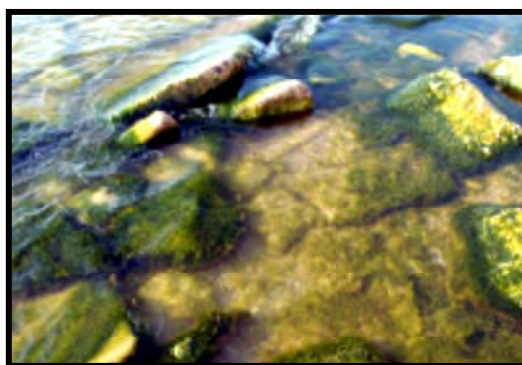
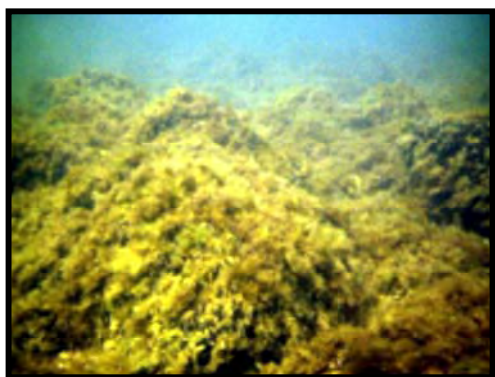


# *Cladophora* Research and Management in the Great Lakes

Proceedings of a Workshop Held at the  
Great Lakes WATER Institute, University of Wisconsin-Milwaukee,  
December 8, 2004



GLWI Special Report No. 2005-01

*This workshop was funded in part by the Wisconsin Coastal Management Program and the National Oceanic and Atmospheric Administration, Office of Ocean and Coastal Resource Management under the Coastal Zone Management Act, Grant # NA04NOS4190062.*

# **CLADOPHORA RESEARCH AND MANAGEMENT IN THE GREAT LAKES**

**Proceedings of a Workshop Held at the  
Great Lakes WATER Institute, University of Wisconsin-Milwaukee  
December 8, 2004**

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## **Workshop Sponsors**



**UW-Milwaukee Great Lakes WATER Institute Special Report No. 2005-01**

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## Table of Contents

Introduction.....	1
<i>Cladophora</i> confounds coastal communities – public perceptions and management dilemmas. V. Harris, University of Wisconsin Sea Grant .....	5
<i>Cladophora</i> – How is it handled? R. Stauffer, Milwaukee Community Service Corps.....	15
Why filamentous green algae dominated benthic habitats after the zebra mussel invasion in Saginaw Bay, Lake Huron. R. Pillsbury, University of Wisconsin – Oshkosh.....	17
<i>Cladophora</i> and the Beach: Implications for Public Health. G. Kleinheinz and E. Englebert, University of Wisconsin – Oshkosh.....	19
The interaction of two nuisance species in Lake Michigan: <i>Cladophora glomerata</i> and <i>Dreissena polymorpha</i> . W. Stankovich, University of Wisconsin – Platteville.....	31
The weakest link and what makes it stink. S. Davis-Foust and J. Janssen. University of Wisconsin-Milwaukee .....	37
Molecular phylogeography and species discrimination of freshwater <i>Cladophora</i> (Cladophorales, Chlorophyta) in North America. K. Müller, University of Waterloo.....	39
Nuisance <i>Cladophora</i> in Milwaukee urban streams - habitats, seasonality, productivity, nutrient trapping, heavy metal sponging - the ultimate foodweb bottleneck. C. Sandgren, University of Wisconsin-Milwaukee.....	43
Modeling <i>Cladophora</i> growth: A review of the Auer-Canale framework. M. Auer, Michigan Technological University .....	57
The contribution of <i>Dreissena</i> to the resurgence of <i>Cladophora</i> in Lake Erie. S. Higgins, University of Waterloo.....	63
Systematic survey of <i>Cladophora</i> and water quality on the Wisconsin Lake Michigan shoreline. P. Garrison and S. Greb, Wisconsin Department of Natural Resources .....	73
Temporal and spatial patterns of <i>Cladophora</i> biomass and nutrient stoichiometry In Lake Michigan. H.A. Bootsma, E.B. Young, and J.A. Berges, Wisconsin-Milwaukee.....	81

Can activity of enzymes involved in nutrient assimilation be useful as indices of nutrient status in *Cladophora*? E.B. Young, J.A. Berges, and H.A. Bootsma, University of Wisconsin-Milwaukee. ....89

Discussion forum: Reaching a consensus on what is known, and setting future research priorities. E.B. Young and J.A. Berges, University of Wisconsin-Milwaukee .....95

Appendix 1. Workshop program.....103

Appendix 2. List of attendees .....105

# *Cladophora* Research and Management in the Great Lakes

## Introduction

Excessive growth of the filamentous green alga, *Cladophora* sp., was one of the most obvious symptoms of eutrophication in the Great Lakes between the 1950s and 1970s<sup>1</sup>. During the latter part of this period, a large amount of research was conducted to determine the causes of excessive *Cladophora* growth. While various factors, including nitrogen<sup>2</sup>, phosphorus<sup>3,4,5</sup>, temperature<sup>6,7</sup> and irradiance<sup>8,9</sup> were found to influence *Cladophora* growth, phosphorus appeared to be the key factor responsible for excessive growth, and phosphorus abatement was seen as the most effective method of solving the problem. This approach appeared to be validated by the decline in the abundance of *Cladophora* and other algae in the 1980s following the removal of phosphorus from detergents, improved phosphorus removal by sewage treatment plants, and changes in agricultural practices designed to reduce phosphorus runoff from land – actions that resulted from a 1983 amendment to the Great Lakes Water Quality Agreement.

In the past five to ten years, excessive *Cladophora* growth has re-emerged as a management problem in parts of the Great Lakes. This has resulted in public complaint, generally related to the decline in aesthetic conditions near the lakeshore. Other negative impacts include human health hazards (e.g. *Cladophora* mats may promote the growth or retention of pathogens), the clogging of water intakes (including those of power plants), the loss of recreation opportunities, and declining lakefront property values. In addition to direct impacts on humans, excessive *Cladophora* growth may have significant impacts on ecosystem functions and properties such as nutrient cycling, energy flow and food web structure. These impacts have received very little attention to date.

Until recently, the *Cladophora* problem has been observed and addressed primarily at the local scale. However, as the research and management community has become more aware of the problem, it has become increasingly obvious that this is not just a local problem, but is a larger, Great Lakes issue that may require a large-scale solution. Because the research community has only recently recognized the magnitude of the problem, there has been little communication among researchers and managers in the Great Lakes region regarding research and management activities related to *Cladophora*. A primary objective of this workshop was to share current perspectives and promote ongoing communication among researchers, managers and the public regarding the *Cladophora* problem.

From the historic perspective, the recent resurgence of *Cladophora* in the Great Lakes is puzzling. It certainly was not anticipated, as phosphorus concentrations in all of the Great Lakes except Lake Erie are at or below the target levels set by the Great Lakes Water Quality Agreement. Because there has been a 20-year hiatus in research on nearshore algae, attempts to understand the current *Cladophora* problem are based largely on what was learned in earlier studies. Those studies were largely successful in identifying the causes of and solutions to the *Cladophora* problem at the time. But the fact that this problem has resurfaced following the implementation of management

strategies that were previously successful at controlling it suggests that there are fundamental changes occurring in these large aquatic ecosystems and extrapolation from an earlier understanding of the problem to the current situation may be misleading. While the *Cladophora* resurgence is of concern in itself, it may be symptomatic of larger changes that are affecting chemical and biological dynamics at the whole-lake scale. These changes need to be identified and understood to determine whether current management strategies remain valid or need to be modified.

A second objective of this workshop was to assess our current knowledge related to *Cladophora* and the causes of its resurgence, in the hope that this assessment will be used to guide further research and management efforts. An additional goal was to promote collaboration and a Great Lakes comparative approach to understanding the problem. A number of hypotheses have been presented regarding causes of the recent *Cladophora* resurgence, including climate change, increased nutrient inputs, lake level fluctuations, changes in water clarity, and alteration of internal nutrient cycles. The ability to test these hypotheses will be improved by comparing results from lakes that differ with regard to chemistry, hydrodynamics, climate, bathymetry, biotic composition and geology.

Much of the public living near the Great Lakes has become made aware of the problem through observation or the media, but has been poorly informed of the nature of the problem. There are general misconceptions that foul-smelling beaches are the direct result of sewage washing ashore – an understandable misconception considering the appearance and smell of rotting algae and its associated invertebrates. Even when it is understood that algae are the direct cause of aesthetic problems on beaches, the public is unaware or misinformed of potential causes of excessive algal growth. We hope the information collected by this workshop will be used to educate the public, so that managers, researchers and the public can work toward understanding the causes of the problem and the best management strategies.

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## ***Cladophora* Confounds Coastal Communities – Public Perceptions and Management Dilemmas**

Victoria A. Harris

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### **INTRODUCTION**

For the past five years, large accumulations of algae, predominantly *Cladophora sp.*, have been fouling the shores of Lakes Michigan, Huron, Erie and Ontario. Unpleasant conditions associated with algae decay have led to mounting public complaints and demands for resolution.

The purpose of this paper is to provide background on the nature and extent of *Cladophora* problems along Wisconsin's Lake Michigan shoreline, to address the human dimensions of public perception and to identify possible management actions. The information reported comes from the literature, a November 2004 WDNR survey of beach managers and health officials, communications with resource managers and personal observations.

### **NATURE OF THE PROBLEM**

*Cladophora* is a filamentous green alga that grows attached to hard substrates within the littoral zone. A common component of freshwater ecosystems, *Cladophora sp.* provide food and shelter for invertebrates and small fish. Problems arise when the conditions of light, nutrients, temperature and substrate are favorable for luxuriant growths over extensive areas.

During midsummer and fall, *Cladophora sp.* senesce, slough off their substrate and drift with waves and currents for some distance before a portion washes ashore and decays. The beached algae may accumulate in mats mixed with decaying zebra mussels, other invertebrates and fish. The combination results in unsightly, malodorous conditions that drive visitors away from popular beaches and force homeowners to keep their windows shut.

Piles of decaying *Cladophora* are more than an annoyance to people strolling on the beach. They may lower property values. *Cladophora sp.* have been linked to taste and odor problems in drinking water and may exacerbate levels of *E. coli* and enterococci bacteria in beach sand and swimming waters, raising questions about beach safety. *E. coli* bacteria are an indicator of fecal contamination, and high numbers prompt managers to close beaches. Recent research shows the stranded *Cladophora* mats may sustain or even nourish the growth of bacteria that come from gull droppings, sewage overflows, or runoff from urban and agricultural areas (Whitman *et al.* 2003; Paul *et al.* 2004).

### **EXTENT OF THE PROBLEM**

In Wisconsin, nuisance conditions have been reported at many sites ranging from northeastern Green Bay and the tip of Door County to Kenosha. *Cladophora* has been observed growing on hard substrates all along the lakeshore, even on Rock Island, a wilderness state park far removed from anthropogenic sources of nutrients. Assessment of the spatial distribution of *Cladophora*

beds along Wisconsin's Lake Michigan coast is underway, but more work is needed to understand the factors controlling distribution and abundance of *Cladophora sp.* and to identify areas at risk for nuisance accumulations.

*Cladophora* strandings extend to Lake Michigan shores in Illinois, Indiana and Michigan and also to portions of Lakes Huron, Erie and Ontario. Only Lake Superior appears to be spared. Clearly, nuisance growths of *Cladophora sp.* in the Great Lakes are not an isolated problem due merely to localized conditions.

The biomass of *Cladophora* that washes ashore varies between years and locations. Mats of stranded algae may be feet thick in some areas – often embayments where waters are calmer and materials tend to collect (e.g. Hika Bay, Cleveland, WI). In other areas, accumulations are minor or non-existent, or the decaying algae may remain submerged offshore or confined to the swash zone. The location, frequency and severity of algae accumulations are likely dictated by a combination of factors including offshore production, prevailing winds, water currents, storms, and nearshore morphology.



Figure 1. Ron Schaper of rural Cleveland, WI stands shin-deep in decaying algae stranded on the shore near his home, October 20, 2003. Photo by Gary C. Klein, Courtesy of the Sheboygan Press



Figure 2. *Cladophora* coats the rocks and beach at Newport State Park, August 26, 2004. Photo by Victoria A. Harris, UW Sea Grant Institute

## PUBLIC PERCEPTIONS

Up and down the Wisconsin coast of Lake Michigan, people are upset, bewildered and frustrated by the noxious accumulations of *Cladophora* on the shoreline. In advanced stages of decomposition, it may be difficult to recognize the decaying algae as plant material. Because of its septic odor, the organic mess has been mistaken for manure or sewage from failing septic

systems or municipal sewer overflows. In the swash zone, the algae may turn into a brown-black organic soup with an oily sheen, prompting some people to suspect an industrial waste or oil spill.

Worsening conditions in 2003 led to numerous complaints to public officials, state legislators and the WDNR and to growing demands for action. Fingers were pointed at a few notable point sources of phosphorus, such as Milwaukee MSD and large feedlot operations. This is understandable because of frequent media coverage of Milwaukee's combined sewer overflows and concern over pending permits for feedlot expansions. It is prudent to control individual sources of pollution to the lakes. However, excessive *Cladophora sp.* production has become a system-wide problem, requiring a better understanding of its causes and a system-wide approach to management.

### **FACTORS CONTROLLING *CLADOPHORA* ABUNDANCE AND THEIR MANAGEABILITY**

Four essential environmental conditions are needed for *Cladophora sp.* to flourish (Hiriart-Baer *et al.*): hard substrate; water temperatures in the range of 10-25°C; adequate light; and nutrients, particularly phosphorus. Determining which of these conditions have changed to promote *Cladophora* production in the lake(s) is essential for limiting nuisance algae accumulations along shorelines.

The dolomite bedrock and boulders of western Lake Michigan have historically provided ample substrate for *Cladophora sp.* and the expansion of zebra and quagga (*Dreissena polymorpha* and *Dreissena bugensis*) mussel beds may provide some additional substrate for algae attachment. The potential influence of lower lake levels on the amount of hard substrate within the littoral zone or on nearshore nutrient fluxes is not clear. Excessive *Cladophora* production has coincided with periods of low lake levels, both now and in the 1960s, leading to speculation about lake level influence on controlling factors. However, lake levels will continue to fluctuate and are not feasibly managed. Summer water temperatures of the lake are not known to have changed appreciably, nor are they logistically manageable.

Dreissenid mussels are generally believed to have increased water clarity in parts of the Great Lakes, including Lake Michigan, allowing light to penetrate to greater depths and expanding the area habitable by *Cladophora sp.* However, long term data sets of secchi depths from the western and central basins of Lake Erie (Barbiero and Tuchman 2004) and from lower (southern) Green Bay (Qualls 2003, Harris *et al.* 2005) show no persistent increases in transparency, likely due to weak stratification in these shallower basins and substantial loading and resuspension of suspended solids. Initially, water clarity increased in Lake Erie and Green Bay immediately following zebra mussel invasion, but increased secchi depths have persisted only in the deeper eastern basin of Lake Erie and northern regions of Green Bay.

Regardless of their impacts on water transparency and expansion of littoral zones, zebra and quagga mussels are now permanent inhabitants of the Great Lakes with no known means of control. Therefore, efforts to reduce *Cladophora* are focused on phosphorus levels in the lakes and the potential for limiting inputs.

## PHOSPHORUS IN LAKE MICHIGAN

Problems with *Cladophora* in the Great Lakes date back to the mid-1950s and 60s, when nutrient levels, particularly phosphorus, were considerably higher. Both localized and wide-spread eutrophic conditions were evidenced by abundant benthic and planktonic algae. Following the 1972 Amendments to the Clean Water Act, municipal wastewater discharges of phosphorus were limited to 1 mg/l and the allowable phosphate content of household detergents was reduced. Phosphorus levels in the lakes declined and nuisance algae blooms largely subsided by the early 1980s (Hiriart-Baer *et al.*).

Since 1983, levels of total phosphorus in offshore waters of the Great Lakes have been relatively stable, except for Lake Ontario where levels are declining (Fig. 3) (D. Rockwell, GLNPO, U.S. EPA, pers. comm.). With the exception of some exceedances in Lake Erie, all the lakes are attaining their respective target concentrations established by the International Joint Commission's Water Quality Board in 1987, under the Great Lakes Water Quality Agreement. In particular, total phosphorus in Lake Michigan is consistently below its target of 7 ug/l.

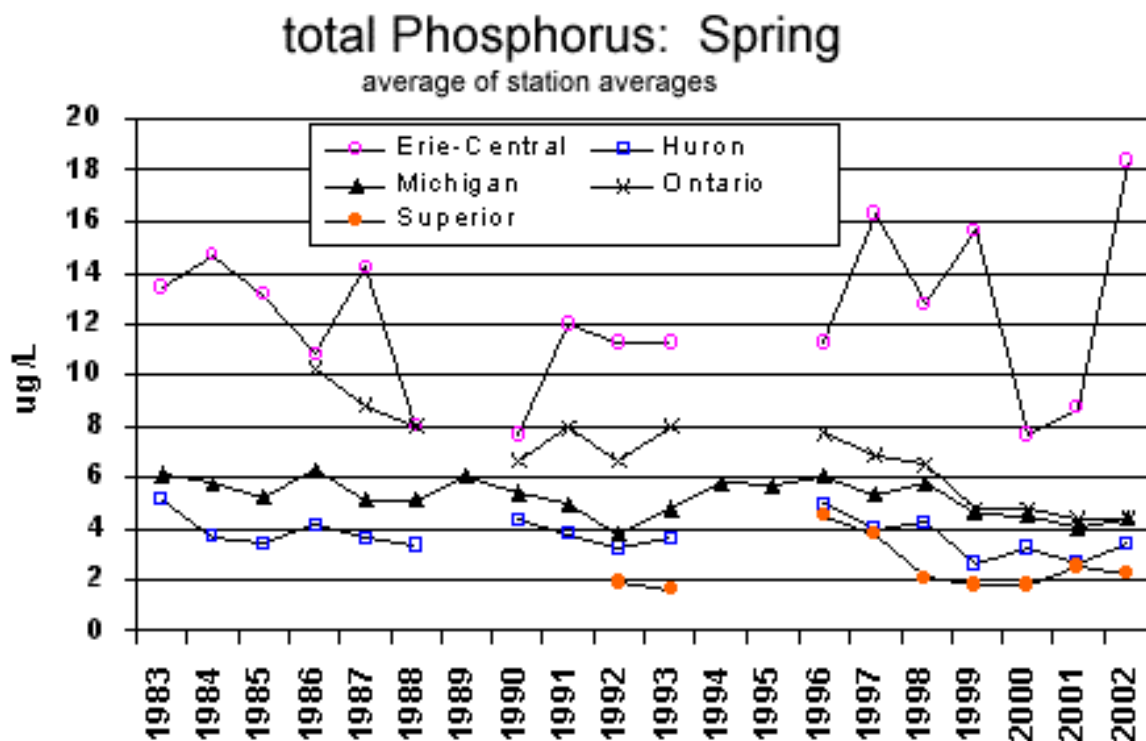


Figure 3. Average spring concentration of total phosphorus in offshore waters of the Great Lakes. From David Rockwell, GLNPO, U.S. EPA.

Hecky *et al.* (2004) propose a conceptual model, the nearshore shunt, to explain the apparent contradiction between high benthic productivity in the nearshore and low phosphorus concentrations in the offshore waters. They suggest that dreissenid mussels have re-engineered the bio-physical environment of the littoral zone and altered nutrient recycling, packaging and

transport. Through their extraordinary filtering capacity, the mussels capture and retain nearshore inputs of phosphorus, redirecting nutrient and energy flow to the nearshore benthic community and away from the offshore pelagic zone. Before the invasion of dreissenid mussels, they suggest offshore concentrations of phosphorus more closely reflected nearshore inputs.

In contrast to the open lake, high concentrations of total phosphorus have persisted in the waters of Green Bay of Lake Michigan (Fig. 4), causing hypereutrophic conditions in the southern bay, grading toward oligotrophic conditions in the northern bay (Richman *et al.* 1984, Harris *et al.* 2005). Levels remain well above the target recommended by the Science and Technical Advisory Committee (STAC) for the Lower Green Bay Remedial Action Plan. In fact, summer average total phosphorus concentrations increased by 22.5% in lower Green Bay following the invasion of zebra mussels ( $p < 0.0001$ ), due mainly to increases since 1999 (Qualls 2003, Harris *et al.* 2005). Estimates of annual Fox River phosphorus loads since 1999 do not indicate significant change (D. Robertson, USGS, pers. comm.). Only a portion (34 - 49%) of the increased total phosphorus concentration may be explained by reduced water volume in the lower bay due to lower lake levels (Harris *et al.* 2005). We attribute the remaining increase to other influences on internal nutrient fluxes, possibly zebra mussels.

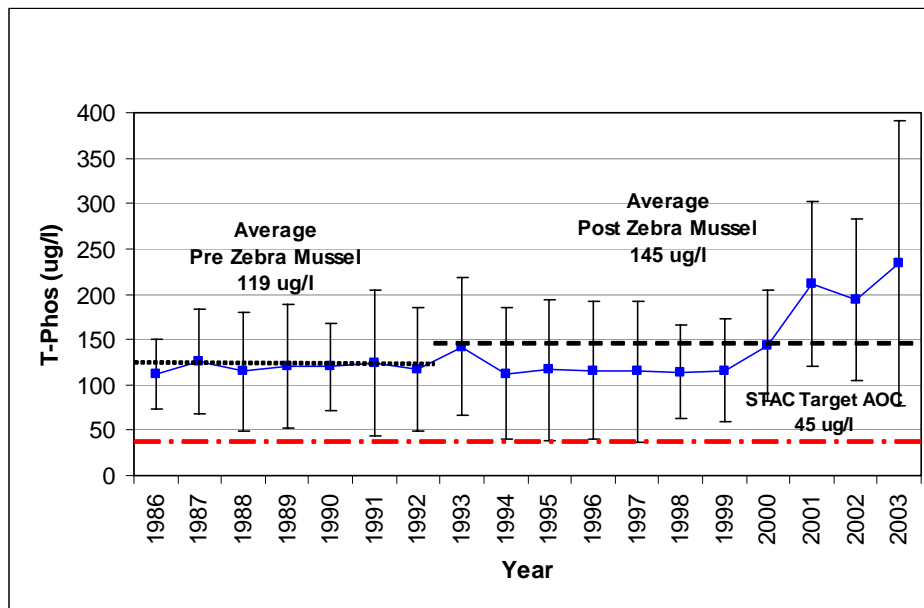


Fig. 4. Average summer total phosphorus for lower Green Bay (zones 1-3) before and after zebra mussel invasion (Qualls 2003, Harris *et al.* 2005).

Phosphorus levels in Green Bay are important to Lake Michigan. A phosphorus budget for the lake was developed as part of the Lake Michigan Mass Balance Study (Fig. 5). While the largest source of phosphorus to Lake Michigan waters is internal recycling from sediments, Green Bay contributes a substantial portion of the external loading to the lake (Kreis *et al.* 2004). The rest comes from direct tributary loading to the lake and atmospheric deposition. Of all the major tributaries monitored in 1994-95, the Fox River discharged the largest phosphorus load (Fig. 6). Therefore, any strategy to reduce phosphorus inputs to Lake Michigan must address Green Bay and the Fox River, as well as other tributaries.



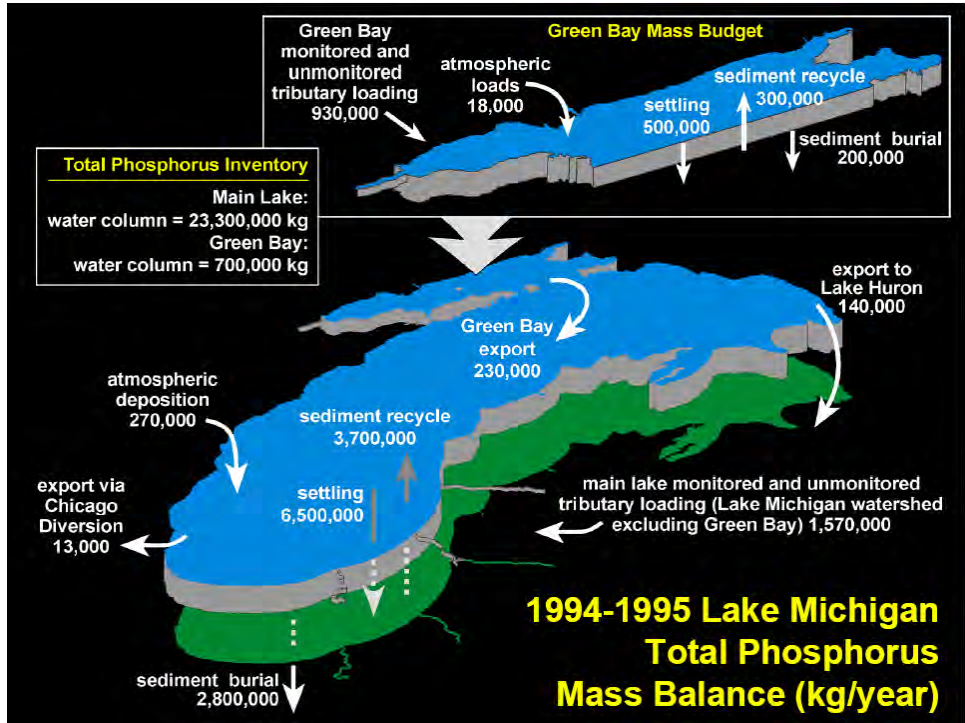


Fig. 5. 1994-1995 Lake Michigan total phosphorus mass balance. (Kreis *et al.* 2004)

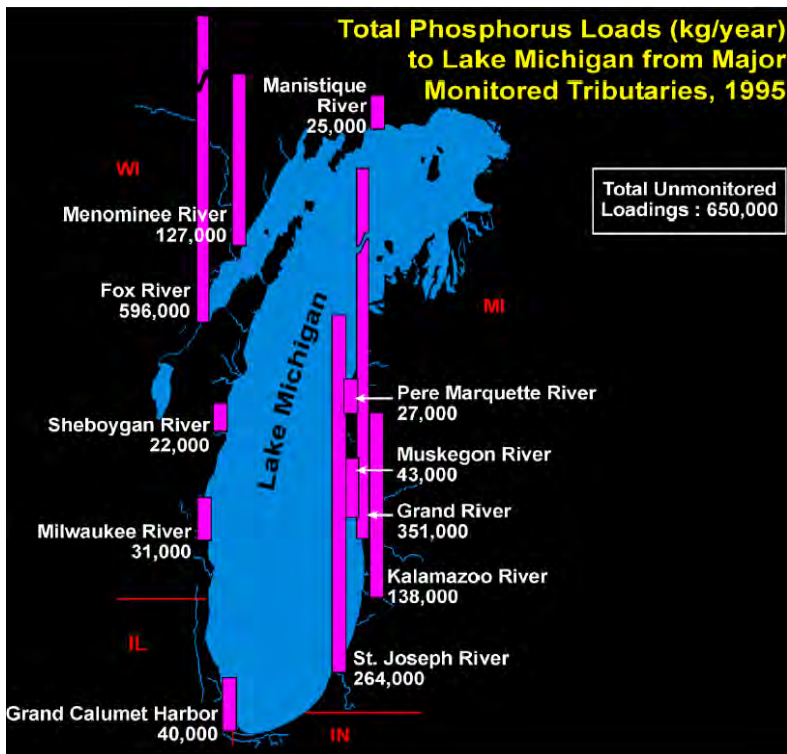


Fig. 6. Total phosphorus loads to Lake Michigan from major monitored tributaries, 1995. (Kreis *et al.* 2004)

While total annual phosphorus loading to Lake Michigan declined from the early 1970s through 1995 (Fig. 7) (Kreis *et al.* 2004), it is not known whether loading may have increased in recent years. Increasing population, burgeoning land development and the trend toward large feedlots and factory farms in the basin may be expected to lead to larger phosphorus loads. In addition, climate change associated with the warmest decade on record is evidenced by reduced ice cover, longer growing seasons, and intensified storm runoff. The cumulative effects of these changes on phosphorus loading needs to be assessed through updated tributary load studies.

