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dissertation entitled

OXYGEN DYNAMICS IN PERIPHYTON COMMUNITIES
AND ASSOCIATED EFFECTS ON
PHOSPHORUS RELEASE FROM LAKE SEDIMENTS

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Zoology

Major professor

Date 12 September 1986

OXYGEN DYNAMICS IN PERIPHYTON COMMUNITIES AND ASSOCIATED EFFECTS ON PHOSPHORUS RELEASE FROM LAKE SEDIMENTS

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

1986

ABSTRACT

OXYGEN DYNAMICS IN PERIPHYTON COMMUNITIES
AND ASSOCIATED EFFECTS ON
PHOSPHORUS RELEASE FROM LAKE SEDIMENTS

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Periphyton is typically a heterogeneous assemblage of filamentous and single celled photoautotrophic and heterotrophic microorganisms suspended in a mucopolysaccharide matrix which they produce. By definition, the assemblage is attached to a substratum such as rock, sediment, or plant in an aquatic environment. Periphyton communities are generally thin (< 1 mm thick) and, when exposed to sufficient light and nutrients, very productive. Therefore, microtechniques with high spatial and temporal resolution are required in order to define metabolic interactions among the heterotrophic and autotrophic constituents, and between periphyton and its environment.

This study used oxygen sensitive microelectrodes with tip diameters of < 30 μm to investigate the effects of photosynthesis and respiration on the oxygen dynamics of several diverse periphyton communities both in situ and in laboratory microcosms. A novel flow-through system that utilized ^{32}P radiotracer and that permitted manipulation of the velocity, flushing rate, and oxygen concentration of overlying water was developed to investigate the role of photosynthetic oxygen production on

the phosphorus dynamics in lake sediments colonized by epipelic periphyton.

Direct measurements with oxygen microelectrodes demonstrated that the distribution of dissolved oxygen in periphyton communities varied diurnally and was markedly different among periphyton types. During illumination, photosynthesis in periphyton resulted in oxygen supersaturation in microzones that were subsaturated or anoxic during darkness. The fate of oxygen produced within periphyton depended on the relative rates of production and consumption, the diffusion characteristics of the periphyton, physical and chemical interactions with the substratum, and the transport rate across the boundary layer, which was affected significantly by water currents. During constant environmental conditions, steady state equilibria of oxygen distribution occurred within periphyton, but seldom was equilibrium with the surrounding water found.

Data obtained with the flow-through system showed that epipelic algae mediated the release of phosphorus from lake sediments to overlying water via the daily formation and breakdown of the oxidized microzone. During daylight, the surficial sediments became oxidized, and some of the phosphorus diffusing from deeper sediment layers was trapped. During darkness the microzone became anoxic and phosphorus was released at an accelerated rate. The short-term result was a diel fluctuation in the efflux rate of phosphorus. More importantly, however, over the period of weeks to months, the daily photosynthetic activity of epipelic periphyton resulted in the conservation of phosphorus in the sediments.

For Pam...

who taught me love and inspired me to do better.

I am deeply grateful to Dr. Robert G. Wetzel for being given the opportunity to pursue my goals as a limnologist. His guidance and encouragement, blended with his tolerance and patience, made my graduate research a productive and pleasurable experience. My colleagues Richard Losee, William Taylor, and JoAnn Burkholder were of great assistance in many phases of my research, especially in the field work. Dr. Michael J. Klug was very generous with his laboratory facilities and equipment, and provided excellent constructive criticism, both for my research and for my dissertation. I am thankful to my other committee members, Dr. William E. Cooper and Dr. Thomas R. Burton, for their contributions to my graduate program. Dr. Robert Moeller was helpful in field and lab work and contributed ideas which were instrumental in the formation of my hypotheses and proposed research. Dr. Michael Coveny was always ready with quick, but thoughtful, answers to my many questions. Last, but not least I want to thank Anita Johnson for her smile, her sense of humor, and her friendship.

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INTRODUCTION

Periphyton is typically a heterogeneous assemblage of filamentous and single celled photoautotrophic and heterotrophic microorganisms suspended in a mucopolysaccharide matrix which they produce. By definition, the assemblage is attached to a substratum in an aquatic habitat. Periphyton communities are commonly found attached to sediments (epipelic), rocks (epilithic), plants (epiphytic), and animals (epizoic). The biological diversity of periphyton communities and the variety of habitats in which periphyton occurs are well documented. The relationships among the microbial constituents of periphyton, and between periphyton and its environment are, however, poorly studied. Recent advances in microelectrode technology together with novel uses of isotope tracer methods are enabling detailed investigations of the metabolism of periphyton and of the role of periphyton in the nutrient dynamics of aquatic ecosystems.

Dominant features of the physical structure of periphyton communities that vary greatly within and among periphyton types include the thickness of the layer, the ratio of living to dead and inorganic material in the layer, the nature of the substratum, and the extent of development of the matrix. Each characteristic has an effect on the diffusive properties of the periphyton layer. The relevance lies in the fact that the transfer of solutes within periphyton occurs by diffusion in the interstitial water. Because diffusion is a slow process,

wherever the rates of production and consumption of a compound are not balanced, the compound will either accumulate or become depleted; this is important because diffusion and uptake rates of solutes and substrates are concentration dependent.

Before the development of microelectrodes for environmental research, knowledge of the dynamics of oxygen, pH, sulfide and other compounds in periphyton and sediments was based on changes measured in the adjacent aqueous mileau. For example, a rock or small plant with attached periphyton would be placed in a chamber and incubated for a time, during which the concentrations of various solutes would be periodically measured. However, within the periphyton layer, some of the oxygen that is produced photosynthetically is consumed by heterotrophs, and some of the carbon dioxide produced by microbial respiration is consumed by autotrophs. Furthermore, chemical equilibrium seldom exists between the interstitial water of periphyton and the adjacent aqueous environment. Therefore, measurements made in the water surrounding a periphyton community only provide approximations of the metabolic activity of the community. As a result of photosynthesis in periphyton, oxygen can accumulate to concentrations far exceeding atmospheric equilibrium. During darkness the same microzones become anoxic because the rate of diffusion of oxygen into the layer from the overlying water is exceeded by the oxygen demand of the community. The implications for microbial metabolism in periphyton are manifold, but few studies of these phenomena exist.

The productivity of most freshwater ecosystems in North America is limited by the availability of phosphorus. Both the concentration and the regeneration rate of phosphorus in a lake are crucial to the primary

productivity of the system. In many lakes the regeneration and subsequent release of phosphorus from the sediments (internal loading) is a major fraction of the annual loading of phosphorus to the water column. Numerous studies concerned with eutrophication reversal and lake restoration have addressed the phenomenon with the goal of finding ways to reduce the efflux of phosphorus from the sediment. When the bottom water of a lake becomes anoxic the oxidized microzone at the sediment-water interface becomes chemically reduced. The reduction of phosphorus-containing Fe (III) compounds, such as iron hydroxyphosphate, a precursor to hydroxyapatite, releases the phosphate which does not precipitate with Fe (II). The result is a marked increase in the rate of phosphorus release to the overlying water. Measurements with oxygen microelectrodes have demonstrated, however, that sediments colonized by epipelic algae are, on the average, more intensely oxidized than sediments not receiving sufficient light for epipelic algal photosynthesis. Therefore, a primary goal of this study was to define the oxygen dynamics of lake sediments on a diel basis and determine the effect of epipelic algal photosynthesis on the efflux of phosphorus to the water column.

CHAPTER I

DISTRIBUTIONS AND FATES OF OXYGEN IN PERIPHYTON COMMUNITIES

INTRODUCTION

The biological diversity of periphyton communities and the variety of habitats in which epiphytic, epilithic, epipelic, and epizoic periphyton occur are well known (Lund 1942; Hutchinson 1975; Round 1981; Wetzel 1983). In recent years the relationships among the constituents of periphyton, and between periphyton and its environment have received increased attention (e.g., Allen 1971; Gruendling 1971; Kairesalo 1977; Gons 1982; Björk-Ramberg 1983; Cuker 1983; Losee and Wetzel 1983; Reuter et al. 1986). Because periphyton is a heterogeneous assemblage of autotrophic and heterotrophic organisms, investigation of the internal metabolism of the community is difficult. Before the development of oxygen microelectrodes for environmental research (reviewed by Revsbech and Jørgensen 1986), knowledge of oxygen production and consumption in periphyton communities was based on measurements made in the surrounding aqueous milieu (e.g., Hunding 1973; Schindler et al. 1973). However, Revsbech and Jørgensen (1983) and Lindeboom et al. (1985) showed that such measurements underestimate oxygen production and consumption in periphyton communities, because chemical equilibrium seldom exists between the interstitial water of the periphyton and the adjacent aqueous environment. Results from measurements with oxygen microelectrodes in investigations of periphyton have demonstrated that the distribution and fates of oxygen in periphyton communities are controlled by four dominant factors: i) the extent of photosynthetic oxygen production, ii) the diffusion characteristics of the microenvironment, which are governed by water currents and the physical structure of the periphyton community, iii) physical interactions with the substratum on which the periphyton grows, and iv) respiratory

activity of the microflora within the periphyton and its microenvironment.

METHODS

Microelectrodes

Two types of polarographic oxygen sensitive microelectrode were constructed for this study. The simpler electrode (the design of Revsbech 1983) consisted of a fine platinum wire encased in glass and gold plated at the exposed tip (cathode surface). Cathode diameters ranged from 2 to 10 µm with overall electrode diameters of 7 to 20 µm in the sensing region. Construction and use are further described in Jørgensen et al. (1979) and Revsbech et al. (1981). This cathode-type (Revsbech and Jørgensen 1986) of oxygen microelectrode requires a separate reference electrode (anode), which must be immersed in the same fluid. Two common characteristics of polarographic sensors are i) they drift for at least a few minutes after the polarizing voltage is first applied, and ii) their calibration changes unpredictively if the couple becomes depolarized, which occurs when electrical continuity is not maintained within the solution between the cathode and the anode. Calibration of the electrode couple must therefore be made in the same fluid body where the measurements are to be made.

The second type of electrode consisted of the same basic components discussed above except that the cathode and anode are encased within an electrolyte-filled, glass housing with a tip diameter ranging from 15 to 40 µm (Revsbech and Ward 1983). This micro-Clark style of electrode maintains constant electrical continuity between the cathode and anode via the electrolyte solution. Therefore, the electrode can be moved

from one fluid to another without depolarization, which permits calibration and experimental measurements to be made in numerous solutions. However, because O_2 must diffuse through a membrane and then through ca. 5 μm of electrolyte, the response time is slower than the cathode-type of electrode.

Calibration of the cathode-type of electrode was necessarily accomplished in the same solution where the measurements were made. This calibration required determining electrode response (picoamperes/$[O_2]$) at two known oxygen concentrations in order to establish the slope and y-intercept of the linear calibration curve. The chemical microsensor (Transidyne General, Ann Arbor, Michigan) used supplied the polarizing voltage of -0.75 V and was calibrated to read out in percent saturation (percent of atmospheric equilibrium concentration). The meter was set to read 100% when the electrode tip was positioned ca. 100 μm below the air-water interface. Zero percent was taken as the asymptotic minimum of microprofiles measured in known anoxic media, such as sediments (Revsbech and Jørgensen 1983).

The micro-Clark electrode allowed more versatility for experimental use and in calibration options. The meter was calibrated at 0 and 100 percent of air-saturation while the electrode was positioned in either nitrogen-saturated or air-saturated water, respectively, at temperatures equal to the experimental systems. This capability was necessary when measurements were to be made in systems where at least two known oxygen concentrations did not occur (essentially all periphyton assemblages except epipelic). Therefore, many of the data from epipelic communities were obtained with the cathode-type of electrode and most other measurements were made with the micro-Clark style.

Aside from breakage during use, the electrodes had a typical life span of ca. 1 to 2 months; therefore, numerous electrodes were used during this study. The electrodes were positioned in the laboratory with micromanipulators, which permitted accurate movement and alignment with a resolution of < 50 µm. A waterproofed fiberscope (American Optical) was used for initial alignment of the electrode tip at an interface appropriate for the periphyton under study (Carlton and Wetzel 1985). Readings were made at 0.2-mm intervals (except above the sediment-water interface); all microprofiles shown are means of duplicate determinations. The high reproducibility of microprofile measurements has been demonstrated by Jørgensen and Revsbech (1985). Percent saturation values were converted to oxygen concentration ($[O_2]$) as micromoles O_2 l^{-1} to simplify comparison of microprofiles measured at different temperatures. All light intensity measurements were made with a LiCor model LI-185 meter with a cosine corrected quantum sensor (Lambda Instruments).

Epiphytic Periphyton

Periphyton attached to submersed aquatic plants was the most difficult to handle. Extreme care had to be taken during sampling and laboratory handling in order to ensure the structural integrity of the epiphyte-macrophyte complex. Samples of Potamogeton illinoensis and Scirpus subterminalis with representative midsummer epiphytic periphyton development were retrieved using SCUBA from the littoral of Lawrence Lake, Michigan (described in Rich et al. 1971). The samples were maintained in aerated, 17°C-lake water for < 4 days before measurements were made. The plants were exposed to 40-80 µEinst $m^{-2}s^{-1}$ of

photosynthetically active radiation (PAR) on a 12:12 LD cycle. An identical mecury vapor lamp, which could be raised or lowered to alter intensity, was used for illumination during microelectrode measurements on these samples.

