Cladophora Abundance and Physical / Chemical Conditions in the Milwaukee Region of Lake Michigan

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Harvey A. Bootsma[1], Erica B. Young[2],
and John A. Berges[2]

[1]Great Lakes WATER Institute
University of Wisconsin-Milwaukee
600 E. Greenfield Ave.
Milwaukee, WI 53204

[2]Department of Biological Sciences
University of Wisconsin-Milwaukee

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1. Executive Summary

In the Milwaukee region of Lake Michigan there has been a recent resurgence of nuisance blooms of benthic algae, dominated by the filamentous green alga, *Cladophora glomerata*. Negative impacts of the excessive growth of this alga include unsightly and foul smelling beaches, potential health risks from bacterial growth, clogging of water intakes, impaired drinking water quality, decline in lakeshore recreational quality, and property depreciation. There may be additional ecological impacts within the lake, though these have not been well documented.

In response to nuisance algal blooms, including *Cladophora* blooms, in the Great Lakes between the 1950s and 1980s, phosphorus reduction programs were put in place. The decline in *Cladophora* that followed the reduction of phosphorus inputs suggested that phosphorus control was the key to algal control, and that nutrient abatement measures were having the desired effect. However, the fact that *Cladophora* abundance has recently increased, while phosphorus inputs have reportedly continued to decrease, raises uncertainties about whether the phosphorus limitation remains applicable. The cause(s) of the current resurgence in *Cladophora* abundance are not obvious.

In order to assess the magnitude of the *Cladophora* problem in the Milwaukee region, and to determine potential causes of the problem, a field study was conducted from June 2003 to October 2004. Specific objectives of the study were: 1) to obtain quantitative data on in-lake biomass and nutrient composition of *Cladophora*, and assess the role of environmental factors (nutrients, light, temperature) in regulating *Cladophora* abundance; 2) to evaluate the role of nearshore currents as a factor influencing the distribution of nutrients and *Cladophora*; 3) to assess the potential role of zebra mussels in promoting *Cladophora* growth; 4) to provide a database on *Cladophora* abundance that will facilitate the development of management targets and allow evaluation of any implemented management strategy.

The results of this study provide strong evidence that *Cladophora* growth in the Milwaukee region of Lake Michigan is phosphorus limited. However, light and temperature are also major factors influencing *Cladophora* growth. Prior to May and after October, nearshore lake temperatures are too low for *Cladophora* to grow. Between July and September, temperatures are frequently optimal for *Cladophora*, although temperatures fluctuate greatly as a result of lake internal waves. An analysis of historic temperature records indicates that the average nearshore temperature has risen by approximately 3°C in the past 30 years, likely as a result of changes in prevailing summer wind direction, and as a result the duration of time during which nearshore temperatures are optimal for *Cladophora* growth has increased.

Historic analysis reveals that nearshore water clarity has also increased, most likely due to the filtration of water by zebra mussels, which invaded Lake Michigan in the early 1990s. Data collected in 2003 indicate that water clarity is the primary factor controlling light levels on the lake bottom, where *Cladophora* grows. *Cladophora* is currently found in large quantities to depths of at least 10 meters (33 feet). While there are little historic data for comparison, a comparison of underwater light levels with *Cladophora* light requirements indicates that *Cladophora* is currently abundant to a depth twice as great as it was in the early 1990s. In addition, *Cladophora* that was always present at shallower depths can now grow at faster rates, due to increased light availability.

A comparison of *Cladophora* abundance and nutrient content with ambient dissolved nutrient concentrations in the Lake Michigan nearshore area suggests that dissolved phosphorus concentrations are not sufficient to support the observed biomass of *Cladophora*. This suggests that there is a benthic (lake bottom) source of phosphorus. The results of both in-lake studies and experimental work suggest that zebra mussels are the likely source of this phosphorus. From a management perspective, an important question to answer is whether zebra mussels ultimately derive their phosphorus from the lake's internal pool in the form of plankton, or from suspended particulate material delivered to the lake from rivers. Mass balance calculations indicate that both of these sources are potentially important, depending on the time of year. Spatial patterns of nearshore currents, phosphorus distribution, and *Cladophora* nutrient content indicate that the discharge of nutrients from rivers through Milwaukee Harbor likely has some influence on *Cladophora* growth. However, estimated *Cladophora* phosphorus demand is greater than phosphorus input from rivers, and therefore a substantial portion of *Cladophora* production must be supported either by the in-lake phosphorus pool or by efficient nearshore recycling of phosphorus delivered by rivers. Comparison of *Cladophora* biomass and nutrient content at a number of locations on the Wisconsin coast of Lake Michigan indicates that, while *Cladophora* phosphorus content is greater in the immediate vicinity of Milwaukee Harbor, biomass is high along much of the coast, and the *Cladophora* problem is not confined to the Milwaukee region.

Based on the results of this study, the recent resurgence of *Cladophora* in the Milwaukee area of Lake Michigan appears to be a result of the combined effect of increased light availability, increased summer nearshore temperatures, and increased phosphorus availability. Critical information required for the formulation of a *Cladophora* management strategy includes a quantitative evaluation of the relative importance of river-borne versus in-lake phosphorus sources for zebra mussels, and an evaluation of the potential impact resulting from the on-going establishment of quagga mussels, another invasive species, which have the potential to cover larger areas of lake bottom than zebra mussels. In addition, a revision of a previously developed *Cladophora* growth / abundance model, to account for phosphorus inputs from zebra mussels, will allow for a quantitative assessment of the relative importance of light, temperature and phosphorus as factors promoting *Cladophora* growth. Such a model will also help to predict the benefits of various management options. In the short term, a better understanding of the mechanisms causing *Cladophora* sloughing (detachment from the lake bottom) will allow for better prediction of the times and locations of *Cladophora* accumulation on beaches. This information will also improve the accuracy of a *Cladophora* growth / abundance model.

2. Purpose

The Milwaukee Metropolitan Sewerage District (MMSD) is currently beginning its Facility Planning effort for the year 2020. This planning effort is utilizing a comprehensive watershed-based approach to evaluate questions and provide answers regarding the impacts to water quality and the benefits of potential improvements to the MMSD's wastewater collection, conveyance, treatment and watercourse systems. The 2020 Facility Planning effort will need answers to questions that deal with a broad range of water quality issues, including those of nuisance algal growth in area waterways. Currently there is public concern regarding nuisance growth of the filamentous green alga, *Cladophora* sp., along Milwaukee's lakeshore. Excessive growth of this algae results aesthetic degradation of beaches and other shorelines, potential health risks, clogging of water intakes, and alteration of the Lake Michigan nearshore ecosystem. The objective of this study was to assess the magnitude of the problem in the Milwaukee region of Lake Michigan, to determine the potential causes of the problem, and to make recommendations as to how *Cladophora* growth might be managed. Specific objectives were:

1. Provide quantitative data on the in-lake biomass and nutrient composition of *Cladophora*, and the influence of nutrient inputs on these properties.

2. To evaluate the role of nearshore currents as a potential factor influencing the distribution of nutrients and *Cladophora*.

3. To assess the potential role of the zebra mussel (Dreissena polymorpha) in promoting *Cladophora* growth.

4. To provide a database on *Cladophora* abundance and nutrient content that will both facilitate the development of management targets and allow for an assessment of the efficacy of any implemented management strategies.

3. Overview of Sampling and Analytical Methods

Standard methods were used to collect and analyze samples in this study. These methods apply to several of the sub-studies reported in this document, and rather than repeat the description of the methods within each sub-section of the report, they are described here.

River water collection

River water samples were collected using a clean polyethylene bucket. For smaller rivers (Oak Creek and Root River during low flow), sampling was done by wading into the center of the river, where a surface sample was collected. In the larger rivers, samples were collected by suspending the sampling bucket from an overpass and collecting water from mid-channel. Samples were returned to the laboratory within three hours, where they were immediately filtered through Whatman GF/F glass fiber filters (nominal pore size = 0.7 μm). Filtered samples were stored at 4^oC and analyzed for dissolved nutrients within 48 hours. Particulate nutrients (collected on filters) were stored in a desiccator until analysis.

Lake water collection

Lake water samples were collected at various depths either by using a Niskin sampling bottle deployed from a boat, or by direct sampling into a polyethylene bottle opened at depth by a SCUBA diver. Near-bottom samples were collected by SCUBA divers using polyethylene syringes. Samples were stored on ice, in the dark, until they were returned to the laboratory, where they were filtered and processed in the same manner as river samples. All sampling bottles were pre-cleaned in an acid bath (5% HCl) followed by copious rinsing with distilled, deionized water.

Cladophora collection

All benthic algae samples were collected by SCUBA divers. To determine areal *Cladophora* concentrations, on each date and at each sampling depth four replicate samples were collected, each from a rock surface area of 20 X 20 cm (400 cm^2). Making temporal and spatial comparisons of benthic algal abundance in lakes is notoriously difficult, due to the large amount of spatial heterogeneity. In order to minimize this heterogeneity, and facilitate comparisons among dates and locations, samples were collected only from large rocks and boulders with a horizontal surface area of between 1,000 and 4,000 cm^2. Visual observations indicated that these surfaces tended to have a more homogeneous distribution of algae than vertical surfaces and gravelly / irregular substrata. When sampling, a

1X1 m square grid consisting of 25 20X20 cm sections was placed on top of a rock and the grid closest to the center of the rock was sampled. Sampling was done by gently hand-picking the *Cladophora* within a grid and placing it in a plastic whirlpak bag. Replicate sample bags were stashed in a submersible cage that was attached by a line to a boat at the surface. Following the completion of a dive, the cave was pulled on to the boat and the samples were immediately stored on ice.

On return the laboratory, each *Cladophora* sample was removed from its bag and placed in a tray with a small amount of distilled water. Large invertebrates, shells and stones were manually removed from the *Cladophora*, after which it was rinsed under a stream of flowing distilled water to remove all non-algal material. Each sample was then placed in a freeze dryer to remove all water. Following freeze drying, samples were weighed to the nearest 0.1 mg, and then subsampled for the analysis of chlorophyll a, phaeophytin, particulate carbon, particulate nitrogen and particulate phosphorus.

Nutrient Analyses

Analyses for soluble reactive phosphorus (primarily phosphate) and nitrate were performed following the methods of Stainton et al. (1977) and Palmer et al. (1984). Dissolved ammonium was measured by derivitization with sodium sulfite and o-phthalaldehyde followed by fluorometric detection (Genfa and Dasgupta 1989). Dissolved organic N and P were measured by photo-oxidizing samples in the presence of peroxide, followed by analysis as described above for inorganic N and P (Stainton et al. 1977). Particulate phosphorus was measured by combusting filter samples for 2 hours at 550^{o}C, followed by heating to 100^{o}C for 1 hour in a dilute hydrochloric acid solution, followed by analysis for inorganic phosphate (Stainton et al. 1977). Particulate carbon and nitrogen concentrations were measured on a Carlo Erba EA 1108 CHN Analyzer. Prior to analyses, particulate C / N samples were treated with a dilute solution of HCl to remove any inorganic carbon that might be present in the form of carbonates.

4. *Cladophora* Abundance and its Relation to Light, Temperature and Nutrients

Previous research has shown that *Cladophora* growth responds in a predictable fashion to nutrient availability, light and temperature (Auer and Canale 1982a, 1982b; Graham et al. 1982; Canale and Auer 1982). Therefore, by monitoring these variables in the nearshore environment, along with *Cladophora* biomass, it is possible to determine the relative influence of each variable on *Cladophora* growth rate and abundance.

We selected a station several km north of Milwaukee, offshore of Atwater Beach, to monitor *Cladophora* biomass and environmental conditions (Fig. 1). The monitoring station was at a depth of 10 meters (32 feet), in a region where the lake bottom was a mixture of large boulders, smaller rocks, gravel, and a small amount of soft, sandy bottom. This site was selected because it has a predominantly hard, rocky substratum suitable for *Cladophora* growth, it is within easy boating distance of the Great Lakes WATER Institute, and it is close enough to the city of Milwaukee to potentially be influenced by Milwaukee Harbor effluent, while being outside of the area of heavy boat / ship traffic in the immediate vicinity of the harbor. A depth of 10 m was selected based on previous reconnaissance dives, during which abundant *Cladophora* had been observed at this depth. While *Cladophora* is also more abundant at shallower depths, 10 m seemed ideal because *Cladophora* is not as prone to be affected by turbulence-induced sloughing as it is at shallower depths, and therefore *Cladophora* abundance and nutritional status are more likely to be related to ambient nutrient concentrations, irradiance (light), and temperature.

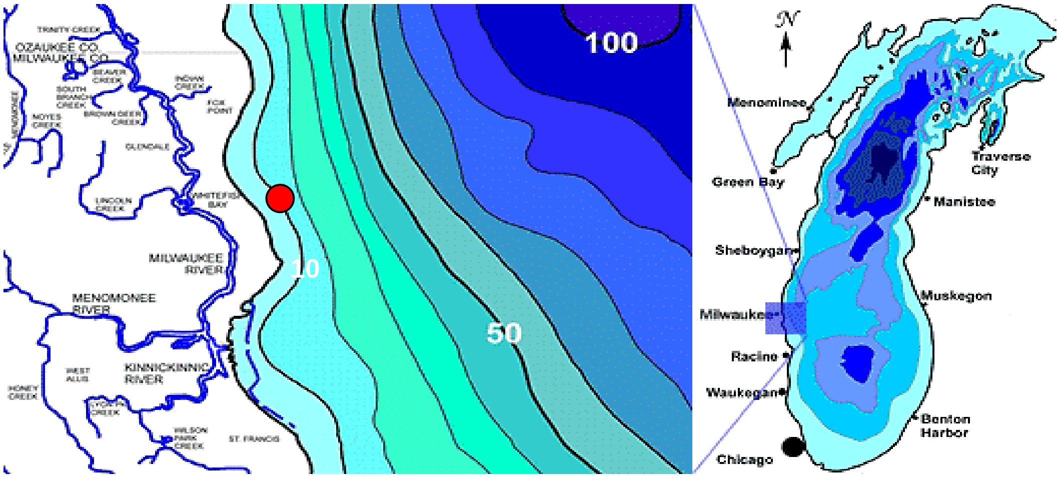


Figure 1. Atwater sampling site at 10 m depth off the coast north of Milwaukee Harbor. Depth contours are given in 10-meter intervals.

Methods employed for *Cladophora* collection are described in the methods overview section at the beginning of this report. To monitor temperature, a HOBO Water Temp Pro Logger® was fixed at 10 m, set to record temperature at 30-minute intervals (accuracy = 0.2°C, resolution = 0.02°C). Irradiance was recorded by mounting a HOBO® light logger inside a transparent, waterproof housing. The logger was pre-calibrated against a PAR (photosynthetic available radiance) sensor, so that measurements could be converted to approximate PAR values, which are more relevant for the purpose of comparing irradiance with *Cladophora* growth and abundance.

On each sampling date, water samples were collected just above *Cladophora* beds for the analysis of dissolved nutrients. While dissolved nutrient concentrations provide some indication of the potential for *Cladophora* growth, algal growth is more directly related to cellular internal nutrient concentrations (Auer and Canale 1982b). Therefore, on each sampling date *Cladophora* samples were collected and returned to the lab, where they where *Cladophora* carbon, nitrogen and phosphorus content was measured.

4.1 Temporal Trend of *Cladophora* Biomass, *Cladophora* Phosphorus Content, Water Column Nutrients, and Nutrient Uptake Enzymes

On the first sampling date in late spring (June 15, 2003), *Cladophora* biomass was relatively high, despite the fact that water temperatures were between 7.3°C (45°F) and 10°C (50°C) during the month of May. At this time, the mean *Cladophora* phosphorus content was 1.4 μg / mg (0.14%) (Fig. 2). However, by the end of June P content had decreased to 0.8 μg / mg, with a further decrease to 0.7 μg / mg in July. The low P content in July was accompanied by a low biomass concentration of 32 g / m^2 (dry weight). In early August, the P content increased to a level similar to that in early June, and levels remained relatively high on the sampling dates in September and October.

Cladophora biomass was also compared to concentrations of dissolved nutrients in the water. In addition, nutrient uptake / assimilation enzymes were measured to further ascertain which nutrient was most limiting to *Cladophora* growth. Water samples were analyzed from our standard 10 m deep sampling station off Atwater beach north of Linnwood intake facility (Fig. 1).

The seasonal patterns of soluble nutrients in the water column at the Atwater sampling site are shown in Fig. 3. There were some oscillations in available inorganic N with levels of NO_3^- consistently high enough to support algal growth. Ammonium (NH_4^+) levels were lower, possibly as a result of preferential uptake by phytoplankton and benthic algae. Soluble phosphate (PO_4^{3-}) availability was consistently very low over the growing season, conditions which will induce P

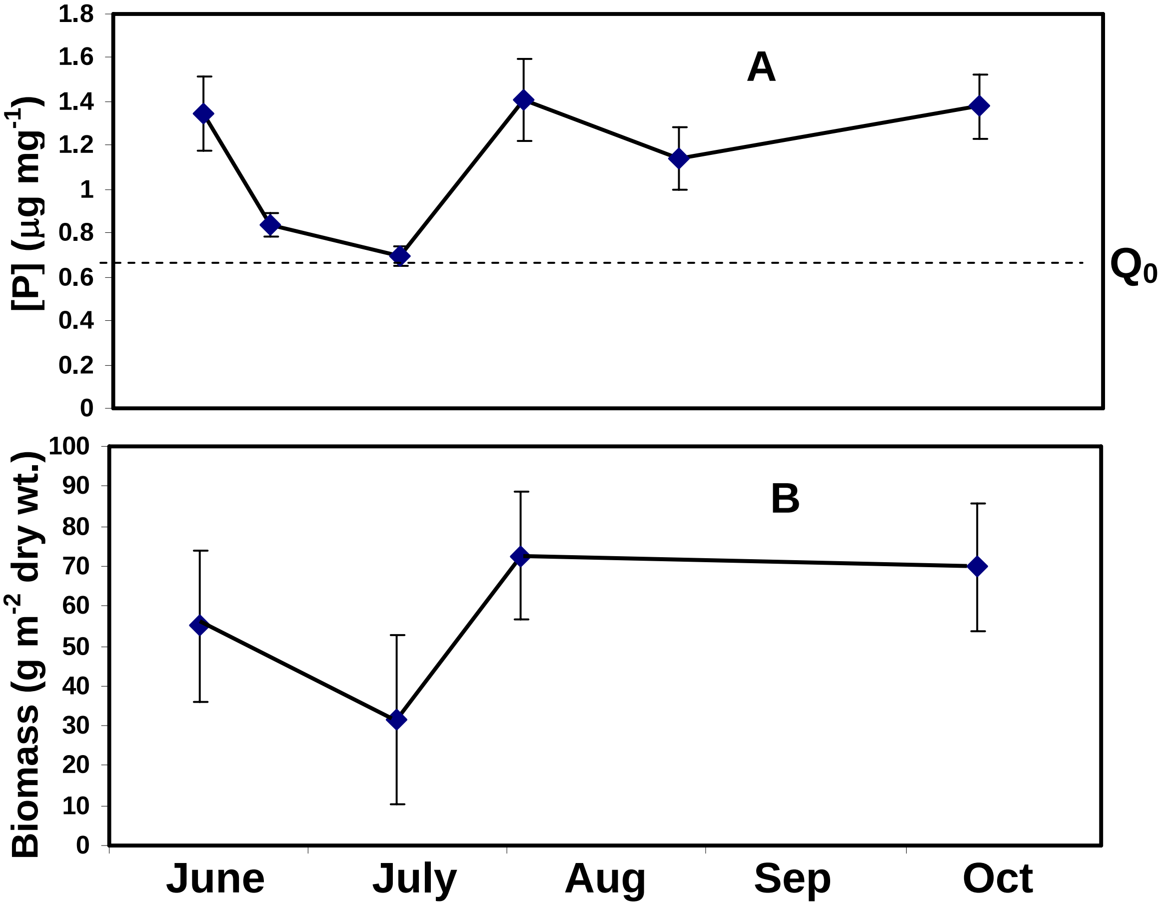


Fig. 2. Temporal trend of (A) *Cladophora* tissue phosphorus content, and (B) *Cladophora* at the Atwater monitoring station in 2004(depth = 10 m). Q_0 = the minimu tissue P content required for positive net growth, based on lab experiments conducted by Auer and Canale (1982b).