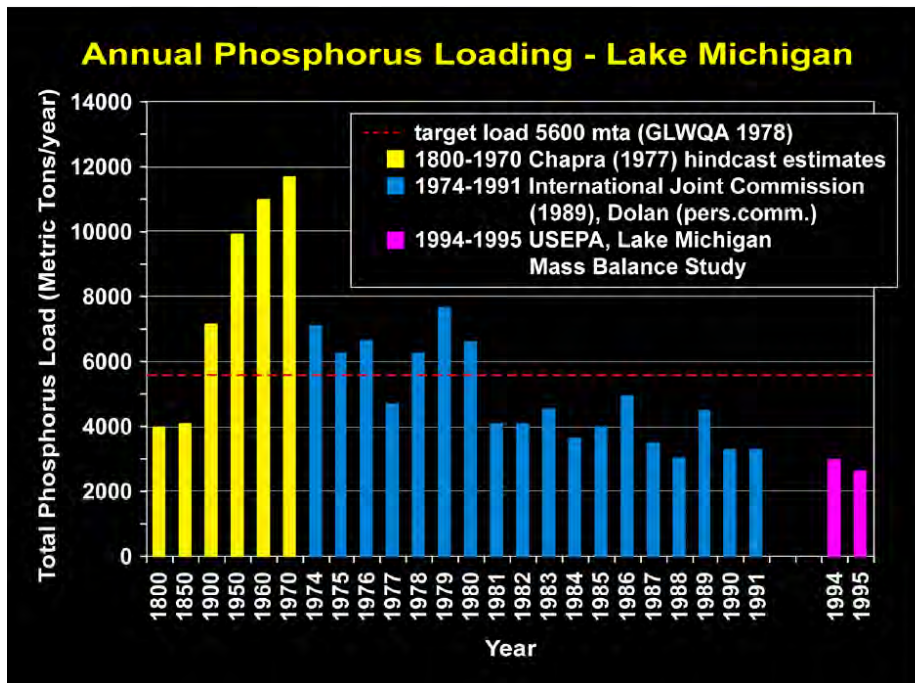


Fig. 7. Annual phosphorus loading to Lake Michigan, 1800-1995. (Kreis *et al.* 2004)

## MANAGEMENT OPTIONS

**Control Options** – Considering the principal environmental factors that control *Cladophora sp.* production (substrate, temperature, light and nutrients), the only feasible option for limiting productivity and macroalgae standing crop over the long-term is to reduce phosphorus inputs to Lake Michigan and the other Great Lakes. A dilemma arises if decreased phosphorus loads are insufficient to overcome the apparent influence of dreissenid mussels on *Cladophora* abundance. However, there are multiple benefits to the lakes, streams and communities in the Great Lakes basin from reducing point and nonpoint sources of pollution and from managing stormwater runoff.

A plethora of cost-effective best management practices exists for reducing runoff and nutrient inputs from urban and agricultural areas. Advanced wastewater treatment technologies also are available to address point source loads of phosphorus. Another dilemma for managing phosphorus is that most municipal and industrial discharge limits are based on effluent concentrations, not mass loads. As municipal populations and industrial outputs increase, a larger



volume of wastewater will deliver a greater mass of total phosphorus to receiving waters. The total maximum daily load (TMDL) approach used for persistent bioaccumulative toxic contaminants should be considered for phosphorus as well.

Most watersheds in the Great Lakes basin have plans on record that recommend strategies for controlling point and nonpoint sources of pollution. Additionally, many governments offer a variety of cost-share programs to assist with implementing some practices. However, greater public, private and political commitments and additional resources are necessary to further implement source reductions.

***Mitigation/Clean Up Options*** – In the short term, removal of *Cladophora* accumulations can help to mitigate offending conditions. A variety of removal methods have been used with some degree of success. For smaller accumulations, frequent hand raking and composting or land filling is feasible, albeit a tedious task. For large accumulations, particularly on public beaches, mechanical removal has been accomplished with front-end loaders, backhoes or beach grooming equipment. However, some cautions must be noted. Monitoring of indicator bacteria in beach sand has shown that heavy equipment may grind the decaying algae down into the moist sand, creating conditions that promote higher counts of *E. coli* bacteria. Also, care must be taken to avoid damaging sensitive beach vegetation and nearshore habitat. There are no state guidelines as yet for *Cladophora* removal and disposal and further assessment of alternative techniques is needed to identify cost-effective and environmentally acceptable methods.

The key to successful cleanup is vigilance in removing the algal mats as soon as they wash ashore. After only a few days in the warm sun, the algae begin to decay into an organic soup that is extremely difficult to collect and remove.

## CONCLUSIONS

The recent resurgence of *Cladophora sp.* in the Great Lakes is most likely the response of a dynamic ecosystem to changes in nutrient flux and water transparency. The relative importance of dreissenid mussels on water clarity and nearshore phosphorus concentrations vs. the importance of phosphorus inputs from tributaries and controllable anthropogenic sources needs to be better understood. The influence of water level changes is also unclear. Future monitoring and research to sort out the complex set of controlling factors will assist in developing management strategies. While it is not feasible to reduce dreissenid mussels or to manage water temperatures, substrates or water levels, effective controls for point and nonpoint sources of phosphorus are available and implementable to reduce tributary loading to Lake Michigan. In addition, the use of such best management practices will provide multiple benefits to local streams and communities in the Lake Michigan basin.

Beach managers and property owners need information on environmentally sound and cost-effective methods of algae removal and odor reduction. Finally, a source(s) of financial assistance for public beach cleanup likely will be necessary for communities to employ effective techniques.

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## ***Cladophora*: How is it Handled?**

Rebekah Stauffer

Milwaukee Community Service Corps

### **Introduction**

By now we are all aware of the *Cladophora* problem on our beaches. But what is being done to clean it up and what happens to it after it is taken off the beach? I will attempt to answer these questions by touching on four main points: the factors that affect the amount of *Cladophora* produced, the role of Milwaukee Community Service Corps in the cleanup process, how *Cladophora* is disposed of, and some ideas for beneficial uses for *Cladophora*.

### **What affects the amount of *Cladophora* produced?**

*Cladophora* growth is promoted by nitrogen and phosphorus. Nitrogen and phosphorus come from two main sources. The first is non-point source pollution. Run-off from roads, pesticides, herbicides, fertilizer from fields and farms, and bird feces are all components of non-point source pollution. The second potential supplier of nitrogen and phosphorus is sewage overflow. Any amount of sewage that makes its way into the waterways provides *Cladophora* with the nitrogen and phosphorus it needs to survive.

Another potential contributor to the amount of *Cladophora* produced is zebra mussels. Zebra mussels are an invasive species that eat nutritious algae but spit out algae that contain toxic compounds. Zebra mussels may contribute to *Cladophora* growth by filtering water, thus improving water clarity. Better water clarity allows light to penetrate to deeper depths, which increases the area available for *Cladophora* growth.

The amount of *Cladophora* washed ashore on a daily basis is affected by two factors. The first of the two determining factors is the weather conditions on any given day. For instance, on rough days when there is an onshore wind, *Cladophora* surface mats can extend more than twenty feet out into the lake. The second factor is the water currents of the lake. In the Milwaukee region, lake water flows from north to south. Therefore, *Cladophora* that grows north of Milwaukee breaks free and can wash up on Milwaukee beaches. What can be done to clean *Cladophora* off the beaches?

## **The role of Milwaukee Community Service Corps**

There is no easy solution to the *Cladophora* problem but sometimes the simplest act can make a difference. Milwaukee Community Service Corps has been cleaning *Cladophora* off the beaches since summer of 2002. Crews are sent out the Milwaukee's lakefront three to five times per week with pitchforks, shovels, and garbage bags to clean the *Cladophora* off Bradford Beach, Picnic Point, and North Point. The project runs from May to October. The work that Milwaukee Community Service Corps does on the lakefront is visible to the community. The importance of this is that other people are encouraged to become educated about *Cladophora* and learn what they can do to help. Approximately twenty five tons of *Cladophora* were picked up in 2004 by the corps. If that much *Cladophora* was cleaned up by Milwaukee Community Service Corps alone, imagine how much more could be cleaned off the beaches if people from the community would donate their time to help.

Milwaukee Community Service Corps also heads up a phytoremediation project. Phytoremediation removes toxic chemicals from the ground and prevents them from running off into lakes and rivers. The corps currently has one phytoremediation test cell at Pier Milwaukee at which zucchini, poplars, and willows are planted. All of the plants are growing very well and appear to be healthy. Hopefully, another test cell will be started at the Lake Michigan ferry site in the near future.

## **How is *Cladophora* disposed of?**

The most important part of *Cladophora* cleanup is what happens to it after it is put into trash bags. The trash bags are dragged up onto the grass and then the Milwaukee County Parks System takes the bags to Orchard Ridge Recycling Center. Yard waste is composted. However, algae is treated as solid waste and dumped into a landfill. There is a need to consider other more beneficial uses for *Cladophora*.

## **Beneficial uses for *Cladophora***

When *Cladophora* is placed in a landfill, gases released from the decaying waste are used to power homes. Another idea for *Cladophora* disposal would be to sell it to area farmers for fertilizing crops because of the fact that the nitrogenous and phosphoric wastes found in *Cladophora* act as fertilizers. However, before this is considered, the potential effects of other chemicals, and possibly metals, contained in *Cladophora* need to be considered.

## **Conclusion**

*Cladophora* has become an unpleasant and smelly nuisance to residents of Milwaukee. The best short term solution is to educate people about the potential causes of the problem and current management actions, with the hope of increasing volunteer activities. Long term solutions will require a better understanding of the factors affecting the growth, detachment and beaching of *Cladophora*.

## Why Filamentous Green Algae Dominated Benthic Habitats After the Zebra Mussel Invasion in Saginaw Bay, Lake Huron

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### Abstract

We conducted a series of nutrient manipulation experiments over the first 5 years of zebra mussel colonization in Saginaw Bay, Lake Huron, to evaluate benthic algal nutrient limitation and community composition. We placed nutrient-diffusing substrata in the littoral zone of the Bay during 1991 (early *Dreissena* colonization) and from 1992 to 1995 (post-*Dreissena* colonization). The treatments consisted of P, N, and P+N additions, and a control. Benthic chlorophyll *a* and benthic algal biomass decreased through time from 1992-1995. Phosphorus limited biovolume only in 1994. Treatments with P additions had significantly ( $p < 0.05$ ) more chlorophyll *a* than the controls each year after 1992. This result was consistent with an observed decrease in dissolved P throughout the study. Nitrogen additions had no significant effect throughout the 5 year period. Major shifts in species composition did not result from nutrient additions but rather seemed consistent with changes in light penetration and *Dreissena* herbivory. Our data demonstrated that the pre-*Dreissena* benthic algal community was dominated by tychoplanktonic diatoms (i.e., *Aulacoseira granulata* and *Tabellaria fenestrata*), which would be susceptible to filter-feeding *Dreissena*. Early post-invasion conditions were marked by an increase in light penetration (probably due to a decrease in phytoplankton by *Dreissena* grazing), and benthic algae were dominated by filamentous green algae (mostly *Spirogyra* sp. and *Cladophora* sp.). This type of algae typically responds well to

increases in light. Late post-invasion conditions were marked by a reduction of light caused by planktonic blooms of the blue-green alga *Microcystis* sp., which were resistant to zebra mussel herbivory. The benthic algal dominance shifted to periphytic diatoms (i.e. *Gomphonema clevei*), which were also resistant to zebra mussel filter-feeding since they were attached to the substrata and thus not part of the plankton. A new equilibrium may have developed where *Dreissena* herbivory limits tycho planktonic diatoms, which promotes *Microcystis* blooms (through lack of competing algal taxa), which in turn limits *Dreissena* filtering rates.

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***Cladophora* and the Beach: Implications for Public Health**

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**KEYWORDS**

*E. coli*, *Cladophora*, beach monitoring, recreational water quality, Door County, Lake Michigan, Lake Superior



**ABSTRACT**

There has been a recent reemergence of *Cladophora* and associated microbial communities at numerous beach locations throughout the State of Wisconsin. These communities form microbial mats and have become an unsightly, malodorous problem at many Great Lakes beaches. Recent research has found very high levels of *E.coli* associated with these algal mats (>39,000 CFU per gram of DW algal mat). *Cladophora* mats have emerged as a possible source of *E.coli* and a possible mechanism for survival of *E.coli* in recreational water. *E.coli* is an indicator organism used to evaluate recent fecal inputs into recreational waters. Thus, these algae-*E.coli* effects have the potential to have profound impacts on beach managers and the many people that utilize the water resources of the Great Lakes. More research is needed to assess the relationship between *E.coli* and these algal mats, as well as the relationship of the mats and pathogens released into the water. By addressing the aforementioned questions we can better understand the risk to public health posed by *E.coli* associated with the algal mats observed at recreational beaches. The Wisconsin tourism industry is a 12 billion dollar a year industry making water quality issues in locations such as Door County not only a local problem, but also an important state-wide issue.

## INTRODUCTION

In recent years the beaches and recreational waters of Lake Michigan have seen a resurgence of algal growth. There is no clear reason for this resurgence, however, such factors as increased phosphorus runoff, zebra muscle invasion, and global warming have been implicated as causes. Whatever the reason, it is clear that this increase in nuisance algae is causing problems throughout the Great Lakes. The only Great Lake without a noticeable increase in algal content is Lake Superior. One of the most predominant algal genera found in recreational water is the green alga *Cladophora*. While *Cladophora* is a major visual component of the algae found at public beaches it is only one of many microorganisms found within the algal masses at these locations. These masses are usually associations of the *Cladophora* and other microorganisms that form a complex floating microbial community, or *Cladophora* mat.

Recent research has found high levels of *E. coli* in *Cladophora* mats throughout Lake Michigan (Table 1). Furthermore, it has been suggested that these algal mats may harbor the water quality indicator *Escherichia coli* (*E.coli*) for longer periods of time than ambient water (Whitman et al, 2003 and Byappanahalli et al, 2003). *E.coli* is used as the standard for water quality assessments in Wisconsin, and many states, and is considered an indicator of recent fecal contamination (US EPA, 1986). *E.coli* levels at beaches are used as the justification for beach closures on the premise; if *E. coli* is found at a beach it represents a recent input of fecal material to the water and consequently an increased risk to public health of water-borne disease. This premise was the original basis for the selection of this organism as an ‘indicator’ organism for beach monitoring (Cabelli, 1983). Thus, the question as to the transient, or stable, nature of these *E.coli* populations associated with algal mats is not well studied.

## **SURVIVAL IN THE ENVIRONMENT**

Indicator organisms, such as *E.coli*, are commensal organisms in warm-blooded animals. These animals constitute their primary habitat and provide a warm and stable environment with relatively high levels of nutrients. The doubling time for *E.coli* in a primary host can be between 12-48 hours (Brettar and Hofle, 1992).

Once indicator microorganisms are released into the environment they are in a constant battle for survival. The aforementioned recreational water would be considered a secondary habitat of *E.coli*. While *E.coli* may be ubiquitous in the environment (Byappanahalli et al., 2003), conditions in this secondary habitat do not favor prolonged survival or growth. Secondary habitats generally limit survival times of *E.coli* due to low nutrients, increased predation, variable temperature, pH, and moisture, and sunlight killing effects (Figure 1). Some research has shown that suspended particles such as sand and organic matter can increase survival times in water environments (Bogosian et al., 1996 and Brettar and Hofle, 1992) and it is plausible that this effect would extend to other compounds present in a water secondary habitat.

## **CLADOPHORA IMPACT ON BEACHES**

The paradigm of *E.coli* accurately indicating recent fecal contamination could be challenged should the recent research regarding *Cladophora* promoting *E.coli* growth hold true on many locations. It is plausible that *Cladophora* mats provide an environment suitable for microbial survival and growth. The decaying mats, containing a myriad of microorganisms, likely provide an increased level of nutrients relative to the surrounding aquatic environment. In addition to potentially increasing nutrients in the water at beaches, it is obvious that the mats would decrease death from ultraviolet radiation due to the dense nature of the mats. These algal

mats would also provide a warmer and more moisture-stable environment than the sand or rock beach environment.

Should the above hold true in future studies, it would drastically change the environmental effects on *E.coli* in the environment at-large. It is then plausible that *E.coli* survival would be prolonged when many of the stresses on bacterial survival are removed or decreased. Furthermore, if these stresses are removed to a sufficient level and nutrients are plentiful enough, *E.coli* may have the opportunity to reproduce and not just survive. Should this be the case it would challenge the paradigm that *E.coli* is a good indicator of recent fecal contamination since *E.coli* may in fact survive for long periods of time when associated with algal mats.

While the prolonged survival, and possible growth, of *E.coli* may challenge current dogma it also may impact the swimming status of many Great Lake beaches. That is, if *E.coli* survive longer it may lead to elevated levels of *E.coli* being found at beaches. Additionally, since these algal mats move in and out from beaches they may contribute to the flux of *E.coli* into the water column. For example, if the mats move in and out from the beach area they may bring *E.coli* with them and deposit the *E.coli* on the beach sand or in the beach water that is monitored. Thus, when beach managers are monitoring a beach for *E.coli* they may not realize that the *E.coli* they are finding in the beach water is not due to a recent fecal influx, but rather a more stable *E.coli* population being emitted from transient algal mats. Should the latter be the case the actual risk posed to public health (i.e., cases of gastrointestinal illness per 1000 swimmers) by a particular *E.coli* level would likely not be the same as the original epidemiological studies used to establish beach monitoring criteria. At a minimum the public health risk is not understood should the *E.coli* populations be stable as opposed to transient.

To complicate matters further, in order to utilize *E.coli* as an indicator of pathogenic organisms associated with fecal pollution, one would have to know the impact of algal mats on pathogenic microorganisms associated with fecal materials. That is, pathogens such as *Salmonella*, *Shigella*, *Campylobacter*, Norovirus, *Girardia*, and *Cryptosporidium* would need to be evaluated in order to truly assess the impacts that these mats have on the risk to public health. In summary, it would be imperative to assess the survival of true pathogens, and not just *E.coli*, in the presence of algal mats in order to determine what the levels of algal-associated *E.coli* has on probable cases of gastroenteritis.

### **CLADOPHORA MONITORING IN DOOR AND KEWAUNEE COUNTY**

Many beach managers realized the potential impacts of *Cladophora* mats on beach status during the last several beach seasons. In order to begin an evaluation of *Cladophora* prevalence at beaches the Wisconsin Department of Natural Resources (WI DNR) asked beach managers and researchers to monitor relative amounts of observable algae. The algae evaluation criteria included a relative scale of 0-3, with 0 being no observable algae and 3 being excessive growth on the beaches. This scale was applied to 30 Door County beaches (27 Lake Michigan, and 3 inland lake beaches) and 2 Kewaunee County beaches (both Lake Michigan). In addition to the *Cladophora* observations, *E.coli* levels were monitored at these beaches as part of the routine beach monitoring in these counties each day an observation was made.

Figure 2 illustrates the relationship between observable *Cladophora* mats and *E.coli* levels. In most cases there was no relationship between *Cladophora* levels. However, a weak relationship was observed at Crescent beach (Kewaunee County), Kewaunee City Park (Kewaunee County), Murphy Park (Door County), and at Portage Park (Door County). While these relationships were observed for the 2004 beach monitoring season it is important to

emphasize that this data is preliminary and there are numerous other environmental factors impacting *E.coli* levels at these locations. Additional research is required to assess the detailed relationship between the observed *E.coli* levels and *Cladophora* mat quantities.

In addition to Door and Kewaunee counties, 3 counties on Lake Superior (Ashland, Bayfield, and Iron), as well as Vilas and Oneida County also participated in the same study. In these counties there were only two days of a beach with observable (n=44 beaches) algal growth and the *E.coli* level was <10 MPN/100mL (MPN=most probable number). Thus, the *Cladophora* issues did not appear to be a significant issue in these locations of Wisconsin.

## **FUTURE WORK**

There is a great deal of future research that needs to take place to further understand the relationship between algal mats and *E.coli*, as well as how this relationship translates to a health risk for recreational water users. Specifically, future research initiatives will focus on the use of genetic techniques (such as repPCR) for the evaluation of *E.coli* populations recovered from the algal mats to determine if these populations are transient, or clonal (i.e., relatively stable). In addition, spatial and temporal studies will be conducted at specific location to determine the effects that these dynamic mats have on the flux of *E.coli* in and out of the water column at beaches. Finally, both laboratory and field evaluations will look at the effects of these algal mats on pathogens and their survival (or lack thereof) when associated with these algal/microbial communities.

## **CONCLUSIONS**

The recent reemergence of *Cladophora* and associated microbial communities at beach locations has become an unsightly and malodorous problem at many Wisconsin beaches. However, while these issues may be unsightly or problematic for beach goers a new and more

problematic relationship with *Cladophora* mats has emerged: the mats' effects on *E.coli* survival and possible growth in recreational water. This effect would have profound impacts on beach managers and the many people that utilize these resources. The next question to answer in the relationship between *E.coli* and algal mats is the one related to the relationship between observable *E.coli* levels and risk to public health. To answer this question more research needs to be conducted on the relationship between *E.coli* and these mats as well as the mats and pathogens that may enter our waters due to fecal releases. The Wisconsin tourism industry is a 12 billion dollar a year industry. Thus, solving water quality issues in locations such as Door County is not only of local concern, but also an important state-wide issue.

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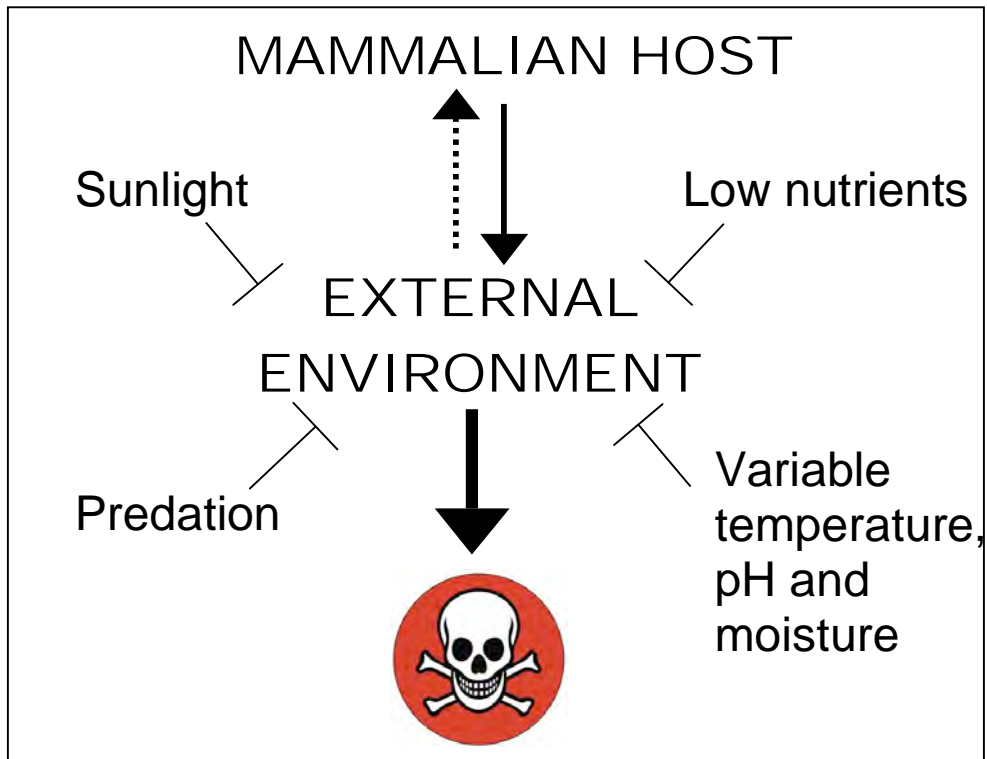
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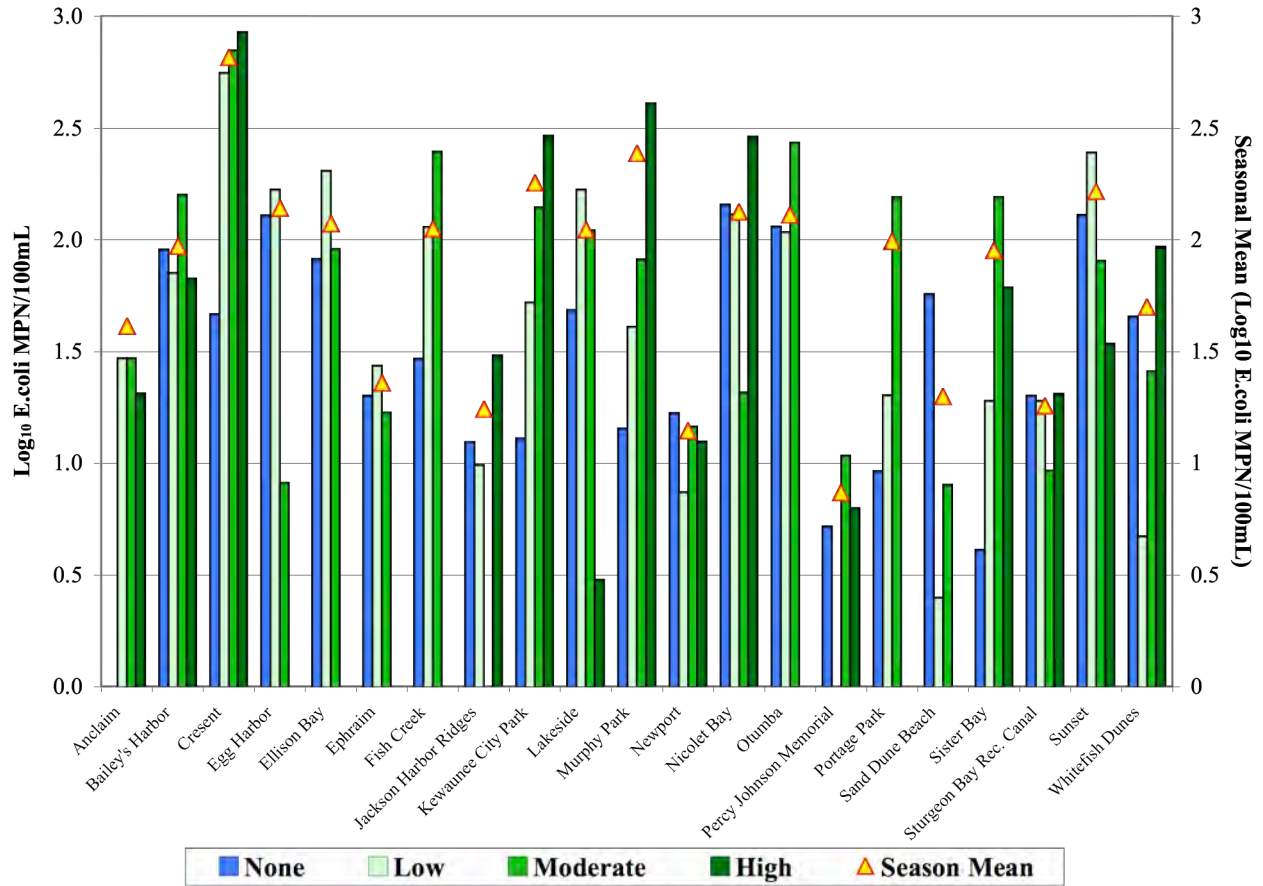


**Table 1.** Summary of *E.coli* recovered from *Cladophora* mats at select Wisconsin beaches during the summer of 2004. “On shore” refers to mats found on-shore and H<sub>2</sub>O refers to mats floating in the water at the beach location. Methods for the recovery of *E.coli* isolates are described in Whitman *et al.*, 2003. (Some data courtesy of Richard Whitman, USGS, Porter, IN)

<b>Beach</b>	<b><i>E. coli</i> (CFU/g DW)</b>
Anclam South (on shore)	110
Baileys Harbor (on shore)	1,729
Lakeshore (H <sub>2</sub> O)	470
Menomonie Park (H <sub>2</sub> O)	16,790
Menomonie Park (on-shore)	31,288
Murphy (on shore)	17,670
Murphy (H <sub>2</sub> O)	22,515
Sister Bay (H <sub>2</sub> O)	10,874
Sunset (H <sub>2</sub> O)	1,254
Whitefish Dunes (H <sub>2</sub> O)	89
Whitefish Dunes (on shore)	39,501



**Figure 1:** Environmental effects on *E.coli* once released from the primary habitat of a mammalian host to the external environment. (Adapted from Winfield and Groisman, 2003)



**Figure 2:** *Cladophora* observations using the WI DNR study during the summer of 2004. Log<sub>10</sub> *E.coli* counts observed on those days with and observable *Cladophora* are presented on the y axis. Season Log<sub>10</sub> *E.coli* mean from beach is presented on the yy axis. Only beaches with multiple days with the same level of algae observation are included. (n=>2,100 *E.coli* samples)

## The Interaction of Two Nuisance Species in Lake Michigan: *Cladophora glomerata* and *Dreissena polymorpha*

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The filamentous green algae *Cladophora glomerata* (L.) Kutz. and the zebra mussel *Dreissena polymorpha* (Pallas) are two of the most obvious nuisance species along the Milwaukee, WI portion of the Lake Michigan shoreline, as well as throughout much of the Laurentian Great Lakes. The biology of *C. glomerata* and *D. polymorpha* make them excellent coinhabitants of North American freshwater systems, including the littoral zone of Lake Michigan. *C. glomerata* reached nuisance levels along the Milwaukee, WI shoreline during the 1990's, soon after the introduction of zebra mussels into the Laurentian Great Lakes in 1985. Zebra mussels modify the benthic ecosystem through their filter feeding activity, which transfers nutrients from the water column to the benthos, and thereby promote changes that include altering the benthic flora and fauna. In order to determine if zebra mussels influence the growth of *Cladophora* by concentrating nutrients in the benthos, a laboratory experiment was initiated.

