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TOXIC Microcystis IN WESTERN LAKE ERIE: ECOTOXICOLOGICAL
RELATIONSHIPS WITH THREE NON-INDIGENOUS SPECIES INCREASE
RISKS TO THE AQUATIC COMMUNITY

DISSERTATION
Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of
Philosophy in the Graduate School of The Ohio State University

By
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The Ohio State University
2000

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ABSTRACT

Cyanobacteria blooms have recurred in western Lake Erie. Recent blooms have been dominated by *Microcystis aeruginosa*, a non-nitrogen fixing species that produces hepatotoxins. Bloom dominance by non-nitrogen fixing species is significant because it has happened after declines in external sources of phosphorus loading led to significant declines in cyanobacteria blooms dominated by nitrogen-fixing species in the 1970's. Recent blooms coincide with the introduction of non-indigenous mussels, *Dreissena polymorpha* and *D. bugensis*, to Lake Erie. In ecologically similar lake systems invaded by dreissenids, bloom formations dominated by *M. aeruginosa* have also occurred. My examinations of historical and present trends in cyanophyte abundance and species composition suggest an interconnection between high nitrate concentration, selective filtration, and excretions of ammonia and phosphate by dreissenids, and extended periods of summer temperatures in the range of 23 to 26° C.

Further, concern about *M. aeruginosa* blooms in western Lake Erie is founded in the production of cyclic heptapeptide hepatotoxins (microcystins) by this species. We examined the fate of microcystin produced by *M. aeruginosa* in western Lake Erie by testing whether microcystin toxin is passed through and/or accumulated by three non-indigenous, interconnected species that have become established in Lake Erie. Dreissenid
mussels, amphipods (*Echinogammarus ischnus*), and round gobies (*Neogobius melanostomus*), served as a model trophic cycle in which to follow the fate of microcystin. Our surveys of Lake Erie water showed that microcystin was present in water and cell material at low levels during *Microcystis* blooms in 1995, 1996 and 1998. Mussels, amphipods and several fish species collected during blooms accumulated the toxin in guts and/or liver tissue. We determined that a novel mechanism making microcystin available to benthic organisms such as *E. ischnus* is the deposition of microcystin-laden pseudofeces + feces by *Dreissena* species.

Further indirect effects caused by the introduction of dreissenids may be negative feedback effects associated with toxic *Microcystis* blooms that they play a part in encouraging. To determine the potential for feedback effects on mussels, I developed a dynamic programming model of *D. polymorpha* filtration behavior to determine if fitness is influenced by blooms of *M. aeruginosa*. In the model, bioenergetic parameters of mussels were influenced by assumed ecological conditions and probabilities. Model outcomes suggest small mussels experiencing bloom conditions will have reduced fitness. An additional implication of this outcome is that size-frequency of populations may be shifted toward dominance by larger sizes having a potentially greater algal consumption and pseudofeces production rates. Thus, population-level feedback effects on dreissenids induced by toxic *Microcystis* blooms may further aggravate their ecological impacts.
Dedicated to Mark, Dana and Benjamin.
ACKNOWLEDGMENTS

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INTRODUCTION

Toxic *Microcystis* in Western Lake Erie: Ecotoxicological Relationships with Three Nonindigenous Species Increase Risks to the Aquatic Community.

Cyanobacteria blooms have recurred in Lake Erie. Recent blooms have been dominated by *Microcystis aeruginosa*, a non-nitrogen fixing species that produces a hepatotoxin, microcystin. In this dissertation, I consider the causes and ramifications of *M. aeruginosa* blooms in western Lake Erie. I also examine the potential risks to the aquatic community associated with the occurrence of microcystin in conjunction with the feeding habits of three new non-indigenous species, a dreissenid mussel (*Dreissena polymorpha*), the round goby (*Neogobius melanostomus*), and an amphipod (*Echinogammarus ischnus*).

*Mechanism of action for the Microcystis toxin microcystin:*

Recent blooms of cyanobacteria, especially *Microcystis aeruginosa*, in Lake Erie have raised potential human and ecosystem health concerns. Seventy to eighty percent of *Microcystis* blooms occurring in the United States produce liver toxins termed microcystins (Carmichael et al. 1990; Carmichael 1994).
Microcystins are toxic to a variety of organisms including humans. The mechanism of action for toxicity of microcystins occurs at the molecular level within all livers and hepatopancreatic tissues possessing protein phosphatase activity. Microcystin is a potent inhibitor of serine/threonine protein phosphatases 1 and 2A (PP1 and PP2A), causing increasing levels of cellular protein phosphorylation, inducing changes in signal transduction and ultimately causing structural damage that results in severe liver damage or death (Bagu et al. 1997; Runnegar et al. 1995). Runnegar et al. (1995) determined that the liver becomes the target due to selective uptake by specific carrier-mediated transport into liver cells (hepatocytes). Once inhibition by covalent bonding to microcystin has occurred in the catalytic subunits of the phosphatase, it is kinetically irreversible. More research is needed to determine the mechanism of toxicity in invertebrates, especially since the observed effects are more or less limited to changes in behaviors, such as decreased ingestion rates, fecundity and growth rates, although lethal toxicity is known to occur (DeMott and Moxter 1991; DeMott et al. 1991; Kaya 1996).

**Threats to drinking water sources and humans:**

Microcystins have been detected in drinking water sources from all over the world including Portugal (Vasconselos et al. 1993), Russia (Sivonen et al. 1992), Brazil (Azevedo et al. 1994), China (Zhang et al. 1991), Japan (Park and Watanabe 1996), and Finland (Namikoshi et al. 1992; Lindholm et al. 1989), among others. In western Lake Erie, a *Microcystis aeruginosa* bloom was observed to begin in August of 1995, and microcystins were first detected in water samples taken from Hatchery Bay, Lake Erie, in
October 1995 and in June through September during 1996 (Brittain et al., 2000). Historically, *Microcystis* species have frequently been detected in Lake Erie, but were not typically the dominating species during cyanophyte blooms of the 1970s (Munawar and Munawar 1976). *Microcystis* spp. pose a new threat to drinking water in Lake Erie because they produce hepatotoxins (microcystins) and have become a dominant feature of recent cyanophyte blooms. Blooms of the past were typically dominated by *Aphanizomenon* spp., which can produce neurotoxins (anatoxin and saxitoxin). Most water treatment systems are incapable of removing algal toxins from water without the addition of activated carbon filtration or ozonation (Lambert et al. 1994; Velzeboer et al. 1995). Chlorination processes are successful only if used properly for long enough periods (Tsuji et al. 1997). Furthermore, pre-chlorination, or treatment with copper sulfate, practices commonly used to control algae growth and zebra mussel settlement in water treatment ponds and pipes, may cause cell lysis, which releases the toxin to water and makes it more difficult to remove by normal filtration processes (Kenefick et al. 1993; Velzeboer 1995).

Biological filters (relying on microbial degradation) may not be effective at removing toxins either. A 1991 study of biodegradation of cyanobacterial toxins (Kiviranta et al. 1991) found that microcystins were not biodegraded after a 90-day incubation with naturally occurring microorganisms. In more recent studies, researchers have found that activities of natural bacterial communities and heterotrophic nanoflagellates effectively degraded microcystins dissolved in waters of both laboratory
and field microcosms, causing an exponential decline of the toxins within a matter of days (K. Christofferson 1998, pers. comm.). Whether biofiltration systems could work fast enough to prevent toxins from occurring in drinking water supplies if that were the only treatment, as is true for some people living near the shores of Lake Erie. They rely solely on sand-filtration beds to filter drinking water derived directly from the Lake, possibly allowing microcystins to occur in their drinking water. Even at low-levels, long-term exposure to microcystins could pose a risk to humans (Solter et al. 1998). Solter et al. (1998) have shown that rats infused constantly for 28 d with low levels (0, 3, 6, or 9 µg microcystin d⁻¹) showed dose-dependent hepatotoxic effects that included decreased ser/thr PP-ase activity and histopathological effects in the livers.

Low levels of microcystins in drinking water also pose a potential threat to humans because of their action as liver tumor promoters (Nishikawa-Matsushima et al. 1992; Falconer 1996). Higher incidences of hepatocellular carcinomas in human populations may be associated with Microcystis contamination of their drinking water supplies (Falconer and Humpage 1996). High levels of microcystins can occur if a treatment system fails entirely. An especially dramatic occurrence of acute human liver toxicity was documented near Recife, Brazil, in February 1996 (Barreto et al. 1996). Water collected from a reservoir with a heavy bloom of cyanobacteria was inadequately treated (filtered through a spent carbon, sand and cation/anion exchange system) before it was used in hemodialysis treatments. Microcystin was confirmed to be present in the carbon from the filtration system and in the livers of patients. Of the treated patients, 136
exhibited symptoms of microcystin toxicity and 70 of them died within two months of exposure. The results of these studies show that toxins of cyanobacteria may directly threaten human health through consumption of drinking water or medical use of inadequately treated water. It remains to be determined whether humans are threatened by microcystin exposure through consumption of contaminated aquatic organisms.

**Threats to Aquatic Organisms and Implications for Lake Erie:**

Microcystins are known to have a range of competitive, inhibitory and toxic effects on algae, protozoa, zooplankton, fish, birds and mammals (Murphy and Lean 1976; Keating 1978; Christoffersen 1996; DeMott et al. 1991; Jungmann 1992; Reinikainen et al. 1994; Smith and Gilbert 1995; Berthon and Brousse 1995; Råbergh et al. 1991; Carmichael 1992 and 1994). For example, the ability of *Mytilus galloprovincialis* and *Mytilus edulis*, estuarine mussels, to accumulate the toxins from *Microcystis aeruginosa* without apparent harm shows that there is a potential threat through food chain transfer (Vasconcelos 1995; Williams et al. 1997). The known ability of mussels to accumulate microcystin indicates that zebra mussels and quagga mussels (*Dreissena polymorpha* and *D. bugensis*) in Lake Erie are likely to accumulate the toxin as well. Therefore, exposure routes and levels of accumulation of the toxin in aquatic organisms, especially mussels, in Lake Erie should be examined and quantified.
Zebra mussels as a route of microcystin transfer through the aquatic food web:

Two natural routes of microcystin exposure are known to occur in invertebrates and vertebrates: one is by direct ingestion of whole cyanobacterial cells in water or from surface scums that wash ashore; another is ingestion of water containing soluble toxin after cells have decomposed. A third, less explored possibility is transfer of toxins through the food web. Human exposure to cyanobacteria toxins is most likely to occur through drinking water (Carmichael and Falconer 1993), but could also occur through eating contaminated shellfish, fish, birds or mammals. The following studies show that there are mechanisms by which zebra mussels and other introduced species could be altering the fate of microcystins in the aquatic environment of western Lake Erie and may ultimately provide a route of exposure to other organisms and humans through the aquatic food web.

Much research has focused on potential ecosystem impacts of zebra mussels and quagga mussels, species introduced to the Great Lakes from Europe circa 1986 (Hebert et al. 1989). Potential impacts include changes in internal nutrient dynamics (Gardner et al. 1995; Arnott and Vanni 1996; James et al. 1997), competition with zooplankton, other bivalves and fish for algae (Makarewicz et al. 1999), increased water clarity and sedimentation (Klerks et al. 1996), and changes in the cycling and fate of toxic contaminants (Bruner et al. 1994). The zebra mussel is known to accumulate metals (Klerks et al. 1997) and organic contaminants in body tissues and has been shown to pass these contaminants along to its feces and pseudofeces (Fisher et al. 1993; Bruner et al. 1994). In turn, pseudofeces (filtered particles that are rejected before ingestion) tend to
have a higher concentration of pollutants than does natural suspended matter, particularly when pollutants are sediment-bound (Reeders and Bij de Vaate 1992). Since zebra mussels may also reject *Microcystis* as pseudofeces, it is possible that the toxin is maintained in the pseudofecal pellet. Algae-associated hydrophobic contaminants have been shown to accumulate in zebra mussel feces and pseudofeces (Gossiaux et al. 1998). Therefore, microcystin could be accumulating in zebra mussels, as well as passing to their pseudofeces and feces.

Typically, concern over bioaccumulation and biomagnification in aquatic organisms focuses on hydrophobic contaminants. For example, hydrophobic contaminants have been shown to biomagnify in amphipods (*Gammarus fasciatus*) feeding upon contaminated feces and pseudofeces from zebra mussels (Bruner et al. 1994). Microcystin is not a very hydrophobic toxin (log $K_{ow} < 3.0$), nor is it typically considered a pollutant in the United States; however, its association with cyanobacterial cells which are rejected in the pseudofeces of zebra mussels and quagga mussels could result in microcystin cycling to sediments in higher concentrations than occurred through natural settling when zebra mussels were not present. Hence, zebra mussels may have become a novel route of transfer of microcystins to the benthic community. Animals feeding directly on zebra mussels, e.g. several fish species (French and Bur 1993; Morrison et al. 1997; Ray and Corkum 1997), diving ducks (Hamilton et al. 1994), and crayfish (MacIsaac 1994), or feeding on their feces and pseudofeces, e.g. amphipods and
other benthic invertebrates (Thayer et al. 1997; Dermott et al. 1998), could therefore serve as routes for bioaccumulation in these organisms, transferring microcystins throughout the aquatic food web.

In particular, an interdependent relationship has developed recently in Lake Erie among three new non-indigenous species: the dreissenid mussels, an amphipod (Echinogammarus ischnus), and the round goby (Neogobius melanostomus). As will be shown in this dissertation, these three organisms have particular relationships with one another that result in a new link between the benthic and pelagic community in Lake Erie. The ramifications of relationships between these organisms will be examined in relation to causes of Microcystis blooms, the possible re-routing of microcystin toxins and risks to the aquatic community, and possible feedback effects on zebra mussel feeding behavior and fitness.

Issues to be addressed in this dissertation:

In this dissertation, therefore, I examine three aspects of the recurrence of Microcystis in Lake Erie: (1) how the recent recurrences of Microcystis may be associated with internal nutrient changes brought about by zebra mussels; (2) how zebra mussels may be altering the cycling of microcystin to the benthic community; and (3) how changes in feeding behavior, and thus fitness, of zebra mussels may be affected by toxic Microcystis blooms.
In Chapter 1, I determine how recent recurrences of *Microcystis* blooms in western Lake Erie may be associated with internal nitrogen and phosphorus loading changes brought about by zebra mussels. I also examine how changes in external loading in western Lake Erie may or may not have influenced cyanophyte blooms since the introduction of *Dreissena* species to Lake Erie. I first review historical occurrences of different cyanobacteria in western Lake Erie and their relationships to available data on nitrogen and phosphorus levels, and then examine a recent (1986 - 1999) data set for Maumee River loading of nitrogen and phosphorus to the western basin relative to *Microcystis* abundance. I also examine the influence of spatial heterogeneity in internal loading of nitrogen and phosphorus on *Microcystis* distribution and density by analysis of phytoplankton and nutrients collected from 44 stations in western Lake Erie during summer 1996.

In Chapter 2, I examine how zebra mussels may be altering the cycling of microcystin within the benthic community. I have determined whether zebra mussels accumulate microcystins in their bodies and whether they pass microcystin to their feces and pseudofeces, using a series of microcosm experiments where I exposed the zebra mussels to different concentrations of toxic *Microcystis* from both cultured and natural sources. I also determined whether the amphipod (*Echinogammarus ischnus*) accumulates the toxin from contaminated pseudofeces/feces or from water and whether the round goby (*Neogobius melanostomus*) accumulates the toxin from water or from feeding on contaminated zebra mussels. In addition to these laboratory experiments, field
collections of zebra mussels, quagga mussels, pseudofeces/feces, amphipods and several fish species (including round gobies) were made to determine accumulation of microcystins in Lake Erie organisms. I relate these data to levels of microcystin in Lake Erie cyanobacteria from metered plankton samples, and interpret the relative rates of accumulation from water to organisms.

In Chapter 3, I modeled how filtration behavior, potentially affected by toxic Microcystis blooms, may influence maximum lifetime fitness of zebra mussels. I modeled a range of possible behaviors using a dynamic programming technique, including varying conditions of temperature, seston concentrations and seston quality, over four sequential time periods. The environmental conditions I chose fall within the range of conditions known to occur in western Lake Erie during typical summer seasons. Known ranges of zebra mussel bioenergetic parameters (metabolic rates, clearance rates, assimilation efficiency, pseudofeces production rates, and excretion rates) were used in a dynamic programming equation to generate a suite of responding filtering behaviors that allow determination of whether zebra mussels grow to a critical length (maximizing their seasonal fitness).
CHAPTER I

INTRODUCTION

*Occurrences of Cyanobacterial Blooms in the Western Basin of Lake Erie: Influence of Temporal and Spatial Variation in External and Internal Loading of N and P.*

Nutrient cycling (N and P) in western Lake Erie is affected by several factors, including external inputs from diffuse (non-point) sources, from rivers and streams, from atmospheric deposition, and by internal physical and biological processes. During the recent history of Lake Erie, cyanobacteria did not become a major component in the western basin phytoplankton community until the late 1950's (Verduin 1964; Davis 1964). The appearance of cyanobacteria, as a major factor in the phytoplankton community, coincides historically with rising external loads of nitrogen (N) and phosphorus (P) in the 1960's and 70's from municipal sewage outfall and increasing agricultural runoff. External loading of N and P from tributaries is an important factor governing nutrient inputs to the lake because the Lake Erie watershed is dominated by agricultural land use (Logan 1987; Baker 1993). A downward trend in tributary phosphorus loading may be attributed to eutrophication reduction programs initiated...
under the 1972 Great Lakes Water Quality Agreement between Canada and the United States. However, diffuse (non-point) sources of N and P are still problematic and difficult to measure, but the relative contribution of N and P from these sources is likely to have remained somewhat constant. The downward trends in phosphorus from tributaries over the last 25 years (Richards and Baker 1993; Dolan 1993) have resulted in recovery of western Lake Erie such that cyanobacterial blooms are presumed to occur with lower frequency, and with lower magnitude. Certainly, observances of long-term surface scums from cyanophyte blooms have been virtually absent in recent years. In fact, water clarity has noticeably increased, especially since introduction of the dreissenid mussels. Internal nutrient flux has become a relatively more important influence on phosphorus availability and determination of phytoplankton community composition. The goal of this study is to determine the relative impact of internal nutrient dynamics, external loading, and temperature, on the recent trend toward increasing frequency of Microcystis blooms in western Lake Erie.

Recently, the frequency of cyanophyte blooms, dominated by non-nitrogen fixing species, suggest a relationship with Dreissena. The blooms consist largely of Microcystis aeruginosa and have occurred in Saginaw Bay, Lake Huron (Lavrentyev et al. 1995), in Oneida Lake, New York (Horgan and Mills 1999), and in the western basin of Lake Erie (our data). All three of these lake regions can be characterized as shallow and eutrophic, with high densities of Dreissena species.
The possibility that *Dreissena* spp. encourage blooms of *M. aeruginosa* is important because this cyanophyte may be able to out-compete other algae and nitrogen-fixing cyanophytes when changes occur in internal N and P loading from the mussels. In addition, *M. aeruginosa* produces a hepatotoxin (microcystin) that is quite undiscriminating in its toxicity to organisms ranging from invertebrates to mammals (Carmichael and Falconer 1993). Therefore, it is important to determine if there is a relationship between the presence of dense colonies of *Dreissena* spp. and the increasing occurrences of *M. aeruginosa* blooms in shallow eutrophic lakes and bays.

Internal sequestering and excretion of nutrients by zebra mussels and other organisms are potentially important influences on phytoplankton composition, especially by crustacean zooplankton. Weisgerber (1999) compared crustacean zooplankton N and P excretion rates from the mid-1970’s and 1995 – 1997 and found a linear decline in N and P loading to western Lake Erie via this source. However, when Weisgerber calculated excretion rates of *Dreissena* during 1995 – 1997, she found that N and P loading from mussels increased in magnitude equal to the decline from crustacean zooplankton. Therefore, recent internal N and P loading to the western basin is similar to the mid-1970’s in spite of declines in the internal pool of nutrients available.
Importance of changes in nutrient dynamics and water temperature patterns to phytoplankton composition:

Abundant nutrients, warm water temperatures, and high light levels all favor cyanophyte blooms. For example, ratios of total inorganic nitrogen (TIN: $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_3^+$) to total phosphorus (TP) or soluble reactive phosphorus (SRP), have been emphasized as important factors in determining the composition of the phytoplankton community (Smith 1982; Pick and Lean 1987). Commonly, the phytoplankton is dominated by cyanophytes when TIN:TP is less than 30 (by weight), with optimum ratios for bloom-formers (*Anabaena*, *Aphanizomenon*, and *Microcystis*) being even lower (Chorus and Bartrum 1999). Temperature (> 25 °C) and high light penetration are also important factors influencing dominance of cyanophytes (Tilman et al. 1986; Robarts and Zohary 1987; Pick and Lean 1987; Fujimoto 1997). According to Reynolds (1997), the optimal temperature for growth of cyanobacteria is higher than for green algae and diatoms. In addition, *Microcystis* spp. and other cyanobacteria are capable of regulating buoyancy, and thus their depth in the water column, in order to gain optimal light or nutrient conditions, giving them another competitive advantage. Nevertheless, nutrient, temperature and light conditions that favor cyanobacteria must occur simultaneously for a bloom (a dense population dominated by a single genus) to occur. Jacoby et al. (1999) studied environmental factors associated with a toxic *Microcystis aeruginosa* bloom in Steilacoom Lake, Washington, in 1994 as compared to 1995. This lake is also shallow (mean depth 3.5 m) and eutrophic. They found that the success of *M. aeruginosa* in 1994 was associated with higher surface water temperatures and pH, decreased water
transparency and lake flushing, higher total phosphorus, low N:P, low nitrate-nitrogen and sufficient ammonium-N. In addition, they found a positive correlation between microcystin concentrations and increasing soluble reactive phosphorus. Steilacoom Lake is not reported to have zebra mussels. However, some of the environmental factors these researchers found to be associated with *M. aeruginosa* and microcystin concentrations, such as increasing soluble reactive phosphorus (SRP) and ammonium nitrogen (NH$_4$-N) are factors associated with dense zebra mussel populations.

