HOW THE FEEDING ECOLOGY OF NATIVE AND EXOTIC MUSSELS AFFECTS FRESHWATER ECOSYSTEMS

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ABSTRACT

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As zebra mussels expand their range in North America new ecosystems become invaded. Beginning with the Great Lakes and continuing through the Mississippi River drainage network, the zebra mussel invasion is currently spreading among small inland lakes. Due to the swiftness and magnitude of this invasion and the effects zebra mussels have had on previously invaded ecosystems, it is imperative that the effects of zebra mussels on inland lakes be investigated. In addition, the zebra mussel invasion represents the functional replacement of the multispecies assemblage of native bivalves which typically disappears after invasion. This dissertation examined the ecology of native unionid bivalves (Unionoidae) and exotic zebra mussels (Dreissena polymorpha) in order to learn what North American freshwater ecosystems are losing and gaining.

I examined a community of unionids in a stream by using both natural and experimentally enriched stable isotopes of nitrogen as a spin-off project of the Lotic Intersite Nitrogen eXperiment (LINX). This is the first and only isotopic enrichment of this taxon. The evidence suggests, contrary to conventional wisdom, that native freshwater mussels they may not be exclusively suspension feeders as adults.

The potential effects of zebra mussels on fish are poorly understood. I examined how larval bluegill growth and survival could be affected by zebra mussels in an experimental mesocosm setting. The hypothesized mechanism of competition between mussels and bluegill for food in the form of microzooplankton was supported.

Competition between zebra mussels and obligate planktivores may contribute to ecological harm done to small inland lakes as zebra mussels spread throughout North America. The effects of zebra mussels on microzooplankton reproduction was studied as part of a large mesosoem ZEbra mussel EXperiment (ZMEX), in order to evaluate the relative importance of direct predation on microzooplankton by zebra mussels and indirect competition for resources (phytoplankton as food) causing reduced fecundity.

Another anticipated impact of zebra mussels is the alteration of phytoplankton community structure, including the promotion of the toxic alga Microcystis. I surveyed 60 lakes in the lower peninsula of Michigan to see whether the phytoplankton community has changed in the presence of zebra mussels. Standing stocks of phytoplankton have clearly been reduced in the presence of zebra mussels. While the relative abundance of Microcystis appears to have increased in the presence of zebra mussels there has been little effect on overall phytoplankton community structure.

Nutrient regeneration and benthic-pelagic coupling are important issues concerning zebra mussels. I measured ammonium (NH_4), soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC) concentrations, and the composition of biodeposits in the mesocosm experiment and survey. While increased SRP was detected in the mesocosm experiment, no evidence of increased SRP was found in natural systems. Zebra mussels have, however, evidently reduced the concentrations of DOC in lakes. This is important because DOC attenuates UV-B radiation, and thus invaded systems may be more susceptible.

For my parents, who introduced me to the family business.

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Chapter 1

INTRODUCTION: BIOLOGICAL INVASIONS, ZEBRA MUSSELS, AND THE REPLACEMENT OF NATIVE UNIONIDS

Overview

One of the most profound scientific advances of the twentieth century was the recognition that the biosphere is increasingly a human-dominated ecosystem. While ozone depletion, habitat destruction, chemical pollution, large-animal extinction and serious risk of global climate change are perhaps the most well known environmental outcomes of this domination, numerous others abound. One such issue is biological invasion, defined as the introduction of a species to an area previously uninhabited by that species. Man is directly and indirectly responsible for thousands of such introductions. Some of those introduced species become established as viable populations, and some of those viable populations bloom into ecological and economic pests. The general concept of invasive species is perhaps less well known than specific case studies, but represents a problem as old as global human migration itself.

People have been moving other species around the planet since prehistoric times (Elton 1958, Williamson 1996). This "aboriginal phase", as I call it, consisted of transporting animals and plants as food necessary for survival. Polynesians, for example, intentionally brought pigs and rats with them to hunt as they colonized Pacific islands. Meso-americans brought plants with them as they migrated across North America. A later "European colonial phase" occurred when explorers of the Pacific intentionally released goats and pigs to islands so future sailors would have something to eat and

unintentionally introduced animals including rats and mosquitoes. Plants introduced to the New World often spread faster than people did, such that later colonizers thought the plants were native. In the 19th century a "romantic phase" of species introduction occurred through the work of acclimatization societies established for the purpose of species introduction. Their motivations were both quaint and practical, spanning the desire to introduce all the birds mentioned by Shakespeare to wanting something familiar to hunt. Today we live in the "modern economic phase" of species introductions, where organisms are transported and released unintentionally through the activities of global commerce such as air traffic, commercial container traffic, and ballast water exchange (Vitousek et al. 1996).

One of the most famous biological invasions that has occurred in recent times the arrival of the zebra mussel (Dreissena polymorpha) to North America. The first discovery of the zebra mussel in North America occurred in 1988, when Herbert et al. (1989) found large populations in Lake St. Clair and western Lake Erie. A release of larval mussels during the ballast exchange of a single commercial cargo ship travelling from the north shore of the Black Sea has been deduced as the likely vector of introduction (McMahon 1996). The zebra mussel has since dispersed throughout the Great Lakes, Mississippi River drainage network and other eastern rivers of North America, and inland lakes including those of Michigan.

Zebra mussels are famous for their biofouling effects on industrial sites (Mackie et al. 1989, Kovalak et al. 1993). Water intakes, heat exchangers, holding tanks and other structures of hydroelectric and other plants have been dramatically clogged, producing densities as high as 750,000 ind./m^2. As of the mid 1990's, industrial mitigation of zebra

mussels cost over $100 million annually in the United States (ZMIS 1996). Biological invasion differs from many environmental problems in that direct monetary costs can be obvious and large.

This dissertation examined several effects of zebra mussels on freshwater ecosystems. Among the impacts zebra mussels have had is the near extirpation of native unionid mussels. Thus the invasion of zebra mussels to North America is not simply the addition of a new species, but the functional replacement of one type of freshwater mussel with another. By also examining the ecology of native unionid bivalves in this dissertation I sought to address what North American freshwater ecosystems are gaining and losing. Much less is known about the basic ecology of unionids than zebra mussels (see chapter 2 for a review of the state of knowledge concerning unionids). Thus while this dissertation advances our knowledge of both native and exotic freshwater mussels, my contribution to the understanding of unionids represents information of a much more fundamental nature. The rest of this introduction describes some of the basic ecology of zebra mussels and unionids, impacts that zebra mussels have had on previously invaded systems, and further motivations for studying these topics.

Life history and physiology

The life history of zebra mussels differs greatly from most endemic Great-Lakes region bivalves (Pennak 1989, Mackie and Schlosser 1996). Exotic dreissenids are dioecious, with fertilization occurring in the water column. Endemic bivalves are monoecious, dioecious or hermaphroditic, some internally fertilized by filtering sperm from the water column. Under natural thermal regimes, zebra mussel oogenesis occurs in

autumn, with eggs developing until release and fertilization in spring. In thermally polluted areas, reproduction can occur continually through the year.

The quagga mussel (Dreissena bugensis), a second invader named after an extinct species of zebra, was discovered in 1991. Large populations of this mussel exist in Lake Erie and Lake Ontario, and invasion of other areas such as the Mississippi River has begun (Mills et al. 1996). One endemic genera of dreissenidae exists, Mytilopsis, which occupies coastal estuaries from New York to the Gulf of Mexico (Pathy and Mackie 1993). It is not known whether Mytilopsis occurs in North America due to early European introduction (Morton 1993). The false mussel, Mytilopsis leucophaeata, has been found in the upper Mississippi, and may pose a similar threat as does the zebra mussel (Mackie et al. 1989).

Most endemic freshwater bivalves have an obligatory parasitic larval stage called a glochidium which temporarily infests the gills of fish. The larval stage of Dreissenidae is a free-swimming pelagic veliger that does not rely on any host. The only other North American freshwater bivalves with pelagic veliger larvae are fingernail clams, Sphaeridae, which burrow into sediment, and the Asiatic clam, Corbicula, another exotic invader which favors running waters causing its own host of ecological and industrial problems (Morton 1997). The zebra mussel displays high fecundity, annually producing 30,000-40,000 eggs. Veligers typically begin to appear in the water column in June at 13°C, and remain pelagic for 8-15 days. Veligers can occupy the water column for the entire summer and even into winter depending on differential gamete production and release.

Once the veliger undergoes morphological changes including development of the siphon, foot, organ systems and blood, it is known as a postveliger. Further subdivision of the larval stage has been delineated: (veliger) preshell, straight-hinged, umbonal, (postveliger) pediveliger, plantigrade, and (juvenile) settling stage (ZMIS 1996). The settling stage attaches to a substrate via protienaceous threads secreted from the byssal gland. The vast majority of veliger mortality (99%) occurs at this stage due to settlement onto unsuitable substrates. Sensitivity to changes in temperature and oxygen are also greatest at this stage. Once attached, the life span of D. polymorpha is variable, but can range from 3-9 years. Maximum growth rates can reach 0.5 mm/day and 1.5-2.0 cm/year. Adults are sexually mature at 8-9 mm in shell length (i.e. within one year).

The rapid invasion of North American waterways has been facilitated by the zebra mussel's ability to disperse during all life stages. Passive drift of large numbers of pelagic larval veligers allows invasion downstream. Yearlings are able to detach and drift for short distances. Adults routinely attach to boat hulls and floating objects and are thus anthropogenically transported to new locations. Transporting recreational boats disperses zebra mussels between inland lakes. In addition, speculation exists that waterfowl can disperse zebra mussels, but this has yet to be conclusively demonstrated.

While byssal threads develop in the larvae of some non-dresissenid endemic bivalves and are used to attach to fish gills, there are no endemic freshwater bivalves with byssal adult stages. This adaptation has been important to the zebra mussel's success in invading North America. Zebra mussels attach to any stable substrate in the water column or benthos: rock, macrophytes, artificial surfaces (cement, steel, rope, etc.), crayfish, unionid clams, and each other, forming dense colonies called druses.

Long-term stability of substrate affects population density and age distributions on those substrates. Within Polish lakes, perennial plants maintained larger populations than did annuals (Stanczykowska and Lewandowski 1993). Populations on plants also were dominated by mussels less than a year old, as compared with benthic populations. These populations of small individuals allow higher densities on plants. In areas where hard substrates are lacking, such as a mud or sand, zebra mussels cluster on any hard surface available. Given a choice of hard substrates, zebra mussels do not show a preference, indicating that veligers cannot discriminate between substrates (with the exception of substrate rejection due to contaminants). Research on danish lakes shows that factors exist, however, that cause substrate to be unsuitable for both initial and long term colonization: extensive siltation, some sessile benthic macroinvertebrates, macroalgae, and fluctuating water levels exposing mussels to desiccation (Smit et al. 1993).

The dispersion of zebra mussels within a lake is controlled by physical conditions including wind strength, lake/shore morphometry, and current patterns (Stanczykowska and Lewandowski 1993). These conditions affect both spatial patterns of pelagic veliger density and benthic adult dispersion. Population density of benthic adults has been observed to vary as widely as two orders of magnitude (e.g. <100 to >1500 individuals/m^2) within individual Polish lakes due to these physical conditions.

Tolerance limits of physical and chemical parameters are well known (Sprung 1993, Vinogradov et al. 1993, McMahon 1996). Although discrepancy exists when comparing temperature tolerance limits of North American and European populations, this is probably due to the American population being founded by mussels from the

southern limit of the European population's range. Most work in Europe has been done in the northern range.

North American populations are generally adapted to warmer temperature regimes than their European counterparts. Although shell growth has been reported to occur at temperatures as low as 3° C, Lake St. Clair populations and some European populations display shell growth at 6-8° C. Eggs are released when the environmental temperature reaches 13° C and release rate is maximized over 17° C. The optimal temperature range for adults extends to 20-25° C, but D. polymorpha can persist in temperatures up to 30° C. Short term tolerance of temperatures up to 35° C is possible if the mussels were previously acclimated to high temperatures. Rapid warming of shallow lakes has been hypothesized to detrimentally affect reproductive rates in Danish populations (Smit et al. 1993).

Oxygen demands are similar to those of other freshwater bivalves including unionids. Tolerance of "anaerobic" conditions has been reported for short time periods under certain temperatures and sizes, but zebra mussels cannot persist in hypoxic conditions. The lower limit of pO_2 tolerance is 32-40 Torr at 25° C. Zebra mussels have been found in the hypolimnetic zone of lakes with oxygen levels of 0.1-11.2 mg/l, and in the epilimnetic zone with oxygen levels of 4.2-13.3 mg/l. Zebra mussels are described as poor O_2 regulators, possibly explaining their low success rate in colonizing eutrophic lakes and the hypolimnion.

Zebra mussels can tolerate only slight salinity. Although some populations of European zebra mussels can be found in estuaries, their persistence has been speculatively attributed to reduced tidal fluctuation. Upper limits of freshwater bivalve

salinity tolerance reach 8-10 ‰, and populations of European zebra mussels have been found to tolerate and range of salinities, from 0.6 ‰ (Rhine River) to 10.2 ‰ (Caspian Sea). North American populations generally tolerate salinity up to 4 ‰.

In European populations, calcium concentrations of 24 mg Ca^{2+}/l allow only 10% larval survival due to inhibition of shell development. Optimal calcium concentrations ranges from 40-55 mg Ca^{2+}/l, but North American populations have been found in lakes with lower concentrations. North American populations require 10 mg Ca^{2+}/l to initiate shell growth and 25 Ca^{2+} mg/l to maintain shell growth. Larval development is inhibited at pH < 7.4. Higher rates of adult survival occur at a pH of 7.0-7.5, but populations have been found in the hypolimnetic zone of lakes with a pH of 6.6-8.0, and in the epilimnetic zone with a pH of 7.7-8.5. Optimal larval survival occurs at a pH of 8.4, and optimal adult growth occurs at pH 7.4-8.0.

Ecological Impacts Observed and Hypothesized

Dreissenids are gregarious, epibenthic, byssal filter feeders. As such, zebra mussels primarily consume phytoplankton, but other suspended material is filtered from the water column including bacteria, protozoans, zebra mussel veligers, other microzooplankton and silt. Large populations of zebra mussels in the Great Lakes and Hudson River reduced the biomass of phytoplankton significantly following invasion. Diatom abundance declined 82-91% and transparency as measured by Secchi depth increased by 100% during the first years of the invasion in Lake Erie (Holland 1993). As the invasion spread eastward during 1988 to 1990, successive sampling stations recorded

declines in total algae abundance from 90% at the most western station to 62% at the most eastern (Nicholls and Hopkins 1993). In Saginaw Bay, sampling stations with high zebra mussel populations experienced a 60-70% drop in chlorophyll-a and doubling of Secchi depth (Fahnenstiel et al. 1993). Phytoplankton biomass declined 85% following mussel invasion in the Hudson River (Caraco et al. 1997). Whether the species composition of the phytoplankton community can be altered by zebra mussels in unresolved (see chapter 4).

Increased water clarity allows light to penetrate further, potentially promoting macrophyte populations. As macrophytes can be colonized by veligers, the macrophyte community may be altered if such colonization proves detrimental. Indeed, this may be occurring in Spring Lake, O ttowa County Michigan, USA with Vallisneria colonized to a lesser extent than other plants (Theresa Lauber personal communication). Increased light penetration may also cause water temperatures to rise and thermoclines to become deeper, but these effects have not yet been documented. As Chl-a drops as a result of the phytoplankton consumption, the DOC concentration may drop. Macrophytes could eventually compensate for this since they are also a source of DOC, but there may be a lag period between the time when phytoplankton biomass is down and macrophytes proliferate. This could produce a period of time when UV-B light penetrates deeper into the water column.

Zebra mussels are able to filter particles smaller than 1μm in diameter, although they preferentially select larger particles (Sprung and Rose 1988) . Thus bacteria may represent an important food source (Cotner et al. 1995, Silverman et al. 1996). At a 90% efficiency rate, zebra mussels are much more efficient at filtration of such small particles

than are Unionids and Asiatic clams. Filtering rate is highly variable, depending on temperature, concentration of suspended matter, phytoplankton abundance, and mussel size (reviewed by Noordhuis et al. 1992). Although European zebra mussels are less active in winter, this seasonal pattern is temperature driven. No diel patterns of filtration rate have been found. During spring, filtration rates rise dramatically between 5 and 10° C, then level off with respect to temperature, and may be inhibited at temperatures over 20° C. Increased suspended matter can reduce filtration activity to a minimum required to maintain oxygen demand. A sigmoidal relationship exists with filtration rate and size, but this may be an affect of aging.

Material filtered by zebra mussels is either ingested or expelled as feces or pseudofeces, which is mucus covered. True fecal pellets are chemically altered, larger and more dense. Pseudofeces production increases with increasing suspended solid concentration, as well as increasing temperature, albeit to a much lesser extent (Noordhuis et al 1992, MacIsaac and Rocha 1995). The rate of biosedimentation through pseudofeces production was very high (28mg/cm^2 day at a density of 1180 individuals/m^2) under turbid conditions in Lake Erie, lending support to the hypothesis that zebra mussels are responsible for increased water clarity observed since mussel introduction (Klerks et al. 1996). Filtration rate was not related to seston composition (POC:TSS, chl:TSS) in Saginaw Bay (Fanslow et al. 1995).

Veligers also filter material, but their impact is far less than that of sessile adults. Settled mussels exerted 10^3 times the grazing rate of veligers in western Lake Erie, for example (MacIsaac et al. 1992). Microzooplankton, e.g. rotifers and veligers, are ingested

by zebra mussels, but larger zooplankton are not eaten (MacIsaac et al. 1991, MacIsaac et al. 1995).

It has been speculated that benthic deposition of feces and pseudofeces may aid bacterial productivity, thus producing a source culture that zebra mussels can feed upon (Silverman et al. 1996). It has also been speculated that biodeposition of feces and pseudofeces might cause observed increases in benthic macroinvertebrate populations (Stewart and Haynes 1994). Biomagnification of PCBs was observed in Gammarus associated with zebra mussels, indicating concentration of pollutants in zebra mussel feces/pseudofeces and transfer to other trophic levels (Bruner et al. 1994). In an experimental study, however, Botts et al. (1996) found greater abundances of macroinvertebrates associated with both living and non-living (i.e. empty shell) zebra mussel druses compared with their no-druse treatment. Thus the increased physical habitat complexity of a mussel colony may benefit macro-invertebrates rather than deposition of feces and pseudofeces.

Zebra mussels can reduce filtration rates (more frequent interruption of filtering or slower pumping rates) and/or produce pseudofeces above an incipient limiting concentration (ILC) of algae to maintain a constant consumption rate (Sprung and Rose 1988, Fanslow et al. 1995 MacMahon 1996,). Feeding activity can be described by the clearance rate (percentage of algal biomass removed from the water column over time), biomass of cleared algae (BCA), feces production and pseudofeces production (μg F or P/BCA). For example, Berg et al. (1996) examined the effects of zebra mussel size and algae species and concentration on zebra mussel feeding activity. Clearance rates were constant over varying concentrations of pure cultures of Chlamydomonas reinhardtii, a

spherical unicellular species of 7.42 μm (± 0.13μm) in diameter. This indicates that the concentrations used in experiments were below the ILC. Clearance rates decreased, however, with increasing concentrations of Pandorina morum, a species made up of colonies with varying numbers of cells that are individually as large as C. reinhardtii. This indicates that the concentrations used in experiments were above the ILC. Large zebra mussels (20-25 mm in length) displayed a higher clearance rate across all concentrations of C. reinhardtii than did small mussels (10-15 mm). ILC differed in this study from previous studies done with European populations. Thus zebra mussel size, phytoplankton species and regional population differences affect clearance rates, ILC and feces/pseudofeces production.

Zebra mussels produce pseudofeces to avoid ingesting non-food material (e.g. clay), as a mechanism to deal with overabundance of food (e.g. algal concentrations above the ILC), and possibly as a way to reject unpalatable algae. Zebra mussels readily reject blue-green algae, such as Microcystis, as pseudofeces (Vanderploeg et al. 2001). The presence of this cyanobacterium does not inhibit filtering, except in mass abundances such as a bloom (Noorhuis et al. 1992, Lavrentyev et al. 1995). Zebra mussels can select material for rejection through pseudofeces production internally, perhaps identifying cyanobacteria by chemical cues (Ten Winkel and Davids 1982).

Understanding of the fate of pseudofeces once it expelled is poor. Zebra mussels removed metals from the water column of Lake Erie and deposited it to the bottom at high rates (Klerks et al. 1997). Pseudofeces production is a key feature in the still experimental use of zebra mussels as biofilters of undesired pollutants, including mitigation of cyanobacterial blooms (Noordhuis et al. 1992, Reeders and Bij de Vaate

1992). Roditi et al. (1997) found that the biodeposits of zebra mussel were organically enriched, including 3.9% live algae by weight. Resuspension of this material occurred in their system, a tidal estuary, reducing the potential impact of biodeposition to the benthos.

Less well known is the fate of live algae bound into pseudofeces. Bastviken et al. (1998) speculate that phytoplankton that survives the pseudofeces process must be resuspended in order for long term survival, a process less likely to occur in inland lakes than in tidal estuaries. If survivorship following filtration is equal between phytoplankton species then community species composition can remain unchanged. Other factors may affect the phytoplankton community, however, including increased light.

The zooplankton community has also been affected by the invasion of zebra mussels (see also chapters 3, 5). Zooplankton abundance dropped 55-71% following mussel invasion in Lake Erie, with microzooplankton more heavily impacted (MacIsaac et al. 1995). Mean summer biomass of zooplankton decreased from 130 to 78 mg dry wt. m^{-3} between 1991 and 1992 in the inner portion of Saginaw Bay. The total biomass of zooplankton in the Hudson River declined 70% following mussel invasion, due both to a reduction in large zooplankton body size and reduction in microzooplankton abundance. These effects can be attributed to reduction of available food (phytoplankton) and direct predation on microzooplankton. Increased competition in the zooplankton community for newly limited food should result from zebra mussel infestation. The size of individual zooplankters might decrease. Hypotheses can be formulated specifying which species will prevail based on knowledge of competitive ability.

Effects should continue through the food web to fish (see chapter 3). Reductions in zooplankton biomass should cause increased competition, decreased survival and decreased biomass of planktivorous fish. Alternatively, because microzooplankton are more heavily impacted by zebra mussels the larval fish population may be more greatly affected than later life stages. This may be especially important to inland lakes with populations of pelagic larval fish such as bluegills. Benthic feeding fish may benefit as opposed to planktivorous fish, or behavioral shifts from pelagic to benthic-feeding may occur. In addition, proliferation of macrophytes may alter fish habitat.

Other effects include the extirpation of native unionid clams through epizootic colonization (Schloesser et al. 1996, Baker and Hornbach 1997). Zebra mussels restrict valve operation, cause shell deformity, smother siphons, compete for food, impair movement and deposit metabolic waste onto unionid clams. To date, unionids have been extirpated from Lake St. Clair and nearly so in western Lake Erie. Many species of bird known to be predators of zebra mussels also occur in the inland lakes of Michigan. While a new food source may benefit such predators, biomagnification of toxins into both fish and birds is possible.

Some effects have been hypothesized as worst-case scenarios. For example, zebra mussels may cause a shift from pelagically to benthically-based food webs in inland lakes. Zebra mussels may also shift lakes from a turbid and phytoplankton-dominated state to clear and macrophyte-dominated state, i.e. between alternative stable equilibria (Scheffer et al. 1993).

Past Research Efforts

A long tradition of zebra mussel study exists in Europe and the former Soviet Union, where the zebra mussel has been present for 150 years (see Mackie et al. 1989 for an annotated bibliography of European references). Work includes spatial distribution patterns, demography, tolerance limits for physical and chemical parameters, and physiology. Studies of food web interactions and nutrient regeneration are conspicuously lacking. Extensive ecological work in the United States began soon as the zebra mussel was discovered and peaked in the early 1990's. The literature on ecosystem and community-level effects of zebra mussels consists primarily of work investigating Lake Erie, Saginaw Bay, the Hudson River, and Oneida Lake (e.g. Fahnenstiel 1993, Holland 1993, Pace et al. 1998, Idrisi et al., 2001).

Further motivations for this dissertation

Comprehensive investigation of the effects of zebra mussels on North American inland lakes is currently lacking. Despite the quantification of ecosystem effects in the Great Lakes and Hudson River ecosystems, predicted effects of zebra mussels cannot be simply transplanted to inland lakes. Important differences between the Great Lakes and Hudson River exist that should affect ecosystem response. For example, in both Lake Erie and Saginaw bay, ecosystem effects caused by the rapid expansion of zebra mussel populations were localized. In Saginaw Bay, the outer bay with low zebra mussel populations behaved independently of the inner bay with high zebra mussel populations (Fahnenstiel et al. 1993). In lake Erie, the eastward expansion of the invasion could be

charted in the successive reduction of phytoplankton at stations along a transect (Nicholls and Hopkins 1993). Due to their sheer size, the Great Lakes do not respond as whole units to ecosystem perturbation. Inland lakes are much smaller and may respond to zebra mussel invasion both more quickly and as whole units. The morphology of inland lakes, with a larger proportion of littoral zone, should affect ecosystem response, as should the greater macrophyte populations relative to lake size.

Lastly, Native freshwater bivalves were originally ubiquitous in North American rivers and streams, but have increasingly fallen victim to anthropogenic pressures such as over-harvesting, waterway impoundment, pollution and exotic species infestation (Ricciardi and Rasmussen 1999). Bivalve conservation efforts have included translocation, but mortality rates have averaged ~50% (Cope and Waller 1995). Captive rearing programs show highly variable mussel survival rates (Dunn and Layzer 1997). Incomplete understanding of the feeding ecology of freshwater bivalves impedes successful conservation efforts. Thus investigation of the basic ecology of these organisms is sorely needed.

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Chapter 2

BIVALVE DIETS IN A MIDWESTERN U.S. STREAM: A STABLE ISOTOPE ENRICHMENT STUDY

with Stephen K. Hamilton

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Abstract

This study examined a community of stream bivalves (unionids and fingernail clams) in a second-order woodland stream in southern Michigan, USA using both the natural abundance of ^{15}N and a six-week whole-stream ^{15}N enrichment experiment, as part of the Lotic Intersite Nitrogen eXperiment (LINX). Objectives included addressing what made up the diet of these bivalves and whether suspended algae consumed by bivalves were derived from pelagic phytoplankton imported from an upstream lake or attached algae sloughed from instream surfaces. Within the examination of bivalve diets we considered whether suspension and/or deposit feeding modes were employed and whether bivalves selectively assimilated the algal and microbial portions of bulk material they ingested. All 12 unionid species reached a level of ^{15}N enrichment greater than the bulk suspended organic matter. Sphaerium striatinum (Sphaeriidae) were enriched to levels greater than all presumed food sources. Suspended algae were derived both from

sloughed epilithon and pelagic phytoplankton originating from lentic waters upstream. A mixing model suggested that unionids were consuming 80% deposited and 20% suspended material. Alternatively, these bivalves were preferentially assimilating the highly enriched living component of suspended and/or benthic organic matter rather than assimilating the bulk material. These results advance our understanding of freshwater bivalve feeding ecology, which is necessary if conservation efforts of these increasingly threatened organisms are to succeed.

Introduction

Freshwater bivalves were originally ubiquitous in North American rivers and streams, but have increasingly fallen victim to anthropogenic pressures such as over-harvesting, waterway impoundment, pollution and exotic species infestation (Ricciardi and Rasmussen 1999). Bivalve conservation efforts have included translocation, but mortality rates have averaged ~50% (Cope and Waller 1995). Captive rearing programs show highly variable mussel survival rates (Dunn and Layzer 1997). Incomplete understanding of the feeding ecology of freshwater bivalves impedes successful conservation efforts.

There are several ways to better understand the diet of organisms including direct observation of feeding behavior, gut contents analysis, and examination of chemical constituents such as stable isotopes or nutrients within the tissues of organisms compared with their potential food sources. Direct observation of the feeding behavior of bivalves has primarily involved either measurement of filtering rates of artificial seston by adults

(e.g. Kryger and Riisgård 1988; Silverman et al. 1997) or examination of filtering morphology (e.g. Ward et al. 1993). Gut contents analyses of freshwater bivalves are rare and may be misleading because the method cannot distinguish ingested material that is not assimilated. Algae, for example, can survive passage through the digestive tract, and fecal material can have a high nitrogen content due to ingested material by-passing the stomach gland and moving directly to the intestinal tract (Hawkins et al. 1983; Miura and Yamashiro 1990). Also rare are energy budgets for freshwater bivalves. An energy budget for the fingernail clam Sphaerium striatinum was reported by Hornbach et al. (1984), who estimated that 35% of its energy was derived from suspension feeding, and the rest possibly from deposit feeding.

