also methodological endeavoring to develop a more convenient method for *in situ* epiphyte studies and to encourage further investigation of the *Lobelia*-epiphyte complex.

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Structure and function of a cyanophytan mat community in an acidified lake

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The structural and functional properties of a cyanophytan mat in an extremely oligotrophic acidified lake were studied. The mat forms a physiological unit well adapted to acid conditions in the lake. Two to three millimetres of the uppermost stratum consists of photosynthetically active filaments of *Lyngbya* with an intrusion of other benthic algae. Relatively high rates of algal primary productivity and low rates of N_2 fixation were recorded in the mat.

LAZAREK. S. 1982. Structure and function of a cyanophytan mat community in an acidified lake. Can. J. Bot. **60**: 2235–2240.

L'objet de notre recherche a été l'étude des propriétés de structure et de fonction d'une couverture de cyanophytes d'un lac acidifié extrêmement oligotrophe. La couverture forme une unité physiologique bien adaptée aux conditions acides du lac. Deux à trois millimètres de la couche supérieure consistent en filaments de *Lyngbya* qui photosynthétisent, de même qu'en d'autres algues benthiques. On a pu enregistrer des taux relativement forts de production primaire des algues et des taux faibles de fixation de N_2.

Introduction

Calcareous environments, high temperatures, and pH levels are believed to favour the growth of the blue-green algae, which are the principal components of benthic mat communities. On the basis of his own studies and other reports, Brock (1973) concluded that pH 4 is the physiological acid limit below which growth of these prokaryotic organisms fails. He also suggested that cyanophytes are absent in waters with pH 5 or lower. However, recent reports on well-developed cyanopytan mats in acidified (pH < 5) lakes in Sweden (Lazarek 1980), in the United States (Hendrey and Vertucci 1980), and in Canada (P. Stokes, personal communication) question this acid barrier.

Progressive acid precipitation in southwestern Sweden has drastically altered the physical and chemical characteristics of freshwaters. It has resulted in low pH and an increased dissolution of metals, particularly aluminum. An increased water transparency and reduced biomass of phytoplankton have been documented for some shallow oligotrophic and humus-rich lakes of this region.

One of the most conspicuous effects of acidification observed in Sweden is the impoverishment of planktonic communities. Synchronously, the importance of benthic and epiphytic algae as primary producers increases. The relatively little information available on the sediment–water interface and the ecology of organisms living there may lead to miscalculations of their importance to the functioning of the lake ecosystem as a whole.

This study describes the structural and functional properties of the blue-green algal mat community found in the acidified phosphorus-limited Lake Gårdsjön, in southwestern Sweden. Based on studies of the lake mat and the available literature an attempt is made to explain the development and growth of this community in low pH conditions and its potential importance to the metabolism of an impoverished lake ecosystem.

Study area

The algal mat in the acidified dimictic Lake Gårdsjön (58°3′ N, 12°3′ E) was studied in 1980–1981. Gneiss and granite–granodiorit rocks form the bedrock of the lake. The region is characterized by soils with highly resistant minerals and a weak buffering capacity and by mixed deciduous and coniferous forests. The littoral of the lake is dominated by isoetids and *Sphagnum subsecundum* Nees. The bottom of the lake, down to 5–6 m, is covered by a more or less compact mat formation. The pH of the interstitial water is just above 4, increasing to 5 at a depth of 2 cm in the sediment and stabilizing at around 6 below that. Some physicochemical characteristics are presented in Table 1. Lake Gårdsjön is classified as an extremely oligotrophic lake owing to the low concentrations of nitrogen, phosphorus, and chlorophyll *a* in the water (cf. Forsberg and Ryding 1980).

Material and methods

Structural studies were performed on fresh and fixed algal mat. The material was collected from a 0.5 m depth in the littoral of Lake Gårdsjön. For taxonomical purposes, algae were cultivated under constant light conditions (55 $\mu E \cdot m^{-2} \cdot s^{-1}$) in Petri dishes with sediment and lake water. Species were identified with reference to the nomenclature of Starmach (1966) and Bourrelly (1970). Scanning electron microscopy (SEM) studies were performed on samples dehydrated in ethanol and freon and dried by the critical-point method (Mercer and Birbeck 1972).

Concentration of chlorophyll *a* (665 nm), corrected for phaeophytin *a* and turbidty, was determined spectrophotometrically according to the method of Marker *et al.* (1980), in mat cores 5 mm thick and 15 mm in diameter. Total carotenoid

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content (450 nm) was estimated in accordance with Jensen (1978). Because of the rapid light attenuation within the mat (see Results), studies of the functional parameters were performed on 5-mm-thick cores only.

Primary productivity rates were calculated on the basis of *in situ* ^{14}C experiments run in July and August 1980–1981, at a depth of 0.5 m, where the mat is usually uniform and compact. In July 1981, some experiments were also performed along the depth gradient in the littoral of the lake. In all cases, mat cores 65 mm in diameter were cut and incubated in 500-mL Plexiglas chambers (100 mm in diameter). One pair of light chambers plus one dark chamber were used in each experiment. The chambers were filled with filtered (Sartorius Membranfilter) lake water. One millilitre of $NaH^{14}CO_3$ (4 μCi, 1 Ci = 37 GBq) was injected via a rubber septum. The initial concentration of DIC (dissolved inorganic carbon) was 1.0 $mg \cdot L^{-1}$. Incubation time was 2 h. After exposure, subsamples 15 mm in diameter (1.8 cm^2 surface area) were cut. Two randomly chosen subsamples, constituting one sample unit, were taken for productivity rate calculations. Some subsamples were frozen and approximately 2-mm sections were cut from them to test the vertical distribution of ^{14}C incorporated in the mat. Samples were homogenized prior to counting, as suggested by Brock and Brock (1967). To distinguish bacterial from algal photosynthesis, some samples were incubated in the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyl urea), with a final concentration of 1×10^{-5} *M*. DCMU is a specific inhibitor of photosystem II (Rippka 1972). The incubated material was digested in scintillation vials for approximately 5 h at 45°C with 1.0 mL Soluene-350 (Packard Instruments Co. Inc.). The brown coloring was removed by the addition of 200 μL isopropanol and 200 μL 30% H_2O_2, with subsequent warming at 45°C for 30 min. Dimilume-30 (Packard Instruments Co. Inc.) was added (9 mL) and counting was done with a Packard automatic Tri-Carb liquid scintillation counter.

The DIC content in the water just above the mat surface was determined in an infrared Beckman carbon analyzer. Water samples were collected in 15-mL Vacutainers (Becton-Dickinson Co., NJ) and kept cold and dark until the analysis was performed.

Light intensity was measured using a quantum meter (LI-185, Lambda Instruments Co.) combined with an underwater silicon photodiode quantum sensor (LI 192S). It was assumed that PAR (photosynthetically available radiation) equals 46% of the total solar radiation at the surface of the lake. A constant 10% loss of PAR due to surface reflection was considered in calculating productivity rates.

On two occasions, N_2-fixation rates were estimated in mat cores (15 mm in diameter) by a modified version of the acetylene-reduction technique (Stewart *et al.* 1967), at a depth of 0.5 m. Material was incubated in 5.0 mL lake water in test tubes, which were closed with stoppers and screw caps. Two millilitres of acetylene, cleaned in H_2SO_4 and water, were injected into the test tubes. The experiments were carried out in triplicate, and two "blank" tubes containing material preserved in 0.15 mL Lugol's solution were included in each of the experimental series. Biological ethylene production, not derived from nitrogenase activity, was tested using samples incubated without the acetylene additions. After 2 h, acetylene reduction was stopped by the injection of 0.15 mL Lugol's solution. Loss of ethylene due to solubility and chemical reaction with Lugol's solution was considered (Leonardson 1980). To convert C_2H_2 to NH_4, the theoretical molar ratio of 1.5:1.0 was used.

TABLE 1. Some limnological parameters of Lake Gårdsjön, 1980–1981

Lake area (km^2)	0.3
Mean depth (m)	4.9
Transparency (m)	9.5
pH	4.3–4.7
Conductivity ($mS \cdot m^{-1}$)	6.2–8.1
$NO_2 + NO_3 - N$ ($\mu g \cdot L^{-1}$)	110
$NH_4 - N$ ($\mu g \cdot L^{-1}$)	56
Total P ($\mu g \cdot L^{-1}$)	6.2
DOC ($mg \cdot L^{-1}$)	3.6
DIC ($mg \cdot L^{-1}$)	0.7–1.2
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	0.89
Al ($mg \cdot L^{-1}$)	0.30

Results

The algal mat in Lake Gårdsjön forms a perennial cohesive blanketlike cover over the organic matter-rich sediment. The surface of the undisturbed mat shows a reticular structure (Fig. 1A), and the brown to bright green color may relate to the phototactic movement of the algal filaments in response to changing light conditions. Sections of 10-mm-thick mat observed under SEM show distinct structural stratification. Two to three millimetres of the uppermost stratum consists of a dense network of motile filaments of *Lyngbya bourrellyana* Compère (=*Phormidium ambiguum* Gomont) with less abundant filaments of *Oscillatoria geminata* Meneghini (Fig. 1B). Other benthic organisms (e.g., cyanophytes *Merismopedia* sp. and *Aphanothece* spp. and pennate diatoms such as *Frustulia rhomboides* (Ehrenb.) De Toni and *Anomoeoneis serians* (Bréb.) Cl.) are also abundant within the filamentous matrix. The surface of the mat is often occupied by chironomid larvae, while nematodes are present in the lower layer of the hyphae-like stratum of dead algal filaments and partially decomposed plant remains (mainly *Sphagnum*).

Algal composition has remained almost unchanged since the first samples of the mat were examined in 1978. Large gas-filled blister formations indicate that the diffusion of gases (N_2, CH_4) built during sediment decomposition is hindered by the poor permeability of the mat. A dense algal filament network effectively binds detrital particles (Lazarek 1980). This creates a resilient seal, severely restricting the exchange processes between the sediment and the overlying water. In nearby Lake Hästevatten (pH 5.1 to 5.7), a cyanophytan mat composed mainly of *Hapalosiphon fontinalis* (Ag.) Born. occurs. Lake Hästevatten and Lake Gårdsjön are connected, but no filaments of *Lyngbya* were found in the Lake Hästevatten mat community.

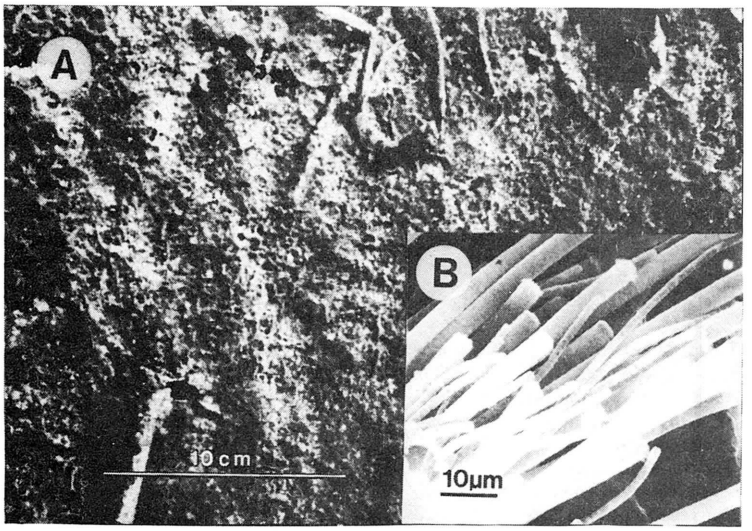

FIG. 1. Reticular structure of the Lake Gårdsjön mat surface (A) and the filamentous matrix of *Lyngbya* and *Oscillatoria* (B).

The most characteristic submerged macrophyte in Lake Gårdsjön is *Lobelia dortmanna* L. In places where the mat covers *Lobelia* plants, a complete depigmentation of the leaves is observed. This probably contributes to the elimination of this plant from the sheltered parts of the littoral. Despite the proximity of *Lobelia* with the sediment, none of the motile cyanophytes were found growing epiphytically on the plant.

The surface of the mat is aerobic year round owing to photosynthetic activity in the mat and O_2 released in the rhizospheres of the abundant isoetids. The pH measured within the mat was higher than in the overlying water, and ranged from 5.5 to 6.2, depending on the time of day.

The average chlorophyll *a* content in the mat was about 20 times higher than the maximum measured in epiphytic algae per surface area of the host plant. The ratio (percentage) of chlorophyll *a* to dry weight equaled 0.4 and was lower than that found for epiphytic algae (>0.7) in the lake (S. Lazarek, unpublished data). The content of degradation products (phaeophytin *a*) in the mat varied from 36.3 to 58.2% of the total chlorophyll *a* content. The absolute chlorophyll *a* values are on the other hand much higher than reported elsewhere for mat-forming algal communities (Moss 1968). This would suggest a highly productive community even in this acidified, extremely oligotrophic lake.

The total carotenoid content follows the chlorophyll *a* microdistribution pattern within the 5-mm-thick cores, being highest in the uppermost 3-mm stratum.

Only 0.026% (0.4 $\mu E \cdot m^{-2} \cdot s^{-1}$) of the incident PAR was measured under the 4-mm-thick mat cores at a depth of 0.5 m, in summer 1981. This means that as little as 0.014% of the PAR was available for algal photosynthesis below that layer. Assuming after Jørgensen *et al.* (1979), that photosynthesis above the compensation point in the prokaryotic communities takes place at light intensities down to 0.1% of full sunlight, the photic zone in the Lake Gårdsjön mat would be restricted to the uppermost 2–3 mm. Results of the functional study of the mat are summarized in Table 2.

The distribution of photosynthetic activity within the mat cores confirms the structural lamination of the mat. As much as 90% of the total activity (disintegrations per minute) was recorded in the homogenized uppermost 2-mm layer. The average value for samples collected on four dates in 1980 and 1981 was 32.8 ± 12.9 mg $C \cdot m^{-2} \cdot day^{-1}$. Productivity rate values showed no significant variation on the different sampling occasions (*t*-test, sample size = 15, $P > 0.1$). Simultaneously incubated epiphytes on *Lobelia* and *Equisetum* showed average rates of 75.0 ± 12.8 and 49.1 ± 23.2 mg $C \cdot m^{-2} \cdot day^{-1}$, respectively. Specific productivity (percentage) of the mat was comparable with that of the epiphytes in the lake (Lazarek 1981).

Light is a principal factor limiting benthic algal photosynthesis (Goldman *et al.* 1963; Priddle 1980). The total daily radiation measured at the water surface during *in situ* productivity studies ranged between 9.4×10^6 in 1980 and 52.4×10^6 $\mu E \cdot m^{-2} \cdot day^{-1}$ in 1981. The average photosynthetic rates between 2 years were not significantly different however (*t*-test on means at 95%

TABLE 2. Mean values in 5-mm-thick mat cores (surface area 1.8 cm^2) for pigment content, biomass, productivity, specific productivity (percent), and nitrogen fixation in Lake Gårdsjön, 1980–1981. Number of samples in parentheses

Measured parameter	Mean ± SD	Units
Chlorophyll *a* (9)	81.3±3.0	$\mu g \cdot cm^{-2}$
Total carotenoids (9)	18.8±6.8	$\mu g \cdot cm^{-2}$
Ash-free dry weight (9)	21.3±3.6	$mg \cdot cm^{-2}$
Productivity rate (60)	32.8±12.9	$mg\ C \cdot m^{-2} \cdot day^{-1}$
Specific productivity (15)	4.1±1.6	$mg\ C \cdot mg\ Chl^{-1} \cdot day^{-1}$
N_2 fixation (16)	14.2±5.8	$\mu g \cdot m^{-2} \cdot h^{-1}$

TABLE 3. Dominating algae in cyanophytan mat communities from freshwater, oligotrophic lakes

Dominating algae	pH	Lake (reference)
Lyngbya bourrellyana (=*Phormidium ambiguum*)	4.3	Gårdsjön, Sweden (present study)
Phormidium tenue	4.8	Colden, U.S.A. (Hendrey and Vertucci 1980)
Oscillatoria sp.	5.8	Mirror, U.S.A. (Moeller and Roskoski 1978)
Microcoleus lyngbyaceus (=*Lyngbya aestuarii*)	7.4	Lake A,* Antarctica (Zaneveld 1969)
Phormidium sp.	—	Changing, Antarctica (Priddle 1980)

*Salinity, 0.3%.

confidence level). This may indicate light-saturated photosynthesis on both occasions. Also, samples incubated at a depth gradient from 0.5 to 2.0 m did not show significant differences in productivity rate values.

The inhibition of the O_2-evolution step of photosynthesis by DCMU enables evaluation of the bacterial contribution to the mat metabolism. The DCMU-sensitive CO_2 assimilation, which is algal photosynthesis, was reduced during 2 h incubation by more than 90%. The remaining was close to the average dark ^{14}C uptake recorded in the course of this study.

The rates of N_2-fixation were very low (Table 2), much lower than that reported for similar cyanophytan mats in the oligotrophic Mirror Lake (Moeller and Roskoski 1978). On both occasions, the values obtained were not significantly different (*t*-test, sample size = 16).

Discussion

The presence of a well-developed, low-diversity cyanophytan mat in low pH conditions is of particular ecological interest. As the biomass and productivity of phytoplankton in the hypolimnion of Lake Gårdsjön are very low (0.89 μg chlorophyll $a \cdot L^{-1}$ and <10 mg $C \cdot m^{-3} \cdot day^{-1}$ respectively; O. Grahn, personal communication), the contribution of the benthic mat to the total primary production of the lake may be quite high. However, diurnal variations in the mat metabolism and the unknown pool of CO_2 and its turnover time in the mat do not allow for a calculation of the absolute benthic production in the lake. The 2-h incubation period used could also be too long, as CO_2 turnover time in cyanophytan mats can be very short. The CO_2 turnover time reported by Jørgensen *et al.* (1979) was only 10–20 min. In an oligotrophic lake, the production of algal mats with extremely low specific productivity rates (0.02) was reported to be 25 to 45 times higher than that of phytoplankton (Grobbelaar 1974).

The autotrophic bacterial production, especially that of sulfur bacteria, may be significant in small nutrient-poor lakes (Parkin and Brock 1981), but as the littoral of Lake Gårdsjön is oxygen-oversaturated, this type of metabolism is of minor importance. Anaerobic conditions may, however, develop locally under the ice cover or be owing to the oxygen consumption by H_2S and Fe oxidation.