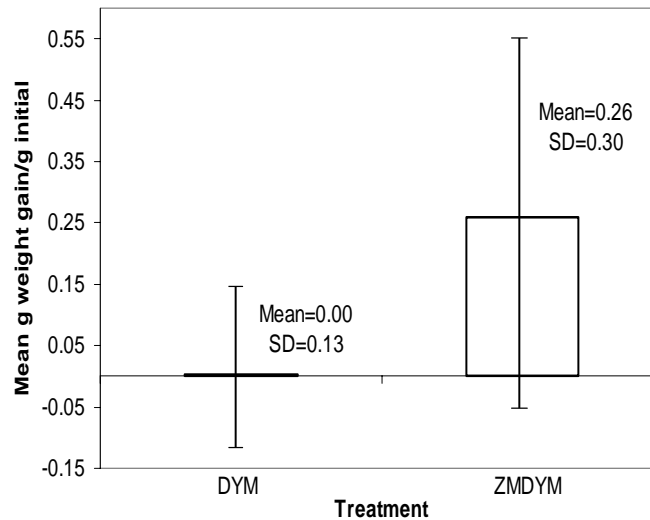
Past studies have not provided evidence of a mechanism for a direct link between zebra mussels and *Cladophora* growth. This study was designed to determine if the recent increase in *C. glomerata* growth in Lake Michigan is linked to enhanced nutrient availability caused by *D. polymorpha*. The objective of this study was to observe the affect of zebra mussel pseudofeces/feces, as well as soluble zebra mussel secretions/excretions, on the growth of *Cladophora*.

Live zebra mussels and *Cladophora* filaments were collected from the Milwaukee, Wisconsin shoreline, cleaned, and then maintained under laboratory condition of 18°C and 35  $\mu\text{M photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of light. *Cladophora* filaments were incubated in the above conditions for 14 days in one of four treatments, each with nine replicates. The first treatment consisted of *Cladophora* in DYM algal growth (DYM) media that mimicked the chemical composition of Lake Michigan. This was used as the control when observing the affects of the ZMDYM treatment. The second treatment, Zebra Mussel DYM (ZMDYM), contained soluble zebra mussel secretions/excretions. The third treatment consisted of *Cladophora* in filtered *Chlorella* DYM (CHLDYM), and was used as the control when observing the affects of the PFDYM treatment. CHLDYM contained any soluble materials that may be found in DYM containing chlorella, which was used for zebra mussel feeding. Treatment 4 was *Cladophora* in Pseudofeces DYM (PFDYM), which was DYM that was supplemented with zebra feces and pseudofeces.

*Cladophora* biomass, tissue nutrients, and median nutrients were measured before and after the incubation to provide the following results.

Experiment determined that *Cladophora* grown in DYM media supplemented with soluble zebra mussel secretions and excretions grew at a faster rate than did *Cladophora* grown in straight DYM media (figure 1). This shows that in a small, closed environment, the presence of zebra mussels can increase the growth of *Cladophora*.

**Mean Grams Wet Weight Gain of *Cladophora* Tissue per Gram Initial Wet Weight (DYM v. ZMDYM)**



**Figure 1.** Mean grams wet weight gain of *Cladophora* tissue per gram initial wet weight over 14 days (DYM v. ZMDYM)

The nutrient analysis of the initial medium shows that the ZMDYM contains nearly twice the level of nitrate/nitrite as the straight DYM ( $22\mu\text{M N}$  vs.  $14\mu\text{M N}$ , respectively) (table 1), and 11 times the level of SRP ( $11\mu\text{M P}$  vs.  $1\mu\text{M P}$ , respectively) (table 2). This suggests that both the higher nitrate/nitrite concentration and higher soluble reactive phosphorus (SRP) concentration in the ZMDYM may have contributed to the increased growth.

**Table 1.** Concentration of nitrate/nitrite in media before experiment ( $\mu\text{M}$ )

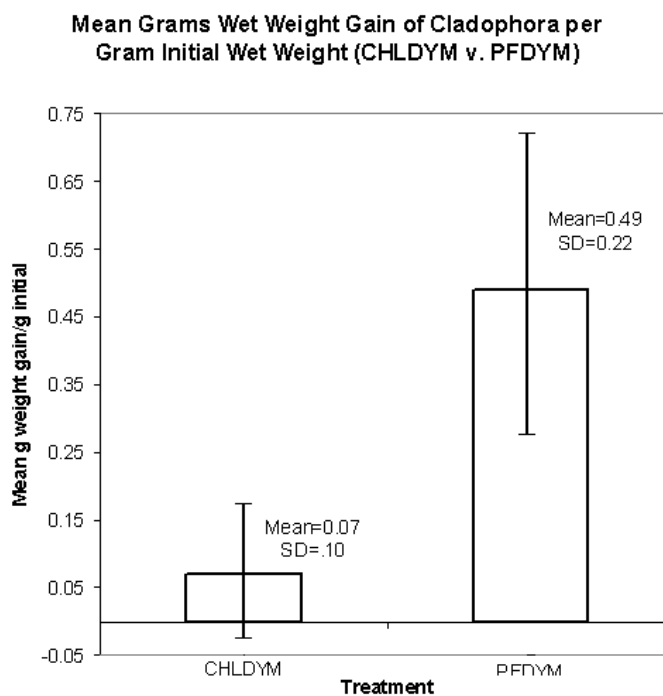
	DYM	ZMDYM	CHLDYM	PFDYM
N of cases	3	3	3	3
Mean	14.526	22.078	15.060	15.504
Standard Dev	0.574	0.776	0.071	0.468

**Table 2.** Concentration of SRP in media before experiment ( $\mu\text{M}$ )

	DYM	ZMDYM	CHLDYM	PFDYM
N of cases	3	3	3	3
Mean	1.009	11.085	5.841	1.232
Standard Dev	0.008	0.149	0.006	0.071

In a broader view, these results suggest that in an open system, such as Lake Michigan, the presence of zebra mussels may increase the biomass of *Cladophora* in close proximity to mussel beds by increasing concentration of both available nitrogen and available phosphorus.

It was also determined that *Cladophora* grown in medium that was fertilized with pseudofeces/feces grew at a faster rate than *Cladophora* grown in medium which contained nutrients that may have solubilized from dried *Chlorella* as it was soaked in DYM (figure 2). This shows that under laboratory conditions such as those described here, the presence of zebra mussel pseudofeces/feces can increase the growth of *Cladophora*, even after correction for nutrients that may have been derived from un-ingested *Chlorella* in the container.



**Figure 2.** Mean grams wet weight gain of *Cladophora* tissue per gram initial wet weight over 14 days (DYM v. ZMDYM)

The nutrient analysis of the media showed that the initial PFDYM and CHLDYM contained similar levels of nitrate/nitrite (15  $\mu\text{M}$  N each) (table 1), and the initial CHLDYM medium contained a higher level of SRP than did the PFDYM (5 $\mu\text{M}$  P vs. 1 $\mu\text{M}$  P) (table 2). These data do not suggest a reason for the increased growth in the PFDYM treatment versus the CHLDYM treatment, since the PFDYM media contained similar or lower levels of the nutrients analyzed.

These data suggest that in an open system, zebra mussel pseudofeces/feces can contribute to a fertilization effect on *Cladophora* in close proximity. At this time it is unclear as to which compounds from the pseudofeces/feces contributed by the zebra mussel are responsible for the observed affect. Nutrient analysis showed that dried *Chlorella* and pseudofeces/feces contained similar levels of carbon and nitrogen per unit mass (approximately 165 mg carbon/g sample and 82 mg nitrogen/g sample, respectively), although the dried *Chlorella* contained nearly twice the level of phosphorus as did the pseudofeces/feces (204 mg phosphorus/g sample v. 123 mg phosphorus/g sample, respectively). This suggests that the mucus that holds the pseudofeces together may contain high levels of carbon and nitrogen, and/or that the mussels

could be assimilating a larger proportion of phosphorus than the other nutrients from the dried *Chlorella*, decreasing the proportion of phosphorus in their feces.

Analysis showed that the carbon and nitrogen levels in *Cladophora* were not significantly different between the straight DYM treatment and the treatment with DYM media supplemented with soluble zebra mussel secretions, or between the *Cladophora* grown in media that was fertilized with pseudofeces/feces and *Cladophora* grown in media which contained nutrients that may have solubilized from dried *Chlorella* as it was soaked in DYM. This can be explained by the fact that nitrogen levels in each of the treatments, 14.52 to 22.08  $\mu\text{M N}$ , are near or above the 16  $\mu\text{M}$  concentration of nitrate/nitrite that would limit the growth of *Cladophora* (Gerloff and Fitzgerald, 1976). *Cladophora* within each treatment was able to take up as much nitrogen as needed from the respective media.

In contrast to the carbon and nitrogen levels, tissue phosphorus levels were significantly higher in the ZMDYM treatment than the DYM treatment, and higher in the *Chlorella* DYM treatment than the PFDYM treatment. The levels found in the ZMDYM treatment versus the DYM treatment can be explained by the fact that the ZMDYM media, before addition to the experimental beakers, contained 11 times the SRP levels of the DYM. Some reasons for this can be phosphorus present in any of the secretion or excretions of the zebra mussels, or a slight contribution from the *Chlorella* used to feed the zebra mussels. Higher tissue phosphorus levels in the CHLDYM treatment versus the PFDYM can be explained by the fact that the initial CHLDYM media contained 5 times the level of SRP as the PFDYM. The reason for this could be that the zebra mussels integrated a higher proportion of phosphorus than the other nutrients from the dried *Chlorella*, and so there was less phosphorus in the pseudofeces/feces to contribute to a high media concentration of SRP.

*Cladophora* C:N:P ratios were also determined from the tissue nutrient analyses. The *Cladophora* grown in straight modified DYM media had a C:N:P ratio of 90:5:1, the *Cladophora* grown in DYM supplemented with zebra mussel excretions/secretions had a ratio of 50:3:1, *Cladophora* grown in DYM supplemented with solubilized *Chlorella* nutrients had a ratio of 54:3:1, and the *Cladophora* grown in DYM fertilized with zebra mussel pseudofeces/feces had a ratio of 82:5:1. This indicates that *Cladophora* grown in DYM was able to integrate less phosphorus than the *Cladophora* grown in ZMDYM, although nitrogen levels were similar, and *Cladophora* grown in CHLDYM was able to integrate more phosphorus than did the *Cladophora* grown in PFDYM, although the tissue from these two treatments also contained similar levels of nitrogen. These tissue nutrient ratios appear to indicate that nitrogen may have been limiting in this experiment

These experiments have shown that, in a small, closed system, the presence of zebra mussel pseudofeces/feces and soluble secretions and excretions cause an increase in *Cladophora* biomass. This suggests that in a large, open system, such as near-shore regions of Lake Michigan, the introduction of zebra mussels during the late 20<sup>th</sup> century may have contributed to the increase in *Cladophora* biomass by concentrating nutrients in the benthos, in contrast to the period of high *Cladophora* during the 1960's, which was attributed to high phosphorus levels in the lake during that time. The results of the current study support the work of Lowe and Pillsbury (1995), who found that the presence of zebra mussels does increase benthic algal

biomass in the Laurentian Great Lakes. As of 2004, no known control method has been developed to solve the *Cladophora* problem along the Milwaukee shoreline, and at this point, it is difficult to suggest any suitable mechanism to control *Cladophora* in a large water body such as Lake Michigan. Unless a control method is developed, the *Cladophora* problem is not expected to wane at any time in the near future.

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## The Weakest Link and What Makes It Stink

Shannon Davis-Foust and John Janssen

Besides modifying nearshore nutrient cycles and enhancing *Cladophora* growth, zebra mussels may play a role in causing more *Cladophora* to be released from the bottom than would naturally occur. There are two detachment points for *Cladophora*; one is at the base of the filament itself, and the other is by the byssal threads of the mussels that *Cladophora* is attached to. Possible mechanisms for mussel detachment are presently being explored. It is possible that a *Cladophora* canopy depresses either filter feeding or gas exchange/waste removal by the mussels, and the mussels detach either by deterioration of health or by some intentional mechanism to obtain more nutritional resources. The algal filaments may promote this detachment by acting as a drogue in the water currents.

Regardless of the mechanism that causes the mussel detachment, mussels, both living and dead, invariably wash up on the shore with *Cladophora* attached to their shells. While mussels have been washing ashore since they became established in the Great Lakes, washed up mussels do appear to be smaller than they were prior to the increased *Cladophora* levels. Also, sometimes it is possible to pull mussels from the substrate by pulling on heavy growths of *Cladophora* that are attached to them, making the mussel attachment the 'weakest link'.

Mussel reefs may provide additional hard substratum for *Cladophora* to attach to, but it has been recently observed that quagga mussel shells may not be as conducive to *Cladophora* attachment, possibly because they have smoother than zebra mussels. Indeed, it appears that zebra mussels are more likely to detach and wash ashore with *Cladophora* attached. The ratio of quagga mussels to zebra mussels in the littoral zone of Lake Michigan appears to be on the rise, and this trend is likely to continue because quagga mussels are more efficient at filter feeding. These changing ratios could impact *Cladophora* dynamics. (Check with Russell about quagga filter feeding; my impression was that they are not as efficient at filtering as zebras)

Rotting vegetation is often blamed for the odor that beached *Cladophora* is associated with. We have observed that these odors are likely enhanced by the presence of rotting animal material, such as mussels, crustaceans, fish, etc. This idea is substantiated by the presence of waterfowl, which congregate at wash up locations for feeding. Also, there are many reports of washed up *Cladophora* that does not stink.

### Creating a Wisconsin Coastal Imagery Database

The potential for *Cladophora* accumulation on beaches is a function of geomorphology, biomass distribution, and nearshore hydrodynamics. Our current efforts are focused on determining *Cladophora* spatial distribution, locating areas of high biomass, and assessing the roles of zebra and quagga mussels in augmenting nuisance levels of *Cladophora*.

In order to assess large-scale *Cladophora* distribution patterns along the Wisconsin coast of Lake Michigan, we have conducted an aerial survey of the Wisconsin coastline on Lake Michigan. During summer 2004, aerial photographs of the nearshore coastline were taken from Racine to about the middle of the Door Peninsula. Combining the aerial imagery, which provides clues to the nearshore bottom type, with ground-truthing via scuba, snorkeling, drop video camera, and unmanned submersible observations as well as bathymetric data, will improve our knowledge of *Cladophora* distribution in relation to the geomorphology of the western Lake Michigan coastal environment.

## Molecular Phylogeography and Species Discrimination of Freshwater *Cladophora* (Cladophorales, Chlorophyta) in North America

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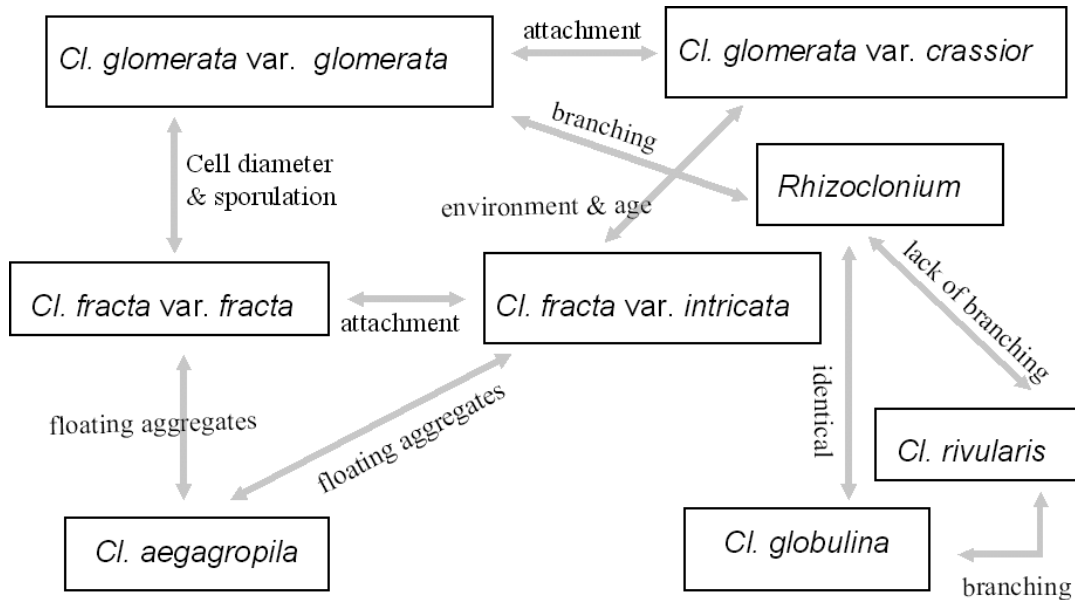
The genus *Cladophora* (Cladophorales, Chlorophyta) is cosmopolitan in temperate and tropical regions in freshwater, brackish and marine habitats (Marks and Cummings, 1996; John, 2002). The freshwater species, *Cl. glomerata*, is frequently observed in eutrophic waters and can cause nuisance blooms under favorable conditions (van den Hoek, 1963) and considerable research has been done (and is ongoing) to prevent or reduce such problem blooms (Reynolds et al. 2000). Despite the considerable research on the ecological and physiological aspects of freshwater *Cladophora*, very little has been done to address the complex taxonomic issues within this genus. In fact, a practical and meaningful taxonomic framework for species within the genus *Cladophora* has yet to be determined (John, 2002). The “species” is the fundamental unit in taxonomic biology and many studies assume correct species identification. However, as in the case with many algal groups species are not easily delineated. Species exist because they fill discrete ecological niches and possibly differ in their physiological responses to the environment. Hence, being able to determine species (or genotype) is essential for interpreting both ecological and physiological studies as well as comparison between studies. This may have a considerable impact on management of nuisance blooms of members of this genus in different bodies of water.

In 1753, Linnaeus established the genus *Conferva* and subsequent to this, Kützing (1843), moved a large number of this genus to a new genus, *Cladophora*. With the renaming came a large number of new species, subspecies and varieties. In fact there are over 650 existing names for species of *Cladophora*. However, the genus *Cladophora* demonstrates a great degree of phenotypic plasticity due to varying environmental factors and age of the alga and therefore there are only 170 currently recognized species epithets. In 1963, van den Hoek reviewed the European species of this genus largely through the use of cultured *Cladophora* material and type specimens using both morphological and reproductive characteristics. He determined that there were 11 separate freshwater species belonging to six sections. The freshwater species listed were composed of six separate sections (species shown in brackets): *Aegagropila* [*Cl. aegagropila* (L.) Rabenh.], *Glomeratae* [*Cl. fracta* var. *fracta* (Mull. Ex Vahl) Kutz., *Cl. fracta* var. *intricata* (Mull ex Vahl) Kutz., *Cl. glomerata* var. *glomerata* (L.) Kutz., and *Cl. glomerata* var. *crassior* (L.) Kutz. ], *Cladophora* [*Cl. rivularis* (L.) v.d. Hoek], *Cornuta* (*Cl. cornuta* Brand), *Affines* (*Cl. kosterae* Hoffm. & Tild.) and *Basicladia* [*Cl. basiramosa* Schmidle, and *Cl. pachyderma* (Kjellm.) Brand.].

The morphological characteristics used to differentiate these six sections and eleven species by van den Hoek (1963) include thallus organization, pattern and amount of branching, type of main axes growth (intercalary or acropetal), mode of reproduction, length/width ratio of main axes cell, apical cell diameter, attachment organ and finally if the organism is attached to a substrate or free-floating. For example, section *Aegagropila* is usually densely and irregularly branched, taking on a ball like formation and section *Cladophora* is composed of usually free-floating plants, which are sparsely branched. In addition, section *Glomeratae* demonstrates the greatest amount of phenotypic plasticity as they can be densely branched, or not; free-floating, or not; with different types of growth, main axes organization, and cell length-width ratios.

Closer inspection of these morphological traits shows that there are a number of overlapping taxonomic characteristics that are to be used to distinguish species within and among these different species and sections (Figure 1). This confusion is most evident in the *Glomeratae* section. *Cl. glomerata* var. *glomerata* differs from *Cl. glomerata* var. *crassior* in the fact that one is attached and one is floating, but each is readily confused with *Cl. fracta* var. *fracta* and *Cl. fracta* var. *intricata* which also only differ

based on their attachment or lack thereof. *Cl. glomerata* has been misidentified as *Cl. fracta* var. *fracta*, another genus, *Rhizoclonium*, and *Cl. barismosa*, all in their unbranched form. Though cell size can be used as a determinant, certain environments may permit each to have a very similar main axes cell diameter. It is very well known and has been for many years that the *Cladophora* species display a high degree of phenotypic plasticity, which is the ability of an individual's genotype to respond to environmental influences and generating different phenotypes.



**Figure 1. Diagram illustrating how the taxonomic characters used for identifying species of *Cladophora* are overlapping. Many of these characteristics are dependent on the environmental conditions under which the plant is observed.**

Considering the tremendous morphological diversity and the issues arising with species identification, it is necessary to re-examine the taxonomic status of these freshwater species using molecular markers. Molecular markers are polymorphic protein or DNA sequences that can be used as indicators of genome-wide variations. There are a number of advantages to using molecular markers, namely that markers and marker variation can be quantified with great precision. Secondly the use of molecular markers allows for qualitative statistical analysis. Initially, we examined the internal transcribed spacers (ITS 1 and ITS 2) of the nuclear ribosomal DNA (rRNA) cistron to determine sequence divergence among North American populations of *Cladophora*. Typically, the ITS regions are beneficial marker for differentiating species and has been used extensively in other algal phyla to address such relationships (Bakker *et al.*, 1992). However, we noted that all sequences were identical or nearly identical. Hence, we are now examining the utility of other molecular markers. This is similar to what was noted by Marks and Cummings (1996) who examined freshwater *Cladophora glomerata* in Europe. Although analysis of ITS regions is used for phylogenetic relationships, it is still a conserved region compared to analysis of the intersimple sequence (ISSR) molecular marker. The ISSR marker is used to determine genetic variation within and among populations. In fact we have observed that there do appear to be differences among populations in the Great Lakes as well as populations from other lakes and streams in North America. While the preliminary data looks promising we have not yet completed this study.

By far the most informative molecular markers currently being used are Microsatellites. Microsatellites are simple sequence repeats, and are tandemly repeated nucleotide stretches of DNA. The repeats may be mono-, di-, tri-, tetra-, or even penta-nucleotide units (i.e. GCGGCGGCGGCG) and are located throughout the genome. Variability is determined by polymorphic length variation. This method

is used extensively for DNA fingerprinting (Queller *et al.*, 1993) and is extremely sensitive and are frequently used to differentiate varieties or individuals, and can reveal parentage and identity (Karp *et al.*, 1996; Wattier *et al.*, 1997).

The primary focus of our research is to create a usable taxonomic scheme by addressing the following objectives: 1) Determine if *Cladophora* in the Great Lakes is all one clone or if different species/varieties are present, 2) Determine the relationship of Great Lakes *Cladophora* with other North American collections, 3) Delineate species of Freshwater *Cladophora* using morphology, chromosome and molecular analyses. We believe that delineation of the species of *Cladophora* that is in the Great Lakes may aid in helping to understand the recent resurgence of blooms as well as the origin of new populations.

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## Nuisance *Cladophora* in Urban Streams: habitats, seasonality, morphology, production, nutrient composition, heavy metals, foodweb bottleneck

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### Introduction

*Cladophora* and other filamentous green algae are natural components of temperate streams and the shorelines of some lakes. Their abundance and seasonal periodicity are influenced by: substrate type and availability (Curry et al 1981, Power 1990a, 1992, Dudley & D'Antonio 1991), water depth and current velocity (Pfieffer & McDiffert 1975, Ronnberg & Lax 1980, Fisher et al. 1982, Power & Stewart 1987, Biggs & Close 1989, Usher & Blinn 1990, Dodds 1991a, 1991b, Marks et al. 2000), light, shading and temperature (Moore 1977, Graham et al. 1982, Graham et al. 1985), chemical water quality (Whitton 1970, Wong & Clark 1976, Spencer & Lembi 1981, Auer & Canale 1982, Rosemarin 1982, Gerloff & Muth 1984, Hill & Knight 1988, Lester et al. 1988, Welch et al 1989, Dodds & Gudder 1992, Parr et al. 2002), grazing by invertebrate animals (Power 1990b, Feminella & Resh 1991, Dodds & Gudder 1992, Hart 1992, Creed 1994, Kupferberg 1997, Pinowska 2002), natural reproductive cycles (Graham et al. 1985, 1986), and sufficient historical time to allow the interactions with these factors to play out. Nuisance blooms of these highly visible green algae result when one or more of these natural checks and balances fails. This has recently become the situation in coastal Lake Michigan and Lake Ontario, resulting in considerable public concern. Similar nuisance blooms have also recently occurred in urban streams within Milwaukee County. Study of green algal blooms in these streams can provide insight for management of nuisance *Cladophora* in urban streams and in the Great Lakes.



Urban Stream Architecture

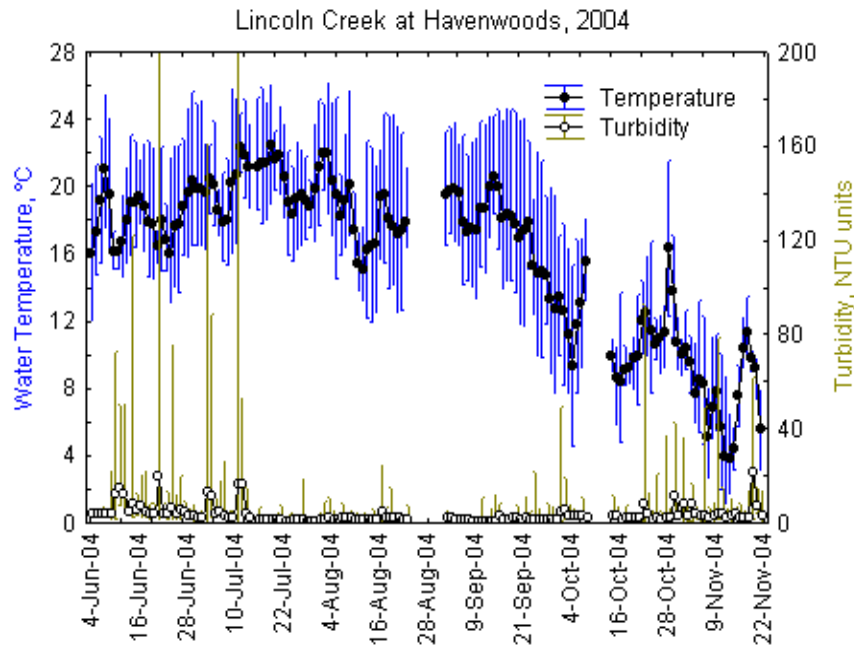
Fig. 1 (left). Underwood Creek near the intersection of Highway 41 and Mayfair Road. Fig. 2 (right). Lincoln Creek in the Havenwoods Nature Center near Sherman Boulevard and Mill Road. One of the temporary holding ponds constructed for Lincoln Creek is located in the upper left corner of Fig. 2. A combination of residential neighborhoods and industrial sites line almost the entire stream course of both.



