

**TOXICITY, ECOLOGICAL IMPACT, MONITORING, CAUSES AND
PUBLIC AWARENESS OF *Microcystis* BLOOMS IN LAKE ERIE**

FINAL REPORT TO THE LAKE ERIE COMMISSION

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Toxicity, Ecological Impact, Monitoring, Causes, and Public Awareness of *Microcystis* Blooms in Lake Erie

Abstract: The large blooms of *Microcystis* that occurred in the western basin of Lake Erie during August - October of 1995 were unexpected. Over the last 25 years cyanobacterial blooms have decreased in frequency while water quality of Lake Erie has increased. *Microcystis* can produce virulent toxins that may affect Lake Erie's ecosystem, including man. This report summarizes the results of a collaborative, comprehensive study to delineate the interactions of this alga and its toxins with Lake Erie, monitor the frequency and distribution of the blooms, examine their causes, and communicate the results of our research to the lay public, Lake Erie managers, and the scientific community.

We adopted a collaborative approach, involving 13 investigators from 7 institutions, plus many additional cooperators from federal, state, provincial, and university institutions. We studied toxicity and ecosystem effects at a common series of sample sites and dates in the Bass Islands. We monitored *Microcystis* abundance both past and present by reviewing samples collected by ship and remote sensing techniques. A series of field and laboratory experiments was performed to determine the causes of *Microcystis* blooms in Lake Erie. *Microcystis* was cultured by W. Carmichael and associates (Wright State University). Extensive toxicity analyses were performed by Carmichael for all the various portions of the project. We began working in June 1996, performing most field data collections and experiments in 1996, followed by an additional period of sampling in 1997, and, to a smaller extent, in 1998, for a total duration of 30 months.

Preliminary findings discussed in this report:

Goal I. Assess Risks of Toxicity from Microcystins to All Trophic Levels

- 1) Six species of fish are being analyzed for microcystin content.
- 2) Microcystin content of Lake Erie was typically <3 ng/L for 1996, although there was a single value of 21.7 ng/L in August 1996, a low *Microcystis* year. Samples from the 1998 bloom year yielded values as high as 50 ng/L, but these are still only 5% of the concentration (1 µg/L) at which the Australian government suggests action should be taken to protect drinking water supplies.
- 3) Microcystins from a strain cultured from Lake Erie (Strain LE-3) are primarily microcystin-LR, the most common form of the toxin, and the most toxic.
- 4) Livers from 11 sick ducks from Lake Erie (1996) showed no histological evidence of microcystin toxicity, nor was *Microcystis* found in the ducks' digestive tracts.
- 5) Zebra mussels were found to be a new route of transfer and biomagnification of microcystin in the aquatic food web through accumulation of microcystin and concentration of toxic algae in their feces and pseudofeces.
- 6) Two newly introduced species, an amphipod (*Echinogammarus ischnus*) and a fish (*Neogobius melanostomus*, the round goby), are important in microcystin transfer. The amphipods consume contaminated feces+pseudofeces and the gobies eat zebra mussels.
- 7) Assays of cytotoxicity of microcystins -LR and -YR on cultured human liver cells yielded LC₅₀ estimates of 500 and 250 µg/ml, respectively, for 24 h exposures, suggesting that acute toxic effects occur at concentrations higher than from typical lake water exposures.

small non-toxic algae such as *Cryptomonas*. Toxic *Microcystis* not occurring in colony form is eaten, however, but ingestion of toxic *Microcystis* appears to inhibit zebra mussel grazing activities on all algae.

9) No significant differences were found between growth rates for zebra mussels during a year when *Microcystis* bloomed (1995) relative to years when it did not (1996 and 1997).

Goal III. Monitor the Occurrence of Blooms of Microcystis and Other Toxic Algae

10) *Aphanizomenon* and *Anabaena* were much reduced from 1970 levels by 1983, whereas *Microcystis* has bloomed at least twice (1995 and 1998) since zebra mussels have become established. The first two taxa are nitrogen fixers, whereas *Microcystis* is not.

11) High abundance of cyanophytes (including *Microcystis*) in Lake Erie in 1996 was associated with stations with total inorganic Nitrogen to total Phosphorus ratios below 25, and total inorganic Nitrogen to soluble reactive phosphorus levels below 400. Highest abundances occurred in August and September, when prevailing water temperatures were highest.

12) A near real-time display of daily surface reflectance (related to surface algae and other particulate matter) for all of Lake Erie, based on AVHRR satellite data has been made available via a NOAA web site (<http://www.glerl.noaa.gov/cw/cw.html>).

13) Calibration of 1995 satellite data with ground truth data enabled following the development in August and disappearance in October of the 1995 *Microcystis* bloom showed that AVHRR satellite data provide an inexpensive means of monitoring the extent of surface blooms in Lake Erie. The SeaWiFS satellite may also be useful for this purpose, since it detects light near the fluorescence wavelength of chlorophyll.

Goal IV. Determine the Causes of Microcystis Blooms in Lake Erie.

14) Low nitrate to ammonia ratios and warm water temperatures in August and September may have helped initiate the *Microcystis* bloom in 1995, but these conditions were also found in four other years without blooms in Hatchery Bay, South Bass Island.

15) External loading of phosphorus and nitrogen was not correlated with the occurrence of a bloom of *Microcystis* in 1995.

16) Algae growing near zebra mussels are less phosphorus-limited than are those living further away, suggesting that phosphorus excretion (recycling) by zebra mussels may in part stimulate algal growth and *Microcystis* blooms.

17) Nitrogen excretion by zebra mussels in Lake Erie were 1.3 to 2.9 times that in Saginaw Bay, while their phosphorus excretion was 20 to 80 times higher than that of Saginaw Bay mussels. Lake Erie zebra mussels excreted N and P at N:P ratios lower than the seston being consumed.

18) High light reaching the bottom of the lake may trigger growth of *Microcystis* cells lying on the bottom, initiating a bloom.

Extensive presentations to the scientific community, Lake Erie managers, and the lay public have been made by various members of the *Microcystis* research project team. Numerous publications are in various stages of preparation, submission, review, and publication. Additional research is being performed on various aspects of the project and will contribute significantly to our understanding of the causes and effects of toxic algal blooms in the Great Lakes and beyond.

PROBLEM IDENTIFICATION

The large blooms of *Microcystis* that occurred in the western basin of Lake Erie during August - October of 1995 were unexpected. Over the last 25 years cyanobacterial blooms have decreased in frequency while water quality of Lake Erie has increased (Makarewicz, 1993). While levels of nitrogen-fixing cyanobacteria genera such as *Anabaena*, *Aphanizomenon*, *Nostoc* and *Nodularia* have remained low since 1990, *Microcystis aeruginosa*, a non-nitrogen-fixing species, has suddenly increased suggesting that one or more factors having to do with phosphorus or N/P ratio changes, and/or activities by filter-feeding zebra mussels may be involved. Because *Microcystis* can produce virulent toxins that may affect Lake Erie's ecosystem, including man, we prepared a collaborative, comprehensive study plan to delineate the interactions of this alga and its toxins with Lake Erie, monitor the frequency and distribution of the blooms, examine their causes, and communicate the results of our research to the lay public, Lake Erie managers, and the scientific community. This report summarizes the results of that study as of December 1998, but many of the research initiatives begun with this collaboration are continuing in the laboratories listed above.

COLLABORATIVE APPROACH TO THE STUDY OF TOXIC CYANOBACTERIA

Because we cannot perform replicated manipulative experiments with the one Lake Erie, we adopted a combination of laboratory and field experiments supported by historical approaches to study *Microcystis* in the lake. We studied toxicity and ecosystem effects (Culver and Babcock-Jackson (OSU), Vanderploeg, Johengen, and Beeton (GLERL), MacIsaac (U. Windsor), Carmichael (Wright State U.), Heath and Wickstrom (Kent State U.), and Wu (Mount Union C.)) at a common series of sample sites and dates in the Bass Islands. We coordinated our temporal and spatial variability of plankton and nutrient monitoring with the Ohio Division of Wildlife's intensive field collections of young-of-year fish (YOY) (41 stations in the western basin). These same cruises were used for collecting "ground truth" data for satellite monitoring of algal blooms (Budd (Michigan Technological University) and Leshkevich (GLERL)). Similarly, David Baker and Peter Richards (Heidelberg College) contributed data from their Maumee and Sandusky River nutrient input studies (1968-1995), some of which have been supported by the LEPP, and performed nutrient analyses from the ODW samples. All of the investigators listed above had historical data on nutrient conditions and/or biological characteristics of Lake Erie that were re-examined and combined with data collected in this project in an effort to understand changes occurring in the western basin that may have encouraged the *Microcystis* bloom. Wayne Carmichael (Wright State University) brought extensive experience with toxic cyanophytes and the measurement of their toxins to this project. We began working in June 1996, performing most field data collections and experiments in 1996, followed by an additional period of sampling in 1997, and, to a smaller extent, in 1998, for a total duration of 30 months.

Continued monitoring and experiments about the causes and effects of the blooms are still needed, but we have tested a broad range of hypotheses in this project to facilitate design of future work. These 12 hypotheses are clustered under four goals:

Goal I. Determine the risks of toxicity from microcystins released into Lake Erie to all trophic levels

Goal II. Assess the ecological impacts of *Microcystis* blooms to the Lake Erie ecosystem.

Goal III. Monitor the occurrence of blooms of *Microcystis* and other toxic algae.

Goal IV. Determine the causes of *Microcystis* blooms in Lake Erie

An integral fifth goal was to communicate the results of our research to the lay public, Lake Erie managers, and the scientific community. Each of our study goals addresses one of the objectives in the original Special Call for Grant Proposals from the Lake Erie Protection Fund. Our activities were organized as a series of 12 hypotheses to be tested, followed by 18 study objectives and their specific methods. This report is arranged in the same order, with the specific researchers involved in each segment identified at its beginning. The report was compiled by Ms. Lisa Babcock-Jackson, doctoral student at The Ohio State University, from materials submitted by the individual investigators, and was edited by David A. Culver.

RESULTS

Goal I. Determine the Risks of Toxicity from Microcystins Released into Lake Erie to All Trophic Levels

Hypothesis 1. Ingestion of or contact with Microcystis microcystin adversely affects wildlife in western Lake Erie.

Objective 1a. Examine wildlife for evidence of liver damage in fish due to microcystins.

Babcock-Jackson and Culver (Ohio State Univ.), Carmichael, et al. (Wright State Univ.)

Weather patterns and other factors influenced conditions in Lake Erie to prevent the formation of large blooms of cyanobacteria during the 1996 and 1997 field seasons, therefore fish were not collected during those years. However, conditions were such that a large *Microcystis* bloom did occur during August of 1998 and fish were collected by trawl during the exponential growth phase (August) and the decline phase (October) of the bloom. Gizzard shad, young-of-year (YOY) yellow perch, adult yellow perch, *Morone* spp., walleye, round gobies and creek chub have been collected. Intestines, stomachs and livers have been removed and frozen and are currently undergoing enzyme-linked immunosorbent assay (ELISA) analysis for microcystin content at Wright State University.

Objective 1b. Determine the toxicity of cyanobacteria in phytoplankton samples from the western basin of Lake Erie.

Babcock-Jackson, Culver, Carmichael, et al.

Samples of zooplankton containing concentrated cyanobacteria were collected and frozen for microcystin analysis from several sites in the western basin of Lake Erie during August and September of 1996 (a low *Microcystis* year) and August of 1998 (a high *Microcystis* year). Samples of the cyanobacteria from both years have been analyzed for microcystin levels by enzyme-linked immunosorbent assay (ELISA). All sites sampled in 1996 showed low levels of microcystin (< 25 ng/L) with spatial variability in microcystin levels (Figures 1a, 1b) usually corresponding to the biomass of cyanobacteria present. Samples collected in 1998 were much higher (Figure 1c). All samples were analyzed at Wright State University for toxin levels using ELISA methods described by An and Carmichael (1994).

In addition live samples were collected for isolation and culture at Dr. Carmichael's lab at WSU. Isolation of a Lake Erie strain (LE-3) of *Microcystis aeruginosa* was accomplished and large-scale cultures are currently available. Freeze-dried cells from the LE-3 cultures were used in some 1998 toxicity experiments described under Hypothesis 2. Microcystin chemical structures and toxicity of the LE-3 *Microcystis* strain have been determined by Dr. Carmichael and his associates as follows.

Carmichael et al. (Wright State University)

INTRODUCTION

Microcystins (MCYSTs) are a group of cyclic heptapeptide liver toxins produced by several genera of cyanobacteria (blue-green algae), including *Anabaena*, *Hapalosiphon*, *Microcystis*, *Nostoc* and *Oscillatoria* (Carmichael 1997). MCYSTs are potent inhibitors of protein phosphatase I and 2A, and are tumor promoters (Nishiwaki-Matsushima, et al. 1992). The related cyclic pentapeptide nodularins (NODLN), produced by *Nodularia spumigena*, are thought to be carcinogenic (Ohta, et al. 1994). In addition, MCYSTs are implicated in animal deaths (Skulberg, et al. 1989), human sickness (Falconer, et al. 1983), and 'Caruaru syndrome', which caused the deaths of over 60 hemodialysis patients from liver toxicosis (Jochimsen, et al. 1998). These algal toxins, therefore, present a health risk to both animals and humans.

Over 60 microcystin structural variants have been isolated from both cultured and bloom material (Rinehart, et al. 1994, Namikoshi et al. 1998). The general structure of MCYSTs is cyclo(D-Ala-X-D-MeAsp-Y-Adda-D-Glu-Mdha), where X and Y are variable L-amino acids at positions 2 & 4 (Figure 2). MCYSTs are named according to the L-amino acids present, by using one letter amino acid designations for X and Y as a suffix (Carmichael et al. 1988). MeAsp (amino acid 3) is D-erythro- β -methylaspartic acid, D-Glu (amino acid 6) is D- γ -linked glutamic acid, Mdha (amino acid 7) is N-methyldehydroalanine and Adda (amino acid 5) is an unusual C₂₀ β -amino acid, (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4E,6E-dienoic acid, which is important in imparting toxicity

(Rinehart, et al. 1994). Common structure variation includes demethylations at amino acids 3 (D-MeAsp) and 7 (Mdha), modification of the Adda C9 methoxy, and substitution at X and Y.

Detection of MCYSTs is accomplished using several analytical techniques including high performance liquid chromatography (HPLC) (Harada 1996), capillary electrophoresis (CE) (Onyewuenyi and Hawkins 1996), and protein phosphatase inhibition and immunoassay (An and Carmichael 1994). Structure characterization is usually based on amino acid analyses, nuclear magnetic resonance (NMR), and mass spectra. Not since 1966, when MCYSTs were known as 'fast death factor', has a toxin-producing *Microcystis* bloom been reported in Ohio (Maloney and Carnes 1966). This study reports the first isolation/characterization of MCYSTs from a Lake Erie, Ohio, *Microcystis* strain.

MATERIALS AND METHODS

Field Collection

Microcystis aeruginosa strain LE-3 was isolated from Put-In-Bay, Lake Erie, using a 49 μm mesh zooplankton net, vertically hauled at a 10 meter depth. Samples were collected midway between South and Middle Bass Islands at the Peach Point Marker buoy (Figure 3). Samples were refrigerated until cultured.

Laboratory Cultures

LE-3 was isolated from field samples and batch cultured with aeration using BG-11 media (Watanabe 1996) at 26°C under continuous light of approximately 25 $\mu\text{E}/\text{m}^2/\text{sec}$. Cells were harvested in 3-4 weeks and lyophilized.

Toxin Extraction

Lyophilized cells were extracted overnight with double-distilled water/methanol/n-butanol (25:4:1 v/v)(50 mL/g) while stirring. The supernatant was collected after centrifugation, and organic solvent was removed by placing the extract under an air stream overnight, and then applied the aqueous extract to a C18 (octadecyl) solid phase extraction (SPE) cartridge, which is then washed with water, 20% aqueous methanol and finally eluting the toxin with 80% aqueous methanol.

High Performance Liquid Chromatography (HPLC)

The SPE eluent was then chromatographed on an analytical HPLC column (Zorbax SB-C18 5 O_m , 4.6 x 250 mm) (MacMod Analyt., Chadds Ford, PA) with 65:35 0.1% (v/v) aqueous TFA/ACN + 0.1% TFA (1 mL/min). HPLC was performed using a Waters 2690 separations module, 996 photodiode array detector and Millennium™ 2010 chromatography manager (Milford, MA). Peaks were identified and collected for structure analysis based on UV spectra matching, monitored at 238 nm.

Amino Acid Analysis (AAA)

Samples (50-250 pmoles) were hydrolyzed overnight (6N, 110°C) and analyzed using a pre-column fluorescence derivitization HPLC technique (Waters Acc-Q-Tag™) and compared to amino acid standards. Adda and mdha are destroyed during hydrolysis and are not detected using this method. Calibration was accomplished using internal standardization.

Mass Spectrometry

FAB mass spectra were obtained on a VG ZAB-SE (Fisons, Manchester, U.K.) using xenon atoms (8 keV) and the 'magic-bullet' matrix (dithiothreitol/dithioerythritol). High resolution FABMS (HRFABMS) data were collected by peak matching on the first half of the four-sector mass spectrometer (Resolving power $M/\Delta M=10,000$ at 10kV). ESI mass spectra were obtained on a VG Quattro by loop injection with a carrier solvent of 1:1 $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ at 10 $\mu\text{L}/\text{min}$.

RESULTS

Toxicity Determination

The isolated strain of Lake Erie *Microcystis aeruginosa*, LE-3, was found to contain 2.2 mg/g microcystins by immunoassay (enzyme-linked immunosorbent assay, ELISA) (An and Carmichael, 1994), and 1.9 mg/g by analytical HPLC. For both methods, MCYST-LR was used as the calibration standard.

Isolation and Characterization of microcystins

The toxin fraction separated by solid phase extraction (SPE) was then chromatographed using analytical HPLC, and compounds 1-3 were collected based on their characteristic 'microcystin-like' UV spectra on the photodiode array detector (Figure 4). Compound 1 (10.4%) yielded Asp, Glu, Arg, Ala and Leu upon amino acid analysis. FABMS data show a molecular ion $(M+H)^+$ of 981.6, HRFABMS=981.5423, corresponding to molecular formula, $\text{C}_{48}\text{H}_{72}\text{N}_{10}\text{O}_{12}$ ($\Delta=1.3$ mda) (Table 1). This evidence indicates that 1 is [D-Asp³] MCYST-LR. Compound 2 (87.7%), the most abundant MCYST, was identified as MCYST-LR, by peak matching (retention time) with the chromatographic program. Amino acid analysis revealed Glu, β -MeAsp, Arg, Ala and Leu. FABMS, $(M+H)^+=995.6$, with the HRFABMS molecular ion corresponding to $\text{C}_{49}\text{H}_{74}\text{N}_{10}\text{O}_{12}$ ($\Delta=0.1$ mda), which corroborates that 2 is MCYST-LR. The minor peak 3 (1.9%) contained Glu, β -MeAsp, Arg (2) and Ala by AAA. FABMS was inconclusive, and ESIMS yielded $(M+H)^+=975$. This result probably represents a Na⁺ adduct $(M+H+Na)^+$, and would therefore correspond to MCYST-AR.

DISCUSSION

MCYST-producing freshwater cyanobacteria (i.e., *Microcystis aeruginosa*) present a hazard in both recreational and drinking supply waters (Lambert, et al. 1994), and may contribute to elevated levels of primary liver cancer in China (Yu 1989, Ueno et al. 1996). MCYST-LR, the most commonly encountered microcystin, has been implicated in many poisonings, and was found to be predominant in strain LE-3 by HPLC and mass spectrometry (Figure 5). It is apparent that monitoring and analysis for these toxins (particularly MCYST-LR) is essential for determining risk and maintaining safe water supplies.

This study demonstrates the isolation and analysis of MCYSTS from a Lake Erie collected strain of *Microcystis aeruginosa* using high performance liquid chromatography and mass spectrometry, and is the first documentation of a potent and toxic strain of cyanobacteria in Ohio waters.

Objective 1c. Determine whether *Microcystis* blooms are responsible for waterfowl deaths in Lake Erie

H. MacIsaac et al., University of Windsor.

METHODS

Eleven ducks were collected from dock areas adjacent to Put-In-Bay, Ohio, during summer 1996. Some of the ducks were (freshly) dead when collected from the lake's surface, though others were collected in a near-death condition by field assistants or by staff affiliated with Stone Lab. Ill ducks were maintained in darkened chests on ice until they were euthanized in a CO₂ chamber; these individuals died within 1.5 min exposure in the CO₂ chamber.

Ducks were dissected in the laboratory for diet and liver examinations. The esophagus, proventriculus and gizzard were excised, following which the diet contents were collected. Diet contents were examined with a dissecting microscope for evidence of *Microcystis* cells or colonies.

RESULTS AND DISCUSSION

Waterfowl livers were excised for examination. Microcystins primarily affect liver cells, causing them to shrink and allowing blood to pool within the liver (Carmichael 1997; Lambert et al. 1994; Nishiwaki-Matsushima et al. 1992); therefore, livers were examined for evidence of gross pathology. Ducks that were near-death when collected were unable to hold their head above the water line. Digestive tracts of all waterfowl examined contained very little particulate material other than gravel in gizzards. Examination of liquid extracted from digestive tracts revealed no indication of *Microcystis* cells or colonies. Cause and effect patterns are difficult to establish in this instance; waterfowl may have had empty digestive tracts because they were very ill, or may have been very ill because of earlier exposure to cyanobacteria toxins or water-borne pathogens. However, because none of the livers were abnormal in appearance (e.g. no evidence of blood pooling, nodules or discoloration) when examined, evidence of microcystin poisoning was lacking.

Alternative mechanisms including avian botulism should be considered. Waterfowl populations have periodically experienced large kills on Lake Erie over the past century, possibly in association with avian botulism. The kills reported during summer 1995 and 1996 thus must be placed in the context of historical patterns.

Hypothesis 2. Microcystis and other toxic cyanobacteria interact with zebra mussels to increase the risk of microcystin accumulation and/or toxicity in the Lake Erie food web, including humans.

Objective 2a. Assess microcystin bioaccumulation potential in zebra mussels and the potential of zebra mussels to act as a route of transfer and biomagnification of microcystin in the aquatic food web.

Babcock-Jackson, Culver, Carmichael, et al.

INTRODUCTION

A complex set of ecological relationships has been created by the invasion of the Great Lakes by three new interdependent species, the zebra mussel, the round goby and an amphipod. Zebra mussels (*Dreissena polymorpha*) were introduced into the Great Lakes via ship ballast water circa 1986 (Hebert et al., 1989) and have become the dominant benthic species in the western basin of Lake Erie (Leach, 1993). Following their introduction, two other species which are closely associated with zebra mussels have also invaded the Great Lakes. These other species are an amphipod (*Echinogammarus ischnus* (Witt, et al. 1997; Dermott et al., 1998)), which prefers living among clusters of zebra mussels and feeds upon zebra mussel excreta, and the round goby (*Neogobius melanostomus* (Jude et al., 1992)), a demersal fish which prefers zebra mussels (Ray and Corkum, 1997) and other benthic organisms as food. The goby also uses the complex benthic habitat created by zebra mussels to avoid predators and spawn; often displacing native fish through aggressive territorial behavior (Jude et al., 1995). This new set of ecological relationships is generally influencing a redirection of energy (Madenjian, 1995; Stoeckmann and Garton, 1997), nutrients (Heath et al., 1995; Gardner et al., 1995; Holland et al. 1995, Arnott and Vanni, 1996; James et al., 1997) and contaminant cycling (Bruner et al., 1994) to the benthic community.

Zebra mussel-mediated changes in nutrient dynamics (and thus in the phytoplankton community (Lavrentyev, et al., 1995)) may be favoring hepatotoxin (microcystin) producing *Microcystis* (see Objectives 8,10a and 10b of this report). Selective filtration and rejection of toxic *Microcystis* by zebra mussels (see Objectives 3 and 12 of this report) may indirectly result in a novel trophic transfer pathway through zebra mussels, cycling microcystin into the benthic community. Bivalves have a unique ability to accumulate microcystins (Vasconcelos, 1995; Watanabe et al., 1997) and other contaminants (Fisher et al., 1993) and survive relatively high doses. Vasconcelos (1995) has suggested that elimination of microcystins in pseudofeces may be a mechanism by which an estuarine species of *Mytilus galloprovincialis* rids itself of microcystins. Since zebra mussels also produce pseudofeces in response to undesirable seston we thought this would be a new route for cycling the toxin to the benthic fauna. The round goby is a benthic feeder which feeds on zebra mussels, and piscivory of the round goby is already known for smallmouth bass and freshwater drum (observations at Stone Lab). In addition native fish including freshwater drum and yellow perch have also adapted to zebra mussels as a food source (Morrison et al., 1997). Predation of larger fish on zebra mussels, other benthic fauna and round gobies in turn may result in cycling of microcystins back to the pelagic community and humans through these sport fish (Figure 6).

We hypothesized that the presence of toxin-producing *Microcystis* and the new ecological relationships developing among zebra mussels, amphipods, and round gobies in western Lake Erie will result in cycling of microcystins from the water column into the benthic community and back to the pelagic community. The purpose of this study was to test this hypothesis using laboratory microcosm experiments and field collections. We had 3 objectives:

(2a-1) To determine whether microcystin toxin is transferred to sediments via zebra mussel biodeposition (selective filtration and rejection as pseudofeces) in western Lake Erie and in microcosm experiments.

(2a-2) To determine whether microcystin is accumulated in zebra mussels and amphipods from exposure to toxic *Microcystis* or microcystin-contaminated pseudofeces.

(2a-3) To determine whether microcystin is accumulated in round gobies from feeding on microcystin-contaminated amphipods and zebra mussels or from direct exposure to toxic *Microcystis*.

To date, we have completed objectives 2a-1 and 2a-2. The experiments relating to objective 2a-3 are completed but samples are still being analyzed for microcystin content at Wright State University.

MATERIALS

Organisms

Zebra mussels, Amphipods and Round Gobies

Zebra mussels (*Dreissena polymorpha*) were collected by SCUBA divers from rocky substrates surrounding the field station (F. T. Stone Laboratory, Put-in-Bay, OH, Lake Erie). Amphipods (*Echinogammarus ischnus*) were collected by rinsing them off of zebra mussel covered rocks from the littoral zone around the field station. Round gobies (*Neogobius melanostomus*) were collected using a 50 ft bag seine from the same littoral areas. All were held in tanks containing filtered Lake Erie water at ambient temperature for a 24 h acclimation period prior to beginning experiments.

Cyanobacteria and algae

In early experiments (1996), laboratory cultured, freeze-dried *Microcystis aeruginosa* strain UV-027 (producing mainly microcystin-RR) was provided by Wayne Carmichael's Laboratory at Wright State University, Dayton, Ohio. In 1997 and 1998 experiments with zebra mussels, amphipods and gobies, a cultured Lake Erie strain of *M. aeruginosa* (LE-3), producing mainly microcystin-LR, was provided by Dr. Carmichael. Cultures were grown in modified BG-11 media as described in Objective 1b of this report. Freeze-dried cells were re-suspended in filtered lake water prior to use in experiments and supplies renewed as needed during the experiments. Live mixed species of Lake Erie cyanobacteria, consisting primarily of *M. aeruginosa*, were collected near Stone Laboratory, Put-in-Bay, Ohio, using vertical hauls of a 49 μ m mesh zooplankton net. Green algae used in experiments were either included in the natural mixed populations from lake water or from monocultures of

Chlamydomonas reinhardtii and *Chlorella vulgaris* raised in amended Bold's Basal medium (Nichols and Bold, 1965).

METHODS

Statistical Analyses

Experimental results were analyzed using SAS software, ANOVA, General Linear Model procedures to test for significance of effects of experimental parameters (time and treatment) and their interactions in nested models of the response variables (microcystin content or production of feces/pseudofeces).

Microcystin analysis

Analyses of microcystin levels in animal tissues, water and feces/pseudofeces from the experiments and field-collected material were performed at Wright State University, Dayton, Ohio. Microcystins were detected using ELISA (Enzyme-Linked Immunosorbent Assay) according to the methods of Chu, *et al.* (1989, 1990) as modified by An and Carmichael (1994), also described in Objective 1b of this report.

Experiments

Objective 2a-1. Sedimentation traps containing live zebra mussels, or no mussels, were placed in Hatchery Bay, western Lake Erie during the summer 1996 before and during periods when cyanobacteria were verified to be present by water sampling. Zebra mussels from an area equal to the screen support area of the sediment trap (133 cm²) were scraped from rocks at the prime sampling area by South Bass Island. Thus the numbers, size distribution, and developmental stage of mussels in the traps matched that in the field surrounding the traps. Sediments/biodeposited materials were collected from the traps several times throughout the summer and analyzed for microcystin levels and amount of sediment. Biodeposited materials (feces/pseudofeces) were also collected for toxin analysis during microcosm experiments to determine both the toxicity of the materials and any change in the rate of pseudofeces production in response to levels of microcystin which were higher than those naturally present in Lake Erie at that time.

To determine whether zebra mussels were filtering and/or selectively rejecting *Microcystis*, we fed zebra mussels different mixtures of toxic *Microcystis* and natural seston collected from Lake Erie while they were held in a flow-through apparatus described below (Figure 7). We then compared removal rates with those of zebra mussels fed a mixture of cultured green algae. The amount (as percent biomass) of each algal component removed or left by groups of 30 zebra mussels was determined by collecting and counting samples of the algae mixtures initially and after allowing the mussels to filter for 24 h.

Objective 2a-2. We measured the accumulation of microcystins in zebra mussels fed different concentrations of a lab-cultured strain of *Microcystis aeruginosa* relative to controls fed either green algae, a mixture of green algae and *M. aeruginosa*, or lake-collected mixed cyanobacteria in flow-through microcosms (Figure 7). We then exposed amphipods to either treatment water from zebra mussel experiments or to zebra mussel biodeposited materials (pseudofeces and feces) from those treatments. Amphipods were observed to eat the biodeposited materials provided during each 24 h period. Amphipods were sacrificed for

determining microcystin accumulation after 72 hours.

Objective 2a-3. We exposed amphipods and zebra mussels to the toxic Lake Erie strain of *Microcystis* in static containers containing 2 L of filtered Lake Erie water for a 24 h period prior to feeding them to round gobies (10 each to each goby every 24h for 7 days). Amphipods and zebra mussels were sampled for microcystin analysis to determine amounts being fed to gobies. For this long-term (7 d) exposure, we held gobies in 4 L containers (1 goby per container) with a continuous flow-through of filtered Lake Erie water. We fed a control group uncontaminated amphipods and zebra mussels. All gobies were sacrificed for microcystin analysis at the end of 7 days.

In a separate experiment gobies were also exposed directly to waterborne toxic *Microcystis* in static 4 L containers while being fed uncontaminated food. These gobies were sacrificed for toxin analysis at 0, 2, 6, 18, 24, 48 and 72 h. All experiments were conducted at ambient temperature (24 -26°C) under subdued natural lighting and with constant aeration. Fish were prevented from seeing outside of their tanks by dark plastic shields.

RESULTS

Objective 2a-1: Zebra mussels delivered microcystin-contaminated seston to the benthos in Lake Erie at higher rates than in no-mussel controls simply by producing more contaminated sediment (Figure 8). While the concentration of toxin per unit of sediment remained similar with or without mussels present, the amount of feces + pseudofeces + seston produced by zebra mussels was higher than background deposition by as much as 68%, and therefore resulted in the deposition of up to 49% greater amounts of microcystin-contaminated sediment per unit area than occurred without zebra mussels (Figure 8). The maximum concentration of microcystin in the lake water (0.54 ng/l) occurred during the same period (mid-August) that the zebra mussel-facilitated delivery of material to the benthos was highest, and also coincided with the peak period of cyanophyte abundance, but not the time of peak seston concentration (see Objective 6). Furthermore, when ZMs were fed Lake Erie cyanobacteria in microcosms they produced more pseudofeces than they did when fed green algae (Figures 9 and 10) and the toxicity of feces + pseudofeces was significantly higher (Figures 11 and 12).

When zebra mussels were fed the different mixtures of toxic *Microcystis* and green algae (Treatments A-D in Figure 13), the chlorophytes were always the phytoplankton group whose biomass was reduced the most after 24 h of filtration by zebra mussels; the chrysophytes and cryptophytes remained relatively unchanged, but the cyanophytes usually became the dominant portion of the biomass left in the treatment water. This was particularly the case for surface water from Lake Erie (Treatment B), which was the natural source of food for the zebra mussels.

Objective 2a-2: Zebra mussels and amphipods tended to accumulate more microcystin upon exposure to higher concentrations of toxic cyanobacteria in lake water or from pseudofeces, such as might be present in a heavy bloom, than they did at lower concentrations (Figures 14-17). However, zebra mussels rejected a great deal of toxic cyanobacteria in biodeposited materials (Figures 11 and 12) resulting in an order of magnitude higher concentrations of

microcystin in pseudofeces than in body tissues and may reflect the mechanism by which the tissue levels of microcystin fluctuated in zebra mussels (Figures 14 and 15). The accumulation of toxin in the tissues of the zebra mussels was dependent on the treatment and was significantly related to an interaction of time and treatment ($p < 0.05$). Accumulation of microcystin in amphipod tissues was significantly related to treatment as well ($p < 0.05$) with feeding on more highly contaminated pseudofeces resulting in a higher accumulation of toxin (Figures 16 and 17).

Objective 2a-3: While the samples for this section are still under analysis for microcystin content we were able to perform some very rough calculations of microcystin uptake and depuration rate constants (Figures 18 and 19) in zebra mussels from the results of experiments described above (objective 2a-2, Figures 14 and 15). The calculations we performed are only for the conditions under which we ran the experiment (i.e. at these temperatures, algal abundance, pH, etc.) and are based on a passive diffusion mode of action for a toxin (Landrum and Lydy 1991). Microcystin is actually taken up by facilitated transport, so these are preliminary estimates of the bioconcentration factor (BCF) and depuration rates. We calculated a BCF of 12,435 for an environmental concentration of $13 \mu\text{g/l}$ microcystin which would not be unreasonable for a heavy bloom (Figure 18). We calculated the half-life ($T_{1/2}$) for depuration of microcystin from zebra mussels in clean water to be 12.5 days.

DISCUSSION

Lake Erie cyanobacteria do contain highly toxic microcystins (see Objective 1b) and zebra mussels are depositing microcystin-contaminated seston onto the benthos at high rates. Mussels and amphipods show accumulation of low levels of microcystins in their body tissues. Zebra mussels and amphipods accumulated more microcystin upon exposure to high concentrations of microcystin-containing cyanobacteria, such as might be present in a heavier bloom than we observed in 1996.

More significantly, zebra mussels rejected a great deal of toxic cyanobacteria in pseudofeces and feces, resulting in one order of magnitude higher concentrations of microcystin in these materials than in body tissues. Rejection of toxic *Microcystis* in pseudofeces is probably the mechanism by which resulting levels of toxin in the zebra mussels tended to stabilize and then decrease over time. We note that the toxin levels in the pseudofeces from the highest three exposure mixtures all followed a pattern of initial increase then leveling off, indicating that mussels responded to the presence of the toxin by increasing their production of pseudofeces, perhaps maintaining a steady-state concentration as long as environmental concentrations remain high. Zebra mussels consistently produced more pseudofeces when toxin levels in the algal/cyanobacteria feeding mixtures were high in the laboratory exposures, indicating that this might also be the case in the field during bloom conditions. Toxin levels of the pseudofeces and amounts produced were much higher for the higher exposure levels in the algae/cyanobacteria feeding mixtures regardless of whether the cyanobacteria were cultured, freeze-dried, and non-native *Microcystis* or live *Microcystis* concentrated from Lake Erie. One major difference between the cultured and field-collected *Microcystis* was the formation of large colonies in the latter, while cultures were composed of single cells. Our results suggest that the toxicity of the material may be more important to stimulating the production of pseudofeces than is the size and shape of colonies, or the alga's

occurrence as single cells. Microcystin contents in those pseudofeces were significantly higher than those produced from exposure to green algae suspended in filtered Lake Erie water which contained low levels of dissolved microcystin. These results all support the hypothesis that zebra mussels are providing a route of transfer for microcystins to the benthos.

Amphipods accumulated microcystin from feeding on contaminated pseudofeces which were collected from the zebra mussels exposed to microcystin contaminated cyanobacteria. Levels of microcystin in the amphipods were higher in those fed the more toxic pseudofeces, regardless of the source of toxin (cultured *M. aeruginosa* or Lake Erie live material). Tissue levels of microcystin in amphipods were roughly ten times higher than tissue levels in zebra mussels on a per weight basis indicating that a kind of biomagnification of microcystin is occurring when amphipods feed on toxic pseudofeces. A fish predator eating equal weights of zebra mussels or amphipods would then receive a much greater toxin dose from amphipods. More fish species eat amphipods than zebra mussels in Lake Erie, but many fish species eat both. Hence, zebra mussels are providing a novel route for trophic transfer of microcystins to the benthic community and this provides support of our hypothesis that they transfer microcystin to upper trophic levels through the food web. When analysis of our fish monitoring samples and our uptake experiments in zebra mussels and fish is completed, we should be able to calculate more accurate bioconcentration factors for exposure to both microcystin-contaminated water and food which will be useful in making predictions about community effects of toxic *Microcystis* in Lake Erie and other aquatic systems.

Objective 2b. Determine the cytotoxicity and genotoxicity of microcystins (LR, YR) commonly associated with *Microcystis aeruginosa* to human liver cell lines (HepG2).

MacIsaac et al., University of Windsor.

METHODS

Cytotoxicity and genotoxicity of two of the most common and toxic microcystins, LR and YR, were determined using human liver cell assays. The cytotoxicity test involved exposure of the cell line to Neutral Red dye, the uptake of which indicates healthy cells (Ali et al. 1994). Briefly, a 96-well tissue culture microplate was treated with 0.2 ml of complete cell growth medium, containing approximately 3×10^4 HepG2 cells. The microplate was incubated at 37°C for 48 h prior the treatment with microcystin. Cell cultures were obtained by Dr. K. Adeli, University of Windsor.

Microcystin-LR and -YR crystals were obtained from Dr. W. Carmichael, Wright State University, or purchased from Calbiochem/Novabiochem Inc (microcystin-YR). Crystals were dissolved into complete medium, and then sonified to ensure that all crystals had dissolved. This medium was used to treat cell cultures in a dilution series (1000, 750, 500, 250, 125, 62.5, 31.25, and $15.625 \mu\text{g}\cdot\text{mL}^{-1}$). Treated cells were incubated for 24 hours at 37°C.

The treatment medium was removed and the cells covered with complete medium containing Neutral Red ($50 \text{ mg}\cdot\text{L}^{-1}$ complete medium), and left to incubate for 1 h. The microplate was washed with a fixative (4% v/v formaldehyde, 1% CaCl_2 in distilled water),

following which Neutral Red contained in cells was extracted by addition of 0.2 mL 1% acetic acid/50% ethanol in water to each cell. After agitation, the absorbance of cells was read with a microplate reader outfitted with a 540 nm filter.

Positive and negative control controls were conducted along with the Neutral Red exposure experiment. The negative control consisted of untreated cells, and reflected normal growth and uptake of dye by healthy HepG2 cells. The positive control was treated, before dye exposure, with digitonin to kill all of the cells in the well. These controls set the upper and lower range, respectively, that test absorbances should fall within. To determine the effect that various concentrations had on the HepG2 cells, mean absorbance for each test concentration was calculated. From this value the mean positive control was subtracted before the result was divided by the negative control and multiplied by 100. All assessments were, therefore, relative to controls. LC_{50} values were calculated using nonlinear regression in SigmaPlot software.

A second series of experiments (alkaline unwinding assay for single-strand breaks) were conducted to assess the genotoxic effects of microcystin LR and YR. Briefly, these assessments consisted of measuring the amount of double-stranded DNA as a fraction of total DNA in cells exposed to microcystins. Because cells exposed to genotoxic compounds suffer from an elevated incidence of DNA breaks, experiments were conducted to determine whether microcystin-exposed cells exhibited an enhanced concentration of single-stranded DNA and a lower ratio of double stranded to total DNA.

HepG2 cells were initially treated with tritiated thymidine to label DNA. Cells were then exposed to different concentrations of microcystin-LR and -YR. Under alkaline conditions, DNA unwinds at breaks induced by the toxin, with the amount of damage (single strand formation) being directly proportional to the degree of toxicity. An F-value was calculated based on the ratio of double- to total DNA. Control tests were run in the absence of microcystin.

RESULTS

HepG2 cells grown in complete medium were used to detect cytotoxicity of microcystin-LR and -YR. Both microcystins showed a decrease in Neutral Red uptake at concentrations $>200 \mu\text{g}\cdot\text{mL}^{-1}$, with microcystin-YR appearing to be more toxic (Figure 20). Once cells began to be affected by the microcystin, there was an exponential decrease in Neutral Red uptake (ie. cells died). Microcystin-LR and -YR LC_{50} estimates for HepG2 cells were 500 and $250 \mu\text{g}\cdot\text{mL}^{-1}$, respectively.

Genotoxicity was assessed by surveying the incidence of single-stranded DNA in HepG2 cells exposed to microcystins. F-values approaching 1.0 indicate a low incidence of single-stranded DNA in exposed cells, and low likelihood of genotoxicity. Relative to controls, neither microcystin appeared to induce genotoxicity in exposed human liver cell lines (Figure 21). F-values appeared to increase with increasing concentration of both microcystins, though values for microcystin-LR were consistently above those (ie. it was less toxic) of microcystin-YR.

Because genotoxicity was not detected at concentrations anywhere near those possible in Lake Erie (ie. $<10^9$), the third test in our toxicological series -- DNA repair -- was not conducted.

DISCUSSION

During the summers of 1995 and 1996, a bloom of *Microcystis aeruginosa* developed in the western basin of Lake Erie. These blooms reached a density of <20 ng L⁻¹ during the summer of 1996 (Babcock-Jackson, personal communication). Associated with *Microcystis aeruginosa* are a family of 32 microcystins (Carmichael, 1996), of which microcystin-LR and -YR are two of the most toxic (Carmichael, 1997). Microcystin-LR is extremely stable in water, resistant to pH extremes and temperature up to 300°C, though it breaks down in the presence of sunlight (Jones and Orr, 1994). Studies have shown that the toxin is taken up through the intestines and transported mainly to the liver, however both intestinal and kidney cells may show signs of toxic stress (Tencalla et al., 1994; Lambert et al., 1994; DeVries et al., 1993). Microcystin also affects tumor inhibitors and promoters (Lambert et al., 1994; Nishiwaki-Matsushima et al., 1992). Microcystin seems to have an ability equivalent to benzene in terms of chromosome breakage. Also, Nishiwaki-Matsushima et al. (1992) reported that microcystin is one of the most powerful liver tumor promoters.

Microcystin-LR and microcystin-YR were produced by *M. aeruginosa* in western Lake Erie (W. Carmichael, personal communication). To determine whether humans could be adversely affected by exposure to these compounds in western Lake Erie, we determined whether or not they were capable of producing cytotoxic or genotoxic effects on a human liver cell line (HepG2). We exposed a human liver cell line to microcystin-YR and -LR to determine whether or not there was a possible cause for concern about human health. Neither of the tests conducted here (cytotoxicity and genotoxicity) revealed an imminent problem with human exposure to microcystins in Lake Erie. For example, based on a toxicity threshold estimate of 250 $\mu\text{g}\cdot\text{ml}^{-1}$, lake *Microcystis* concentration of 5 ng L⁻¹, and dry mass to microcystin conversion value of 10^{-3} , we estimate that lake concentrations of toxin are $\sim 5 \times 10^{-10}$ times below the human liver toxicity threshold. This value should be treated cautiously, however, owing to the nature of the estimates and likelihood of appreciable spatial and temporal variation in *Microcystis* (and microcystin) concentrations in the lake. A second caveat also applies to these findings. Our studies addressed only acute cyto- and genotoxicity. It is possible that microcystins could affect wildlife and/or humans in other, less detectable ways. For example, subtle but significant behavioral shifts could result from chemical interference, while chronic exposure to lower concentrations of microcystins in the lake could affect organisms in a manner not assessed by these studies. Nevertheless, our results indicate that lake concentrations of microcystins are unlikely to produce cytotoxic or genotoxic effects in humans.

In the western basin of Lake Erie, exposure routes for humans and wildlife most likely would be oral or dermal (during recreational use), although contaminated drinking water is another possibility (Carmichael, 1996). Assuming that someone fell into the lake during a bloom condition, it is possible to determine cell densities that would be required to harm humans. The cell density required then can be calculated by dividing the LC₅₀ determined divided by the concentration of microcystin within the cells and assuming that 0.1 L was

swallowed. Using the cellular concentration for microcystin-LR determined by Jungmann and Benndorf (1994) of 1.16 to 4.3 μg microcystin-LR/mg *Microcystis*, it is likely that lake concentrations in 1996 would have not posed a threat to humans. Given that the 1996 bloom reached a maximum concentration of 20 ng/L (L. Babcock-Jackson, personal communication), it appears that humans would not be exposed to concentrations capable of causing acute liver damage. However, the Lake Erie (LE-3) strain cultured by W.W. Carmichael showed a toxin level of 1.9-2.2 μg microcystin/mg of *Microcystis* indicating that microcystin levels could get that high in the Lake and in fact did so in 1998. Given that microcystin-LR is shown to promote the formation of tumors (Nishikawa-Matsushima et al., 1992), and is also linked to increased liver cancer rates in China where microcystins are regularly found in drinking water (Yu, 1989; Chen and Yu, 1996), chronic exposure at low concentrations also needs to be considered.

Upon treating the human cell line with Neutral Red, percent uptake values ranged between 107 to -4.4 and 100.2 to -4.0 for microcystin-LR and -YR, respectively. These results appear to indicate an analytical problem since percentages >100 and <0 occurred. However, these results may have occurred if cell densities varied across test wells. We feel that this is unlikely since each test was replicated 4 times, and because treatments were randomly assigned. A second possibility for this result relates to the pathway by which the toxin affects the liver cells, and the properties of the Neutral Red assay. The Neutral Red assay is based on the principle that live cells actively take up dye when they are extracting essential nutrients from the medium. At the same time, some natural uptake occurs *via* diffusion, and this is why some control HepG2 cells were killed with digitonin. The mean absorbance of the digitonin killed cells was subtracted from the absorbance of the test wells to compensate for this diffusion into dead cells. In HepG2 cells that were treated at high microcystin concentrations, the absorbance was lower than that of the digitonin treated cells. Digitonin kills the HepG2 cells but does not interfere with their adhesion to the microplate well. However, microcystin is believed to interfere with the reverse phosphorylation of intermediate filaments; this may have caused the HepG2 cells to become dislodged from the microplate walls (DeVries et al., 1993). Therefore, cell loss may have occurred even though care was taken to ensure that they were not removed when the medium was washed and changed. The increase in uptake of Neutral Red to greater than 100% of that of the control may be explained by a secondary property of the toxin: that microcystins may act as tumor promoters or initiators. Nishiwaki-Matsushima et al. (1992) found that microcystin-LR promoted the formation of tumor loci within rats at concentrations of $10 \mu\text{g}\cdot\text{kg}^{-1}$. This could occur at the lower concentrations tested ($10 \mu\text{g}\cdot\text{mL}^{-1}$ or less), particularly for microcystin-LR.

Microcystin-LR and -YR had LC_{50} values of 500 and $250 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. Both toxins exhibited sharp dose-responses, with the majority of the lethal dose applied before any measurable effect was evident (Carmichael, 1996). The dose-response for microcystin-YR also appeared sharper than that of -LR.

GOAL II. Assess the Ecological Impacts of Microcystis Blooms on the Lake Erie Ecosystem.

Hypothesis 3. Toxic Microcystis inhibits the feeding activities of zebra mussels, altering the impact of zebra mussels on the phytoplankton community.

Objective 3. Measure the relative importance of level of toxicity and colony size on the feeding inhibition of zebra mussels.

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INTRODUCTION

A series of replicated (4 experimental beakers with 4 mussels in each 2-liter beaker plus 3 control beakers), traditional feeding experiments was done with natural seston from Lake Erie and Saginaw Bay, Lake Huron, and with pure cultures of toxic and non-toxic strains of *Microcystis aeruginosa*, including Carmichael's LE-3 isolate from the Lake Erie bloom. These experiments were designed in response to earlier work we had done on Saginaw Bay and on Lake Erie during the extensive *Microcystis* bloom of September 1995. We show some of these results for comparison. At the same time as the feeding experiments, video observations were made with our "Critter Cam" to evaluate the mussels' behavioral response to the various algae.

MATERIALS, METHODS AND RESULTS

Feeding experiments with natural seston

Filtering rates were expressed in terms of amount of chlorophyll removed (F) from the water column as well as that assimilated (F_A). The latter filtration variable is the most important one in terms of the mussels using the algae. We used a size-fractionated method to measure chlorophyll concentration and feeding rate in the $> 53 \mu\text{m}$ size fraction, $< 53 \mu\text{m}$ size fraction, and total (sum) of size fractions. This allowed us to distinguish between filtration on *Microcystis* and other algae because *Microcystis* usually dominated the $> 53 \mu\text{m}$ size fraction because of *Microcystis*' tendency to form colonies of hundreds or thousands of cells per colony. We also expected that pseudofeces produced by the mussels would end up in the $> 53 \mu\text{m}$ size fraction.

Experiments with natural seston included experiments in which *Rhodomonas* or *Cryptomonas* — small (5 and 8 μm) highly digestible and nutritious cryptophytes — were added to seston or were added to seston $> 53 \mu\text{m}$ (produced by screening) to create a mixture in which the small cryptophyte was found in the $< 53 \mu\text{m}$ fraction and *Microcystis* was found in the $> 53 \mu\text{m}$ fraction (Table 2). F_A on the $> 53 \mu\text{m}$ fraction, the *Microcystis* fraction, was very low or zero. In all experiments, F_A on the $< 53 \mu\text{m}$ fraction was quite high for experiments where *Cryptomonas* or *Rhodomonas* was added to the seston. F_A for the $< 53 \mu\text{m}$ fraction of the LE seston experiment (21 September 1995) was somewhat lower than that for the LE seston plus *Rhodomonas* (22 September 1995), and F_A for the $< 53 \mu\text{m}$ fraction of SB seston alone (11 July 1995) was essentially zero, in contrast to the high value in the "SB seston $> 53 \mu\text{m}$ plus *Rhodomonas*" experiment. Filtering rates on the cryptophytes in many of these experiments approached values seen for these algae when offered alone (Table 3).

Feeding experiments with pure cultures

Experiments with pure cultures of *Microcystis*, *Cryptomonas*, and mixtures of *Microcystis* and *Cryptomonas* (Table 3) showed that the inhibition effect of *Microcystis* was size and strain specific. Both CCAP1450/11 and PCC 7820 strains were ingested and filtered at high rates in both size classes as well as the total (sum) of both fractions. Note the very high carbon assimilation rates (39 - 43 % d⁻¹), which are higher than values found in the literature for any algae but are similar to results we obtained with *Cryptomonas* (Table 3). Note most of the biomass of these *Microcystis* strains was found in the < 53 µm size category despite CCAP 1450/11 being described as retaining its colonial form in culture. Tests done by Wayne Carmichael to determine microcystin content of these 2 strains showed that CCAP 1450/11 was non-toxic and PCC 7820, a known toxic strain, was highly toxic (Table 3). The somewhat lower filtering rate for the total of the size fractions for the CCAP strain compared to the PCC strain resulted from its very high concentration, which is considerably above the incipient limiting concentration. The high negative filtering rates seen in this and some other experiments for the > 53 µm fraction is caused by pseudofeces production and low initial concentration of algae in this size fraction. Experiments 3A, 3B, and 4B in Table 3 show that when the Lake Erie isolate alone is offered alone the overall (total) F_A is very low or zero. Experiments 3A and 4A are very much alike in terms of total concentration of chlorophyll and distribution of the chlorophyll in the < 53 µm size class. This distribution was created by shaking the culture to break up the *Microcystis* into small colonies. A somewhat higher F_A (9.08) was seen for Experiment 3B, which had a lower total chlorophyll concentration evenly distributed between the 2 size classes. We attribute the higher filtering rate to a lower *Microcystis* concentration, which may have exposed the mussels to a lower toxin ingestion rate.