Judging from the glucose concentration in the lake sediment (0.1–2.0 $mg \cdot L^{-1}$), which is higher than in other acidified, nutrient-poor lakes of the region, heterotrophic growth of the mat cannot be excluded. The microbial glucose uptake in Lake Gårdsjön was found to be unusually high (0.2–2 $mg \cdot L^{-1} \cdot h^{-1}$ (G. Gahnström, unpublished data)). The aspect of possible heterotrophy is not considered in this study.

Among factors governing the growth of the benthic algal community, light conditions and availability of P and DIC should be considered. The filamentous construction of the mat and a high content of detritus and

carotenoids provide effective photoprotection of the superficial algal cells. Detritus is a main source of both particulate N and P in the lake. The N/P ratio in the lake water equals 63. According to Forsberg and Ryding (1980), P is the growth-limiting element at N/P ratio values above 17. High phosphatase activity in the lake water reported by Jansson *et al.* (1981) indicates a shortage of P, which either precipitated effectively or is bound in the benthic mat. The DIC content in the water above the mat surface ranged from 0.7 to 1.2 mg $C \cdot L^{-1}$ in samples collected at midday. Practically all inorganic carbon in the water is in the form of free CO_2. Most of the cyanophytes have an ability to utilize CO_2 as the sole source of inorganic carbon for photosynthesis, so it is unlikely that DIC is limiting photosynthesis in the mat in Lake Gårdsjön.

The growth of a single cell is governed at the enzymatic level. Most enzymes are surface-bound and are the first to be affected by low external pH or a high content of active metal ions. Low pH will also have a direct destructive effect on the superficially located chlorophyll in cyanophytes. It may also affect the availability of nutrients, especially CO_2, Fe, and organic acids. Why then, is *Lyngbya* dominating in the Lake Gårdsjön benthic mat?

Lyngbya is either an acid-resistant organism or it was present in the lake long before the pH dropped to the present level. In any case, *Lyngbya* may have initiated a long-term succession of other benthic forms by considerably modifying the sediment–water microenvironment. This leads to the formation of the present community as a physiological unit which is in homeostasis with the physicochemical environment. By its structural and physiological properties this unit achieved "maximum protection." The idea of treating the mat community as a unit is not new (cf. Koizumi 1980; Priddle 1980). The ability of *Lyngbya* to produce a dense mucilage matrix was observed in Lake Gårdsjön. Mucilage substances are composed mainly of polysaccharides, which can chelate metal ions, including Al (Tanaka *et al.* 1974; Lasik and Gordiyenko 1977). The soluble polysaccharides can also be used by organisms in times of nutrient depletion (Wilkinson 1958).

Turning now to similar mats appearing in oligotrophic lakes (Table 3), we see that they are composed of filamentous cyanophytes belonging exclusively to the family Oscillatoriaceae. They are observed in small, shallow, and nutrient-poor lakes with high transparency and a low phytoplankton population and are situated in the cold or temperate parts of the world.

Our knowledge of the physiological mechanisms of algal adaptation to low pH conditions is still poor and based mainly on laboratory experiments. This study suggests that benthic cyanophytan mats may be an important component in the metabolism of acidified, nutrient-poor lakes. It will hopefully encourage further studies of the nonplanktonic algal communities appearing in impoverished, low-pH waters.

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MICROSTRUCTURE AND METAL CONTENT OF THE *LOBELIA*-EPIPHYTE COMPLEX IN ACIDIFIED LAKES

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Lobelia-epiphyte Complex In Acidified Lakes

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SUMMARY. The microstructure and the phosphorus and metal content of the epiphytic complex associated with *Lobelia dortmanna* was studied in the acidified Lake Gårdsjön, Sweden. The acidified and limed Lake Högsjön was used as a reference.

The epiphytic microstructure and the retention of some elements in the tissue of the *Lobelia* and epiphytes are discussed in relation to extremely low concentration of dissolved inorganic carbon and phosphorus and the high content of some metals in the water.

Compared to other components of the epiphytic complex, mucilage retains the greatest variety of metals and had the highest content of calcium, sodium and aluminium. The aluminium content in leaves with epiphytic cover was twice as high as that of the leaves alone. In both lakes leaf tissue was poor in zinc and aluminium.

The epiphytic complex is a stable microbial association where the structural adaptation of the organisms composing the complex is intrinsic to the various components' metabolism.

KEY WORD INDEX: *Lobelia dortmanna*, *Eunotia*, epiphytes, microstructure, metals, mucilage, acid lakes, limed lakes.

MICROSTRUCTURE AND METAL CONTENT IN THE *LOBELIA*-EPIPHYTE COMPLEX IN ACIDIFIED LAKES

S. Lazarek

Introduction

In contrast to the extensive research on marine epiphytic covers and microbial fouling (Marshall *et al.* 1971: DiSalvo & Daniels, 1975; Dempsey, 1981), little attention has been paid to freshwater algal fouling. Allanson (1973) stressed the importance of the structural relationships between the various components of the epiphytic complex and suggested that the structural relationships are closely implicated in the metabolism of the complex as a whole.

The settlement and colonization of microorganisms on submerged plant surfaces is a natural process involving competition, succession and modifications of the plant-epiphyte microenvironment. Microorganisms which are attached to plant surface benefit from the enriched nutrient status at the solid-liquid interface, especially in nutrient-poor aquatic ecosystems (Marshall & Bitton, 1980). Such interfaces may provide a nutrient and pH level sufficient to support rich epiphytic growth.

The progressing acidification of freshwaters has caused severe damage to dystrophic and oligotrophic lakes (*Lobelia*-lakes) in temperate regions of Northern Europe and North America. Changing physico-chemical conditions in acidified lakes in different parts of the world are followed by similar characteristic biological responses (Hendrey *et al.*, 1976; Wright & Gjessing, 1976; Stokes, 1982). Laake (1976) reported the gradual extinction of *Lobelia dortmanna*

L. via inhibition of production and flowering at pH 4, when epiphytic and metaphytic algal growth increased. His observations were supported by the conspicuous epiphytic cover on *Lobelia* leaves in acidified lakes in Sweden (Lazarek, 1982). In lakes unaffected by acidification, *Lobelia* is almost barren of eukaryotic, firmly attached epiphytes.

This study was designed to provide information on the spatial arrangement and development of the *Lobelia*-epiphyte complex in acidified lakes. One objective was to show to what extent the composition of elements in the epiphytic microlayers may influence results from routine chemical analyses of the plant material. Another objective was to identify and localize metals and phosphorus within the *Lobelia*-epiphyte complex. Due to the reduced biomass of phytoplankton in acidified lakes, benthic and epiphytic algae become the prime food source for grazers which may accumulate metals (Bistricki & Munawar, 1982). Studies on the content and localization of metals in algae are therefore highly desirable.

Material and Methods

Lobelia plants with their associated epiphytes were sampled from 1.0 m depth in August 1980 and 1981. Samples were taken from Lake Gårdsjön (pH 4.2 to 4.7) and Lake Högsjön (4.3 to 5.4, but after liming in 1978 5.6 to 6.6). Both lakes are situated in south-western Sweden. More detailed characteristics of these lakes are given by Jansson *et al.* (1981) and Lazarek (1982).

In these lakes *Lobelia dortmanna* forms underwater meadows

in the littoral showing light-dependent zonation with other isoetids. The *Lobelia* leaves support heavy growth of epiphytes with many species common to both lakes.

a) *microstructure*

Studies of the microstructure of the *Lobelia*-epiphyte interface were done on leaf sections from plants growing in the lakes as well as epiphyte-free land forms of *Lobelia*, which were exposed in the lakes in a separate study of epiphytic colonization rates (Lazarek, in prep.).

Leaves with associated epiphytes were dehydrated in a series of ethanol and preserved in a 10% solution of phosphotungstic acid (PTA) and in 0.5% of uranyl acetate (UA) in absolute ethanol. The material was than embedded in Neoprene. Sections were cut on a LKB Ultramicrotome. Analysis was performed on transmission electron microscope (TEM) Philips EM 300. Light microscopy was carried out using a Leitz ORTHOLUX phase-contrast microscope.

b) *X-ray microanalysis*

The specimens viewed under the scanning electron microscope (SEM) were dehydrated in a series of ethanol and freon and dried by the critical point drying method. One set of samples was mounted on carbon specimen holders (Liljesvan & Roomans, 1976) and coated with a conductive carbon layer to facilitate thermal and electric conductivity. Metals were localized. Qualitative and semi-quantitative microanalyses were performed on the epiphytic structures which were dominating or common for both lakes and on the *Lobelia* leaf surfaces. A Kevex (EDX) energy dispersive X-ray spectrometer in combination with a Cambridge Stereoscan SEM was used.

Specimens were viewed at 30 Kv. The electron beam was kept stationary on the spot chosen for analysis and counts were made over 100 secs. The spots where X-ray analysis was performed are marked with asterisks on the figures. Another set of samples from the same *Lobelia* plants was prepared on aluminium holders and covered with a layer of gold/palladium in preparation for high contrast micrographs of the structures which had been analysed by X-ray.

c) *elemental analyses*

During 1980 and 1981, a total of forty plants was collected from each lake. Element analyses were performed on the dried ($60^{o}C$) material, which was divided into three groups: leaves with epiphytic cover, leaves from which the epiphytes had been brushed off and the roots. For leaves with epiphytic cover the ash-free dry weight was 84.3%, for leaves from which the epiphytes had been removed it was 87.8% and it was 88.0% for the roots. The metal content of the above mentioned components was converted to % of the ash-free dry weight. Total-P and metals were analysed after digestion with a perchloric-nitric acid mixture (1:3) and filtration through Whatman GF/C filters. The metals Ca, Mg, Fe, Mn, Cu, Zn and Al were analysed by atomic absorption spectrophotometry (Perkin Elmer 560). The flame photometer (Eppendorf) was used for analysis of Na and K.

Due to the thick cuticula, brushing did not damage the outer epidermal layer of the leaves. This was confirmed by SEM observations.

Results

The epidermal outer wall of the *Lobelia* leaf has a uniform, smooth surface (Fig. 1). Cross-sections of healthy leaves

observed under LM and TEM, revealed a primary mucilaginous film on epidermis which is probably secreted by the plant as the thickness of the film is equal on the upper and lower sides of the leaf and ranges from < 2 µm in L. Gårdsjön to 3–6 µm in L. Högsjön. Bacterial and cyanophytan organisms (Fig. 2–5) probably initiated settlement by secreting an adhesive substance on which the first compact layer of epiphytes was formed. In both lakes, this layer was built up of rod-shaped bacteria (Fig. 2). Observation of microorganisms under high-magnification revealed colloidal-size fibrils which mediate in the attachment process (Fig. 3) and consolidate the settling cells (Fig. 4). The electron-opaque material was stained with ruthenium red which, according to Fletcher & Floodgate (1973), is specific for the primary acidic polysaccharide. Void areas surrounding cells are considered by Jones *et al.* (1969) to be places of storage for diffusible nutrients. The secondary polysaccharideous material was excreted by the following settlement of the diatoms *Eunotia rhomboidea* Hust. and *E. veneris* (Kütz.) O. Müller (Fig. 5). SEM revealed that mucilaginous matrix covers the bacterial and cyanophytan cells (Fig. 6). The side-to-side attachment of *Eunotia* cells leaves very little space for other algae. Fig. 7 shows the effective utilization of space when a germinating filament of blue-green alga grew down through the adhesive pad. *Eunotia* cells with flattened mucilaginous adhesive around the periphery of their lower valves are often covered with a mesh-like fibrillar substance (Fig. 8), which could have been involved in the attachment of the colonial *Merismopedia* and *Aphanothece*, and pseudo-filamentous *Johannesbaptistia* (R. Nordin, personal communication) shown in Fig. 9 and 10. Some coccoidal bacteria were also

observed growing on frustules of *Eunotia* (Fig. 11). In L. Gårdsjön, the extensive release of a polysaccharideous substance by loosely attached epiphytic algae forms a coating which coalesces with that produced by the underlying bacterial and *Eunotia* microlayers (Fig. 12). This formation harbours benthic diatoms, detrital particles and sedimenting planktonic organisms. In shallower parts of the littoral, mineral particles contribute to the high ash content of the epiphytic complex.

Since 1978, firmly attached *Eunotia* in L. Högsjön has been replaced by *Achnanthes minutissima* (Kütz.) and *Gomphonema constrictum* Ehrenb. These two diatoms, which are attached by means of mucilaginous stalks (Fig. 13 and 14), made the epiphytic construction a more suitable microenvironment for unicellular green algae, particularly desmids.

Loosely attached filamentous algae contributed most to the total biomass of epiphytes in these lakes. These algae were made up of *Bulbochaete* spp. attached by discoidal holdfast cells and unbranched filaments of *Oedogonium* spp. (Fig. 15 and 16). Filaments of *Mougeotia* spp. and *Binuclearia tectorum* (Kütz.) Berger in Wichm., interwoven with diatoms, were characteristic for the epiphytic complex in both lakes and the metaphytic algal clusters observed in the littoral of these lakes. Bacterial colonization was observed on filaments of *Bulbochaete* and *Binuclearia* (Fig. 17 and 18), while other green algal filaments were almost barren of bacteria.

Metal analyses results from *Lobelia* plants in L. Gårdsjön and L. Högsjön are given in Table 1. The metal content values of the different *Lobelia* components were ranked and the values for the two lakes were compared. Spearman's

coefficient of rank correlation, r_s (Bishop, 1981) was calculated. Agreement was found between the total metal content in the leaves with epiphytes (r_s= 0.65, p= 0.05) and in the roots (r_s= 0.93, p= 0.01). The content of metals in leaves without epiphytic cover differed significantly between the two lakes (r_s= 0.31, p= 0.01).

In L. Gårdsjön, leaves with epiphytes showed significantly different levels of metals compared to leaves without epiphytes (r_s= - 0.29, p= 0.05) and to root tissue (r_s= 0.16, p= 0.05). In this lake, root tissue had the highest levels of calcium and magnesium. A surprisingly low amount of manganese was found in the leaves with epiphytic cover.

In L. Högsjön, which was limed, a considerable amount of calcium, magnesium and manganese seems to have been retained in the epiphytic cover.

In both lakes leaf tissue was poor in zinc and aluminium. These elements clearly tended to accumulate in the epiphytic cover and roots. In L. Gårdsjön, the level of aluminium in the leaves with epiphytes was 99% higher than in the leaves alone, and 19% higher than in the roots. Most of the sodium was accumulated in root tissues while iron was found precipitated on the root surface. The highest content of potassium and phosphorus was found in leaves without epiphytic cover. The copper content was equal in all the analysed components.

SEM X-ray microanalysis of the *Lobelia*-epiphyte complex was done to complement the atomic absorption analysis. The elements detected cover the range from sodium (K_α- 1.03 eV) to iron (K_α- 6.41 eV). In general, a greater variety of metals was found in the epiphytic structures from L. Högsjön than in those from L. Gårdsjön. The greatest variety of metals and the highest content of calcium, sodium and aluminium were detected in mucilaginous extracellular structures (Table 2). In L. Gårdsjön metals such as titanium,

manganese and zinc were identified only in the mucilaginous structures. Phosphorus was localized in the mucilage and in the cell wall of green algae, but was not detected in the outer walls of the *Lobelia* leaves, which suggests high intracellular accumulation of this element. The proportion of calcium (based on percentage contribution to the total counts of the metal spectrum) in the scanned leaf surfaces was 8.8% in L. Gårdsjön and 31.4% in L. Högsjön. A higher proportion of sulfur, chlorine, potassium and iron was found in the leaf surfaces from L. Gårdsjön. The spectrum for the filamentous green alga showed accumulation of calcium (up to 36.6%) and chlorine. Apart from the high proportion of silica (82-89%), diatoms retained considerable amounts of calcium and iron. The spectrum showed 5% of iron and 2.7% of aluminium for blue-green algae from L. Gårdsjön. Despite high concentrations of heavy metals in the L. Gårdsjön water (see Discussion), metals such as vanadium, chromium, nickel, cobalt, cadmium and lead were below the detection limits for X-ray microanalysis of the epiphytic structures.

Discussion

Extreme conditions influence algal growth in L. Gårdsjön. 1) Low concentration of dissolved inorganic carbon (DIC). The DIC content above the sediment surface at 1.0 m depth ranged from 0.8 to 1.2 mg l^{-1}. 2) Low concentration of dissolved inorganic phosphorus in the lake (< 1 µg l^{-1}). An extremely high phosphatase activity was measured in the lake (Jansson *et al.*, 1981). 3) High content of some metal ions especially aluminium (350 µg l^{-1}).

From a physical point of view, the epiphytic complex can

be considered as an association of microstrata which have strong chemical gradients in terms of accumulated cations and anions. The settlement of particles and living cells on the leaf surface of *Lobelia* leads, according to Ramamoorthy & Leppard (1976), to a rearrangement of the distribution of ionic charges on the leaf surface. The *Lobelia* rosettes, which grow in the proximity of the sediment, harbour large amounts of detritus, despite the smooth surface of their leaves. The initial phase in the development of the epiphytic cover may be seen as a passive, non-specific settlement of particles. Due to the surface charges of the particles, nutrients in low supply can be attracted to and concentrated on the particles. Such a concentration, with additional enrichment of particles by attached microorganisms, is believed to be common in extremely oligotrophic waters (Paerl, 1975). The detrital material associated with the epiphytic complex certainly includes bacteria and the dissolved substances produced by microorganisms and used by them or other members of the complex. Because the attached microorganisms represent different forms of metabolism, chemical and physical modifications of the *Lobelia*-epiphyte interface are likely to lead to the formation of a stable epiphytic system.

Observations of the epiphytic cover under SEM and TEM revealed the existence of electron-opaque fibrils which are the physical units connecting and consolidating the attaching cells. The importance of the fibrillar formations has only recently been brought to light (Massalski & Leppard, 1979). The secretion of insoluble polysaccharidic materials in L. Gårdsjön may be a defence reaction against the critical factors mentioned above. It has been suggested

(Paerl, 1980) that the magnitude of the fibrillar web formation reflects the trophic state of the lake and is especially noticable on organic carbon-rich particles. The excretion and formation of webs and appendages serve other functions besides attachment. Both algal and bacterial excretions accumulate growth-limiting nutrients and bind and chelate trace elements, especially iron (Murphy *et al.*, 1976). The content of iron in the epiphytic cover in L. Gårdsjön was high.

Another function of mucilage excretions, suggested by Dempsey (1981), was that of binding enzymes and providing a larger absorption and digestion surface.

Attached bacterial flora was represented in L. Gårdsjön by two morphologically distinct types, while in L. Högsjön the variety of bacteria was greater and included filamentous bacteria. Marszalek *et al.* (1979) reported initial colonization by rod-shaped bacteria in bacterial fouling. Dempsey (1981) documented that primary periphytes are predominantly chemo-organotrophic, Gram-negative rods, which provide specialized organic nutrients. Most of the living algal cells in the studied material supported bacterial growth.