Most stable isotope studies of food webs can be classed as either surveys of natural isotope abundance or experimental isotope enrichments. Natural abundances of stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) have increasingly been used to examine the relative importance of various potential autotrophic sources in supporting food webs that include mollusks (Incze et al. 1982; Peterson et al. 1985; Thorp et al. 1998). Consumers tend to be enriched with the heavier stable isotope of nitrogen (^{15}N) relative to their diet (Minagawa and Wada 1984). Accounting for this trophic enrichment, unionids have been used to indicate the isotopic composition of the base of the food web in studies of lakes because they live long, have slow metabolism, and presumably utilize primary producers (phytoplankton) as their dominant food source. Unionids should thus integrate potential short-term fluctuations in primary producer isotope signatures over time (Cabana and Rasmussen 1996). This concept has been used to study the trophic

position of fish in Canadian lakes and effects of exotic species on food webs (Vander Zanden et al. 1997; Vander Zanden et al. 1999).

In an isotope enrichment experiment, the abundance of the normally rare isotope is increased in the ecosystem by the introduction of a nutrient enriched in the rare isotope, allowing a more detailed analysis of the food web. Tracer ^{15}N experiments have been conducted in the Kuparuk River, AK, USA, Walker Branch, TN, USA and Hugh White Creek, NC, USA to elucidate food web relationships in streams (Peterson et al. 1997; Hall et al. 1998; Mulholland et al. 2000a, b). The present study and the latter two cited above were part of the Lotic Intersite Nitrogen eXperiment (LINX), a multi-site examination of nitrogen cycling within stream ecosystems across North America. The study reported here is the first experimental enrichment of stable isotopes in an ecosystem containing a high abundance and diversity of freshwater bivalves.

We examined two questions of bivalve feeding ecology: 1) What is the diet of stream bivalves? and 2) To what extent is suspended material available to filter feeders derived from benthic algae suspended within the stream or from a subsidy of pelagic phytoplankton derived from upstream? Within the context of bivalve diets we considered whether bivalves employed deposit and/or suspension feeding modes and whether bivalves selectively assimilated the algal and microbial portions of bulk material ingested. To evaluate the potential food sources for bivalves in a woodland stream of the Midwestern U.S., we examined unionids, sphaeriids, and their potential food sources using both natural N isotopic abundances and a nitrogen isotope (^{15}N) enrichment experiment.

Methods

Study Site- This study examines a 500m reach of Eagle Creek, Fort Custer State Recreation Area, Michigan, USA near the city of Battle Creek. Originating as an outflow from an artificial impoundment 1200m upstream known as Eagle Lake, the stream flows through secondary forest and wetland upstream of the study reach, where there are several beaver dams. Below the study reach the stream enters the Kalamazoo River. The study reach has little wetland and is largely shaded by deciduous forest. Hydraulic and geomorphological features are summarized in Table 1.

^{15}N Tracer Experiment- Experimental design and sampling methods that were standardized among LINX sites are cursorily described here; details are provided in Mulholland et al. (2000*a*, *b*) and Hamilton et al. (2001). Isotope measurements are expressed as δ values in units of ‰ :

$$\delta^{15}N = [(R_{sample}/R_{standard})-1] \times 1000 \quad (1)$$

where $R = {}^{15}N:{}^{14}N$ ratio and $R_{standard}$ (N^2 in air) = 0.003663. Ammonium chloride enriched with ^{15}N was dripped into the stream for 42 days beginning on 16 June 1998 with the intent of raising the $\delta^{15}N$ of ammonium at least +250 ‰ above natural background levels. Enrichment experiment data were corrected for background $\delta^{15}N$ by subtracting natural delta values measured either just before the experiment or during the experiment at an upstream reference site (see below).

Sampling- Sampling stations were established at 7 locations downstream of the dripper as well as at a reference reach just above the dripper. Potential food sources for bivalves included epipsammon (EPS, or detritus and possibly algae mixed with sand) and suspended particulate organic matter (SPOM). EPS was collected using a turkey baster to suck organic matter from the surface of sand deposits, and EPS samples likely contained a substantial portion of mobile fine benthic organic matter (FBOM). SPOM was sampled by filtering water pumped from the middle of the water column. Samples of EPS and SPOM were collected on Whatman GF/F filters (nominal pore size = 0.7 μm). Additional ecosystem compartments that served as primary food sources for other organisms in the stream included Coarse Particulate Organic Matter (CPOM, collected as leaf fragments), small pieces of rotten wood, and Epilithon (EPI, scraped from rocks and collected on GF/F filters). Filamentous macroalgae and macrophytes were present at very low abundances and were thus unlikely to be significant to food webs. Composite samples were made from several locations within sampling stations. Samples for natural isotope abundance were obtained from the reference station. Samples were collected weekly beginning on 15 June 1998 at all or at selected sample stations, including food sources and various macroinvertebrates. Bivalves were sampled under a different schedule.

To monitor temporal patterns of ^{15}N enrichment in bivalves, we collected three individuals of Pleurobema sintoxia from a single station 396 m downstream from the dripper on the seventh day of the experiment (day 7), as well as on days 14, 28, 35, and 42. We also collected 3 individuals of P. sintoxia from the same station for temporal analysis of ^{15}N turnover rate the day after the dripper was turned off (post-day 1), as well as on post-days 7, 14, and 28. We studied spatial patterns of unionid isotopic enrichment

by collecting 3 P. sintoxia from each station on the day of maximum enrichment (day 42) and spatial patterns of Sphaerium striatinum enrichment by collecting several individuals from various stations on day 42. We collected unionids of other species from two adjacent stations on day 42 for interspecific comparisons. To test for seasonal variation in natural ^{15}N abundance, we collected bivalves from the reference station on day 42 for comparison with bivalves collected the day before the dripper was turned on (day -1). We evaluated natural isotope abundance by comparing mussels collected from the reference station on day 42 to values for food sources collected at that station averaged over the course of the enrichment experiment. We froze unionids for storage and preserved S. striatinum in ethanol until analysis (Peterson et al. 1993).

We surveyed unionids visible on the stream bottom to estimate abundance and biomass. A more invasive subsurface survey would have disturbed the substrate, altering seston composition and invertebrate drift. The unionid population at the surface is also that portion most directly interacting with the water column, and therefore the most relevant to food web interactions discussed in this study.

Replicate samples of suspended chlorophyll a (Chl a) were collected on GF/F filters from several points along the stream on two dates for measurement by flourometry (Welschmeyer 1994). Suspended algae were sampled on 20 August 1998 at 4 locations: at the Eagle Lake outlet, the outflow of wetlands 400 m upstream of the study reach, 20 m downstream of the dripper and 460 m downstream of the dripper. Samples were concentrated by sedimentation and preserved in Lugol's solution for enumeration. Algae were identified to species and ranked as either more prevalent in benthic habitats (scored as 1), more prevalent in pelagic habitats (scored as -1), or equally prevalent in both

pelagic or benthic habitats (scored as 0). The rankings were used to calculate a weighted average index from -1 to 1 (pelagic to benthic, respectively; Zelinka and Marvan 1961). These rankings were made by Dr. R. J. Stevenson of Michigan State University.

Tissue analysis- We selected individual unionid mussels for $\delta^{15}N$ analysis and did not pool any unionid samples. Due to the long nitrogen turnover times in bivalves, we analyzed three "tissues" rather than whole-body samples (Hawkins 1985). The stomach gland was sampled to reflect short-term assimilation of nutrients because it is a major site of nutrient absorption (Pechenik 1985). Foot muscle was sampled to integrate long-term nutrient sources. Gut contents from the intestinal tract were sampled because they contain partially undigested material. We dissected tissues from frozen specimens and dried and pulverized the samples for isotopic analysis. Shells were used for species identification. The entirety of S. striatinum soft tissue was dried and up to five individuals pooled per sample station for isotopic analysis due to the limited amounts of soft tissue per individual. The ^{15}N turnover rates for bivalve tissues and food sources were estimated from the slope of the $\ln(\delta^{15}N)$ over time after the dripper was turned off (i.e. from the depletion of tracer ^{15}N).

To estimate ash-free dry mass (AFDM) and %N, we dried and ground the soft tissues (gut contents removed) of 10 whole unionids of various species. We measured dry weight and % ash gravimetrically and %N using a Carlo Erba elemental analyzer. Isotopic analysis of bivalves was done by continuous-flow isotope-ratio mass spectrometry with a Carlo Erba Elemental analyzer connected to a Finnigan Delta Plus Spectrometer at the Center for Environmental Science and Technology, University of

Notre Dame, Notre Dame, Indiana, USA. The remaining Eagle Creek LINX $\delta^{15}N$ measurements were made at that facility or using similar equipment at the Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts, USA.

Results

Unionids dominated consumer biomass in Eagle Creek (Table 2). A mean abundance of 1.9 individuals m^{-2} was present at the surface. Although the collection effort was not designed to systematically quantify species richness, we found 12 unionid species: Anodontoides ferussacianes, Actinonais ligamentina, Elliptio dilitata, Fusconaia flava, Lampsilis cardium, Lasmigona compressa, Lasmigona costata, Pleurobema sintoxia, Pyganodon grandis, Strophitus undulatus, Venustaconcha ellipsiformis, and Villosa iris. Sphaeriids were serendipitously sampled, usually from underneath rocks, and thus the biomass of sphaeriids is not known.

FBOM was abundant in the study reach, with 164 g AFDM m^{-2} in the upper cm of the stream bottom and 922 g AFDM m^{-2} in the upper 5 cm (Hamilton et al. 2001). The mass ratio of organic carbon to Chl *a* in surficial FBOM was 841, and the C:N mass ratio was 14. The stream carried a mean SPOM concentration of 3.3 mg AFDM L^{-1} in the study reach; the C:Chl *a* ratio of this material was 571. The results of the longitudinal survey of suspended matter above and within the study reach showed that the Chl *a* concentrations increased from the lake outflow (1200m upstream) to the wetland outflow (immediately upstream), then decreased through the study reach (Table 3). A similar decrease in Chl *a* concentrations through the study reach was observed on 14 July 1998,

falling from 2.85 (± 0.19 SD) to 2.18 (± 0.08 SD) μg L^{-1} between the dripper and the 461-m station. The species composition of algae in the suspended matter collected in the longitudinal survey showed that benthic algae predominated in all samples, with an increase in the relative abundance of benthic algae along the course of the stream (Table 3).

The unionid Fusconaia flava was discovered during processing of P. sintoxia specimens; these two species are extremely similar in external appearance. Although we believe the majority of bivalves we had originally classified as P. sintoxia were correctly identified, we cannot rule out the possibility that a few were actually F. flava; we thus describe this group hereafter as P. sintoxia + F. flava.

Natural isotope abundances- Natural $\delta^{15}N$ values for the unionid community and food resources, as measured just upstream of the enriched reach, are shown in Figure 1. EPS and FBOM had identical mean values (3.6‰) and were enriched relative to SPOM (1.3‰). Unionid muscle tissue (4.8‰) was 1.6 ‰ enriched with respect to stomach gland tissue (3.3‰), 1.3‰ enriched with respect to EPS and FBOM, and 3.5‰ enriched relative to SPOM. S. striatinum was 3.1 ‰ enriched relative to SPOM.

Isotope enrichment experiment- The addition of ^{15}N -labeled NH_4^+ to the stream elevated the $\delta^{15}N$ of food resources and consumers. Unionid stomach gland and gut contents became measurably enriched with ^{15}N during the addition ($P < 0.0001$, $P < 0.0001$, respectively) and then became depleted over time after the dripper was turned off

($\underline{P}$ = 0.0007, $\underline{P}$ = 0.0034, respectively, Fig. 2). Estimated turnover rates for ^{15}N in the stomach gland and gut contents were similar at 78 and 85 d, respectively. Unionid stomach gland tissue was on average 0.8‰ (± 0.7 SD) enriched relative to gut contents during the enrichment experiment. Muscle tissue from the foot responded very slowly to the addition of ^{15}N -enriched NH_4^+ ($\underline{P}$ = 0.02, Fig. 2). Although muscle tissue did become depleted after the dripper was turned off, the depletion curve was not significant ($\underline{P}$ = 0.16), making the estimated turnover rate of 357 d questionable. These turnover rates may be overestimated by the possible consumption of enriched FBOM after the dripper was turned off. FBOM, however, had a N turnover rate of 7 d (Hamilton et al. in review) and this rapid rate would have minimized the influence of enriched FBOM following termination of tracer addition. Based on the greater response to experimental ^{15}N enrichment of stomach gland compared with muscle, the stomach gland was used for seasonal, species and mixing model analyses within the enrichment experiment. Muscle tissue was used for mixing model analysis of natural abundance data.

The potential food sources EPS and SPOM also became enriched during the ^{15}N addition (Fig. 3A). The temporal pattern of enrichment for EPS and SPOM followed the same general pattern as the calculated $\delta^{15}N$ enrichment of the NH_4^+ in the stream water (Fig. 3B). The peak in calculated tracer $\delta^{15}N$ NH_4^+ between days 25-30 was caused by a combination of lower discharge and lower ambient NH_4^+ concentrations, resulting in less dilution of the added ^{15}N (Fig. 3B; Hamilton et al. 2001).

It was not clear whether unionid tissues approached isotopic equilibrium during the ^{15}N enrichment period. Gut contents may have reached a plateau by the end of the enrichment experiment (Fig. 2). Enrichment of stomach gland tissue appeared to still be rising on day 42, although variability in $\delta^{15}N$ values of this tissue increased near the end of the experiment.

The stomach gland $\delta^{15}N$ values from various unionid species were similar between day -1 and day 42 at the station above the dripper (mean for community: 3.4‰ ± 0.6 SD and 3.3‰ ± 0.4 SD, respectively). The $\delta^{15}N$ values of S. striatinum were also similar between day -1 and day 42 at the station above the dripper (4.4‰ and 4.3‰, respectively). Thus no seasonal isotopic change occurred in either the unionid community or S. striatinum. The various unionid species reached similar levels of ^{15}N enrichment (Fig. 4).

The $\delta^{15}N$ for EPS, SPOM and unionid stomach gland varied over the length of the stream on day 42 (Fig. 5). Enrichment of EPS appeared to increase in the downstream part of the reach, as did the $\delta^{15}N$ of unionid stomach glands. S. striatinum varied widely between locations with an average enrichment of 13.9‰, which exceeds that of EPS and SPOM, the presumed potential food sources (Fig. 5).

Discussion

The simultaneous consideration of natural abundance of stable isotopes with the results of our isotope enrichment experiment yields several insights into the feeding ecology of stream bivalves that are not available from other kinds of studies, but also raises some interesting new questions that challenge our traditional view of freshwater bivalves as primarily suspension-feeders. In the following discussion we address the evidence regarding dietary sources of nitrogen for the bivalves, the role of bivalves in the overall cycle of nitrogen in the stream, and the implications for conservation.

Patterns of ^{15}N enrichment in bivalves- The observed enrichment of ^{15}N in bivalve gut contents and tissues was due to the experiment rather than seasonal changes in natural isotope abundance. The community was remarkable in its similar response suggesting that all unionid species were utilizing similar food resources. The small response of unionid muscle compared with stomach gland during the enrichment experiment indicated that muscle tissue N integrates food resources over a much longer time period than does the stomach gland.

Turnover times for muscle calculated in this study agree well with a 333 d whole-body N turnover time calculated for Mytilus edulis by Hawkins (1985). Studies utilizing unionids as a baseline for comparison of food webs between ecosystems would benefit from analysis of isolated muscle tissue rather than the whole body because of longer-term integration of dietary sources within muscle, whereas studies needing shorter-term dietary information should use the stomach gland.

Food resources reached isotopic equilibrium or nearly so during the experiment (Hamilton et al. in review), as illustrated by the similar patterns of changing $\delta^{15}N$ values between the tracer, SPOM, and EPS (Fig. 3). This equilibrium reflected the rapid turnover rates and direct utilization of suspended ammonium by algae and heterotrophic microbes within SPOM and EPS. Unionids did not clearly reach isotopic equilibrium during the course of the enrichment experiment. Two explanations of unionid food resource utilization are thus possible: a diet of both EPS and SPOM (their presumed potential food resources), and/or differential assimilation of food resource components within the SPOM rather than uniform assimilation of the bulk organic material.

Sources of suspended algae- The total amount of algae decreased along the study reach as indicated by Chl *a*, while the relative abundance of benthic algae increased. This pattern can be explained by settling and active removal of suspended pelagic algae by bivalves and macroinvertebrate filtering collectors, coupled with sloughing of benthic algae from the stream bottom. Lake outlet streams have long been recognized to support a high density of filter feeders including unionids (Brönmark and Malmqvist 1982; Richardson and Mackay 1991). While the study reach was not strictly a lake outlet because there are also wetlands below the lake, the importation of high quality food from lentic environments might explain the observed bivalve abundance and diversity. Thus, within the suspension mode of feeding, the bivalves of Eagle Creek could be supported both by constant re-suspension of material from deposited pools and an ecological subsidy of pelagic phytoplankton supplied by the upstream lake and wetland. The concept of an

ecological subsidy could also be extended to organic detritus exported from the wetland and entering the steam (Polis et al. 1996).

Food resource mixing model- The relative abundance of multiple food sources in the diet of organisms can be quantified using an isotope mixing model:

$$\delta^{15}N_{consumer} = \sum f_i \delta^{15}N_i \quad (2)$$

where f_i and $\delta^{15}N_i$ are the fractional contributions and $\delta^{15}N$ values of each food resource i, and $\sum f_i = 1$. Use of a mixing model assumes both the organism and potential food resources to be at isotopic equilibrium (Gannes et al. 1997).

Bulk SPOM could not be the predominant food resource for bivalves in the stream because we observed a consistent enrichment of stomach gland tissue to levels greater than that of SPOM (Fig. 5). The simplest explanation of food resources for unionids then becomes a mixture of SPOM and EPS. In the enrichment experiment each sampling station was treated as a different test of unionid food resource partitioning. The ^{15}N enrichment for stomach gland slightly exceeded that of EPS at the 251m station (5.8‰ and 5.7‰ respectively), and the 461m station (8.4‰ and 7.2‰ respectively). Unionids were assumed to have consumed EPS exclusively at these stations for the purposes of mixing model analysis. The mixing model using stomach gland data from the enrichment experiment suggested unionids consumed 80% EPS and 20% SPOM on average (Table 4).

Application of the mixing model to natural muscle isotope abundance data required correction of consumer $\delta^{15}N$ values due to fractionation that occurs in

consumers relative to their food resources. In the enrichment experiment, trophic fractionation was accounted for by the subtraction of background (natural) $\delta^{15}N$ values from the measured $\delta^{15}N$ values. A 3.4‰ enrichment per trophic level is commonly used in food web studies, but this is an average value for a wide variety of animals (Minigawa and Wada 1984). Data on marine bivalves from the literature suggest that bivalves tend to be about 1.7‰ enriched with ^{15}N relative to suspended food resources (calculated from Minagawa and Wada 1984; Fry 1988). Application of the mixing model to natural-abundance muscle $\delta^{15}N$ values, assuming a trophic enrichment of 1.7‰, indicated that unionids consumed 81% EPS and 19% SPOM. Using the same trophic correction of 1.7‰ with whole-body S. striatinum $\delta^{15}N$ values, the mixing model indicated that S. striatinum consumed 64% EPS and 36% SPOM. Application of the mixing model to natural isotope abundance data for S. striatinum agreed well with Hornbach et al.'s (1984) energy budget for this species that indicated greater reliance on deposit feeding than suspension feeding.

Suspension vs. deposit feeding- Suspension feeding is the removal of suspended particles including phytoplankton from the water column. Deposit feeding is the consumption of particles from the sediment. Descriptions of unionid behavior and the role of unionids in aquatic ecosystems emphasize the suspension mode of feeding (e.g. McMahon 1991; Strayer et al. 1994; Strayer et al. 1999, Box and Mossa 1999).

Use of the deposit feeding mode by freshwater bivalves has generally been described within specific contexts in which direct feeding upon sediment by adults is not usually considered to be typical. Examples include: 1) Illustrations of exceptional

deposit-feeding behaviors (Way 1989 cited in MacMahon 1991; see also Reid et al. 1992); 2) Burial of sphaeriids in the substrate (e.g. Pennak 1989; MacMahon 1991); and 3) Pedal transport of sediment by juvenile unionids (Reid et al. 1992; Yeager et al. 1994). Re-suspended sediment has been examined as a factor affecting suspension-feeding behavior and clearance rates, but this is not an example of deposit feeding (e.g. Kiørboe et al. 1980; Bricelj et al. 1984).

In the scientific literature, descriptions of adult unionids as both suspension and deposit feeders are uncommon (e.g. Singh et al. 1991), while the belief that adult unionids are exclusively suspension feeders is pervasive yet not supported by empirical evidence. Ecological studies often examine unionids within the context of suspension feeding only (Kramer 1979, Brönmark and Malmqvist 1982, McCall et al. 1995, Parker et al. 1998). If citations are given in reference to stating that unionids are suspension feeders, the citations are usually not of studies that quantify freshwater bivalve feeding ecology (Brönmark and Malmqvist 1982, Yeager et al. 1994). To state that adult unionids are exclusively suspension feeders is therefore an inductive conclusion.

The mixing model used in this study suggested a predominance of the deposit mode of feeding in unionids, with good agreement between model results obtained with natural abundances and results obtained from the enrichment experiment. Indeed, application of a mixing model might be considered a standard procedure in a study of this kind. Use of the model, however, requires satisfaction of several conditions: isotopic equilibrium and assimilation of bulk material. The ^{15}N enrichment of gut contents appeared to reach a plateau over the 42-day addition, but the stomach gland became more variable at the end of the experiment. These results obscure evaluation of whether

unionids reached isotopic equilibrium. If unionids were near equilibrium by the end of the experiment, then the mixing model is still reliable. If not, the model yields a maximal estimate of the importance of the relatively ^{15}N -depleted SPOM because the $\delta^{15}N$ of unionid tissues would have continued to rise had the experiment been longer.

The duration of the present study was insufficient to definitively evaluate feeding mode preference. Future in-situ isotope enrichment studies of bivalves will need longer periods to accommodate slow N turnover rates. Laboratory experiments, however, should prove more valuable in determining the extent to which adult freshwater bivalves employ the deposit mode of feeding (Gannes et al. 1997). Laboratory isotope enrichment experiments could entail use of smaller amounts of ^{15}N, thereby costing less, and different levels of isotopic enrichment between suspended and deposited organic matter could be produced in the laboratory.

Preferential assimilation of microbes and algae- Pennak (1989) describes the diet of freshwater bivalves as consisting chiefly of fine organic detritus dislodged from the substrate, with phytoplankton of minor importance. In contrast McMahon (1991) lists phytoplankton, bacteria and fine detritus as the chief components of the freshwater bivalve diet, with deposit feeding as a potential, and perhaps underestimated, supplement. This dichotomy of opinion should not be surprising given the common assertion that the ecology of freshwater bivalves is poorly understood (e.g. Bogan 1993; Neves 1993, 1997; Box and Mossa 1999).

In LINX experiments in other streams, macroinvertebrate consumers of epilithon often became more highly labeled than their food resources (e.g. Tank et al. in press).

This could only have occurred if the organisms were assimilating highly labeled algal components of epilithon, rather than the bulk material that included algae, mucilage and entrained detritus. Unionids could also have preferentially assimilated the microbial and algal components of the bulk material they ingested. Nichols and Garling (2000) found that unionids in a Michigan river and lake fed primarily on bacteria and concluded that the unionids were selectively assimilating bacteria from ingested detritus. The enrichment of S. striatinum to levels greater than that of bulk potential food sources supports the preferential assimilation explanation (Fig. 5). Due to small body size relative to unionids, S. striatinum likely had higher N turnover rates, resulting in greater enrichment during the course of the experiment.

If bivalves differentially assimilate living components of bulk fine organic material, we cannot use the $\delta^{15}N$ data of bulk samples to distinguish between suspension and deposit feeding. This is because SPOM represented suspended material of multiple origins including lentic phytoplankton exported to the stream, attached algae sloughed from surfaces, and FBOM in constant exchange between suspension and deposition. Lake phytoplankton, however, passed through the study reach very quickly (~32 min) due to high current velocities and this short residence time may have prevented much uptake of labeled ammonium by plankton that entered the study reach already in suspension. Labeled microbial portions of SPOM were more likely derived from resuspended stream sediments and benthic algae. In light of the probability of preferential assimilation of microbial and algal components of food, the mixing model becomes questionable. The possibility of preferential assimilation does not, however, eliminate the potential for deposit feeding to exist in this community. The bivalves of Eagle Creek may to a

substantial extent feed directly on sediments and likely derive their nutrition from the living microbial and algal components of fine organic material on the sediment surface.

Role of bivalves in stream N cycling- Due to their large size and abundance, unionids represented the largest pool of consumer N in the stream ecosystem. The present study shows that the slow N turnover rate of unionids, however, effectively prevented N bound in biomass from cycling in the short term. The importance of this pool of N may appear upon the death of a unionid by releasing large amounts of N to the sediment in a localized area. Predation upon bivalves by terrestrial mammals such as raccoons represents a loss of this N from the stream.

Implications for conservation- Both the mixed diet of EPS + SPOM and differential assimilation explanations of patterns seen in this study could improve our understanding of the relationship between stream bivalves and sediment. For example, while improvements in the success of freshwater bivalve translocation efforts have been made by refining handling techniques, limited understanding of unionid ecology still impedes optimal site selection (Dunn and Sietman 1997). Poor success of translocation efforts might be explained in part by differences between locations in the microbial and algal components of sediment and seston. A radical change in sediment characteristics can be encountered by unionids during a translocation project, and living components of sediment may differ in abundance or palatability in the new location. Such differences in the sediment would affect deposit feeding directly and suspension feeding indirectly by altering one source of suspended organic matter. Changes in the microbial community of

sediment and seston may also explain why greater translocation success has been seen in projects that minimize the distance mussels are moved, especially to new locations in the same river (Valovirta 1998). Such alteration might not need to occur in the stream itself to have an impact if an upstream wetland or lake were providing an ecological subsidy of suspended organic material. Clearly we should consider how alteration of the sediment impacts the food sources of unionids within the context of the general ecology and conservation of these increasingly threatened organisms.

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Table 1. Hydraulic and geomorphological features of Eagle Creek, MI.

Slope[a]	0.6 %	
Water velocity[b]	24 cm s^{-1}	
Water depth[b]	19 cm	
Channel width[a]	5 m	
Discharge[b]	202 L s^{-1}	
Substrate[a]:	Sand and/or FBOM	54 %
	Gravel	35 %
	Cobble	10 %
Habitat[a]:	Riffle	90 %
	Run	10 %

a: Mean for study reach.

b: Mean during enrichment experiment.

Table 2. Biomass and nitrogen content of consumers in Eagle Creek, MI.

	g AFDM m^{-2}	g N m^{-2}
Unionids	1.93	0.196
Fishes	0.83	0.074
Macroinvertebrate Functional Feeding Groups:		
Shredders	0.11	0.010
Scrapers	0.07	0.007
Collectors	0.04	0.005
Filterers	0.02	0.002
Predators	0.08	0.010

Table 3: Chlorophyll *a* and relative abundance of planktonic and benthic algae in Eagle Creek, MI, on 20 Aug 1998. The wetlands occur between the lake outflow and the dripper.

	1200m upstream (Eagle Lake Outflow)	400m upstream of dripper	20m downstream of dripper	460m downstream of dripper
Chl a (mean $\mu g\ L^{-1}$)	2.15	5.72	3.15	2.14
(SD)	(0.02)	(0.87)	(0.34)	(0.20)
Cell density (cell L^{-1})				
Bacillariophyta	110	136	60	20
Chlorophyta	181	122	200	170
Chrysophyta	1	144	48	20
Cryptophyta	126	141	122	168
Cyanoprokaryota	10601	3862	2796	4393
Euglenophyta	0	9	3	0
Pyrrophyta	1	1	0	0
Total	10731	4157	2973	4594
Benthic-Pelagic Index	-5.4 E-02	-5.3 E-04	-1.9 E-06	-9.3 E-08

Table 4: Food resource partitioning for bivalves in Eagle Creek, MI, based on observed ^{15}N enrichment on day 42 of the experiment. Stomach gland $\delta^{15}N$ was used for bivalves unless otherwise noted in text.