Individual mussel behavior

The observation of mussel behavior was important for understanding the mechanisms that zebra mussels use to cope with *Microcystis*. Four mussels treated and acclimated the same way as the mussels used in the feeding experiments, were each observed for 15 to 30 min to record time budgets for the behaviors shown in Figure 22. In all but one experiment with natural seston (Table 4), the mussels spent little time in behaviors that interrupted filtering, and the dominant non-filtering behavior (DNFB) was pseudofeces expulsion. In the case of the exception, the SB seston experiment of 11 July 1995, the mussels spending a high (46%) but variable time in filtering-interrupting behaviors, primarily siphons open but not creating a filtering current.

The behavior of the mussels (Table 4) reflected the F_A values obtained for the mussels on the < 53 µm size fraction and explained how the mussels cope with large *Microcystis*. High F_A values were correlated with mussels that are filtering most of the time (Table 4), and large *Microcystis* were expelled as loosely consolidated pseudofeces (Figure 23). In experiments with cultured algae (Table 5), F_A is again correlated with time not spent in filtering-interrupting behaviors. In particular, mussels offered the LE strain of *Microcystis* alone—either as small (< 53 µm) or large (> 53 µm) colonies (Experiments 3A, 3B, and 4B)—spent a lot of time in the filtering-interrupting behaviors, with two siphon retraction (TSR) being the DNFB. It is interesting that there appears to be a behavioral carry over effect in mussels first acclimated to *Microcystis* (Experiments 3A and 3B in Tables 3 and 5)

and then briefly acclimated (0.9 h) to *Cryptomonas*. Behavior of mussels in this case was more similar to that of mussels in *Microcystis* than that of mussels long acclimated to *Cryptomonas* (Experiment 4A) both in terms of percent of time in filtering-interrupting behavior and DNFB. Both DNFB and percent of time in filtering-interrupting behaviors for the CCAP and PCC strains were very similar to that for the fully acclimated mussels feeding on *Cryptomonas*.

DISCUSSION AND CONCLUSIONS

Microcystis in both Saginaw Bay and Lake Erie was toxic and occurred as large multicellular colonies > 53 μm . Our experiments showed as long as *Microcystis* colonies were large, the mussels filtered at quite high rates on small edible algae if available. When the LE strain of *Microcystis* was offered alone, F_A was low or zero. The mixture experiments with small sized colonies (< 53 μm) of *Microcystis* and *Cryptomonas* showed *Microcystis* depresses feeding on other algae. Lastly, F_A and behavioral observations showed that the mussel's response was highly strain specific: both the non-toxic CCAP and toxic PCC strains were fed upon at high rates. Because *Microcystis* usually occurred as large colonies in Saginaw Bay and Lake Erie it is unlikely *Microcystis* inhibits feeding of mussels on other algae if this other algae is abundant.

Hypothesis 4. *Microcystis* blooms will decrease growth rate of zebra mussels.

Objective 4. Compare growth rates of zebra mussels in cages for years with and without *Microcystis*.

Babcock-Jackson, Culver, et al.

INTRODUCTION

Occurrences of toxic blooms of *Microcystis* species and other cyanobacteria in Saginaw Bay, Lake Huron and in the western basin of Lake Erie in 1995 and 1996 were associated with regions of high densities of *Dreissena*. Recent results from Johengen and Vanderploeg (Objective 10b) showed that zebra mussels excrete high levels of $\text{NH}_4\text{-N}$ and P in Lake Erie. Heath and Wickstrom have shown that *Microcystis* in enclosure experiments with high densities of zebra mussels were less P-limited than those without zebra mussels (Objective 10a). It is probable that zebra mussels are influencing the return of potentially toxic *Microcystis* through excretion of $\text{NH}_4\text{-N}$ and SRP in amounts and ratios that favor its growth (see objectives 10a and 10b). In objective 2 we showed that zebra mussels and amphipods accumulate the toxin microcystin when exposed to toxic *Microcystis*, thereby providing a potential for transfer of the toxin into the benthic community and upper trophic levels. Assuming that the presence of toxic *Microcystis*, and accumulation of toxins, would have negative energetic effects on the mussels by decreasing consumption (see Objectives 3 and 12) and increasing metabolic costs, we hypothesized that the presence of high concentrations of toxic *Microcystis* would negatively affect the growth of zebra mussels as compared to years when *Microcystis* is low.

METHODS

Zebra mussels were collected by SCUBA divers from Peach Point, South Bass Island, Lake Erie (near Stone Laboratory). Mussels were removed from rocks by severing byssal threads with a scalpel. They were subsequently washed with lake water and separated into groups of 33 mussels consisting of equal numbers size range groups (5 - 25 mm). Each mussel in the group was pre-measured and tagged with a small colored and numbered "bee tag" so that it had a color and number code by which it could be tracked. Several groups of mussels were measured and soft tissues dried (65°C) to obtain a length vs. dry weight regression to be used for estimating initial weights for all mussels of known length collected at the same time for use in the experiment. The rest of the mussel groups were placed in cylindrical plastic mesh cages which were attached to lines at 0.25 or 0.5 m intervals. The cages were labeled with colored beads to indicate their respective depths (or position on the line). Two replicate lines of cages were made and were anchored at a depth approximately 5.0 m below the surface of Lake Erie near Stone Laboratory. Replicate lines were placed approximately 10 m apart (Figure 24). The lines with cages remained out for the duration of the summer with the experiment being repeated for summers of 1995, 1996 and 1997. The shell lengths and soft-tissue dry weights of all of the remaining tagged mussels were measured at the end of each summer to obtain growth rates ($\mu\text{m}/\text{d}$ or $\mu\text{g}/\text{d}$) for each year.

RESULTS AND DISCUSSION

Resulting measures of growth in length added ($\mu\text{m}/\text{d}$) or in tissue dry weight added ($\mu\text{g DW}/\text{d}$) were compared by depth, by year and by depths across years for all cages of mussels remaining at the end of the experimental period using SAS General Linear Models procedures. Due to severe storm events in 1995 and 1996 some of the cages near the surface and at the bottom were lost from one or both of the replicate lines, therefore comparisons of growth with depth between years are limited to cages from the bottom 2 m.

Comparison of Yearly Mean Growth Rates for Depths Combined

Through the comparison of yearly mean growth rates we discovered that mean shell growths for all depths combined were not significantly different between years, but tended to be highest in 1996 (Figure 25). The means of soft tissue growth rates for all depths combined were significantly higher in 1996, while 1995 and 1997 were not significantly different (Figure 26). These results do not support the hypothesized outcome of negative effects on growth in a high *Microcystis* year (1995) as compared to relatively low *Microcystis* years (1996 and 1997). This may be explained by the lack of data for near surface growth rates and that variability in growth at different depths is obscured by this type of analysis. The unfortunate loss of the near surface cages prevented us from including the depths which would have been more likely to show greater impact since *Microcystis* is more likely to be found near the surface. However when we consider that zebra mussels are almost always found near the bottom, and Wu has found that *Microcystis* is often found at or near the bottom (see Objective 11b), our average yearly growth measurements for the bottom 2 m should be realistic.

Comparison of Mean Growth Rates at Different Depths between Years

A comparison of growth rates at different depths for each of the years reveals an overall pattern of decreasing growth rates with increasing depths for both shell and tissue

growth (Figures 27 and 28). Again there are no significant differences in shell growth between years at similar depths (Figure 27). Our most significant findings can be illustrated by the comparison of soft tissue growth between 3 and 4 m depth for the three years (Figure 28). Here a significantly different (year and depth are significant at $p < 0.01$) and lower growth rate is observed for the high *Microcystis* year (1995) as compared to low *Microcystis* years (1996 and 1997). Whether the lower tissue growth rate at this depth range is due to increased energy demands on the mussels caused by the presence of toxic *Microcystis* alone cannot be absolutely determined since many factors in the field cannot be controlled between years.

A combination of factors such as temperature, food quality and availability, and reproductive status can affect seasonal variations in metabolic costs for zebra mussels (Stoeckmann and Garton, 1997) in any given year. Thus it is quite difficult to sort out which effects, or combination of effects, may have reduced tissue growth rate in our 3 - 4 m depth mussels in 1995. However, it is quite likely that a large proportion of overall consumption by zebra mussels would be lost to pseudofeces when toxic *Microcystis* is present and assimilation efficiency would be reduced (experiments to measure the relationship of assimilation efficiency to the presence of toxic *Microcystis* were conducted in 1997 and samples have yet to be analyzed). The mussels would then have less energy available to direct toward somatic growth and reproduction resulting in lower growth rates and possibly lower reproduction. In addition, poor food quality and increased demands on the gill to sort out and expel the "undesirable" colonies of *Microcystis* would result in higher metabolic costs (oxygen consumption). It is therefore reasonable to attribute, at least in part, the decrease in growth rate of zebra mussel soft tissues to the presence of a large bloom of toxic *Microcystis* in western Lake Erie in 1995. It is also reasonable to hypothesize that there may have been more negative effects on some size-classes than others, or on overall recruitment success of the zebra mussels. We are currently exploring these possibilities using the data we have accumulated on growth rates of different size classes of mussels and abundances of *Dreissena veligers*.

GOAL III. Monitor the Occurrence of Blooms of *Microcystis* and Other Toxic Algae.

Hypothesis 5. Toxic cyanobacterial blooms occurred regularly in the 1970's and 1980s but disappeared in the early 1990s until 1995.

Objective 5. Determine the historical occurrence and duration of cyanobacterial blooms in the western basin of Lake Erie.

Babcock-Jackson and Culver.

INTRODUCTION

It was our intention to re-analyze historical data on phytoplankton from our own collections and historical literature. The comparison of multiple sources of data collected and reported in different manners has proven to be a major obstacle and has prevented us from entirely fulfilling the objective. However, we have been able to gather sufficient data to give a general idea about recent historical trends in cyanophyte abundances in Lake Erie. It is difficult to determine just how prevalent *Microcystis* was before 1990 since the few sources we found either did not mention it at all or identified it as *Anacystis* and *Microcystis*. Since these genera are now considered one and the same (Taft and Taft, 1971), we reported both of them when found in the same data set. In other cases cyanophytes were lumped together without identification of all genera present, or with only those contributing greater than 5% of the biomass mentioned.

RESULTS AND DISCUSSION

Cyanophytes did not become a major component in the phytoplankton community until the late 1950's (Table 6) when nitrogen, and phosphorus in particular, were significantly higher than before 1950 (Verduin, 1964; Davis, 1964). It appears that cyanophytes then became a major component of the community in summer and fall from at least 1970 through the mid-1980's. However, it is difficult to determine exactly how much *Microcystis* contributed to the cyanophyte biomass since the data are rather sparse, but it is apparent that *Aphanizomenon* and *Oscillatoria* dominated in the years for which we have data in the 1970's. Presuming that *Anacystis* is the same as *Microcystis* in the data for the 1980's, we can see that it became the dominant cyanophyte along with a good mix of others. Note that in 1984 there were no zebra mussels present in Lake Erie. Further analyses are needed to determine the percentage of phytoplankton biomass that was *Microcystis* in the years beyond 1986 when zebra mussels were introduced to the system. Makarewicz (1993) reviewed changes in phytoplankton biomass and species composition in Lake Erie that occurred from 1983 to 1987 with a comparison to 1970. He found a general reduction in biomass and a shift towards more mesotrophic species assemblages, reporting that *Oscillatoria* and *Aphanizomenon* biomasses in the western basin were much greater than the central and eastern basins. The relative abundance of all cyanophytes in the western basin on average for 1983 to 1987 was 6.86 % of the biovolume of phytoplankton. Makarewicz observed that *Aphanizomenon* had declined in the western basin during the 1980's by 92% as compared with 1970. No major blooms of any cyanophyte were observed in the western basin from 1990 until 1995 when the bloom of *Microcystis* occurred. However, if we compare the maximum *Microcystis aeruginosa* observed in 1995 (8,400 cells/ml) by Babcock-Jackson is

spatial and temporal highs in cyanophyte biomass and the relative ratios of N:P in 1996.

RESULTS AND DISCUSSION

Our analyses of phytoplankton distributions, biomass, chlorophyll *a*, and nutrient relationships support our hypothesis that spatial distributions of phytoplankton, especially cyanophytes, are influenced by chemical variables. The abundance of cyanophytes for the summer of 1996 never reached what we could consider to be a "bloom" condition as compared with 1995 and 1998 in which highly apparent surface blooms did occur. The most likely factor which prevented a "bloom" from occurring that summer, and the following summer, was that water temperatures never reached sustained highs ($>24^{\circ}\text{C}$) for an extended period which coincided with relatively calm weather conditions. This condition has been one most often related to *Microcystis* blooms after appropriate nutrient levels to support growth are already present (Tilman et al., 1986; Pick and Lean, 1987; Fujimoto et al., 1997). Regardless of whether a large scale "bloom" occurred in 1996, highs in cyanophyte biomass relative to other phytoplankton did occur during the months of August and September when higher temperatures are expected and when the TN:TP ratios found at the majority of sites were below 25 (Figures 47 and 48) and TN:SRP ratios for the majority of sites were below 400 (Figures 51 and 52).

The abundances of cyanophytes in June 1996 remained quite low at less than 20% of the total phytoplankton biomass and the TN:TP ratios for nearly all the stations were above 25 (Figure 45), but TN:SRP still remained below 400 (Figure 49). In July we began to see localized highs in cyanophytes (Figure 31). TN:TP for most stations, including some high cyanophyte, remained above 25 (Figure 46), but TN:SRP for the high cyanophyte stations were below 400 (Figure 50). These results indicate that high relative cyanophyte abundances were generally well correlated to $\text{TN:TP} < 25$ or $\text{TN:SRP} < 400$ in the months of August and September (Figures 47, 48, 51 and 52) when water temperatures were higher. High cyanophyte abundances in localized areas in July are not well correlated with $\text{TN:TP} < 25$, but are better correlated with $\text{TN:SRP} < 400$. This may be related to temperature effects or other factors. Further analyses relating water temperatures with cyanophyte abundance are needed. In addition an analysis of the influences of prevailing water currents and relative abundances of zebra mussels to localized highs in N, P and cyanophytes is necessary to further test our hypothesis by extending the possible influences on spatial heterogeneity to physical and biological factors.

Our results have shown support for the well-known hypothesis that cyanophytes will dominate the phytoplankton when N:P ratios are less than 30, or simply when there is enough phosphorus available (Smith, 1982; Pick and Lean, 1987). However, it is more difficult to determine whether zebra mussel mineralization, filtration and/or external loading of nitrogen and phosphorus are better correlated to the majority of stations in the western basin where N:P ratios favor high cyanophyte abundance. Accurate estimates of zebra mussel densities and their excretion and filtration rates and external loading data are needed to assess these factors. We plan to address these issues to some extent under objectives 8, 9 and 10 and in future work.

Objective 7a. Correlate satellite images with the *Microcystis* abundance in surface samples collected for Objective 6.

G. A. Leshkevich, Great Lakes Environmental Research Laboratory, NOAA.

INTRODUCTION

During a *Microcystis* bloom, a change (increase of 3-5%) in surface reflectance caused by volumetric and surface scattering can be detected in NOAA/AVHRR (Advanced Very High Resolution Radiometer) satellite imagery (Leshkevich, et al. 1993). AVHRR channel 1 (visible - 580-680 nm) and channel 2 (near infrared - 720-1000 nm) satellite images were used in this study. Although the AVHRR may not be the optimum sensor to use owing to its broad visible (red) and infrared bandpasses and its relatively coarse spatial resolution (~1.3 km), it has a high temporal resolution (daily coverage) and is available free of charge from the Great Lakes CoastWatch Program, thus making it well suited for monitoring. Moreover, imagery from the SeaWiFS (Sea Viewing Wide Field of View Sensor) was not available at the onset of this project. As an ocean color sensor, the SeaWiFS bandpasses are better positioned for algal bloom detection and although the spatial and temporal resolutions are similar, the cost of near realtime imagery is prohibitive for monitoring.

ACCOMPLISHMENTS

Early in the program, at the request of other researchers who wanted a near realtime, synoptic view of where *Microcystis* was likely to be located when a bloom occurred (for ship sampling and monitoring purposes) a reflectance product was developed and put on the Great Lakes CoastWatch web site (<http://www.glerl.noaa.gov/cw/cw.html>) and was made known to other investigators. The reflectance product is a result of the subtraction of AVHRR channel 2 from channel 1 (see Figures 53 - 55). The radiance or signal in the visible band is a result of volumetric and surface scattering and atmospheric effects (attenuation, scattering, absorption) and path radiance. Theoretically, water absorbs all energy in the near infrared, thus any signal in that band is the result of atmospheric effects. Thus, if the atmospheric signal largely representing Rayleigh scattering and path radiance is subtracted out, the results should represent the signal (reflectance) due to the volumetric and surface scattering. Optimally, a correction should be made for aerosol scattering. Aerosols are highly variable in time and space. As this correction, based on the satellite imagery itself is highly interactive, it is hard to automate. The reflectance images have not been aerosol corrected pending the development of an automated procedure. However, the subtraction algorithm results in a very useable product, especially for monitoring. Since the product is created using the same algorithm, changes (patterns) are easily detected and tracked.

PROBLEMS

Several problems were encountered which affected progress including equipment problems, weather problems, and third party problems. An important part of remote sensing algorithm development is obtaining concurrent measurement of remote sensing reflectance and *Microcystis* abundance or density during a satellite (AVHRR) overpass on a cloud-free day(s). A radiometer (belonging to Judy Budd of Michigan Technological University) initially used to obtain reflectance measurements did not function properly. Measurement data collected by a contractor to the Charleston Services Center (a NOAA National Ocean Service (NOS) office)

have not as yet been received.

Late in the 1997 season, after receiving the Satlantic instrumentation we had ordered, attempts to make measurements were foiled due to high winds. The same situation occurred during the bloom that occurred in August/September 1998. High winds and rough seas precluded deploying instrumentation and only water samples were collected. Although we finally got reflectance measurements late in the season, the bloom was over and the sky conditions were only marginally clear.

FUTURE WORK

Plans for future work include the continuation of the development of an algorithm for bloom detection and monitoring using NOAA/AVHRR and/or the new SeaWiFS satellite sensor. As an ocean color sensor, SeaWiFS should be better able to "identify" biological from non-biological (eg. Sediment) reflectance patterns. In addition, through coincident satellite and surface measurements, some measurement of surface intensity or density can be assigned to the reflectance values.

RADARSAT or ERS-2 Synthetic Aperture Radar (SAR) data coincident with an intense bloom and with surface sampling was not obtained during this study but is still planned. The capability of SAR to detect surface blooms (owing to dampening of surface capillary waves) is a real possibility and should be explored. Can C-band SAR (~5.3 cm) detect this dampening effect or is X-band SAR more useful? Data are needed during a large, intense bloom and await future opportunity.

Objective 7b. Estimate distribution of *Microcystis* blooms in time and space from satellite images and *Microcystis* abundance estimates in samples collected for Objective 6.

J.W. Budd, (Michigan Technological University), A. Beeton (GLERL/NOAA), D. Culver.

INTRODUCTION

Cyanobacteria and coccolithophore blooms are characteristically highly reflective and can be documented through satellite images (Ackleson et al. 1989, Brown & Yoder 1994, Kahru et al. 1993). Multi-sensor satellite reconnaissance of western Lake Erie permitted monitoring of high reflectance regions believed to be surface blooms of the bluegreen alga *Microcystis* over a six year period from 1993-1998. We used three satellite remote sensors in this study, the Advanced Very High Resolution Radiometer (AVHRR) for time series imagery of surface reflectances, Landsat Thematic Mapper (TM) imagery to obtain a chlorophyll map of fine scale features of surface blooms, and the Sea-Viewing Wide Field-of-View Sensor (SeaWiFS) for ocean color imagery collected on a daily basis from September 1997 to October 1998.

THEORY AND METHODS

A satellite detects the total radiance (L_s) from the water (L_w), plus that from the water surface or sunglint (L_g) and from atmospheric or path radiance (L_a). Together these are summarized as

$$L_s(\lambda) = T(I)L_w(I) + T(I)L_g(I) + L_a(I)$$

where T is the transmission coefficient of the atmosphere from the earth to the satellite. The retrieval of remote sensing reflectance (RSR) and pigment (as chlorophyll plus phaeopigments) concentrations from satellite imagery is the result of the scattering of light with surface particles. In fresh water, this is approximately equal to the ratio of backscattered light to absorption ($L_w \approx b_b(\lambda)/a(\lambda)$), where λ is the wavelength (Gordon et al. 1975). The absorption term is small for RSR, so the particles, which are usually inorganic suspended sediment or organic particles (e.g., detritus or living cells), are strongly influenced by backscattering; whereas pigments strongly influence absorption and have little or no effect on backscattering (Gordon et al. 1975). Reflectances can be correlated linearly with scattering ($b_b(\lambda)$) over modest ranges of the data to obtain the concentration of materials in water. For the AVHRR, band 1 at 580-680 nm provides the reflectance, while band 2 (725-1000 nm), which has significantly lower or negligible reflectance in water, is used to estimate and remove the effects of atmospheric aerosols and atmospheric gases (including water vapor and ozone) (Stumpf & Pennock 1989, Stumpf 1992, Bukata et al. 1995). For SeaWiFS and Landsat TM, a ratio of water leaving radiance at two wavelengths ($L_w(\lambda_2)/L_w(\lambda_1)$) is typically used to obtain estimates of pigment abundances, which is inversely proportional to the ratio of the associated in situ absorption coefficients.

Image processing. AVHRR and SeaWiFS images were obtained free of charge through government archiving services within NOAA and NASA. The Landsat TM image was obtained from a commercial vendor at a cost of \$3100/scene. A comparison of the sensor characteristics for the three instruments is given in Table 7. The AVHRR images were obtained from the NOAA CoastWatch Active Access System (NCAAS), an online archiving service supported by the National Environmental Satellite and Data Information Service (NESDIS). We examined six years of AVHRR imagery altogether representing data from the pre-*Microcystis* (1993-1994) and post-*Microcystis* periods (1995-1998). DECCON, a freeware program developed for use with the CoastWatch data sets (Townesley & Stumpf 1996), was used to strip off the header information and to convert the data from 11-bit to 8-bit format for importing into the image processing software. Commercial image processing software packages were used to process the CoastWatch and Landsat images. SeaWiFS data were obtained from the Goddard Distributed Active Archive Center (DAAC) and processed with IDL/SeaDAS. SeaDAS is a freeware image software program developed by NASA for use with the commercial IDL (Interactive Data Language) software.

Our procedure for classifying AVHRR pixels as bloom or water was analogous to the ad hoc procedure outlined in Kahru et al. (1993), although we used average conditions in the western basin to establish thresholds for classification since empirical determinations were not available. Mean reflectance for western Lake Erie on cloud-free days during August through September from 1991-1994 was 2.3%. Thus, pixels with values greater than or equal to 2.3%

were classified as bloom while pixels with values less than 2.3% were classified as water. In their studies, Kahru et al. (1993) determined empirically that the band 1 albedo of cyanobacterial accumulations for the Baltic Sea was between 2.3% and 4.0%, which also agrees well with our findings.

In situ Sampling. Seston and Secchi disc depths (SD) were collected in western Lake Erie on a monthly basis in 1995 at forty-one stations (Figure 29) using standard methods by the Ohio Division of Wildlife in collaboration with the Ohio State University. Note that the regular sampling season ended in mid-August, just prior to the first reports of *Microcystis* by anglers. Linear regressions of the 1995 water quality data against RSR were significant with r^2 coefficients of 0.83 (seston) and 0.81 (SD), respectively ($p < 0.0001$). For seston, the regression equation was $Refl = 0.61 + 0.16(\text{Seston})$. The predicted value of reflectance for pure lake water is 0.61 percent. A 1 mg/L increase in seston yields a 0.16 percent increase in reflectance. Reflectance was linearly related to the reciprocal of Secchi disc depth as $Refl = 0.33 + 2.44*(1/SD)$.

RESULTS

1995 time series. Time series AVHRR images of the changes in reflectances in western Lake Erie illustrate the potential to map algal blooms and estimate the areal extent (Fig. 56). In our original 1995 study of western Lake Erie, the high reflectance regions were detected in AVHRR imagery over a six week period during bloom conditions. Mean reflectances in the Maumee Bay region of western Lake Erie doubled from approximately 2 percent to greater than 4.5 percent (representing an eleven-fold increase in seston abundance: range 1.4 to 16.1 mg/L). By associating satellite reflectances with *Microcystis* cell concentrations, AVHRR imagery provides an inexpensive means of monitoring algal blooms.

RSR increased in the western basin during late August and remained at elevated levels until early October, coincident with the occurrence of the *Microcystis* blooms reported by anglers. The images show the movement of high reflectance waters associated with the blooms from Maumee Bay east to the Bass Islands (see Figure 29, location map). Low reflectances in the western basin (similar to reflectances found in central Lake Erie throughout the year) during the two week period prior to the bloom, suggest that a clearance event may have precipitated the bloom (Figure 56a). Increased reflectances near the Maumee River confluence were first detected on August 26, two days after *Microcystis* was reported by anglers in western Lake Erie. Over the course of two weeks the high reflectance regions spread north and east toward the Bass Islands, nearly doubling in area. After September 27, RSR associated with the *Microcystis* blooms dissipated (Figure 56g-h).

Mean reflectances for Maumee Bay and western Lake Erie more than doubled during the seven week period (Figure 57a). Reflectances on August 11, 21, and 23 believed to be the early states of the bloom were around two percent (seston=1.4 mg/L; SD=4.9 m). Mean reflectance in Maumee Bay increased to over three percent (seston=7.3 mg/L; SD=1.6 m) on August 26, then dropped slightly during the week of August 30 to September 5. Peak mean reflectances of 3.1 percent (seston=7.9 mg/L; SD=1.7 m) and 4.6 percent (seston=16.7 mg/L; SD=0.9 m) for the western basin and Maumee Bay, respectively, were observed on September 15. Average reflectances declined dramatically from September 27 to October 2. Figure 57b

shows the change in the area of the bloom for Maumee Bay from August 26 to October 2. Area estimates follow the same pattern as mean reflectance. Initially on August 26, the estimated bloom area was approximately 300 km², but then dropped to 250 km² during the first week of September. The greatest areal extent of approximately 1000 km² was observed on September 24.

Six year time series. Remote sensing reflectances were highly variable on a daily basis over the six year period (Fig. 58). In the pre-*Microcystis* imagery, RSRs were generally below the threshold of 2.2% for the three month period from August to October, while reflectances were higher during the same period for the other years. Certain general patterns were discernible in the post-*Microcystis* imagery. For example, reflectances generally were higher in late August through September, particularly in the Maumee Bay region of western Lake Erie. RSR increased above threshold in Maumee Bay in early to mid August in 1996-1998 with peak reflectances observed around the third week of September.

Landsat TM and SeaWiFS analyses. While the AVHRR is useful for obtaining daily estimates of seston abundances, it is not possible to differentiate pigment from sediment due to the limited number of visible bands. We used Landsat TM and SeaWiFS (after August 1997) imagery to obtain chlorophyll maps. Figure 59a-b provides a comparison of AVHRR RSR with a Landsat TM false color composite to illustrate differences in the spatial resolution of the two sensors (~1 km² for AVHRR and 30 m² for TM). The AVHRR image is highly "pixelated" in comparison to the Landsat imagery, however, the images show strikingly similar surface turbidity patterns. Comparison of Landsat TM RSR and chlorophyll maps on September 23, 1995 (Figure 59c-d) indicate that the southern basin of western Lake Erie had much higher chlorophyll levels than the turbid northern portion of the basin. The comparison of SeaWiFS RSR and pigments in Figure 59d-e indicates very high levels of turbidity at the confluence of the Detroit River and along the southern shore of western Lake Erie. The SeaWiFS chlorophyll map had low pigment abundances at the mouth of the Detroit River, and high pigments in the southern basin, north of Pelee Island toward Point Pelee, and east of the Bass Islands.

DISCUSSION

A major limitation of ship-based research programs arises when unexpected events of short duration (such as cyanobacteria blooms) preclude *in situ* monitoring. Alternatively, satellite monitoring of novel events, depends upon *in situ* measurements for comparison with the spatial data. AVHRR RSRs provide valuable information about the areal extent and concentration of materials in fresh water, while Landsat TM and SeaWiFS imagery provide actual estimates of pigment abundances. In western Lake Erie, Landsat TM's high cost and low return rate (once in 16 days) and the fact that the SeaWiFS sensor was operational in the fall of 1997 limited the applicability of these sensors in the study.

In addition to western Lake Erie, a separate empirical comparison allowed construction of a time series of *Microcystis* blooms in Saginaw Bay, Lake Huron during 1993-94 (Budd 1997). Reflectance peaks associated with *Microcystis* were observed in Saginaw Bay during 1993 by late June (two months before a bloom appeared in western Lake Erie) through late

September. In Chesapeake Bay, comparison of morning vs. afternoon AVHRR images revealed that surface reflectances decreased in the region of a red tide bloom (*Heterocapsa triquetra*), due to vertical migration of the dinoflagellate (Tyler and Stumpf, 1989). In the latter study, changes in surface abundances were compared with shipboard data. Kahru, et al. (1993) used AVHRR imagery to construct annual distributions of cyanobacterial accumulations over a twelve year period in the Baltic Sea. Gower (1995) applied a nonquantitative method to detect "brightenings" in band 1 data associated with plankton blooms and river plumes off the unproductive British Columbia coast. A combination of AVHRR thermal and visible data was used to investigate blooms when shipboard sampling data were limited.

Because of its temporal frequency and long mission history, AVHRR satellite imagery has potentially widespread application in freshwater and marine studies of algal blooms. These data are useful for enumerating mesoscale processes usually of 100-1000 km². Temporal and synoptic spatial coverage of operational satellites afford good replication, provided cloud cover is minimal. Moreover, on-line archiving services, such as the NOAA CoastWatch Program, provide free access to AVHRR data on a near real-time basis. Additional analyses are planned that will examine long-term trends in water clarity over a 10 year period (1989-1998), prior to the establishment of monitoring programs. In these analyses, seasonal and monthly trends in water clarity will be examined to show seasonal and interannual variations in water clarity following the procedure outlined in Stumpf and Frayer (1997).

Satellite remotely sensed imagery and shipboard monitoring complement each other. The relationship of the *in situ* water quality variables to AVHRR RSR in this case may be specific for western Lake Erie. For example, surface films associated with blue-green algal scums may produce different regressions, depending upon circumstances. In addition, the regression coefficients may change because factors, such as the size of the colonies could alter the specific absorption and backscatter coefficients of suspended materials (Stumpf 1987). For example, the optical grain size of suspended materials may shift to larger sizes in the presence of *Microcystis*. An increase in optical diameter will result in a decrease in reflectances per mass of material due to the lowered surface area per unit volume of the colonies. This may lower the coefficients during bloom events (i.e., lower reflectance for the same concentration).

Remotely sensed data are now routinely used in oceanographic studies to investigate physical and biological processes of ocean waters; however, applications of remote sensing tools for monitoring large lakes is not as commonly employed (Bukata et al. 1995). Advanced ocean color instruments, such as SeaWiFS, will improve our ability to estimate primary productivity and turbidity in large lake and coastal systems. This operational sensor has sampling frequencies analogous to the AVHRR instruments and greatly improved spectral resolution suitable for estimating total suspended solids and phytoplankton abundance in coastal waters.

GOAL IV. Determine the Causes of *Microcystis* Blooms in Lake Erie.

Hypothesis 8. Microcystis blooms have recurred because of increased availability of phosphorus under a condition of increasing nitrogen availability.

Objective 8. Analyze a series of historical samples of algal composition and nutrients collected weekly at a site in Hatchery Bay, South Bass Island.

T. Johengen and H. Vanderploeg (GLERL/NOAA).

INTRODUCTION

A weekly monitoring program for nutrient concentrations, water transparency, and algal composition was established in Hatchery Bay, South Bass Island region of Lake Erie in March 1984 to examine the effects of phosphorus control programs. Year round, continuous weekly sampling was conducted through September 1987. The monitoring program was subsequently re-established in April 1990 to initiate further studies on the effects of zebra mussel colonization. The extended monitoring program was then run continuously between April 1990 and September 12, 1995. Details and results of these studies are described in Holland (1993) and Holland et al. (1995). In summary, the authors concluded that the filtering activities of zebra mussels resulted in a significant decrease in diatom abundance, a significant increase in water transparency, and significantly increased levels of dissolved nutrients including phosphate (SRP), ammonium (NH_4^+), nitrate (NO_3^-), and silica (SiO_2).

Serendipitously, the last two sampling events of this study in September 1995 coincided with the peak of the first *Microcystis* bloom known to have occurred in Lake Erie since the phosphorus control programs first met point-source discharge targets in 1982 and 1983. Consequently the monitoring data from Hatchery Bay provides a detailed record of water quality conditions just prior to the development of the bloom. Additionally, this data set provides a valuable record of water quality conditions throughout the 1990's that can be used in comparison against the conditions in 1995 under which the bloom developed.

RESULTS AND DISCUSSION

Weekly results of water quality conditions in 1995 are presented in Figures 60-62. Results show that concentrations of NH_4^+ and SRP were substantially higher during July and August just prior to the *Microcystis* bloom, and peaked at levels of 80 and 8 $\mu\text{g/l}$ respectively (Fig. 60). NO_3^- concentrations declined continuously throughout the summer months and reached levels below 0.3 mg/l just prior to the bloom (Fig. 61a). Although NO_3^- was still the dominant form of dissolved inorganic nitrogen, $\text{NO}_3^-:\text{NH}_4^+$ ratios were at their annual minimum throughout August and September (Fig. 61b). It has been previously reported by Blomqvist et al. (1994) that higher $\text{NH}_4^+:\text{NO}_3^-$ ratios favor non-nitrogen fixing cyanophytes such as *Microcystis*. Therefore, the combination of elevated SRP levels and changes in the ratios of the dissolved inorganic nitrogen species may have, in part, promoted *Microcystis* growth. In addition, Secchi depth values were also at annual maxima in August 1995 just prior to the bloom (Fig. 62a) and were typically greater than 4 m for most of the summer which approaches the nominal depth of the water column at this site. Lastly, water temperatures during July and August of 1995 were 25 °C or greater (Fig. 62b) and would

again favor the growth of blue-green algae because of their tendency to have higher temperature optimums than other classes of phytoplankton.

In general, water quality conditions in the summer of 1995 were quite favorable for promoting *Microcystis* growth. These results, however, in no way suggest a direct causal relationship between these water quality parameters and the bloom. A more complete analysis of the possible correlation between water quality conditions and *Microcystis* growth can be obtained by comparing conditions in 1995 against those from the previous four years when there was no indications of significant levels of *Microcystis*. Monthly means of water quality conditions in Hatchery Bay during 1991 through 1995 are described in Figures 62-65. It should be noted that in 1995 monitoring data ends in mid-September and that conditions for this month most likely reflect changes brought about by the developing bloom. Seasonal trends for NH_4^+ and SRP are quite similar among all five years and indicate that concentrations typically increase in July and August and actually continue to increase throughout the remainder of the year (Fig. 63). Seasonal trends in NO_3^- and the corresponding $\text{NO}_3^-:\text{NH}_4^+$ ratio were slightly more variable among years, however, conditions in 1993 and 1994 were very similar to those in 1995 (Fig. 64) and suggest no major deviations. Secchi depth values during the summer of 1995 were more variable than during previous summers and actually varied between being the maximum in June and August, and the minimum in July and September (Fig 65a). In general, however, the pattern was consistent among all years and transparency always tended to increase in late summer. Given the shallowness of the water column, variation in light penetration was probably not a major factor in promoting the bloom conditions in 1995. Seasonal trends in water temperature followed a consistent bell-shaped curve in all the years, however, the water temperature in August 1995 was noticeably higher than in any of the other four years (Fig.65b). It is difficult to estimate whether this small difference in temperature could possibly have had any influence on the development of a *Microcystis* bloom in 1995. Furthermore, we do not know to what extent this difference in temperature was observed throughout the western basin.

CONCLUSIONS

In conclusion, water quality conditions in Hatchery Bay in the summer of 1995 were quite favorable for supporting high rates of phytoplankton growth. However, because these conditions were not atypical for this site when compared to the previous four years during which there were no reported findings of elevated *Microcystis* levels, it is doubtful that differences in water quality conditions were a main factor causing the development of the *Microcystis* bloom in 1995. Satellite observations (see objective 7) indicate that the bloom initiated in the western end of the basin near Maumee around August 26th, and continued to develop and spread eastward toward South Bass Island over the next several weeks. At present, we have not compared how well water quality conditions in Hatchery Bay correlate with those from rest of the western basin. Therefore it is difficult to say whether water quality conditions in the region where the bloom initiated played a more significant role, or may have indicated greater differences from previous years. The expanded monitoring program of the present study (see Objective 6) will help determine the amount of spatial variation in water quality conditions within the western basin and provide evidence whether conditions in Hatchery Bay are reflective of those throughout the western basin. Additionally,

variations in external nutrient loading rates are being evaluated under objective 9, and will help to distinguish between the possible influence of external inputs from the major tributary systems draining into the western basin.

Hypothesis 9. Microcystis blooms are a result of increased external loading of phosphorus and nitrogen.

Objective 9. Compare 1995 nutrient loading data (P, N, N:P) with previous years when cyanobacterial blooms (including *Microcystis*) did and did not occur.

Richards, Baker, Babcock-Jackson and Culver.

RESULTS AND DISCUSSION

Richards and Baker (Heidelberg) have provided annual data for discharge loading of N and P from the Maumee and Sandusky Rivers to the western basin of Lake Erie for 1986 through 1995 (Table 8). A monthly comparison of discharges and TN:TP of discharge for those years is provided in Figures 66 and 67. Statistical comparisons were still incomplete at the time of this report, but are underway. Examination of the monthly plots shows that discharge is generally greater from the Maumee (Figure 67) than the Sandusky (Figure 66), but trends of high and low flows are similar for the same months within the same years. However N:P of the discharge shows a slightly different pattern for the two rivers. For instance, in 1995, which is of interest in this study since a *Microcystis* bloom occurred, N:P in the Sandusky River (Figure 68) remained below 30 after the Spring thaw which might suggest that external loading may have influenced conditions to promote cyanophytes. N:P in the Maumee River (Figure 69) fluctuated near 30 with greater variation. Examination of satellite images for 1995 (see Objective 7 of this report) shows that high reflectance values began from Maumee Bay in late August and progressed out into the western basin coinciding with high biomass of cyanophytes (see Objective 6 of this report). The evidence would seem to suggest that external loadings of N and P in ratios that tend to promote cyanophytes was a major factor in promoting the *Microcystis* bloom of 1995 and yet 1986 through 1991 showed similar seasonal trends in the N:P of discharges from the Maumee and Sandusky Rivers and blooms of *Microcystis* did not occur. In fact a historical analysis of loading N:P and N and P relative to annual discharges (Frost, 1997) indicates a trend of rising N:P since 1976, with all years still remaining below 30 (Figures 70 and 71a and b). Further statistical analysis of the loading data are needed, but it appears that external loading alone cannot account for the bloom of *Microcystis* in 1995.

Hypothesis 10. Populations of Microcystis sp. bloom because of nutrient enrichment provided by mineralization activities of zebra mussels.

Objective 10a. Compare the relative levels of phosphorus limitation for Lake Erie phytoplankton communities in areas with and without zebra mussels.

Heath and Wickstrom, Kent State University.

INTRODUCTION

A plausible synthesis of our earlier observations in the western basin of Lake Erie (WB-LE) and Saginaw Bay is that zebra mussels (ZM) are "keystone remineralizers", releasing sufficient P and N to relieve nutrient limitations to algal growth. Although substantial portions of phytoplankton may be grazed, the remaining phytoplankton populations may be able to grow at their "theoretical" maximum growth rates (Heath, et al. 1995). Large inputs of nutrients, especially those with low N:P (Arnott and Vanni 1996), are likely to favor differential growth of cyanobacteria such as *Microcystis* (Sommer 1989). Specifically, we hypothesized that P-limited phytoplankton communities near dense populations of ZM may be altered by large inputs of available P, relieving them from P-limitation, favoring growth of "eutrophic" species (e.g. cyanobacteria), shifting the community especially toward those species inedible to ZM (e.g. *Microcystis*). Based on this hypothesis it would be reasonable to find in the vicinity of dense populations of ZM:

- increased concentrations of available P (detected as "soluble reactive P")
- phytoplankton communities with diminished degrees of P-limitation
- diminished ZM grazing of *Microcystis* in preference to other phytoplankton.

Goals and Objectives

The purpose of this study was to test this hypothesis using both field and laboratory studies. We had three objectives:

(10a-1) To examine WB-LE phytoplankton communities collected from near the surface and from near the bottom in areas with and without dense populations of ZM. We examined these samples for recognized physiological and community indicators of P-limitation: low SRP, high alkaline phosphatase activity (Heath and Cooke 1976), high P-debt (Healey and Hendzel 1980), short phosphate turnover time (Lean, et al. 1987), and low PDI (Lean and Pick 1981).

(10a-2) To compare ZM grazing on naturally occurring populations of *Microcystis* with ZM grazing rate on other naturally co-occurring phytoplankton populations.

(10a-3) To observe differential growth of P-limited and P-luxury axenic cultures of *Microcystis aeruginosa* grown in the lab, placed into dialysis chambers and suspended in Lake Erie at sites adjacent to dense populations of ZM (or not, as controls).

RESULTS

Work completed on Objective 10a-1 supported the hypothesis that ZM released significant quantities of SRP and these relieved phytoplankton populations of P-limitation. We examined regions with and without ZM present, comparing communities in surface waters with communities immediately above ZM beds at the same site. We found that the water chemistry and the physiological characteristics of phytoplankton communities were significantly different between surface and bottom waters but only in regions occupied by dense populations of ZM. A site near Stone Lab in 4.5 m of water, studied extensively by Lisa Babcock-Jackson, was typical of examination of sites occupied by ZM.

Water samples were drawn from 0.5 m below the surface and 0.5 m above the bottom. Figure 72 shows that the SRP content was significantly higher ($p < 0.05$) in the bottom waters over ZM beds; we detected somewhat higher chlorophyll concentrations in the bottom waters. Figure 73 shows the physiological characteristics of phytoplankton communities in these samples. The surface community appeared to be a P-limited community, typical of such communities we have observed during the past ten years in WB-LE. Whereas, the communities sampled from immediately above ZM beds were significantly less P-limited. P-limited communities are characterized by having detectable P-debts, indicative that the phytoplankton could store more P in their "excess storage compartment" if sufficient P was available (Healey and Hendzel 1980). They also show rapid phosphate uptake rates, characteristic of P-limited communities of the Great Lakes (Lean, et al. 1987). The Phosphorus Deficiency Index (PDI) provides a graded response to P-limitation. The PDI is determined as the optimum photosynthetic rate divided by the maximum possible uptake rate of phosphate (P_{opt}/V_{max}). Under P-limitation the P_{opt} declines and the V_{max} of phosphate uptake increases: the lower the PDI, the greater the P-limitation of the community. Under extreme P-limitation PDI can be less than 10; PDI greater than 30 indicates little P-limitation (Lean et al. 1987).

Work completed on Objective 10a-2: Our intention to examine natural communities during *Microcystis* blooms was frustrated by the absence of major blooms of this cyanobacterium during the tenure of the grant. A small bloom of *Microcystis* occurred late in 1996, none in 1997. Experiments conducted in the field using naturally occurring phytoplankton populations indicated that ZM differentially avoid grazing on *Microcystis*, preferentially grazing on other phytoplankton present (e.g. diatoms and chlorophytes). In 1996 we concentrated *Microcystis* by gravity flotation-filtration procedures. Naturally occurring phytoplankton assemblages in 8 L enclosures were amended with this concentrate of naturally occurring *Microcystis* spp. (*aeruginosa* and *ichthyobleba*). The enclosures were then amended with 30 ZM (1.3 - 1.8 cm) and incubated under ambient conditions for 16 hours. Net removal rate (loss due to grazing - increase due to growth) was determined as the difference in *Microcystis* biovolume between experimental and controls per unit time; ZM net grazing rate on all other algae was determined in the same way. Figure 74 shows that while *Microcystis* spp. were not significantly grazed by ZM, significant quantities of "all other algae" were significantly removed. Enclosures containing ZM showed 90 percent of the *Microcystis* biovolume shown in the control (no ZM) enclosures. On the other hand, enclosures containing ZM showed only 15 percent of the biovolume of "other algae" found in the control enclosures.

Work completed on Objective 10a-3: Despite many efforts over the duration of this proposal we were unable to culture *Microcystis aeruginosa* in sufficient quantities to conduct planned experiments. In addition, the Lake Erie *Microcystis* (LE-3) cultured by W.W. Carmichael was not yet available when we were planning to conduct these experiments.

CONCLUSIONS

The most significant finding of this study was that ZM populations in the field appear to affect the degree of P-limitation of the phytoplankton communities. Further studies are warranted to determine whether ZM may relieve phytoplankton in the WB-LE from P-limitation, or whether this is more localized effect, limited only to those populations immediately adjacent them. Such an expanded study was beyond the scope of this present study but is the topic of another study currently underway. A second finding was that populations of ZM appear to behave in the field as in the laboratory, rapidly grazing other taxa of phytoplankton in preference to *Microcystis*. These findings support our hypothesis that ZM can alter the P-limitation of natural populations of phytoplankton and differentially graze *Microcystis*. **This indicates that ZM may be a causative factor in the sudden reappearance of this cyanobacterium in WB-LE.**

Besides providing data in support of this hypothesis, this study provided insight into the next questions that need to be asked in order to understand the impact of zebra mussels at the community and ecosystem levels of organization. Figure 75 shows a model based on the findings of this study. In this model the salient features of this study are provided: (1) ZM differentially graze on different phytoplankton, "edible" and "inedible" algae, (2) ZM release a portion of the algae they graze as dissolved nutrients, e.g. phosphate, (3) increased concentrations of phosphate cause increased growth of P-limited algae. This model has been expressed in a competition model and is currently being investigated by R.T. Heath and Prof. Per Enflo, Dept. Mathematics and Computer Science, Kent State University. **Our analysis to date indicates that grazing by ZM would under most conditions lead to unstable phytoplankton communities which would oscillate between dominance by "edible" and "inedible" algae (e.g. *Microcystis*).** The model also indicates that the most stable appearance of communities dominated by "edible" algae would appear when P-loading is at its lowest; conversely, increase of external P-loading would lead to increased frequency of blooms of "inedible" algae and to increased populations of ZM.

Observation that ZM diminish and/or relieve phytoplankton communities from P-limitation implies that energy flow into phytoplankton may not be as severely constrained as loss of chlorophyll alone may imply. Previous workers have been alarmed at the great decline of phytoplankton standing stocks with the advent of ZM, suggesting a concomitant decline in energy flow into the base of the food web, with less energy available to support higher trophic levels. Our studies suggest that the decline in energy flow may not be so severe as decline in chlorophyll may imply. Rather, the remnant phytoplankton may not be as severely P-limited, and so may be able to photosynthesize more rapidly than under the severe P-limitation evidenced before the ZM. Determining the energy flow to phytoplankton in WB-LE was beyond the scope of this study, but our findings indicate that future studies need to examine this possibility.

Objective 10b. Determine the release of N and P by zebra mussels as a function of changes in the composition and quality of their food source (seston).

T. Johengen and H. Vanderploeg, GLERL/NOAA.

INTRODUCTION

Nutrient excretion experiments were conducted on zebra mussels from Hatchery Bay, Lake Erie in July 1996, September 1996, and June 1997. On each occasion, these experiments were conducted in parallel with experiments on zebra mussels from Saginaw Bay to highlight potential differences in the rates and ratios of nutrient excretion as a function of seston quantity and composition. In addition, during September and July transplant experiments were conducted whereby mussels from one site were incubated in water from the opposite site for between 40 - 120 hours and then excretion rates were re-measured using identical procedures and identical mussels. These transplant experiments were conducted to help verify that observed differences in excretion rates and ratios were the result of differences in food source and not simply differences among two different populations of mussels. It should be noted that western Lake Erie is more eutrophic than Saginaw Bay, and contains higher levels of total phosphorus (annual means approx. 30 $\mu\text{g P/L}$ vs. 20; Johengen, unpublished data) and dissolved phosphorus (annual means approx. 6.0 $\mu\text{g P/L}$ vs. 1.0; Johengen, unpublished. data). Correspondingly, the N:P ratios of seston in Lake Erie tend to be significantly lower than for seston in Saginaw Bay. These underlying differences in eutrophy provided the impetus for comparing zebra mussel excretion rates within these two ecosystems. In addition, shortly after zebra mussels colonized Saginaw Bay, *Microcystis* began to dominate its summer phytoplankton community (Vanderploeg, unpublished data).

METHODS

Nutrient excretion rates were determined by measuring changes in nutrient concentrations during short-term incubations (2-8 hrs) in bottles filled with 0.2 μM filtered site water. Mussels were collected by divers and transported to the laboratory in chilled coolers while covered with damp paper towels. In the lab, mussel shells were thoroughly cleaned of all visible material and then the mussels were acclimated in a 30 L aquarium filled with site water in a temperature controlled room for between 12-18 hours prior to the experiments. The water was exchanged several times during the acclimation period to maintain near ambient particle concentrations. All experiments were conducted in the temperature controlled room at in situ temperatures for the day of collection. Reported values are averages from 3 - 5 replicate bottles, containing 3-5 mussels each depending on the size category. Soft-tissue dry weights were obtained from all mussels used in the experiments and were used to normalize excretion rates. Measured excretion rates are compared to measured filtering and assimilation rates that were determined in parallel run feeding experiments. Details of these experiments are described under Objective 3. Filtering rates ($\text{ml/cm}^2/\text{h}$) are expressed in terms of the amount of chlorophyll removed from the water column per unit time, normalized per unit surface area of zebra mussel gill. Assimilation rates ($\%\text{C/d}$) are expressed as the percentage of the total carbon content of the mussel that has been assimilated by the mussel per day. Assimilation values reported here have also been adjusted to reflect the initial ambient chlorophyll concentrations.

RESULTS AND DISCUSSION

Excretion rates varied considerably among the three experiments, however, in all cases excretion rates by Lake Erie mussels were significantly greater than for Saginaw Bay mussels (Tables 9-11). The most pronounced difference among the two sites occurred in amount of phosphorus (P) excreted by zebra mussels. Nitrogen (N) excretion rates for zebra mussels from Lake Erie ranged between 1.3 and 2.9 times higher than for excretion rates for Saginaw Bay mussels. In contrast, P excretion rates for Lake Erie mussels ranged between 20 - 80 times higher than for Saginaw Bay mussels. For two of the experiments, P excretion rates by Saginaw Bay mussels were below detection using the current methods. For the basis of comparison, we will sometimes use the average P excretion rate of 0.001 $\mu\text{gP}/\text{mgDW}/\text{h}$ for Saginaw Bay mussels. This rate was measured during several independent experiments and represents the methodological level of detection. The substantially higher rates of P excretion by Lake Erie mussels most likely resulted from the fact that Lake Erie seston had significantly lower N:P ratios and the mussels from Lake Erie had substantially higher assimilation rates. The N:P ratios of seston in Lake Erie ranged between 4.3 - 8.5 during the course of the experiments, whereas the N:P ratios of seston in Saginaw Bay ranged between 8.3 - 57.7. The significantly greater levels of P excretion by Lake Erie mussels naturally resulted in significantly lower N:P ratios of the excreted nutrients in Lake Erie versus Saginaw Bay. It is interesting to note that mussels from Lake Erie excreted nutrients at N:P ratios lower than that in the seston (Table 12) and appeared to be satiated with respect to their phosphorus needs. In contrast, mussels from Saginaw Bay always excreted nutrients at N:P ratios much greater than that for the seston and P appeared to be tightly conserved by the mussels. Details of the individual experiments are described below.

In July 1996, N and P excretion rates for Lake Erie mussels were 2.4 and 80 times greater respectively, than for Saginaw Bay mussels (Table 9). Ambient chlorophyll concentrations were also 2.9 times greater in Lake Erie and appear to reflect the difference in N excretion rates, but cannot account for the huge difference in P excretion. An additional factor promoting the much greater levels of P excretion by Lake Erie mussels was most likely that seston from Lake Erie had a substantially lower N:P ratio than that for Saginaw Bay. However, the magnitude of this difference again seems insufficient to explain the observed difference in excretion ratios. Results of the feeding experiments indicate that mussels from both sites were filtering at similar levels, however, mussels from Lake Erie were assimilating algal carbon at a rate nearly 7-fold greater than that for Saginaw Bay mussels (Table 9). It is apparent from these results that nutrient excretion rates are controlled by a variety of competing factors that include the amount of available food, the nutritional content of the food, and perhaps most importantly, the palatability of the food. However, these experimental values only provide an instantaneous snapshot of conditions, and it may also be important to consider additional time history effects in the composition of food particles and the physiological needs of the mussels.