**Impact of Dreissena on internal N and P loading:**

Zebra mussels are capable of influencing internal N and P dynamics in shallow, eutrophic systems like the western basin of Lake Erie (Arnott and Vanni 1996). James et al. (1997) have shown that zebra mussels excrete NH$_4$-N and soluble reactive phosphorus (SRP). NH$_4$-N is more easily oxidized by bacteria, and NH$_4$-N and dissolved P are more readily available for uptake by phytoplankton. Culver et al. (1999) showed that zebra mussels in Lake Erie excrete NH$_4$-N at 1.3 to 2.9 times, and P at 20 to 80 times, the rates of mussels in Saginaw Bay, Lake Huron, indicating that local nutrient pools affect levels of nutrient excretion. Lake Erie mussels also excreted N and P at N:P ratios lower than the seston being consumed in that study. Non-nitrogen fixing cyanobacteria such as *M. aeruginosa* tend to be favored under conditions of higher N in the form of ammonia (Smith 1982; Tilman et al. 1986; Pick and Lean 1987). *Dreissena* contributes both to the available pools of ammonia and SRP.
Recent occurrences of toxin-producing blooms of *Microcystis* species, and other cyanobacteria, in Saginaw Bay, Lake Huron, and in the western basin of Lake Erie were associated with regions of high densities of *Dreissena*. Studies by Heath et al. (1995), Lavrentyev et al. (1995), and Gardner et al. (1995), in Saginaw Bay Lake Huron, and by Holland et al. (1995) and Arnott and Vanni (1996) in western Lake Erie, have shown that zebra mussels do mediate changes in nutrient cycling and influence phytoplankton composition. Culver et al. (1999) have shown that *Microcystis* nearer to high densities of zebra mussels in Lake Erie were less phosphorus-limited than *Microcystis* farther away from zebra mussels. Thus, it is quite likely that zebra mussels are influencing the return and dominance of potentially toxic *Microcystis* in shallow, well-mixed, eutrophic lake regions through changes in internal nutrient dynamics in addition to selective filtration (Lavrentyev et al. 1995).

In this study, I examined several factors potentially influencing increasing trends in non-nitrogen fixing *Microcystis* blooms in western Lake Erie, including external tributary loading of N and P, internal loading of N and P, spatial and temporal trends in N and P concentration, and temporal trends in water temperature. I compared a 1996 data set including cyanophyte biomass, N and P concentrations, and temperature in western Lake Erie, to available historical data for the same region (Davis 1964; Verduin 1964; Munawar and Munawar 1976; Munawar and Burns 1976; Burns 1976; Fraser 1987; Wu and Culver 1991; Holland 1993 and Holland et al. 1995; Makarewicz 1993; and Makarewicz et al. 1999). I also examined whether changes in external loading of N and P
may have been responsible for the major 1995 *Microcystis* bloom in western Lake Erie, and other blooms occurring the 1990's, by looking for coinciding tributary loadings of N and P, internal N and P concentrations and N:P, and higher water temperatures, over a 15 year span encompassing the introduction of *Dreissena*. I examined these factors using the following working hypotheses and objectives.

**WORKING HYPOTHESES:**

(1) (a) Cyanophyte blooms, dominated by non-nitrogen fixing species, have become more frequent, but are lower in magnitude than historical blooms of nitrogen-fixing cyanobacteria in western Lake Erie since introduction of dreissenid mussels; (b) Recent and historical cyanophyte blooms are associated with low N:P and higher surface water temperatures.

(2) During the summer of 1996, spatial and temporal distributions of cyanophyte biomass in western Lake Erie were associated with internal environmental factors: N, P, N:P, or temperature.

(3) Cyanophyte blooms occurring in the western basin of Lake Erie since 1983 result from peaks in external N and P loading.
The hypotheses were tested with the following objectives.

OBJECTIVES:

(1) (a) Determine the frequency, magnitude, and compositional changes of western Lake Erie cyanophyte blooms for years prior to and after introduction of dreissenid mussels to Lake Erie in relation to temperature, and the ratio of N:P;

(b) Examine the roles of temperature, internal concentrations of inorganic N and P (TP and SRP), and the ratio of N:P in spatial and temporal variation in cyanophyte biomass in 1996.

(2) Examine the relative associations of external and internal N and P loading rates with historical occurrences of cyanobacteria blooms in western Lake Erie.
METHODS

Objective (1) Temperature, N and P, and Occurrence of Cyanobacteria Blooms.

Historical phytoplankton, nutrient and water temperature information:


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A "bloom" in our study, was defined as a cyanobacterial abundance exceeding 2000 cells ml\textsuperscript{-1} or a biomass $\geq 0.2 \text{ mm}^3 \text{ l}^{-1}$ as biovolume or 200 $\mu\text{g} \text{ l}^{-1}$ as biomass wet weight. These criteria are based on the World Health Organization (WHO 1998) guideline for microcystin-LR, stating that water, or water with cell-bound microcystin, should not exceed 1 $\mu\text{g}$ microcystin-LR l\textsuperscript{-1} for human and animal safety. At a density of 100,000 cells ml\textsuperscript{-1}, Microcystis is likely to produce up to 20 $\mu\text{g}$ microcystins l\textsuperscript{-1} and form scums (Chorus and Bartram 1999). Since this value is 20 times the safety level recommended by the WHO, Chorus and Bartram (1999) recommend the 2000 cells ml\textsuperscript{-1} threshold. Because our concern over recurring blooms of cyanophytes in Lake Erie is associated with Microcystis dominance and its potential for microcystin production, we decided to use this standard threshold for definition of a bloom if it was surpassed at any of our sampling sites or in data from literature we reviewed.

**Collection sites and methods for historical data:**

Samples of phytoplankton collected by Munawar and Munawar (1976), Wu and Culver (1991), Pontius and Culver (unpublished), Makarewicz (1993) and Makarewicz et al. (1999) were collected from different sites in the western basin of Lake Erie (Figure 1). All samples were identified and enumerated on an inverted microscope using the same method (Utermöhl 1958). The data from summer 1970 (July - September) for phytoplankton (Munawar and Munawar 1976) and nutrients (Munawar and Burns 1976) were collected at 4-week intervals from five stations in the western basin. The samples, collected by Van Dorn bottle from 1 and 5 m, were mixed them together and subsamples
were taken for plankton identification and enumeration, or for nutrient analyses (Munawar and Burns 1976). Temperature and nutrient data for 1968 were obtained from Hartley and Potos (1971). We averaged their TN and TP data (each of their data points were an average of 6 – 7 samples) for summer, 1968, from Maumee Bay, southern nearshore, mid-basin and northeast sector of the western basin. D. Van Keuren’s unpublished data (held at the Center for Lake Erie Area Research (CLEAR)) for 1979 cyanophyte biomass were collected from 12 stations in the western basin. Wu and Culver (1991) sampled two sites on a weekly basis (1988, 1989); one near Kelleys Island (8 m depth), and one in East Harbor (5 m depth). They used a 5 cm diameter tube sampler lowered to 2.5 m. We used the average of data collected from the two sites for 1988 – 1989 summer cyanophyte biomass (late June through August). Pontius and Culver (unpublished) used the same sampling technique, on a weekly basis, to sample from the channel marker buoy at Put-in-Bay, South Bass Island, in 1991 and 1992. Makarewicz (1993) and Makarewicz et al. (1999) analyzed data from samples taken offshore using an 8 - 1 Niskin bottle mounted on a General Oceanics Rosette sampler in August 1983 – 1993. The number of sampling stations varied in each year ranging from 15 to 60. Samples collected in the western basin were taken at 1 m, mid-depth and 1 m above the bottom and 1-liter was preserved from each. We used some summer cyanophyte averages and standard deviations from the figures in Makarewicz et al. (1999) for years for which we had no data.
Lake Erie sampling and nutrient analyses for 1995 – 1998:

During 1995 – 1998, phytoplankton and water quality samples were collected by the Ohio Division of Wildlife (Lake Erie Fisheries Research Unit, Sandusky, OH, Roger Knight, Supervisor) during fish recruitment surveys of 44 sites and by the Canadian Center for Inland Waters (CCIW) monthly surveys of 6 or 7 sites (Figures 2 and 3) in western Lake Erie from May - September, 1996 and 1997. Water samples for nutrient analyses were collected and refrigerated until delivery to the Heidelberg College Water Quality Laboratory (WQL), Tiffin, Ohio, or the CCIW. Water samples were analyzed for nutrients (NO$_3^-$, NO$_2^-$, NH$_4^+$, TP, and SRP). Personnel at the WQL used Technicon autoanalyzers and EPA standard methods to analyze water samples and used extensive quality control programs. Total and dissolved phosphorus were analyzed there using the Murphy-Riley ascorbic acid technique, and nitrate was analyzed using cadmium reduction.

Phytoplankton sampling and biomass determination:

ODNR and CCIW phytoplankton samples were collected using a tube-sampler lowered to 2 times the Secchi depth (1 – 5 m) and were preserved in Lugol’s iodine solution. Identification (Taft and Taft 1971; Desikachary 1959) and enumeration was accomplished by counting transects of concentrated (Utermöhl 1958) subsamples on an inverted microscope (400X; Wild Heerbrug). Identification to genus or species and biovolume estimates of phytoplankton were conducted in our lab. Average biovolume of cells, or colonies, of each species of algae and cyanobacteria, were determined by
inserting the average dimensions from at least ten representatives of each taxon into an appropriate volume calculation. Volume formulae most resembling the shape of the cell or colony were used (sphere, cylinder, etc.). Biovolumes (\(\mu m^3 l^{-1}\)) were converted to wet weight biomass (mg m\(^{-3}\) or \(\mu g l^{-1}\)), assuming a specific gravity of 1.0 for phytoplankton (\(mm^3 l^{-1} = mg l^{-1} = g m^3\)).

Phytoplankton samples collected from the Put-in-Bay channel marker buoy (Figure 2) were either obtained as described above (1996 - 1998) or by using a metered 0.5 m diameter zooplankton net (49 \(\mu m\) mesh) vertically hauled to a depth of 10 m (1995). Samples collected by net were preserved in sugared formalin, then enumerated and measured on a dissecting scope (Wild; 50X) in our lab. Biovolumes were calculated and transformed to biomass as described above. The net-collected samples were used to fill in missing cyanophyte abundance data for August and September of 1995; and is likely to have resulted in underestimates of biomass of small phytoplankton species. Only colonies and cells of cyanobacteria large enough to be maintained in the net were counted. However, the net method sampled the whole water column and concentrated a much larger volume of water (~1.5 m\(^3\)), therefore it resulted in a more accurate picture of biomass for rarer larger species and colonies of cyanophytes (\(Microcystis\) spp., \(Aphanizomenon\) spp. and \(Anabaena\) spp.) often found below 2 m (Edwards et al. in review).
Station locations in western Lake Erie in 1996:

We examined the spatial variation in summer cyanophyte biomass, N, P, N:P and surface temperature in August and September, 1996, by grouping data collected from sampling stations that had similar influences, such as being near a tributary, near islands and reefs, or offshore. Station numbers used by the ODNR (Figure 4) provide a convenient way to summarize the groupings we chose. Stations influenced by the Sandusky River (SR): 1 – 8, 41; or the Maumee River (MR): 36 – 40 and 42 – 44; were treated as two separate groups influenced by tributaries. Offshore stations (OS): 25 – 35 were grouped together because they were in water > 5 m deep and not near islands, reefs or tributaries, although there may be influences from the Detroit River on some of these stations. Island and reef stations (IR): 9 – 24 were grouped together because they were near islands or reefs. The IR stations were presumably more influenced by dense beds of Dreissena than the other groups.

Data Analysis:

Spatial variation of cyanophyte biomass, NO$_2$ + NO$_3$, NH$_4$, TP, SRP, TIN:TP ratios and temperature among the region groupings (SR, MR, OS, IR) were analyzed by least squares regression (GLM procedure, SAS, version 6) using an alpha level of 0.05. Significant ($p < 0.05$) differences between regions for any of the environmental parameters above were further examined using Tukey’s multiple comparisons (SAS, version 6) of the least squares means.
Means of parameters for a station group were significantly different from other groups if their 95% confidence intervals did not overlap. A Pearson correlation matrix (SAS, version 6) was produced to determine if there were correlations between any of the environmental parameters, region or cyanophyte biomass in August and September. We further examined relationships between the monthly (June – September) nutrient ratios (TIN:TP) and the proportion of cyanophyte biomass out of the total phytoplankton biomass for all stations. Temperatures for all sampling stations were also examined in relation to time and cyanophyte biomass.

Objective (2) External and Internal N and P Loading Rates and Cyanobacteria Blooms.

External loading influences on cyanophyte blooms:

Western Lake Erie N and P loading and discharge variation were examined over time (water-years 1986 – 1999). Richards (pers. comm., Heidelberg Water Quality Laboratory, Tiffin, Ohio) indicated that the Maumee and Detroit rivers were the main tributary influences on N and P loading in the western basin. We assumed a constant loading of approximately 3500 metric tons P yr⁻¹ from the Detroit River (Fraser 1987; D. Culver, Ohio State University, pers. comm.). Fraser (1987) states that the Detroit River was the dominant P-loading source for western Lake Erie up to 1977, after which other tributary sources became more important. This conclusion is supported by downward trends in P-loading reported by Dolan (1993).
Therefore, we assumed that N and P loading from the Maumee River 1986 - 1999 accounts for the majority of external loading variation since then. We used Maumee loading data to accomplish our objective of determining if there were patterns of influence by tributary sources of N and P on cyanophyte blooms.

Nitrogen and phosphorus loading and discharge volumes for the Maumee River were kindly provided by Richards and Baker (Heidelberg Water Quality Laboratory) for the years 1986 through 1999. Nutrient analyses were performed at the WQL as described above for the samples collected from Lake Erie. The historical cyanophyte biomass data we found under Objective 1 were used to indicate evidence of major blooms occurring. We used our criterion for a bloom (> 200 µg l⁻¹ cyanophyte biomass at any site, anytime) to decide which years had significant blooms out of the years data were available. Using this information, we examined temporal trends in N and P loading from the Maumee River during bloom years as opposed to years with lower cyanophyte abundance.

Estimates of internal nutrient contribution by crustacean zooplankton and zebra mussels:

Seasonal averages of internal loading in the western basin of Lake Erie from excretion of N and P by crustacean zooplankton plus Dreissena spp. for 1995 – 1997 were 35.26 µg N l⁻¹ d⁻¹ and 16.72 µg P l⁻¹ d⁻¹ (Weisgerber 1999). We used her estimate of the seasonal average zooplankton excretion (15.83 µg N l⁻¹ d⁻¹ and 8.32 µg P l⁻¹ d⁻¹) as constant background input since 1995.
We calculated our own local *Dreissena* excretion rates based on weight-specific N and P excretion rates measured by Arnott and Vanni (1996), and measurements of *Dreissena* spp. biomass at Peach Point, South Bass Island, in 1995. Average excretion rates determined by Arnott and Vanni (1996) were converted to daily areal excretion rates and multiplied by average mussel biomass. These estimates were compared with known N and P concentrations measured in Hatchery Bay in 1995 (Holland et al. 1995).

We estimated the contribution of zebra mussels to internal loading at a nearshore, tributary-influenced region using Custer and Custer's (1997) estimates of Maumee Bay zebra mussel biomass in 1993 with the weight-specific excretion rates of Arnott and Vanni (1996). Estimates of mussel excretion were then compared to estimates of 1993 Maumee River N and P loading to Maumee Bay (Richards and Baker).
RESULTS

Objective (1) Temperature, N, and P, and the Occurrence of Cyanobacteria Blooms.

**Historical context:**

Our review of the historical literature revealed that cyanobacteria were a major component of the phytoplankton community of western Lake Erie in summer and fall from at least 1970 through the mid-1980's (Table 1). Since 1970, summer cyanobacteria biomass peaked in 1988, with relatively lower levels through 1993 (Figure 5). A shift from dominance by nitrogen-fixers (*Aphanizomenon flos aquae* and *Anabaena* spp.) to non-nitrogen fixers (*Anacystis* and *Microcystis* spp.; Table 1) has occurred in recent years. For the years 1995, 1997 and 1998, *Microcystis aeruginosa* was the dominant cyanophyte from August through September (Figures 6 and 7). However, in 1996, *Aphanizomenon* dominated in August and September. According to Makarewicz (1987), *Aphanizomenon flos aquae* was the dominant cyanophyte species during blooms of the 1980's, and in the 1970's as well. According to our criterion for a bloom (> 200 µg l⁻¹ cyanophyte biomass), significant cyanophyte blooms occurred in at least 1970, 1985, 1987, 1988, 1989, 1993, 1995, and 1998 (Figure 5). The blooms of 1988, 1995 and 1998 were similar or greater in magnitude, to the 1970 bloom that occurred before controls on external phosphorus loading were established.
Therefore, hypothesis (1 a) is both supported and refuted: cyanophyte blooms may have become more frequent compared with the early 1980's (after P-loading controls, but before dreissenids). However, the blooms that have occurred since introduction of dreissenids are not lower in magnitude. In addition, the composition of blooms has changed so that non-nitrogen fixing *Microcystis* spp. have, in general, become the dominant cyanophyte.

Hypothesis (1 b), stating that recent and historical cyanophyte blooms were associated with low N:P and high surface water temperatures, is supported by the historical data: average temperatures during bloom seasons (Aug – September) for 1970, 1995 and 1998 were between 25 – 26 °C, and N:P ratios during bloom seasons for 1968 and 1995 were below 10 (Figure 8). Makarewicz (1987) and Makarewicz et al. (1999) unfortunately did not provide any detailed nutrient or temperature data for specific years in the 1980's and we were unable to find data elsewhere for this period. However, Makarewicz et al. (2000) do report that N:P and ammonia levels have, on average, increased from 31.7 (pre- zebra mussel years 1983-1988) to 40.1 (post-zebra mussel years 1989-1993), and from 12.9 μg NH$_3$-N l$^{-1}$ (pre-) to 15.4 μg NH$_3$-N l$^{-1}$ (post-) during summer, respectively. Therefore, we relied on nutrient data available for 1968 (Hartley and Potos 1971) and the 1990's (Holland et al. 1996; Babcock-Jackson and Culver this document), and we assumed the 1968 N:P ratio was likely to be similar for 1970. We showed that average temperatures in the 1990's were similar to 1970 (Figure 8), disregarding the low average temperature for 1992 because Mount Pinatubo (Philippines)
erupted (resulting volcanic ash in the atmosphere lowered temperatures world-wide).

When N:P ratios were higher than 15 in 1991, 1992, 1996 and 1997, no significant blooms occurred. This is still a quite low ratio of N:P, therefore other factors must have been involved.
Temporal and Spatial Variation in Cyanophyte Biomass, N, P and Temperature in Western Lake Erie during August – September, 1996 (Hypothesis 2):

Temporal variations:

Cyanophyte biomass began to increase in mid-July, peaking in August and declining through September, 1996 (Figure 9). Surface water temperatures ranged from 22 to 28 °C during this period (Figure 10). Cyanophyte biomass maxima occurred when temperatures ranged from 23 to 26 °C (Figure 11). However, temperature was not correlated with cyanophyte biomass in August, or any other parameter that we examined, but in September temperature was correlated with high cyanophyte biomass (r = 0.44), ammonia (r = 0.40), and negatively correlated with SRP (r = -0.45). High nitrate + nitrite were correlated with high cyanophyte biomass in August (r = 0.43), and with region (r = 0.37), TP (r = 0.35) and SRP (r = 0.50) in September. Ammonia was correlated with temperature and TP (r = 0.34) in September, but not with any parameters in August. Low N:P was correlated only with region (0.47) in September. However, low N:P appears to have a relationship with the number of stations having a high proportion of cyanophyte biomass in the phytoplankton during August and September, but not in June and July (Figures 12 – 15). Nevertheless, large variation in cyanophyte biomass confounded all attempts to find correlation by regression analysis of N:P with proportion of cyanophytes in August and September.
**Spatial variation:**

There was no statistically significant spatial difference in cyanophyte biomass, temperature, or SRP among the four designated regions in August or September, 1996 (Table 2). However, variations occurred between regions for nitrate + nitrite and ammonia for August and September. For example, nitrate + nitrite and ammonia in August and for total phosphorus and the ratio of total inorganic N to total phosphorus in September all varied with location (Table 3). Island and reef stations had higher nitrate + nitrite than the Sandusky River and Offshore stations in August (Table 4), and the Maumee River stations were higher in nitrate + nitrite than all other regions in September (Table 5). TP at Sandusky River stations was significantly higher than at offshore stations. At the offshore stations, TP was significantly lower than the Maumee River stations in September (Table 5). N:P at the Sandusky River stations was significantly lower than at the Maumee River stations in September. Mean N:P for all regions in August was > 36, and was < 21 in September.