Excised leaves were transferred without emersion to a shallow pan and securely suspended between two rubber stoppers in approximately 1 liter of lake water. This arrangement permitted contact of the bulk water with all surfaces of the leaves in the region where measurements were made. The water was continuously aerated and mixed with an airstone bubbler located ca. 20 cm distant from the leaf.

Oxygen concentration microprofiles were measured by visually aligning the electrode tip at the surface of the periphyton and then advancing incrementally through the layer, with readings taken at various intervals. Upon reaching the leaf surface an erratic output signal was received (thus revealing the thickness of the periphytic layer). This symptom was verified by observing electrode response when the tip was lowered onto bare sections of leaf surface.

The glycocalyx (mucopolysaccharide) in epiphytic periphyton tended to foul the electrode tips after measuring a few profiles. This interference decreased electrode sensitivity and invalidated previous calibration by altering membrane characteristics (Hale 1983). Microscopic examination showed that layers of material would form over the electrode tip. Dipping the electrode in 3 N HCl quickly removed the fouling material. This problem was also encountered during measurements in epilithic periphyton, but not in other periphyton communities.

Epilithic Periphyton

Algae attached to rocks was simplest to sample because of its secure attachment to the substratum. Rocks used for this study were of sedimentary origin (ca. 5-10 cm diam.), and were encrusted with a carbonate layer at the base of the periphyton; they were taken from the eulittoral of Gull Lake, Michigan (described in Moss 1972). Measurements were made in essentially the same manner as in the epiphytic periphyton, except that detection of the substratum interface with the electrode tip was a more delicate and critical operation. Light was supplied as was done for epiphytic periphyton.

Epipelic Periphyton

All samples of periphyton growing on sediments were taken from Lawrence Lake by a SCUBA-diver using the box corer-microcosm method described in Carlton and Wetzel (1985), except that the acrylic transporting caddy was replaced with an aluminum box with a watertight, hinged lid. This device sampled > 700 cm^2 of sediment surface area with a volume > 29 liter (sediment + overlying water). Sediment profile samples were 15 to 20 cm thick with 20 to 25 cm of overlying water. The corer design permitted transport of the sample to the laboratory and transfer to an acrylic sleeve in an aquarium filled with lake water, with essentially no disturbance of the sediment profile or of conditions at the sediment-water interface. The aquaria were maintained at environmental temperatures by a circulating temperature controller which could also be used to provide circulation over the core samples. These sediment "microcosms" were exposed to ambient light levels and photoperiods which were simulated with height-adjustable banks of

timer-controlled, fluorescent lamps (Luxor, natural light simulating). This apparatus permitted observations and measurements to be made throughout simulated diurnal periods.

Epizoic Periphyton

Eggs of the spotted salamander Ambystoma maculatum appear green due to the presence of symbiotic chlamydomonad algae inhabiting the egg envelope. Egg masses containing late term embryos were collected from a shallow woodland pond in southeastern lower Michigan and maintained for 10 days in an aquarium containing continuously aerated well water at 15-17°C. The eggs were exposed to indirect sunlight and full-spectrum fluorescent light for ca. 12 h d^{-1}. Microelectrode measurements were made inside eggs containing live embryos. Prior to the measurements the egg mass was isolated from light for 3 h. An electrode was inserted into an egg and then the system was exposed to 105 μEinst m^{-2} s^{-1}. Oxygen concentration within the egg was monitored for 34 min. Other pertinent information can be found in Bachmann et al. (1986).

RESULTS

Epiphytic periphyton

The periphyton community present on leaves of Potamogeton illinoensis during this study consisted of a ca. 2-mm-thick layer of algae, bacteria, particulate detritus, and calcium carbonate suspended in a mucoid matrix with high water content (Losee and Wetzel 1983). Algal cell numbers were dominated by the genera Scenedesmus (40%), Fragilaria (9%), Achnanthes (9%), Gomphonema (6%), and Pinnularia (6%). Steady state oxygen concentration microprofiles measured in the

periphyton layer are shown in Fig. 1. After each increase in illumination, a steady state microprofile occurred in ca. 25 min; this time was similar to that observed by Sand-Jensen et al. (1985) for epiphytic periphyton on Potamogeton crispus. During darkness the rate of oxygen consumption in the layer exceeded the rate of diffusion into the periphyton from the surrounding water, producing a weak negative gradient. Illumination of the assemblage with 7 μEinst $m^{-2}s^{-1}$ for 0.5 h produced a very slight increase in oxygen concentration. At steady state under 20 μEinst $m^{-2}s^{-1}$ there was essentially no gradient of $[O_2]$, indicating that oxygen production was balanced by respiratory consumption within the layer, and that there was no net diffusion of O_2. Further increases in intensity of PAR resulted in increased $[O_2]$ throughout the periphyton layer, with the maximum change occurring at the leaf surface, i.e. from 97 μM in darkness to 842 uM at 80 μEinst $m^{-2}s^{-1}$. In terms of extent of atmospheric saturation these concentrations represented a change from 33 to 290 percent.

Oxygen concentration microprofiles were also measured in periphyton epiphytic on Scirpus subterminalis. This periphyton community was thinner (ca. 0.8 mm), less calcareous and less dense with algal cells than the community on P. illinoensis, and allowed a greater fraction of the incident irradiation to reach the leaf surface. Very little difference was observed between microprofiles measured on colonized and uncolonized regions of the leaf after 8 h of illumination at 20 μEinst $m^{-2}s^{-1}$ (Fig. 2B). After several hours of darkness $[O_2]$ at the leaf surface beneath the periphyton layer was depleted to only 228 μM.

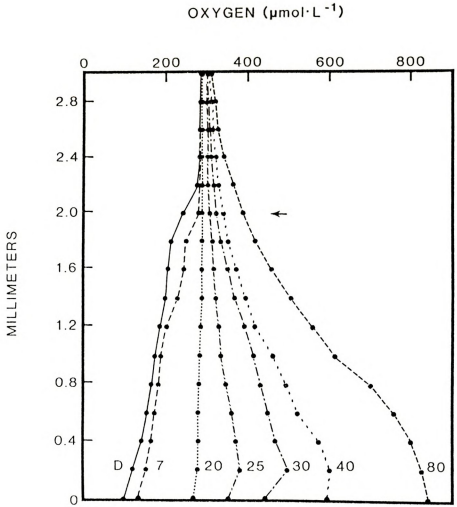

Figure 1. Short term steady state oxygen distributions at increasing light intensities (D = dark, numbers are μEinst m^{-2} s^{-1}) in periphyton epiphytic on a leaf of Potamogeton illinoensis. Vertical scale is distance from leaf surface. Arrow indicates surface of periphyton layer.

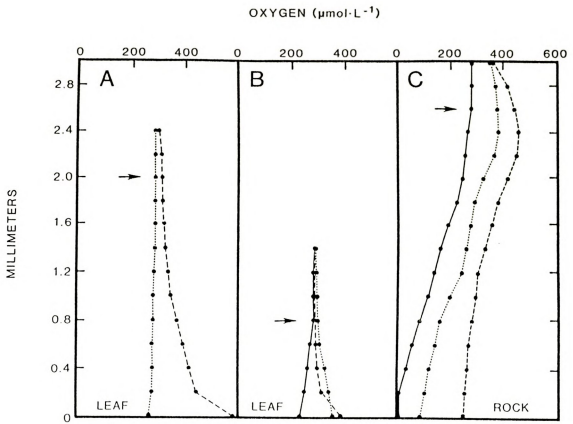

Figure 2. A: Short term (●·····●) and long term (●—●) steady state O_2 microprofiles in periphyton epiphytic on a leaf of Potamogeton illinoensis exposed to 20 μEinst m^{-2} s^{-1}. B: Oxygen microprofiles measured during darkness (●—●) and at an irradiance of 20 μEinst m^{-2} s^{-1} (●·····●) in periphyton epiphytic on a leaf of Scirpus subterminalis, and above a bare section of leaf at the same irradiance (●—●). C: Oxygen microprofiles in epilithic periphyton during darkness (●—●) and after 1 h (●·····●) and 2 h (●—●) of illumination with 40 μEinst m^{-2} s^{-1}. Vertical scale is distance from leaf or rock surface. Arrows indicate surfaces of periphyton.

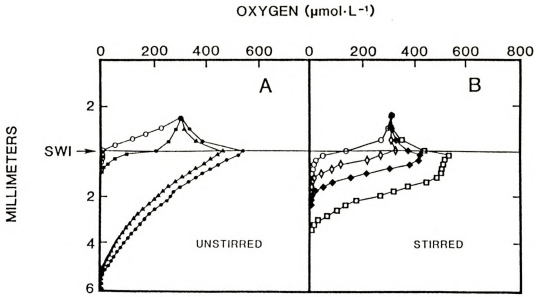

Figure 3. Oxygen microprofiles in sediments colonized by epipelic microalgae. A: During darkness (○—○), and after 1 h (■—■), 8 h (▲—▲), and 10 h (●—●) illumination with 10 µEinst m^{-2} s^{-1}; overlying water not stirred. B: During darkness (○—○), and after 8 h of illumination with 10 µEinst m^{-2} s^{-1} (◇—◇), 30 µEinst m^{-2} s^{-1} (■—■), and 45 µEinst m^{-2} s^{-1} (□—□); water stirred. Vertical scale is distance from sediment-water interface.

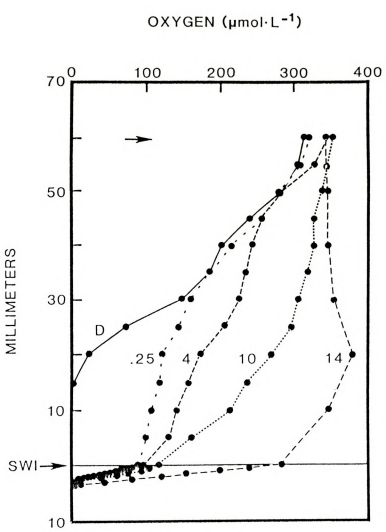

Figure 4. Oxygen concentration profiles in and above sediments colonized by epipelic Dichotomosiphon tuberosus illuminated with 46 μEinst m^{-2} s^{-1}. D = 12 h of darkness, numbers are cumulative time of irradiance in hours. Vertical scale is distance from sediment-water interface. Arrow indicates surface of periphyton.

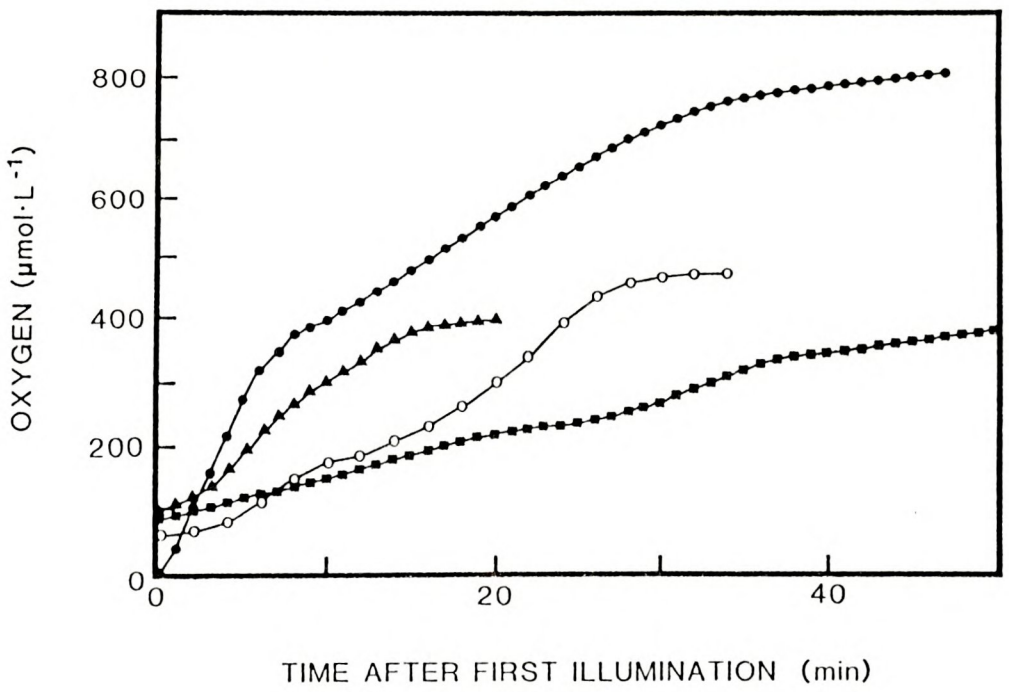

Figure 5. Oxygen concentration change through time during illumination following darkness in four periphyton communities: epilithic exposed to 80 µEinst m^{-2} s^{-1} (●—●), epiphytic exposed to 40 µEinst m^{-2} s^{-1} (▲—▲), epizoic exposed to 105 µEinst m^{-2} s^{-1} (○—○), and epipelic exposed to 20 µEinst m^{-2} s^{-1} (■—■).