In September 1996, experiments were conducted at each site and a transplant experiment was conducted with mussels from Lake Erie being incubated in Saginaw Bay water. For this experiment, N excretion rates for Lake Erie mussels were only 30% higher than for Saginaw Bay mussels (Table 10). The difference in P excretion rates between sites was again much greater. The P excretion rate for Saginaw Bay mussels was actually below

detection, whereas P excretion for Lake Erie mussels was 0.022 $\mu\text{gP}/\text{mg}/\text{h}$. The N and P excretion rates by Lake Erie mussels in September were 40 and 73% lower respectively compared to rates measured in July. The lower rates may have resulted from the 88% decline in chlorophyll concentrations (2.3 versus 10.3 $\mu\text{g}/\text{L}$). Results from the feeding experiment for Lake Erie mussels show that in contrast to decreased levels of chlorophyll, filtering rates were actually 4 times greater than rates observed in July (Table 10). Surprisingly, the assimilation rate was also 4 times greater in September, indicating that the algae that were present were highly digestible to the mussels. Results of the feeding experiment for Saginaw Bay mussels were very different. Filtering rates in September were nearly 40% lower than in July. This difference may have resulted from the 4-fold increase in chlorophyll concentration in Saginaw Bay, if mussels were becoming satiated (Table 10). The fact that the assimilation rates of this food were actually below zero, however, suggests that the algae present were highly undesirable, and filtering rates were likely suppressed due to the algae's lack of palatability. The lack of assimilation helps to explain why P excretion by the Saginaw Bay mussels was undetectable despite chlorophyll levels nearly 6 times greater than in Lake Erie. These results again indicate that excretion rates reflect a balance between the amount of food, its nutrient content, and the palatability of the food.

For the transplant experiment in September 1996, the same Lake Erie mussels used in the initial experiment were re-acclimated in water from Saginaw Bay for 40 hours, and then excretion rates were re-measured. At the time of the transplant experiment an intense bloom of *Microcystis* occurred in Saginaw Bay. Consequently, chlorophyll concentrations increased from 13.5 to 57.7 $\mu\text{g}/\text{L}$ in less than a week. The extensive bloom also caused severe P limitation in the algae, and seston N:P ratios increased to 44.9. After Lake Erie mussels were incubated in the Saginaw Bay water, N and P excretion rates decreased by 60 and 70 percent from initial rates (Table 10). Results of the feeding experiment showed that decrease in the filtering rate was even more dramatic, with rates only 4% of those observed when incubated in their own site water. Furthermore the assimilation rate of this food dropped below zero, similar to that for Saginaw Bay mussels. Interestingly, the excretion N:P ratio of the transplanted mussels only increased from 6.5 to 8.9 after acclimation with Saginaw Bay water. It appears that the Lake Erie mussels maintained sufficient internal supplies to allow for P excretion much above that provided for in their immediate food source. Overall, results of the transplant experiment clearly indicate that excretion rates can be altered on relatively short-term time scales to reflect major shifts in the quality of the food particles. Presumably, had the incubation period in the transplant experiment been longer, the Lake Erie mussels would have continued to show an even greater change and have eventually approached a pattern of excretion similar to that of Saginaw Bay mussels.

In June 1997, experiments were conducted at each site and a transplant experiment was conducted where Saginaw Bay mussels were acclimated in Lake Erie water. Differences in excretion rates between sites were similar to those in the previous two experiments. N excretion rates by Lake Erie mussels were nearly 3-fold greater than for Saginaw Bay mussels (Table 11). Differences in P excretion were nearly identical to those observed in September. P excretion rates for Lake Erie mussels averaged 0.02 $\mu\text{gP}/\text{mg}/\text{h}$, whereas rates were below detection for Saginaw Bay mussels. P excretion by Saginaw Bay mussels was undetectable even though the seston had a relatively low N:P ratio of 8.3. Overall, excretion rates for both

sites were the lowest observed during the study. N excretion was only 35 and 47 % of previous highs for Saginaw Bay and Lake Erie respectively. The lower excretion rates most likely resulted from a significant reduction in the amount of food available. Chlorophyll concentrations at both sites had declined to only 0.6 $\mu\text{g/L}$. Additionally, excretion rates may also have been lower because of the lower water temperatures at this time. Previous experiments have shown that excretion rates are strongly temperature dependent. Results from feeding experiments indicate that even though the seston from Saginaw Bay had a more balanced nutrient content, the mussels still did not assimilate these food particles (Table 11). This result suggests that the seston was either not palatable or digestible to the mussels, and again helps to explain the lack of observed P excretion. In contrast, filtering and assimilation rates were again very high for Lake Erie mussels. These results agree with the previous experiment, and again suggest a modification in feeding behavior.

For the June 1997 transplant experiment Saginaw Bay mussels were acclimated in Lake Erie water for 120 hours. The Lake Erie water used in this experiment had a lower seston N:P ratio (5.6 vs. 8.3) and a higher chlorophyll concentration (1.6 vs. 0.6 $\mu\text{g/L}$) than the original water from Saginaw Bay (Table 11). Nutrient excretion rates of the Saginaw Bay mussels were significantly different after exposure to the Lake Erie water. N excretion increased by 63% from initial rates and P excretion went from below detection to 0.013 $\mu\text{gP/mg/h}$. The P excretion rate represents a greater than 10-fold increase from levels typically observed for Saginaw Bay mussels and was only 50% lower than the rates observed for Lake Erie mussels. Interestingly, the N:P excretion ratio by the transplanted mussels was lower than seston N:P ratio (Table 11). This result indicates that the mussels became satiated with P rather quickly and began excreting surplus amounts, similar to what has been observed for the Lake Erie mussels. Results from the feeding experiment with Saginaw Bay mussels and Lake Erie water confirm that Lake Erie seston was a much more desirable food source. Filtering rates increased from 21 to 124 $\text{ml/cm}^2/\text{h}$ and the assimilation rate went from below zero to greater than 10 %C/d. The greater assimilation rate and lower N:P ratio of the Lake Erie seston lead to higher excretion rates, especially for P.

Results from the current study have been averaged and compared to previously published results by Arnott & Vanni (1996) for Lake Erie mussels (Table 12). Results for Lake Erie mussels agree quite well between the two studies, and substantiate our experimental approach as well as our results. It should also be noted that results for Saginaw Bay mussels presented in this study are consistent with results from an expanded two-year study by the authors where excretion rates were measured 6-7 times per year. Lastly, results from the two transplant experiments confirm that observed differences in excretion rate were directly correlated to differences in food quantity and quality. Even after fairly short-term exposure to a new food source, mussels from either site began to exhibit modified excretion rates and ratios that were more similar to the mussels from the site of water.

CONCLUSIONS

In conclusion, results from these experiments demonstrate that the rates and ratios of nutrients excreted by zebra mussels depend on both the quantity and the quality of their food source. For this study we have defined food quality in terms of the nutrient content of the

seston, as well as, the palatability of the food as determined by filtering rates and assimilation rates. Lake Erie is a relatively phosphorus rich ecosystem, and consequently produces relatively high amounts of phytoplankton biomass with relatively low N:P ratios. Filtering and assimilation rates indicate that Lake Erie seston is highly edible and the observed assimilation numbers are some of the highest reported within the literature.

The high assimilation rates, combined with the low N:P ratios in the seston, appear to satiate the P demands of the mussels and result in substantial rates of P excretion. Consequently, nutrient excretion by zebra mussels in Lake Erie represents a significant source of dissolved ammonia and phosphorus to the water column and could, in part, promote high growth rates of *Microcystis*. However, comparisons between water quality conditions and excretion rates of mussels from Saginaw Bay to those in Lake Erie suggest that additional factors, other than nutrient excretion by zebra mussels, must also be important in promoting the development of blue-green algal blooms. *Microcystis* blooms have been observed in Saginaw Bay to various extent since mussels became abundant in the fall of 1991, and yet, the inputs of P from mussel excretion appears to be insignificant. The contribution of nutrient inputs from zebra mussel excretion may promote higher phytoplankton growth rates, but this input must be balanced against other nutrient sources and sinks. Additionally, the increased water clarity produced by the mussels filtering activity would also promote higher growth rates for any phytoplankton remaining in the column. Ultimately, the development of bloom conditions depends on the ability of phytoplankton to maintain growth rates much greater than their loss rates. We believe that in Lake Erie, filtering by zebra mussels is the main sink for phytoplankton. Results from feeding experiments clearly demonstrate that *Microcystis* is not filtered by mussels at the same rates as for more desirable forms of phytoplankton. Consequently, the major role that zebra mussels may play in promoting the development of *Microcystis* blooms is most likely related to alterations in feeding behavior and the specific rejection of *Microcystis* over other types of phytoplankton.

Hypothesis 11. Microcystis blooms in western Lake Erie are occurring because of recent increases in light penetration and elevated ammonia and phosphorus levels above beds of zebra mussels.

Objective 11a. Measure *in situ* effects of zebra mussels on algal growth and productivity.

H. MacIsaac *et al.*, University of Windsor.

METHODS

A study site was established on rocky substrate off the north shore of North Bass Island in approximately 3m water during July 1996 during a cyanobacteria bloom (mainly *Lyngbya*, with lesser amounts of *Microcystis*). *Dreissena* colonies were videotaped using SCUBA and an underwater video camera. A high resolution black and white Sony (VCL-4800) camera with a Sony 3.6-mm lens (VCL-SO3XM) was housed in a water-proof housing, and adapted to a Toshiba (KV-6300A) time-lapse S-VHS videorecorder operating at 5 frames s⁻¹. The videorecorder and camera were powered by a marine 12V battery, while visual observation in the boat was provided by a Sony Watchcam (EIA/CCIR System). Two divers placed the camera at a pre-determined focal distance (~20cm) using a cinder block and camera-support system.

RESULTS

Despite extensive manipulation of the camera system, and moderately high (ie. mid- to late afternoon) illumination at the site, we were unable to focus the camera on the mussel bed owing to large quantities of particulate matter in the water column. Moreover, because of concern about exposure to water-borne cyanobacteria toxins, this procedure was not repeated. Following consultation with colleagues, it was determined that all behavioral studies would be limited to laboratory assessments by Dr. H. Vanderploeg.

Objective 11b. Examine the contribution of the recruitment of benthic resting cells to the development of a blue-green algal bloom in Lake Erie.

L. Wu, Mount Union College.

INTRODUCTION

Summer algal blooms are of special interest because they are often dominated by toxic cyanobacterial species such as *Microcystis*, *Anabaena*, *Nodularia*, etc. In general, algal blooms occur due to environmental conditions that lead to a high rate of biomass increase (Legendre 1990). Some blue-green algae have been shown to develop on the sediments and then ascend into the water column when light levels become adequate (Barbiero and Welch 1992). In western Lake Erie, *Microcystis*-dominated summer algal blooms have occurred in the past years. Recent changes such as increase in zebra mussel abundance and light intensity may have altered environmental conditions of the lake to favor certain algal species. The objective of this study was to examine the effect of light intensity on the benthic recruitment to summer algal bloom in western Lake Erie.

MATERIALS AND METHODS

Sampling was conducted biweekly between June and August in 1996. Water samples were collected using a clear, horizontal 1-L bottle every meter from the surface to the bottom at two sites in western Lake Erie. The shallow site (41° 40' 03" N, 82° 49' 61" W) has a rocky bottom with maximum depth of 5 m. The deep site (41° 40' 30" N, 82° 49' 64" W) has a muddy bottom with maximum depth of 13 m. The samples were preserved with Lugol's solution for later phytoplankton identification and enumeration (see Wu and Culver 1991 for detail procedures).

Light intensity was measured every meter at both sites using a LI-COR (LI-1000) meter with underwater quantum sensor. Water temperature and dissolved oxygen were also measured.

RESULTS

Temperature changed similarly at both shallow and deep sites during June through August in 1996 (Figure 76, bottom panels). No thermal stratification was observed at either sites. At the shallow site, light intensity of 200 $\mu\text{mol}/\text{m}^2/\text{s}$ reached 4 m in depth (Figure 76, upper panels). Light intensity became minimal beyond 6 m in depth at the deep site.

A summer bloom of blue-green algae (cyanophytes) was observed on the surface of the shallow site (Figure 77, left panels). Abundant blue-green algae were also found on the bottom of the shallow site. Similarly, abundant blue-green algae were found on the bottom of the deep site (Figure 77, right panels). But no surface bloom occurred at the deep site during summer of 1996. Most of blue-green algae during the bloom were *Microcystis* spp. (Figure 78).

DISCUSSION

Algal growth is influenced by light attenuation, temperature, mixing depth, nutrient supply, and biological interactions (Sommer 1989, Bleiker and Schanz, 1997). Wasmund (1997) demonstrated that a cyanobacterial bloom in the Baltic Sea required conditions of $>16^{\circ}\text{C}$ in water temperature, $>500 \mu\text{mol}/\text{m}^2/\text{s}$ in light intensity and $<6 \text{ m}/\text{s}$ in wind speed. Results in this study reveal that light intensity of $500 \mu\text{mol}/\text{m}^2/\text{s}$ reached 3 m in depth in the shallow site. The deep site, however, was dark beyond 6 m in depth. Abundant *Microcystis* cells were found on the bottom at both sites, but only the shallow site had a surface bloom. Thus enough light reaching the bottom may trigger growth of *Microcystis* cells on the bottom of the shallow site.

Hypothesis 12. Selective filtration/rejection by zebra mussels promotes blooms of Microcystis that have large colony size and high toxicity in Lake Erie.

Objective 12. Evaluate the role of zebra mussels in selecting for *Microcystis* strains of large colony size and high toxicity in the laboratory and in the field.

H. Vanderploeg and T. Johengen, GLERL/NOAA.

DISCUSSION AND CONCLUSIONS

The experiments presented and discussed under Objective 3 were also designed to evaluate Objective 12; therefore, we present only discussion and conclusions to be drawn from experiments discussed under Objective 3. Toxin concentrations from *Microcystis* in Saginaw Bay and the isolate from the Lake Erie bloom (Tables 2 and 3) were highly toxic as measured by ELISA assay for microcystin content by Wayne Carmichael and as measured by the mussels' behavioral and feeding responses. The mussels did not like the LE strain when offered alone (Experiments 3A, 3B, and 4B, Tables 3 and 5) or the Saginaw Bay seston of 11 July 1995 (Tables 2 and 4) as witnessed by their low or zero filtering rates as well as long periods of time spent with their siphons retracted or siphons open but with no filtering current. Note that the response was strain specific in that the CCAP and PCC strains were readily ingested. The experiments with Saginaw Bay and Lake Erie seston indicated that zebra mussels could selectively reject large toxic colonies while ingesting great quantities of small edible algae. The video observations showed the rejection occurred via the mechanism of pseudofeces. Thus, the observed feeding and feeding mechanisms are consistent with the hypothesis that selective rejection promoted *Microcystis* blooms in Lake Erie and Saginaw Bay selecting for large toxic colonies. *Microcystis* blooms have occurred in Saginaw Bay, Lake Erie, and Gull Lake a number of years after the zebra mussels became established. Perhaps a selection for a particular strain was necessary before blooms could develop.

INFORMATION DISSEMINATION

Extensive presentations to the scientific community, Lake Erie managers, and the lay public have been made by various members of the *Microcystis* research project team. Various members of the research team have been interviewed by the media, and articles containing their comments have appeared widely in the print media and on radio. Numerous publications are in various stages of preparation, submission, review, and publication. Additional research is being performed on various aspects of the project and will contribute significantly to our understanding of the causes and effects of toxic algal blooms in the Great Lakes and beyond. Below is a listing of some of the information dissemination activities undertaken by the research team members.

Manuscripts Based on the *Microcystis* Project:

- Babcock-Jackson, L.K., J. An, W.W. Carmichael and D.A. Culver. 1999a. Role of Zebra Mussels in Accumulation and Trophic Transfer of Cyanobacterial Toxins in Lake Erie: The interaction between zebra mussels and amphipods. Manuscript in preparation.
- Babcock-Jackson, L.K., J. An, W.W. Carmichael and D.A. Culver. 1999b. Role of Zebra Mussels in Accumulation and Trophic Transfer of Cyanobacterial Toxins in Lake Erie: The interaction among zebra mussels, amphipods and round gobies. Manuscript in preparation.
- Babcock-Jackson, L.K. and D.A. Culver. 1999a. A toxicokinetic model of uptake and depuration of the cyanotoxin microcystin in zebra mussels (*Dreissena polymorpha*) and round gobies (*Neogobius melanostomus*). Manuscript in preparation.
- Babcock-Jackson, L.K. and D.A. Culver. 1999b. The effects of toxic *Microcystis* on Zebra Mussel (*Dreissena polymorpha*) growth in western Lake Erie. Manuscript in preparation.
- Babcock-Jackson, L.K., D. Baker, R. Knight and D.A. Culver. 1999. Temporal and spatial variation in cyanophyte abundance relative to phosphorus, nitrogen and zebra mussel densities at 41 stations in the western basin of Lake Erie for May - September, 1996-1998. Manuscript in preparation.
- Babcock-Jackson, L.K., J. An, W.W. Carmichael and D.A. Culver. 1999. Spatial variation in hepatotoxin (microcystin) concentrations associated with *Microcystis aeruginosa* at several sampling stations in western Lake Erie in a low *Microcystis* year (1996) and a high *Microcystis* year (1998). Manuscript in preparation.
- Beeton, A. M. and J. Hageman. 1998. Changes in zooplankton in western Lake Erie after establishment of *Dreissena polymorpha*. Verh. int. verein. Limnol. 27: Manuscript in review.

Brittain, S.M., J. Wang, L.K. Babcock-Jackson, W.W. Carmichael and D.A. Culver. Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins, from a Lake Erie strain of *Microcystis aeruginosa*. Manuscript in preparation.

Budd, J. W., A. M. Beeton, R. P. Stumpf, D. A. Culver, and W. C. Kerfoot. 1999. Satellite observations of *Microcystis* blooms in western Lake Erie and Saginaw Bay, Lake Huron. Verh. int. verein. Limnol. 27: Manuscript in review.

Enflo, P. and R. T. Heath. 1999. Possible effects of dreissenid mussels on stability of phytoplankton community composition: Analysis of a simple mathematical model. Manuscript in preparation.

Heath, R. and X. Gao. 1999. Zebra mussels (*Dreissena polymorpha*) may be "keystone remineralizers" in Lake Erie. Verh. int. ver. Limnol. 27: Manuscript in review

Heath, R.T., X. Gao, C. E. Wickstrom, and D. Casamatta. 1997. Evidence that zebra mussels (*Dreissena polymorpha*) can stimulate growth of *Microcystis*, a nuisance blue-green alga. Ohio J. Science 97 (2): A-23.

Heath, R.T., X. Gao, C. E. Wickstrom, and D. Casamatta. 1999. Effects of the zebra mussel (*Dreissena polymorpha*) on P-limited phytoplankton communities in Lake Erie. Manuscript in preparation.

Johengen, T., H. Vanderploeg, and J. Liebig. 1999. Effects of zebra mussels on nutrient cycling in two diverse ecosystems within the Laurentian Great Lakes. Verh. int. ver. Limnol. 27: Manuscript in review.

Pontius, R., and D. A. Culver. 1999. Estimating zebra mussel impact on pelagic food webs: The role of size-specific grazing rates. Verh. int. ver. Limnol. 27: Manuscript in review.

Wickstrom, C.E., D. Casamatta, X. Gao, and R.T. Heath. 1997. Preliminary studies on algal responses to nutrient enrichment of Lake Erie water with or without zebra mussels and *Microcystis*. Ohio J. Science 97 (2): A-12.

Presentations from the *Microcystis* Project at Professional Meetings and Communications to the public:

*Babcock-Jackson, L.K., J. An, W.W. Carmichael and D.A. Culver. Zebra Mussels and Toxic *Microcystis*: Do Zebra Mussels provide their own negative feedback loop? Oral presentation at the Seventh International Zebra Mussel and Other Aquatic Nuisance Species Conference, January 28-31, 1997. New Orleans, Louisiana.

- *Babcock-Jackson, L.K., J. An, W.W. Carmichael and D.A. Culver. 1997. Role of Zebra Mussels in Accumulation and Trophic Transfer of Cyanobacterial Toxins in Lake Erie. Poster presentation at GLAERC Colloquium, April 29, 1997. Wright State University, Ohio.
- *Babcock-Jackson, L.K., J. An, W.W. Carmichael and D.A. Culver. 1997. Role of Zebra Mussels in Accumulation and Trophic Transfer of Cyanobacterial Toxins in Lake Erie. Poster presentation at The Society of Environmental Toxicology and Chemistry, November 16-20, 1997. San Francisco, California.
- *Babcock-Jackson, L.K., J. An, W.W. Carmichael and D.A. Culver. 1998. Lake Erie *Microcystis*: Trophic interactions and ecological relationships to the Zebra Mussel, *Dreissena polymorpha*. 4th International Conference on Toxic Cyanobacteria. August 30 - September 3, 1998. Duke Marine Laboratory, Beaufort, North Carolina.
- *Beeton, A. M. and J. Hageman. 1998. Changes in zooplankton in western Lake Erie after establishment of *Dreissena polymorpha*. INVITED PRESENTATION as part of the "Ecosystem effects of invasive nuisance species" held as part of the program at the XXVII Congress of the Societas Internationalis Limnologiae (SIL). Dublin, IRELAND. August 1998.
- Budd, J. W., A. M. Beeton, R. P. Stumpf, D. A. Culver, and W. C. Kerfoot*. 1999. Satellite observations of *Microcystis* blooms in western Lake Erie and Saginaw Bay, Lake Huron. INVITED PRESENTATION as part of the "Ecosystem effects of invasive nuisance species" held as part of the program at the XXVII Congress of the Societas Internationalis Limnologiae (SIL). Dublin, IRELAND. August 1998.
- *Culver, D. A. and R. A. Pontius. 1996. Lake Erie zebra mussels: Ecosystem effects and the future. North American Benthological Society Annual Meetings. Kalispell, Montana. 7 June 1996. Invited presentation.
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- Culver, D. A. 1997. Changes in the Lake Erie Ecosystem: The Role of Zebra Mussels. Michigan Sea Grant Research Meeting, Pt. Huron, MI 21 Jan. 1997. Invited.

- Culver, D. A. Changes in the Lake Erie Ecosystem: Phosphorus, Zebra Mussels and Toxic Blue-green Algae. Lake Erie Forum, Windsor, Ontario. 24-25 January 1997. Invited Presentation.
- Culver, D. A. 1997. The Importance of Phosphorus Changes in Lake Erie - Panel Discussion, The Lake Erie Forum, USEPA, May 30, 1997, Toledo, OH. Invited Presentation.
- Culver, D. A. 1997. Toxic Algae and Lake Erie Ecological Change. Ohio Lake Erie Conference. Cleveland, OH. September 18, 1997. Invited presentation.
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- *Frost, P. and D. A. Culver. 1997. Zooplankton and phytoplankton responses to ecological change within western Lake Erie. American Society of Limnology and Oceanography Aquatic Sciences Meeting. Santa Fe, NM, February 10-14, 1997.
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- Heath, R.T. and X. Gao. 1998. Zebra mussels may be "keystone remineralizers" in Lake Erie. INVITED PRESENTATION as part of the "Ecosystem effects of invasive nuisance species" held as part of the program at the XXVII Congress of the Societas Internationalis Limnologiae (SIL). Dublin, IRELAND. August 1998.
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- *Johengen, T.H., and H.A. Vanderploeg. Effects of food quantity and quality on nutrient excretion by zebra mussels. Annual Meeting of the International Association of Great Lakes Research, Buffalo, NY, June 3, 1997. International Association of Great Lakes Research.

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- Vanderploeg, H.A. Exotics in the lakes: Top-down and bottom agents of instability. Great Lakes Environmental Journalism Training Institute, East Lansing, MI, May 22, 1997. Great Lakes Environmental Journalism Training Institute.
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Table 1. HPLC, amino acid analysis and mass spectrometric data of Compounds 1-3 from *Microcystis aeruginosa* strain LE-3.

PEAK	t _r *	% Area (HPLC)	AMINO ACIDS	m/z(M+H) HRFABMS, Δ [†] COMPOSITION	PROPOSED STRUCTURE C ₄₈ H ₇₂ N ₁₀ O ₁₂
1	14.7	10.4	Asp(1), Glu(1), Arg(1), Ala(1), Leu(1)	981.6 981.5423, 1.3 C ₄₈ H ₇₂ N ₁₀ O ₁₂	[D-Asp ³] MCYST-LR
2	15.5	87.7	Glu(1), β-MeAsp(1), Arg(1), Ala(1), Leu(1)	995.6 995.5567, 0.1 C ₄₉ H ₇₄ N ₁₀ O ₁₂	MCYST-LR
3	20.6	1.9	Glu(1), β-MeAsp(1), Arg(2), Ala(1)	975.0 ND ND	MCYST-AR

*Analytical HPLC retention time

†High resolution fast atom bombardment mass spectrometry, Δ=difference (mda) from calculated value

Table 2. Zebra mussels feeding on naturally occurring *Microcystis* in seston from Lake Erie (LE) and Saginaw Bay (SB) and on mixtures with *Rhodomonas minuta* and *Cryptomonas ozolini*. *Rhodomonas* and *Cryptomonas*, because of their small size (5 and 8 μm , respectively), occur only in the $< 53 \mu\text{m}$ size fraction. F is filtering rate calculated for removal of chlorophyll from the water column, and F_A refers to chlorophyll actually assimilated. Both rates are normalized per unit surface of mussel gill area.

Experiment	Date	Microcystin concentration ($\mu\text{g} \cdot [\mu\text{g Chl } a]^{-1}$)	Size fraction (μm)	Avg. Chl a concentration ($\mu\text{g} \cdot \text{l}^{-1}$)	F ($\text{ml} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$)	F_A ($\text{ml} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$)	Carbon assimilation ($\% \cdot \text{d}^{-1}$)
SB seston	11 July 95	0.17	> 53	19.35 ± 0.05	0.76 ± 0.45	1.11 ± 0.08	3.39 ± 0.29
			< 53	4.37 ± 0.03	-4.19 ± 1.18	-5.79 ± 2.11	-4.04 ± 1.47
			Total	23.71 ± 0.07	-0.12 ± 0.49	-0.18 ± 0.43	-0.66 ± 1.61
SB seston $> 53 \mu\text{m}$ plus <i>Rhodomonas</i>	12 July 95	0.17	> 53	17.09 ± 0.09	-1.50 ± 0.86	-1.50 ± 0.86	-6.54 ± 3.79
			< 53	6.10 ± 0.14	53.56 ± 3.39	53.56 ± 3.39	37.55 ± 2.00
			Total	23.20 ± 0.16	12.83 ± 0.78	12.93 ± 0.79	31.01 ± 4.29
LE seston	21 Sept. 95	-	> 53	36.61 ± 1.14	1.72 ± 6.43	2.06 ± 3.26	8.58 ± 13.58
			< 53	1.28 ± 0.03	43.34 ± 4.26	22.18 ± 3.22	3.09 ± 0.45
			Total	37.90 ± 1.16	3.14 ± 6.30	2.71 ± 3.26	11.66 ± 14.05
LE seston plus <i>Rhodomonas</i>	22 Sept. 95	-	> 53	16.61 ± 0.75	2.82 ± 9.24	1.71 ± 1.43	3.22 ± 2.68
			< 53	3.47 ± 0.05	81.74 ± 4.63	79.80 ± 4.50	14.15 ± 0.82
			Total	20.08 ± 0.79	18.16 ± 8.62	15.04 ± 1.70	17.37 ± 2.81
SB seston $> 153 \mu\text{m}$ plus <i>Cryptomonas</i>	15 Aug. 97	-	> 53	1.91 ± 0.08	10.19 ± 4.33	2.67 ± 1.61	1.36 ± 0.80
			< 53	2.84 ± 0.10	59.38 ± 5.58	58.01 ± 5.13	11.56 ± 0.92
			Total	4.75 ± 0.04	35.82 ± 1.19	35.15 ± 2.36	12.92 ± 1.22
SB seston $53\text{--}153 \mu\text{m}$ plus <i>Cryptomonas</i>	15 Aug. 97	-	> 53	1.74 ± 0.06	19.74 ± 4.54	4.52 ± 1.10	1.91 ± 0.44
			< 53	3.16 ± 0.09	64.25 ± 3.92	59.63 ± 6.66	13.03 ± 0.75
			Total	4.90 ± 0.12	45.79 ± 3.57	40.07 ± 3.94	14.94 ± 0.87

Table 3. Zebra mussels grazing on pure cultures of *Cryptomonas ozolini* and toxic and non-toxic *Microcystis aeruginosa* offered alone or together. Experiments with same number but different letter designations were run with the same mussels on the same or consecutive days in indicated order. *Cryptomonas*, because of its small size (8 μm), occurs only in the < 53 μm size fraction. F is filtering rate calculated for removal of chlorophyll from the water column, and F_A refers to chlorophyll actually assimilated. Both rates are normalized per unit surface of mussel gill area.

Strain or mixture	Exp.	Acclimation period (h)	Temp. ($^{\circ}\text{C}$)	Microcystin concentration ($\mu\text{g} \cdot \mu\text{g Chl } a^{-1}$)	Size fraction (μm)	Avg. Chl a concentration ($\mu\text{g} \cdot \text{l}^{-1}$)	F ($\text{ml} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$)	F_A ($\text{ml} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$)	Carbon Assimilation ($\% \cdot \text{d}^{-1}$)
CCAP 1450/11 ^a	1	4.1	25	0	> 53	0.99 \pm 0.03	-8.84 \pm 6.59	-67.56 \pm 12.77	-8.25 \pm 1.58
					< 53	10.79 \pm 0.20	25.31 \pm 3.81	45.43 \pm 4.10	47.98 \pm 4.55
					Total	11.77 \pm 0.19	23.56 \pm 3.33	33.03 \pm 2.19	39.52 \pm 2.82
PCC 7820 ^b	2	70.0	17	0.44	> 53	0.51 \pm 0.05	83.88 \pm 25.43	71.14 \pm 10.12	3.80 \pm 0.55
					< 53	3.87 \pm 0.34	109.20 \pm 17.98	109.67 \pm 12.51	39.30 \pm 4.46
					Total	4.38 \pm 0.36	105.29 \pm 16.56	104.39 \pm 11.93	43.05 \pm 4.91
LE ^c	3A	17.9	20	0.66 ^d	> 53	0.61 \pm 0.04	-22.74 \pm 5.91	-44.91 \pm 6.67	-6.15 \pm 0.84
					< 53	4.64 \pm 0.11	14.87 \pm 2.74	14.67 \pm 7.50	7.22 \pm 1.38
					Total	5.26 \pm 0.11	5.91 \pm 2.32	1.89 \pm 2.40	1.21 \pm 1.49
LE ^c	3B	1.9	20	0.66 ^d	> 53	0.68 \pm 0.02	19.84 \pm 3.36	2.34 \pm 7.59	0.25 \pm 0.67
					< 53	0.80 \pm 0.02	18.21 \pm 3.29	16.67 \pm 5.31	1.25 \pm 0.37
					Total	1.49 \pm 0.02	18.92 \pm 1.81	9.08 \pm 3.82	1.50 \pm 0.63
<i>Cryptomonas</i>	3C	0.9	20	0	-	7.76 \pm 0.19	54.83 \pm 2.84	50.16 \pm 3.64	37.67 \pm 3.27
<i>Cryptomonas</i>	4A	18.2	20	0	-	2.98 \pm 0.11	98.21 \pm 5.83	92.93 \pm 5.44	30.66 \pm 1.06
LE ^c	4B	18.2	20	0.66 ^d	> 53	0.04 \pm 0.00	-3.64 \pm 9.15	-86.61 \pm 23.16	-0.43 \pm 0.12
					< 53	3.81 \pm 0.03	-2.54 \pm 0.97	-3.12 \pm 1.34	-1.55 \pm 0.70
					Total	3.84 \pm 0.03	-2.57 \pm 0.89	-3.92 \pm 1.42	-1.97 \pm 0.76
50:50 Mixture of LE and <i>Cryptomonas</i>	4C	19.0	20	0.66 ^e	> 53	0.07 \pm 0.02	-40.30 \pm 24.93	-81.32 \pm 22.88	-0.69 \pm 0.20
					< 53	4.13 \pm 0.12	20.86 \pm 3.58	15.08 \pm 2.27	7.51 \pm 1.09
					Total	4.20 \pm 0.11	19.51 \pm 3.11	13.46 \pm 2.57	6.85 \pm 1.28

^aCulture Collection of Algae and Protozoa, Ambleside, United Kingdom

^bPasteur Culture Collection, Paris, France

^cLake Erie isolate of Wayne Carmichael

^dMicrocystin concentration in LE strain measured in Experiment 4C

^eMicrocystin concentration in *Microcystis* fraction

Table 4. Video observations of 4 individual zebra mussels in feeding experiments (Table 2) with naturally occurring seston from Lake Erie (LE) or Saginaw Bay (SB): dominant non-filtering behavior (DNFB) and total percent of time spent in behaviors that interrupted feeding. DNFB is the non-filtering behavior which has the longest duration summed over all 4 mussels. Behaviors examined (Fig. 2a) included ESR (excurent siphon retraction), TSR (two siphon retraction), ONF (siphons open or extended, but not filtering), and PFE (pseudofeces expulsion).

Experiment	Date	Avg. Chl <i>a</i> concentration ($\mu\text{g} \cdot \text{l}^{-1}$)	DNFB	% of time not filtering by mussel				$\bar{X} \pm SE$
				1	2	3	4	
SB seston	11 July 95	23.71 \pm 0.07	ONF	8.07	46.33	84.24	-	46.21 \pm 31.1
LE seston	21 Sept. 95	37.90 \pm 1.16	PFE	1.53	4.59	4.32	10.78	5.30 \pm 3.38
SB seston >153 μm plus <i>Cryptomonas</i>	15 Aug. 97	4.75 \pm 0.04	PFE	4.22	6.13	6.07	8.95	6.34 \pm 1.70
SB seston 53-153 μm plus <i>Cryptomonas</i>	15 Aug. 97	4.90 \pm 0.12	PFE	2.42	6.83	0.28	2.11	2.91 \pm 2.41

Table 5. Video observations of 4 individual zebra mussels in feeding experiments (Table 3) with pure cultures of *Cryptomonas ozolini* and toxic (Strains PCC 7820 and LE) and non-toxic (Strain CCAP 1450/11) *Microcystis aeruginosa*: dominant non-filtering behavior (DNFB) and total percent of time spent in behaviors that interrupted filtering. DNFB is the non-filtering behavior which has the longest duration summed over all 4 mussels. Behaviors examined (Fig. 2) included ESR (excurrent siphon retraction), TSR (two siphon retraction), ONF (siphons open or extended, but not filtering), and PFE (pseudofeces expulsion).

Strain or mixture	Exp.	Avg. Chl <i>a</i> concentration (µg l ⁻¹)	DNFB	% of time not filtering by mussel				$\bar{X} \pm SE$
				1	2	3	4	
CCAP 1450/11 ^a	1	11.77	ESR	5.45	3.27	15.21	9.01	8.24 ± 4.52
PCC 7820 ^b	2	4.38	ESR	2.04	10.47	3.90	8.06	6.12 ± 3.33
LE ^c (< 53µm)	3A	5.26	ESR	31.25	39.97	62.76	5.18	34.79 ± 20.60
LE ^c	3B	1.49	TSR	14.91	100.00	10.30	14.17	34.84 ± 37.66
<i>Cryptomonas</i>	3C	7.76	TSR	2.34	100.00	100.00	1.63	50.99 ± 49.01
<i>Cryptomonas</i>	4A	2.98	ESR	1.78	0.44	8.60	20.03	7.78 ± 7.86
LE ^c	4B	3.84	TSR	2.85	30.48	57.97	100.00	47.82 ± 35.88
50:50 Mixture of LE and <i>Cryptomonas</i>	4C	4.20	TSR	14.60	100.00	34.83	85.46	58.92 ± 35.13

^aCulture Collection of Algae and Protozoa, Ambleside, United Kingdom

^bPasteur Culture Collection, Paris, France

^cLake Erie isolate of Wayne Carmichael

Table 6. Some Historical Trends in Nitrogen, Phosphorus and Cyanophyte abundances in western Lake Erie.

Year	General phosphorus ranges (TP mg/l)	General nitrogen ranges (TN and NH ₃ mg/l)	Cyanophytes as % of total phytoplankton biomass or cells/ml maxima of species as available	Source
Before 1950	0.105 (as PO ₄) in Maumee Bay	1.4 (as NO ₃) in Maumee Bay	No cyanophytes are listed as dominant in the phytoplankton before 1958.	Verduin, 1964; Davis, 1964.
Late 1957 - 1958	0.45 (as PO ₄) in Maumee Bay	2.2 (as NO ₃) in Maumee Bay	<i>Microcystis</i> , <i>Anabaena</i> and <i>Aphanizomenon</i> listed as major components of PP community in July and August 1957-58 (1- 10 µl/L)	Verduin, 1964.
1970	0.024-0.05	0.14-0.518 TN 0.042-.07 NH ₃	5-35%, max in September.* <i>A. flos aquae</i> max Aug- Oct. <i>Microcystis</i> < 5% all samples	Burns, 1976; Munawar and Munawar, 1976; DePinto et al, 1986.
1975	N.A.	N.A.	9.36% max in August. ^b	Reuter, 1979.
1979	N.A.	N.A.	66 - 7702 cells/ml <i>A. flos aquae</i> Aug- Oct. 22 - 18,454 cells/ml <i>Oscillatoria spp.</i> Aug- Oct. 500 cells/ml max. <i>Microcystis</i> Aug. ^c	D. Van Keuren, 1979 CLEAR records.
1984	.028 annual mean	N.A.	50% max in July ^d <i>Anabaena</i> max 1162 cells/ml <i>A. flos aquae</i> max 2643 cells/ml <i>Merismopedia</i> 6218 cells/ml <i>Oscillatoria</i> max 5179 cells/ml * <i>Anacystis</i> max of 22.253 cells/ml (avg 1052)	GLNPO Report #88-3, 1988.

Year	General phosphorus ranges (TP mg/l)	General nitrogen ranges (TN and NH ₃ mg/l)	Cyanophytes as % of total phytoplankton biomass or cells/ml maxima of species as available	Source
1983 - 1987	N.A.	N.A.	Average biovolume of Cyanophytes 6.86%. <i>Microcystis</i> , <i>Anacystis</i> and <i>Oscillatoria</i> (3600 - 22,253 cells/ml) species all very prevalent along with <i>Anabaena</i> , <i>Aphanizomenon</i> and others	Makarewicz, 1993.
1991	.02-.06	0.5-1.4 TN 0.0-0.043 NH ₃	57.3% max cyanophyte biomass in Aug ^e	Beeton et al., 1996; Pontius and Culver, unpublished.
1992	0.01-0.04	0.4-1.2 TN 0.02-0.065 NH ₃	0.3% max cyanophyte biomass in Aug ^e	Beeton et al., 1996; Pontius and Culver, unpublished.
1995	0.02-0.06	0.12-0.38 TN 0.018-0.054 NH ₃	8400 cells/ml max ^e <i>Microcystis aeruginosa</i> in Sept.	Beeton et al., 1996; Babcock-Jackson unpublished.
1996	see Objective 6	see Objective 6	182 cells/ml max <i>M. aeruginosa</i> in late Aug. ^e	Babcock-Jackson, unpublished;
1997	N.A.	N.A.	11 cells/ml max <i>M. aeruginosa</i> in Aug. ^e	Babcock-Jackson, unpublished.

^a Combined cyanophyte data for 5 stations sampled in the western basin of Lake Erie, April - December, 1970.

^b Cyanophyte data combined from 5 stations sampled nearshore in the eastern basin of Lake Erie, July, 1975 -June, 1976 (only available data for that year).

^c Cyanophyte data compiled from 12 stations sampled in the western basin of Lake Erie, Aug - Oct., 1979. Records held at Center for Lake Erie Area Research, Columbus, Ohio.

^d Details of sampling methods may be found in report. Cyanophytes are % biovolume. **Anacystis* is considered to be *Microcystis* by Taft and Taft (1971).

^e Cyanophyte data from samples collected in Hatchery Bay or from Peach Point marker buoy, both at South Bass Island, western Lake Erie. N and P measurements were not made at this site, but detailed N and P measurements for 41 stations in the western basin are provided in Objective 6 of this report.

N.A. = not available.

Table 7. Comparison of sensor characteristics and band widths of various satellite instruments.

	Spatial Res. (km)	Repeat Time (days)	Scene Width (km)	# of Bands Refl./therm.	Orbital Characteristics
AVHRR	1.1	.25-1.0	2000	2.0/3.0	Polar
Landsat TM	0.06	16.0	180	4.0/2.0	Polar
SeaWiFS	1.0	1.0	2800	8.0/0	Polar

	Band Width (micrometers)					
	Blue	Green	Red	Near-IR	Mid-IR	Thermal-IR
AVHRR			0.58-0.68	0.72-1.00	3.50-3.90	10.5-11.3 11.5-12.5
Landsat TM	0.45-0.52	0.52-0.60	0.63-0.69	0.76-0.90 1.55-1.75	2.08-2.35	10.4-12.5
SeaWiFS	.402-.422 .433-.453 .480-.500	.500-.520 .545-.565	.660-.680	.745-.785 .845-.885		

Table 8. Estimates of annual total phosphorus load to Lake Erie, and amounts from Ohio tributaries monitored by Heidelberg College (*italic numbers*), and from IJC estimates (**bold numbers**) (metric tonnes/water year). Water year is the 12 months previous to October of the year given. * = our estimates of total lake loading.

Year	Maumee River	Sandusky River	Total Ohio Monitored Tributary (our est)	Total Ohio Tributary Loading (IJC)	Total Tributary Loading- (IJC Graph)	Total Lake P Loading
1987	<i>1063.5</i>	<i>359.2</i>	2283	3331	3222	6,293
1988	<i>813.3</i>	<i>125.9</i>	1507	2199	2726	4,612
1989	<i>1956.3</i>	<i>313.8</i>	3643	5315	4709	9,238
1990	<i>3070.7</i>	<i>482.8</i>	5702	8320	6939	13,696
1991	<i>3492.1</i>	<i>573.4</i>	6524	9519	6691	15,476
1992	<i>1579.7</i>	<i>325.9</i>	3058	4462	4462	7,971
1993	<i>3493.3</i>	<i>556.6</i>	6499	9483	none avail.	15,422*
1994	<i>1322.0</i>	<i>272.9</i>	2559	3735	none avail.	6,890*
1995	<i>1139.2</i>	<i>395.9</i>	2464	3595	none avail.	6,685*

Table 9. Results of nutrient excretion experiment for Saginaw Bay and western Lake Erie mussels in July 1996. Reported values are the means and standard errors of 6 replicate bottles with 3-5 mussels per bottle. Fluxes were calculated over a 3 hr interval. Temperature was 22 °C for Saginaw Bay and 25 °C for Lake Erie.

	Saginaw Bay mussels	Lake Erie mussels
NH ₄ Flux (ugN/mg/h)	0.098 (.007)	0.236 (.021)
SRP Flux (ugP/mg/h)	0.001 (.0003)	0.082 (.001)
Excreted N:P ratio	98	2.9
Seston N:P ratio	12.3	8.5
Chlorophyll (ug/L)	3.5	10.3
Filtering rate (ml/cm ² /h)	26.8 (2.3)	28.4 (4.1)
Assimilation rate (%C/d)	1.6 (0.8)	11.1 (1.7)

Table 10. Results of nutrient excretion experiment for Saginaw Bay and Lake Erie mussels in September 1996. Rates were first determined for mussels using ambient site water and then the experiment was repeated for Lake Erie mussels after being acclimated in aquaria with water from Saginaw Bay for 38 hours. Reported values are the means and standard errors of 3-6 replicate bottles with 3 mussels per bottle. Rates were determined using a 3 hr incubation interval. Experiments were conducted at 21 °C for Saginaw Bay and 25 °C for Lake Erie.

	Saginaw Bay mussels	Lake Erie mussels	Lake Erie mussels in Saginaw Bay water
NH ₄ Flux (ugN/mg/h)	0.108 (.009)	0.140 (.013)	0.056 (.004)
SRP Flux (ugP/mg/h)	Below Detection	0.022 (.002)	0.006 (.001)
Excreted N:P ratio	ND	6.5	8.9
Seston N:P ratio	19.1	ND	44.9
Chlorophyll (ug/L)	13.5	2.3	57.7
Filtering rate (ml/cm ² /h)	16.8 (11.3)	117.6 (13.8)	4.4 (5.7)
Assimilation rate (%C/d)	-0.4 (4.8)	44.6 (9.1)	-4.2 (1.9)

ND: Not Determined

Table 11. Results of nutrient excretion experiment for Saginaw Bay and western Lake Erie mussels in June 1997. Mussels from each site were tested in ambient water and Saginaw Bay mussels were tested after being transplanted into flow-through tanks at Stone Lab and acclimated for 120 hours. Reported values are the means and standard errors of 4 replicate bottles with 4 mussels per bottle. Fluxes were calculated over a 4 hour interval and all experiments were done at 19 °C.

	Saginaw Bay mussels	Lake Erie mussels	Saginaw Bay mussels in L. Erie water
NH ₄ Flux (ugN/mg/h)	0.038 (.002)	0.111 (.006)	0.062 (.005)
SRP Flux (ugP/mg/h)	Below Detection	0.020 (.001)	0.013 (.003)
Excreted N:P ratio	ND	5.6	4.8
Seston N:P ratio	8.3	4.3	5.6
Chlorophyll (ug/L)	0.6	0.6	1.6
Filtering rate (ml/cm ² /h)	21.1 (3.3)	140.4 (4.5)	123.8 (10.6)
Assimilation rate (%C/d)	-2.0 (1.3)	23.0 (1.4)	10.5 (0.9)

ND: Not Determined

Table 12. Averaged results for zebra mussel excretion experiments for Saginaw Bay (Lake Huron), and Hatchery Bay, Lake Erie in this study compared to previously reported excretion rates for mussels from western Lake Erie.

	Saginaw Bay	Lake Erie (This Study)	Lake Erie (Arnott & Vanni 1996)
NH ₄ Flux (se)	0.081	0.162	0.074
SRP Flux (se)	0.001 ^a	0.041	0.032
Excreted N:P ratio	80	4.0	2.3
Seston N:P ratio	21.2	6.1	5.0

^a Value includes results from additional experiments not described here, when rates were measurable.

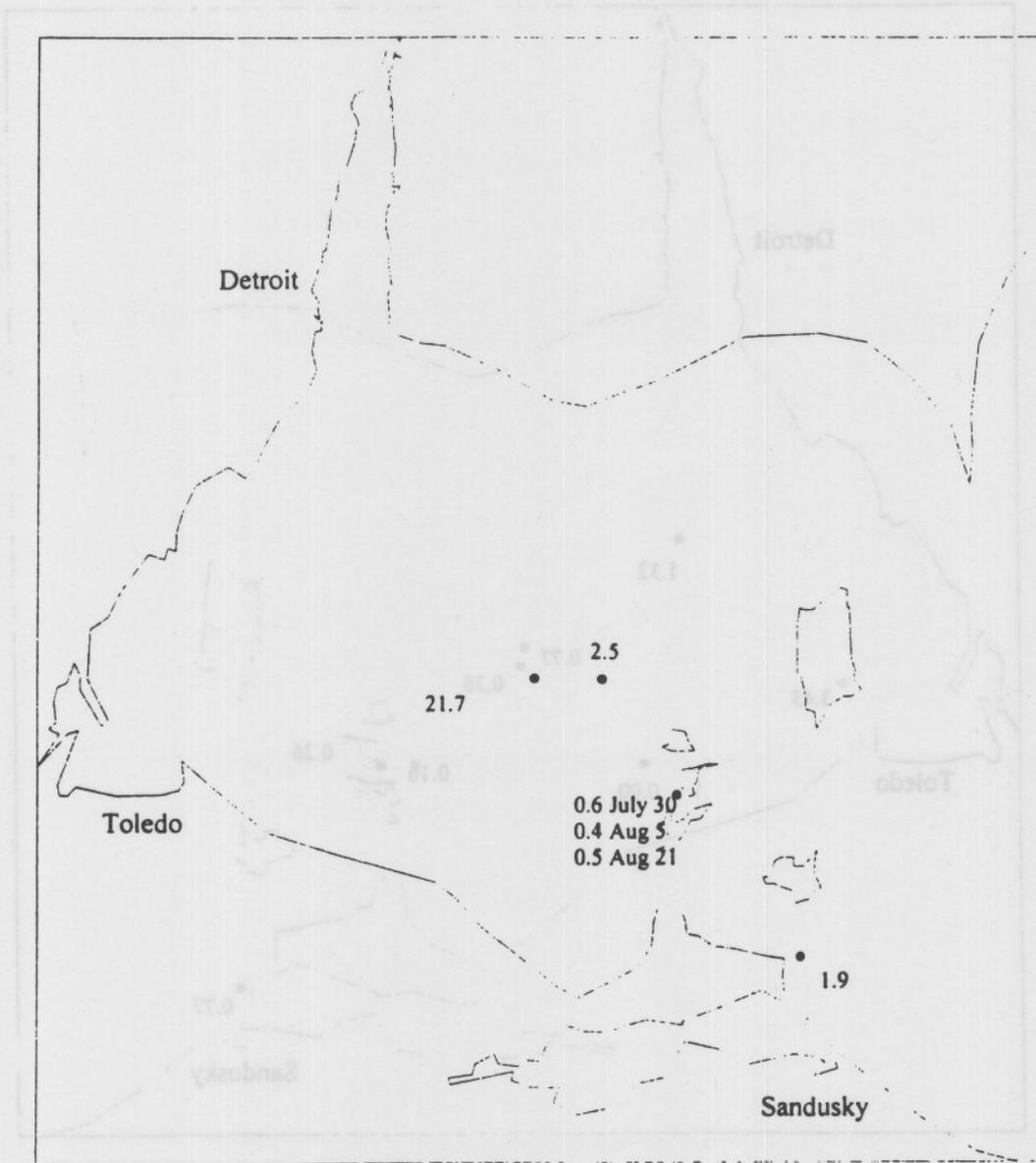


Figure 1a. Microcystin concentrations (ng/l) at several sites in the western basin of Lake Erie in August, 1996.

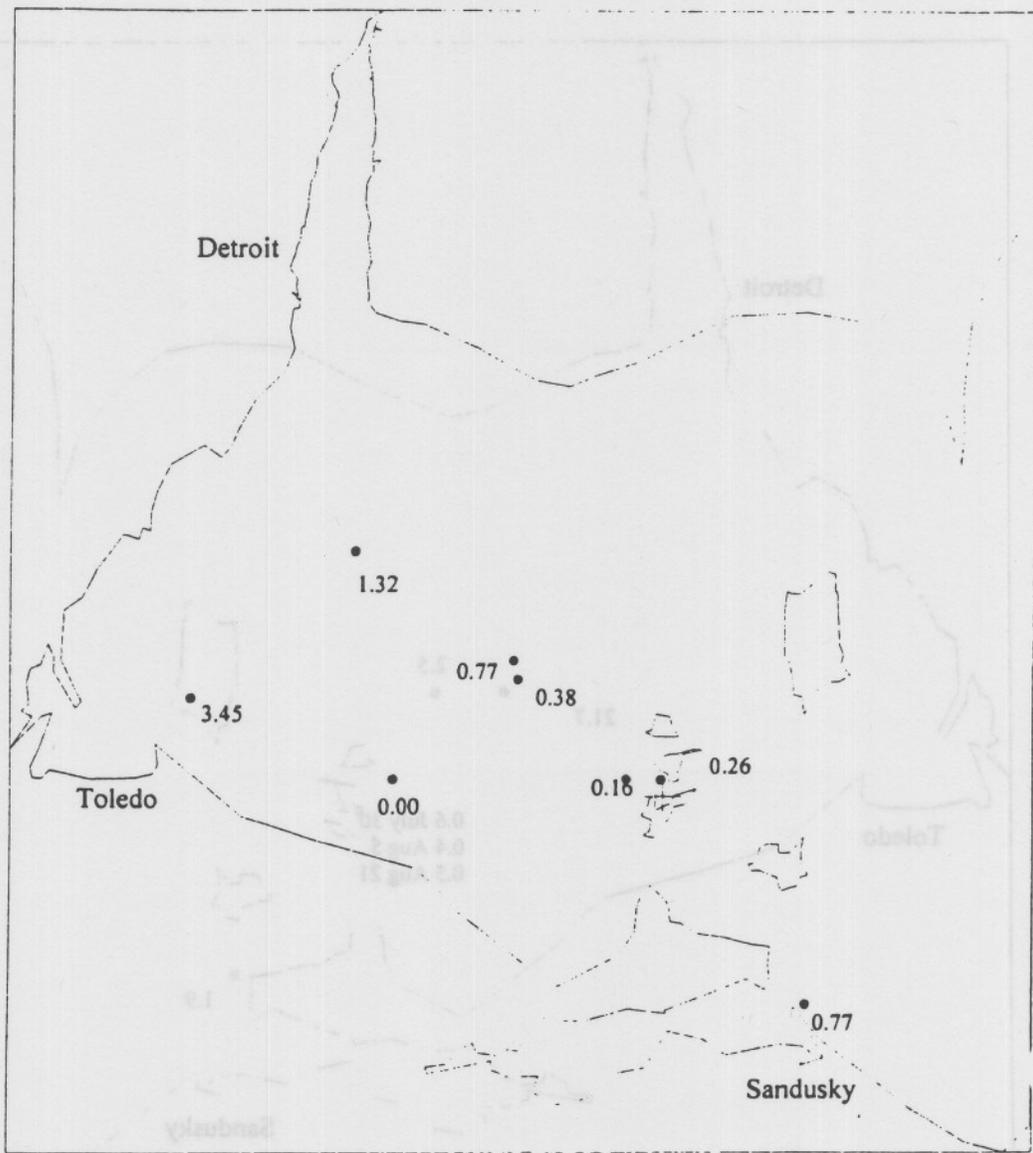


Figure 1b. Microcystin concentrations (ng/l) at several sites in the western basin of Lake Erie in September, 1996. [+] Indicates one sample collected on July 12, 1996 = 9.5 ng/l; zooplankton toxicity = 6.1 μ g/g DW from the same sample.