Many urban streams were converted to straight channels and then lined with concrete during the growth of cities in the past century. This was done as an attempt to control flooding by quickly moving water through the urbanizing areas. Underwood Creek in Milwaukee County, Wisconsin is an example of this type of stream (Fig. 1). Flow regimes in such channels tend to be flashy, with low base flow interrupted with brief episodes during which large volumes of sediment-laden storm water scours algae off the concrete surfaces. A more recent approach to flood control has promoted increasing the short-term storage potential of urban streams by establishing cobble stream beds with meanders and adding small temporary holding ponds along the stream course. Flow in such streams is still flashy but the intensity of flushing events is reduced because of the added capacitance of the system and increased resistance to flow. Less sediment is transported because some settles out in holding ponds. Lincoln Creek in Milwaukee County, which was the object of a massive engineering project sponsored by the Metropolitan Milwaukee Sewerage District completed in 2000, is an example of such a restored stream (Fig. 2). Added benefits of the restoration projects were expected to be reestablishment of natural stream biota and greatly increased aesthetic and recreational appeal to residents.

### The Urban Stream Habitat

Streams differ greatly from large lakes as a habitat for algae. Moving water, which can rip algae from the substrate, is directional in streams, whereas waves and long-shore currents along the shores of lakes result in complex flow regimes that create not only shear stress, but also lift and torsion. While it may be possible to develop a functional relationship between water velocity and shearing of algae off substrate (which leads to beach or downstream aggregations) for streams, this would be a much more formidable task for nearshore algae in lakes.



Sha Fig. 3. Mean daily water temperature in Lincoln Creek, Summer 2004. Vertical lines (daily standard deviations) indicate the large day-night cycles in water temperature resulting from solar heating. Turbidity spikes, caused by suspension of fine sediment during storms, are used here to illustrate the frequency and seasonal pattern of rain events which can scour stream algae and reset the “successional clock” for foodweb development. e can

grow luxuriously for periods of time, but storms represent unpredictable major disturbances that dramatically change the biota by scouring or burial. Although generally unpredictable, there is a seasonal pattern to storm events in the study streams, with greater frequency in the spring and autumn (Fig. 3). Significant summer storms have been rare during the years of our study, but may have major biological consequences (*see below*). Storm frequency is correlated with water depth in the streams. The summer dry period is a time of very low water levels and water velocity. Water temperatures (Fig. 3) are much higher in these streams than in the coastal Great Lakes, and solar heating produces marked day-night cycles (4 – 8 °C) that can cause significant physiological stress on stream biota.

### Nuisance Algae in Urban Streams

Algae in streams can be segregated into three morphological categories based upon their size, habitat, and function in the foodweb. *Microperiphyton* are microscopic algae that grow attached to substrate in the stream. They are integral to stream foodwebs, representing the primary source of “plant” material to feed stream insects and other invertebrates, which in turn feed fish. *Suspended* microscopic algae are typically a mix of phytoplankton forms and microperiphyton cells that have been ripped from their substrate location. These represent food for small herbivorous animals that glean particles from the flowing water; they are typically present in very low abundance relative to microperiphyton (*see below*). Microperiphyton and suspended microalgae are critical for the establishment and maintenance of stream invertebrates and a diverse natural stream foodweb. Macroperiphyton are mostly filamentous algae that are visible to the eye, are attached by one end to substrate, or may be floating in areas with low flow. Although these morphologically large filamentous algae can be eaten by some invertebrates (e.g. crayfish, amphipods), grazers can affect growth of these algae primarily when the filaments are very short, essentially when they are the same size as microperiphyton. When invertebrates are unable to “manage” the macroperiphyton, these filamentous algae can develop very dense populations. In the Milwaukee urban streams, *Cladophora glomerata*, the same species found along the Great Lakes shoreline, is the most abundant member of the macroperiphyton assemblage during much of the summer, and it produces massive growths comparable to those found along the shores of Lakes Michigan and Ontario.

*Cladophora* is not necessarily a nuisance alga. Species are present in all local streams. It becomes extremely abundant in urban streams because these represent high-light and nutrient-rich environments in which *Cladophora* has a competitive advantage. The basis of this competitive advantage remains to be identified. Lincoln Creek, after the restoration project was completed in 2000, initially represented an algae-free environment characterized by nutrient-rich water flowing over newly-mined, white limestone cobble. Within two years, this stream course became choked with massive *Cladophora* blooms that have clearly interfered with the engineering project goal of restoring natural ecological function to the stream. This situation continues to date.

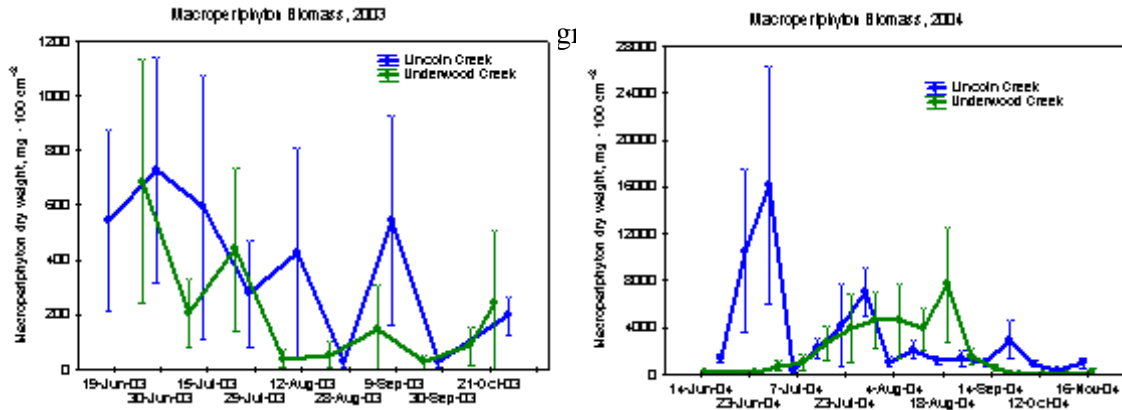


Fig. 4. Seasonal dynamics of macroperiphyton abundance in Lincoln Creek and Underwood Creek at the reference sampling stations (*illustrated above*). Data represent means of 8 spatial subsamples and are corrected for percent cover. Variability among subsamples is indicated by standard deviation (the vertical bars).

*Cladophora* can produce very large amounts of biomass in these streams (Figs. 4, 5). The period of maximum abundance and highest growth rate is generally from mid-May until mid-July, but *Cladophora* plants persist throughout the summer and autumn, becoming increasingly covered with small epiphytic algae and fine sediment particles. *Hydrodictyon*, a web-forming green alga that appears to be filamentous, replaces *Cladophora* as the macroperiphyton dominant in Lincoln Creek during July and August with similarly dense growths. This seasonality and succession pattern is clearly different that what is observed in Underwood Creek and on the shores of Lakes Michigan and Ontario. The specific environmental or biological factors that define this pattern remain unclear. *Cladophora* abundance is somewhat regulated by storm events which can rip loose the filaments. This accounts for much of the variability through time in biomass (Fig. 4). There is also a great deal of spatial variability in the abundance of macroperiphyton; this is represented by the large error estimates (standard deviations) at each sampling date. The standing crop of *Cladophora* has continued to increase in Lincoln Creek each of the four summers since the restoration project was completed (note the different vertical scales on the 2003 and 2004 panels of Fig. 4).

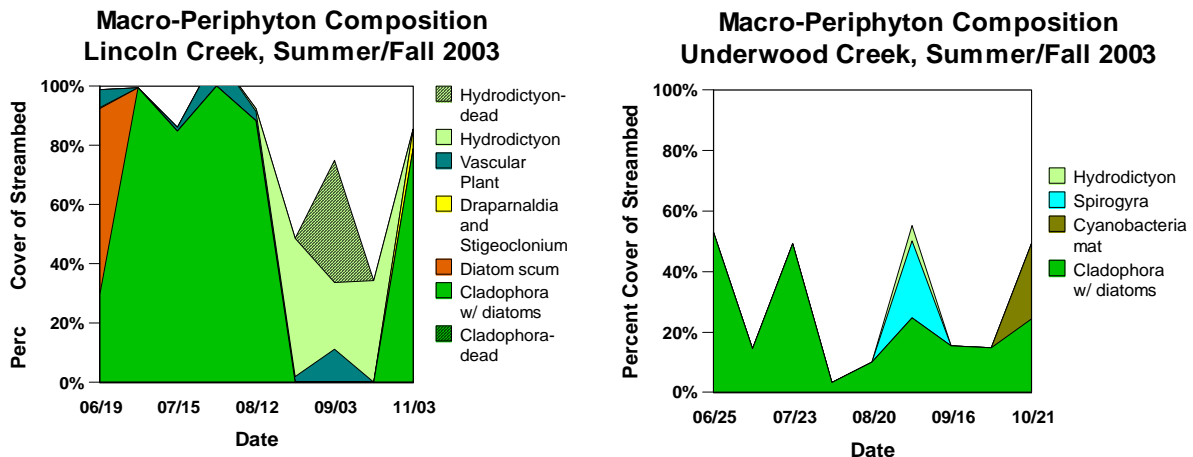


Fig. 5. Seasonal dynamics of macroperiphyton genera, expressed as percent cover of the available stream substrate during Summer 2003. Open space above the colored strips indicates bare substrate.



Fig. 6. The same segment of Lincoln Creek in Havenwoods Nature Center as observed on July 4 and July 5, 2004, straddling a storm event that raised the stream level by 1.1 m. Note also how quickly the stream has returned to near-baseflow conditions.

There is almost always more bare substrate present in Underwood Creek than in Lincoln Creek as a result of great water velocity and sediment scouring in this concrete-lined channel (Fig. 5). The dramatic effects of storm-driven scouring are easily observed in photos of Lincoln Creek at our study before and after last summer's major storm on July 4<sup>th</sup> (Fig. 6). The effects on biomass (Fig. 5, right panel) and on growth rate (Fig. 7) are also striking.

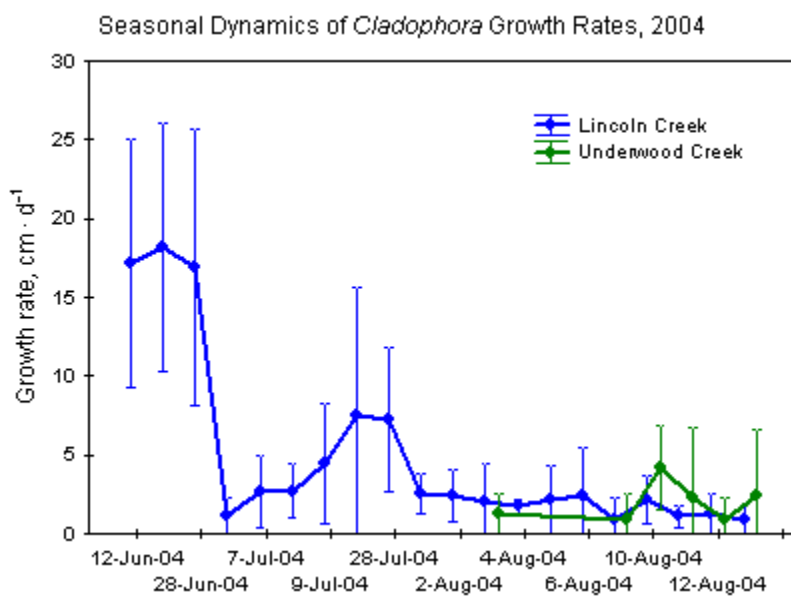


Fig. 7. Seasonal shift in growth rates of *Cladophora* in Lincoln Creek in 2004, measured as changes in the length of short portions of the plants.



high growth rates (Fig. 7). These rates decreased dramatically in July following a major storm scouring event and never recovered. *Cladophora* remained the dominant filamentous form for approximately one more month (Fig. 5), and the stream cobble remained completely covered with algae during this time because there were no large storm events to scour off the algae (Fig. 3). Both streams demonstrate similar seasonal growth kinetics for the period in which field data are available (Fig. 7). Although we only have growth rate data for 2004, observations of the seasonal biomass pattern suggest that this pattern has been repeated each of the past three summers. The seasonal change in growth rate is accompanied by striking changes in plant morphology as well.



**Cl** Fig. 8. Morphological changes of *Cladophora* in Lincoln Creek during the growing season. *Upper left*: basal crusts of winter and early spring. *Upper right*: branched morphology of late spring.

**Th** *Lower left*: long unbranched filaments of June. *Lower right*: detritus-covered, short branched filaments of late summer and autumn.

**co** s with small patches of cold-resistant green filaments (*Draparnaldia* and *Stigeoclonium*). *Cladophora* is present as extensive green crusts with little upright development (Fig. 8). In late Spring, these

mats develop bright green, highly branched upright filaments (5-25 cm. length) similar to those found along the Great Lakes shorelines (Fig. 8). Subsequently, the tips of these filament systems generate extremely long, unbranched filaments which may be 2-4 meters in length (Fig. 8). It is these long, unbranched filaments that dominated the streams during the period of very high growth rates in June 2004 (Fig. 7). These changes in morphology are correlated with the seasonal increase in water temperature and decrease in water velocity. The long *Cladophora* hairs largely cease growing in early July. They may persist under continued low flow conditions, or they may be washed downstream by storms, as occurred in 2004. In the absence of storm scouring, they turn golden-brown and die, presumably from damage caused by intense solar radiation in the very shallow water. Living *Cladophora* in the late summer or autumn is represented by short, branched tufts that are densely covered with detritus and epiphytic microperiphyton (Fig. 8). The potential for high growth rates (e.g. Fig. 7) has apparently been lost. Such filaments are very readily removed from the rock substrate.

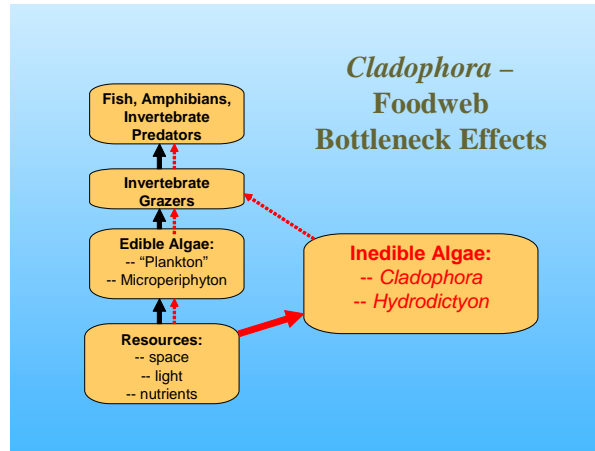
*Cladophora* in these streams can become covered with a layer of calcification. This is presumably due to very high rates of primary production which causes an increase in pH on the outside of filaments that is sufficient to precipitate calcium carbonate in these very hard water streams. This phenomenon was observed in both study streams but was most pronounced in areas of high light and intermediate water velocity in Underwood Creek (Fig. 9). The effects of calcification and of growth form on *Cladophora* growth rates or photosynthetic rates require additional study.



***Cladopho*** Fig. 9. Weir at Underwood Creek. The dark green algal zone contains highly branched *Cladophora* with little calcification. The surrounding light green areas represent *Cladophora* covered with a crunchy layer of calcification.

The comb directional flow over semi-porous substrate (limestone or concrete) seems to represent a highly favorable habitat in which *Cladophora* can become the competitively dominant algal filament. The extensive growth of *Cladophora* in these streams not only represents an aesthetic nuisance, it also prevents the establishment of a more diverse stream biological community characteristic

of a natural stream ecosystem. Resources that could be utilized by edible microalgae, and then subsequently used to feed herbivores and predators in a complex stream foodweb (Fig. 10, *black arrows*), are instead being tied up in macroperiphyton biomass, which is largely inedible, and unsightly (Fig. 10, *red arrows*).



The dominance of macroperiphyton biomass in Lincoln Creek is illustrated by comparison to the two microalgal fractions for Summer 2002 (Table 1). Despite the dominance of macroperiphyton biomass, the microperiphoton assemblage still makes a substantial

**Table 1. Comparison of Biomass and Productivity of Algal Functional Groups in Lincoln Creek, Summer 2002. Macroperiphyton primary production was estimated with in situ incubations. Microperiphyton and suspended algal production was estimated independently in laboratory incubators.**

	Macroperiphyton (including <i>Cladophora</i> )	Microperiphyton	Suspended Microalgae
<b>% of Total Algal Biomass</b> (POC, $\mu\text{g} \cdot \text{cm}^{-2}$ )	<b>99.9733 %</b>	<b>0.00018 %</b>	<b>0.00009 %</b>
<b>% of Primary Production</b> ( $\mu\text{g C} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ )	<b>26.3 %</b>	<b>71.8 %</b>	<b>1.8 %</b>
<b>Resulting Efficiency of Carbon Turnover</b> ( $\% \text{C} \cdot \text{hr}^{-1}$ )	<b>0.001 %</b>	<b>21 %</b>	<b>1 %</b>



contributions to total stream primary productivity. The ratio of production to biomass suggest that the attached microalgae could potentially develop much larger populations sizes, were the space, light and nutrients not being sequestered into the comparatively inert mass of large filamentous algae. Reduction of the macroperiphyton biomass should result in increased microalgal growth, which, in turn, could promote the establishment of a more diverse animal community within these streams.

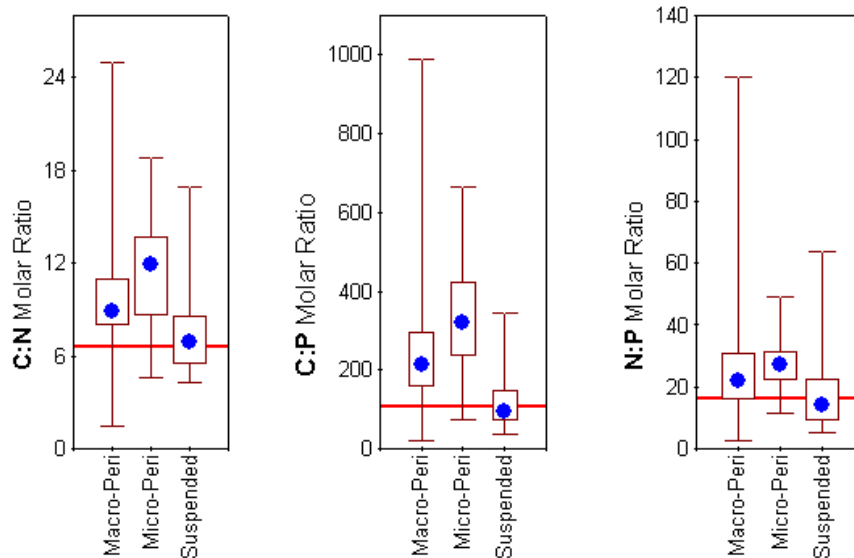


Fig. 11. Nutrient content of algal biomass expressed as the molar ratio of carbon, nitrogen and phosphorus. Data are 2002 summer seasonal aggregates for Lincoln Creek. The blue dot represents the seasonal median value, the brown boxes enclose the central 50% of all data, and the “T” lines indicate the total range. The red bars indicate the Redfield values for each ratio.

A comparison of the nutrient content of the three functional groups of algae further illustrates the impact of *Cladophora* and other macroperiphyton on the physiological condition, and hence potential growth rates, of the microperiphyton (Fig. 11). Ratios of potentially growth-limiting nutrients, carbon, nitrogen and phosphorus, have been widely used to identify nutrient stress. Data are frequently compared to the Redfield Ratios, a construct developed for marine microscopic plankton in the 1940’s, and identified by the red lines in Fig. 11. Although there has been a great deal of discussion regarding the specific values appropriate for different kinds of organisms (e.g. Sterner & Elser 2002), there is agreement that, for algae, values significantly above the Redfield Ratio (i.e. red lines in Fig. 11) indicate serious depletion within the organisms of the second nutrient in each ratio. Field data (unpublished) suggests that there is always excess dissolved phosphorus and nitrogen in the stream water. The data from Lincoln Creek (Fig. 11) indicate that the suspended microalgae, which are continually surrounded by such water, demonstrate no indication of nutrient limitation based on these ratios. The micro- and macroperiphyton components demonstrate depletion of cellular pools of both nitrogen and phosphorus which is indicative of nutrient-limited growth. As attached forms, these algae have



to deal with laminar flow boundary layers which can greatly reduce nutrient uptake rates, particularly under condition of low flow. The preponderance of macroperiphyton biomass exacerbates the situation of the microperiphyton, which demonstrate the most severe nitrogen and phosphorus stress as C:N and C:P molar ratios.

The large biomass of *Cladophora* and other macroperiphyton forms in these streams not only restricts potential growth of more edible algae by preemption of resources, but also creates a very physiologically stressful environment for other algae and animals as a result of macroperiphyton basic metabolism. Day-night oscillations in macroperiphyton primary production and respiration generate wide oscillation of stream water pH and dissolved oxygen (Fig. 12).

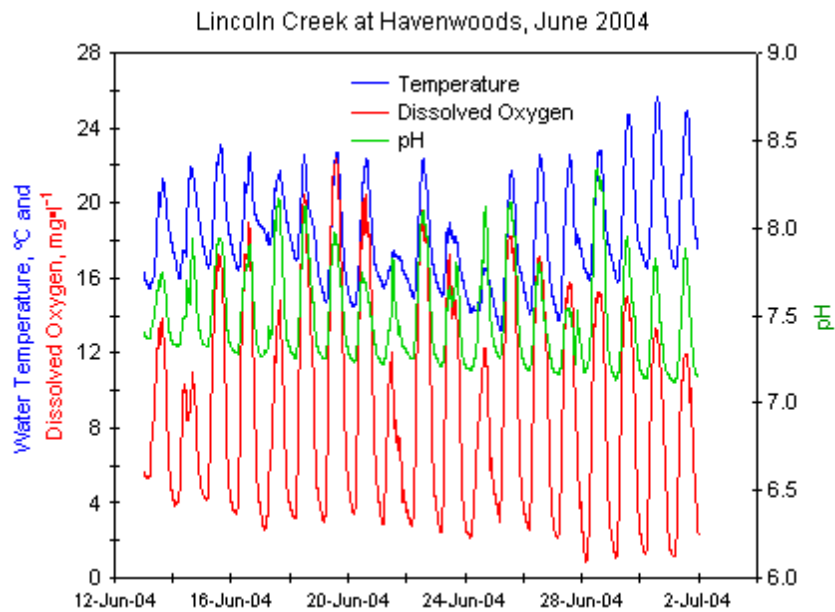


Fig. 12. Day-night oscillation in water temperature, dissolved oxygen and pH in Lincoln Creek during a two-week period in June 2004. Such oscillations occur throughout the summer. They are disrupted by storm events, but quickly become reestablished.

Oxygen often becomes severely depleted at night as a result of respiratory consumption exceeding the supply rates from photosynthesis and equilibration with the atmosphere. Data from in situ sondes suggest that subsurface water may actually become anaerobic for periods of time in late summer when stream flow rates are low. Calculations from 2003 in Lincoln Creek suggest that the stream water near the sampling site contains less than  $0.5 \text{ mg} \cdot \text{l}^{-1}$  dissolved oxygen (the EPA suggested threshold for fish physiological stress) for 16% of the total time during the three summer months. Some types of desirable stream invertebrates and fish are not tolerant of such conditions. Similar oscillations may be dampened entirely in the coastal Great Lakes near *Cladophora* beds by the vast volume of lake water, but they may exist in close proximity to the plants. Cycles in pH and dissolved oxygen and dissolved carbon could affect growth rates of coastal *Cladophora* and zebra mussels.

### Heavy Metals Associated with *Cladophora* in Urban Streams

Despite stringent regulatory efforts, dissolved metals enter urban streams from street runoff, industrial sites and groundwater flushing of landfills. In Lincoln and Underwood Creeks, such contaminants eventually enter Lake Michigan through Milwaukee Harbor. Stream algae, particularly the macroperiphyton, play a major role in intercepting these metallic ions. For most of the metals under study, 90% or more of the metals associated with algae in the streams are associated with the macroalgal fraction (Fig. 13). This would be expected from the overall biomass dominance of the macroperiphyton. Therefore, it is noteworthy that 25-40% of the total sequestered mass of some metal isotopes ( $^{55}\text{Mn}$ ,  $^{56}\text{Fe}$ ,  $^{63}\text{Cu}$ ) is tied up with the much smaller biomass pools of microperiphyton.

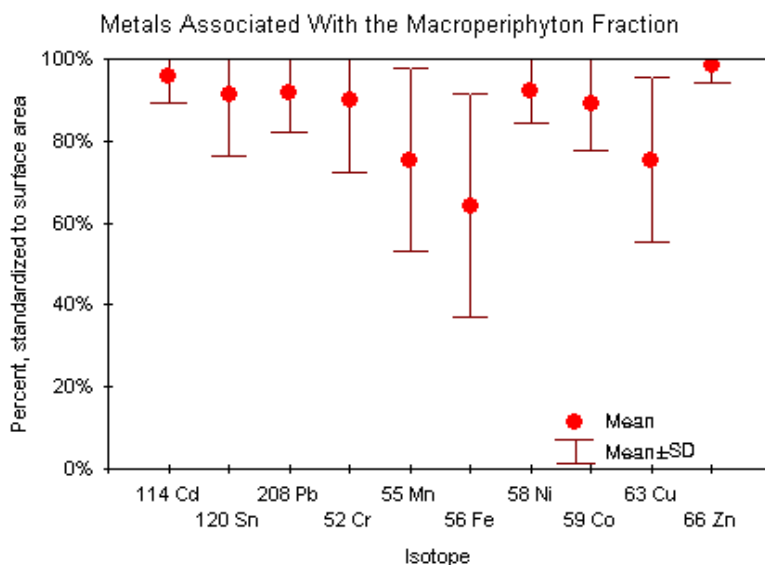


Fig. 13. Mass of metal isotopes bound to periphytic algae, analyzed by inductively-coupled mass spectrometry (ICP-MS).

Despite the differences in land use and size of the subwatersheds drained by these two urban streams, the macroalgal loads of metals are remarkably similar (Fig. 14). The concentrations of  $^{55}\text{Mn}$ ,  $^{56}\text{Fe}$ ,  $^{58}\text{Ni}$  and  $^{66}\text{Zn}$  (*lefthand panel*) are particularly elevated. Some metals, those with wide error bars in the figure, demonstrated clear seasonal oscillations in concentration with peak values occurring in early to mid-July. These changes in concentrations were not correlated with macroperiphyton biomass, suggesting either that the supply rate was not constant or that the physiological condition of the algae may influence their ability to sequester metal isotopes. Data suggest that most (53-80%) of the metal load associated with the macroperiphyton in these streams is *adsorbed* onto the surface of filaments rather than actually being *absorbed* into the cells. Transport of metals in these streams may, therefore, be sensitive to both the physiological condition of the algae and the severe day-night oscillations of dissolved oxygen, temperature and pH.