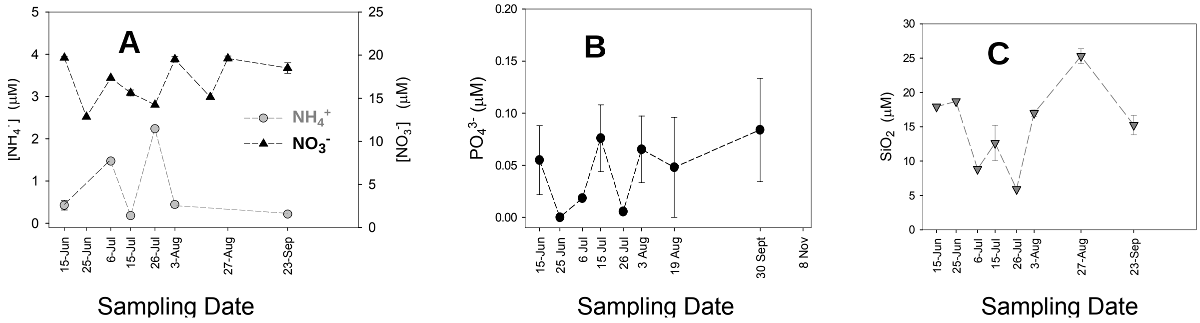


Fig. 3. Seasonal variation in soluble nutrients in the water column at Atwater 10 m site, 2004. **A**. Inorganic nitrogen (NO_3^- + NO_2^-, NH_4^+) **B**. soluble reactive phosphate (PO_4^{3-}). **C**. Silicate (SiO_2).

limitation of algal growth. There was no evidence for draw-down of P from algal uptake over the growing season with slightly higher levels in Aug - Sept than at points earlier in the season. Silicate was consistently high enough to support growth of silicate-requiring algae (i.e. diatoms).

Earlier lab experiments by Auer and Canale (1982b) have indicated that *Cladophora* growth rate is strongly related to internal P content. At optimum irradiance and temperature levels, a maximum net growth rate of approximately 0.6 day^{-1} is achieved when internal cellular content of P is 3 μg / mg or greater. At this growth rate, *Cladophora* will increase its mass by approximately 60% per day. At internal P concentrations below 3 μg / mg, growth rate is P dependent, and at a P content of 0.6 μg / mg or less, *Cladophora* growth stops. At a depth of 10 m in Lake Michigan, growth rates will likely be less than those reported above, due to the effects of lower irradiance and temperatures (Graham et al. 1982). The range over which *Cladophora* growth is strongly related to P content may be expected to vary to some degree with irradiance and temperature conditions. However, Auer and Canale observed that although maximum net growth rates in field experiments were influenced by irradiance and temperature, the minimum P quota of 0.6 μg / mg measured in lab experiments is similar to that measured in the field. Therefore the P content range of 0.6 to 3 μg / mg appears to be a consistent range within which *Cladophora* growth rate is strongly influenced by P availability. The measurements of P content made at the Atwater standard station, as well as those made at other locations in Lake Michigan (see below), are all within the lower portion of this range (Fig. 4). This has two consequences related to the management of *Cladophora* in the nearshore waters of Lake Michigan. First, if there is any further increase in the availability of P within the nearshore zone, there will be a concomitant increase in *Cladophora* growth rates, which will likely result in an increase in *Cladophora* abundance. This is because the P content of *Cladophora* is currently below that required to produce maximum growth rate. The second consequence is that small reductions in P availability may result in large reductions in *Cladophora* growth rate and biomass. Based on the results of Auer and Canale (1982b), a reduction of *Cladophora* P content from 1.3 to 0.8 μg / mg would result in approximately a 50% decrease in *Cladophora* growth rate.

If a small reduction in P availability might lead to a large reduction in *Cladophora* abundance, the obvious question is: What should the target level be for nearshore P concentration, and are the specific management interventions that can help to achieve that target. Current P concentrations in Lake Michigan *Cladophora* are generally between 1.0 and 1.6 μg / mg. Determining the target level of ambient soluble reactive P (SRP) concentration required to reduce *Cladophora* P content to 0.8 μg / mg is not simple, because the relationship between ambient soluble P concentration and *Cladophora* P content is influenced by light and temperature, which change with depth, location and time. Based on experimental and model results (Canale and Auer 1982), at near-optimal light and temperature conditions a reduction in ambient soluble reactive

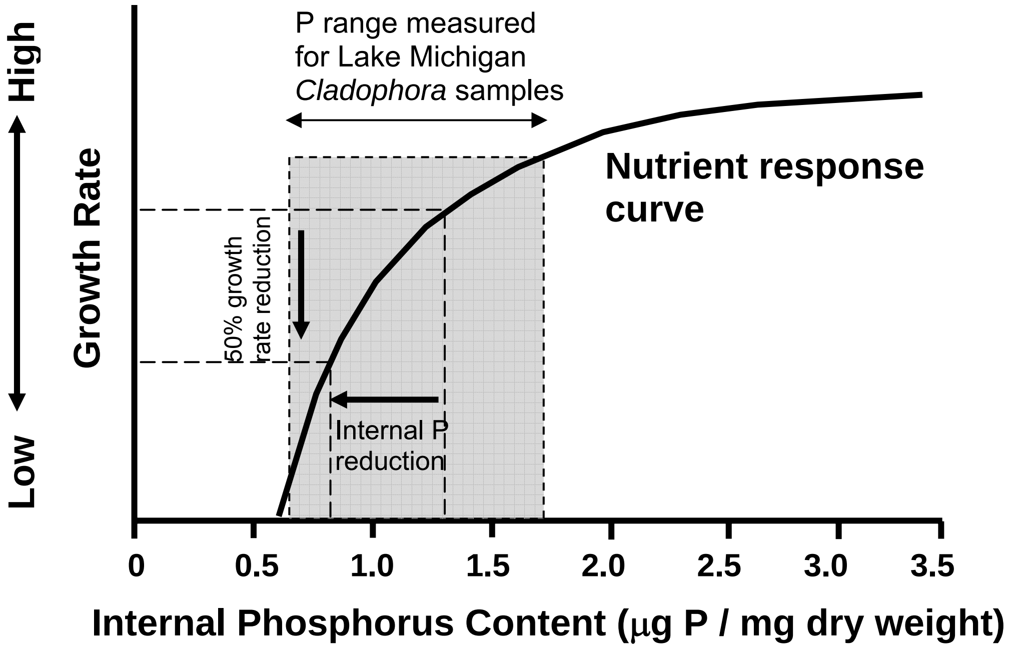


Fig. 4. P content of Lake Michigan *Cladophora* measured in this study (shaded area) compared with experimentally determined relationship between *Cladophora* P content and growth rate (Auer and Canale 1982b). Dashed lines illustrate the reduction in growth rate that would result from a reduction in *Cladophora* internal P content from 1.3 µg/mg to 0.8 µg/mg.

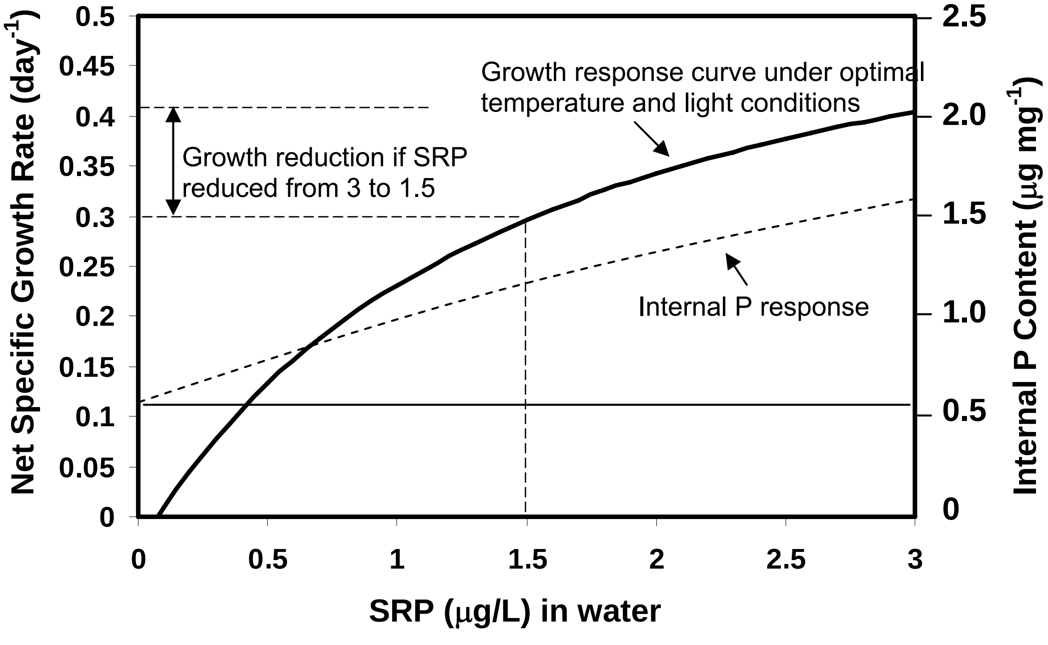


Fig. 5. Expected relationship between the concentration of soluble reactive phosphorus concentration in water and *Cladophora* growth rate under optimal temperature and light conditions, based on experimental and model results of Canale and Auer 1982).

phosphorus concentration from about 3 μg / L to about 1.5 μg / L will result in a *Cladophora* content P decrease from around 1.6 to 1.1 μg / mg, and a growth rate decrease from around 0.4 day^{-1} to 0.3 day^{-1} (Fig. 5). At lower temperature and light levels, such as those generally observed at the Atwater *Cladophora* monitoring station, a P content of 0.8 μg / mg should be achievable at SRP concentrations between 1.5 and 2.5 μg / L. However, at the Atwater monitoring station, where SRP concentrations in lake water averaged 1.5 μg/L during the study period, *Cladophora* P content is generally greater than 0.8 μg / mg. This suggests that *Cladophora* P content in Lake Michigan may not be entirely controlled by ambient dissolved P concentrations, and that there may be another source of P for *Cladophora*. The most likely candidate is the zebra mussel. The potential impact of the zebra mussel is further discussed in section 5.

The rationale for examining enzyme activity in *Cladophora* is that enzyme activities in algae are regulated in response to available nutrients and are thus a physiological index of nutrient status (Beardall et al. 2001). Nitrate reductase (NR) catalyses the initial reduction of nitrate (NO_3^-) to nitrite (NO_2^-), the rate-limiting step in uptake and assimilation of NO_3^- into amino acids and proteins by algae. NR activity is regulated in response to available NO_3^- and is suppressed by high ammonium (NH_4^+) concentration (Berges et al. 1995; Young et al. 2005).

Much of the P available in the aquatic environment is not available for uptake by algae because it is bound to organic chelators. A widely distributed enzyme which cleaves orthophosphate from the organic chelator, releasing soluble phosphate (PO_4^{3-}) for uptake, is alkaline phosphatase (AP). The expression of AP activity is greatly elevated under conditions of low P availability and is suppressed under adequate P supply (Dyhrman and Palenik 1997). Thus AP activity can be used as an index of P limitation in algae.

For the measurement of nitrate reductase activity, *Cladophora* was frozen in liquid nitrogen at the sampling site and stored until assaying for enzyme activity. There was some indication that NR activity in *Cladophora* is greater when nitrate concentrations are lower (Fig. 6).

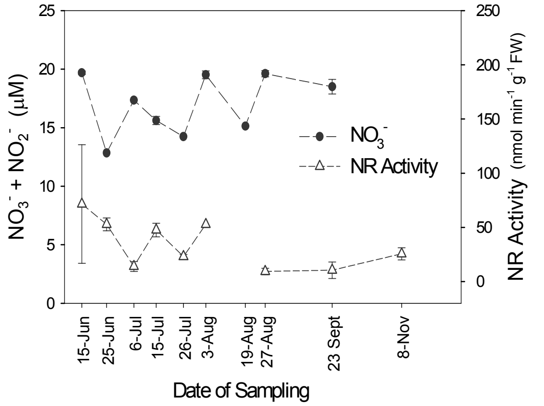


Figure 6. Seasonal variation in nitrate reductase (NR) activity in *Cladophora* and water column NO_3^- concentration at Atwater 10 m site. Points are mean ± standard deviation, n ≥ 2 for NO_3^-, n = 8 for NR Activity..

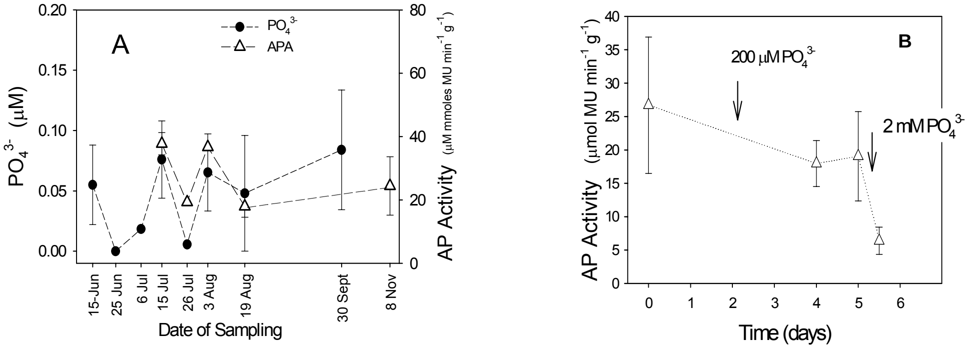


Figure 7. **A.** Seasonal variation in alkaline phosphatase (AP) activity in *Cladophora* and water column soluble PO$_4^{3-}$ from Atwater 10 m site. Points are mean ± std dev, $n \le 2$ (PO$_4^{3-}$), $n = 8$ (APA). **B.** Suppression of *Cladophora* alkaline phosphatase activity by PO$_4^{3-}$- enrichment in laboratory culture. Points are means ± std dev, $n = 8$.

For alkaline phosphatase AP activity, measurements were made in freshly collected *Cladophora* from the Atwater site over the summer growing period. Alkaline phosphatase activity (APA) was consistently measured in *Cladophora* sampled from the Atwater 10 m site (Fig. 7A). As APA is an index of P limitation, this suggested that *Cladophora* at that site were growth-limited by P. APA in *Cladophora* was demonstrated to be responsive to seasonal changes in water column available P. This was verified in laboratory tests. When 2 mM PO$_4^{3-}$ was added to the growth medium for freshly collected *Cladophora*, APA was significantly reduced from 20 to 6 μmol MU min^{-1}g^{-1} FW within 5 hours (Fig. 7B).

Conclusions about enzymes

- Nitrate reductase activity oscillates with available NO$_3^-$. There was no evidence for inorganic N limitation of *Cladophora* growth.
- Alkaline phosphatase activity (APA) in *Cladophora* is responsive to available P, with rapid suppression of APA following PO$_4^{3-}$ enrichment of growth medium.
- Elevated alkaline phosphatase activity in *Cladophora* indicates P limitation of *Cladophora* growth mid - late summer.
- In conjunction with other indicies of nutrient availability and internal tissue nutrient concentrations, enzyme activity can be a useful additional parameter for examining nutrient status of *Cladophora* (and other algae).
- P limitation is evident in *Cladophora* based on

    - Consistently very low soluble phosphate available in water
    - continual alkaline phosphatase enzyme activity
    - Nutrient stoichiometry

4.2 Spatial Trend of *Cladophora* Abundance and Nutrient Status

If river inputs of nutrients have a significant influence on *Cladophora* growth, then it is reasonable to expect that *Cladophora* nutrient status and abundance will vary according to proximity to river nutrient sources. To test this hypothesis, a longshore survey of *Cladophora* biomass and nutrient content in the Milwaukee regions was conducted in September 2004. Samples were collected by SCUBA divers from a depth of 8-10 m at all stations, which included Cudahy, Oak Creek, Green Can Reef (approximately 4-5 km southeast of Milwaukee Harbor), Bradford Beach, Atwater Park and Fox Point. In addition, as part of a separate study, samples were collected at several other locations between Milwaukee and the Door Peninsula.

In the Milwaukee region, *Cladophora* biomass was significantly greater north of the city than it was south of the city (Fig. 8A). This was surprising, since a similar survey in September 2003 had revealed the opposite trend. However, this trend does not appear to be related to phosphorus supply, as *Cladophora* south of the city had a P content similar to or greater than that for *Cladophora* north of the city (Fig. 8B). This is suggestive of a moderate river influence on P availability, as prevailing north-to-south currents are expected to result in the Milwaukee Harbor plume flowing more to the south than the north.

On a larger spatial scale, benthic algal biomass at three stations north of Milwaukee (Sheboygan, Algoma and Bailey's Harbor) was similar to or greater than that measured near Milwaukee (Fig. 8A). At the Bailey's Harbor site, algae was dominated by another genus of green algae, *Chara* sp., with only small amounts of *Cladophora* being present. This was likely a result of the mixed hard / soft lake bottom at this site, since *Chara* grows better in soft sediment. Despite high biomass at the three northern stations, *Cladophora* P content at these sites was similar to or lower than that measured near Milwaukee (Fig. 8B).

These large-scale results support earlier reports of *Cladophora* problems along many parts of Lake Michigan's western shore, and indicate that the problem is not confined to the Milwaukee region. However, because all sample sites (with the exception of the soft-bottom Bailey's Harbor site) were collected near the mouths of major rivers, it is not yet possible to conclusively determine the extent to which P inputs from rivers may be responsible for excessive *Cladophora* growth. Further sampling planned for 2005 at non-river sites should help to resolve this question.

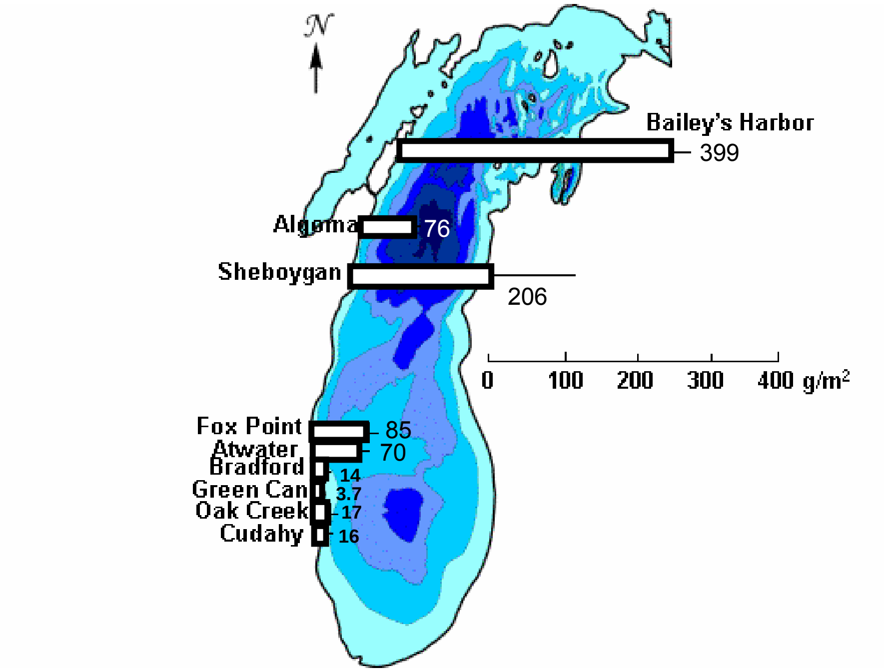


Fig. 8A. Longshore distribution of *Cladophora* biomass in September, 2004.