Although bacterial degradation in acid conditions is believed to be slow (Andersson *et al.*, 1978), any, even localized nutrient enrichment, would be of importance in a nutrient-poor milieu. Epiphytes would benefit from degradation products, especially PO_4^{-3} and CO_2. Enrichment with the organic phosphates which derive from dying algae (Stewart, 1978) is also likely to be important. The existence of heterotrophic and photosynthetic cells would replenish the CO_2 content within the epiphytic complex and

make it less dependent on the low external supply. In L. Gårdsjön, the dissolved organic carbon (DOC) content in the water is relatively high (2-4 mg l^{-1}) and epiphytic bacteria may assimilate DOC. In the functional sense, this would represent a form of harvesting of the fixed carbon in the environment that might otherwise be lost.

Compared with L. Högsjön, *Lobelia* plants at the same depth in L. Gårdsjön receive 50% more of the photosynthetically available light (Lazarek, 1982). In L. Gårdsjön the epiphytic cover is especially compact and mucilage-rich. This could mean that the compact epiphytic cover affords light protection for its algal components. The disappearance of *Lobelia* from acidified lakes would then be at least partly related to the development of compact epiphytic covers which reduce the light available to *Lobelia*. In this case, the growth of the host and that of the epiphytes would be counteractive. Decreased production of mucilage and the replacement of *Eunotia* by the stalk-forming diatoms *Achnanthes* and *Gomphonema*, is considered to be one of the long-term effects of the added bicarbonates. The increase of DIC to 8 mg l^{-1} seems to benefit desmids.

My results indicate that epiphytes have a considerable capacity to accumulate metals. This supports Stokes's *et al.* (1982) findings that the epiphytic and metaphytic algae are able to immobilize toxic metal ions in the lake. Paerl & Lean (1976) discussed the retention of phosphorus in colloidal-size particles and microbes. In the phosphorus-poor conditions of L. Gårdsjön, it may be advantageous for the epiphytes to take up and use the inorganic phosphorus bound to polysaccharideous structures. In this way cells

would build up their own pool of phosphorus. The studies of Jansson *et al.* (1981) indicated that most phosphatase activity was derived from dissolved enzymes and those associated with particles < 5 µm. It has recently been shown (Carignan & Kalff, 1982) that the impact of phosphorus recycling within the epiphytic complex is of greater importance than phosphorus transferred from macrophytes. Carignan & Kalff observed that 90% of the diurnal release of phosphorus derived from the epiphytes alone. Only firmly attached epiphytes were considered in my study and it remains unknown how big a fraction of phosphorus is accumulated in the loosely attached green algae. My results suggest that *Lobelia* leaves are a substantial source of phosphorus in the lake. Moeller (1978) found a similar content of phosphorus in *Lobelia*, with a slightly higher content in the roots of fruiting plants. The ratio N:P in his study was 22:1 for leaves of sterile plants which corresponds to the N:P ratio of 175:1 in this study. This reflects the high N:P ratio in the epilimnion of L. Gårdsjön (71).

Using plants to study metal ion concentrations in acidic waters was suggested by Leland *et al.* (1979). My study demonstrates however that the metal concentration in plant material which includes a thin layer of epiphytes may be severely overestimated as regards sodium, iron, aluminium and calcium. On the other hand, elements such as phosphorus, magnesium, potassium, and manganese may be underestimated. Patrick & Loutit (1977) found that the concentration of metals in *Alisma* leaves was markedly reduced when their epiphytic bacteria were removed. The methods used to remove the epiphytes from plant surfaces are inefficient,

although some progress in this field has recently been made (Booth, 1981).

Briand *et al.* (1978) found that the binding capacity of low-nutrient lake water for metal ions is related more to the algal species composition and less to the total algal biomass or the physico-chemical conditions of the water. In their study, green algae, diatoms and chrysomonads that constituted a small percentage of the total algal biomass accounted for most of the binding. One of the structural changes in the attached assemblage following additions of zinc was the formation of mat made up of fungal strands, sheathed bacteria and blue-green algae which have a heavy secretion of mucilage (Williams & Mount, 1965). Similarly, Foster (1982) observed that many of the species found at metal-polluted sites were mucilaginous (including *Batrachospermum* and green algae), which convinced her that the production of slime is a common algal response to toxic metals.

The high levels of aluminium associated with the epiphytic cover can be explained by biological uptake of this metal by diatoms. The catalytic effect of aluminium on the removal of SiO_2 was proved in seawater (Stoffyn, 1979). Foy & Gerloff (1972) found a positive relationship between aluminium tolerance and the ability of algae to raise the pH of water.

In the SEM X-ray microanalysis, it is difficult to distinguish metals bound to the cell and those which precipitated outside the cell. X-ray analysis of metals in attached microorganisms and mucilaginous covers from marine

environments are numerous because of their direct technological application (Daniel & Chamberlain, 1981). Metal analysis on *in situ* plant material is necessary as metal-tolerance for the same species growing in the laboratory is different (Stokes *et al.*, 1973).

The uppermost layer of the L. Gårdsjön sediment has higher levels of calcium, iron and copper due to the formation of complexes with organic compounds (Renberg, in press). The content of both calcium and iron was high in the *Lobelia* roots. The high iron content of the roots is probably a result of an intensive precipitation of Fe^{+2} as Fe-III oxides (Larcher, 1980) on the root surfaces. A large amount of iron oxides was present on those parts of the leaves which were closest to the sediment. This accounts for the high iron content for leaves with epiphytes. According to Hutchinson (1975) the content of sodium in tissues of aquatic angiosperms is nearly always lower than potassium which is also the case in this study. It is difficult to explain on the basis of two determinations why the content of sodium in the leaf tissue from L. Gårdsjön was below detection limits. Nevertheless, the results for 1980 and 1981 were consistent.

It may be suggested that the appearance of acid and metal resistant algal species in acidified environments is a result of their capacity to chemically and physically modify the existing environment. Their structural adaptations as well as the close metabolic relationships (cometabolism) between the different components of the epiphytic complex, enable such species to grow successfully in acid and metal-rich environments.

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TABLE 1. Mean metal and total phosphorus content in epiphyte-free leaves (L-E) and roots (R) of sterile *Lobelia* plants, and in *Lobelia* leaves with a firm epiphytic cover (L+E). Values represent percentages of ash-free dry weight of the cumulative samples (for each lake n= 20 in 1980 and 20 in 1981).

	L. Gårdsjön			L. Högsjön		
	L+E	L-E	R	L+E	L-E	R
Tot-P	0.101	0.177	0.080	0.079	0.157	0.132
Ca	0.361	0.293	0.488	0.733	0.561	0.385
Mg	0.180	0.196	0.229	0.244	0.147	0.168
Na	0.918	n.d.	1.608	0.918	0.878	2.502
K	3.348	5.739	1.061	2.490	5.128	1.732
Fe	0.465	<0.01	1.870	0.552	<0.01	2.201
Mn	0.026	0.176	0.039	0.721	0.033	0.045
Cu	0.001	0.001	0.001	0.001	0.001	0.000
Zn	0.013	0.009	0.015	0.011	0.005	0.018
Al	0.543	0.006	0.438	0.208	0.012	0.083

n.d. = not detected

TABLE 2. Sequence of the X-ray detected metals in the *Lobelia*-epiphyte complex in Lake Gårdsjön and Lake Högsjön in 1980 and 1981. Based on percentage contribution to the total energy counts.

L. Gårdsjön	
leaf surface	Cl > Al > S > K > Ca > Fe
mucilage	Ca > Na > Al > K > Cl > S > P > Fe > Mn > Zn > Mg > Ti
blue-green algae	Si > Cl > Fe > S > Ca > Al
Eunotia	Si > Cl > Al > S > Fe > Ca > K
green algae	Ca > Cl > Fe > Al > S > K > P > Mg
L. Högsjön	
leaf surface	Ca > Cl > Si > S > K > Mn > P > Al > Fe
mucilage	Ca > Na > Al > Cl > S > Si > P > K > Fe > Mg > Mn
blue-green algae	Ca > Si > Fe > Cl > S > Al > Mn
Eunotia	Si > Ca > Cl > S > K > Mn > Al > Mg > Fe
Achnanthes	Si > Ca > S > Cl > Fe
Achnanthes stalk	Ca > S > Cl > Al > Si > P > K > Fe > Mn > Na > Mg > Ti
Gomphonema	Si > Cl > S > Na > Ca > Al > Fe > K
green algae	Ca > Cl > Si > K > S > Mn > Al > Fe

Figure 1. SEM micrograph of the *Lobelia* leaf surface showing the concave outer walls of the epidermal cells.

Figure 2. SEM micrograph showing a colony of rod-shaped bacteria attached to the epiphyte-free surface of a *Lobelia* leaf. Note the mucilaginous adhesive strings (arrows).

Figure 3. High-magnification TEM micrograph showing a coccoidal microorganism attaching to the *Lobelia* leaf surface (LS) by means of colloidal-size fibrils (F) originating from the cell. Numerous intracellular vesicles are present (V). Section stained with ruthenium red.

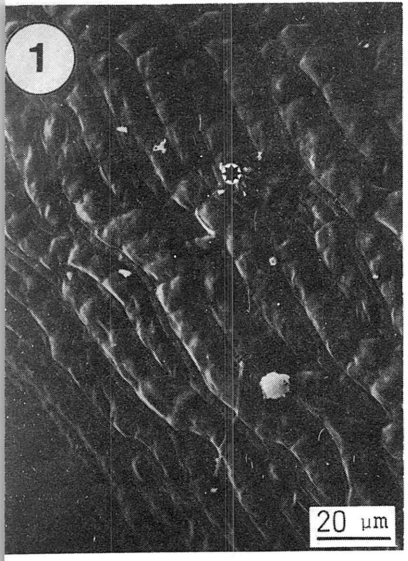
Figure 4. High-magnification TEM micrograph showing microorganisms surrounded by interlacing electron-dense strands of polysaccharide-like material (P). Note the void areas (VA) enclosing the cells. Section stained with ruthenium red.

Figure 5. LM micrograph from L. Gårdsjön showing the detached primary mucilaginous film (arrows) with embedded microorganisms. (E) *Eunotia*, (C) cyanophytes.

Figure 6. SEM micrograph showing firmly attached frustules of *Eunotia* and a colony of bacteria embedded in mucilaginous matrix.

Figure 7. SEM micrograph of the opening left in the secondary mucilaginous film by a detached blue-green algal filament. Note the arrangement and utilization of space by *Eunotia*.

Figure 8. SEM micrograph showing the mesh-like formation of mucilaginous material on the upper valve of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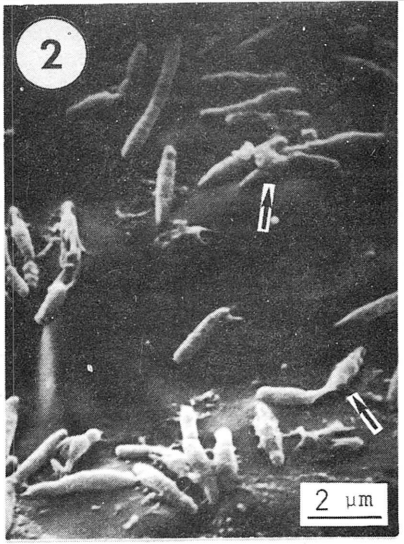
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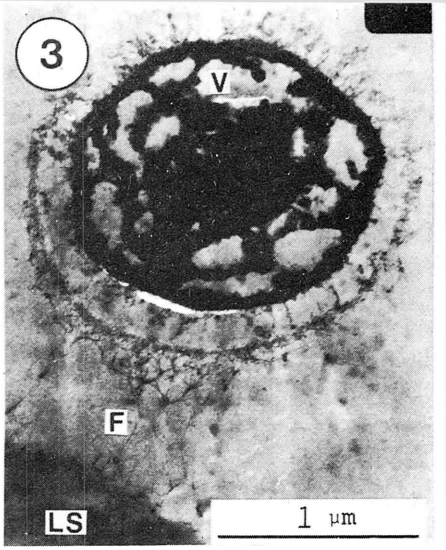
3
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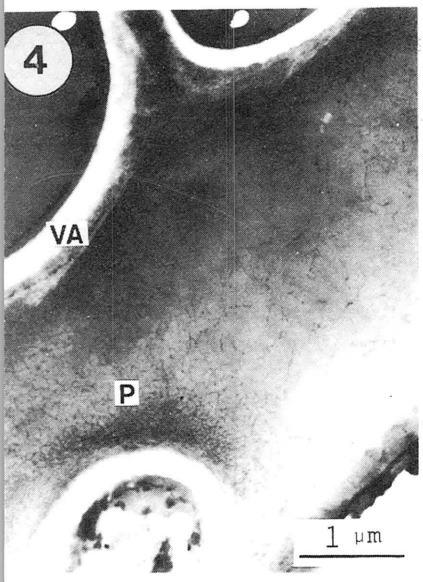
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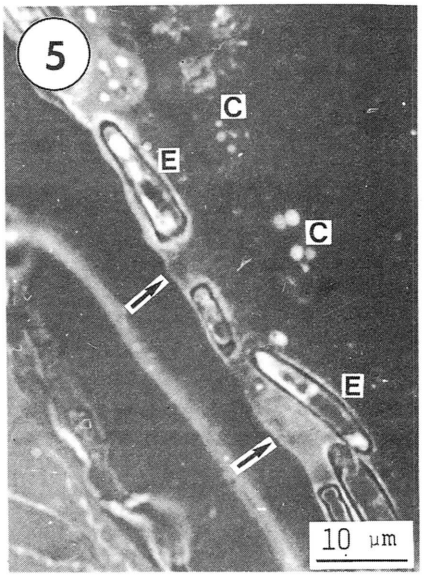
5
C
E
C
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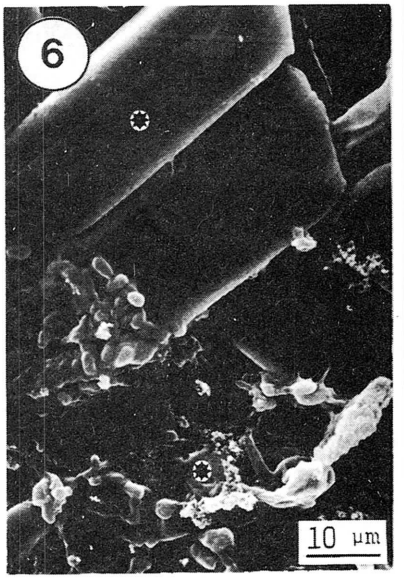
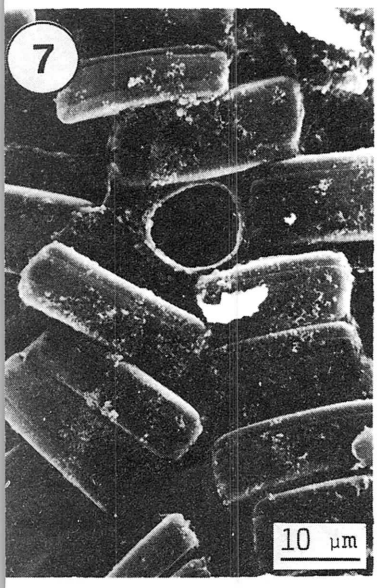

6
10 µm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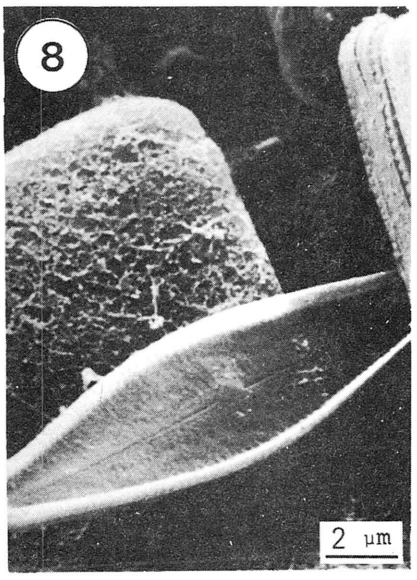
8
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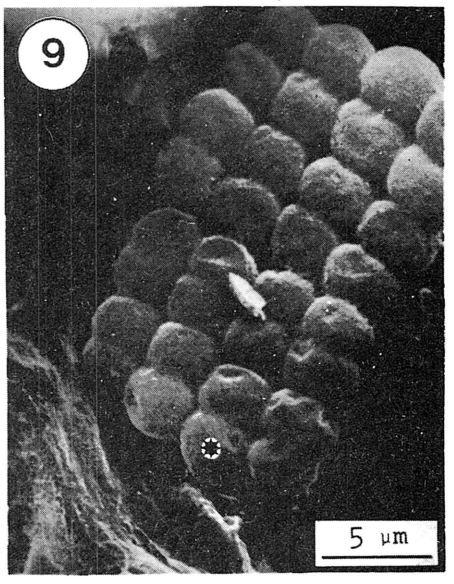

9
5 µm

Eunotia.

Figure 9. SEM micrograph showing a flat colony of blue-green alga *Merismopedia* settled on a strand of mucilaginous material within the firm layer of attached *Eunotia*.

Figure 10. SEM micrograph showing a pseudofilamentous blue-green alga growing between two diatom frustules. Note the extensive extracellular material (arrow).

Figure 11. SEM micrograph showing a frustule of *Eunotia* infested with coccoidal bacteria. Note the mucilaginous adhesive strings (arrows) anchoring the bacteria to the *Eunotia* frustule.

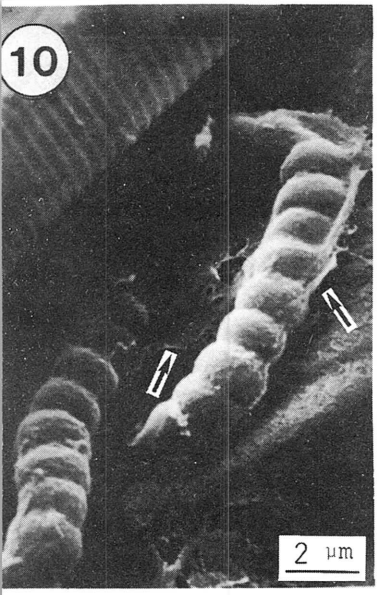
Figure 12. SEM micrograph of the mucilaginous matrix covering the firmly and loosely attached components of the epiphytic complex on a *Lobelia* leaf in L. Gårdsjön.

Figure 13. SEM micrograph showing the diatom, *Achnanthes*, attached by means of a mucilaginous stalk.