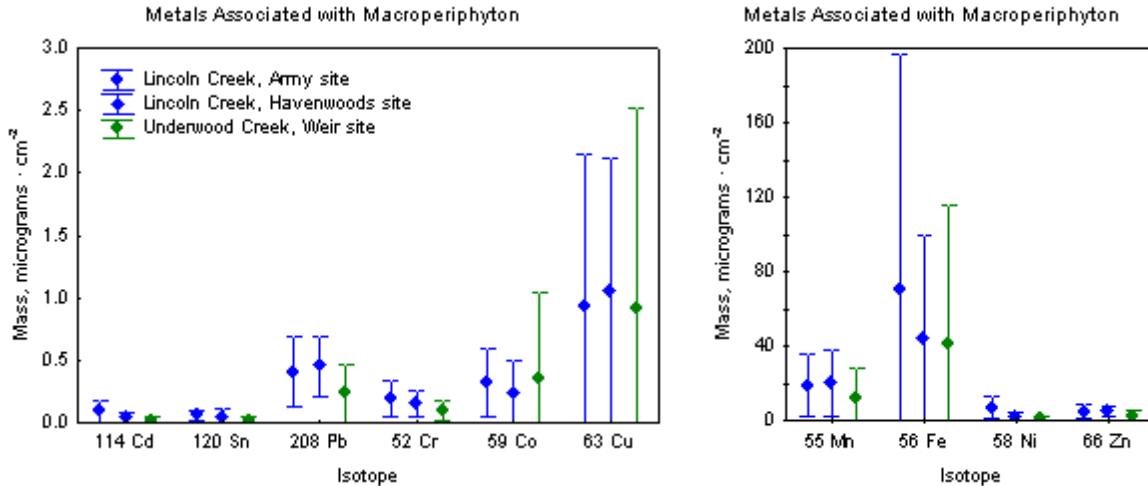


Fig. 14. Mean summer mass ( $\pm$  SD) of metal isotopes measured from collections of macroperiphyton taken from two sites in Lincoln Creek and one site in Underwood Creek in 2004. Note the difference in vertical scale of the two panels. Samples were analyzed by ICP-MS in the Chemistry Department, University of Wisconsin-Milwaukee.

## Conclusions

1. The same *Cladophora* creates nuisance blooms in Milwaukee-area urban streams as well as along the shores of Lakes Michigan and Ontario.
2. Algal biomass in these urban streams is highly sensitive to storm events that can cause scouring of algae and subsequent downstream transport.
3. The blooms of *Cladophora* in these streams are restricted to several months during the late spring and early summer. Reasons for this remain unclear.
4. These blooms create a “food web bottleneck” which prevents the development of species-rich natural foodwebs in restored urban streams. Massive *Cladophora* growths sequester essential resources (space, nutrients, light), and also generate severe day-night oscillations in chemical conditions that create physiological stress for other aquatic organisms.
5. *Cladophora* demonstrates dramatic seasonal changes in growth form which differ from what is typically observed in the coastal Great Lakes. Causes of this, e.g. water velocity, nutrient concentrations, water temperature, light intensity, have yet to be adequately evaluated. The impact of these morphological changes on algal primary production and growth rates are being investigated.
6. *Cladophora* and other filamentous green algae sequester large amounts of metals in these

urban streams. Appropriate management of flow rates may offer methods to remove both the *Cladophora* biomass and the metal contamination from the stream course. Metal-*Cladophora* coupling continues to be investigated.

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## Modeling *Cladophora* Growth: A Review of the Aure-Canale Framework

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### Introduction

To address the problem of excessive growth of filamentous algae in the Great Lakes in the 1970s, a three-year study was conducted. Specific objectives of the study were: 1) to identify environmental factors mediating the growth of *Cladophora*, 2) to develop mathematical algorithms describing these relationships, 3) to incorporate these algorithms into a mechanistic model, 4) to test the model in a demonstration program, and 5) to apply the model to determine target levels of limiting nutrients. Field work for this study focused on Harbor Beach, Lake Huron, where *Cladophora* was locally abundant due to the discharge of nutrients from a municipal wastewater treatment plant. Presented below is a summary of the results of this study. The details of this effort, as well as the results of other earlier research on filamentous algae in the Great Lakes, are available in a special issue of the Journal of Great Lakes Research (Volume 8(1), 1982).

### Model Overview

#### Model Conceptual Framework

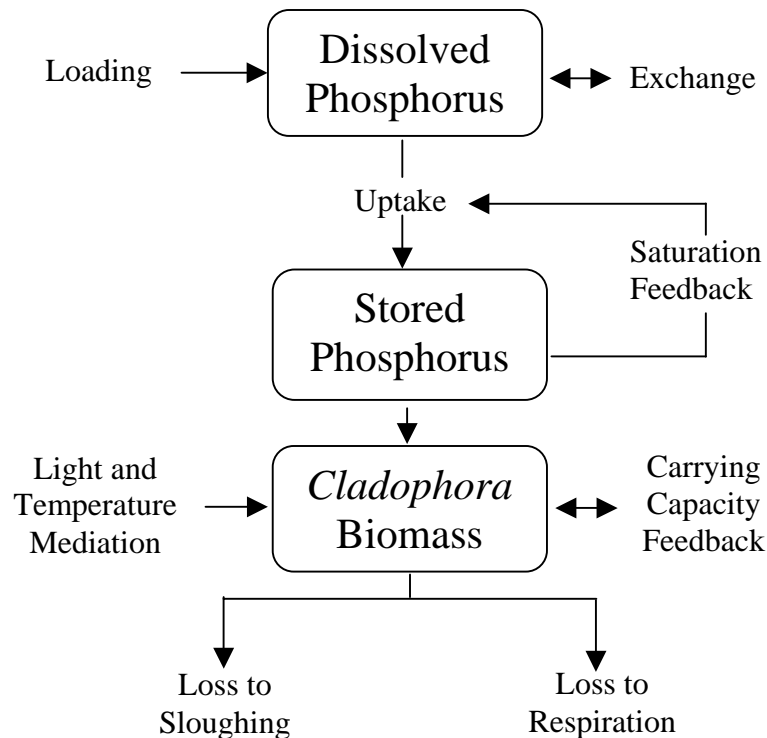


Fig. 1. Conceptual framework of model to simulate phosphorus dynamics and *Cladophora* biomass.

The model takes a mass balance approach, in which two major phosphorus pools (dissolved phosphorus and phosphorus stored in *Cladophora*) and *Cladophora* biomass are calculated by simulating the gain and loss processes for each of these state variables. For dissolved P, the gain processes include loading and mass transport within the lake, while the loss processes are uptake by *Cladophora* and mass transport. For *Cladophora* P content, P uptake is the main gain mechanism while dilution through growth is the loss process. This portion of the model accounts for the feedback between *Cladophora* P content and P uptake (Fig. 2). P uptake is modeled as a function of dissolved P, stored P and temperature. For *Cladophora* biomass, growth is the gain processes, while losses result from respiration and sloughing. Growth is modeled as a function of light, temperature, stored P, and carrying capacity.

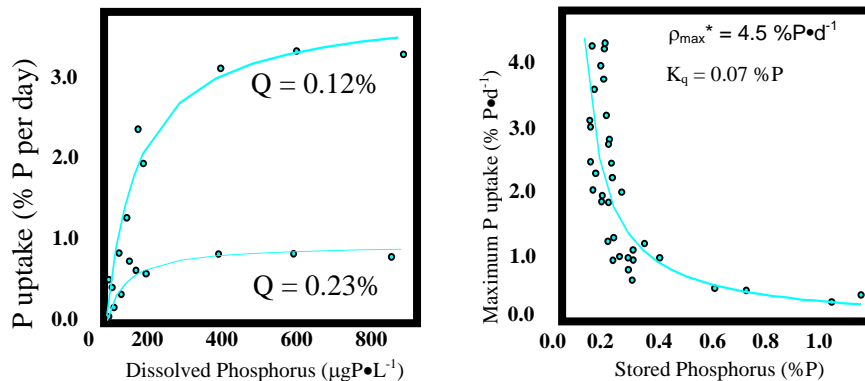


Fig. 2. Plots illustrating opposite effects of dissolved P and stored P on P uptake.

Laboratory experiments were conducted to determine the relationships between the state variables and forcing factors. The results of these experiments were then used to parameterize the various algorithms used in the model (Figs. 3, 4, 5). Sloughing proved to be the most difficult process to model, and there remains a need to better understand the mechanisms that influence the timing and magnitude of sloughing.

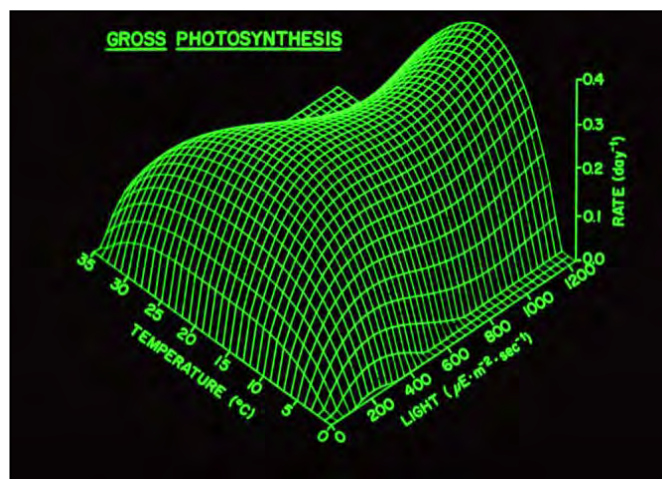


Fig. 3. The interactive effect of temperature and light on *Cladophora* photosynthetic rate.

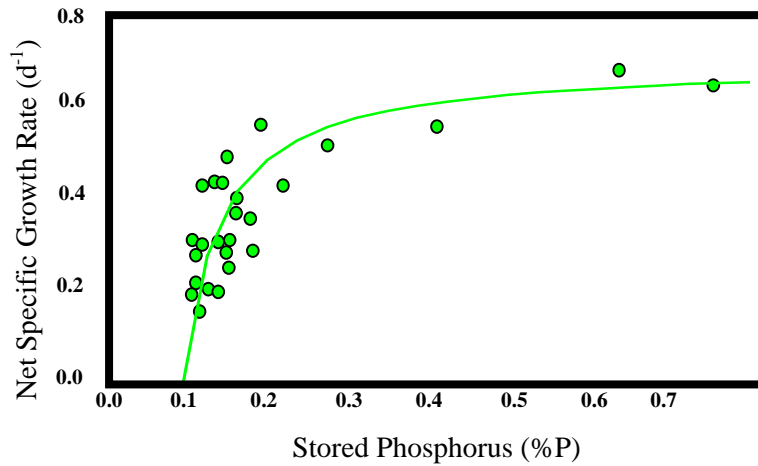


Fig. 4. *Cladophora* growth rate as a function of *Cladophora* P content.

**Model Verification**

By improving P removal at the wastewater treatment plant and monitoring phosphorus concentrations and *Cladophora* biomass in the lake, it was possible to compare model simulations of dissolved P, stored P and *Cladophora* biomass with in-lake measurements, and to determine the efficacy of P removal as a *Cladophora* management strategy.

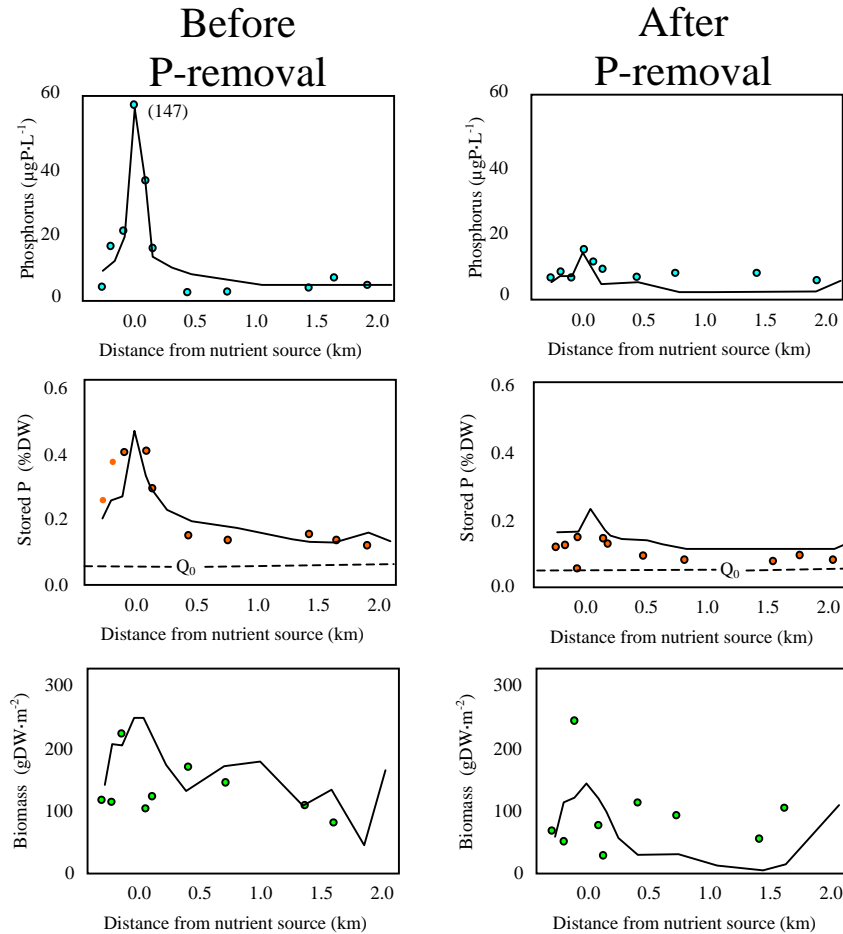


Fig. 5. Comparison of model results (lines) and in-lake measurements (symbols) before and after P removal.



In general, model results agreed well with field observations (Fig. 5). A reduction in P loading from  $1.35 \text{ kg day}^{-1}$  to  $0.20 \text{ kg day}^{-1}$  resulted in significant reductions in *Cladophora* production and standing crop density (Fig. 6), and in areal distribution (Fig. 7).

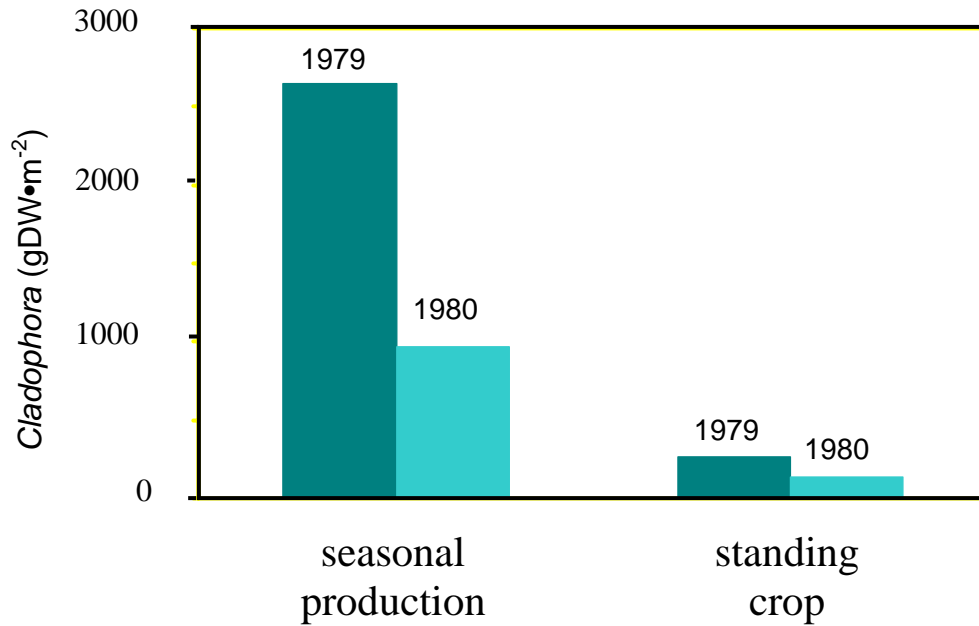


Fig. 6. Changes in *Cladophora* production and standing crop following a reduction of phosphorus input from a wastewater treatment plant.

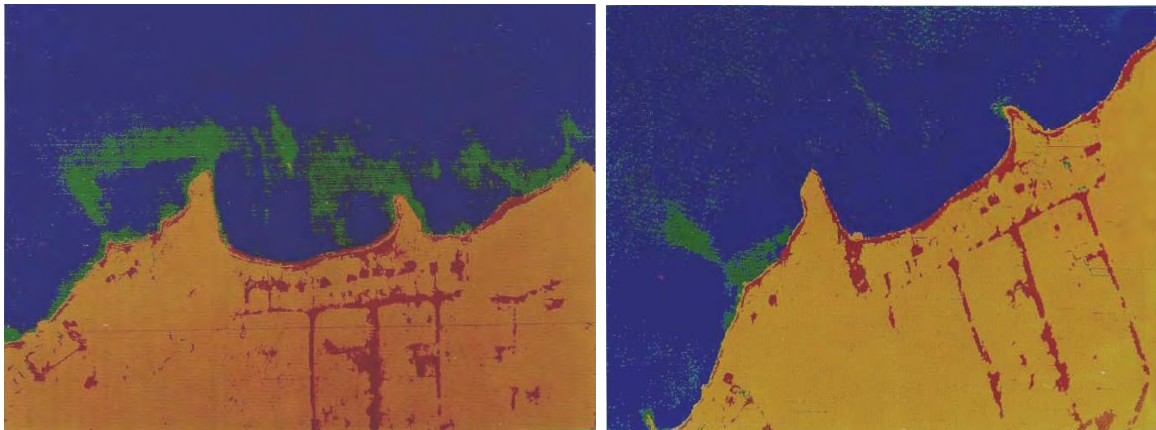


Fig. 7. Areal coverage of *Cladophora* on July 21, 1979 and August 4, 1980. *Cladophora* = green; water = blue; land = yellow and red. For details of remote imagery, see Lekan and Coney (1982).

### **Future Applications**

The work conducted in Lake Huron demonstrated the utility of the *Cladophora*-phosphorus model for simulating *Cladophora* dynamics and designing management strategies. Since this research was carried out, a number of changes have occurred in the Great Lakes. Most notably, the nearshore benthic environment in which *Cladophora* and other filamentous algae grow is now inhabited by dreissenid mussels. It is evident that filtration of particulate material by dreissenids has resulted in increased water clarity, and may also be altering nutrient supply to the nearshore benthos (Hecky et al. 2004). The model described above may be used to assess the potential effect of these and other changes, such as temperature change, on *Cladophora* growth and abundance.

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## The Contribution of *Dreissena* to the Resurgence of *Cladophora* in eastern Lake Erie.

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### Introduction

From 1995 to 2003 widespread *Cladophora* blooms were documented along the rocky coastlines of eastern Lake Erie (Higgins et al. in press). *Cladophora* blooms have also been recently reported in Lake Ontario (T. Howell Ontario Ministry of the Environment, pers. com., Hiriart-Baer et al. submitted, S. Malkin, University of Waterloo, unpublished data), Lake Michigan (Byappanahalli et al. 2003; H. Bootsma, this report), and portions of Lake Huron (T. Howell, pers. com.). The presence of widespread algal blooms in the coastal zones of these lakes (or lake basins), all of which are considered oligotrophic by offshore total phosphorus concentrations, are highly troublesome and may be a symptom of fundamental shifts in water quality caused by the invasive Zebra and Quagga mussels (*Dreissena polymorpha* and *D. bugensis* respectively).

*Cladophora* blooms were a significant ecological phenomenon in these Laurentian Great Lakes during the 1960's through to the early 1980's (Bellis and McClarty 1967; Herbst 1969; Shear and Konasewich 1975; Auer et al. 1982; Millner and Sweeney 1982). During the late 1970's a significant modeling effort to understand the dynamics of *Cladophora* growth and biomass accrual resulted in the development of a mathematical growth model specific to *Cladophora* (Auer and Canale 1980; Auer et al. 1982; Canale and Auer 1982a, b; Auer and Canale 1982a, b; Graham et al. 1982). The *Cladophora* growth model (subsequently referred to as the 'Canale and Auer' model), which relates growth and biomass accrual to several dynamic parameters including light, temperature, dissolved phosphorus, and carrying capacity was successfully validated on field populations of *Cladophora* in proximity to a sewage treatment outfall in Lake Huron (Canale and Auer 1982b). Recently, the 'Canale and Auer' model was revised, brought into a user-friendly computer-modeling platform (Stella) and successfully validated on field populations of *Cladophora* in eastern Lake Erie (Higgins et al. submitted).

The revised 'Canale and Auer' model is used here to determine the relative importance of highly variable ecological data to the current growth and biomass accrual in eastern Lake Erie, to demonstrate the contribution of *Dreissena* induced changes in water quality to the resurgence of *Cladophora*, and to provide necessary information for the management of *Cladophora* in eastern Lake Erie.

## Methods

### Site Description

Approximately 77% of the northern shoreline of Lake Erie's eastern basin consists of bedrock and cobble lake bottoms of low slope (Rukavina and St. Jacques 1971; St. Jacques and Rukavina 1973). During 1995, 16 sites were sampled for *Cladophora* community characteristics, tissue nutrient status, and biomass once during the peak growing season (Howell 1998). During 2001 and 2002 an additional 8 sites were added, and five of these sites were chosen for intensive seasonal studies of *Cladophora* and water quality parameters required for modeling purposes. All sites chosen had hard rocky lake bottoms that were colonized by *Dreissena*. Current mean *Dreissena* densities in the littoral zone of eastern Lake Erie range from 4,000 – 11,000 ind./m<sup>2</sup> (Patterson et al. submitted).

### Field Observations/Collections

Field observations and collections were conducted by snorkeling or SCUBA. At each site at least 3 quadrats (0.5m x 0.5m) were sampled using an airlifting device described by Barton and Hynes (1978). The collection of meteorological data and water quality data for modeling purposes is described in detail elsewhere (Higgins et al. submitted; Higgins et al. in press).

## Results and Discussion

### Current Status

The mean peak biomass of *Cladophora* during 1995, 2001, and 2002 was similar to historical values in the eastern and western basins of Lake Erie (Figure x) during the 1960's and 1970's, a period where the International Joint Commission (IJC) declared *Cladophora* a 'serious problem' to the lower Laurentian Great Lakes (Shear and Konanewich 1975). Unfortunately, very little data exists for *Cladophora* biomass in eastern Lake Erie during the 1980's, a period where total phosphorus concentrations were greatly reduced, but prior to the invasion of the Dreissenid mussels. In Lake Ontario, tissue P concentrations and *Cladophora* biomass were reduced through the mid-1980's (Painter and Kamaitis 1987), and it is likely that *Cladophora* growth and bloom formation in Lake Erie was also reduced.

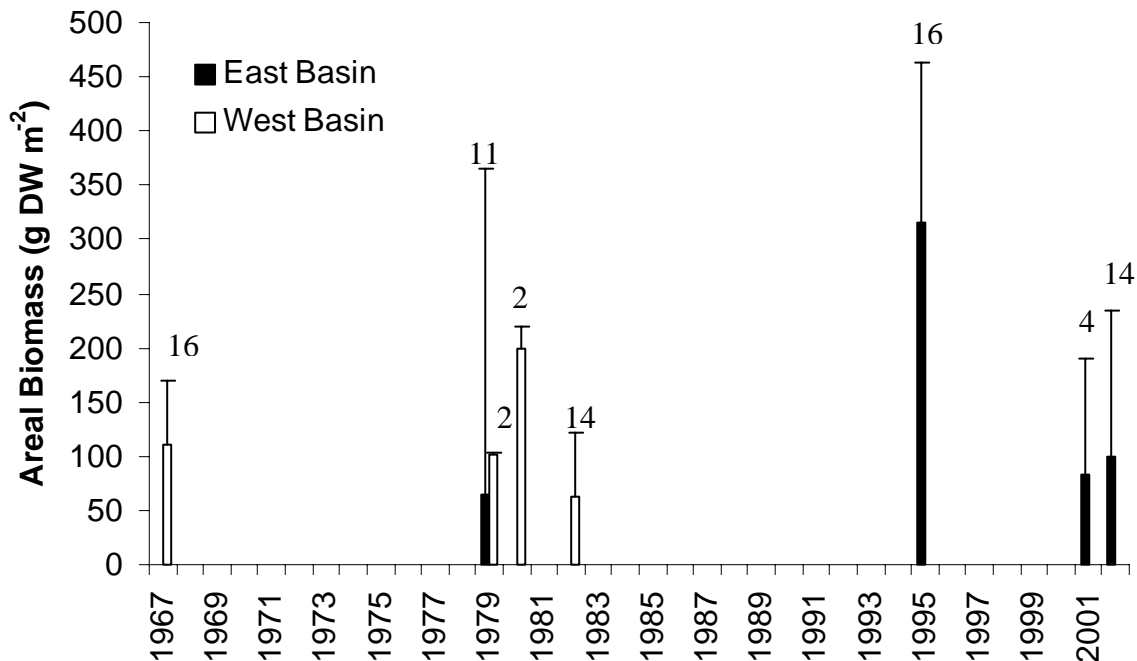


Figure 1. *Cladophora* biomass at shallow depths (<3m) during the peak biomass period in Lake Erie. Methods of collection differ between years. Pre-1995 data from Mantai et al. 1982, Neil and Jackson 1982, Kishler in Taft 1975, Lorenz and Herdendorf 1982, Monaco 1985. Data from 1995 from Howell et al. 1998. Data from 2001 and 2002 from Higgins et al. in press. The number above each bar represents the number of sites sampled and averaged.

### Factors controlling growth and biomass

Although many factors are required for *Cladophora* growth, several factors have been identified as being most important to controlling both the growth rates and biomass accrual of *Cladophora* in the Laurentian Great Lakes. In eastern Lake Erie *Cladophora* blooms are a common feature of the northern shoreline, and the median areal coverage over suitable substrata is approximately 96% at depths less than 5m. From 1995 to 2002 the northern shoreline of Lake Erie's eastern basin supported a maximum standing crop of approximately 12,000 tonnes dry mass (DM) of *Cladophora*. Presumably, if more suitable substratum were available the total standing crop would also increase.

The mean standing crop of *Cladophora* over depth is strongly correlated with the availability of light (Figure 2). At shallow depths, where irradiance is highest, small variations in irradiance have little effect on the standing crop. At these shallow depths (<3m) *Cladophora* growth and biomass accrual is limited by factors other than the quantity of light. At deeper depths (>3m) light becomes increasingly important to controlling growth rates and small variations in irradiance have large effects on the standing crop (Figure 2).

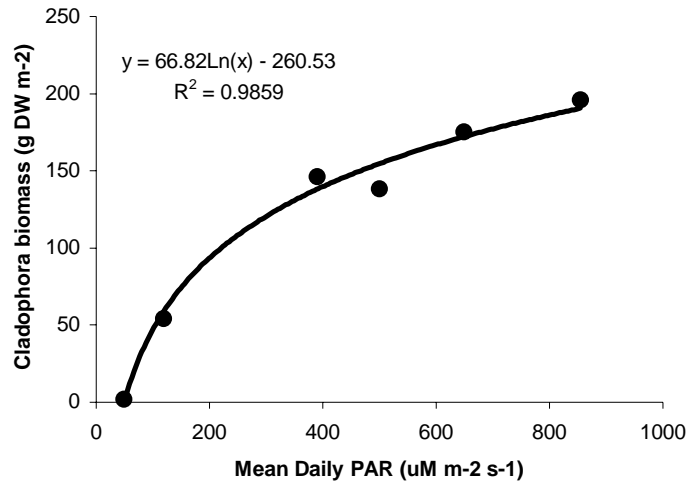


Figure 2. *Cladophora* biomass in eastern Lake Erie as a function of mean daily PAR. *Cladophora* biomass values are from Table 1. Mean daily PAR calculated from mean daytime surface irradiance and water column transparency during the spring growth period (see Higgins et al. in press for calculations).