**Objective (2) Examine the relative associations of external and internal N and P loading rates with the occurrence of cyanobacteria blooms in western Lake Erie.**

**External loading of N and P:**

Inorganic nitrogen and total phosphorus loading patterns closely followed river discharge patterns from October 1985 – December, 1999 (Figures 16 – 18). The N:P ratios of discharge throughout this entire period remained largely below 30 during July -
September (Figure 19). Upon closer examination of N:P during the three major bloom years 1988 (Figure 20), 1995 (Figure 21) and 1998 (Figure 22) the pattern of low discharge N:P during the bloom period remains true. However, there were major inflows of nitrogen prior to August and September in 1995 and 1998. In the more minor bloom years 1992 (Figure 23) and 1996 (Figure 24), and in 1997 (Figure 25) when no bloom occurred, the N:P also remains low during the same summer periods and again, there are pulses of high nitrogen before them. If these years are examined from earliest to most recent, it is apparent that there has been a small, but steady increase in the N:P ratio of the discharge during August and September. However, the trend seems minor in comparison to large temporal variations in N and P loading on a monthly basis. Therefore, tributary loading may account for a generally low summer N:P in the western basin assuming that the Detroit River and smaller tributaries follow a similar pattern based on annual rainfall and point source controls on P. If we also assume that diffuse (non-point) sources of N and P follow similar patterns year to year based on annual rainfall, then variations in summer cyanophyte blooms must be attributable to other factors such as temperature and internal loading.
**Estimates of internal N and P loading by zebra mussels:**

At our Hatchery Bay site in 1995, *Dreissena* spp. were most likely an important source of P-loading and a less important source of N-loading. Average concentrations of N and P in Hatchery Bay during August and September, 1995, when a *Microcystis* bloom was occurring, were 0.271 mg TN l⁻¹ and 0.04 mg TP l⁻¹ with N:P of 9.5 (Holland et al. 1995). Average temperature during the same period was 25.5 °C, with relatively calm conditions prevailing in Hatchery Bay (our observations). The results of our estimates for *Dreissena* excretion there are as follows. Nitrogen and phosphorus excretion rates from zebra mussels were determined experimentally (Amott and Vanni 1996) to be, on average, 5.26 μmol g DW⁻¹ h⁻¹ for NH₄⁺-N and 1.02 μmol g DW⁻¹ h⁻¹ for PO₄⁻-P, at temperatures of 18 to 25 °C. At our sampling site in 1995 (Peach Point, at the entrance to Hatchery Bay, South Bass Island, Lake Erie), we found an average of 17,857 *Dreissena* individuals m⁻² with an average soft tissue dry weight of 147.8 g dw m⁻². Average depth of Hatchery Bay is about 3 m. Using Arnott and Vanni’s excretion rates (above) for NH₄⁺-N and PO₄⁻-P, multiplied by our average mussel biomass, we calculated excretion rates for Hatchery Bay to be 18,658.3 μmol NH₄⁺-N m⁻² d⁻¹ (260 mg NH₄⁺-N m⁻² d⁻¹) and 3618.14 μmol PO₄⁻-P m⁻² d⁻¹ (112.2 mg PO₄⁻-P m⁻² d⁻¹). This would provide about 87 μg NH₄⁺-N l⁻¹ d⁻¹ and 37 μg PO₄⁻-P l⁻¹ d⁻¹ in addition to any nutrients excreted by other organisms. We conclude that dreissenids contributed significantly to P-loading since our estimate of P-excretion (0.037 mg PO₄⁻-P l⁻¹ d⁻¹) closely matches the average P concentration measured in Hatchery Bay (0.04 mg TP l⁻¹) for August and September, 1995.
Total nitrogen in Hatchery Bay for that period was 0.271 mg l⁻¹, so dreissenids were excreting an amount of ammonia equivalent to 32% of the N concentration and 92.5% of the P concentration in the Bay each day.

We further examined the contribution of zebra mussels to internal loading at a nearshore, tributary-influenced region using Custer and Custer's (1997) estimates of western basin nearshore zebra mussel biomass and the average *Dreissena* excretion rates of Arnott and Vanni (1996). The measurements of mussel biomass ranged from 0 to 3611 g ww m⁻² in and near Maumee Bay in 1993. Custer and Custer's site numbers 8 – 11 are found at the mouth of Maumee Bay (mean water depth of those sites was 1.8 m). Using the mean biomass of mussels for all 11 of their nearshore sites (379.4 g shell-free ww m⁻², estimated at 37.94 g dw m⁻², assuming dw is roughly 10% of ww) multiplied by the N and P excretion rates of Arnott and Vanni, we estimate N and P excretion rates of 4789.5 μmol N m⁻² d⁻¹ (0.067 mg N m⁻² d⁻¹) and 928.8 μmol P m⁻² d⁻¹ (0.029 mg P m⁻² d⁻¹).

Using the mean water depth of 1.8 m in the nearshore zone of the Maumee River, we get a mussel contribution of 3.72 *10⁻⁵ mg N l⁻¹ d⁻¹ and 1.61*10⁻⁵ mg P l⁻¹ d⁻¹. Maumee River discharge for August, 1993, averaged 0.68 mg P l⁻¹ d⁻¹ and 3.073 mg N l⁻¹ d⁻¹ (calculated from data provided by Richards and Baker, Figures 17 and 18). We conclude that mussels do not account for any significant amount of the N and P loading near the Maumee outfall.
DISCUSSION

*Trends in occurrence, magnitude and composition of algal blooms:*

Makarewicz (1993) and Makarewicz et al. (1999) reviewed changes in phytoplankton biomass and species composition in Lake Erie that occurred from 1983 to 1987 with a comparison to 1970; and again from 1983 to 1993. They found a general reduction in phytoplankton 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 during the early years. The average relative abundance of all cyanophytes in the western basin for 1983 to 1987 was 6.86% of the total average biovolume of phytoplankton. Makarewicz observed that Aphanizomenon had declined in the western basin during the 1980's by 92% as compared with 1970. According to our data, blooms of cyanophytes were observed in the western basin after the zebra mussel invasion during 1988, 1989, 1993, 1995 and 1998. A major Aphanizomenon bloom occurred in 1988, but this was before Dreissena spp. were significantly established in the western basin. Nicholls and Hopkins (1993) report a major impact by zebra mussels on all phytoplankton classes by 1989, with equal impacts on all classes at the Canadian municipal water intake sites they sampled. However, our literature review and sampling of offshore and nearshore sites in the western basin
showed that a major change in bloom composition from the 1980's to the 1990's has been the switch in dominance to non-nitrogen fixing species in the Cyanophyta. This may in part be explained by grazing activity of zebra mussels as Nicholls and Hopkins suggest, but they assume equal impacts on all classes of algae. Culver et al. (1999) were able to show that zebra mussels graze other classes of algae, or do not graze at all, in the presence of toxic Microcystis, indicating an unequal impact on algae classes. Before 1988, Microcystis was not the dominant cyanophyte during blooms, and impacts by dreissenids would have increased as they spread and increased in density. Perhaps the impacts on algal classes observed by Nicholls and Hopkins were in part because Microcystis was not yet a dominant factor, and in part because all of their sampling was at nearshore municipal water intakes.

The magnitude of blooms in the 1990s is similar to, or greater than, that of 1970 and the 1980s. The range (1963 ± 3000 µg liter⁻¹) of M. aeruginosa biomass observed by Babcock-Jackson in 1995 was similar to the average biomass (3279 µg liter⁻¹) of M. aeruginosa observed by Makarewicz 1983-1987, and very similar to the average biomass of the 1970 bloom (1193 µg liter⁻¹). Our review of the literature (Table 1) indicates that a reduction in total phytoplankton biomass and cyanophyte abundance has been accompanied by a relatively larger reduction in nitrogen-fixing cyanophyte genera than in non-nitrogen fixers. Densities of Aphanizomenon flos-aquae and Anabaena spp. are reduced greatly as compared to the 1970's and 1980's.
We also found that years with major blooms of the 1990s shared characteristic
temperature and N:P averages to the bloom of 1970.

**Spatial and temporal associations of cyanophyte biomass with internal environmental parameters:**

The establishment of zebra and quagga mussels (*D. polymorpha* and *D. bugensis*
respectively) in western Lake Erie (Griffiths et al. 1991) has altered the internal cycling
of nutrients in ways that are incompletely understood. *Dreissena* spp. have densely
colonized most available hard substrates in western Lake Erie (MacIsaac et al. 1991) and
have been spreading to soft substrates as well (Custer and Custer 1997; Berkman et al.
1998). Thus, the spread of *Dreissena* spp. to soft substrates is increasing their potential
impacts on this shallow, well-mixed system. Previous work on ecological changes
associated with *Dreissena* generally concerns the influences they have on phytoplankton
dynamics from either a top-down or bottom-up approach. There seems to be little
consensus among researchers except that changes in phytoplankton composition are
probably due to both processes.

For instance, Holland et al. (1995) concluded that a lack of reduction in total
phosphorus (given downward trends in external P loading), and increasing trends in
soluble reactive phosphorus, silica, nitrate-N and ammonium-N after *Dreissena* became
established in Hatchery Bay, Lake Erie, have resulted in a state of biological oligotrophy
where nutrients are available, but are not being utilized because of zebra mussel-induced
declines in algae. They postulated that the combination of *Dreissena* removal of algae from water and increased biodeposition through production of feces and pseudofeces by *Dreissena* are causing increased water clarity and reduced nutrient demand. By their conclusion, biodeposition is then directing nutrients and organic matter to the sediments in pseudofeces and feces. However, since this system is well mixed and shallow, loosely consolidated feces and pseudofeces are easily re-suspended. In fact, Roditi et al. (1997) found that *Dreissena* biodeposits were re-suspended by 50% less bed stress than background deposits. Thus, re-suspension of nutrient-rich biodeposits should be a greater factor in regulating nutrient flux from the benthic zone where *Dreissena* is present, but this has not yet been measured *in situ*. Examination of algal biomass profiles by Edwards et al. (in review) at the same Hatchery Bay sampling site we used in 1995 reveals that during August and September, 1995, *M. aeruginosa* were present in abundance just above mussel beds. Therefore, because *Microcystis* spp. are capable of regulating buoyancy, mussels not only have access to it, but *Microcystis* spp. also have access to nutrients released by the mussels.

We determined from estimates of *Dreissena* excretion at the Hatchery Bay site that mussels could have accounted for most of the soluble phosphorus and a third of the ammonia available to the *Microcystis* occurring there. However, we found the contribution by *Dreissena* at sites near tributary out-falls was insignificant relative to the total loading. Richards and Baker’s measurements of N and P loading were taken upstream of the mouth of the river.
However, river discharge of N and P probably still far outweighs the contribution by mussels even with significant dilution in the Bay. In addition, a river plume carrying higher N and P concentrations into the lake would be present. However, when dense colonies of zebra mussels are found farther from river discharge and in relatively shallow water, such as at the Bass Islands or on offshore reefs, our estimates of excretion indicate that mussels may be a significant source of ammonium-N and/or SRP. James et al. (1997) estimated rates of soluble phosphorus release from zebra mussels at 0.3 to 2.5 mg PO₄-P m⁻² d⁻¹ at mussel densities ranging from 170 to 1300 individuals m⁻² based on their microcosm experiment results. The phosphorus excretion rate we calculated (112.2 mg PO₄-P m⁻² d⁻¹) is an order of magnitude higher than the range reported by James et al. (1997), but so is the average number of individuals at our sampling site. Our estimate of N-loading (0.087 mg NH₄⁺-N l⁻¹ d⁻¹) falls below the average measured N concentration (0.271 mg TN l⁻¹) in Hatchery Bay, August - September, 1995. Therefore, mussels accounted for far less of the TN, much of which may have been from a sewage out-fall there, in addition to contributions by zooplankton and other organisms.

Based on our estimations of mussel excretion we expected that ammonia and SRP might show significant spatial variation near the island and reef stations (IR) in 1996 as opposed to the offshore (OS) and river stations (MR and SR), or be correlated with temporal and spatial highs in cyanophyte biomass. Unexpectedly, we did not find significant relationships between high cyanophyte biomass and ammonia or SRP,
or spatial variation in ammonia and SRP that might be more or less associated with Islands and reefs (and by inference, *Dreissena* beds) in 1996. Unfortunately, 1996 is the only year out of the four we examined for cyanophyte species ratios that had dominance by a nitrogen-fixing species (*Aphanizomenon* *flos aquae*), and this may in part explain why we did not find the expected correlations of SRP and ammonia with cyanophyte biomass or location. Therefore, the trend toward non-nitrogen fixers in 1995, 1997 and 1998 may still be related to internal contributions of SRP and ammonia from dreissenids. Another possibility is that we did not detect soluble forms of N and P, which would have readily been taken up by algae and therefore, were not accounted for in our samples due to high turnover. The surprising result that there was no statistical correlation between low N:P and cyanophyte biomass was countered by an apparent graphical relationship between low N:P and a large number of stations with a high proportion of cyanophytes in August and September. However, this may have just been a reflection of the differences in species composition and quantity at the different sites. During their manipulations of N:P ratios in experimental lakes, Levine and Schindler (1999) found that non-heterocystous (non-N\textsubscript{2} - fixing) cyanophytes dominated more when N:P was high (33:1), temperatures were higher and TP was high, and that heterocystous (N\textsubscript{2}- fixing) cyanophytes dominated more when N:P was low (4:1), pH was high, and light was subdued. In our 1996 survey, high NO\textsubscript{3} + NO\textsubscript{2} – N was correlated with cyanophyte biomass in August and with temperature and SRP in September. Perhaps the NO\textsubscript{3} + NO\textsubscript{2} – N portion of the TN contributed more to dominance by *Microcystis* at some sites, in part explaining the graphical relationships we observed.
However, further comparisons of nutrient and phytoplankton factors in a bloom year dominated by *Microcystis* spp., such as 1998, are needed to test whether this relationship holds true in western Lake Erie. Resulting correlations of SRP with high microcystin concentrations discovered by Jacoby et al. (1999) in Steilacoom Lake, Washington, suggest that we should examine the possibility that this correlation exists in Lake Erie as well. Brittain et al. (2000), and our data (unpublished), show temporal and spatial relationships of microcystin concentrations with cyanophyte biomass, but correlations with other environmental parameters have not yet been examined.

**Conclusions**

Clearly, it is difficult to determine whether a specific factor, or set of factors, has the greatest impact on the re-appearance of cyanophyte blooms in western Lake Erie. A link between internal loading from *Dreissena* spp. and *M. aeruginosa* blooms requires more information regarding locations and densities of *Dreissena* species. In addition, many processes have not been adequately measured such as density, depth and site-specific variability of excretion and filtration rates of *Dreissena* in the field. There are also the unknown influences of horizontal currents and vertical mixing on directing tributary and internal sources of nutrients and concentrations of plankton. However, our spatial and temporal analysis of N, P, N:P and temperature in relation to cyanophyte abundance in 1996 suggest that temperatures between 23 –26° C in combination with complicated internal nutrient dynamics are influential factors on the dominance of
cyanophytes in the PP. Our historical examination of phytoplankton abundance and species composition supports our observation of a trend toward dominance of non-nitrogen fixers in recent blooms. In addition, we found that blooms occurring most recently are of the same magnitude as blooms occurring in 1970 before phosphorus controls, and in the 1980's before *Dreissena* spp. were introduced to Lake Erie.

Examination of tributary loading of N and P from the Maumee River indicates that though the river is a source of low N:P and often huge pulses of nitrogen prior to blooms, this source could not account for temporal and spatial variations in bloom occurrences. Regardless of the causes of recurring cyanophyte blooms in western Lake Erie, the dominance of these blooms by hepatotoxin-producing *Microcystis aeruginosa* is cause for new concerns about the risks this family of cyanotoxins poses to humans and the ecosystem.
CHAPTER 2

INTRODUCTION

Fate of a Cyanobacterial Hepatotoxin, Microcystin, is Mediated by a Novel Trophic Pathway in Western Lake Erie.

A novel set of ecological relationships has recently emerged in western Lake Erie with the introduction of several non-indigenous and interdependent species. Zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*) were introduced to the Great Lakes *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 closely associated with zebra mussels have also invaded the Great Lakes, an amphipod, *Echinogammarus ischnus*, (Witt et al. 1997; Dermott et al. 1998), and the round goby, *Neogobius melanostomus*, (Jude et al. 1992). *E. ischnus* prefers living among clusters of *Dreissena* and feeds upon their feces and pseudofeces, among other items (Gonzales and Downing 1998). The round goby is a demersal fish and intermittent spawner preferring *Dreissena* (Ray and Corkum 1997) and other benthic organisms, including *E. ischnus*, as food. Round gobies also use the
complex benthic habitat created by *Dreissena* colonies on rocks for refuge and nesting sites, often displacing native fish through aggressive territorial behavior (Jude et al. 1995).

*Dreissena*-mediated changes in nutrient dynamics and in the phytoplankton community (Lavrentyev et al. 1995) may be favoring hepatotoxin (microcystin) -producing *Microcystis aeruginosa* in the Great Lakes (Heath et al. 1995). Selective filtration or rejection of toxic *Microcystis* by zebra mussels (Bastviken et al. 1998) may indirectly result in a novel trophic transfer pathway through zebra mussels, depositing microcystin into the benthic community. Bivalves have a unique ability to accumulate and survive relatively high body burdens of microcysts (Eriksson et al. 1989; Vasconcelos 1995; Watanabe et al. 1997) and other contaminants (Fisher et al. 1993; Klerks et al. 1997). Vasconcelos (1995) has suggested that elimination of microcystins in pseudofeces (the portion of filtered material that is ejected before ingestion) may be a mechanism by which an estuarine species of bivalve, *Mytilus galloprovincialis*, rids itself of microcystins. Since *Dreissena* mussels also produce pseudofeces in response to undesirable particles in the water they filter, it is reasonable to hypothesize that this is a new pathway allowing greater access to microcystin by the benthic fauna in Lake Erie. Though settling of neurotoxin-laden cyanobacteria (*Aphanizomenon, Oscillatoria*, and *Anabaena* spp) must have occurred during past blooms of cyanobacteria in Lake Erie, recent blooms are dominated by microcystin-producing hepatotoxic species and may be specifically directed to the benthos by dreissenid mussels.
This is a specifically new pathway for directing toxin to the benthic community, and it may be complicated and amplified by the introduction of the two other non-indigenous benthic species, *Echinogammarus ischnus* and *Neogobius melanostomus*.

Direction of microcystin to the benthic fauna via *Dreissena*-mediated deposition could result in bioaccumulation of the toxin in pelagic fauna feeding upon them. Many other fish species are also benthic feeders at some or all parts of their life cycles, in turn serving as food for larger fish. Round gobies are eaten by smallmouth bass (*Micropterus dolomieu*) and freshwater drum (*Aplodinotus grunniens*) (observations by fishermen at Put-in-Bay, Ohio, Lake Erie). In addition, fish including freshwater drum and yellow perch (*Perca flavescens*) have recently adapted to eating zebra mussels (Morrison et al. 1997). Predation by larger fish on *Dreissena*, amphipods and round gobies in turn may result in passage of microcystins back to the pelagic community and humans who eat Lake Erie sport fish.

Ecological changes wrought by introduction of *Dreissena* spp. include redirection of energy from the pelagic to the benthos (Madenjian 1995; Stoeckmann and Garton 1997), increased internal nutrient loading (Heath et al. 1995; Gardner et al. 1995; Holland et al. 1995, Arnott and Vanni 1996; James et al. 1997), and increased deposition of sediment (Klerks et al. 1996) and contaminants (Bruner et al. 1994b; Klerks et al. 1997) to the benthic community.
However, *Dreissena* were not present in Lake Erie in the 1970s and early 1980s when blooms of neurotoxin and hepatotoxin-producing cyanobacteria were commonplace. Before external controls on point-source phosphorus inputs became relatively successful, large cyanophyte blooms included *Aphanizomenon*, *Anabaena* and *Oscillatoria* spp. that produce neurotoxins and hepatotoxins in addition to *Microcystis* spp. producing hepatotoxins. Therefore, it is unlikely that microcystins were concentrated in the benthos or passed to benthic fauna at that time without a directing agent, and microcystin is less likely to have been passed back into pelagic fauna from the benthos. In addition, the presence of two other nonindigenous species (*E. ischnus* and *N. melanostomus*) known to be closely dependent on *Dreissena* for food and habitat, has increased the number of possible sources for trophic transfer of microcystin to the pelagic community. Therefore, we hypothesized that benthic-pelagic coupling relationships are developing among *Dreissena* mussels, *E. ischnus*, and round gobies in western Lake Erie that result in a series of novel pathways for microcystin mediated by *Dreissena* (Figure 26).

The purpose of this study was to test this hypothesis using field collections and laboratory microcosm experiments by way of four objectives:

1. **Measure the relative concentrations of microcystin in plankton, mussels, amphipods, and fish in western Lake Erie during blooms of *Microcystis aeruginosa***.
(2) Measure the rate of microcystin transfer to sediments via zebra mussel biodeposition.

(3) Determine whether microcystin is accumulated in zebra mussels and amphipods from environmental exposure to *Microcystis* spp. and microcystin-contaminated pseudofeces.

(4) Determine whether round gobies accumulate microcystin from feeding on microcystin-contaminated zebra mussels, or from exposure to water containing *Microcystis* species.
METHODS

Organisms

Zebra mussels, amphipods and round gobies for laboratory experiments:

SCUBA divers collected zebra mussels from rocky substrates surrounding F. T. Stone Laboratory, Put-in-Bay, OH, Lake Erie (Figure 39), and rinsed amphipods (*E. ischnus*) from the mussel-covered rocks collected above. Round gobies (*N. melanostomus*) were collected by fishing with barbless hook and line from the same littoral areas. A protocol for experimental use of gobies was approved (amended ILACUC protocol 97A0110, approved July 10, 1998, The Ohio State University). All animals were held in tanks containing filtered Lake Erie water (49 µm mesh screen to remove most colonies of cyanobacteria and other organisms) for a 48 h acclimation period prior to beginning experiments. Water in the tanks was held at ambient lake temperature (24 – 26º C).

Cyanobacteria and algae:

We performed microcystin uptake and rejection experiments using three different sources of *Microcystis*. First, a freeze-dried *M. aeruginosa* culture (strain UV-027, producing mainly microcystin-RR) was provided by Wayne Carmichael (Wright State University, Dayton, Ohio).
Subsequently, a Lake Erie strain (LE-3) was isolated by Carmichael and grown in large cultures in modified BG-11 media (Stanier et al. 1971; Carmichael 1986). Preliminary analysis of the LE-3 strain by Scott Brittain in W.W. Carmichael’s lab has shown that it is toxic and produces mainly microcystin-LR (Brittain et al., 2000). Freeze-dried or live cells (UV-027 or LE-3) were re-suspended in filtered lake water prior to use in experiments and supplies renewed as needed. Finally, collections of live, mixed species of Lake Erie cyanobacteria, consisting primarily of *M. aeruginosa*, were collected from Lake Erie near F.T. Stone Laboratory using vertical hauls of a metered 49 μm mesh plankton net. Live cyanobacteria were allowed to float to the top of concentrated samples and then were siphoned off for use in experiments. Green algae used in experiments included either natural mixed populations from Lake Erie water, or were from monocultures of *Chlamydomonas rheinhardtii* and *Chlorella vulgaris* raised in amended Bold’s Basal medium (Nichols and Bold 1965).