Figure 14. SEM micrograph showing the colonial diatom, *Gomphonema*, attached by means of an elongated mucilaginous stalk.

Figure 15. SEM micrograph showing loosely attached blue-green algal filaments (BG) and a *Bulbochaete* filament with the holdfast cell (H) visible.

Figure 16. SEM micrograph of the holdfast cell of a green algal filament.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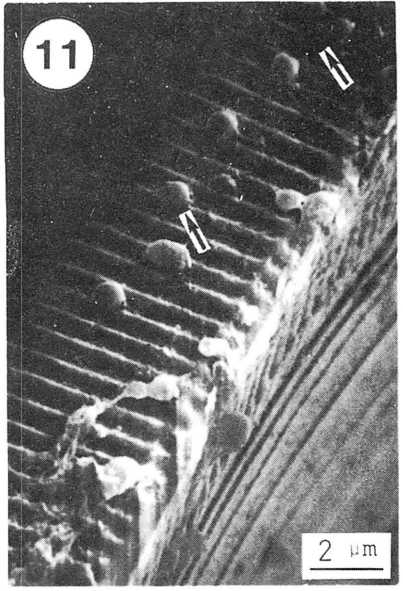
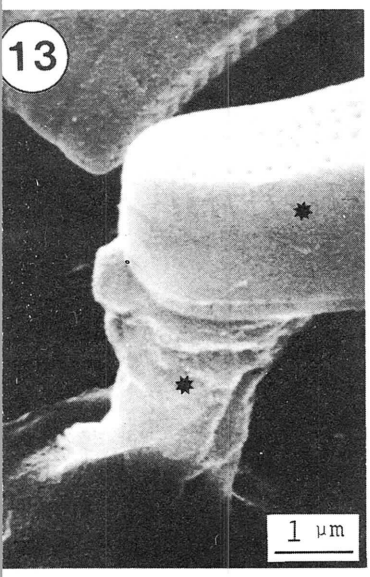

11
2 µm


12
20 µm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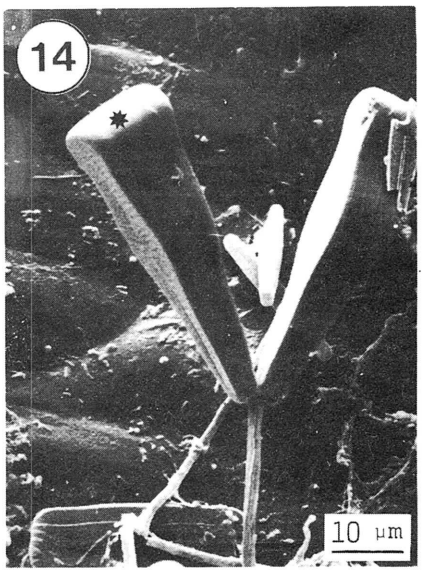
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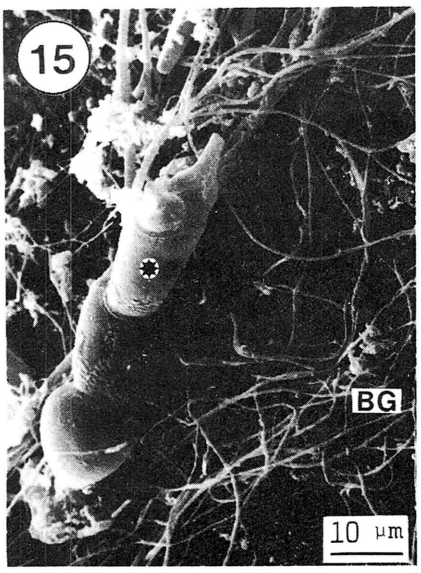
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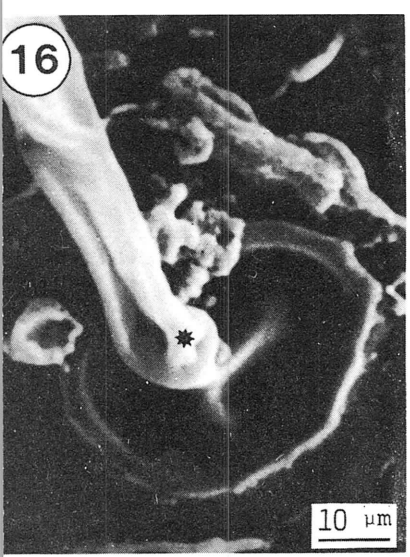
BG
10 µm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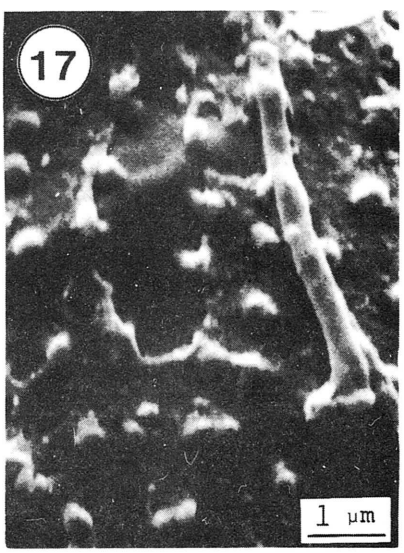
17
1 µm

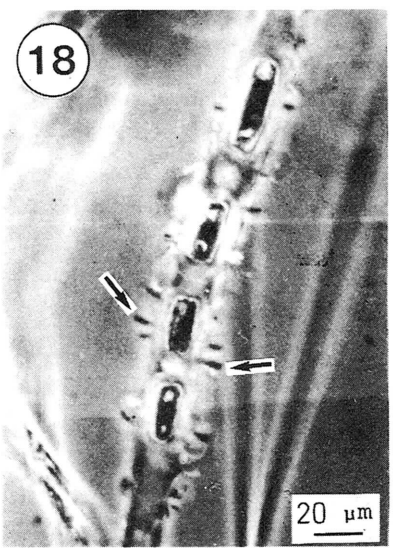
18
20 µm

Figure 17. SEM micrograph showing coccoidal bacteria embedded in the mucilaginous film covering the surface of a *Bulbochaete* filament.

Figure 18. LM micrograph of a loosely attached filament of *Binuclearia* with blue-green algae polarly attached as a secondary epiphytes (arrows).

NOTE: Figure 1–12, and 15–18, L. Gårdsjön.
Figure 13–14, L. Högsjön.

EPIPHYTIC ALGAL PRODUCTION IN THE ACIDIFIED LAKE GÅRDSJÖN, SOUTH WESTERN SWEDEN

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Abstract

The increase in the growth of benthic filamentous algae is one of the most visible effects of acid precipitation on oligotrophic lakes. It is still unclear which factors are involved in this process.

This paper takes up the structural characteristic and production capacity of the epiphytic algae associated with Lobelia dortmanna L. Studies were done in the acidified Lake Gårdsjön, south-western Sweden.

Productivity values were significantly correlated with the chlorophyll a content, total surface area and volume of the epiphytic algal cells. Optimal conditions of production for the Lobelia-epiphyte complex were found at 1.0 m depth. Daily productivity correlated best with number, surface area and volume of E. veneris and E. rhomboidea cells. These diatoms formed a firmly attached cover on Lobelia leaves, which also included sessile bactera and blue-green algae. This epiphytic cover had a high photosynthetic capacity. The slow-growing green algal species, Mougeotia and Binuclearia, were main components of a loosely attached community with a low production capacity. This supports suggestions that the accumulation of benthic algal biomass observed in the littoral of the lake is not a result of their high net production, but rather, is an effect of reduced grazing intensity and reduced rates of decomposition.

Introduction

Macrophyte-epihyte associations in the littoral of lakes are important contributors to this highly structured zone between terrestrial and aquatic habitats. The availability of plant surfaces and their topography influence the composition and growth of epiphytic flora (Pieczyńska, 1976; Cattaneo & Kalff, 1980). Eminson & Moss (1980)

documented specific influence of the host-plant on the nature of attached algae, especially in nutrient-poor waters. Direct nutrient transfer between the host-plant and epiphytes is an open question (Harlin, 1973; McRoy & Goering, 1974).

Acid precipitation has reduced the autotrophic productivity of phytoplankton in lakes, and simultaneously caused an increase in benthic algal biomass (Hendrey et al., 1976). Various explanations have been put forward (cf. Hendrey et al., 1980).

Our knowledge of algal metabolism in low pH conditions is poor and mainly derived from laboratory studies. Many authors have discussed acidic biota in relation to specific factors such as levels of hydrogen (Müller, 1980) or metal ion concentrations in open water (Sheath et al., 1982). However, in nature it is always a combination of factors which determines growth rates, and the survival or elimination of algal species. Furthermore, it is difficult to interpret biological changes resulting from decreased levels of pH solely on the basis of correlation analysis or lists of species. As pointed out by Allen (1977) and Hoagland et al. (1982), microalgal systems are most appropriate for examining general ecological concepts in relation to the changing environment. The gradually altering physicochemical parameters of lakes affected by acid precipitation ought to be reflected by changes in the structure and function of the epiphytic complex.

This paper takes up the production capacity of the epiphytic algae associated with Lobelia dortmanna L., a common hydrophyte of shallow oligotrophic lakes in Scandinavia. The aim of this investigation is

to estimate by means of *in situ* experiments the seasonal and annual production of epiphytes in the acidified, low-productive and phosphorus limited (Jansson *et al.*, 1981) ecosystem of Lake Gårdsjön, south-western Sweden. An attempt is made to interrelate the production and structure of epiphytic assemblages.

Material and methods

Studies were performed in Lake Gårdsjön during the periods May to October, 1980, and June to November, 1981. Detailed chemical and morphometric characteristics of the lake are presented in other papers published within the Lake Gårdsjön Project (Jansson *et al.*, 1981; Nilsson; 1983).

Epiphytic primary production was estimated on the basis of *in situ* ^{14}C uptake experiments run at 0.5, 1.0 and 2.0 m depth. Incubation chambers made from 3 mm plexiglass were placed over *Lobelia* plants with the help of SCUBA diving. The DIC (dissolved inorganic carbon) content in the water above the sediment surface was determined in an IR Beckman Carbon Analyzer. The incorporated ^{14}C activity was counted in a Packard Automatic Tri-Carb Liquid Scintillation Counter. Sampling methods and treatment of the samples followed procedures described earlier (Lazarek, 1981).

Epiphytic productivity per m^2 of the macrophyte leaf surface area was calculated on the basis of the mean ^{14}C activity resulting from the sum of activities counted for the tip, middle and base of the leaf. It was assumed that distribution of epiphytes on the upper and under surfaces of *Lobelia* leaves was not significantly different.

Loosely attached algae were separated in accordance with a method suggested by Cattaneo & Kalff (1980). Tightly attached algae were not removed but were treated together with the leaf they were growing on. It was proved that Lobelia without epiphytes does not absorb CO_2 from the water but that it satisfies its CO_2 supply from the sediment (cf. Wium Andersen, 1971).

Continuous light measurements on the shore permit a reconstruction of the light climate and calculations of the daily production rates from the ratio of photons received during 4 h incubation, over the total photons for that day. The daily depth-integrated (0.5 to 2.0 m) productivity was extrapolated to seasonal and annual production. No correction was made for photoinhibition or light reduction due to ice and snow cover. The lake-wide production of epiphyton, expressed per m^2 of the littoral and lake surface area, was calculated, assuming after Grahn & Lydén (1983) that dry weight biomass of Lobelia including roots, is 6.8 +- 1.6 g m^{-2} for the whole lake area and 36.0 +- 8.4 g m^{-2} for the littoral zone. A biomass turnover rate of 38 % per year (according to Grahn & Lydén, 1983) was considered in the calculations. The biomass of roots was found to be 40 % of the total biomass of infertile Lobelia plants at the depth 0.5 to 2.0 m. Lobelia contributes 34 % of the total macrophyte biomass in the lake (Grahn & Lydén, 1983).

Epiphytic productivity was also measured on petioles of Nuphar lutea (L.) Sibth. et Sm. on internodes of Equisetum fluviatile L. and leaves of Isoetes lacustris L. The intention was to givean idea of the photosynthetic capacity of epiphytes on other host-plants in the lake.

Conversion of the Lobelia biomass to leaf surface area is based on the linear regression $Y = 487\ X + 1.5$ ($r = 0.91$), where X represents dry weight in grams and Y stands for leaf surface area of Lobelia available for epiphytic colonization per m^2 of the littoral zone, was 1.45 m^2, or 0.27 m^2 for the entire lake area.

Counts of tightly and loosely attached cells were performed separately. Counting of loose cells was done according to Cattaneo & Kalff (1980). Tightly attached cells were counted directly using a thin collodion-film technique (Lazarek, 1983).

Results

Daily productivity rates are presented in Fig. 1. The maximum value of 69.6 mgC per m^2 of Lobelia leaf surface area and day was found in July, 1981, at 1.0 m depth. Highest rates were recorded at 1.0 m depth throughout the sampling period, except in August, 1981, when the highest productivity was recorded at 0.5 m depth.

Correlation analysis was used to determine relationships between productivity and the measured biotic and abiotic parameters. When values for 1980 and 1981 were combined, a significant correlation was found between productivity, epiphytic chlorophyll a content, total surface and volume of the algal cells ($r = 0.67$ to 0.77, $P < 0.01$). However, the best correlation was found between daily productivity rates and the number, surface and volume of tightly attached cells of diatoms Eunotia veneris (Kütz.) O. Müller and E. rhomboidea Hust. ($r = 0.74$ to 0.85, $P < 0.01$). This suggests a high photosynthetic capacity of the Eunotia population. The rate of ^{14}C

uptake by the algal components within the epiphytic complex differs markedly, as was found in another study of the Lobelia-epiphyte complex in Lake Gårdsjön (Lazarek, 1983). The tightly attached community of Eunotia and small sessile cells of blue-green algae and bacteria (Fig. 2A) were responsible for approximately 80 % of the total ^{14}C uptake during 4 h. This pattern changed little during the 1980-81 sampling period. Blue-green algae in the tightly attached category were represented by colonial forms, e.g. Merismopedia, Aphanothece, Microcystis. The number of Eunotia cells remained relatively stable throughout the sampling period (Fig. 3). This diatom formed an almost pure population on overwintering leaves of Lobelia, as was cheched by sampling this plant under the ice (Lazarek, unpubl.).

Seasonal changes in occurrence of the more common species are shown in Fig. 3. Important with regard to algal biomass, but not productivity, was the community of Mougeotia spp. (at least two species) and Binuclearia tectorum (Kütz.) Berger in Wichm. These algae were the main components of the filamentous flocculent mat (metaphyton) appearing in warmer periods of the year in shallow parts of the lake. It is interesting that filamentous blue-green algae were not represented in this metaphytic formation, although they form a perennial mat on the sediment proper (Lazarek, 1982).

The filamentous green algal cells of Mougeotia and Binuclearia made up from as little as 0.2 % to 39.4 % of the total number of epiphytic cells (Fig. 3). These two slow-growing forms contributed from 6 % to 96 % of the total algal surface area, and from 10 % to 97 % of the total algal volume (Fig. 4). However, their contribution to the

total epiphytic production was not great. In August, 1981, when Mougeotia alone made up > 96 % of the total algal volume, the productivity rate of the epiphytic complex was low (Fig. 1). The decreased daily light intensity from 602 uE m^{-2} s^{-1} during incubation time in July, to 285 uE m^{-2} s^{-1} in August, 1981, may have partly contributed to such low productivity rates.

The number of Eunotia cells did not follow the changes in community biomass. Two morphologically close species of Eunotia formed from 5 % to 45 % of the total cell number. Their percentage contribution to the total algal surface area ranged from 17 % to 62 % (Fig. 4A). The volume of Eunotia cells ranged from 5 % to 47 % of the total (Fig. 4B).

Rough calculations of the annual epiphytic production on Lobelia leaves were made for the period June, 1980 to June, 1981. Production was equal to 1.8 gC m^{-2} leaf surface area yr^{-1}, which corresponds to 2.6 gC m^{-2} littoral area yr^{-1}, or 0.4 gC m^{-2} lake area yr^{-1}. The amount of carbon produced by epiphytes on Lobelia alone would be about 127 kg per year in Lake Gårdsjön. Adding the primary production of epiphytes on other plants present in the lake (Table 1), the total production of epiphyton would approximate 1.2 gC m^{-2} lake area yr^{-1}.

It should be remembered that the total benthic primary production in the lake is incomplete as the production of the cyanophytan mat is not included. The spring and summer mass appearance of filamentous metaphyton in the shallow parts of the lake makes an estimation of the absolute benthic production even more difficult. An accurate

method for measuring the production of these algal assemblages is urgently needed.

Discussion

Physiological sensitivity to low pH and chemical changes in oligotrophic lakes undergoing acidification, results in the elimination of certain species. About 60 % of all epiphytic algal species in Lake Gårdsjön was classified as acidophilic (Lazarek, 1979). High biomass of benthic algae associated with the Lobelia beds in the lake confirms an earlier observation of Laake (1976) that attached algae are espeically plentiful on this isoetid in low pH conditions. In non-acidified lakes, however, isoetid vegetation usually supports low epiphytic growth (Moeller & Roskoski, 1978).

Epiphytic biomass in the lake was highest in spring, declining through summer (Lazarek, 1981), but the seasonal rates of productivity were not as regular. It is difficult to establish which factors determine epiphyton productivity in Lake Gårdsjön. Incomplete seasonal productivity measurements and the subsequent physico-chemical analyses of water in the littoral, hinder reliable assessment of the results.

It appears that the daily productivity of epiphytes in Lake Gårdsjön compare favourably with the values presented by Søndergaard & Sand-Jensen (1978) from their studies on epiphytes associated with the isoetid - Littorella in the oligotrophic Lake Kalgaard, Denmark. Jones (1980) obtained somewhat higher values of production for

epiphytes associated with Myriophyllum in the hardwater, eutrophic Lake Wingra.

Several factors could influence the accuracy of the results obtained in this study. An underestimation of production values could have derived from a discrepancy between the estimated and the photosynthetically available amount of inorganic carbon in the water. The amount of DIC measured in this study ranged from 0.8 to 1.5 mgC l^{-1}. The DIC content in other Swedish lakes where comparative productivity studies were performed (Table 2) ranged from 1.7 mgC l^{-1} (Tjärnesjön) to 4.9 mgC l^{-1} (Högsjön).