The growth rates and maximum biomass accrual of *Cladophora* at shallow depths in eastern Lake Erie are strongly phosphorus limited (Higgins et al. in press). The relationship between tissue P concentrations and maximum growth rates has been determined for *Cladophora* using the Droop model (Auer and Canale 1982b). The Droop model illustrates that during the peak biomass period, maximum growth rates were strongly controlled by tissue phosphorus concentrations at depths shallower than 5m (Figure 3). At depths greater than 5m, tissue P concentrations were higher and maximum growth rates became increasingly insensitive to small shifts in tissue P. Seasonally, the tissue P concentration declined from 0.25 % DM during late May 2002 to values approaching 0.06 % DM by late June 2002 (Higgins et al. in press). The decline in tissue P concentrations over this period corresponds to a decrease in lake wide SRP concentrations (Higgins et al. in press). The decline in tissue P during this period indicates that SRP from *Dreissena* and other sources was insufficient to maintain high tissue P concentrations and maximal growth rates.

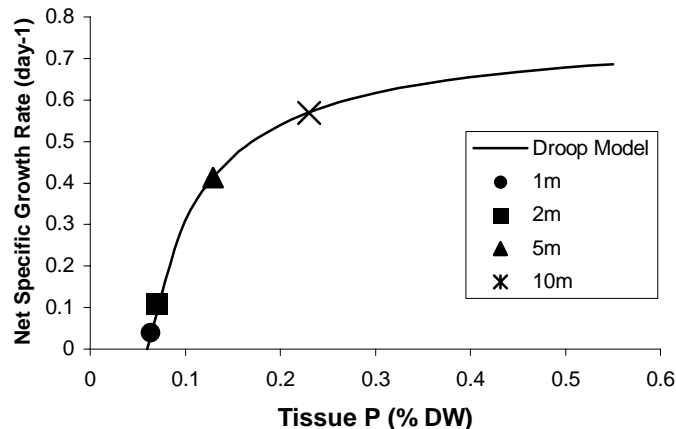


Figure 3. The relationship between tissue phosphorus concentrations and net specific growth in *Cladophora* as predicted by the Droop equation (Auer and Canale 1982). Tissue P data from eastern Lake Erie from the early summer period (July).

In order to confirm P limitation in *Cladophora* tissues an *in situ* P addition experiment was performed using P-enriched and non-P enriched agar. While the experiment was fraught with methodological problems, the effects of P enrichment were clearly noted in several trials. In these successful trials, *Cladophora* that received the P treatment overgrew the sampling container and were bright green in colour, while *Cladophora* in the non-P treatment showed stunted growth and appeared an unhealthy dull green colour.

In addition to other factors, the growth rates and seasonality of *Cladophora* are mediated by temperature (Graham et al. 1982). In eastern Lake Erie visible growths of *Cladophora* were noted as water temperatures approached 10 C, and maximum accumulation of biomass occurred as temperatures approached 20 C. In 2002 the midsummer-sloughing event occurred as temperatures approached 23.5 C.

### **Modeling growth and biomass**

The 'Canale and Auer' model provides an excellent framework for understanding the complex interactions of highly dynamic ecological variables that control growth rates and biomass accrual. The revised model can also be used to estimate how changes in single or multiple model parameters (e.g. surface irradiance, water clarity, temperature, dissolved phosphorus) affect growth rates. For eastern Lake Erie the model was used to evaluate the contribution of *Dreissena* to the resurgence of *Cladophora*, and how current shifts in SRP concentration would affect depth integrated biomass. The modeling effort indicated, based on shifts in water clarity (Howell et al. 1995) and spring SRP concentrations (Makarewicz et al. 2000) that occurred over *Dreissena* invasion, that *Dreissena* induced changes in water quality were responsible for increasing basin wide *Cladophora* biomass from approximately 3,000 tonnes DM (pre-*Dreissena*) to 12,000 tonnes DM (post-*Dreissena*)(Figure 5). Under current post-*Dreissena* conditions, the model indicated that depth-integrated biomass was highly sensitive to increases or decreases in ambient dissolved phosphorus concentrations.



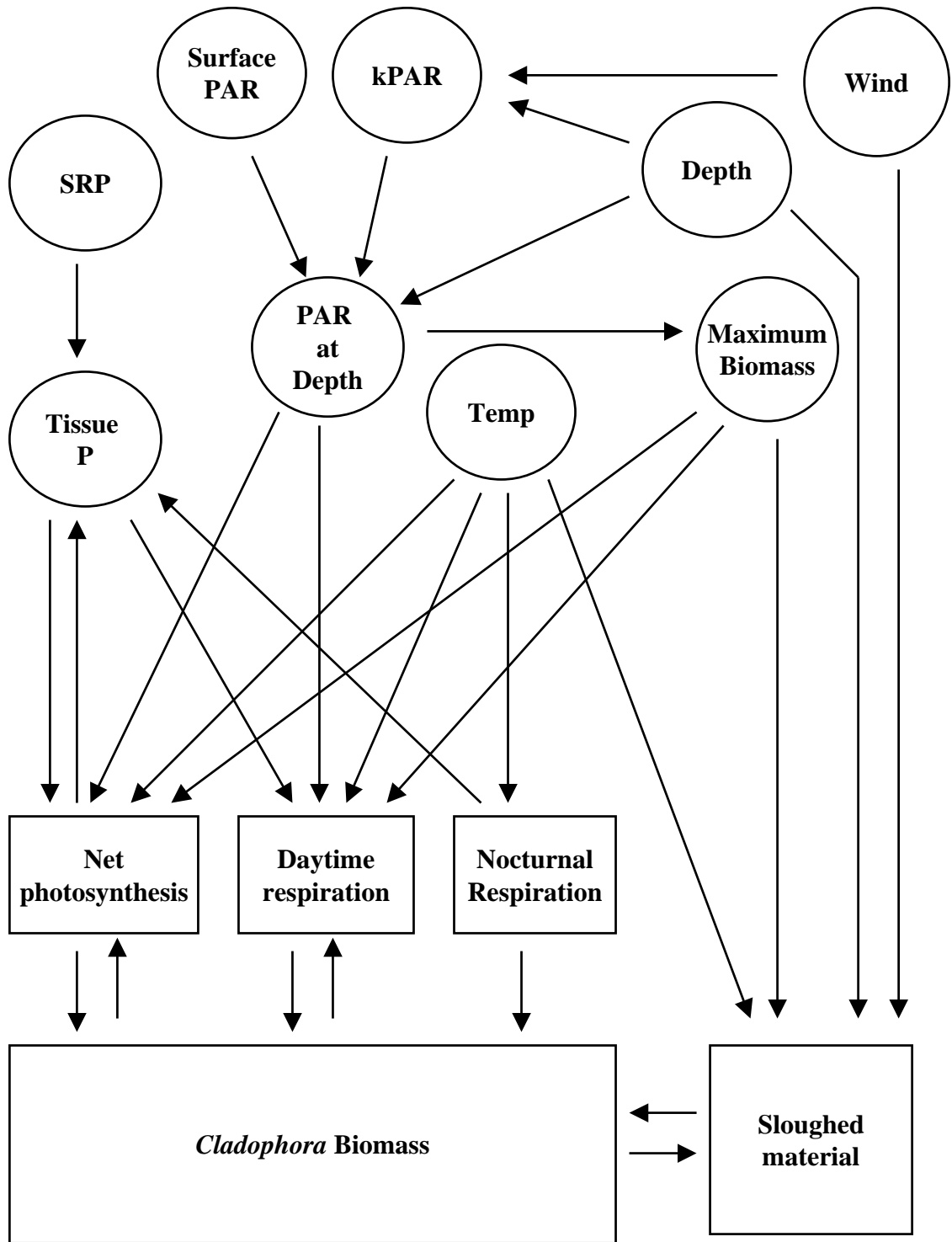


Figure 4. Simplified diagram of the revised *Cladophora* growth model (Higgins et al. submitted). Arrows represent mathematical equations linking model parameters.

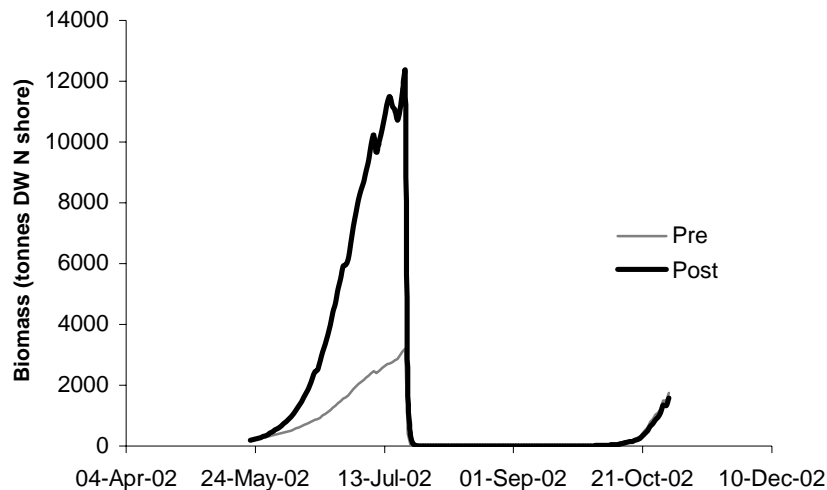


Figure 5. Model simulated depth-integrated *Cladophora* biomass on the northern shoreline of Lake Erie's eastern basin pre and post-*Dreissena* invasion. Post *Dreissena* conditions are based on data collected during 2002 by Higgins et al. (submitted). Pre-*Dreissena* model simulations are based on SRP concentrations (Makarewicz et al. 2000) and water clarity (Howell 1998) immediately prior to *Dreissena* invasion while holding other input parameters identical to post-*Dreissena* values.

## Conclusion

The 'Canale and Auer' model provides an excellent framework for understanding the interactions of highly dynamic ecological variables that control growth rates and biomass accrual of *Cladophora*. The 'Canale and Auer' model was revised, brought into a user-friendly computer modeling platform (Stella 2001), successfully validated in eastern Lake Erie (Higgins et al. submitted), and used to estimate the contribution of *Dreissena* to the resurgence of *Cladophora* and to determine how *Cladophora* would respond to shift in dissolved P concentrations. Under current post-*Dreissena* conditions in eastern Lake Erie, depth integrated *Cladophora* biomass is highly sensitive to dissolved phosphorus and small increases or decreases can have dramatic effects on basin wide *Cladophora* production. Phosphorus released by *Dreissena* was insufficient to meet *Cladophora* growth requirements on short time scales. Increased spring SRP concentrations due to *Dreissena*, however, appeared to be the main cause of the dramatic resurgence of *Cladophora* and the widespread bloom formations across the northern shoreline.

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## ***Cladophora* and Water Quality of Lake Michigan: A Systematic Survey of Wisconsin Nearshore Areas**

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Shaili Pfeiffer, DNR Office of the Great Lakes

### **Introduction**

In recent years *Cladophora* has increased along Lake Michigan and been deposited in large quantities on Lake Michigan beaches. The presence of rotting *Cladophora* on Lake Michigan beaches presents aesthetic and odor problems that impairs recreational use of Lake Michigan. In addition, the rotting algae may provide adequate conditions for bacterial growth and crustaceans deposited on the beach with the decaying *Cladophora* may attract large flocks of gulls resulting in increased *E. coli* concentrations from gull fecal material.

In the spring of 2004 the Wisconsin DNR initiated a working group to address the nuisance algal problem on Lake Michigan. The working group includes representatives from the DNR Northeast Region, Southeast Region, Integrated Science Services, and Central Office. The group's objectives include researching environmental factors causing the algal blooms to assist with developing long term management plans, identifying short term beach clean up and odor mitigation options, and addressing public information needs. The *Cladophora* working group is addressing these objectives by working in conjunction with others around the state including DNR programs, UW Extension, UWM WATER Institute, UW Sea Grant, Wisconsin Coastal Management Program, county health departments, and Centerville Cares – a citizen organization in Manitowoc County.

The working group developed a monitoring program for the summer of 2004 to observe the density, distribution, and associated water quality of *Cladophora* along Wisconsin's Lake Michigan shoreline. This investigation was intended to test sampling techniques and inform long term monitoring plans and research needs.

### **Methods**

Sampling sites were systematically chosen along Wisconsin's Lake Michigan shoreline at every other township boundary, 12 miles apart (See Figure 1). These 16 sites were sampled in June and late August/early September. At each location, water samples were taken at the 2 m and 10 m depth contour. At the 2 m depth contour samples were taken 1 m below the surface. At the 10 m depth contour samples were taken 1 m below the surface and 1 m above the bottom. Samples were analyzed for chlorophyll-a, total Kjeldahl nitrogen, ammonia, nitrate, total suspended solids, total phosphorus, total dissolved phosphorus, and soluble reactive phosphorus. These data were compared with historic nearshore water quality data from EPA monitoring in 1963 and 1974 and Milwaukee Metropolitan Sewage District monitoring from 1980 to present.

In addition to water quality sampling, a survey of *Cladophora* distribution and density was conducted. In June at each sampling location at 2 m, 4 m, 6 m, 8 m, and 10 m depth contours the bottom substrate, percent cover, and zebra mussel presence were noted. At most locations an underwater video camera was used to observe the bottom. Initially Door and Kewaunee sampling locations were observed using an aquascope, which proved to be inadequate for bottom

observation. Two sites, in Door and Kewaunee counties, were revisited in mid July and resurveyed using an underwater video camera. In September, the distribution and density survey was repeated at 2 m and 10 m, and a 15 m depth contour survey was added. Alga samples were also collected as part of the September monitoring at each sampling location and identified to determine what taxa other than *Cladophora* were present.

Beach monitors, as part of their monitoring of *E. coli* concentrations in beach water for the federal BEACH Act, noted *Cladophora* accumulation on Lake Michigan beaches for all except Kenosha County beaches and three Milwaukee County beaches. Beach monitors recorded data from a fixed sampling location 1-5 times per week between Memorial Day and Labor Day. They rated *Cladophora* accumulations on a scale of none, low, moderate and high using a picture scale distributed by the DNR prior to the 2004 beach monitoring season. These observations of alga accumulation were analyzed to determine the percentage of days with moderate and high alga accumulation on monitored Lake Michigan beaches.

## Results

Minimum, maximum, median concentrations and percent of samples below the detection limit for each water quality variable are reported in Table 1. In June, total phosphorus ranged between <0.005 and 0.018 mg L<sup>-1</sup> with a median of 0.009 mg L<sup>-1</sup>. In September, total phosphorus ranged between <0.005 and 0.046 mg L<sup>-1</sup> with a median of 0.006 mg L<sup>-1</sup>. One outlying total phosphorus concentration measured in 2004, 0.046 mg L<sup>-1</sup>, was from a sample off northern Sheboygan County south of Hika Bay during the algae sloughing period in early September. This higher concentration, in relation to other values observed, is likely due to the abundance of decomposing algae present in the water. The corresponding elevated SRP (0.025 mg L<sup>-1</sup>) is consistent with this hypothesis.

	TSS	Chlor-a	TKN	NH <sub>3</sub> -N	NO <sub>3</sub> -N	TP	TDP	SRP
	--June--							
Min	<2	<0.28	<0.14	<0.015	0.155	<0.005	<0.005	<0.002
Max	7	4.58	0.97	0.042	0.368	0.018	0.012	0.005
Median	<2	0.70	0.23	<0.015	0.270	0.009	0.005	<0.002
% Non-detect	89	9	19	69	0	27	40	81
	--September--							
Min	<2	<0.28	<0.14	<0.015	0.183	<0.005	<0.005	<0.002
Max	17	1.80	0.34	0.129	0.327	0.046	0.026	0.025
Median	<2	0.56	<0.14	<0.015	0.249	0.006	<0.005	<0.002
% Non-detect	56	32	54	74	0	18	85	74

Table 1. Lake Michigan nearshore nutrient concentrations measured 6/22/04 – 6/29/04 and 8/25/04-9/2/04. All concentrations are reported in mg L<sup>-1</sup>.

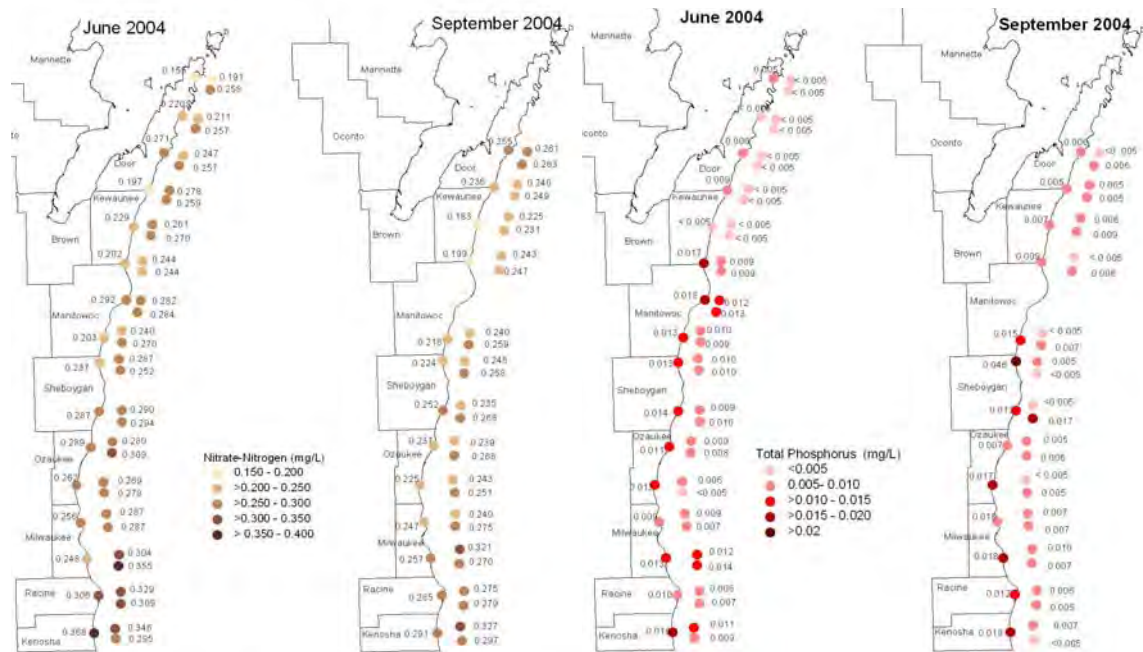
Paired signed rank tests for significant different between the surface samples at 2 m and 10 m depth contours, between the surface and bottom samples at the 10 m depth contour, and between the June and September were conducted (see Table 2). These results show higher concentrations of particulate related water quality variables, i.e. total phosphorus, Kjeldahl-nitrogen, total suspended solids, in the shallower water. No significant difference was observed between the surface and bottom samples at 10 m except for nitrate-nitrogen. Higher

concentrations of nitrogen species and total dissolved phosphorus were observed in June than in September. Total phosphorus was also found to be higher in June than in September when compared between samples taken from the Illinois border to Kewaunee.

Comparison	TSS	Chlor-a	TKN	NO <sub>3</sub> -N	TP	TDP
2 m surface vs. 10 m surface	<b>0.0003</b> (2 m)	0.076	<b>0.03</b> (2 m)	<b>0.008</b> (10 m)	<b>0.001</b> (2 m)	0.11
10 m surface vs. 10 m bottom	0.62	0.25	0.17	<b>0.048</b> (bottom)	0.86	0.56
June sampling vs. Sept. sampling	**	0.077	<b>0.0001</b> (June)	<b>0.0001</b> (June)	<b>0.059</b> Illinois	<b>0.038</b> (June)

Table 2. Paired signed rank test for Lake Michigan nearshore water chemistry concentrations measured 6/22/04 – 6/29/04 and 8/25/04-9/2/04. Values in bold are considered significant ( $P \leq 0.05$ ). The grouping with the statistically greater value is listed below the P value. \*\*indicates insufficient data. NH<sub>3</sub> and SRP are not included because of insufficient data.

The subsequent analysis of the water chemistry results focuses on nitrate-nitrogen and total phosphorus, because these variables had low rates of non-detection. Figures 1 and 2 display the nitrate-nitrogen and total phosphorus concentrations measured at each sampling location along Lake Michigan.



Samples were taken at two meter and ten meter water depths. At ten meters samples were taken one meter below the surface and one meter above the bottom. The surface sample is shown above the bottom sample on these maps. Points display general sampling location, but do not correspond to exact location. This figure is for informational purposes only.

Samples were taken at two meter and ten meter water depths. At ten meters samples were taken one meter below the surface and one meter above the bottom. The surface sample is shown above the bottom sample on these maps. Points display general sampling location, but do not correspond to exact location. This figure is for informational purposes only.

Figure 1. Geographic distribution of nitrate-nitrogen concentration reported as mg L<sup>-1</sup> in June and September 2004.

Figure 2. Geographic distribution of total phosphorus concentrations reported in mg L<sup>-1</sup> in June and September 2004.



Nitrate-nitrogen and total phosphorus showed decreasing trends from south to north (Figure 3).

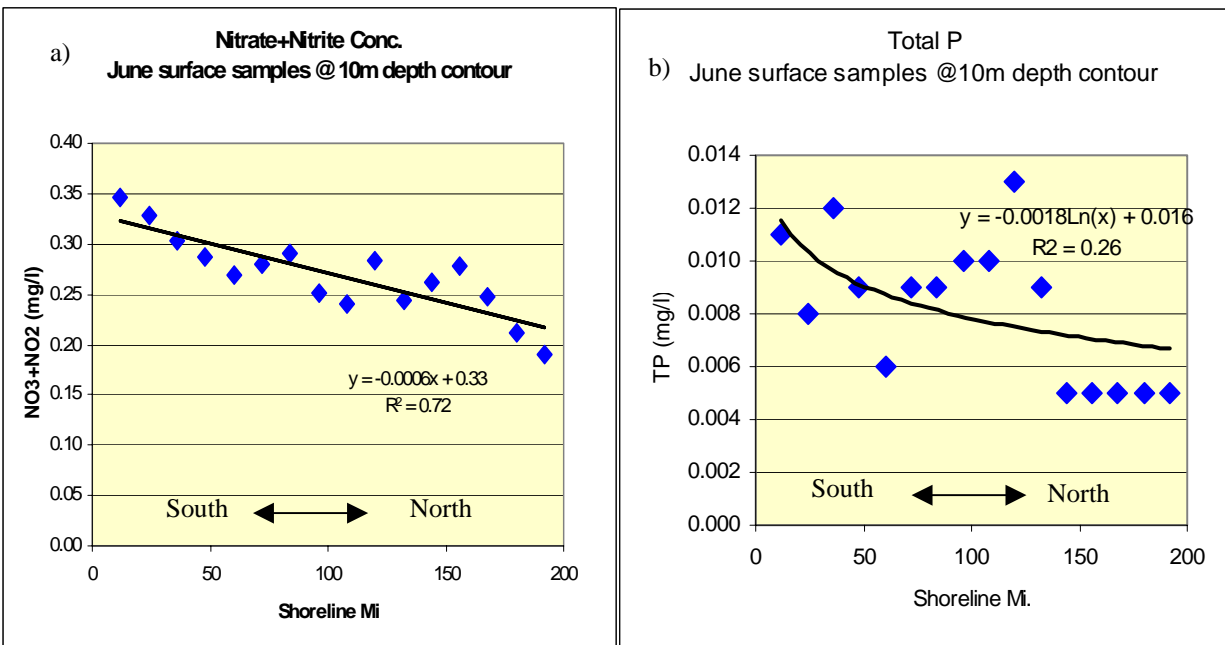


Figure 3. a) Nitrate-nitrogen shows a decreasing trend from south to north in the June surface samples at the 10 m depth contour. June and September results from the 2 m, 10 m surface, and 10 m bottom samples all show a similar trend. b) Total phosphorus shows a decreasing trend from south to north in the June surface sample at the 10 m depth contour. June samples from 2 m, 10 m surface, and 10 m bottom samples and September samples from 2 m and 10 m surface samples all show the same trend. The September 10 m bottom sample shows no trend.

A comparison of nitrate-nitrogen and total phosphorus concentrations observed in 2004 with historic data from Ozaukee, Milwaukee, Racine and Kenosha counties show no increasing or decreasing trends (Figure 4).

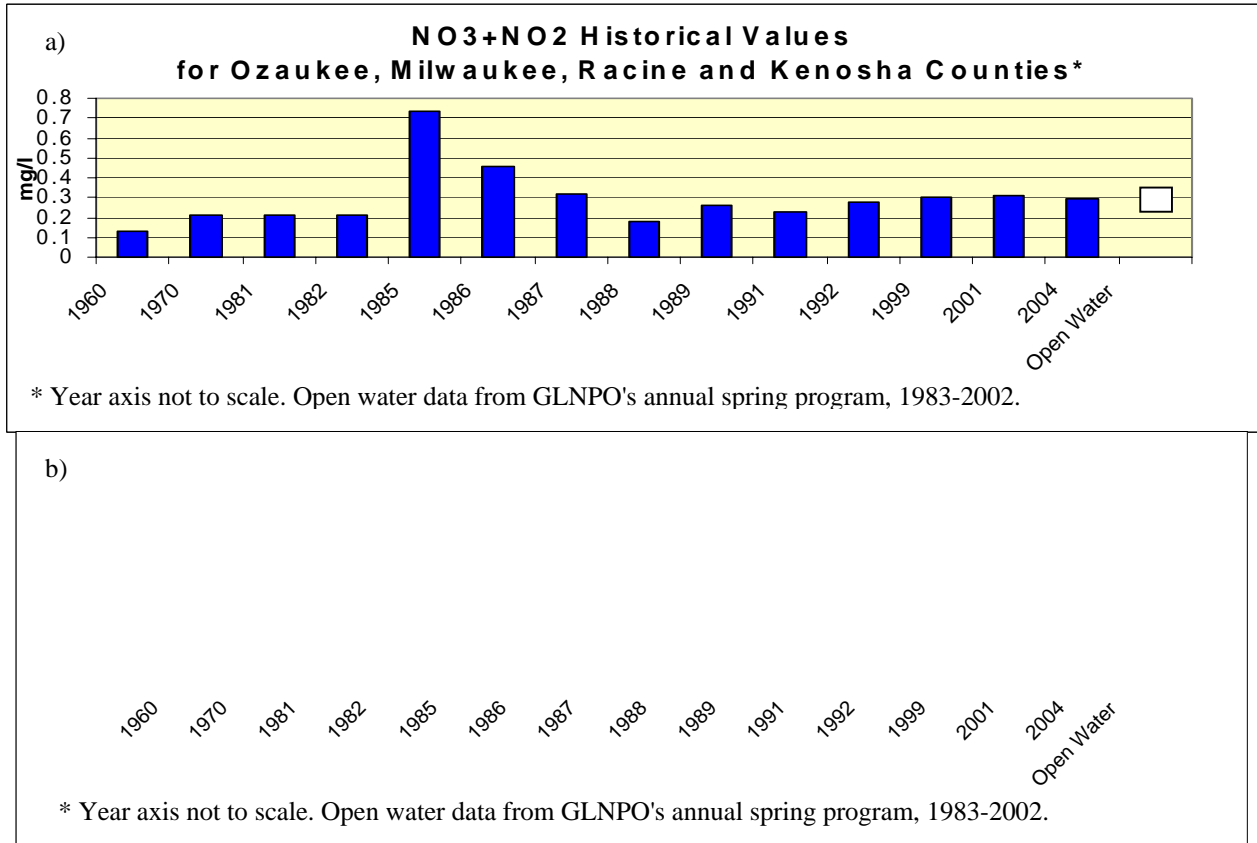


Figure 4. a) Historic nitrate-nitrogen concentrations. b) Historic total phosphorus concentrations. Data from USEPA STORET, MMSD, and 2004 DNR Lake Michigan sampling.

The distribution of *Cladophora* observed during the survey is reported in Figure 5. *Cladophora* coverage is dependent on substrate, with greater than 80% coverage in areas with rock substrate and less than 10% coverage in areas with sand substrate.

