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Production, regulation and ecophysiology of periphyton in shallow freshwater lakes

PhD thesis

Lone Liboriussen

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National Environmental Research Institute

Preface

This Ph.D. thesis is submitted to the Faculty of Science, University of Aarhus, Denmark. It represents my work since 1998 at Department of Freshwater Ecology, the National Environmental Research Institute (NERI), Silkeborg, and at Department of Plant Ecology, University of Aarhus (AU). My research supervisors were Research Professor Erik Jeppesen (NERI/AU) and Professor Tom Vindbæk Madsen (AU). The project has been financially supported by the Danish Research Agency, the National Environmental Research Institute and the Faculty of Science, University of Aarhus.

I would like to thank all the people who inspired, helped and encouraged me during my project. In particular, I am grateful to my two supervisors Erik Jeppesen and Tom Vindbæk Madsen for their encouragement, enthusiasm and valuable scientific discussions. A special acknowledgement goes to the people at the two departments were my work has been conducted. First I enjoyed the inspiration and kindness from the people at Department of Plant Ecology (AU), and during the last two year I had the pleasure of being part of the supportive and encouraging atmosphere of the Lake Group (NERI). I would also like to thank Søren E Larsen for statistical assistance and Tinna Christensen for refinements of the figures used in the papers and the synopsis.

Silkeborg, December 2002

Lone Liboriussen

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Summary

Periphytic communities are important components of shallow freshwater lakes, not only because they can be very productive food web components, but also because they may serve as chemical modulators and restrict the distribution of aquatic vegetation. Knowledge of the regulation mechanisms controlling abundance, composition and production of the periphytic communities may hence be valuable tools in lake management and biomanipulation.

To stress the importance of production by attached microalgal communities and furthermore explore the effect of pelagic nutrients and season on the distribution between habitats, the production of pelagic and sediment-associated (epipelic) microalgae were measured over a year in a pre-defined area (depth 0.5 m) in each of two lakes: one nutrient-rich and turbid and one less nutrient-rich and with clear water. Total annual primary production was only 34 % higher in the nutrient-rich than in the less nutrient-rich lake. Epipelic microalgae were the dominant primary producers in the clear lake (77%), whereas phytoplankton accounted for most of the production (96%) in the turbid lake. Seasonality of the proportional distribution between habitat differed considerably between the lakes. In the turbid lake the epipelic algae contributed more in winter, when the water was relatively clear, than during summer, when the water was more turbid. By contrast, the epipelic contribution in the clear lake was slightly reduced by phytoplankton blooms in spring and autumn relative to the rest of the year. Production by the epiphytic algal communities within the reed stands was estimated to be insignificant compared to that of phytoplankton and epipelon. Our results support the general theory of a redistribution of the microalgal production from the sediment to the water column with increasing pelagic nutrient concentrations rather than a proportional increase in primary production with eutrophication. A conceptual model of the seasonal variation in the relative contribution of epipelic primary production to total primary production in shallow lakes of contrasting nutrient state was proposed from the results. In nutrient-rich turbid lakes pelagic production is expected to dominate throughout the year, while the clear lakes with lower nutrient levels may be dominated by epipelic production. At some intermediate nutrient level a lake may be expected to shift from mainly epipelic production during the clear winter period to dominance of phytoplankton production in summer, because the self-promoting processes of epipelic production collapse in spring when a threshold phytoplankton density and temperature is exceeded.

The relationship between the pelagic nutrient concentration and the potential for periphytic growth and production on chemically inert substrata in the littoral zone (0-1 m) was studied in July and September along an inter-lake gradient in total phosphorus (TP) ranging from 11 to 536 µg l^{-1} (summer mean). Mean periphytic algal biomass related unimodally to the inter-lake TP gradient in both July and September, and lakes of intermediate TP (60 - 200 µg l^{-1}) pos-

sessed the optimum combination of physical, chemical and biological conditions for periphytic growth. A shift from nutrient to light limitation seemed to occur along the inter-lake TP gradient in July, whereas nitrogen control may have been important in the most nutrient-rich lakes in September. Rates of community respiration, maximum light-saturated photosynthesis (P_{max}) and production related better to the periphyton biomass than to the pelagic TP concentration. The periphytic communities generally became more heterotrophic from July to September, and mean net production (24 hours) was positive in most lakes in July and negative in all lakes in September. Net production was not related to the inter-lake TP gradient in July, but decreased with TP in September. The results stressed that the nutrient concentration alone is a poor predictor of the standing biomass and production of periphyton.

Periphyton may be regulated simultaneously by bottom-up (resources) and top-down (herbivory) forces, but the interactive effect of these forces is largely unknown. In long-term enclosure experiments conducted simultaneously in a clear and a turbid shallow lake, light-mediated bottom-up and fish-mediated top-down forces strongly controlled the biomass of periphyton and phytoplankton. By contrast, the light- and fish-mediated effects on the benthic macro-grazers were highly dependent on the structural composition of the community. Fish reduced the biomass and abundance of snails in the clear lake, and of ostracods in the turbid lake, but had no effect on chironomids in the turbid lake. The results implied that the snails in the clear lake were, at least partly, reduced by indirect mechanisms, because both adult and juvenile snails were reduced, and only the latter were assumed to be within the size range vulnerable to predation. Light had no effect on the total biomass of benthic grazers in the clear lake, but strongly influenced life history traits of the snail community. The light-mediated bottom-up regulation of the primary producers was generally stronger in the turbid than in the clear lake, but the light regulation did not unambiguously cascade to the primary consumer level. Top-down regulation of the benthic grazer community was stronger in the clear lake than in the turbid lake, and in both lakes strong cascading effects on periphyton were seen. In conclusion, both light-mediated bottom-up and fish-mediated top-down forces controlled the biomass of periphyton, while the reductive impact of the two factors on the benthic grazer community was highly dependent on the structural composition of the community.

Although light and temperature are usually considered sub-optimal for algal growth in winter, periphyton growth and standing biomass may be higher in winter than in late spring. A study of monthly winter-spring growth and accrual rates of periphyton revealed that the periphyton accrual rates were higher from January to February than during any other months from February to May. The ratio of productivity to respiration and the chlorophyll-specific productivity of periphyton were negatively correlated with community biomass, and though low periphytic algae densities were developed under the ice, the community had a high productive potential. The study showed a high potential for periphytic growth and production in winter-spring.

Dansk resumé

Perifytiske mikrosamfund kan være af stor betydning for de økologiske samspil og interaktioner i mange lavvandede søer, dels fordi de kan bidrage betydeligt til søens totale primærproduktion, men også fordi de kan regulere udbredelsen af neddykket vegetation og påvirke forskellige fysio-kemiske forhold. En øget viden om de reguleringsmekanismer, der kontrollerer den temporale og spatiale udbredelse og produktion af perifyton, kan derfor bidrage til forståelsen af lavvandede søers økologi og kan anvendes i forbindelse med sørestaurering og biomanipulation.

Med henblik på at belyse hvordan næringsindholdet i de frie vandmasser påvirker den totale mikroalgeproduktion og forholdet mellem, samt sæsonvariationen af, bentisk og pelagisk produktion i lavvandede søer, blev primærproduktionen på søbunden og i vandfasen beregnet syv gange inden for et år i en næringsrig, uklar sø og en mindre næringsrig, klar sø. Produktionsfordelingen var meget forskellig i de to søer. Fytoplankton stod for størstedelen (96%) af den årlige primærproduktion i den uklare sø, mens de bentiske alger dominerede (77%) i den klare sø. I begge søer var det relative bidrag fra bentiske alger størst i vinterhalvåret, mens der i den klare sø ligeledes var et stort bidrag i de næringsstofbegrænsede sommermåneder. Mikroalger knyttet til tagrørsoverflader bidrog med <1% af den totale årlige produktion i begge søer. Produktionsomfordelingen fra sediment til vandfase med stigende næringsindhold resulterede i, at den totale produktion kun øgedes 34% fra den mindre næringsrige, klare sø til den meget næringsrige, uklare sø. Ud fra resultaterne blev der foreslået en model af sæsonvariationen i de bentiske algers relative bidrag til den totale mikroalgeproduktion i søer med forskellig næringsindhold. I næringsrige, uklare søer antages den relative andel af bentisk produktion at være lav igennem hele året, mens den i mindre næringsrige søer formodes at være høj. I søer med et intermediært pelagisk næringsindhold kan et skifte fra dominans af bentisk produktion om vinteren til pelagisk produktion om sommeren forventes, fordi de positive feed back processer, der medvirker til at opretholde en betydelig bentisk produktion, kollapser i forårsmånederne når fytoplanktonbiomassen og temperaturen overstiger et vist niveau.

Relationen mellem vandfasens næringsindhold og potentialet for perifytisk produktion og biomasse akkumulering på en neutral overflade (vanddybden 0-1 m) blev undersøgt i juli og september i 13 søer, der tilsammen repræsenterede en total fosforgradient fra 11 til 536 µg l^{-1} (sommergennemsnit). Den gennemsnitlige perifyton biomasse, indsamlet 0,1, 0,5 og 0,9 m under overfladen, var unimodal relateret til total fosforgradienten, og den største biomasse blev fundet i søer med en total fosforkoncentration mellem 60 og 200 µg l^{-1}. Et skifte fra næringsbegrænsning af perifyton i de mindst fosforrige søer til lysbegrænsning i de mest fosforrige søer synes at finde sted i juli, mens indikationer på kvælstofbegrænsning i de mest fosforrige søer blev fundet i september. Raterne for perifytisk primærproduktion, respiration og maksimal lysmættet fotosyntese (P_{max}) kunne bedre beskrives ud fra perifytons biomasse end udfra søens fosforkoncentration. De perifytiske samfund blev generelt mere heterotrofe fra juli til september, og netto-produktionen for et døgn var positiv i de fleste søer i juli, men negativ i alle søer i september. Undersøgelsen viste, at vandfasens næringsindhold som enkelt parameter er utilstrækkelig til at bestemme potentialet for den perifytiske produktion og biomasse i den littorale zone.