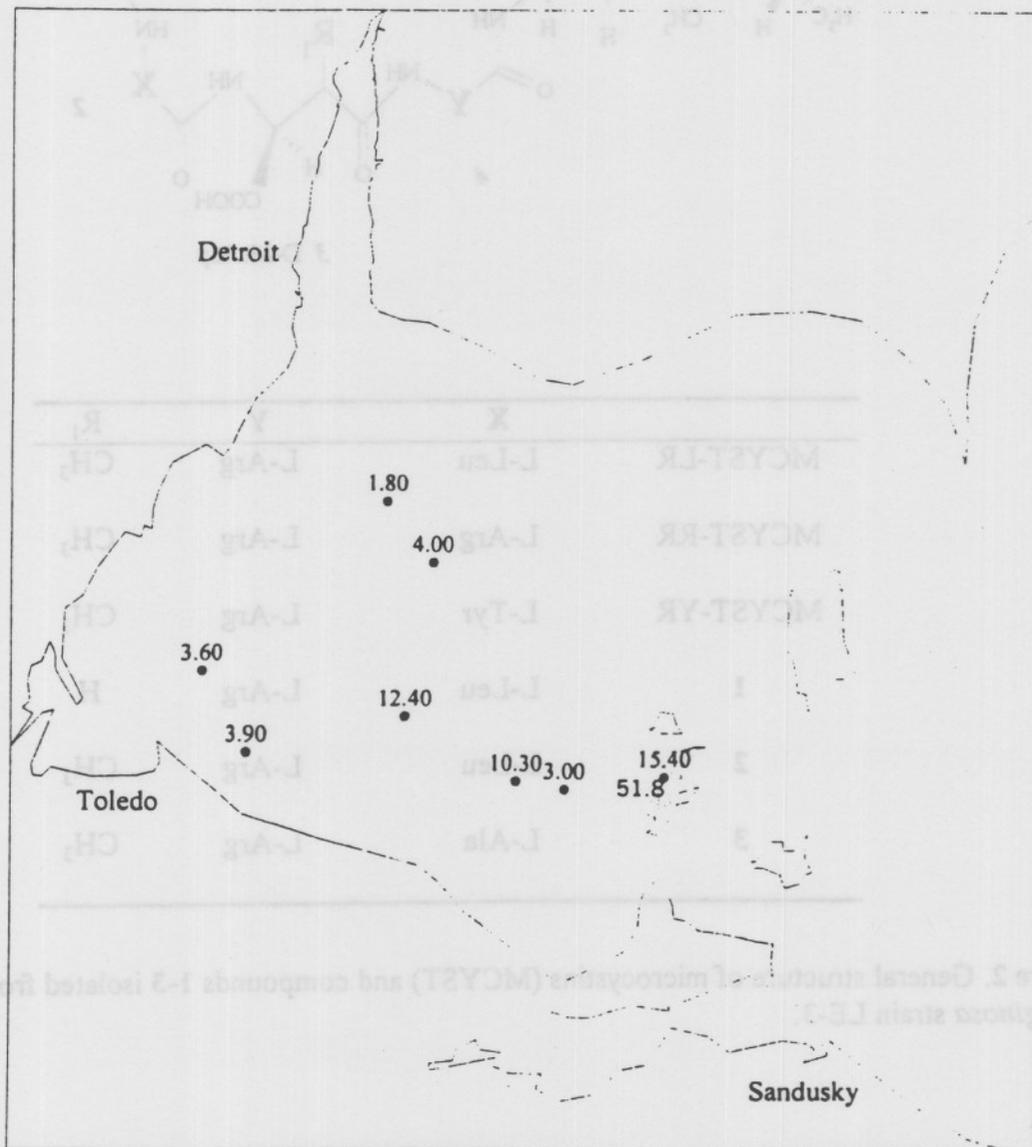
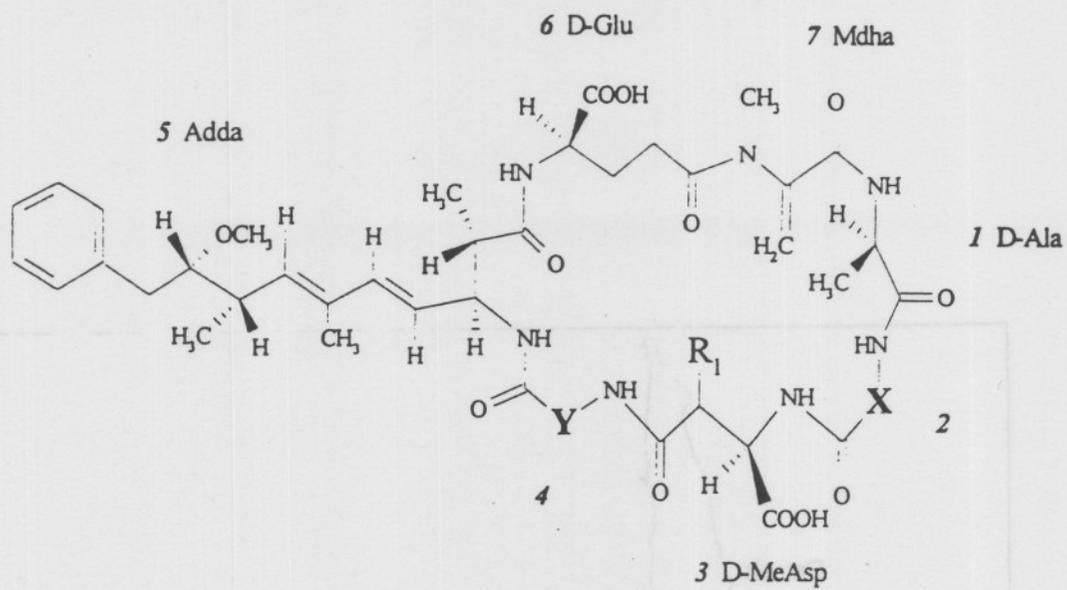


Figure 1c. Microcystin concentrations (ng/l) at several sites in the western basin of Lake Erie on August 20, 1998. The *Microcystis* bloom condition progressed further into September and October, but no samples for toxin analysis are available for those months.



	X	Y	R ₁
MCYST-LR	L-Leu	L-Arg	CH ₃
MCYST-RR	L-Arg	L-Arg	CH ₃
MCYST-YR	L-Tyr	L-Arg	CH ₃
1	L-Leu	L-Arg	H
2	L-Leu	L-Arg	CH ₃
3	L-Ala	L-Arg	CH ₃

Figure 2. General structure of microcystins (MCYST) and compounds 1-3 isolated from *M. aeruginosa* strain LE-3.

Figure 1c. Microcystin concentrations (ng/l) at several sites in the western basin of Lake Erie on August 20, 1992. The microcystin bloom condition progressed further into September and October, but no samples for toxin analysis are available for those months.

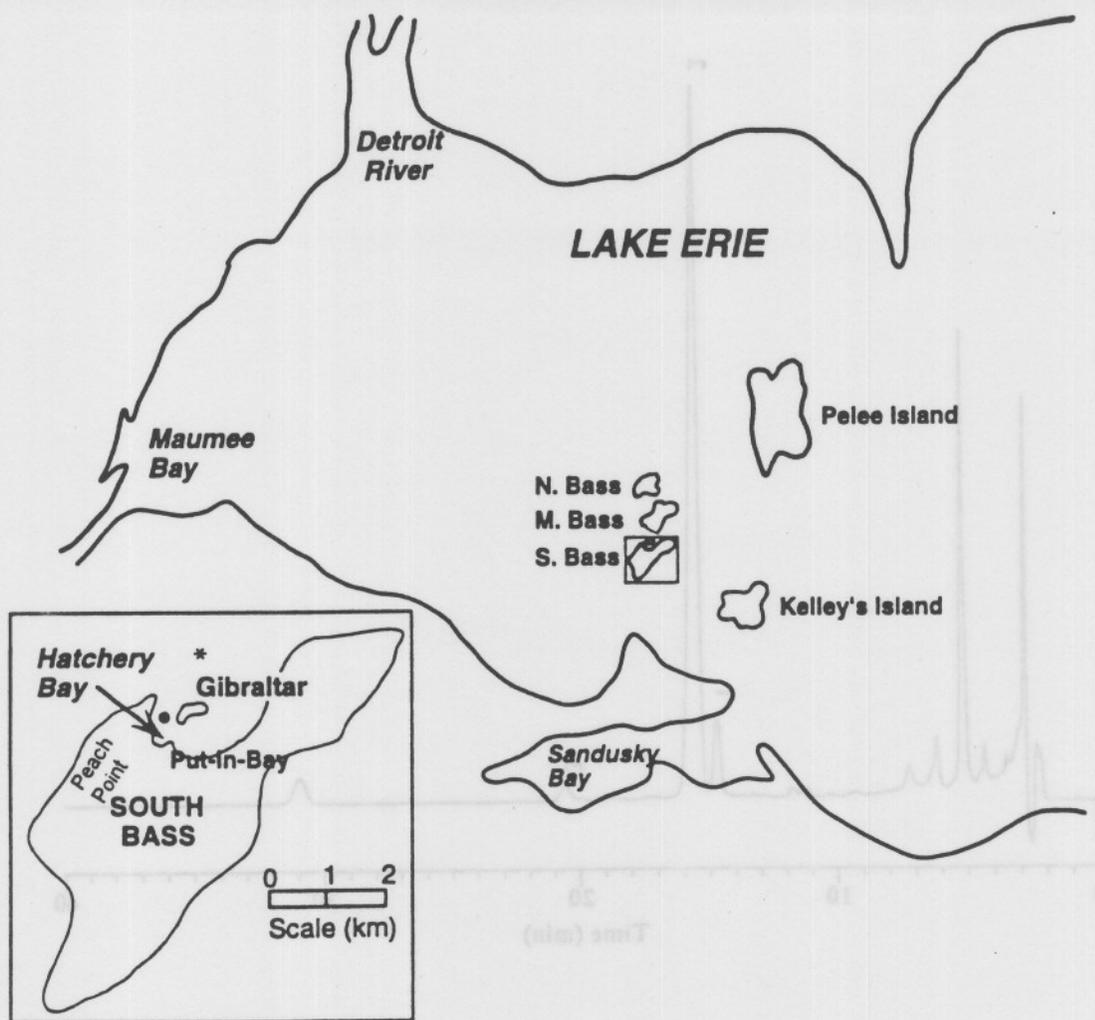


Figure 3. The western basin of Lake Erie showing the location of South Bass and Gibraltar Islands. Detail of Hatchery Bay (●) and Peach Point marker buoy (*) sampling sites.

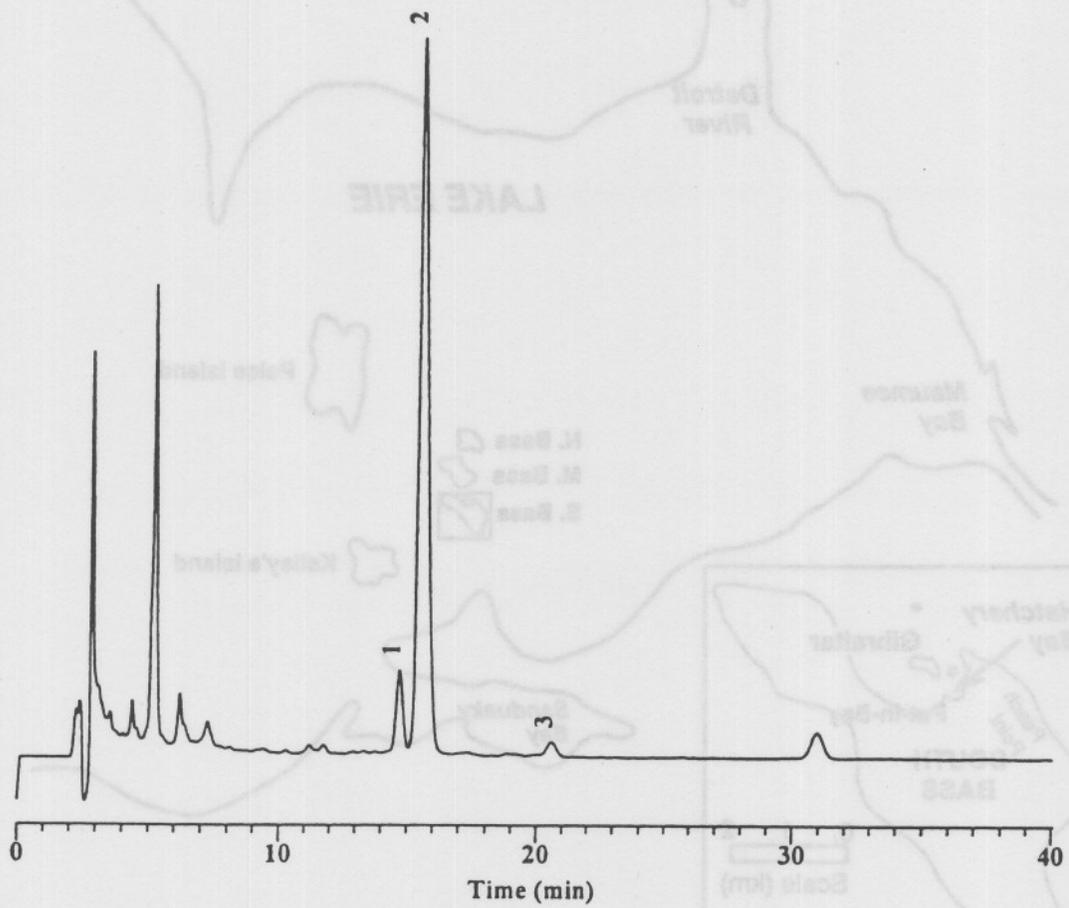


Figure 4. High performance liquid chromatography (HPLC) of LE-3 toxin fraction.

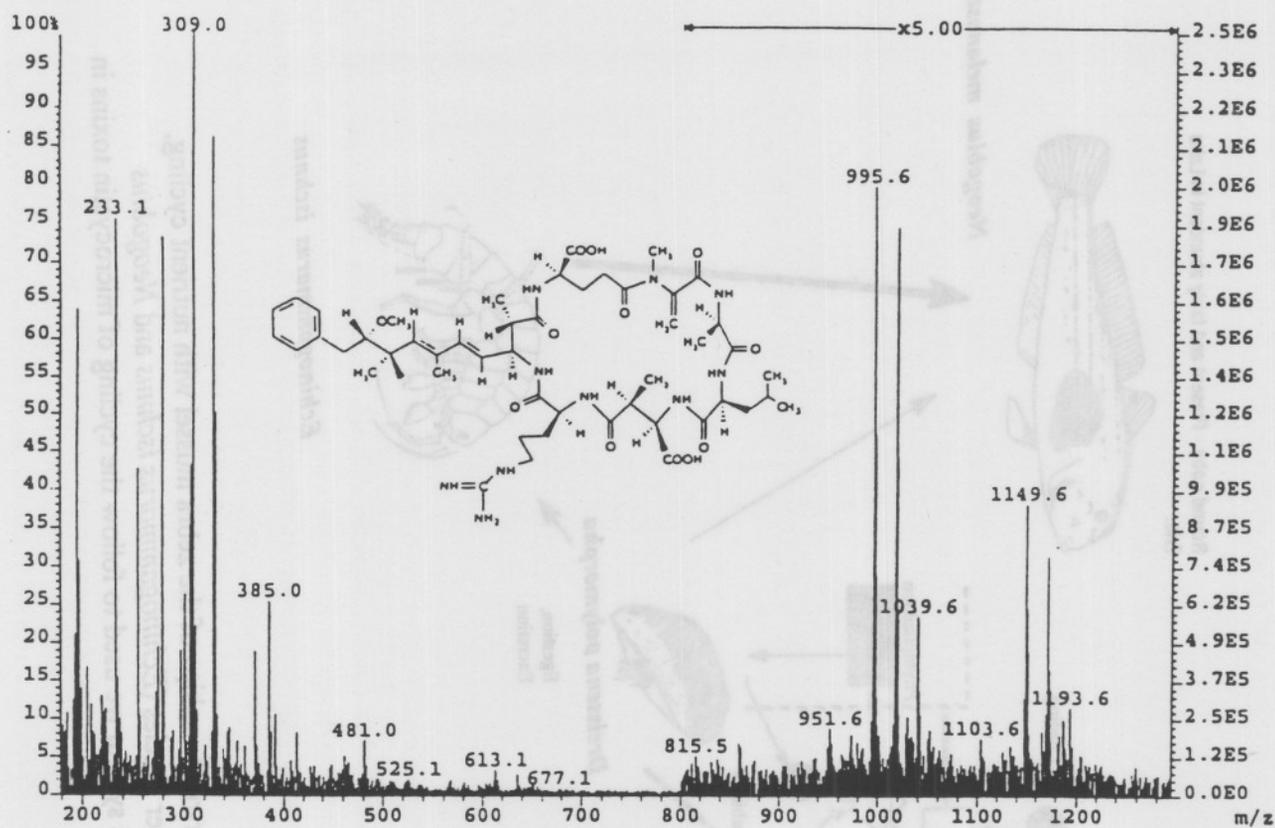


Figure 5. FABMS identification of 1 as MCYST-LR (M+H)⁺ = 9995.6 (x-axis = mass/charge ratio, y-axis = intensity as percent of base peak).

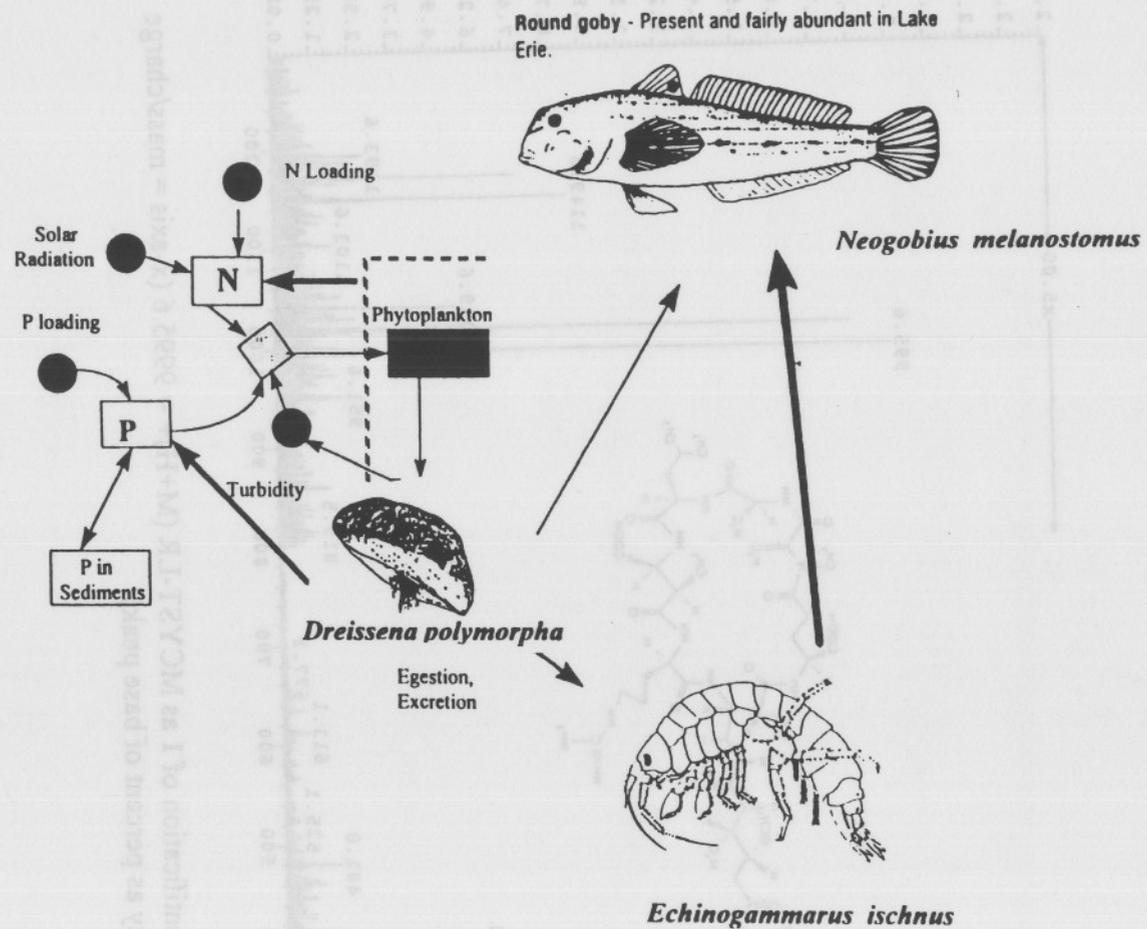


Figure 6. Diagram showing the inter-relationships of the zebra mussel with nutrient cycling, phytoplankton, and two other invader species (*Echinogammarus ischnus* and *Neogobius melanostomus*) in the model trophic system we used to follow the cycling of microcystin toxins in the Lake Erie food web.

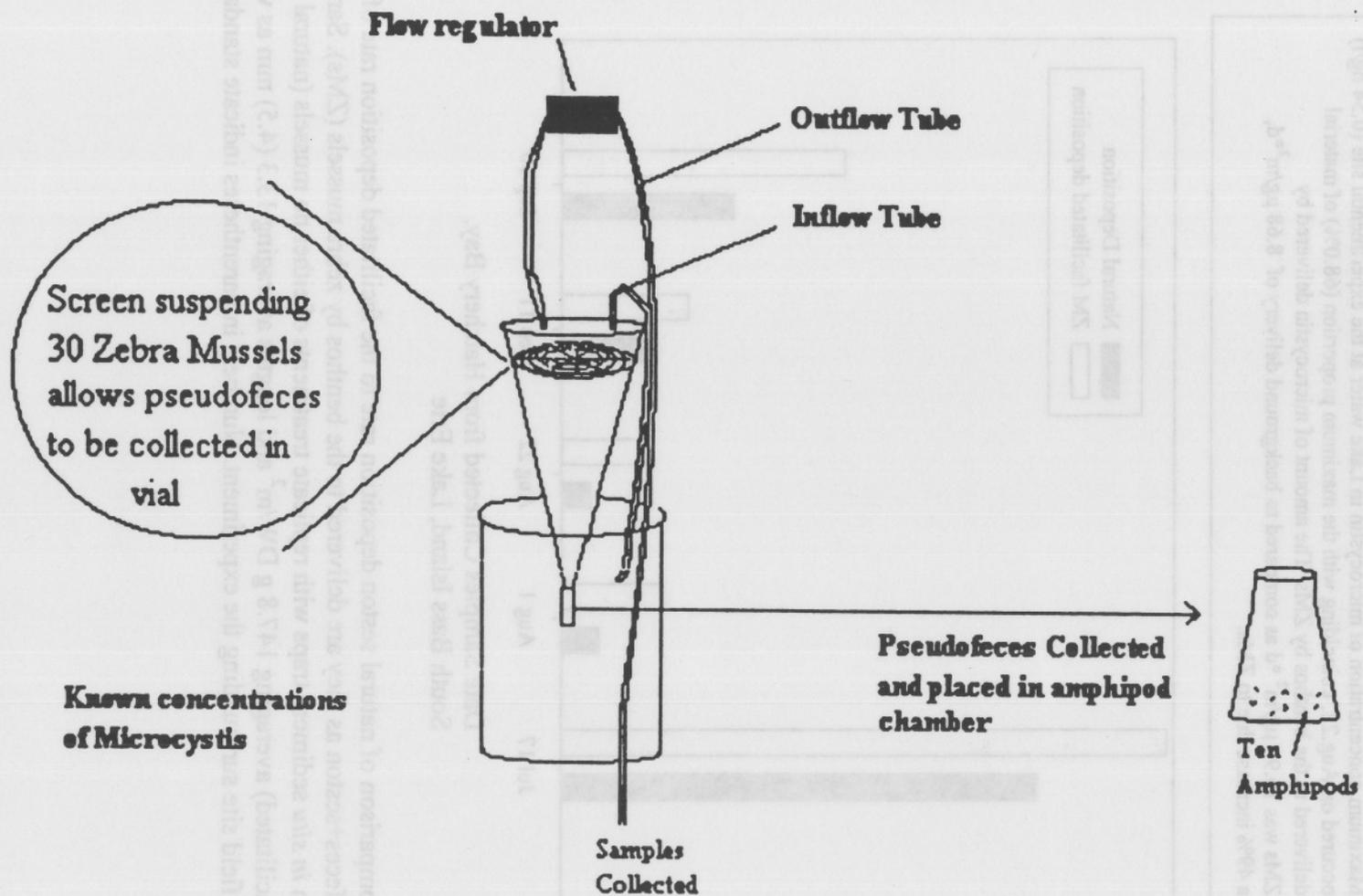


Figure 7. Detail of experimental set-up showing one of the 1-liter Imhoff cone microcosms used to expose replicate zebra mussels to toxic *Microcystis* treatments and to collect feces/pseudofeces produced during the exposures. Replicate samples of the feces and pseudofeces were collected for toxin analysis and to use in feeding amphipods. Samples of inflowing and outflowing water were also collected to determine the clearance of algae from the treatment water by the zebra mussels during exposure.

NOTE:

Maximum concentration of microcystin in Lake water at the experimental site (0.54 ng/l) occurred on Aug.22, coinciding with the maximum proportion (68.0%) of material delivered to the benthos by ZMs. The amount of microcystin delivered by ZMs was $16.94 \mu\text{g}/\text{m}^2 \cdot \text{d}$ as compared to background delivery of $8.68 \mu\text{g}/\text{m}^2 \cdot \text{d}$, a 49% increase due to ZMs.

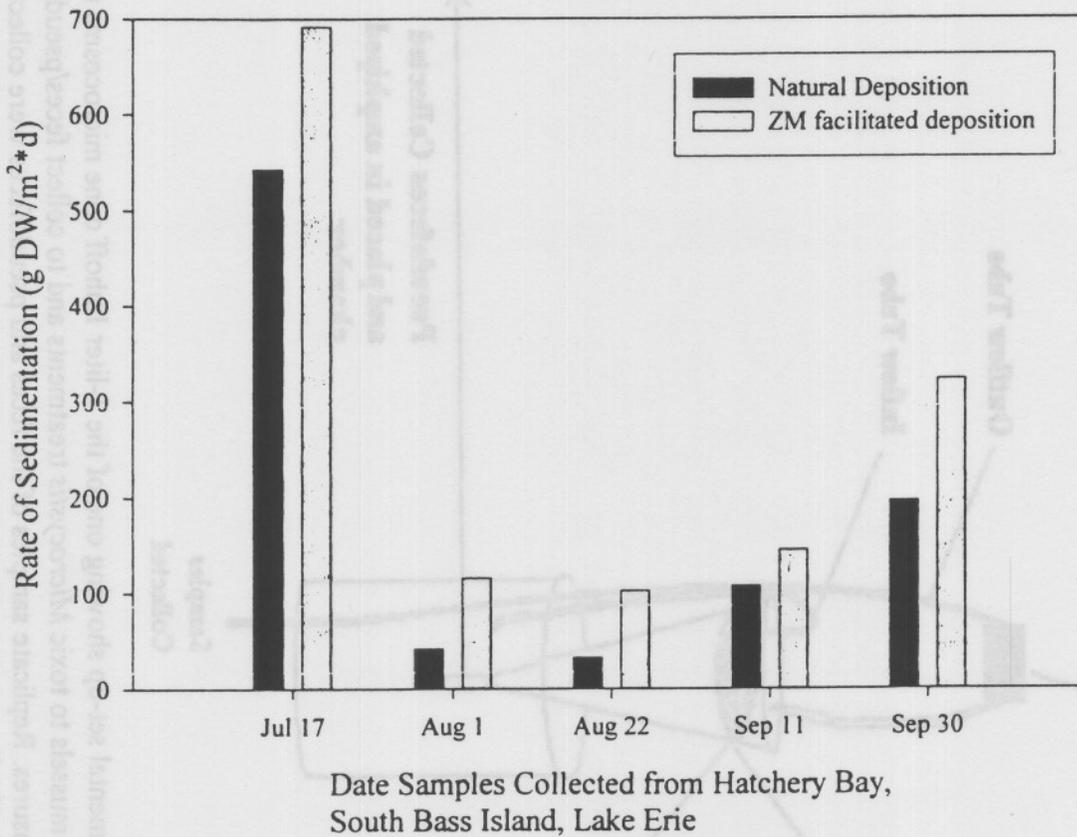


Figure 8. A comparison of natural seston deposition rate to the facilitated deposition rate of feces+pseudofeces+seston as they are delivered to the benthos by zebra mussels (ZMs). Samples were collected from *in situ* sediment traps with replicate treatments of either no mussels (natural deposition) or mussels (facilitated) averaging $147.8 \text{ g DW}/\text{m}^2$ and lengths averaging $13.3 (4.5) \text{ mm}$ as were present at the field site surrounding the experiment. Numbers in parentheses indicate standard deviations.

- Lake Erie surface water (80 ng/L microcystin content)
- Cultured green algae suspended in filtered lake Erie water (100 ng/L microcystin content)
- Cultured *M. aeruginosa* suspended in filtered Lake Erie water (200 ng/L microcystin content)
- Cultured *M. aeruginosa* suspended in filtered Lake Erie water (2000 ng/L microcystin content)
- Cultured *M. aeruginosa* suspended in filtered Lake Erie water (6900 ng/L microcystin content)
- Cultured *M. aeruginosa* suspended in filtered Lake Erie water (13,000 ng/L microcystin content)

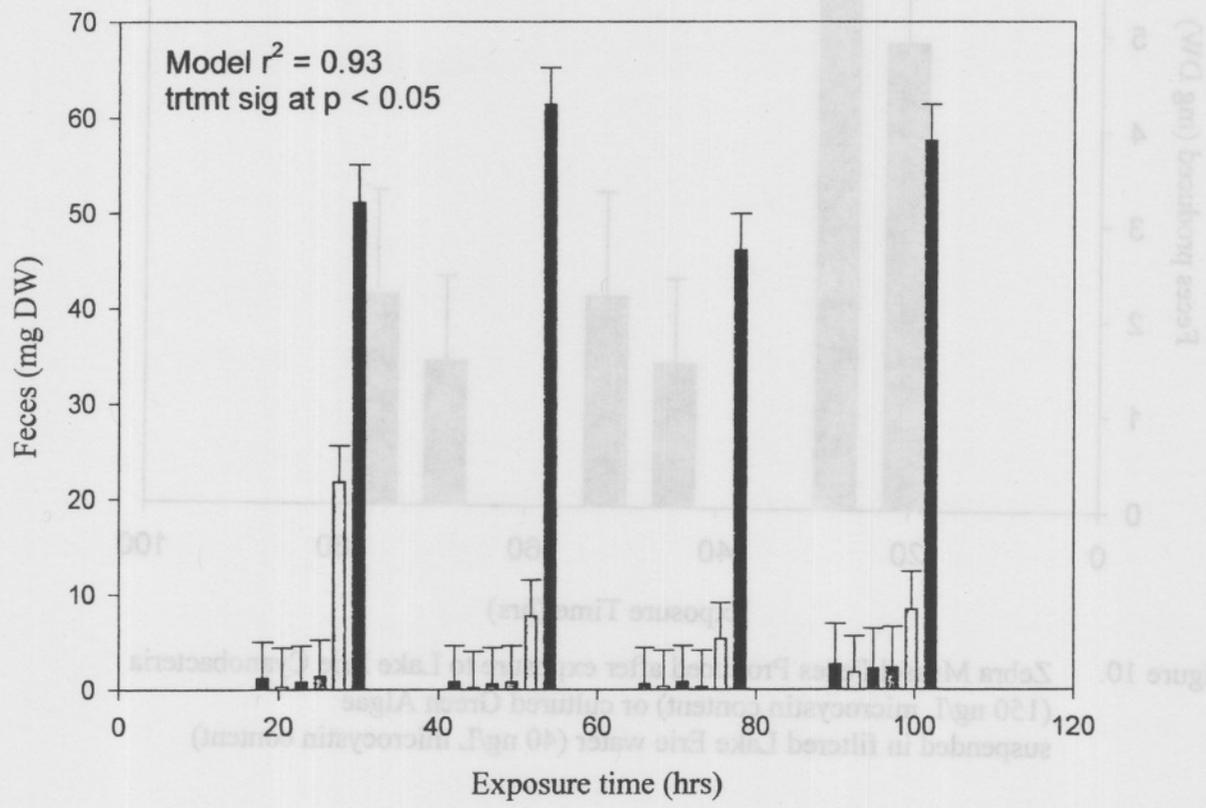


Figure 9. Amounts of Zebra Mussel feces produced during exposure to different mixtures of natural or cultured cyanobacteria and green algae suspended in filtered Lake Erie water.

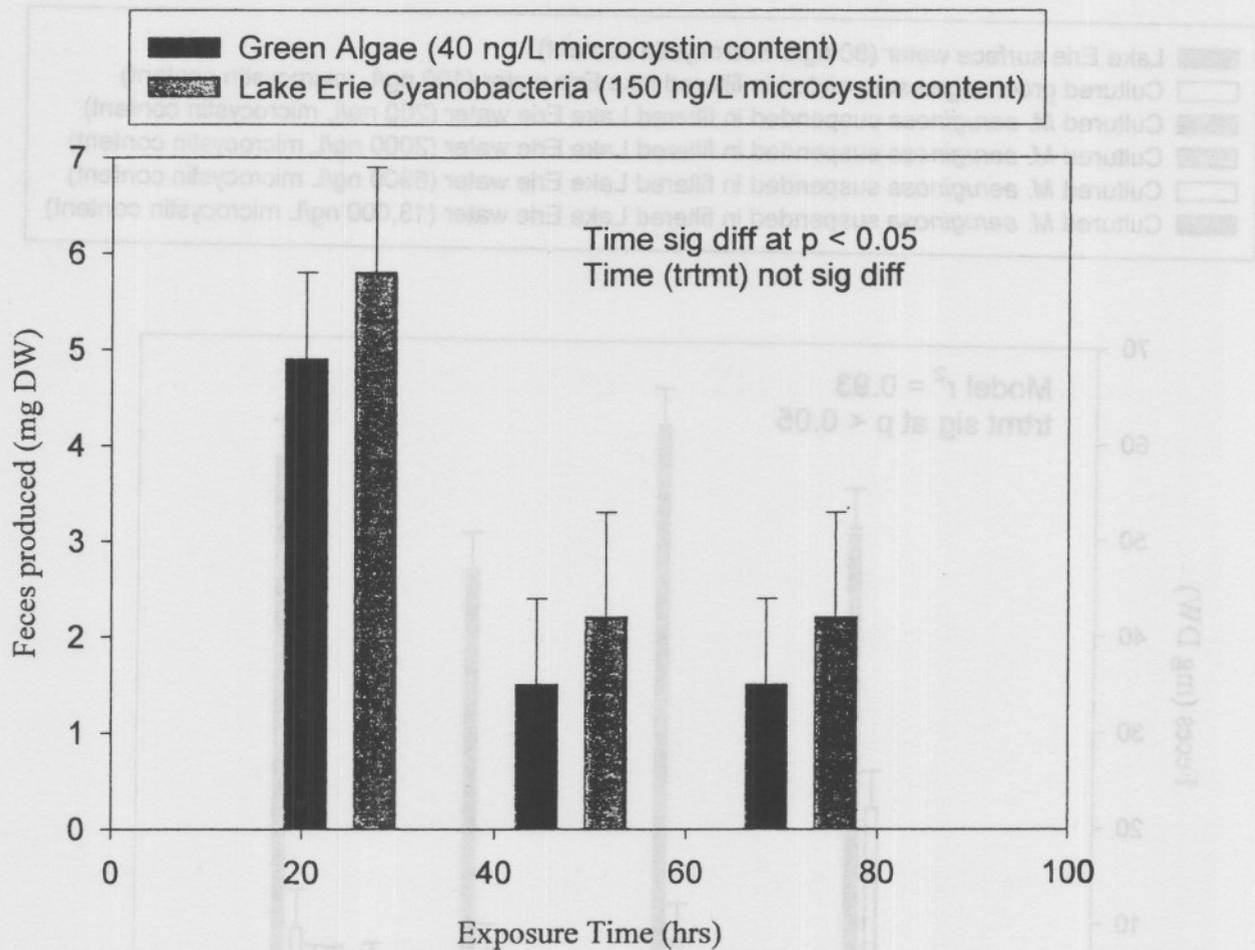


Figure 10. Zebra Mussel Feces Produced after exposure to Lake Erie Cyanobacteria (150 ng/L microcystin content) or cultured Green Algae suspended in filtered Lake Erie water (40 ng/L microcystin content)

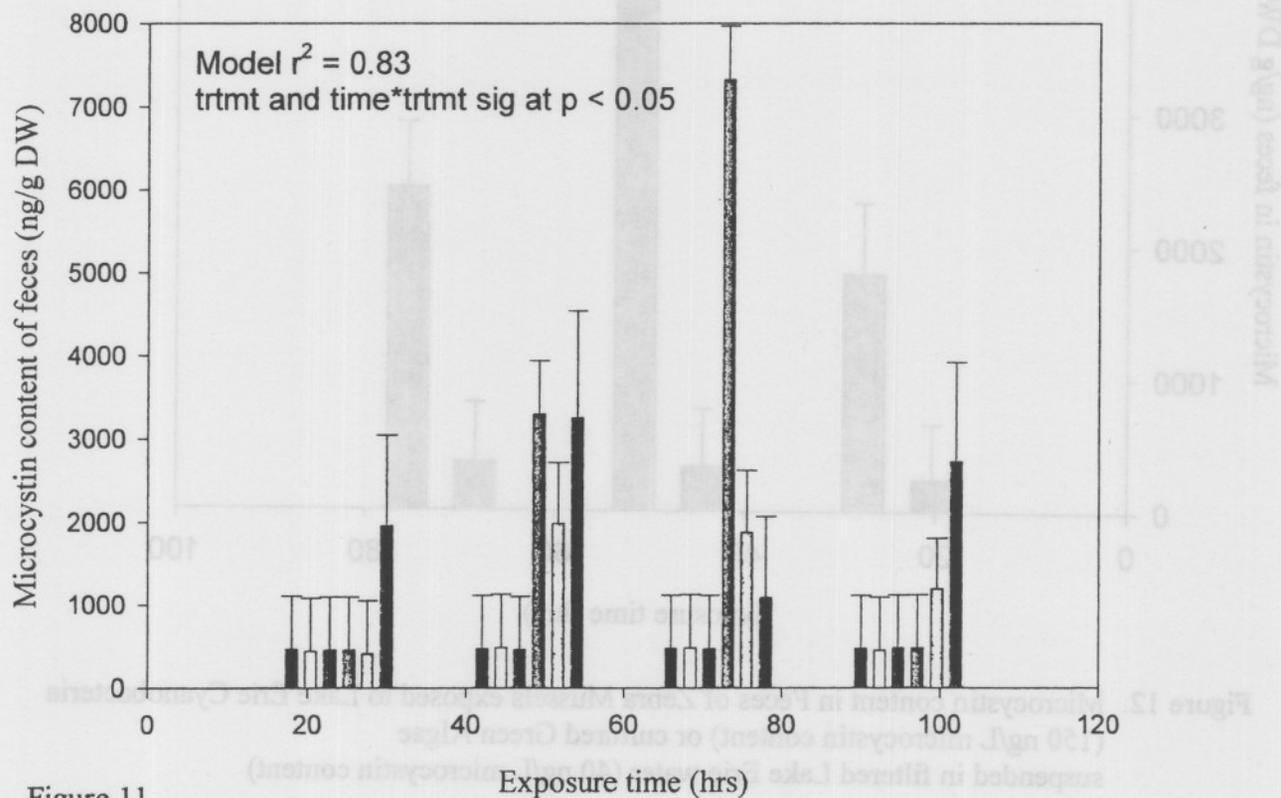
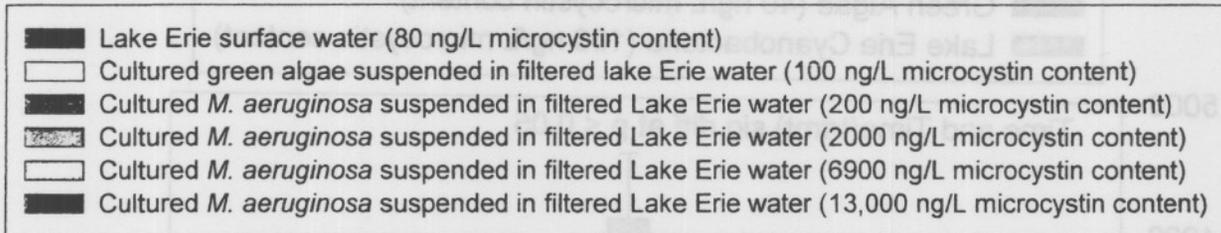


Figure 11.

Amounts of microcystin in Zebra Mussel feces during exposure to different mixtures of natural or cultured cyanobacteria and green algae suspended in filtered Lake Erie water

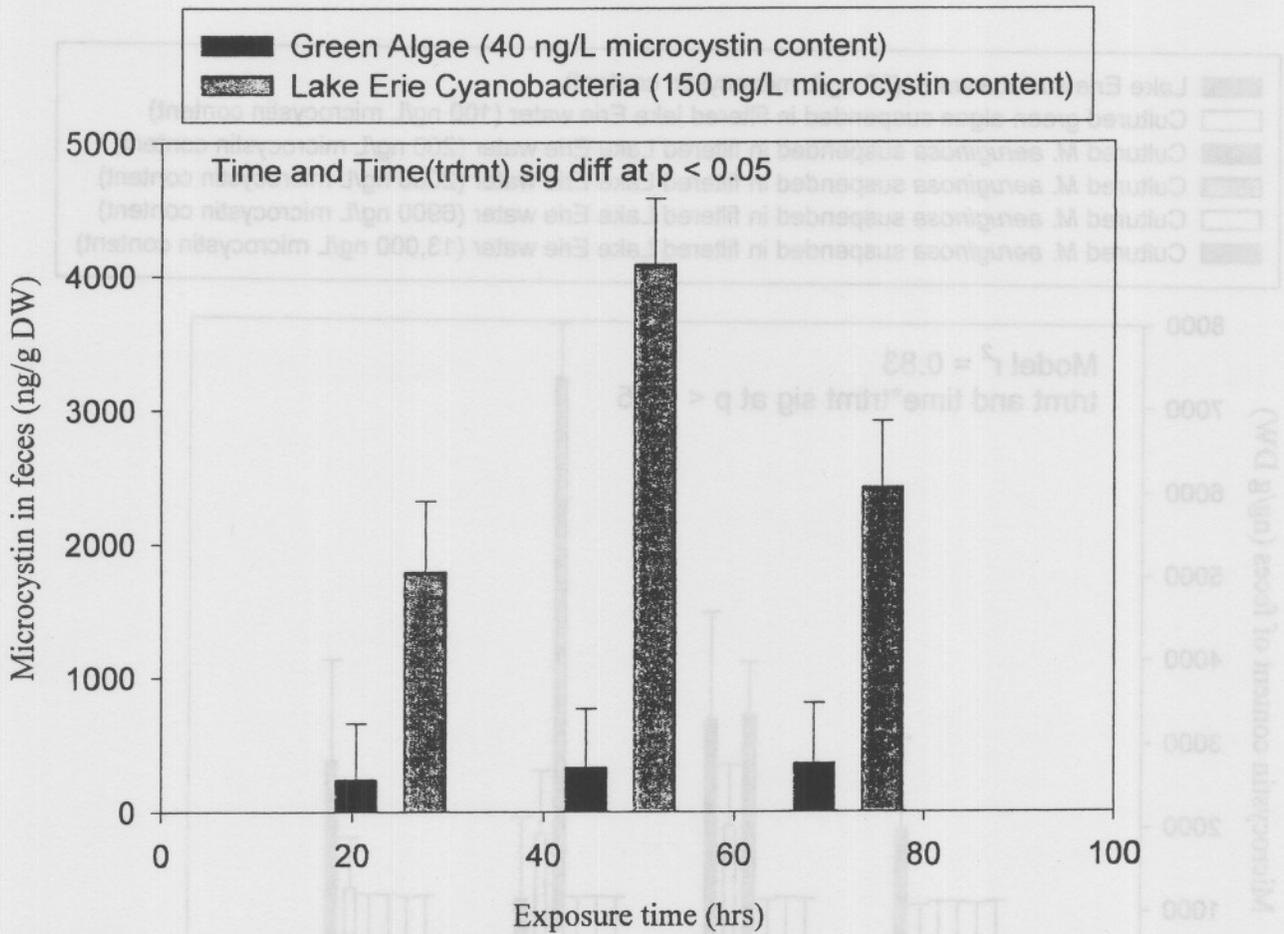


Figure 12. Microcystin content in Feces of Zebra Mussels exposed to Lake Erie Cyanobacteria (150 ng/L microcystin content) or cultured Green Algae suspended in filtered Lake Erie water (40 ng/L microcystin content)

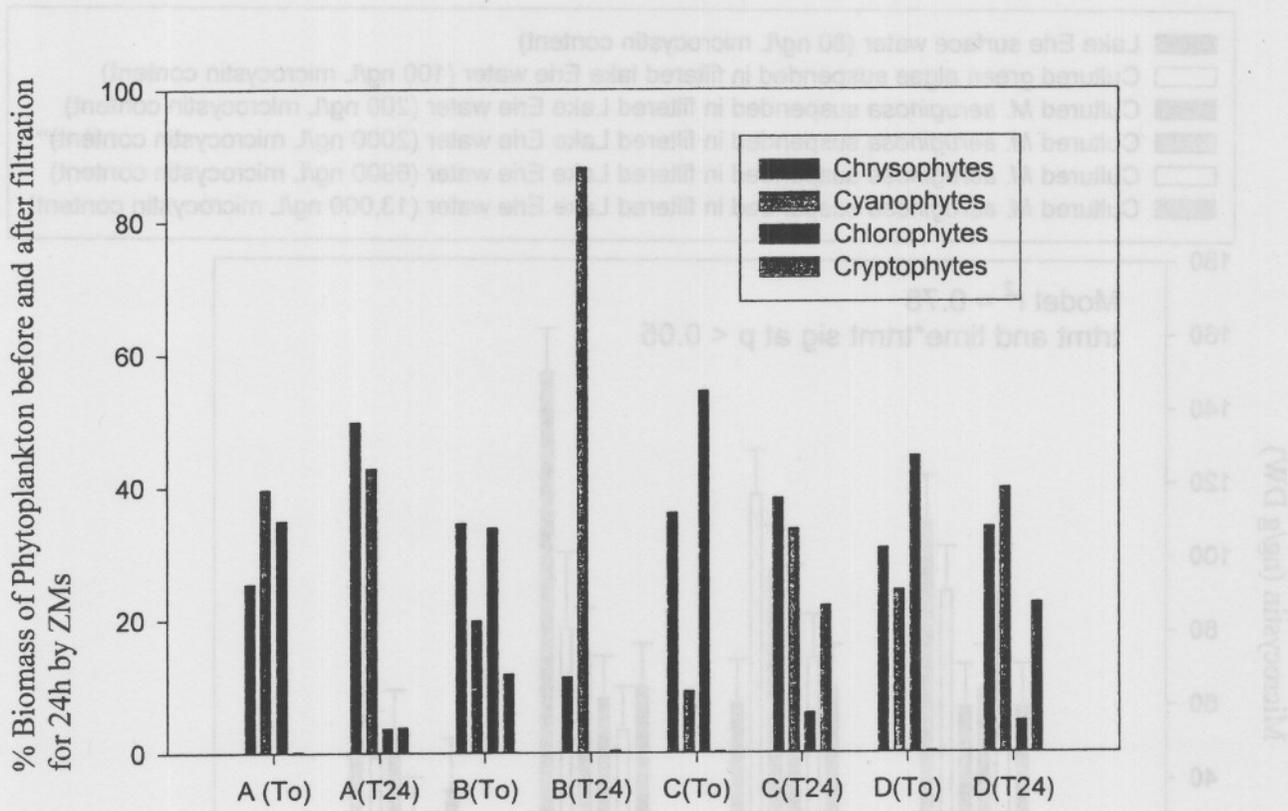


Figure 13. Percentages of total Phytoplankton Biomass remaining after Zebra Mussels filtered different mixtures of algae and cyanobacteria in Lake Erie water for 24 hrs.