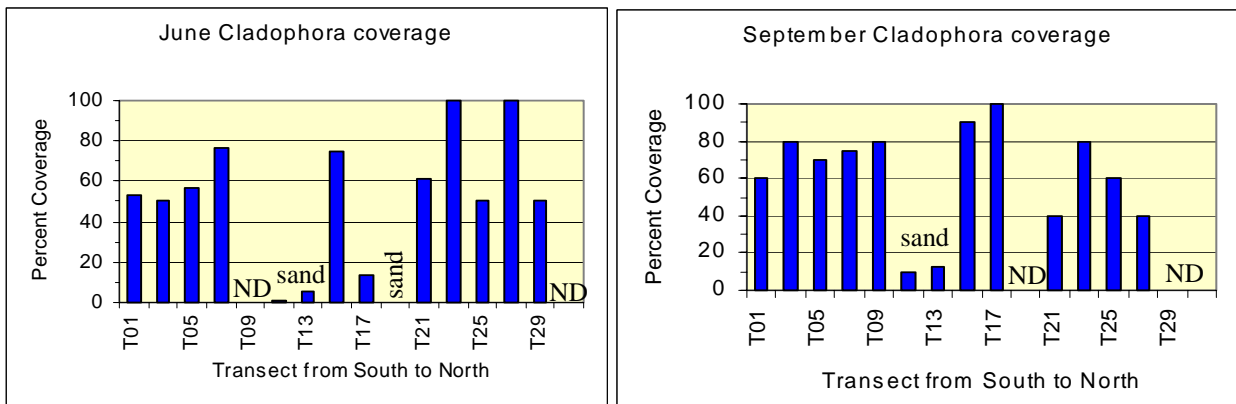


Figure 5. Average percent coverage of *Cladophora* on transects conducted along Wisconsin's Lake Michigan shoreline in June and September. (ND = no data)

Identification of the algae samples collected with the September monitoring indicated that *Cladophora* was the predominant filamentous algae at most survey sites. *Dichotomosiphon*

and *Tolypella* were also identified as important filamentous algae at a few sites. While hard substrate is critical for *Cladophora* growth, in Door County *Tolypella* was observed growing abundantly in some locations (such as Lily Bay) in soft sediment. Taxa other than *Cladophora* appear to be a significant component of the nuisance alga problem in Door County, however green algae growth in soft sediment was not observed outside of Door County. Zebra mussel presence or absence was often difficult to note using an underwater video camera due to complete coverage of hard surfaces by algae growth.

Analysis of the monitoring data from the BEACH ACT program shows that 45 of 66 Wisconsin Lake Michigan beaches had moderate to high accumulations of *Cladophora* on the beach less than 20 percent of days monitored between Memorial Day and Labor Day. Two beaches in Milwaukee County and one in Door County had moderate to high accumulations more than 60% of days monitored (Table 3).

Percentage of Season Affected by Moderate to High to Algae Onshore	Number of Beaches	Beach Locations and Names
> 60	3	McKinley Beach, Bradford Beach - Milwaukee; Newport Bay Beach - Door
>40 – 60	5	Door (4) and Kewaunee (1)
>20 – 40	13	Ozaukee (1), Milwaukee (1), Manitowoc (1), Kewaunee (1), Door (7), Brown (2)
≤ 20	45	All Counties

Table 3. Percentage of summer monitoring season nuisance algae was present in moderate to high quantities on Wisconsin's Lake Michigan beaches.

## Discussion

The results of the survey conducted by the DNR indicate that *Cladophora* growth is abundant along Wisconsin's entire Lake Michigan shoreline. Local differences in *Cladophora* abundance appear to be most likely due to differences in percentage of hard substrate. The 2004 nearshore phosphorus and nitrogen species concentrations are low. Median phosphorus concentrations were 0.009 mg L<sup>-1</sup> in June and 0.006 mg L<sup>-1</sup> in September. By comparison the long term (1988 – present) median total phosphorus concentrations in Lake Michigan tributaries Kewaunee River, Manitowoc River, Sheboygan River and Milwaukee River are 0.090, 0.168, 0.120, and 0.140 mg L<sup>-1</sup>, respectively. This comparison shows that the nearshore concentrations are an order of magnitude lower than the tributary concentrations. Examination of the historic data from 1963, 1974, and 1980-present collected along Wisconsin's southern nearshore area shows no distinct trend over time for total phosphorus and nitrate-nitrogen concentrations. The 2004 nearshore phosphorus and nitrogen species concentrations are within the range of variability observed in historic data sets. Not surprisingly, because of near-shore particulates, the total phosphorus concentrations are slightly lower, approximately 0.004 mg L<sup>-1</sup>, in the open water region of the lake.

Generally a decreasing trend from south to north in June and September of total phosphorus and nitrate concentrations was observed at 2 m and 10 m surface sample sites,

however observations of *Cladophora* density did not follow this trend. In fact, the results of the BEACH Act program beach monitoring suggest that moderate to high accumulations of *Cladophora* were most frequently found on Door County and Milwaukee beaches. Note that the BEACH Act monitoring ends on Labor Day, yet *Cladophora* sloughing at the end of August make September a time of potentially high algae accumulation on beaches that is not captured by the beach monitoring data set. Further exploration of the hypothesis that *Cladophora* distribution is based primarily on the availability of hard substrate (rather than on local nutrient availability) is merited. In addition, analyses of alga tissue nutrient concentrations in future surveys are also recommended to further determine if there are distinct differences in nutrient availability along the Lake Michigan coastline that was not captured in the 2004 summer sampling. These results will be used to develop monitoring plans for 2005.

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## Temporal and Spatial Patterns of *Cladophora* Biomass and Nutrient Stoichiometry in Lake Michigan

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### Introduction

Although there has been a noticeable increase in the amount of benthic algae fouling beaches on the western shoreline of Lake Michigan in recent years, there have been no quantitative studies of the distribution of benthic algal biomass, or the temporal fluctuations in biomass abundance. Such studies can provide useful information regarding factors that may potentially influence benthic algal growth. For example, the distribution of biomass in relation to river mouth location and prevailing nearshore currents may help to determine whether river nutrient inputs have a direct influence on algal abundance, while depth distribution will reflect the significance of light as a controlling factor.

In the 1970s, when *Cladophora* also grew to nuisance levels in parts of all the Great Lakes except Lake Superior, a large amount of research was conducted to determine the causes of this excessive growth. There was no consensus in these studies, with different emphasis being given to the role of light (Adams and Stone 1973; Mantai 1974; Graham et al. 1982), temperature (Bellis 1968; Moore 1978; Taft 1975; Mantai 1974), nitrogen (Hopkins and Carney 1972; Mantai 1976), inorganic carbon (Wood 1968), and phosphorus (Neil and Owen 1964; Herbst 1969; Lin and Blum 1973; Gerloff and Muth 1979) as controlling factors. In fact, more than one of these factors may operate simultaneously to control *Cladophora* growth. Perhaps the most definitive studies were those of Auer, Canale and colleagues (Auer and Canale 1982; Canale and Auer 1982; Graham et al. 1982), who used experimental data to develop calibrated models to simulate the combined influence of temperature, light and phosphorus on *Cladophora* growth.

To determine which factors might be responsible for the recent resurgence in *Cladophora* abundance on the western shores of Lake Michigan, we monitored *Cladophora* abundance, *Cladophora* nutrient content, and environmental conditions at a 10 m deep station immediately north of Milwaukee during the summer and fall of 2004. In addition, on one occasion we measured *Cladophora* abundance at 5 m intervals between 5 and 20 m, and we conducted a shoreline survey to compare abundance and nutrient composition at nine locations. Our objective was to compare measurements of biomass, nutrient content, temperature, and irradiance with the results of earlier experimental and modeling exercises cited above to determine which factors may be responsible for the recent resurgence of *Cladophora* in Lake Michigan. In addition, by comparing *Cladophora* biomass among sites, and comparing *Cladophora* nutrient demand with river nutrient

input, we attempted to determine the relative importance of river nutrient input as a factor controlling *Cladophora* growth.

### Temporal Fluctuations in Biomass and Phosphorus Content

In considering measurements of *Cladophora* biomass and nutrient content, it needs to be noted that throughout the study period, large amounts of epiphytic diatoms were observed on *Cladophora*. At times, diatom biomass exceeds *Cladophora* biomass (see Fig. 1). These diatoms are included in all measurements of biomass and nutrient content presented below.



**Fig. 1.** *Cladophora* filament with epiphytic diatoms. Diatoms have not yet grown on the faster growing filament branches, but on the main filament diatom biomass is greater than *Cladophora* biomass.

*Cladophora* phosphorus content, expressed as  $\mu\text{g}$  of P per mg of *Cladophora* dry weight, decreased from early June into late June and July, but then increased on sampling dates between early August and October (Fig. 2). A comparison with the experimental data of Auer and Canale (1982) indicates that P content in late June and July were near the minimum quota to permit growth ( $Q_0$ ), while P content on other dates, while still suboptimal, was sufficient to support growth, providing temperature and irradiance were sufficient. A comparison of *Cladophora* P content with water temperature (Fig. 2) indicates that the June-July decline in P content coincided with a temperature increase to levels that are optimal for *Cladophora* growth (Graham et al. 1982). *In situ* irradiance data (which are only available for dates after mid-July) suggest that irradiance at 10 m during July was suboptimal for growth, but was sufficient for growth for much of August and September. Despite the low irradiance in late July, we suggest that the increase in temperature in June – July allowed *Cladophora* to grow more rapidly, exhausting its internal phosphorus supply and becoming more P limited. *Cladophora* growth at 10 m was likely temperature- and light-limited prior to June, and P limited later in the summer. Biomass measurements during the study period varied between 30 and 70  $\text{g m}^{-2}$  (dry weight). Lowest biomass was recorded in mid-July, when *Cladophora* P content was lowest. Hence, while growth rate likely increased with temperature in late June, it appears that sloughing rate also increased, resulting in a net loss of biomass.

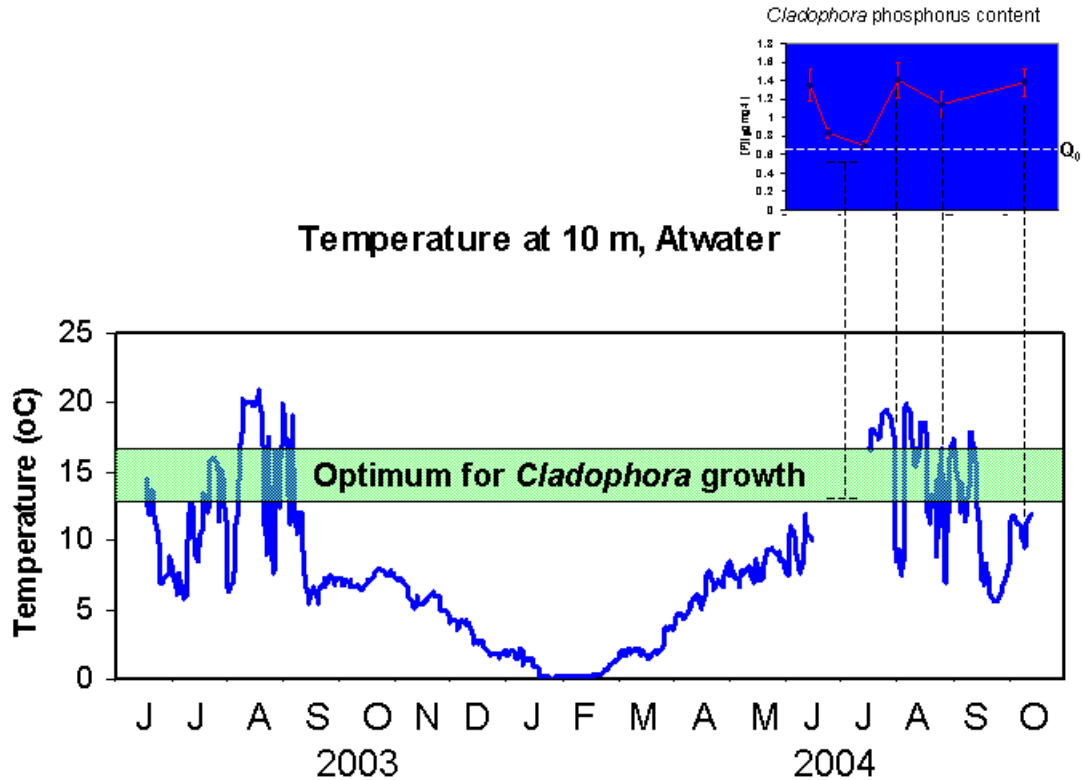


Fig. 2. Temperature and *Cladophora* phosphorus content at the Atwater station.

***Cladophora* Depth Transect**

The depth transect of *Cladophora* biomass and P content indicated a large decline in biomass between 10 and 15 m, and a significant increase in *Cladophora* P content over the same depth interval (Fig. 3). Using the minimum P quotas of Auer and Canale (1982), *Cladophora* at depths of 10 m or shallower appeared to be P limited, while *Cladophora* at depths of 15 m or greater are not P limited, but are likely limited by low irradiance. The growth rate of *Cladophora* at depths greater than 10 m will respond positively to any further increases in water clarity in Lake Michigan.

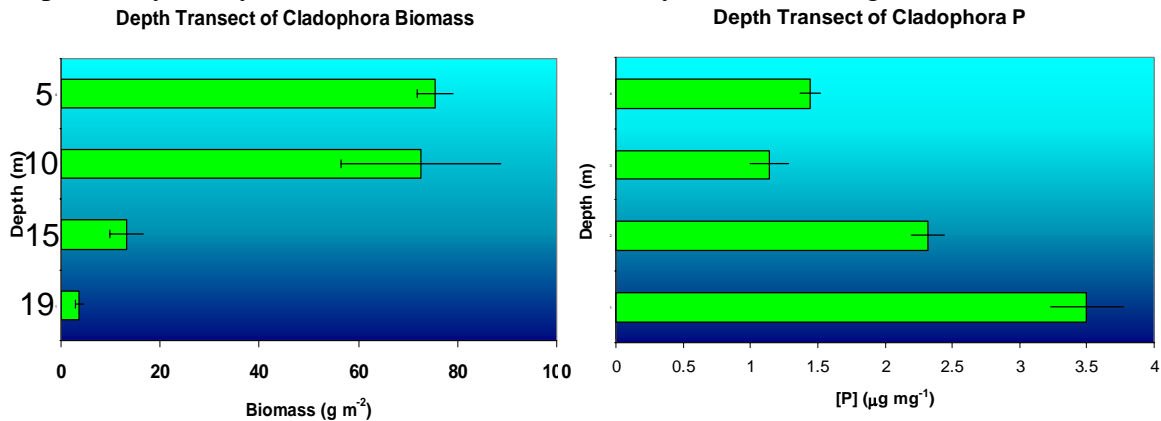
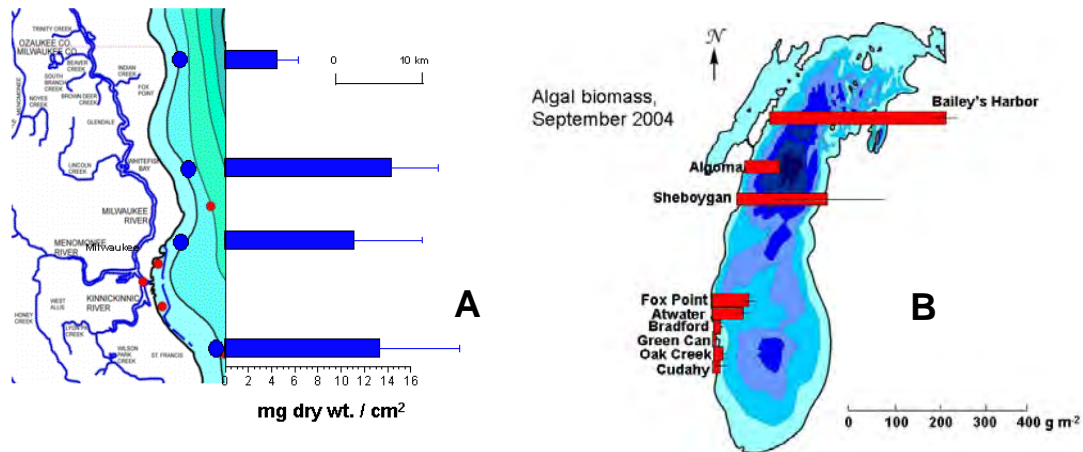


Fig. 3. Depth transect of *Cladophora* biomass and P content, August 2004.



### *Cladophora* Shoreline Survey

A September 2003 survey of *Cladophora* abundance at four 10 m deep sites in the Milwaukee region indicated that biomass was greater near the Milwaukee Harbor than at a location approximately 15 km north of the city (Fig. 4a). This suggested that nutrient output from the harbor may have a significant influence on *Cladophora* biomass. However, a second transect conducted in September 2004 indicated that *Cladophora* abundance north of the city was greater than that south of the city (Fig. 4b). This was not expected, since current in this part of Lake Michigan are generally from the north to the south (Beletsky et al. 1999), carrying nutrients released from Milwaukee harbour south along the western shoreline of Lake Michigan.



**Fig. 4.** *Cladophora* biomass distribution near Milwaukee in September 2003 (A) and between Cudahy and Door Peninsula in September 2004 (B).

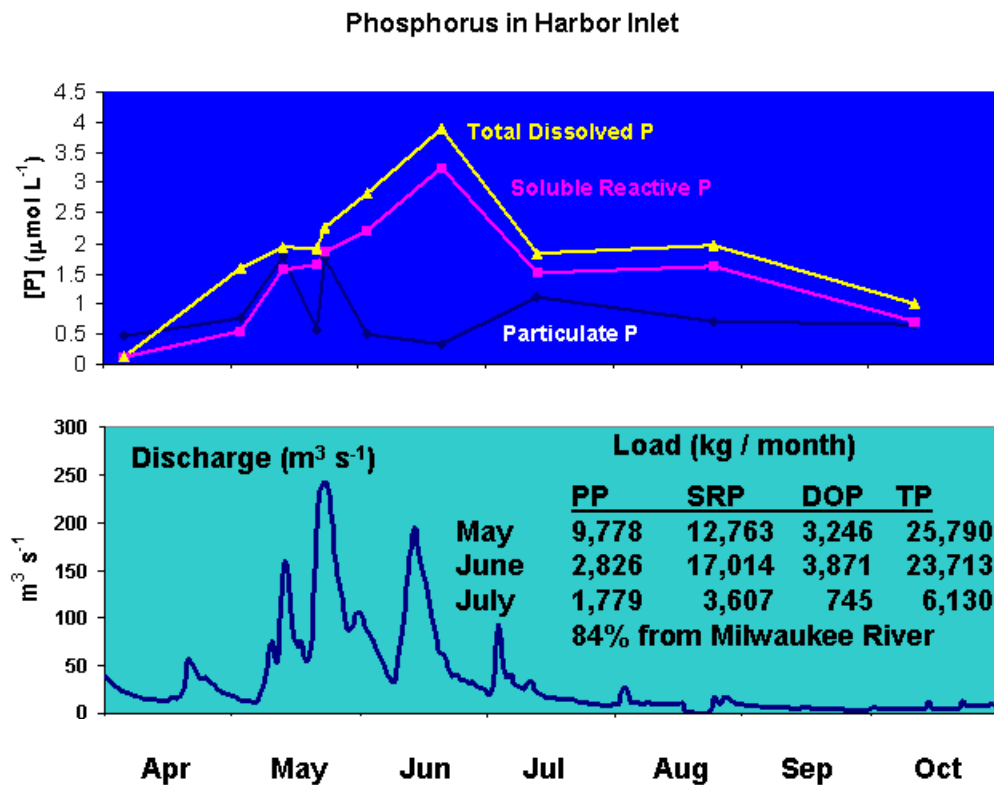
*Cladophora* biomass at other stations further to the north was also high, although biomass at the Bailey's Harbor on the Door Peninsula was dominated by another green alga, *Chara* sp. (probably due to the soft substratum at this sampling site).

Despite the lower biomass south of Milwaukee, *Cladophora* P content was generally higher in this area, supporting the hypothesis that the Milwaukee Harbor outflow serves as a nutrient source to regions south of the harbor outflow. We suspect that sloughing in this region prior to the sampling date may have resulted in the low biomass measurements, and further measurements both north and south of the harbor will be required to confirm the potential effect of harbor discharge on *Cladophora* growth and abundance.

### River Phosphorus Loading and *Cladophora* Phosphorus Demand

A second approach to assess the importance of river input as a nutrient supply for *Cladophora* is to compare estimates of *Cladophora* P requirements with P loading from rivers. Between April and October 2004, samples were collected from the three major rivers that converge in Milwaukee (Milwaukee River, Menomonee River, Kinnickinnic

River), as well as from the inflow to Milwaukee Harbor (immediately below the Hoan Bridge) and the three gaps connecting the outer harbor to the open lake. Nutrient concentrations measured at the inlet to the harbor, as well as water discharge (determined as the combined discharge of the three rivers as reported by the USGS) are shown in Fig. 5. The large rain events of May 2004 were accompanied by high particulate P concentrations, but there was a lag in the concentration of dissolved P, which peaked in June. In all months, P loads were dominated by soluble reactive phosphorus (primarily phosphate), which is the form of P most available for uptake by algae, and most of the P load originated from the Milwaukee River. Note that these data do not allow us to reach any conclusions regarding the source of these nutrients, which will be an unknown mixture of urban runoff, agricultural non-point sources, and other point sources. These measurements simply allow us to estimate how much P is entering the lake from all sources via the three rivers and Milwaukee harbor.



**Fig. 5. Water discharge rate and nutrient concentrations measured at the confluence of the Milwaukee, Menomonee and Kinnickinnic Rivers prior to entering Milwaukee Harbor.**

A comparison of phosphorus concentrations in the water flowing into the harbor (harbor inlet) with that flowing out of the harbor’s main gap to the lake indicates that there was a retention of both particulate and dissolved P in the harbor. These data suggest that the harbor is serving as a net sink for phosphorus, most likely due to phosphorus burial in harbor sediments.

In order to determine the importance of the river P load in supporting *Cladophora* growth in Lake Michigan, an estimate of *Cladophora* P demand is necessary. An approximate estimate was derived by selecting an area that is potentially influenced by outflow from the Milwaukee Harbor, and determining the amount of P that is required to support *Cladophora* growth within that area. We selected an area spanning a distance from approximately 15 km north of Milwaukee to 25 km south of the city. The only other tributary within this area is Oak Creek, but its discharge and P load are very small compared to that of the Milwaukee Harbor. Using bathymetric charts and aerial imagery to determine the distribution of *Cladophora*, assuming that mean biomass within the area is equal to the mean measured at the regular monitoring station north of Milwaukee, and applying the mean P content of  $1.1 \mu\text{g mg}^{-1}$ , we derived a conservative estimate of *Cladophora* P mass of 20,680 kg for the 0 to 10 m depth range (area = 188 km<sup>2</sup>). P demand was determined as total P mass multiplied by the *Cladophora* growth rate. Auer and Canale (1982) observed that at a P content of between 1 and  $2 \mu\text{g mg}^{-1}$ , *Cladophora* growth rate is generally between 0.1 and 0.25 day<sup>-1</sup>. Using the conservative estimate of 0.1 day<sup>-1</sup> and the above estimate of 20,680 kg of P in *Cladophora* within this area, the P supply required to support *Cladophora* growth in the area is approximately 2,068 kg day<sup>-1</sup>. In comparison, the average P load from the Milwaukee Harbor to the lake for the period April – October 2004 was 249 kg day<sup>-1</sup>. This comparison suggests that P loading from the rivers near Milwaukee can only provide a fraction of the P required to support *Cladophora* growth. The above estimate of river P supply is likely conservative, since it does not account for P retention in Milwaukee Harbor. These comparisons strongly suggest that internal P recycling processes within the lake are providing much of the P to support *Cladophora* production.

The above analysis does not mean that river P inputs have no influence on *Cladophora* growth. Depending on the rate of dispersion of river plumes after entering the lake, river nutrient input may have a significant local effect, and the higher P content of *Cladophora* observed south of Milwaukee in September suggests that there is some river influence. But on a larger spatial scale there must be other processes within the lake that affect nutrient supply to benthic algae. An obvious possibility is nutrient excretion / egestion by dreissenid mussels. Two questions requiring further investigation regarding the role of mussels are: 1) What is the *in situ* nutrient supply rate from mussels relative to allochthonous nutrient input rate? and 2) To what degree do mussels rely directly on allochthonous nutrient inputs as opposed to the existing large nutrient pool within the lake? The answers to these questions are necessary to determine whether reduction of nutrient loads from rivers will have any impact on *Cladophora* growth.

The earlier work of Auer and Canale (1982) demonstrated that *Cladophora* growth responds strongly to internal P concentrations between 1 and  $2 \mu\text{g mg}^{-1}$ , similar to those measured in this study. Within this range, a small increase in *Cladophora* P content may result in a relatively large increase in growth rate, and *vice versa*. Therefore, while reduction of P input from rivers may have a small effect on total P availability, it may have a significant effect on *Cladophora* growth rate. Likewise, any changes in the lake's internal nutrient cycle that result in a small increase in *Cladophora* P content may result in significant increases in *Cladophora* growth rate and abundance.

## Conclusions

1. At depths of less than 10 m, *Cladophora* will respond to changes in P availability, irradiance and temperature.
2. At depths greater than 10 m, *Cladophora* is light and / or temperature limited.
3. Lakeshore surveys suggest that river nutrient discharge does not strongly influence *Cladophora* abundance on a large scale.
4. While river phosphorus input may support *Cladophora* production near river mouths, a comparison of estimated *Cladophora* phosphorus demand with river phosphorus inputs suggests that most *Cladophora* production is supported by phosphorus cycling processes within Lake Michigan. Future research needs to focus on identifying and quantifying these internal cycling processes.

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## Can Activity of Enzymes Involved in Nutrient Assimilation be Useful as Indices of Nutrient Status in *Cladophora*?

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The recent resurgence of benthic algal growth in Lake Michigan near Milwaukee has caused public outrage and concern about ecosystem health. During the late 1970's - 1980's, blooms of the invasive *Cladophora* appeared in Great Lakes nearshore regions and research pinpointed increasing P inputs as the major causative factor.

Consequently, with the 1990's reoccurrence of *Cladophora* blooms, it was initially assumed that algal growth was being stimulated by increasing nutrient inputs to the lake from sewage, agricultural and industrial runoff. Freshwater habitats are usually considered to be P limited, and with the relationship between *Cladophora* growth and P found during the 1980's, the role of P was considered pivotal. However, P inputs to Lake Michigan have not increased significantly since the 1980's, rather with the implementation of P control measures, the P inputs have decreased over the last decade. Clearly a broader picture of nutrients and other factors need to be considered in understanding the current *Cladophora* problem in Lake Michigan near Milwaukee.

Our research addressed the issue of whether *Cladophora* growth is controlled by P availability? To do this, we targeted the following questions :

1. Does growth of *Cladophora* become seasonally limited by nutrients?
2. What is the water column availability of macronutrients P and N ?
3. Is there a seasonal variation in nutrient status in *Cladophora*? To assess this third question, we used three approaches -
  - o Changes in nutrient content (stoichiometry)
  - o Changes in photosynthetic 'capacity'
  - o Variation in expression of enzymes of nutrient assimilation
    - Nitrate reductase – regulated by N availability
    - Alkaline phosphatase – only present when P is limiting

In addressing the question of what nutrients are available to *Cladophora*, we collected water samples over the summer growing period, from our 10 m site off Atwater beach, north of Linnwood intake facility (Fig. 1). We also assessed nutrient inputs to Lake Michigan via Milwaukee harbour, the largest point-source of nutrients in the region. Survey results are presented in Bootsma, Young and Berges (this volume).

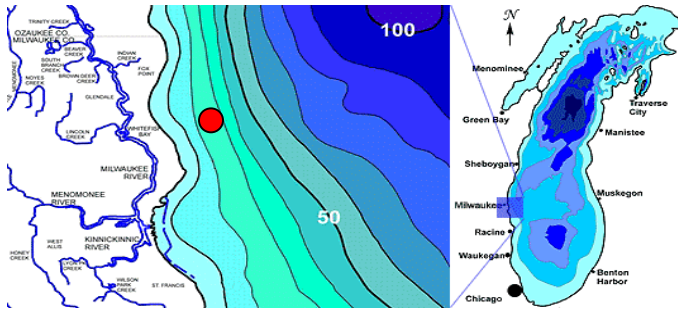


Figure 1. Atwater sampling site at 10 m depth off the coast N of Milwaukee harbor.