**Microcystin analysis:**

Analyses of microcystin levels in animal tissues, water and feces/pseudofeces from the experiments and field-collected material were performed at Wayne Carmichael’s laboratory, 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).
Sequential methanol extractions of tissue and pseudofeces/feces samples were performed on previously dried materials in 1996, while in 1998 and 1999, extractions were done on frozen and thawed materials not previously dried.
Field and experimental methods:

Objective 1. Determine whether microcystin can be found in field-collected plankton, zebra mussels, quagga mussels, amphipods and fish.

Concentrated samples of zooplankton and phytoplankton were obtained using a front-weighted 0.5 m diameter zooplankton net (49 μm mesh) hauled vertically to and from a 10 m depth near a channel-marker buoy anchored at 12 m between Middle and South Bass Islands (41° 39.8410’ N, 82° 49.2722’ W, by GPS) from June through October, 1996, 1997 and 1998 (cross symbol on map in Figure 2, star symbol on Figure 27). In addition, plankton samples were collected from several locations in the western basin of Lake Erie (Figures 2 and 3), from July to September, 1996 and 1997, and on August 20, 1998, by Ohio Department of Natural Resources (ODNR) staff using the same methods. Two replicate plankton samples from some ODNR collections were taken in 1996 and 1998 so that one sample could be preserved in sugared formalin for plankton identification and enumeration and another frozen for microcystin analysis. The frozen samples from July to September, 1996, and from August to October, 1998, were analyzed for microcystin concentrations using ELISA as described above (Brittain et al. 2000).

Phytoplankton samples were also taken by ODNR and at the Put-in-Bay channel marker buoy using a tube sampler lowered to twice the Secchi depth (1 – 5 m). Phytoplankton samples were preserved in Lugol’s iodine. Identification and enumeration of these samples were performed on transects of concentrated whole water samples (Utermöhl 1958) using an inverted microscope (Wild Heerbrug; 400X).
Biomass was estimated by using volumetric conversions (appropriate to cell shapes) of algal cell measurements and densities, assuming a specific gravity of 1.0 for phytoplankton (mm$^3$ l$^{-1}$ = mg l$^{-1}$ = g m$^{-3}$).

Field collections of zebra mussels and quagga mussels from Peach Point, South Bass Island (Figure 27) were made by SCUBA divers in August, 1998. Field collections of various fish species were made from Schoolhouse Bay near Middle Bass Island (Figure 27), western Lake Erie, using an otter trawl from the RV Biolab during August and October, 1998. Gizzard shad (Dorosoma cepedianum), young-of-year (YOY) and adult yellow perch (Perca flavescens), YOY white perch and white bass (Morone spp.), small walleye (Stizostedion vitreum), and creek chub (Semotilus atromaculatus) were sampled in August, 1998. Round gobies, and YOY yellow perch and white perch were collected in October, 1998. All of the collected fish were measured and weighed wet prior to dissection of the livers and stomach/intestinal tracts (gut). Livers and guts were weighed and frozen separately in pre-weighed glass vials, with some vials containing organs from several fish of a given species. The livers and guts were analyzed for microcystin content using ELISA as previously described.
Objective 2. Microcystin is transferred to sediments via zebra mussel biodeposition.

In Situ sedimentation experiments:

The experimental design was based on the assumptions that mussels feed by filtering all suspended particles non-selectively, then sort particles on the gill for ingestion or expulsion in pseudofeces through the inhalant siphon (Sprung and Rose 1988; Roditi et al. 1996; Berg et al. 1996). Based on observations by researchers (Culver et al. 1999) that zebra mussels produce more pseudofeces when exposed to toxic Microcystis, we used sediment traps to measure in situ deposition of microcystin in pseudofeces + feces (PF) from zebra mussels during the summer of 1996. The sediment traps (also described in Yu and Culver 1999) consisted of two replicate crates each containing four 1-L cones modified with screens (113 cm²) to hold zebra mussels and plugged at the bottom to collect biodeposited or sedimented materials. The traps were anchored 10 m apart at 5 m depth, on rocky benthic substrate in Hatchery Bay near the shore of Gibraltar Island (detail Figure 27). Mussels were collected from rocks from Hatchery Bay, Lake Erie. Clumps of mussels were removed from the rocks, rinsed and added to cones at natural densities (approximately 17,700 mussels m⁻² or 200 mussels on a 113 cm² screen). Average biomass of mussels in each trap was estimated from the regression of the lengths vs. dry weights of 10 groups of mussels collected from the same measure of surface area at the same collection time and location.
The regression was:

\[ dw = 5.0 \times 10^{-6} L^{2.7958} \]  

where \( dw \) = soft tissue dry weight (g), and \( L \) = shell length (mm).

Mussels had a length range of 12.0 ± 4.8 mm and average biomass of mussels per trap was 1.48 g soft tissue dry weight (dw). Each trap consisted of two cones with zebra mussels and two cones without zebra mussels (to measure background sedimentation). Yu and Culver (1999) found no significant difference between sedimentation in cones containing empty zebra mussel shells and those having no mussels or shells. Therefore, we did not add empty shells to the controls. Sediments and biodeposited materials were sampled from the cones at five semi-monthly intervals from July through September, 1996. The deposited materials were then dried to a constant weight (65°C) on pre-weighed filter paper for 48 h and re-weighed. Sub-samples of the sediment were removed, dried, and frozen for toxin analysis at Wright State University.

**Microcosm biodeposition experiments:**

We measured biodeposition rates of zebra mussels, in response to different cyanobacteria and algae treatments, in lake-side laboratory microcosm experiments in 1996. During the experiments, adult zebra mussels (15 - 20 mm) were exposed to different concentrations of a lab-cultured strain of *M. aeruginosa* (Carmichael strain UV-
027) or green algae, a mixture of green algae and M. aeruginosa, or living Lake Erie cyanobacteria suspended in filtered lake water (Table 6). The microcosm apparatus consisted of twenty 1-liter plastic Imhoff cones modified with screens to hold zebra mussels near the top of the wide opening of the cones. A small glass vial was attached to the bottom of each cone to collect material deposited by the zebra mussels (Figure 28). The tops of the cones were sealed with lids to which tubes were attached to allow lake water containing various concentrations of Microcystis to be pumped in and out of the cone over the mussels. The cones were suspended from a rack above 20-liter supply buckets from which the exposure water was pumped using two 10-channel peristaltic pumps. Exposure water was constantly aerated to keep dissolved oxygen high (> 7.0 mg l\(^{-1}\)) and to keep algae suspended. Water in the cones was kept at ambient lake temperature (24 - 26° C) by a constant flow of lake water surrounding the exposure water supply buckets. Cartridges of the peristaltic pump were adjusted to 80 - 85 ml h\(^{-1}\), sufficient to renew the water in each cone approximately twice per day. Exposure waters in the 20-liter buckets were renewed daily with their respective treatments. All experiments were conducted at room temperature (24 - 26° C) and under subdued natural sunlight through shaded windows (approximately 14 h light: 10 h dark).
At the start of an experiment, groups of forty mussels were placed in each of three replicate cones per treatment. Biodeposited pseudofeces + feces (PF) were collected from glass vials attached to each cone at 24 h intervals, then dried and weighed on tared glass fiber filters prior to toxin analysis. For some experiments, the entire treatment regimen was replicated so that replicates of the biodeposited PF could be collected and used for feeding to amphipods in the set of experiments described under Objective 3.

**Objective 3. Microcystin accumulation in zebra mussels and amphipods.**

In July, 1996, we measured the accumulation of microcystins in zebra mussels fed different dilutions of lab-cultured strain of freeze-dried *M. aeruginosa*, or live Lake Erie cyanobacteria, with or without green algae added (Table 6). Green algae were added to some cyanophyte treatments to determine if toxin uptake or deposition rates were altered when mussels were offered both. In addition, filtered lake water controls had green algae added to control for the number of particles added to cyanophyte treatments. Mussel accumulation experiments were performed simultaneously with PF production experiments in the flow-through microcosm described under Objective 2 (Figure 28). Samples of each treatment water type were taken for toxin analysis. Samples of mussels (ten from each microcosm) were taken from each of three replicates of every treatment every 24 h, for up to a 96 h exposure period. The mussel lengths were recorded, soft tissues were removed and weighed, and then dried to a constant weight at 65° C. Soft tissues were sent to Wright State University for extraction and toxin analysis.
In a congruent 1996 experiment, amphipods (20 adult, male and female *E. ischnus* in each of three replicates) were exposed to either the treatment waters from the above zebra mussel microcosm experiments, or to zebra mussel PF collected from those treatments. Experiments took place in 250 ml plastic containers (Figure 28). A piece of plastic mesh for amphipods to cling to was provided in each container. Respective treatments were renewed in a clean exposure vessel every 24 h using new zebra mussel treatment water or the PF collected from the zebra mussels during that interval. The PF was added to filtered Lake Erie water to expose the amphipods to a contaminated food source. Amphipods were observed to see if they ate the PF provided during each 24 h period. After 72 h, amphipods were sacrificed and frozen for drying, weighing and determining microcystin accumulation.

Amphipods (adult male and female *E. ischnus*) were also exposed to live *M. aeruginosa* (dilutions of strain LE-3) in the presence of zebra mussels in August, 1999. These amphipods (twenty per replicate, two replicates per concentration) were exposed together with fifteen zebra mussels, in 2.5 l of aerated exposure water at 26°C for 48 h. Treatments consisted of a series of increasing concentrations of the toxic *M. aeruginosa* LE-3 (0, 20, 40, 60 and 80 µg l⁻¹ nominal equivalents of microcystin-LR). The live *M. aeruginosa* LE-3 we used to make the dilutions contained 0.327 µg microcystin-LR ml⁻¹. Animals were checked for mortality every 24 h, then frozen for analysis of microcystin content as described previously.
Uptake, elimination and bioconcentration of microcystin:

In August 1998, a zebra mussel microcystin accumulation/depuration experiment was conducted in order to determine uptake and elimination rates, and bioconcentration factors for microcystin. A mass-based kinetic model was used to estimate zebra mussel uptake clearance (after Fisher et al. 1993). We assumed that (1) the mass of microcystin did not change in the experimental system; and (2) that microcystin was not biotransformed. Since microcystin has a low affinity for lipids (log $K_{ow} < 3.0$), we did not expect loss of microcystin mass by sorption to glassware or plastics used in the microcosms. Biotransformation within the organisms was not expected since microcystin-LR was found to be excreted unchanged in bile of rainbow trout (Sahin et al 1996). However, because microcystin may be taken up by a facilitated mechanism and not passively, it is unclear whether the kinetic model was appropriate. Bury et al (1998) suggest that intestinal transport of microcystin-LR from intestinal mucosa to serosa is a passive process, but carrier-inhibition studies strongly suggest that microcystin uptake by hepatocytes is facilitated (Runnegar et al. 1991). We had insufficient information (no protein phosphatase inhibition assays of mussel tissue) to determine more appropriate Michaelis-Menten kinetic constants, therefore we fit a mass-based kinetic model to the data.
Kinetic model

Experimental uptake clearance ($k_u$) was determined by fitting a 1st order exponential to the relationship of zebra mussel soft tissue microcystin concentrations ($C_{zm}$) over time ($t$) to a single water concentration ($C_w = 20 \mu g \ l^{-1}$) of microcystin.

$$C_{zm} = C_w \left( \frac{k_u}{k_e} \right)^{-k_e t}$$

(2)

where $C_{zm} =$ Microcystin concentration in the zebra mussel tissue (ng g$^{-1}$ ww).

$C_w =$ concentration of microcystin in exposure water (ng ml$^{-1}$).

Elimination data were fit to a first order elimination model in the following form:

$$\ln C_{zm} = \ln C_{zmi} - k_e t$$

(3)

where $C_{zm} =$ Microcystin concentration in the zebra mussel at time $t$ (ng g$^{-1}$ ww).

$C_{zmi} =$ Microcystin concentration in the zebra mussel at the beginning of the elimination period (ng g$^{-1}$ ww).

The elimination rate constant ($k_e$) is determined from the slope of the regression line for $C_{zm}$ vs. $t$. 
Water to zebra mussel bioconcentration factors (BCFs) were calculated from the ratio of the uptake clearance and elimination rate constants:

\[
\text{BCF} = \frac{k_u}{k_e}
\]  

(4).

**Uptake/Elimination experiment**

During the uptake/elimination experiment, zebra mussels (350, 15 - 20 mm) were exposed to lab-cultured *M. aeruginosa* (LE-3) (5.0 mg microcystin-LR g\(^{-1}\) dw) diluted to a concentration of 20 ng microcystin ml\(^{-1}\) in an aerated 40-l exposure tank. Zebra mussels (30) were sampled at 0, 2, 6, 18, 24, 48 and 72 h intervals. A depuration period was allowed, wherein zebra mussels, having been exposed for 72 h, were removed to net-filtered (49 μm mesh) water for 48 h. Zebra mussels (30) were again sampled at 24 and 48 h during depuration. Mussels were assessed for mortality by checking for a lack of resistance to opening, or by clear evidence of dead tissues. Live mussels were dissected from shells, and wet weights taken in three groups of ten mussels. The mussels were frozen until toxin analysis was performed at Wright State University, after methanol extractions of microcystins from frozen wet tissues, instead of dry tissues as in the 1996 experiments.
Objective 4. Accumulation of microcystin in round gobies from environmental exposure or food.

In August 1998, an accumulation/depuration experiment was performed to determine uptake and elimination rates for microcystin in gobies. Experimental uptake and elimination rates, and BCFs, for microcystin in the livers and guts of round gobies were determined using the kinetic model described above for zebra mussels. During the experiments, twenty gobies were exposed, one goby per tank, two replicates per exposure/elimination period, to re-suspended waterborne toxic *M. aeruginosa*. We used *M. aeruginosa* strain LE-3 (5.0 mg microcystin-LR g\(^{-1}\) dw) diluted to the nominal equivalent of 20 ng microcystin ml\(^{-1}\) in 4-l aerated tanks for 72 h. A depuration period, using net-filtered (49 \(\mu\)m mesh size) water in separate tanks, of an additional 72 h was also allowed for the six gobies remaining after 72 h exposure. Two replicate gobies were sacrificed and frozen for toxin analysis at 0, 2, 6, 18, 24, 48 and 72 h during the exposure period and at 24, 48 and 72 h during the depuration period. Time 0 gobies served as controls. All experiments were conducted at ambient temperature (26\(^\circ\)C) under subdued natural lighting and natural photoperiod (approx. 14 h light: 10 h dark), with constant aeration (D.O. > 8.0 mg l\(^{-1}\)). Fish were prevented from seeing outside of their individual tanks by dark plastic shields on each tank. Water containing the toxic *Microcystis* was renewed by emptying, cleaning and refilling the tanks every 24 h to maintain a more constant microcystin concentration in the exposure media. Each goby was anesthetized in ice water and frozen prior to dissection to remove the liver and gut for weighing and analysis (by ELISA) for microcystin (ng g\(^{-1}\) ww).
Trophic transfer:

In August 1999, we tested for trophic transfer of microcystin toxin to gobies from microcystin-contaminated zebra mussels. After starving for 48 h, gobies were fed one of three mussel treatments daily for 7 days: 1) toxic zebra mussels exposed to 1 µg microcystin l⁻¹, or 2) 10 µg microcystin l⁻¹ (from live *M. aeruginosa* LE-3) for 24 h prior to feeding to gobies, or 3) starved mussels. Two gobies were sacrificed initially after being starved for 48 h to determine their microcystin levels at the beginning of the experiment. During the experiment, individual gobies were kept in screened and darkened 4-liter tanks submerged within a living stream receiving a constant flow of filtered Lake Erie water held at 26°C. Lake Erie water was net-filtered (49 um mesh) and then passed through activated carbon to remove ambient cyanobacteria and microcystins. Each tank holding a goby was removed from the flow-through system during feeding so the goby could be observed. Daily, 10 mussels (1.5 - 2.0 cm) were fed to each goby and 10 were frozen for determination of microcystin content. Zebra mussels were fed to gobies "on the half-shell" (one valve was removed to encourage gobies to eat the mussels). This was done because a preliminary experiment (1998) showed that gobies did not consume enough mussels in a 24 h period to detect tissue-level changes in microcystin when mussels were given whole or in small (0.5 cm) sizes. The number of zebra mussels eaten was determined by counting how many empty shells were left after each 24 h interval. Shells and any remaining mussels were removed before adding new
ones. All gobies were sacrificed (anesthetized with ice water, then frozen) prior to dissection at the end of seven days. Each goby that survived until the end of the experiment was measured, weighed and then the liver, gut and a muscle tissue sample were removed. The three types of tissue samples were weighed separately in pre-weighed glass vials, then frozen until microcystin extraction and analysis procedures were performed as previously described. Whole-fish toxin levels were estimated by averaging toxin levels in each tissue type (in ng microcystin g⁻¹ ww), then multiplying by the wet weight of the tissue type. This estimate leaves out any toxin that may have been associated with other organs in the fish. However, microcystin is not expected in other tissues because it is a specific inhibitor of protein phosphatases (Runnegar et al. 1995).

**Data analysis:**

Experimental results were analyzed using a least squares regression procedure (SAS version 6.12, General Linear Model) to test for significant correlations (α = 0.05) of experimental factors (microcystin concentration in exposure medium, length of exposure), and their interactions, with dependent variables: microcystin concentration in animal tissues, pseudofeces and feces (PF), and amount of PF produced. Tukey’s multiple comparisons (α = 0.05) of least squares means were used if significant correlations between treatment and response variables were found. The comparisons allowed the determination of which treatments gave significantly different responses.
RESULTS

Objective 1. Microcystin in plankton, mussels and fish in western Lake Erie.

A *Microcystis aeruginosa* bloom occurred in the late summer of 1998 in the western basin of Lake Erie. Microcystin (< 1 μg l⁻¹) was detected in all field samples of net plankton collected near the Bass Islands during the bloom. Microcystin was also detected in all samples collected on August 20, 1998, from several stations located in the western basin, with the highest levels detected near the Bass Islands (Figure 27). Cell densities of cyanobacteria (dominated by *M. aeruginosa*) peaked near the Bass Islands in early September at 2100 cells ml⁻¹ (Figure 29) coinciding with increasing densities of cryptophytes. Concentrations of microcystin detected in metered plankton-net collected cell material (whole water column) peaked several times in August and September and ranged from 1.6 to 85.3 ng microcystin l⁻¹ of original lake water (Figure 30). Microcystin concentrations rose following peaks in cyanobacteria cell density (Figure 29) and biomass (Figure 31) occurring in early August and early September.
Mussels and fish collected near the Bass Islands in August and October, 1998, also had detectable levels of microcystin (Table 7). *Dreissena* collected in August, from Peach Point, South Bass Island, had microcystin concentrations similar to those of quagga mussels (dry weight concentrations would be roughly ten times higher). Amphipods collected from the same location averaged 200 ng microcystin g^{-1} dw. Mean gut concentrations of microcystin were similar for the walleye, small *Morone* spp., larger yellow perch, creek chub and round gobies, but were much higher in gizzard shad. In addition, it is noteworthy that for fish collected in both August and October (young of the year (YOY) yellow perch and *Morone* spp.), the mean microcystin concentrations in guts and livers increased from August to October. For all fish except gizzard shad and walleye, the concentration of microcystin in liver tissue was similar to or higher than concentrations in the gut. The concentration of microcystin in gizzard shad livers was lower than their respective gut concentrations, but still relatively higher than that found in most other fish species collected. Microcystin was not detected in the three walleye livers.

**Objective 2. Microcystin is transferred to sediments via zebra mussel biodeposition.**

**Field experiments:**

Zebra mussels significantly enhanced rates of deposition of sediment (p = 0.0254) and microcystin (p = 0.0334) in the sediment traps during the late July and early August incubation periods for the summer of 1996 (Table 8). Periods of maximum delivery coincided with periods when cyanobacteria biomass (900 μg ww l^{-1}) and microcystin
concentrations (as integrated whole water column) in lake water (0.54 ng l⁻¹) were highest (Table 8). Zebra mussels accounted for the greatest percentage of sediment and microcystin delivery during late July and early August. However, maximum delivery of microcystin occurred in late August, when background deposition was the dominating influence on microcystin deposition. No microcystin deposition was detected in September, but sediment deposition was greater when zebra mussels were present.

Microcosm experiments:

(a) Different sources of toxic Microcystis:

In our microcosm experiments, rates of deposition of pseudofeces + feces (PF) and its microcystin content depended on concentrations of microcystin in exposure water and, in some cases on length of exposure. At lower levels of microcystin (below 6900 ng l⁻¹), there were no significant differences between deposition rates of mussels exposed to UV-027, LE-3 or lake-collected cyanobacteria. Rates of PF deposition ranged from 2 – 600 ng PF dw mussel⁻¹ d⁻¹ (1.8 – 530 µg PF dw m⁻² d⁻¹) for all sources. However, PF deposition (Figure 32) increased significantly only when the levels of microcystin reached 6900 or 13,000 ng l⁻¹ and after 96 h of exposure using the UV-027 Microcystis. Microcystin deposition (in PF) rates for these same mussels (Figure 33) also depended on microcystin exposure concentration, but not always length of exposure (p = 0.2723). Microcystin deposition was significantly higher (p = 0.0054) at 72 h for 6900 ng microcystin l⁻¹, and at 24, 72 and 96 h for 13,000 ng microcystin l⁻¹.
Rates of microcystin deposition in PF ranged from 1 – 19 ng microcystin mussel\(^{-1}\) d\(^{-1}\) (0.89 – 16.8 µg microcystin m\(^{-2}\) d\(^{-1}\)). Microcystin deposition rates for UV-027- exposed mussels were not different from simultaneous Lake Erie *Microcystis* – exposed mussels at the lower exposure concentrations (≤ 2000 ng microcystin l\(^{-1}\)) where little or no microcystin was deposited in PF.