- A= Concentrated Lake Erie Cyanobacteria suspended in filtered Lake Erie water
- B= Surface water from Lake Erie
- C= Concentrated *Chlamydomonas reinhardtii* and *Chlorella vulgaris* suspended in filtered Lake Erie water
- D= Treatments A and C combined

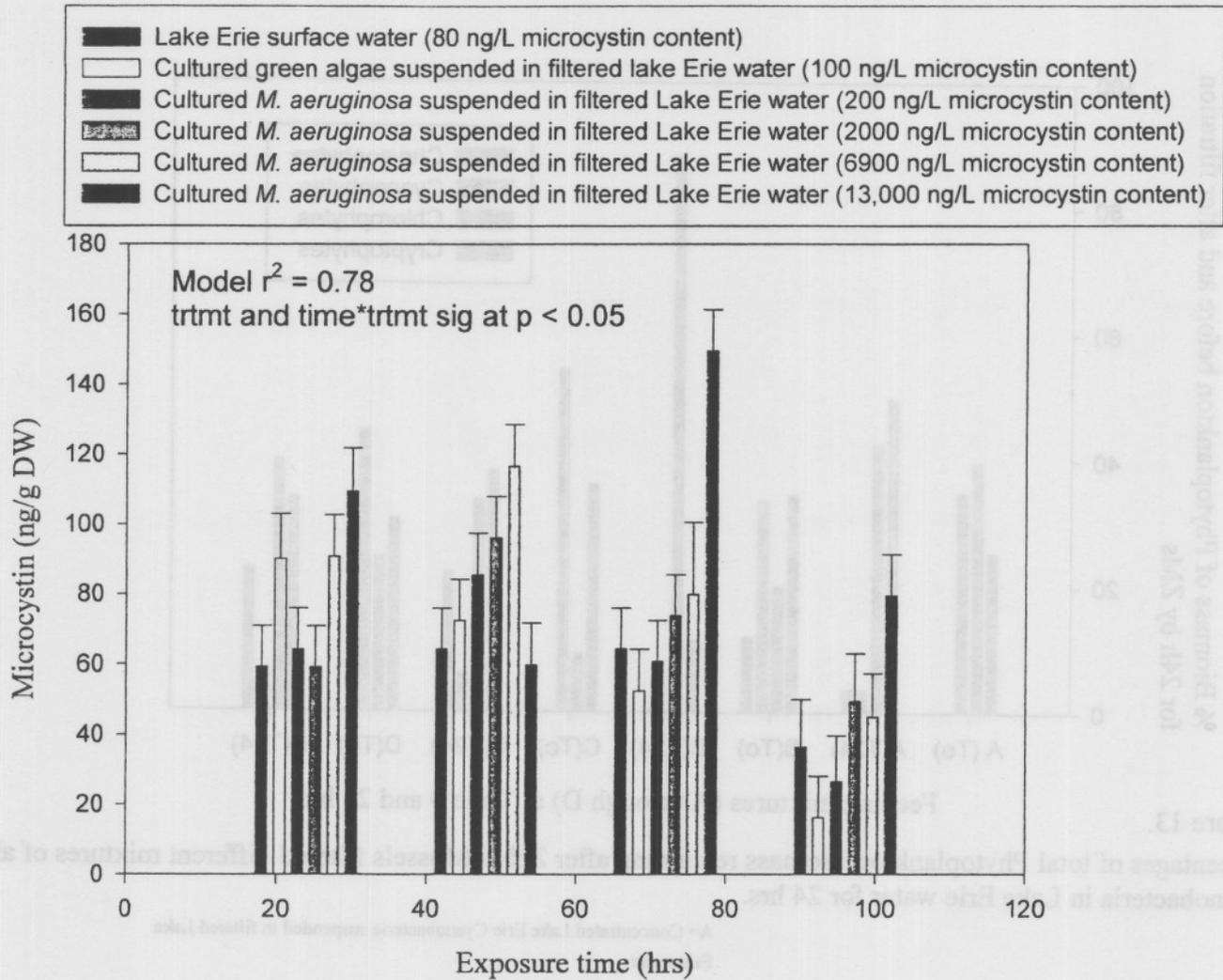


Figure 14. Amounts of microcystin in Zebra Mussel soft tissues during exposure to different mixtures of natural or cultured cyanobacteria and green algae suspended in filtered Lake Erie water

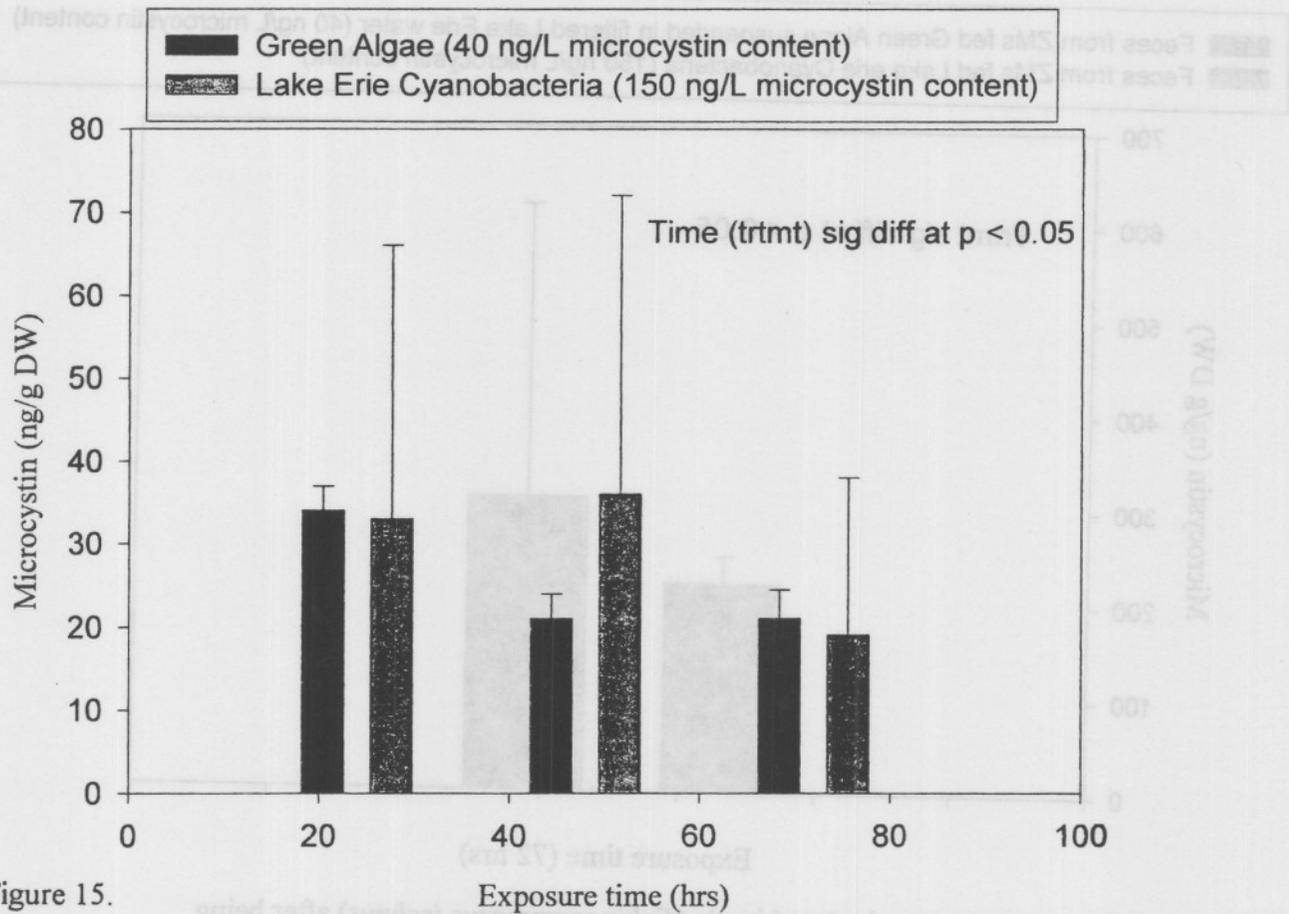


Figure 15.

Microcystin content in Zebra Mussel tissue after exposure to Lake Erie Cyanobacteria (150 ng/L microcystin content) or cultured Green Algae suspended in filtered Lake Erie water (40 ng/L microcystin content).

Feces from ZMs fed Green Algae suspended in filtered Lake Erie water (40 ng/L microcystin content)
 Feces from ZMs fed Lake erie Cyanobacteria (150 ng/L microcystin content)

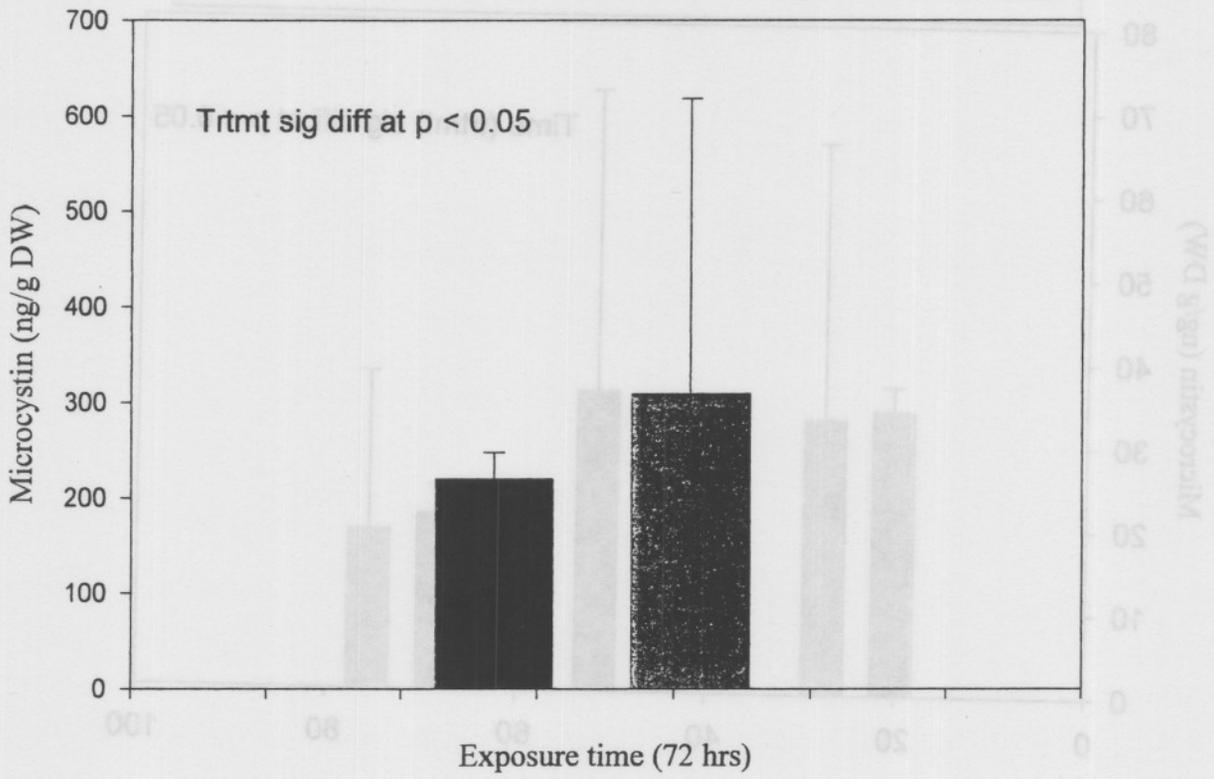


Figure 16. Microcystin content in Amphipods (*Echinogammarus ischnus*) after being fed Zebra Mussel feces contaminated with microcystin from different sources

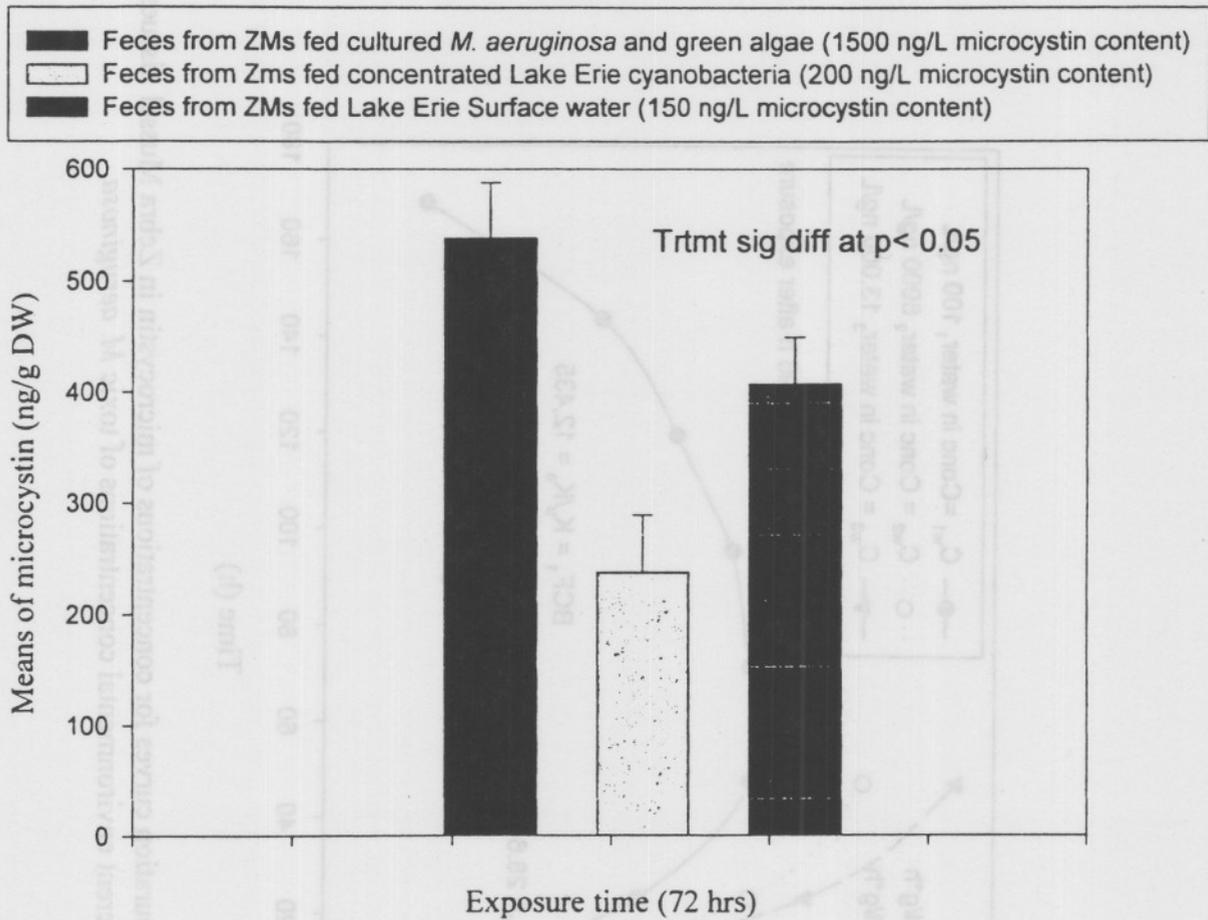


Figure 17.

Microcystin content of Amphipods (*Echinogammarus ischnus*) after being fed Zebra mussel feces contaminated with microcystin from different sources.

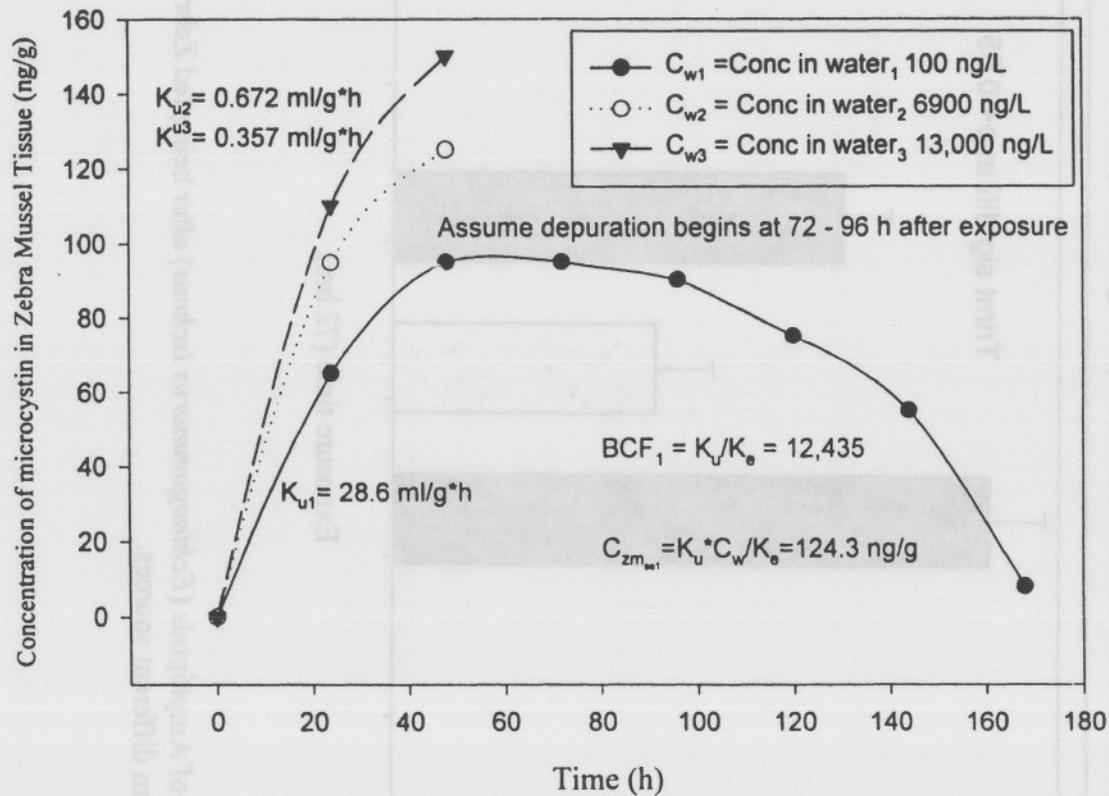


Figure 18. Uptake and depuration curves for concentrations of microcystin in Zebra Mussel tissues exposed to different environmental concentrations of toxic *M. aeruginosa*.

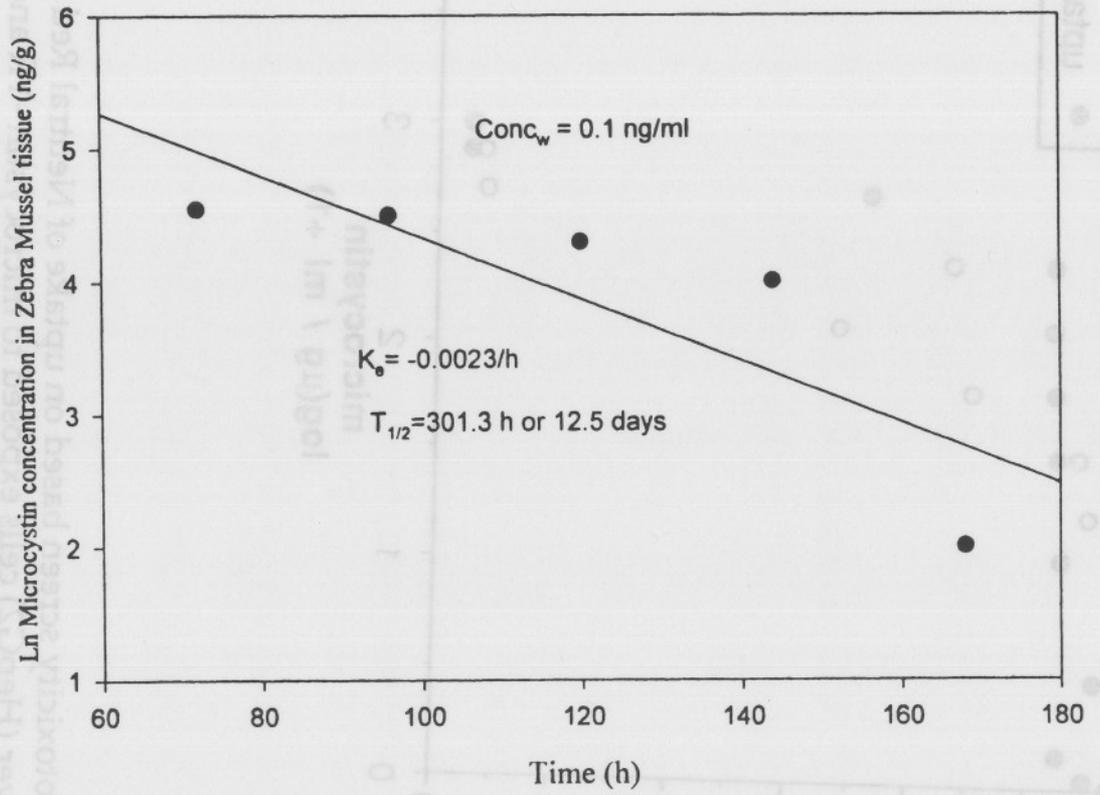


Figure 19. Depuration curve for Ln concentration of microcystin in Zebra Mussel tissues.

**Cytotoxicity tests for microcystin
Lr and Yr using HepG2 human liver cell line**

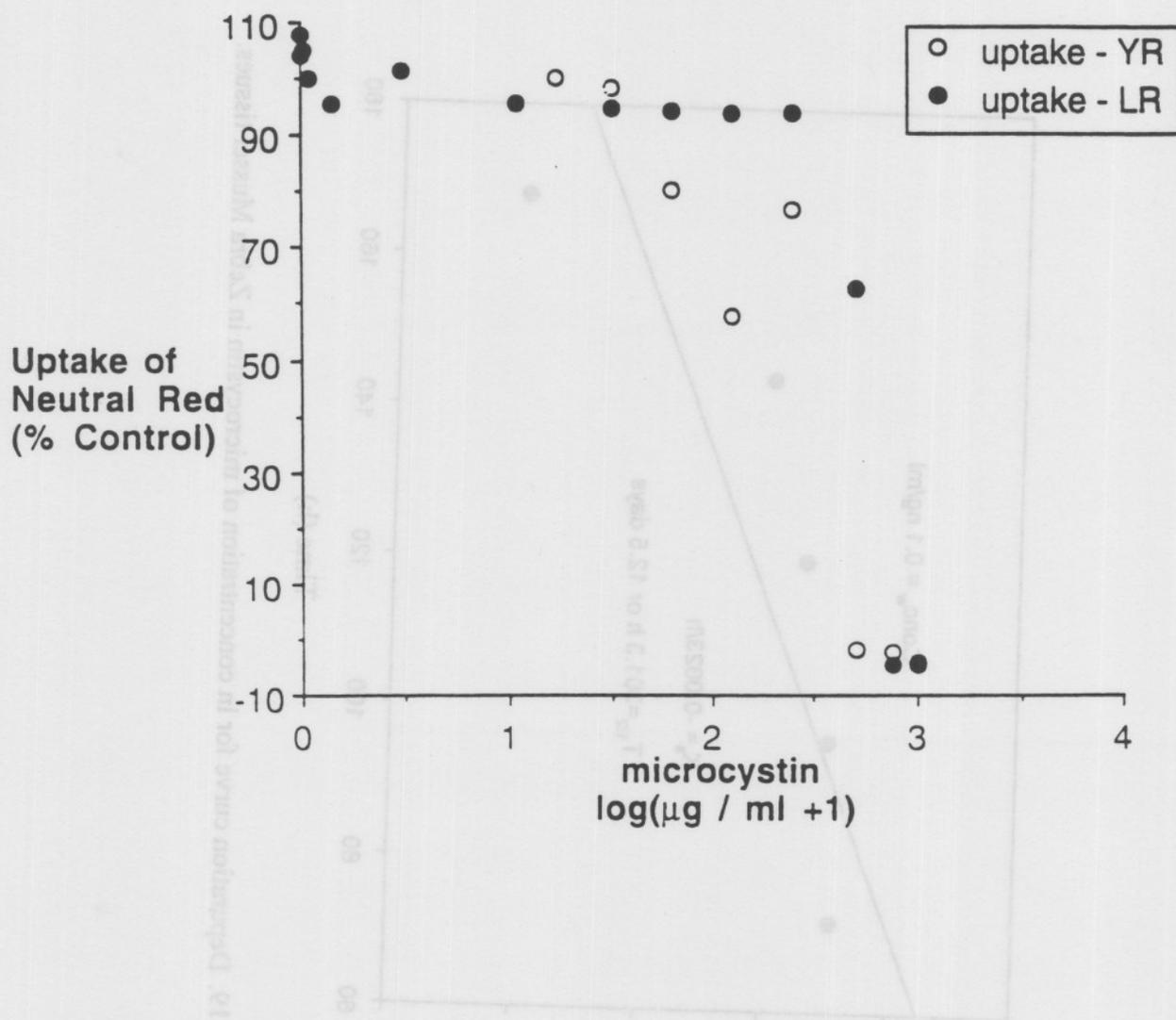


Figure 20. Cytotoxicity screen based on uptake of Neutral Red dye by human liver (HepG2) cells exposed to microcystin -YR and -LR. Values are corrected for diffusive uptake by dead cells. Results were analyzed using nonlinear regression in Sigmaplot.

Genotoxicity tests on human liver cell line (HepG2)

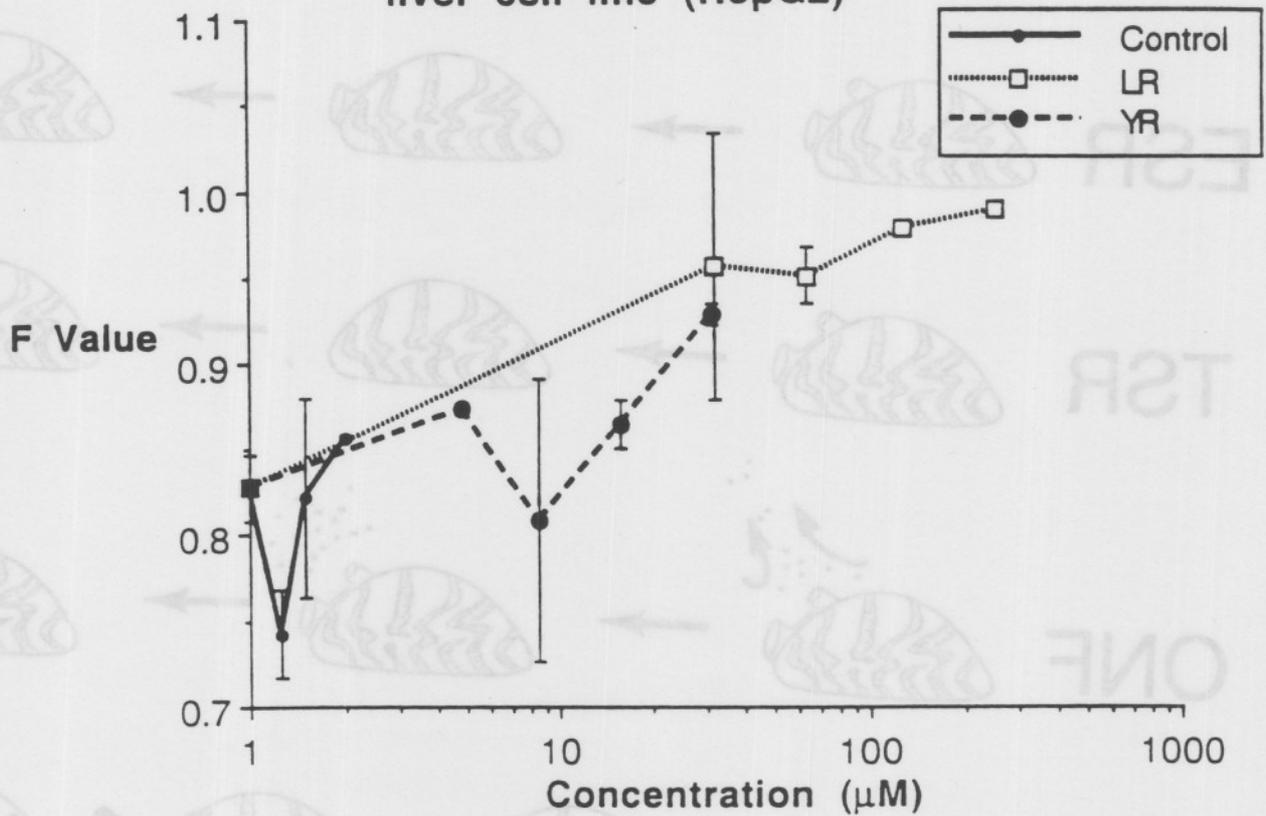


Figure 21. Assessment of genotoxicity of microcystin -YR and -LR based on exposure of DNA to suspected genotoxins. F-values represent the ratio of double-stranded DNA to total DNA. Lower values indicate greater presence of single-stranded DNA (ie. greater genotoxicity).

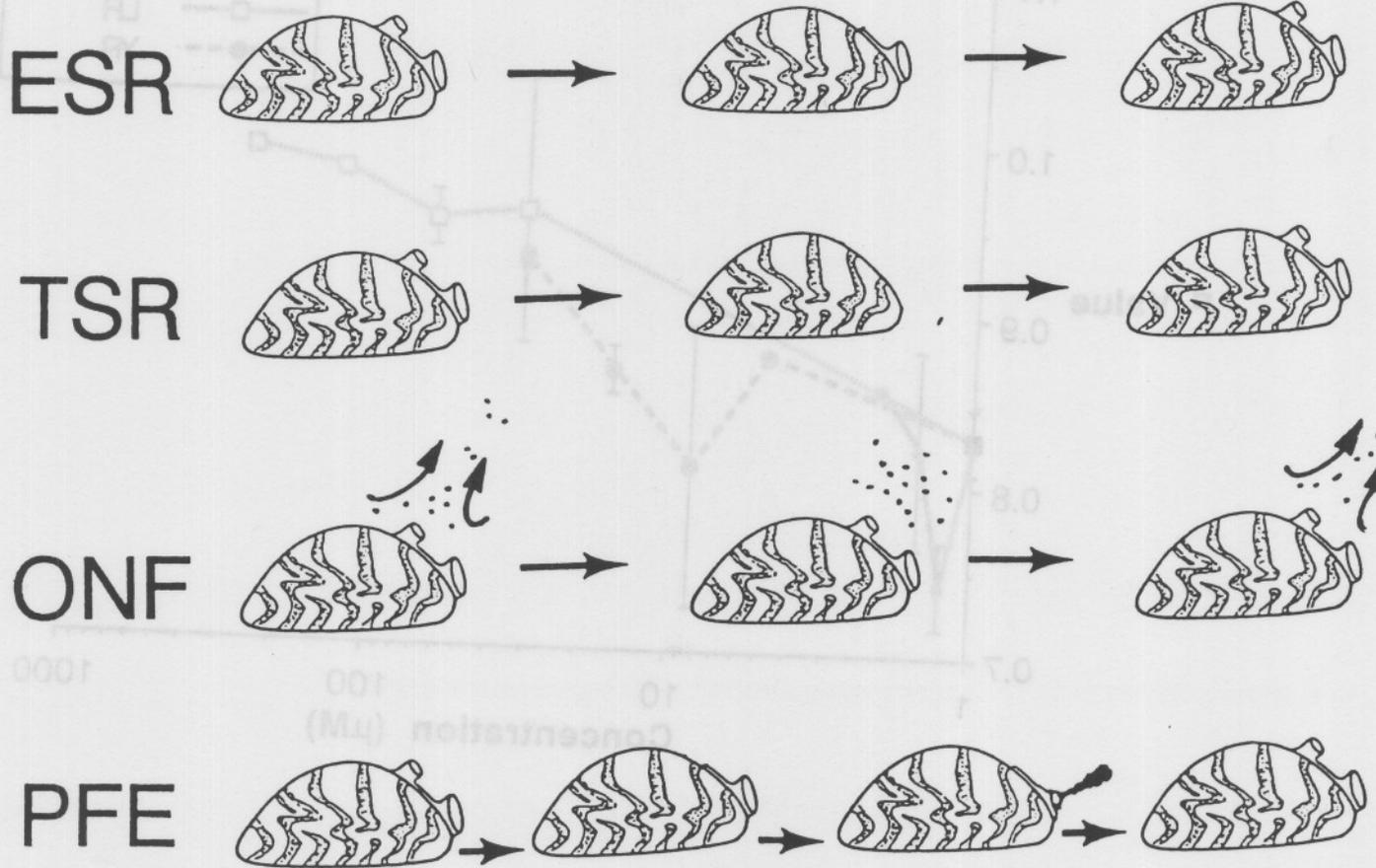
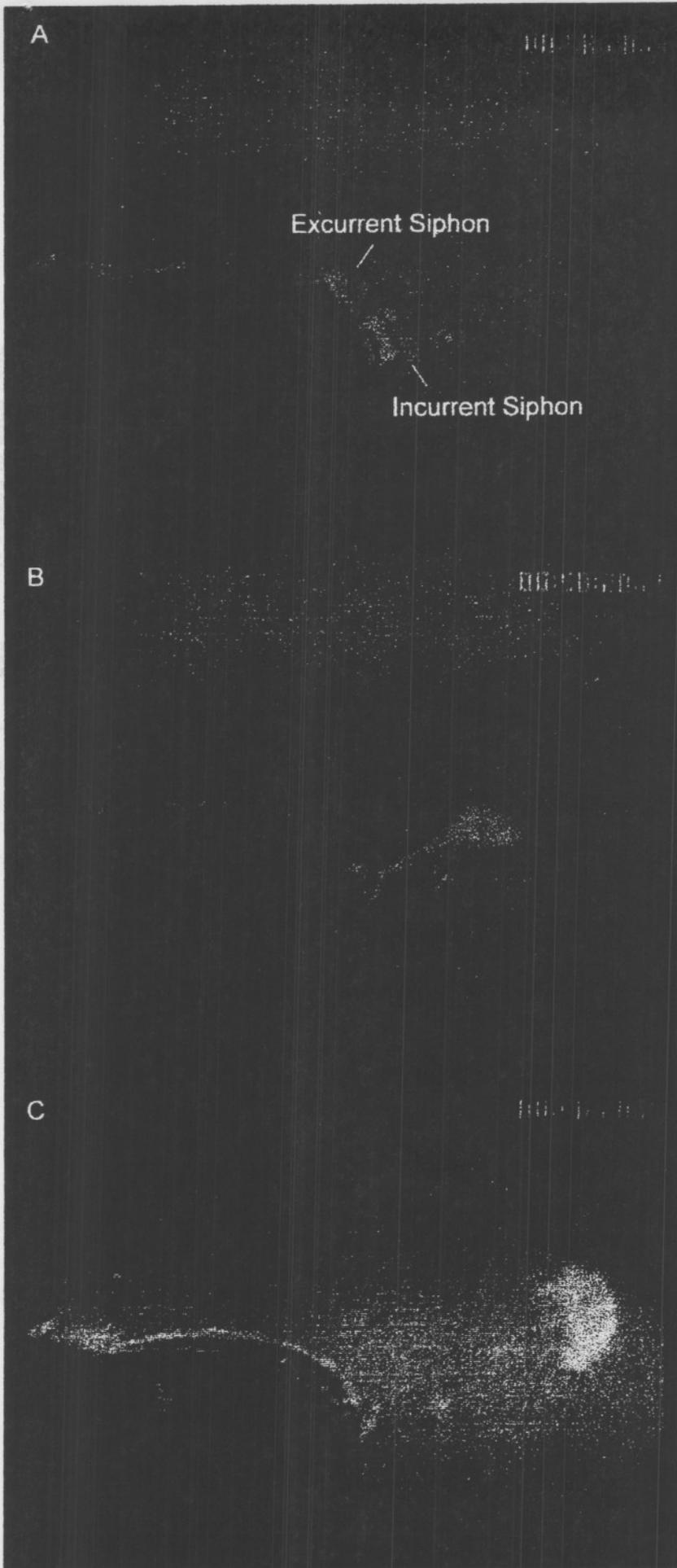


Figure 22. Non-filtering behaviors recorded in time-budget analyses of 1-2 h videotapes of mussel behavior. The diagram shows the mussel in the normal, open state, then moving into the behavior, and then returning to the normal state. Behaviors recorded were ESR (excurrent siphon retraction), TSR (two siphon retraction, which can include complete closure of valves), ONF (open but not creating a filtering current), PFE (pseudofeces expulsion). Most ESRs and TSRs were complete, but we also recorded significant partial retractions.

Figure 23.

Zebra mussel expelling *Microcystis* as pseudofeces.
(A) mussel filtering with siphons in normal position.
(B) Excurrent siphon retracted and incurrent siphon starting to expell the *Microcystis* as pseudofeces. (C) Pseudofeces ejected.



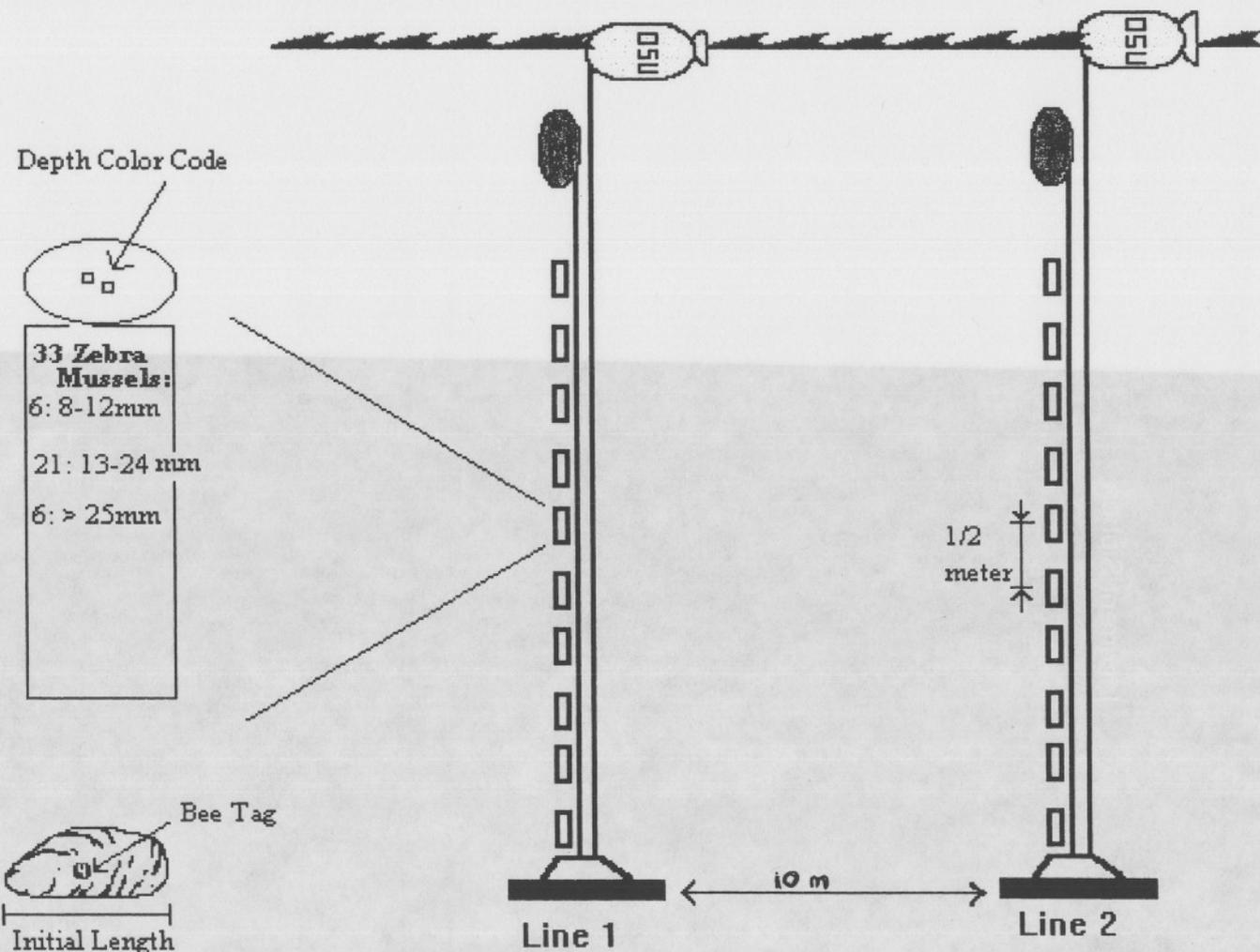


Figure 24. Diagram of the experimental set-up designed to measure zebra mussel growth in years 1995-1997. Shown are the growth cages attached to lines and placement on the lines. Also shown is a detail of a tagged mussel and the size-class distribution of 33 mussels placed in each cage.

Figure 24. Diagram of the experimental set-up designed to measure zebra mussel growth in years 1995-1997. Shown are the growth cages attached to lines and placement on the lines. Also shown is a detail of a tagged mussel and the size-class distribution of 33 mussels placed in each cage.

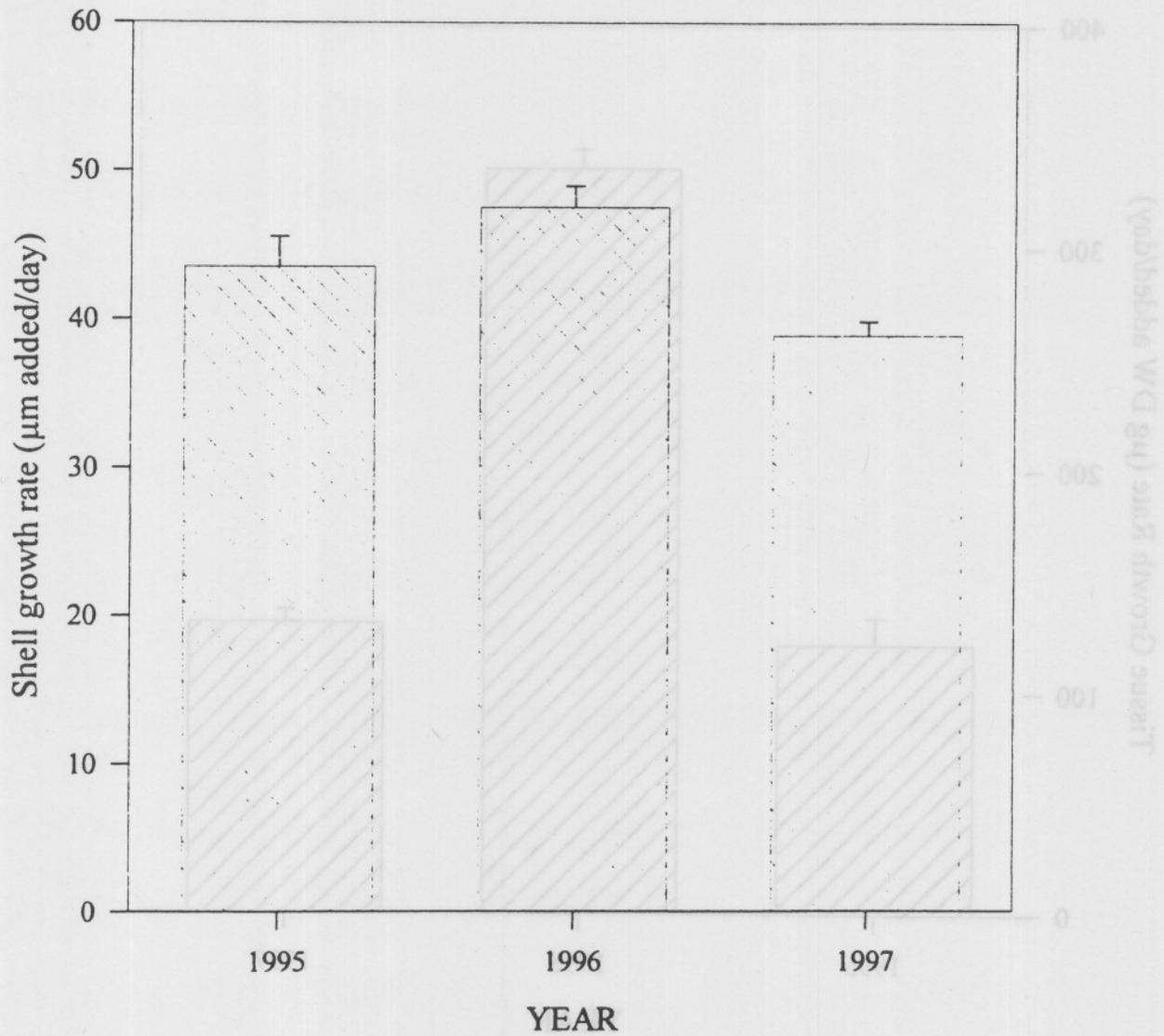


Figure 25. Means of zebra mussel shell growth for all depths combined within each year.

Figure 25. Means of zebra mussel shell growth for all depths combined within each year.

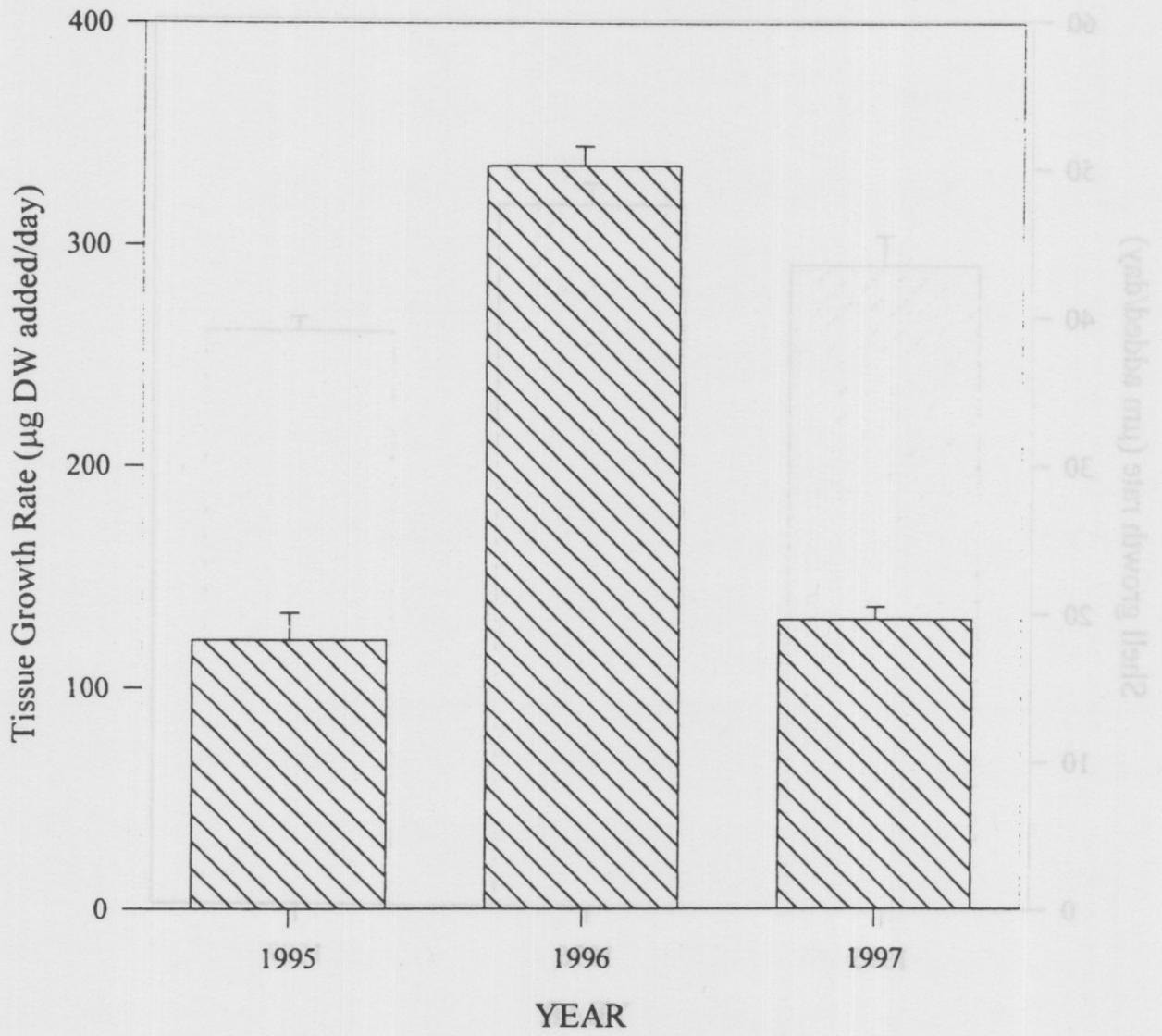


Figure 26. Means of zebra mussel soft tissue growth for all depths combined within each year.

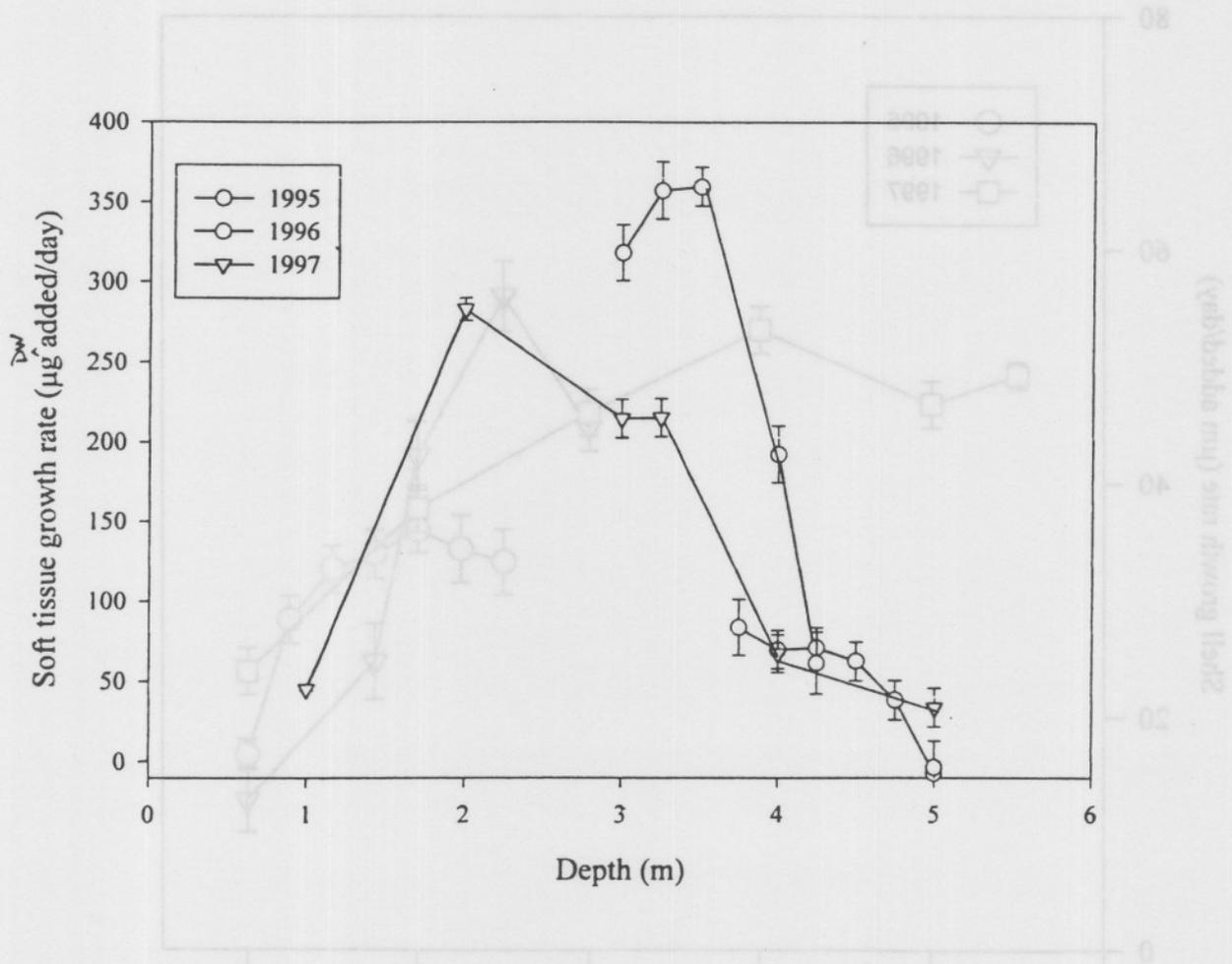


Figure 27. Means and SE of zebra mussel soft tissue growth for all sizes combined at each depth for each year. 1996 and 1997 zebra mussel tissues have significantly higher growth rates than 1995 at 3.5 m below the surface. All show zero or negative growth at the bottom and similar growth rates within 1.5 m from the bottom. Cages above 3.5 m were lost in 1995.

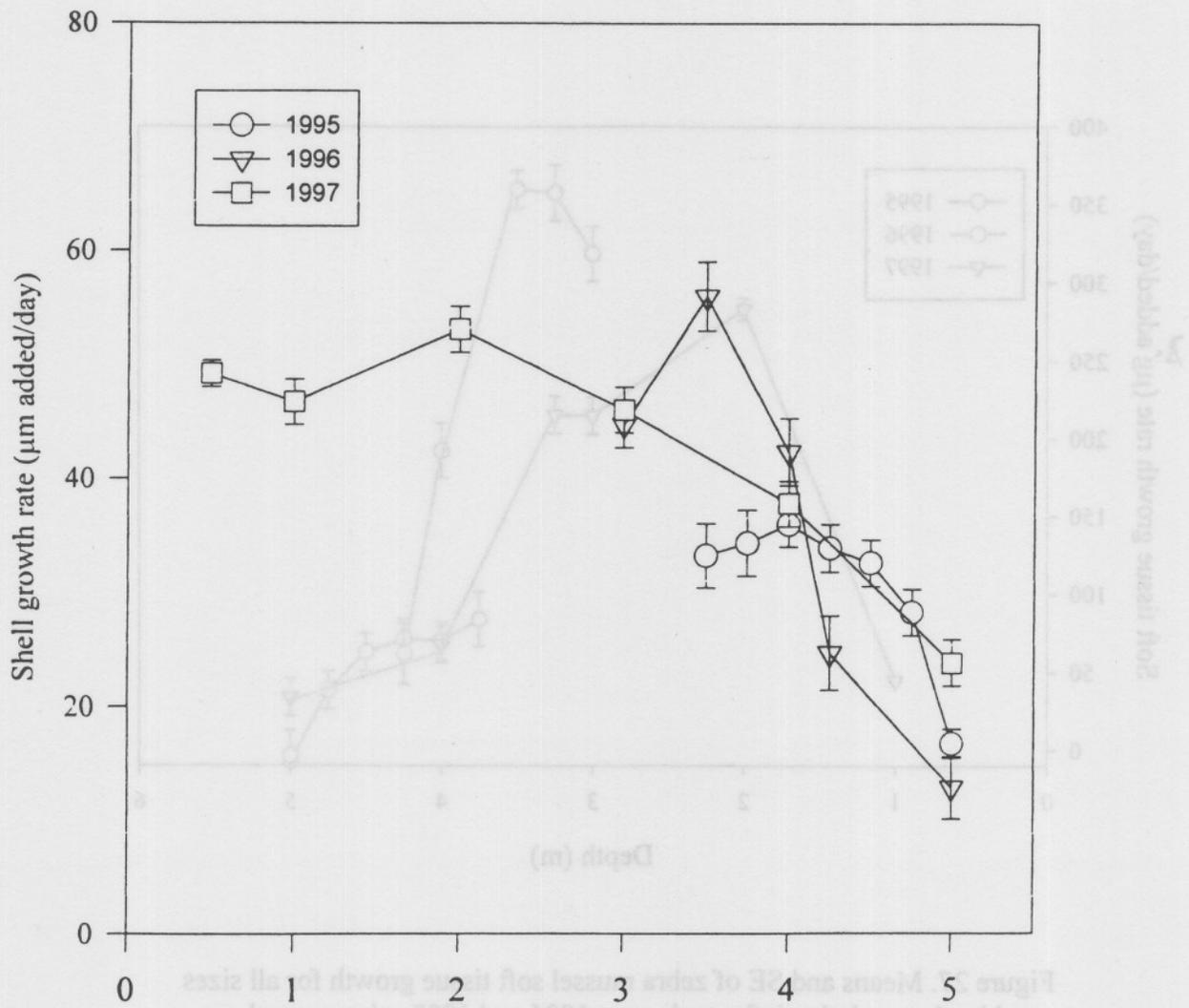


Figure 28. Means of zebra mussel shell growth for all sizes of mussels combined at each depth for each year.