Ressourcetilgængelighed (bottom-up regulering) og græsning (top-down regulering) kan regulere perifyton samtidig, men kendskabet til de interaktive effekter af disse reguleringsmekanismer er begrænset. I et indhegningsforsøg udført samtidig i en klar og en uklar sø blev biomassedynamikken for fytoplankton, perifyton og de perifyton tilknyttede makro-græssere fulgt ved forskellige grader af skygning og enten i nærvær eller fravær af fisk. Både lysforhold og fisk havde en markant effekt på biomassen af primærproducenter i begge søer, skygning reducerede biomassen, mens tilstedeværelsen af fisk øgede den. Effekterne af skygning og fisk på de bentiske græssersamfund var afhængig af samfundenes strukturelle sammensætning. En reduktion af lyset, og dermed lavere perifyton biomasse, havde ingen effekt på den totale biomasse af bentiske græssere i den klare, sneglедominerede sø. Derimod var sneglenes reproduktion påvirket af lyset, og tidligere reproduktion blev observeret under naturlige lysforhold og let skygning end under kraftig skygning. Fisk reducerede biomassen og antallet af snegle i den klare sø, og ostracoder i den uklare sø, men havde ingen indflydelse på forekomsten af dansemyglarver i den uklare sø. Både antallet af juvenile og voksne snegle var lavere i indhegninger med fisk end i dem uden fisk, selvom direkte prædation på voksenindivider sandsynligvis var umulig. Dette indikerer, at fiskenes påvirkning, i det mindste delvis, foregik via indirekte mekanismer. Den skygge-medierede bottom-up regulering af primærproducenterne var generelt større i den uklare sø end i den klare sø, men en generel kaskadevirkning fra primærproducent-niveau til græsser-niveau blev ikke observeret. Den fiske-medierede top-down regulering af det bentiske græssersamfund var størst i den klare sø, men i begge søer blev en tydelig kaskadevirkning til primærproducent-niveauet observeret. Samlet viste undersøgelsen, at både skygning og tilstedeværelsen af fisk påvirkede perifyton biomassen, mens deres effekt på det bentiske græssersamfund var afhængig af samfundets strukturelle sammensætning.

Selvom vinterens lys- og temperaturforhold ofte er suboptimale for algers vækst og produktion, kan perifytons vækst og biomasse være større end i sommermånederne. En undersøgelse af perifytons vinter-forårs-dynamik fandt en højere biomasse akkumuleringsrate fra januar til februar end i månederne februar til maj. Den biomasse-specifikke produktivitet og ratioen mellem produktivitet og respiration var negativt korrelerede med perifyton biomassen, og selvom kun en lav biomasse blev udviklet under isdække, havde perifyton samfundet alligevel et højt produktionspotentiale. Resultaterne understreger betydningen af perifytonproduktion i vinter-forårsmånederne.

Structure of the thesis

The thesis consists of a synopsis and four papers (A-D). Literature and subjects relevant to the topics are presented in the synopsis just as some of the results presented in the four papers are integrated and discussed. I have additionally taken the opportunity to go more detailed into a discussion of some basic and theoretical considerations concerning my work.

The material in the papers is based on four separate research projects, each presented in one of the included manuscripts. All four projects, however, included aspects of temporal and spatial dynamics of the periphytic communities. Most of the investigations have been made by empirical samplings of periphyton developed on either natural or artificial substratum and subsequently analysing composition, densities, production rates and physiological responses of the communities in the laboratory. Only the project presented in Paper C has involved direct manipulation of some of the ecological conditions under which periphyton was developed.

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Synopsis

1 Introduction

The liquid-solid interface of virtually all submerged surfaces will shortly after submersion be colonised by bacteria and diatoms from the water column. By secreting mucilaginous polymeric substances, adhesive molecules and enzymes, these organisms will modify the surface to a complex matrix upon which organic and inorganic material settles and bacterial and algal growth occurs (Otten & Willemse, 1988; Wetherbee, Lind & Burke, 1998). Some mature communities have a three-dimensional structure similar to that of terrestrial vegetation, with low-profile and prostrate algal taxa as the lowest layer, upright or stalked taxa as the central layer and filamentous taxa extending from the mat and forming an upper layer. Most people know these layers as woolly, slimy and slippery, brownish or greenish coats on stones, plants and the boardwalk in the lake where they swim or in their garden pond, and most people find them annoying and redundant. Aquatic scientists, however, have an extensive recognition of the importance of these layers, which may act as both structural and functional components of the lake communities.

1.1 Definition

Browsing the literature reveals that the terminology of these attached microbial communities is both inconsistent and highly confusing. Terms such as biofilms, microlayers, aufwuchs, periphyton and benthos have been used synonymously for several decades, but many of these terms have also been linked to more specific definitions varying with time and geography, among research groups (Weitzel, 1979) and even between publications from the same author. Today, the term most often used in the aquatic scientific literature is '*Periphyton*', but still the definitions vary considerably and may specify either microfloral communities or entire microcommunities (including both living and dead components) attached to substrata that are either natural or artificial. Based on recognition of the structural complexity and the tight couplings of many heterotrophic and autotrophic processes within the periphyton, I have chosen to define 'Periphyton' according to Wetzel's opening remarks in the book "*Periphyton of Freshwater Ecosystems*" (1983). After a short discussion of the inconsistencies related to the terminology he concludes "…that it is perhaps best to simply accept the word periphyton, as it is widely understood: a complex community of microbiota (algae, bacteria, fungi, animals, and inorganic and organic detritus) that is attached to substrata. The substrata are inorganic or organic, living or dead."(Wetzel, 1983). Yet, whenever possible I have tried to specify the terminology both in relation to which fraction of the community I refer to (e.g. periphytic algae, periphytic dry weight, periphytic microorganisms etc.), but also in relation to the type of substratum colonised counting macrophytes (epiphyton), wood (epixylon), stone (epilithon), sandy sediments (episammon) and muddy sediments (epipelon). Although I have chosen a broad and general definition of the periphytic community, most of the studies in this thesis focus on the periphytic algae because of their significant role in energy and nutrient transformation in many lakes.

1.2 Functional and ecological role of periphyton

It is not unusual to find submerged vegetation and other underwater surfaces covered by periphyton densities of several hundreds micrograms of algal chlorophyll *a* per square meter. Frequently, the supporting surfaces are so densely coated that they are actually impossible to see. Intuitively, it is easy to imagine that such massive coverings may have a vast impact on the surrounding environment. Moreover, the central position on the interface zone between the overlaying water column and the substrate beneath allows periphyton to affect both environments. When considering periphyton-mediated effects on the ecosystem, attention is usually drawn to three major areas: regulation of submerged vegetation, nutrient dynamics and energy input to food webs.

1.2.1 Regulation of submerged vegetation

Several studies have found that increased epiphyton loads are detrimental to the success of the host plant (Sand-Jensen, 1977; Brönmark, 1985; Jones *et al.*, 2002; Williams, Moss & Eaton, 2002). It has therefore been suggested that epiphyton is one of the most important regulators of abundance and depth distribution of submerged vegetation in lakes (Phillips, Eminson & Moss, 1978; Sand-Jensen & Borum, 1984; Sand-Jensen & Borum, 1991). Epiphyton may suppress the host plant by shading, and the light reaching the photosynthetic tissue under a dense epiphyton mat may be reduced by more than 80% (Sand-Jensen & Søndergaard, 1981) just as the quality of the light may be changed (Losee & Wetzel, 1983). In addition, epiphyton may restrict some host plants by acting as a barrier to up-take of inorganic carbon from the water column (Sand-Jensen, 1977; Jones *et al.*, 2002). Low rosette-forming isoetids obtain most carbon dioxide from the sediment (Søndergaard & Sand-Jensen, 1979) and the epiphyton-mediated suppression is almost certainly caused by a reduced availability of light (Sand-Jensen & Søndergaard, 1981; Sand-Jensen & Borum, 1984). Taller macrophytes, however, take up most inorganic carbon from the water column (Madsen & Sand-Jensen, 1991), and epiphyton may hence suppress the host plant by interception of inorganic carbon (Sand-Jensen, 1977; Jones *et al.*, 2002) as well as light. Attached epiphyton can also create O_2 and pH regimes unfavourable to the macrophytes (Simpson & Eaton, 1986) just as it could be expected to be a mechanically annoyance to the host plant. Various authors have suggested that submerged vegetation may derive some benefit from being covered by epiphyton, because grazing invertebrates are diverted from consuming the plant tissue just as pathogen attach may be prevented (Hutchinson, 1975; Rogers & Breen, 1981). However, these benefits are most frequently of minor importance relative to the light, carbon, and mechanical stress simultaneously exerted by the epiphyton on the host plant. Addressed on a larger ecological scale suppression of aquatic vegetation by epiphyton may encourage a lake to shift from a clear water stable state with high habitat heterogeneity to a turbid state with a more homogeneous environment, and thereby induce major changes on the ecosystem.

1.2.2 Nutrient dynamics

Periphyton can both directly and indirectly serve as a major regulator of the nutrient dynamics in lakes (Wetzel, 1990; Wetzel, 2001). A direct mechanism is by uptake and assimilation by which the available nutrient pool of both water column and sediment is reduced (Hansson, 1989). Periphyton may, however, also directly enrich a relatively nutrient-poor water column by export of dissolved or particulate organic matter originally sequestered from the substrata. Indirectly the pelagic nutrient pool may be affected by photosynthetic active periphyton on the sediment, because the produced oxygen may immobilize several dissolved elements and reduce the chemical fluxes at the sediment-water interface (Carlton & Wetzel, 1988; Hansson, 1989; Woodruff *et al.*, 1999). These processes are only some by which periphyton affects the nutrient dynamic in a lake, while introduction of new nitrogen by N_2 fixation or removal of excessive nitrogen by denitrification are others. Considered in a general ecosystem perspective, periphytic communities and their coupled metabolism typically improve the capacity of a lake to retain externally loaded nutrients just as they may reduce internal loading of phosphorus from sediment pools (Wetzel, 1999).

1.2.3 Energy input to food webs

Periphyton may contribute substantially to whole-lake primary productivity in shallow lakes (Wetzel, 1964; Burkholder & Wetzel, 1989; Vadeboncoeur, Lodge & Carpenter, 2001; Liboriussen & Jeppesen, 2003) and thus provide an important energy input to both detritus and grazing food chains (Gresens, 1995). A wide range of fish and benthic invertebrates including snails, chironomids, mayflies, oligochaetes and several groups of crustaceans include periphyton in their diet (Jones, Moss & Young, 1997). While some species only switch to feeding on periphyton when their preferred food items are unavailable, others exploit it as their main food source. Quality, quantity and community composition of the periphyton can therefore have a significant effect on growth, distribution, life history parameters and composition of the grazer community. Accordingly, most studies manipulating periphyton abundance reveal higher growth rates and densities of benthic grazers at high-periphyton conditions (Feminella & Hawkins, 1995), just as patch preference and spatial distribution of grazers often are related to the species composition of the periphytic algal community (Gresens & Lowe, 1994). The importance of periphyton as an energy base for food webs cannot readily be predicted from the standing biomass. Even when biomass is low, periphyton communities can be exceedingly important as a food resource because of their high productivity and turnover rates (McIntire *et al.*, 1996). Direct observations of gut contents and analysis of stable isotope ratios may, however, be used to evaluate the overall contribution of periphytic primary production to higher consumer levels. By the use of these methods it was shown that more than half of the energy consumed by fish in north-temperate lakes of North America was derived from zoobenthos that had originally exploited periphytic algae as their main food source (Vander Zanden & Vadeboncoeur, 2002). Even fish species generally thought of as pelagic were partly supported by carbon fixed by periphytic algae. Also other studies using stable isotopes have provided evidence that periphytic algae are major contributors to fish production in lakes throughout the world (Hecky & Hesslein, 1995). As stated by the stable isotope studies, high rates of periphytic primary production will not only be of importance for the benthic primary consumers and benthic food webs in general, but may also provide a central energy basis for pelagic food webs (Vander Zanden & Vadeboncoeur, 2002).