(b) Addition of green algae to treatments.

Exposures of mussels to high concentrations of *M. aeruginosa* UV-027 increased the rates of PF deposition over green algae-only treatments and low-level Lake Erie sources of *Microcystis*. The green algae added, *C. vulgaris* and *C. rheinhardtii*, are single-celled green algae, and the lab-cultured *M. aeruginosa* we added to treatments was also single-celled, but much smaller in diameter. We originally assumed that the concentration of cell material added for *M. aeruginosa* treatments was controlled for by the green algae additions to a filtered water treatment. However, when dry weights were taken of the *M. aeruginosa* and green algae, we found that the highest *M. aeruginosa* treatments still had more cell material in them than the green algae treatment. Therefore, we cannot be certain increased PF deposition in the 13,000 ng microcystin l\(^{-1}\) treatment was the result of high toxin concentration in the exposure water or from the amount of dry cell material added to the water to achieve the necessary toxin concentration.
However, PF deposition remained low for the green algae controls, but increased in the 6900 ng microcystin l⁻¹ UV-027 treatment (Figure 32). At that level, green algae additions were sufficient to act as controls for the cell material added in the UV-027 treatment.

In further laboratory experiments, the addition of green algae to the Lake Erie source of cyanobacteria (concentrated from net collections) influenced the rates of PF and microcystin deposition. At 72 h, PF deposition rates ranged from 12 – 48 ng PF dw mussel⁻¹ d⁻¹ (10.6 – 42.5 µg PF dw m⁻² d⁻¹). When green algae are present with concentrated Lake cyanophytes, mussels decreased the rate of PF deposition (150 ng microcystin l⁻¹ treatment in Figure 34). This may indicate that mussels ingest more green algae and Microcystis when both are offered. PF deposition rates were higher when green algae were not present (the concentrated 60 ng microcystin l⁻¹ treatment in Figure 34) with concentrated cyanobacteria, even though microcystin concentration was higher in the concentrated Microcystis treatment with green algae (150 ng microcystin l⁻¹ treatment in Figure 34). PF deposition was significantly affected by length of exposure (p = 0.0329), but less significantly by whether green algae were added to cyanobacteria sources (p = 0.0587).

Conversely, microcystin deposition (in PF) rates were significantly higher (p = 0.0001) when green algae were present with concentrated Microcystis (Figures 35). At 72 h, microcystin in PF deposition rates ranged from 0.08 – 0.6 ng microcystin mussel⁻¹ d⁻¹ (0.07 – 0.531 µg microcystin m⁻² d⁻¹). In addition, length of exposure was not a
significant factor for microcystin deposition from mussels exposed to the Lake Erie source \((p = 0.3587)\).

However, since PF includes both feces and pseudofeces, it is uncertain whether mussels were rejecting microcystin-laden cyanobacteria in pseudofeces before ingestion, or excreting microcystin in feces after ingestion. Therefore, we can speculate that the greater rate of microcystin deposition when green algae are offered at the same time is either an indication of selective ingestion of green algae and rejection of *Microcystis*, or an increased ability to excrete microcystin through digestive processes.

**Comparing field and microcosm biodeposition:**

A comparison of our microcosm PF and microcystin deposition rates to field deposition rates reveals that rates of PF deposition in the field are roughly a magnitude higher than microcosm rates on an areal basis (Table 9). However, the number and size-distributions of mussels m\(^{-2}\) differed significantly between these two experiments. In microcosms, the number of mussels m\(^{-2}\) was about 3540 and all the mussels were 15 – 20 mm in length. In the field, there were approximately 17,700 mussels m\(^{-2}\) ranging from 7.2 to 16.8 mm. However, areal microcystin deposition rates measured in the field fall within the ranges measured in microcosms for the Lake Erie source of microcystin and very close to the ranges measured for the UV-027 source (Table 9). On a per mussel basis, the ranges of PF deposition from mussels exposed to UV-027 and Lake Erie sources in microcosms were similar, but microcystin deposition rates were lower for the Lake Erie
exposed mussels (Table 10). PF deposition per mussel by the field-exposed mussels is roughly 400 times higher than the microcosm mussels. However, the ranges of microcystin deposition per field-mussel are roughly 75 times lower than the UV-027 microcosm mussels, and are in the lower end of the UV-027 range for the microcosm mussels exposed to Lake Erie cyanophytes. Variability in deposition was probably caused by differences in size-frequency distribution of the mussels, different seston concentrations between microcosms and the Lake, and composition of seston in the Lake compared to the microcosms. For example, there were 9-10 mg dw l⁻¹ lake seston composed of organic and inorganic particles; compared to around 0.064 mg dw l⁻¹ for concentrated Lake Erie cyanophytes in microcosms, and a maximum of 32 mg dw l⁻¹ for UV-027 *Microcystis* in microcosms.

**Objective 3. Microcystin accumulation in zebra mussels and amphipods.**

Accumulation of microcystin in zebra mussels depended on source and concentration of microcystin, but not on length of exposure. When mussels were exposed to freeze-dried *M. aeruginosa* UV-027, accumulation in mussel tissue was not significantly higher than that for mussels exposed to live Lake Erie cyanobacteria until microcystin concentrations reached 6900 ng l⁻¹ or higher (p = 0.0001). Mean concentrations of microcystin in mussel tissues for 24 h intervals were pooled because length of exposure had no significant effect (p = 0.2120) on microcystin accumulation for UV-027 after 24 h (Figure 36).
Mussels exposed to Lake Erie sources of microcystin for 72 h had significantly (p = 0.0001) lower accumulation when mussels were exposed to filtered Lake Erie water with green algae added than to cyanobacteria in surface water, or concentrated in filtered water (Figure 37). Accumulation was somewhat higher in mussels exposed to the concentrated cyanobacteria without green algae added than with it. Again, the mean concentrations of microcystin in mussels for 24 h intervals were pooled since length of exposure had no significant (p = 0.4328) effect on accumulation.

Zebra mussels statically exposed to increasing concentrations of live Lake Erie *M. aeruginosa* LE-3 for 48 h all accumulated microcystin at the same rate for concentrations ranging from 20 to 80 ng microcystin l⁻¹, averaging 135 ng g⁻¹ ww (from 5 to 30 ng mussel⁻¹) (Figure 38). Amphipods exposed simultaneously with those mussels averaged 138 ng microcystin g⁻¹ ww (3 to 7 ng microcystin amphipod⁻¹) for all exposure concentrations except control (Figure 39). All mussels and 98 % of amphipods survived the static exposure even at the highest concentrations of microcystin. Mussels were observed to be filtering throughout the exposure for all concentrations.

Zebra mussels took up microcystin rapidly, reaching equilibrium within 24h. Microcystin uptake rates in zebra mussels fit (adjusted R² = 0.80) a 1st order exponential relationship (Figure 40) with experimental rate constants estimated for uptake: k_u = 47.45 ml g⁻¹ h⁻¹ and elimination: k_e = 0.0533 h⁻¹. Tissue concentrations of microcystin in zebra mussels exposed to freeze-dried *M. aeruginosa* LE-3 (nominally 20 ng microcystin l⁻¹)
averaged 22 ng g\(^{-1}\) ww (about 2.0 ng microcystin mussel\(^{-1}\)) by 72 h and appeared to reach a steady state at that concentration. After 48 h of depuration, concentrations of microcystin in mussels still living averaged 7.6 ng g\(^{-1}\) ww (about 0.6 ng mussel\(^{-1}\)). Not enough mussels survived to continue depuration past 48 h and decay of tissue allowed release of microcystin from dead mussels. Surviving mussels maintained a low level of microcystin, with a depuration half-life \(T_{1/2} = \ln 2/k_e\) estimated at 13 h in net-filtered water. Resulting uptake/elimination rates yield a bioconcentration factor (BCF) of 890 for zebra mussel uptake of microcystin from \(M. \ aeruginosa\) UV-027 in water.

Amphipods accumulated microcystin in nearly equal amounts whether exposed to \(M. \ aeruginosa\) suspended in water alone, or by exposure to contaminated PF alone, or when exposed together with zebra mussels. Assuming the amphipods consumed all of the PF they were given (they were observed doing this), they were able to accumulate microcystin from direct doses of microcystin contained in the PF (Figure 41). Amphipods also accumulated microcystin from exposure in water (Figure 41). The resulting amphipod microcystin concentrations are paired in Figure 41 according to the direct water exposure of the amphipods and the exposure to PF from mussels exposed to that water concentration. Amphipods began with roughly 200 ng microcystin g\(^{-1}\) dw. Whole-body accumulation of microcystin after 72 h exposure ranged from 200 to 675 ng microcystin g\(^{-1}\) dw (about 20 to 67.5 ng g\(^{-1}\) ww). This range was similar whether amphipods were exposed to Lake Erie cyanobacteria (20 – 200 ng microcystin l\(^{-1}\)) or to PF sources of Lake Erie microcystin. However, microcystin accumulation was
significantly (p = 0.0001) higher for amphipods given PF from zebra mussels exposed to *M. aeruginosa* UV-027 at 1500 ng microcystin l\(^{-1}\) (treatment code 6 in Figure 41) than to the lower concentrations presented in Lake Erie *Microcystis*.

**Objective 4. Determine whether microcystin is accumulated in round gobies from feeding on microcystin-contaminated zebra mussels or from environmental exposure to toxic *Microcystis*.**

Field collections of gobies showed they accumulate microcystin from their environment (Table 7). Whether they accumulate it mainly from exposure to toxic algae in water or from food sources remains unclear. Gobies accumulated microcystin in their guts and livers when exposed to a nominal concentration of 20 \(\mu\)g microcystin l\(^{-1}\) from *M. aeruginosa* LE-3 for 72 h at 24\(^\circ\) C. Uptake rates in guts (\(k_u = 1360.6\) ml g\(^{-1}\) h\(^{-1}\)) and livers (\(k_u = 4152\) ml g\(^{-1}\) h\(^{-1}\)), were high, fitting a 1\(^{st}\) order exponential relationship (adj. \(R^2 = 0.865\) for guts and 0.678 for livers) (Figure 42). Elimination rates were estimated from the plots for guts (\(k_e = 0.2194\) h\(^{-1}\)) and livers (\(k_e = 0.3974\) h\(^{-1}\)) giving depuration half-lives of 3.2 h for guts and 1.7 h for livers. These elimination rates appear to be underestimates, because livers reached an average microcystin concentration of 282.3 ng g\(^{-1}\) ww after 72 h of exposure, and after 72 h depuration had an average of 89.2 ng microcystin g\(^{-1}\) ww. Similarly, the gut concentration of microcystin averaged 143.6 ng g\(^{-1}\) ww after 72 h, and after 72 h depuration still had 90.2 ng microcystin g\(^{-1}\) ww. From the resulting uptake/elimination rates for microcystin in the livers and guts, bioconcentration factors (BCFs) were estimated at 10,450 for livers and 6200 for guts.
Field-collected gobies still had high initial concentrations of microcystin even after we starved them for 48 h (Figure 43), confounding experimental results of the feeding experiment with microcystin-contaminated zebra mussels. If all gobies started out with similar concentrations to those measured in the initial ones, then gobies did not have a net gain in microcystin from eating contaminated mussels. After 7 days of eating contaminated zebra mussels, however, tissue concentrations remained, on average, higher in those eating more microcystin-contaminated zebra mussels. Tissue (gut, liver and muscle) concentrations were related to the cumulative amount of microcystin ingested (based on the number of toxic zebra mussels each fish ate) (Figure 44). Levels of toxin in livers ($p = 0.0008$) and guts ($p = 0.0046$) were significantly affected by amount of toxin eaten, but toxin in muscle ($p = 0.9108$) was not. In fact, only four fish had detectable levels of microcystin in their muscle tissue (the two initial fish and two from the experiment). Average amounts of microcystin that might be found in a goby eating 10 mussels per day exposed to 0, 1 or 10 $\mu$g microcystin l$^{-1}$ were obtained from averages of total toxin in livers and guts from each fish (Figure 45). Toxin content of muscle tissue was not included because it was not significantly influenced by the amount of toxin eaten, nor were there very many fish that had toxin in muscle tissue. In addition, because microcystin is a specific inhibitor of protein phosphatases in the liver tissue, and gut content of microcystin is most likely from food present in the gut, it is unlikely that large amounts of microcystin would accumulate in other organs of the fish.
DISCUSSION

Our hypothesis that *Dreissena* spp. are mediating the fate of microcystin in a new trophic cycle has been supported. Our experiments showed that zebra mussels deposit PF at higher rates than background, and with higher concentrations of microcystin, in response to exposure to toxic cyanobacteria in the field or in microcosms. Thereby, zebra mussels made microcystin available to benthic detritivores such as the amphipod *E. ischnus*. In addition, accumulation of microcystin was detected in all organisms comprising the inter-related food web of *Dreissena* spp., *Echinogammarus ischnus* and *Neogobius melanostomus* (Figure 26). Microcystin was also detected in several other fish species collected from the western basin of Lake Erie during a *Microcystis aeruginosa* bloom in 1998 and in sediment deposited by zebra mussels in Lake Erie in 1996. Our microcosm experiments clearly demonstrated that these organisms accumulate microcystin from environmental exposure to living, or freeze-dried, Lake Erie *Microcystis* spp. or from a different cultured strain (UV-027).

Comparisons of field and microcosm PF and microcystin deposition rates (per mussel or areal rates) revealed that PF deposition rates were much higher in the field, but microcystin deposition rates (microcystin in the PF) were similar for field and microcosm
mussels exposed to Lake Erie *Microcystis*. However, these rates were much lower than those measured for mussels exposed to UV-027 *Microcystis*. Microcystin in Lake Erie generally occurred in lower concentrations than in our laboratory experiments. In addition, the field densities of mussels were much higher (17,700 m\(^2\)) than in microcosms (885 to 3540 m\(^2\)). Therefore, in the field many more mussels were competing for a much more dilute source of *Microcystis* containing a much lower concentration of microcystin in addition to other more palatable food sources and inorganic particles. This may explain why PF deposition rates were much higher in the field, but microcystin deposition rates were much the same between the field and microcosms for Lake Erie *Microcystis*. As demonstrated in the microcosm experiments, the microcystin concentration, length of exposure, and the presence, or absence, of green algae all influenced the rates of deposition of PF and microcystin to sediments. From our comparison of field and microcosm results, we can conclude that the number of zebra mussels present, time of year, and seston composition all influence the rates PF and microcystin deposition.

The results of our trophic transfer experiments were less conclusive because we could not start experiments with microcystin-free gobies. Because the gobies started with high microcystin loads, it is unclear how the rates, or amounts, of uptake of toxin from contaminated food were influenced. Inhibition of protein phosphate sites in the liver is presumably limited by the number of sites available, so uptake rate in the liver could have been slowed if fewer open sites remained.
The uptake rate could also have been influenced by the amount of contaminated food in the gut and the rate of passage of the microcystin from the gut to the liver. Gobies in the natural environment are known to eat much higher numbers of zebra mussels. They would also receive microcystin from other food sources, and from water across the gills. The fact that our initial gobies had such high microcystin concentrations in the livers, even after 2 days of starvation, indicates that they had been receiving microcystin in the field. It also indicates that toxin elimination is a slow process. The fact that gobies being fed contaminated mussels over 7 days had lower concentrations of microcystin, albeit dependent on the amount of mussels they ate, indicates that any elimination of the toxin may require active digestive processes. Williams et al. (1997) found that elimination of microcystin from Mytilus edulis fed toxic M. aeruginosa occurred rapidly, within 4 days for the covalently bound portion of microcystin, but “free” microcystin (that detected by MeOH extraction and protein phosphatase detection) elimination occurred over a much longer 53 day period.

Detection of microcystin in mussels and amphipods was limited to whole body analysis, and presumably constituted a measure of unbound microcystin in these organisms. In fish, we were able to detect microcystin in guts and livers and, in a few fish, muscle tissue. The method we used for detection of microcystins (ELISA) may limit detection to unbound microcystin (Williams et al. 1997) and it is quite likely that most microcystin in the livers was in a covalently bound (PPase-microcystin complex) state.
Williams et al. (1997) compared several detection methods, including Lemieux oxidation gas chromatography-mass spectrometry (GCMS), protein phosphatase assay, and radiolabelling, to show that greater than 60% of microcystin is covalently bound in mussel and salmon tissues. They determined that less than 0.1% of bound microcystin was extracted using MeOH and it therefore, may go undetected by ELISA. Therefore, our analyses of microcystin concentrations in body tissues were probably proportional to the greater concentrations that were present in these organisms but, the bulk of microcystin covalently bound in the livers or hepatopancreatic tissues may have gone undetected. If the estimates of Williams et al. (1997) are correct, then the bound microcystin content in mussels, amphipods and fish from our experiments could have been up to 99.9% higher than we measured. Nevertheless, we showed that there is a significant potential for accumulation of microcystins from water to mussels, amphipods and fish. The probability that accumulation of microcystin is much higher than we detected indicates that the risk of trophic transfer of toxic doses through food organisms is high.

Determination of the main source of contamination in these organisms, food or cyanobacteria in water, presented greater difficulties. Our attempts at feeding amphipods microcystin-contaminated pseudofeces, and feeding fish microcystin-contaminated zebra mussels, resulted in somewhat ambiguous findings. Amphipods accumulated similar amounts of microcystin (200 – 700 ng g⁻¹ dw) from being exposed to toxic cyanobacteria in water or from eating contaminated PF when they were exposed to these sources separately.
When they were exposed, together with zebra mussels, to living toxic *Microcystis* they accumulated higher levels of microcystin (an average of 138 ng g\(^{-1}\) ww or an estimated 1380 ng g\(^{-1}\) dw). In this situation, we presumed that the amphipods were getting microcystin from both food and water, and would therefore predict that accumulation would have been higher. The exposure concentration of microcystin (nominally 20 µg l\(^{-1}\)) was higher for the combined zebra mussel and amphipod experiment, so the higher concentration cannot be ruled out as the sole reason accumulation was higher. Regardless of the main source of microcystin contamination, the zebra mussels and amphipods in our experiments showed a high tolerance for microcystin and remained alive even when they retained high levels of microcystin in their bodies. It is probable that these organisms, having evolved with microcystins in their environment, have developed tolerances for microcystins and/or mechanisms for ridding themselves of them.

*Zebra mussels apparently decrease the rate of assimilation of the toxin by making pseudofeces. Evidence in support of this mechanism is that zebra mussels in our experiments suffered little or no mortality, and deposited PF at higher rates, and with higher toxin content, in response to higher environmental concentrations of toxin-containing *Microcystis*. Zebra mussels rejected less microcystin when more palatable green algae were present in water since mussels produced PF at higher rates when given concentrated cyanobacteria without green algae. Zebra mussel accumulation of toxin appeared to reach a steady state. Therefore, there may be a limit to the amount of *Microcystis* mussels can reject under high concentrations.
Berg et al. (1996) found that zebra mussel clearance rates declined in relation to increasing concentrations of algal particles, and that pseudofeces production only increased once a threshold algal concentration was reached. Both of these processes were also dependent on the type of algae and size of mussel. Sorting of particles on the gill for ingestion or rejection is therefore limited in part by particle concentration and type of particle. It is likely that particle concentration in our experiments with UV-027 at 13 ng microcystin ml⁻¹, or 32 mg cell dw l⁻¹, had reached the level where sorting on the gills was limited and PF production was maximized. Because we did not control for particle density in this highest UV-027 exposure concentrations, it is unknown whether direct microcystin toxicity, or crossing a threshold for high concentrations of undesirable food particles may have played a part in PF production. However, at lower concentrations, where we did control for particle concentration, PF and microcystin deposition rates were apparently more related to toxin concentration. More experiments controlling for particle density vs. toxin concentration are needed to clarify this issue.

Apparently, microcystin accumulation in the mussels reached equilibrium, or binding sites became saturated, in nearly all of our experiments. In addition, there was no apparent lethal effect on the zebra mussels. Accumulation of MeOH - extractable microcystin did not get higher than about 22 ng g⁻¹ ww regardless of the source. Vasconcelos (1995) reported that 3.5 cm long mussels (Mytilus) he exposed to toxic Microcystis also suffered low mortality and produced pseudofeces.
Vasconcelos also found that concentration of microcystin in mussels (using the same detection technique we used) responded rapidly (within 24 h) to changes of microcystin concentration in the exposure water. He discovered an apparent microcystin saturation point around 11 μg g⁻¹ dw occurring around Day 10 of exposure, 500 times the apparent saturation level of 22 ng g⁻¹ ww we found for the much smaller dreissenid mussels we exposed to Lake Erie *Microcystis*. Other physiological differences are likely as well. The average amount of microcystin he fed to his mussels on a daily basis (153 ± 74 μg microcystin-LR in 20 liters of seawater or about 7.65 μg l⁻¹) was higher than most of our exposure levels, though two of our UV-027 microcystin-RR concentrations were similar (6.9 and 13 μg l⁻¹). Vasconcelos also concluded that the levels of microcystin maintained in mussels caused no apparent ill effects on them while providing a source of trophic transfer risk to organisms eating the mussels.