Western Basin

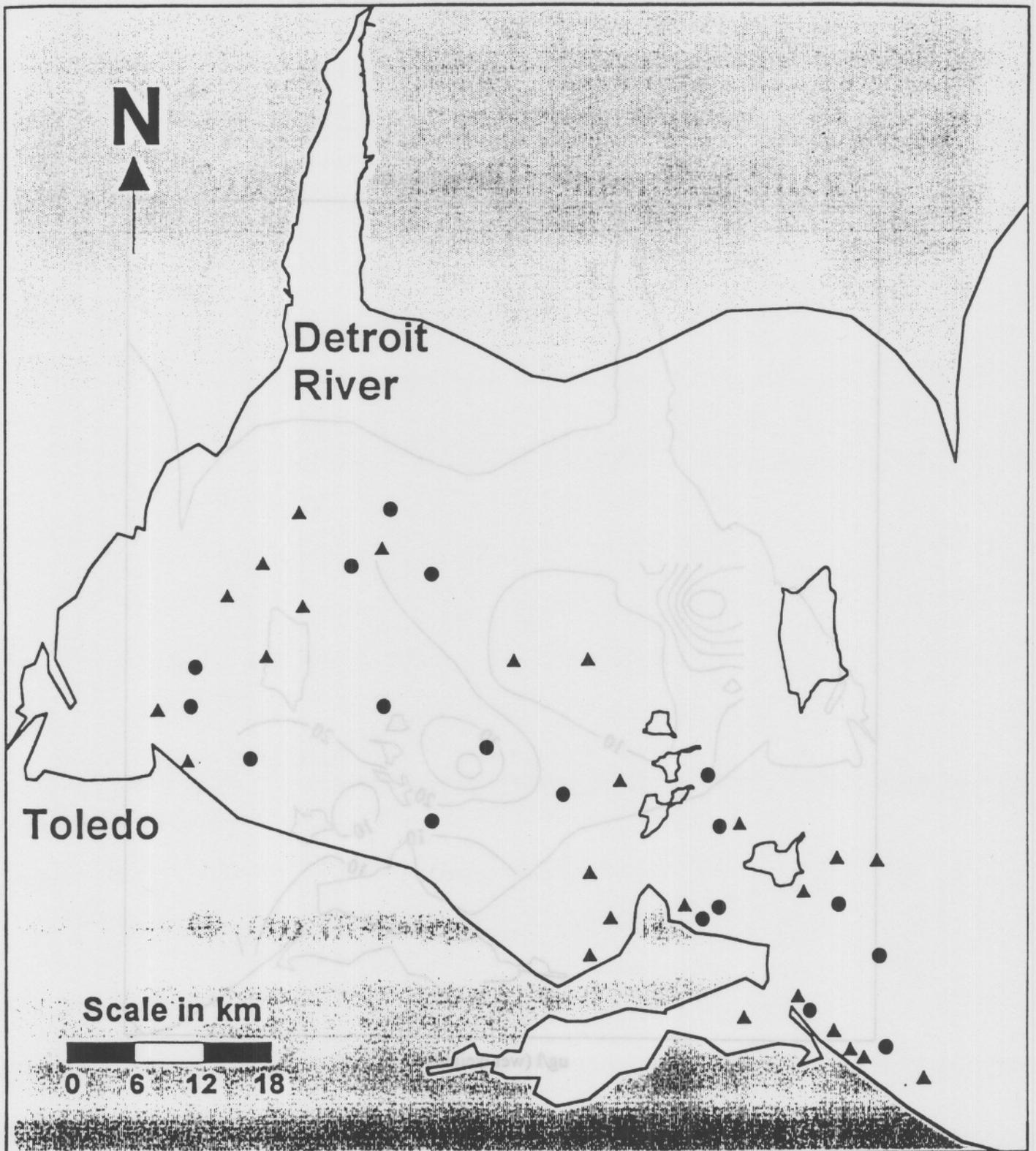


Figure 29. Sites from which plankton samples were taken in 1996 in the western basin of Lake Erie by ODNR field crews.

1996 Sampling Stations
weekly stations ●
monthly stations ▲

June 1996 Cyanophyta Biomass

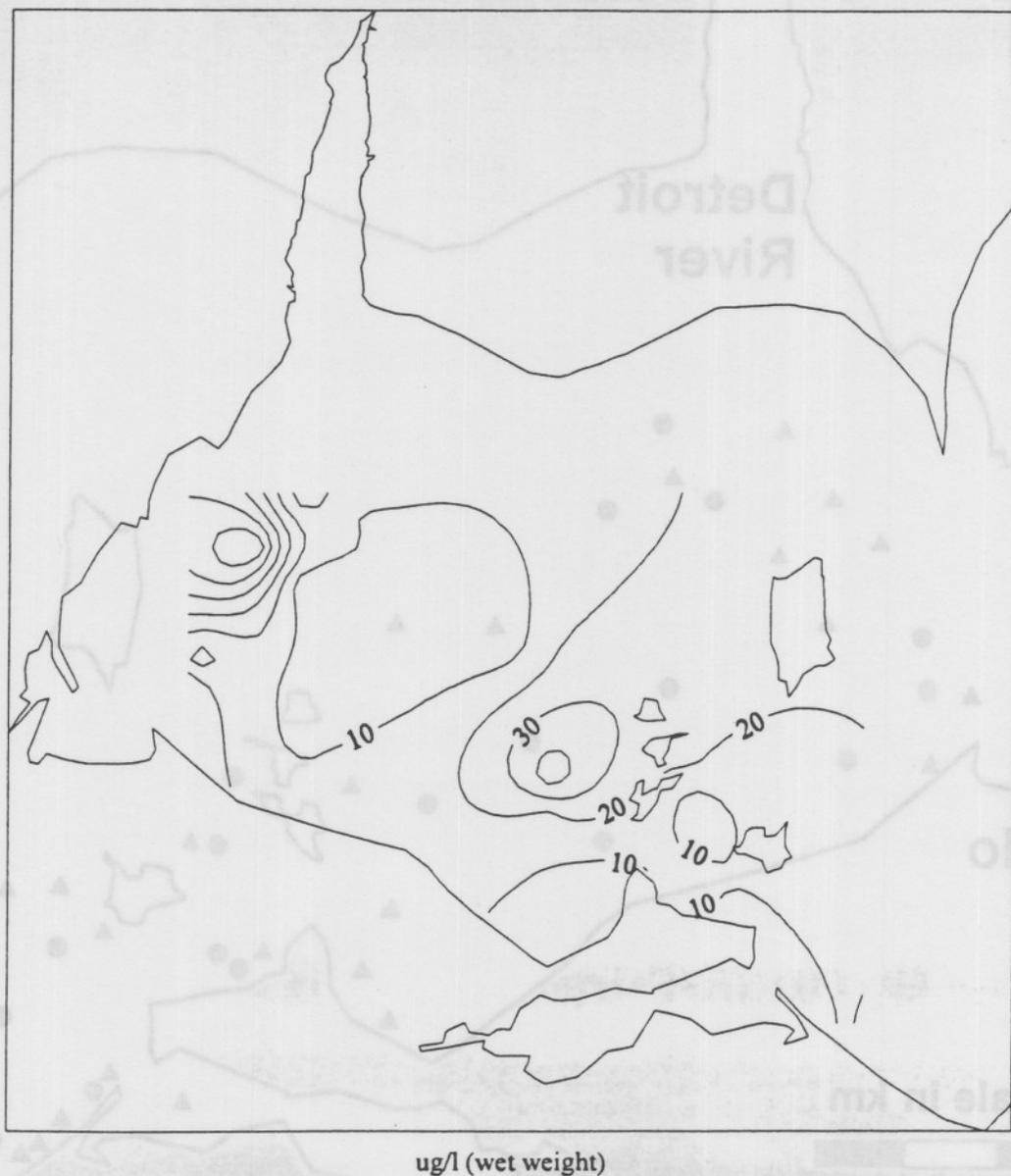


Figure 30. Distribution of cyanophytes in the western basin of Lake Erie for June, 1996. Contour interval is $10 \mu\text{g/l}$.

July 1996 Cyanophyta Biomass



Figure 31. Distribution of cyanophytes in the western basin of Lake Erie for July, 1996. Contour interval is 100 $\mu\text{g/l}$.

August 1996 Cyanophyta Biomass



Figure 32. Distribution of cyanophytes in the western basin of Lake Erie for August, 1996. Contour interval is 150 $\mu\text{g/l}$.

September 1996 Cyanophyta Biomass



Figure 33. Distribution of cyanophytes in the western basin of Lake Erie for September, 1996. Contour interval is $30 \mu\text{g/l}$.

September 1996 Cyanophyta Biomass

June 1996 Total Phytoplankton Biomass

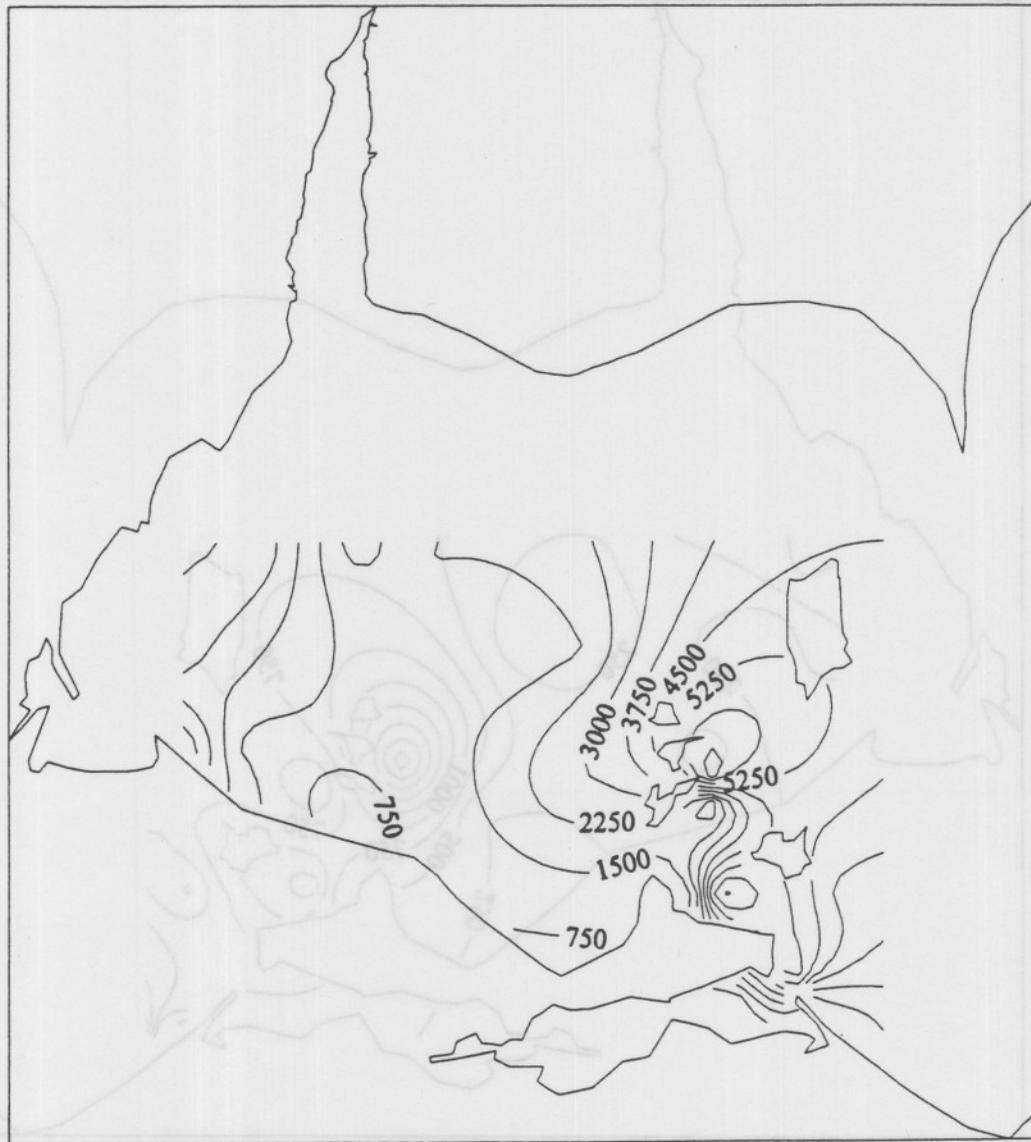


$\mu\text{g/l}$ (wet weight)

$\mu\text{g/l}$ (wet weight)

Figure 34. Distribution of phytoplankton in the western basin of Lake Erie for June, 1996. Contour interval is 250 $\mu\text{g/l}$.

July 1996 Total Phytoplankton Biomass



$\mu\text{g/l}$ (wet weight)

Figure 35. Distribution of phytoplankton in the western basin of Lake Erie for July, 1996. Contour interval is 750 $\mu\text{g/l}$.

August 1996 Total Phytoplankton Biomass

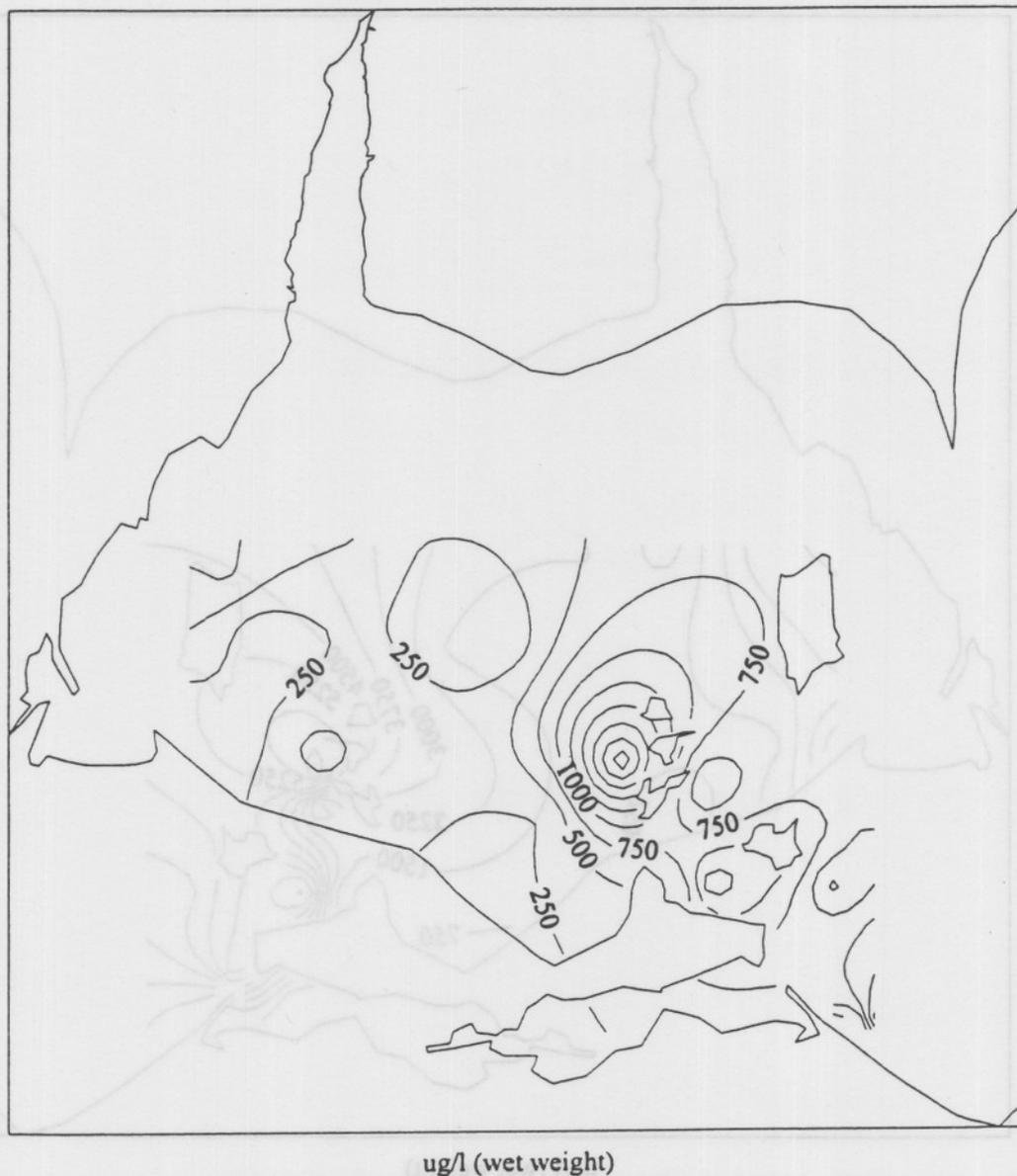
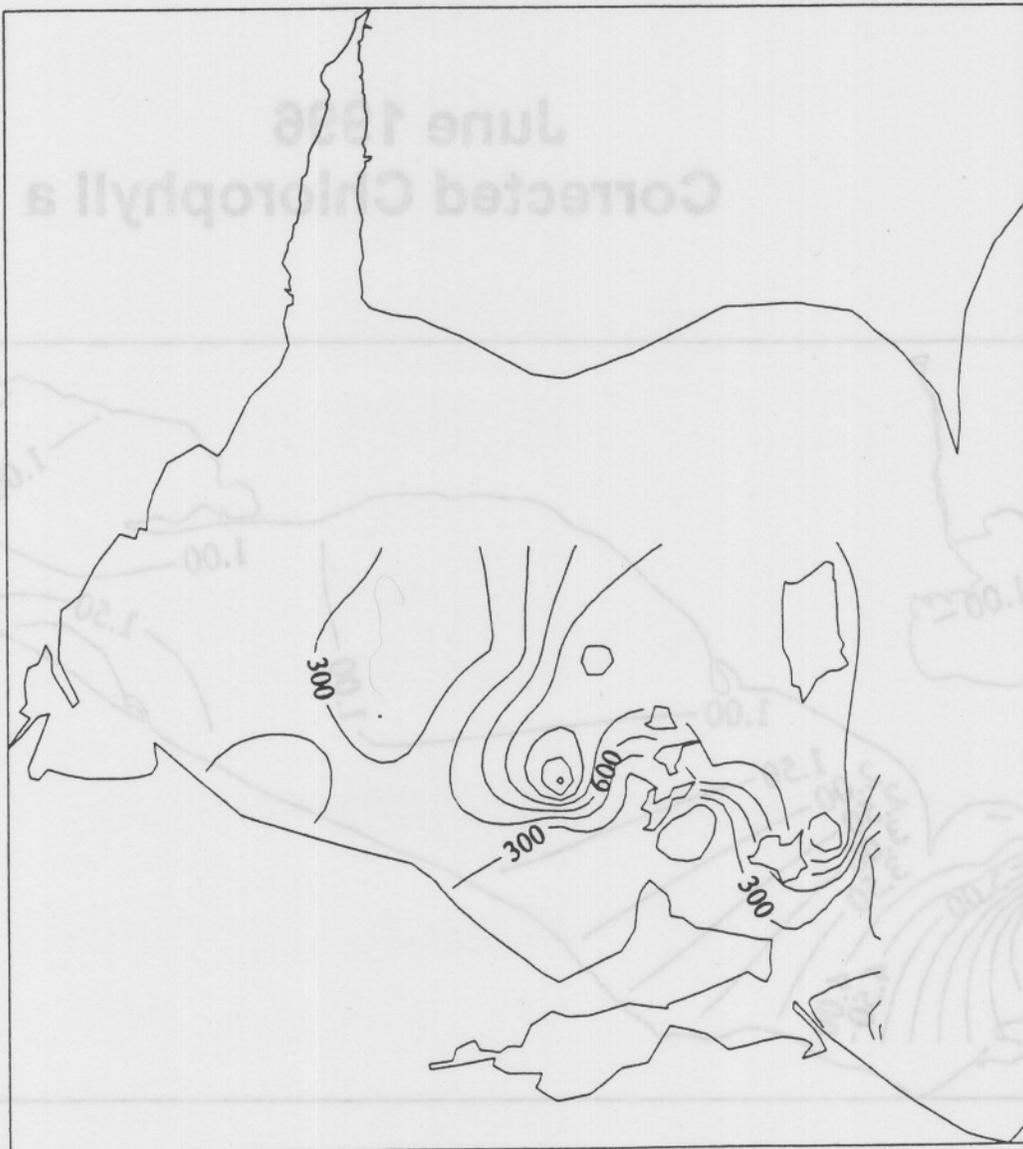


Figure 36. Distribution of phytoplankton in the western basin of Lake Erie for August, 1996. Contour interval is 250 $\mu\text{g/l}$.

September 1996 Total Phytoplankton Biomass

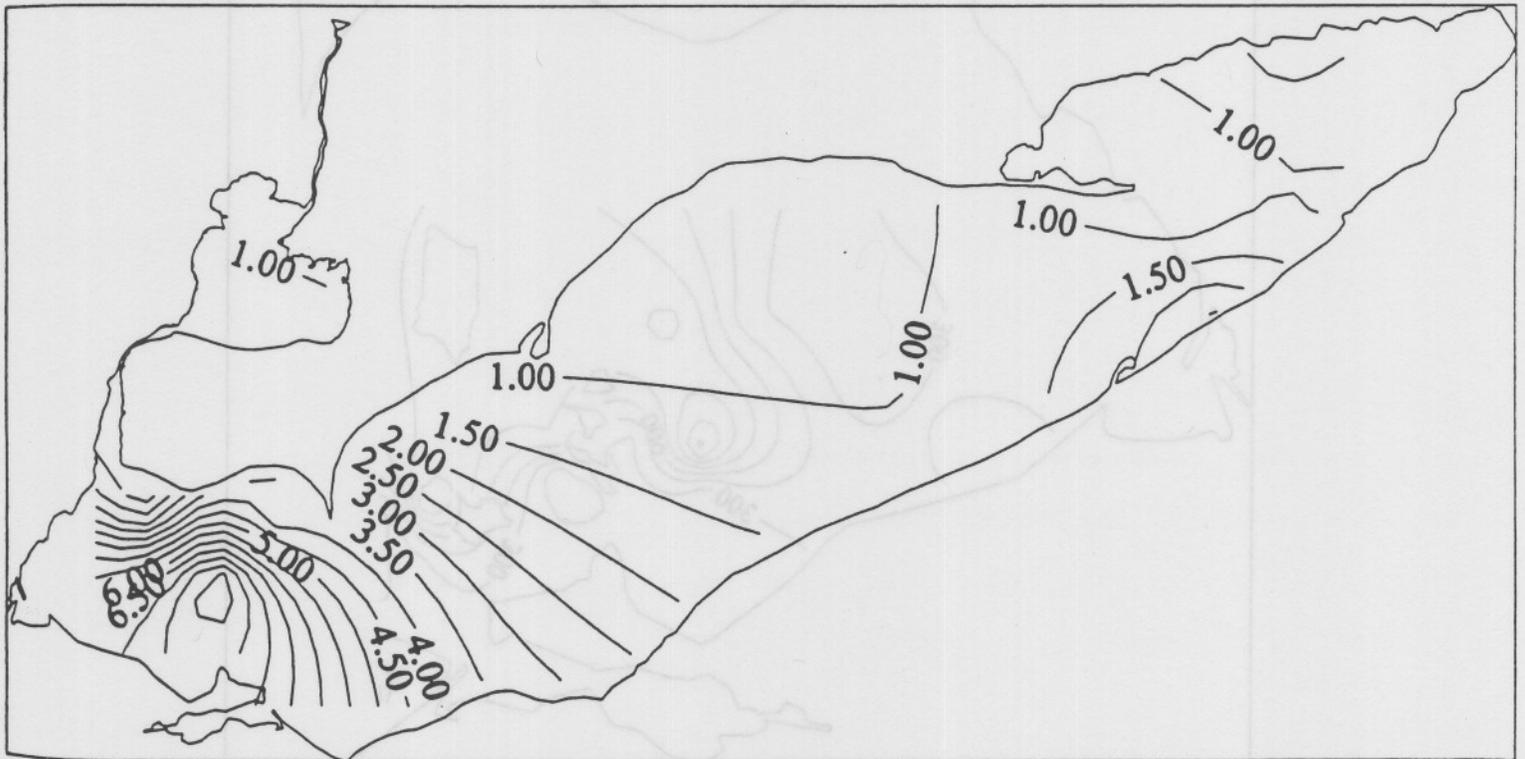


ug/l (wet weight)

Figure 37. Distribution of phytoplankton in the western basin of Lake Erie for September, 1996. Contour interval is 150 $\mu\text{g/l}$.

September 1996 Total Phytoplankton Biomass

June 1996 Corrected Chlorophyll a

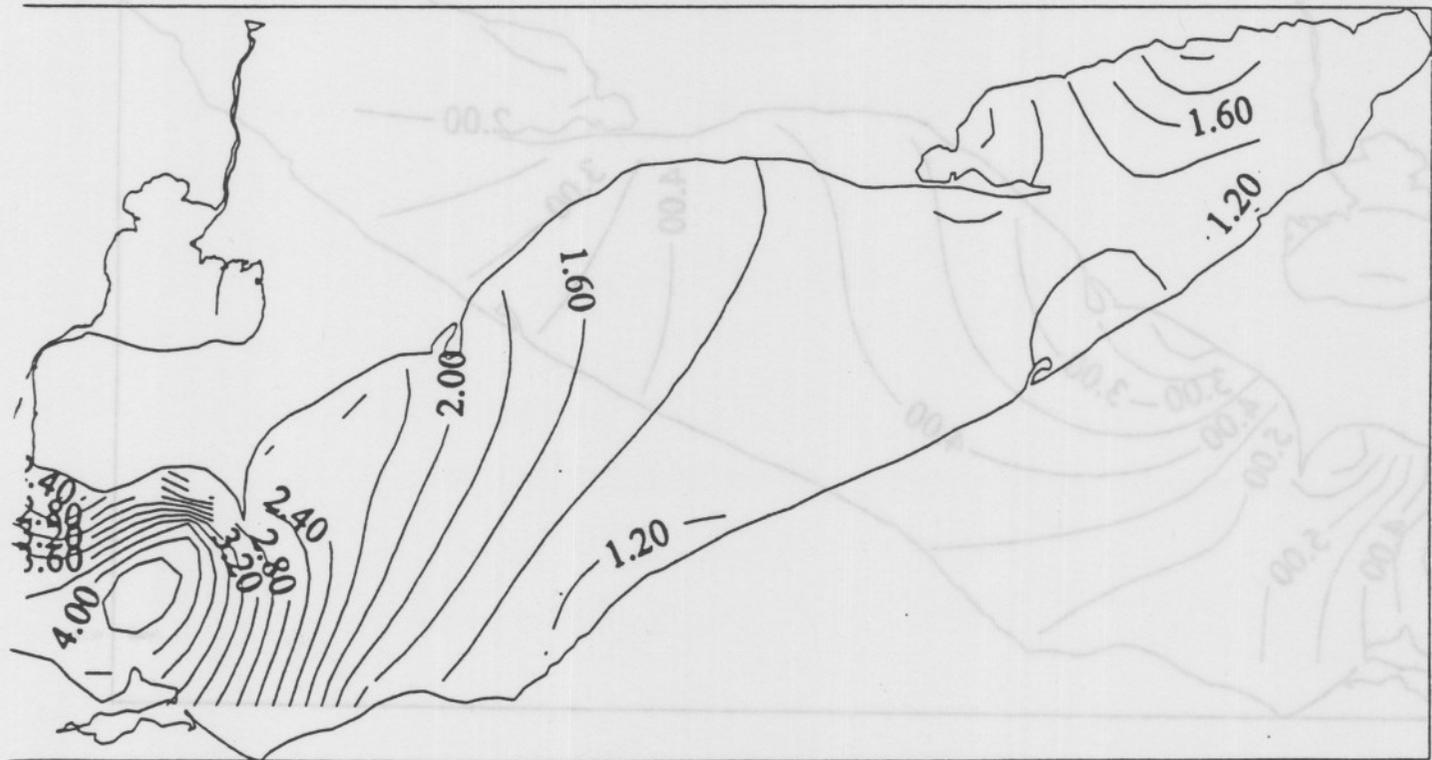


ug/l

Figure 37 Distribution of phytoplankton in the western basin of Lake Erie for September, 1996. Contour interval is 150 $\mu\text{g/l}$.

Figure 38. Distribution of Chlorophyll a in Lake Erie, June 1996. Contour interval is 0.5 $\mu\text{g/l}$. The maximum observation was 8 $\mu\text{g/l}$.

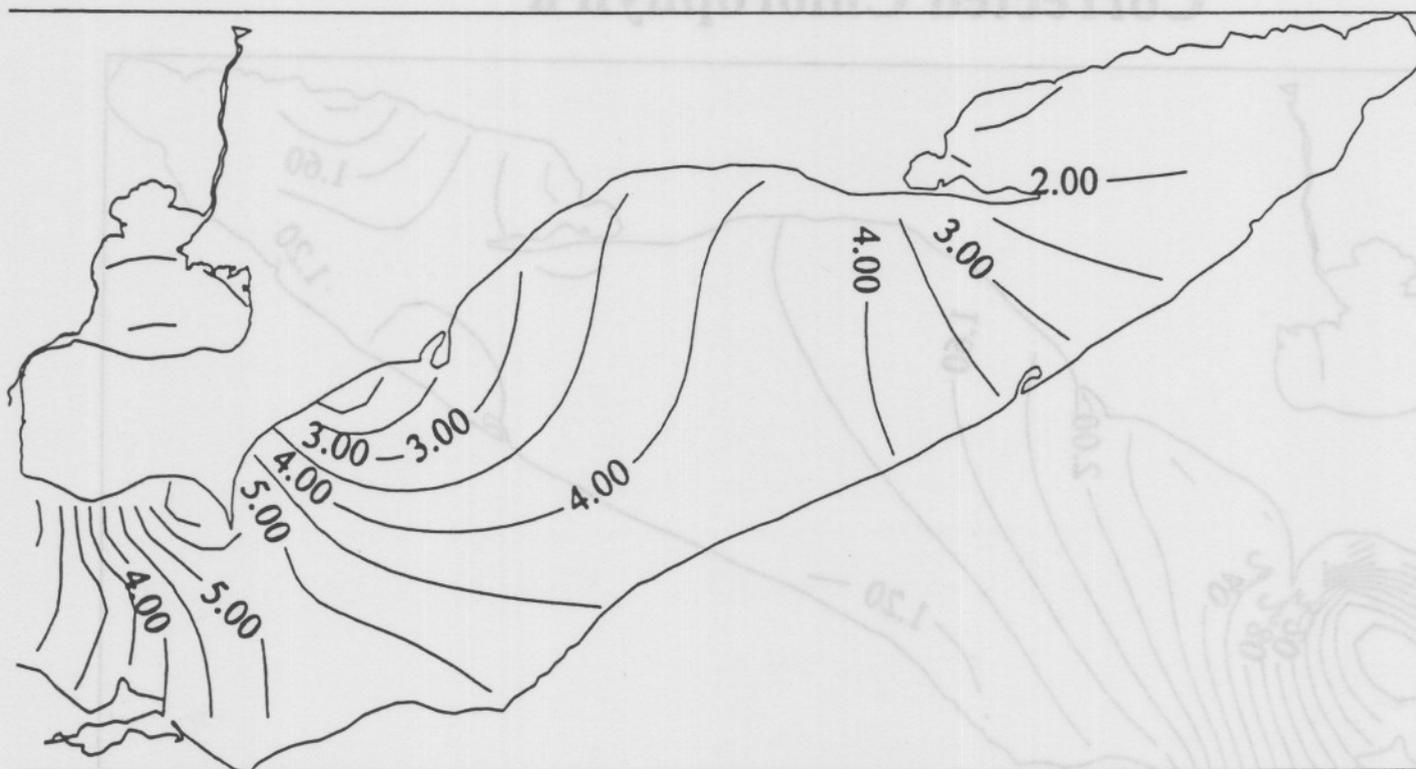
September 1996
August 1996
Corrected Chlorophyll a



ug/l

Figure 39. Distribution of Chlorophyll *a* in Lake Erie, August 1996. Contour interval is 0.2 $\mu\text{g/l}$. The maximum observation was 4.2 $\mu\text{g/l}$.

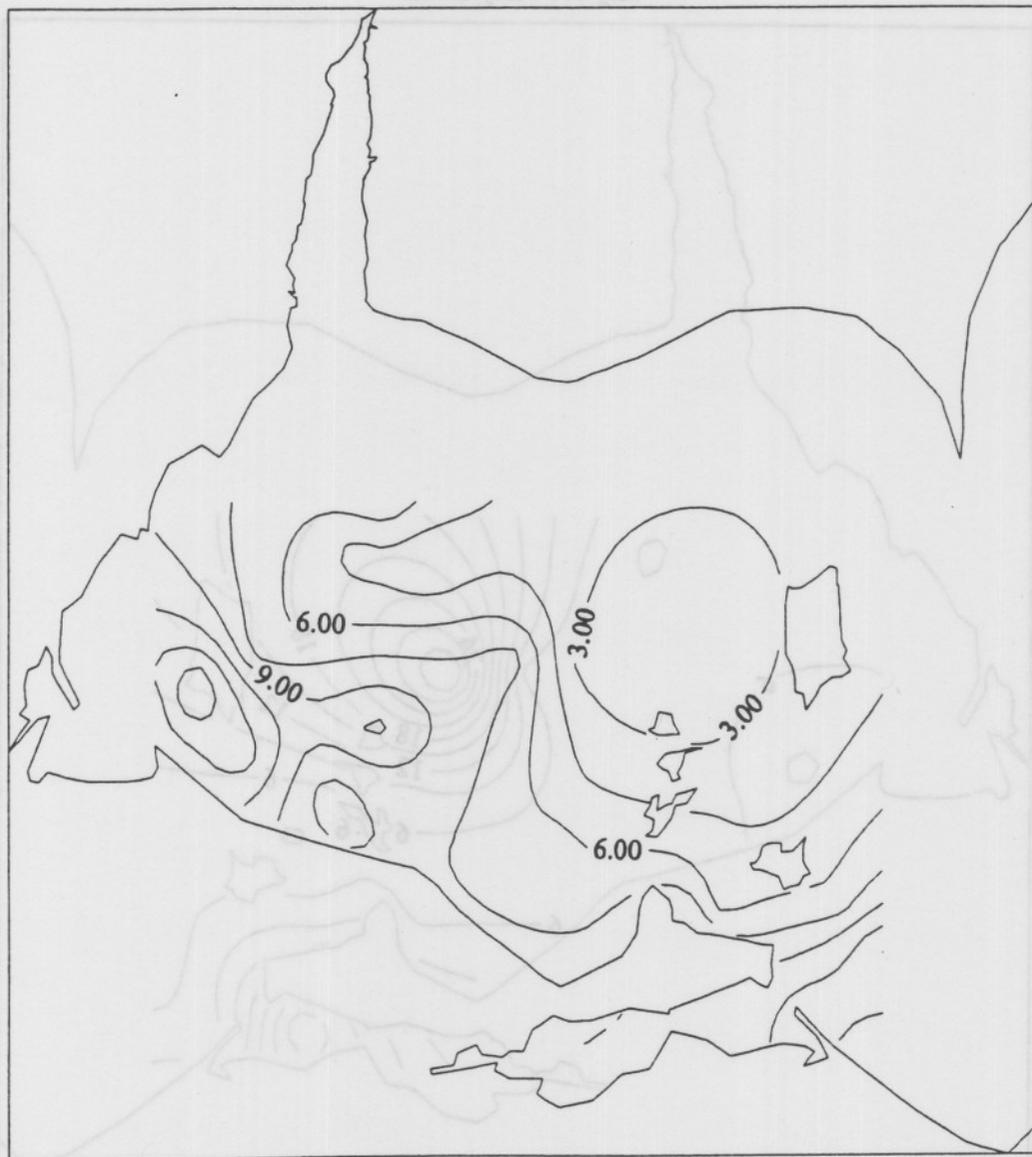
September 1996



Corrected Chlorophyll a ($\mu\text{g/l}$)

Figure 40. Distribution of Chlorophyll *a* in Lake Erie, September 1996. Contour interval is 0.5 $\mu\text{g/l}$. The maximum observation was 6.2 $\mu\text{g/l}$.

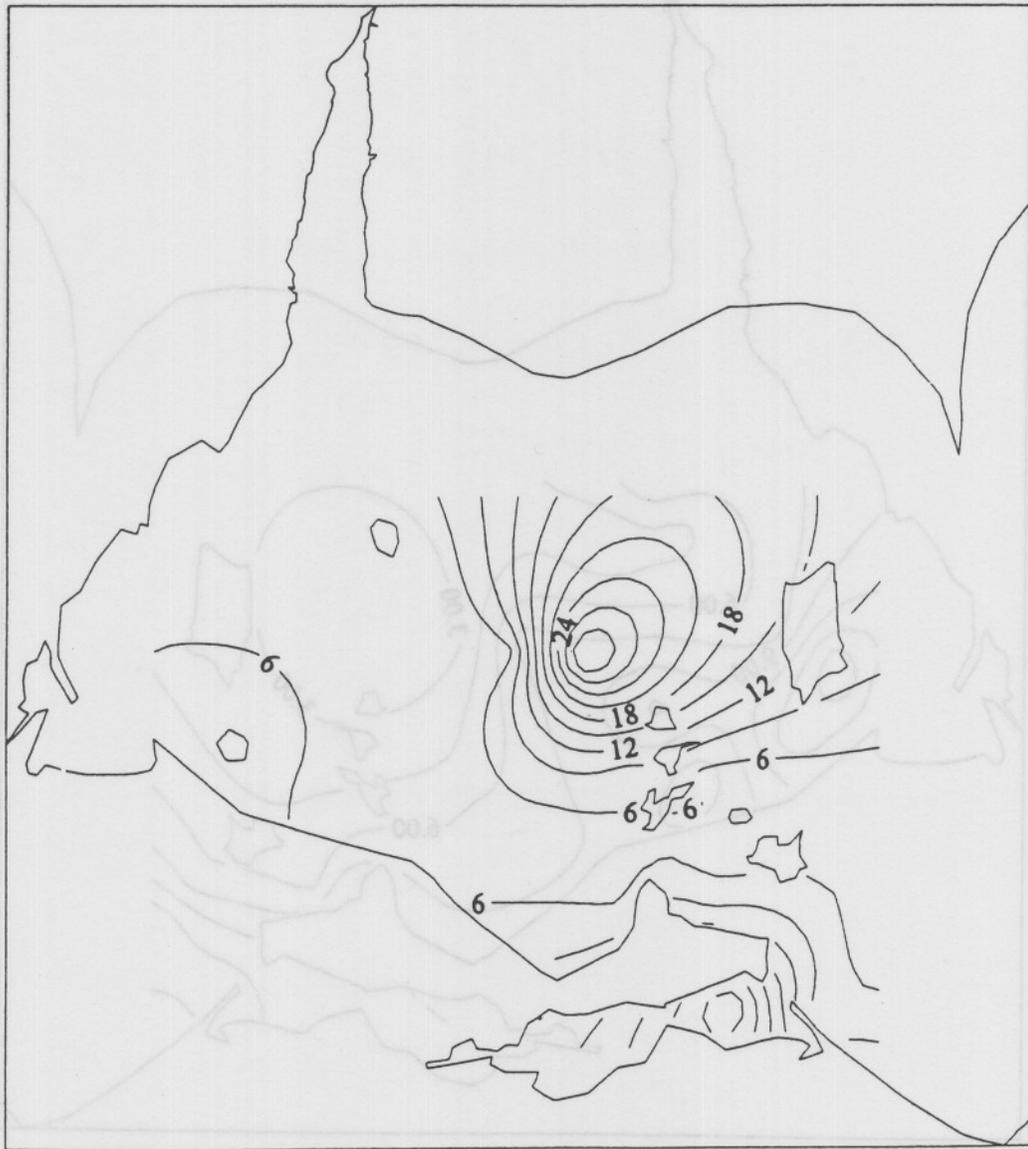
June 1996 Dry Seston



mg/l (dry weight)

Figure 41. Distribution of seston in western Lake Erie for June, 1996. Contour interval is 1.5 mg/l.

July 1996 Dry Seston



mg/l (dry weight)

Figure 42. Distribution of seston in western Lake Erie for July, 1996. Contour interval is 3.0 mg/l.

August 1996 Dry Seston



mg/l (dry weight)

Figure 43. Distribution of seston in western Lake Erie for August, 1996. Contour interval is 10.0 mg/l.

September 1996 Dry Seston



mg/l (dry weight)

Figure 44. Distribution of seston in western Lake Erie for September, 1996. Contour interval is 2.0 mg/l.

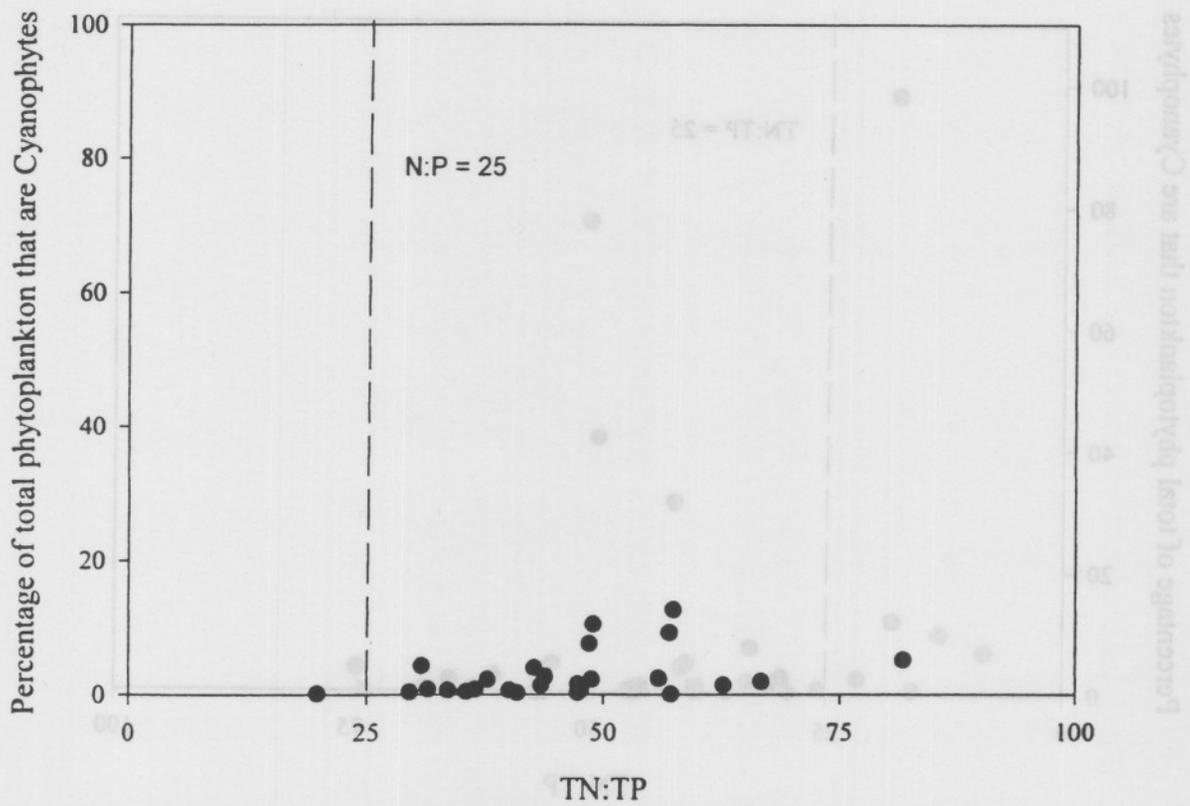


Figure 45. Relationship of Cyanophyte abundance to TN:TP in western Lake Erie for June, 1996.

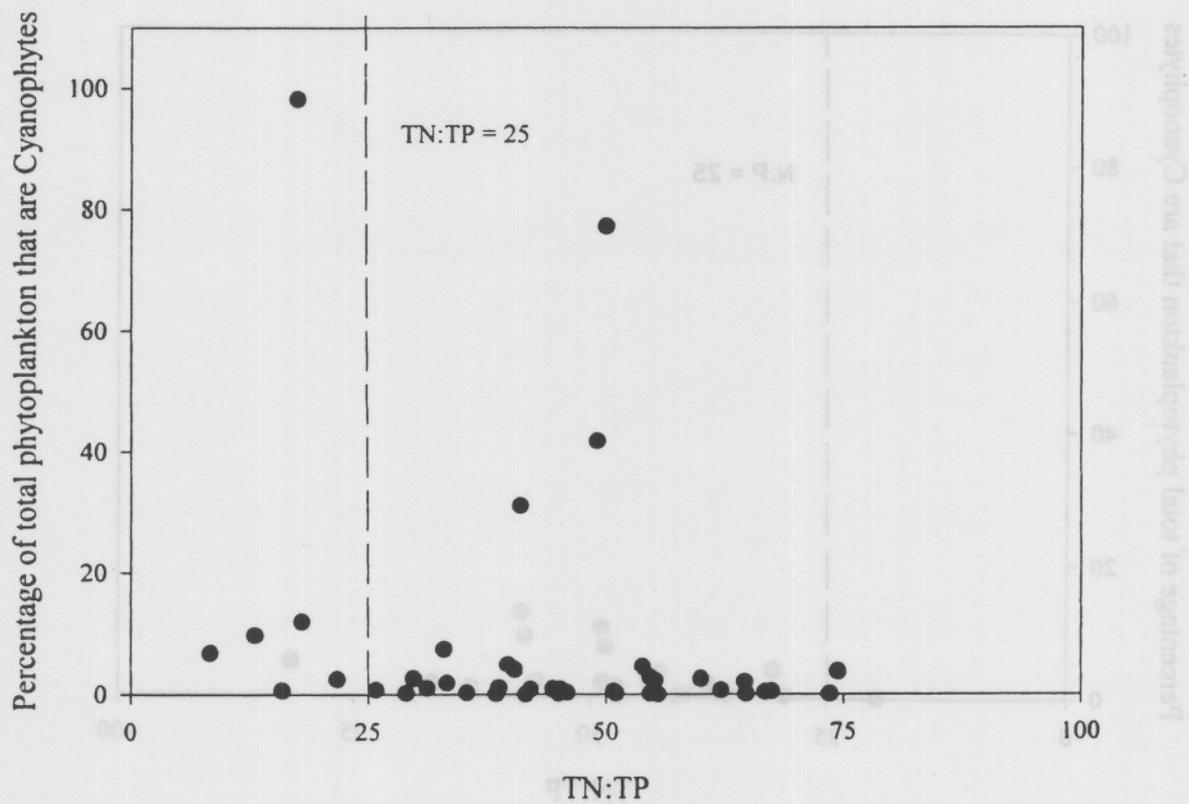


Figure 46. Relationship of Cyanophyte abundance to TN:TP in western Lake Erie for July, 1996.

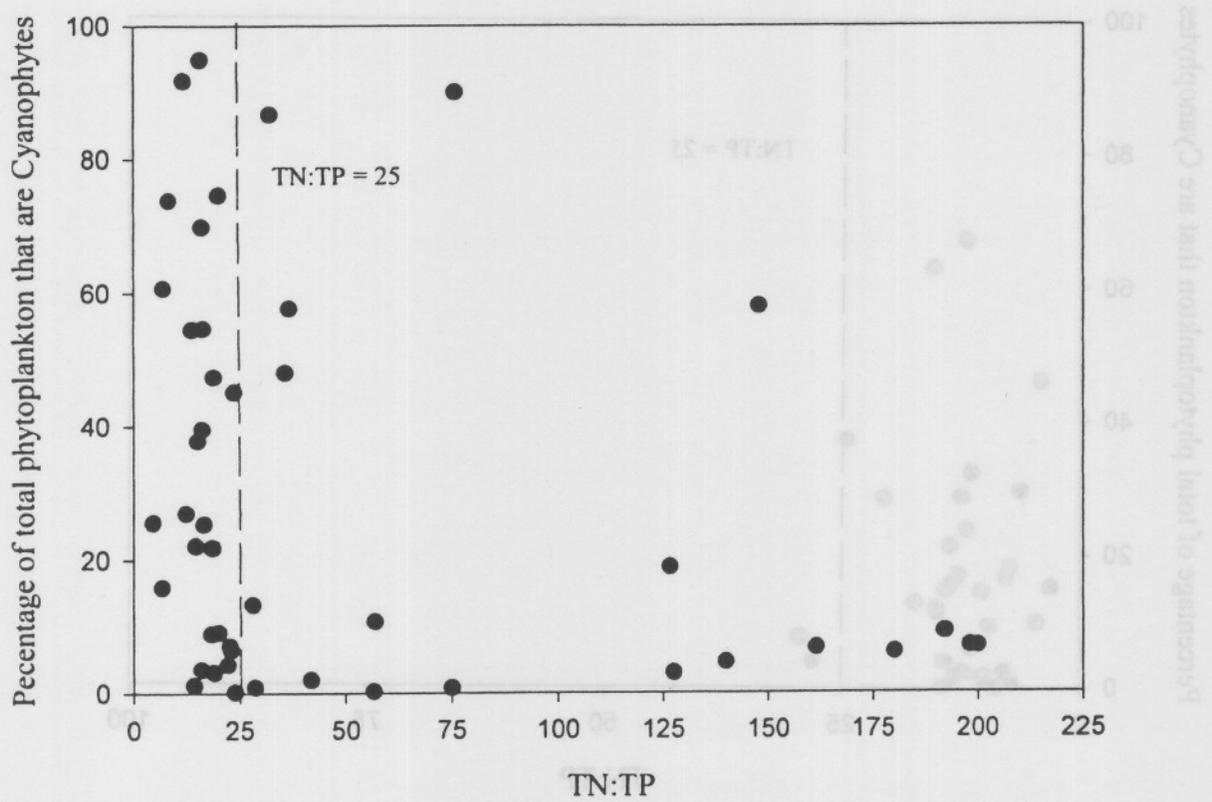


Figure 47. Relationship of Cyanophyte abundance to TN:TP in western Lake Erie for August, 1996.