1.3 Objectives of the thesis

The key role of periphyton and periphyton-mediated processes in many ecological associations and interactions emphasises the importance of knowledge on the regulating mechanisms controlling the temporal and spatial dynamics of these communities. Despite an increasing interest in periphyton over the past decades, the knowledge of lentic periphyton communities still lags far behind that of lotic periphyton and even farther behind the knowledge on phytoplankton. The overall objective of my work has therefore been to study how primary production and abundance of periphytic communities are regulated in shallow freshwater lakes. Furthermore, it has aimed to improve the understanding of how seasonal changes and ecological differences may affect interactions between periphytic and pelagic primary production and the metabolism of the periphyton in general. An improved understanding of the complex mechanisms structuring the periphytic communities may serve as a valuable analytical tool for modelling how the ecosystem of shallow lakes will respond to changes in the physical, chemical or biological conditions associated with for instance biomanipulation of lakes or environmental changes in general.

1.4 Shallow lakes versus other aquatic environments

My studies have focused on periphyton in shallow freshwater lakes for two reasons: shallow lakes are the most common lake type in Denmark, and the potential for periphyton growth and production is higher than in deeper lakes due to the high ratio of benthic to pelagic habitats. In a meta-analysis of 85 experiments with epilithic and epiphytic communities, Hillebrand (2002) found that both the effects of nutrient addition and grazer removal were positive and highly consistent in running and standing freshwater habitats and in coastal environments. He concluded that the strength of the biotic interactions acting on the periphyton may be relatively similar across different aquatic environments, and that the observed variation in the outcome of single experiments is not a result of general large-scale differences between environments (e.g. marine versus freshwater or lotic versus lentic), but rather a result of factors acting on a smaller scale. Although I am fully aware that extrapolation of research results from one type of aquatic ecosystems to another is problematic, I occasionally find it valuable to include and draw parallels to observations and classical experimental results from streams, deep lakes and marine waters in the discussion of my results.

2 Methodical considerations

2.1.1 Variation - variabilitet

Major difficulties may be encountered when assessing naturally occurring periphyton biomass and production. One of them being that spatial variability of periphyton biomass is often rather high. In a review of variability in freshwater periphyton Morin & Cattaneo (1992) found that periphytic chlorophyll *a* can span more than 5 orders of magnitude (from 0.01 to 2385 mg m^{-2}), whereas dry mass and ash-free dry mass span more than 3 orders of magnitude. The variability is often particularly evident in contrasting substratum types such as mud, sand, rocks and vegetation (Uehlinger, 1991), but even on relatively homogeneous substrates great local variability may occur. Colonisation time, and thus community succession, has also been found to be an important source of variation for biomass, metabolic parameters as well as community composition (Wilhm & Long, 1969; Hill & Boston, 1991). Due to high natural variability a very intense sampling effort with numerous replications is often needed to get a discriminating power sufficiently high to distinguish the response of a treatment effect from a temporal variation. Reviewing periphyton variability Morin & Cattaneo (1992) concluded that depending on the periphyton variable sampled, means have to differ by a factor ranging from two (biovolume) to eight (dry mass) to have a 95% probability of being significantly different when five replicates are taken.

2.1.2 Artificial substrata

To secure standard means of comparison among periphyton in different lakes and eliminate variability due to differences in colonisation time and surface structure, artificial substrata were used in most of my studies. The use of artificial substrata allowed sampling from the same depth in all lakes (Paper B+D) and at all sampling occasions (Paper A+B+D), and thus ensured a relatively high and uniform sample size within each treatment or seasonal observation. Employing artificial substrata also facilitated comparative physiological studies of community metabolism among temporally, spatially and physico-chemically different environments, because the communities could readily be transferred to incubation chambers and measured as intact communities without disturbance of the community or interference of the substrata.

In order to resemble the flexibility and upright position of most macrophytes and additionally include incubation depth as a factor of investigation, I chose to use vertically orientated green PVC strips (Max Tape, Max Co. Ltd – width 11 mm) as substratum for most of the experiments. This substratum was chosen because its surface is slightly structured and hence facilitates periphytic attachment. Moreover, the green material transmits only 10 - 15% light and both surfaces are hence exposed to light conditions resembling those in natural vegetation. Furthermore, chlorophyll *a* extractions could be made directly on the strips without interference on the spectrophotometric readings, just as freezing and freeze-drying did not affect the material. Strip segments could also easily be pooled at low biomasses or subdivided at high biomasses to obtain the most favourable absorbances or densities for the measurements.

A major consideration in the use of the artificial substrata was whether the periphyton abundance systematically differed from that of natural substrata. Comparative tests of epiphyton naturally developed on macrophytes or reeds and periphyton associated to the artificial substrata were therefore performed simultaneously with the experiments. Although these tests did not always corresponded well with the periphyton load on the two types of material, no systematic difference was detected. In three independent experiments (approximate incubation time: 1, 2 and 3 months), the algal accrual rates on the strips were, additionally, tested against that of glass slides and that of the PVC material used for the enclosures in Paper C. These tests revealed that the plastic materials generally accumulated higher algal densities than the glass slides ($p=0.04$, ($n=135$)), but the colonisation of the two plastic substrates did not differ systematically ($p=0.26$, ($n=90$). Prior to all incubations, the strips were rinsed and submerged in demineralized water for at least 24 h, to wash off residual substances from the surfaces, if any.

To allow colonisation by benthic invertebrates the artificial substratum was always kept in direct contact with the sediment. Snails were especially abundant on the strips in the study of periphytic winter dynamics (Paper D), whereas chironomids and oligochaetes occasionally occurred at high densities in both the study of periphyton abundance among lakes of different TP concentration (Paper B) and the investigation of primary production in lakes of contrasting nutrient availability (Paper A). These observations assured that the artificial substratum did not categorically repel the benthic invertebrates. However, though several attempts were made, employing various exclosure designs, to estimate the actual grazing pressure assessed to the periphyton communities, I have no information on whether the grazing pressure on the periphyton on the artificial substratum was comparable to that of natural periphyton.

Despite the fact that the use of artificial substrata in periphyton surveys is sometimes questionable, since it omits effects of the periphyton-substrate interaction (Cattaneo & Amireault, 1992), the advantages over other experimental methods are often numerous, especially in comparative studies across different environments (Wetzel, 1983; Aloi, 1990). The use of artificial substratum potentially limits the applicability of the results to natural periphyton communities in shallow lakes, and I therefore fully agree with the conclusion of Cattaneo & Amireault (1992) that the use of naturally occurring substrata is preferable where feasible. However, neither the inter-lake study of periphyton distribution along a phosphorus gradient (Paper B) nor the winter dynamics study (Paper D) aim to determine the actual *in situ* periphytic abundance and primary productivity. Instead, the studies were conducted to compare the potential dynamics of periphyton given a set of interacting environmental conditions, and identical start and substratum conditions were therefore essential.

3 Regulation of periphyton

Periphyton communities are important structural and productive components of freshwater ecosystems; yet factors regulating their temporal and spatial abundance are difficult to predict. Many environmental factors such as disturbance, resources, environmental conditions and grazing pressure collectively interact and influence both distribution and species composition of periphyton. Due to this complex regulation, the relative importance and contribution that each factor may exert in shaping the periphytic community is difficult to evaluate and quantify (Lowe, 1996). Moreover, the periphytic communities habitually include various species that all respond uniquely to changes in the environment, which consequently further blurs the interpretation of the regulation at the community level (Biggs, Stevenson & Lowe, 1998).

3.1 Regulation by light

Light is the ultimate energy source for primary production and algal growth. Variation in primary production within a localised area and across geographical regions can be attributed to variations in the light availability, just as the temporal variation (from minutes to seasons) of the light has been shown to be of major importance (Harris, 1980). Several attempts have been made to determine primary productivity directly by integration of the standing periphytic algal biomass and the light environment, because these parameters are fairly easily obtained. Algae may, however, adjust their pigmentation, cell size and metabolic capacity to the prevailing environmental factors (Richardson, Beardall & Raven, 1983; Falkowski & LaRoche, 1991), just as high rates of grazing may reduce the biomass, thus making the standing biomass a rather poor estimate of the primary production. Direct measurement of the photosynthesis by ^{14}C uptake or changes in dissolved oxygen concentrations are, however, more common and recognised methods used to assess periphyton productivity (Stevenson, 1996), and I have used these methods in Paper A+D and in Paper B, respectively.

3.1.1 Photosynthesis-irradiance curves

The short-term response of photosynthesis to changes in the light intensity can be described by photosynthesis-irradiance curves (P-I curves). Combined with information on the actual local light climate P-I curves may be employed to estimate *in situ* photosynthesis (Paper A+B). However, because the parameters of these curves may change with light, temperature, grazing and the structure of the autotrophic community (Jasper & Bothwell, 1986; Henley, 1993; Uehlinger, König & Reichert, 2000), they can also be used to evaluate ecophysiological community adaptations in relation to the prevailing environment. Two contrasting strategies of photosynthetic adaptations by autotrophs to light conditions are generally advanced for terrestrial vegetation, higher macrophytes and phytoplankton (Richardson *et al.*, 1983; Salisbury & Ross, 1991). Exposure to consistently high light levels would favour plants with high maximum light-saturated photosynthetic rates (P_{max}), while plants with a high efficiency to use light at subsaturating intensities (α) are more advantageous in low light environments. Accordingly, shade adaptation or acclimation is usually manifested in increased α, lower P_{max} and respiration and the need for lower light intensities to compensate for respiration and saturate the photosynthesis. While dynamic P-I responses, in which photosynthesis does not only depend on the existing environmental conditions, but also on the prior history of conditions, are known for phytoplankton, such responses are largely unexplored for intact periphytic communities developed in the field (Hill, 1996). However, because periphyton communities are sessile and thus fixed to a specific depth and, within certain limits relatively consistent environmental conditions, they could be expected to exhibit distinct P-I responses when developed in different environments.