Location (m)	% EPS	% SPOM
116	50	50
176	77	23
251	100[a]	
301	48	52
351	96	4
396	86	14
461	100[a]	
Mean	80	20
Unionid Muscle[b]	81	19
S. striatinum[b]	64	36

a: $\delta^{15}N$ of tissue slightly exceeded $\delta^{15}N$ of EPS at this location.

b: Natural abundance $\delta^{15}N$ with 1.7‰ correction for trophic level enrichment.

Figure 1. Natural abundance of ^{15}N in food resources and bivalves of Eagle Creek, MI. EPI = Epilithon, EPS = Epipsammon, FBOM = Fine Benthic Organic Matter, SPOM = Suspended Particulate Organic Matter, SPH STR = Sphaerium striatinum. EPS and SPOM are presumed to be the potential food resources for bivalves. Data for food resources are means for 8 collection dates at the reference station during the course of the enrichment experiment. Gland and muscle are isolated tissues from Pleurobema sintoxia + Fusconaia flava. The datum for Sphaerium striatinum is a composite of the whole soft tissue of several individuals. Error bars are ± 1 standard deviation.

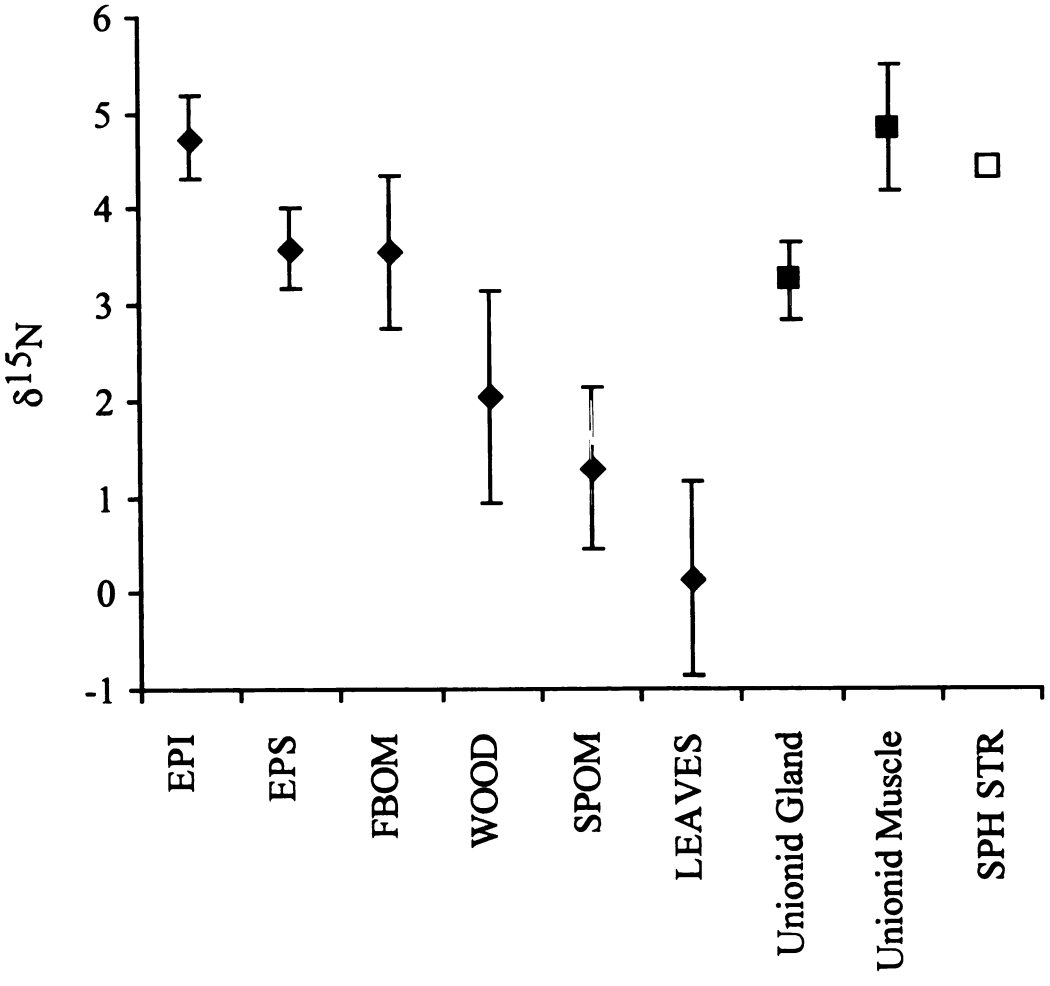

6
5
4
3
2
1
0
-1
δ15N
EPI
EPS
FBOM
WOOD
SPOM
LEAVES
Unionid Gland
Unionid Muscle
SPH STR

Figure 2. Enrichment of unionid tissues over the course of the ^{15}N tracer experiment, with $\delta^{15}N$ values corrected for background $\delta^{15}N$. Error bars are ± 1 standard deviation.

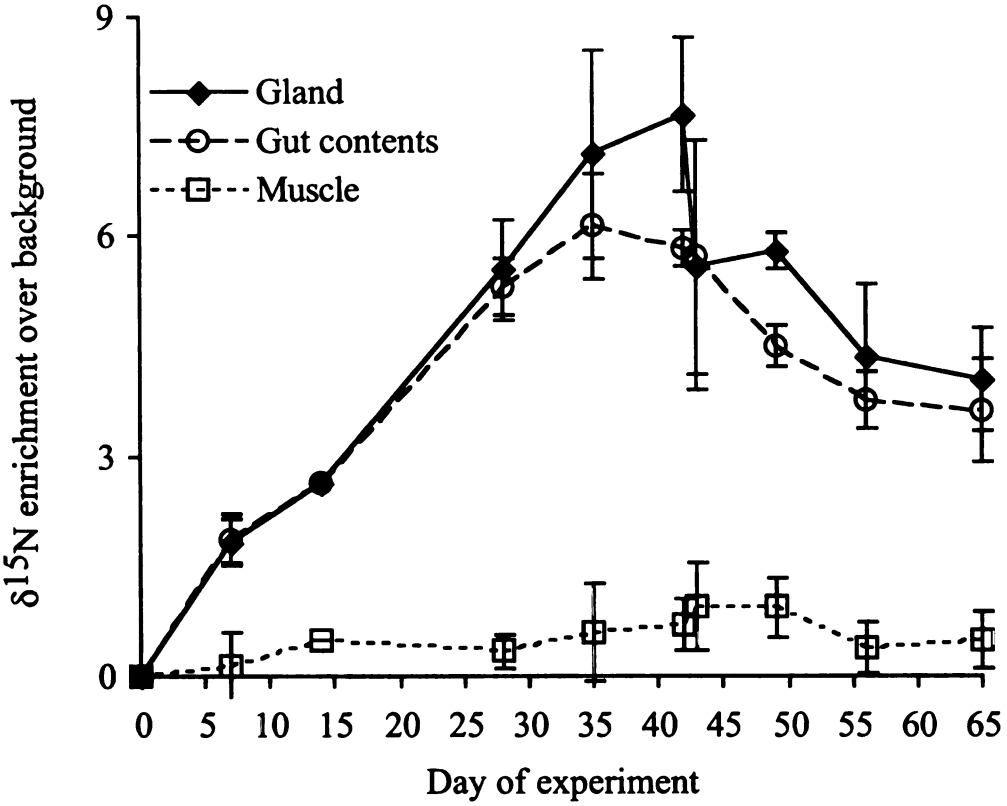

9
6
3
0
Gland
Gut contents
Muscle
δ15N enrichment over background
0 5 10 15 20 25 30 35 40 45 50 55 60 65
Day of experiment

Figure 3. A) Enrichment of potential food resources of bivalves over the course of the ^{15}N tracer experiment, with $\delta^{15}N$ values corrected for background $\delta^{15}N$. The last SPOM datum was calculated by extrapolating the decay rate. Error bars are ± 1 standard deviation. B) Calculated ^{15}N enrichment of dissolved NH_4^+ during the experiment, at the dripper. The enrichment decreased across the study reach as the $^{15}NH_4^+$ was assimilated or nitrified, and at the main bivalve sampling site the $\delta^{15}N$ of $^{15}NH_4^{+}$ was 70-75% that of the dripper (Hamilton et al. 2001).

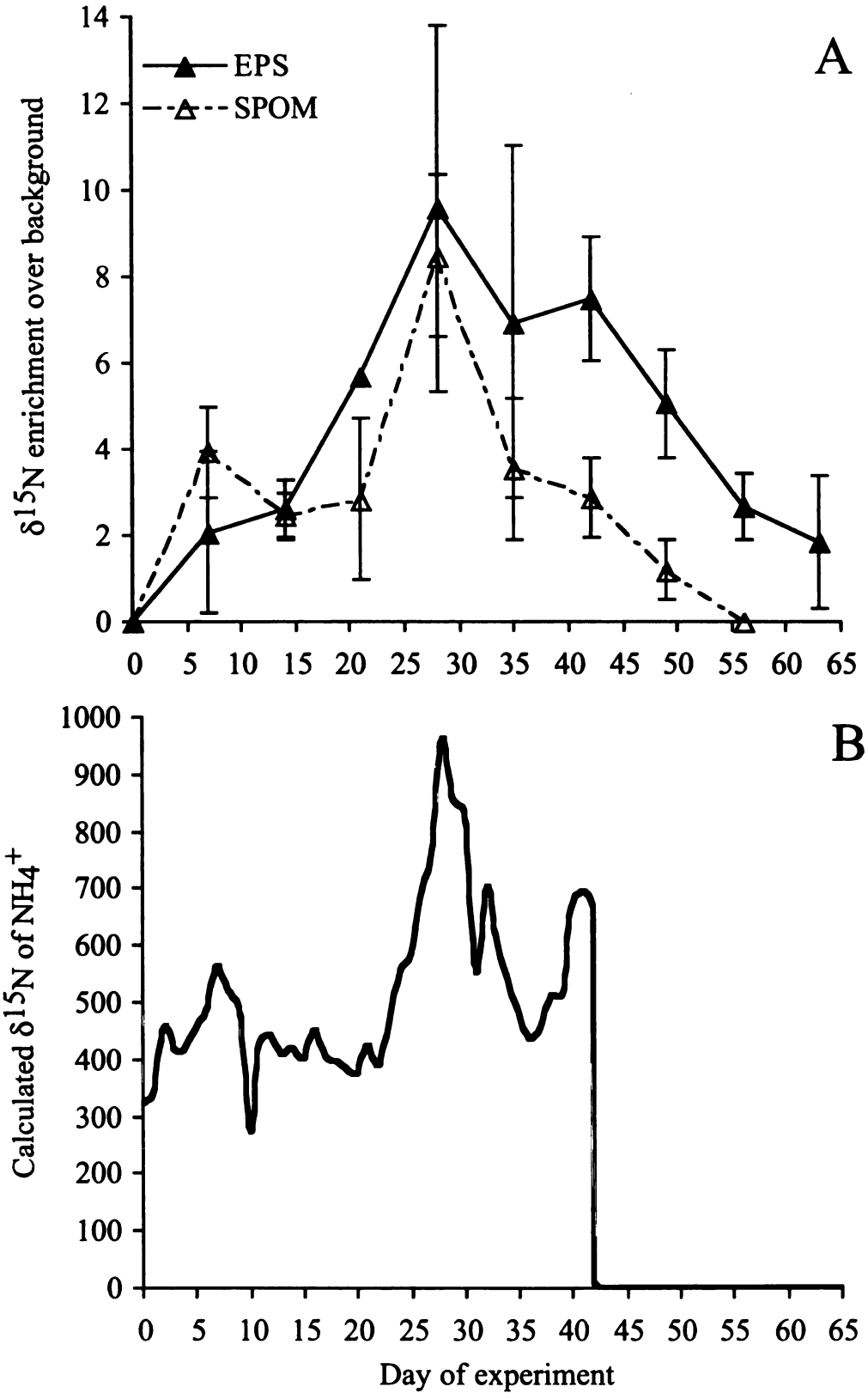

A
EPS
SPOM
δ15N enrichment over background
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65
B
Calculated δ15N of NH4+
0
100
200
300
400
500
600
700
800
900
1000
0 5 10 15 20 25 30 35 40 45 50 55 60 65
Day of experiment

Figure 4. Comparison of ^{15}N enrichment in unionid stomach glands and gut contents on day 42 ($\delta^{15}N$ not corrected for background levels of enrichment). PLE + FUS = Pleurobema sintoxia + Fusconaia flava; ELL DIL = Elliptio dilitata; STR UND = Strophitus undulatus; LAM CAR = Lampsilis cardium; VEN ELL = Venustaconcha ellipsiformis; ACT LIG = Actinonais ligamentina; LAS COS = Lasmigona costata; VIL IRI = Villosa iris.

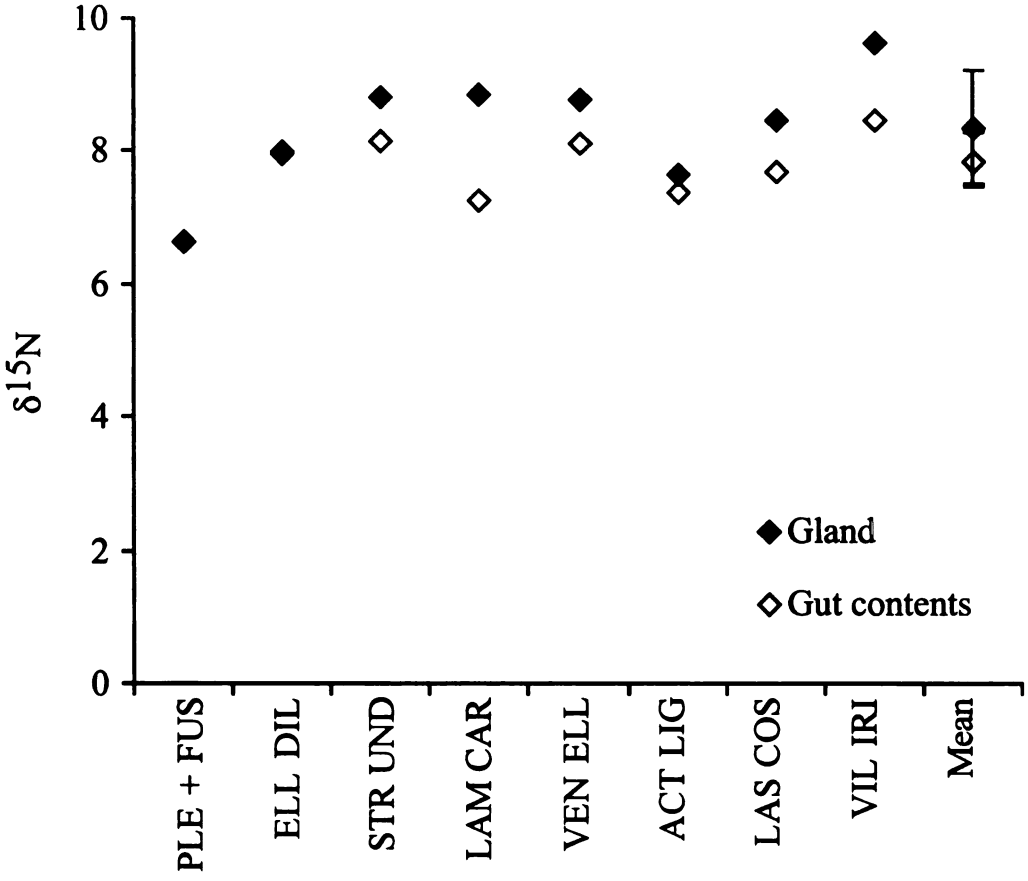
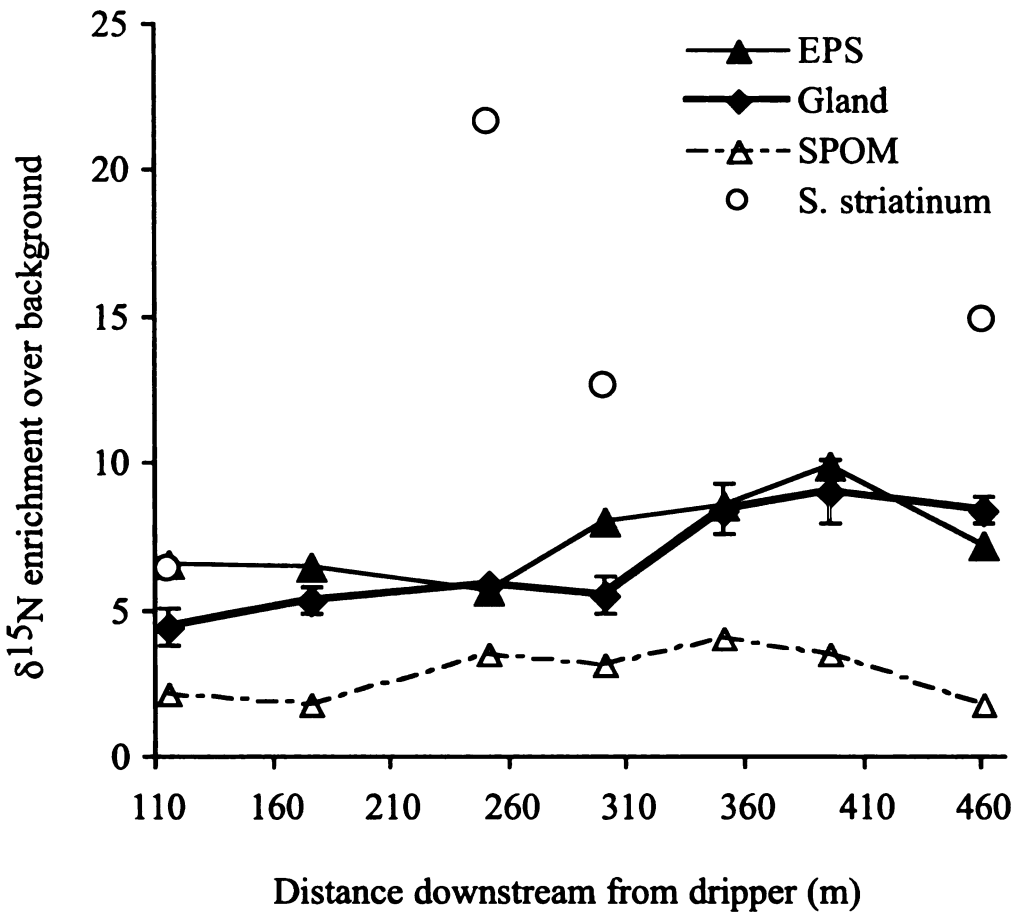

10
8
6
4
2
0
δ15N
Gland
Gut contents
PLE + FUS
ELL DIL
STR UND
LAM CAR
VEN ELL
ACT LIG
LAS COS
VIL IRI
Mean

Figure 5. Longitudinal patterns of ^{15}N enrichment for unionid stomach-gland samples, Sphaerium striatinum whole-body samples, and potential food resources across the study reach on day 42 of the ^{15}N enrichment experiment (values corrected for background $\delta^{15}N$). Error bars are ± 1 standard deviation.


25
20
15
10
5
0
δ15N enrichment over background
EPS
Gland
SPOM
S. striatinum
110
160
210
260
310
360
410
460
Distance downstream from dripper (m)

Chapter 3

FOOD WEB INTERACTIONS BETWEEN ZEBRA MUSSELS, ZOOPLANKTON, AND LARVAL FISH

Abstract

Food web interactions between exotic invasive zebra mussels (Dreissena polymorpha), zooplankton, and native larval bluegill (Lepomis macrochirus) were examined with a mesocosm experiment. Hatchling larval bluegill collected from nests were reared in the presence of size-structured populations of zebra mussels in 1,500 L limnocorrals suspended in an artificial pond for two weeks. Chlorophyll a and other limnological variables, and zooplankton abundance and biomass (including copepod nauplii and rotifers) were monitored over time. During their first two weeks of life, larval fish reared in the presence of mussels grew 24% more slowly than fish reared alone. Competition between mussels and bluegill for food in the form of microzooplankton can explain the differential growth rates, but also likely was an indirect competition via starvation of the zooplankton community as zebra mussels consumed the phytoplankton. Either direct or indirect trophic competition between zebra mussels and obligate planktivores may result in ecological harm as zebra mussels spread throughout inland lakes of North America.

Introduction

As zebra mussels (Dreissena polymorpha) expand their range in North America new ecosystems become invaded. Beginning with the Great Lakes and continuing through the Mississippi River drainage network, the zebra mussel invasion is currently spreading among small inland lakes. The southern peninsula of the State of Michigan is at the forefront of this invasion due to its proximity to the Great Lakes, its high density of inland lakes, and its high rate of inter-lake recreational boat traffic (Buchan and Padilla 1999). Over 150 of Michigan's inland lakes are known to have zebra mussels and over 75% of Michigan's thousands of inland lakes possess suitable pH and calcium to support zebra mussels (Ramcharan et al. 1992, Klepinger 2000).

Among the ecological concerns of zebra mussel invasion are food web interactions. Zooplankton communities, for example, have been negatively affected by the invasion of zebra mussels in the Great Lakes and Hudson River. Zooplankton abundance dropped 55-71% following mussel invasion in Lake Erie, with the smallest of these animals more heavily impacted (MacIsaac et al. 1995). The total biomass of zooplankton in the Hudson River declined 70% following mussel invasion, due both to a reduction in large zooplankton body size and reduction in small zooplankton abundance (Pace et al. 1998). These effects can be attributed to reduction of available food (phytoplankton) due to competition with zebra mussels and because the mussels feed directly on microzooplankton such as copepod nauplii and rotifers. Less well understood are interactions between zebra mussels and higher trophic levels including fish.

Although the feeding habits of the larval stages of many fish species are not well understood, larvae are most often visually-oriented predators of zooplankton during the earliest life stage, and they experience high mortality rates (Gerking 1994). Body size is generally believed to affect survival because larger size confers advantages in starvation resistance, swimming ability, predation avoidance, foraging ability, and overwinter survival (Adams et al. 1982, Werner and Gilliam 1984, Blaxter 1986, Crowder et al. 1987, Miller et al. 1988, Pepin 1991, Schindler 1999). Prey availability can also affect larval size development (Mills et al. 1989, Rettig and Mittelbach in press). It is thus logical that if zebra mussels can affect the abundance or size structure of zooplankton communities, larval fish growth or survival can be diminished (Richardson and Bartsch 1997, Thayer et al. 1997, Trometer and Busch 1999). No studies to date have employed experiments to examine interactions between zebra mussels and larval fish.

One fish species that might compete for food with zebra mussels in inland lakes is bluegill (Lepomis macrochirus). In early summer, bluegill hatch from eggs in nests built in the littoral zone, migrate to the limnetic zone, then return to the littoral zone in the fall (Faber 1967, Werner 1967, 1969). Larval bluegill feed on microzooplankton such as copepod nauplii and rotifersas well as small-bodied cladocerans while in the limnetic zone (Seifert 1972, Barkoh and Modde 1987, Bremigan and Stein 1994, Welker et al. 1994).

The purpose of this study was to test whether zebra mussels negatively impact the growth and survival of larval bluegill in an experimental setting using limnocorrals suspended in a pond as mesocosms. A negative impact of zebra mussels on larval bluegill could occur by several mechanisms: 1) The entire zooplankton community might decline

due to starvation if algal biomass [as indicated by chlorophyll a (chl a) concentrations] decline in the presence of zebra mussels; 2) Microzooplankton abundance might decline in the presence of zebra mussels independently of macrozooplankton dynamics if zebra mussels heavily consumed microzooplankton and thus competed directly with larval bluegill for food and if microzooplankton were less tolerant of lower algal concentrations; 3) Toxic conditions might be produced by zebra mussels through excretion of ammonia or consumption of oxygen.

Methods

Twelve 1.5-m deep, 1-m^2 limnocorrals holding 1,500 L of water each were installed in an artificial pond at the Experimental Pond Facility of the W. K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI, USA. The artificial pond measured 29 m in diameter and was 1.8-m deep, and did not have zebra mussels but contained redear sunfish (L. microlophus) that maintained a zooplankton community dominated by small-bodied cladocerans and copepods. A balanced factorial design of zebra mussels (ZM) x bluegill (BG) with three replicates each of the four treatments (ZM, BG, ZM+BG, control) was used. Limnocorrals were arranged in two parallel rows and treatments assigned in a stratified random fashion with one replicate of each treatment at the end of a row. The limnocorrals were filled with pond water screened through 100-μm mesh and then stocked with natural densities of zooplankton collected from the pond with a 100-μm net and mixed in a large container before removing aliquots for each replicate.

Zebra mussels were collected from nearby Gull Lake, then held in a tank with continuous flow-through of Gull Lake water and allowed to attach to Plexiglass sheets for two weeks. Size-structured populations of 200 mussels per treatment were created: 25 of 20-30 mm, 75 of 10-20 mm, and 100 of <10-mm, for a total of approximately 2 g AFDM / m^2 of the mussel's soft tissue (Young et al. 1996). The Plexiglass sheets with attached zebra mussels were suspended vertically 50 cm below the water surface in the limnocorrals. Sediment traps were attached immediately under the Plexiglass sheets. Control treatments received Plexiglass sheets and sediment traps without any attached mussels. Dead mussels were removed from the experiment when detected, but any change in mussel abundance within a replicate was disregarded in the data analysis due to a very low mortality rate.

Hatchling bluegill were collected directly from nests in nearby Warner Lake (Barry County) and reared in the lab for several days. When the fish reached the swimming stage they were fed brine shrimp nauplii. Fish that had fed upon nauplii were then selected from a single cohort in one tank for use in limnocorrals. The standard length of larval fish from the cohort used for the experiment was 5.5 mm (SD ± 0.16) estimated by measuring 20 fish not used in the experiment but from the same cohort. Twenty larval fish were used per treatment. Control treatments had no fish. Bluegill and zebra mussels were added to the mesocosms at the same time three days after zooplankton had been added.

Samples were taken approximately every three days from 9 July to 22 July 1999. The experiment was terminated after two weeks in order to focus on the period when larval fish and zebra mussel diets overlap. Temperature, O_2, pH, and specific

conductance were measured with a YSI multisensor. Sestonic chl a was measured by flourometry (Welschmeyer 1994). Measurements and sampling of water were performed before moving Plexiglass plates and sediment traps to prevent sediment from being resuspended and thereby contaminating the water column. Transparency was measured with a light meter. A two-tiered regime was used to sample zooplankton: 2 1-m tows using a 10-cm diameter 100-μm net for macrozooplankton and the filtration of 4 L of water through 30-μm mesh for microzooplankton. All zooplankton were stored in 95% ethanol. To collect the larval bluegill at the conclusion of the experiment, a 1-m diameter 500-μm net was pulled through each limnocorral until three consecutive tows yielded no fish. Fish were placed into containers, put on ice, and measured the day of collection.

Zooplankton were enumerated with a dissecting microscope. Body size was measured for macrozooplankton only using a digitizing pad. Entire zooplankton samples were enumerated, but only up to 50 individuals of a species were measured for calculation of biomass using published length-mass regressions (McCauley 1984).

Several measures were undertaken to ensure that zebra mussels did not escape from the experiment (Reid et al. 1993). The tops of mesocosms were covered with gray fiberglass window-screen to prevent animals from entering the limnocorrals and transporting water potentially infested with zebra mussel veligers to other experimental ponds. After the experiment, the limnocorrals were treated with Rotenone and chlorine before the bags were cut away from their frames. Lastly, the entire pond was drained (with the water infiltrating nearby soil) and allowed to dry completely before being refilled.

Time series measurements were analyzed by Repeated Measures Analysis of Variance (rmANOVA) using SAS for Windows v.8 (von Ende 1993). Only days 3 through 13 were used for statistical analysis because day zero represented initial conditions, i.e. those measurements were taken before treatment factors were in place. In rmANOVA terminology, between-subjects effects refer to differences between the means of treatments for the time period and within-subject effects refer to differences between treatments in the temporal pattern of response (i.e. the time x treatment interactions). If a sphericity test on orthogonal components was rejected then Greenhouse-Geisser corrected rmANOVA results were used for within-subject effects, otherwise Multivariate Repeated Measures ANOVA (MANOVAR) results were used (SAS Institute 1999). Larval fish sizes were compared with a pooled-variance t-test.