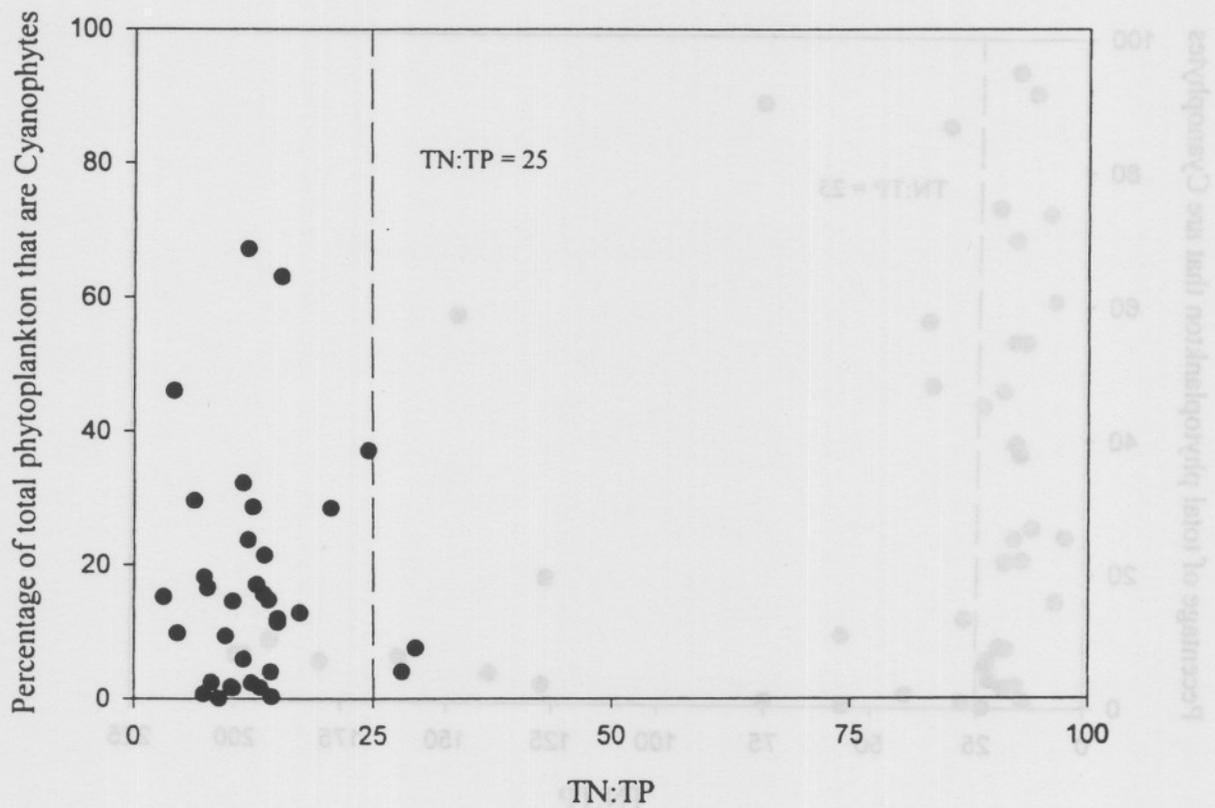


Figure 48. Relationship of Cyanophyte abundance to TN:TP in western Lake Erie for September, 1996.

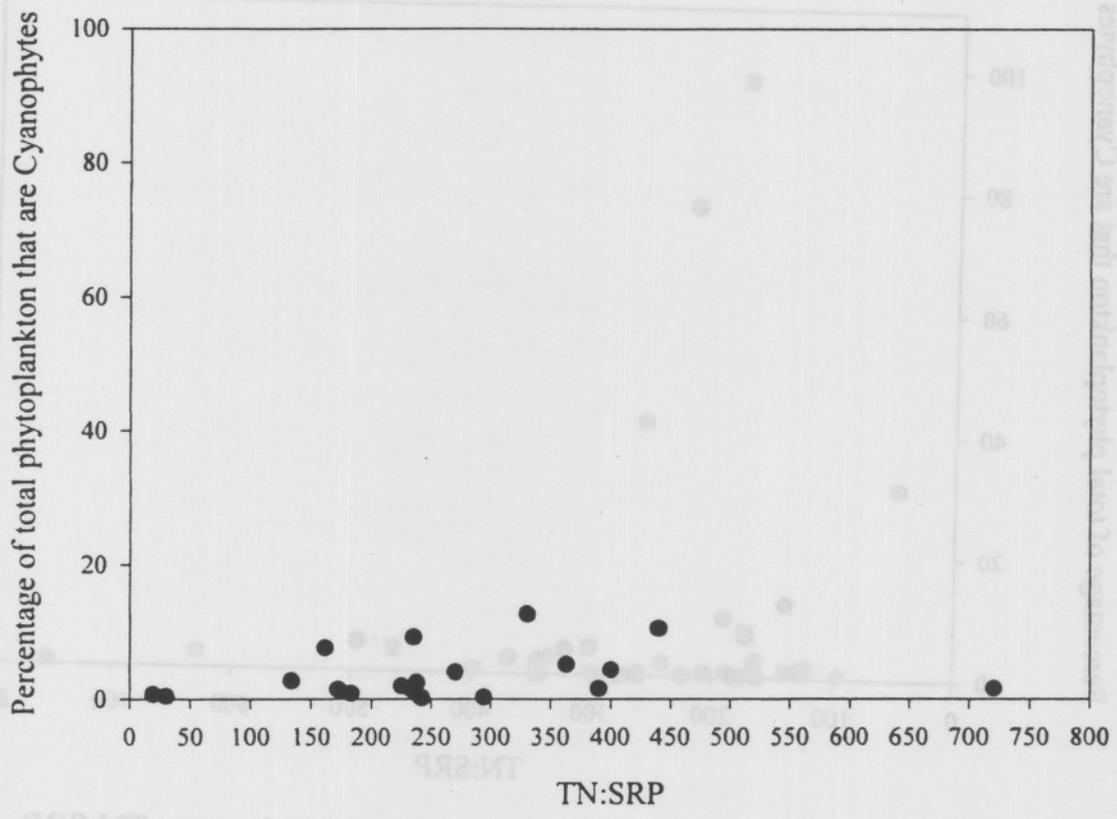


Figure 49. Relationship of Cyanophyte abundance to TN:SRP in western Lake Erie for June, 1996.

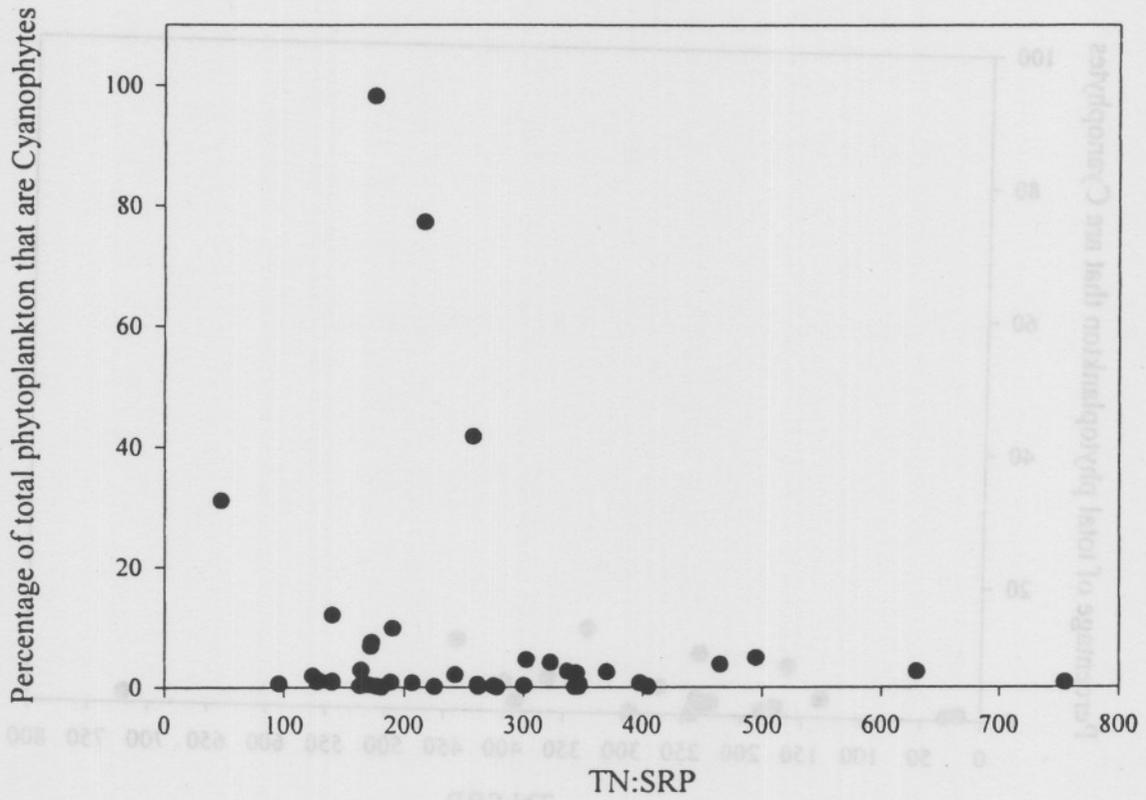


Figure 50. Relationship of Cyanophyte abundance to TN:SRP in western Lake Erie for July, 1996.

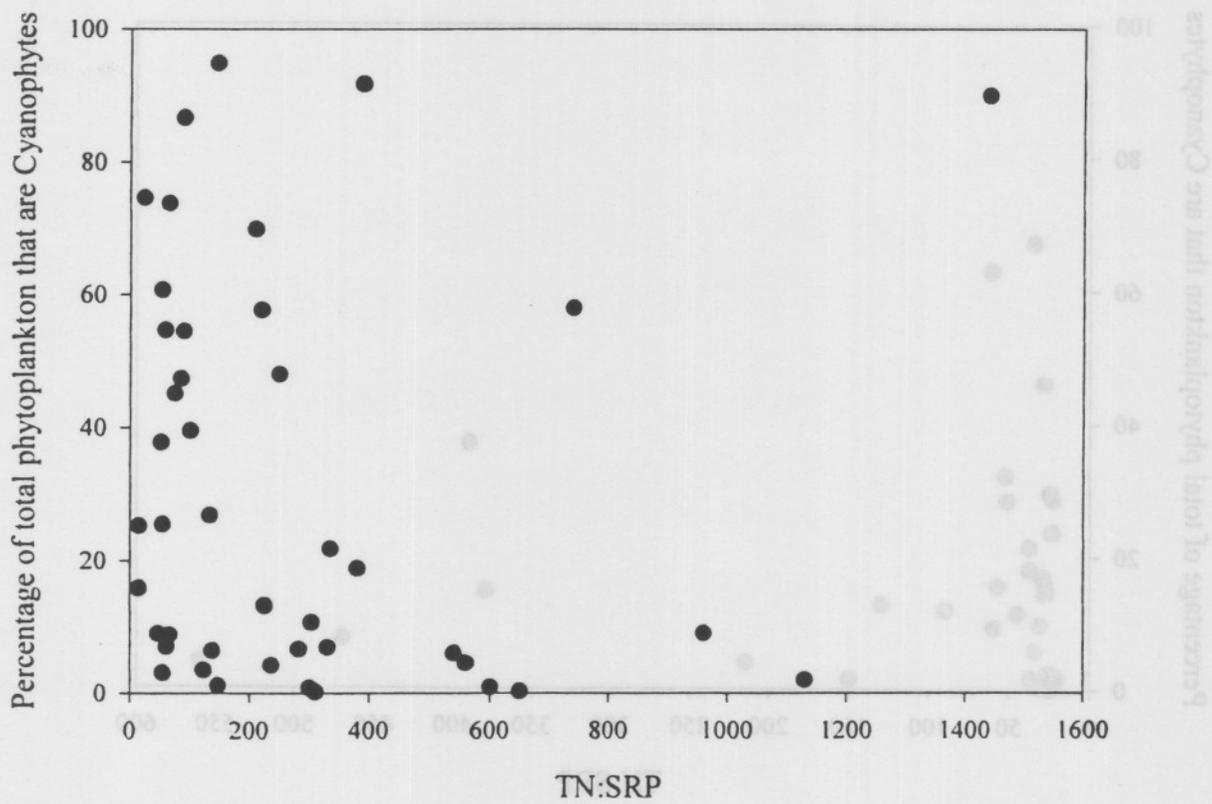


Figure 51. Relationship of Cyanophyte abundance to TN:SRP in western Lake Erie for August, 1996.

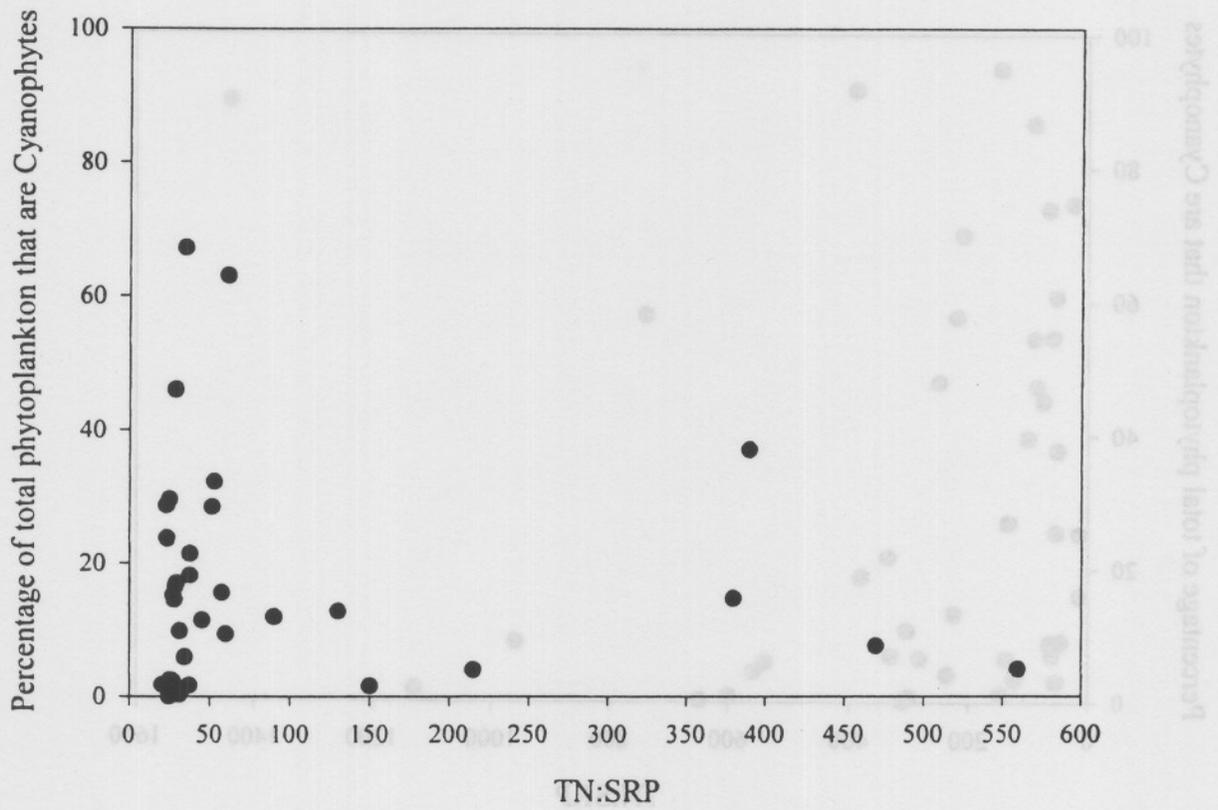


Figure 52. Relationship of Cyanophyte abundance to TN:SRP in western Lake Erie for September, 1996.

Lake Erie Reflectance
Reflectance Range: 0.0 - 6.5 %

g9822522.ec1 - g9822522.ec2

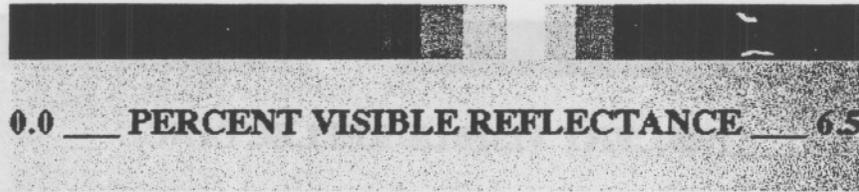
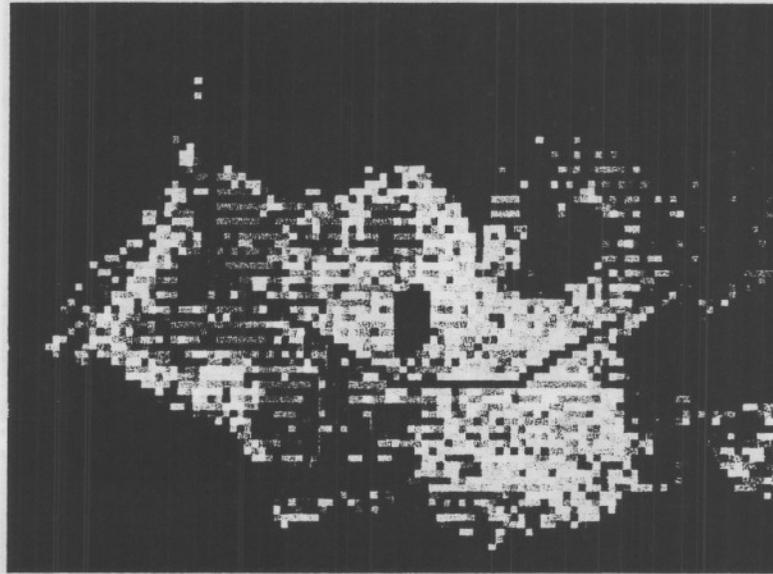


Figure 53. Lake Erie visible reflectance image for the western basin on August 13, 1998.

Lake Erie Reflectance
Reflectance Range: 0.0 - 6.5 %

g9822618.ec1 - g9822618.ec2

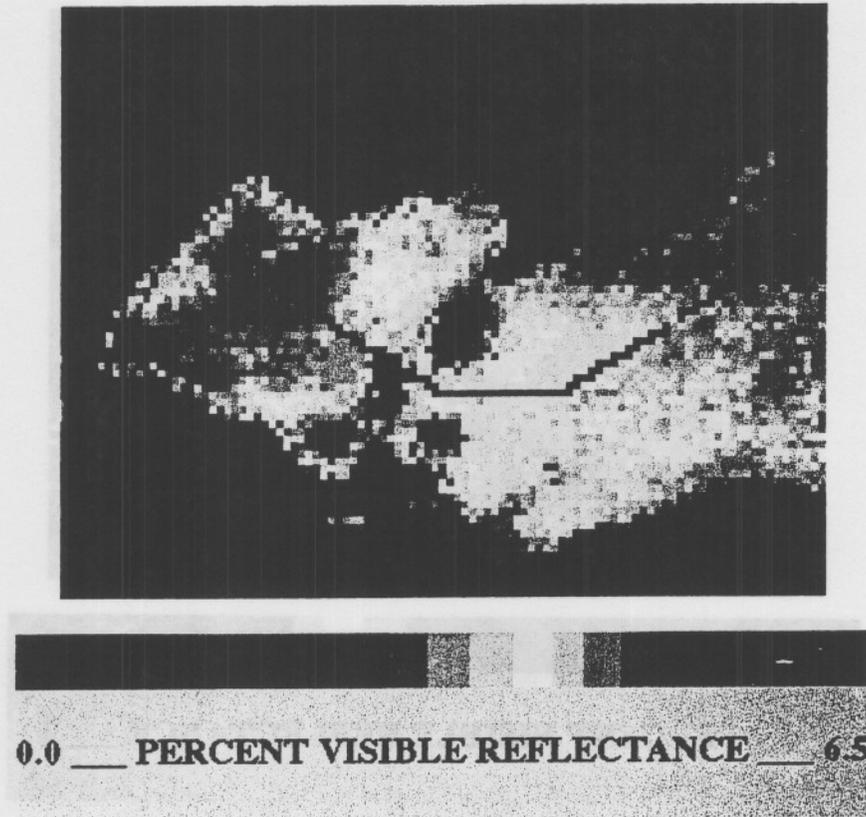


Figure 54. Lake Erie visible reflectance image for the western basin on August 14, 1998.

Lake Erie Reflectance
Reflectance Range: 0.0 - 6.5 %

g9822811.ec1 - g9822811.ec2

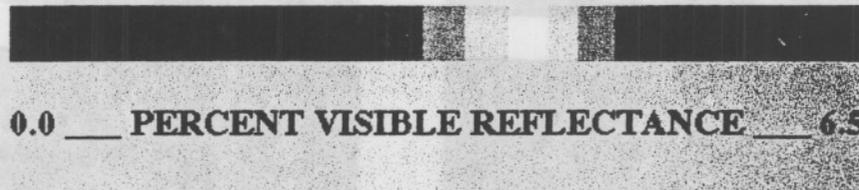
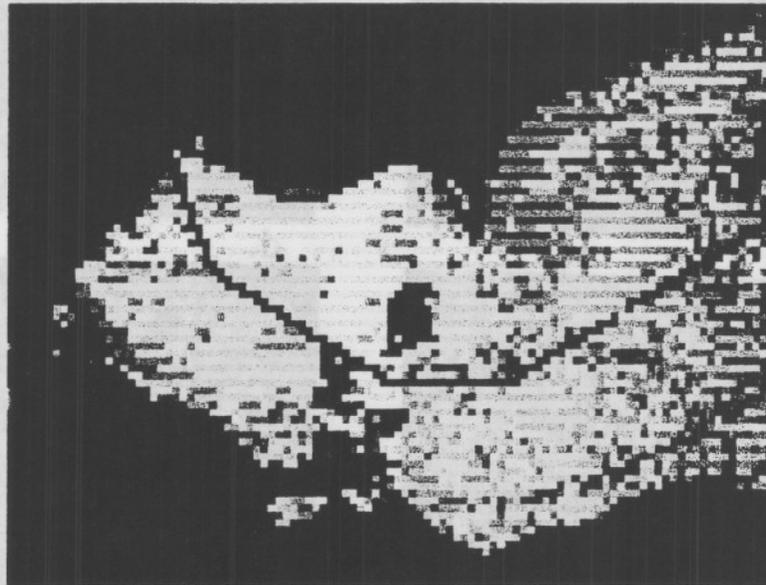


Figure 55. Lake Erie visible reflectance image for the western basin on August 16, 1998.

Figure 56. False color composite of AVHRR reflectance images illustrate the spatial extent of Microcystis blooms in western Lake Erie

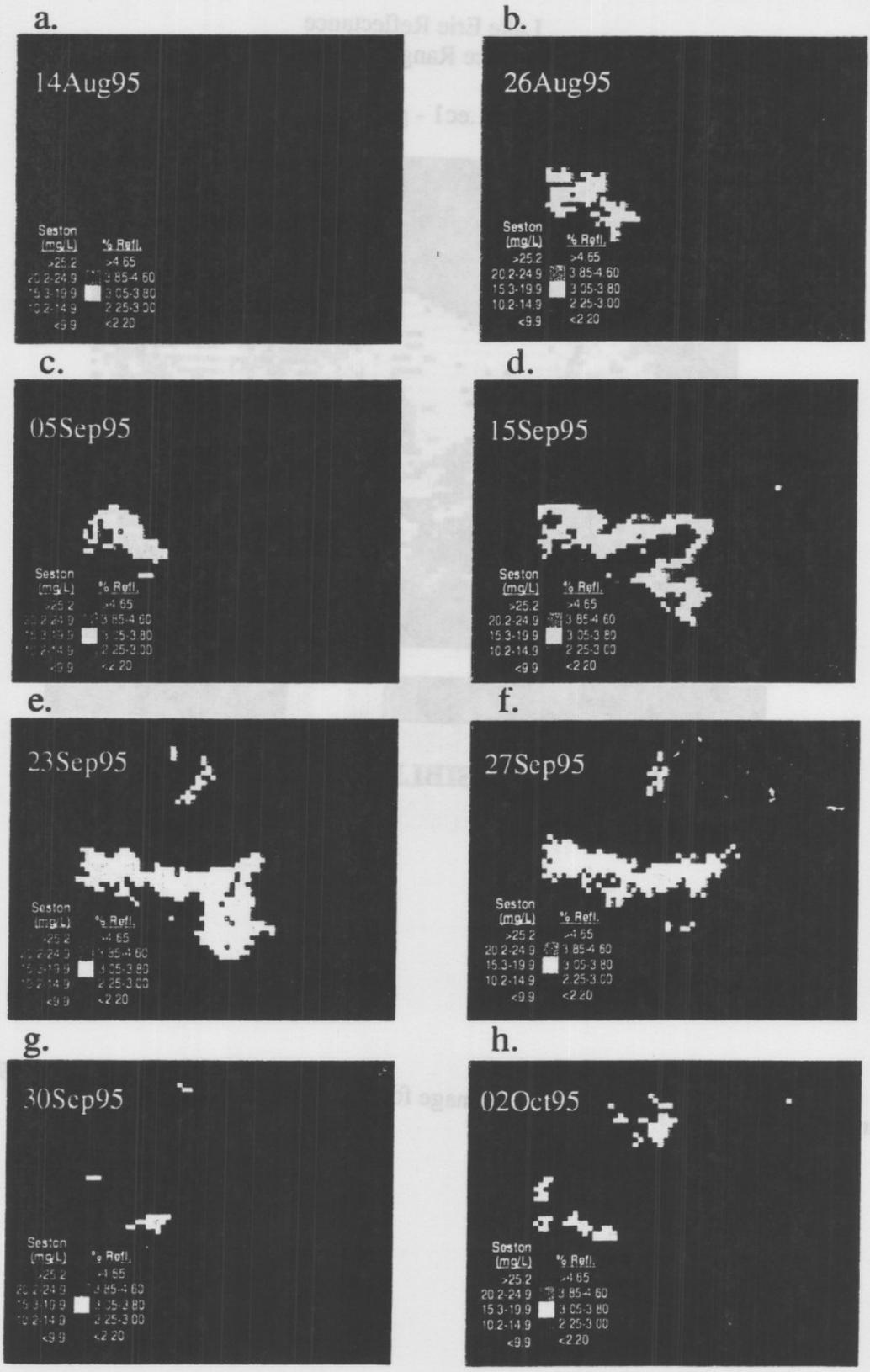


Figure 56. False color composites of AVHRR reflectance imagery illustrate the areal extent of Microcystis blooms in western Lake Erie

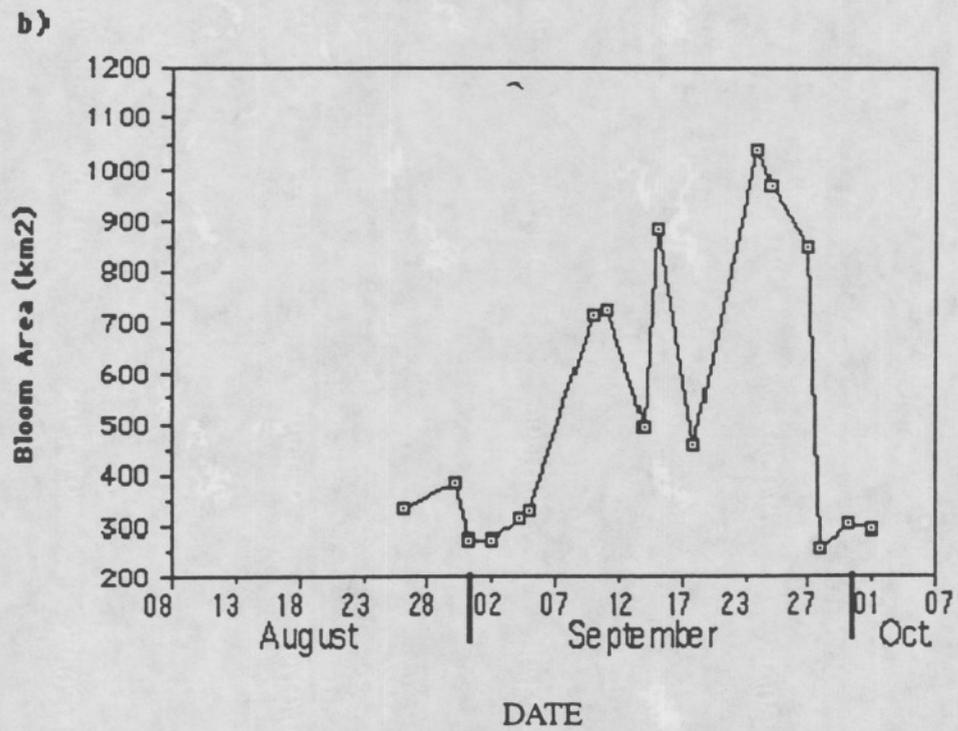
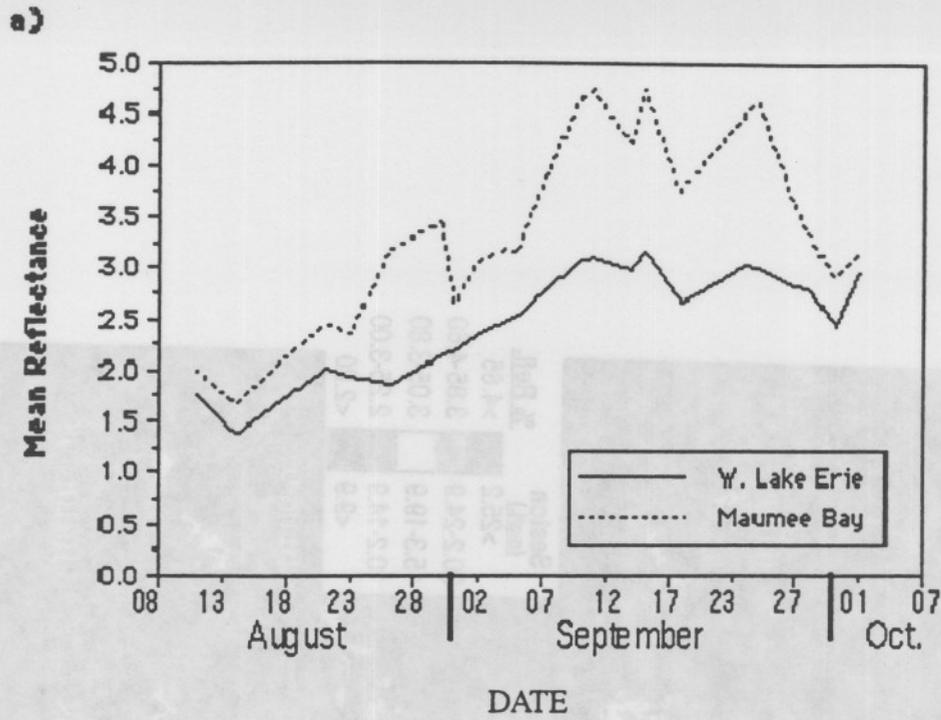


Figure 57. a) Mean RSR for western Lake Erie and Maumee Bay from August 11 to October 2, 1995; b) Estimates of bloom area over time.

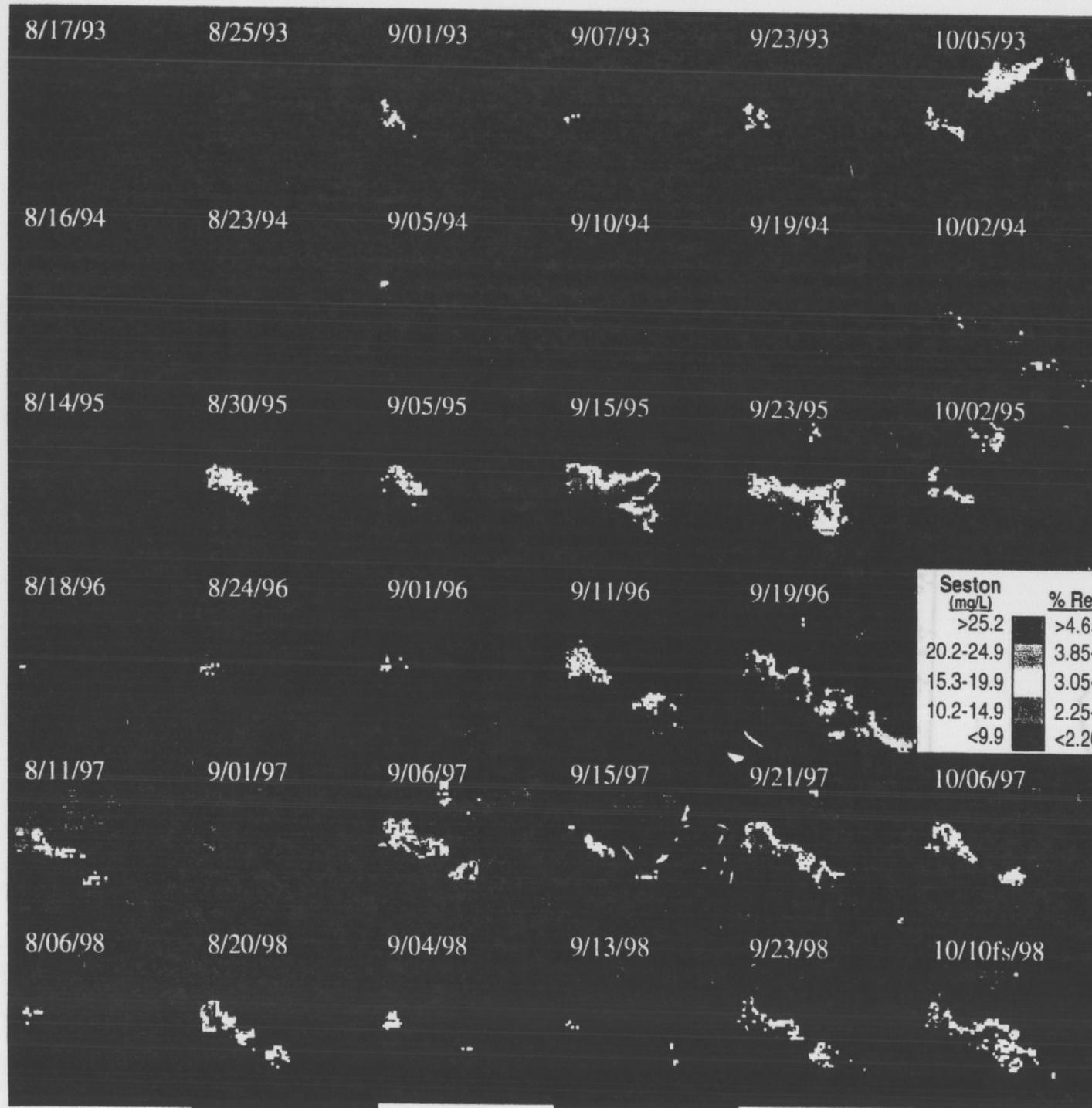
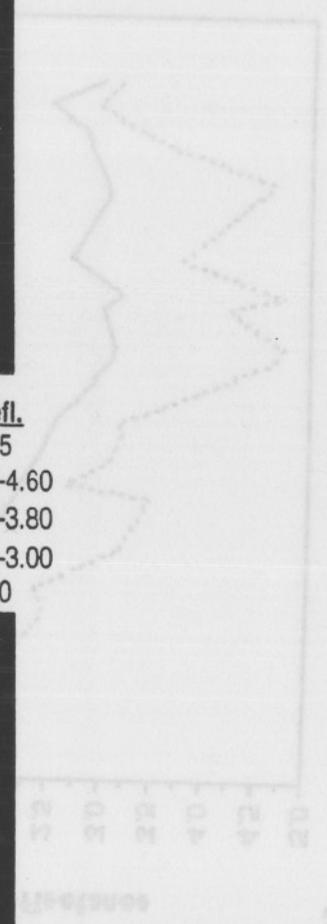


Figure 58.
Six year
time series of
AVHRR imagery
from August to
October 1993-
1998.



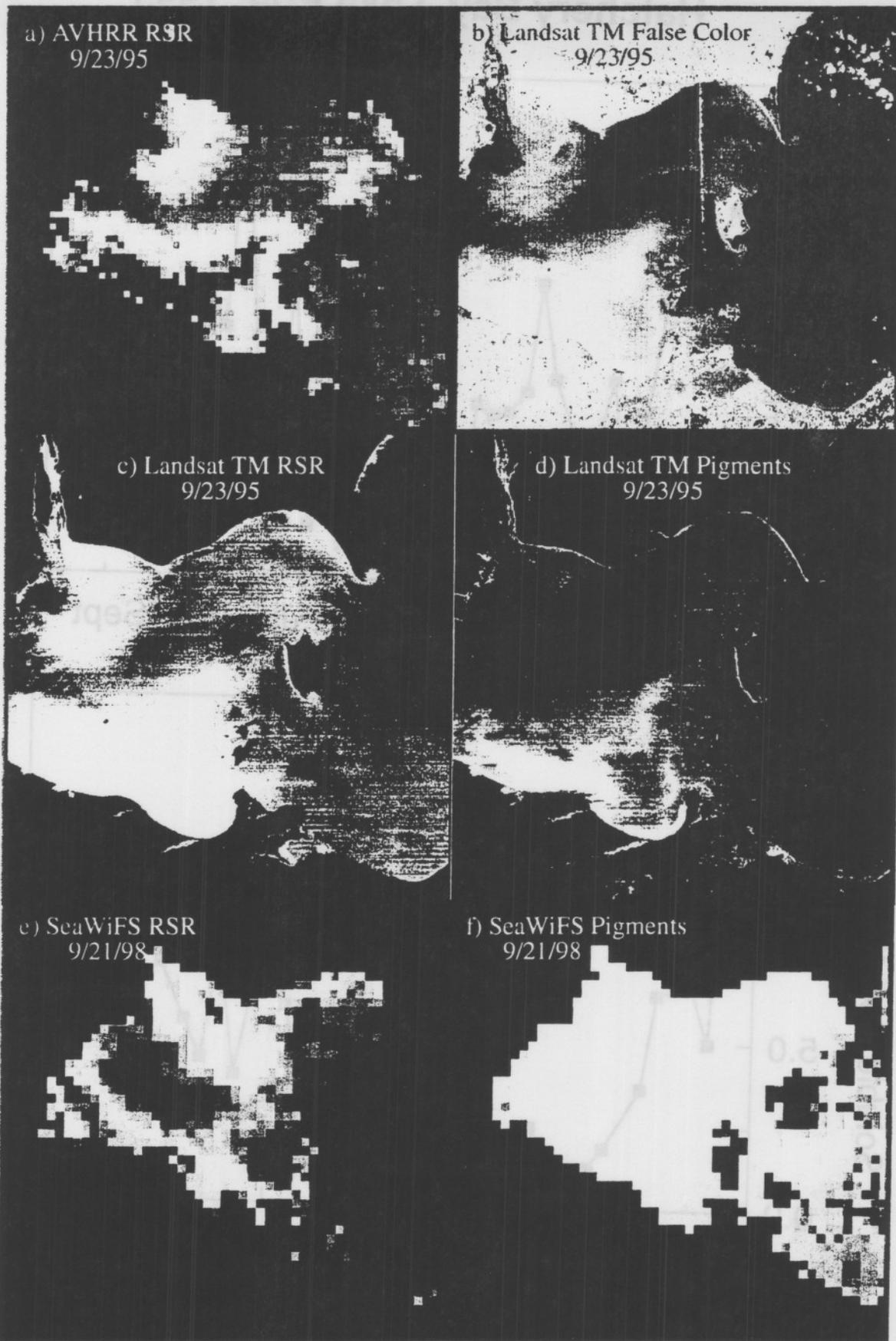


Figure 59. Different types of images available from satellites.

Hatchery Bay, Lake Erie: 1995

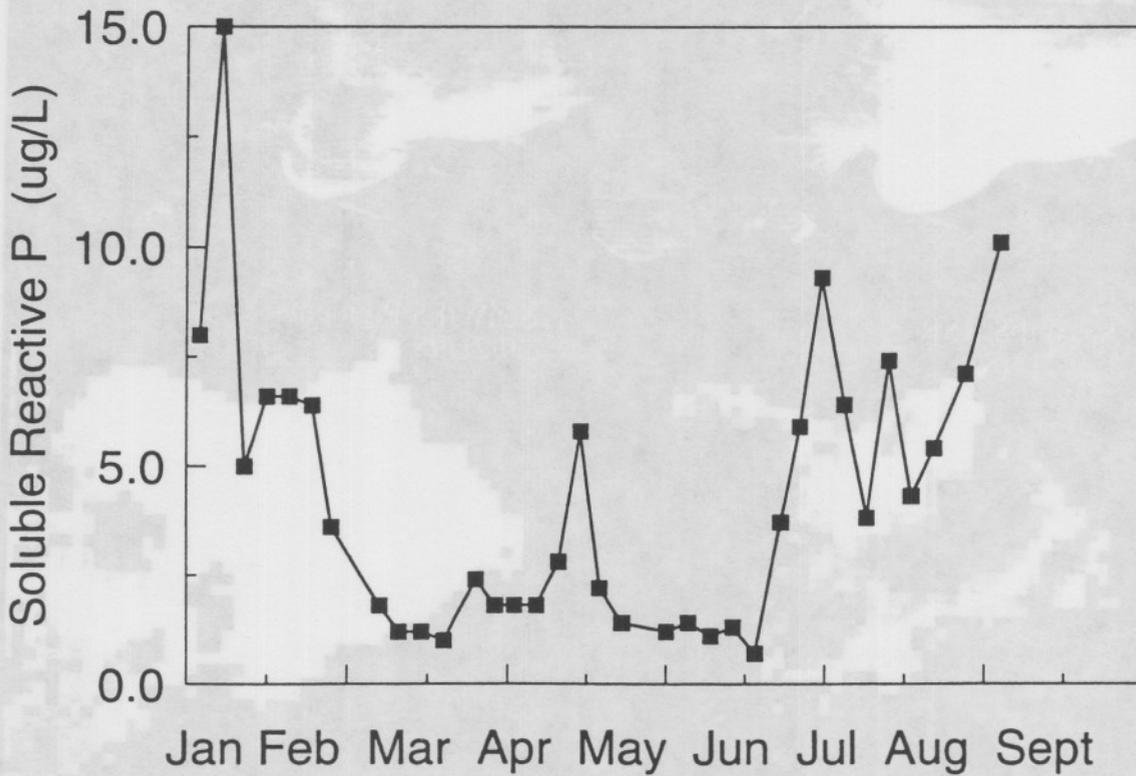
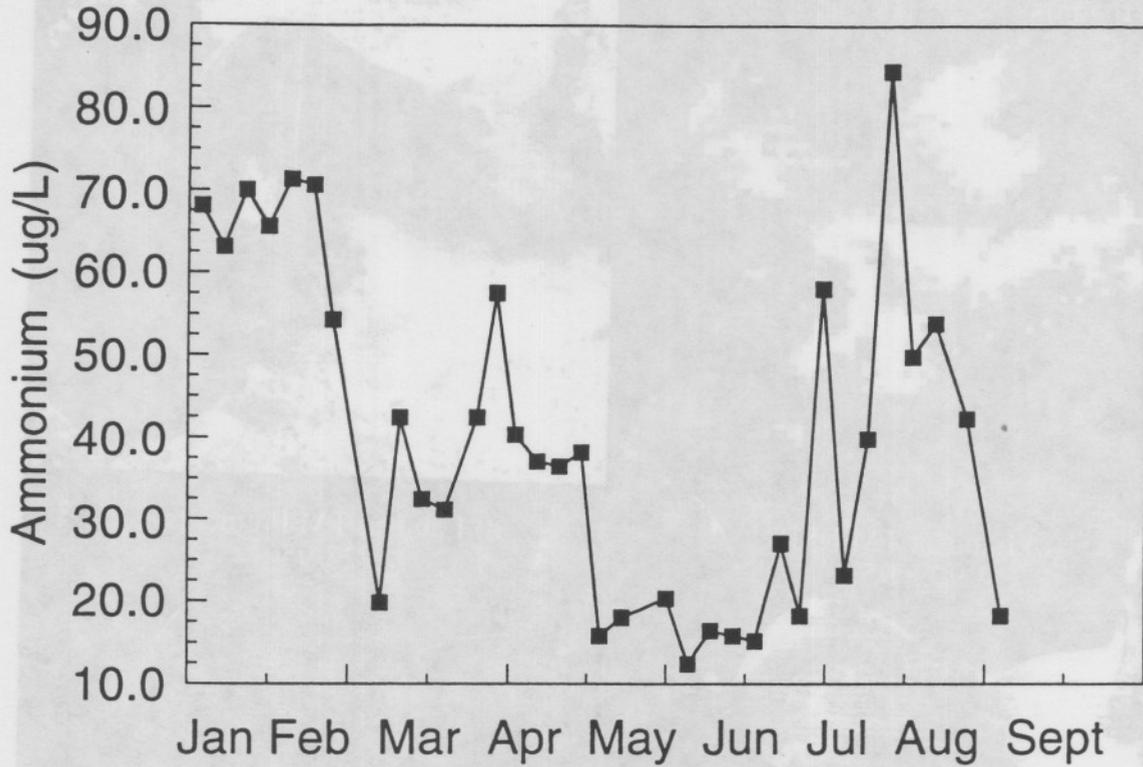


Figure 60. Ammonium and soluble reactive phosphorus concentrations in Hatchery Bay, Lake Erie determined from weekly sampling during January 4 - September 12, 1995.

Hatchery Bay, Lake Erie: 1995

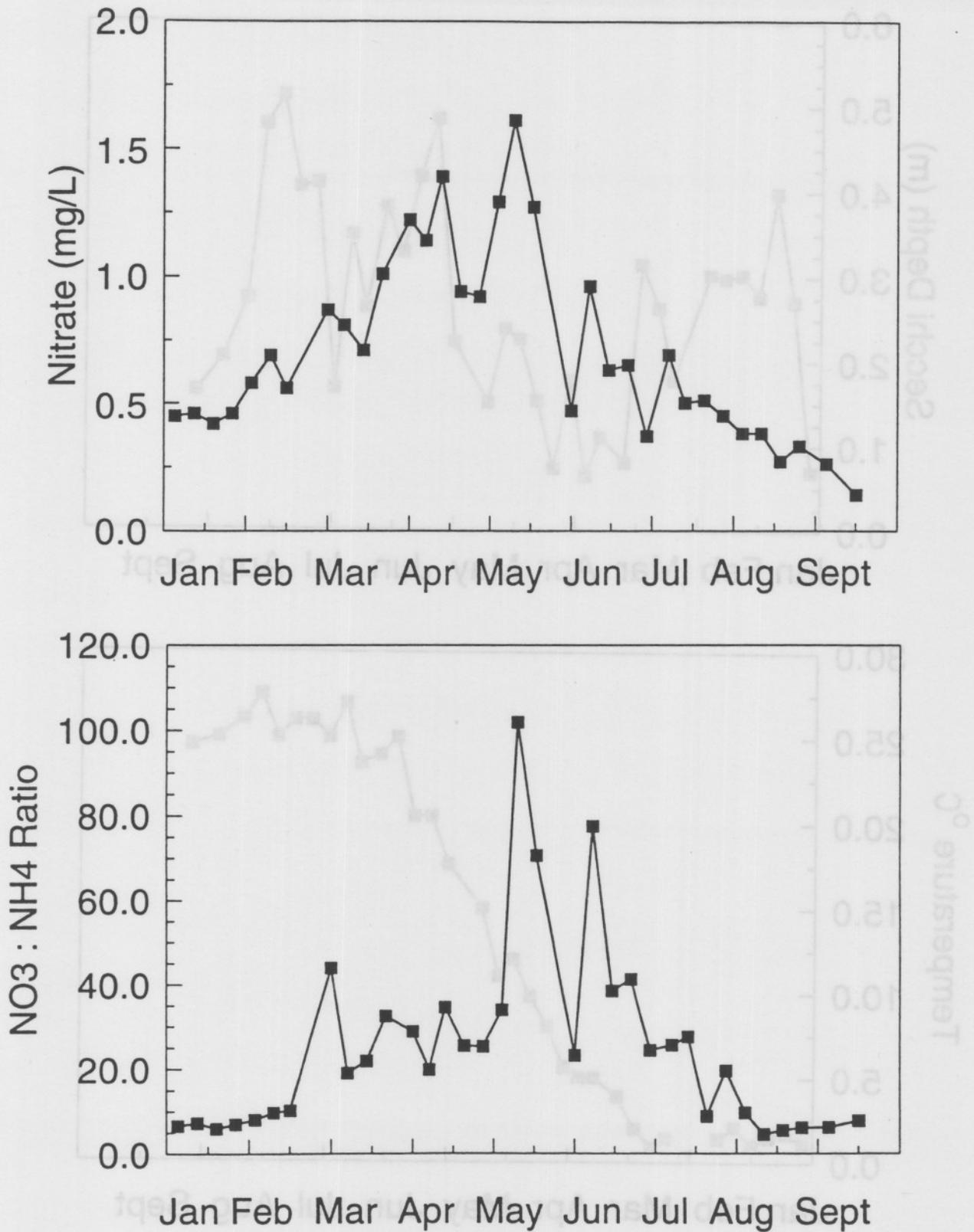


Figure 61. Nitrate concentrations and nitrate to ammonium ratio in Hatchery Bay, Lake Erie determined from weekly sampling during January 4 - September 12, 1995.

Hatchery Bay, Lake Erie: 1995

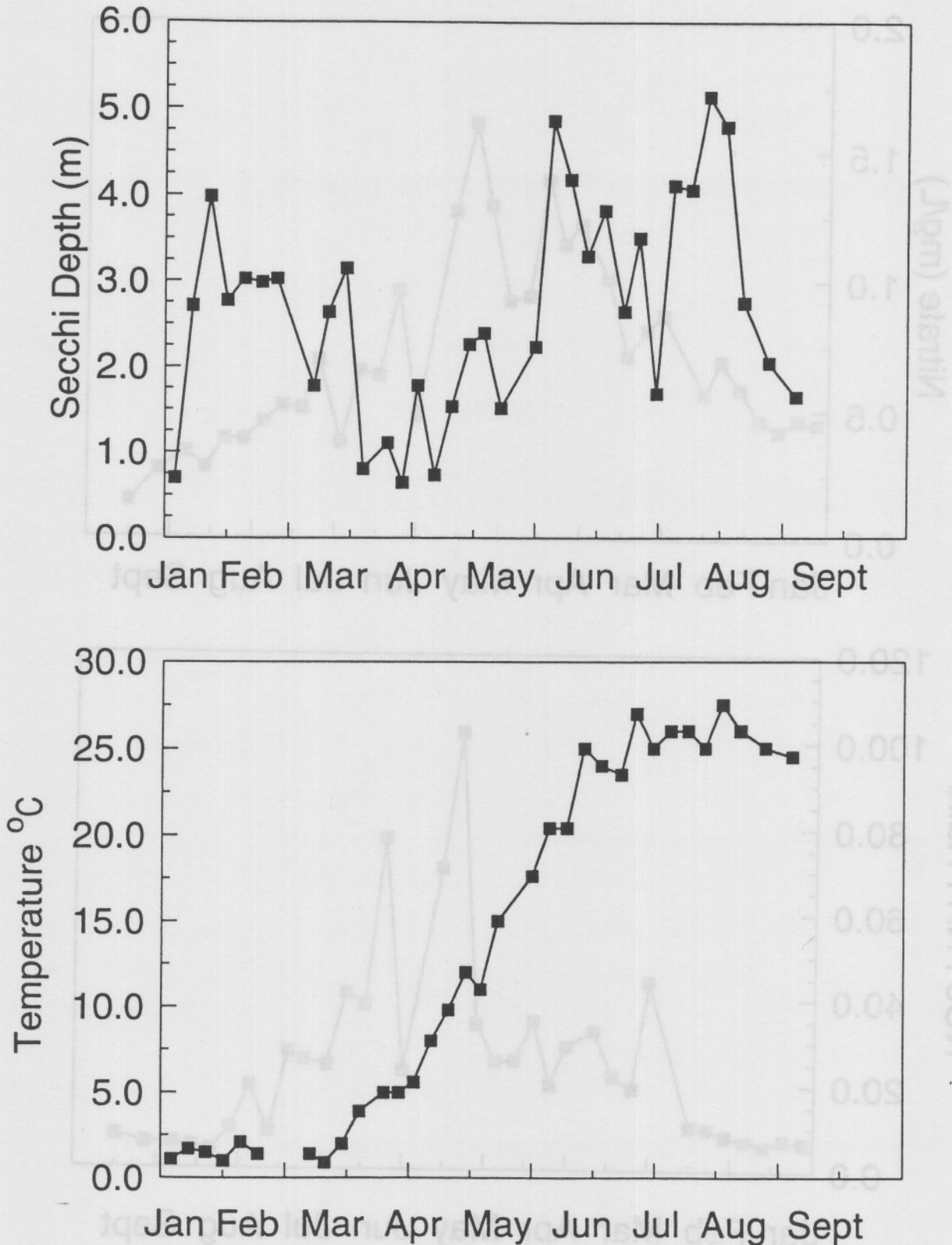


Figure 62. Secchi depth and temperature in Hatchery Bay, Lake Erie determined from weekly sampling during January 4 - September 12, 1995.