3.1.2 Periphytic P-I responses in relation to depth, nutrients and season

The P-I response of periphyton at different incubation depths and across an inter-lake total phosphorous (TP) gradient (mean TP_{summer} range: 11 - 536 µg l^{-1}) was studied to elucidate if the theory of sun-shade adaptations could be detected on the inter- or intra-lake scale (Paper B)(Fig 1 list of the lakes in the study). Community P-I responses were measured on periphyton developed on artificial substrata 0.1, 0.5 and 0.9 m below the water surface in July and September after 8 and 15 weeks of incubation, respectively. The results indicated that neither respiration nor P_{max} correlated to the light conditions in the lake or changed systematically with depth as might be expected from the theory of sun- shade adaptations. This was evident in July as well as in September, and both when the parameters were related to a long-term light average estimated from the Secchi depth and when related to the specific light attenuation determined on the day of sampling. In contrast, the community respiration rate (R) and P_{max} were better correlated to periphytic algal biomass, the correlation being positive when the parameters were expressed per unit of surface area (R^A, $P^A{}_{max}$) and negative when expressed per unit of algal biomass (R^B, $P^B{}_{max}$). Moreover, the overall relationships of metabolic parameter versus periphyton biomass were the same at all three depths. These results are in accordance with those modelled and experimentally described by Dodds *et al.* (1999), who found that the whole-community photosynthetic properties of periphyton mats were functions of the community thickness. Yet, in micro-scale measurements they demonstrated that photoacclimation (e.g. increased α with depth) can occur with depth within some periphyton mats, but

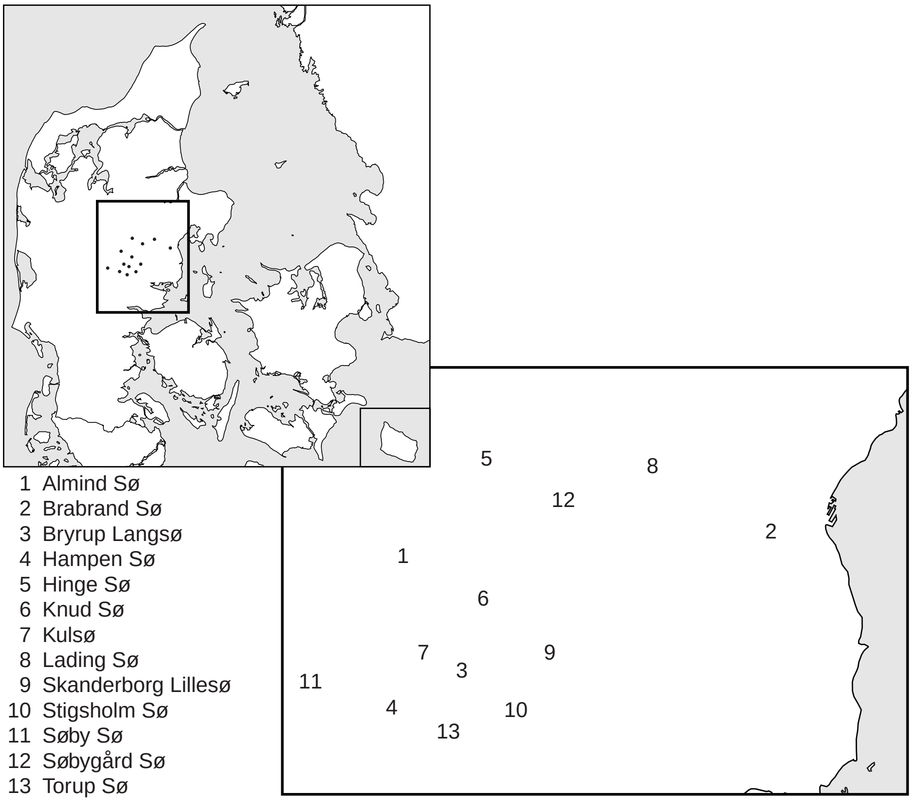

Fig 1 Map of Jylland, Denmark, showing the location of the thirteen lakes investigated in Paper B.

because a whole-community measurement is an integration of all the P-I curves in the mat such differences may not be evident on the community scale. A link between the P-I parameter and assemblage thickness agrees with the general trend described for a range of phototrophic organisms by Enríquez *et al.* (1996). They noted a strong intercorrelation between the P-I parameters and a negative relationship between thickness and P^B_{max} and α^B (per unit carbon) and concluded that these trends reflect broad-scale patterns in the photosynthetic metabolism rather than adaptive responses. The analysis by Enríquez *et al.* (1996) presumably only included pelagic forms of microalgae with a single cell maximum thickness of 100 µm. It was therefore interesting to find the same overall trend for whole-community periphyton counting several algae species within a mat many fold thicker. Hill *et al.* (1995), however, registered shade adaptations on whole-community periphyton. They found that periphyton from heavily shaded sites had a two times greater α than that from open sites. Interestingly, they also registered the highest gross P_{max} at the shade sites, but argued that this could be explained by a possible lack of photoprotective pigments. A possible explanation of the lack of shade adaptation with incubation depth in my study may be that the differences existed at a level below the detection limit of the experiment. If so, such differences had to be detected by finer-scaled techniques or across a larger gradient in light. Revealing and interpreting shade adaptation at the community level is furthermore made complex by potential genetic differences and co-occurrence of several different species with specific response patterns. Although the metabolic parameters of the periphyton did not differ according to the sun-shade theory, the community metabolism changed towards a more heterotrophic community between July and September.

Generally, higher respiration rates rather than changes in P_{max} accounted for this shift. It was suggested that this metabolic shift was mainly caused by a changed community structure and biomass whereas changes in the external physical environment e.g. in temperature was of minor importance. In a study describing monthly changes in the metabolic potential and community dynamics of periphyton during winter and spring (Paper D), I accordingly found a more heterotrophic community at high community densities than at low densities. The metabolic measurements in this study were made under standardised temperature and light conditions, and can therefore not be directly applied to that found at natural condition. However, it is worth noticing that the seasonal changes in the overall community metabolism were mainly caused by changes in P_{max} rather than in respiration. Another striking result was that periphyton grown under ice, and thus presumably adapted to low temperatures and low light intensities, still had a relatively high capacity to respond to the favourable light and temperature conditions offered under the incubations.

Seasonal changes in P-I parameters were also addressed in the study comparing the distribution of primary producers in a nutrient-rich, turbid lake to that in a less nutrient-rich, clear lake (Paper A). In each of the two lakes the P-I responses for phytoplankton and epipelon were determined 7 times in one year within a defined area with a water depth of 0.5 m. We found that P^A_{max} of the microalgal communities changed more than an order of magnitude over the year from maximum rates in spring-summer to minimum rates in winter (Fig 2). Epipelic P^B_{max} and α^B were also higher in summer than in winter, while P^B_{max} and α^B for phytoplankton did not always change with season. The productive capacity of the two communities furthermore differed substantially between the lakes. In the turbid lake phytoplankton had a much higher P^A_{max} than epipelon, while the epipelon had the highest P^A_{max} in the clear lake (Fig 2). P^A_{max} and α^A of the dominant algal community in each lake were inter-correlated and correlated to water temperature and incident light intensity. A direct correlation to light and temperature were, however, not seen for the subdominant algal communities. Through an interactive effect of stimulation by higher temperatures and depression by higher turbidity in summer, light and temperature may still be the most important regulators of the epipelic P-I parameters in the turbid lake. By contrast, our results suggest that the decoupling of phytoplankton production from light and temperature in the clear lake was due to nutrient limitation or a high grazing rate in summer.

3.1.3 Photoinhibition

The trade-off between incubating at irradiances high enough to saturate whole-community photosynthesis and at the same time low enough to prevent damages on the metabolic apparatus is sometimes problematic when measuring photosynthesis of autotrophs. Although the periphyton communities during measurements of photo-

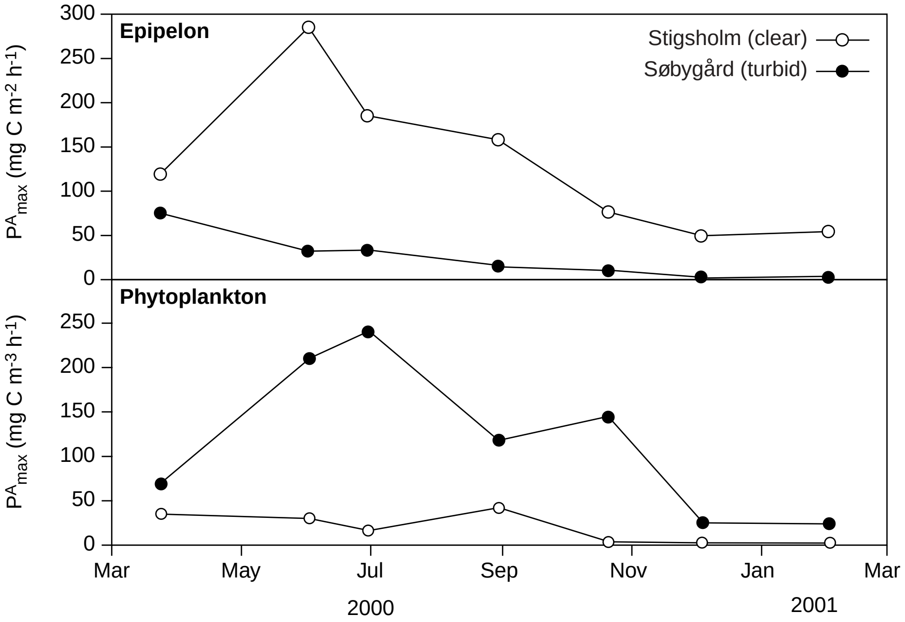

Fig 2 Seasonality in the light saturated productive capacity (P^A_{max}) of the epipelic and pelagic microalgal communities in Lake Stigsholm and Lake Søbygård. P^A_{max} was estimated from laboratory measurements run at *in situ* temperatures and saturating light intensities.

synthesis were occasionally exposed to light intensities higher than they had ever encountered under field conditions, photoinhibition was never observed, neither for epipelon (Paper A) nor for periphyton (Paper B). When working with periphyton, photoinhibition is often referred to as a decrease in photosynthetic rate at irradiances higher than that needed for maximal photosynthesis (Hill, 1996). Photoinhibition of intact periphyton communities is suggested to be a rare phenomenon (Dodds *et al.*, 1999; Uehlinger *et al.*, 2000), and it has almost exclusively been reported during short-term, closed chamber incubations (Meulemans, 1988; Hill & Boston, 1991; Hill *et al.*, 1995). Though photoinhibition may occur in the upper surface of the periphyton mats, a simultaneous stimulation of the photosynthesis in deeper layers of the mat is likely to compensate for and balance the photoinhibitory reduction. Surface layer cells and cells in the subsurface layers may hence receive inhibitory, saturating or subsaturating irradiances and localized photoinhibition can thus be hidden in the whole-community metabolism, because the actual measurement is an integration of many separate P-I curves. The same mechanism is likely to be responsible for the relatively high light intensity that is often needed to saturate photosynthesis of most attached microalgal communities.