Schindler et al. (1980) found that reduced DIC levels down to 0.8 mgC l^{-1} had no effect on algal photosynthesis. Ohle (1981) observed that in an acidic pond with a DIC content similar to that of Lake Gårdsjön, small differences in CO_2 content influenced photosynthesis to a lesser degree and that the supply of phosphate ions effected photosynthesis. The important role of animals orgnisms in the regeneration of carbon was also stressed. Conway & Hendrey (1981) suggested that it is the shift from HCO_3 - to CO_2 (96.2 % at pH 5), rather than total DIC content in the water, which influences algal productivity and structure. The CO_2 is supposed to determine the growth of certain algae (Ruttner, 1960) but the metabolism of the entire algal assemblage must be controlled in a more complex way.

When the epiphytes were categorized as loosely attached or tightly attached, it appeared that ^{14}C uptake was higher for the tightly attached cells of Eunotia, blue-green algae and bacteria, although they contributed little to the total biomass of the complex. This

observation agrees with results obtained by Cattaneo & Kalff (1979). who found that tightly attached epiphytes were responsible for 87.9 % to 92.3 % of the total epiphyte productivity in Lake Memphremagog. In the same lake, the production of loosely attached epiphytes was significantly correlated with the total P in the water (Cattaneo & Kalff, 1980). The role of P in the metabolism of Lake Gårdsjön epiphyton is unknown.

Tightly attached epiphytes in Lake Gårdsjön were seasonally less variable than the loosely attached algae. The loose filamentous forms were represented by a slow-growing species of green algae, notably Mougeotia spp., Binuclearia sp., Oedogonium spp., and Bulbochaete sp. A high diversity community of benthic diatoms and blue-green algae was interwoven in that filamentous assemblage. Only Binuclearia and Bulbochaete filaments supported a growth of secondary epiphytes, mainly bacteria. Loosely attached algae, in contrast to those tightly attached, showed low productivity which may indicate that the observed accumulation of benthic algal biomass in the littoral of the lake is not a result of the high production of these algae, but rather a result of reduced grazing intensity and reduced rate of decomposition. Information on the rates of these processes in acidic waters is needed.

The production at 1.0 m depth was significantly higher than at other sampled depths (Mann-Whittney test, P = 1 %). At this depth stable light conditions and reduced wave action, compared with the shallower parts of the littoral, may provide optimal conditions for epiphytic photosynthesis. At 2.0 m depth high content of organic particles within the epiphytic complex could have reduced the available radia-

tion by the effect of shading. Declining biomass of Lobelia at this depth provided less substrate for algal colonization. This resulted in the extremely low production values calculated on the basis of the littoral area.

In acidic conditions like those in Lake Gårdsjön, an accumulation of filamentous algae may lead to the suppression of submerged macrophytes similar to what has been observed in progressively eutrophic conditions (Phillips et al., 1978; cf. also studies of Sand-Jensen & Søndergaard, 1981). Goldman & Amezaga (1975) compared periphytic complexes to grassland, suggesting that moderate grazing may favour the plants' productivity. Reduced grazing as a result of the poorer diversity of algae and invertebrates in acid conditions may lead to reduced epiphytic productivity.

It is possible that part of the intersticial CO_2 assimilated by Lobelia is transferred to its epiphytic cover. If this were proved, it would mean that epiphytes play a substantial role in the carbon metabolism of the littoral and that their photosynthesis in the lake is not carbon limited.

Conclusions

Epiphytic assemblages in Lake Gårdsjön had a production capacity similar to that of other oligotrophic, nutrient-poor lakes.

Most favourable conditions for production of the Lobelia-epiphyte complex existed at 1.0 m depth.

The epiphytic complex consists of a structurally defined layer of side-to-side attached Eunotia cells and abundant sessile prokaryotic cells, covered predominantly by filamentous green algae and dislodged benthic algal cells.

The ^{14}C uptake was relatively high and stable in the firmly attached layer of epiphytes.

Acknowledgements

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Table 1. Daily productivity rates of the epiphyton on four macrophytes in Lake Gårdsjön. Measurements performed 29 - 30 August, 1981. Daily sub-surface PAR = 1.0 to 1.2 10^7 uE m^{-2} day^{-1}. Values expressed as mgC assimilated by epiphyton per m^2 macrophyte surface area. n = 5 for each macrophyte.

Macrophyte	Mean+-range (mgC m^{-2} day^{-1})
Lobelia dortmanna	22.1 +- 6.3
Isoetes lacustris	28.5 +- 4.5
Nuphar lutea	59.2 +- 6.2
Equisetum fluviatile	11.5 +- 2.8

Table 2. Comparative daily (average for the period June 1980 - August 1981) and annual epiphytic production in some oligotrophic Swedish lakes and other selected aquatic ecosystems where production was measured with ^{14}C method. Values are expressed per m^2 of macrophyte surface area and day, and per m^2 littoral area and year. Production values per m^2 lake area are given in brackets.

Macrophyte	Lake	pH	Production		Reference
			mgC $m^{-2}day^{-1}$	gC $m^{-2}yr^{-1}$	
Lobelia	Gårdsjön, Sweden	4.5	39	2.6(0.4)	present study
Lobelia	Tjärnesjön, Sweden	4.8	43	n.d.	Lazarek, unpubl.
Lobelia	Högsjön, Sweden	6.3	97	n.d.	Lazarek, unpubl.
Scirpus	Crescent Point, Canada	8.0	32	44(0.2)	Hooper & Robinson (1976)
Nuphar	Ohrid, Yugoslavia	8.2	887	37	Allen & Ocevski (1981)
Myriophyllum	Wingra, USA	8.2	?	29(1.4)	Jones (1980)
Potamogeton	Memphremagog, Canada	8.4	9	?	Cattaneo & Kalff (1980)

Figure 1. Seasonal changes in the primary productivity rates of epiphytic algae on Lobelia leaves in Lake Gårdsjön. Rates expressed as mgC assimilated per m^2 of the plant surface area and day. Bars represent S.E. n = 5 for each depth.

Figure 2. Epiphytic algae on Lobelia leaves in Lake Gårdsjön.

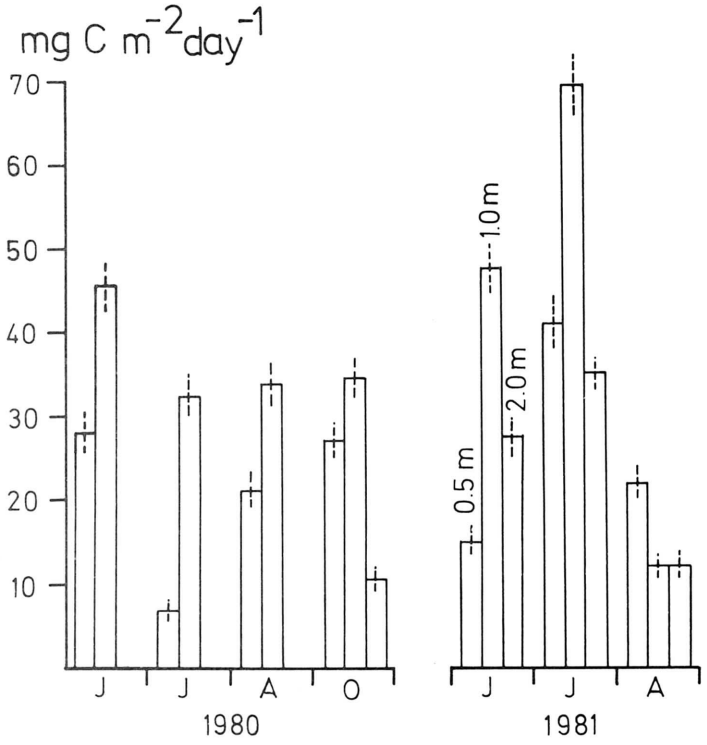
A. Tightly attached bacteria (b), blue-green algae (bG) and diatom Eunotia (e).

B. Loosely attached algae, Binuclearia (Bi), Mougeotia (M) and Tabellaria (T).

Figure 3. Seasonal abundance of the ten most common epiphytic algal species in 1980 and 1981, expressed as number of cells per cm^2 of Lobelia leaf surface area. Depth 1.0 m.

Figure 4. Seasonal changes in the surface area (A) and volume (B) of epiphytic algal cells on Lobelia leaves, expressed as cm^2 per cm^2 of leaf surface area and as mm^3 per cm^2 of leaf surface area. The bars represent Eunotia rhomboidea and E. veneris (black), Mougeotia and Binuclearia (dashed) and the sum of the remaining epiphytic algal species (white).

Figure 1.



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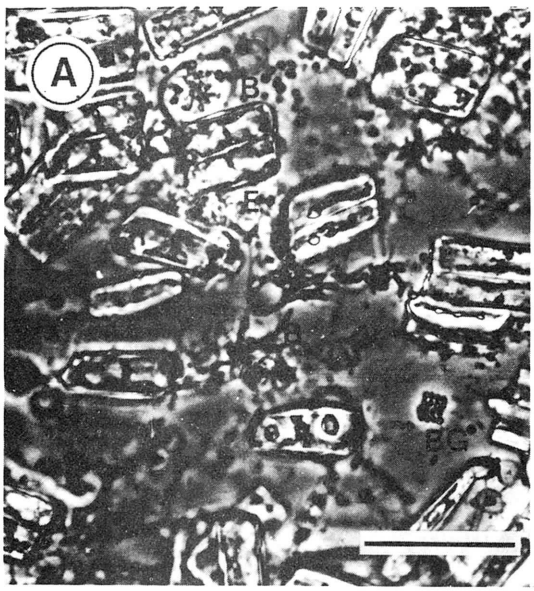


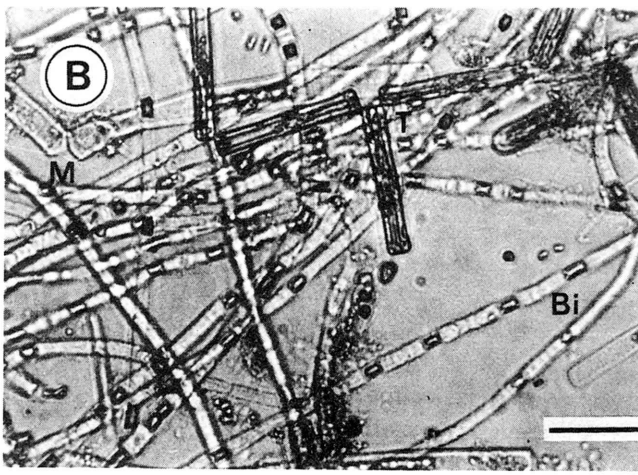
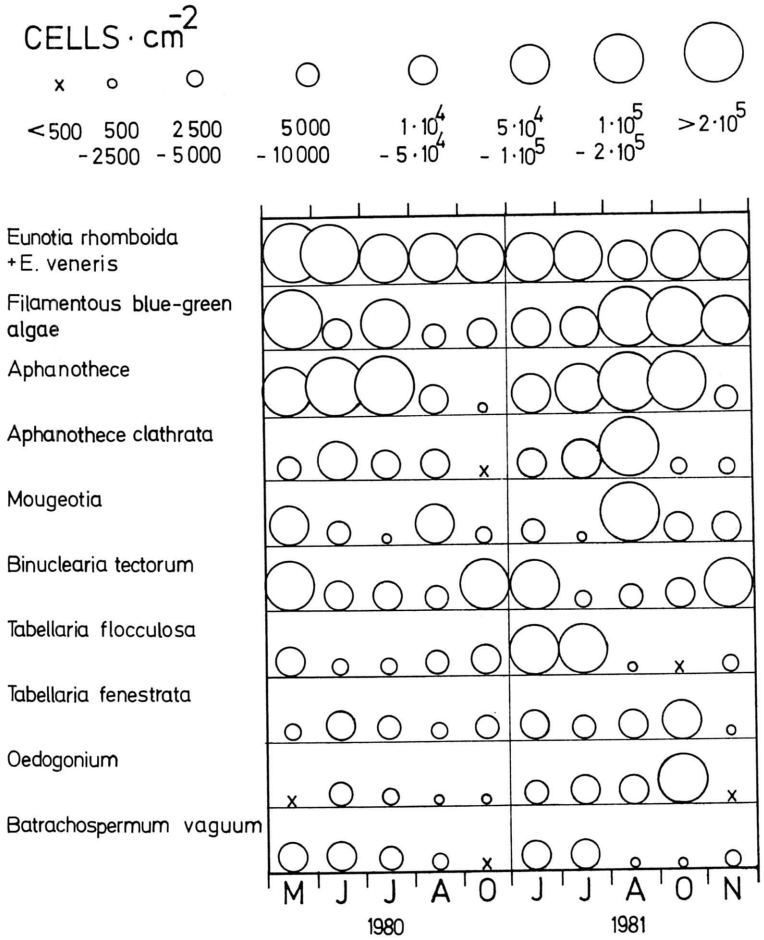


Figure 3.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1·10^4 - 5·10^4
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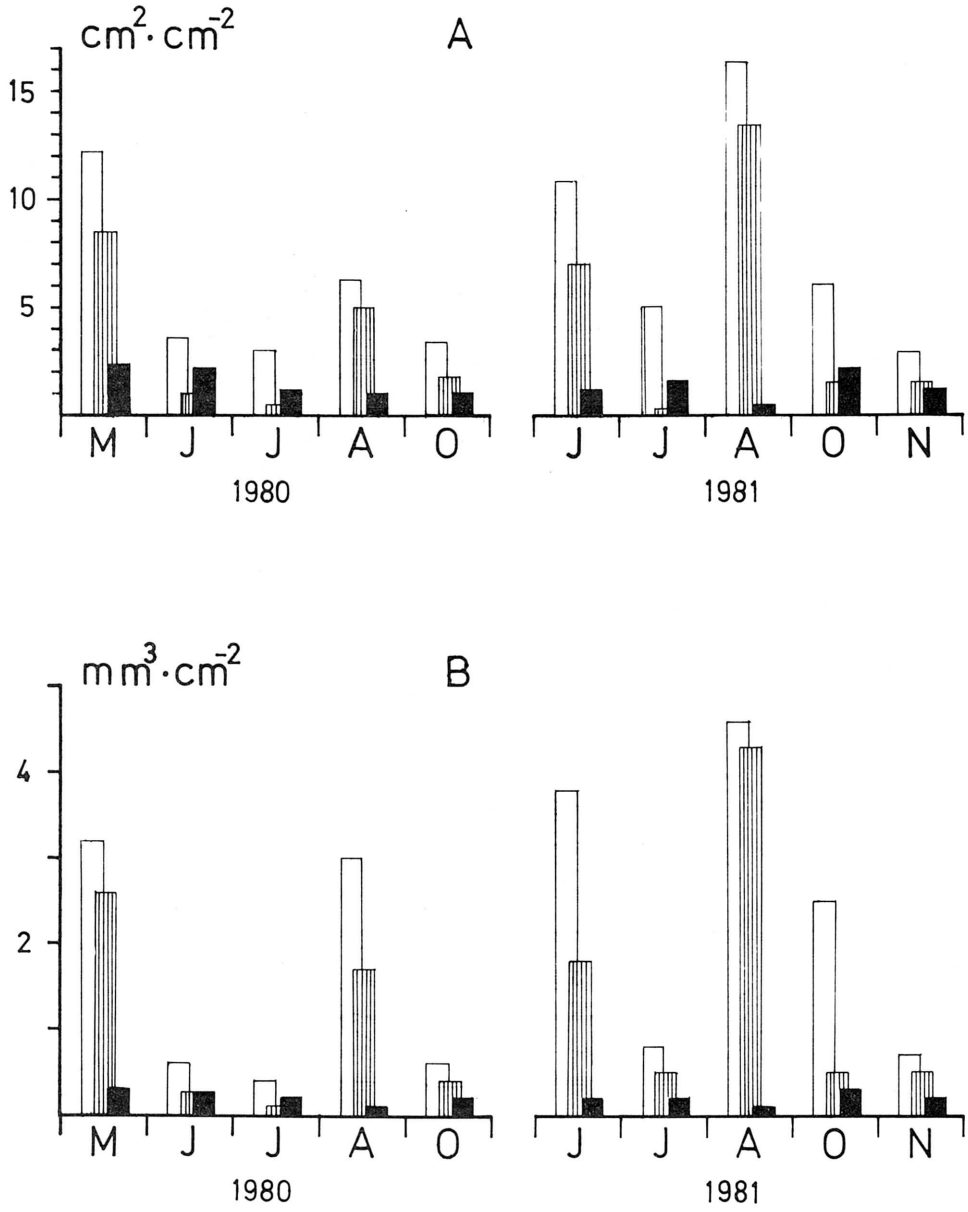
Eunotia rhomboida +E. veneris
Filamentous blue-green algae
Aphanothece
Aphanothece clathrata
Mougeotia
Binuclearia tectorum
Tabellaria flocculosa
Tabellaria fenestrata
Oedogonium
Batrachospermum vaguum
M J J A O J J A O N
1980
1981

Figure 4.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EPIPHYTIC COLONIZATION OF *LOBELIA DORTMANNA* L. IN THE ACIDIFIED AND LIMED LAKE GÅRDSJÖN, SOUTHWESTERN SWEDEN

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Summary

An experiment on the epiphytic colonization of epiphyte-free *Lobelia* plants was performed in 1981, in the acidified Lake Gårdsjön, Sweden. The experiment was repeated in 1982, four months after liming of the lake.

Colonization of the barren leaf surfaces was rapid. The process was initiated (after one day), by a passive attachment of algal cells (*Mougeotia*, *Tabellaria*) and an active attachment of bacteria. After four weeks of exposure the epiphytic structures that had developed were similar to the epiphytic structures associated with *Lobelia* growing naturally in L. Gårdsjön.

Liming resulted in the replacement of *Eunotia* in the firm layer of epiphyton, by *Achnanthes* and *Synedra*. The recruitment of new species was most evident in the loose layer of epiphyton. The new species that appeared were represented by desmids, diatoms and filamentous bacteria.

The diversity indices of epiphyton had low values due to the domination of a few taxa. The epiphytic structures that developed before and after the liming, shared 68.4% of the maximum community similarity.

EPIPHYTIC COLONIZATION OF *LOBELIA DORTMANNA* L. IN THE ACIDIFIED AND LIMED LAKE GÅRDSJÖN, SOUTHWESTERN SWEDEN

S. Lazarek

Introduction

The development of the periphytic community in eutrophic conditions resembles the successional stages in the development of higher plant communities (Hoagland *et al.* 1982). Jordan & Staley (1976) pointed out the significance of heterotrophic bacterial attachment in the initial stage of periphytic colonization in Lake Washington. Both these studies stressed the importance of the morphological structures of attaching microorganisms for successful colonization of bare substrates. This includes their extracellular polysaccharideous products.