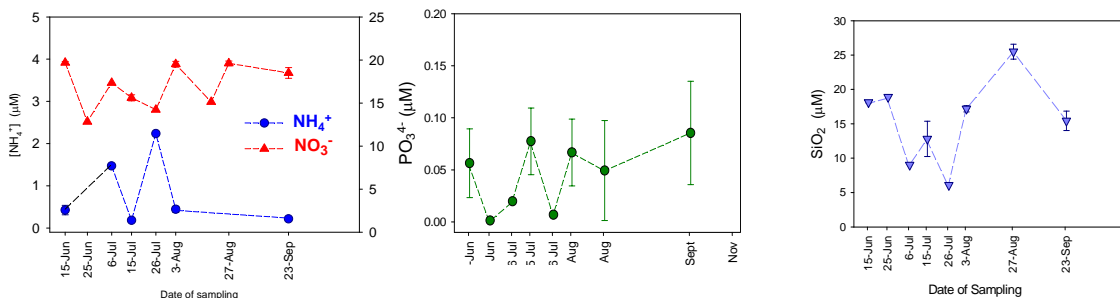


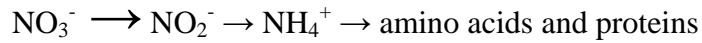
Figure 2. Seasonal variation in soluble nutrients in the water column at Atwater 10 m site. **A.** Inorganic nitrogen ( $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{NH}_4^+$ ) **B.** ortho-phosphate ( $\text{PO}_4^{3-}$ ). **C.** Silicate ( $\text{SiO}_2$ ).

The seasonal patterns of soluble nutrients in the water column at the Atwater sampling site are shown in Fig. 2. There were some oscillations in available inorganic N with levels of  $\text{NO}_3^-$  consistently high enough to support algal growth. The ammonium levels were lower, possibly as a result of preferential uptake by phytoplankton and benthic algae. Soluble phosphate availability was consistently very low over the growing season, suggesting possible limitation in P for 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).

**Enzyme Analyses:**

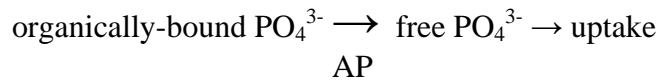
The rationale for examining enzyme activity in *Cladophora* is that enzyme activities in algae are regulated in response to available nutrients and are thus a physiological index of nutrient status (Beardall et al. 2001).

Inorganic N assimilation by algae follows this pathway :

**NR**

Nitrate reductase (NR) catalyses the initial reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , which is believed to be the rate-limiting step in uptake and assimilation of  $\text{NO}_3^-$  into amino acids and proteins. The activity of NR is regulated in response to available  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$ ; NR expression requires the presence of  $\text{NO}_3^-$  and light and is suppressed by high ambient concentrations of  $\text{NH}_4^+$ , in most algae (Berges et al. 1995; Young et al. 2005).

Phosphate acquisition similarly involves expression of enzymes. 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 helps cleave orthophosphate from the organic chelator is alkaline phosphatase (AP). It has been shown in algae that the expression of AP activity is greatly elevated under conditions of low P availability (Dyhrman and Palenik 1997). Thus AP activity can be used as an index of P limitation in algae.

**Enzyme assays - how they work**

**Nitrate Reductase :** *Cladophora* is frozen in liquid nitrogen at the sampling site and stored until assaying. Tissue samples are ground in liquid nitrogen and extracted into a buffer containing constituents to preserve protein function (Young et al. 2005). Small volumes of the extract are incubated in replicate tubes with the substrates  $\text{NO}_3^-$  and NADH supplied in excess. The reaction is stopped at various times. The concentration of  $\text{NO}_2^-$  is measured spectrophotometrically in clarified extracts. The increase in  $\text{NO}_2^-$  concentration is plotted and linear rate is proportional to the activity of NR enzyme.



**Alkaline Phosphatase:** Freshly collected *Cladophora* tissue is stored in Lake Michigan water. Intact pieces of *Cladophora* are incubated with artificial fluorometric substrate, methyl umbelliferone-  $\text{PO}_4^{3-}$  (MUP). The change in fluorescence over time is measured and is indicative of cleavage of the  $\text{PO}_4^{3-}$  from MUP. AP enzyme activity is proportional to rate of fluorescence change, compared with a standard curve.

Seasonal patterns in NR and AP activity were measured in *Cladophora* from the Atwater site over the summer growing period (Figs. 3, 4). There was some correlation between  $\text{NO}_3^-$  available in the water column, and NR activity in *Cladophora* (Fig. 3).

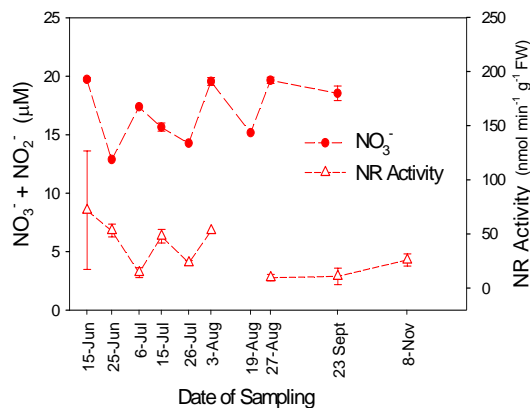


Figure 3. Seasonal variation in nitrate reductase (NR) activity in *Cladophora* and water column  $\text{NO}_3^-$  at Atwater 10 m site. Points are mean  $\pm$  standard deviation,  $n \geq 2$  ( $\text{NO}_3^-$ ),  $n = 8$  (APA).

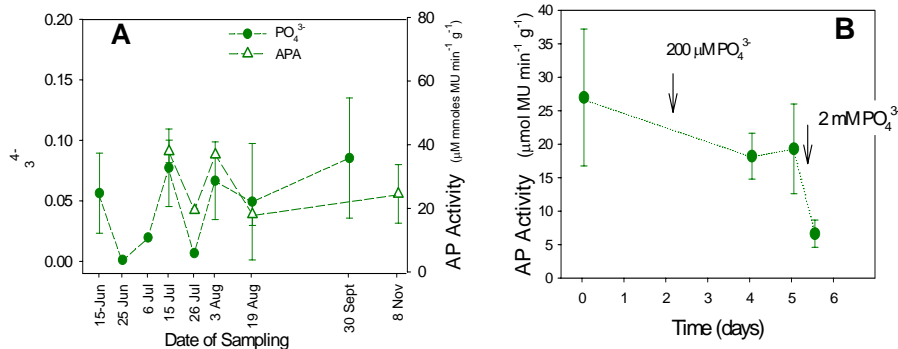


Figure 4. **A.** Seasonal variation in alkaline phosphatase (AP) activity in *Cladophora* and water column soluble  $\text{PO}_4^{3-}$  from Atwater 10 m site. Points are mean  $\pm$  std dev,  $n \leq 2$  ( $\text{PO}_4^{3-}$ ),  $n = 8$  (APA). **B.** Suppression of *Cladophora* alkaline phosphatase activity by  $\text{PO}_4^{3-}$ -enrichment in laboratory culture. Points are means  $\pm$  std dev,  $n = 8$ .

There was a correlation between AP activity and the soluble  $\text{PO}_4^{3-}$  available in the water column (Fig. 4A). AP activity in *Cladophora* was demonstrated to be responsive to water column available P. In laboratory tests, when 2 mM  $\text{PO}_4^{3-}$  was added, APA was significantly reduced from 20 to 6  $\mu\text{mol MU min}^{-1}\text{g}^{-1}$  FW within 5 hours (Fig. 4B).

### Conclusions about enzymes

- Nitrate reductase activity oscillates with available  $\text{NO}_3^-$  - 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  $\text{PO}_4^{3-}$  enrichment of growth medium.
- Elevated alkaline phosphatase activity in *Cladophora* indicates P limitation of *Cladophora* growth mid - late summer
- In conjunction with other indices of nutrient availability and internal nutrient concentrations, enzyme activity can be a useful additional parameter for examining nutrient status of *Cladophora* (and other algae).

### Estimating Photosynthesis using Chlorophyll *a* Fluorescence

The relationship between photosynthesis and available light was measured using a rapid *in situ* measurement of *Cladophora* using a pulse amplitude modulated fluorometer (PAM; Walz GmbH, Germany). Photosynthesis vs Irradiance curves were modelled to derive maximum photosynthesis rate, the initial slope of the P v I curve (alpha) and the irradiance at which the onset of light limitation occurs ( $I_k$ ) (Fig. 5).

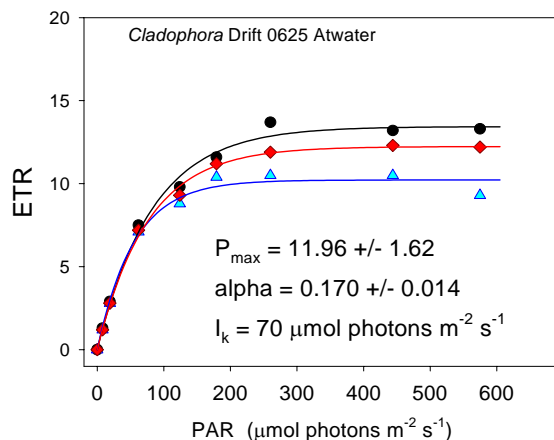


Figure 5. Three replicate measurements of P v I curves of *Cladophora* at the 10 m Atwater site on 25 June, 2004. The lines represent models of P v I relationship from Webb et al. (1974). Values of  $P_{\max}$ , alpha and  $I_k$  are modelled means (std dev) of the three replicate curves.

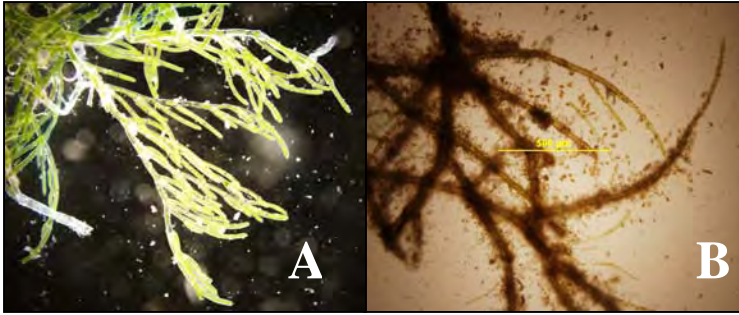


Figure 6. A. Healthy *Cladophora* collected from Bradford Beach Nov 2004. B. *Cladophora* collected from Atwater 10 m site Aug 2004, encrusted with epiphytic diatoms.

When we collected *Cladophora* from Lake Michigan at the 10 m Atwater site, macroscopically it appeared to be brown, rather than green. When observed under a microscope, the filaments of *Cladophora* were shown to be heavily epiphytised with, predominantly, diatoms (Fig. 6). This should be considered when thinking about what the *Cladophora* represents - a community of organisms rather than just the macroalga. The heavy epiphyte load may represent a stress to *Cladophora* as the epiphytes will shade light, and may strip 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.

### Overall Conclusions

- P limitation is evident in *Cladophora* based on
  - Consistently low soluble phosphate
  - AP activity
  - Nutrient stoichiometry (see Bootsma et al. chapter)
- If there are excess P inputs to nearshore Lake Michigan
  - it is being taken up immediately by benthic algae or phytoplankton
  - it is still insufficient to support unlimited *Cladophora* growth
  - P cycling within the benthos may be more important
- Light is probably limiting to *Cladophora* photosynthesis for the majority of the growing period
- Heavy epiphyte loads will further increase light limitation for *Cladophora* and may exacerbate nutrient limitation.

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## Discussion Forum: Reaching a Consensus on What is Known, and Setting Future Research Priorities

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### Introduction

Examining research into and management of *Cladophora* problems in the Great Lakes is complicated by the diversity of organizations and people involved, and the many different levels at which the issues can be approached. At the December 8, 2004 workshop, we closed the day with a relatively informal discussion session in order to survey and consolidate our present understanding of the problems, and to see if we could reach consensus about future research directions.

The discussion was organized around six major questions. In this summary, we provide a brief overview of the discussions and major points of consensus or dispute. In some cases, where points were raised by particular workshop participants, we have included their names in order to facilitate follow-up by readers of this report. In other cases, we have made reference to individual workshop presentations.

We have also provided a brief, annotated bibliography of sources in print and available on the web that are of potential use to readers.

### 1. What are impacts of *Cladophora* on ecosystem and human health?

Participants generally agreed that it is the aesthetics of the blooms that draws most attention, especially the odors from the rotting material, and the appearance on the shore and in the nearshore water. Such problems clearly have effects on property values, but they also affect tourism and recreational uses. While the effects of large quantities of *Cladophora* on the shore are obvious, much of the *Cladophora* probably settles deeper in the lakes; the effects of this biomass on the lake benthos are not known. It is also not clear if the decay of *Cladophora* blooms can affect oxygen levels and whether it is therefore implicated in fish kills.

Additional risks are posed by the tendency of blooms to clog water intakes. Such events can be quite serious. There have been power outages caused by fouling of power plants in Wisconsin, and shut-downs of nuclear power plants in Lake Ontario have been caused by *Cladophora* blooms (Hecky).

In terms of direct effects on human health, there is some evidence of increases in botulism due to decreases in O<sub>2</sub> levels in Lake Ontario, which may be related to *Cladophora* decay. It is clear that the moist, protected environment created by *Cladophora* mats could aid in survival of some pathogens on recreational beaches, though the real importance of this process is not known (Kleinheinz talk). The ability of *Cladophora* to take up and sequester heavy metals was noted (Sandgren talk) and there was speculation about the degree to which decaying *Cladophora* might release these toxic metals. This could also pose a problem for disposal of *Cladophora* after clean-operations. Curiously, there has also been interest in looking for bioactive compounds in marine *Cladophora* species (Ref. 10).

On the other hand, abundant *Cladophora* growth may have the potential to increase biomass of invertebrates by providing habitat and refuges. In turn, this may provide better resources for vertebrate predators. There is relatively little evidence of this, but some studies have demonstrated large increases in invertebrates associated with *Cladophora* growth (Pillsbury talk).

## **2. What is the magnitude of the current *Cladophora* problem relative to the past?**

Participants agreed that this was difficult to assess and largely subjective. Lake Ontario had huge problems in the late 60's-70's, and Lake Erie problems peaked in the 1970's. In Lake Michigan, problems received considerable media attention in the 1960's. Wisconsin DNR files detail homeowners' and fishermen's concerns and include photographs of *Cladophora*-laden front-end loaders on beaches (circa 1968).

Current problems in Lake Michigan may be worse than in the past because they seem less localized to point sources (Auer). Effects of fouling on nuclear plants on Lake Ontario appear to be worse judging by increased frequency and intensity of breakdowns in the last few years (Hecky). In Lake Huron, problems seem to have become much worse since the dreissenid mussel invasions.

## **3. What do we know about the spatial distribution of *Cladophora* in Lake Michigan and other Great Lakes?**

Large scale distributions of *Cladophora* are not known, and the correlation between regions of high *Cladophora* growth and shore deposition has not been established. The availability of a suitable, hard substratum for *Cladophora* is probably important, and might help explain why the Michigan shores of Lake Michigan are generally much less affected than the Wisconsin side. However, south-western Wisconsin and Lake Co. Illinois share similar substrata, yet Illinois's *Cladophora* problems are apparently less severe. Water clarity also plays a role; in Green Bay problems are much worse in the clearer northern part of the bay, as opposed to the southern areas where sediment loads are higher and there is more sediment resuspension. There are fewer obvious connections between nutrient point sources and areas of heavy *Cladophora* growth. Recently, aerial photography of shorelines has been attempted in Lake Michigan and this may provide a means to establish distributions on a wider scale (Janssen & Bootsma).

## **4. Is there evidence for the causes of the problems? Is it the same for all the Great Lakes?**

In the past, nutrients, especially phosphorus were identified as the critical variable controlling *Cladophora*. Stoichiometric data does indicate that *Cladophora* in Lake Michigan is phosphorus-limited in most cases (Bootsma talk), and this may indicate that increased availability of phosphorus is the cause of recent problems. The source of this phosphorus is not clear. Budgets of P-loading to the lakes provides some evidence for higher P inputs by some Wisconsin rivers in the short-term, but it seems unlikely that rivers can provide all the P necessary to drive the blooms (Bootsma talk). It is important to recognize that there are large reserves of phosphorus in Great Lakes sediment, and

particular chemical processes control P-availability in Lake Michigan (Brooks); the extent to which these are driving the problems is unknown.

Another cause of the problems seems likely to be the dreissenid mussels (the zebra mussel, *Dreissena polymorpha*, and the quagga mussel, *D. bugensis*), now well-established in all the Great Lakes except Superior. Mussels filter-feed on plankton, increasing water clarity which favors growth of benthic algae. For example, there were large increases in Secchi depth on the western side of Green bay due to mussels in 1993-4 (Harris). In addition, by filtering P-containing particulate matter from the whole water column and excreting P near the bottom, they may effectively be enriching P in the benthic regions where *Cladophora* grows (Maybruck, Stankovich and Higgins talks).

It is also important to recognize that *Cladophora* growth by itself may not be the only cause of problems. From the late 1980's-1990's when the problems of shoreline *Cladophora* deposition apparently disappeared in Lake Michigan there is no evidence that *Cladophora* growth and biomass distributions changed. Instead, it may be that it is the mechanisms that control *Cladophora* losses and wash-ups on the shore that are relevant. Basic questions remain unanswered such as the factors that cause *Cladophora* to detach (physical stress, nutrient deprivation, substratum characteristics), and the best ways to assess the state of health of *Cladophora* populations (Young talk). There are reports of growth associated with dolomite and limestone substrate (Colorado streams), and in places such as Waukegan where bedrock dominates, algae are bleached-looking and easy to pull off. Because *Cladophora* attaches to mussels, detachment may relate to the state of the mussels as well as the algae (Davis-Foust & Janssen talk).

There is clear evidence of differences in *Cladophora* problems between lakes, for example, in seasonal patterns of growth and loss. A late summer die-off in *Cladophora* beds in Lake Ontario (Higgins) has not been clearly observed in Lake Michigan populations (Bootsma, Young, Berges). Moreover, epiphyte loads (mostly diatoms) on growing *Cladophora* are markedly higher in Lake Michigan (Bootsma, Young talks) than in Lake Ontario and Lake Erie.

## **5. What management strategies are possible and which are being applied?**

Manual cleaning of washed-up *Cladophora* from the shore has been attempted in several cases (Stauffer talk), but it is labor intensive, and impractical on a large scale; the Lakes contain vast biomasses of *Cladophora*. There has been local success at harvesting from the shallow water by essentially 'corralling' the algae and using hydraulic pumping to remove it (Harris).

Other suggestions range from identifying beds from which *Cladophora* might detach and putting in some form of containment (e.g. 'snow-fence'-like netting; Pillsbury), or harvesting algae from the lake bottom using commercially-available machinery, as is done to prevent blooms in some marine systems (e.g. Po River estuary, Italy; Berges). Anecdotal evidence from fishermen suggests that trawl nets are effective in collecting *Cladophora*.

Use of chemical compounds was also discussed. Algaecides such as copper sulphate were used in the 70's in Lake Ontario but were not very successful. Participants doubted whether chemical treatment would be effective in large bodies of water and questioned whether environmental regulations would permit it in any case. Alternatively,



researchers at Clemson University (SC) are using test plots to examine whether application of enzymes could help to speed *Cladophora* degradation once on the shore and help avoid odour problems. In South Africa, there have also been attempts to find biological agents (bacteria, fungi) that could decrease growth or speed decomposition (Ref. 3).

Another strategy that might be effective would be to characterize the hydrodynamics of susceptible regions and use computer models to predict where detached *Cladophora* is likely to end up. With predictions, warnings could be given and clean-up efforts could be better organized and targeted (Ref. 5).

## 6. What are the priorities for future research?

Concerns were raised about the identity of the organisms responsible for the blooms. Macroscopically, *Cladophora* can be confused with other green algae such as *Ulothrix* and *Spyrogira* species. The current taxonomy of *Cladophora* itself is problematic, and we do not have a clear idea of which species (or even how many species) we are dealing with (Muller talk, Ref. 6). Since species can differ markedly in physiology, it is an open question whether differences observed between lakes and over time might relate to taxonomic differences.

More work is needed to assess P-inputs to lake systems, and the relative importance of point sources versus non-point sources. It is troubling that recent estimates of river P loading (USGS, Lake Michigan Mass Balance Program, Bootsma talk) to Lake Michigan differ by such large amounts. River discharges should be the simplest to quantify, but there may be autochthonous offshore sources of P as well. Deep waters may serve as a reservoir of P which can be introduced/regenerated in spring and thus feed the algal growth for the summer. One approach that has not been well-explored is the use of detailed comparisons of 'then vs. now' nutrient budget estimates to determine whether there have been changes over time that can be related to changes in *Cladophora* problems. It would also be wise to determine whether the existing water-treatment infrastructure is adequate for treating effluents and whether improvements to these systems (e.g. addition of storm-water treatment) are feasible and cost-effective.

In terms of benthic processes and the role of dreissenid mussels, it will be important to understand how P is incorporated in and regenerated from the benthos. We need clearer estimates of P excretion and cycling rates from dreissenids, and in particular whether the more recent invaders, quagga mussels, differ substantially from the zebra mussels in terms of processes such as excretion and production of pseudofaeces.

There is a clear need for better mapping of the spatial distribution of *Cladophora* between sites and within different lakes. Methods such as sonar mapping (Ref. 9) and spectral remote sensing (Ref. 8) should be considered in addition to aerial photography. Global Information System techniques could then be applied to determine relationships with substratum and known nutrient sources.

In terms of management options, better information about lake hydrography, coupled with hydrodynamic modeling will be essential in order to identify likely places for *Cladophora* to accumulate on shores, and thus to provide early warning and direct clean-up efforts effectively.

Participants felt strongly that coordinated work that uses similar approaches to study different lakes and lake/stream environments will be needed to improve our understanding of factors controlling *Cladophora*. For example, it may be significant that there are regular die-offs of *Cladophora* in August in Lake Ontario, but apparently not in Lake Michigan. Moreover, studies of *Cladophora* in river systems have significant advantages in terms of our ability to make manipulations.

### **Selected sources for further information on *Cladophora* and nuisance algal blooms**

- (1) The U. Wisconsin Milwaukee-Great Lakes Water Institute *Cladophora* site. Basic overview and discussion of Lake Michigan issues. <http://www.uwm.edu/Dept/GLWI/cladophora/>
- (2) Ontario Water Works Consortium. Discussion of *Cladophora* issues in Lake Ontario and U. Waterloo research efforts.. <http://www.owwrc.com/AA.htm>
- (3) The Potential Biological Control Agents of *Cladophora glomerata* that Occur in Irrigation Schemes in South Africa Report No 669/1/99. Discussion of possible application of fungi and bacteria to reduce blooms in irrigation waters. <http://www.fwr.org/wrcsa/669199.htm>
- (4) Centre for Aquatic Plant Management, UK. Notes on chemical control of algae, including *Cladophora*. <http://www.rothamsted.bbsrc.ac.uk/pie/JonathanGrp/InformationSheets/Chemical%20control%20of%20algae.pdf>
- (5) Use of modeling for algal bloom prediction. Green algal blooms in Ortobello lagoon, Italy. [http://www.iemss.org/iemss2002/proceedings/pdf/volume%20tre/355\\_marsilii.pdf](http://www.iemss.org/iemss2002/proceedings/pdf/volume%20tre/355_marsilii.pdf)
- (6) AlgaeBase website. Taxonomic overview of *Cladophora*, including nomenclature and literature references. [http://www.algaebase.org/generadetail.lasso?genus\\_id=37&-session=abv3:44F904F21daa007C40yVi152753B](http://www.algaebase.org/generadetail.lasso?genus_id=37&-session=abv3:44F904F21daa007C40yVi152753B)
- (7) Bioaccumulation of metals in *Cladophora* in a refinery waste lagoon in Bratislavia, eastern Europe. [http://jagor.srce.hr/ccaaa/CCA-PDF/cca2001/v74-n1/cca\\_74\\_2001\\_135-145\\_Chmielewska.pdf](http://jagor.srce.hr/ccaaa/CCA-PDF/cca2001/v74-n1/cca_74_2001_135-145_Chmielewska.pdf)
- (8) Efforts to apply remote sensing: hyperspectral imaging of *Cladophora* in Lake Ontario . A. Vodacek, Rochester Institute of Technology. <http://www.cis.rit.edu/research/dirs/pages/embayment/>
- (9) National Oceanic and Atmospheric Administration's (NOAA) Delaware's experience with marine green algal blooms (in this case, largely *Ulva* species). Comments and protocols for benthic mapping using side-scan sonar. [http://www.csc.noaa.gov/crs/rs\\_apps/issues/sb\\_ulva.htm](http://www.csc.noaa.gov/crs/rs_apps/issues/sb_ulva.htm)
- (10) Possibility of bioactive compounds in algae (including *Cladophora*) being developed for pharmaceuticals. <http://chapmanlab.lsu.edu/digitalalgae/GulfAlgae/MMSBiotech.html>
- (11) Lake Ontario Algae Cause and Solution Workshop Proceedings. <http://www.monroecounty.gov/documentView.asp?docID=2351>



**Workshop Program**

8:30	Welcome	Dr. J. Val Klump, UWM Great Lakes WATER Institute
8:45	Program Overview, Goals and Objectives	Harvey Bootsma, UWM Great Lakes WATER Institute
9:00	<i>Cladophora</i> Confounds Coastal Communities – Public Perceptions and Management Dilemmas	Vicky Harris, Wisconsin Sea Grant Rebekah Stauffer, Milwaukee Community Service Corps
9:30	<i>Cladophora</i> and the Beach: Public Health Implications	Gregory Kleinheinz, University of Wisconsin-Oshkosh
10:00	<b>Break</b>	
10:15	The Influence of <i>Dreissena polymorpha</i> Ejecta on Bacterial Nutrient Remineralization and Growth Efficiency	Brian Maybruck, University of Wisconsin-Milwaukee
10:45	Why Filamentous Green Algae Dominated Benthic Habitats After the Zebra Mussel Invasion in Saginaw Bay, Lake Huron	Robert Pillsbury, University of Wisconsin-Oshkosh
11:15	The Interaction of Two Nuisance Species in Lake Michigan: <i>Cladophora glomerata</i> and <i>Dreissena polymorpha</i>	Wendy Stankovich, University of Wisconsin-Plattville
11:30	The Weakest Link and What makes it Stink	Shannon Davis-Foust, John Janssen, UWM Great Lakes WATER Institute
12:00	<b>Lunch</b>	
1:00	Molecular Phylogeography and Species Discrimination of Freshwater <i>Cladophora</i> (Cladophorales, Chlorophyta) in North America	Kirsten Muller, University of Waterloo
1:30	Nuisance <i>Cladophora</i> in Milwaukee Urban Streams - Habitats, Seasonality, Productivity, Nutrient trapping, Heavy Metal Sponging - the Ultimate Foodweb Bottleneck	Craig Sandgren, University of Wisconsin-Milwaukee

- 2:00 Modeling *Cladophora* Growth: A Review of the Auer-Canale Framework Martin Auer, Michigan Technological University
- 2:30 The Role of *Dreissena* in the Resurgence of *Cladophora* in Eastern Lake Erie Scott Higgins, University of Waterloo
- 3:00 **Break**
- 3:10 Systematic Survey of *Cladophora* and Water Quality on the Wisconsin Lake Michigan Shoreline Paul Garrison and Steve Greb, Wisconsin Department of Natural Resources
- 3:30 Temporal and Spatial Patterns of *Cladophora* Biomass and Nutrient Stoichiometry in Lake Michigan Harvey Bootsma, Erica Young, John Berges University of Wisconsin-Milwaukee
- 4:00 Can Activity of Enzymes Involved in Nutrient Assimilation be Useful as Indices of Nutrient Status in *Cladophora*? Erica Young, John Berges, Harvey Bootsma, University of Wisconsin-Milwaukee
- 4:15 Discussion Session: Reaching a Consensus on What is Known, and Setting Future Research Priorities Erica Young, John Berges, University of Wisconsin-Milwaukee

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