Summary - light

Conclusively, the studies of the periphytic P-I relationships demonstrated that both when compared over the season and across an interlake depth scale community P_{max}, α and respiration correlated strongly to algal biomass. When expressed per unit area the correlation was positive, while the correlation was negative when expressed per unit algal biomass. Furthermore, the P-I parameters of the most dominant microalgal producers were intercorrelated and exhibited a distinct seasonal variation, which corresponded to that of light and temperature.

3.2 Regulation by nutrients

Within the context of decades with eutrophication of many lakes, there has been a natural interest focused on how aquatic primary production is related to the available nutrient pool. Enhanced nutrient input tends to favour algae (phytoplankton and attached algae) over vascular plants because algae obtain nutrients primarily from the water column. In addition, microalgae have more rapid rates of nutrient uptake and growth (Enríquez *et al.*, 1996), and their compensation irradiance is generally low (1-10 µmol photons m^{-2} s^{-1}) (Richardson *et al.*, 1983) compared to that of vascular plants (10-100 µmol photons m^{-2} s^{-1})(Duarte, 1995), hence making them better competitors under non-limiting nutrient conditions.

3.2.1 Biomass

The relationship between water chemistry and periphytic algal abundance is far from simple and more complex than that of nutrients and phytoplankton biomass. Phosphorus is generally stressed as being the prime nutrient limiting algal growth in lakes, and experimental as well as field studies focusing on the pelagic habitat have repeatedly found that phytoplankton increases with phosphorus (Schindler, 1978; Elser, Marzolf & Goldman, 1990; Jeppesen *et al.*, 2000). Inconsistent data, however, exist on the relationship between nutrient availability and periphytic algal abundance (Vadeboncoeur, Vander Zanden & Lodge, 2002). Single-lake investigations and experimental nutrient enrichment assays of enclosures often find that periphyton biomass and production are influenced by the nutrient concentration (Cattaneo & Kalff, 1980; Mazumder, Taylor & McQueen, 1989; Vadeboncoeur *et al.*, 2001). By contrast, inter-lake comparisons across a natural trophic gradient frequently report no or weak correlations between periphyton and phosphorus (Hansson, 1988; Lalonde & Downing, 1991; Fairchild & Sherman, 1993). In an inter-lake study of 10 lakes within the TP range from 4 to 72 µg l^{-1}, periphytic algal biomass increased with the TP concentration (Cattaneo, 1987). Accordingly, Lalonde & Downing (1992) found an increased periphyton biomass up to a TP concentration of ~ 39 µg l^{-1}, but at higher TP periphyton decreased. Other inter-lake studies found sediment-associated periphyton to correlate negatively with pelagic phosphorus and explained this by increased light attenuation from dense phytoplankton growth (Hansson, 1988; Vadeboncoeur *et al.*, 2003). A reduction in epiphytic abundance in high nutrient lakes was, however, ascribed to reduced macrophyte density and hence lower availability of host substratum on which to attach (Moss, 1976). Furthermore, a comparative study of epiphyton on *Littorella uniflora* from five lakes with similar TN and TP concentrations demonstrated higher epiphytic densities in silicate-rich lakes than in silicate-poor lakes (Sand-Jensen & Søndergaard, 1981).

To improve the understanding of nutrients as a regulating factor for the distribution and the total quantity of periphyton biomass in shallow lakes, we conducted an inter-lake comparison among thirteen lakes covering a natural summer mean TP gradient from 11 to 536 µg l^{-1} (Paper B). This investigation was conducted by incubating vertically arranged artificial substratum in the littoral vegetation-free zone of the lakes for 8 weeks before measuring biomass (July). To clarify if the overall periphyton density and distribution changed as the community aged, the investigation was repeated in the same lakes after approximately 7 weeks (September). The study showed that lakes with intermediate TP levels (TP_{summer}: 60-200 µg l^{-1}) could potentially accumulate higher periphyton densities in the upper 0-1 m of the water column than lakes with both higher and lower TP levels. In July this unimodal distribution was proposed to represent a shift from nutrient limitation at low TP levels to light limitation at high TP levels, because the vertical distribution of periphyton differed among lakes. In the most nutrient-rich and turbid lakes periphyton decreased with depth, whereas no general distribution pattern was seen among lakes of lower TP (Fig 3). Although most lakes were more turbid in September, 8 of the 13 lakes had a uniform depth distribution of the periphyton. Phosphorus limitation presumably still accounted for the increase in biomass among the low-TP lakes, whereas the decreased biomass at the highest TP levels was associated with a taxonomic shift in the periphytic algal community. In the two most TP-rich lakes, the algal composition had shifted towards dominance of a diatom, *Epithemia* sp., usually characteristic of nitrogen limited habitats (Deyoe, Lowe & Marks, 1992). Yet, equivalent distribution patterns along the inter-lake TP gradient were observed at the two sampling dates, but the key element controlling

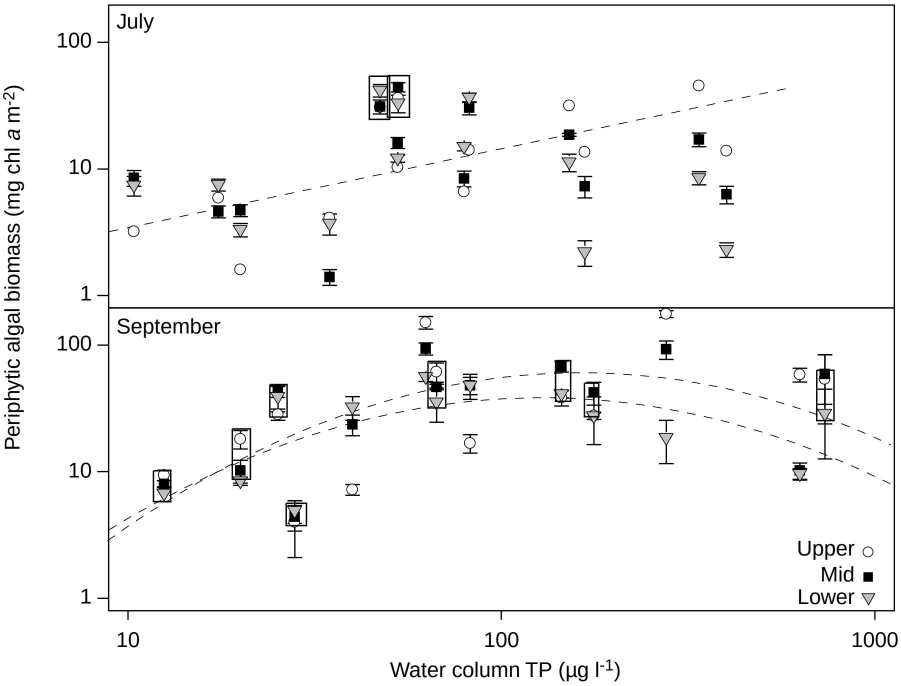

Fig 3 Depth distribution of the periphytic algal biomass (n=5) along an inter-lake (13 lakes) TP concentration gradient in July and September. The biomass was determined 0.1 m (Upper), 0.5 m (Mid) and 0.9 m (Lower) below the surface on communities developed on artificial substrate. Boxes denote lakes were biomass did not significantly differ among depths. Mean (± SE). Significant relationships: July; Upper: $\log y = -0.089 + 0.624 \log x$, $r^2=0.46$, $p=0.011$ (n=13). September; Mid: $\log y = -2.263 + 3.655 \log x – 0.826 (\log x)^2$, $r^2=0.49$, $p=0.034$ (n=13), Lower: $\log y = -1.871 + 3.278 \log x – 0.778 (\log x)^2$, $r^2=0.47$, $p=0.043$ (n=13).

periphyton in the high TP lakes may have changed from light in July to nitrogen in September.

Occasionally, periphyton fails to respond to changes in the external nutrient supply, even when the nutrient concentration in the water column lies within the potentially limiting range (Mulholland *et al.*, 1991) and when no alternative nutrient source could be exploited. Besides being an effect of co-limitation by other resources or an insufficient nutrient change to induce a detectable response in the periphyton, nutrient cycling within the periphyton community is a plausible explanation (Wetzel, 1996). The typical structural composition of periphyton communities where living and dead bacteria and algal cells, detritus and inorganic particles are tightly integrated within a matrix of organic extra-cellular polysaccharides (Lock *et al.*, 1984) facilitates nutrient cycling within the communities, and the nutrient conditions within the periphytic mat have been found to differ considerably from those of the pelagic (Riber & Wetzel, 1987). Internal nutrient cycling mechanisms, however, take time to become established because of the need to build up enough community biomass to assure proximity of processes and build up a considerable boundary-layer to repress mass transfer of remineralized nutrients away from living periphyton cells. It may therefore be assumed that periphytic communities on chemical inert substrata are most sensitive to differences in the pelagic nutrient pool during the establishing phase.