Results

Larval bluegill reared in the presence of zebra mussels were shorter at the end of the experiment than larval bluegill reared alone (initial standard lengths were 5.5 mm ± 0.16, final standard lengths were 8.4 mm ± 0.03 SE in ZM+BG treatments and 9.3 mm ± 0.15 SE in BG treatments, $P = 0.03$, $t = 5.33$, Table 1). Thus bluegill growth rates were about 24% lower in the ZM+BG compared to the BG treatments (0.22 mm/d and 0.29 mm/d, respectively). There was no significant difference in survival among treatments (initial count was 20 fish, final counts were 10.7 ± 1.7 SE fish in ZM+BG treatments and 12.0 ± 2.6 SE fish in BG respectively, $P = 0.69$, $t = 0.43$, Table 1).

Macrozooplankton included calanoid and cyclopoid copepods, as well as the cladocerans Daphnia ambigua, Bosmina longirostris, Diaphanosoma spp., Scaphloberis

spp., Simocephalus spp., Chydorus spp., and Sida spp. Although significant between-subjects effects of zebra mussels and bluegill were found for total macrozooplankton biomass ($P = 0.0021$, $F = 19.82$; $P = 0.0076$, $F = 12.53$, respectively), greater initial macrozooplankton densities were present in control treatments due to the presence of greater numbers of Bosmina and copepods in spite of attempts to equalize densities across all enclosures (Fig. 1A). This difference in initial conditions confounds interpretation of zebra mussel effects on macrozooplankton (between-subjects effects). Total macrozooplankton biomass fell over time during the experiment (within-subject effect, G-G Time $P = 0.0103$, $F = 6.51$) but zebra mussels and bluegill had no within-subject effect (Table 2).

Microzooplankton included copepod nauplii and the rotifers Keratella cochlearis and Polyarthra vulgaris. Initial microzooplankton densities were less variable among all treatments (Fig. 1B). Both zebra mussels and bluegill caused significant between-subjects effects, reducing the abundance of microzooplankton ($P = 0.0011$, $F = 24.91$; $P = 0.0300$, $F = 6.94$, respectively, Table 3). Temporal dynamics of the major microzooplankton taxa were variable. Copepod nauplii dominated the microzooplankton count and drove the temporal pattern seen in figure 1B, where nauplii increased over time in control treatments, declined quickly in ZM and ZM + BG treatments, and were affected by fish late in the experiment in BG treatments. Keratella abundance dropped to below detectable levels in all treatments by the third day. Polyarthra was reduced to low levels by day three and held at low constant levels in ZM and ZM+BG treatments while increasing in abundance in control treatments.

Within the control treatment, chl a fell steadily from a mean of 2.70 μg/L to a mean of 1.21 μg/L (Fig. 2A). Zebra mussels further decreased the concentration of chl a (between subjects $P < 0.0001$, $F = 415.39$). Zebra mussels increased the transparency of the water column (between subjects $P = 0.0001$, $F = 46.27$, Fig. 2B). Most of the impact of zebra mussels occurred within the first three days. Transparency decreased in all treatments between days 3 and 6.

Specific conductance (corrected to 25°C) fell from an initial experiment-wide mean (± SD) of 315 (± 1.3) μS/cm to 311 (± 1.7) μS/cm on day 3 and then remained level. Dissolved oxygen fell from an initial experiment-wide mean (± SD) of 75 (± 4) % saturation to 54 (± 7) % saturation and then remained level. The pH of all treatments rose from an experiment-wide mean (± SD) of 7.67 (± 0.03) to 7.75 (± 0.05) by day 9 (day 13 pH data were not available). Temperature did not vary among treatments and ranged between 23 and 26 °C during the experiment.

Discussion

Zebra mussels substantially reduced the growth of bluegill during the critical larval stage. The most likely cause of this effect was competition with fish for consumption of microzooplankton. A reduction in the abundance of algae probably also impacted the microzooplankton community. Toxic conditions were not produced by zebra mussels.

The temporal pattern of microzooplankton abundance suggested direct consumption by zebra mussels. Temporal patterns of rotifer abundance mirrored those of

phytoplankton with an initial decline followed by low abundances, although Keratella abundance was below detectable levels after day 3. Sustained feeding by zebra mussels appeared to prevent nauplii abundance from increasing as seen in the control treatment.

Although the susceptibility of microzooplankton including the rotifers Keratella and Polyarthra to predation by zebra mussels has been conclusively demonstrated by previous researchers (Shevtsova et al. 1986, MacIsaac et al. 1991, MacIsaac et al. 1995), additional interactions may have existed between zebra mussels and microzooplankton in the present study. Microzooplankton could have been incorporated into pseudofeces instead of being consumed by mussels. The net effect of pseudofeces incorporation of microzooplankton would, however, have been the same for larval fish, i.e. exploitative competition for resources. Less probable was either overall or selective consumption of algae by zebra mussels having caused selective suppression of microzooplankton through exploitative competition for algal food resources. Zebra mussels preferentially feed on particles 5 to 45 μm in size, a range which overlaps that which rotifers consume (4 to 17 μm) and that which copepods consume (<1μm to 1mm) (Gilbert 1985, Sprung and Rose 1988, Williamson 1991). Zebra mussels are also able to feed on a larger range of particles, from <1 μm to 750 μm including bacterioplankton, Ankistrodesmus cells and cyanobacterial filaments (Ten Winkel and Davids 1982, Cotner et al. 1995, Horgan and Mills 1997). Zebra mussels were thus unlikely to have selected a size range of particles that was detrimental to microzooplankton and simultaneously not detrimental to macrozooplankton.

Macrozooplankton abundance did not decline to a greater degree in the presence of zebra mussels. This result is consistent with the idea that zebra mussels directly

consumed microzooplankton and did not counsume macrozooplankton. Larger zooplankton, however, may have tolerated possible starvation conditions for longer than smaller zooplankton, and hence may not have declined on the short time scale of the experiment. While chl a concentrations were reduced by zebra mussel consumption within the first three days, macrozooplankton biomass fell relative to initial values in all treatments. In the ZM treatment, macrozooplankton biomass actually rose between days 6 and 13. The difference in initial macrozooplankton biomass densities could be attributable to uneven stocking despite attempts to add equal amounts. While this confounds comparison of between-subjects effects, the lack of a statistically significant within-subjects effect of zebra mussels on macrozooplankton biomass shows that the decline in macrozooplankton biomass in treatments with zebra mussels occurred with the same temporal pattern as in other treatments.

It is possible that reduced phytoplankton abundance in treatments with zebra mussels reduced available energy to copepods, and that as a result copepod reproduction rates might have been lower in treatments with zebra mussels (Williamson 1991). While the average biomass of an individual copepod increased in all treatments, it is not known whether this reflects the growing of organisms or the culling of small individuals from the population.

No toxic conditions were produced by zebra mussels. Oxygen concentrations were not reduced in any treatment. While ammonium concentrations changed during the experiment, rising during the first half then falling during the second half, zebra mussels had no effect. Moreover, ammonium concentrations never exceeded toxic levels.

The potential for zebra mussels to affect bluegill growth in natural systems should occur during the earliest life stages when bluegill are most restricted in their diet. Richardson and Bartsch (1997) studied zebra mussel-bluegill food web interactions in mesocosms using omnivorous juvenile bluegill (34-mm long) that were not limited to microzooplankton, and found that the growth of bluegill was not affected by the presence of zebra mussels. Competition with zebra mussels should not occur during later life stages when bluegill display more omnivorous feeding behavior. Several questions, however, affect the potential for and biological significance of bluegill-zebra mussel competition in natural systems.

The obvious question is whether zebra mussels have actually had an effect on microzooplankton abundance in inland lakes. MacIsaac et al. (1991) speculated that vertical zonation of zooplankton and poor water column mixing might ameliorate the impact of microzooplankton predation by benthic zebra mussels in the Great Lakes, and the same could be true of small inland lakes. The epilimnion of a small inland lake, however, is arguably in greater contact with the benthos than the epilimnia of the Great Lakes. An exception would be where the Great Lakes are shallow, like the western Basin of Lake Erie and Saginaw Bay. If obligatory feeding on microzooplankton is restricted to a short enough time period then larval fish might survive long enough to outgrow reliance on the size class of zooplankton fed upon by zebra mussels (Lazzaro 1987). This ontogenetic niche shift may include feeding on benthic or littoral invertebrates and may help to explain why detrimental impacts on fish populations have not yet been observed in inland lakes invaded by zebra mussels (Gopalan et al. 1998, Rutherford et al. 1999, Trometer and Busch 1999, Mayer et al. 2000, Idrisi et al. 2001).

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Table 1. Pooled-variance t test of zebra mussel (ZM) effects on larval bluegill (BG) growth and survival.

	Treatment	N	Mean	SE	df	t	P
BG Length (mm)	ZM + BG	3	8.5	0.03	2.2	5.330	0.0274
	BG	3	9.3	0.15			
BG Survival (count)	ZM + BG	3	10.7	1.7	3.4	0.426	0.6956
	BG	3	12.0	2.6			

Table 2. Univariate repeated measures ANOVA test for effects of zebra mussels and larval bluegill on macrozooplankton biomass over the course of the experiment (days 3 to 13).

	Source	df	MS	F	P
Between-subjects	ZM	1	0.021	19.82	0.0021
	BG	1	0.013	12.53	0.0076
	ZM X BG	1	0.002	2.54	0.1493
	Error	8	0.001		
Within-subject	Time	3	0.002	6.51	0.0103*
	Time X ZM	3	0.000	2.08	0.1621*
	Time X BG	3	0.001	3.21	0.0720*
	Time X ZM X BG	3	0.000	2.06	0.1640*
	Error	24	0.000		

* Greenhouse-Geisser corrected value.

Table 3. Univariate repeated measures ANOVA and multivariate repeated measures ANOVA (MANOVAR) testing for effects of zebra mussels and larval bluegill on microzooplankton abundance over the course of the experiment (days 3 to 13).

	Source	df	MS	F	P
Between-subjects	ZM	1	116328.5	24.91	0.0011
	BG	1	32396.0	6.94	0.0300
	ZM X BG	1	15950.5	3.42	0.1018
	Error	8	4670.6		
Within-subject*	Time	3		5.00	0.0452
	Time X ZM	3		18.37	0.0020
	Time X BG	3		19.92	0.0016
	Time X ZM X BG	3		18.57	0.0019

* MANOVAR (sphericity test of orthogonal components: Mauchly's Criterion = 0.07, $\underline{X}^2$ = 17.8, $\underline{P}$ = 0.0032).

Figure 1. A) Total macrozooplankton biomass response over the course of the experiment in the presence of zebra mussels and larval bluegill. Shown are means ± standard deviation. B) Total microzooplankton abundance response over the course of the experiment in the presence of zebra mussels and larval bluegill. Shown are means ± 1 standard deviation.

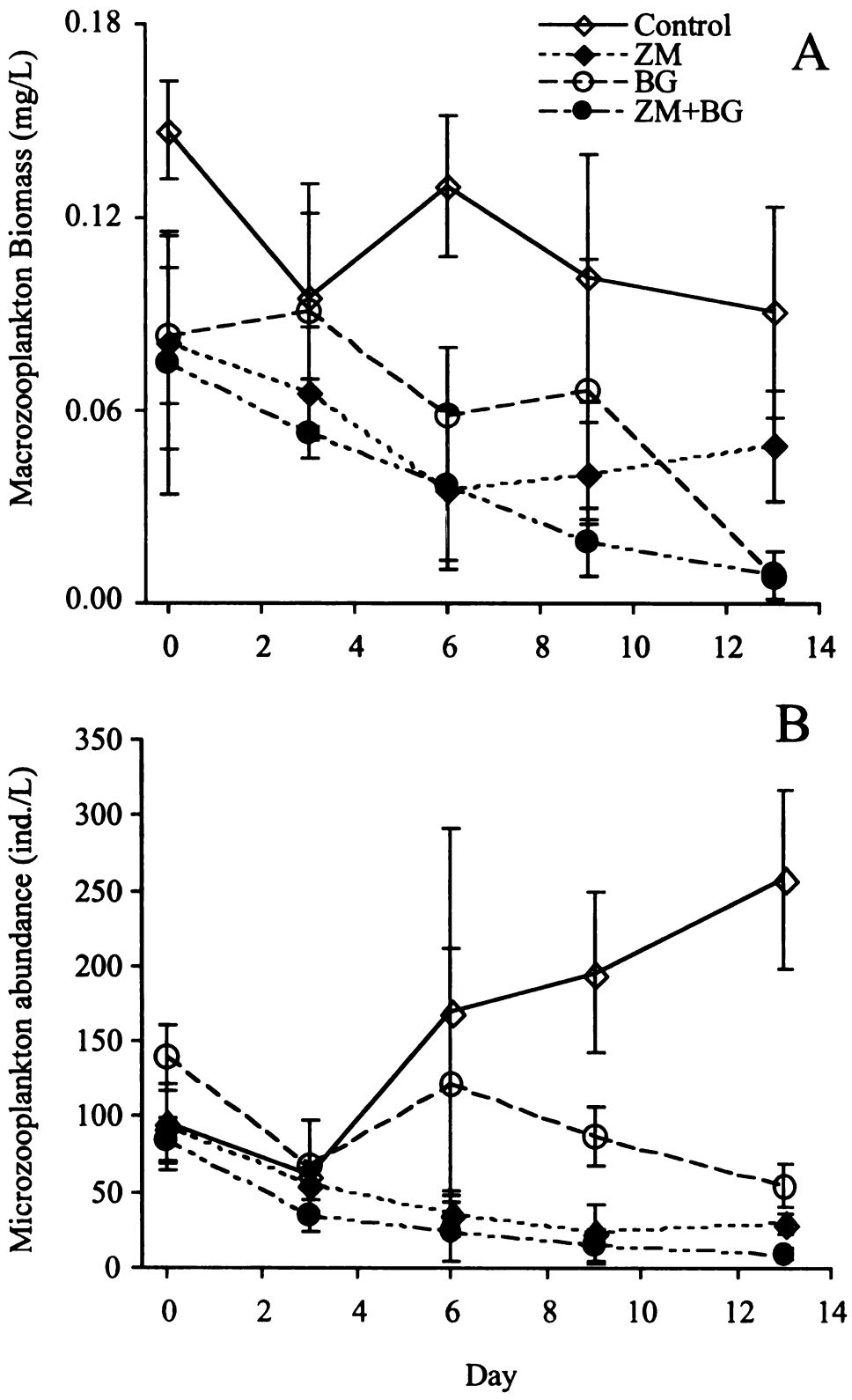
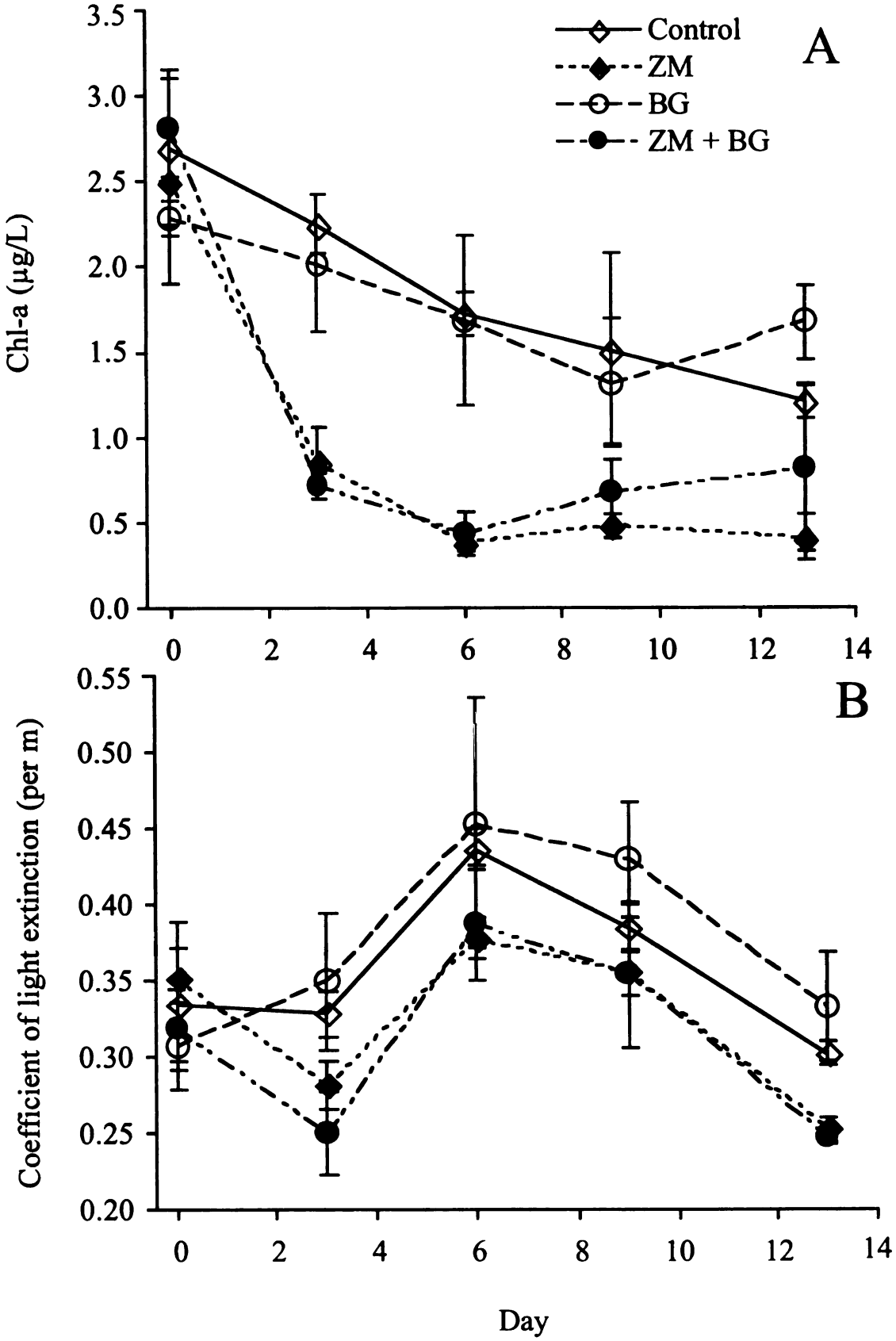

Control
ZM
BG
ZM+BG
A
B
Macrozooplankton Biomass (mg/L)
Microzooplankton abundance (ind./L)
0.18
0.12
0.06
0.00
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
Day

Figure 2. A) Chlorophyll-a response over the course of the experiment in the presence of zebra mussels and larval bluegill. Shown are means ± standard deviation. B) Transparency response over the course of the experiment in the presence of zebra mussels and larval bluegill. Transparency decreases with an increasing coefficient of light extinction. Shown are means ± standard deviation.


Control
ZM
BG
ZM + BG
A
B
Chl-a (µg/L)
Coefficient of light extinction (per m)
Day
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0
2
4
6
8
10
12
14

Chapter 4

ALTERATION OF NUTRIENT CYCLING BY ZEBRA MUSSELS

Abstract

The effects of the exotic zebra mussel (Dreissena polymorpha) on nutrient cycling in small inland lakes was investigated in a mesocosm experiment. Dissolved and particulate nutrients and sedimented material were monitored for two weeks in 1,500 L limnocorrals stocked with zebra mussels and/or larval bluegill sunfish (Lepomis macrochirus). Zebra mussels reduced concentrations of sestonic carbon, nitrogen and phosphorus and increased concentrations of total dissolved phosphorus (TDP) and soluble reactive phosphorus (SRP). Zebra mussels did not affect the relative abundance of nutrients as measured by dissolved inorganic nitrogen (DIN):TDP, sestonic N:sestonic P, and Sestonic C:Sestonic N. The ratio of DIN+sestonic N:total P increased in non-zebra mussel treatments following rain events that contained high levels of nitrogen, reflecting the flow of introduced nitrogen to periphyton due to removal of phytoplankton by zebra mussel filtration. Sedimented material in zebra mussel treatments varied over time in abundance and chemical composition. Alteration of foodweb structure by zebra mussels had a more important impact on nutrient cycling than nutrient regeneration or biodeposition.

Introduction

Small inland lakes in the lower peninsula of Michigan are currently being invaded by zebra mussels at an alarming rate due to their proximity to the Laurentian Great Lakes and high rate of inter-lake recreational boat traffic (Buchan and Padilla 1999). Most research of North American zebra mussel invasions, however, has been carried out in the Laurentian Great Lakes, large rivers, and the inland Lake Oneida. Ecosystem effects of zebra mussel invasions that have been documented in larger water bodies do not necessarily apply to small inland lakes. Important differences between the Great Lakes, large rivers and inland lakes exist that should affect ecosystem response. In Saginaw Bay of Lake Huron, for example, the outer bay hosted low zebra mussel populations and behaved independently of the inner bay, which hosted high zebra mussel populations (Fahnenstiel et al. 1993). In Lake Erie, the eastward expansion of the invasion could be charted in the successive reduction of algae at stations along a transect (Nichols and Hopkins 1993). Thus, due to their sheer size, the Great Lakes do not respond as whole units to ecosystem perturbation. Inland lakes may respond to zebra mussel invasion more rapidly and homogeneously. Inland lake morphometry, with a larger proportion of littoral zone, might also affect ecosystem response, as should larger and more diverse communities of macrophytes in the littoral zone. Large river systems such as the Mississippi and tidal Hudson River are distinct from inland lakes because of their flow regimes and inorganic suspended matter. Such differences might produce radically different responses to zebra mussel invasion in inland lakes compared to previously observed ecosystems.

Zebra mussels remove phytoplankton and other seston from the water column and biodeposit much of this material to the benthos in altered forms. This benthic-pelagic coupling, along with excretion of dissolved materials into the water column, has fueled speculation that zebra mussels might significantly alter nutrient cycling regimes and food webs in lakes (Ludyanskiy 1993, MacIsaac 1996). Such speculation has ranged from simply promoting benthic communities to shifting lakes from a stable equilibrium of high turbidity and phytoplankton domination to another stable equilibrium of clear water and macrophyte domination (Scheffer et al. 1993).

Studies of zebra mussel biodeposition began with examinations of factors affecting rates of pumping, filtration, clearance, and pseudofeces production (Morton 1971, Walz 1978). This knowledge has been refined through studies of the effects of specific algal species and concentrations on biodeposition (Sprung and Rose 1988, Berg et al. 1996), effects of clay on biodeposition (MacIsaac and Rocha 1995), in situ biodeposition rates (Klerks et al. 1996), effects of biodeposition on contaminant cycling and food web transfer of contaminants (Bruner et al. 1994, Klerks et al. 1997), use of biodeposition by zebra mussels for water quality management (Reeder and Bij de Vaate 1990, 1992, Noordhuis et al. 1992), and effects of biodeposition on benthic macro-invertebrate abundance (Thayer et al. 1997). Less common are investigations of biodeposit composition. Ten Winkel and Davids (1982) noted that pseudofeces pellet composition from individual mussels varied greatly in the relative abundance of algae and the species present. Roditi et al. (1997) examined in situ zebra mussel biodeposit composition in depth and found that sediment deposited by zebra mussels contained more

organic matter, more live algae, higher rates of bacterial production, and lower C:N ratios than passively deposited material.

When large populations of zebra mussels become established in small inland lakes, biodeposit composition might become important to the overall nutrient cycling. For example, Mellina et al. (1995) speculated that biodeposition played a small role in the removal of nutrients from the water column of a microcosm, but that assimilation of nutrients into zebra mussel biomass and resuspension of biodeposits were important processes for nutrient cycling. Holland et al. (1995) speculated that resuspension of zebra mussel biodeposits may alter relative amounts of nutrients in the water column during seasonal turnover events in lakes. Mixing events might differentially alter pelagic nutrient availability due to episodic or seasonal resuspension of biodeposits. Temporal variation in biodeposit composition might affect benthic communities differently at different times.

Observations of the alteration of dissolved nutrient concentrations by zebra mussels include increases in Dissolved Inorganic Nitrogen (DIN, i.e. ammonium and nitrate) seen in experimental systems, Lake Huron, and Lake Erie (Gardner et al. 1995, Heath et al. 1995, Holland et al. 1995, Johengen et al. 1995), and increased Total Dissolved Phosphorus (TDP) and Soluble Reactive Phosphorus (SRP) concentrations in experimental systems and Lake Erie (Heath et al. 1995, Holland et al. 1995, James et al. 1997, James et al. 2000). Unresolved is whether such zebra mussels can alter the relative abundance of nutrients through regeneration.

The purpose of this study was to examine how zebra mussels affect nutrient cycling. Sedimented material and dissolved nutrients were monitored over time in a mesocosm experiment stocked with zebra mussels, zooplankton and larval fish. Two

primary questions were addressed: 1) Can zebra mussels alter the relative availability of potentially limiting nutrients as indicated by pelagic nutrient ratios? 2) How does biodeposit content change as the mussels deplete available food?

Methods

This study was conducted in an artificial pond 29 m in diameter and 1.8-m deep at the Experimental Pond Facility of the W. K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI, USA. The pond did not have zebra mussels but contained redear sunfish (L. microlophus) that maintained a zooplankton community dominated by small-bodied cladocerans and copepods. Twelve 1.5-m deep, 1-m^2 limnocorrals holding 1,500 L of water each were installed, filled with pond water screened through 100-µm mesh, and stocked with natural densities of zooplankton collected from the pond with a 100-µm net and mixed in a large container before removing aliquots for each replicate. Limnocorrals were arranged in two parallel rows and the treatments with zebra mussels (ZM), bluegill (BG), ZM+BG, and control assigned in a stratified random fashion with one replicate of each treatment at the end of a row.

Zebra mussels collected from Gull Lake were held in a tank with continuous flow-through of Gull Lake water, placed on Plexiglass sheets, and allowed to attach for two weeks. Approximately 2 g AFDM (soft tissue) / m^2 of mussels represented by size-structured populations of 200 mussels were used in each treatment: 25 of 20-30 mm, 75 of 10-20 mm, and 100 of <10-mm (Young et al. 1996). Sediment traps were attached to

vertically oriented acrylic sheets with attached zebra mussels. The sheets were then suspended in limnocorrals 50 cm below the water surface. Control treatments received acrylic sheets without mussels and sediment traps.

Hatchling bluegill collected directly from nests in Warner Lake were reared in the lab for several days until reaching the swimming stage. Fish were then fed brine shrimp nauplii. Fish (standard length 5.5 ± 0.16 mm, estimated by measuring 20 fish not used in the experiment but from the same cohort) that consumed nauplii were selected from one tank for use in limnocorrals. Twenty larval fish were used per treatment. Control treatments received no fish. Bluegill and zebra mussels were added to the mesocosms at three days after zooplankton had been added. Samples were taken from 9 July to 22 July 1999. Temperature, O_2, pH, and specific conductance were measured with a YSI multisensor. Measurements and water sampling were performed prior to collecting sedimented material to prevent accidentally resuspended sedimented material from contaminating water samples.

Dissolved organic carbon (DOC) was measured with a high-temperature combustion DOC analyzer. Nitrate was measured with an ion chromatograph (detection limit of ca. 15 µg/L NO_3-N). Ammonium, particulate phosphorus (PP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), and phosphorus content of sedimented material were analyzed colorimetrically, after persulfate oxidation in the case of total P (Langner and Hendrix 1982, Aminot et al. 1997). Dissolved calcium was measured with a flame atomic absorption spectrophotometer.

Seston was collected on Gelman AE glass-fiber filters. Sestonic carbon, carbon content of sedimented material, sestonic nitrogen and nitrogen content of sedimented

material were measured with a CHN analyzer. Inorganic carbonates were removed from seston samples on filters by acid fumigation prior to CHN analysis. Sestonic $CaCO_3$ and associated P were measured by extraction of the material on AE filters overnight in dilute HNO_3 (0.5%), followed by analysis of dissolved Ca and SRP in the extract. Total sestonic phosphorus (TP) was calculated by summing PP and TDP. Sestonic chl a and chl a content of sediment were measured by flourometry (Welschmeyer 1994).