Epilithic Periphyton

The periphyton used in this part of the study was composed largely of filamentous diatoms and green algae, which had received 80-120 μEinst $m^{-2}s^{-1}$ of irradiance for ca. 6 h in its natural environment. At that time $[O_2]$ throughout the entire 2.6-mm-thick epilithic layer was in excess of 400% of atmospheric saturation (i.e. > 1200 μM O_2); ebullition was abundant. The periphyton was then shielded from light in the laboratory. After 6 h of darkness the metabolic activity of the community had produced suboxic conditions throughout the periphyton layer and anoxia occurred at the rock surface (Fig. 2C). Subsequent illumination with 40 μEinst $m^{-2}s^{-1}$ resulted in an increase in $[O_2]$ at the rock surface to 84 and 248 μM after 1 and 2 h of illumination, respectively. Increasing the irradiance to 80 μEinst $m^{-2}s^{-1}$ resulted in bubble formation and extreme supersaturation throughout the profile, as had been observed when the sample was first retrieved.

Epipelic Periphyton

At the time of sampling, the epipelic periphyton of the profundal in Lawrence Lake was dominated by diatoms, in particular Navicula. Maximum midday irradiance at that depth in the lake ranged from 10 to 20 μEinst $m^{-2}s^{-1}$ depending on weather conditions. Water currents immediately above the sediments in the lake were negligible; therefore, no circulation was supplied in the laboratory. After 12 h of darkness $[O_2]$ was < 10 μM at the sediment-water interface (SWI) and O_2 was absent below 1.0-mm depth into the sediment (Fig. 3A). After 1 h of illumination with 10 μEinst $m^{-2}s^{-1}$, O_2 concentration at the SWI increased to 203 μM, but did not penetrate farther into the sediment.

A total of 8 h of illumination resulted in a marked increase in $[O_2]$, which was maximal at the SWI (467 µM), and in penetration of oxygen to a depth of 5.5 mm. After 10 h of illumination, $[O_2]$ at the SWI had increased to 542 µM, with a penetration depth of 6 mm. Based on the $[O_2]$ gradient above the SWI at that time the boundary layer was apparently thinner than after the previous dark period. It is possible that convection currents occurred during illumination and reduced the thickness of the boundary layer. This phenomenon has been observed in other experiments where the overlying water was not intentionally circulated (Carlton, unpublished).

In order to assess the effects of currents the water in the microcosm was subsequently circulated, generating a current velocity of ca. 0.5 cm s^{-1} above the sediments (determined by observing suspended particles). After 12 h of darkness $[O_2]$ at the SWI was 136 µM, and the boundary layer was ca. 0.5 mm thick (Fig. 3B). On three successive days, the system was exposed to 10, 30 and 45 µEinst $m^{-2}s^{-1}$, respectively, with a 12-h dark period each night; microprofiles were measured after 8 h of illumination (Fig. 3B). An illumination intensity of 10 µEinst $m^{-2}s^{-1}$ resulted in only slight oxygen supersaturation at the SWI (330 µM O_2), with maximum oxygen penetration of 1.8 mm. At 30 and 45 µEinst $m^{-2}s^{-1}$ the maximum $[O_2]$ occurred at a depth of 0.2 mm in the sediment, rather than at the SWI, and oxygen penetrated to 2.4 and 3.4 mm, respectively. These distributions differ greatly from those in the unstirred situation (Fig. 3A).

During summer, sediments between the 5.5 and 7-m depth contours in Lawrence Lake become heavily colonized by a dense population of Dichotomosiphon tuberosus. This branched, filamentous, green alga

anchors itself with rhizoids which penetrate several millimeters into the sediment. During the period of maximum biomass the filaments extended 4-8 cm above the SWI. Oxygen dynamics were investigated in microcosms in the laboratory. The 6-cm-thick periphyton layer was exposed to 43 μEinst $m^{-2}s^{-1}$ on a 12:12 LD cycle for 3 d before measurements were made. After 12 h of darkness anoxic conditions existed for 15 mm above the SWI (Fig. 4). Brief (15 min) illumination produced an increase in $[O_2]$ at the SWI from 0 to 90 μM. However, after 4 and 10 h of illumination the $[O_2]$ at the SWI increased only to 96 and 115 μM, respectively. After a total of 14 h of illumination $[O_2]$ at the SWI had increased to 283 μM (90% saturation) and slight supersaturation existed above 10 mm above the SWI. At that time oxygen had penetrated to a depth of 4.5 mm which was just 1.0 mm deeper than after only 15 min of illumination. Following these measurements the light was extinguished and 4 h later the distribution of oxygen in the periphyton was virtually identical to the profile measured after 4 h of illumination (data not shown).

The change in $[O_2]$ through time at a single point in each of four different periphyton communities after exposure to light is shown in Fig. 5. In each case the periphyton was isolated from light for 3-6 h prior to illumination, which was supplied at intensities typical of the habitats from which the samples were taken. The $[O_2]$ near the base of a 2-mm-thick layer of epilithic periphyton exposed to 80 μEinst $m^{-2}s^{-1}$ increased from 0 to 302 μM during the first 5.5 min of illumination and continued to rise, although at a slower rate, until bubble formation became visible after ca. 45 min. The change in $[O_2]$ in the middle of a 2-mm-thick layer of epiphytic periphyton on a leaf of P. illinoensis was

measured during illumination with 40 µEinst $m^{-2}s^{-1}$. The $[O_2]$ increased in 18 min from an initial value of 102 µM to an asymptotic maxmimum of 395 µM. At the surface of a layer of epipelic microalgae (in a microcosm similar to that used for the data in Fig. 3A; overlying water not stirred) exposed to 20 µEinst $m^{-2}s^{-1}$, $[O_2]$ increased from 86 to 289 µM (100% sat.) in 32 min. After 50 min of illumination the $[O_2]$ was 380 µM and was still increasing. The fourth curve in Fig. 5 shows the effect of photosynthesis by epizooic algae living symbiotically in the egg wall of the spotted salamander Ambystoma maculatum (for further details see Bachmann et al. 1986). During illumination with 105 µEinst $m^{-2}s^{-1}$ the $[O_2]$ inside an egg containing a live embryo increased from 63 µM to an asymptotic maximum of 476 µM in 32 min.

DISCUSSION

The distribution of oxygen in periphyton is a function of the rate of oxygen production, the rate of oxygen consumption by respiration and chemical oxidation, and the rates of diffusion into and out of the system. The direct effects of each of these functions upon oxygen concentration can be evaluated from oxygen microprofile data collected under different irradiance intensities and through time. Interactions between the productive and consumptive processes in periphyton have not yet been addressed quantitatively, but it is known that oxygen consumption in sediments varies in a nonlinear fashion with oxygen concentration (Hunding 1973; Lindeboom et al. 1985; Revsbech et al. 1986). The diffusive flux of oxygen at the surface of the periphyton layer can be calculated using Fick's first law of diffusion (Crank 1983):

[1] $$J = -D_s \cdot (dC/dx)$$

where flux J is equal to the product of the diffusion coefficient D_s in and the concentration gradient dC/dx across the boundary layer adjacent to the periphyton. The slope of the linear gradient within the boundary layer is defined by i) the oxygen concentration of the bulk surrounding water, ii) the thickness of the boundary layer, and iii) the oxygen concentration at the surface of the periphyton. The boundary layer thickness results largely from the viscosity and velocity of the aqueous medium, but it can also be affected by the topography of the periphyton (Vogel 1981). The $[O_2]$ at the surface of the periphyton is a function of the production, consumption, and diffusion of oxygen within the periphyton layer. These rates are difficult to determine when a gradient exists within the periphyton layer. However, when the gradient equals zero no net diffusion occurs and the rates of photosynthesis and respiration are balanced, providing a situation in which photosynthesis and respiration can be determined (method described by Sand-Jensen et al. 1985). Gradients in which dC/dx = 0 have not been observed in other types of periphyton. Periphyton epiphytic on healthy lacunate hydrophytes are unique in that their substratum produces oxygen when sufficiently illuminated, and it possesses internal gas spaces (lacunae) which provide for rapid transport of gases within the plant (the diffusion of oxygen in air is ca. 10,000 times faster than in water [Vogel 1981]), and between the plant and the aqueous environment (Westlake 1978). Therefore, the concentration of oxygen and other gases in epiphytic periphyton (particularly near the base of the community)

can be significantly affected by photosynthesis, respiration, and gas transport within the host plant. As part of another study in Lawrence Lake, it was observed that in situ lacunar oxygen concentrations in stems of Potamegeton praelongus (a species morphologically similar to P. illinoensis) at depths of 3 to 5 m changed from 23.9 ± 1.4 (S.E.) % (v/v) in early morning to 30.9 ± 1.23 % in midafternoon, (Carlton and others, unpublished), but direct effects on $[O_2]$ in epiphytic periphyton have not been established.

Solute flux in epilithic periphyton can only occur horizontally within the layer and vertically between the periphyton and the surrounding aqueous environment. This functional difference between epiphytic and epilithic periphyton communities resulted in markedly different oxygen distributions during darkness and after periods of illumination (Figs. 1 and 2C, respectively). After 6 h of darkness oxygen was fully depleted at the rock surface, but in periphyton epiphytic on P. illinoensis the $[O_2]$ was 98 μM at the leaf surface; in this study anoxic conditions did not occur in any of the epiphytic periphyton samples. After 2 h of illumination at 40 μEinst $m^{-2}s^{-1}$ oxygen was still subsaturated for 1 mm above the rock surface (Fig. 2C), but in the epiphytic periphyton oxygen was supersaturated throughout the steady state microprofile measured at 40 μEinst $m^{-2}s^{-1}$ (Fig. 1). This comparison is, however, confounded by the inability to separate the effects of different diffusivities and photosynthetic efficiencies of the communities. The epilithic periphyton was adapted to higher light intensities, which was demonstrated by the fact that $[O_2]$ near the base of the epilithic layer increased from < 10 to 100 μM in 1 h during exposure to 40 μEinst m^{-2} s^{-1} (Fig. 2C), but when exposed to 80 μEinst

m^{-2} s^{-1} a similar change in concentration occurred in ca. 2 min (Fig. 5).

The structural relationships between epipelic periphyton and its substratum differs markedly from that of epiphytic and epilithic periphyton. Except among habitats characterized by quiescent water and high light intensities, where thick algal mats can develop, there often is no obvious demarcation between the epipelic periphyton community and the sediment. Conversely, epiphytic and epilithic periphyton are easily distinguishable from their substrata. Therefore, the prefix "epi-" can be an inappropriate misnomer, and "endopelic" may be a more suitable term for many situations. The semantics, however, are of less importance than recognition of the functional relationship of these microflora to their environment.

Comparison of the distribution of oxygen in different periphyton communities reveals some of the functional differences that result from the structural relationships between periphyton and their respective substrata. In epiphytic and epilithic periphyton [O_2] exceeded 100% saturation at the base of the community after a period of illumination (Figs. 1 and 2). However, regardless of the extent of photosynthetic activity of epipelic periphyton, metabolic activity within the substratum always resulted in total depletion of oxygen at some depth below the depth of maximum O_2 concentration (Fig. 3A and 3B). Direct measurement of photosynthetic oxygen production in epipelic periphyton has shown that O_2 is produced throughout the upper few millimeters in high light situations (Jørgensen et al. 1983; Revsbech and Ward 1984), and in the upper few tenths of a millimeter under low light conditions (Carlton and Wetzel, unpublished). The effects of photosynthesis and

respiration on the flux and distribution of oxygen are described mathematically by a modified version of Fick's second law of diffusion (Crank 1983; Revsbech et al. 1981):

$$[2] \qquad dC(x)/dt = D_s \cdot (d^2C/dx^2) + [P(x) - R(x)]$$

where $C(x)$ is the oxygen concentration, D_s is the diffusion coefficient (assumed to be constant within the sediment), $P(x)$ is the rate of oxygen production, and $R(x)$ is the rate of oxygen consumption, at depth x.

The penetration depth of oxygen is affected greatly by the relative magnitudes of oxygen production and consumption in the sediment profile, i.e. $P(x)\big|_x$ and $R(x)\big|_x$, respectively. During darkness when $P(x)\big|_x = 0$, oxygen diffuses from the overlying water, through the diffusive boundary layer, and into the sediment. Therefore, in theory, although the diffusion coefficient of O_2 in sediment is ca. 30% lower than in the boundary layer (Revsbech et al. 1986), if no consumptive processes existed (i.e. $R(x)\big|_x = 0$) the gradient dC/dx would eventually equal zero throughout the sediment and the boundary layer. In reality, the magnitude of $R(x)$ in each stratum (x) varies as a function of the biomass and types of organisms present, the concentration of oxidizable substrates (e.g., H_2S or reduced carbon compounds), the temperature, and the concentration of oxygen. While the interdependence of these factors is obvious, the dominating role of photosynthesis by epipelic periphyton can only be appreciated by comparing the distribution of oxygen during darkness and after illumination (e.g., Fig. 3A). This formation during day and deterioration during night of an oxidized microzone at the sediment-water interface produces a situation that has previously been

thought to be a seasonal rather than a diel phenomenon (Mortimer 1941, 1942; Gorham 1958). Associated effects on microbial metabolism and chemical reactions in sediments are largely unknown. However, it has been determined that the diel fluctuation of $[O_2]$ in sediment colonized by epipelic algae causes a diel fluctuation in the release of phosphorus from the sediment to the overlying water (Carlton and Wetzel, unpublished).