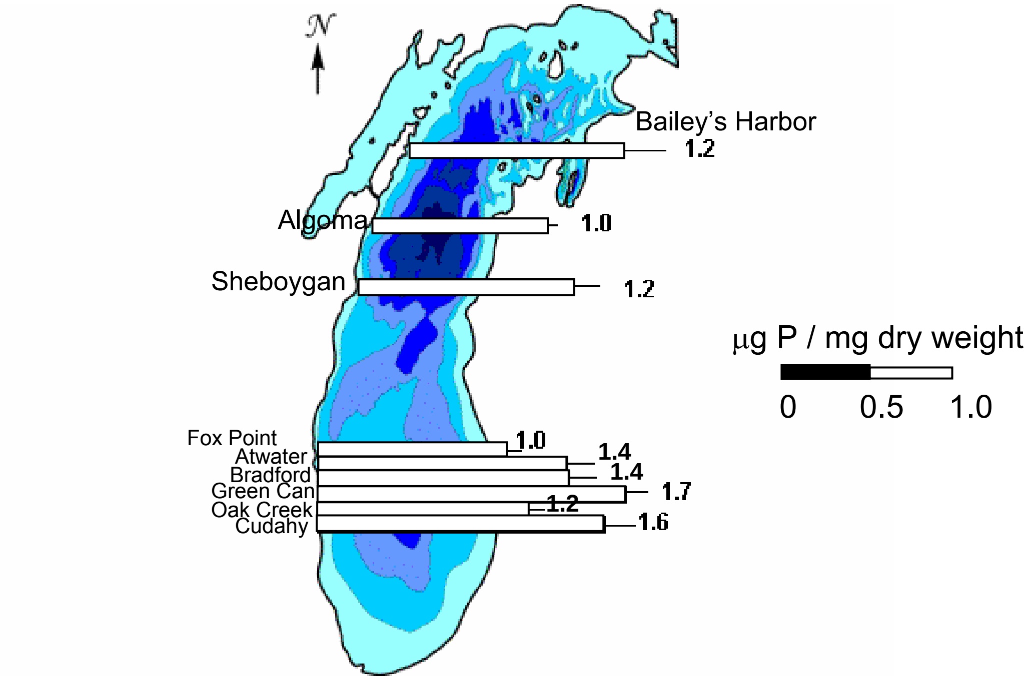


Fig. 8B. Longshore distribution of *Cladophora* internal phosphorus content, September 2004.

4.3 The Influence of Temperature on *Cladophora* Growth

Fig. 9 presents the temperature record for the period June 2003 to October 2004 at a depth of 10 m, along with indications of *Cladophora* temperature requirements based on the experiments of Graham et al. (1982). Prior to mid-April, temperature was too low to allow *Cladophora* growth. In May growth was possible, although temperatures were well below optimum. Although the *in situ* temperature logger was not functioning in late June and early July, the temperature trend indicates that optimum growth temperature was probably reached in early July. A comparison with *Cladophora* internal cellular P content (Fig. 2) indicates that it was during this period that the lowest P content measurements were made. P content is a function of P uptake and *Cladophora* growth rate. P uptake will increase P content, while growth results in the distribution of internal P into a larger biomass pool. It appears that the warmer temperatures in late June and early July resulted in an increased growth rate. Growth during this period relied to some extent on internal P stores, so that *Cladophora* P content was reduced due to a fixed amount of internal P being diluted into a large biomass pool.

Had internal P content remained at its late June – early July level, growth would have been minimal for the remainder of the summer. However, P content measured on other dates between July and October was moderately high. Because samples were not collected at a high frequency, it is difficult to determine whether P content was continuously high during this period, or if it occasionally reached levels as low as those in July. Between July and September, temperatures in the nearshore zone fluctuate widely, probably as a result of internal waves and wind-induced upwelling. Coincidentally, all sampling was done on days when water temperature was low. During this period, slowed growth may have resulted in P accumulation in *Cladophora*, similar to that observed in early June. However, *Cladophora* samples collected at other locations on warmer days (Fig. 8B) also had internal P concentrations between 1.0 and 1.7 μg/mg, suggesting that there is a continuous supply of P in the latter half of summer that is supporting *Cladophora* growth.

Fig. 9 illustrates that nearshore temperatures in Lake Michigan during the summer fluctuate between optimal and suboptimal for *Cladophora* growth. While temperatures are occasionally greater than optimal, they are more frequently less than optimal. Hence, an increase in average summer temperature might be expected to increase *Cladophora* growth rates. This begs the question: might temperature be responsible for the increase in *Cladophora* abundance that has been observed in the past decade?

An examination of temperature records for the water intake at the Linnwood Water Treatment (approximately 3 km north of Milwaukee Harbor) indicates that, while average summer water temperature can vary by more than 3^{o}C from one

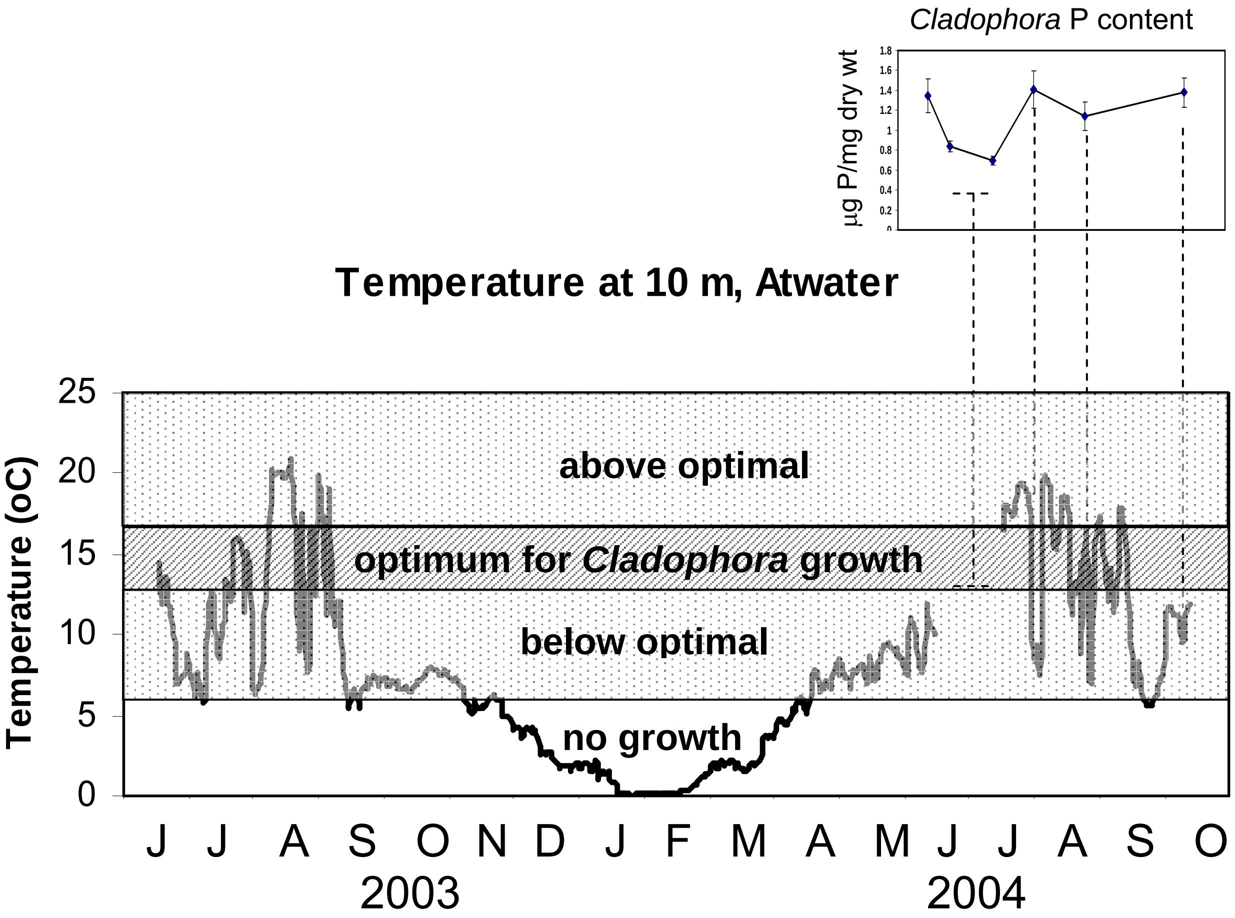


Fig. 9. Temperature record at a depth of 10 m, Atwater monitoring station. *Cladophora* P content during 2004 is shown for comparison. No data are available for mid-June to mid-July 2004.

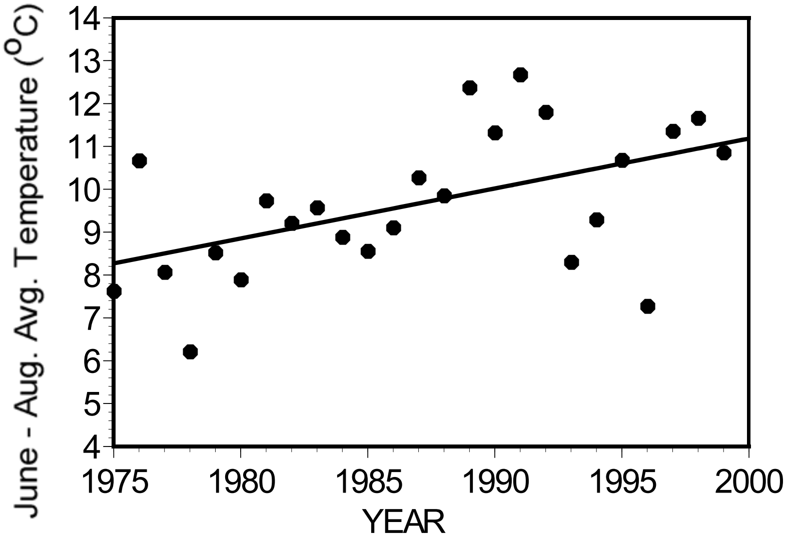


Fig. 10. Temperature trend for the Milwaukee nearshore area from 1975 to 2000. Data provided by Milwaukee Water Works for the Linnwood Water Treatment Plant. Data are for measurements of water drawn from a depth of ~15 m.

year to the next, there has been a general increasing trend, with average June-August temperature increasing from around 8.2°C in the mid 1970s to around 11°C in 2000 (Fig. 10). A precise average June-August temperature for 2004 is not yet known, since our temperature logger did not function for part of June and July, but if a linear trend is assumed between mid-June and mid July, the mean June – August temperature for 2004 is 13.95 °C, suggesting the warming trend has continued since 2000. The magnitude of this trend is too large to be the result of air temperature warming over this period. Rather, it is likely the result of changing lake circulation patterns, which may be related to changes in summer wind patterns (Waples and Klump 2002).

While this temperature change is relatively large, it is uncertain whether it has resulted in a large increase in *Cladophora* growth. The *Cladophora* response may depend in part on light availability. Graham et al. (1982) demonstrated that, at an irradiance level of 150 μmol photons/m^2/s, *Cladophora* photosynthetic rate at 10°C was nearly equal to that at 15°C. But at an irradiance of 75 μmol photons/m^2/s (which is similar to average daily irradiance during the summer at the 10 m sampling site), net photosynthetic rate at 10°C is less than half of what it is at 15°C. This suggests that the historic temperature increase may have had a greater effect on *Cladophora* at depths of around 10 m than at shallower depths.

4.4 The Influence of Light on *Cladophora* Growth

A. Estimating Photosynthesis using Chlorophyll a Fluorescence

To measure the relationship between *Cladophora* photosynthesis and light, we used a rapid *in situ* method with an underwater pulse amplitude modulated chlorophyll fluorometer (PAM; Walz GmbH, Germany). This method uses the chlorophyll in *Cladophora* to give a very sensitive measure of photosynthetic activity.

- Photosynthesis vs Irradiance (P v I) responses were measured on *Cladophora* at the standard 10 m Atwater site
- Photosynthesis-irradiance data were modeled to derive light-dependent photosynthesis rate and maximum photosynthesis rate, and the light intensity at which the onset of light limitation occurs (I_k) (Fig. 11).
- Light saturation levels in *Cladophora* were compared with light logger data to estimate extent of light limitation of photosynthesis of *Cladophora in situ.*

The P v I curves suggested that the onset of light saturation of photosynthesis by *Cladophora* occurred at ~ 70 μmol photons m^{-2} s^{-1}. (Fig. 11).

For comparison with the light logger data (Fig. 12), the 70 μmol photons m^{-2} s^{-1} is equivalent to ~3 mol photons m^{-2} d^{-1} (assuming 12 hours of light) marked on

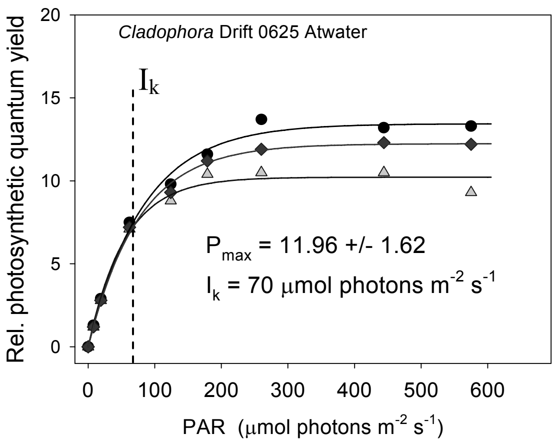


Figure 11. Three replicate measurements of P v I curves of *Cladophora* at the10 m Atwater site on 25 June, 2004. The lines represent models of P v I relationship from Webb et al. (1974). Values of P_{max} and I_k are modelled means (std dev) of the three replicate curves. Ik is the irradiance below which *Cladophora* photosynthesis begins to be light limited. Note: 70 μmol photons m^{-2} s^{-1} = 6.1 mol photons m^{-2} d^{-1}.

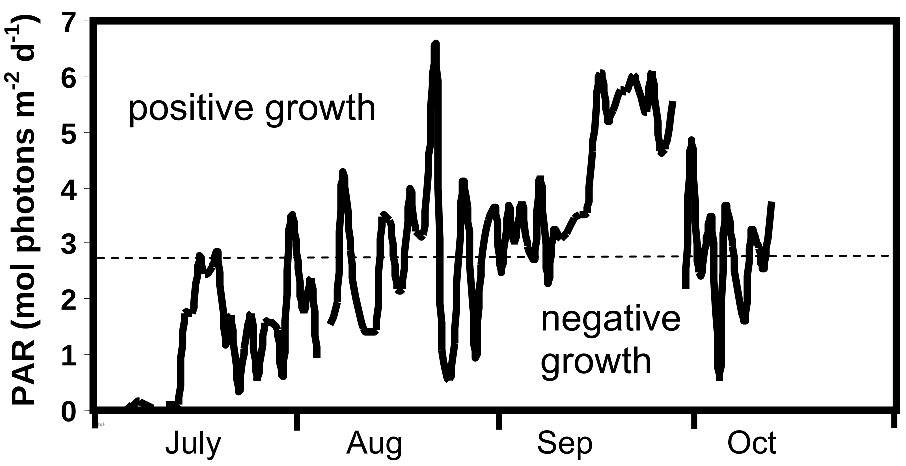


Fig. 12. Irradiance (light) levels recorded at a depth of 10 m at the Atwater monitoring station in 2004. The horizontal dashed line represents the threshold below which there is insufficient light for positive net growth, based on previous observations by Graham et al. (1982). PAR = Photosynthetic Available Radiation (the total flux of photons within the wavelength range of approximately 400 to 700 nm.

Figure 12. At the 10 m Atwater site, in over 86% of daylight hours there was less light than is required to saturate photosynthesis of *Cladophora*. This suggests that for the majority of time during the growing season, an increase in light reaching the benthic substratum at this site would have resulted in more photosynthesis by *Cladophora*.

Cladophora collected from Lake Michigan at the 10 m Atwater site, macroscopically appeared brown, rather than green. Microscopic examination showed the filaments were heavily epiphytised with, predominantly, diatoms (Fig. 13). The heavy epiphyte load may represent a stress to *Cladophora* by shading light, and possibly stripping the water of nutrients and inorganic carbon, reducing availability of those resources for *Cladophora*. This may be a stress factor contributing to the detachment of *Cladophora* from the hard substratum during the summer.

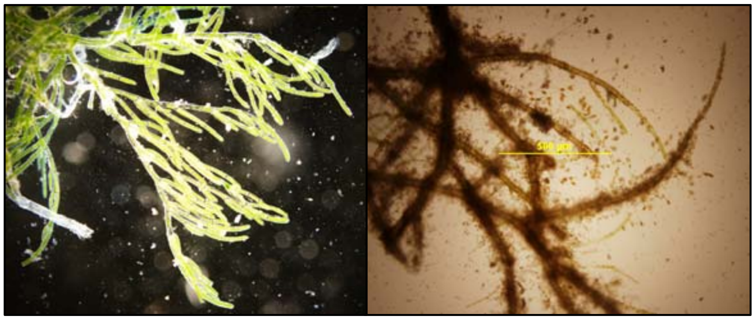


Figure 13. **Left:** Healthy *Cladophora* collected from Bradford Beach Nov 2004. **Right**: *Cladophora* collected from Atwater 10 m site Aug 2004, encrusted with epiphytic diatoms.

Conclusions about Photosynthesis - Light relationships

- Light is probably limiting to *Cladophora* photosynthesis for the majority of the summer growing period
- Heavy epiphyte loads will further increase light limitation for *Cladophora* and may exacerbate nutrient limitation.

B. Underwater Light and *Cladophora* Depth Distribution

Light conditions at the 10 m site from mid-June to mid-September are shown in Fig. 12. Despite the fact that the solar angle at this latitude is high in late June and early July, light levels were relatively low in this period, becoming greater in August. A comparison of these recorded underwater irradiance levels with atmospheric irradiance measurements made at the Great Lakes WATER Institute indicates that the daily underwater fluctuations apparent in Fig. 12 are not related to surface solar radiation, and therefore they are primarily the result of variations in water clarity, with clarity generally being greater in late summer. As discussed above, the decrease in *Cladophora* P content in late June – early July is thought to have been the result of increased growth rates during that period. If light levels were as low during that period as they were in the second half of July, this would not have been possible. However, if water clarity was sufficient during that period, when atmospheric irradiance is at its annual maximum, there would have been more than enough light at a depth of 10 m to support growth. The decrease in *Cladophora* P content suggests that this was the case. The recorded irradiance levels for the second half of July were at or below the level required for positive *Cladophora* growth. This likely was a causative factor in the observed increase of *Cladophora* P content in late July, as *Cladophora* would have continued to take up P, but in the absence of growth this P accumulated within the existing *Cladophora* biomass. The apparent positive effect of temperature on growth rate in late June – early July, and the negative effect of low irradiance in late July illustrate the strong roles that both temperature and light appear to play in regulating *Cladophora* growth.