Measures were taken to prevent the spread of zebra mussels to other ponds at the facility (Reid et al. 1993). Mesocosms were covered with gray fiberglass window-screen to prevent animals from entering the limnocorrals and transporting water potentially infested with zebra mussel veligers. After the experiment, Rotenone and chlorine were added to limnocorrals before bags were cut away from frames. The entire pond was drained directly to soil and allowed to dry before being refilled.

Data from days 3 through 13 were used for time series analysis with Repeated Measures Analysis of Variance (rmANOVA) using SAS for Windows v.8 (von Ende 1993). Between-subjects effects refer to differences between the means of treatments for the time period and within-subject effects refer to time x treatment interactions. Greenhouse-Geisser (G-G) corrected rmANOVA results were used for within-subject effects if a sphericity test on orthogonal components was rejected, otherwise Multivariate Repeated Measures ANOVA (MANOVAR) results were used (SAS Institute 1999).

Results

Algal biomass and nutrients in the water column - Chlorophyll a fell steadily in the control treatment from a mean of 2.70 μg/L to a mean of 1.21 μg/L (see Chapter 3). The concentration of chl a fell to about 0.5 μg/L and stabilized in zebra mussel treatments after day 3 (between subjects $P < 0.0001$, $F = 415.39$). Transparency of the water column increased in treaments with zebra mussels (between subjects $P = 0.0001$, $F = 46.27$).

Prior to the addition of fish and zebra mussels, mean concentrations (± SD) of sestonic C, N, and P among all limnocorrals were 0.64 (± 0.17) mg/L, 23.3 (± 16.5) μg/L, and 3.47 (± 0.96) μg/L, respectively. Zebra mussels reduced sestonic carbon (between subjects $P = 0.0054$, $F = 14.29$, Fig. 1A) and nitrogen (between subjects $P < 0.0001$, $F = 101.32$, Fig 1B). Some sestonic phosphorus samples became contaminated due to the use of P-contaminated filters and were removed from statistical analysis. Subsequently time series analysis could not be used for sestonic phosphorus or nutrients ratios including sestonic phosphorus; instead, ANOVA was used to analyze only Day 3 data. Zebra mussels reduced mean sestonic phosphorus on Day 3 (ANOVA $P < 0.0001$, $F = 49.34$).

Our measurements failed to demonstrate an appreciable increase in acid-soluble calcium and phosphate isolated from seston samples. Both sestonic and dissolved calcium dropped by day 3 in all treatments, perhaps indicating calcium precipitation. overall temporal patterns, however, did not conform to expectations and there was no relation between acid-soluble Ca and P in the seston.

Other nutrients showed changes over the course of the experiment but with little or no effect of zebra mussels. Dissolved inorganic nitrogen (DIN) consisted chiefly of

ammonium as nitrate was not found above detectable levels. Ammonium followed a similar temporal pattern as DOC starting at a mean of 4.5 (± 0.4) µg/ L, peaking on day 6 at 37.4 (± 14.3) µg/L, and falling to 17.8 (± 11.9) µg/L on day 13, but zebra mussels had no effect on ammonium. The concentration of TDP fell in non-zebra mussel treatments from an initial experiment-wide mean of 6.1 (± 1.3) µg/L to 5.2 (± 0.4) µg/L by day 13, while TDP in zebra mussel treatments increased slightly (between subjects, $\underline{P} = 0.0049$, $\underline{F} = 14.74$). The concentration of SRP increased in all treatments from an initial experiment-wide mean of 1.2 (± 0.2) µg/L to an experiment-wide mean of 1.8 (± 0.6) µg/L; zebra mussel treatments on day 13 had concentrations of SRP that were < 1 µg/L greater than non-zebra mussel treatments (between subjects $\underline{P} = 0.0228$, $\underline{F} = 7.70$). Zebra mussels had no effect on TP on Day 3. Treatments with zebra mussels had different DIN + Sestonic N:TP molar ratios (between subjects, $\underline{P} = 0.0032$, $\underline{F} = 17.26$, Fig. 2), but zebra mussels did not significantly affect relative amounts of nutrients as measured by molar ratios of DIN:TDP, Particulate N:Particulate P (Day 3 only), and Particulate C:Particulate N. Dissolved organic carbon (DOC) increased from a mean (SD) of 7.4 (± 0.7) µg/L across all replicates on day 0 to 8.5 (± 0.8) µg/L on day 9 before falling back to 7.5 (± 0.8) µg/L on day 13 (within-subjects Time G-G $\underline{P} = 0.0091$, $\underline{F} = 6.23$), but zebra mussels had no effect on DOC concentrations. Mean (± SD) concentrations of dissolved calcium among all limnocorrals prior to the addition of fish and zebra mussels was 51.3 (± 1.3) mg/L. Mean dissolved calcium levels dropped in the control and BG treatments (by 5% and 2.5%, respectively) and rose slightly in the ZM and ZM+BG treatments (by 0.2% and 0.3% respectively) between day 0 and day 3, but returned to previous levels by the end of the experiment.

Sedimented Material- Zebra mussels produced copious amounts of sediment in the first three days of the experiment, but then reduced their sedimentation rate to a constant level slightly above that in control and BG treatments (Fig. 3A). Treatments with zebra mussels had higher rates of C, N, and P sedimentation (between subjects for carbon $P < 0.0001$, $F = 276.2$; for nitrogen $P < 0.0001$, $F = 113.9$; for phosphorus $P < 0.0001$, $F = 981.0$) following temporal patterns essentially the same as in Figure 3A. Sedimentation rates of chl a were more complex with zebra mussel x bluegill effects, i.e. there was more chl a in sediment from ZM+BG treatments (between subjects ZM $P < 0.001$, $F = 241.5$; BG $P = 0.0097$, $F = 11.38$; ZM x BG $P = 0.0168$, $F = 9.07$; Fig. 3B).

Analysis of nutrient ratios within sedimented material (C:N, N:P, and C:Chl-a) using repeated measures ANOVA on the entire time period showed varying influences of zebra mussels (between subjects for C:N $P = 0.0550$, $F = 5.04$; for N:P $P = 0.6658$, $F = 0.20$; for C:Chl a $P < 0.001$, $F = 61.03$). Sediment sampling dates represent collection of material that sedimented over the time interval since the previous sampling, with the first sampling representing days 0-3. Temporal patterns indicated that sedimented material collected on day 3 consisted of material substantially different than sediment collected on days 6, 9 and 13 (Fig. 4). Sedimented material in zebra mussel treatments had higher C:N on day 3 (within-subjects effect, time x ZM $P = 0.0009$, $F = 7.75$), lower C:Chl a from day 3 onward (within-subjects effect, time x ZM $P = 0.0041$, $F = 5.76$), but no discernable differences in N:P throughout the experiment (within-subjects effect, MANOVA, time x ZM $P = 0.0792$, $F = 3.75$).

Discussion

Nutrients in the water column: Observed nutrient cycling effects by zebra mussels were generally consistent with previous studies. Reduction in suspended carbon, nitrogen, and phosphorus concentrations was attributable to the general filtering activity of zebra mussels. Increased TDP and SRP concentrations were attributable to zebra mussel excretion and were consistent with observations in previous experimental systems as well as Lake Erie (Heath et al. 1991, Holland et al. 1995, James et al. 1997, James et al. 2000). Fahnenstiel et al. (1995) and Johengen et al. (1995) reported reductions in total phosphorus in areas of Lake Huron with large zebra mussel populations but no effect on TP was observed in the present experiment. Incorporation of phosphorus into zebra mussel biomass and increased SRP availability through remineralization may represent the most important alterations of nutrient availability by zebra mussels for inland lakes, considering the high frequency of phosphorus limitation of algal growth in inland lakes (Wetzel 2001).

Zebra mussels evidently prevented a change in DIN+PN:TP ratios that was observed in non-zebra mussel treatments on days 3 and 6. The temporal patterns of DIN+PN:TP ratios in all treatments, including the lower ratios seen in treatments with zebra mussels, and the pattern of changes in transparency might be partially explained by the precipitation of calcium carbonate from the water column. Through this mechanism, the reduction of algae and thus photosynthetic activity in zebra mussel treatments reduces the rate of calcium carbonate ($CaCO_3$) precipitation, the whiting effect (light attenuation)

of such precipitation, and the removal of dissolved phosphorus through adsorption (Kelts and Hsü 1978). Thus the calcium carbonate explanation was not supported.

A possible explanation for the temporal pattern of DIN+PN:TP exists with rain entering the enclosures. The temporal pattern of DIN+PN:TP was driven by DIN+PN, which itself was largely driven by sestonic nitrogen (Fig. 3B). Any explanation must then account for greater nitrogen present in living plankton in treatments without zebra mussels (BG and control treatments). Rain fell on 9 July 1999 (day 0) after initial conditions were measured and fish and zebra mussels added (2.82 cm, 0.54 mg/L NH_4, 1.95 mg/L NO_3, Kellogg Biological Station #MI26, National Atmospheric Deposition Program 2001). Each 1m x 1m limnocorral therefore received an infusion of 24.3 mg of DIN in 28.2 L of water, raising the concentration of DIN in the entire 1,500 L mesocosm by approximately 16 µg/L. Rain also fell between days 6 and 9 (0.27 cm, 0.43 mg/L NH_4, 3.64 mg/L NO_3) which raised the concentration of DIN by approximately 2µg/L. In addition, the mean annual concentration of dissolved organic nitrogen in Kalamazoo County precipitation is 0.43 mg/L (Rheaume 1990), and this form could add up to 50% more nitrogen to precipitation. Thus enrichment can at least partially account for the increase in total N seen in non-zebra mussel treatments on days 3 and 9.

Nitrogen entering the mesocosms through rain could have been taken up primarily by phytoplankton in non-zebra mussel treatments and periphyton in zebra-mussel treatments. Reduced phytoplankton biomass in zebra mussel treatments would have provided less nutrient-uptake competition for periphyton. Moreover, less turbid conditions in zebra mussel treatments would have provided more light and thus better conditions for periphyton growth.

The present experimental pond system, however, may have been limited by nitrogen, as indicated by a low initial DIN + Sestonic N:TP ratio (< 10). Although an increase in ammonium concentration has been observed in the presence of zebra mussels in experimental systems, Lake Huron, and Lake Erie (Gardner et al. 1995, Heath et al. 1995, Holland et al. 1995, Johengen et al. 1995), ammonium did not increase in the presence of zebra mussels in the present study. Rapid utilization of ammonium by phytoplankton and periphyton within limnocorrals could have masked an increase in ammonium regeneration produced by zebra mussels. Rapid uptake of nitrogen introduced in rain would also be consistent with a nitrogen-limited system.

Biodeposition- Temporal patterns in the nutrient ratios of sedimenting material indicate that sediment produced by zebra mussels in the first 3 days differed substantially from sediment produced later. The sediment produced by zebra mussels from days 0 to 3 had higher C:N ratios and similar N:P and C:Chl a ratios compared with the other treatments. After day 3, nutrient ratios in sedimenting material did not differ among treatments, except for lower C:Chl a in zebra mussel treatments, yet the zebra-mussel treatments consistently yielded larger quantities of sediment.

Temporal patterns in the content of sedimenting material in the zebra-mussel treatments might be explained by two mechanisms: 1) greater production of feces which changed in composition as the seston changed, or 2) pseudofeces production during the first three days. The initial concentration of sestonic C in limnocorrals (0.64 ± 0.17 mg C/L) was greater than a previously documented threshold for beginning pseudofeces production (~0.2 mg/L) but less than the threshold for reduced filtering rates (incipient

limiting concentration, ~2.0 mg C/L) (Walz, 1978). While pseudofeces production is expected to begin at the incipient limiting concentration for pure algal cultures, pseudofeces are also produced in order to reject inedible inorganic or organic particles (Sprung and Rose 1988, MacIsaac and Rocha 1995, Berg et al. 1996). Thus zebra mussels could have been producing pseudofeces early in the experiment.

Conclusions- Indirect effects on nutrient cycling by zebra mussels through alteration of foodweb structure were more important than direct effects via dissolved nutrient regeneration or biodeposition in this experiment. Specifically, the increase in DIN + SestonicN:TP ratios in non-zebra mussel treatments was evidence that removal of phytoplankton by zebra mussels allowed nitrogen naturally introduced by rainfall to be taken up by periphyton. Biodeposits produced by zebra mussels varied substantially over short time periods in terms of relative nutrient composition. In natural systems temporal change in biodeposit composition might occur both due to seston availability and seston content including phytoplankton abundance or community structure, and seasonal changes in biodeposit composition may occur in response to phytoplankton succession. A trophic cascade can explain why greater amounts of chl-a were found in sedimented material in enclosures with zebra mussels and fish, compared to sedimented material in enclosures with zebra mussels and no fish. Models of zebra mussel alteration of nutrient cycling including stoichiometric relationships in natural systems must include flow paths to benthic communities produced by indirect food-web effects.

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Figure 1. Nutrient responses over the course of the experiment in the presence of zebra mussels and larval bluegill. Shown are means ± 1 standard deviation. A) Sestonic carbon. B) Sestonic nitrogen.

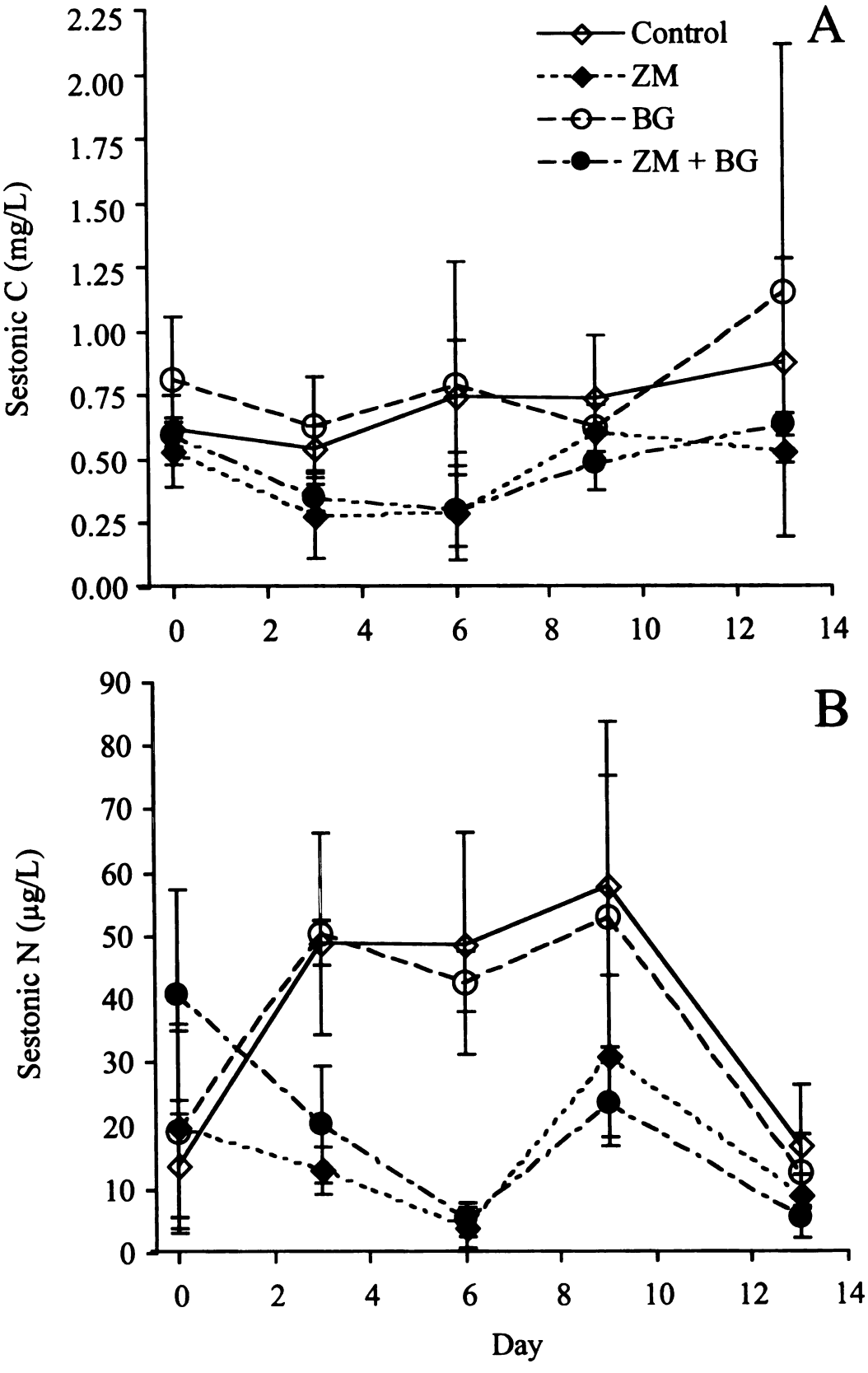

A
Control
ZM
BG
ZM + BG
Sestonic C (mg/L)
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
12
14
B
Sestonic N (µg/L)
90
80
70
60
50
40
30
20
10
0
Day

Figure 2. A) DIN+sestonic N:Total P molar ratio, uncontaminated replicates. B) DIN+sestonic N:Total P molar ratio, all days. Some replicates on days 6, 9 and 13 were slightly underestimated due to phosphorus contamination and were excluded from statistical analysis (see text).

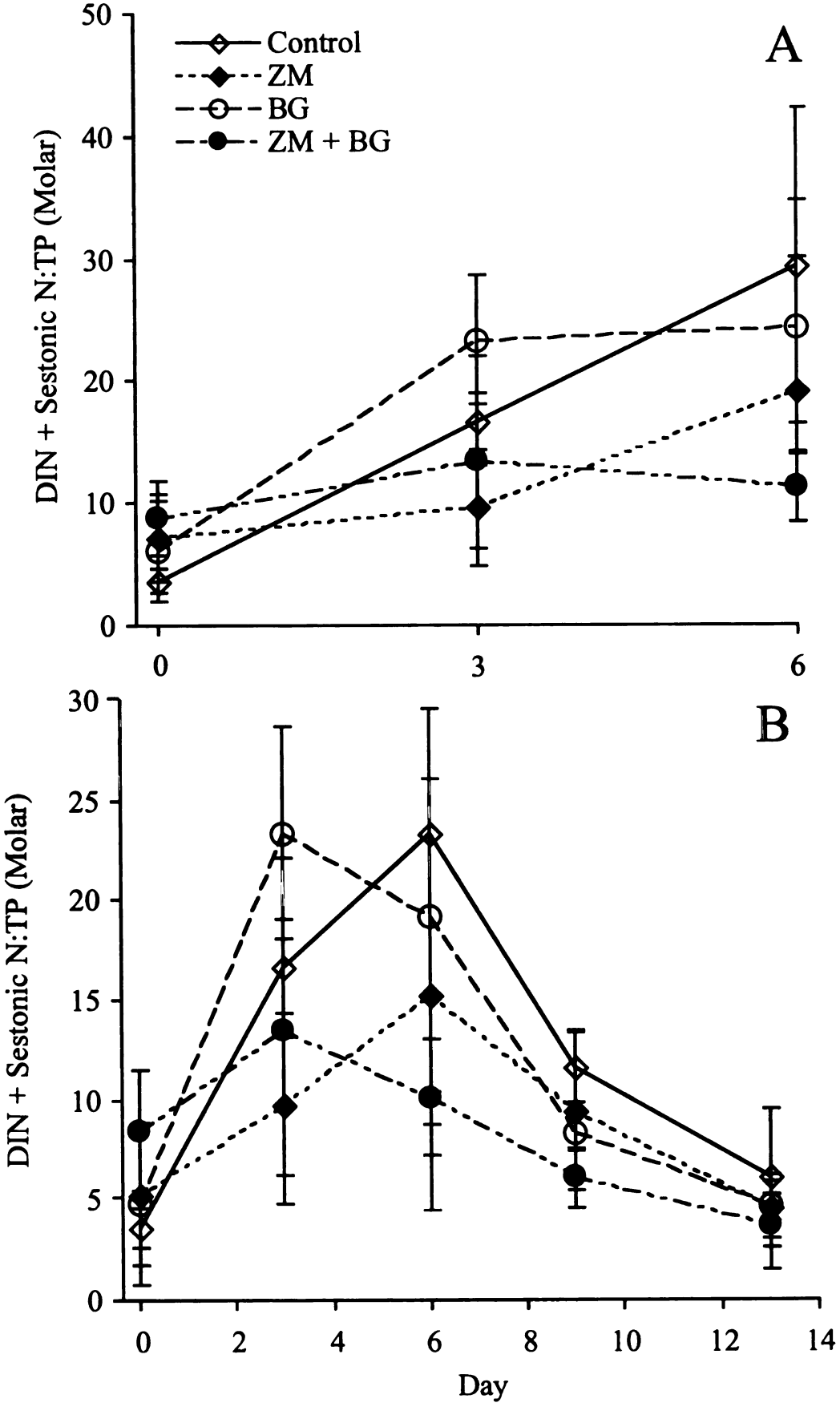

A
Control
ZM
BG
ZM + BG
DIN + Sestonic N:TP (Molar)
50
40
30
20
10
0
0
3
6
B
DIN + Sestonic N:TP (Molar)
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
Day

Figure 3. Sedimentation response over the course of the experiment in the presence of zebra mussels and larval bluegill. In both graphs the sampling dates represent sedimentation over the preceding time interval. Shown are means ± 1 standard deviation. A) Sedimentation rate. B) Sedimentation rate of chlorophyll a.

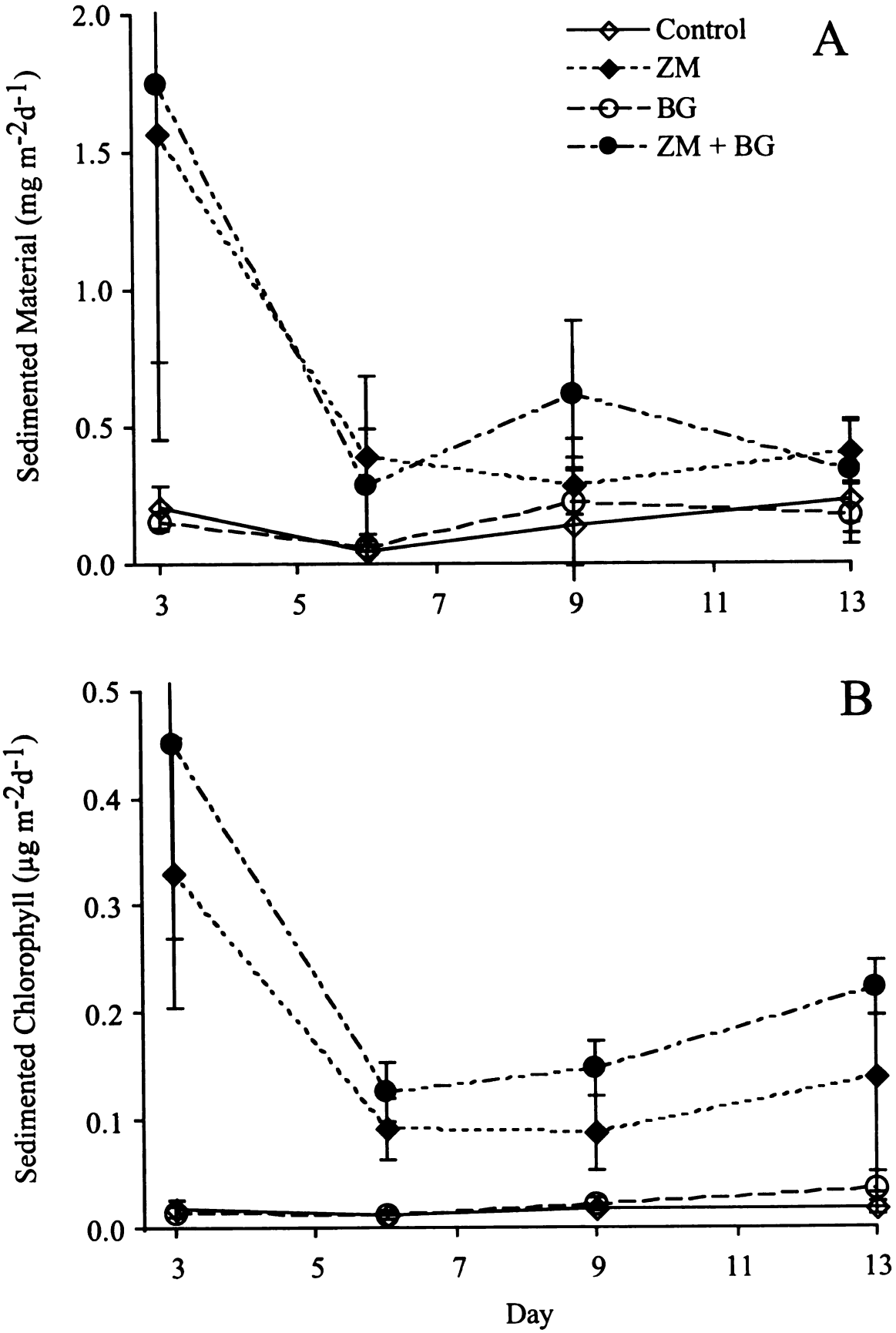

A
Control
ZM
BG
ZM + BG
Sedimented Material (mg m-2d-1)
2.0
1.5
1.0
0.5
0.0
3
5
7
9
11
13
B
Sedimented Chlorophyll (µg m-2d-1)
0.5
0.4
0.3
0.2
0.1
0.0
3
5
7
9
11
13
Day

Figure 4. Nutrient ratio in sediment response over the course of the experiment in the presence of zebra mussels and larval bluegill. Shown are means ± 1 standard deviation. A) N:P molar ratio of sediment. B) C:N molar ratio of sediment. C) C:Chlorophyll a mass ratio of sediment.