A portion of the oxygen produced in epipelic periphyton photosynthesis diffuses through the diffusive boundary layer to the overlying water. The flux rate is inversely proportional to the thickness of the boundary layer, which decreases with increasing velocity of the overlying water (Vogel 1981). The increased flux rate of O_2 from illuminated sediment overlain by moving water results in decreased oxygen penetration and lower $[O_2]$ in the sediment; this is demonstrated by comparing the microprofiles in Fig. 3A (overlying water not stirred) and Fig. 3B (free stream velocity of water above the SWI was ca. 0.5 cm s^{-1}). (Boundary layer thicknesses are not accurately presented due to sampling resolution above the SWI). After 8 h of illumination at 10 μEinst m^{-2} s^{-1}, the O_2 content in the sediment was 9.74 X 10^{-2} μmol cm^{-2} vs. 1.80 X 10^{-2} μmol cm^{-2} in unstirred vs. stirred conditions, respectively; at 45 μEinst m^{-2} s^{-1} under stirred conditions, the sediment oxygen content (10.29 X 10^{-2} μmol cm^{-2}) was only slightly greater than at 10 μEinst m^{-2} s^{-1} without stirring. During darkness the transport of oxygen into the sediment was enhanced by currents in the overlying water (Fig. 3B), but under stagnant water the sediment was virtually anoxic and a stronger negative gradient existed above the SWI (Fig. 3A). The effects of currents on periphyton oxygen dynamics are

even more pronounced in streams, where water velocities can exceed 50 cm s^{-1}. Even in highly productive epilithic stream periphyton the concentration of oxygen is not significantly higher than in the moving water because of rapid transport across the thin boundary layer. But, if the current is halted oxygen accumulates rapidly in the periphyton layer and ebullition quickly ensues. Therefore, the use of microelectrodes to investigate oxygen dynamics in highly productive periphyton in high-flow-rate environments is limited.

The physical structure of the epipelic alga D. tuberosus resulted in markedly divergent oxygen dynamics compared to those of epipelic microalgae. Although the filaments were not sufficiently dense to alter the diffusion coefficient as sediments do, the macro-structure of the alga produced a 60-mm-thick stagnant boundary layer above the SWI which, by virtue of its volume, also provided a sink for oxygen produced there. The time course of oxygen distribution in sediment colonized by D. tuberosus (Fig.4) contrasted markedly with that of diatom dominated sediments (Fig. 3) where the advance of the oxic zone was slow, but continuous during the illumination period. It is likely that the oxygen distribution during illumination of D. tuberosus resulted from translocation of oxygen from the thalli to the rhizoids at the onset of photosynthesis, but that during the ensuing photoperiod the rate of translocation did not exceed the net oxygen demand of the rhizosphere. The role of rhizoids in a variety of rhizophytic algae is discussed by Raven (1981), and recently, ammonium uptake by rhizoids with subsequent translocation to thalli has been demonstrated in Caulerpa cupressoides, a marine siphonous green alga (Williams 1984). However, physiological studies of solute translocation in D. tuberosus are unknown, and

therefore no further conclusions concerning internal oxygen dynamics of the alga can be made.

During photosynthesis in four distinctly different periphyton communities $[O_2]$ increased most rapidly in epilithic periphyton and slowest in epipelic periphyton (Fig. 5). Although the epizoic periphyton received the highest irradiance intensity the $[O_2]$ there increased more slowly than in epilithic and epiphytic periphyton. These results are congruous with what would be expected, because in the epipelic and epizoic periphyton the thickness of the algal layer was only a few tenths millimeter, which was quite small relative to the volume of the adjacent sinks (i.e. the boundary layer and subtending sediment for epipelic algae, and for epizoic algae the vitelline fluid and the gelatinous mass in which the eggs are suspended), where considerable oxygen demand existed via sediment microbial metabolism or respiration of the salamander embryo. Therefore, oxygen produced in epipelic and epizoic periphyton was probably more quickly diluted or consumed. Conversely, in the relatively thick epilithic periphyton layer there was but one path for the escape of oxygen, and the $[O_2]$ increased most rapidly there. The diffusion regime and $[O_2]$ distributions in epiphytic periphyton was intermediate to that of the others. Oxygen could diffuse from the layer in two directions, but the subtending leaf probably did not supply as great an oxygen demand as did sediments on the epipelic periphyton.

A general understanding of photosynthetic oxygen production and oxygen dynamics in a limited variety of periphyton systems has been developed in recent years, primarily as a result of the increased availability of microelectrode technology. In this comparative study we

specifically addressed the physical differences in the structure of periphyton communities and their substrata, and described the attendant functional aspects which govern the distribution and fates of oxygen. The results show i) that through the diurnal period periphyton of all types act alternately as sources and sinks for oxygen, and ii) that the substrata for epiphytic, epilithic, epipelic, and epizooic periphyton vary greatly in their function in the oxygen dynamics of their respective communities. Of equal importance were the observations that the structural modification by periphyton of their microenvironments can cause significant reduction in the transport rates of oxygen between their substrata and the aqueous environment, with the result that microzones which are anoxic during darkness can become hyperoxic after exposure to light. Observations on epipelic microalgae revealed that even at very low irradiances photosynthesis was sufficient to significantly oxidize surficial sediments, but that the extent of this effect was moderated by the presence of currents. The dominating role of diffusion, and the chemical disequilibrium between the interstitial water of the periphyton layer and the surrounding aqueous environment were also demonstrated. In conclusion, it is apparent that periphyton communities are more complex than is commonly realized, and it is suggested that the interrelatedness of structure and function be considered in future investigations of periphyton ecology.

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CHAPTER II

PHOSPHORUS FLUX FROM LAKE SEDIMENTS:
EFFECT OF EPIPELIC ALGAL PHOTOSYNTHESIS

INTRODUCTION

Microbial metabolism in aquatic sediments regenerates inorganic phosphate which accumulates in the sediment interstitial water forming concentration gradients. Subsequent diffusive transport to the overlying water can be retarded by a number of processes which either temporarily or permanently immobilize phosphate. Mortimer (1941, 1942) demonstrated that the presence of an oxidized microzone at the sediment surface inhibited phosphorus release, but that a decrease in redox potential of the microzone following the onset of anoxic conditions in the overlying water stimulated the reduction of iron (III), thus releasing phosphate bound in hydrous oxides and gels at the sediment surface. This key role of oxygen has been substantiated in numerous studies in a variety of lake and sediment types (Kamp-Nielsen 1974, Patrick and Khalid 1974, Frevert 1980). Also identified as factors affecting the rate of P flux from sediments are pH, temperature, ebullition, bioturbation, nitrate concentration, microbial activity, and macrophyte root exudates (reviewed by Bostrum et al. 1982, and by Ryding 1985). However, because of the diversity of lake sediment characteristics, few broadly applicable generalizations can be formulated. Increased temperature, by virtue of its effect on rates of microbial oxygen consumption in sediments, has been universally shown to result in increased P flux (e.g., Kelderman 1984, Kamp-Nielsen 1975). Conversely, pH and nitrate concentration have been demonstrated to exert both positive and negative effects on P efflux rates (Andersen 1975, Andersen 1982, Bostrum 1982, Bostrum 1984, Tiren and Pettersson 1985). For obvious reasons short-term P release is typically enhanced by ebullition and bioturbation.

Germane to this study are the effects of pH and oxygen concentration ($[O_2]$) in surficial sediments. Even at low light intensities (<50 μEinst m^{-2} s^{-1}) photosynthesis by epipelic microalgae can be sufficient to produce marked changes in the distribution of pH and oxygen in surficial sediments (Carlton and Wetzel in press; Revsbech et al. 1983). The shift from oxic conditions in the upper few millimeters during day to nearly anoxic conditions at night results in daily formation and breakdown of the oxidized microzone, a phenomenon that was previously thought to be seasonal in lake sediments (Mortimer 1941, 1942; Gorham 1958). Although the diel change in pH as a result of epipelic algal photosynthesis is not as spatially extensive as the change in $[O_2]$, the magnitude of the increase is significant. We show here that i) during periods of illumination, sediments colonized by epipelic periphyton released less P than during darkness, and ii) the metabolic activity of epipelic periphyton produced a conserving mechanism for phosphorus in lake sediments, thus reducing the net flux of P to the overlying water.

EXPERIMENTAL PROCEDURES

The periphyton-sediment samples used in this study were obtained from Lawrence Lake, a hard water, oligotrophic, P-limited lake in southwest lower Michigan. The samples were taken at a depth of 8 m by a SCUBA diver using a box corer which sampled a surface area of > 700 cm^2 with a combined height of 42 cm for the sediment and overlying water (Carlton and Wetzel 1985). The samples were transferred without disturbance to laboratory aquaria and maintained as short-term microcosms in which the water was gently stirred and aerated by a stream

of rising air bubbles, and temperature and photoperiod could be controlled. In all microcosms and subsequent flow-through experiments the illumination intensity was 30 $\pm$ 3 µEinst m^{-2} s^{-1}.

The effects of photosynthesis and sediment microbial respiration on the distribution of oxygen and pH in the sediment were determined with high spatial resolution at various times during the simulated photoperiod using microelectrodes constructed by R. Carlton. The oxygen microelectrodes were the micro-Clark style with a cathode diameter of 3 to 7 µm and an outer diameter of 15 to 40 µm in the sensing region (Revsbech and Ward 1983). A chemical microsensor (Transidyne General, Ann Arbor) supplied polarizing voltage (- 0.75 V) and processed the electrode output signal. Calibration was accomplished with volumes of 0 and 100% air-saturated lake water at the temperature of the experimental systems. The pH electrode (Thomas 1978) had a diameter of ca. 100 µm in the sensing region. A Beckman pHi 55 ph meter and buffer solutions of known pH were used to perform a two-point calibration of the electrode. During measurements in the microcosms the microelectrodes were positioned with a micromanipulator (0.05-mm resolution), and were aligned initially at the sediment-water interface by remotely viewing position through a water proofed fiberscope (American Optical). Measurements were made at 0.2-mm intervals.

The algal species assemblage of the periphyton community was determined by removing the thin layer with a micro-siphon that was positioned with a micromanipulator. Samples were preserved with acid Lugol's solution and then settled before observation with an inverted microscope. Samples for chlorophyll a content of the periphyton were obtained with the micro-siphon by removing ca. 1.5 mm of the upper

sediment layer within a 1-cm-high plastic ring (area = 1.65 cm^2) placed on the sediment surface. The microflora-sediment slurry was immediately passed through a 47-mm-diameter glass fiber filter (Gelman GF/F, retention size 0.7 µm), that was immediately frozen at -20°C, and later stored at -70°C. The filters were ground in a 9:1 mixture of chilled acetone and water in a tissue grinder, and then centrifuged. The pheophytin-corrected chlorophyll a concentration was determined spectrophotometrically by the method of Strickland and Parsons (1972).

The phosphate flux experiments were conducted in flow-through chambers which permitted manipulation of i) the diffusive efflux rate of phosphate across the sediment-water interface, ii) the volume and flushing rate of the overlying water, iii) the oxygen concentration of the overlying water, iv) the photoperiod and light intensity to which the epipelic algae was exposed, and v) the temperature of the system. Each experimental unit consisted of an 8–12-mm-thick layer of sediment and its intact periphyton isolated on a supported nylon membrane (Ultipor N_{66}, 0.45 µm pore size) which separated the upper flow-through chamber from the lower artificial pore water (APW) chamber. In a test for permeability of PO_4^{3-} in flow-through chambers without sediment the $Nylon_{66}$ material was found to be superior to teflon and polycarbonate membranes. Periphyton-sediment samples were transferred from the microcosms to the chambers by the method depicted in Fig. 1. The cylinders of the flow-through chambers (O.D. = 45 mm, I.D. = 38 mm, height = 50 mm) and the O-ring-sealed piston (diam. = 37 mm, height = 60 mm) were constructed of translucent acrylic plastic. The base of the flow-through chamber, and the cylinder and base of the APW chamber

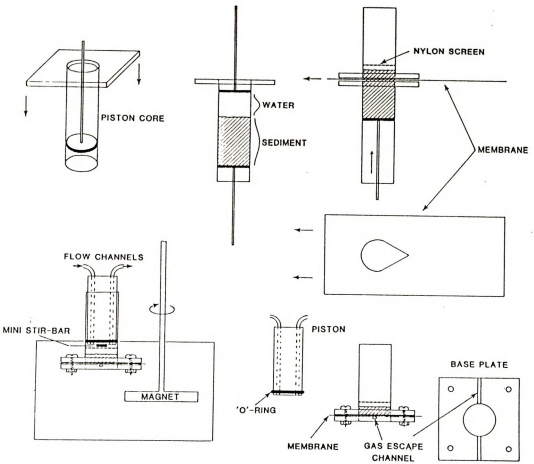

Figure 1. Diagram of apparatus used to isolate surficial sediment section on a membrane. Scheme flows clockwise from upper left. After sampling sediment with the piston corer (I.D. = 38 mm) the hole in the membrane and the upper chamber are aligned and the sample is pushed upward to the desired height. The membrane is pulled across severing the sediment, the lower section of which is discarded. Then the base plate is secured and the piston is installed.