Hatchery Bay, Lake Erie

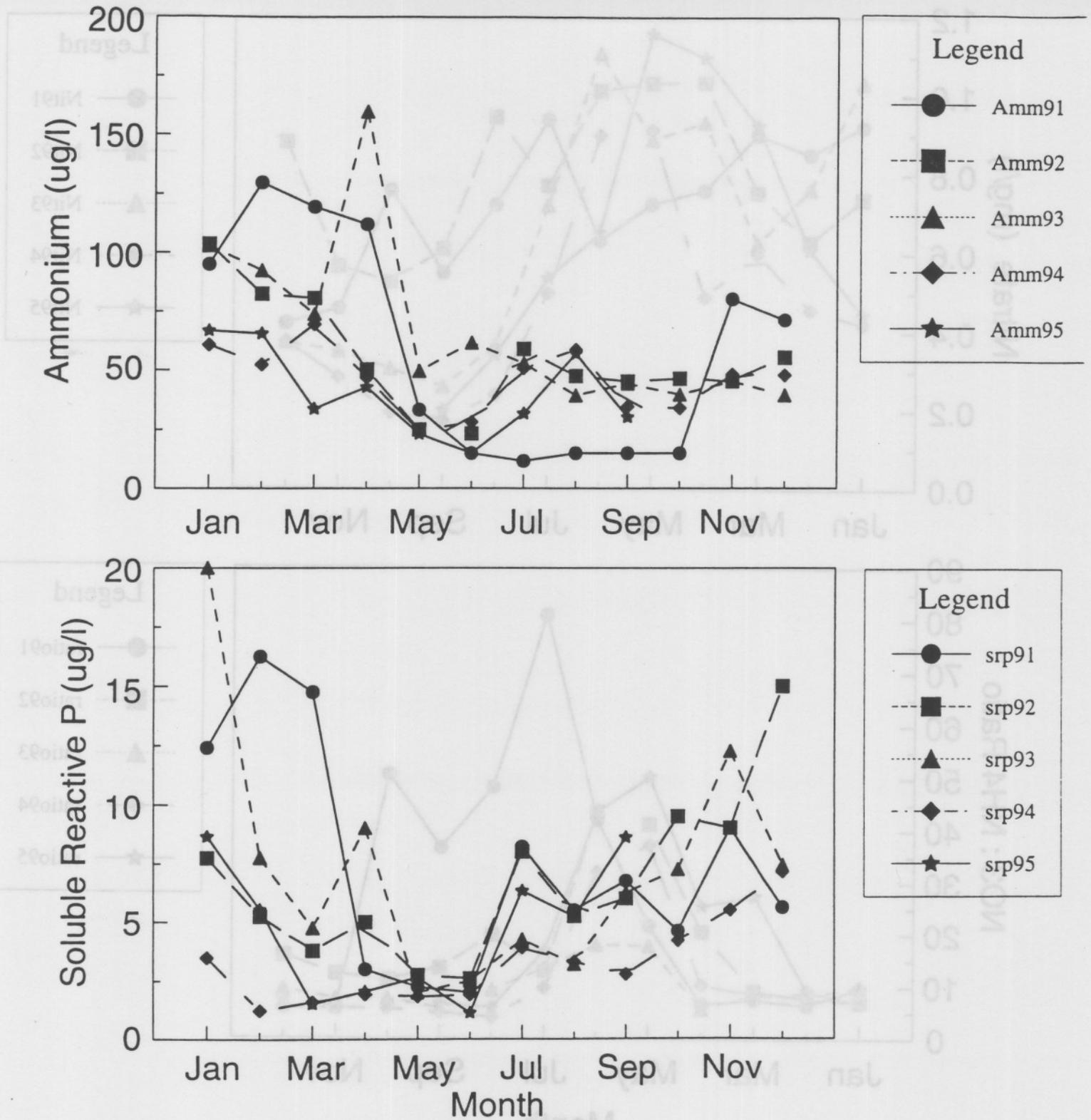


Figure 63. Monthly mean concentrations for ammonium and soluble reactive phosphorus in Hatchery Bay, Lake Erie determined from weekly sampling during January 1991 - September 1995

Hatchery Bay, Lake Erie

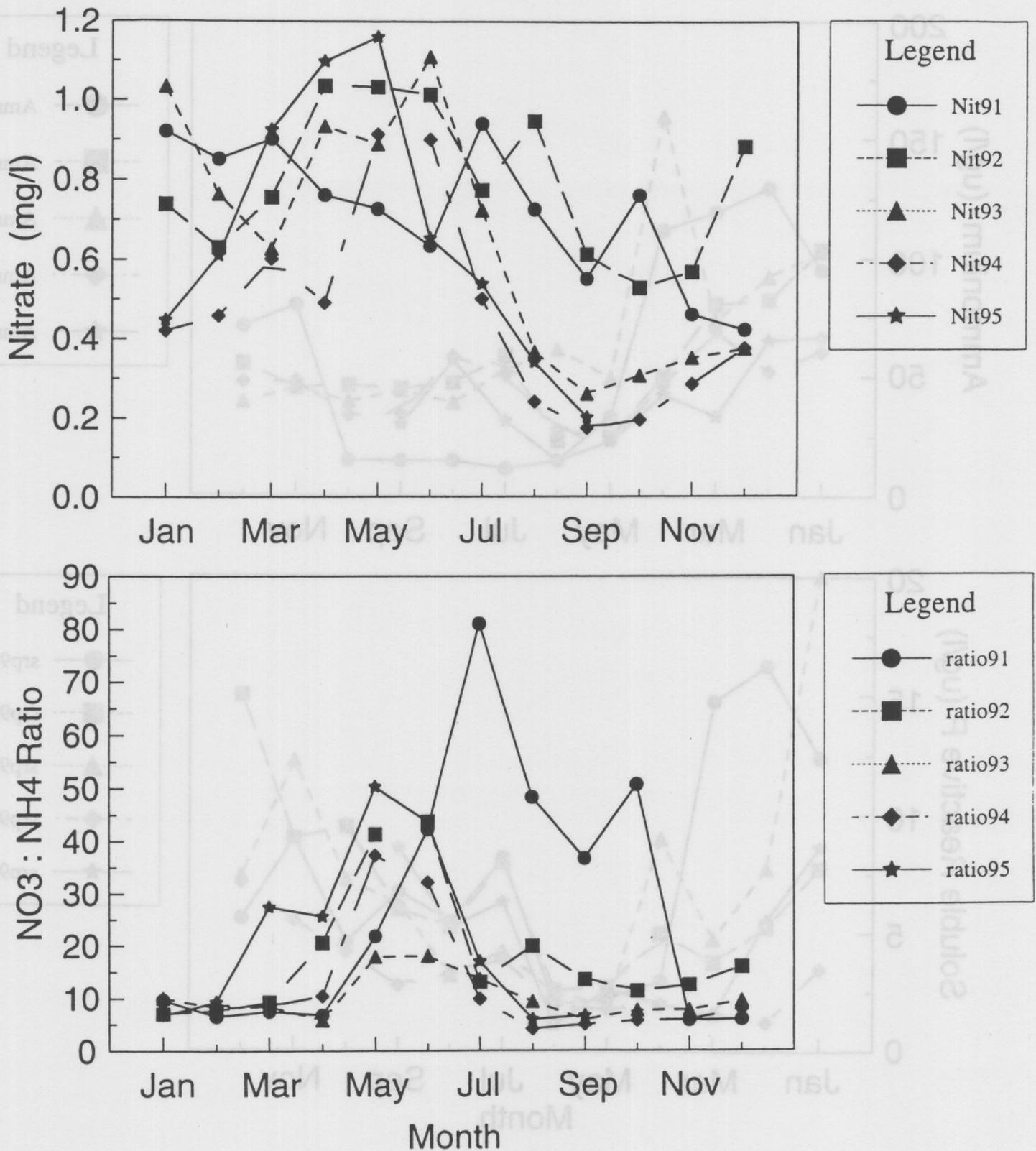


Figure 64. Monthly mean nitrate concentrations and nitrate to ammonium ratio in Hatchery Bay, Lake Erie determined from weekly sampling during January 1991 - September 1995.

Hatchery Bay, Lake Erie

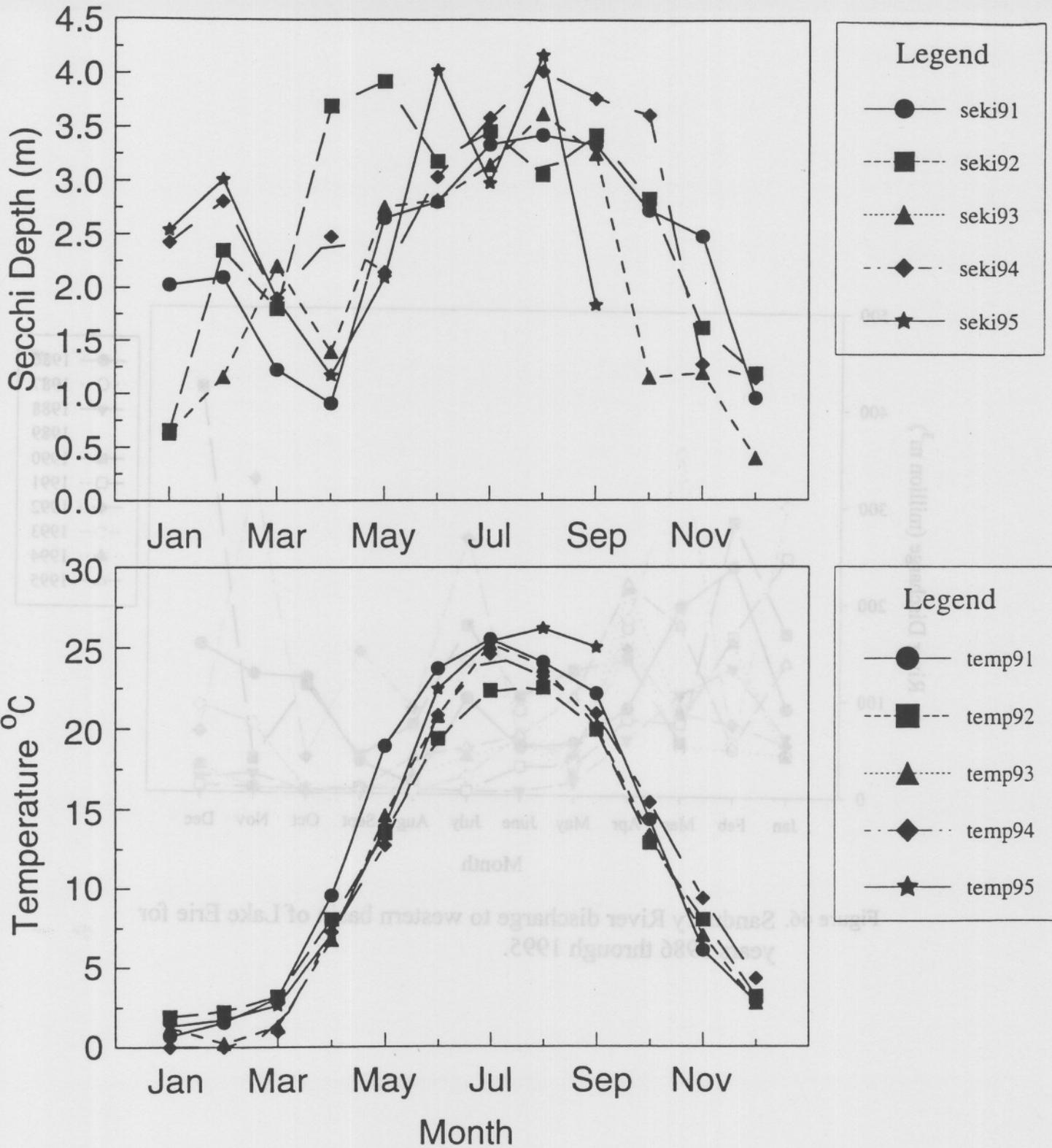


Figure 65. Monthly mean concentrations for secchi depth and temperature in Hatchery Bay, Lake Erie determined from weekly sampling during January 1991 - September 1995.

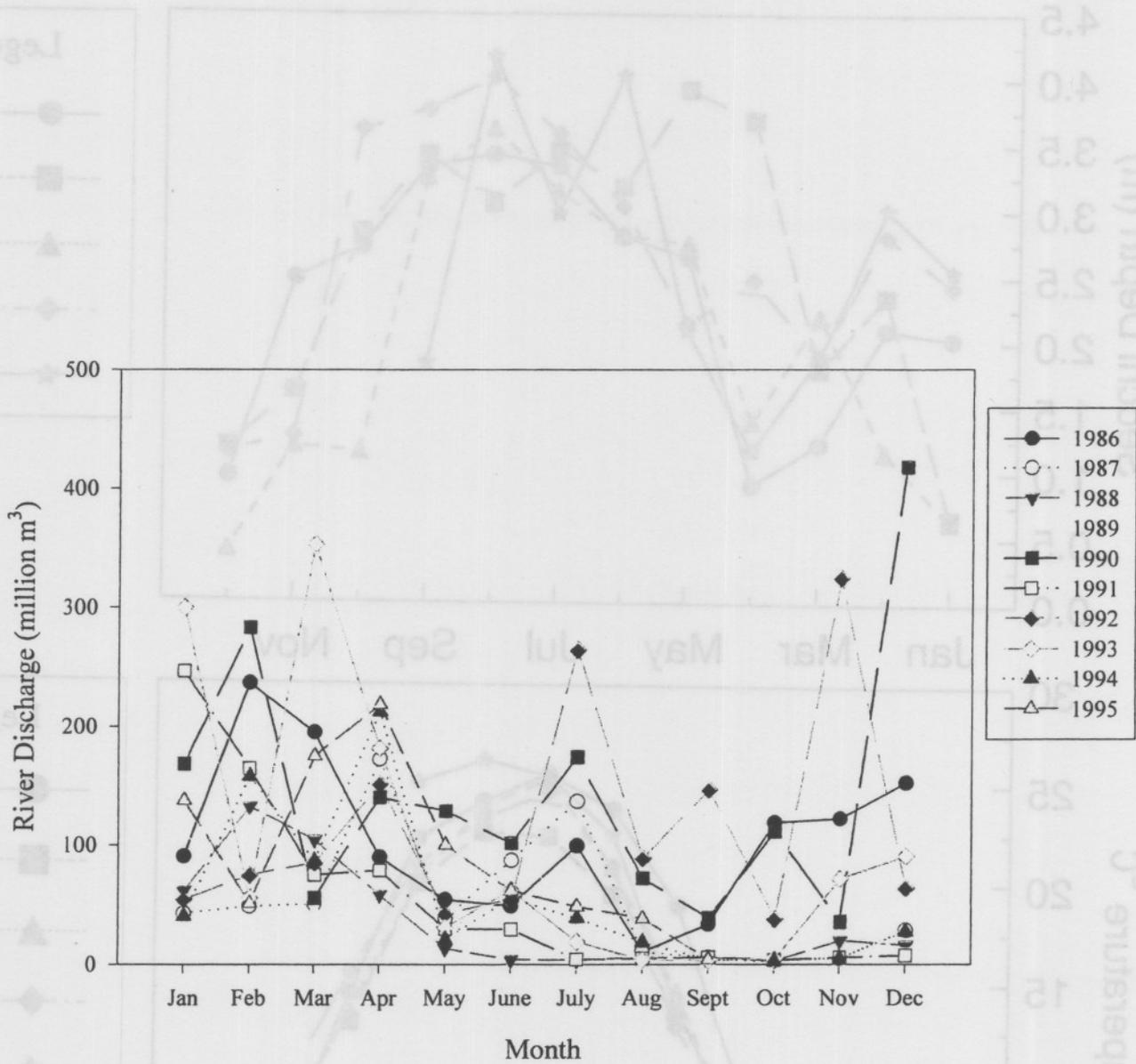


Figure 66. Sandusky River discharge to western basin of Lake Erie for years 1986 through 1995.

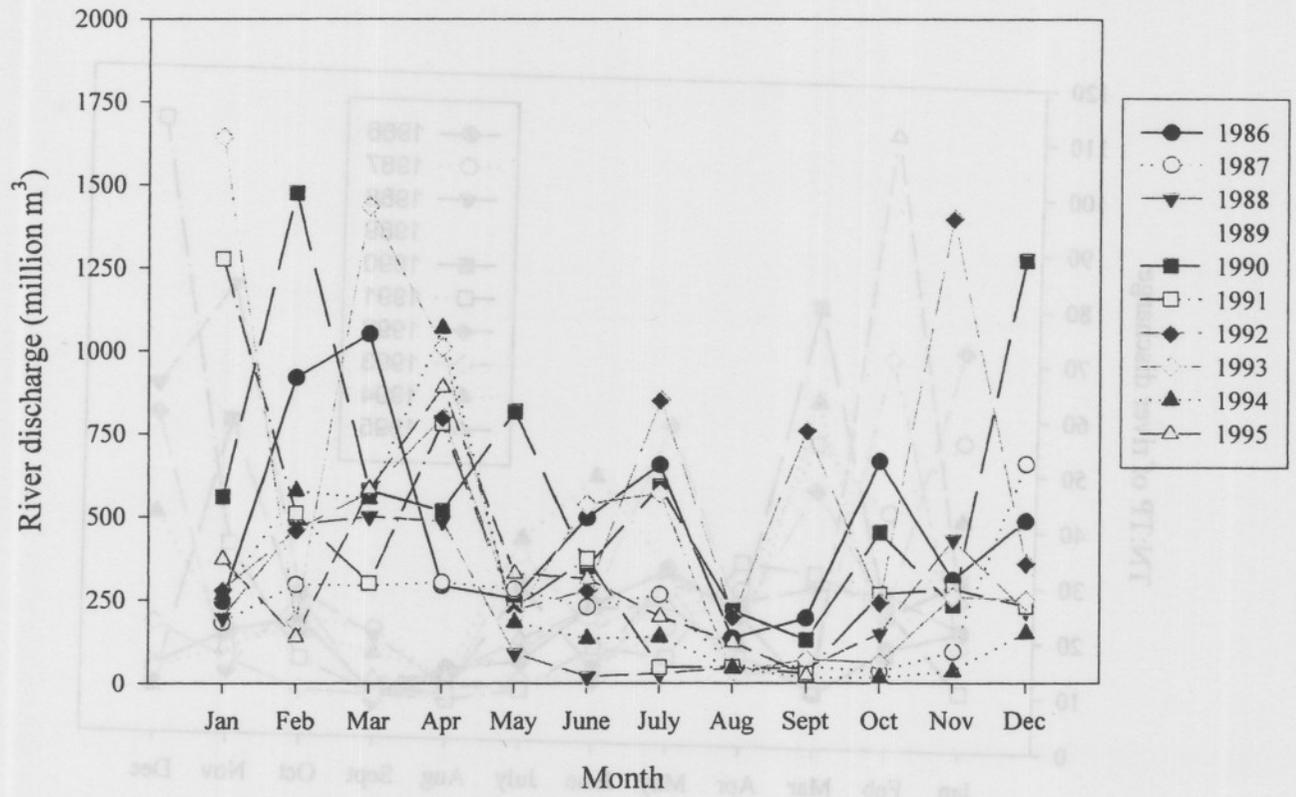


Figure 67. Maumee River discharge to the western basin of Lake Erie for years 1986 through 1995.

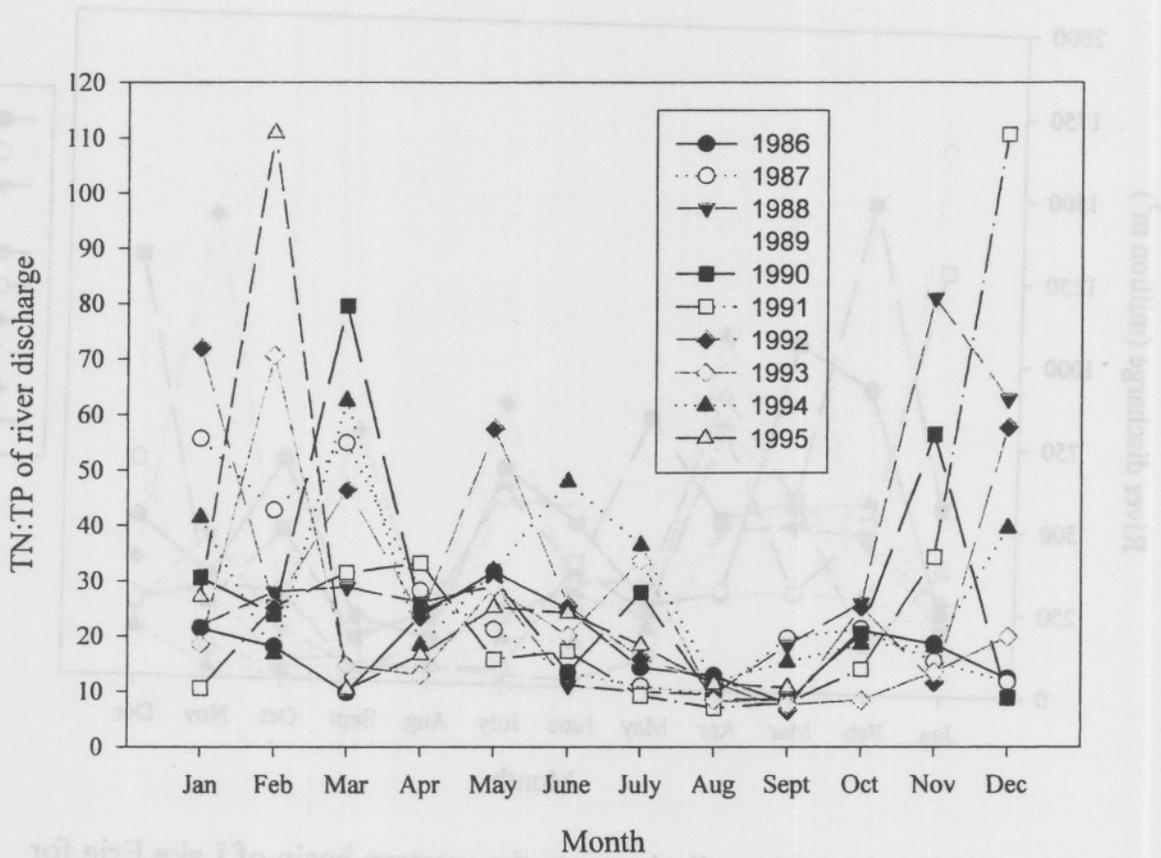


Figure 68. Sandusky River monthly TN:TP of discharge to the western basin of Lake Erie for years 1986-1995.

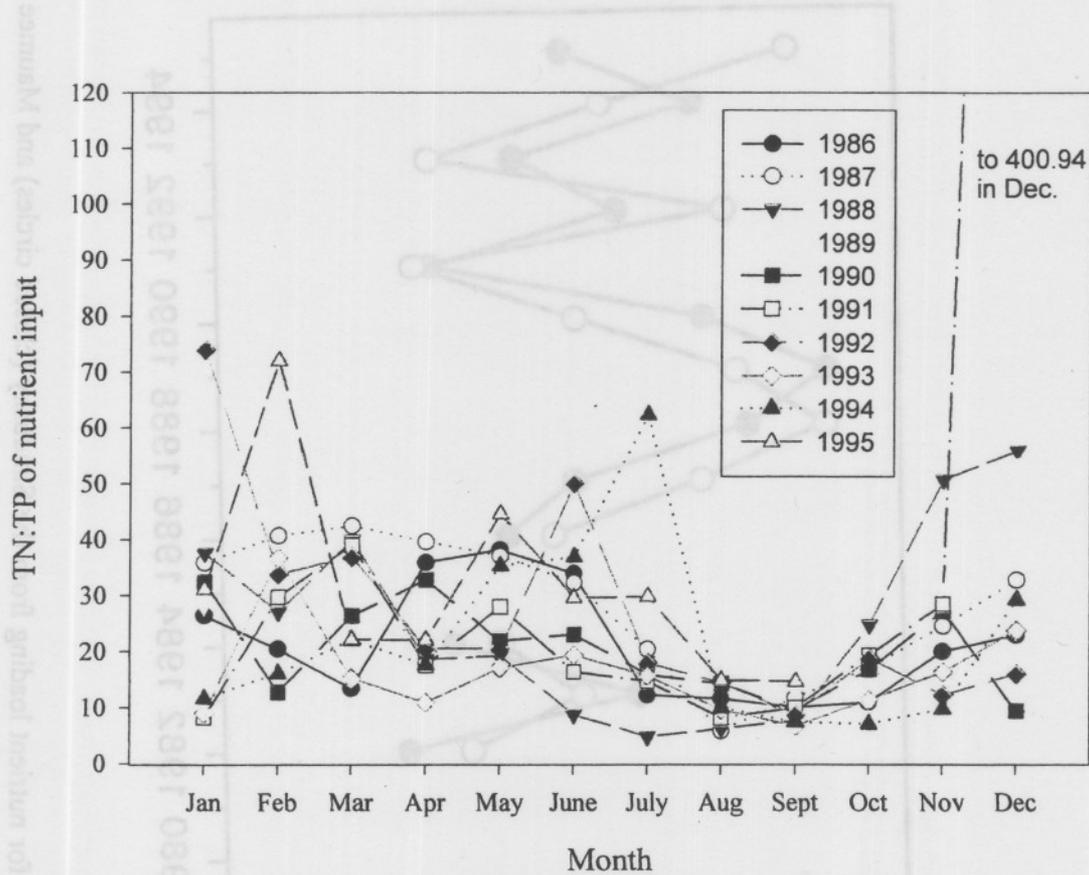


Figure 69. Maumee River monthly TN:TP of discharge to the western basin of Lake Erie for years 1986 through 1995.

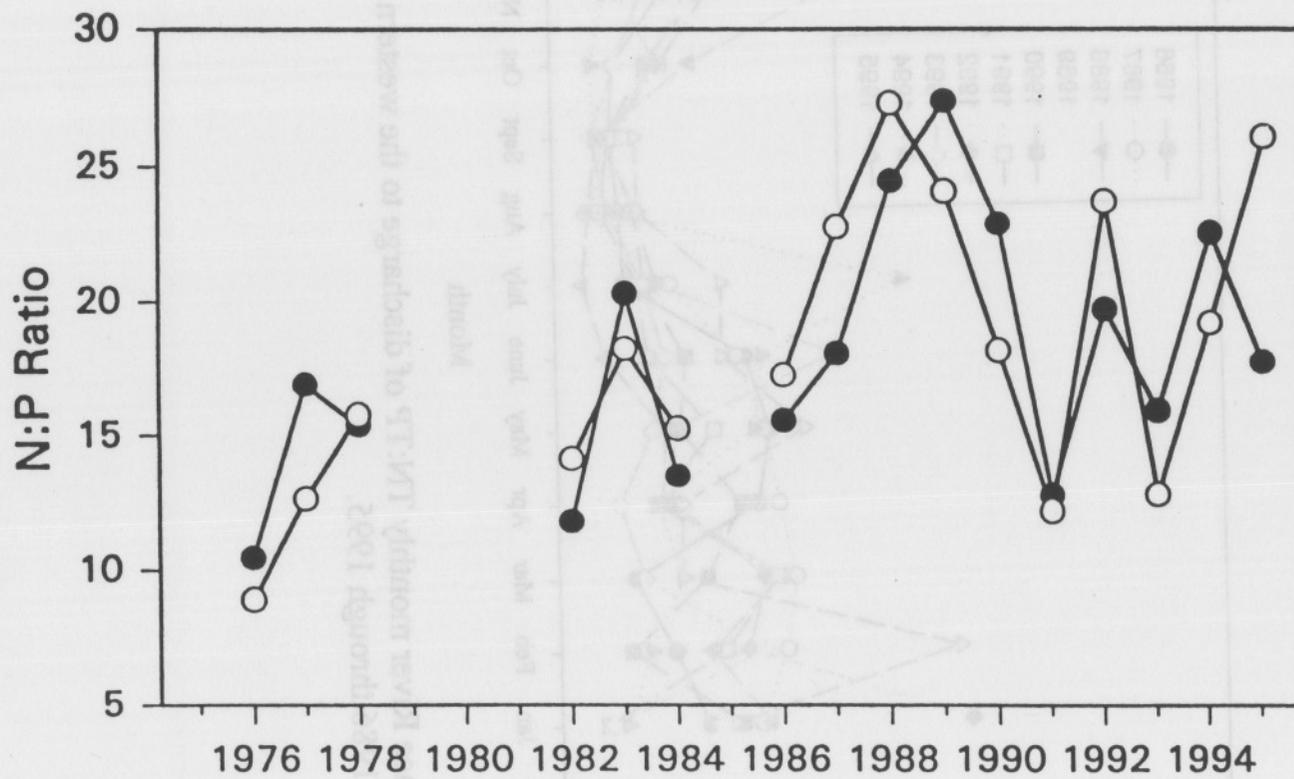


Figure 70. Annual N:P ratios for nutrient loading from the Sandusky (closed circles) and Maumee (open circles) rivers. Nutrient loading data from Baker (1995 a,b).

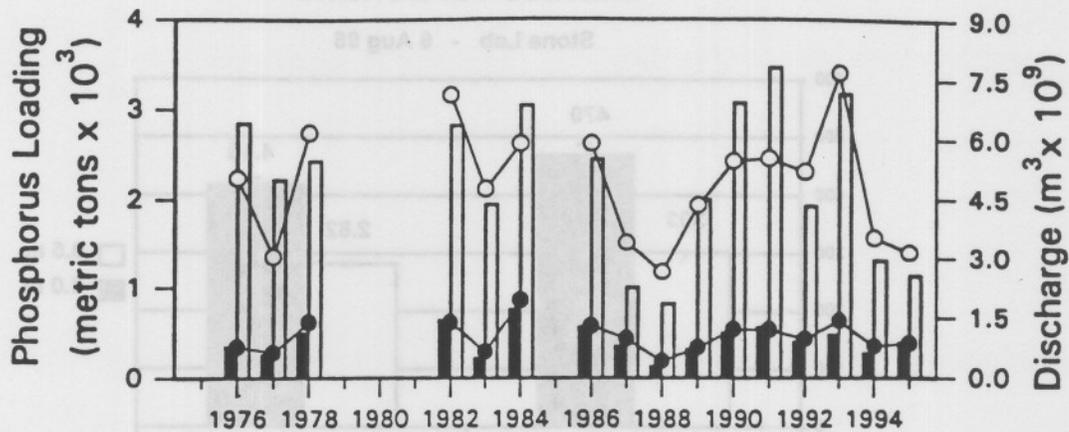


Figure 71b. Recent trends in annual external phosphorus loading (bars) relative to annual water discharge (circles) to western Lake Erie from the Sandusky (shaded bars and closed circles) and Maumee (open bars and open circles) rivers. Data obtained from Baker (1995 a,b).

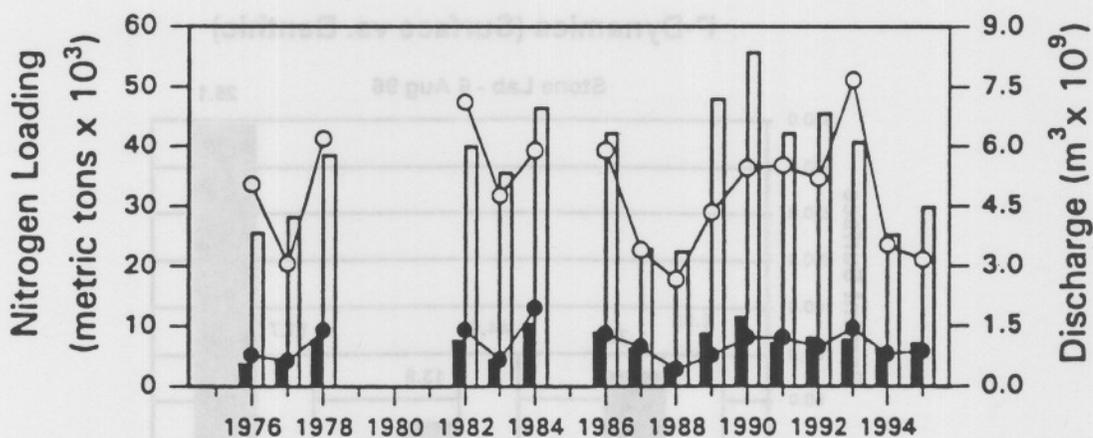


Figure 71a. Recent trends in annual external nitrogen loading (bars) relative to annual water discharge (circles) to western Lake Erie from the Sandusky (shaded bars and closed circles) and Maumee (open bars and open circles) rivers. Data obtained from Baker (1995 a,b).

Surface vs. Benthic

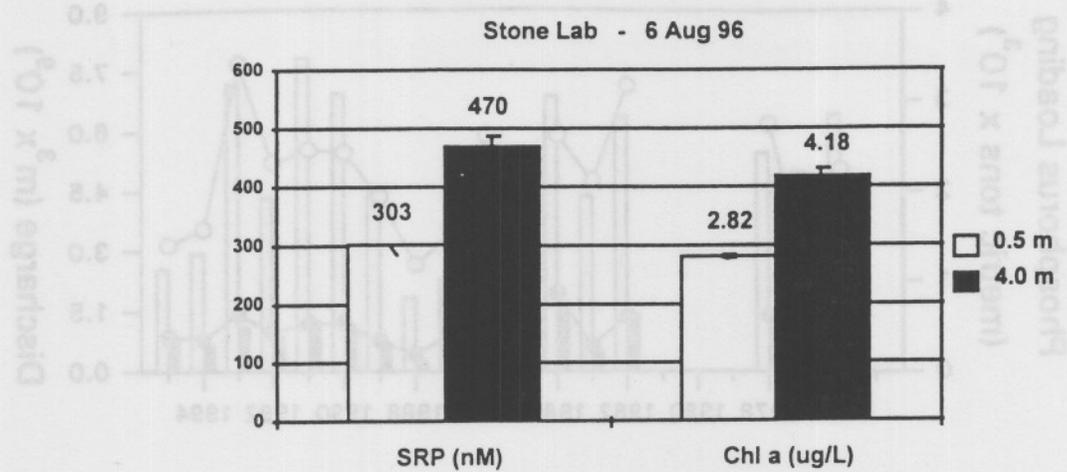


Figure 72. Relative amounts of soluble reactive phosphorus (SRP) and chlorophyll *a* at 0.5 m and 4.0 m depths in an area over a zebra mussel bed at 4.5 m.

P-Dynamics (Surface vs. Benthic)

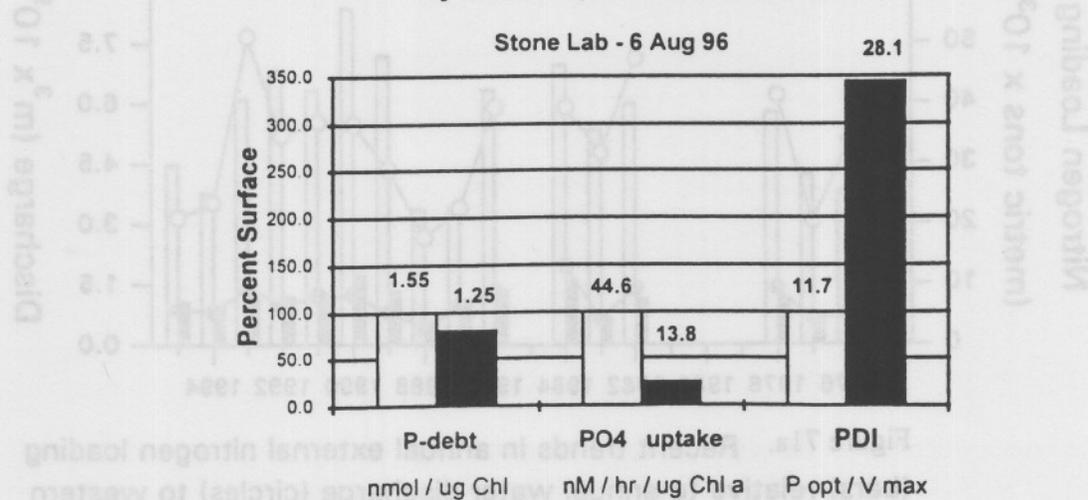


Figure 73. Relative P-debt, phosphate uptake rate, and phosphorus deficiency index for water within 0.5 m of the surface and at 4.0 m (0.5 m of zebra mussels bed). All three indices suggest algae near the zebra mussels had a greater supply of phosphorus than those near the lake surface.

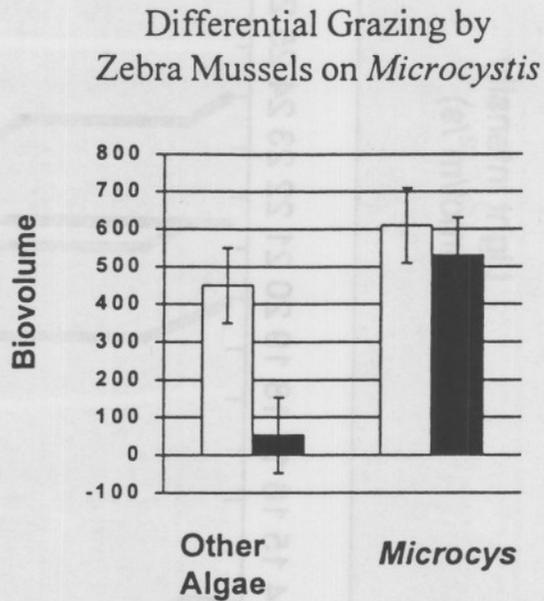


Figure 74. Biovolume of microcystis and "other" algae remaining after 16 h incubation with zebra mussels (solid bars) and without (clear bars).

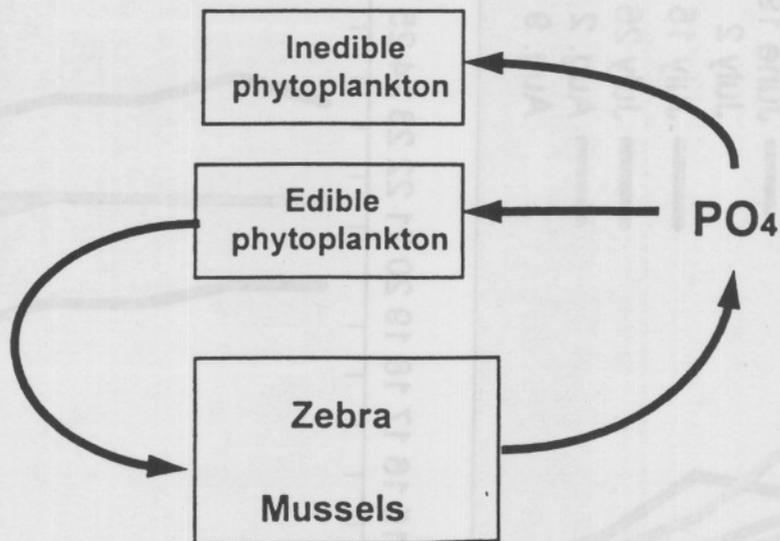


Figure 75. A simple model illustrating that the presence of zebra mussels favors the production of a system dominated by inedible algae through selective grazing and phosphate excretion by zebra mussels.

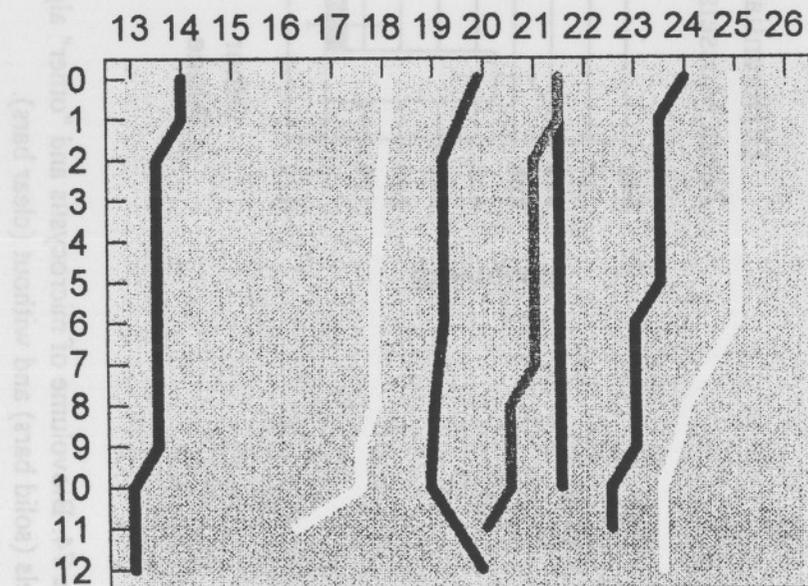
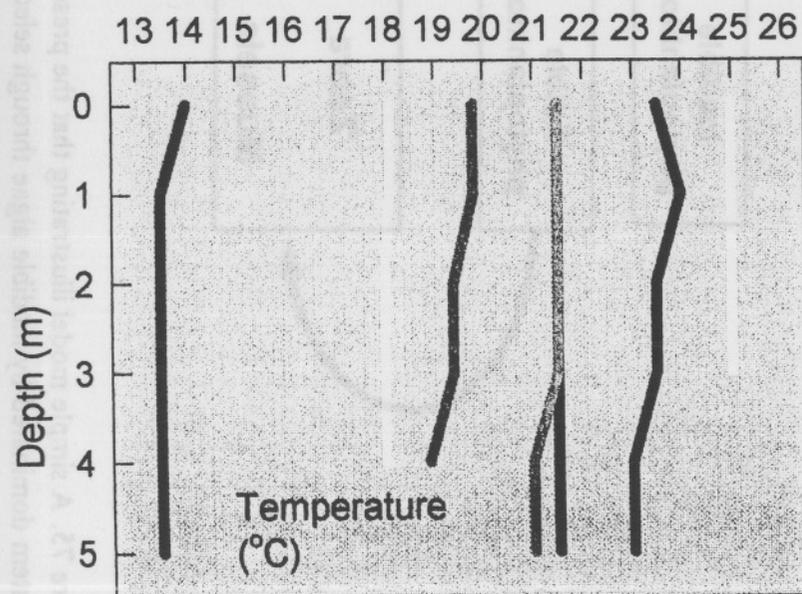
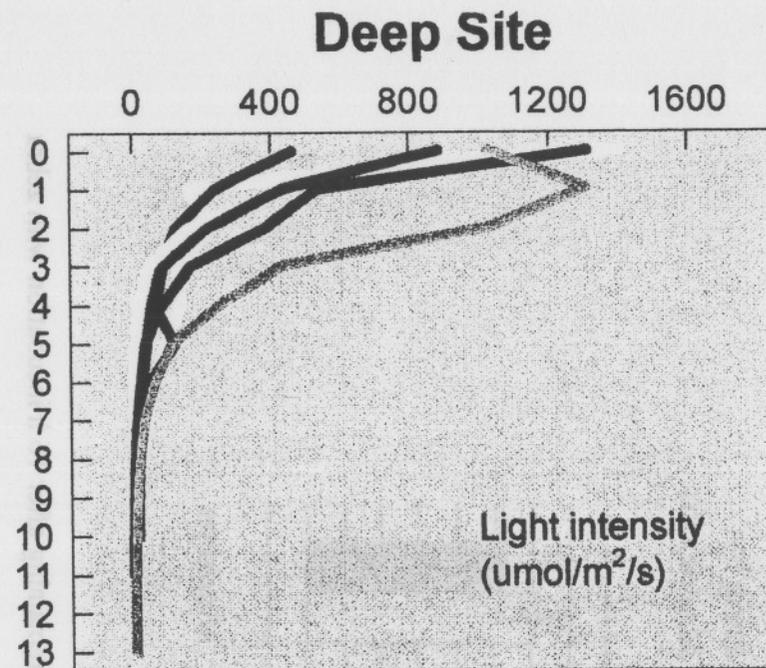
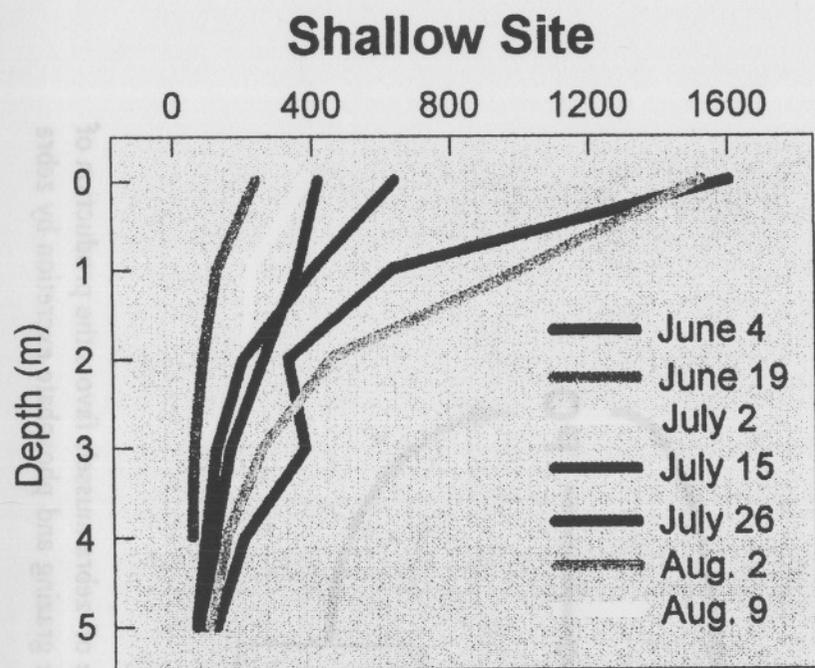


Figure 76. Vertical profiles of light intensity ($\mu\text{mol}/\text{m}^2/\text{s}$) and temperature ($^{\circ}\text{C}$) at two sites in western Lake Erie, 1996.

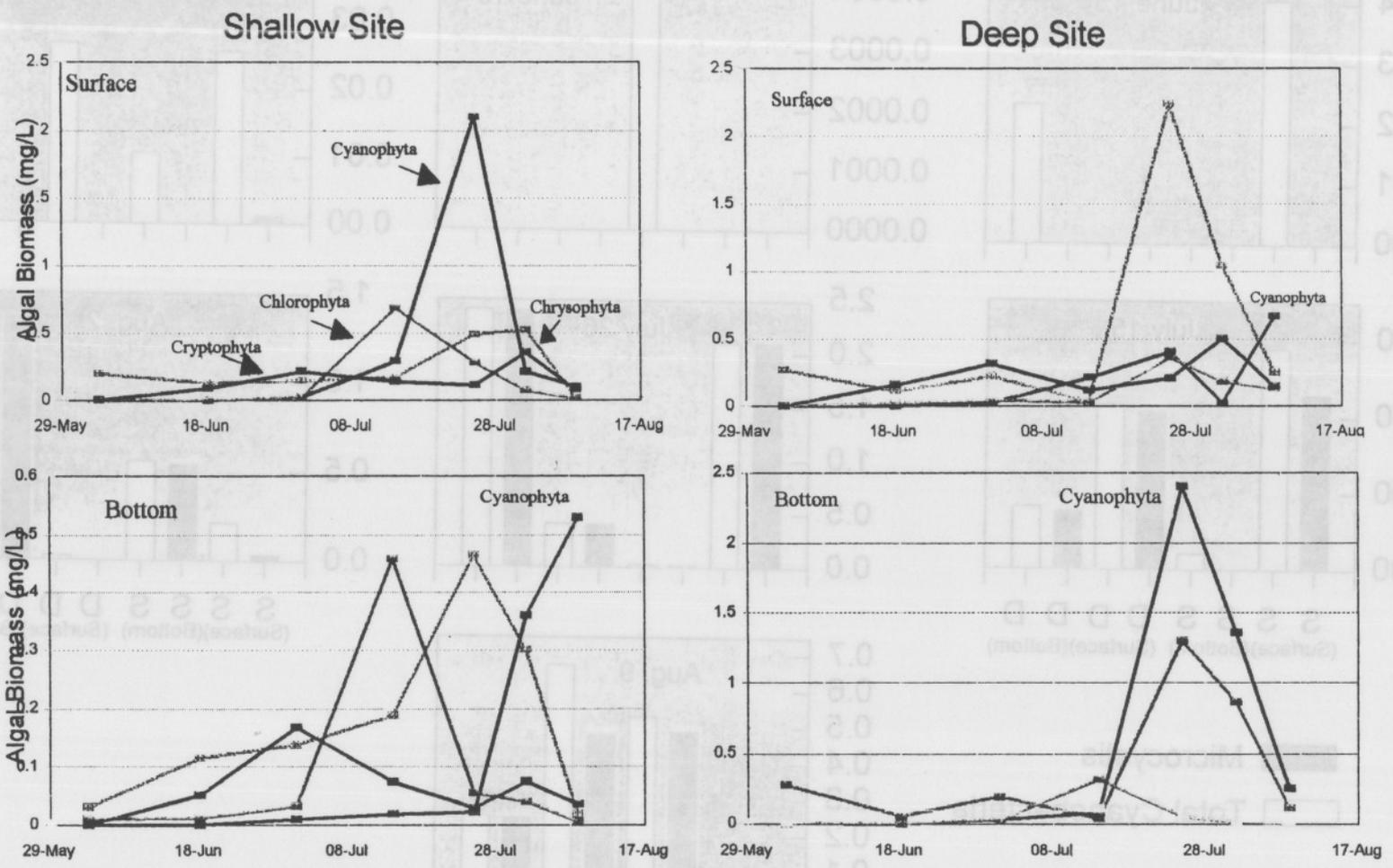


Figure 77. Algal abundance (mg/L) at the surface and on the bottom of two sites sampled in western Lake Erie, 1996.

Figure 78. A comparison of Microcystis vs total Cyanobacteria in algal samples from two sites collected on several dates in western Lake Erie, 1996. S = shallow site; D = deep site.

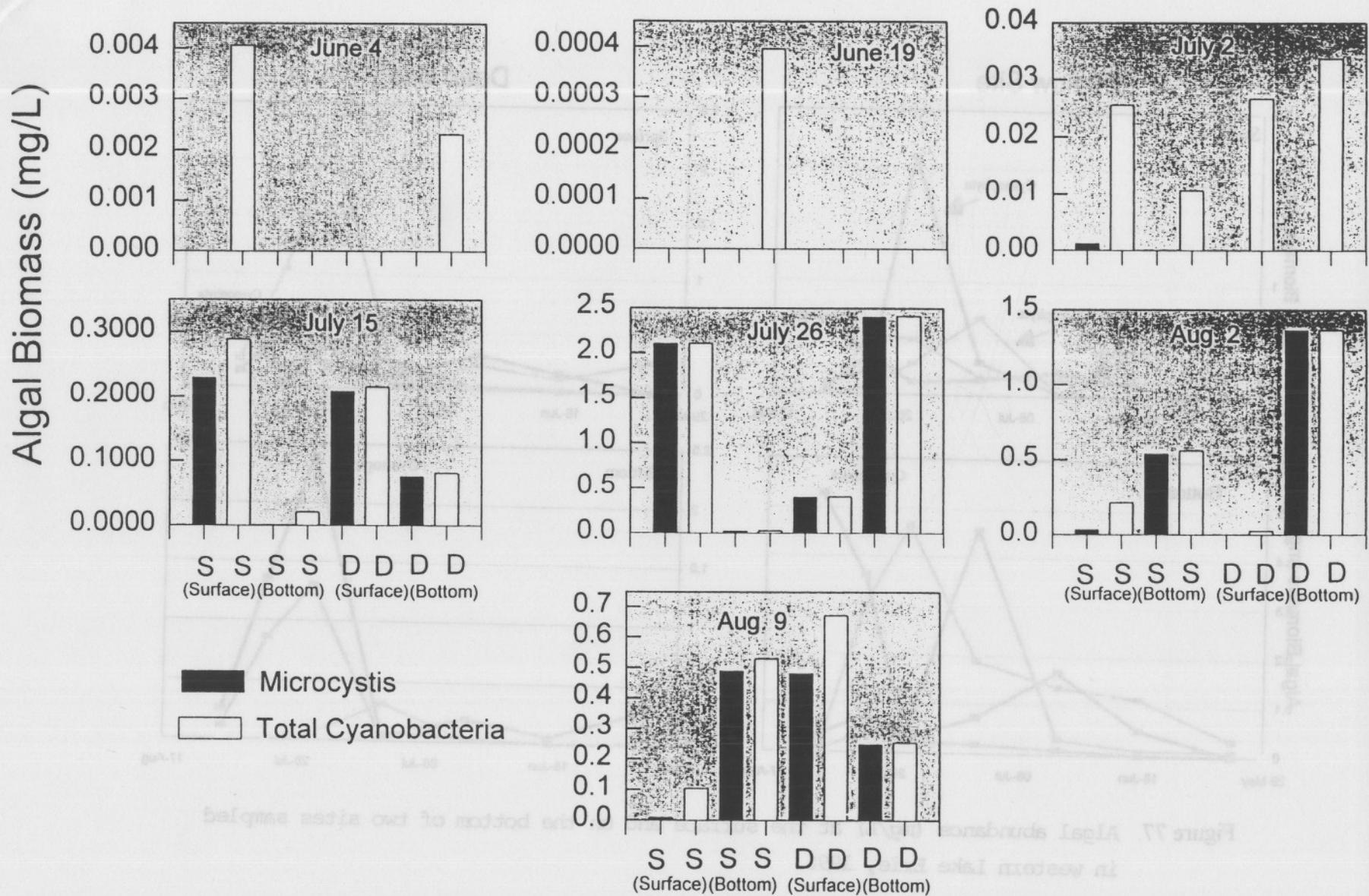


Figure 78. A comparison of *Microcystis* vs. total Cyanobacteria in algal samples from two sites collected on several dates in western Lake Erie, 1996. S = shallow site; D = deep site.