In August 2004, a depth transect at the Atwater site was sampled to determine the depth distribution of *Cladophora*, and the effect of depth on *Cladophora* nutrient content. Sampling depths were 5, 10, 15 and 19 m. *Cladophora* abundanced was similar at depths of 5 and 10 m, but decreased greatly between 10 and 15 m, and was very low at a depth of 19 m (Fig. 14A). The opposite trend was apparent for *Cladophora* P content, which was greater below 10 m than it was at 10 and 5 m (Fig. 14B). The combination of less biomass and greater P content at depths below 10 m suggests that at these depths, *Cladophora* is no longer P-limited, but is likely light-limited. Below 10 m, low levels of light results in slow growth rates, and *Cladophora* is able to uptake P at a rate sufficient to support these slow growth rates. This transition from P-limitation at depths of 10 m or less to light limitation at depths greater than 10 is in agreement with the observed light data recorded at 10 m, which was sufficient for positive growth for parts of August and much of September (and presumably June), but insufficient at other times. Between 10 and 15 m, light levels are likely sufficient to support growth only on the occasional day when water clarity is very high and the sky is cloudless.

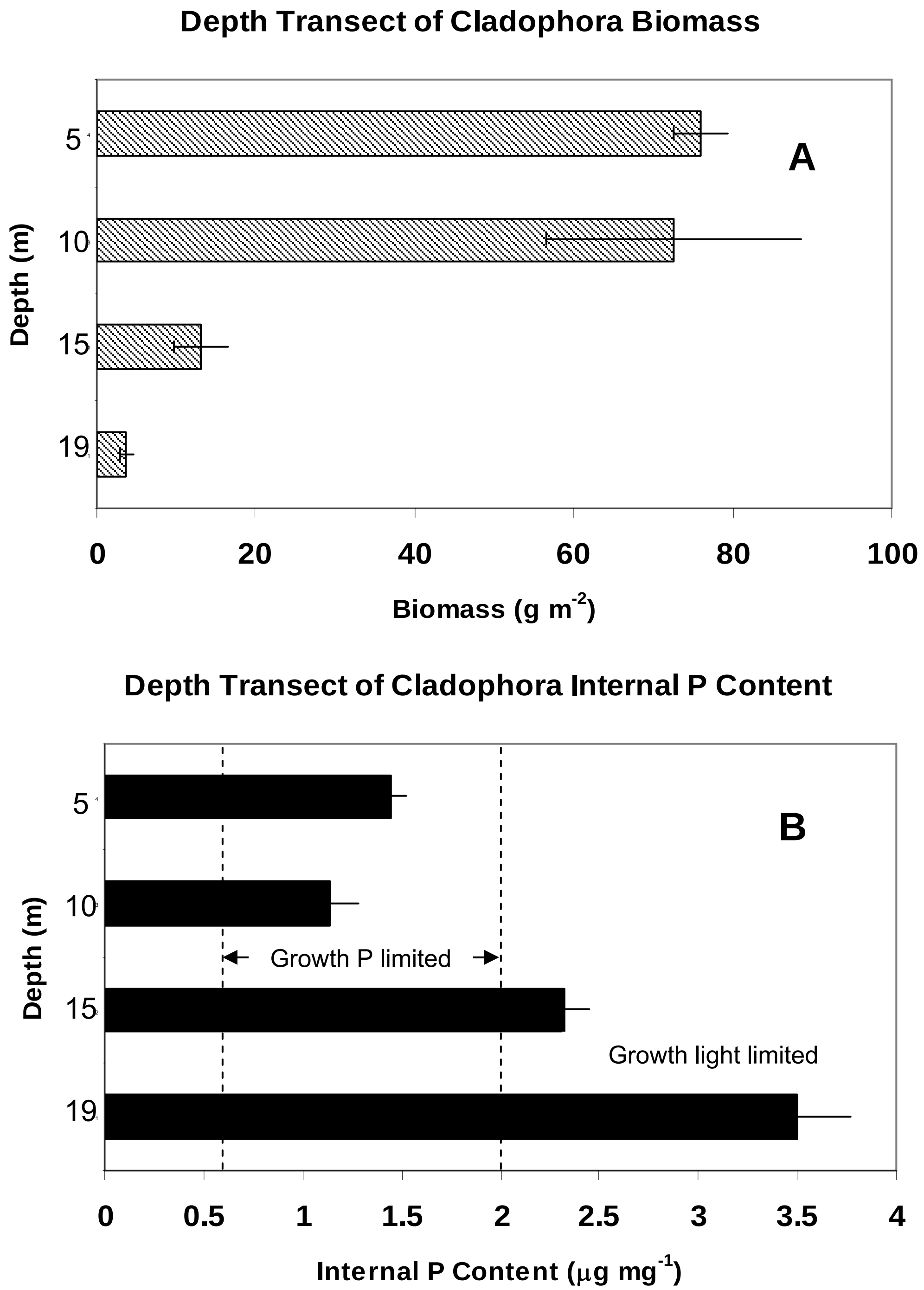


Fig. 14. Depth distribution of *Cladophora* biomass (A) and *Cladophora* internal P content (B) at the Atwater monitoring site in August 2003. Vertical dashed lines in plot B define the range of P content over which growth is expected to be P limited, based on the experimental results of Auer and Canale (1982b). Below a P content of 0.6 μg/mg, no growth is expected.

Historic water clarity data (provided by MMSD, Fig. 15) indicates that nearshore water clarity in the Milwaukee region of Lake Michigan has increased markedly since 1990. This increased clarity is almost definitely the result of the establishment of the zebra mussel (*Dreissena polymorpha*). Individual zebra mussels can filter up to 1 liter of water per day, and can reach population densities of several hundred thousand per square meter (Klerks et al. 1996). As a result, they are capable of filtering a large portion of the water volume in the nearshore zone over relatively short time periods, resulting in removal of particles from the water, and increased water clarity. Similar increases in water clarity have been observed in other lakes following the establishment of zebra mussels (Fahnenstiel et al. 1995), and in some cases (e.g. Lake St. Clair) the increased depth of light penetration has resulted in the proliferation of benthic algae and macrophytes (Nalepa et al. 1996).

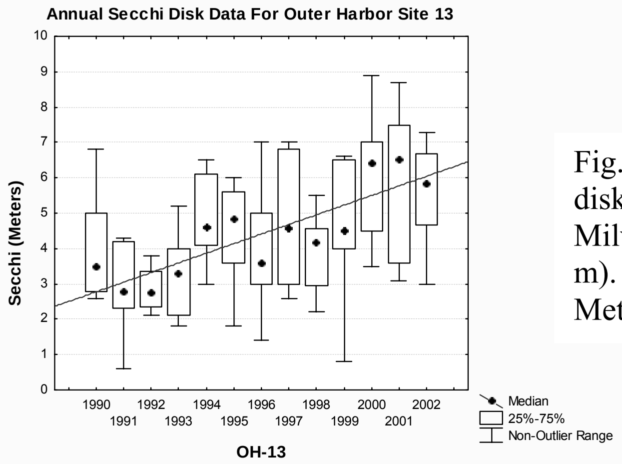


Fig. 15. Temporal trend of secchi disk depths measured just south of Milwaukee, 2km offshore ($Z_{max} = 10$ m). Data provided by Milwaukee Metropolitan Sewerage District.

The effect of decreased Secchi disk depth on underwater irradiance can be determined by converting the Secchi disk depth to a light extinction coefficient. This can be approximated using the relationship

$$k = 1.7 / SD$$

where k = extinction coefficient (units = 1/m, or m^{-1}), and SD = Secchi depth (m).

Based on this relationship, a Secchi depth of 3 m (representative of the early 1990s) is equivalent to an extinction coefficient of 0.57 m^{-1}, while a Secchi depth of 6 m (post-2000) is equivalent to an extinction coefficient of 0.28 m^{-1}. The influence of extinction coefficient on irradiance at a given depth is expressed as

$$I_z = I_o e^{-kz}$$

Where I_z = Irradiance at depth z
I_o = Surface irradiance

Applying the above equation and the different k values for the early 1990s and post-2000, the ratio of the irradiance at depth in the early 1990s to that for the post-2000 period can be determined as

$$\frac{e^{-k_1 z}}{e^{-k_2 z}}$$

Where k_1 and k_2 are the extinction coefficients for the early 1990s and post-2000, respectively. Multiplying this ratio by the measured irradiance values recorded at 10 m in 2004 provides an estimate of what the irradiance levels would have been in the early 1990s, had atmospheric irradiance been identical. The results are shown in Fig. 16. As can be seen, in the early 1990s at a depth of 10 m there would have been insufficient light to support *Cladophora* growth. The increase in water clarity over the past decade will have had a positive effect on *Cladophora* abundance in two ways. At shallow depths, where *Cladophora* has always been present, the increased light levels will have resulted in increased growth rates. This effect is compounded by the increased depth range of *Cladophora*, due to an increase in the compensation depth, i.e. that depth at which light levels are such that photosynthetic and respiratory rates are equal, resulting in zero net growth. Increase water clarity has resulted in greater *Cladophora* growth rates, and a larger area of the lake bottom within which *Cladophora* can grow.

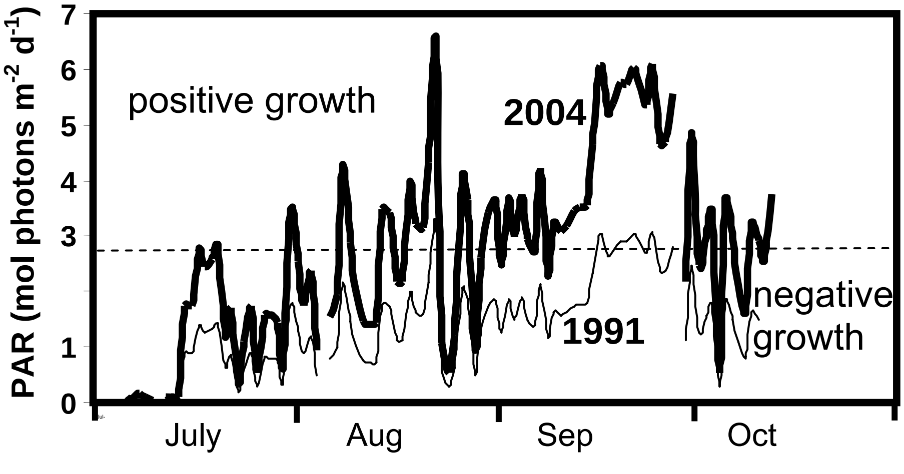


Fig. 16. Irradiance (light) levels recorded at a depth of 10 m at the Atwater monitoring station in 2004 (thick line), and re-constructed irradiance levels at the same location under water clarity conditions observed in 1991 (thin line). See Figure 11 legend for further description of units.

5. The Potential Role of Zebra Mussels as a Nutrient Source for *Cladophora*

A possible alternative nutrient source for *Cladophora* is zebra mussels. Mussels may provide nutrients either by direct excretion of soluble nitrogen and phosphorus (Heath et al. 1995; Mellina et al. 1995), or by egestion of feces and pseudofeces, which are then decomposed by bacteria and invertebrates.

A preliminary test of this hypothesis was made by measuring the P content of different segments of *Cladophora* filaments. If mussels serve as a significant nutrient source for *Cladophora*, then the P content of *Cladophora* may be influenced by its proximity to mussels. The initial approach to this question was to sample *Cladophora* growing both in the presence and absence of zebra mussels. However, it proved nearly impossible to find *Cladophora* growing in the absence of zebra mussels, due to the ubiquity of the mussels. During one dive in June 2004 at the Atwater monitoring site, we were able to find one small patch of *Cladophora* growing on small rocks with no zebra mussels. This was sampled, along with *Cladophora* from nearby rocks with mussels. *Cladophora* biomass concentration on the rocks without zebra mussels was much less than that on rocks with mussels (Fig. 17). However, P content of the *Cladophora* with mussels was not significantly greater than that for *Cladophora* without mussels.

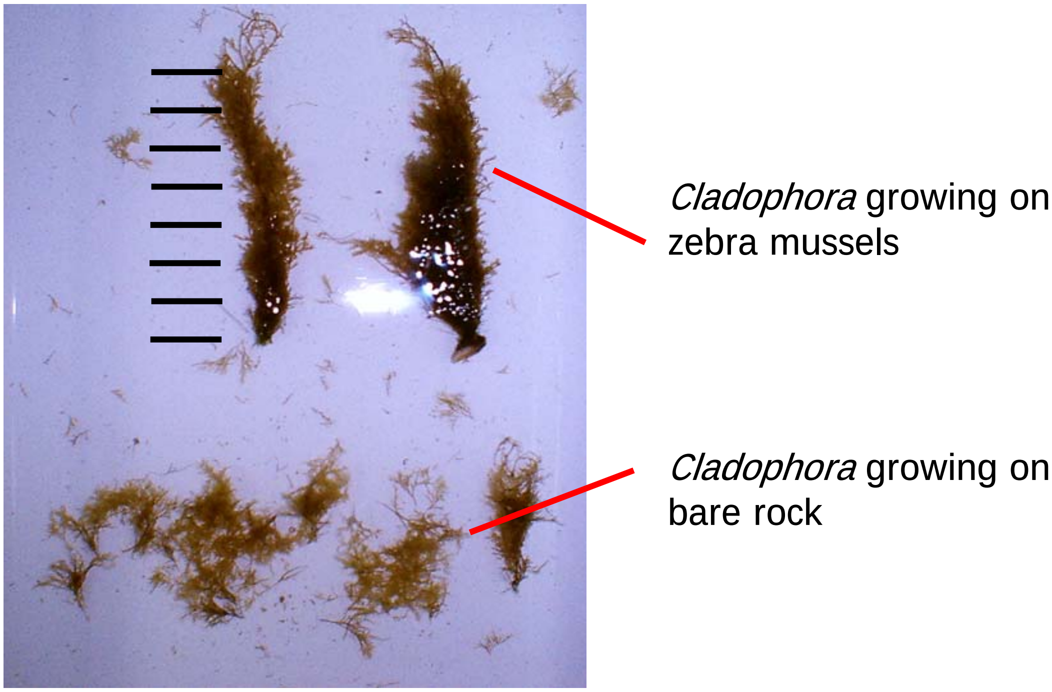


Fig. 17. *Cladophora* collected from rocks with and without mussels in June 2004 at the Atwater monitoring station.

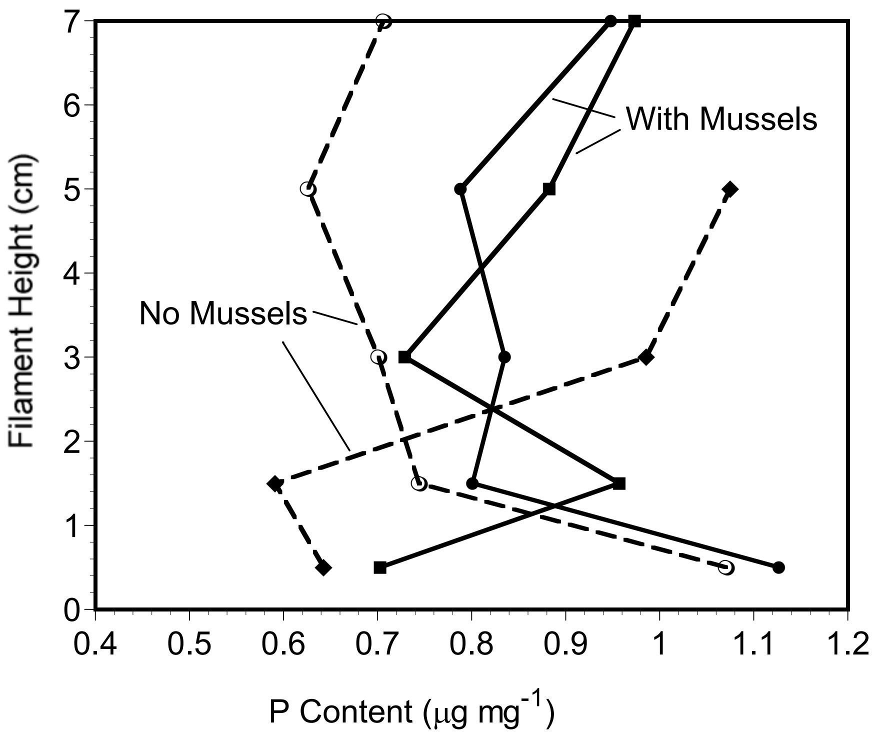
A second approach taken was to measure P of different sections of *Cladophora* filaments, the hypothesis being that lower parts of filaments, which are in closer proximity to zebra mussels, would have a higher P content than more distal parts. The results of these measurements were inconclusive. One of the two "with-mussel" *Cladophora* filaments had higher P content near its base, but one of the "no-mussel" strands did also (Fig. 18). On a subsequent sampling date in July, four separate *Cladophora* filaments were collected from separate horizontal rock surfaces, each of which was also covered with zebra mussels. Three of the four filaments had higher P contents in their lowest 1-cm sections, while the fourth filament had a higher P content in its 1-2 cm section, but low P content in its 0-1 cm section. These results support the possibility that mussels are serving as a phosphorus source to *Cladophora*, but they may also reflect other mechanisms. For example, lower sections of filament will be shaded by higher sections, and therefore the growth rate of lower sections may be less than that of higher sections. These lower growth rates my allow P to accumulate in lower sections, while P in higher sections is rapidly utilized to produce new biomass. Nevertheless, results of recent laboratory experiments (Stankovich 2005) provide strong evidence that zebra mussels have a positive influence on *Cladophora* growth rates.

6. Nutrient Input from Rivers

Within the nearshore region of Lake Michigan, rivers are the primary source of new nutrients, with atmospheric deposition, direct surface runoff and groundwater inflow being possible secondary sources. Therefore nutrient inputs from rivers may have a significant impact on nearshore nutrient concentrations and the production of phytoplankton and benthic algae. The impact of river nutrient inputs on nearshore nutrient concentrations and algal production is determined by 4 factors: 1) river nutrient concentration; 2) river discharge rate; 3) the physical path of the river plume as it enters the lake; 4) the modifying effect of lake temperature and irradiance (light) on algal growth rate.

Within the Milwaukee region there are three main tributary rivers that discharge into Lake Michigan – the Milwaukee River, Menomonee River and Kinnickinnic River. Other rivers of near enough proximity to have a potential impact on nearshore waters in the region include Oak Creek, which drains into the lake approximately 25 km south of Milwaukee Harbor, and the Root River, which drains a catchment just west and south of Milwaukee, entering the lake at Racine.