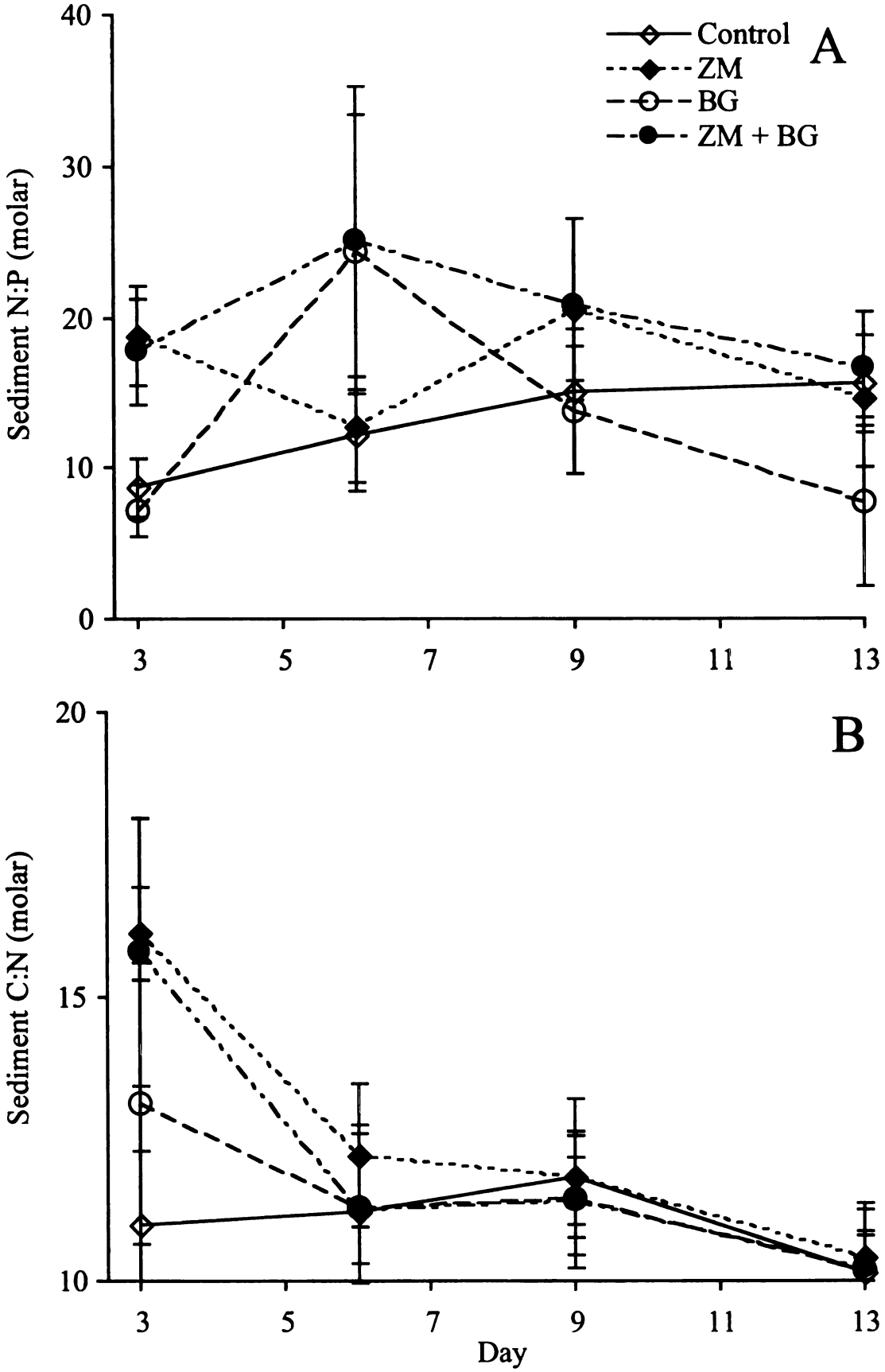
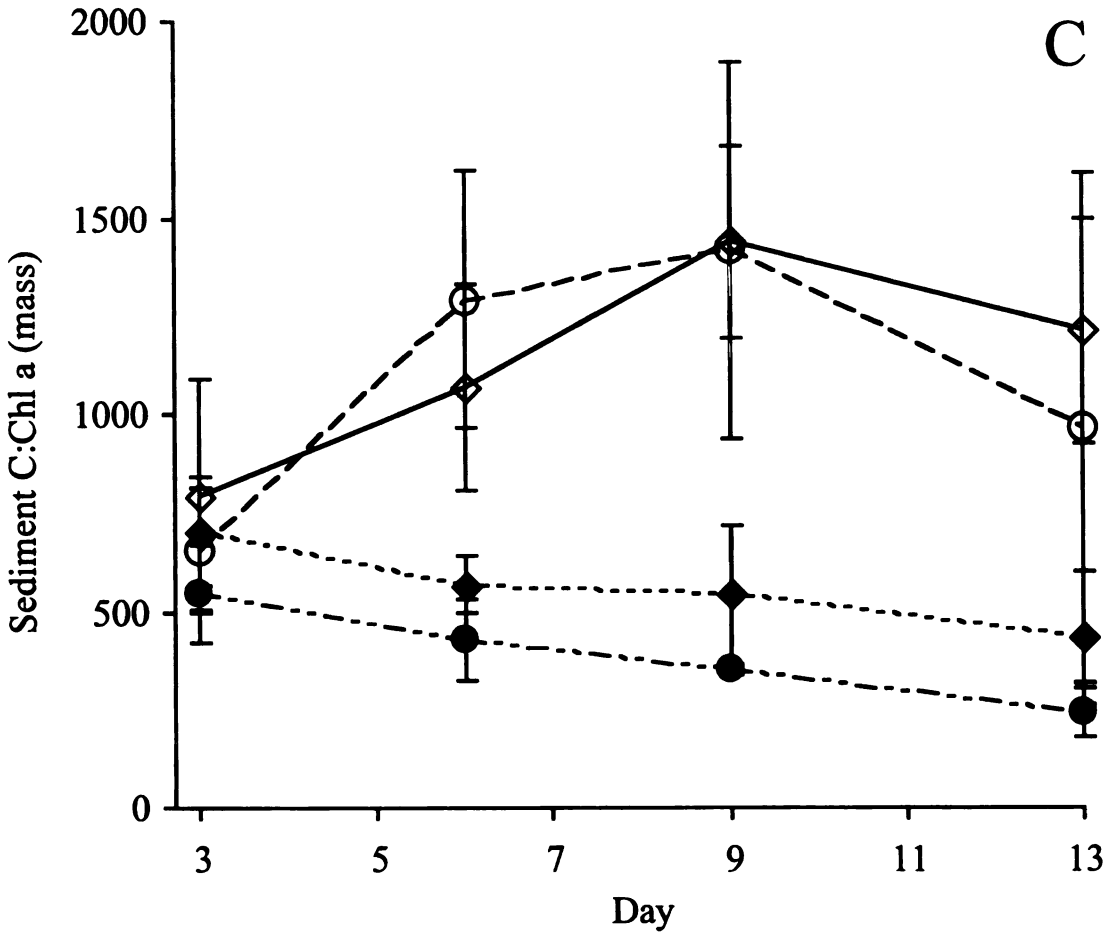

Control
ZM
BG
ZM + BG
A
B
Sediment N:P (molar)
Sediment C:N (molar)
40
30
20
10
0
15
3
5
7
9
11
13
Day

Figure 4. Continued.


C
2000
1500
1000
500
0
Sediment C:Chl a (mass)
3
5
7
9
11
13
Day

Chapter 5

ZEBRA MUSSEL IMPACTS ON THE PHYTOPLANKTON COMMUNITIES AND WATER CHEMISTRY OF INLAND LAKES

Abstract

A survey of 61 Michigan inland lakes spanning a wide phosphorus gradient was conducted in 1998 and 1999 to assess the impact of zebra mussels on phytoplankton abundance, phytoplankton community structure, lake transparency, and nutrient concentrations. Lakes with zebra mussels had lower phytoplankton abundances as measured by chlorophyll a concentration and total algal biovolume, differences in phytoplankton community structure, and lower dissolved organic carbon concentrations than lakes without zebra mussels. Lakes with and without zebra mussels were indistinguishable in their transparencyand concentrations of ammonium and soluble reactive phosphorus. Total phytoplankton abundance and abundance of cyanobacteria were positively related to total phosphorus. Most differences in phytoplankton communities were subtle but there were marked increases in the relative abundances of the colonial cyanobacteria Microcystis at low total phosphorus concentrations (i.e., < 25 _g P/L). Recent invasion by zebra mussels may have directly contributed to Microcystis blooms recently observed in several Michigan inland lakes. The reduction of dissolved organic carbon may render inland lakes more susceptible to UV-B radiation.

Introduction

Zebra mussels (Dreissena polymorpha) are notorious for their ability to disperse widely and quickly (Johnson and Carlton 1996). Since their introduction to North America zebra mussels have invaded the Great Lakes, the Mississippi drainage network and other rivers, and numerous small inland lakes. Less is known about the ecological effects of zebra mussels in North American inland lakes compared with other ecosystems including the Laurentian Great Lakes. While important limnological differences between inland lakes and the larger lakes and rivers that have been invaded may result in different ecological impacts, observations in those other systems do provide a basis for hypotheses about what may take place in smaller lakes.

Effects of the invasion on phytoplankton communities have been of primary ecological concern due to the intense filtering activity of zebra mussels (MacIsaac 1996, Ram and McMahon 1996). Some effects of zebra mussels on phytoplankton communities have occurred in all invaded ecosystems while others have been system-specific. Chlorophyll a (chl a) concentrations, for example, have been reduced following zebra mussel invasion in the Great Lakes (MacIsaac et al. 1992, Holland 1993, Nichols and Hopkins 1993, Fahnenstiel et al. 1995), the Hudson River (Caraco et al. 1997), and the inland Oneida and Hargus Lakes (Yu and Culver 2000, Idrisi et al. 2001). Increases in transparency have often accompanied these reductions in phytoplankton abundance (Holland 1993, Skubinna et al. 1995, Caraco et al. 1997, Yu and Culver 2000, Idrisi et al. 2001).

The effects of zebra mussels on cyanobacteria and Microcystis in particular have been of particular interest due to the undesirable nature of cyanobacterial blooms as well as the potential toxicity of Microcystis to vertebrates including humans if ingested in large quantities (Carmichael and Falconer 1993). Algal blooms are unslightly, malodorous and reduce the recreational value of freshwaters. Filamentous and colonial cyanobacteria (blue-green algae) are the most important taxa causing blooms because they increase turbidity, enhance oxygen depletion in bottom waters, and can produce toxins that threatens the health of livestock and humans when ingested (Fogg et al. 1973, Paerl 1988, Chorus and Bartram, 1999). In addition, cyanobcteria are often avoided or poorly assimilated by herbivores reducing planktonic food chain efficiency and thus can negatively impact pelagic-based fisheries (Gilwicz and Pijanowska 1989, DeMott 1986). Aesthetic degradation and public health threats are greatest when floating scums of algae accumulate at the shore, and therefore scum-forming species that potentially produce toxins, such as Microcystis aeruginosa, are the most notorious of the noxious cyanobacteria.

Smith et al. (1998) documented a striking shift in phytoplankton dominance from cyanobacteria to diatoms following zebra mussel invasion of the Hudson River. In contrast, Microcystis aeruginosa blooms were observed soon after zebra mussel invasion of Saginaw Bay in Lake Huron (Vanderploeg et al. 2001), and Aphanizomenon blooms were observed soon after zebra mussel invasion in Oneida Lake (Horgan and Mills 1997), although in Oneida Lake long term trends in phytoplankton community composition have not shown consistent change following invasion (Idrisi et al. 2001). No change in the relative abundance of dominant phytoplankton taxa was observed immediately following

zebra mussel invasion in the nearshore waters of Lake Erie (Nichols and Hopkins 1993), and phytoplankton community composition in offshore waters of Lake Erie was minimally impacted during the first years of zebra mussel invasion (1989-1993, Makarewicz et al. 1999). In 1995, however, a Microcystis bloom occurred in the western basin of lake Erie, where zebra mussel densities are greatest (Budd et al. 2001).

The long history of research in Europe and the former Soviet Union on zebra mussels yields little information on shifts in phytoplankton community composition following zebra mussel invasion (Karatayev et al. 1997). One exception is the exploration of the use of zebra mussels as bioprocessors to control effects of eutrophication including cyanobacterial blooms (Reeder and Bij de Vaate 1990). Noordhuis et al. (1992), for example, demonstrated the suppression of Aphanizomenon and Oscillatoria blooms following experimental introduction of zebra mussels to a hypereutrophic pond.

Experimentation has yielded conflicting results concerning the ability of zebra mussels to alter phytoplankton community structure. Diatoms were more heavily reduced by zebra mussels while cyanobacteria were relatively unaffected in a short mesocosm experiment in Lake Huron (Heath et al. 1995). Nanoplanktonic algae were selectively removed while Microcystis was not in a microcosm experiment using Lake Huron plankton (Lavrentyev et al. 1995). Cyanobacteria were suppressed at high zebra mussel densities and diatoms promoted at mid-level densities in an in-situ experiment on the Ohio River (Jack and Thorp 2000). Some experimentation has shown that zebra mussels can cause change through selective rejection and subsequent survival of algae including Microcystis in feces and pseudofeces. Bastviken et al. (1998), for example, demonstrated filtering of all algae from the water column (gross clearance) by zebra mussels in

microcosms and alteration of phytoplankton community composition upon resuspension of biodeposited material (net clearance), including an increase in the relative abundance of Microcystis colonies. Vanderploeg et al. (2001) showed rejection of Microcystis in pseudofeces depending on whether the algae strains produced the toxin microcystin. In contrast, Baker et al. (1998) demonstrated preferential ingestion of Microcystis by zebra mussels in the Hudson River.

In addition to Microcystis blooms following zebra mussel invasion reported in the literature, Microcystis blooms have been observed following zebra mussel invasion in several inland lakes in the vicinity of the Kellogg Biological Station, Michigan State University. For example, in 1996 one of us (Hamilton) observed a Microcystis bloom in Gull Lake, where the Kellogg Biological Station is located, two [three?] years after zebra mussels were first observed in the lake, and similar blooms have since been observed in nearby Gun Lake; neither lake had experienced such blooms in the recent past.

Most phytoplankton taxa become more abundant with increasing total phosphorus concentration (Watson et al. 1997). Total phosphorus has been further demonstrated as an excellent predictor of cyanobacterial biomass (Trimbee and Prepas 1987) and the potential for bloom formation (Paerl 1998). The response of cyanobacterial biomass to total phosphorus concentrations is nonlinear, however, dramatically increasing at total phosphorus concentrations above 20-30 μg/L (Downing et al. 2001). Blooms observed in Gull and other local inland lakes were thus surprising due to their low total phosphorus concentrations (<25μg/L).

Alteration of nutrient cycling by zebra mussels is also only partially understood. Regeneration of and subsequent increases in concentrations of total dissolved phosphorus

(TDP), soluble reactive phosphorus (SRP), and ammonium by zebra mussels have been demonstrated under laboratory conditions and observed in Lake Erie (Heath et al. 1991, Holland et al. 1995, James et al. 1997, James et al. 2000). Mellina et al. (1995), however, observed variable phosphorus trends over time with increasing zebra mussel density in microcosms. Zebra mussel excretion of ammonium may contribute to altered biogeochemical cycles in lakes (Lavrentyev et al. 2000). Zebra mussels have also recently been shown to be able to directly assimilate dissolved organic carbon (DOC, Roditi et al. 2000). Potential reduction of DOC is of particular concern because DOC attenuates ultraviolet-b (UV-B) radiation in aquatic ecosystems, and this radiation has been shown to detrimentally impact freshwater ecosystems by inhibiting photosynthesis and increasing amphibian mortality (Bothwell et al. 1994, Kiesecker and Blaustein 1995, Schindler et al. 1996).

The objectives of this study were to evaluate the effects of zebra mussels on inland lake phytoplankton communities and nutrient availability. We examined several questions: 1) Do lakes with zebra mussels differ from lakes without zebra mussels in algal biomass and transparency across a range of phosphorus availability? 2) Do lakes with zebra mussels differ from lakes without zebra mussels in phytoplankton community composition, specifically Microcystis and other cyanobacteria? 3) Do lakes with zebra mussels differ from lakes without zebra mussels in nutrient availability, particularly ammonium, SRP, and DOC concentrations? A survey of 61 lakes preselected along a total phosphorus (TP) gradient and classed by zebra mussel presence or absence was conducted in the lower peninsula of the State of Michigan to address these hypotheses.

Methods

In Michigan, zebra mussels were known to inhabit 150 inland lakes by the late 1990's (Kraft and Johnson 2000, Klepinger 2000). A list of lakes infested by zebra mussels was obtained from the Michigan Sea Grant. The Sea Grant list also included the year zebra mussels were first sighted, and from this the age of invasion at time of sampling was estimated. For lakes adjacent to Lake Michigan, the age of invasion was estimated based on when zebra mussels were sighted along the shore of Lake Michigan near the lake that was sampled, since numerous boats travel between Lake Michigan and those lakes. Lakes to be sampled were chosen to represent the range of total phosphorus concentrations common in Michigan Lakes, but only included lakes with pH and calcium concentrations suitable for zebra mussels (Ramcharan et al. 1992). Lakes sampled had similar distributions of TP levels in each category. Historical data on total phosphorus concentrations in Michigan inland lakes were obtained from the Michigan Department of Environmental Quality. Data on maximum depth and surface area were used to find lakes of roughly similar morphometries with and without zebra mussels. Most lakes sampled were thermally stratified.

Lakes were sampled in late summer (August or early September) of 1998 and 1999. Phytoplankton samples were analyzed from all lakes sampled in 1998 but from only selected lakes sampled in 1999 with mid-range TP due to time restraints (n = 40 lakes analyzed). Late summer was chosen to maximize the potential of measuring cyanobacterial dominance of the phytoplankton community (Wetzel 2001). Samples were taken by boat at the deepest area of the lake as indicated by a bathymetric map and depth

finder. Transparency was evaluated by Secchi depth. A vertical profile of temperature, oxygen, pH, and specific conductance was measured to locate the thermocline using a YSI multisensor. An integrated water sample of the epilimnion was then taken with a tube sampler and mixed in an opaque cooler. From this integrated sample, 250 ml of water was preserved in Lugol's fixative as a phytoplankton sample and 1 L was put on ice for later chemical analysis.

Dissolved organic carbon (DOC) was measured with a Pt catalyzed high-temperature-combustion C analyzer. Ammonium, particulate phosphorus (PP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) were analyzed colorimetrically (Wetzel and Likens 1991, Aminot et al. 1997, Langner and Hendrix 1982). Total phosphorus (TP) was calculated by summing PP and TDP. Dissolved calcium was measured with a flame atomic absorption spectrophotometer. Sestonic chl a was collected on Gelman AE glass-fiber filters and measured by fluorometry (Welschmeyer 1994).

Phytoplankton were settled in 10 ml subsamples for several days prior to enumeration. The circular field of the settling chamber was divided into an inner disk and outer ring of equal areas. Plankton were examined using an inverted microscope at 160x, 400x, and 1000x. Within each of the inner and outer areas 30 random fields were examined for a total of 60 fields per sample at each magnification. Phytoplankton were identified to genus. Relative abundance was estimated by calculating the biovolume using measurements of cell size at 1000x. Biovolume of Microcystis was estimated by counting the number of grid squares occupied by colonies and estimating the number of cells per grid square before measurement of individual cells.

Analysis of covariance (ANCOVA) was used to compare the following characteristics in lakes with and without zebra mussels using TP as a covariate: NH_4^+, SRP, DOC, Secchi depth, chl-a, biovolume of phytoplankton, biovolume and relative abundance (as % of total phytoplankton biovolume) of all cyanobacteria, bloom-forming cyanobacteria (sum of Anabaena, Aphanizomenon, Microcystis, and Oscillatoria), colonial coccoid cyanobacteria (sum of Aphanocapsa, Chroococcus, Merismopedia, Microcystis), filamentous cyanobacteria (sum of Anabaena, Aphanizomenon, Lyngbya, Oscillatoria), Microcystis, and other selected genera if correlated with multivariate functions. Data from 1998 and 1999 were pooled for all analyses and log-transformed ($\log_{10}+1$), including TP. Values for lakes sampled in both years were the average of both years.

The ANCOVA was preceded by a test for homogeneity of slopes (ZM*TP interaction). If the interaction term was not significant at $P \leq 0.05$ we ran ANCOVA. Principal Components Analysis (PCA using the covariance matrix), Correspondence Analysis (CA), and Multi-dimensional Scaling (MDS using Euclidean distances) of biovolume and relative abundance were used to evaluate alteration of phytoplankton community composition by ordination (PCA of relative abundance excluded, Jackson 1997). Simple Linear Discriminant Analysis (LDA) was used to attempt classification of lakes based on phytoplankton biovolume and relative abundance. Rare genera (<5% of total biovolume in any lake or present in only one lake) were removed from the data set used for ordination and classification. Analyses were conducted with SAS for Windows version 8 (ANCOVA, PCA, CA), Systat version 9 (MDS), and SPSS version 10 (LDA).

Results

Selection of lakes- In 1998, 22 lakes without zebra mussels and 12 lakes with zebra mussels were surveyed. In 1999, 17 lakes without zebra mussels and 21 lakes with zebra mussels were surveyed. Ten lakes were surveyed in both years. The final data set consisted of 33 lakes with zebra mussels and 28 lakes without zebra mussels. In the lower peninsula of Michigan, lakes with zebra mussels are concentrated in the southeast and southwest corners and along the western coast, and the distribution of lakes with zebra mussels chosen in this study reflects those geographic patterns (Fig. 1). Total phosphorus in surveyed lakes ranged from 12.4 to 112 μg/L and did not differ between lakes with and without zebra mussels (Kolmogorov-Smirnov, $D = 0.135$, $P = 0.9444$, Fig. 2).

Phytoplankton abundance and transparency- The ANCOVA results showed that lakes with zebra mussels had lower abundances of phytoplankton, as indicated by chl a concentrations (ZM $P = 0.0028$, TP $P < 0.0001$, Fig. 3A). Total algal biovolume was also lower in lakes with zebra mussels (ZM $P = 0.0489$, TP $P < 0.0001$, Fig. 3B). Secchi depth decreased with increasing TP but lakes with zebra mussels did not differ in Secchi depths from lakes without zebra mussels (ZM $P = 0.1722$, TP $P < 0.0001$, Fig. 3C).

Cyanobacteria- Lakes with zebra mussels did not differ from lakes without zebra mussels with respect to biovolume of all cyanobacteria (ZM $P = 0.6231$, TP $P = 0.0024$), biovolume of bloom-forming cyanobacteria (ZM $P = 0.9430$, TP $P = 0.0087$), or biovolume of filamentous cyanobacteria (ZM $P = 0.0680$, TP $P = 0.0002$). Lakes with

zebra mussels had greater biovolumes of coccoid colonial cyanobacteria (ZM P = 0.0403, TP P = 0.4948). Lakes with zebra mussels did not differ from lakes without zebra mussels with respect to the relative abundance of all cyanobacteria (ZM P = 0.6889, TP P = 0.2734), relative abundance of bloom-forming (ZM P = 0.7399, TP P = 0.0899), or relative abundance of filamentous cyanobacteria (ZM P = 0.0817, TP P = 0.0010). The relative abundance of coccoid colonial cyanobacteria was greater in lakes with zebra mussels (ZM P = 0.0009, TP P = 0.0771).

The relative abundance of Microcystis did not vary linearly with total phosphorus in lakes with or without zebra mussels precluding the use of ANCOVA (Least squares regression P = 0.5127, P = 0.3961, respectively Fig. X). Similarly, biomass of Microcystis did not vary linearly with total phosphorus in lakes with or without zebra mussels (Least squares regression P = 0.2193, P = 0.7928, respectively). The relative abundance of Microcystis, however, appeared to be greater in lakes with zebra mussels at lower TP levels (Kolmogorov-Smirnov, D = 0.437, P = 0.0507), and this result was clearly seen when lakes were grouped into low TP (< 25 μg/L) and high TP (>25μg/L) classes (Two-way ANOVA, ZM, F = 5.42, P = 0.0255, Fig. 4). No relationship was found between relative abundance of Microcystis and the time since initial observations of zebra mussel invasion (P = 0.1584). While the abundance of phytoplankton as indicated by total algal biovolume increased with increasing TP concentrations, the strength of the positive relationship weakened within nested subsets of total algal biovolume. For example, total algal biovolume, cyanobacteria, colonial cyanobacteria, and Microcystis responded to TP with P values of < 0.0001, 0.0024, 0.4948, and 0.4835, respectively.

Phytoplankton community structure- Enumerated genera are listed in Table 1. Principal Components Analysis did not efficiently reduce the data. Component 1 of the PCA using biovolume by genus, for example, explained only 22% of the variance and principal component 2 explained 12%. Data reduction using Correspondence Analysis was similarly poor. The first factor in the CA using relative abundance by genus, for example, explained only 11% of the variance, and the second factor explained 10%. Thus ordination employing PCA and CA failed to separate lakes with and without zebra mussels. Multi-Dimensional Scaling also failed to separate lakes with and without zebra mussels using genera and division biovolume (R^2 = 0.76 and R^2 = 0.92, respectively) and relative abundance (R^2 = 0.74 and R^2 = 0.96, respectively).

Linear Discriminant Analysis using algal division biovolume and relative abundance failed to classify lakes with and without zebra mussels (Wilk's Lambda = 0.919, df = 5, P = 0.701, and Wilk's Lambda = 0.914, df = 5, P = 0.669, respectively). Linear Discriminant Analysis using genera biovolume and relative abundance succeeded in classifying lakes with and without zebra mussels (Wilk's Lambda = 0.188, df = 23, P = 0.005 and Wilk's Lambda = .226, df = 23, P = 0.018, respectively, Fig. 5). Canonical discriminant function 1 of the LDA using genera biovolume was correlated with the biovolumes of Anabaena (R = 0.43, P = 0.0055), Merismopedia (R = -0.54, P = 0.0003), and Scenedesmus (R = -0.71, P < 0.0001). Canonical discriminant function 1 of the LDA using genera relative abundance was correlated with the relative abundances of Anabaena (R = -0.32, P = 0.044), Coelospherium (R = -0.42, P = 0.0072), Merismopedia (R = 0.40,

P = 0.0110), Microcystis (R = 0.36, P = 0.0230) and Scenedesmus (R = -0.0.38, P = 0.0170).

Nutrients - Concentrations of DOC increased with increasing TP in lakes without zebra mussels, but zebra mussels altered this relationship by decoupling the TP-DOC relationship (i.e. a non-significant relationship between TP and DOC, P = 0.5086, Fig. 6). Thus the test for homogeneity of slopes was not passed, and ANCOVA was not used (ZM*TP P = 0.0227). Lakes with zebra mussels had lower concentrations of DOC (ANOVA, F = 18.72, P < 0.0001). Concentrations of ammonium (NH_4^+) increased with increasing TP but lakes with zebra mussels did not differ from lakes without zebra mussels (ZM P = 0.2819, TP P = 0.0160). Concentrations of SRP showed the same pattern (ZM P = 0.0764, TP P < 0.0001).

Discussion

This study is the first interlake comparison of the effects of zebra mussels on phytoplankton communities. Because the invasion of inland lakes by zebra mussels is ongoing at the present time, the results of this study serve as a snapshot of ecological conditions in ecosystems at or near the beginning of colonization. In the following discussion we evaluate the impact zebra mussels have had on phytoplankton communities and nutrient concentrations in inland lakes of Michigan, and relate these impacts to those seen in previously invaded systems.

Phytoplankton abundance- Zebra mussels have clearly reduced standing stocks of phytoplankton in inland lakes as evidenced by the reduction of chl a and total algal biovolume. Reduction of algal abundance thus remains a universal effect of zebra mussels in all ecosystems they invade. Mellina et al. (1995) hypothesized that upon invasion of inland lakes by zebra mussels, the Dillon-Rigler relationship (Dillon and Rigler 1974) of chl a to TP in inland lakes might be "decoupled" (a term they used incorrectly to mean changing the shape of the relationship), but found no evidence of such an effect in European inland lakes. The reduction of algal abundance in Michigan inland lakes by zebra mussels has changed the Dillon-Rigler relationship, but there was no discernable difference in the slopes of the response curves of chl a to TP in lakes with and without zebra mussels.

Transparency- Contrary to our hypothesis we did not observe an effect of zebra mussels on transparency. This result was surprising given the observed effect on algal abundance, and could be due in part to the poor sensitivity of the transparency measurement method (Secchi depth). Directly measuring light extinction with a quantum meter might have revealed an effect of zebra mussels on transparency. Secchi depth has been used, however, to demonstrate increased water clarity following zebra mussel invasion in other systems (e.g. Idrisi et al. 2001). In the Hudson River resuspension of material including clay and detritus biodeposited by zebra mussels may have offset the large reductions in phytoplankton abundance, resulting in only a modest increase in transparency (Baker et al. 1998). Mixing within the epilimnion might also resuspend biodeposited material in

inland lakes, thus contributing to turbidity despite reductions in phytoplankton abundance.

Phytoplankton community structure- With the exception of Microcystis abundance in low-P lakes, zebra mussels had surprisingly subtle effects on late summer phytoplankton community composition. Several multivariate ordination techniques failed to separate lakes into classes of zebra mussel presence and absence, suggesting little overall change in the phytoplankton community. The magnitude of this result stands in contrast to the tidal Hudson River, where Smith et al. (1999) demonstrated dramatic changes in the phytoplankton community following zebra mussel invasion. Poor data reduction by the ordination techniques employed here likely reflects the multiple controlling factors that interact to determine differences between phytoplankton communities in lakes. Most of the genera encountered in surveys were not discernibly different in abundance in lakes with and without zebra mussels. None of the genera classified as more abundant in Michigan inland lakes with zebra mussels benefited from zebra mussel invasion in the tidal Hudson River (Smith et al. 1999). Linear Discriminant Analysis successfully classified lakes based on genera biovolume and relative abundance. While differences in the overall phytoplankton communities in lakes with and without zebra mussels were small, some genera displayed differential abundances.

The relative abundance of the colonial cyanobacterium Microcystis was greater in low-P lakes with zebra mussels. The observation that absolute abundance (as biovolume) of Microcystis did not differ while relative abundance did suggests several possibilities. Microcystis might have avoided or survived ingestion (including encapsulation in pseudofeces) rather than being directly promoted in some way in the presence of zebra

mussels. Alternatively, in lakes with zebra mussels, Microcystis, Merismopedia, and Scenedesmus might have had rates of production greater than other genera, enabling them to restock their populations at a higher rate. This possibility is unlikely, however, given that laboratory determinations of Microcystis growth rates are relatively slow (Reynolds 1989). In addition, Baker et al. (1998) demonstrated selective feeding in zebra mussels that included the active rejection of Scenedesmus. Thus rejection of mucilaginous colonial cyanobacteria and Scenedesmus could account for the changes in phytoplankton community structure observed here, with the effects being most marked at lower algal abundances typical of lower-P lakes, where grazing pressure might be more influential.