3.2.2 Primary production

For decades studies of primary production in lakes have mainly focused on the pelagic habitat (Vadeboncoeur *et al.*, 2002). It is therefore sometimes implicitly assumed that an inter-lake phosphorus gradient is equivalent to an ecosystem level primary productivity gradient, thereby either ignoring periphytic primary production or assuming it to be proportional to that of phytoplankton. Several studies have, however, emphasised that production by attached microalgal communities can account for a substantial proportion of total lake productivity. For example, Burkholder and Wetzel (1989) estimated that between 70 and 85% of the lake primary productivity in Lawrence Lake could be ascribed to epiphyton, while Allen (1971) found epiphyton to contribute with 24% in a small temperate lake. The proportional distribution among habitats varies greatly with, for instance, lake morphometry, sediment characters, photic zone and lake trophy. Accordingly, an analysis of estimates of whole-lake primary production from 27 lakes varying in size and trophy showed that periphytic algae accounted for 1-92% (median 43%) of whole-lake production (Vadeboncoeur & Steinman, 2002). A general model on the relationship between lake trophy and periphytic production has been proposed by Sand-Jensen & Borum (1991), suggesting that periphyton is favoured in nutrient-poor lakes when having access to the nutrient pool in the substrata (e.g. from the sediment). Increased shading by phytoplankton will reduce the periphytic production along a trophic gradient and in nutrient-rich lakes the production will be almost exclusively pelagic. Due to seasonal changes in turbidity, the absolute and relative distribution of primary producers among habitats can also be expected to change in relation to lake trophy, these seasonal changes have, however, only rarely been addressed or empirically investigated.

In Paper A, we aimed to study how the relative distribution of primary producers among habitats changed in relation to lake trophy and over the season. This was done by measuring phytoplankton and epipelic microalgal production 7 times over an annual cycle in the same two 0.5 m deep areas located in clearwater Lake Stigsholm (mean TP_{annual} = 97 µg l^{-1}) and in turbid Lake Søbygård (mean TP_{annual}= 274 µg l^{-1}), respectively. In accordance with the model by Sand-Jensen & Borum (1991) epipelic microalgae were the dominant primary producers in clear Lake Stigsholm (77%), whereas almost all primary production occurred in the water column in turbid Lake Søbygård (96%). The seasonal patterns of the proportional distribution among habitats differed considerably between the lakes. In the turbid lake the contribution was higher in winter (11%-25%), when the water was relatively clear, than during summer (0.7%-1.7%), when the water was most turbid. In the clearwater lake, the relative contribution of epipelon was high both in winter, when the water was most clear, and in mid-summer, when phytoplankton production was constrained either by nutrients or grazing. Based on our observations a conceptual model of the seasonal relationship between lake trophy and the relative proportion of benthic production was proposed (Fig 4). With plentiful nutrients (solid line) high phytoplankton production in the water column suppresses the epipelon production throughout the year. A lower phytoplankton production in winter may slightly increase the relative proportion of epipelon production (example: Lake Søbygård). Lakes with poor nutrient supply (dashed line) tend to have a low phytoplankton production, clear water and thus high proportions of epipelon production throughout most of the year. Phytoplankton blooms in spring or autumn may, however, slightly reduce the proportion of epipelic production (example: Lake Stigsholm). At some intermediate nutrient levels (dotted line) lakes can be expected to shift from epipelon dominance in winter to phyto-

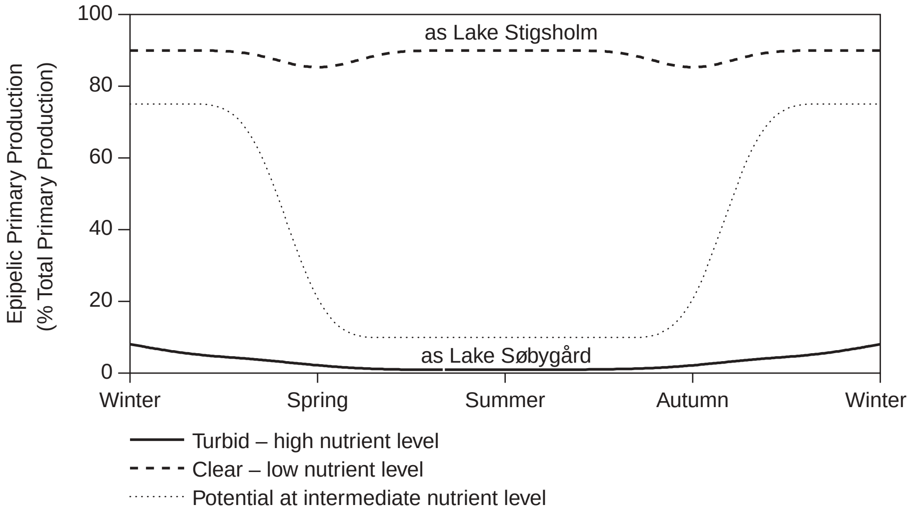

Fig 4 Tentative conceptual model showing the seasonal variation in the relative contribution of epipelic primary production to total primary production in shallow lakes of different nutrient levels and without submerged macrophytes. See text for description of the model.

plankton dominance in summer, because both phytoplankton and epipelon, within certain limits, promote their own existence. Despite the major difference in the proportional distribution of the primary producers among habitats, total annual production was only 34% greater in the turbid than in the clear lake. Hence, rather than finding a proportional increase in primary production with trophy, as seen if phytoplankton is employed as the only predictor for productivity, a redistribution from the attached communities to phytoplankton dampens the overall productive increase. Since total primary production and the relative distribution of the primary producers modulate basic ecosystem properties, shifting production from the benthic to the pelagic habitat may affect energy pathways within the lakes and mediate major changes in both species competition and consumer food web structure (Power, 1992).

Summary - nutrients

The complexity between water chemistry and periphytic algae makes it difficult to draw explicit conclusions on the relationship. However, the result presented here indicates that within the investigated nutrient range eutrophication has a greater effect on the temporal and spatial distribution of periphyton than it has on total primary production within a lake.

3.3 Seasonal regulation

In temperate regions phytoplankton communities usually follow a distinct seasonal pattern (Round, 1971; Reynolds, 1984; Sommer *et al.*, 1986). The phytoplankton biomass is typically low in winter, and as it responds to increasing temperature and light, the biomass increases during spring when the nutrient availability is still high. Small and fast-growing diatom species often dominate this spring bloom (Sommer *et al.*, 1986). In early summer zooplankton grazing or nutrient limitation may reduce the biomass and hence produce a clear water state in the lakes. Usually a second biomass maximum is reached in late summer when the grazing pressure from zooplanktonic species is declines. This peak initially consists of small forms of especially cyanobacteria and green algae. Later in summer large planktonic species capable of avoiding grazing become abundant and, ultimately, diatom abundance increases again with the progress of autumn.

Seasonality of periphyton communities has, by contrast, not yet been investigated so intensively as to allow general patterns to be proposed, but densities varying several orders of magnitudes from one month to the next are typical for many temperate lakes. In accordance to the seasonal patterns of phytoplankton most studies report dominance of diatoms in winter, spring and autumn and a greater abundance of periphytic green algae and cyanobacteria during summer (Gons, 1982; Cattaneo, 1987; Burkholder & Wetzel, 1989; King, Jones & Barker, 2002). Yet, colonisation time and thus the seasonal turnover of submerged surfaces are of importance to the community structure, implying that diatoms often dominate early successional communities, whereas filamentous green algae become increasingly abundant in older communities (Steinman, 1996). As for phytoplankton, increasing light and temperatures encourage periphytic algal growth in spring-summer just as higher grazing rates reduce the abundance. However, as previously stressed dense phytoplankton populations overlaying the periphyton may competitively depress the growth of the attached communities, and consequently interact with the actual resource availability or the turnover rate of the substratum in shaping the seasonal distribution and production rates of periphyton. A depression of sediment-associated periphyton production by phytoplankton was seen in the study of seasonal distribution of primary producers in a clear and a turbid lake (Paper A). In this study epipelon in the turbid lake had a low and almost constant primary production in summer and winter although both temperature and incident light were much higher in summer. Increased phytoplankton production in summer, however, reduced the light availability at the sediment surface and constrained the epipelic production. By contract, the epipelic production increased significantly from winter to summer in the clear lake where summer pelagic production was lower and more light reached the sediment.

Most studies of periphyton dynamics focus on the main growing season from spring to autumn, whereas winter dynamic is often neglected (Sand-Jensen & Søndergaard, 1981; Carpenter & Kitchell, 1987). Several studies have, however, reported that periphytic assemblages are present throughout winter, (Meulemans, 1988; Burkholder & Wetzel, 1989; Gustina & Hoffmann, 2000) but few have explored dynamics and relative changes in these winter communities. I therefore made a study where I monthly followed the temporal and vertical dynamics of periphyton biomass, primary productivity at saturating light and standard temperature, respiration and community composition from January to May in the littoral zone of the eutrophic Danish freshwater Lake Torup (Paper D). In the study two community types developed on a short-time (1 month) and a long-time (3-7 months) incubated substratum were compared at three incubation depths (5-15 cm, 35-45 cm and 65-75 cm below the surface). The long-time incubated community had a very low algal biomass accrual during the ice-covered winter months (Fig 5). The biomass increased rapidly from January to February and attained an almost constant density until April, before a dramatic decline took place in May. Colonisation and growth of algae on the short-time incubated substratum accordingly showed the highest rate of biomass accrual from January to February, whereas the accumulation potential was much lower later in spring. Spring bloom of phytoplankton usually occurs later (March-April) than the timing of peak periphyton accrual observed in this study. The early timing of a high productive capacity may be a major advantage for periphytic algae, because the community may be established prior to any competitive depression from phytoplankton.

A taxonomic shift occurred from dominance of diatoms in all short-time - and the January long-time incubated communities to dominance of chlorophytes in later successive spring communities with

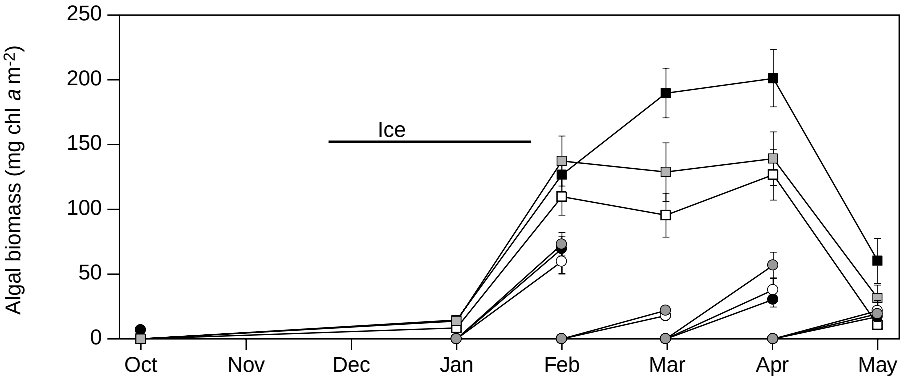

Fig 5 Seasonal algal biomass accrual rates 5-15 cm (solid symbols), 35-45 cm (grey symbols) and 65-75 cm (open symbols) down the water column in two periphytic communities (n=12)(± SE). The total-incubated (square) community was incubated from October to sampling (3-7 months), while the interval-incubated (circle) community was incubated for 1 month exactly. A vertical bar shows the ice-covered period.

high algal biomass. Whereas biomass of the diatom-dominated communities was equally distributed vertically, it frequently decreased with depth in those dominated by chlorophytes, indicating that different regulation mechanisms controlled the two communities.