It was suggested that the addition of lime to the water of an acidified lake introduces structural changes in the existing *Lobelia*-epiphyte complex without increasing the primary production capacity of the complex (Lazarek, 1982). The replacement and recruitment of new algal species were observed after the liming had been performed. Further, it was noted (Lazarek, 1983a) that the production of mucilage, which is important for nutrition, attachment and consolidation of the epiphytic complex, was higher in low pH conditions.

Liming of the acidified Lake Gårdsjön, created an excellent opportunity of carrying out simple short-term experiments where the epiphytic colonization and its

successional stages could be followed before and after the addition of lime.

My intention was to quantify the successional stages of the developing epiphytic cover in terms of species diversity, dominance and biomass, with special attention paid to the initial step of the colonization process. This study was primarily designed to monitor the structural reorganization of epiphyton in response to the environmental perturbation which liming of the lake caused.

The notorious problems arising from the heterogeneity of substrates and the separation of attached microorganisms from substrates have brought about the adoption of artificial substrate sampling techniques, as Müller (1980) and Stokes (1982) have demonstrated in their investigations of the acid impact on lakes. A modern approach to the problems related to sampling with artificial substrates was provided by Cairns (1982).

In this study, I used the land-form of *Lobelia dortmanna* L. which was first reported in Sweden by Sylven (1903) and in Germany by Hoeppner (1932). The idea of using epiphyte-free land forms of aquatic plants for studies of epiphyton was introduced by Brown (1974) but has so far not been given the attention it warrants.

Material and Methods

a) *material*

The experiments were performed in Lake Gårdsjön, south-western Sweden. A map of L. Gårdsjön showing the distribution of *Lobelia* and the sampling sites, is presented together with information about the limnology of the lake in Appendix A and B of the thesis.

Land forms of *Lobelia* were collected from the eulittoral of L. Gårdsjön and planted in plastic flower pots in sand from the lake. The pots were kept in aquaria with the water level reaching half-way up the sides of the pots. After three months of acclimatization in a green-house, the pots were re-transferred to L. Gårdsjön. Before starting the experiments, the leaves of the plants were carefully washed using filtered water to remove any microorganisms that could have developed on the leaf surface while the plants were in the aquaria.

Five pairs of *Lobelia* plants (in their pots) were embedded in the sand on the bottom of the lake at a depth of 0.5 m, at the end of July 1981, and exposed to epiphytic colonization for about four weeks. One pair of plants was sampled the following day to check for the presence of pioneer organisms. One pair of plants was collected each week and the plants were kept separately in jars with formalin-aceto-alcohol fixative (Grimstone & Skaer, 1972). The experiments were repeated in 1982, from the end of July through August. L. Gårdsjön had been limed in April 1982.

b) *microscopic analyses of algae*

Analyses began at the end of August, after all the plants had been collected. The total number of leaves on each

plant ranged from 12 to 25. Only healthy leaves were taken for analyzing species composition and abundance of epiphytic cells. The abundance of organisms was determined on the basis of their number of cells per cm^2 of the leaf surface area. The cells were counted in the following way:

Two leaves from the same plant were placed in separate vials with two milliliters of distilled water and shaken vigorously about 100 times. The algal fraction which became detached is referred to as 'loosely attached' algae (analysed on Sedgwick-Rafter counting cell), while the remaining cells comprise the 'tightly attached' assemblage (Cattaneo & Kalff, 1978) analysed by the collodion film method. These two fractions were treated separately.

The suspension of loosely attached algae was transferred to two Sedgwick-Rafter counting cells (S-R). The recommended (Woelkerling *et al.*, 1976) settling time was 10 mins. The cells were counted at a magnification of X250. Five to ten fields on two S-R cells were counted along the transversal and longitudinal axes. Results were transformed to numbers of cells per cm^2 of the surface area of the *Lobelia* leaf, determined planimetrically.

The values presented in Table I were used to check the precision of the method. The method was tested using the Kolmogorov-Smirnov two-sample test (Siegel, 1956, p. 127-136). The largest discrepancy (K_D) between scores on A and B cells, was equal to 2. At this value (α= .01 of the two-tailed K.S. test) the null hypothesis that scores obtained on the two S-R cells represent the same population, could not be rejected. The total number of cells per cm^2 of the leaf surface area consists of mean estimates on both sides of the leaf.

TABLE I. The comparative counting of diatoms in samples from five different *Lobelia* leaves was performed simultaneously on two S-R cells (designated as A and B).

leaves	I	II	III	IV	V
A	680	850	3000	2500	120
B	1100	300	450	2350	100

Scanning electron microscopy (SEM) and epifluorescence techniques could not be used routinely in this work, due to financial and accessibility difficulties. Not wanting to loose the information inherent in the undisturbed patterns of the epiphytic microdistribution, I applied the collodion film technique first suggested by Wenzl (1940) for fungal epiphyll studies. Margalef (1949) used the method for studying aquatic attached communities. The procedures were the following:

The leaf was dehydrated in a series of ethanol and diethyl ether for about 30 mins. It was stained with 5% iron haematoxylin (Grimstone & Skaer, 1972) and then submerged for a few secs in 4% collodion (E. Merck, Darmstadt) dissolved in ethanol (96%) and ether. The transparent film (about 50 µm thick) which formed after drving was peeled off the leaf. The effectiveness of the method was checked by repeatedly covering the leaf with collodion and then examining the film obtained under a microscope. Cells were removed to almost 100% by the first application of collodion. A few mm^2 of the film from both sides of the *Lobelia* leaf was placed on the microscope slide with a drop of Histoclad synthetic microscopic mounting medium (Clay Adams, Parsippany, N.J., refractive index = 1.54). Due to

the thickness of the film and its transparency, high-contrast micrographs could be taken at a magnification of X1250. The sides of the leaf were easily recognizable by the presence or absence of stomata impressions in the film.

Similar techniques were described by Dickinson *et al.* (1974) who used cellulose film for the removal of bacterial epiphyton, and by Cattaneo (1978) who used a cosmetic 'facial mask' to peel epiphytes off leaves. Cosmetic film could not be satisfactorily applied in my work because it gave a highly granulated background on micrographs taken at magnifications > X500, which severely hindered the counting of bacteria.

The embedded diatoms and pseudofilamentous blue-green algae were counted with the help of a Whipple ocular reticule which is divided into fields arranged in blocks. One block consists of 25 fields. The number of blocks which were counted depended on the density of the epiphytic cover. Microdistribution of the epiphytic cells was also registered on micrographs taken under a phase-contrast microscope and, in some cases under a scanning electron microscope (SEM).

c) *analysis of bacteria and filamentous blue-green algae.* Bacteria and filamentous blue-green algae were counted with the help of the collodion film impressions of the leaves which contained tightly attached epiphytes. It was technically possible to count the organisms which formed distinct colonies or filaments. Bacteria were counted from the collodion film impressions, as the configuration of the S-R cell inhibits the use of a high-power microscope objective making identification of organisms smaller than 15 µm ~~was~~ difficult or impossible. The procedures were as

follows:

Several bacterial colonies on randomly chosen parts of the collodion film from five leaves were photographed at X1250, using an immersion oil objective. This gave an estimation of the average number of bacteria per colony for each of the sampling days. All the micrographs were taken with ILFORD PANF 50 ASA black and white film.

To calculate the number of bacteria per cm^2 of leaf surface area, the same collodion film impressions were viewed under X1000 magnification and the number of microcolonies on randomly selected microscope fields was counted.

The filamentous algae appeared on the first day of colonization, almost exclusively in the loosely attached fraction. In order to count the filaments accurately, I modified the Burnham *et al.* (1973) method as follows:

The total length of any filament was tallied on 50 randomly selected fields from the 1000 fields of a S-R cell at a magnification of X250. This was designated as C. The number of cells per 50 µm of the filament was calculated under a phase-contrast microscope, using a magnification of X1000. This was designated as B. The mean number of cells per field of the S-R cell (A) was found using the equation $A = C \cdot B/2500$. By multiplying A by 1000 the number of cells per 1 ml (the volume of S-R cell) was obtained. This value was then transformed to number of cells per cm^2 of the leaf surface area.

All stages of counting on S-R cells were performed under a Reichert light microscope, while a phase-contrast

ORTHOLUX microscope was used for the remaining analyses and for taking the micrographs.

d) *diversity statistics*

I used the simplest measure of diversity, the Shannon-Wiener function

$$DI = -\sum_{i=1}^{s} p_i \log p_i, \text{ (Pielou, 1977)}$$

To obtain a representative sample of the population, 200 organisms were counted on the first day of colonization, and from 1000 to 2000 individuals on the subsequent sampling dates. For bacteria, morphological type of cell was assumed to be equivalent to species (Jordan & Staley, 1976) and one bacterial colony was considered as an individual.

The species eveness index was calculated as $J = DI/\log S$ where DI is the index of diversity and S stands for number of species (Marcus, 1980).

A quantitative comparison of epiphytic communities was made using a similarity index *SIMI* (McIntire & Moore, 1977) calculated as:

$$SIMI_{(a,b)} = \frac{\sum_{i=1}^{s} P_{ai} P_{bi}}{\sqrt{\sum_{i=1}^{s} P_{ai}^2} \sqrt{\sum_{i=1}^{s} P_{bi}^2}}$$

SIMI is the measure of similarity between the communities on the corresponding days in 1981 (a) and 1982 (b), where P_{ai} and P_{bi} represent proportions of individuals represented by i-th species in 1981 and 1982 respectively. S stands for the total number of species in the two communities. *SIMI* has a minimum value of 0 when the two compared communities had have no species in common, and a maximum value of 1 when their species composition and proportions were identical.

Results

The average daily surface water temperature during the experiments (July-August) ranged from 16.5°C to 19.0°C in 1981, and from 19.5°C to 22.5°C in 1982. In 1981, the pH was 4.6 and after liming, in 1982, the pH increased to 7.6.

In both years, filamentous and chain-forming organisms predominated in the initial stage of epiphytic succession. The percentage of cells shared by the six dominating and/or characteristic (present in all samples) taxa on the exposed leaves, ranged from 77% to 86% in 1981 (Fig. 1A). In 1982, the number of dominating algal taxa increased from three on the first day, to six, in the fourth week of exposure. Pooled, the contribution of the dominating taxa to the total amount of settled cells ranged from 91% on the first day to 93.5% at the end of the experiment (Fig. 1B).

The six dominating taxa made up 86.5% in July to 98% in August 1981, of the total number of cells on the naturally growing plants (Fig. 2). The values for the corresponding months in 1982 ranged from 91.5% to 88.5%.

In both years, filamentous Chlorophyceae *Mougeotia sp.*, *Oedogonium spp.*, and the chain-forming diatom *Tabellaria flocculosa* (Roth) Kütz. appeared on the first day of exposure. *Tabellaria* showed little change in abundance ($0.5\ 10^4$ to $2.7\ 10^4$, $\bar{X}= 1.4\ 10^4$ cells cm^{-2}). Its relative contribution to the total number of cells declined progressively during the experimental period, due to the settlement of unicellular algae.

In 1981, *Dinobryon divergens* Imhof. was an important component of the epiphytic biomass. It constituted 10% of the total number of cells on the first day, and 28% after

three weeks of exposure. On naturally growing *Lobelia* it ranged from 2.5% in July to 28.5% in August, 1981. The contribution of *Dinobryon* and *Oedogonium* in 1982 was neglegible.

In 1982, the firmly attached diatom *Eunotia** (Fig. 4A-B) which dominated in 1981, declined and was succeeded by the two codominant genera *Achnanthes* and *Synedra* (Fig. 4C-D). *Achnanthes* was not found on *Lobelia* after one day of exposure, but then the number of cells increased rapidly (Fig. 1B), reaching a maximum after two weeks ($4.0\ 10^4$ cells cm^{-2}). On naturally growing *Lobelia*, the number of *Achnanthes* cells ranged from $4.0\ 10^4$ in July, 1982, to $2.4\ 10^5$ cells cm^{-2} in August, 1982.

In the 1981 experiment, *Eunotia* contributed 2%-33% of the total number of cells. In 1982, its contribution was reduced to only 2.5% at the end of the experiment. On naturally growing *Lobelia*, it still made up from 11% in July to 24% in August, 1982, compared to 33% and 27% for the corresponding months in 1981.

The Spaerman rank correlation test, run on untransformed variables, indicated a significant disparity in the microdistribution of the two diatoms, *Achnanthes* and *Eunotia* (Fig. 3). *Achnanthes* was significantly more abundant on the lower side of the leaf ($r_s = 0.33$, $p = 0.05$). *Eunotia* showed a clear preference for settling on the upper side of the leaf ($r_s = 0.75$, $p = 0.05$).

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* Based on the morphological similarities (cf. van Dam et al. 1981), in the text below, *Eunotia* refers to *E. rhomboidea* and *E. veneris*, and *Achnanthes* to *A. microcephala* and *A. minutissima*.

The settlement of bacteria on exposed leaf surfaces was a rapid process owing to the morphology of the organisms. Only rod-shaped microorganisms could be recognized in the collodion film peeled off leaves incubated for one day in 1981 (Table II). The numbers of microorganisms were considered significantly different when their 95% confidence limits did not overlap. The density of rod-shaped organisms increased in the first week of exposure in 1981, after which the numbers stabilized. Rods were significantly more abundant in 1981, when the average number of cells per colony in the third week of exposure was 18.

Distinct microcolonies of coccoids appeared on the leaf surface among the already settled *Eunotia* (Fig. 4A) within one week in 1981. The density of coccoids increased continuously during the three weeks of exposure (Table II), with the number of cells per colony ranging from 10 to 50. No filamentous bacteria were observed in 1981.

In 1982, three morphological types of bacteria were represented from the first day of exposure (Table II). The density of coccoids was significantly higher in the first two weeks than it had been in the same period in 1981. The number of cells per colony ranged from 12 to 80. Rod-shaped organisms were significantly less abundant in 1982, although there were more cells per colony than in 1981 (12 to 40, compared with 4 to 18).

In 1981, the blue-green algae *Tetrachloris sp.* (Fig. 4B), probably *T. merismopedioides* Skuja, and *Aphanothece* appeared on the exposed leaves on the first day. In both experiments, the size of the colonies of *Aphanothece elabens* (Bréb.) Elenkin and *A. stagnina* (Spreng.) A. Br.

(Fig. 4E) increased rapidly. *Aphanothece* appeared in significantly higher numbers in 1981 than in 1982. *Tetrachloris* was not present in the 1982 samples. In 1982, *Pseudoanabaena sp.*, *Gloeocapsa sp.* and filamentous bacteria resembling *Vitreoscilla sp.* (Godinho-Orlandi & Jones, 1981) were observed on initially epiphyte-free leaves. The pseudofilamentous blue-green alga (*Johannesbaptistia sp.*, R.N. Nordin, personal communication) shown on Fig. 4F, appeared in the firmly attached layer of epiphytes in the 1981 experiment. This little known microorganism was a characteristic component of the firm layer of epiphyton in the lake before liming.

Few species were represented on the exposed leaves during the four weeks of exposure and the number of species was similar in both years (Table III). The structure of the epiphyton by the fourth week resembled that of the naturally existing assemblages. In both years, the highest number of epiphytic taxa on the naturally growing *Lobelia* was found in July. Fifteen taxa were common in the pooled samples of the epiphyton from naturally growing *Lobelia* in July and August 1981 and 1982. On exposed plants, six taxa were common for the epiphyton developing after one day of exposure in 1981 and 1982.

The diversity and evenness indices (Table III) show similarly low values for both experimental periods. A continuous increase in diversity values was noted in the 1981 experiment, while in 1982 the values were less consistent. The number of taxa common to both experimental periods increased to eight by the fourth week. Most other taxa occurred occasionally and in low numbers.

The calculated similarity values ranged from 0.488 to

0.727. Comparison of the *SIMI* values for the epiphytic structures that developed in 1981 and 1982, revealed that they shared 68.4% of the maximum similarity (Table III).

Some new species appeared on naturally growing *Lobelia* in 1982 (Table IV). These were represented mainly by desmids and diatoms. The recruitment of new species was more pronounced in the loosely attached fraction of epiphyton, but as these species appeared in low numbers they had little effect on the similarity and diversity indices.

So far, there is little evidence that any epiphytic taxa disappeared after liming. *Binuclearia* had been an important component of the epiphytic and metaphytic biomass in the lake before liming (Lazarek, 1983b), but five months after liming only a few cells were found.

Discussion

Epiphytic colonization was rapid even in extremely oligotrophic conditions and at low pH levels. The initial stage of epiphytic colonization in both experiments could be described as: a) passive entanglement of "pioneer" algal organisms such as *Mougeotia* and *Tabellaria*. This process was probably determined by the morphological features of the settling organisms and their abundance in the littoral, and by water movement. b) active attachment of bacterial cells by means of mucilaginous strings (Lazarek, 1983a). The collodion technique revealed that coccoidal cells colonized those areas of the leaf surface that were free of epiphytes. This technique also allowed for observing the compact bacterial microcolonies that must have originated from a settled single cell. The growth of coccoidal microorganisms

on *Eunotia* frustules (Lazarek, 1983a) must have occurred at a later stage of epiphytic development.

Later colonization was characterized by: a) passive attachment of diatoms, which subsequently developed specialized modes of attachment e.g. flattened mucilaginous adhesive around the lower valves (*Eunotia*) or mucilaginous stalks (*Achnanthes*) and pads (*Synedra*). b) passive attachment of colonial blue-green algae (*Merismopedia*, *Tetrachloris*) and desmids. This process was largely mediated by the topography of the leaf surface and the presence of organic substances released by previously settled epiphytes (Lazarek, 1983a).

The principal criteria for comparing the epiphyton that developed before and after liming were: 1) taxonomic composition. 2) the sequence of the species in the colonization process. The influence of light, believed to determine the taxonomic composition of epiphyton (Brown, 1976), was minimized by performing the experiments at the same depth.

The addition of lime influenced the taxonomic composition of epiphyton, as some algal organisms were eliminated and new algae were recruited. These processes were clearly observed on the exposed *Lobelia* but were less evident on the naturally growing plants. This may indicate that environmental conditions did not have the same effect on the naturally existing epiphytic assemblages as on the developing epiphyton.

The structural reorganization of the existing epiphytic assemblages was much slower than on the newly colonized surfaces. It is notable that *Eunotia*, three months after

the experiment in 1982, remained the dominating component of the firm layer of epiphyton on naturally growing *Lobelia*. On the initially epiphyte-free leaves, *Eunotia* was rapidly exchanged for *Achnanthes* and *Synedra*. Paleolimnological studies by Renberg & Hellberg (1982) showed that these two diatoms were present in the lake before the acidification process began. The reappearance of the diatoms is one of the most evident biological effects of liming. The qualitative pattern of changes induced by liming seems consistent with earlier observations from the limed Lake Högsjön (Lazarek, 1982). Moore (1977) noticed that *Achnanthes* has several physiological forms and suggested that this contributes to a high adaptability. The number of cells in the *Eunotia* population increased slowly but continuously, while the number of *Achnanthes* cells decreased slightly in the two weeks following exposure in 1982. This may indicate competition for space which resulted in the mechanical removal of *Achnanthes* by the growing cells of *Eunotia*.