(volume = 1.0 L) were 12.8-mm-thick, opaque acrylic. The volume of water above the sample was adjusted to 15 ml by inserting the piston to a predetermined level. The flushing water entered and left the flow-through chamber via channels inside the piston; a three-way valve mounted on the inlet to each chamber allowed switching among different source reservoirs. The space above the sediment was divided horizontally by a 1-mm-mesh nylon screen on which a magnetic stir bar (2 x 8 mm) rested. A second, slightly larger stir bar was suspended in the APW chamber on a thin nylon line 2 cm below and 1 cm to the side of the lower face of the membrane. Each experiment typically utilized three units that were submersed in a temperature-controlled water bath; two units (A & B) were exposed to simulated photoperiods, and the third (control, C) was shielded from light. The gentle motion of the stir bars, which was induced by a magnet rotating at 60 rpm between the three chambers, decreased the thickness of boundary layers at the sediment-water interface and at the lower surface of the membrane, and decreased streaming of the flow-through water between the inlet and outlet ports. A septum at the top of the inlet channel in the piston permitted removal of gas bubbles from the flow-through chamber; a septum on the top of the APW chamber was used for removal of bubbles from the APW and for the injection of $^{32}PO_4^{3-}$.

The flow-through water was prepared by filtering water from Lawrence Lake (SRP concentrations < 2.0 μg L^{-1}) through a series of filters (10 μm, 1 μm, 0.45 μm, and 0.22 μm). The desired oxygen concentration was obtained by vigorous sparging with either air, oxygen, or nitrogen for > 4 h. The pH (7.9) and alkalinity (4.4 meq L^{-1}) of the water did not change significantly during sparging. After a given

treatment the water was transferred to a flexible polypropylene container (Cubitainer) which, during the experiment, was suspended in the bath and which collapsed as the water was removed, thus preventing gas exchange with the atmosphere. Problems of gas exchange through the wall of the container were minimized both by its immersion in the bath and by changing the reservoir fluid daily.

The artificial pore water was prepared by mixing several liters of lake water and sediment (ca. 3:1 vol. ratio) continuously for 3 days under a nitrogen purged head space. The slurry was allowed to settle for several seconds before transferring 1.0 L to each APW chamber. The concentration of soluble reactive phosphorus in each APW chamber was adjusted to 50 μg PO_4^{3-}-P L^{-1}, and 3-5 mCi $^{32}PO_4^{3-}$ were added.

A peristaltic pump downstream of the flow-through chambers continuously pulled flushing water through at a rate of 7.5 ml h^{-1}, thus flushing each unit once per 2 h. Each effluent stream from that pump was continuously sampled by a second peristaltic pump at a rate of 0.8 or 1.6 ml h^{-1}; this liquid was collected in 20-ml liquid scintillation vials mounted on the deck of a fraction collector timed to advance at intervals of 1 or 2 h. Therefore, 12 or 24 samples were obtained from each flow-through unit each day.

Light was supplied to units A and B through fiberoptic wands. The timer-controlled source was a projector lamp (Sylvania EJY), and the light was diffused at the distal end of the wand by passage of the beam through a piece of nylon membrane material. The intensity of irradiance incident on sediment within a flow-through chamber was measured in units assembled without sediment with a LiCor model LI-185 meter with a cosine corrected quantum sensor (Lambda Instruments). Tests were conducted

which showed that no temperature changes occurred in the chambers as a result of illumination.

The radioactivity was determined in each vial by adding ca. 14 ml of aqueous counting coctail and counting for 20 min in a Beckman LS-8000 liquid scintillation counter. Quench was insignificant and the counting efficiency was essentially 100%. Background radiation (52 cpm) was subtracted from sample counts before plotting.

The effect of periphyton photosynthesis on the distribution of PO_4^{3-} in surficial sediments of Lawrence Lake was determined by comparing the distribution of SRP in surficial sediments isolated from light with that in unshaded plots. Shortly after the spring ice thaw a 6 m x 7 m area of sediment at a depth of 9 m in Lawrence Lake was shielded from illumination by suspending a sheet of black polyethylene on thin wood posts ca. 0.6 m above the sediment surface. Two nearby control plots each consisted of an area (2 m x 2 m) covered by transparent polyethylene sheet that was similarly suspended. Downwelling light was only slightly attenuated by the clear sheet, which was cleaned periodically of sedimented seston and periphyton. Light intensity within the darkened plot was < 0.1 μEinst m^{-2} s^{-1} when ca. 30 μEinst m^{-2} s^{-1} reached the sediment surface in the control plots. Sediment pore water in the plots was sampled with dialysis samplers (Hesslein 1976) which were inserted by a SCUBA-equipped diver 2 weeks after installation of the plots. Each sampler consisted of an acrylic plastic body with four 8-ml chambers spaced at 1.7-cm vertical intervals. The surface area of the nylon membrane (described previously) over each chamber was 5 cm^2. The chambers were filled with purified (Millapore Q) water, and the membranes were secured in a manner

which excluded bubbles from the chambers. Then the samplers were deoxygenated by immersion for 2 d in a bath of purified water that was continuously sparged with N_2. The samplers were left in situ for 50 d, with two in each control plot, and three in the dark plot. Just prior to removal from the sediment the face of each sampler was covered with a thin acrylic plate to inhibit oxygenation of the samples which could affect subsequent SRP analysis (Bray et al. 1973). SRP was determined using the method of Murphy and Riley (1962) within 1 h by withdrawing 4 ml from each chamber and diluting it with 4 ml of 0.2 N HC1 (to achieve a higher final acidity; John 1970) before adding the mixed reagent. A color blank was measured on the remaining 4-ml in each cell by the same procedure, except ascorbic acid was not added to the mixed reagent.

In order to determine if algal oxygen production and subsequent oxygen dynamics in sediments were altered during preparation of the sample and assembly of the flow-through chamber, a chamber was constructed that permitted insertion of an oxygen microelectrode. A simulated flow-through experiment (no ^{32}P) was performed with a photoperiod of 6:8 LD. Measurements were obtained at 0.2 and 1.0 mm below the SWI.

RESULTS

In each of the flow-through experiments reported here, the $CaCO_3$-rich sediment was colonized by a thin periphyton community which was dominated by pennate diatoms, especially of the genera Navicula and Nitzschia. Pheophytin-corrected chlorophyll a content of the periphyton was 30.2 $\pm$ 8.3 mg m^{-2} (pooled mean $\pm$ s.d.; n = 9, 3 samples per

experiment).

Except for minor differences due to temperature, the temporal and spatial distributions of oxygen were similar in all of the sediment microcosms used. Fig. 2 is presented as an example. After 6 h of darkness [O_2] at the SWI was < 50% saturated, and the sediment was anoxic below 0.8 mm. Illumination for two hours resulted in supersaturated conditions in the upper 0.9 mm, and in increased oxygen penetration. After 6 h of illumination the zone of supersaturation extended to a depth of 1.9 mm, and oxygen penetration occurred to a depth of 4.0 mm. Under similar light and temperature conditions in similar sediment microcosms (taken from the same area of the lake) where the overlying water was not stirred, oxygen maxima were greater and oxygen penetrated farther into the sediment (Carlton and Wetzel, in press). The temporal and spatial distributions of O_2 in membrane-mounted sediment were similar to that in the microcosms (Fig. 3). Measurement of pH microprofiles was possible only for the third experiment. During darkness a negative gradient of pH occurred across the SWI, but after 6 h of illumination a positive heterograde existed in the thin photosynthetic layer, with a maximum pH of 8.9 (Fig. 4).

In the first flow-through experiment (temperature = 17°C, influent water saturated with air) two chambers (A & B) were exposed to a 12:12 LD photoperiod, and a third was kept dark as a control. The efflux rate of ^{32}P oscillated in a pattern coincident with the photoperiod (Fig. 5). Although the magnitude of the effect was different in the two replicates the rate of ^{32}P efflux always increased during darkness and decreased during illumination. There was no diel oscillation in ^{32}P efflux in the dark control.

The experiment was repeated with identical conditions except the temperature was lowered to 11°C (Fig. 6). Although the background ^{32}P efflux rate was similar to the first experiment at 17°C, no obvious diel pattern existed. This absence of diel periodicity of P efflux was observed in two similar experiments at 10 and 12°C (Carlton and Wetzel, unpublished).

The third experiment was performed identically to the first for three days during which the diel pattern occurred in the illuminated treatments (Fig. 7). At the end of the third day, when the light was extinguished, the air-saturated water influent to all three flow-through chambers was replaced by water saturated with pure oxygen (45-50 mg L^{-1}). This treatment resulted in a decrease in ^{32}P efflux in all three flow-through chambers; the greatest effect occurred in the dark control. After 16 h the oxygen-supersaturated water was replaced with deoxygenated water and the ^{32}P efflux rate increased in all three chambers. These manipulations were repeated again with similar results. After 12 h of anoxic influent water, chambers A and B were again exposed to light (for the first time in 54 h), but with no obvious effect. The deoxygenated water was maintained for two more photoperiods, during which no response to illumination occurred in chamber A, but in B a response was apparent. The ^{32}P efflux rate in the control (never exposed to light) remained nearly constant until after 52 h of treatment with anoxic flow-through water it increased rapidly to the highest level observed in any chamber during that experiment. However, upon switching to superoxygenated water the ^{32}P efflux in the control decreased precipitously. Similar decreases occurred in replicates A and B, and no

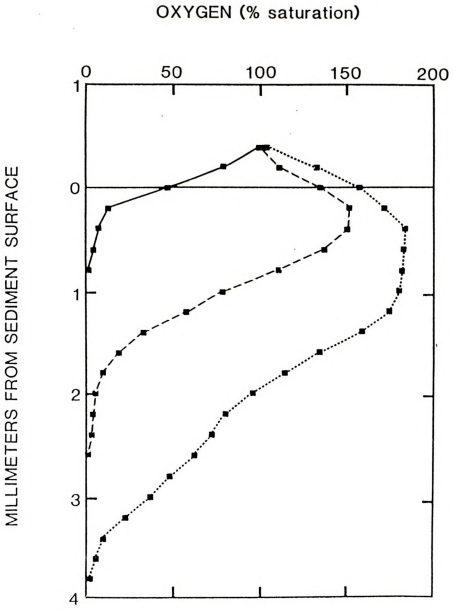

Figure 2. Microprofiles of oxygen concentration in sediments prior to their use in the flow-through system; after 6 h of darkness (solid line), after 2 h of illumination(dashed line), and after 6 h of illumination (dotted line). Light intensity = 30 μEinst m^{-2} s^{-1}; temperature = 17°C; equilibrium air saturation = 302 μmol O_2 L^{-1}.

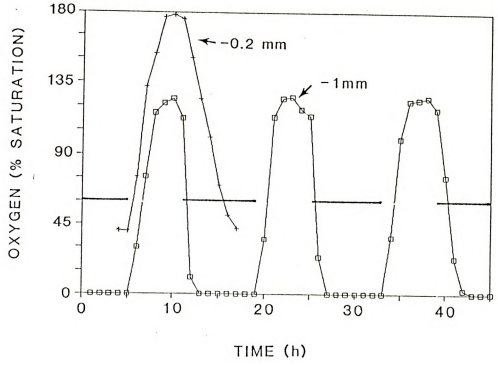

Figure 3. Oxygen concentrations through time at depths of 0.2 and 1.0 mm below the sediment surface in a sediment section isolated on a membrane within a flow-through chamber. Black bars represent dark period. Light intensity = 30 µEinst m^{-2} s^{-1}, temperature = 17°C, equilibrium air saturation = 302 µmol O_2 L^{-1}.

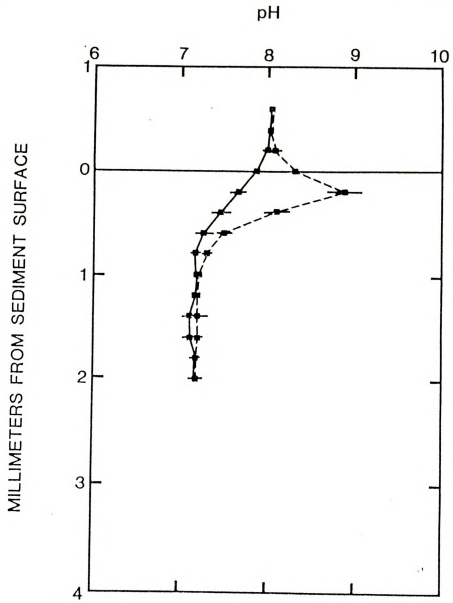

Figure 4. Distribution of pH in sediments used for flow-through experiments; after 6 h of darkness (black line) and after 6 h of illumination (hatched line) with 30 μEinst m^{-2} s^{-1} at a temperature of 17°C. Error bars are one standard error of the mean on either side of the mean (n = 3).

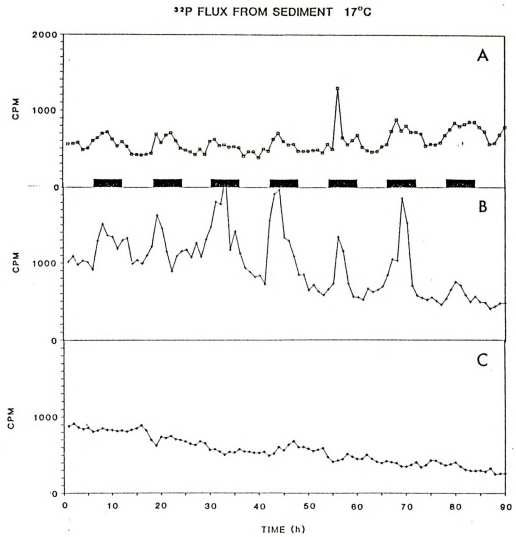

Figure 5. Phosphorus flux experiment with time course of ^{32}P release (CPM) from sediments exposed to a 6:6 LD cycle. Light intensity during illumination of chambers A & B = 30 µEinst m^{-2} s^{-1}, C = control (permanently dark). Temperature = 17°C. Bars designate dark period.