Indistinct seasonal regulation patterns of periphyton may furthermore arise because of sloughing. A sudden dramatic decline in the periphyton biomass sometimes occurs for thick and old communities because the algae mat deteriorates and sloughs from the substrate. During sloughing large patches of the periphyton mat may be lifted and eventually detached from the substratum, a process often involving underlain gas bubbles created under highly productive conditions. Moreover, senescence and death of algae in the lower layer of the mat reduces the resistance of the periphyton to shear stress and grazer-induced dislodgement. The density at which the community deteriorate or the physical and productive conditions under which sloughing happens differ greatly. Experimental studies have shown that low-growing and tightly-adhering diatom taxa are much more resistant to sloughing than taller-growing filamentous green algae (Peterson & Stevenson, 1992). Additionally, changes in resistance may be associated with seasonal changes in, for instance, the light regime. Biggs *et al.* (1999) found that a reduction in maximum daily light levels to < 400 µmol m^{-2} s^{-1} caused a significant increase in the sloughing resistance of a diatom-dominated periphyton community. This occurred through a change in physiology and physical structure of the mat rather than through taxonomic changes of the algal community. Although I have no direct evidence or visual observations of sloughing in my studies, it could have been responsible for the dramatic biomass declines observed from April to May in the study of winter dynamics (Fig 5)(Paper D). However, increased grazing rates cannot be rejected as being partly responsible for the decline.

Summary - seasonal regulation

In winter, spring and autumn, periphyton is often dominated by diatoms, whereas a greater abundance of green algae and cyanobacteria is typically found in summer. As for biomass, this taxonomic composition is, however, strongly dependent on colonisation time. Periphytic algae are generally stimulated by the same resources as pelagic algae, but may demonstrate different seasonal patterns because phytoplankton may intercept light and hence alter the light availability at the substratum surface relative to that in the water col umn. Although developed at low temperature and low light, winter communities still have high productive capacities, and relatively high periphytic biomass densities may accordingly develop during winter.

3.4 Regulation by top-down and bottom-up forces

Like other primary producer communities, periphyton may be controlled both by growth limiting resources (bottom-up forces) and via consumption by herbivores (top-down forces). The previous sections have mainly focused on regulation of periphyton by resources, but grazer-control may also be of vital importance for lentic periphyton (reviewed by Jones *et al.*, 1997 and Steinman, 1996).

3.4.1 Grazing

Grazing is usually based on differences in biomass after experimental enclosure or exclosure of grazers, and is thus measured as the net effect of grazers. More correctly such estimates should be defined as ’removal rates’ to indicate that they comprises of both ingestion and loss of material due to grazer activity within or on the surface of the periphyton mat. However, to allow consensus with most research papers I will use the term grazing to describe effects of grazer communities on periphyton.

Periphyton standing crop typically decreases with increased grazing pressure (Feminella, 1995). Yet, low or moderate grazing may occasionally increase the biomass and stimulate area-specific production, by shifting the taxonomic composition of algal community toward more productive species or by improving the nutrient supply to the individual algal cells (Steinman, 1996). In a review of the literature, Cattaneo & Mousseau (1995) found that periphyton grazing rates increased with grazer body mass and food availability and decreased with grazer abundance, and that these parameters explained 65%, 7% and 6% of the variation in grazing rates, respectively. Additionally, they found that grazing rate, corrected for body size, was similar among all grazer taxa expect from amphibians, which tended to have lower rates.

3.4.2 Bottom-up versus top-down regulation

Traditionally, most studies have considered either the importance of resource-mediated or consumer-mediated regulation (Lamberti, 1996) but as stressed by several investigations bottom-up and top-down forces may act simultaneously on primary producer communities (Carpenter *et al.*, 1987; Hansson, 1992; Rosemond, Mulholland & Elwood, 1993; McCollum, Crowder & McCollum, 1998). One approach to explore the conditions under which top-down and bottom-up forces operate and interacts is to experimentally manipulate resources and consumers simultaneously in factorial investigations. Rosemond *et al.* (1993) used this approach to study effects of nutrients and snail grazing on periphyton. They found that both biomass and productivity of periphyton were stimulated by nutrients and reduced by grazing, but also that the top-down and bottom-up effects, alone, were less important than their combined effects. Seasonal changes in the relative importance of resources and grazing in controlling periphyton have also been reported (Rosemond, Mulholland & Brawley, 2000). By investigations of the relative response of periphyton biomass to single- and combined-factor manipulations of light, nutrients and snail grazing, Rosemond *et al.* (2000) concluded that nutrients were the most important factor constraining stream periphyton in spring while grazing was more important in summer and fall. In general, many factorial two trophic level (periphyton-grazers) experiments manipulating resources and consumers simultaneously have shown that periphyton productivity, is ultimately bottom-up controlled, but that top-down forces exerts proximate control over the standing periphyton biomass (Cuker, 1983; Steinman, Mulholland & Kirschtel, 1991; Hill, Boston & Steinman, 1992).

Experiments involving three trophic levels (fish-grazers-periphyton) often report that fish may stimulate periphyton either by increasing available nutrients via nutrient recycling or by indirect cascading effects via a negative impact on the grazer community (Mazumder *et al.*, 1989; McCormick & Stevenson, 1989; McCollum *et al.*, 1998; Biggs *et al.*, 2000; Flecker *et al.*, 2002). In a classical experiment on fish-snail-periphyton interactions Brönmark *et al.* (1992) found that predation by pumpkinseed sunfish reduced the snail biomass and thus indirectly stimulated periphyton by reducing the grazing pressure. However, McCollum *et al.* (1998) reported that although fish had only visual and olfactory contact with snails, their presence negatively affected snail life-history traits and their grazing activity so that twice as many snails were produced in the absence of fish. Species-specific feeding behaviour and predation strategies of fish (Biggs *et al.*, 2000) and the structural composition of the grazer and periphyton communities may furthermore be of vital importance for the cascading regulation mechanisms in the three trophic level food webs.

To explore the interaction of fish-mediated top-down regulation and light-mediated bottom-up regulation of enclosure wall periphyton and the associated macro-grazer community, we conducted simultaneous long-term (October-August) enclosure experiments in clear Lake Stigsholm and turbid Lake Søbygård (Paper C). The two lakes had structurally different grazer communities; the clear lake being dominates by snails and *Asellus aquaticus* and the turbid by chironomids and ostracods. A factorial set of treatments involving presence-absence of fish (roach; *Rutilus rutilus*) and four light levels (100%, 55%, 7% and <1% of the incoming light) were applied to 24 enclosures in each lake.

A light reduction strongly reduced the primary producers biomass at the most shaded treatments (Fig 6), whereas a 45% reduction of light only reduced periphyton in winter. Although lower periphyton densities and thus impoverished food conditions for the benthic grazers were found in the most shaded enclosures, light had no overall effect on the total biomass of grazers. However, snail breeding was delayed in the most shaded and periphyton-poor enclosures relative to that in the enclosures with better light conditions and superior food resources. Furthermore the abundance of chironomids in the turbid lake in summer was also positively correlated to the periphyton biomass, and hence indirectly stimulated by high light conditions. In accordance to a simple model of cascading trophic effects fish significantly reduced the total biomass of benthic grazers, thereby indirectly increasing the biomass of periphytic algae (Fig 6). The fish reduced the biomass and abundance of snails in the clear lake, and of ostracods in the turbid lake, but had no effect on chironomids in the turbid lake. Fish may have reduced the benthic grazer community both directly through consumption and indirectly by inducing a shift in habitat. Our results implied that the snails in the clear lake, at least partly, were reduced by indirect mechanisms, because both adult and juvenile snails were reduced, and only the later was assume to be within the size range vulnerable to predation.

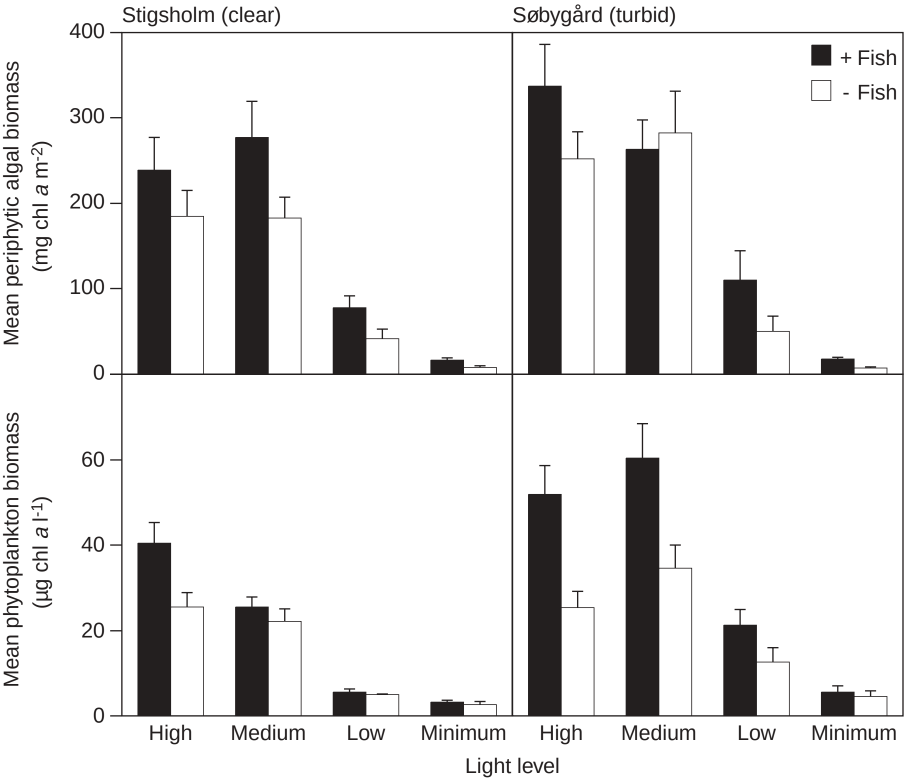

Fig 6 Mean (± SE) biomass of periphytic algae (November-August) and phytoplankton (October-August) from light (high, medium, low and minimum light levels) and fish (+ fish and - fish) manipulated enclosures in clear Lake Stigsholm and turbid Lake Søbygård.