Amphipods (*E. ischnus*) also accumulated the toxin in our experiments. They survived while eating all PF provided during exposures, and likely eating the provided algae and cyanobacteria in lake water exposures as well. We did not, however, measure whether microcystin leached from PF to water, so do not know whether the main route of microcystin uptake in amphipods is from water exposure or from food intake. Nevertheless, amphipods exposed to increasing concentrations of living LE-3 in the presence of zebra mussels, would have received microcystin from both routes, and accumulated an average of 138 ng microcystin g⁻¹ ww; a level even higher than was obtained from either route alone in the previous experiment.
Liras et al. (1998) found that crayfish (*Pacifastacus leniusculus*) accumulated microcystin in the hepatopancreas during field exposures to *Oscillatoria sancta* and in feeding trials when exposed to toxic *Planktothrix agardii*. These authors concluded that the microcystin had no negative impact on the crayfish and that they could pass the toxin up the food chain. They found that crayfish were actually eating the cyanobacteria, whereas we observed that amphipods ate contaminated PF. This suggests that food may be a significant source of microcystin contamination for many Crustacea.

The tolerance of microcystin by other crustacean species suggests that they must have mechanisms for avoiding assimilation of the toxin, detoxifying and/or eliminating it, or being insensitive to it. Thostrup and Christoffersen (1999) found that toxic *Microcystis* accumulates in *Daphnia magna*, having some toxic effects on their survival and reproduction, but still allowing many to survive. Demott and Moxter (1991) found that copepods could discriminate against toxic cyanobacteria in feeding experiments and thereby avoid it. Shi et al. (1999) discovered that some strains of *M. aeruginosa* have protein phosphatases that are insensitive to the microcystin produced by toxic strains. It is possible that some Crustacea, or other invertebrates, may have evolved protein phosphatases that are insensitive to microcystins as well. Kotak et al. (1996) observed microcystin accumulation in zooplankton and gastropods, *Lymnaea stagnalis*, from Canadian lakes where blooms of toxic *M. aeruginosa* were occurring. However, they did not observe accumulation in nine other macroinvertebrates, including benthic detritivores such as the amphipod, *Gammarus lacustris* and *Chironomus* spp., or in northern pike.
(Esox lucius) and white suckers (Catostomus commersoni). In contrast, we found microcystin accumulation in E. ischnus and several fish species in western Lake Erie during the M. aeruginosa bloom of 1998. However, the authors do not mention the presence of Dreissena spp. in their study lakes. Assuming Dreissena spp. were not present, their absence may be a significant factor contributing to a lack of availability of microcystin to detritivores, and their predators, in those lakes.

Our findings of microcystin accumulation in fish species occupying different trophic levels in Lake Erie allow us to make several additional observations. Gizzard shad accumulated more microcystin than the other fish species, accumulating more toxin in their guts than in livers, probably a reflection of their filter-feeding habit. They would have received toxin directly from eating toxic phytoplankton and zooplankton, as well as across the gills from water. Gizzard shad are often prey of walleye and other piscivores and would therefore, pose a risk to them during toxic Microcystis blooms. We found microcystin in known Dreissena predators such as round gobies and yellow perch collected in the field. Since the levels of accumulation in these fish increased from August to October, when water concentrations were decreasing, we might conclude that they were receiving toxin from food sources that still maintained higher levels of toxin. Researchers (Kent et al. 1996) studying net-pen liver disease in pen-reared Atlantic salmon (Salmo salar) determined that the disease, caused by an unknown source of microcystin-LR, was most likely the result of the fish eating contaminated food organisms that came into their pens and not from the seawater itself.
However, the small (95 – 120 mm) walleye we collected did not show any toxin accumulation. Even at that relatively small length the walleye would surely have been feeding on smaller fish such as the YOY yellow perch and Morone spp. we found to have microcystin loads. Therefore, we cannot conclusively say that trophic transfer of microcystin was occurring in piscivores. The walleye, and our sample size, were small and collections of more, and larger, piscivorous fish and analysis of their gut contents for toxin-containing fish are needed to clarify this issue.

Clearly, smaller fish that are prey to larger sport fish do accumulate microcystin in their livers and guts. Since only a few gobies were known to have microcystin in muscle tissue, and we did not examine muscle tissue in the other fish, we do not know whether humans eating contaminated sport fish are at risk. However, large fish and bird predators eating whole fish certainly are at risk. Using the estimated body concentrations from livers and guts, an individual goby, eating only ten contaminated mussels per day for a week, could deliver up to 200 ng of microcystin to a bigger fish or bird that might eat it. This toxin concentration is higher than is likely to be found in the water surrounding an animal in western Lake Erie and it is delivered in a direct dose. However, Råbergh et al. (1991) found that intraperitoneal doses of 130-330 μg microcystin–LR kg⁻¹ to common carp (Cyprinus carpio) for seven days were sublethal, but caused severe liver damage and some kidney damage. The lowest lethal dose these researchers found for the carp was 550 μg kg⁻¹.
Therefore, eating microcystin-contaminated food organisms from Lake Erie is likely to result in much lower doses than the carp received. A 1 kg fish would have to eat an unlikely 2700 or more adult-sized gobies a day to receive the lethal dose for carp. A piscivore would have to eat 6 gobies to receive 1 µg of microcystin if mussels were being exposed to 10 µg microcystin liter$^{-1}$ for 1 day. A benthic feeder would have to eat 66 2.0 cm mussels or 166 adult amphipods to receive 1 µg of microcystin if mussels were being exposed to 20 µg microcystin liter$^{-1}$ for 1 day. These numbers are not out of range for predation rates of piscivores and benthic feeders on these types of organisms in Lake Erie, especially over periods longer than a day. Since microcystin is likely to accumulate in a cumulative fashion over long periods of exposure, predators on these food organisms could still be at risk even at much lower exposure concentrations. We found microcystin levels in field collected amphipods, fish and mussels that were near the levels we measured for the 10 and 20 µg microcystin liter$^{-1}$ single day exposures of these organisms. Therefore, there is evidence that this level of contamination of food organisms is already occurring in Lake Erie, even when measured microcystin levels from whole water samples are only in the ng liter$^{-1}$ range.

It remains to be determined whether organ damage was caused in any of the Lake Erie fish exposed to toxic *M. aeruginosa*. Apparently, gobies in our feeding experiment were able to rid themselves of the toxin even though they were still receiving it from eating contaminated mussels.
The gobies initially had an average of 280 ng microcystin g⁻¹ in their livers, but after seven days of eating contaminated mussels these levels declined in a manner dependent on the number of mussels the fish ate. Upon dissection, we discovered large amounts of green liquid in the gall bladders of fish that ate many contaminated mussels. This was incidentally also true for fish exposed directly to *Microcystis* in the uptake experiment. Sahin et al. (1996) reported that rainbow trout (*Oncorhynchus mykiss*) gavaged with 4.6 mg microcystin-LR g⁻¹ dw eliminated microcystin in bile fluid collected from their gall bladders. Tencalla and Dietrich (1997) showed that a transient inhibition of protein phosphatases 1 and 2A in the liver occurred within 3 hrs of gavage of rainbow trout with microcystin. While the inhibition was transient, liver damage still progressed. Therefore, our observation of green fluid in the gall bladders of gobies eating microcystin-contaminated mussels may be an indication that gobies also excrete microcystin in bile, but may still suffer liver damage. Subsequent research should specifically examine bile in these fish for microcystin content. Long-term effects on gobies, and other species receiving low doses of microcystins over long periods, may be significant.

**CONCLUSION**

Our quantitative study of the accumulation and fate of natural sources of microcystin in a natural (though non-indigenous) food web shows the potential for a key species (*Dreissena*) to have far-reaching indirect effects on an ecosystem. The invasion of the Great Lakes by non-indigenous species has certainly wrought havoc with any equilibrium that might theoretically become established in an undisturbed system.
Arguably, an ecosystem is never really in a state of equilibrium and is never completely undisturbed. *Dreissena* spp. in particular, have greatly upset the balance between benthic-pelagic coupling processes in Lake Erie. We have shown that the mechanism of biodeposition of microcystin-contaminated PF by zebra mussels results in greater availability of the toxin to benthic feeders. Detritivores and benthic-feeding fish are particularly at risk. Further studies of microcystin accumulation in higher predators are needed to conclusively determine whether trophic transfer is a significant factor posing a risk to them or to humans. The potential for direct toxic effects on other indigenous organisms via this route and long-term disturbances in food webs needs to be determined.
CHAPTER 3

INTRODUCTION

Toxic Microcystis Blooms in Western Lake Erie have the Potential to Impact Zebra Mussel Energetics and Influence Size-Frequency Dynamics of Zebra Mussel Populations.

When studying the potential impacts of an invader organism on an ecosystem, it is necessary to understand its biology in terms of its habitat requirements, energetic requirements, and reproductive and dispersal strategies (Suter 1993). The zebra mussel, Dreissena polymorpha, is an example of an invader species that has the potential to profoundly change the ecosystems it invades. It has a great filtering capacity for removal of algae (Madenjian 1995; Stoeckmann and Garton 1997) and excretes wastes that can change benthic-pelagic nutrient relationships (James et al. 1997) and deposition rates (Klerks et al. 1996). Reproductive and dispersal strategies, and genetic plasticity, have allowed zebra mussels to become established throughout many lacustrine and riverine habitats in the eastern United States ranging from the Great Lakes to Louisiana in a matter of roughly ten years.
Economic and ecological impacts since the establishment of this invader (Mackie et al. 1989) include the clogging of power plant intake pipes by aggregates of attached mussels, and the decimation of native bivalve populations by settlement on the surfaces of shells (Mackie 1992; Haag et al. 1993). These types of impacts affect industry or organisms directly. In this study, however, I am concerned with specific, but indirect effects that zebra mussel feeding-behavior may have on the ecosystem and how its fitness may in turn be affected by the changes it has wrought on the ecosystem.

**Ecological impacts of feeding behavior:**

Knowledge of feeding behavior is important in understanding the kinds of impacts zebra mussels may have on the availability of food to competing native organisms, such as zooplankton or other bivalves, and on changes in nutrient and contaminant cycling which may impact the whole system. Recent studies of zebra mussel related changes in Saginaw Bay, Lake Huron (Heath et al. 1995; Gardner et al. 1995), and the western basin of Lake Erie (Arnott and Vanni 1996), have shown that zebra mussel mediated changes in nutrient cycling (James et al. 1997) and selective filtering abilities (Larentyev et al. 1995), could be major factors in the return of noxious, and sometimes toxic (Brittain et al. 2000) *Microcystis aeruginosa* (cyanobacteria). Cyanobacteria are an undesirable food for most organisms that filter feed because of size, shape and potential toxicity. Therefore, *Dreissena* spp. may be mediating potentially negative changes in lake ecosystems affecting other organisms and themselves.
Environmental factors may affect the fitness of the zebra mussel by determining its feeding strategies and growth. Understanding potential effects of environmental factors on zebra mussel populations would help modelers predict how populations might increase, stabilize or decline in a given water body. Therefore, it is the goal of this project to model how the fitness of zebra mussels, in terms of ability to grow to a critical size and survive, will be impacted by changes in filtering behavior brought by a bloom of toxic cyanobacteria.

**Bioenergetics of mussels:**

Bayne et al. (1989) found that growth in a marine bivalve, *Mytilus edulis*, was limited mainly by constraints on feeding behavior. Scope for growth (the energy left over for growth after metabolic needs are met) was related to feeding processes such as filtration rate, ingestion rate, assimilation efficiency and pseudofeces production (that portion of filtered particles that are rejected before ingestion). These factors changed under different conditions of particle concentration (seston) and food quality. Madon et al. (1998 and 1999) studied zebra mussel bioenergetics in a range of simulated and natural river conditions and obtained comparable results. However, Madon et al. determined that the main factor influencing feeding processes in relation to food quality was the organic to inorganic carbon ratio (O: IO) of the particulate matter in the seston. They also found a relationship between increasing seston concentrations and decreasing filtration rates. Recent experiments have shown that zebra mussels produce significant quantities of pseudofeces (Babcock-Jackson, unpublished) and decrease their filtration
rates and assimilation efficiencies in the presence of toxic *Microcystis aeruginosa*, even when more palatable algae are available (Culver et al. 1999). When cyanobacteria are in a bloom condition, seston levels are typically high, with an artificially high apparent food quality (according to the above criterion of a high O:IO). However, the feeding behavior of the mussels does not follow the pattern previously observed (i.e.; increased ingestion rates and assimilation efficiencies when POM is high; Culver et al. 1999). Therefore, I hypothesized that the relationship of zebra mussel clearance rates to food quality (as defined by O:IO) and seston concentration will not follow previously observed patterns when toxic *Microcystis* is present in a simulated bloom. I predicted that because of *Microcystis* - induced changes in feeding behavior, the presence of high concentrations of toxic *Microcystis* would negatively affect the growth of zebra mussels as compared to years when *Microcystis* is low. The hypothesis was tested with the following objective:

(1) Model how zebra mussel feeding behavior, given certain environmental conditions and bioenergetic costs and benefits of other behaviors, determines growth to a critical size of 6 mm and 10 mm, the respective sizes to survive a winter and spawn.

*The Dynamic Programming Model*

The model was developed according to the dynamic modeling approach detailed by Mangel and Clark (1988) where it is assumed that animal behavior is governed by a Darwinian model of fitness.
Therefore, to obtain maximum fitness the organism makes optimal choices among a set of decision options having costs and benefits attached to them. The dynamics of the system come into play as the state of the organism changes over time according to the decisions it makes, with the ultimate goal of getting the maximum lifetime expected fitness. In the present model, the changing state of the zebra mussel is length (serving here as an index of growth), which is determined by the starting size and conversion of calories to somatic and shell growth. Intake of calories is achieved from filtering of particulate organic matter from water. Because zebra mussels are fixed, sedentary bivalves, the only way they can control their intake of food is by changing their filtration rate (the decision variable) and pseudofeces production rate (Foster-Smith 1975). Costs and benefits of the filtering decisions (in terms of energy gained or lost) were set; assigning values to parameters that affect the filtering decision, namely assimilation efficiency, pseudofeces production rates and metabolic rates. The values these parameters received were based on temperature, seston concentrations and seston organic content during four time periods in a simulated growing season. Filtering decisions were modeled during two simulated 12-week summer growing seasons, one modeling the effects of a toxic *Microcystis* bloom on mussels, and the other when no bloom occurs.
METHODS

Parameters for the Model

Each 12-week growing season was divided into four time periods (T), each with a representative seston concentration and organic carbon ratio one might find in western Lake Erie. In reality, these conditions change continually, however a simplification of the continuum into discrete time blocks makes it easier to program and interpret the model outcome. The model parameters are defined in Table 11. Different values for the parameters were assigned to time periods 1 through 4. Time periods 3 and 4 either simulated a *Microcystis* bloom (MC) or not (No MC). The parameter values affecting the filtration decision variable clearance rate (CR), values for metabolic rates (MR), assimilation efficiencies (AE), and pseudofeces production rates (pp) under different environmental temperatures and conditions, were obtained from literature (Reeders and Bij de Vaate 1989 and 1992; Schneider 1992; Madenjian 1995; Klerks et al. 1996; Madon et al. 1998 and 1999; Stoeckmann 1997; and Culver et al. 1999). The values assigned for AE and pp during time period 4 (MC) were within the range of actual values observed in experiments on zebra mussels responding to toxic *Microcystis aeruginosa* (Culver et al. 1999).
Estimates of predation probabilities ($P_l$) for different predators on zebra mussels such as crayfish (MacIsaac 1994; Martin and Corkum 1994), freshwater drum and yellow perch (French and Bur 1992; Morrison et al. 1997), round gobies (Ray and Corkum 1997) and diving ducks (Hamilton et al. 1994) were used to generate probabilities of predation in the model (Table 12).

**Model Assumptions**

Zebra mussels used in the simulation were assumed to be from a new cohort. The new cohort could spawn (maximizing their lifetime fitness) during the growing season of 12 weeks only if they reached a critical size of 10 mm. If they did not reach that size, then they must reach a critical size of 6 mm in order to survive the winter with the potential to spawn the next season. These critical sizes were chosen based on observations of serotonin-induced spawning success observed by myself and others (A. Stoeckmann and J. Stoeckel) for various size ranges of zebra mussels. Typically, zebra mussels must be at least 10 mm and in good condition to induce release of gametes using serotonin. My own field observations of zebra mussels in early June reveal that survivors of the last settling event from the previous year are typically in the 5 to 10 mm range. Therefore, I chose the 6 mm and 10 mm critical sizes for the model. I also assumed zebra mussels were already settled at the beginning of the simulated growth period and started at a size of 3 mm (a size at which they have already established a byssal attachment, but are still capable of releasing it to move short distances). Then, I tested for survival and fitness by starting individuals at each successively larger size (4, 5, 6, 7 mm, etc.).
zebra mussels are attached, they cannot avoid predators even when they do not feed. All
the predators used for the model calculations eat zebra mussels whole or are able to break
open the shells. The model equation was written such that the probability of being eaten
\( \beta_L \) depended on the starting size \( L \) of the mussel during each time step. I assumed that
the mussel must first survive being preyed upon before it could obtain calories for growth
by filtering.

I assumed that temperature and food concentration/food quality changed between,
but not within each of the 4 time periods occurring in a growing season. Correspondingly,
I also used a constant zebra mussel respiration rate during each time period, in agreement
with observations by Stoeckmann (1997) and Madon et al. (1999). I also assumed that
food concentration is always > 0, that if zebra mussels do not filter they engage in
anaerobic respiration and no growth occurs, and that filtration rates ranged from 0 to 2.4
liters d\(^{-1}\).

**The Dynamic Programming Equation**

Maximum expected fitness \( F(L, t, T) \) of a zebra mussel of length \( L \) on week (\( t \))
of the simulation during the two simulated growing seasons varied temporally. Therefore,
for each value of \( t \) within a period, different parameter values were assigned.
The maximized relative fitness of zebra mussels using an optimal strategy of filtration rate (CR) at different starting sizes was then determined by backwards iteration (Mangel and Clark 1988) from a terminal fitness function $F (L, T, T)$, or the maximum expected fitness accrued from $T - 1$ to $T$; $(T-1 = t)$ for each backwards iteration from terminal time $T$ defined below (Equation 5). At starting time $t$, the state of the zebra mussel is $L(t) = L$, at that size $L(t) = L$, the mussel experiences some probability of being eaten $\beta_L$; if it survives, then the zebra mussel has a decision option of filtration rate (CR), a range of CR (0 to 2.4 liters d$^{-1}$). Thus, the subscript for $F$ tells us that fitness is maximized on the decision variable CR within a range of decision options. The CR decision option chosen is the one that allows maximum fitness to be accrued, given costs and benefits of the decision (particles are cleared from water, calories are gained or lost through bioenergetic processes, and net gains are converted to somatic growth). Somatic growth is converted to length added.

$$F (L, t, T) = (1 - \beta_L) \times \max_{0 \leq CR \leq 2.4} F (L', t + 1, T; L'' t + 1, T) \quad (5)$$

If the mussel engages in aerobic respiration and filters, then length ($L'$) (equation 6) at time ($t$) is determined by starting size ($L$) plus the length added (fitness accrued) which is determined by caloric gains ($Y$) minus losses ($\alpha$) during time step ($t$) converted to length by converting calories of soft tissue growth to length (Figure 46).
Caloric gains are obtained from filtering rate (CR = liters mussel$^{-1}$ d$^{-1}$) multiplied by POM concentration (mg l$^{-1}$), and assimilation efficiency (AE, a proportion of POM that is ingested and turned into tissue), and E (the caloric conversion of mg POM assimilated). Losses include metabolic costs (MR, calories d$^{-1}$ consumed by respiration) and the proportion of calories lost from engaging in filtration, and the proportion of filtered particles lost to pseudofeces production (1 - pp). The factor 0.064 is the assumed proportion of assimilated ration lost to excretion for a standardized zebra mussel (Schneider et al. 1998). If the mussel engages in anaerobic respiration, then length (L") at time (t) is determined by caloric gains (Y$_{ar}$) minus caloric losses (α$_{ar}$). Under these conditions I assumed that no growth occurs, so Y$_{ar}$ is zero and L = L" (equation 7).

\[
\begin{align*}
L' &= (L + Y_t - \alpha_t) \text{ for } 0.1 \leq CR \leq 2.4; \\
L'' &= (L + Y_{ar} - \alpha_{ar}) \text{ for } CR = 0.
\end{align*}
\] (6) (7)

Where

\[
Y_t = \{(CR \times POM_t \times AE_t \times (1 - pp_t) \times E) \};
\]

\[
\alpha_t = (MR_t + [0.064 \times (CR \times POM_t \times AE_t \times (1 - pp_t) \times E)])
\]

Y$_{ar}$ = caloric gains from anaerobic respiration.

E = 0.00531 cal mg$^{-1}$ POM$_t$, is the number of calories for each mg of POM ingested (caloric value of POM from Schneider et al 1998).

α$_{ar}$ = caloric losses from anaerobic respiration.

β$_L$ = probability of being eaten at size L during time step t.
POM_t = seston concentrations (mg dw l^{-1}) at time (t) multiplied by 0.10 ratio (%
organic carbon) at time t.

AE_t = assimilation efficiency of mussel during time interval t.

pp_t = proportion of filtered material lost to pseudofeces during time interval t.

MR_t = metabolic rate during each period at time t.