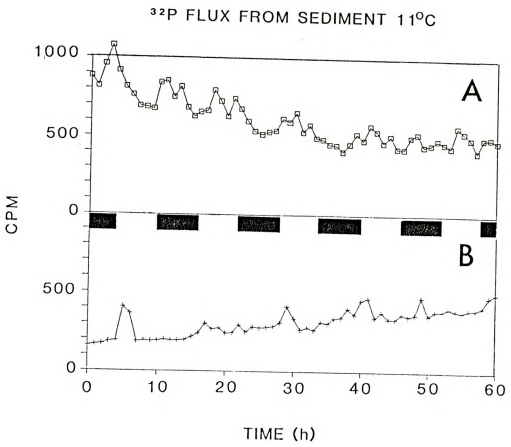

Figure 6. Phosphorus flux experiment with time course of ^{32}P release (CPM) from sediments exposed to a 6:6 LD cycle. Light intensity during illumination of chambers A & B = 30 μEinst m^{-2} s^{-1}. Temperature = 11°C. Bars designate dark period.

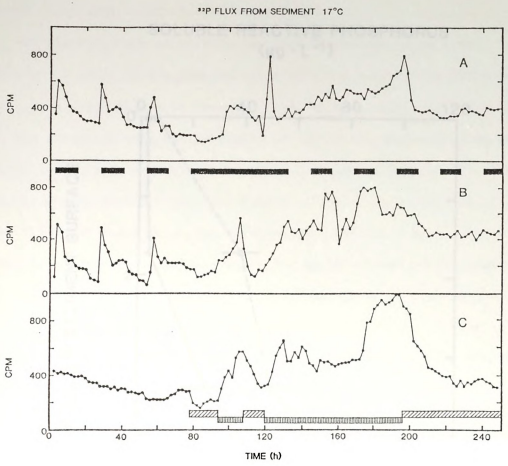

Figure 7. Phosphorus flux experiment with time course of ^{32}P release (CPM) from sediments exposed to a 12:12 LD cycle. Light intensity during illumination of chambers A & B = 30 μEinst m^{-2} s^{-1}. Black bars indicate dark periods for chambers A & B; the control (C) was permanently dark. Round symbols on data points designate period during which gases in flow-through water were in atmospheric equilibrium; square symbols start at point where $[O_2]$ was manipulated in the flow-through water. Diagonally hatched bars at bottom indicate periods when flow-through water was superoxygenated; bars with vertical hatching indicate anoxic flow-through water. All three chambers received same quality of water. Temperature = 17°C.

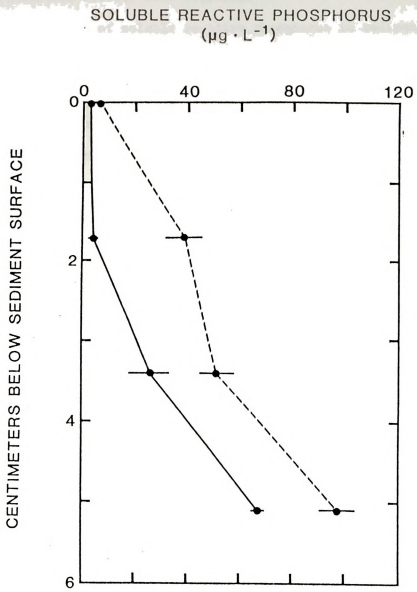

Figure 8. In situ distribution of soluble reactive phosphorus in interstitial water in sediments of Lawrence Lake in a plot which was permanently shielded from light (black line) and in control plots which received normal light intensities (hatched line). Error bars are one standard error of the mean on either side of the mean (n = 4 for control, n = 3 for dark plot).

further illumination effects were observed.

The distributions of soluble reactive phosphorus in sediment interstitial water in the unshaded (control) and permanently darkened plots, at a depth of 9 m in Lawrence Lake, were markedly different (Fig. 8). The concentrations and gradients of SRP in the unshaded plots were similar to previous determinations in that region of the lake (Carlton unpublished; R. Moeller, personal communication). In comparison, in the upper 1.7 cm of sediment in the darkened plot SRP was 3 $\mu g\ L^{-1}$ and virtually no gradient existed. Below 1.7 cm the slopes of the gradients were similar, but the concentration of SRP was lower throughout the darkened sediment profile. Visual examination of sediment during retrieval of the interstitial water samplers revealed that the shaded sediments had not developed the coloration evident on the sediment surface in the unshaded plots and elsewhere in the lake.

DISCUSSION

The increase in the efflux rate of ^{32}P from sediment in the flow-through chambers during dark periods probably resulted from the cessation or reversal of one or more chemical or biological processes that had immobilized phosphorus diffusing upward through the sediment during the illumination period. Several chemical processes are known to affect phosphate mobility in sediments as a function of $[O_2]$ and $[OH^-]$, which we have demonstrated to fluctuate greatly in surficial sediments exposed to a light-dark cycle. The rate of formation of iron hydroxide is first order with respect to $[O_2]$, and second order with respect to $[OH^-]$ (Stumm and Morgan 1981). When the sediment surface is oxidized iron (III) hydroxides can bind phosphate as a precursor to the formation

of hydroxyapatite (Syers et al. 1973). However, some investigators have reported increased phosphate flux at high pH and speculated that hydroxide ions competed with phosphate for binding sites on the oxidized iron gels, thus increasing the mobility of PO_4^{3-} (Jacoby et al. 1982). Conversely, by virtue of its effect on calcite formation in hard water, increased pH in overlying water has been shown to reduce the net flux of P from calcareous sediments via coprecipitation (Otsuki and Wetzel 1972) with and adsorption to the surfaces of calcite crystals (Gunatilaka 1982). Gunatilaka also showed that the adsorption reaction was rapid and that the maximum phosphate adsorption occurred at pH 9, which is approximately the value we observed in surficial sediment after 6 h of epipelic algal photosynthesis (Fig. 4). The decreases in oxygen concentration and depth of penetration in sediments which occur during darkness (Fig. 2 and 3) result in breakdown of the oxidized microlayer and lowering of the redox potential. The attendant chemical reduction of oxidized iron gel complexes releases phosphate (Syers et al. 1973). Phosphate adsorbed to calcite can also desorb as the pH in the microzone drops during darkness.

If the mechanisms producing the observed diel P flux variation were strictly chemical in nature the pattern at 17°C should also have existed at 11°C, but with slightly lower magnitude because of the temperature (Kelvin) dependence of chemical reactions. Because a small decrease in temperature resulted in a virtual absence of the phenomenon, it is apparent that the dominant mechanism was biological. Algae are known to absorb excess phosphate under certain conditions and store it as polyphospate (e.g., Shapiro 1968). However, we know of no studies showing subsequent release of the phosphate as a result of darkness.

Conversely, Levin and Shapiro (1965) showed that the microorganisms (e.g., the bacterial genera Zooglea, Escherichia, Bacillus, Flavobacterium, and Pseudomonas, and some ciliated protozoans) in activated sludge in sewage treatment plants absorbed excess phosphate when the suspension was aerated, and then released it when aeration was stopped and the system became anoxic. The reaction was shown to be rapidly reversible, temperature dependent, independent of the external phosphate concentration, and did not involve cell death or growth. Shapiro et al. (1967) reported that it was not oxygen concentration, but redox potential which must be depressed to a sufficiently low level to induce the release, but this was refuted by Randall et al. (1970) who reported that the release began when $[O_2]$ reached zero, and that the lowering of redox potential lagged by 40 to 60 min.

The conditions for induced uptake and release of phosphate by microorganisms are extant in Lawrence Lake sediments. Bacteria and protozoans are common in aquatic sediments (Kuznetzov 1970). Photosynthesis by epipelic periphyton produces a diel fluctuation of $[O_2]$ from zero to ca. 200% saturation in surficial sediments (Fig. 2). Therefore, the periodicity of P flux observed in Lawrence Lake sediments is possibly a result of biological uptake and release of phosphate induced by diel changes in $[O_2]$ and redox potential resulting from epipelic algal photosynthesis.

The $^{32}P\text{-}PO_4^{3-}$ injected into the artifical pore water may have undergone biological transformations prior to diffusion through the sediment layer. However, it is unlikely that significant diffusive fractions were converted to biologically unavailable forms (Bostrum 1984; Cembella et al. 1984). Furthermore, as several nutrient loading

models have shown, lake phytoplankton productivity responds to total P inputs (e.g., Dillon and Rigler 1974). Therefore, the flux of ^{32}P from sediments in the flow-through chambers represents release of phosphorus compounds that probably can stimulate phytoplankton productivity in P-limited systems. Because we do not know the molecular forms involved or the specific activities of the individual pools, we hesitate to quantify net P flux based on the observed ^{32}P flux . However, the existence of the phenomenon implies that the net release of P from sediments is reduced by the presence of epipelic periphyton. Analysis of the interstitial water in the darkened and unshaded plots in Lawrence Lake demonstrated that the concentration of SRP in sediments colonized by an epipelic microflora capable of oxygenic photosynthesis was significantly greater than in permanently darkened sediments (Fig. 8), indicating that conserving mechanisms operating in the unshaded plots were absent in the darkened plot.

Photosynthesis by periphyton epipelic on lake sediments modifies the oxygen and pH conditions of interstitial water in surficial sediments, even at low irradiance intensities. Subsequent chemical or biological reactions that are sensitive to $[O_2]$ or pH mediate the mobility of phosphorus compounds in the sediment interstitial water, thus affecting the release rate of P to the overlying water. The net effect is the conservation of P in the sediment, i.e. P efflux is reduced when an active epipelic algal community is present. This conclusion implies that epipelic periphyton not only contributes to whole-lake primary productivity, but that it may reduce the productivity of phytoplankton by conserving P in the sediments. The ecology of epipelic periphyton in lakes is largely unknown. However, the results

of this study have demonstrated that the function of epipelic periphyton in lake ecosystems is of much greater importance than is presently realized. Further studies of the effect of periphyton on nutrient cycles and flux rates in lake sediments are essential, and will perhaps reveal information which can be used in lake management and restoration.

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CHAPTER III

A BOX-CORER FOR STUDYING METABOLISM OF EPIPELIC MICROORGANISMS IN SEDIMENT UNDER IN SITU CONDITIONS

Limnol. Oceanogr., 30(2), 1985, 422-426

A box corer for studying metabolism of epipelic microorganisms in sediment under in situ conditions[1]

Abstract—The device described here permits sampling of a combined sediment and overlying water volume of about 29 liters (cross-sectional area > 700 cm²) with minimal disturbance of sediment structure. Vertical microprofiles of oxygen concentration measured in littoral sediments before (in situ) and after sampling with the box corer showed good agreement. These data are compared with oxygen microprofiles measured in pelagic sediments sampled with the box corer and kept in a laboratory under in situ conditions. Oxygen production by epipelic algae exposed to very low quantum flux resulted in marked diurnal phasing of oxygen above and within the sediments.

In recent years increased emphasis has been placed on the importance of investigating sediment function under natural conditions. Because of the difficulty of performing experiments in situ, samples are often brought into the laboratory and maintained for short periods; piston corers are usually adequate for this retrieval procedure (reviewed by Wright et al. 1965). A properly used piston corer can retrieve an undisturbed column of sediment and overlying water with little disturbance of the sediment-water interface. These conditions are necessary in studies of, for example, benthic algal productivity, sediment microbial metabolism, and solute diffusion. Large diameter cores may produce less "edge effect" and the increased sediment surface area can be advantageous for many reasons. Sediments at great depth are often sampled with large box corers which are maneuvered with winches (Hessler and Jumars 1974; Bouma and Marshall 1964). However, no presently available hand-held device can sample a large surface area (> 500 cm²) and volume of sediment (and overlying water) without disturbing the sediment profile or destroying conditions at the interface. We describe here a box-corer sampling method, compare oxygen microprofiles measured in littoral sediments before (in situ) and after sampling with the box corer, and present data from pelagic sediments that were sampled with the box corer and maintained as a microcosm. We thank N. P. Revsbech for his tutelage in the skills and knowledge necessary for construction of oxygen microelectrodes.

In the past several years knowledge concerning the dynamics of dissolved oxygen in freshwater and marine sediments has been increased greatly, largely as a result of work by Revsbech and others using oxygen-sensitive microelectrodes (Revsbech 1983; Revsbech et al. 1980, 1981, 1983; Reimers et al. 1984). Some of this work was performed in situ in very shallow water, but sediments underlying deeper water were sampled with corers and analyzed in a laboratory. In situ measurements are important, but often not possible. Further, it can be advantageous or necessary to control environmental conditions. Therefore, to enhance the versatility of oxygen microelectrodes and improve the efficiency of obtaining accurate microprofile measurements, we have developed the following methodology.

The box corer is made of 6-mm-thick acrylic plastic and has a flexible aluminum lower door and a flat 3-mm-thick acrylic upper door, each of which slides in grooves milled in the box walls (Fig. 1). The lower tambour door is made of 0.5-mm aluminum flashing, strengthened with 1.6-mm-thick aluminum crossbars. As this flexible door is pushed downward in the grooves, its leading edge is guided through the sediments around the lower edge of the end wall and across the bottom of the box. Inside dimensions of the box corer are 25- × 28-cm horizontal by 42-cm vertical, giving a total sample volume (sediment + water)

[1] This work was supported by the U.S. Department of Energy (DE-AC02-76EV01599, COO-1599-245) and National Institutes of Health, Division of Research Resources (BRSG Grant 2-S07 RR07049-15). Contribution 546, W. K. Kellogg Biological Station of Michigan State University.