With the collodion film technique, it was possible to quantitatively assess microdistribution of *Eunotia* and *Achnanthes* on the *Lobelia* leaves. Differences in the abundance of these diatoms on the upper and lower sides of the leaf may be attributed to light preference or differences in the metabolism of the upper and lower sides. Cattaneo (1978) found *Eunotia* species more abundant on the lower sides of *Potamogeton* leaves. She related these differences to the metabolism of the host-plant. Düringer (1958) observed a preference in diatoms for colonizing leaf edges. He proposed that this is due to more favourable illumination and nutrient renewal in these areas.

It was noted in both experiments that the firm layer of

epiphytes after the initial fast growth, did not change in taxonomic composition and that the biomass tended to stabilize.

The recruitment of new species of desmids was another visible effect of liming, consistent with observations in Lake Högsjön (Lazarek, 1982). The increase in pH and the content of DIC (from 1.0 to 8.0 mg l^{-1}) in the water seems to favour desmids. Coesel (1978) puts foreward an idea of decreasing diversity of desmids in waters exposed to acidification and oligotrophication. He observed the replacement of desmids by a uniform, excessive development of blue-green algae. Higher abundance of some blue-green algae, particularly *Aphanothece*, *Merismopedia* and *Tetrachloris*, in the 1981 experiment supports Coesel's observations. Moss (1973), discussing factors associated with exclusion of oligotrophic algae from eutrophic waters, considered the following factors important: a) direct effect of pH on the enzyme system of algae. b) toxic effect of CO_3^{--} or OH^- ions. c) the availability of different DIC compounds for photosynthesis which most reasonably explained his laboratory and field observations.

In both experiments, the diversity indices were low due to the domination of a few species. The epiphytic complex that developed after four weeks of colonization can, to some extent, be considered a 'stable system' as it consisted of organisms representing various forms of metabolism. The complexity of the epiphytic structures increased to a certain level, after which the structural parameters such as biomass and diversity stabilized.

The collodion film technique proved to be a practical alternative to SEM techniques which are expensive and

laborious, and cannot be applied in routine studies. Additionally, the use of SEM to observe bacteria is limited (Jordan & Staley, 1976) and the natural distribution of microcolonies may be disturbed by fixing procedures. However the collodion film technique cannot be applied on fragile and old leaves that support heavy epiphytic covers.

Acknowledgements

This study was carried out within the integrated Lake Gårdsjön project, funded by the Swedish National Environmental Protection Board (SNV). I express my thanks to Per Leyton and Stefan Larsson for helping with the sampling of epiphyton. My wife, Thora, corrected the grammar.

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TABLE II. Mean number of bacteria and blue-green algae, (cells 10^3 cm^{-2}) with 95% confidence limits (C.L.), on five initially epiphyte-free *Lobelia* leaves which had been exposed for between 1 and 21 days to epiphytic colonization in L. Gårdsjön, 1981 and 1982*. t= 2.78, four degrees of freedom.

	1981				1982			
Days	1	7	14	21	1	7	14	21
Bacteria:								
coccoidal	------	4.9(0.7)	44.6(8.4)	119.0(18.1)	7.2(0.3)	102.0(24.1)	200.0(91.9)	172.6(63.6)
rod-shape	5.5(2.0)	77.0(54.6)	94.0(46.7)	94.0(32.0)	2.5(0.4)	5.1(1.8)	20.4(9.1)	40.8(14.6)
filamentous x	------	------	------	------	0.3(0.1)	1.5(0.1)	1.7(0.2)	1.4(0.7)
Blue-green algae:								
Aphanothece	23.0(9.4)	96.0(39.2)	170.0(21.7)	294.0(48.4)	0.5(0.1)	12.0(5.8)	25.0(11.7)	23.0(9.1)
Pseudoanabaena	------	------	------	------	5.3(2.3)	8.9(4.7)	14.2(2.1)	16.2(5.6)
Gloeocapsa	------	------	------	------	------	0.5(0.1)	0.6(0.1)	0.8(0.2)
Tetrachloris	0.6(0.2)	1.2(0.4)	1.2(0.3)	7.0(0.4)	------	------	------	------

* After four weeks of exposure, accurate counting was not possible due to the high density of the epiphytic cover.

x Counted and expressed as single filaments

TABLE III. Indices for the epiphyton on naturally growing *Lobelia* plants (N) and for the epiphyton which developed on initially epiphyte-free *Lobelia* plants, exposed for from 1 to 28 days in the L. Gårdsjön experiments. Number of taxa (*S*), diversity (*DI*), median value of the similarity index (*SIMI*) and the evenness index (*J*) are given. The median value of the similarity is derived from the *SIMI* values for the corresponding dates in 1981 and 1982.

	N	1981					N	N	1982					N
	July	1	7	14	21	28	August	July	1	7	14	21	28	August
S	28	12	22	19	20	19	18	26	8	20	20	16	21	22
DI	0.31	0.25	0.58	0.98	1.30	1.12	0.76	0.64	0.78	0.85	0.66	1.04	0.91	1.20
SIMI							0.684							
J	0.21	0.23	0.43	0.77	1.00	0.88	0.60	0.45	0.87	0.65	0.51	0.87	0.69	0.90

TABLE IV. Some characteristic algal species, not observed in 1979-1981, which appeared on the naturally growing *Lobelia* plants in L. Gårdsjön in 1982.

Scenedesmus serratus (Corda) Bohl.

Cosmarium phaseolus Bréb.
Docidium undulatum Bail.
Gonatozygon Brébissonii West & G.S. West
Xanthidium antilopeum (Bréb.) Kütz.

Achnanthes microcephala (Kütz.) Grun.
A. minutissima Kütz.
Neidium bisulcatum (Lagerst.) Cl.
Synedra ulna (Nitzsch) Ehrenb.

Figure 1. Trends in relative abundance of the dominating and/or characteristic taxa on initially epiphyte-free *Lobelia* leaves exposed to colonization for from 1 to 28 days in L. Gårdsjön in 1981 (A) and in 1982 (B). Percentage contribution was estimated using the number of cells of a particular taxa and the total number of algal cells. Bacteria and blue-green algae are not included.

Figure 2. Relative abundance of the dominating and/or characteristic taxa of epiphytic algae on the leaves of naturally growing *Lobelia* in L. Gårdsjön. Diagrams represent the composition of the epiphytic communities sampled at the start and end of the colonization experiments at the same sampling site in 1981 and 1982. Bacteria and blue-green algae are not included.

Figure 3. Logarithmic histogram showing the distribution of the diatoms *Achnanthes* and *Eunotia* on the upper and lower sides of initially epiphyte-free leaves of *Lobelia*, exposed to colonization in L. Gårdsjön, July-August 1982. Bars are enclosed by their corresponding ranges.

Figure 4. Some components of the epiphytic cover which developed on *Lobelia* in L. Gårdsjön, 1981 and 1982. A, B, C, E and F are LM micrographs. D is a SEM micrograph.

A) Collodion film with *Eunotia* and coccoidal microorganisms on the 7th day of colonization, 1981.

B) Collodion film with *Eunotia (e)*, *Tetrachloris (t)*, rod-shaped *(r)* and coccoidal *(c)*

microorganisms on the 7th day of colonization, 1981.
C) Collodion film with *Achnanthes (a)* and *Synedra (s)*, 14th day of colonization, 1982. Note: mucilaginous stalks (arrows).
D) Cells of *Achnanthes* attached to the barren surface of a *Lobelia* leaf on the 7th day of colonization, 1982. Note: mucilaginous stalk (arrow).
E) Collodion film with a colony of *Aphanothece*, 14th day of colonization, 1982.
F) Pseudofilamentous blue-green alga in the collodion film, photographed under a phase-contrast microscope with a Whipple ocular placed in the tubus.

Fig. 1A

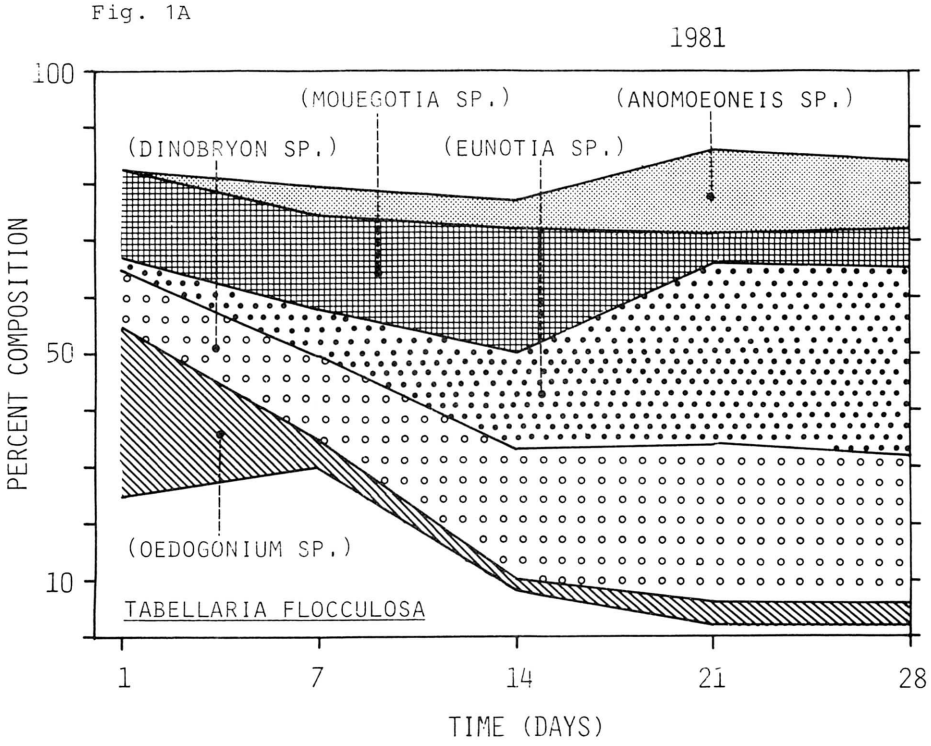

1981
100
50
10
PERCENT COMPOSITION
(MOUEGOTIA SP.)
(ANOMOEONEIS SP.)
(DINOBRYON SP.)
(EUNOTIA SP.)
(OEDOGONIUM SP.)
TABELLARIA FLOCCULOSA
1
7
14
21
28
TIME (DAYS)

Fig. 1B

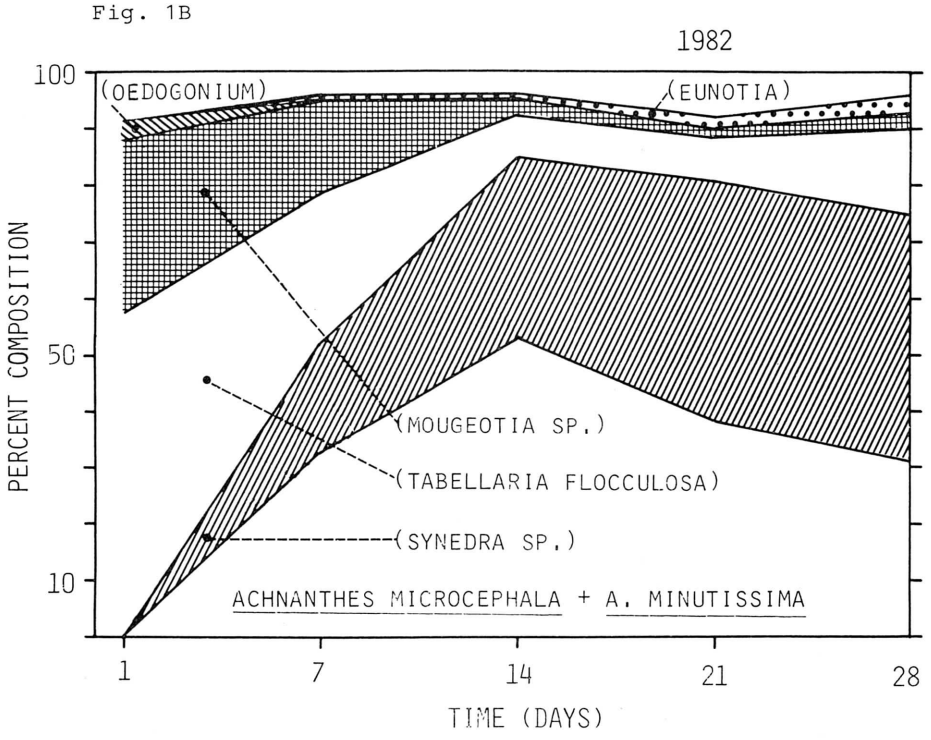

1982
100
50
10
PERCENT COMPOSITION
(OEDOGONIUM)
(EUNOTIA)
(MOUGEOTIA SP.)
(TABELLARIA FLOCCULOSA)
(SYNEDRA SP.)
ACHNANTHES MICROCEPHALA + A. MINUTISSIMA
1
7
14
21
28
TIME (DAYS)

Fig. 2.

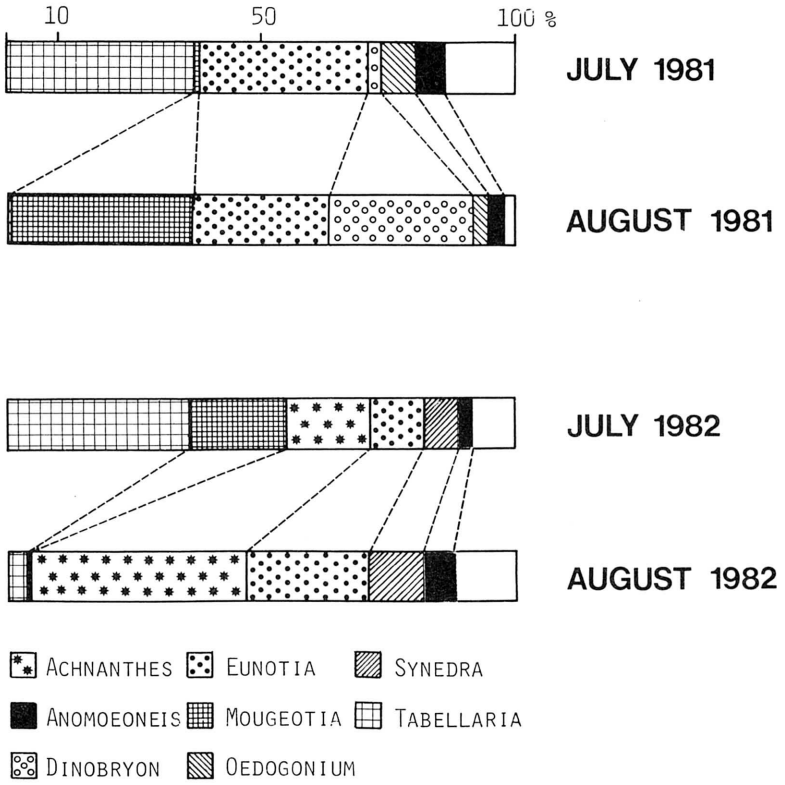

10
50
100 %
JULY 1981
AUGUST 1981
JULY 1982
AUGUST 1982
ACHNANTHES
EUNOTIA
SYNEDRA
ANOMOEONEIS
MOUGEOTIA
TABELLARIA
DINOBRYON
OEDOGONIUM

Fig. 3.