The response of phytoplankton community composition in the inland lakes of Michigan to zebra mussel invasion differed from the responses reported for some other ecosystems. In the Hudson River, for example, cyanobacteria were strongly suppressed (Smith et al. 1999). Aphanizomenon blooms were observed shortly after invasion in Oneida lake (Horgan and Mills 1997).

The conclusions regarding zebra mussel impacts on phytoplankton community structure in this study are limited to late summer communities. The timing of this study was selected because late summer is the typical period of cyanobacterial dominance in stratified lakes, and by sampling then be we were more likely todetect the potential effect of zebra mussels on cyanobacterial abundance. Zebra mussels could, however, affect phytoplankton community structure (including the abundance of cyanobacteria) at other times during the year. For example, Makarewicz et al. (1999) documented seasonal differences in the relative abundance of cyanobacteria in the western basin of Lake Erie

after zebra mussel invasion, where cyanobacteria relative abundance increased in the spring but decreased in the summer.

Dissolved Organic Carbon- Zebra mussels appear to have reduced the concentrations of DOC in Michigan lakes, supporting our hypothesis. This result is consistent with Roditi et al. (2001), who demonstrated the ability of zebra mussels to absorb DOC from the water column and argued that up to 50% of zebra mussel carbon demand could be satisfied by the uptake of DOC. An alternative possibility is that reduced phytoplankton abundance in the presence of zebra mussels is the proximate cause of the reduction in DOC. The reduction of DOC in lake waters by zebra mussels may pose a significant threat to the biota since DOC attenuates UV-B radiation in the water column (Schindler et al. 1996). If the DOC removed by zebra mussels includes those humic substances which contribute most to UV-B attenuation, UV-B radiation will penetrate further in to the water column. The reduction of DOC in aquatic ecosystems has been proposed as a more important factor regulating the exposure of aquatic ecosystems to UV-B than further destruction of the atmospheric ozone layer (Williamson et al. 1996). It is possible, however, zebra mussels might be reducing the concentration of relatively labile forms of DOC that contribute little to UV-B attenuation.

The reduction of DOC concentrations in lakes with zebra mussels was not consistent with the mesocosm experiment (see chapter 4), where zebra mussels did not reduce DOC. Resolution of this discrepancy between studies is difficult due to the heterogeneous composition of what is collectively measured as DOC. To speculate, the pond where the mesocosm experiment was conducted had many macrophytes and large

amounts of macrophytic detritus relative to total pond volume. Thus the pond could have been dominated by DOC derived from macrophyte tissues, rather than DOC derived from phytoplankton. Changes in phytoplankton abundance within limnocorrals would not have affected the chief source of DOC in the pond. Lakes, in contrast, generally had much less macrophyte biomass relative to total volume, and so reductions in phytoplankton abundance could have resulted in reduced sources of DOC.

Nitrogen and Phosphorus - No effect of zebra mussels on the concentration of ammonium was observed, contrary to expectations. This result contradicts experimental observations of ammonium regeneration by zebra mussels (e.g. Heath et al. 1991, James et al. 1997). Lavrentyev et al. (2000) demonstrated increased nitrification rates in the presence of zebra mussels in experimental systems with sediment cores. Loss of ammonium through nitrification might account for the lack of an increase in ammonium in inland lakes with zebra mussels.

No effect of zebra mussels on the concentration of SRP was observed, contrary to expectations. This result corroborates recent observations of the inland Oneida Lake (Idrisi et al. 2001), although increases in SRP concentration have been observed in both the Hudson River and Lake Erie following zebra mussel invasion (Holland et al. 1995, Caraco et al 1997). This result illustrates that inland lakes respond to zebra mussel invasion differently than do other ecosystems. Since Michigan inland lakes are commonly phosphorus limited, SRP regenerated by zebra mussels might be taken up quickly by phytoplankton.

Magnitude of zebra mussel effect on phytoplankton community structure- A potential explanation for the subtle effect of zebra mussels on overall phytoplankton community composition is simply that zebra mussel populations are too small to have a large effect. Mellina et al. (1995), for example, suspected that zebra mussel populations in European lakes might be too small to alter the TP-chlorophyll relationship. Physical factors present in inland lakes that are not present in the Great Lakes or large rivers may contribute to limitation of zebra mussel population sizes (Karateyev et al. 1998). Freezing, for example, kills zebra mussels and inland lakes commonly freeze over entirely. Thus the shallowest areas of inland lake littoral zones may be extirpated of zebra mussels in winter. Soft substrates limit zebra mussel attachment and are also common in inland lakes. In addition, oxygen depletion may be common in deeper waters during summer stratification, and under ice during winter.

The temporal dynamics of zebra mussel populations in lakes we surveyed are not known. In addition, the date of first sighting of zebra mussels in a particular lake is a questionable indicator of the time of colonization. If zebra mussel populations in North American lakes behave as populations have in European lakes, an initial spike in population size may occur after invasion with the population size subsequently stabilizing at a much lower level (Mackie and Schloesser 1996, Karatayev et al. 1997). Thus lakes we surveyed could have had very different population sizes even if they have similar habitat availability. If large populations are required to exert biologically significant effects on phytoplankton community composition, then the greatest potential for such change may occur only shortly after invasion. This may explain why cyanobacterial blooms were observed in inland lakes shortly after invasion, but not observed at later

times, including perhaps during this survey. From a strictly logical perspective, Microcystis blooms observed in two inland lakes prior to this study could have been simply coincidental with zebra mussel invasion and caused by other factors.

Lastly, the response of Microcystis abundance to zebra mussel invasion may be dependent on the alga's toxicity (microcystin production) and/or colony size. In a mesocosm experiment, zebra mussels reduced the relative abundance of unicellular cultured Microcystis while colonies of wild Microcystis increased in relative abundance, but only after resuspension of biodeposited material (Bastviken et al. 1998). Zebra mussels displayed lower feeding rates when toxic strains of Microcystis were present and more readily rejected large colonies in microcosm experiments (Vanderploeg et al. 2001). Thus if small colonies of Microcystis that do not produce toxins are present in inland lakes, zebra mussels may not not actively reject them.

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Table 1. Dominant phytoplankton genera encountered during surveys of Michigan Lakes.

Anabaena
Aphanizomenon
Aphanocapsa
Binuclearia
Ceratium
Chlamydomonas
Chrococcus
Closterium
Coelosphaerium
Dinobryon
Fragilaria
Glenodinium
Gleobotrys
Lyngbya
Melosira
Merismopedia
Microcystis
Mougeotia
Navicula
Oocystis
Oscillatoria
Pediastrum
Rhodomonas
Scenedesmus
Selenastrum
Staurastrum
Tetraedron
Ulothrix

Figure 1. Lakes surveyed in the lower peninsula of the State of Michigan. Black dots indicate lakes invaded by zebra mussels. Grey dots indicate lakes free of zebra mussels at the time of surveying.

Figure 2. Total phosphorus (TP) concentrations in lakes surveyed in 1998 and 1999. Gray bars are lakes without zebra mussels at the time of the survey and black bars are lakes with zebra mussels.

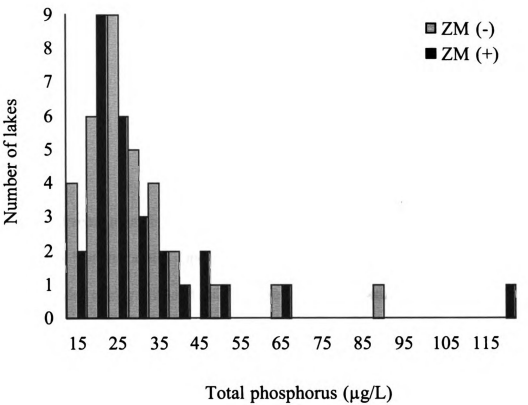

ZM (-)
ZM (+)
Number of lakes
0
1
2
3
4
5
6
7
8
9
15
25
35
45
55
65
75
85
95
105
115
Total phosphorus (µg/L)

Figure 3. Effect of zebra mussels on phytoplankton abundance and transparency in inland lakes of Michigan. Note log scales. Open circles and dashed lines represent lakes without zebra mussels, closed circles and solid lines represent lakes with zebra mussels. A) Chlorophyll a concentrations (n = 61). Lakes without zebra mussels: $y = 1.148x - 0.762$, $P = 0.0003$; lakes with zebra mussels: $y = 1.407x - 1.352$, $P < 0.0001$. B) Total algal biovolume (n = 40). Lakes without zebra mussels: $y = 2.329x + 3.161$, $P = 0.0021$; lakes with zebra mussels: $y = 2.554x + 2.361$, $P = 0.0142$. C) Secchi depth (n = 61). Lakes without zebra mussels: $y = -0.925 + 1.681$, $P < 0.0001$; lakes with zebra mussels: $y = -0.893x + 1.693$, $P < 0.0001$.

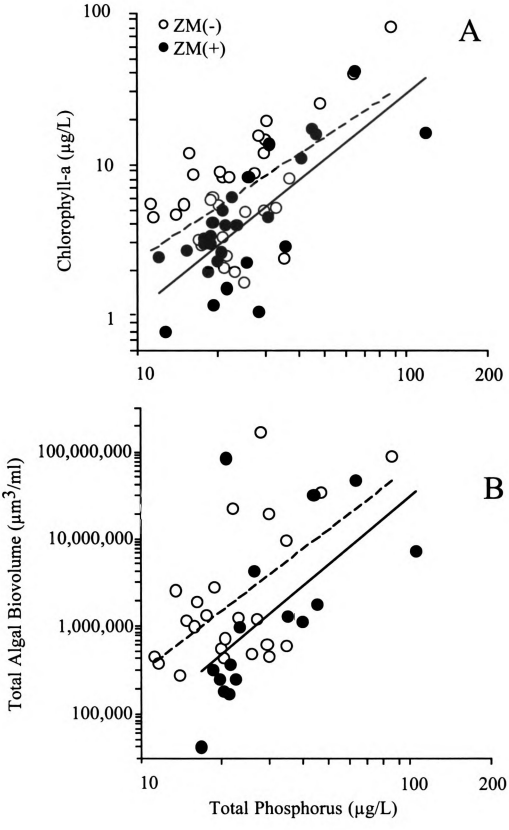

ZM(-)
ZM(+)
A
Chlorophyll-a (µg/L)
100
10
1
10
100
200
B
Total Algal Biovolume (µm3/ml)
100,000,000
10,000,000
1,000,000
100,000
10
100
200
Total Phosphorus (µg/L)

Figure 3. Continued.

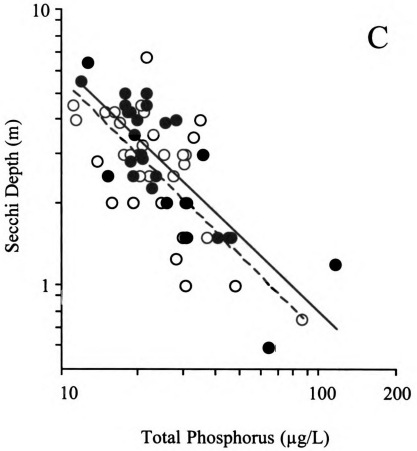

C
10
1
Secchi Depth (m)
10
100
200
Total Phosphorus (µg/L)

Figure 4. Effect of zebra mussels on Microcystis relative abundance and transparency in inland lakes of Michigan. Note log scales. Open circles and dashed lines represent lakes without zebra mussels, closed circles and solid lines represent lakes with zebra mussels.

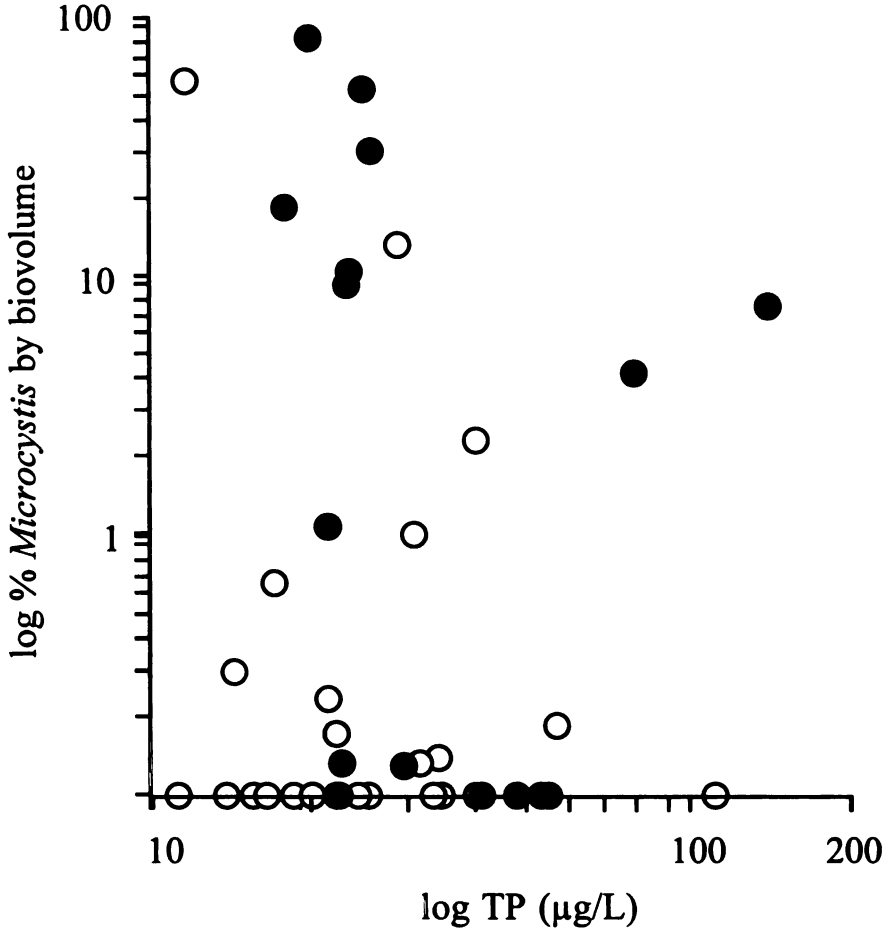

100
10
1
log % Microcystis by biovolume
10
100
200
log TP (µg/L)

Figure 5. Effect of zebra mussels on the relative abundance of Microcystis in lakes grouped into low TP (< 25μg/L) and high TP (> 25 μg/L) classes (Two-way ANOVA, ZM, $F = 5.42$, $P = 0.0255$). Error bars are standard error.

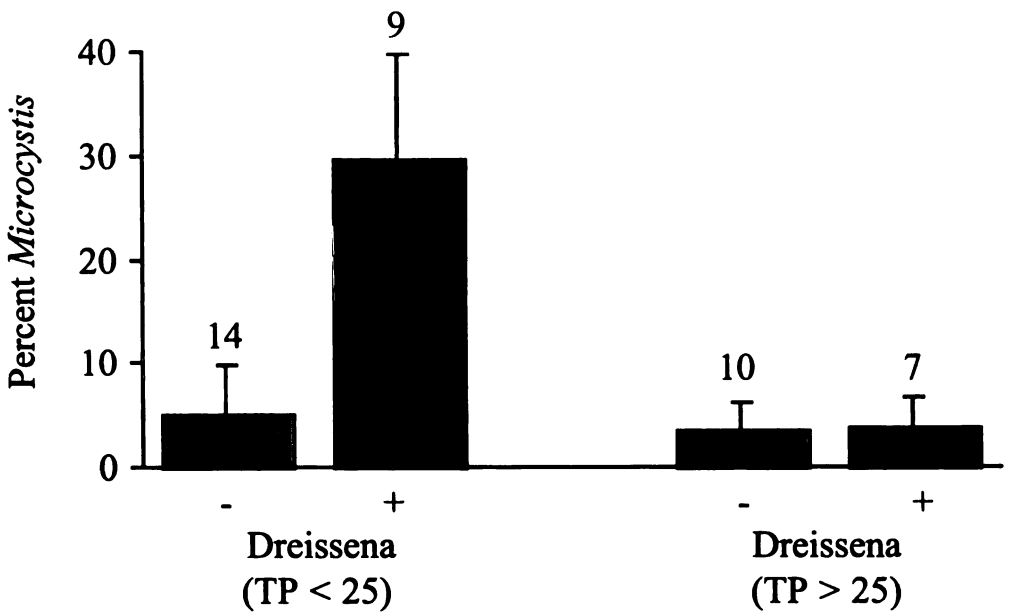

Percent *Microcystis*
40
30
20
10
0
9
14
10
7
-
+
-
+
Dreissena
(TP < 25)
Dreissena
(TP > 25)

Figure 6. Simple Linear Discriminant Analysis of lakes with and without zebra mussels using log-transformed biovolumes at the genus level (eigenvalue = 4.323, canonical correlation = 0.901).

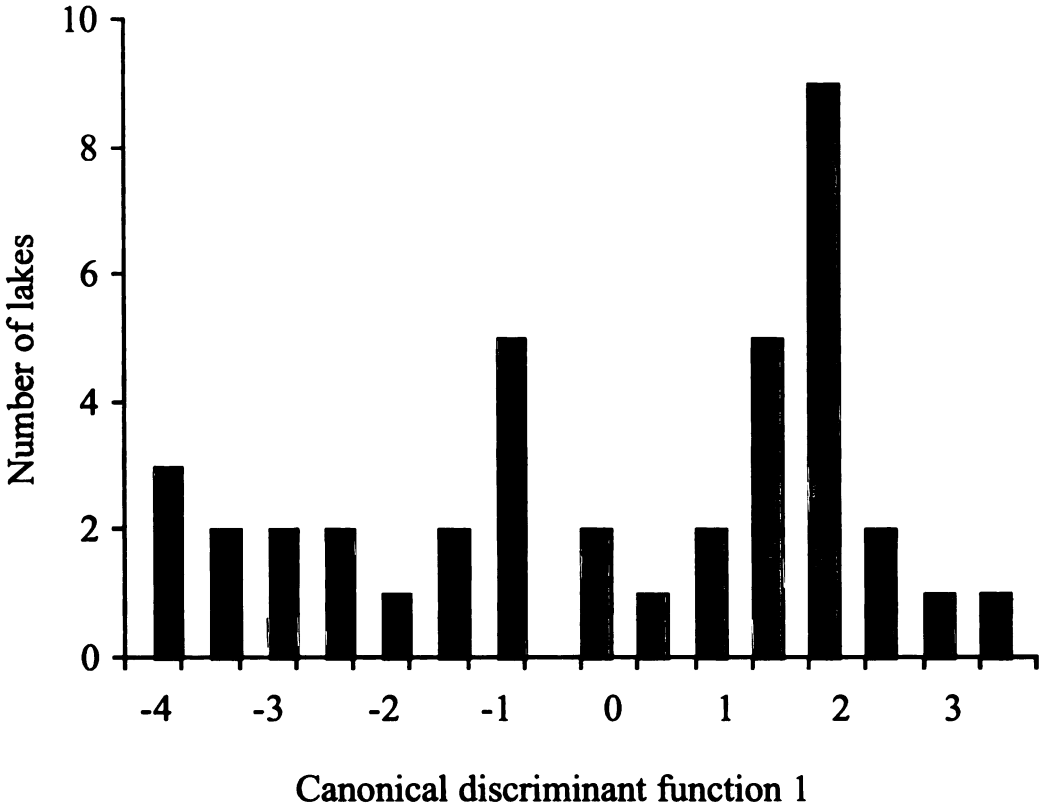
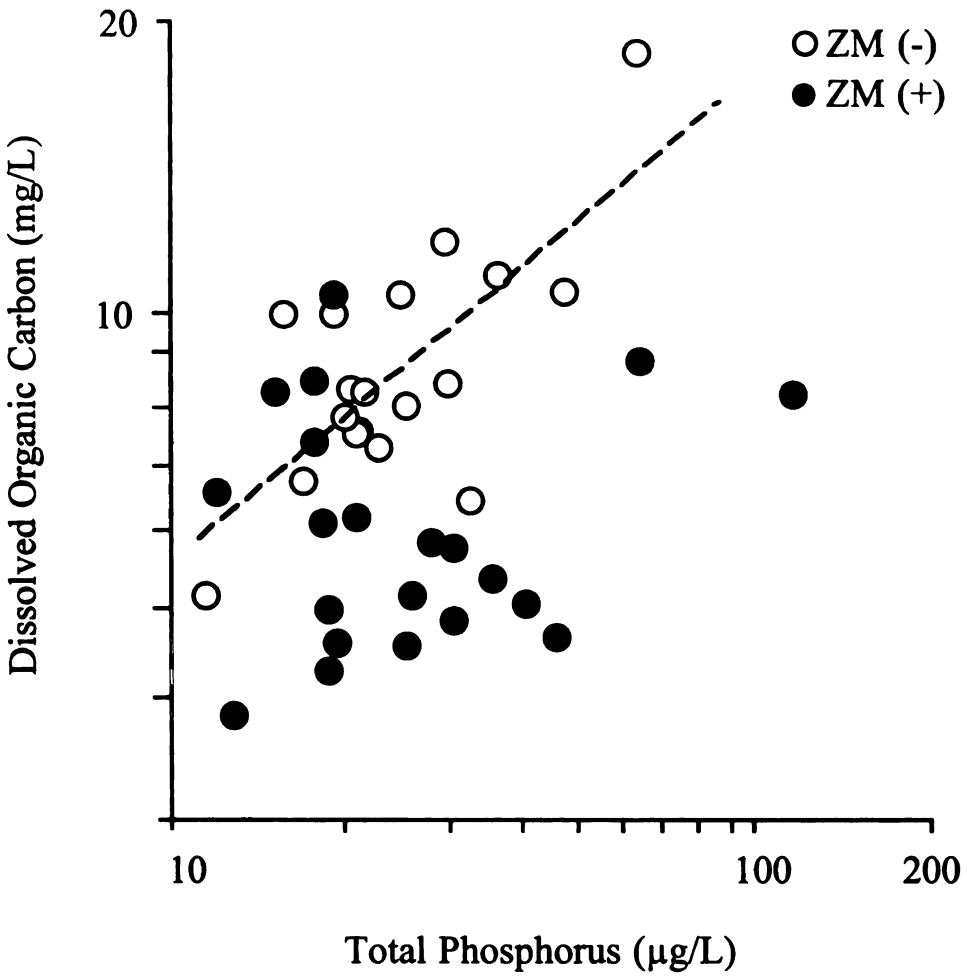

10
8
6
4
2
0
Number of lakes
-4
-3
-2
-1
0
1
2
3
Canonical discriminant function 1

Figure 7. Effect of zebra mussels on Dissolved Organic Carbon (DOC) concentrations in lakes surveyed in 1999. Open circles and dashed line represent lakes without zebra mussels. Closed circles represent lakes with zebra mussels. Note log scale. Lakes without zebra mussels: $y = 0.508x + 0.228$, $\underline{P} = 0.0008$. Lakes with zebra mussels: $\underline{P} = 0.5086$.


20
10
10
100
200
ZM (-)
ZM (+)
Dissolved Organic Carbon (mg/L)
Total Phosphorus (µg/L)

Chapter 6

ZEBRA MUSSEL EFFECTS ON MICROZOOPLANKTON FECUNDITY

Abstract

To assess how zebra mussels might suppress microzooplankton populations via either predation or competition for food resources, microzooplankton fecundity was investigated as part of a larger zebra mussel experiment. Twelve 49,000 L mesocosms were installed in Gull Lake in the summer of 2001 and stocked with zebra mussels to form a gradient of mussel density. If microzooplankton were predator-limited rather than resource-limited then egg ratios would not rise if population size rises in low zebra mussel treatments. Total phosphorus, chl a, Polyarthra abundance, Keratella abundance, nauplii abundance, Polyarthra egg ratio, and Keratella egg ratio, and Keratella and Polyarthra population growth rates were evaluated. No variables were related to zebra mussel density during the experiment (using mean values of days 6 through 19). The addition of phosphorus to the experiment most likely caused an increase in algal abundance which may have limited the potential to evaluate interactions between microzooplankton and zebra mussels. This experiment was not able to demonstrate an effect of zebra mussels on microzooplankton or evaluate the relative importance of competition and predation.

Introduction

Direct effects of zebra mussels (Dreissena polymorpha) on North American aquatic ecosystems are well known. As filter feeders, zebra mussels remove phytoplankton, microzooplankton and other particles from the water column, decreasing the standing stock of algae (Caraco et al. 1997, Idrisi et al. 2001) and microzooplankton (MacIsaac et al. 1995, Pace et al. 1998) and increasing water transparency (Holland 1993, Skubinna et al. 1995). Particulate waste as feces and pseudofeces is deposited, transferring energy and nutrients from the water column to the benthos in an example of pelagic-benthic coupling (Roditi et al. 1997, Ackermen et al. 2001). Epizootic colonization of, and competition for food with, native unionid mussels contributes to their continuing demise (Ricciardi and Rasmussen 1999, Strayer and Malcom 2001). New benthic habitats are created by both living and dead zebra mussel shells which can benefit some benthic macroinvertebrates (Wisenden and Bailey 1995, Botts et al. 1996).

Less well understood are indirect food web interactions involving zebra mussels. While consumption of microzooplankton has been demonstrated (Shevtsova et al. 1986, MacIsaac et al. 1991, MacIsaac et al. 1995), the relative strengths of impacts via predation of microzooplankton compared to impacts via competition for shared food resources (phytoplankton) on microzooplankton are not known. Observations of reduced microzooplankton population sizes following zebra mussel invasion have identified predation as the likely cause, but have not ruled out exploitative competition for food as a contributing factor (MacIsaac et al. 1995, Pace et al. 1998). Jack and Thorp (2000) observed reduced rotifer abundances without reductions in egg ratios in an in situ river enclosure experiment. Daphnia fecundity has been shown to be negatively affected by

reduced phytoplankton abundance following zebra mussel invasion of Oneida Lake (Horgan and Mills 1999).

This purpose of this study was to examine the effects of zebra mussels on the reproductive rates of Polyarthra and Keratella, two rotifers which are commonly found in North American freshwater ecosystems susceptible to zebra mussel invasion, and which are food for larval fish (see chapter 3). This study was part of a larger experiment using a gradient of mussel density in mesocosms arrayed in an meso-oligotrophic inland lake. Although zebra mussels were added to mesocosms, this was a zebra mussel removal experiment because Gull Lake, where the experiment was located, is infested with zebra mussels at densities near the upper end of those stocked in the mesocosms. Thus mesocosms with zebra mussels stocked at densities lower than that present in the lake might recover from zebra mussel impact over time. We hypothesized that the abundance of both phytoplankton and rotifers would increase with decreasing mussel density. If competition for phytoplankton is an important interaction between zebra mussels and microzooplankton, egg ratios of microzooplankton should also increase with decreasing mussel density. If predation by zebra mussels is the primary interaction, then with reduced mussel density the microzooplankton egg ratios should remain constant as microzooplankton population size rises, at least until food resources become limiting to the growing population.

Methods

An array of 12 mesocosms was deployed in Gull Lake, Michigan, USA offshore of the Kellogg Biological Station, Michigan State University from 5 July 2001 to 5 August 2001. Gull lake is a meso-oligotrophic inland lake that is 882 ha in area with a maximum depth of 31 m. Zebra mussels were first observed in Gull Lake in 1994, and it was one of the first in the region to be infested (Klepinger 2000). A previous survey determined a mean zebra mussel density in Gull Lake of 6.4 g dry mass / m^2 (upper 95 % density = 10g dry mass / m^2, O. Sarnelle, unpublished data).

Each mesocosm measured 2.5 m in diameter, ~10 m in depth, with a surface area of 4.9 m^2, and a volume of ~49,000 L. Mesocosms were constructed of 8 mil polyethylene tubes manufactured by Greentek. We folded the tube back onto itself to form a cuff at the top of the tube to reinforce the attachment of the tube to a supporting ring and to prevent holes from forming at the water surface. The mesocosms were left uncovered, not stocked with fish, and filled with Gull Lake water filtered through a 100-µm net. Mesocosms were then stocked with natural densities of macrozooplankton collected from the lake with a 100-µm net and mixed in a large container before removing aliquots for each replicate. Mesocosms were attached to a floating platform constructed using the EZ-Dock modular dock system in a line oriented parallel to the shore (north-south). The platform was located about 100-m offshore and anchored in place with cement blocks.