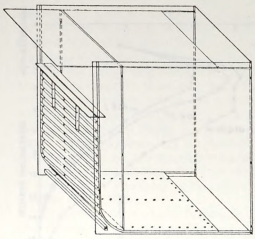

Fig. 1. Perspective diagram of the box corer with the upper and lower doors partially closed. Note the protrusion of side walls on the left to accommodate the vertical travel of the flexible, tambour door. The support rod at the lower left maintains rigidity where the door flexes.

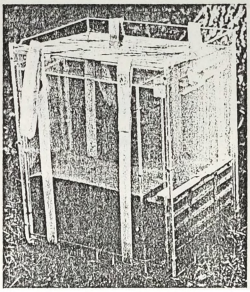

Fig. 2. Sediment core sample within the box corer held within the transport caddy (caddy lid removed).

of >29 liters. The Meiostecher of Thiel (1983) also uses a sliding flexible door, but this unit has a cross-sectional area of <25 cm^2 and was designed for subsampling large box corers.

Our box corer is deployed with SCUBA; while in the water the corer can be operated and maneuvered by one person. A sample is taken by pushing the open box the desired distance into the sediment, sliding the top door in to isolate the sample from disturbance, and then inserting the lower door into the grooves and pushing it until the box is sealed. The loaded corer is transported in a caddy, a watertight acrylic box that has inside dimensions about 1 cm larger than the box corer (Fig. 2). The caddy serves the purposes of reducing stress on the lower door and not permitting air into the top of the sample, which would allow sloshing and aeration of the water during transport. To facilitate later removal of the box corer from the caddy, we secure two nylon straps with loops in the ends in the caddy before loading the corer (Fig. 2). The mass of the sealed caddy is about 65 kg. Two persons can easily maneuver the unit on land after securing two 1-m-long handles to the side of the caddy (not shown). We also have made smaller versions that can be lifted by one person into a small boat.

For our purposes we transport the sealed caddy to a laboratory and transfer the box corer with a rope hoist (hooked through the straps) to an aquarium partially filled with lake water. As the box corer is lowered, the water rises to the top of the aquarium. First, the small amount of mud lying outside the box above the curved portion of the lower door (Fig. 2) is siphoned off. The bottom door is then removed. We allow any slightly disturbed external sediment to settle, remove the top door, and then insert a tight fitting 42-cm-long sleeve (3-mm-thick acrylic) into the top of the box corer (itself a sleeve with the doors removed) and push it to the bottom. The box corer is then withdrawn vertically, without disturbance to the sample, for later use. The resulting system consists of a rectangular solid volume of sediment, with its overlying water intact, enclosed by a system in which environmental conditions can be regulated.

Temperature in the systems was controlled with an external circulating cooler which was also used at times to simulate

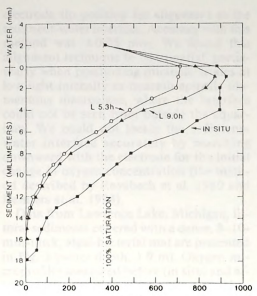

Fig. 3. Comparison of microprofiles of oxygen concentration measured in situ (Lawrence Lake, Michigan; water depth, 1.9 m) and in a box-corer sample of the same sediment in the laboratory after 12 h of darkness followed by 5.3 and 9.0 h of illumination at 150 μEinst m^{-2} s^{-1}. Water temperature was 9.0°C in all cases.

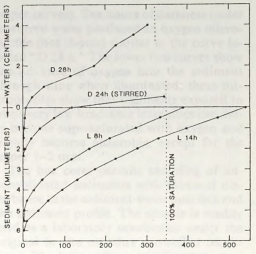

Fig. 4. Microprofiles of oxygen concentration in and above pelagic sediments sampled at 10-m water depth in Lawrence Lake, Michigan, with the box corer and maintained in the laboratory. Water temperature was 14°C for the upper curve, and 11°C for the three lower curves. Light intensity was 10 μEinst m^{-2} s^{-1}. Note change in depth scale at sediment-water interface.

water currents. Light was supplied from either fluorescent (Luxor, natural light simulating) or mercury vapor lamps, with neutral density shadecloth (Chicopee, Ball Seed Co.) used to reduce light intensities to in situ values. Light intensity at the sediment–water interface was determined with a LiCor model LI-185 meter with a cosine quantum sensor.

Microprofiles of dissolved oxygen were measured with polarographic oxygen-sensitive microelectrodes (Revsbech and Ward 1983) with an outer tip diameter <30 μm and a cathode diameter <10 μm. This type has an internal reference electrode, which allows the unit to be moved from one solution to another without depolarizing the cathode. A Transidyne General chemical microsensor supplied polarizing voltage (-0.75 V) to the electrodes and displayed the output signal. Calibration in the laboratory was performed with volumes of 0 and 100% oxygen-saturated lake water equal in temperature to the system. Field calibration was accomplished by assuming 100% saturation in the open water near the lake surface, and taking "zero" as the asymptotic minimum at the bottom of the oxygen microprofile. Readings of percent saturation were later converted to μmol O_2 $liter^{-1}$ (Figs. 3 and 4).

Because of their fragility, these microelectrodes must be positioned carefully using a micromanipulator. For in situ measurements the micromanipulator had to be submerged, stabilized on a framework above the sediment, and operated by a SCUBA diver; because of the difficulty of working with microelectrodes while under water, readings were taken at intervals of only 1.0 mm through the sediment.

For samples in laboratory systems the micromanipulator was mounted on a deck above the water, and a glass rod with a microelectrode secured at the bottom was attached to the micromanipulator. Measurements were taken at 0.5- or 1.0-mm intervals (we have easily made measurements at 0.2-mm intervals). A waterproofed Fiberscope (American Optical) was used to view the

electrode tip position for alignment at the sediment-water interface. Accuracy with this method was ±0.05 mm. We found this alignment technique to be essential, particularly when positioning microelectrodes at low light intensity or beneath epipelic filamentous macroalgae, where the interface could not be seen from outside the aquarium. We could not locate the sediment–water interface accurately by searching downward with the electrode for the initial change in oxygen concentration (the method described by Revsbech et al. 1980 and Reimers et al. 1984).

Data from Lawrence Lake, Michigan, littoral sediments covered with a dense, 8–10-mm-thick, algal-bacterial mat are presented in Fig. 3 (water depth, 1.9 m). Oxygen microprofiles measured before (in situ) and after sampling with the box corer were similar. The in situ measurements were made on 26 April 1984, at 1400 hours, when irradiance at the interface was 480 μEinst m^{-2} s^{-1}. Although this intensity could not be duplicated in the laboratory, a proximally located mercury vapor lamp supplied 150 μEinst m^{-2} s^{-1}; on 27 April, after 9.0 h at this intensity the oxygen concentration 1.0 mm below the sediment–water interface was nearly equal to the observed in situ value of 928 μM O_2. The lower oxygen production and penetration into the sediment in the laboratory system probably resulted from insufficient irradiance at the interface and hydrostatic pressure differences. Bubble formation in the mat was observed in the lake and the laboratory (note supersaturation in surficial strata, Fig. 3), but this presented no problems for microelectrode measurements (cf. Revsbech and Jørgensen 1983).

Pelagic sediments at a depth of 10 m were also sampled with the box corer. The epipelic periphyton community on these sediments consisted of a thin film of diatoms and microflagellates. In the laboratory, the system was maintained at 11°–14°C, with a 14:10 LD cycle at 10 μEinst m^{-2} s^{-1}. Figure 4 shows the effect of water currents above the sediment and the relationship between sediment oxygen distribution and quantum flux to the sediment–water interface. Downward transport of oxygen was accelerated by water currents above the sediment (two upper curves). Ten hours of darkness under unstirred water produced an oxygen microprofile (not shown) similar to the curve labeled "D 28 h." The lower two curves show the advance of oxygen into the sediment through time when illuminated; these microprofiles show that sediments exposed to even very low irradiance intensities can daily become supersaturated with oxygen and nightly become anaerobic, except for the upper 1–2 mm.

The box corer permits sampling of unconsolidated sediments with minimal disturbance to the sediment-water interface and the sediment profile. The sample is readily held as a laboratory microcosm under the original in situ or other controlled conditions. The large sediment surface area provides ample space for the equipment necessary for measuring oxygen microprofiles. The technique should provide samples appropriate for numerous applications, e.g. studies of biogeochemical cycling and solute flux, interactions among benthic populations, and periphyton productivity and nutrient dynamics.

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Submitted: 28 December 1983
Accepted: 10 October 1984

CHAPTER IV

SYMBIOSIS BETWEEN SALAMANDER EGGS AND GREEN ALGAE: MICROELECTRODE MEASUREMENTS INSIDE EGGS DEMONSTRATE EFFECT OF PHOTOSYNTHESIS ON OXYGEN CONCENTRATIONS

Symbiosis between salamander eggs and green algae: microelectrode measurements inside eggs demonstrate effect of photosynthesis on oxygen concentration[1]

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Received December 17, 1985

BACHMANN, M. D., R. G. CARLTON, J. M. BURKHOLDER, and R. G. WETZEL. 1986. Symbiosis between salamander eggs and green algae: microelectrode measurements inside eggs demonstrate effect of photosynthesis on oxygen concentration. Can. J. Zool. **64**: 1586–1588.

Eggs of the spotted salamander, *Ambystoma maculatum*, are usually green because of the presence of symbiotic, chlamydomonad algae which inhabit the envelope of each egg. An oxygen-sensitive electrode was used to measure the effect of algal photosynthesis on oxygen concentration inside eggs and within the gelatinous matrix surrounding them. During darkness, oxygen became severely depleted within the eggs, but upon exposure to light oxygen concentrations increased rapidly. Photosynthetic oxygen production by the chlamydomonads exceeded respiratory consumption by the embryo–algae complex and led to oxygen supersaturation inside eggs, even when water surrounding the egg mass was almost anoxic.

BACHMANN, M. D., R. G. CARLTON, J. M. BURKHOLDER et R. G. WETZEL. 1986. Symbiosis between salamander eggs and green algae: microelectrode measurements inside eggs demonstrate effect of photosynthesis on oxygen concentration. Can. J. Zool. **64**: 1586–1588.

Les oeufs de la salamandre *Ambystoma maculatum* sont ordinairement rendus verts par la présence d'algues chlamydomonades symbiotes qui occupent l'enveloppe de chaque oeuf. Une électrode sensible à l'oxygène a servi à mesurer les effets de la photosynthèse des algues sur la concentration d'oxygène à l'intérieur des oeufs et dans la matrice gélatineuse qui les entoure. A l'obscurité, la concentration d'oxygène contenue dans les oeufs baisse considérablement, mais de retour à la lumière, elle augmente de nouveau rapidement. La production d'oxygène due à la photosynthèse par les algues excède la consommation d'oxygène respiratoire du complexe embryon–algues et entraîne une sursaturation à l'intérieur des oeufs, même lorsque l'eau qui entoure la masse d'oeufs devient presque anoxique.

[Traduit par la revue]

Introduction

The eggs of the spotted salamander, *Ambystoma maculatum* Shaw (Caudata: Ambystomatidae), usually are bright green because of the chlamydomonad algae inhabiting the middle layers of each egg envelope. First reported by Orr (1888), this unusual symbiotic association between green algae and the eggs of a vertebrate was shown by Gilbert (1942, 1944) to result in lower mortality and faster development of embryos and in larger hatchling size than embryos developing without algae. We demonstrate here, in contradiction to an earlier report of Hutchinson and Hammen (1958), that photosynthetic oxygen production by the algal symbionts exceeds the total respiratory demand within the egg mass, even when the surrounding water is nearly anoxic.

Eggs of *A. maculatum* are often found in shallow woodland ponds which are hypoxic because of microbial metabolism of deposits of leaves and other detritus (Collins and Wilbur 1979). Because premetamorphic *A. maculatum* larvae are oxygen conformers, their metabolic rates decline and development slows under reduced oxygen tension (Branch and Taylor 1977). Therefore, embryos developing in hypoxic environments could benefit from an association with algae capable of photosynthesizing at rates sufficient to increase oxygen levels within the egg. In this study we used an oxygen-sensitive microelectrode to directly measure oxygen concentration ($[O_2]$) within eggs and their surrounding gelatinous matrix. Experiments were performed with and without light and with manipulation of the degree of oxygenation of surrounding water.

Materials and methods

Egg masses with late-term embryos of *A. maculatum* were collected in May from a woodland pond in southeastern lower Michigan and maintained in aquaria containing continuously aerated well water at 15–17°C. During the 10 days before the experiment the eggs received indirect sunlight and full-spectrum fluorescent light for approximately 12 h day^{-1}.

To determine algal cell density three eggs were randomly selected and their embryos were excised. The egg envelopes were separately ground using a loose-fitting tissue grinder and the suspensions were settled using the inverted microscopy method of Utermöhl (Lund et al. 1958). One field-width strip per chamber was counted at 600×. Two perpendicular strips were counted in one chamber to determine uniformity of settling.