Cladophora Filament P Content, June 25/04, 10 m, Atwater



Cladophora Filament P Content, July 15/04, 10 m, Atwater

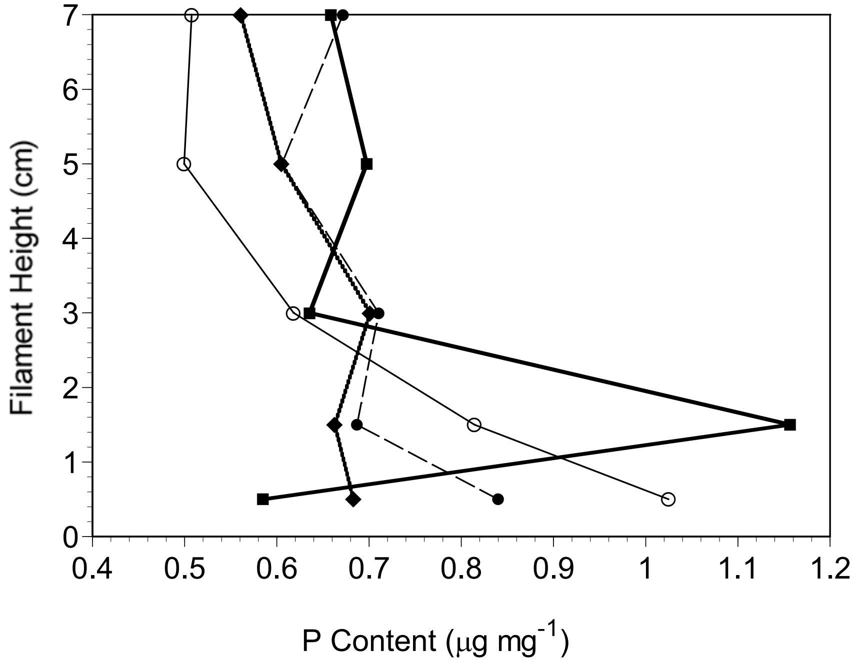


Fig. 18. Phosphorus content of 1 cm sections of *Cladophora* filaments collected at the Atwater monitoring station in June and July 2004.

In order to assess the relative importance of each of these rivers as a nutrient source that might promote *Cladophora* growth in the Milwaukee nearshore region, all 5 rivers were sampled on six separate dates. This allowed a direct comparison of nutrient concentrations under similar meteorological conditions. Rivers were sampled mid-stream either by wading in and collecting with a sampling bucket, or in the case of the larger rivers suspending a sampling bucket from an overpass. In addition, to derive an estimate of nutrient loading to the lake from the three main Milwaukee tributaries, the Milwaukee Harbor Inlet (i.e. the channel connecting the three river mouths to Milwaukee Harbor) was sampled mid-channel from a boat on 10 dates between April and October 2004. This water is a mixture of water entering from the Milwaukee, Menomonee and Kinnickinnic Rivers, which converge upstream of the inlet. On 7 dates, water samples for nutrient analyses were also collected at the three gaps through which Milwaukee Harbor drains to the open lake. Sampling at these sites was done on the harbor (upstream) side of the gaps, in order to ensure that the sample collected did not consist of harbor water that had been diluted with lake water. In most cases, harbor and lake water could be distinguished by a sharp, visible turbidity boundary.

The spring of 2004 was exceptionally wet, with the Milwaukee region receiving more than 8 inches of rain during the month of May. As a result, river discharge was exceptionally high during the second half of May (Fig. 19). Additional large rainfall events in early June resulted in a second discharge peak in that month.

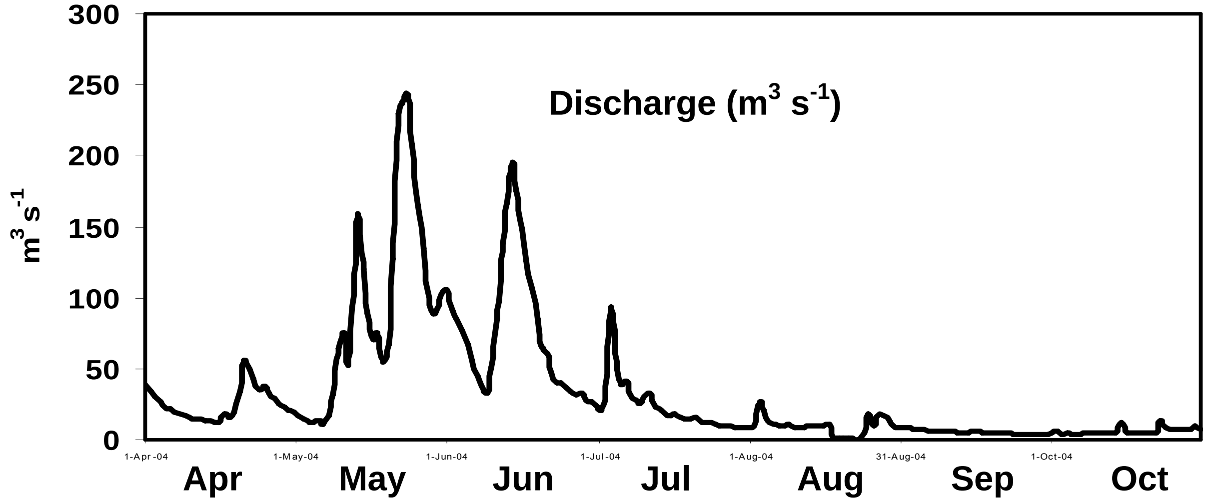


Fig. 19. Combined discharge of the Milwaukee, Menomonee and Kinnickinnic Rivers between April and October, 2004. Data represent discharge for each river at the USGS monitoring station closest to the river mouth. Data obtained from the USGS.

In all five of the sampled rivers, soluble reactive phosphorus concentrations (primarily phosphate) and total dissolved phosphorus concentrations (dissolved inorganic + dissolved organic) concentrations displayed similar temporal trends. Concentrations were low in all rivers in April and May, high during the early summer, and low again in the fall (Fig. 20). The high concentrations in May and June were possibly the result of high sediment erosion rates and/or urban runoff caused by the heavy rainfall during those months. Identifying the nutrient sources is not possible with the current data set, although long-term nutrient records for various sites on these rivers (discussed below) suggest that both agricultural and urban sources may be important.

A comparison of dissolved phosphorus concentrations in the five rivers indicates that summer concentrations were relatively high in the four largest rivers, but remained low in Oak Creek (Fig. 20). Dissolved phosphorus concentrations were generally highest in the Root River. Unlike the other rivers, the Root River was not sampled near its mouth, but approximately 14 km upstream from its mouth. However, land use within most of the watershed downstream from the sampling site is similar to that in the rest of the watershed, and therefore dissolved phosphorus concentrations in the river as it approaches the lake are probably similar to those reported here.

The influence of river-borne phosphorus on algal production in the lake depends in part on the form of phosphorus. Algae are only able to assimilate the inorganic orthophosphate (PO_4^{3-}) form of phosphorus, although some algae are able to indirectly utilize dissolved organic phosphorus by using the enzyme alkaline phosphatase to extracellularly hydrolyze organic phosphorus to PO_4^{3-}. Because this hydrolysis has an energetic cost, PO_4^{3-} is the form that has the greatest influence on algal production. In all the rivers sampled, soluble reactive phosphorus (which is assumed to consist primarily of PO_4^{3-}) made up a significant proportion of the total dissolved P (TDP). The average SRP : TDP ratio for all samples was 0.67 ± 0.23, i.e. more than half of the dissolved phosphorus entering the lake from rivers is immediately available for uptake by algae.

In addition to dissolved phosphorus, particulate phosphorus (PP) made up a significant proportion of the total phosphorus load in rivers (0.4 ± 0.24%). The fate of river-borne particulate phosphorus in Lake Michigan is not well known. Most eutrophication models use total phosphorus as input, assuming that both dissolved and particulate forms are eventually available to algae. However, the fate of particulate phosphorus is influenced by a number of factors, including particulate mineral chemistry, in-lake dissolved PO_4^{3-} and iron concentrations, and redox conditions. Particulate phosphorus that settles in Milwaukee Harbor will likely be exposed to anoxic conditions, which are prevalent in harbor sediments (Klump et al. 2004), and which will promote dissolution of phosphorus. Particulate phosphorus that enters the lake may never become available for algae if it follows a fast track from entry point to deep sediment burial. However,

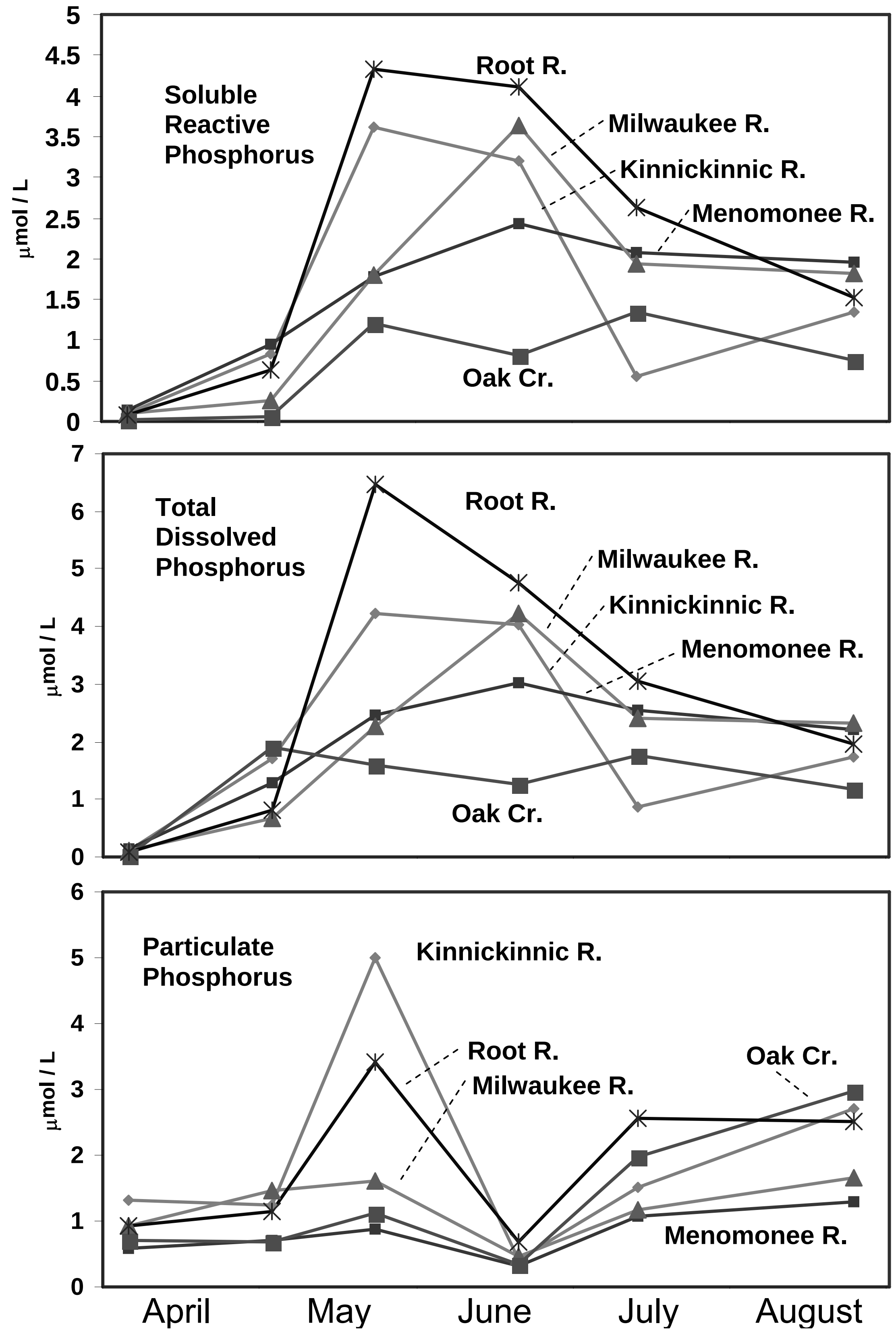


Fig. 20. Dissolved and particulate phosphorus concentration in five Milwaukee area rivers, summer 2004.

this track may have been altered as a result of the establishment of zebra mussels. By filtering large volumes of water, these mussels may promote the retention of particulate phosphorus in the nearshore zone. The mussels may themselves remineralize this phosphorus, making it more available for algae (Arnott and Vanni 1996; James et al. 1997), or they may egest P in the form of feces and pseudofeces, which are then mineralized by bacteria. Considering the relatively large contribution of PP to the total phosphorus load from rivers, a better understanding of the fate of this PP in the lake is required in order to predict its impact on the production of *Cladophora* and other algae.

In order to derive an estimate of phosphorus loading to Lake Michigan from rivers in the Milwaukee area, phosphorus concentrations measured in the inlet to Milwaukee Harbor (representing inflow from the Milwaukee, Menomonee and Kinnickinnic Rivers, Fig. 21) were used along with discharge rate measurements (Fig. 19) to calculate phosphorus loading rates.

Daily loading rates were determined as the product of discharge (daily measurements obtained from the USGS) and river phosphorus concentration. Phosphorus concentrations on days between sampling dates were estimated by interpolation. Estimates of loading derived in this manner are subject to some error due to uncertainty regarding phosphorus concentrations between sampling dates. However, sampling during the period of greatest loading – April to June – was frequent enough to reveal a monotonic trend of increasing phosphorus concentrations during this period, suggesting that interpolation results in reasonably reliable estimates of daily phosphorus concentrations. Loading estimates for the July – October period are less reliable, but because discharge was low during this period, errors in phosphorus concentration estimates have a small influence on total loading estimates for the April – October period.

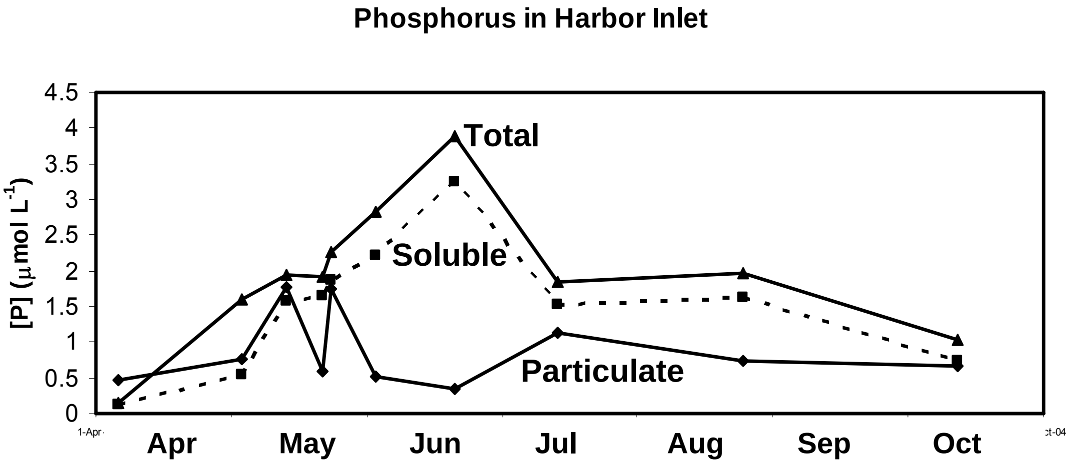


Fig. 21. Concentrations of particulate, dissolved (inorganic + organic) and total phosphorus in the Milwaukee Harbor Inlet in 2004(sampled beneath Hoan Bridge).

Table 1. Estimated phosphorus loading rates (kg/month) to Milwaukee Harbor from April to October, 2004. Part. P = particulate P; SRP = soluble reactive P; DOP = dissolved organic P; TDP = total dissolved P; TP = total P.

Month	Part. P	SRP	DOP	TDP	TP
April	1,196	574	822	1,400	2,596
May	9,778	12,763	3,246	16,012	25,790
June	2,826	17,014	3,871	20,887	23,713
July	1,779	3,607	745	4,351	6,130
August	661	1,266	265	1,530	2,191
September	301	545	137	683	984
October	330	380	148	528	859
April – Oct.	16,871	36,150	9,234	45,392	62,263

Both phosphorus concentrations and discharge rates were high in the months of May and June, and as a result the total P loading during these two months was much greater than for any of the other months, making up 80% of the loading during the 7-month period of sampling. In all months, soluble reactive phosphorus was the largest component of the total phosphorus pool, although in several months particulate P loading was nearly as high as SRP loading. Of the total P load between April and October, approximately 84% was from the Milwaukee River. This is primarily because the discharge of the Milwaukee River is much greater than that of the Menomonee and Kinnickinnic Rivers.

It is interesting to note that while both SRP and PP concentrations tended to be high in May, in June SRP concentrations remained high while PP concentrations were low. The lower PP concentrations in June may be the result of foliage development on natural and cultivated vegetation within the watershed between May and June, which will have a strong influence on erosion rates (e.g. Vought et al. 1995). In addition, PP concentrations may have been lower in June as a result of the flushing effect of storm events in May, which would have removed much of the available PP from land surfaces in the watershed, especially the impervious surfaces in urban areas. The cause of the high SRP concentrations in June is uncertain. It appears that, unlike PP, SRP within the watershed remains readily available even after large rain events such as those that occurred in May, so that subsequent rain events continue to result in large river SRP loads. SRP discharge from rivers may also result from the recycling of particulate P that has previously settled on the riverbeds. Klump et al. (2004) found that SRP flux from river anoxic river sediments is 50 to 90 μmol/m^2/hr, which was two to four times greater than that measured in harbor sediments.

7. Nutrient Retention in Milwaukee Harbor

Prior to entering the lake, water discharging from the three rivers passes through Milwaukee Harbor. The harbor has an approximate volume of 24 X 10^6 m^3. At the highest discharge rate during the study period (240 m^3/s), the residence time of water in the harbor will be approximately 1.2 days. Using the 11-month average discharge rate for January – November 2004 (32 m^3/s), the average harbor residence time is 8.7 days. This residence time allows for substantial changes in the nutrient chemistry of water as it travels from the rivers to the open lake, due to settling of particles to the harbor sediment, and uptake / recycling of nutrients by phytoplankton, bacteria, protozoa and zooplankton within the harbor. In order to determine the potential effect of the harbor on river water quality, on seven dates between October 2003 and October 2004 samples for nutrient analyses were collected at both the harbor inlet and the main (central) gap connecting the harbor to the open lake. On each date, concentrations of particulate P and total dissolved P were markedly lower at the gap than in the channel flowing into the harbor (Fig. 22). Particulate P concentrations in the gap ranged from 31% to 96 % of those in the inlet (mean = 57%), and TDP concentrations in the gap were 19% - 72% of those in the inlet (mean = 46%). While an accurate determination of phosphorus retention within the harbor would require a full annual cycle of frequent sampling at the inlet and all three outlet gaps, the data presented in Fig. 22 indicate that the harbor was acting as a phosphorus sink on each of the sampling dates. The loss of particulate P is not surprising, since P attached to particles will settle as a result of relatively slow flow rates in the harbor. However, the loss of dissolved P was not expected, since the anoxic sediments within the harbor might be expected to promote the dissolution of particulate phosphorus and release into the water column. In addition, phosphorus is added to the harbor in the form of effluent from the sewage treatment plant on Jones Island. This P input is not accounted for in our measurements of P loading for the harbor inlet, and would be expected to result in a P load from the harbor to the lake that is greater than our measurement of P load from the rivers to the harbor. That this is not the case suggests that there is an active phosphorus removal process in the harbor. Two possible mechanisms for dissolved P loss are adsorption onto particles followed by sinking, and uptake by phytoplankton and bacteria, which then are deposited in the harbor sediment. Other studies have found that harbor sediments may serve both as a net source and a net sink for phosphorus (Giblin et al. 1997). In several experiments conducted between July and September 2004, Klump et al. (2004) measured sediment-water SRP flux rates of averaging 20 $\mu mol/m^2/hr$ in Milwaukee Harbor, which over an entire month would be equivalent to a P flux of 1,786 kg to the harbor. During this same period estimates of river input of total phosphorus to the harbor ranged from 984 to 2,191 kg (Table 1). This suggests that in late summer, when river inputs are low, a significant amount of phosphorus may still enter the lake from the harbor, due to recycling from harbor sediments. Improved quantification of Milwaukee Harbor's potential to modify nutrient inputs to the lake will require a more focused study on nutrient inputs and outputs, uptake by biota, sedimentation rates, and sediment-water exchange rates.