Summary - top-down and bottom-up forces

We found that light-mediated bottom-up and fish-mediated top-down forces strongly controlled the biomass of periphyton. By contrast, the light- and fish-mediated effects on the benthic macrograzers were highly dependent on the structural composition of the community.

4 Concluding remarks

The importance of periphytic communities as primary producers and energy source to food webs and as fundamental components in biogeochemical cycles of many, if not most, shallow freshwater lakes makes regulation, ecology, ecophysiology and productivity of periphyton vital areas of research in aquatic ecology. In the investigations presented in this thesis I employed different approaches and perspectives to the studies of periphyton in shallow freshwater lakes.

Paper A focuses on seasonality of the total microalgal production and its relative distribution between the benthic and the pelagic habitat in shallow lakes of contrasting nutrient availability and water clarity. This study showed that epipelic microalgae were the dominant primary producers in the clear lake, whereas phytoplankton accounted for most of the production in the turbid lake. The turbid lake had a slightly higher contribution of benthic production in winter, while the clear lake had high benthic contributions in both winter and summer. A conceptual model of the seasonal variation in the relative contribution of epipelic primary production to total primary production in shallow lakes of different nutrient levels was proposed.

In **Paper B** the relationship between the pelagic nutrient concentration and the biomass, production and vertical distribution of periphyton on a chemical inert substratum was studies across an interlake phosphorus gradient. From this study it was concluded that lakes of intermediate nutrient concentrations had the highest potential for periphytic growth and that the metabolic parameters of the periphyton was not adapted systematically to incubation depth.

Simultaneous manipulation of fish and light in enclosure experiments, conducted in parallel in two lakes with different structural compositions of the benthic grazer communities, was used in **Paper C** to study bottom-up and top-down regulation of periphyton and the periphyton associated grazers. The general conclusion of the experiment was that both the light-mediated bottom-up and the fish-mediated top-down forces controlled the biomass of periphyton, while the reductive impact of the two factors on the benthic grazer community was highly dependent on the structural composition of the community.

Winter-spring dynamics of periphyton was addressed in **Paper D.** This study found that although the light and temperature conditions are usually sub-optimal for algal growth in winter, the periphytic algal may still possess a high potential for growth and production.

Although the research on periphyton has been intensified over the past decades, there is still an essential need for more information within several areas. Relatively few detailed analyses of the periphytic energy budget or energy flow through food webs have been established for whole-lake ecosystems. Existing data imply that periphyton may be an important food resource for both benthic and pelagic food webs, but the general understanding of how the total primary production and the distribution of production between the benthic and pelagic habitat affects food web interactions is still poor. The conditions under which resource- and consumer-control interacts and effects periphyton and periphyton-based food webs are also poorly understood, and could be useful tools in predicting food web dynamics in complex environments. Basic knowledge is also needed on the importance and impact of microconsumers in affecting the production and standing biomass of periphyton in natural ecosystems.

A complete outline of the numerous information still missing on production and regulation of the periphytic communities, will be too extensive for this synopsis, but there are plenty of questions just waiting for an answer.

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PAPER A (for full text, please see: http://www.blackwellpublishing.com)

Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and a turbid shallow lake

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Keywords: benthic-pelagic links, eutrophication, periphyton, P-I parameters, primary productivity

SUMMARY

1. Pelagic and epipelic microalgal production were measured over a year in a pre-defined area (depth 0.5 m) in each of two lakes, one turbid and one with clear water. Further estimates of epiphytic production within reed stands were obtained by measuring production of periphyton developed on artificial substrata.
2. Total annual production of phytoplankton and epipelon was 34% greater in the turbid lake (190 g C m^2 year^1) than in the clearwater lake (141 g C m^2 year^1). However, the ratio of total production to mean water column TP concentration was two fold greater in the clearwater lake.
3. Phytoplankton accounted for the majority of the annual production (96%) in the turbid lake, while epipelic microalgal production dominated (77%) in the clear lake. The relative contribution of epipelic algae varied over the year, however, and in the turbid lake was higher in winter (11-25%), when the water was relatively clear, than during summer (0.7-1.7%), when the water was more turbid. In the clearwater lake, the relative contribution of epipelon was high both in winter, when the water was most clear, and in mid-summer, when phytoplankton production was constrained either by nutrients or grazing.
4. Compared with pelagic and epipelic primary production, epiphytic production within a reed stand was low and did not vary significantly between the lakes.
5. The study supports the theory of a competitive and compensatory trade-off between primary producers in lakes with contrasting nutrient concentrations, resulting in relatively small differences in overall production between clear and turbid lakes when integrating over the season and over different habitats.

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PAPER B (*in preparation*)

Structure, biomass, production and depth distribution of periphyton on artificial substratum in shallow lakes with contrasting nutrient concentrations

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Key words: Primary productivity, eutrophication, P-I parameters

Abstract

To examine how the vertical distribution of periphytic biomass and primary production in the upper 0-1 m of the water column changes along an inter-lake eutrophication gradient, artificial substratum (plastic strips) was introduced into the littoral zone of thirteen lakes covering a total phosphorus (TP) summer mean range from 11 to 536 µg l^{-1}. Periphyton was harvested in July (after 8 weeks) and September (after 15 weeks) at three water depths (0.1, 0.5 and 0.9 m). Periphyton biomass assayed as both chlorophyll *a* concentration and dry weight generally increased with time and, concurrently, the communities became more heterotrophic. Mean periphytic biomass was unimodally related to the pelagic TP concentration, and maximum biomass developed in lakes within the TP range: 60 – 200 µg l^{-1}. A shift from nutrient to light limitation seemed to occur along the inter-lake TP gradient in July, whereas nitrogen control may have been important in the most nutrient-rich lakes in September. Community respiration ®, maximum light-saturated photosynthetic rate (P_{max}) and primary production were more tightly related to periphyton biomass than to TP. Biomass-specific rates of R, P_{max} and production declined with increasing biomass, and depth had no overall effect on this relationship. R was higher in September than in July, whereas gross production did not differ systematically between dates. Mean net periphyton production (24 hours) was positive in most lakes in July (range: –1.8 –2.4 µmol O_2 cm^{-2} d^{-1}), and negative in all lakes in September (range: –4.7- –0.7 µmol O_2 cm^{-2} d^{-1}). Net production was not related to the inter-lake TP gradient in July, but decreased with TP in September. The relative proportion of diatoms decreased from July to September. A taxonomic shift occurred from biovolumic dominance of diatoms and cyanobacteria at low TP levels to dominance of chlorophytes at intermediate-TP levels and diatoms (*Epithemia* sp.) in the two most TP-rich lakes. The results stressed that the nutrient concentration alone is a poor predictor of the standing biomass and production of periphyton.

PAPER C (*submitted*)

Periphyton-macroinvertebrates interactions in light and fish manipulated enclosures in a clear and a turbid shallow lake

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Keywords: algae, herbivory, grazing, top-down and bottom up effects.

Abstract

1. In a clear and a turbid freshwater lake phytoplankton, periphytic algal biomass and periphyton-associated macro-grazers were followed synchronously from October to August in enclosure experiments with presence-absence of fish (*Rutilus rutilus*) and four light levels (100%, 55%, 7% and <1% of the incoming light).

2. Biomass of phytoplankton and periphyton on enclosure walls were generally higher in the turbid than in the clear lake, and fish and light availability affected the biomass in both lakes. A 93% and >99% reduction of light reduced the biomass of primary producers throughout the experiment, while a 45% light reduction only reduced periphyton during winter. Fish had a positive affect on the biomass of primary producers.

3. The total biomass of benthic grazing invertebrates was higher in the clear than in the turbid lake. Snails and *Asellus aquaticus* dominated in the clear lake, while chironomids and ostracods were most abundant in the turbid lake. Light reduction had no effect on the total biomass of benthic grazers in the clear lake. However, snail breeding was delayed in the most shaded and periphyton-poor enclosures compared to the enclosures with better light conditions and superior food resources. Total biomass of grazing invertebrates and the number of snails on the enclosure walls in the clear lake were lowest in enclosures with fish.

4. In the turbid lake, the overall effect of light differed between grazers. Ostracods were not influenced by light, but they were most numerous in fish-free enclosures. The abundance of chironomid larvae correlated to the periphyton biomass in summer, while fish had no effect on the chironomids.

5. The light-mediated bottom-up regulation of primary producers was generally stronger in the turbid than in the clear lake, but the light regulation did not unambiguously cascade to the primary

consumer level. Top-down regulation of the benthic grazer community was stronger in the clear lake than in the turbid lake, and in both lakes strong cascading effects on periphyton were seen. In conclusion, both light-mediated bottom-up and fish-mediated top-down forces strongly controlled the biomass of periphyton, while the reductive impact of the two factors on the benthic grazer community was highly dependent on the structural composition of the community.

PAPER D (*in preparation)*

Periphyton production and biomass dynamics in a shallow lake during winter and spring

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Keywords: Attached algae, winter dynamics, light, biomass accrual, respiration, metabolism

Abstract

Abundance, depth distribution, productivity and respiration of lentic periphyton grown on short-time and long-time incubated substratum were recorded monthly during the winter-spring (January – May) transition. Algal biomass accrual rate was low during the ice-covered winter months (4.2 mg chl *a* m^{-2} $month^{-1}$), but increased and reached a maximum of 112.0 mg chl *a* m^{-2} $month^{-1}$ in February. During February-April, the biomass remained constant (~ 95-130 mg chl *a* m^{-2}) before declining significantly in May. A taxonomic shift was observed, from dominance of diatoms during the ice-covered period and of short-time incubated periphytic communities to dominance of chlorophytes in later successive spring communities with high algal biomass. From winter (January-February) to spring (March-May), the long-time incubated chlorophyte-dominated community shifted from equal distribution in the water column to higher algal biomass near the water surface than near the sediment. Such a shift in depth dependency was not repeated by the short-time incubated diatom-dominated community, suggesting different regulation mechanisms of the two communities. The ratio of productivity to respiration was employed as a community heterotrophy index. Like the chlorophyll-specific productivity, this ratio was negatively correlated with community biomass, the ratio being high in January (2.20 mol C mol O_2^{-1}) and May (1.85 mol C mol O_2^{-1}) and low in March-April (0.78 mol C mol $O_2^{-1}$and 0.44 mol C mol O_2^{-1}, respectively). These metabolic ratios suggest that, opposed to both the January and May communities, the March-April communities did not exhibit positive net organic accumulation. In conclusion, this study demonstrates the existence of high productive capacities of winter periphyton, which may result in the accumulation of relatively high periphytic biomass densities. The occurrence of such high periphyton densities was also recorded in this study.

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