Microscale measurements of oxygen concentration were made with a polarographic oxygen-sensitive microelectrode (internal reference style; Revsbech and Ward 1983) with an outer tip diameter of 30 μm and a cathode diameter of 7 μm. This electrode experienced drift less than 3% h^{-1}, had a 95% response time of <2 s for a 200 μ*M* change in $[O_2]$, and was insensitive to water currents. A Transidyne General chemical microsensor supplied polarizing voltage (−0.75 V) to the electrode, and displayed the output signal. Calibration was accomplished using volumes of 0 and 100% air-saturated water at temperatures equal to those of the experimental systems. The fragile microelectrode was positioned with a micromanipulator. Unless stated otherwise, measurements were made at 17–19°C in eggs containing live embryos. Aerated systems were circulated by air from an air stone.

An egg mass containing ca. 60 eggs at Harrison stage 40 (near hatching) was immersed in 100 mL of air-saturated water in a 250 mL beaker and shielded from light for 3 h at 17°C without further aeration. The initial measurements in and around the eggs were then made under very dim light (5–10 μE m^{-2} s^{-1}), which was unavoidable. Following these measurements, the electrode tip was situated in the center of an egg located approximately 3 mm inside the outer edge of the gelatinous matrix and the system was illuminated. A mercury vapor lamp supplied

[1]Contribution No. 566, W. K. Kellogg Biological Station, Michigan State University, and No. 70, College of Agriculture, Iowa State University.

Printed in Canada / Imprimé au Canada

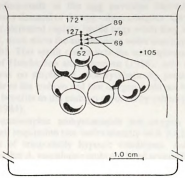

FIG. 1. Oxygen concentration (micromoles of O_2 per litre) in and around eggs of *A. maculatum* after 3 h of darkness in uncirculated water at 17°C. 100% air saturation = 302 μM O_2.

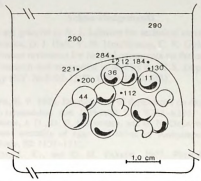

FIG. 2. Oxygen concentration (micromoles of O_2 per litre) in and around eggs of *A. maculatum* after 3 h darkness in continuously aerated water at 19°C. 100% air saturation = 290 μM O_2.

105 $\mu E\ m^{-2}\ s^{-1}$ of photosynthetically available radiation (approximately 8% of full sunlight in summer) as measured by a Licor quantum photometer with a cosine sensor. After $[O_2]$ stabilized at a maximum within the eggs the system was darkened and monitored for another hour. Later the same egg mass was transferred to a 4-L container which was continuously aerated and mixed. This system was darkened for 3 h, after which the initial $[O_2]$ measurements were made in total darkness.

To provide comparative data, even though we lacked algae-free clutches of *A. maculatum*, we measured $[O_2]$ in similar-sized eggs of *Ambystoma tigrinum* (a closely related species which often cocxists with *A. maculatum*; Collins and Wilbur 1979), which did not possess an algal symbiont. *Ambystoma tigrinum* adults collected in southwestern lower Michigan were allowed to mate and the egg masses were reared in the laboratory under the same conditions as the eggs of *A. maculatum*. An *A. tigrinum* egg mass containing ca. 40 embryos at Harrison stage 40 was held in darkness in continuously aerated water for 3 h, and then used for $[O_2]$ measurements as for *A. maculatum*.

Results

The algae were identified as a species of *Chlamydomonas*. During the experiments an average egg contained ca. 83 000 algal cells. Bi- and quadri-flagellate round and ovoid cells ranged from 120 to 900 μm^3 biovolume. Round vegetative cells averaged 3600 μm^3. Algal population density per egg averaged 5.39×10^5 cells cm^{-2} with an average egg surface area of 1.54 cm^2.

Oxygen concentration in water surrounding the *A. maculatum* egg mass was initially in equilibrium with the atmosphere (302 μM O_2), but metabolic activity during the 3-h dark period produced an $[O_2]$ gradient in the unstirred system between the water surface (172 μM) and an egg within the gelatinous matrix (52 μM) (Fig. 1). Mean $[O_2]$ in two eggs at this point was 57 μM, but this value was slightly elevated (as are the initial points on curve A, Fig. 3) as a result of slight photosynthesis from the unavoidable dim light. Subsequent exposure of the egg mass to light produced an increase in $[O_2]$ within an egg capsule from 63 to 476 μM in 32 min (Fig. 3 curve A). One hour after eliminating the light, $[O_2]$ within the egg was 9 μM, while $[O_2]$ in the gelatinous matrix and surrounding water were 160 and 91 μM, respectively.

Later, after 3 h of darkness in continuously aerated water, the concentration of O_2 inside four embryo-occupied eggs averaged 31 ± 14 μM (mean ± SD). A strong gradient of decreasing $[O_2]$ (from 290 to <50 μM) again existed between the open water and the interior of eggs (Fig. 2), except that in this case $[O_2]$ values within the gelatinous matrix were considerably higher

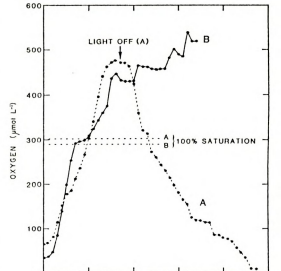

FIG. 3. Oxygen concentration (micromoles of O_2 per litre) over time within illuminated eggs of *A. maculatum* after 3 h of darkness. Light on at time zero. Curve A: egg mass in hypoxic water (Fig. 1) at 17°C. Light turned off after 34 min elapsed time. Curve B: same egg mass in continuously aerated water at 19°C. The steps in curve B were caused by tail movements by the embryo which mixed and broke down the oxygen gradient in the egg fluid.

than in the previous unaerated system (Fig. 1). During 64 min of illumination, $[O_2]$ inside one egg increased from 34 to 540 μM (Fig. 3, curve B).

The mean $[O_2]$ in *A. tigrinum* eggs was 102 ± 15 μM after 3 h of darkness in aerated water. Exposure to light produced no change in $[O_2]$. In this study and another (Anderson et al. 1971), algae were found only in dead *A. tigrinum* eggs. Insertion of the microelectrode through egg capsules of *A. tigrinum* was considerably more difficult than for *A. maculatum* and eventually resulted in breakage of the electrode tip.

Discussion

Our data show that photosynthetic oxygen production by the chlamydomonads in the egg envelope exceeded the total respiratory demand of the egg–algae complex. Oxygen concentrations increased rapidly inside eggs when they were exposed to light, even when the surrounding water was hypoxic (Fig. 3, curve B). This result contradicts the assumptions and conclusions of Hutchinson and Hammen (1958), who reported that there was no surplus of photosynthetically produced oxygen available to the embryo and that oxygen could not be responsible for the benefits to the embryo reported by others (e.g., Gilbert 1942, 1944).

Premetamorphic ambystomatids are oxygen conformers, i.e., their respiration rate varies directly with $[O_2]$, yet they are tolerant of temporarily hypoxic conditions. Adolph (1979) reported that *A. maculatum* embryos lived several hours without oxygen and up to 24 h at low oxygen partial pressures (equivalent to $<50\ \mu M\ O_2$ in our study). Adolph (1979) also presented data that indicated that *A. maculatum* may be more tolerant of anoxia than *A. tigrinum*. In our study, $[O_2]$ in *A. tigrinum* eggs was approximately three times greater than $[O_2]$ in *A. maculatum* eggs after 3 h of darkness in aerated water. This difference could have been a result of different numbers and metabolic rates of embryos, respiration by algae, or differences in diffusive characteristics of the egg envelopes and gelatinous matrices. However, given the conclusions of Adolph (1979) this difference may be moot in terms of embryo survival, although of interest from an evolutionary perspective. Conversely, the difference in $[O_2]$ between *A. maculatum* and *A. tigrinum* after illumination may be important. The ability of oxygen conformers to increase oxygen consumption and metabolic rate at higher $[O_2]$ may confer an advantage to *A. maculatum* in illuminated, hypoxic environments.

Many animal species exist in hypoxic environments, but few are exposed to hyperoxia such as that occurring in *A. maculatum* eggs; even fewer experience the diurnal changes in $[O_2]$ depicted in Fig. 3. The relationship between the algae and embryos is undoubtedly more complex than is presently realized. Further research could investigate the function of pH inside eggs which may vary because of photosynthetically induced changes in H^+ and OH^-, or the possibility that the gelatinous matrix acts as an oxygen storage capacitor for *A. maculatum* embryos. Obviously much more can be accomplished using microelectrodes, which are extremely versatile and appropriate for field measurements (Carlton and Wetzel 1985).

Acknowledgements

We are grateful to A. J. Johnson for technical assistance, and J. Petranka, D. J. Hall, V. H. Hutchinson, C. R. Goldman, and anonymous reviewers for helpful comments on the manuscript. This research was supported in part by the U.S. Department of Energy (EY-76-S-02-1599, COO-1599-245).

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CHAPTER V

SYNOPSIS

Although periphyton is widely recognized as an essential component of primary production in streams, it has historically been relegated a minor role in the metabolism of lakes. Over twenty years ago Wetzel (1964) showed that periphyton in a shallow lake supplied the majority of primary production on an annual basis. Few additional quantitative studies exist, but in lakes with low nutrient content and high water transparency (i.e. those in which much of the basin lies within the euphotic zone) periphyton can contribute significantly to whole-lake primary production.

Relative to the plankton, the ecology of periphyton is poorly understood. Traditional studies evaluating ecological interactions of the planktonic community such as competition for nutrients or light are lacking for lake periphyton. Most research on lake periphyton has simply attempted to enumerate the algal species composition on substrata that could be easily sampled from the surface of the water A recent opinion of some limnologists is that lakes classified as oligotrophic based on the water transparency and the areal chlorophyll and nutrient concentrations of the water column may actually be closer to mesotrophic when the biomass and primary productivity of the epipelic algae are considered.

Perhaps most enigmatic is the nearly total lack of information about the nutrient dynamics of epipelic periphyton. Though it is widely recognized that lake sediments are responsible for internal loading of nutrients, no published research has attempted to elucidate the role of sediments in the nutrient dynamics of epipelic periphyton. Carlton (1981) showed that epipelic algae of the littoral of Castle Lake, California, had the potential to absorb all of the ammonium

diffusing from subtending sediment. Other studies in the same lake demonstrated high nitrogen fixing capability of epilithic periphyton (Reuter et al. 1986) and the mediation of nitrification and denitrification in sediments by epipelic algal oxygen production (Paulsen 1986).

The development of microelectrode technology for environmental research by N. P. Revsbech (reviewed by Revsbech and Jørgensen 1986) has enabled scientists for the first time to make direct measurements of the distribution of oxygen in periphyton and sediments (as well as in many other microenvironments and ecosystems e.g., salamander eggs, rice soils, foraminiferans, insect haemolymph and gut tracts, leaf packs, and partially decomposed, water-logged wood). By making measurements through time, while manipulating light intensity and oxygen concentration in the overlying water, one can determine rates of photosynthesis and respiration in periphyton and sediments (methods described in Chapter I). Use of these techniques in the initial phase of this study demonstrated that periphyton communities of all types were extremely dynamic with respect to oxygen. Even under low light intensities (e.g., 10 uEinst m^{-2} s^{-1}) a thin layer of diatoms on the surface of sediments generated sufficient oxygen to increase the penetration depth of oxygen from < 1 mm to > 6 mm into the sediment and produce oxygen supersaturation near the sediment-water interface.

Because of the difficulty of working in situ with microelectrodes, the box corer/microcosm system (presented in Chapter IV) was designed. This apparatus allowed manipulation of several relevent environmental factors and gave ready access to a large surface area of undisturbed sediment. Up to twelve box corer samples were maintained as microcosms

under simulated environmental conditions. The extensive documentation of the daily formation and breakdown of the oxidized microzone at the surface of Lawrence Lake sediments led to a working hypothesis:

> If the seasonal formation and breakdown of the oxidized microzone results in respective decreases and increases in the flux of phosphorus from lake sediments, then perhaps the diel oxygen dynamics resulting from epipelic algal photosynthesis can cause a diel fluctuation in the efflux rate of phosphorus.

This hypothesis was investigated using sediments from Lawrence Lake (Chapter III), with the result that epipelic algal photosynthesis did indeed mediate the efflux rate of phosphorus on a diel basis. An in situ experiment in Lawrence Lake that compared the distribution of phosphate in sediment interstitial water inside and outside shaded plots demonstrated that oxidation of the surficial sediment microzone also resulted in long-term conservation of phosphorus in sediment. This result implies that the presence of a photosynthetically active epipelic periphyton community may be advantageous by reduction of internal loading to the overlying water column. Furthermore, the phenomenon may be important in maintaining oligotrophic conditions in the water column of transparent lakes. In essence, a positive feedback mechanism is invoked: nutrients regenerated in sediments are conserved there either by utilization by the periphyton or some metabolically induced immobilizing process, with the result that phytoplankton production is reduced and water transparency is maintained, thus allowing sufficient light to continue to reach the sediment surface and support the epipelic community.

For the feedback mechanism to continue to operate as a regulator of internal loading, the external nutrient loading must remain low.

Otherwise, phytoplankton production and biomass increase and water column transparency decreases, short-circuiting the feedback mechanism. Human activities in drainage basins have resulted in increased external loading to thousands of lakes, and we may never know how important epipelic periphyton was to the maintenance of oligotrophic lakes. Few lakes exist that have not been adversely affected by humankind. However, if this feedback mechanism can be substantiated in some of the remaining pristine lakes, perhaps a new awareness of the delicacy of lake ecosystems will emerge.

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