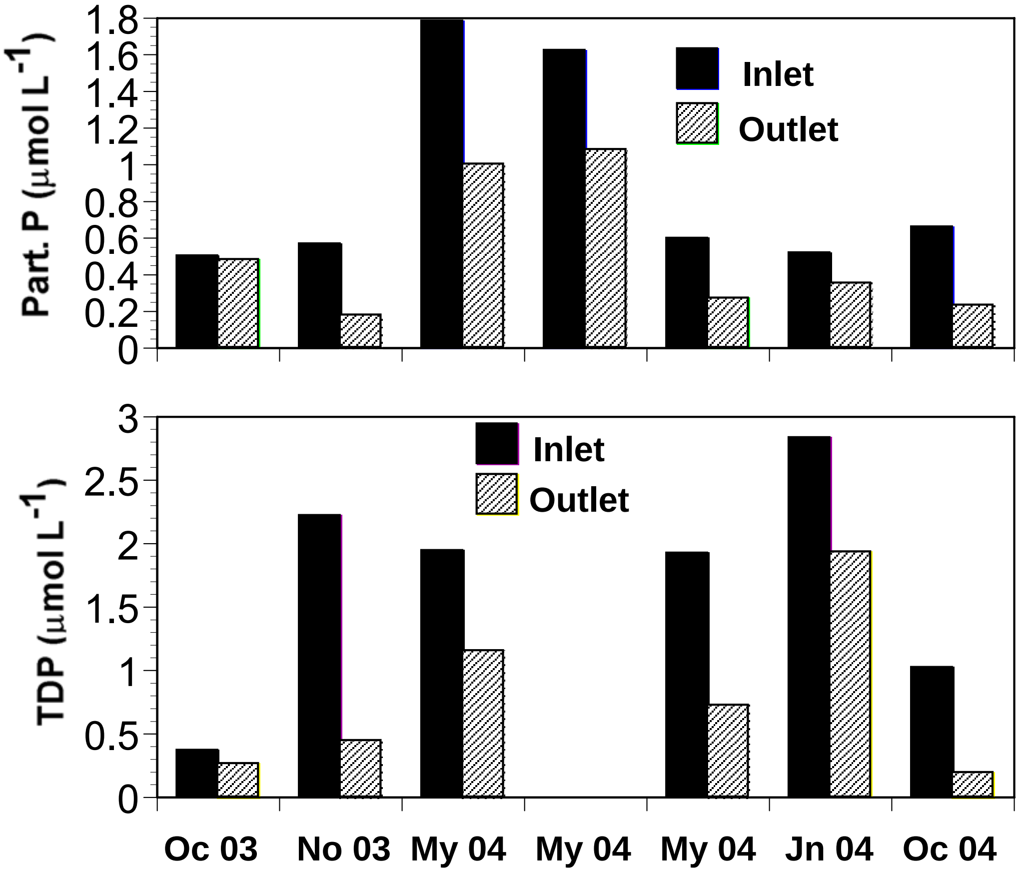


Fig. 22. Comparison of particulate and total dissolved phosphorus concentrations measured in the inflow to Milwaukee Harbor (sampled under Hoan Bridge) and the main (central) gap through which water flows from the harbor tgo the open lake.

8. Nearshore Hydrodynamics and Phosphorus Distribution

On entering the lake, the influence of river-borne phosphorus on *Cladophora* production will be influenced to a large degree by the speed and direction of nearshore currents. In the southern basin of Milwaukee, large-scale currents are generally counterclockwise, resulting in a flow direction from north to south along the lake's western shore (Beletsky et al. 1999). However, nearshore currents may differ in speed and direction from large-scale currents, due to alteration of large-scale flow patterns by nearshore bathymetry and shoreline structure.

In order to determine the degree to which nearshore currents control nutrient distribution, and whether this might influence the production and spatial distribution of *Cladophora* in the Milwaukee region, on three dates the horizontal distribution of currents and soluble reactive phosphorus were measured in the nearshore region north, east and south of Milwaukee Harbor. This study focused on surface waters, and did not attempt to determine the three-dimensional distribution of currents and SRP. However, on all sampling dates the nearshore lake surface temperature was cooler than the harbor water temperature, and therefore it is probable that harbor water persisted as a surface plume within the lake rather than sinking immediately to the lake bottom. At each station, a water sample was collected from a depth of 2.5 m, and current measurements were made at 2.5 m using a Falmouth Scientific 2ACM acoustic current meter with internal tilt gauge and compass. Currents were measured at a frequency of twice per second, averaged over 15-second intervals, for a minimum of 3 minutes. Replicate 15-second measurements were averaged using a vector averaging technique in which individual direction measurements were velocity-weighted, producing a resultant current speed and direction for each location.

On July 17, the nearshore current direction was from south to north, despite a wind direction of WNW (Fig. 23A). As a result of the prevailing northward current, water exiting the harbor was immediately force toward the north. SRP concentrations along most of the nearshore zone were relatively low – usually less than 0.2 μmol/L. However, in the McKinley Beach area, just north of the harbor, SRP concentrations were significantly higher, reaching 0.35 μmol/L. This is still much lower than concentrations within the harbor, but it is sufficiently high to promote rapid *Cladophora* growth rates, providing growth is not limited by low light levels or low temperatures. In earlier field and lab studies, Canale and Auer (1982b) observed that a SRP concentration of 0.35 μmol/L resulted in *Cladophora* internal concentrations of approximately 0.25%, which is sufficient to allow *Cladophora* to grow at near-maximum growth rate (Auer and Canale 1982b).

On October 2, SRP concentrations in the harbor and in the nearshore region of the lake were low (Fig. 23B). This was likely the result of very low river discharge rates during this period. Of particular interest on this date was a high SRP concentration of 0.3 μmol/L measured offshore of Bradford Beach, just south of the Linnwood Water Treatment Plant. The cause of this "hot spot" is uncertain, but it does not appear to originate from the harbor or from the beach, as concentrations at sampling sites to the south and on the shoreward side of this site had low SRP concentrations, and the nearshore current was flowing from north to south. While a concentration of 0.3 μmol/L is not extremely high (river concentrations are usually much higher), it is sufficient to promote rapid *Cladophora* growth when temperature and light conditions are suitable.

Sampling on November 11 followed a moderately wet period with greater river discharge than on the previous sampling dates. On this date, nearshore current

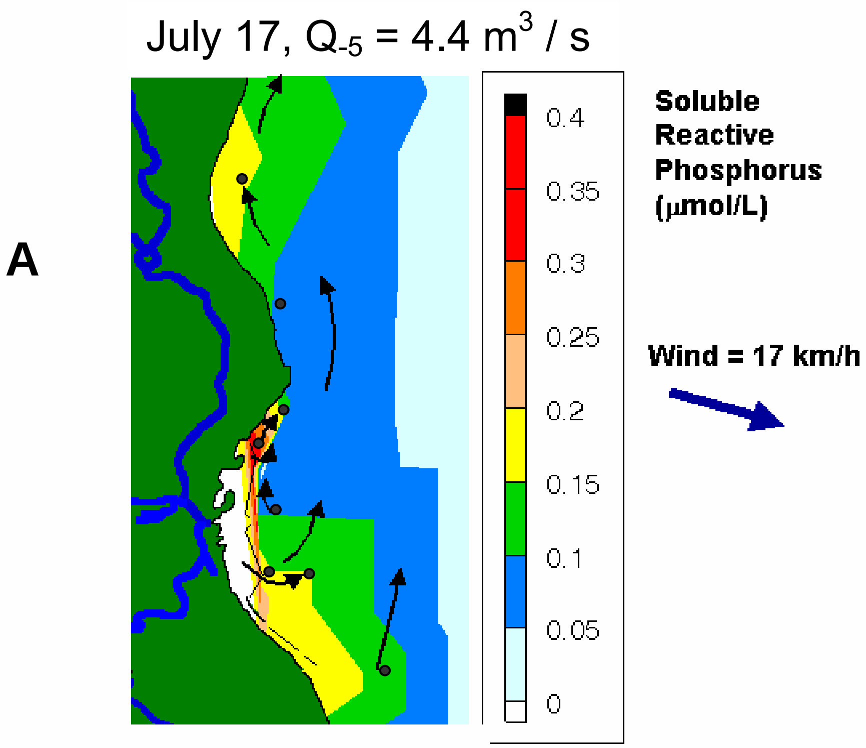


Fig. 23A. Surface current directions and soluble reactive phosphorus distribution on July 17, 2003. No samples were collected within the harbor on this date. Q-5 represents the discharge rate for the Milwaukee River over the 5 days preceding sampling. The blue arrow on the right side of the plot represents wind direction during the sampling period. SRP sampling sites are represented by black dots.

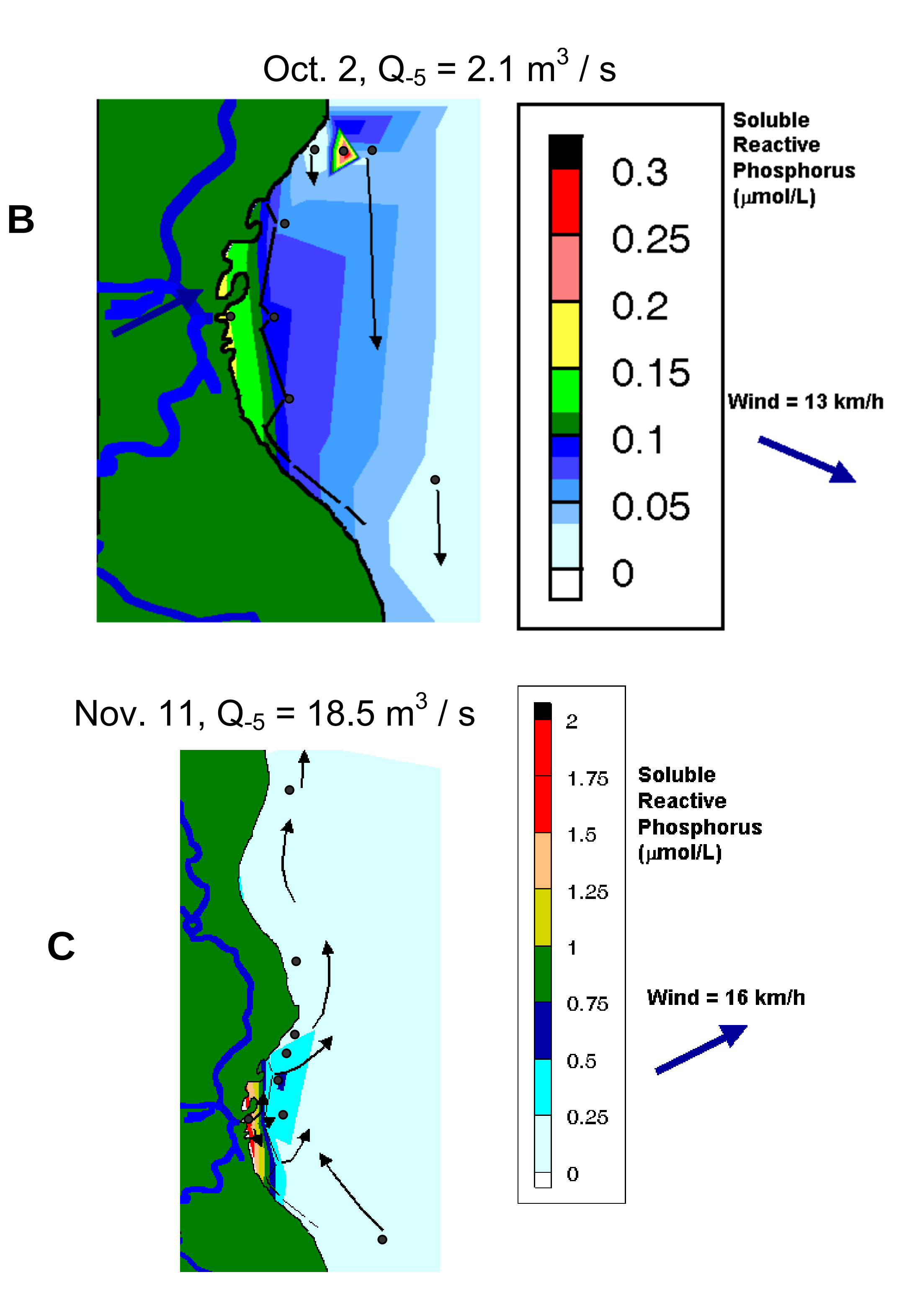


Fig. 23B, C. Surface current directions and soluble reactive phosphorus distribution on October 2, 2003 (**B**) and November 11, 2003 (**C**). Note that the color scales are not identical for all plots.

direction was from south to north. This resulted in high nearshore SRP concentrations (up to 0.6 μmol/L) north of the harbor, directly offshore of McKinley Beach and Bradford Beach (Fig. 23C).

A surprising result of the above measurements is that, on two of the three sampling dates, nearshore currents were flowing from the south to the north. On November 11 this current direction was in concordance with the southwest wind direction, but on July 17 the wind direction was WNW. While the outflow from the harbor does not appear to affect SRP concentrations over a large area of the lake, there is a measurable influence on nearshore waters between Milwaukee Harbor and the Linnwood Water Treatment Plant to the north. On two of the sampling dates, concentrations in this area were sufficiently high to potentially result in high *Cladophora* growth rates. Presumably, a similar area of influence exists to the south of the harbor, between the harbor and Green Can Reef, when nearshore currents are flowing southward and there is sufficient SRP discharge from the harbor. Discharge rates during the 5 days preceding each of the sampling dates were well below the annual average for the Milwaukee River. Therefore, it is reasonable to assume that under average discharge rates the influence of harbor effluent on nearshore phosphorus concentrations extends somewhat north of the Linnwood Intake Plant and south of the Green Can Reef. However, whether this has an impact on *Cladophora* growth rates in these more distant areas will depend on the rate at which this nearshore water exchanges with low-phosphorus water from the lake pelagic zone, and on lake bathymetry, which determines whether areas of the lake that are receiving the harbor plume are of shallow enough depth to allow light sufficient for *Cladophora* growth to reach the lake bottom.

9. Long-term River Phosphorus Trends

Cladophora abundance on the western shore of Lake Michigan was relatively high in Lake Michigan from the 1960s to the early 1980s, decreased during the late 1980s, and has increased during the past 5 to 8 years. An obvious question is whether these fluctuations might be related to changes in nutrient loading from rivers. There is a general consensus that the measures put in place by the Great Lakes Water Quality Agreement, such as improved agricultural practices and improved efficiency of sewage treatment plants, have resulted in a steady decrease in the amount of P entering Lake Michigan and the other Great Lakes (e.g. Barbiero et al. 2002). To determine whether this is indeed the case for the Milwaukee region of Lake Michigan, historic phosphorus concentrations for several river locations near Milwaukee were examined. The results indicate that total and soluble phosphorus concentrations at all six locations for which data were analyzed decreased between 1982 and the late 1980s (Fig. 24). However, since the early 1990s, phosphorus concentrations have increased at all locations. The plot for station RI-15 in Fig. 24 presents the P concentration for the largest source of river water (the combined input of the Milwaukee and Menomonee

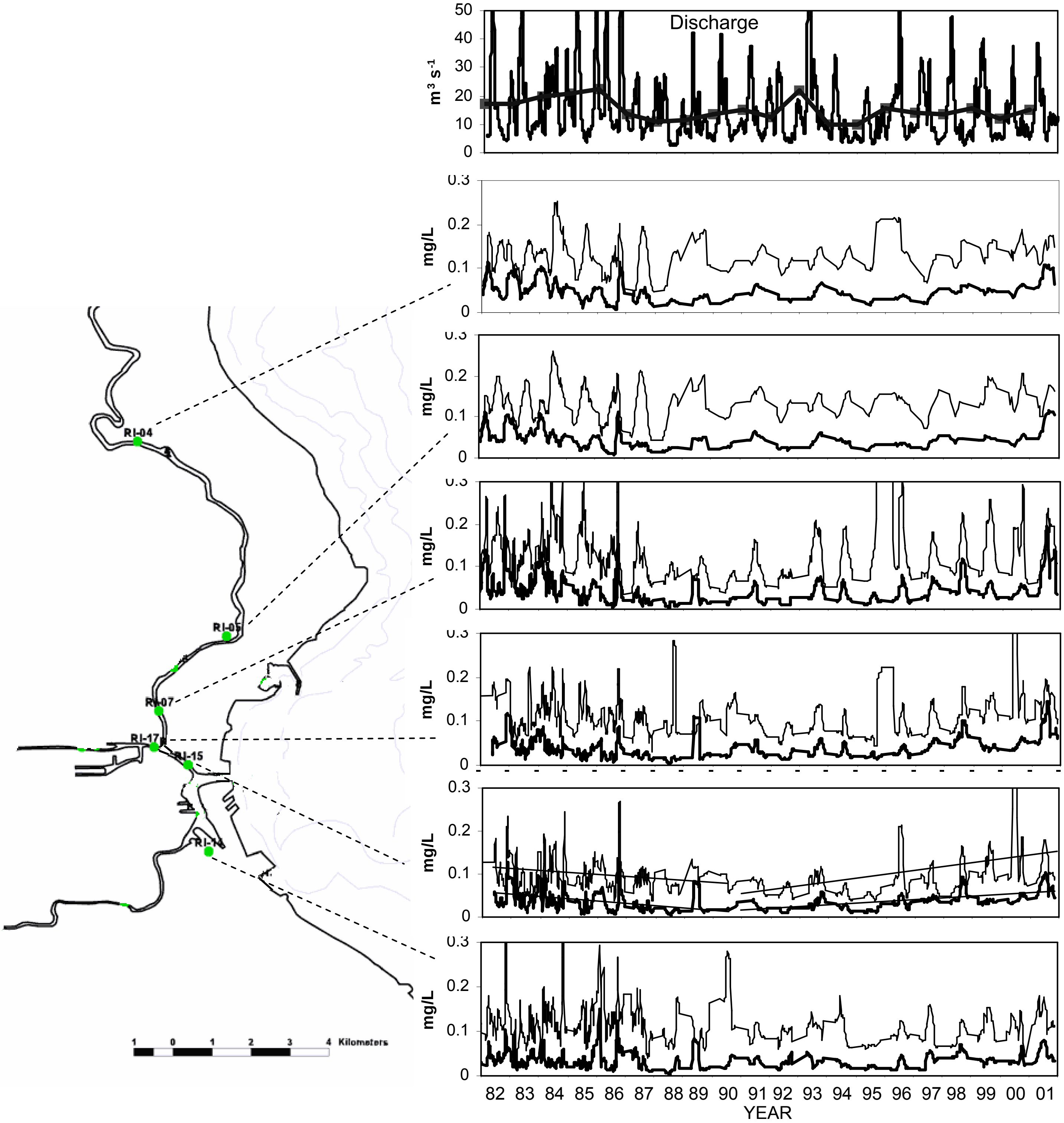


Fig. 24. Concentrations of total phosphorus (thin line) and soluble reactive phosphorus (thick line) at various locations on the Milwaukee, Menomonee and Kinnickinnic Rivers between 1982 and 2001 (data provided by MMSD; 10-point smoothing filter applied). Straight lines in the plot for Station RI-15 indicate the linear trends for the pre- and post-1990 periods. Top plot shows Milwaukee River discharge for this period, smoothed with a 30-day average window and annual means (data obtained from USGS).