The terminal fitness function is defined according to whether the mussel reaches a
critical size of 6 mm or 10 mm by equations (8) and (9) after cycling through the range of
filtering rates. In other words, the terminal fitness function is the value of length (L) at
terminal time step (T). The maximum fitness is obtained if the value of F (L,T,T) reaches
6 mm or 10 mm. I have not yet determined how to run the model using both terminal
fitness definitions at the same time. Caloric gains (C) are converted back to zebra mussel
length according to equation (10), the inverse of the equation obtained from the length to
dry weight regression of zebra mussels measured in the field (Babcock-Jackson
unpublished data). Dry mass was converted from the predicted tissue mass to calories
using Schneider's (1992) conversion 3.87 cal*mg dw^{-1} (Figure 46).

\[ F(L, T, T) = 0 \text{ for } l < 6.0 \text{ mm or 10 mm}; \] (8)

\[ F(L, T, T) = 1 \text{ for } l \geq 6.0 \text{ mm or 10.0 mm.} \] (9)
The sequence of zebra mussel activities used to drive the dynamic programming equation begins with zebra mussels at size “L” in each time step. First they either get eaten (probability of being eaten during time step \( t \) at size \( L \) is \( \beta_L \)) or survive (probability of surviving time step \( t \) at size \( L \), is \( 1 - \beta_L \)). Probabilities of predation for each size class are the summed probabilities of predation estimated from the information provided in literature sources listed in Table 12. Predation depends on size of the predator, density of the predator, and density and size distributions of mussels. Therefore, these probabilities are very rough estimates. After surviving, the mussel encounters conditions defined for each seasonal period (1 to 4, parameters defined in Table 11), “decide” the optimal clearance rate by cycling through the given range of CRs (0.1 to 2.4 liters mussel\(^{-1}\) d\(^{-1}\)), then either grow or not. The length of the zebra mussel at the end of the time interval (\( L' \)), is converted from calories gained or lost to mass and then length by equation (10) determines the probability of survival in the next time interval.
RESULTS

In either simulation, mussels adopt similar feeding strategies (patterns of CR) before period 4 if they start a time step at > than the critical length. Differences in feeding strategies arise in the simulations for mussels starting a time step at < 6.0 mm (critical length to survive winter) or 10 mm (critical length to reproduce) when there is a bloom in the 4th period. Without a Microcystis bloom in the 4th period, the smallest size classes (3 – 6 mm) are able to survive until terminal time with \( F(L,T,T) = 0.6 \), a relatively high terminal fitness level (dotted line in Figure 47). Mussels at sizes > 6 mm (or 10 mm if that is the critical size), have terminal fitness levels equal to mussels experiencing the bloom in the 4th period (dotted line follows the solid line in Figure 47). Survival depends on not being eaten and accruing enough fitness by ingesting and assimilating calories from POM. Therefore, if the mussel does not get eaten, reproduction and the ability to over-winter depend on the feeding strategy the mussel adopts.

The strongest effect of Microcystis is on survival of the smaller mussels where the 3 - 5 mm mussels do not survive the season at all (Figure 47). In both simulations, mussels starting out a time step at smaller lengths are in much greater danger from predation, they experience the highest probabilities of being eaten if they are between 3 – 10 mm, the highest at 8 - mm.
This, in addition to bioenergetic demands in each season, gives mussels starting a time step between 8 – 10 mm the lowest terminal fitness values (Figure 44). However, the small mussels (the surviving ones > 5 mm; or 9 mm depending on critical size) adopt different feeding strategies (optimal CRs for each time step) in response to the risk of predation and bioenergetic parameters determined by whether there is a bloom in the 4th period. If there is a bloom, then AE is lower, pp is higher and therefore energy gained from filtering POM is lower unless the mussel increases CR. Therefore, mussels beginning a time step at 7 – 8 mm must increase their CRs near terminal time in order to accrue the same fitness level as their counterparts in the simulation without a bloom (Figures 48 – 49). The 6 mm *Microcystis* mussels must increase their clearance rates to 2.0 liters d^{-1} and the 7 mm mussels to 0.5 liters d^{-1}, while the same size mussels without *Microcystis* gain enough energy at the minimum clearance rate of 0.1 liters d^{-1}. When terminal critical size was changed to 10.0 mm a pattern of optimal filtering strategies similar to the 6.0 mm terminal critical size was apparent for both simulations (Figures 50 and 51), except that fitness of mussels (Figure 52) less than 11.0 mm was strongly depressed in the *Microcystis* simulation, and mussels less than 9.0 mm at terminal time could not reproduce F(L,T,T = 0). Therefore, the presence of *Microcystis* strongly influenced the potential of small mussels to (L < 9 mm) to achieve reproductive size and over-wintering size (L ≤ 5 mm).

*Sensitivity Analysis*

Changes in model parameters strongly influence the outcome of the model. For example, in the simulation with a bloom deliberate changes made to the parameters of
assimilation efficiency and pseudofeces production in the last four weeks impacted the adopted feeding strategies of small mussels. Clearly, those changes affected the optimal filtering strategies of the mussels as compared to the no-Microcystis simulation.

I also tested the sensitivity of the model to metabolic rate (MR) and predation. Using a constant, low MR (0.00357 cal d⁻¹) across all seasonal periods, the outcomes were much the same as reported above. If each MR for a seasonal period was increased by a factor of 1000, then the mussels always chose the minimum CR = 0.1 liters d⁻¹, and mussels below 6.0 mm (or 10 mm if critical size was increased) did not survive (or reproduce, if critical size was 10 mm) in the Microcystis simulation. Fitness patterns and feeding strategies remained similar to the original model for larger mussels and for all mussels in the no-Microcystis simulation. Therefore, increasing the metabolic costs influences fitness and optimal filtering strategies during a bloom, but not without one.

Changing the probability of predation influenced the model more than changes to metabolic rate. If the probability of predation was reduced by 10 % (leaving critical size at 6 mm), then fitness of all mussels, in either simulation, increased by 7.5%. The smallest (< 5 mm) mussels still did not survive with a Microcystis bloom. If predation was removed from the model, then all mussels from either simulation survived with very high fitness values and adopted the minimum filtration strategy (CR of 0.1 liters d⁻¹).
The small degree of change in fitness resulting from a 10% reduction in probability of predation indicates that the estimated probabilities may not be too far from reality. However, because removing predation from the model also removes all impacts on fitness, further sensitivity analyses are needed on all of the parameters.
DISCUSSION

Under seasonal parameters similar to those used in my model, relative fitness of a new cohort of mussels, determined from the adopted feeding strategy, reveals that survival and reproduction are significantly impacted by the occurrence of a toxic *Microcystis aeruginosa* bloom. In addition, surviving mussels cannot maximize their lifetime fitness even using the highest clearance rates. A series of simulations of a typical summer growing season were run under two conditions (with and without a *Microcystis* bloom), in order to test the hypothesis that the relationship of zebra mussel growth to food quality and seston concentration is not related to the concentration of POM when toxic *Microcystis* is present. Changes in the parameters of POM organic content, assimilation efficiency and pseudofeces production were made during the last 4 weeks of the 12 week season to simulate zebra mussel behavior and environmental conditions under a *Microcystis* bloom, while seston concentrations were kept the same. Organic to inorganic carbon ratios, and thus POM concentrations, were changed in seasonal period 4 to reflect a different organic to inorganic ratio during a *Microcystis* bloom. Results suggest that the feeding strategy for a mussel in the size range for a new cohort (3 ≤ 6 ≤ 10 mm) was more likely to die or have lower terminal fitness value. CR, and hence fitness accrued, was influenced by the changes to assimilation efficiency and pseudofeces production meant to simulate bioenergetic effects of a *Microcystis* bloom.
Small mussels feeding during a bloom do not accrue enough fitness to over-winter or reproduce. Surviving (7 – 8 mm or 11 – 12 mm) mussels must increase their clearance rates in the last 4 weeks in order to obtain enough energy to grow to the critical size due to increased losses from pseudofeces production and decreased assimilation efficiency. Therefore, from a number of perspectives, the model predicts a reduction in the fitness (survival or reproductive potential) of small zebra mussels during a summer growth season ending with a toxic Microcystis bloom.

Experiments by Bayne et al. (1989), Reeders et al. (1989 and 1992), Schneider et al. (1998), and Madon et al. (1999) show exponential declines with increases of bivalve clearance, or filtration rate with seston concentration, and ingestion rate with POM concentrations. Mechanical interference with sorting of particles on the gills by high seston concentrations and a decreased need to filter high volumes at high POM concentrations are usually indicated as the reason for decline in filtration rates. Typically when seston levels are high it is because there is a large influx of inorganic material from some event, such as a storm, that washes material into the water and stirs up sediments. In this case, the ratio of organic to inorganic carbon remains low and thus, the response of the bivalve is to increase filtration rate and pseudofeces production. However, if the ratio of organic to inorganic carbon remains high, indicating higher food quality (Bayne et al. 1989; Schneider et al. 1998; Madon et al. 1999) the predicted response of the bivalve would be to decrease filtration rate. In my model simulations, low organic to inorganic ratio with a high seston concentration was assigned to time period 4 for one model while
the other had a higher organic content with a constant seston concentration, and the responses of the mussels differed. Experiments by Culver et al (1999) showed that zebra mussels have lower assimilation efficiencies and higher pseudofeces production rates when toxic Microcystis is present. The typical response of decreasing filtration if the organic to inorganic ratio is high was not followed even when highly palatable green algae were added to a mixture of toxic Microcystis. The same was true for small mussels. Mussels that accrued enough fitness to over-winter or reproduce had to increase their filtration rates in order to make up for energetic losses induced by lower assimilation efficiency and higher pseudofeces production. However, larger mussels remained largely unaffected. The size range of zebra mussels impacted by Microcystis is strongly influenced by the chosen terminal critical size.

The model predicts that in nature one might find that size class dominance would shift to the larger mussels (> 11 mm) in a year following a bloom. If the bloom is an event which only happens occasionally, which is the case for western Lake Erie, this would only be a temporary set-back since the next cohort spawned by the surviving larger mussels could make up for the losses in the previous year. However, if the bloom conditions were a regular occurrence or persisted for longer periods, which is the case for many water bodies in the world, then the population of zebra mussels might be permanently shifted toward larger mussels and would soon decline if new juveniles were consistently unable to survive to replace dying adults, or grow large enough to spawn. In addition, a population shifted to larger ranges might have significantly different impacts.
on both total consumption of phytoplankton and production of pseudofeces than more variable or smaller size-frequency populations. Young et al. (1996) modeled different size-frequency relationships of zebra mussels and their variable impacts on consumption and pseudofeces production using Schneider’s (1992) bioenergetic parameters. Their models predicted that a population skewed toward larger size ranges (distribution 5 in their results) would have the maximum impact on consumption and pseudofeces production compared with the different size–frequency relationships they modeled. Therefore, further indirect effects on the ecosystem are possible. Annual field surveys of zebra mussel length to weight distributions in lakes where blue-green algae blooms occur occasionally or regularly compared to those that do not could be used to test this prediction.

Another factor strongly influencing the potential dynamics of the population arose from the sensitivity analyses of the model. Predation was the main driving force, which in combination with the losses in energy from reduced assimilation efficiency and increased pseudofeces production, forced the mussels to increase filtration rates in the presence of *Microcystis* and resulted in lower terminal fitness for the 7–8 mm or 11–12 mm mussels. Thus, under strong predation pressure the optimal strategies of filtration were significantly influenced by changes in energetic parameters induced by a blue-green algae bloom. These model simulations suggest that any environmental contaminant having the potential to influence assimilation efficiency and pseudofeces production in
zebra mussels has the potential to influence size and frequency dynamics, and ultimately survival, of zebra mussel populations.
LITERATURE CITED


Table 1. Summary of historical trends in cyanophyte abundance, cyanophyte composition, nitrogen, and phosphorus in western Lake Erie.

\(^a\) 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.

\(^d\) Cyanophytes are % biovolume. *Anacystis* is considered *Microcystis* by Taft and Taft (1971).

\(^e\) Cyanophyte data from samples collected in Hatchery Bay or from Put-in-Bay marker buoy, both at South Bass Island, western Lake Erie.

\(^f\) Cyanophyte data from samples collected for 44 stations in the western basin collected by ODNR personnel.

N.A. = not available.
<table>
<thead>
<tr>
<th>Year</th>
<th>General phosphorus ranges (TP mg/l)</th>
<th>General nitrogen ranges (TN and NH$_3$ mg/l)</th>
<th>Cyanophytes as % of total phytoplankton or biomass maxima of species as available</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 1950</td>
<td>0.105 in Maumee Bay</td>
<td>1.4 TN in Maumee Bay</td>
<td>No cyanophytes are listed as dominant in the phytoplankton before 1958.</td>
<td>Verduin, 1964; Davis, 1964.</td>
</tr>
<tr>
<td>1957-58</td>
<td>0.45 in Maumee Bay</td>
<td>2.2 TN in Maumee Bay</td>
<td>Microcystis, Anabaena and Aphanizomenon listed as major components of PP community in July and August 1957-58 (1-10 µl/l)</td>
<td>Verduin, 1964</td>
</tr>
<tr>
<td>1970</td>
<td>0.024-0.05</td>
<td>0.14-0.518 TN 0.042-.07 NH$_3$</td>
<td>5-35%, max in September. * A. flos aquae max Aug- Oct. Microcystis &lt; 5% all samples</td>
<td>Burns, 1976; Munawar and Munawar, 1976; DePinto et al, 1986</td>
</tr>
<tr>
<td>1984</td>
<td>.028 annual mean</td>
<td>N.A.</td>
<td>50% max in July* dominated by Anacystis, and also present are Anabaena spp., A. flos aquae, Merismopedia, and Oscillatoria spp.</td>
<td>Makarewicz, 1987 EPA report.</td>
</tr>
<tr>
<td>1987</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Aphanizomenon, Anabaena, and Oscillatoria dominate; Microcystis, and Anacystis present.</td>
<td>Makarewicz, 1993.</td>
</tr>
</tbody>
</table>

Table 1 continued
<table>
<thead>
<tr>
<th>Year</th>
<th>General phosphorus ranges (TP mg/l)</th>
<th>General nitrogen ranges (TN and NH₃ mg/l)</th>
<th>Cyanophytes as % of total phytoplankton or biomass maxima of species as available</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>0.02 - 0.06</td>
<td>0.5 - 1.4 TN 0.0 - 0.043 NH₃</td>
<td>57.3% max cyanophytes biomass in Aug* Average 100 µg/l Cyanophyta biomass.</td>
<td>Holland et al., 1996; Pontius and Culver, unpublished. Makarewicz et al. 1999.</td>
</tr>
<tr>
<td>1991</td>
<td>0.01 - 0.04</td>
<td>0.4 - 1.2 TN 0.02 - 0.065 NH₃</td>
<td>0.3% max cyanophyte biomass in Aug* Average 20 µg/l Cyanophyta biomass.</td>
<td>Holland et al., 1995; Pontius and Culver, unpublished.</td>
</tr>
<tr>
<td>1995</td>
<td>0.02 - 0.06</td>
<td>0.12 - 0.38 TN 0.018 - 0.054 NH₃</td>
<td>Average 1964 (sd 2774) µg/l Cyanophyta biomass. Dominated by <em>M. aeruginosa</em>.</td>
<td>Holland et al., 1995; Babcock-Jackson and Culver.</td>
</tr>
<tr>
<td>1996</td>
<td>0.02 - 0.23</td>
<td>0.2 - 6.2 TN</td>
<td>Average 71.4 (sd 408) µg/l Cyanophyta biomass. <em>Aphanizomenon, Anabaena</em> and <em>M. aeruginosa</em> dominates.</td>
<td>Babcock-Jackson and Culver.</td>
</tr>
<tr>
<td>1997</td>
<td>0.002-0.03</td>
<td>0.2 - 0.7 TN</td>
<td>Average 15.3 (sd 47) µg/l Cyanophyta biomass. <em>Aphanizomenon, Anabaena</em> and <em>M. aeruginosa</em> dominates.</td>
<td>Babcock-Jackson and Culver.</td>
</tr>
<tr>
<td>1998</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Average 1088 (sd 1600) µg/l cyanophyta biomass, dominated by <em>M. aeruginosa</em>.</td>
<td>Babcock-Jackson and Culver.</td>
</tr>
</tbody>
</table>
### Table 2. Results of spatial analysis using least squares regression of the dependent variables against location groupings for four regions (SR, IR, OS and MR) in western Lake Erie for August, 1996.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>$R^2$</th>
<th>Coefficient of Variation</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanophyte biomass</td>
<td>0.052</td>
<td>377.2</td>
<td>0.5160</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.0086</td>
<td>13.57</td>
<td>0.9466</td>
</tr>
<tr>
<td>NO$_3$ + NO$_2$</td>
<td>0.311</td>
<td>42.59</td>
<td>0.0012*</td>
</tr>
<tr>
<td>NH$_4$</td>
<td>0.001</td>
<td>90.6</td>
<td>0.0426*</td>
</tr>
<tr>
<td>TP</td>
<td>0.0386</td>
<td>57.7</td>
<td>0.6427</td>
</tr>
<tr>
<td>SRP</td>
<td>0.0585</td>
<td>124</td>
<td>0.4541</td>
</tr>
<tr>
<td>TIN:TP</td>
<td>0.0569</td>
<td>115.2</td>
<td>0.4768</td>
</tr>
</tbody>
</table>

### Table 3. Results of spatial analysis using least squares regression of the dependent variables against location groupings for four regions (SR, IR, OS and MR) in western Lake Erie for September, 1996.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>$R^2$</th>
<th>Coefficient of Variation</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanophyte biomass</td>
<td>0.05</td>
<td>175.1</td>
<td>0.6016</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.048</td>
<td>12.2</td>
<td>0.6483</td>
</tr>
<tr>
<td>NO$_3$ + NO$_2$</td>
<td>0.5123</td>
<td>29.8</td>
<td>0.001*</td>
</tr>
<tr>
<td>NH$_4$</td>
<td>0.1035</td>
<td>47.4</td>
<td>0.3005</td>
</tr>
<tr>
<td>TP</td>
<td>0.359</td>
<td>35</td>
<td>0.0019*</td>
</tr>
<tr>
<td>SRP</td>
<td>0.144</td>
<td>67.8</td>
<td>0.1579</td>
</tr>
<tr>
<td>TIN:TP</td>
<td>0.2409</td>
<td>43.57</td>
<td>0.0264*</td>
</tr>
</tbody>
</table>

Table 2. Results of spatial analysis using least squares regression of the dependent variables against location groupings for four regions (SR, IR, OS and MR) in western Lake Erie for August, 1996.

Table 3. Results of spatial analysis using least squares regression of the dependent variables against location groupings for four regions (SR, IR, OS and MR) in western Lake Erie for September, 1996.
<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Sandusky River (SR)</th>
<th>Island and reef (IR)</th>
<th>Offshore (OS)</th>
<th>Maumee River (MR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanophyte biomass (µg l⁻¹)</td>
<td>75.6 ± 465</td>
<td>471.6 ± 422.1</td>
<td>208.9 ± 710.5</td>
<td>20.7 ± 580</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>24.2 ± 0.4</td>
<td>24.2 ± 0.4</td>
<td>24.0 ± 0.7</td>
<td>24.1 ± 0.6</td>
</tr>
<tr>
<td>NO₃ + NO₂ (mg l⁻¹)</td>
<td>0.41 ± 0.13</td>
<td>0.745 ± 0.12</td>
<td>0.373 ± 0.2</td>
<td>0.620 ± 0.164</td>
</tr>
<tr>
<td>NH₄ (mg l⁻¹)</td>
<td>0.044 ± 0.021</td>
<td>0.041 ± 0.019</td>
<td>0.042 ± 0.032</td>
<td>0.044 ± 0.026</td>
</tr>
<tr>
<td>TP (mg l⁻¹)</td>
<td>0.025 ± 0.008</td>
<td>0.026 ± 0.007</td>
<td>0.018 ± 0.012</td>
<td>0.029 ± 0.016</td>
</tr>
<tr>
<td>SRP (mg l⁻¹)</td>
<td>0.007 ± 0.004</td>
<td>0.004 ± 0.003</td>
<td>0.007 ± 0.005</td>
<td>0.004 ± 0.003</td>
</tr>
<tr>
<td>TIN:TP</td>
<td>37 ± 32</td>
<td>57 ± 29</td>
<td>39 ± 49</td>
<td>73 ± 41</td>
</tr>
</tbody>
</table>

Table 4. Test for spatial differences for cyanophyte biomass and 5 nutrient variables among four regions in western Lake Erie in August, 1996. Least squares means are significantly different from named regions at α = 0.05 (*) if the 95% confidence intervals (±) did not overlap for regions MR, IR, OS or MR. The island and reef region had the highest average cyanophyte biomass, but was not significantly different from other regions due to large variability.
<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Sandusky River (SR)</th>
<th>Island and reef (IR)</th>
<th>Offshore (OS)</th>
<th>Maumee River (MR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanophyte biomass ($\mu g \text{ l}^{-1}$)</td>
<td>$68.6 \pm 60.8$</td>
<td>$24.2 \pm 45.9$</td>
<td>$58.8 \pm 57.2$</td>
<td>$61.3 \pm 70.1$</td>
</tr>
<tr>
<td>Temperature ($^\circ\text{C}$)</td>
<td>$22.6 \pm 2$</td>
<td>$21.8 \pm 1.5$</td>
<td>$21.1 \pm 1.8$</td>
<td>$22.4 \pm 2.2$</td>
</tr>
<tr>
<td>$\text{NO}_3 + \text{NO}_2$ (mg l$^{-1}$)</td>
<td>$0.31 \pm 0.08$</td>
<td>$0.38 \pm 0.06$</td>
<td>$0.26 \pm 0.07$</td>
<td>$0.58 \pm 0.09$ <em>(SR, IR, OS)</em></td>
</tr>
<tr>
<td>$\text{NH}_4$ (mg l$^{-1}$)</td>
<td>$0.06 \pm 0.02$</td>
<td>$0.05 \pm 0.02$</td>
<td>$0.04 \pm 0.02$</td>
<td>$0.07 \pm 0.02$</td>
</tr>
<tr>
<td>TP (mg l$^{-1}$)</td>
<td>$0.044 \pm 0.006$ <em>(OS)</em></td>
<td>$0.017 \pm 0.023$</td>
<td>$0.021 \pm 0.008$ <em>(MR)</em></td>
<td>$0.038 \pm 0.008$</td>
</tr>
<tr>
<td>SRP (mg l$^{-1}$)</td>
<td>$0.011 \pm 0.001$</td>
<td>$0.011 \pm 0.001$</td>
<td>$0.005 \pm 0.005$</td>
<td>$0.012 \pm 0.005$</td>
</tr>
<tr>
<td>TIN:TP</td>
<td>$9 \pm 5$ <em>(MR)</em></td>
<td>$15 \pm 4$</td>
<td>$16 \pm 4$</td>
<td>$20 \pm 5$</td>
</tr>
</tbody>
</table>

Table 5. Test for spatial differences for cyanophyte biomass and 5 nutrient variables among four regions in western Lake Erie in September, 1996. Least squares means are significantly different from named regions at $\alpha = 0.05$ (*) if the 95% confidence intervals ($\pm$) did not overlap for regions (MR, IR, OS or MR). There was no significant difference in cyanophyte biomass between regions.
<table>
<thead>
<tr>
<th>Experiment Type</th>
<th>Cyanobacteria Source(s)</th>
<th>Measured microcystin concentrations (ng l⁻¹)</th>
<th>Green Algae added to any treatments?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biodeposition and zebra mussel toxin accumulation</td>
<td><em>M. aeruginosa</em> UV-027 (lab cultured strain)</td>
<td>UV-027: 200, 2000, 6900, 13,000</td>
<td>A filtered Lake water treatment had concentrated culture of <em>Chlamydomonas</em> + <em>Chlorella</em> added (1.25 mg cell dw l⁻¹).</td>
</tr>
<tr>
<td></td>
<td>Lake-collected cyanophytes.</td>
<td>Lake collected cyanophytes: 80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Filtered Lake water: 100</td>
<td></td>
</tr>
<tr>
<td>Biodeposition of PF for amphipods, and toxin accumulation in mussels.</td>
<td><em>M. aeruginosa</em> UV-027</td>
<td>UV-027: 200, 1500</td>
<td><em>Chlamydomonas</em> + <em>Chlorella</em> added (1.25 mg cell dw l⁻¹) added to UV-027 treatment 200, and to Lake collected cyanophytes 150.</td>
</tr>
<tr>
<td></td>
<td>Lake Collected cyanophytes.</td>
<td>Lake collected cyanophytes: 20, 60, 150</td>
<td>A Lake Erie surface water treatment contained green algae.</td>
</tr>
</tbody>
</table>

Table 6. Comparison of cyanobacteria sources and treatments with green algae used in lake-side biodeposition experiments to determine microcystin accumulation in zebra mussels, rates of pseudofeces and microcystin deposition, and microcystin accumulation in amphipods from environmental exposure or from eating contaminated pseudofeces.