Zebra mussels were collected from Gull Lake and allowed to attach to 27 x 36 cm PVC sheets within tanks fed by a continuous flow-through of Gull Lake water for two

weeks. Feces and pseudofeces were periodically cleaned from tanks. We used mussels 20-mm in length. Each mesocosm was stocked with 12 Plexiglass sheets attached to each other with string and suspended vertically from 50-cm below the water surface to a depth of 7m. One mesocosm received dummy sheets that had no mussels. The other mesocosms were stocked to form a gradient of mussel density with the following densities in g dry mass/m^2 (total number in mesocosm): 6.2 (1260), 5.7 (1152), 5.2 (1056), 4.7 (960), 4.2 (840), 3.7 (744), 3.1 (636), 2.1 (432), 1.1 (216), 0.5 (108), 0.3 (60). The health and activity of zebra mussels were evaluated using a submersible video camera. Zebra mussel mortality was enumerated by a technician.

Phosphorus was periodically added to mesocosms to compensate for loss of phosphorus to uptake by periphyton and isolation from benthic sources. Chlorophyll a (chl a) was measured by fluorometry (Welschmeyer 1994). Microzooplankton was sampled approximately once a week during the experiment as part of the standard sampling regime. A tube sampler was used to collect an integrated sample of the water column to a depth of 8m. From this sample 10 L of water was passed through a 24-μm mesh sieve to collect rotifers, veligers and nauplii. Microzooplankton samples were preserved in a 1% Glutaraldehyde solution. Samples were settled in a graduated cylinder overnight to separate microzooplankton, which sank, from Microcystis colonies, which floated. Microcystis was then collected and stored in a separate container. The supernatant was discarded and the settled microzooplankton collected and placed in a phytoplankton settling chamber overnight. Use of the phytoplankton settling chamber allowed inspection of microzooplankton samples using an inverted microscope. The

entire sample was enumerated including counts of eggs attached to Keratella and Polyarthra.

The analysis of zebra mussel mortality was used to determine when to terminate the experiment. Analysis of the experiment was limited to those days when zebra mussel mortality was at a minimum. Experimental effects were analyzed by averaging response variables following initial conditions and looking for relationships with zebra mussel density recalculated based on mortality data. The following variables were analyzed: total phosphorus, chl a, Polyarthra abundance, Keratella abundance, nauplii abundance, Polyarthra egg ratio, and Keratella egg ratio, and Keratella and Polyarthra population growth rates.

Results

Zebra mussels experienced high rates of mortality by the end of experiment. After five days, 21.5% of mussels had died on average within each enclosure, but most of the mortality occurred after day 18 (Fig. 1). Microzooplankton population size, egg ratios, and population growth rates were evaluated up to day 19. The sheets supporting zebra mussels fell off in the enclosure stocked with 60 mussels, and although it was retrieved and reinstalled soon thereafter only a few mussels remained necessitating its exclusion from analysis.

The community of microzooplankton included the rotifers Keratella cochlearis, Polyarthra vulgaris, Monostyla, spp., Lecane spp., Sychaeta spp., copepod nauplii and zebra mussel veligers. Neither total phosphorus, chl a, nor any of the microzooplankton

variables was related to zebra mussel density on day 0, or during the experiment (using mean values of days 6 through 19). Considering the lack of a treatment effect, all treatments were averaged together. Comparing these experiment-wide means between initial conditions and mean of days 6-19, total phosphorus and chl-a increased (ANOVA, $P < 0.0001$ and $P = 0.0009$, respectively, Table 1). Keratella abundance increased while Keratella egg ratio did not change (ANOVA, $P < 0.0001$ and $P = 0.1207$, respectively). Polyarthra abundance and egg ratio decreased (ANOVA, $P < 0.0001$ and $P < 0.0001$, respectively). Nauplii abundance decreased (ANOVA, $P < 0.0001$). Zebra mussels had no effect on the population growth rates of either Keratella or Polyarthra (0.021 ind./day and –0.024 ind./day, respectively).

Discussion

This experiment was not able to demonstrate an effect of zebra mussels on microzooplankton or evaluate the relative importance of competition and predation. The addition of phosphorus to the experiment most likely caused the very modest increase in algal abundance as measured by chlorophyll-a. The increase in algal abundance possibly limited the potential to evaluate the effects of competition between microzooplankton and zebra mussels. The increase in algal abundance may also have caused an increase in Keratella abundance, again hampering attempts to evaluate hypotheses. The reduction of Polyarthra in all treatments was unexpected and may have reflected an experimental effect of the enclosures. A similar drop in Polyarthra abundance was observed in a

smaller mesocosm experiment (see chapter 3). This particular rotifer may suffer damage during collection and transfer (Alan Tessier, pers. comm.).

I attribute the failure of this experiment to the difficulty in balancing loss of phosphorus in mesocosm experiments with intentional phosphorus additions. An increase in algal abundance reduced competition for food resources. A lack of effect of zebra mussel consumption of microzooplankton at high zebra mussel densities was still surprising, however. Such a lack of pattern may indicate that consumption of microzooplankton by zebra mussels may not be important enough to affect population sizes in pelagic systems. This conclusion, however, would contradict the results and conclusions of the smaller mesocosm experiment described in Chapter 3. If the lack of effect of zebra mussels on microzooplankton population sizes observed in this experiment is real, and not due to methodological issues, the effects seen in the smaller mesocosm experiment might be due to forcing pelagic zooplankton populations into close proximity with benthic consumers. Thus the scale of mesocosm experimentation becomes critical when attempting to evaluate the food web and ecosystem effects of zebra mussels. This is speculation, however, due to the unexpected response of algae in this experiment.

Acknowledgements

The ZMEX project would not have been possible were it not for the daily efforts of Justin Chiotti, Elizabeth Milroy and Allison Schuerer who collected and reared mussels, built bags, assembled the dock, collected samples and made sure the whole thing didn't float away. Thanks also to Alan Wilson and Carrie Scheele. This project was funded by Michigan Sea Grant (project number R/NIS-4) and the Environment Now Fund of the Kalamazoo Community Foundation.

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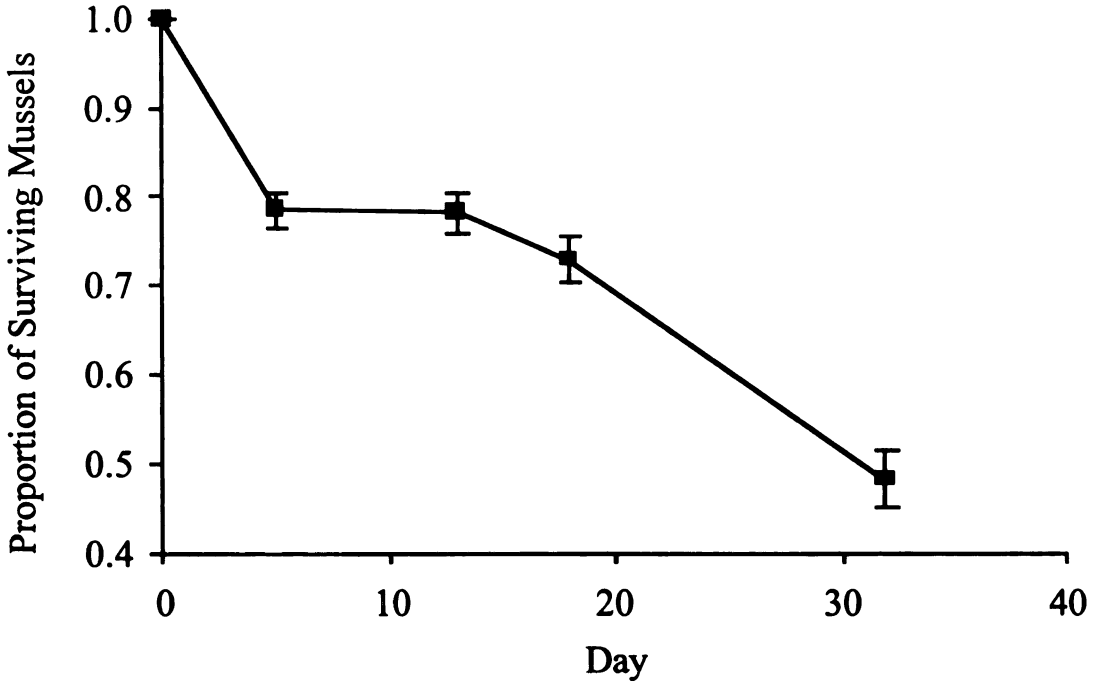
Table 1. Experiment-wide response of variables. All zebra-mussel treatments have been combined because no effect of mussel density on these variables could be detected.

Variable	Unit	Initial (SD)	Mean of days 6-19 (SD)	ANOVA (P)
Total Phosphorus	µg/L	5.0 (0.7)	9.8 (1.7)	<0.0001
Chlorophyll-a	µg/L	3.5 (0.4)	4.8 (1.5)	< 0.001
Keratella Abundance	ind./L	13.8 (6.1)	90.3 (73.6)	< 0.0001
Keratella egg ratio	#/ind.	0.26 (0.1)	0.19 (0.1)	0.1207
Polyarthra Abundance	ind./L	59.5 (22.1)	13.9 (15.0)	< 0.0001
Polyarthra egg ratio	#/ind.	0.08 (0.04)	0.05 (0.07)	< 0.0001
Nauplii	#/L	2.8 (1.0)	0.7 (0.6)	< 0.0001

Figure 1. Zebra mussel mortality during the experiment. Points are means for 11 enclosures. Bars are standard errors.


Proportion of Surviving Mussels
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0
10
20
30
40
Day

APPENDICES

APPENDIX A

METHODS FOR THE DESIGN AND CONSTRUCTION OF LIMNOCORRALS

I used a design commonly used around KBS as described to me by Gary Mittelbach and Chris Steiner (Figure A1). Materials needed to build frames that fit a 140" circumference bag are listed in Table A1. The notes that follow were provided to me by Jessica Rettig:

Building Bags:

- Sweep floor so it is very clean (helps prevent punctures of plastic) and put roll of plastic on wooden stake between two chairs.
- Put a layer of clean plastic on floor (helps protect plastic for the bag).
- Roll out plastic and iron 3-6 inches of the bottom to seal the bottom. Place cardboard under the plastic and notebook paper on top of the plastic so the heat from the iron melts the plastic but doesn't goop up on the iron or the plastic floor cover. set the iron at around 6 power.
- Loosely pleat bottom, once plastic has cooled, and tie a knot. This step takes 2 people! Insert an anchor rope into the knot.
- Measure 2.2m above the knot and cut the plastic from the roll. This length will give you a bag of about 1.5 m. Adjust your cut to get the bag length you need.
- Fold the top into a cuff about 4-6 inches wide and tape the cuff with poly-tape (used 3.5 rolls to tape 24 cuffs). This step works best with two people.
- Survey finished bag for any small nicks or holes and cover with poly-tape.

- Put finished bags into a vat lined with plastic. Stack no more than 8 bags high, with a plastic liner between every 3 or 4 bags (helps prevent punctures to bags).
- I think it can take around 30 minutes to build one bag, but I can't remember for sure.

Pond Prep:

- Cut opening into cattails so you have access to the pond. Opening should be around 3m wide and all cattails should be trimmed to the level of the pond substrate (cattails can puncture the bags if the stems are sticking up under the water).
- Also snorkel the transect upon which you will deploy the frames and remove any stiff vegetation that might puncture the bags.
- set up ropes across the pond, 2 ropes per row of bags. Ropes are tied to metal stakes set beyond the lip or hump of the pond. In the past I've set up 6 ropes for 3 rows of bags using 4 stakes.

Attaching Bags:

- Attaching bags to the frames works best with three people, two of which need to be in the water (the third may be in the water or in a boat leaning out to hold the frame or bag).
- Before using the frames check for rough edges, nails, staples, etc. and remove. Any of these could puncture the bag.
- Pass one frame into the water and have the person in the boat unfold a bag so the bottom can be sent through the center of the frame. Try to keep the edges of the bag from snagging on the frame.

- Stretch the cuff opening along the top of the frame and staple into place. This step works best if two people stretch and staple the cuff at the same time (2 staple guns) while the third person holds the frame steady. Staples should be parallel to the edges of the frame so they are less likely to tear off.
- Find the bottom of the bag (knot) and fold it over the side of the frame and into the bag, so no parts of the bag are dragging as you push the frame and bag through the water.
- Once you have enough frames to fill a row, attach the frames to the ropes using cable-ties. Adjusting the distance between frames as needed.

-Note that working with 3 or 4 people, it can take 1-1.5 hours to deploy 8 bags.

Filling Bags:

- Attach anchors (half brick with rope and clip) to the anchor rope on each bag and set the end of the bag into the water. Try to center the knot under the frame so the bag hangs vertically. Once the bag is filled, it is difficult to adjust how it hangs.
- Put big red pump into boat (fill tank with gas prior to setting into boat and check oil in pump).
- Attach the flexible blue hose to the pump outflow and cable-tie a zooplankton net over the end to filter out zooplankton (if needed). The zooplankton net should be tied shut.

-Attach the stiff gray hose to the intake.

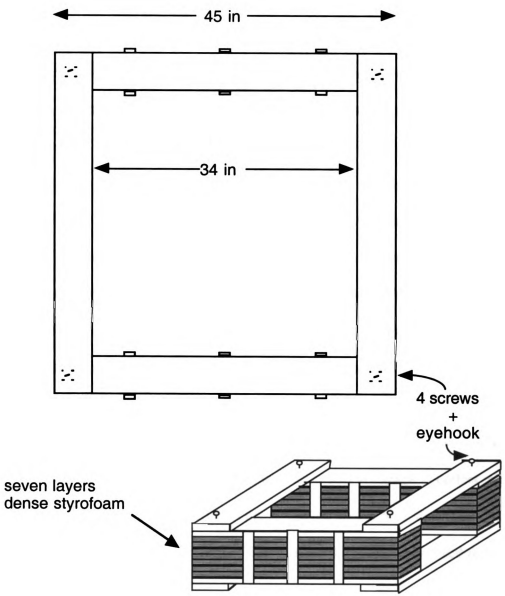
-Prime the pump before starting it by flooding the open end of the gray hose and raising it upwards toward the sky to force water into the pump.

- Start the pump, keeping the gray hose under the water. I tie it to the boat to prevent it from sinking to the pond bottom and sucking up muck.
- Filling bags often works best with two people, one holding the blue hose to fill the bags and the other monitoring the gray intake and holding the boat in position.
- Fill each bag for a similar period of time and check the volume by pressing on the sides of the bag under the frame (does it feel full?). Every 3-5 bags, point the blue hose away from the bags, untie the knot in the zoop net, and rinse the net clean. I filled 22.5 bags 91.5cm deep) with one tank of gas. Pump gets very hot and you may need to let it cool down before adding more gas.

Table A1: Materials needed to build limnocorrals.

Materials for 18 corrals:		Materials for 1 corral:	
72	8' (6x1) boards	4	8' (6x1) board
18	8' (3x1) boards	1	8' (3x1) board
15	4'x8' styrofoam panels	1	4'x8' styrof. panel
576	screws	32	screws
864	nails	48	nails
72	eyehooks	4	eyehooks

Figure A1. Design of Limnocorral Frames.


45 in
34 in
4 screws
+
eyehook
seven layers
dense styrofoam

APPENDIX B

METHODS FOR THE COLLECTION AND REARING OF ZEBRA MUSSELS

Collection: I collected zebra mussels from Gull Lake. There are plentiful populations along the KBS shoreline. For larger specimens you need to snorkel deeper water. Take a razor blade and cut the byssal threads to remove mussels from rocks (don't just pull them off). This prevents injury to the mussels, and should encourage them to secrete new threads. You can use a sieve to sort for a specific size class. Watch for empty shells that are still closed.

Holding: I held mussels in a tank with continuous flow-through of lake water. Place a submersible pump in Gull Lake just under the dock. Run the power cord and a hose into the boat house by fishing them through the fan duct next to the hood. This prevents any windows or doors from being unlocked during operation. Nina allowed the bending of a few bars of the fan guard to accommodate the hose. Put the hose in a bucket into which you have placed coarse grade mesh. Cut a hole in the bucket so that it drains into the oversize tank in the boat house. Position the top hole of the tank over the cistern so that it may drain directly into the pit. This set up will allow fine sediment to collect in the tank, depending on how much wave action is present in the lake. Check the set-up daily and do the following: clear the pump intakes of debris, rinse the mesh, siphon sediment from the mussels.

Substrate: I allowed zebra mussels to attach to Plexiglass sheets for two weeks. Sprinkle zebra mussels onto Plexiglass (or PVC pipe). They will creep around very slowly. The next day some mussels will have begun to attach while others will have wandered off your substrate. Reposition the stray mussels in the vicinity of attached mussels. Allow 7-14 days for the mussels to become affixed. I separated my plexiglass sheets by wedging foam insulation into the tank, creating "walls" between the plexiglass. This allowed me to add a set number of mussels to each plexiglass sheet without fear of them wandering over to another sheet.

Size and Density: I used a size structured population of 200 mussels per treatment. Natural densities impose an upper natural limit, but the actual density used should be less because the corrals are closed systems. In addition to density, size structure of the population must be considered. Large mussels (>30 mm) will exert a large influence on filtering activity compared to smaller individuals. If one large mussel filters at a different rate than another large mussel in another treatment, overall filtering activity will differ between treatments. Very large mussels should thus be excluded.

APPENDIX C

METHODS FOR THE COLLECTION AND REARING OF LARVAL BLUEGILL.

Thanks again to Jessica Rettig for providing valuable advice.

Collection: I collected hatchling bluegill directly from nests in Warner lake (Fig. A2). Bluegill nests are bowl-shaped depressions in soft sediment close to the shore. Up to 50-100 cm wide and 20 cm deep, nests are clustered into colonies. The nests collect organic debris but are cleaned out when active. When hatchlings are present, the nests appear to have a gray or pink "fuzzy" substance within them, sometimes glittering with gold (the gold-eye stage). Adult bluegill guard active nests and so try not to scare fish away from a colony as you approach (guarding fish act as flags to active nests). To collect hatchlings, swim slowly up to a nest and use a turkey baster with its tip cut off. Take two baster's worth and squirt the fish into a jar and cap the jar, all while underwater. Transport jars to the lab in a cooler filled with lake water to prevent the jars from overheating. Don't get greedy during collection. Call Lyle Champion for permission to work in Warner Lake. Lawrence Lake had many colonies that were not active. Middle Three Lakes had one colony that yielded some active nests. Warner Lake is the best. I made collections in late June to practice, fine tune the tanks, and be sure I'd have larvae swimming at all times.

Holding: I reared hatchling bluegill in the lab for 4-7 days. Acclimate jars by placing them into prepared tanks without removing the lids for 30 minutes. A ten-gallon tank can accommodate up to three jars. Add methylene blue to the tank water just until there is a

slight blue tint to the water. Have an air stone running. Use an out-o-tank filter, and wrap the intakes with filter floss. After 30 minutes, open jars and lay them on their side. Place the airstone at the mouth of the jar, but not inside. This creates a gentle current within the jar without disturbing the fish. After a few days, the hatchlings will be swimming in the tank (swimming stage). This method results in several hundred fish in a tank. If something goes wrong and the fish die, their bodies become white.

Feeding: I fed larval bluegill brine shrimp nauplii in the lab. Have a brine shrimp culture prepared. Brine shrimp require a rearing chamber to ensure that nauplii can be collected without their egg shells. The culture also needs to be warm (80°F) and saline. Sea monkeys hatch overnight and are orange. You can tell if the bluegill are eating the shrimp because the bluegill's bodies are translucent and their stomachs turn orange. Also add copepods collected from Duck Lake so the larvae can get some experience feeding on naturally occurring plankton.

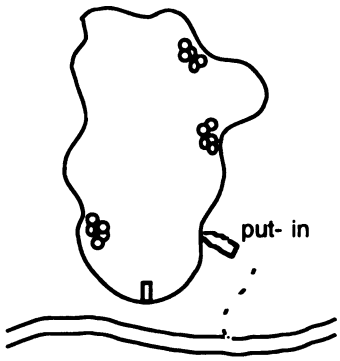
Size and Density: I used 20 fish (7.5mm-long) per treatment. The fish are usable when they begin eating. Since they grow quickly, they must be small to qualify as larval and not juvenile. 20 fish per bag represents a density at the high end of, or just higher than natural density.

Sea Monkey (brine shrimp) rearing tank: Use a 10 gallon tank. Affix two opaque Plexiglass dividers inside the tank, one with space above and one with space below. This forces the nauplii to swim and shed their egg shells. Affixing the light to the side of the

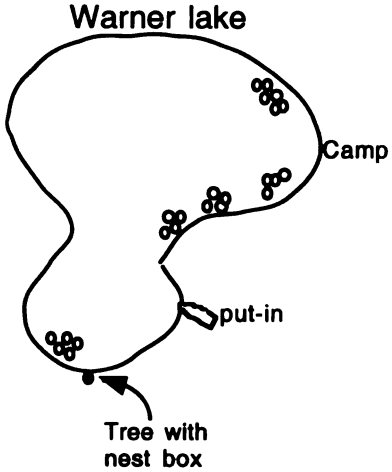
tank heats the tank nicely. Put the eggs in the far right area, and they will swim to the light and congregate. Add eggs every day near the time you need them so you will have nauplii ready for your fish larvae.

Figure A2: Location of some bluegill nest colonies in local lakes.

Lawrence lake



Warner lake



Middle 3 lakes

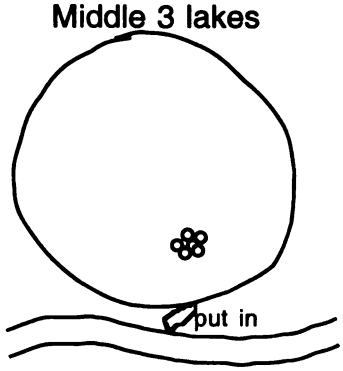
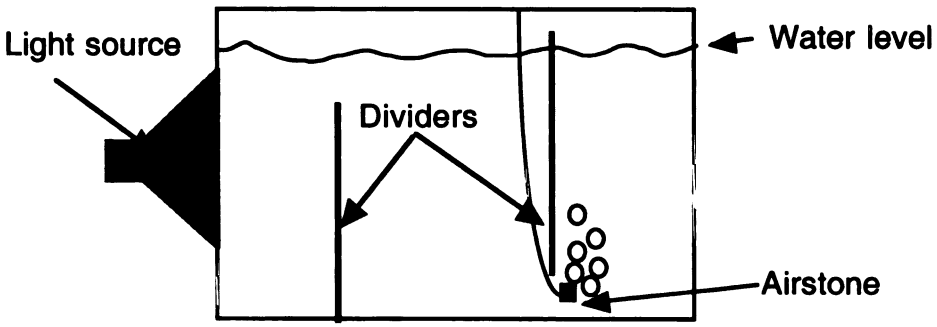


Figure A3. Design of brine shrimp rearing tank.


Light source
Water level
Dividers
Airstone

APPENDIX D

SAS program codes with dummy data

ANCOVA and Linear Regression

```
title ' title';
data logchla;
input ZM        LOGTP        LOGChla;
cards;
0       1.401   1.275
0       1.323   0.782
0       1.584   0.908
1       1.509   0.653
1       1.821   1.606
1       1.331   0.079
proc print data=logchla;
proc glm;
class zm;
model logchla = ZM LOGTP/SS3;
output out=logchla2 r=resid;
proc glm;
class zm;
model logchla = ZM LOGTP ZM*LOGTP/SS3;
proc glm;
model logchla = LOGTP;
by ZM;

run;
quit;
```

Kolmogorov-Smirnov

```
title ' K-S test';
data lakeTP;
input ZM        TP;
cards;

0       18.8
0       28.4
0       16.9
1       30.8
1       17.8
1       64.5


proc print data=lakeTP;
proc npar1way data=LakeTP;
class zm;
run;
quit;
```

Correspondence Analysis

```
Title 'Correspondence analysis';
data 'genera';
input Binuclearia      Oocystis      Scenedesmus Pediastrum    Mougeotia
      Chrococcus       Rhodomonas    Aphanocapsa Dinobryon     Staurastrum
      Gleobotrys       Selenastrum   Merismopedia Fragilaria   Diatom
      Chlamydomonas    Cerratium     Anabeana    Crysophyte    Lyngbya
      Ossilatoria      Microcystis   Coelosphaerium Ulothrix
      Aphanizomenon;
cards;
0     9418  0     0     0     0     14127 0     0     0     0     0
      1056  0     0     0     1420000     8852803     0     1421405
      0     46172 0     0     7539625
0     0     0     0     0     15249 4709  0     0     0     0     0
      0     496353      0     0     94667 63016 0     0     0     0
      0     0     164801
6669  3973  0     0     0     26303 58027 0     14236 0     0     0
      0     0     492505      0     0     134660      0     0     0
      0     327762      0     0
0     0     0     0     0     0     16023 770   27703 0     0     0
      0     0     0     0     189333      143377      0     0     0
      4329  35634 27703 89267
0     0     0     0     0     0     0     6541  21372 0     0     0
      0     0     0     0     189333      0     0     0     0     0
      399479      0     0
proc corresp;
var Binuclearia      Oocystis      Scenedesmus Pediastrum    Mougeotia
      Chrococcus       Rhodomonas    Aphanocapsa Dinobryon     Staurastrum
      Gleobotrys       Selenastrum   Merismopedia Fragilaria   Diatom
      Chlamydomonas    Cerratium     Anabeana    Crysophyte    Lyngbya
      Ossilatoria      Microcystis   Coelosphaerium Ulothrix
      Aphanizomenon;

run;
quit;
```

MANOVA

```
data survey;
input zm        Chloro Chryso Crypto Cyano  Pyrro;
cards;
0       21.12  39.91  24.17  261.40 112.40
0       68.65  79.18  16.76  62.41  45.58
0       22.00  88.52  38.72  78.77  1.00
0       30.26  40.28  25.21  64.90  57.42
0       1.00   89.96  1.00   74.05  57.42
0       1.00   175.60 29.17  248.41 101.36
0       21.77  63.80  40.08  107.58 72.35
0       31.28  57.81  34.59  14.83  1.00
0       1.00   74.93  24.17  65.64  1.00
0       84.54  25.33  1.00   6.93   1.00
0       32.52  1.00   36.33  60.42  56.81
0       71.57  1.00   1.00   41.93  1.00
0       62.41  78.30  36.04  64.09  56.81
0       36.64  73.68  25.45  208.54 45.58
1       34.19  43.64  26.40  33.68  1.00
1       12.44  19.96  1.00   31.88  1.00
1       19.32  26.61  21.12  59.99  1.00
1       1.00   32.07  39.00  43.86  57.42
1       48.52  54.55  26.92  436.89 1.00
1       25.87  28.81  1.00   65.53  1.00
1       24.67  85.29  25.15  147.65 72.35
1       48.22  1.00   24.17  307.78 123.71
1       1.00   1.00   22.87  39.40  45.58
1       37.56  49.56  37.75  60.34  1.00
1       15.68  42.26  1.00   95.68  1.00
1       15.77  46.35  32.31  47.94  3.08
1       26.32  57.62  34.40  189.95 58.00
proc glm;
        class zm;
        model Chloro  Chryso Crypto Cyano  Pyrro= zm/ss3;
        output out=phyto r=resid;
        manova H = zm;

run;
quit;
```

Repeated Measures ANOVA

```
data trans;
input rep zm bg day1 day2 day3 day4;
cards;
1     1     1     0.8     0.6     0.6     0.6
2     1     1     0.6     0.3     0.9     0.5
3     1     0     1.0     0.3     0.4     0.3
4     1     0     1.0     0.4     0.5     0.4
5     0     0     2.4     1.6     1.0     1.1
6     0     0     2.1     1.7     2.1     1.3
7     1     0     0.6     0.4     0.5     0.6
8     0     1     2.3     2.0     1.0     1.4
9     0     1     1.6     2.0     1.7     1.7
10    0     0     2.2     1.9     1.4     1.2
11    1     1     0.7     0.4     0.5     1.4
12    0     1     2.2     1.1     1.2     1.9
proc print data=trans;
proc glm;
class zm bg;
model day1-day4 = zm bg zm*bg/nouni;
repeated time 4 (1 4 7 11) profile/summary printm printe;
run;
quit;
```

APPENDIX E

Complete References with Additions

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