Rivers) just before it enters the harbor, and is therefore a good indication of the trend for the entire drainage basin. It indicates that soluble phosphorus concentration decreased by more than 0.03 mg/L (approximately 1 μmol/L) from 1982 to 1990, but then increased by approximately the same amount between 1990 and 2002. The average soluble phosphorus concentration for the 3-year period 1999-2001 (0.050 mg/L) was similar to that for 1982-1984 (0.045 mg/L), and the average total P concentration for 1999-2001 (0.146 mg/L) was significantly greater ($P<0.01$) than that for 1982-1984 (0.105 mg/L). Therefore, while there appears to have been some success in reducing phosphorus inputs in the decade following the early 1980s, the past decade has seen a reversal of this trend. The long-term fluctuations in river phosphorus concentrations do not appear to be a function of river discharge rate, as discharge rates in the late 1990s and early 2000s were similar to those in the early 1990s. Hence, the increase in river phosphorus concentration between 1990 and 2001 resulted in an increased P loading rate from rivers to the Milwaukee Harbor.

In order to determine the potential impact of this increased P loading rate on *Cladophora* production, it is necessary to compare phosphorus loading data with historic trends for the two physical factors that also have a strong influence on *Cladophora* growth – light and temperature. In addition, phosphorus load must be compared with *Cladophora* phosphorus uptake rates to determine whether the P load can meet P demand by *Cladophora*. These comparisons are made in the next section.

10. Comparing *Cladophora* P Demand with P Inputs

Based on measurements of soluble reactive P, *Cladophora* P content, and enzyme analyses, there is strong evidence that *Cladophora* growth at depths of 10 m and less in Lake Michigan nearshore waters is limited by P availability. Therefore, a useful approach to determining what processes might be contributing to excessive *Cladophora* growth is to quantify the P supply required by *Cladophora*, and compare this supply rate with supply rates.

P uptake (demand) by *Cladophora* is a function of *Cladophora* P content, and growth rate:

$$U_P = u\, P_C$$

Where U_P = uptake rate (μg / mg / day)
u = specific growth rate (day^{-1})
P_C = *Cladophora* P content (μg / mg)

If *Cladophora* biomass concentration is known, an areal P uptake rate can be calculated as:

$U_{P(a)} = U_P B$

where B = biomass concentration (mg / m^2)
and $U_{P(a)}$ has units of µg / m^2 / day

Values for each of the above parameters were determined as follows:

Growth Rate (*u*) and P Content (P_C). *Cladophora* growth rate in Lake Michigan was not measured directly. However, previous studies conducted by Auer and Canale (1982b) have shown that *Cladophora* growth rate can be estimated quite accurately if *Cladophora* P content, temperature and irradiance are known. An approximation of the average *Cladophora* growth rate in the Milwaukee region for the July – September 2004 period can be derived by using P content, irradiance and temperature measurements made during this study.

P content measurements for all samples collected in the Milwaukee region were usually between 1 and 2 µg / mg, with the exception of several measurements of <1 µg / mg in late June – early July. The mean P content for all *Cladophora* samples was 1.13 µg / mg, which was used in the calculations below. Mean temperature from July 1 to September 30 was 15^{o}C. Mean daily irradiance at 10 m from July to September was 2,690 mmol photons / m^2 / day. If the light period of each day is accepted as 12 hours, then this is equivalent to a mean irradiance of 62 µmol photons / m^2 / s at a depth of 10 m. Graham et al. (1985) found that between 5^{o}C and 20^{o}C (similar to the Lake Michigan temperature range in this study), the minimum irradiance required for positive net photosynthesis by *Cladophora* is around 30 µmol photons / m^2 / s. However, in order for *Cladophora* accumulation to occur, daytime photosynthetic rates must be more than sufficient to balance nocturnal respiration rates. Graham et al. found that in this temperature range, gross photosynthetic rates were more than twice respiration rates when there was a minimum irradiance of 35 to 75 µmol photons / m^2 / s. This is in agreement with our observations of large *Cladophora* biomass concentrations at depths of 10 m or less, but low concentration at depths greater than 10 m.

At depths of less than 10 m, the average daily irradiance will be greater than 62 µmol photons / m^2 / s. Assuming a Secchi depth of 6 m (Fig. 15), an extinction coefficient of 0.28 is calculated. Using this extinction coefficient, an irradiance of 62 µmol photons / m^2 / s at 10 m would result from an irradiance of 251 µmol photons / m^2 / s at a depth of 5 m. To derive a conservative value of *Cladophora* growth rate, an irradiance of 75 µmol photons / m^2 / s was applied to the entire 0-10 m nearshore depth range.

Based on the empirical models of Auer and Canale (1982b) and Graham et al. (1982), a P content of 1 µg / mg, a temperature of 15^{o}C, and a 12-hour average daily irradiance of 75 µmol photons / m^2 / s would result in a *Cladophora* growth rate between 0.1 and 0.25 day^{-1}. Again, to derive a conservative estimate of *Cladophora* P demand, a growth rate of 0.1 day^{-1} was assumed.

Biomass Concentration (B). The mean *Cladophora* biomass areal concentration at the Atwater station was 57±19 g/m^2. Biomass at stations south of Milwaukee during the September transect were less than this, but because P content (and estimated growth rate) of those samples was high, and because high biomass was measured in that area in 2003, it is probable that the low biomass on the 2004 sampling date was due to a sloughing event, and biomass in this area is on average likely to be more similar to that at the Atwater site. Therefore the mean Atwater biomass concentration of 57 g/m^2 (57,000 mg/m^2) was applied to determine *Cladophora* P demand.

Using the above values, the areal uptake of P by *Cladophora* is estimated as:

$$
\begin{aligned}
U_P &= u\, P_C B \\
&= 0.1 \cdot 1.13 \cdot 57{,}000\ (\mu g/m^2/day) \\
&= 6{,}441\ \mu g\ P/m^2/day,\ = 6.4\ mg\ P/m^2/day
\end{aligned}
$$

Cladophora P Requirement versus P Supply from Rivers and Mussels

To determine the total P requirement of all *Cladophora* growing in the Milwaukee region, an estimate of the area covered by *Cladophora* is required. The area selected ranged from Fox Point in the north to Wind Point in the south. While there are several other small river phosphorus sources to this region (e.g. Oak Creek), they are negligible relative to the input through the Milwaukee Harbor. Using bathymetric charts, the total area along this length of shoreline between the shore and 10 m (*Cladophora* abundance below 10 m is assumed to be minimal) is determined as 188 km^2. Based on aerial images collected in the summer of 2003, the proportion of lake bottom within the area that is covered by *Cladophora* was conservatively estimated as 75%, resulting in a *Cladophora*-covered area of approximately 141 km^2. This area, multiplied by the estimated *Cladophora* P demand of 6.4 mg P / m^2 / day, results in a total P requirement of 902 kg P / day, or approximately 27,000 kg / month. In comparison, the estimated monthly total phosphorus loading rates from rivers to Milwaukee Harbor for the July – September period ranges from 984 to 6,130 kg / month. It is not known how much of this P is immediately available for uptake by algae, since a significant proportion of it is made up of particulate and dissolved organic P. The loading rate of more readily available SRP is approximately half of the total P loading rate. Regardless of whether the TP or SRP loading rate is used, it is obvious that, despite potential errors in the calculation of *Cladophora* P requirements, river inputs between July and September could only meet a small fraction of this requirement. It should be pointed out that the above loading estimates do not account for retention of P within the harbor, which may be significant (Fig. 22). This strengthens the argument that the flux of P from the harbor to the lake between July and September is insufficient to support *Cladophora* growth during that period.

The above comparison suggests that there must be a source of P other than immediate river inputs that promotes *Cladophora* growth. There are two potential sources of this P. First, P may be provided by zebra mussels, which can excrete SRP directly, and excrete P-containing feces and pseudofeces which may be remineralized. Quantifying the P recycling rate for zebra mussels in Lake Michigan is difficult, because this rate is influenced by a number of factors, including temperature, plankton concentration, plankton species composition, current speeds, and zebra mussel densities. In laboratory experiments, James et al. (1997) found that mussel densities of 1,300 individuals / m^2 resulted in SRP production rates as high as 2.5 mg P/m^2/day, which over 141 km^2 is equivalent to 10,575 kg P/month. Mussel densities in Lake Michigan can be much higher than 1,300/m^2, and therefore this P recycling rate is likely conservative. This rate is greater than the estimated river input of total P between July and September.

An alternative approach is to use the weight-specific P excretion rate of $3.16 \cdot 10^{-5}$ g P/g dry mass/hour measured by Arnott and Vanni (1996). Using the same mussel density as above (1,300/ m^2), a dry weight content of 1 mg/mm (Chase and McMahon 1994), and an average length of 1.5 mm, an excretion rate of 11.7 mg P/m^2/day is calculated (assuming 19 active hours of excretion; Bunnell et al. 2005), equivalent to 49,490 kg P/m^2/month.

Obviously there is a large degree of uncertainty regarding actual P supply rates from mussels, but the above estimates indicate that mussels have the potential to provide a significant portion of the P required to support *Cladophora* growth. The rate at which mussels provide P will ultimately be constrained by the supply of particulate P to mussels in the form of plankton and particulate detritus. The clarification of nearshore waters is a testament to the mussels ability to deplete plankton in the nearshore zone, and therefore food supply in the form of plankton will depend on nearshore plankton production rate and exchange rate with the pelagic zone, which may serve as a source of new plankton. Typical nearshore summer particulate P concentrations are between 2 and 4 μg/L, which is equivalent to a total mass of 2,820 kg P in the nearshore zone described above. Based on the estimated *Cladophora* P requirement of 902 kg/day, the mass of particulate P in nearshore waters is sufficient to support 3 days of *Cladophora* growth if it is completely recycled to SRP. Assuming offshore particulate P concentrations are similar, nearshore water would need to be replaced every 3 days with offshore water to provide sufficient particulate P to support a recycling rate that would be sufficient to support *Cladophora* P demand. This nearshore-offshore exchange rate is realistic, especially during periods of upwelling or downwelling, which occur frequently on Lake Michigan's western shore. However, the above P recycling rate using plankton as a P source should be considered a maximum, since it assumes that mussels are filtering 100% of the plankton from the water as it passes through the nearshore zone. In reality, their filtration efficiency will be significantly lower than this.

Although the process of plankton supply → mussel feeding → mussel P excretion is a feasible mechanism by which P may be supplied to *Cladophora*, the importance of river P inputs cannot be completely discounted. Zebra mussels may facilitate the retention of river-borne particulate P within the nearshore zone by filtering particulates and then depositing them on the bottom in the form of feces and pseudofeces (Hecky et al. 2004). If these bottom deposits are slowly remineralized, then particulate P entering the lake from rivers in spring and early summer may support *Cladophora* growth later in the summer. The above estimates of river P inputs were based on the period July – September, because that is the period for which growth estimates were made. However, as shown in Table 1, total P inputs to the Harbor in May and June were between 24,000 and 26,000 kg/month. If this P was retained and recycled within the nearshore zone, it would have been sufficient to support *Cladophora* growth from July to September.

Our analysis strongly suggests that zebra mussels play an important role in facilitating P supply to *Cladophora*. Where there are large concentrations of mussels and a sufficient supply of particulate P, mussels will provide P to *Cladophora* in the form of direct excretion of SRP and egestion of feces and pseudofeces that will concentrate P in the benthos where *Cladophora* grow. A critical question that remains is the relative importance of plankton versus river-borne sediment as sources of food and phosphorus to zebra mussels.

11. Conclusions and Recommendations

A. Based on a number of approaches, there is strong evidence that *Cladophora* production in the Milwaukee region, as well as other parts of Lake Michigan's western coast, is phosphorus limited. Supporting evidence includes the low concentrations of soluble reactive phosphorus in the water column, the high activity of the enzyme alkaline phosphatase in *Cladophora*, and the low P content of *Cladophora*.

B. In the Milwaukee region, *Cladophora* is abundant to depths of at least 10 m (33 feet). Between a depth of 10 and 15 m, light becomes insufficient for growth.

C. At depths of 10 m and less, temperatures are above the minimum threshold for *Cladophora* growth between May and October, and temperatures are optimal for *Cladophora* growth between July and early September.

D. In the past 30 years, mean nearshore temperature for the period July – September has risen by approximately 3°C. In the past decade, nearshore water clarity (and therefore underwater light intensity) has increased approximately two-fold. There is also evidence that total phosphorus loading rate from rivers to Milwaukee Harbor has increased, perhaps more than two-fold since 1990. All three of these changes have made nearshore conditions more suitable for the growth of *Cladophora*. **While current analyses suggest that each of the three variables – light, temperature and phosphorus – has played a significant role in promoting increased *Cladophora* growth, quantification of the relative roles of these variables will require the incorporation of environmental data on these variables into a calibrated *Cladophora* growth / biomass model. In addition to allowing an analysis of the relative importance of these driving variables, such a model will help to predict the efficacy of various management strategies.**

E. Despite the fact that phosphorus inputs to the lake appear to have increased, measured concentrations of soluble reactive phosphorus in the nearshore region are usually too low to support the observed biomass of *Cladophora* (based on growth vs. P relationships defined in earlier studies). This suggests that there may be a benthic (lake bottom) source of P that support *Cladophora* growth. The most likely benthic source is zebra mussels.

F. Estimates of zebra mussel phosphorus excretion rates suggest that this source may provide an amount of soluble reactive phosphorus equivalent to more than 50% of that required to support observed *Cladophora* growth rates. Measurements of P in *Cladophora* filaments indicate that the lower sections of the filament (which are in closest proximity to mussels) are often more enriched in P. This further supports the suggestion that mussels are providing P to *Cladophora*, although other mechanisms (e.g. shading of lower filament sections) might also produce this result.

G. The phosphorus supplied from zebra mussels may ultimately be derived from plankton growing in the lake or from particulate material entering the lake from rivers and the harbor. During the present study, P input from rivers during the late summer was insufficient to support estimated P requirements of *Cladophora*, and therefore P supply to *Cladophora* must have been supported by plankton or by particulate P that had settled in the nearshore region earlier in the summer. Currently it is not possible to quantify the relative importance of river P inputs versus the lake's internal P pool as P sources for *Cladophora*. Calculations based on lake particulate P concentrations and nearshore-offshore exchange rates suggest that supply of P from the lake's internal pool would be sufficient to support *Cladophora* growth. However, elevated P concentrations in *Cladophora* south of Milwaukee, where harbor influence is likely the greatest, suggest that river inputs of P do have some influence on *Cladophora* growth. **A critical prerequisite to the implementation of a *Cladophora* management strategy is an understanding of the relative importance of river-borne phosphorus versus the lake's internal phosphorus pool as ultimate phosphorus sources to zebra mussels. This will determine whether a reduction of phosphorus inputs from rivers will have a significant effect on *Cladophora* abundance.**

H. Measurements of *Cladophora* phosphorus content made in this study indicate that there is generally sufficient phosphorus to support positive growth at depths of 10 m or less, but that *Cladophora* remain phosphorus-stressed. Small increases in phosphorus availability may result in large increases in *Cladophora* growth and abundance. Conversely, small reductions in phosphorus availability may cause a significant reduction in *Cladophora* growth and abundance.

In the past two years, the quagga mussel (*Dreissena bugensis*) has become abundant in the nearshore waters of Lake Michigan near Milwaukee. In the summer of 2003, the nearshore mussel community was nearly 100% zebra mussels, but in 2004 quagga mussels made up nearly 50% of the dreissenid community in some areas. The potential impact of this latest invasive species on nearshore phosphorus cycling is currently unknown. Quagga mussels appear to have a lower metabolic rate than zebra mussels, but they are not confined to hard bottoms, and therefore may inhabit a larger portion of the lake bottom than zebra mussels do. **It is important that both field and laboratory studies be conducted to determine the influence of quagga mussels on nutrient cycles. This information will help determine whether *Cladophora* growth will increase or decrease if quagga mussels come to dominate the nearshore community.**

Solving the *Cladophora* problem requires that the mechanisms supporting excessive *Cladophora* growth be determined, and that strategies be implemented which will reduce this growth. **However, apart from any attempts to reduce growth rates, management of the *Cladophora* problem may still be improved if there is a better understanding of the mechanisms causing sloughing (detachment) of *Cladophora*. Such an understanding will have management applications in that it will allow for the prediction of sloughing events that lead to accumulation on beaches, and may lead to management actions that could minimize accumulation on beaches. It will also allow for improvement of a previously developed *Cladophora* growth / biomass model, because sloughing affects *Cladophora* standing crop, which in turn affects absolute *Cladophora* production rate**. Possible factors influencing sloughing include wind-induced turbulence, life cycle stage, temperature, irradiance, and the presence of epiphytic diatoms on *Cladophora*. Currently there is little data to indicate the relative importance of these factors.

Appendix 1. Background Literature Review.

In Lake Michigan and the other Great Lakes there has been a recent resurgence of nuisance blooms of benthic algae, dominated by the invasive, green, filamentous alga, *Cladophora glomerata* (Edsall & Charlton 1996; New York Sea Grant 2002; Milwaukee Journal Sentinel 2002). Negative impacts of these algal blooms include unsightly and foul smelling beaches, potential health risks from bacterial growth (Byappanahalli et al. 2003, Whitman et al. 2003), clogging of water intakes and impaired quality drinking water, decline in lakeshore recreational quality and property depreciation. The potential economic and health impacts were recently highlighted when the Kewaunee Nuclear Plant was closed due to clogging of its emergency cooling system with benthic algae (http://www.jsonline.com/bym/news/jan04/201605.asp). In addition, excessive *Cladophora* growth may reduce fish and invertebrate species diversity, and impairs spawning success of some fish species (Oster 1980), by modifying the substratum or by promoting anoxia during decomposition (Hartman 1973).

During the 1950s and the 1970s, *Cladophora*-covered beaches were an obvious symptom of eutrophication in many parts of the Great Lakes, including the western shores of Lake Michigan (IJC 1976). This stimulated a large amount of research in the 1960s and 70s to determine the physical and chemical factors that control the growth of *Cladophora* and other benthic algae. The conclusions of these studies were often inconsistent; some indicated that nutrients were primarily responsible for excessive *Cladophora* growth. In most cases the limiting nutrient was identified as phosphorus (e.g. Neil & Owen 1964; Lin & Blum 1973; Gerloff & Muth 1984), but nitrogen (Hopkins & Carney 1972; Mantai 1976) and inorganic carbon (Wood 1968) were also of potential importance. Others found temperature to be a critical factor controlling *Cladophora* growth and distribution (Bellis 1968; Moore 1978). Superimposed on nutrient and temperature effects was the influence of light (Adams & Stone 1973; Mantai 1974; Graham et al. 1982). In the 1980's and 1990's, the *Cladophora* problem seemed to improve and, as a result, research tapered off. The coincident decline in *Cladophora* along with attempts to reduce phosphorus inputs to the Great Lakes suggested that nutrient control was the key to harmful algal control, and that nutrient abatement measures were having the desired effect. However, in light of the fact that *Cladophora* abundance has increased, while phosphorus inputs have apparently continued to decrease (Barbiero et al. 2002) suggests that this paradigm is now uncertain. The causes of the current resurgence in *Cladophora* blooms are not known.