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<table>
<thead>
<tr>
<th>Species</th>
<th>Month</th>
<th>n =</th>
<th>Length Range (mm)</th>
<th>Gut toxin* (ng g⁻¹)</th>
<th>Liver toxin* (ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zebra mussels</td>
<td>AUG</td>
<td>70</td>
<td>6 - 18</td>
<td>21.3 (3.4)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Quagga mussels</td>
<td>AUG</td>
<td>70</td>
<td>7 - 25</td>
<td>18.4 (5.1)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Amphipods</td>
<td>AUG</td>
<td>60</td>
<td>Adults</td>
<td>200 (34)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Gizzard shad</td>
<td>AUG</td>
<td>15</td>
<td>70 - 97</td>
<td>1191 (229)</td>
<td>297 (80)</td>
</tr>
<tr>
<td>YOY Yellow perch</td>
<td>AUG</td>
<td>15</td>
<td>62 - 76</td>
<td>94.2 (31)</td>
<td>94.2 (50.0)</td>
</tr>
<tr>
<td></td>
<td>OCT</td>
<td>10</td>
<td>81 - 90</td>
<td>191 (210)</td>
<td>230 (77.0)</td>
</tr>
<tr>
<td>Walleye</td>
<td>AUG</td>
<td>3</td>
<td>95 - 120</td>
<td>50 (24)</td>
<td>0.0</td>
</tr>
<tr>
<td>Morone spp.</td>
<td>AUG</td>
<td>15</td>
<td>50 - 84</td>
<td>62 (31)</td>
<td>62.2 (57.0)</td>
</tr>
<tr>
<td></td>
<td>OCT</td>
<td>10</td>
<td>86 - 107</td>
<td>100 (30)</td>
<td>233 (102)</td>
</tr>
<tr>
<td>Creek chub</td>
<td>AUG</td>
<td>4</td>
<td>122 - 170</td>
<td>60 (34)</td>
<td>93.5 (65.0)</td>
</tr>
<tr>
<td>Yellow perch</td>
<td>AUG</td>
<td>7</td>
<td>118 - 180</td>
<td>46 (59)</td>
<td>127.1 (119)</td>
</tr>
<tr>
<td>Round Goby</td>
<td>OCT</td>
<td>10</td>
<td>72 - 150</td>
<td>48 (20)</td>
<td>187 (98)</td>
</tr>
</tbody>
</table>

Table 7. Microcystin levels in organs of mussels and fish collected from western Lake Erie, in August and October, 1998. Mussels were collected by SCUBA divers from rocks on Peach Point, South Bass Island. Fish were collected by otter trawl from Schoolhouse Bay near Middle Bass Island. Microcystin toxin* levels are given as mean and (standard deviations) ng g⁻¹ ww. Organisms were analyzed for microcystin using ELISA in 7 groups of 10⁶, 5 groups of 3⁵, 5 groups of 2², 3 groups of 2⁰, or individually. *Indicates organisms were analyzed whole.
Table 8. Rates of sediment and microcystin deposition in sedimentation traps incubated at 5.0 m depth near Gibraltar Island, Hatchery Bay, western Lake Erie in 1996.

CB = cyanophyte biomass (µg l⁻¹ ww); S = seston (mg l⁻¹ dw); M = microcystin (ng l⁻¹) in 3rd column; NM = no mussel treatment; M = mussel treatment in 6th column.

n.d. = none detected.
Table 9. A comparison of ranges of zebra mussel PF and microcystin areal deposition rates measured in microcosms (M) or in the field (F).

<table>
<thead>
<tr>
<th>Microcystin Source</th>
<th>PF deposition (µg PF dw m⁻² d⁻¹)</th>
<th>Microcystin in PF (µg microcystin m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. aeruginosa UV-027</td>
<td>1.8 - 530</td>
<td>0.89 - 16.8</td>
</tr>
<tr>
<td>Lake Erie cyanophytes</td>
<td>10.6 - 42.5</td>
<td>0.07 - 0.531</td>
</tr>
<tr>
<td>Lake Erie cyanophytes</td>
<td>38,000 - 76,300</td>
<td>0 - 0.771</td>
</tr>
</tbody>
</table>

Table 10. A comparison of ranges of zebra mussel PF and microcystin deposition rates per mussel measured in microcosms (M) or in the field (F).

<table>
<thead>
<tr>
<th>Microcystin Source</th>
<th>PF deposition (µg PF dw mussel⁻¹ d⁻¹)</th>
<th>Microcystin in PF (ng microcystin mussel⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. aeruginosa UV-027</td>
<td>0.005 - 0.15</td>
<td>0.3 - 5.0</td>
</tr>
<tr>
<td>Lake Erie cyanophytes</td>
<td>0.003 - 0.012</td>
<td>0.02 - 0.2</td>
</tr>
<tr>
<td>Lake Erie cyanophytes</td>
<td>2.15 - 4.3</td>
<td>0 - 0.04</td>
</tr>
<tr>
<td>Model Parameters</td>
<td>Seasonal period 1</td>
<td>Seasonal period 2</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td><strong>Time</strong> (t = weeks)</td>
<td>1 - 3</td>
<td>4 - 6</td>
</tr>
<tr>
<td><strong>Temperature (°C)</strong></td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td><strong>Seston concentration</strong> (mg dw liter⁻¹)</td>
<td>0.1</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Organic: Inorganic Carbon ratio</strong></td>
<td>90:10</td>
<td>70:30</td>
</tr>
<tr>
<td><strong>α or POM</strong> (mg liter⁻¹)</td>
<td>0.9</td>
<td>7.0</td>
</tr>
<tr>
<td><strong>Assimilation efficiency (AE)</strong></td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Metabolic rate (MR)</strong> (calories d⁻¹)</td>
<td>0.00047</td>
<td>0.00117</td>
</tr>
<tr>
<td><strong>Proportion filtered material lost to pseudofeces (pp)</strong></td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 11. Model parameter values assigned during 12-weeks of zebra mussel growth divided into four summer periods, beginning with the second week of June (week 1) and progressing through the 4th week of August (week 12). During periods three and four, MC = a *Microcystis aeruginosa* bloom occurred; No MC = no *M. aeruginosa* bloom occurred. During period 3 the bloom would have just gotten started if it occurred, therefore its impact on pp is lower than in period 4.
<table>
<thead>
<tr>
<th>Zebra mussel length range (mm)</th>
<th>Probability of being eaten at size L ($\beta_L$)</th>
<th>Likely predator(s) eating this size range</th>
<th>Source of information</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 – 5</td>
<td>0.39</td>
<td>Crayfish;</td>
<td>MacIsaac 1994; Martin and Corkum 1994;</td>
</tr>
<tr>
<td>6 – 7</td>
<td>0.43</td>
<td>Yellow perch and Freshwater Drum Round gobies.</td>
<td>Morrison et al. 1997.</td>
</tr>
<tr>
<td>8 – 10</td>
<td>0.86</td>
<td>Crayfish;</td>
<td>MacIsaac 1994; Martin and Corkum 1994;</td>
</tr>
<tr>
<td>11 – 14</td>
<td>0.27</td>
<td>Crayfish;</td>
<td>MacIsaac 1994; Hamilton et al. 1994.</td>
</tr>
<tr>
<td>15 – 30</td>
<td>0.09</td>
<td>Diving ducks.</td>
<td>Hamilton et al. 1994.</td>
</tr>
</tbody>
</table>

Table 12. Estimated probabilities of zebra mussel predation by predators on different size classes of mussels used in the model.
Figure 1. Map of western Lake Erie indicating sites where phytoplankton samples were taken by several researchers, from 1970 to 1993. VK = sites sampled by D. Van Keuren for the Center for Lake Erie Area Research in 1979; MM = Munawar and Munawar (1976) sites sampled in 1970; MK = Makarewicz et al. (1999) sites sampled from 1983 to 1993; and WC = Wu and Culver (1991) sites sampled in 1988 and 1989.
Figure 2. Sampling sites used to collect data on phytoplankton and/or environmental factors in western Lake Erie, summer, 1996. ODNR = samples collected by the Ohio Department of Natural Resources; CCIW = samples collected by the Canadian Center for Inland Water; and Channel marker site was sampled by L. Babcock-Jackson (1995 – 1998) and R. Pontius (1991 –1992).
Figure 3. Sampling sites used to collect data on phytoplankton and/or environmental factors in western Lake Erie, summer, 1997. ODNR = samples collected by the Ohio Department of Natural Resources; CCIW = samples collected by the Canadian Center for Inland Water; and Channel marker site was sampled by L. Babcock-Jackson (1995 – 1998) and R. Pontius (1991 –1992).
Figure 4. Site numbers of sites sampled in different regions of western Lake Erie. Four regions were designated for spatial comparisons of environmental factors. MR = Maumee River influenced sites (36 – 44); SR = Sandusky River influenced sites (1 - 8; 41); IR = sites near islands and reefs (9 – 24); and OS = offshore sites with > 5m depth and not near islands, reefs or rivers, (25 – 35).
Figure 5. Occurrence of cyanophyte blooms (> 200 µg l⁻¹) for western Lake Erie before and after introduction of *Dreissena* species (circa 1988). MM = 1970 data from Munawar and Munawar 1976; VK = 1979 data from Van Keuren; MK = 1983-87 data from Makarewicz et al. 1999; WC = 1988-89 data from Wu and Culver; PC = 1991-92 data from Pontius and Culver; BJ = 1995-98 data from Babcock-Jackson and Culver. No data were available for the remaining years. Dashed line indicates threshold for a "bloom".
Figure 6. Ratios of cyanophyte species present in August, 1995 - 1998 in western Lake Erie. Narrow filaments are unidentified filaments.
Figure 7. Ratios of cyanophyte species present in September, 1995 - 1998 in western Lake Erie. Narrow filaments are unidentified filaments.
Figure 8. Average summer (Aug. - Sept.) temperatures and N:P in western Lake Erie. Data for 1968 from Hartley and Potos, (1971); data for 1991-92 and 1995 from Holland et al. (1995); data for 1996-98 from Babcock-Jackson and Culver. No data were available for the remaining years.
Figure 9. Cyanophyta biomass in western Lake Erie 1996.
Figure 10. Surface water temperature at individual stations in western Lake Erie, 1996.
Figure 11. Cyanophyta Biomass in relation to surface water temperatures at individual stations in western Lake Erie, 1996.
Figure 12. Relationship of cyanophyte abundance to TIN:TP at individual stations in western Lake Erie for June, 1996.
Figure 13. Relationship of cyanophyte abundance to TIN:TP at individual stations in western Lake Erie for July, 1996.
Figure 14. Relationship of cyanophyte abundance to TIN:TP at individual stations in western Lake Erie for August, 1996.
Figure 15. Relationship of cyanophyte abundance to TIN:TP at individual stations in western Lake Erie for September, 1996.
Figure 16. Maumee River monthly discharge to western Lake Erie 1985 - 1999.
Figure 17. Maumee River N monthly loading to western Lake Erie 1985 - 1999.
Figure 18. Maumee River P monthly loading to western Lake Erie 1985 - 1999.
Figure 19. Maumee River N:P of discharge to western Lake Erie 1985 to 1999.
Figure 20. N:P in the Maumee River discharge to western Lake Erie in 1988.
Figure 21. N:P in the Maumee River discharge to western Lake Erie in 1995.
Figure 22. N:P in the Maumee River discharge to western Lake Erie in 1998.
Figure 23. N:P in the Maumee River discharge to western Lake Erie in 1992.
Figure 24. N:P in the Maumee River discharge to western Lake Erie in 1996.
Figure 25. N:P in the Maumee River discharge to Lake Erie in 1997.
MCM = Microcystin Concentration measurement or
MRM = Microcystin rate of transfer measurement.

Figure 26. Compartmental diagram indicating trophic components in which microcystin concentrations and transfer rates were measured under Objectives 1 – 4. Filled black arrows and bold compartment labels indicate novel microcystin compartments and transfer pathways created by new non-indigenous species in Lake Erie.
Figure 27. Locations of South Bass Island, Put-in-Bay channel marker buoy (*) where plankton samples were collected; and detail of location of Hatchery Bay and Gibraltar Island sedimentation traps (•).
Figure 28. Detail of experimental microcosm set-up, shows 1-liter Imhoff cone used to expose replicate zebra mussels to toxic Microcystis treatments, and to collect feces/pseudofeces (PF) produced by mussels during exposures. Replicate samples of PF were collected for microcystin analysis and for feeding to amphipods.
Figure 29. Phytoplankton collected June 27 (Julian day 179) to September 18 (Julian day 262), 1998, from Put-in-Bay channel marker buoy between Middle and South Bass Islands, western Lake Erie. Density of cyanophytes peaked in early September.
Figure 30. Concentrations of microcystin from net plankton samples collected in 1998 from Put-in-Bay channel marker buoy. The peak in microcystin concentration coincides with peak cyanophyte density in early September.
Figure 31. Phytoplankton collected June 27 (Julian day 179) to September 18 (Julian day 262), 1998 from Put-in-Bay channel marker buoy. Peaks in cyanophyte biomass occur in mid-August and early September.
Figure 32. Rates of PF deposition by zebra mussels exposed to microcystin in freeze-dried *M. aeruginosa* UV-027 (200, 2000, 6900, and 13,000 ng l\(^{-1}\)), or Lake Erie surface water (100 ng l\(^{-1}\)), or filtered Lake Erie water with green algae added (80 ng l\(^{-1}\)).
Figure 33. Rates of microcystin deposition by zebra mussels exposed to microcystin in freeze-dried *M. aeruginosa* UV-027 (200, 2000, 6900, and 13,000 ng l⁻¹), or Lake Erie surface water (100 ng l⁻¹), or filtered Lake Erie water with green algae added (80 ng l⁻¹).
Figure 34. Rates of PF deposition by zebra mussels exposed to microcystin in Lake Erie cyanobacteria in surface water (20 ng l\(^{-1}\)), concentrated (60 ng l\(^{-1}\)), in filtered lake water (40 ng l\(^{-1}\)) with green algae, or concentrated with green algae added (150 ng l\(^{-1}\)).
Figure 35. Rate of microcystin deposition by zebra mussels exposed to microcystin in Lake Erie cyanobacteria in surface water (20 ng l\(^{-1}\)), concentrated (60 ng l\(^{-1}\)), in filtered lake water with green algae added (40 ng l\(^{-1}\)), or concentrated with green algae added (150 ng l\(^{-1}\)).
Figure 36. Concentrations of microcystin in zebra mussels exposed to microcystin in freeze-dried *M. aeruginosa* UV-027 (200, 2000, 6900, and 13,000 ng l\(^{-1}\)), or Lake Erie surface water (100 ng l\(^{-1}\)), or filtered Lake Erie water with green algae added (80 ng l\(^{-1}\)). Mussels were exposed for 96 h at 24\(^\circ\)C. Data for 24 h intervals were pooled because length of exposure had no significant effect on toxin accumulation in the mussels after 24 h.
Figure 37. Concentrations of microcystin in zebra mussels exposed to microcystin in Lake Erie cyanobacteria in surface whole water (20 ng microcystin l⁻¹), in concentrated cyanobacteria (60 ng microcystin l⁻¹), in filtered lake water with green algae added (40 ng microcystin l⁻¹), or concentrated cyanobacteria with green algae added (150 ng microcystin l⁻¹). Mussels were exposed for 72 h (24°C). Data for 24 h intervals were pooled.
Figure 38. Variation in microcystin content in zebra mussels after 48 h as a function of microcystin concentration in *M. aeruginosa* strain LE-3.
Figure 39. Variation in concentration of microcystin in individual amphipods after 48 h exposure, as a function of microcystin concentrations in live *M. aeruginosa* strain LE-3.
Figure 40. Uptake-clearance kinetics of microcystin in zebra mussels (ZM), determined from 1st order kinetics equation $c_{zm} = c_w (K_u/K_e)^{K_e t}$. Mussels (15 to 20 mm) were exposed for 72 h to toxic *M. aeruginosa* (LE-3) containing primarily microcystin-LR in filtered Lake Erie water (24° C), resuspended to contain 20 μg. microcystin l⁻¹.
Figure 41. Microcystin concentration in amphipods after 72 h exposure to different sources of microcystin from PF or cyanobacteria in water. Treatments 1 - 5 were from Lake Erie sources, treatment 6 was from *M. aeruginosa* UV-027. 0 = initial levels. Water treatment [microcystin]: 1 = 20 ng l⁻¹; 2 = 40 ng l⁻¹; 3 = 60 ng l⁻¹; 4 = 150 ng l⁻¹; 5 = 200 ng l⁻¹; 6 = 1500 ng l⁻¹. PF microcystin content (sd): 1 = 3 ng; 2 = 0 ng; 3 = 17.6 (2.40 ng); 4 = 2.85 (0.2) ng; 5 = 6.6 (2.7) ng; and 6 = 35.7 (3.4) ng.
Figure 42. Microcystin uptake rates in guts and livers of round gobies. Gobies were exposed for 72 h to toxic *M. aeruginosa* (LE-3), re-suspended to 20 μg microcystin l⁻¹ (*C_w*) in filtered Lake Erie water in August, 1998.
Figure 43. Concentrations of microcystin found in three types of tissue (liver, gut and muscle) removed from gobies that were fed the indicated amount of microcystin in zebra mussels over seven days in August, 1999.
Figure 44. Microcystin concentration in three tissue types (liver, gut and muscle) after round gobies ate zebra mussels contaminated with microcystin (*M. aeruginosa* LE-3 source) for seven days, in August 1999. Levels of microcystin eaten reflect number of mussels eaten over the time period of seven days.
Figure 45. Average total microcystin (from gut + liver) in gobies eating 10 zebra mussels d⁻¹ for 7 days. Mussels were exposed to 0, 1 or 10 µg l⁻¹ concentrations of microcystin in *M. aeruginosa* for 24 h prior to feeding them to gobies. Gobies averaged 124.8 (14.3) mm in length and 29.3 (11.8) g ww.
Figure 46. Caloric conversion of zebra mussel lengths (x) to calories (y) using the length to dry weight conversion $Y = 0.005(x)^{2.703}$ calculated from zebra mussels grown over a summer season in western Lake Erie (Babcock-Jackson, unpublished data), multiplied by a zebra mussel mass caloric conversion of 3.87 cal mg dw$^{-1}$ (Schneider, 1992).
Figure 47. A comparison of relative terminal fitness \( F(L, T, T) \) of zebra mussels at the end of simulated summer growing seasons with and without a toxic \textit{Microcystis} bloom in the last four weeks. The fitness values are determined for mussels at different starting sizes. A value of 1.0 is maximum fitness, a value of 0 means the mussel died. Values in between are less than maximum. Dotted line follows the solid line after 6 mm.
Figure 48. Optimal clearance rates for juvenile zebra mussels, *Dreissena polymorpha*, growing for a simulated summer season without a *Microcystis* bloom in the final 4 weeks.
Figure 49. Optimal clearance rate strategies for juvenile zebra mussels, *Dreissena polymorpha*, growing for a simulated summer season with a *Microcystis* bloom in the final 4 weeks.
Figure 50. Optimal clearance rates for zebra mussels, *Dreissena polymorpha*, growing for a simulated summer season without a *Microcystis* bloom in the final 4 weeks. Critical size for reproduction is 10 mm.
Figure 51. Optimal clearance rates for zebra mussels, *Dreissena polymorpha*, growing for a simulated summer season with a *Microcystis* bloom in the final 4 weeks. Critical size for reproduction is 10mm.
Figure 52. A comparison of relative terminal fitness [$F(L,T,T)$] of mussels at the end of simulated summer growing seasons ending with or without a toxic *Microcystis aeruginosa* bloom in the last four weeks. A value of 1.0 is maximum fitness, a value of 0 means the mussel died. Values in between are less than maximum. Dotted line follows the solid line after 6 mm.