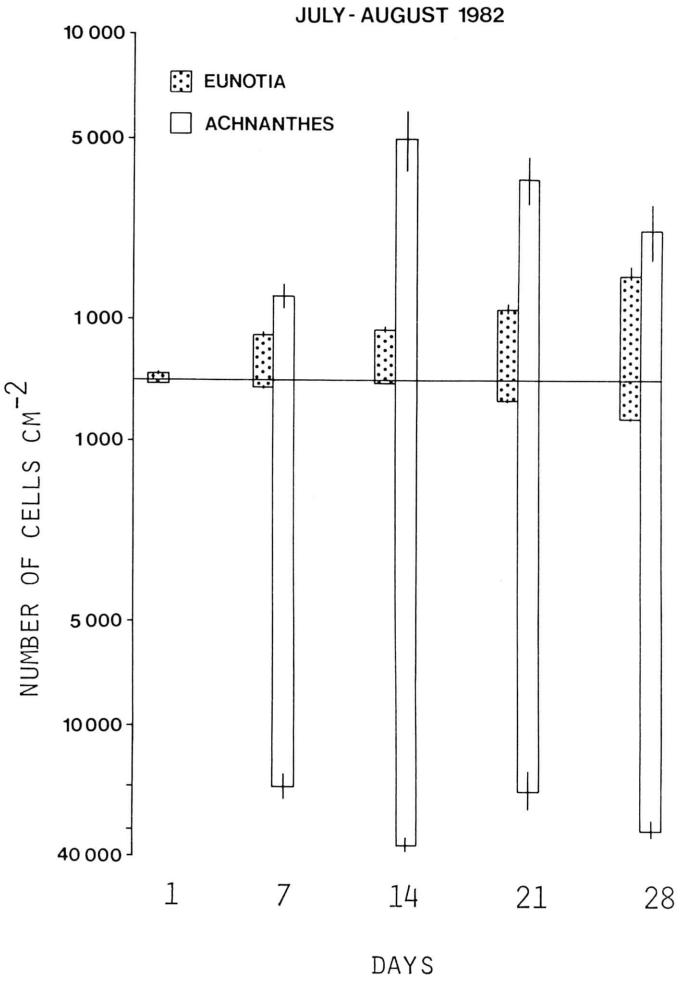
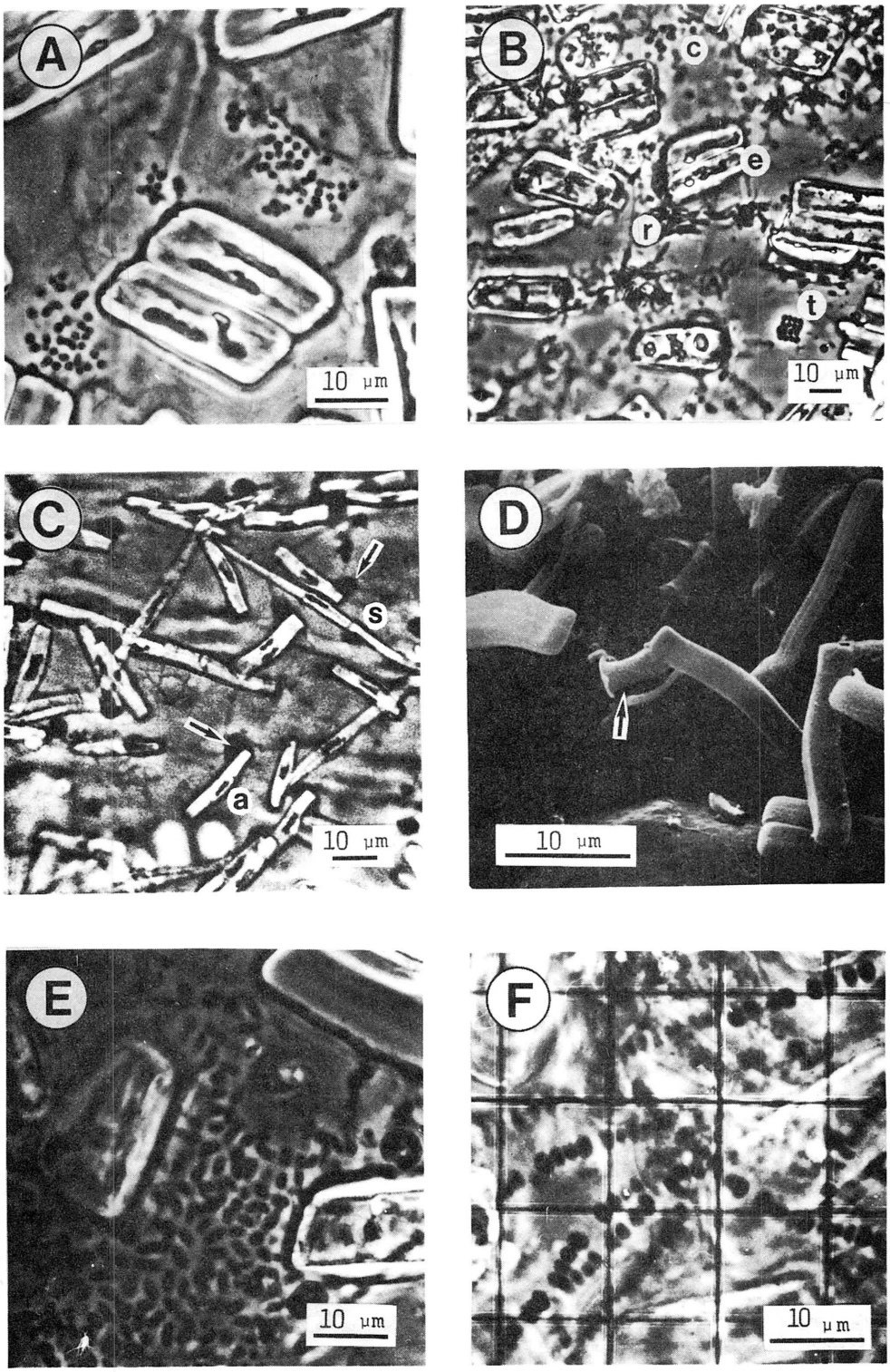
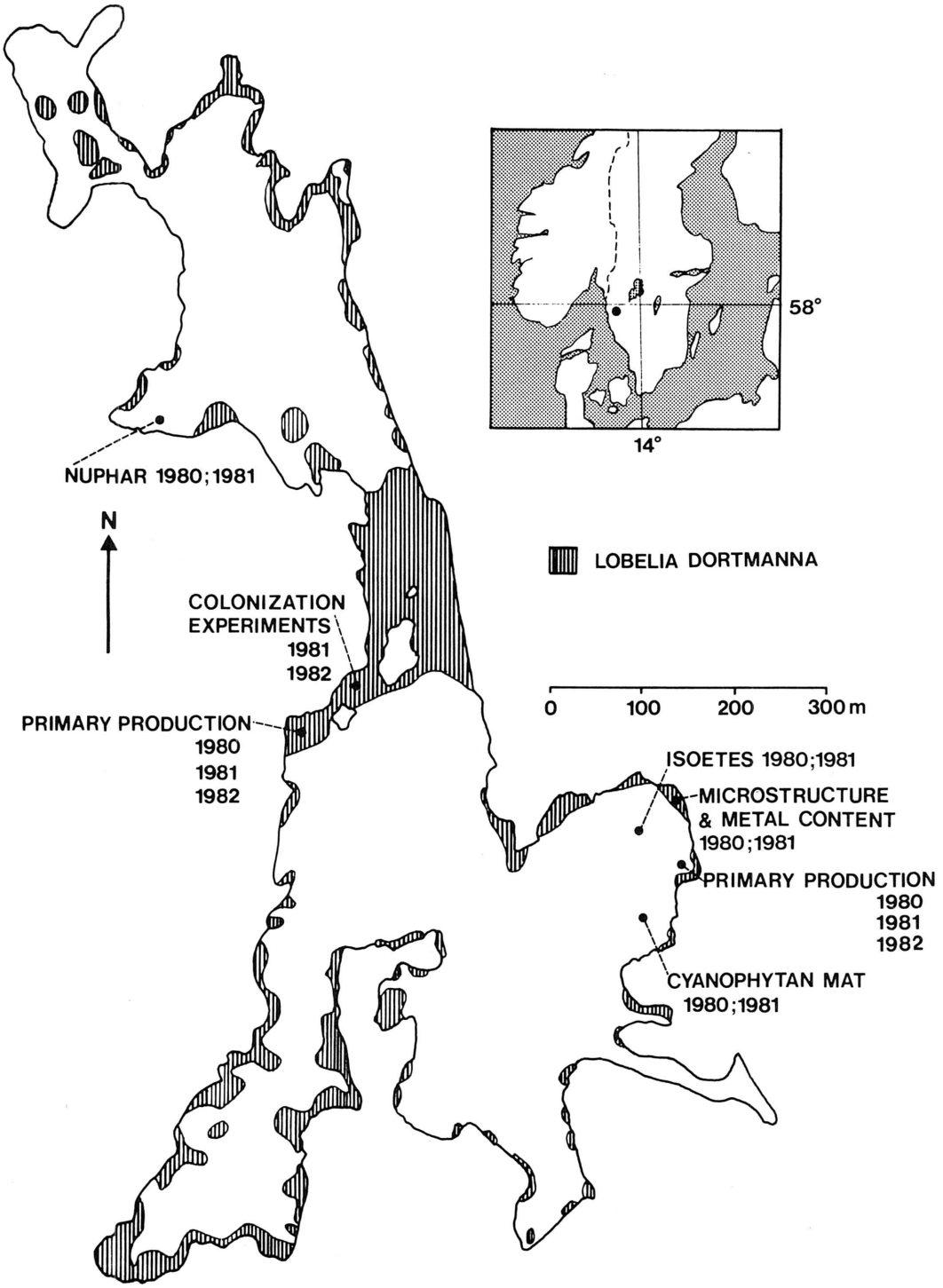

JULY - AUGUST 1982
EUNOTIA
ACHNANTHES
10 000
5 000
1 000
1 000
5 000
10 000
40 000
NUMBER OF CELLS CM-2
1
7
14
21
28
DAYS

Fig. 4.



APPENDIX A


58°
14°
NUPHAR 1980;1981
N
LOBELIA DORTMANNA
COLONIZATION
EXPERIMENTS
1981
1982
0
100
200
300 m
PRIMARY PRODUCTION
1980
1981
1982
ISOETES 1980;1981
MICROSTRUCTURE
& METAL CONTENT
1980;1981
PRIMARY PRODUCTION
1980
1981
1982
CYANOPHYTAN MAT
1980;1981

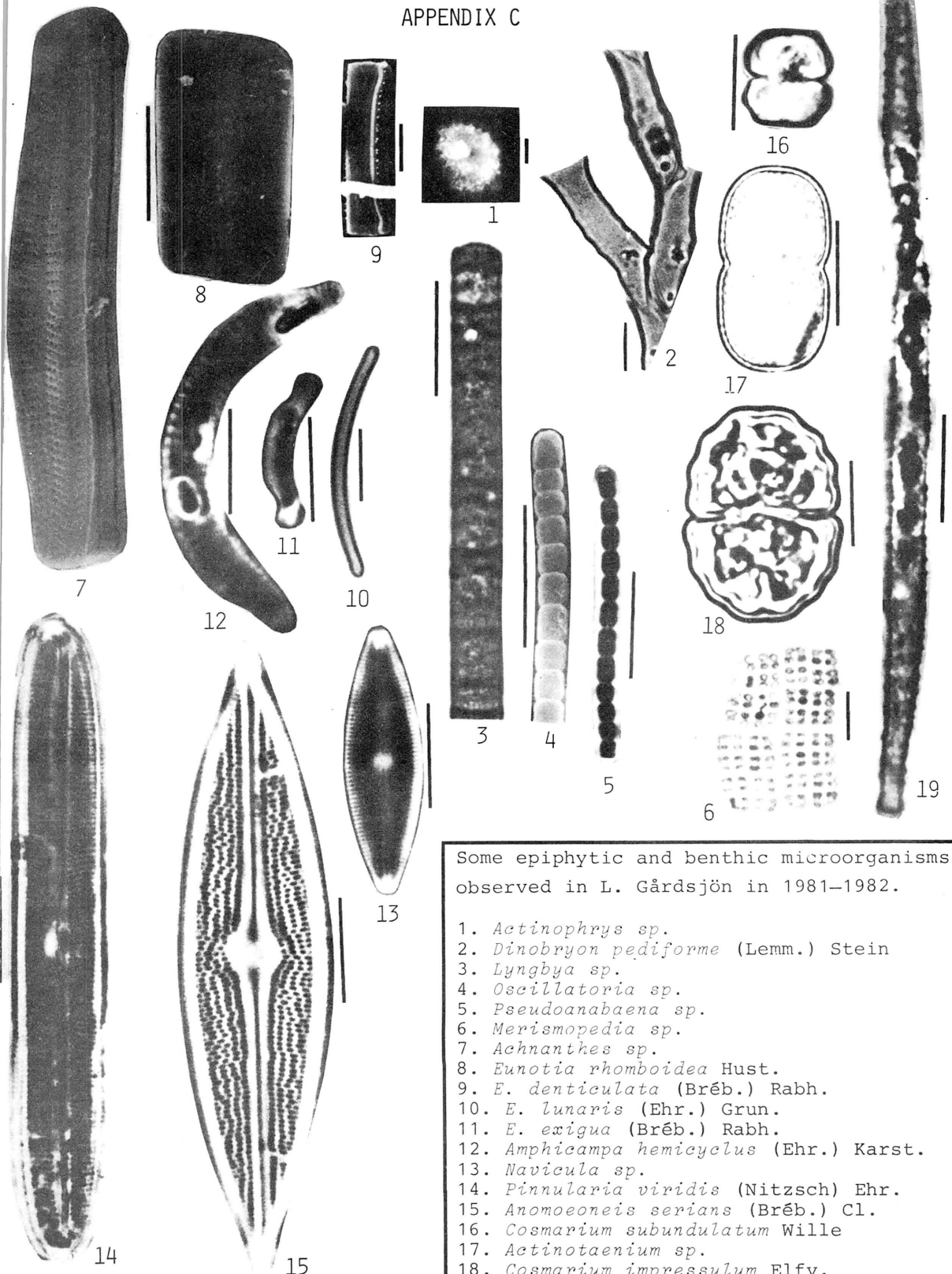
APPENDIX B

Some limnological parameters of Lake Gårdsjön*, expressed as mean values or range of values. The lake was limed in April 1982.

	1980–1981 Jan.–Dec.	1982 May–Nov.
Lake area (km^2)	0.3	
Mean depth (m)	4.9	
Max. depth (m)	18.5	
Transparency (m)	9.5	
Colour ($mgPt \cdot l^{-1}$)	2–6	1–5
pH	4.2–4.7	7.4–7.7
Conductivity ($mS \cdot m^{-1}$)	6.2–8.1	7.6–12.2
Alkalinity ($meq \cdot l^{-1}$)	0.00	0.2–0.7
Total–P ($\mu g \cdot l^{-1}$)	6.2	
PO_4–P ($\mu g \cdot l^{-1}$)	3.7	
Total–N ($\mu g \cdot l^{-1}$)	380	
$NO_2^+ NO_3$–N ($\mu g \cdot l^{-1}$)	110	93–120
NH_4–N ($\mu g \cdot l^{-1}$)	56	26.3–260.0
DIC ($mg \cdot l^{-1}$)	0.7–1.2	4.5–8.6
DOC ($mg \cdot l^{-1}$)	3.6	
Total–Al ($\mu g \cdot l^{-1}$)	350	
Ca ($mg \cdot l^{-1}$)	1.86	7.8–14.6
SO_4 ($mg \cdot l^{-1}$)	10.9	10.8–7.5
SiO_4 ($mg \cdot l^{-1}$)	0.25	
Chlorophyll *a* ($\mu g \cdot l^{-1}$)	1.0	
Net production ($g\ dwt \cdot m^{-2}$ lake area):		
Total macrophytes	8.9	
Lobelia dortmanna	2.6	
Lobelia leaf turnover (%)	38	

* determined within the integrated Lake Gårdsjön project.

APPENDIX C



Some epiphytic and benthic microorganisms observed in L. Gårdsjön in 1981–1982.

1. *Actinophrys sp.*
2. *Dinobryon pediforme* (Lemm.) Stein
3. *Lyngbya sp.*
4. *Oscillatoria sp.*
5. *Pseudoanabaena sp.*
6. *Merismopedia sp.*
7. *Achnanthes sp.*
8. *Eunotia rhomboidea* Hust.
9. *E. denticulata* (Bréb.) Rabh.
10. *E. lunaris* (Ehr.) Grun.
11. *E. exigua* (Bréb.) Rabh.
12. *Amphicampa hemicyclus* (Ehr.) Karst.
13. *Navicula sp.*
14. *Pinnularia viridis* (Nitzsch) Ehr.
15. *Anomoeoneis serians* (Bréb.) Cl.
16. *Cosmarium subundulatum* Wille
17. *Actinotaenium sp.*
18. *Cosmarium impressulum* Elfv.
19. *Gonatozygon Brebissoni* De Bary

Scale lines = 10 µm.

APPENDIX D

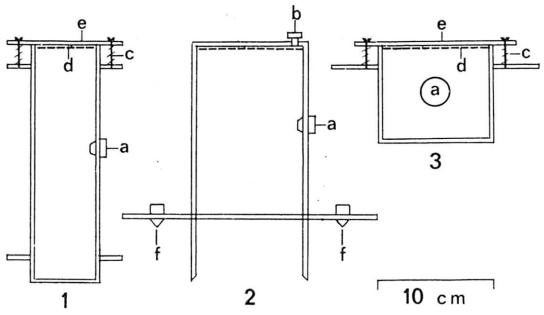
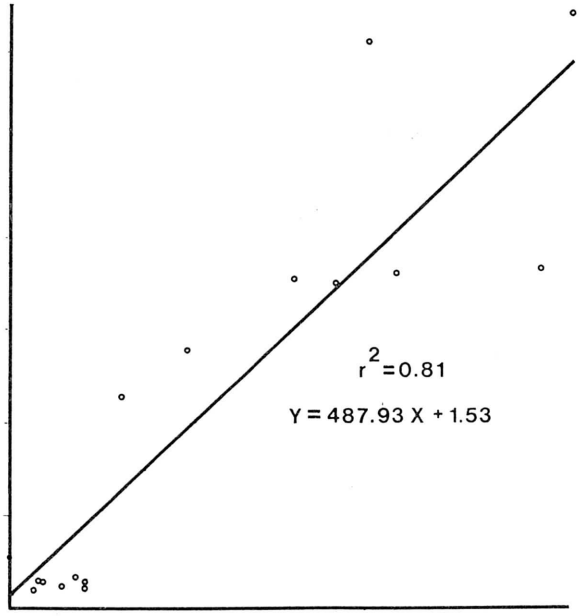


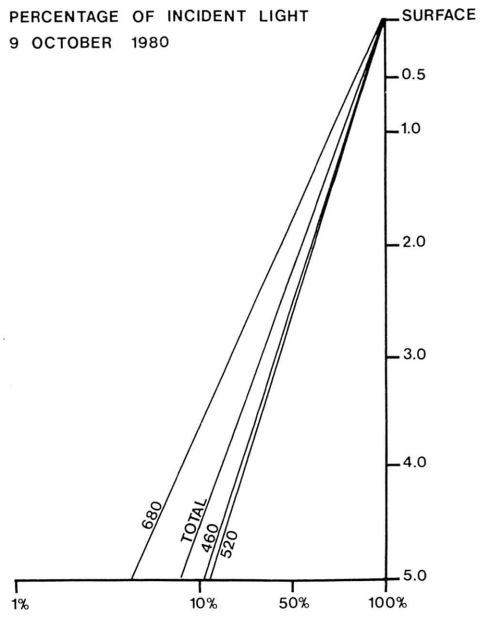
Diagram of the plexiglas chambers used for the *in situ* 14-C uptake experiments. (1) 450 ml, (2) 1000 ml, (3) 600 ml. (a) rubber septum, (b) screw prop to release trapped air and excess water when the chamber was inserted into the sediment, (c) screws, (d) rubber rings, (e) transparent lid, (f) weights.

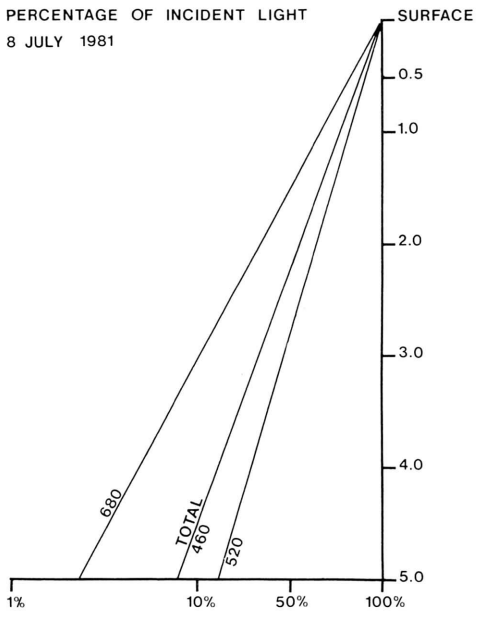


Linear regression for conversion of the *Lobelia* leaf biomass (X) expressed in g dwt, to cm^2 leaf surface area (Y).

APPENDIX E

Semilogarithmic plot of light transmission in the littoral of L. Gårdsjön. Wave length is given in nannometers.





Tryckericentralen
AVC Lund 1983