Phosphorus Input

Excessive *Cladophora* growth in parts of the Great Lakes several decades ago was accompanied by high phytoplankton concentrations, and much research was devoted to understanding the factors responsible. From this work, the phosphorus limitation paradigm arose, in which phosphorus (P) is seen as the critical factor controlling phytoplankton abundance in many temperate lakes (Vollenweider 1976; Schindler 1977). A number of studies (e.g. Herbst 1969; Lin & Blum 1973; Gerloff & Muth 1984; Canale & Auer 1982; Painter & Jackson

1989) have demonstrated that this principle may also apply to *Cladophora* in the Great Lakes. Moreover, a recent study in an English lake reported dramatic reductions in *Cladophora* biomass by remediation of P inputs (Parker & Maberly 2000). If *Cladophora* growth in Lake Michigan is also controlled by P supply, then the recent resurgence of *Cladophora* suggests that either allochthonous P inputs are increasing or there has been a fundamental change in P cycling in the lake. Determining the relative importance of these mechanisms is critical, because management strategies will differ depending on the ultimate source of P that is promoting benthic algal growth.

In Lake Michigan and the other Great Lakes, external P loading has apparently decreased by 50% or more between 1975 and 1990 (Nicholls & Hopkins 1993; DePinto & Narayanan 1997; Barbiero et al. 2002). However, non-point agricultural sources of nutrients, sewage overflows and storm drains, all of which are poorly accounted for, remain a concern. In the case of the Milwaukee River, despite the removal of P from detergents and the increased efficiency of sewage treatment, current P concentrations and P loadings are similar to or greater than during the mid-1970s. Similar trends have been reported for some Lake Erie and Lake Superior tributaries (Nicholls et al. 2001).

Causes of increases in P loading to the Milwaukee River may include greater use of lawn or agricultural fertilizers, changes in land use, and higher sewage loads. Demographic changes may also be a factor: although population density in the river basin has not increased significantly since 1970, there has been a large shift from urban to rural distribution (WDNR 2001), which may result in greater fertilizer application (larger lawns) or loss of natural vegetation cover. Regardless of the ultimate cause, preliminary data on *Cladophora* distribution near Milwaukee suggest that it is related to proximity to the mouth of the Milwaukee River, implying that allochthonous nutrient inputs play a role. In order to assess the potential role of nutrient inputs as a cause of *Cladophora* blooms, it is necessary to be able to quantify the degree to which *Cladophora* populations are limited by nutrients in the lake.

To reduce the impact of urban fertilizers, the state of Minnesota recently enacted legislation to restrict the P content in lawn fertilizers. We have been approached by Wisconsin state legislators wishing to know if a similar action might solve the *Cladophora* problem on the western shores of Lake Michigan. With current data and understanding of *Cladophora* ecology and nearshore nutrient dynamics, we cannot provide reliable advice. There is simply insufficient information to answer the questions crucial for management and/or legislative decisions.

Cladophora – Zebra Mussel Interaction

The apparently greater abundance of *Cladophora* near river discharges and urban centers (Herbst 1969; Auer & Canale 1982) supports the idea that allochthonous inputs control *Cladophora* production. However, the recent resurgence of *Cladophora* in the Great Lakes, even in locations distant from large urban centers (Edsall & Charlton 1996) suggests a more widespread change in ecosystem function could be occurring. Increased benthic nutrient supply through zebra mussel feeding and excretion is a plausible cause of this large scale change. Zebra mussels (*Dreissena polymorpha*) were first noticed in Lake Michigan around 1990. This invasive species filters water extremely efficiently, removing phytoplankton and suspended sediment from the water column (Fahnenstiel et al. 1995; Caraco et al. 1997). It has a strong influence on lake nutrient cycles and has led to increased light penetration through the water column.

The results of several studies suggest that zebra mussel filtration and excretion result in accelerated nitrogen and phosphorus recycling rates (Gardner et al. 1995; Heath et al. 1995), and an increase in dissolved nutrient concentrations (Holland et al. 1995; James et al. 1997; Effler & Siegfried 1998). An increase in benthic algal biomass observed coincident with the establishment of zebra mussels (Lowe & Pillsbury 1995; Fahnenstiel et al. 1995), has been suggested to be related to an increased nutrient supply to the benthos in the form of zebra mussel feces and pseudofeces, and recent models propose that zebra mussels shunt water-column nutrients to the benthos (DePinto & Narayanan 1997). While there is evidence that zebra mussels positively influence benthic algal production (Lowe & Pillsbury 1995; Fahnenstiel et al. 1995), few studies have quantified nutrient fluxes through zebra mussels to benthic algae, so the relative importance of this nutrient source versus allochthonous inputs in promoting *Cladophora* growth is uncertain. In fact, these two factors may be synergistic. By filtering nutrient-rich particles entering from rivers, and phytoplankton that utilize the dissolved nutrients entering from rivers, zebra mussels may promote nearshore benthic retention of allochthonous nutrients that otherwise would be dissipated over a much larger lake area. An understanding of the relative importance of internally cycled nutrients promoting *Cladophora* growth is prerequisite to devising effective management strategies.

A second consequence of filtration by zebra mussels is increased water clarity in the Great Lakes (Holland et al. 1995; Howell et al. 1996). In Lake Michigan, nearshore water clarity has increased dramatically since zebra mussels first entered the lake. This increased clarity may promote *Cladophora* growth and extend its depth distribution. Under nutrient replete conditions in the laboratory, Graham et al. (1982) and Lorenz et al. (1991) found the minimum and maximum photon flux density requirements for net *Cladophora* growth were ~35 μmol m^{-2} s^{-1} and 400 μmol m^{-2} s^{-1}, respectively. If these values are representative of *in situ* conditions, an increase in Secchi depth from 3 m to 6 m could extend the maximum depth of *Cladophora* from 8 to 16 m, and at a depth of 6 m *Cladophora* net photosynthesis may have increased twofold. In contrast, Lester et al. (1974) found higher light compensation levels for *Cladophora* in Green Bay than those reported for Lake Michigan by Lorenz et al. (1991), possibly because of nutrient limitation. More detailed *in situ* measurements of the *Cladophora* growth response to light and nutrients are thus required.

Appendix 2. Literature References

Adams MS & Stone W (1973) Field studies on photosynthesis of *Cladophora glomerata* (Chlorophyta) in Green Bay, Lake Michigan. *Ecoogy*. 54: 853-862.

Arnott, D.L., and M.J. Vanni. 1996. Nitrogen and phosphorus recycling by the zebra mussel (*Dreissena polymorpha*) in the western basin of Lake Erie. Canadian Journal of Fisheries and Aquatic Science 53:646-659.

Auer, M.T., and R.P. Canale. 1982a. Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: 2. Phosphorus uptake kinetics. Journal of Great Lakes Research 8:84-92

Auer, M.T., and R.P. Canale. 1982b. Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: 3. The dependence of growth rates on internal phosphorus pool size. Journal of Great Lakes Research 8:93-99.

Barbiero RP, Tuchman ML, Warrant GJ & Rockwell DC (2002) Evidence of recovery from phosphorus enrichment in Lake Michigan. *Can. J. Fish. Aquat. Sci*. 59: 1639-1647.

Barbiero, R.P., M.L. Tuchman, G.J. Warren, and D.C. Rockwell. 2003. Evidence of recovery from phosphorus enrichment in Lake Michigan. Canadian Journal of Fisheries and Aquatic Science 59: 1639-1647.

Beardall, J. Young, E. and Roberts, S. (2001). Interactions between nutrient uptake and cellular metabolism: approaches for determining algal nutrient status. *Aquatic Sciences.* 63: 44-69.

Beletsky, D., J.H. Saylor, and D.J. Schwab. 1999. Mean circulation in the Great Lakes. J.; Great Lakes res. 25: 78-93.

Bellis VJ (1968) Unialgal cultures of *Cladophora glomerata* (L.) Kütz. In southern Ontario. *J. Phycol*. 3:57-63.

Berges JA, Cochlan WP & Harrison PJ (1995) Laboratory and field responses of algal nitrate reductase to diel periodicity in irradiance, nitrate exhaustion, and the presence of ammonium. *Marine Ecology Progress Series* 124: 259-269.

Brooks AS & Edgington DN (1994) Biogeochemical control of phosphorus cycling and primary production in Lake Michigan. *Limnol. Oceanogr.* 39: 961-968.

Byappanahalli MND, Shively A, Nevers MB, Sadowsky MJ, Whitman RL. 2003. Growth and survival of *Escherichia coli* and enterococci populations in the macro-alga *Cladophora* (Chlorophyta). FEMS Microbiol. Ecol. 46: 203-211.

Canale RP & Auer MT (1982) Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: 5. Model development and calibration. *J. Gr. Lakes Res*. 8: 112-125.

Canale RP, Auer MT & Graham JM (1982) Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: 6. Seasonal and spatial variation in growth kinetics. *J. Gr. Lakes Res*. 8:126-133.

Canale, R.P., and M.T. Auer. 1982. Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: 7. Model verification and system response. Journal of Great Lakes Research 8: 134-143.

Caraco NF, Cole JJ, Raymond PA, Strayer DL, Pace ML, Findlay SEG & Fischer DT (1997) Zebra mussel invasion in a large, turbid river: Phytoplankton response to increased grazing. *Ecol.* 78: 588-602.

Chase, R., and R.F. McMahon. 1994. Effects of starvation at different temperatures on dry tissue and dry shell weights in the zebra mussel, Dreissena polymorpha (Pallas). Proceedings of the fourth international zebra mussel conference, Madison, Wisconsin, March 1994.

DePinto JV & Narayanan R (1997) What other ecosystem changes have zebra mussels caused in Lake Erie: Potential bioavailability of PCBs. *Gr. Lakes Res. Rev*. 3: 1-8.

Dyhrman, S. T. and Palenik, B. P. (1997). The identification and purification of a cell-surface alkaline phosphatase from the dinoflagellate *Prorocentrum minimum* (Dinophyceae). *Journal of Phycology*. 33: 602-612.

Edsall T & Charlton M (1996) Nearshore waters of the Great Lakes. Published by USEPA and Environment Canada. Available at: http://www.on.ec.gc.ca/solec/nearshore-water/paper/part5.html.

Effler SW & Siegfried C (1998) Tributary water quality feedback from the spread of zebra mussels: Oswego River, New York. *J. Gr. Lakes Res*. 24: 453-463.

Fahnenstiel, G.L., G.A. Land, T.F. Nalepa, and T.H. Johengen. 1995. Effects of zebra mussel (*Dreissena polymorpha*) colonization on water quality parameters in Saginaw Bay, Lake Huron. Journal of Great Lakes Research 21:435-448.

Gardner WS, Cavaletto JF, Johengen TH, Johnson JR, Heath RT & Cotner JB (1995) Effects of the zebra mussel, *Dreissena polymorpha*, on community N dynamics in Saginaw Bay, Lake Huron. *J. Gr. Lakes Res*. 21: 529-544.

Genfa, Z., and P.K. Dasgupta. 1989. Fluorometric measurement of aqueous ammonium ion in a flow injection system. Analytical chemistry 61:408-412.

Gerloff GC & Muth JV (1984) Nutritional ecology of Great Lakes *Cladophora glomerata*. EPA-600/3-84-016, 157pp.

Giblin, A.E., C.S. Hopkinson, and J. Tucker. 1997. Benthic metabolism and nutrient cycling in Boston Harbor, Massachusetts. Estuaries 20: 346-364.

Graham, J.M., M.T. Auer, R.P. Canale, and J.P Hoffmann. 1982. Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: 4. Photosynthesis and respiration as functions of light and temperature. Journal of Great Lakes Research 100-111.

Hartmann WL (1973) Effects of exploitation, environmental changes, and new species on the fish habitats and resources of Lake Erie. Tech. Report No. 22. Ann Arbor: Great Lakes Fishery Comm.

Heath, R.T., G.L. Fahnenstiel, W.S. Gardner, J.F. Cavaletto, and S.J. Hwang. 1995. Ecosystem-level effects of zebra mussels (*Dreissena polymorpha*): an enclosure experiment in Saginaw Bay, Lake Huron. Journal of Great Lakes Research 21: 501-516.

Hecky, R.E., R.E.H. Smith, D.R. Barton, S.J. Guildford, W.D. Taylor, M.N. Charlton, and T. Howell. 2004. The nearshore phosphorus shunt: a consequence of ecosystem engineering by dreissenids in the Laurentian Great Lakes. Canadian Journal of Fisheries and Aquatic Science 61:1285-1293.

Herbst RP (1969) Ecological factors and the distribution of *Cladophora glomerata* in the Great Lakes. *Am. Midland Nat*. 82: 90-98.

Holland RE, Johengen TH & Beeton AM (1995) Trends in nutrient concentrations in hatchery Bay, Western Lake Erie, before and after *Dreissena polymorpha*. *Can. J. Fish. Aquat. Sci*. 52: 1202-1209.

Hopkins GJ & Carney E (1972) *Cladophora* bioassays for IFYGL (1972) Report to Toronto Ministry of the Environment, Biology Section.

Howell ET, Marvin CH, Bilyea RW, Kauss PB, & Somers K (1996) Changes in environmental conditions during *Dreissena* colonization of a monitoring station in eastern Lake Erie. *J. Gr. Lakes Res*. 22: 744-756.

International Joint Commission (1976) Great Lakes water quality 1975. Appendix B. Surveillance subcommittee report.

James, W.F., J.W. Barko, and H.L. Eakin. 1997. Nutrient regeneration by the zebra mussel (*Dreissena polymorpha*). Journal of Freshwater Ecology 12:209-216.

Klump, J.V., P.D. Anderson, D.C. Szmania, and K. Weckerly. 2004. Milwaukee Harbor sediment oxygen demand study 2004. Great Lakes WATER Institute Technical Report No. 2004-B1.

Lin CK & Blum JL (1973) Adaptation to eutrophic conditions by Lake Michigan algae. University of Wisconsin Madison, Dept. Botany and Water Resources Center.

Lorenz RC, Monaco ME & Herdendorf CE (1991) Minimum light requirements for substrate colonization by *Cladophora glomerata. J. Gr. Lakes Res*. 8: 42-53.

Lowe RL & Pillsbury RW (1995) Shifts in benthic algal community structure and function following the appearance of zebra mussels (*Dreissena polymorpha*) in Saginaw Bay, Lake Huron. *J. Gr. Lakes Res*. 21: 558-566.

Mantai KE (1974) Photosynthesis in *Cladophora* from Lake Erie. Plant Physiol. 53: 33.

Mantai KE (1976) The physiology of *Cladophora* as a function of Lake Erie environmental conditions. Abstracts, 19th Conference on Great Lakes Research, University of Guelph, Guelph, Ontario.

Mellina, E., J.B. Rasmussen, and E.L. Mills. 1995. Impact of zebra mussel (Dreissena ploymorpha) on phosphorus cycling and chlorophyll in lakes. Canadian Journal of Fisheries and Aquatic Science 52: 2553-2573.

Milwaukee Journal Sentinel (2002) Lake water quality worsens this year. Available at: http://www.jsonline.com/news/Metro/jul02/62274.asp .

Moore LF (1978) Attached algae at thermal generating stations – the effect of temperature on *Cladophora*. *Verh. Internat. Verein. Limnol*. 20:1727-1733.

Nalepa, T.F., D.J. Hartson, G.W. Gostenik, D.L. Fanslow, and G.A . Lang. 1996. Changes in the freshwater mussel community of Lake St. Clair: From Unionidae to *Dreissena polymorpha* in eight years. Journal of Great Lakes Research 22:354-369.

Neil JH & Owen GE (1964) Distribuiton, environmental requirements and significance of *Cladophora* in the Great Lakes. Proc. 7th Conference on Great Lakes Research: 113-121.

New York Sea Grant (2002) Lake Ontario algae cause and solution workshop proceedings. Available at: http://www.monroecounty.gov/documentView.asp?docID=2351.

Nicholls KH & Hopkins GJ (1993) Recent changes in Lake Erie (north shore) phytoplankton: Cumulative impacts of phosphorus loading reductions and the zebra mussel introduction. *J. Gr. Lakes Res*. 19: 637-647.

Nicholls KH, Hopkins GJ, Standke SJ & Nakamoto L (2001) Trends in total phosphorus in Canadian near-shore waters of the Laurentian Great Lakes: 1976-1999*. J. Gr. Lakes Res*. 27: 402-422.

Oster DA (1980) Eutrophication and the Lake Ontario fishery. Lake Ontario Tact. Fish. Plan Resour. Doc. 1: 38 pp.

Painter DS & Jackson M (1989) *Cladophora* internal phosphorus modeling verification. *J.Gr.Lakes Res*.15:700-708.

Parker JE & Maberly SC (2000) Biological response to lake remediation by phosphate stripping: control of *Cladophora*. *Freshwat. Biol*. 44: 303-309.

Parsons, T. R., Maita, Y., Lalli, C. M. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press. Oxford.

Planus D, Maberly SC & Parker JE (1996) Phosphorus and nitrogen relationships of *Cladophora glomerata* in two lake basins of different tropic status. *Freshwat. Biol*. 35: 609-622.

Reuter JE & Axler RP (1992) Physiological characteristics of inorganic nitrogen uptake by spatially separate algal communities in a N-deficient lake. *Freshwat. Biol*. 27: 227-236.

Schindler DW (1977) The evolution of phosphorus limitation in lakes. *Science* 195: 260-262.

Stainton, M.P., M.J. Capel, and F. Armstrong. 1977. The chemical analysis of fresh water, 2nd edition. Canadian Fisheries and Marine Service Special Publication 25.

Stankovich, W. 2005. The Interaction of Two Nuisance Species in Lake Michigan: *Cladophora glomerata* and *Dreissena polymorpha*. Proceedings of a workshop: *Cladophora* Research and Management in the Great Lakes, UW-Milwaukee Special Report No. 2005-01 (http://www.uwm.edu/Dept/GLWI/cladophora/page_report.html).

Vollenweider RA (1976) Advances in defining critical loading levels for phosphorus in lake eutrophication. *Mem. Ist. Ital. Idrobiol*. 33: 53-83.

Vought, L.B.-M., G. Pinay, A. Fuglsang, and C. Ruffinoni. 1995. Structure and function of buffer strips from a water quality perspective in agricultural landscapes. Landscape and Urban Planning 31:323-331.

Waples, J.T., and J.V. Klump. 2002. Biophysical effects of a decadal shift in summer wind direction over the Laurentian Great Lakes. Geophysical Research Letters 29:43-1 – 43-4.

WDNR (2001) The state of the Milwaukee River basin. Wisconsin Dept Natural Resources. Publ. WT 704 2001.

Webb, W.L., Newton, M. and Starr, D. (1974). Carbon dioxide exchange of *Alnus rubra*: A mathematical model. *Oecologia.* 17: 281-291.

Whitman RL, Shively DA, Pawlik H, Nevers MB, Byappanahalli NM. 2003. Occurrence of *Escherichia coli* and enterococci in *Cladophora* (Chlorophyta) in nearshore water and beach sand of Lake Michigan. Appl. Environ. Microbiol. 69: 4714- 4719.

Wood KG (1968) Photosynthesis of *Cladophora* under unnatural conditions. *In* Algae, man, and the environment. DF Jackson (ed.), pp. 121-133. Syracuse Univ Press.

Young, E.B., Lavery, P.S., van Elven, B., Dring, M.J. and Berges, J.A. (2005) Dissolved inorganic nitrogen profiles and nitrate reductase activity in macroalgal epiphytes within seagrass meadows. *Marine Ecology Progress Series*. In Press.