Lawrence University Honors Projects

2014

Effects of Toxic Cyanobacteria (Microcystis Aeruginosa) on the Feeding and Reproduction Ecology of the Copepod Eurytemora Affinis from Green Bay, Lake Michigan

Nicholas J. Barrett
Lawrence University, Nicholas.j.barrett@lawrence.edu

Recommended Citation
Barrett, Nicholas J., "Effects of Toxic Cyanobacteria (Microcystis Aeruginosa) on the Feeding and Reproduction Ecology of the Copepod Eurytemora Affinis from Green Bay, Lake Michigan" (2014). *Lawrence University Honors Projects*. 60.
https://lux.lawrence.edu/luhp/60

Effects of Toxic Cyanobacteria (*Microcystis aeruginosa*) on the Feeding and Reproduction Ecology of the Copepod *Eurytemora affinis* from Green Bay, Lake Michigan

By Nick Barrett

A Thesis Submitted in Candidacy for Honors at Graduation

from Lawrence University

May 2014

Table of Contents

Table of Contents i

Table of Figures iii

Table of Tables iii

Acknowledgements iv

Introduction 1

- Aquatic Ecosystems 2
- Biotic and Abiotic Characteristics 3
- Climate Change: Impacts on Aquatic Ecosystems 5
- Aquatic Ecosystems and Invasive Species 8
- Bottom-up and Top-Down Controls 10
- Phytoplankton: The Foundation of Aquatic Ecosystems 11
- Cyanobacteria as a Food Source 12
- Good Food Sources and Selective Feeding 14
- Harmful Algal Blooms 15
- Phytoplankton Used in This Study 20
- Zooplankton 23
- Study Environment 26
- Study Organism 29
- Significance and Importance of this Study 32

Methods

- Water Acquisition 34
- Animal Acquisition and Sorting 35
- Food Sources 36

- Preliminary Experiments

    - Survivorship in Varying Conductivities 37
    - Water Conditioning 38
    - Survivorship with Varying Levels of *Microcystis* 39
    - Survivorship in Differing Temperatures 39

Focal Experiments

Grazing Experiments 40
Egg Production Experiments 41
Statistical Analysis 43

Results

Preliminary Results

Survivorship in Varying Conductivities 43
Water Conditioning 44
Survivorship with Varying Levels of *Microcystis* 45
Survivorship in Differing Temperatures 47

Focal Results

Grazing Experiment 48
Egg Production Experiment 51

Discussion

Points of Emphasis 54
Grazing Experiment and Related Topics 55
Egg Production Experiment and Related Topics 66
Trade-off: More or Less? 71
Difficulties and Future Experiments 74
Implications and Conclusions 77
References 79

List of Figures

Figure 1: *Microcystis aeruginosa* 21

Figure 2: Microsystin-LR 22

Figure 3: *Scenedesmus quadricauda* 23

Figure 4a: Little Sturgeon Bay 27

Figure 4b: Lake Michigan and Green Bay 28

Figure 5: *Eurytemora affinis* 30

Figure 6: Survivorship in Varying Conductivities 44

Figure 7: Water Conditioning: Centrifugation Technique 45

Figure 8: Survivorship with Varying Levels of *Microcystis* 46

Figure 9: Survivorship at Varying Temperatures 48

Figure 10: Filtering Rate 49

Figure 11: Ingestion Rate 50

Figure 12: Survivorship in Grazing Experiment 51

Figure 13: Average Number of Eggs 52

Figure 14: Average Nauplius Size 54

Figure 15: Reproductive Trade-off: Average Nauplius Size versus Number of Eggs Produced 55

List of Tables

Table 1: Summary of ANOVA- Average Filtering Rate 49

Table 2: Summary of ANOVA- Average Ingestion Rate 50

Table 3: Summary of ANOVA- Average Number of Eggs 51

Table 4: Summary of ANOVA- Average Nauplius Size 52

Acknowledgements

I would first like to thank my advisor, Bart De Stasio, for giving me the opportunity to perform this project as well as for providing me with guidance and support along the way. I would also like to acknowledge the help of Andreas Brutemark, Joanna Engstrom-Öst, and Anna-Karin Almén from the Tvärminne Zoological Station who assisted in previous experiments and experimental set-up that was paramount to this project. I would like to thank Wayne Krueger and JoAnn Stamm from the Lawrence University Lab for all of their help with experimental materials, set-up, and clean up.

INTRODUCTION

Aquatic environments are ever changing systems, with multiple drivers and related effects that can change in time and space. The alteration of aquatic systems is an interesting and important area of scientific study. By gaining more knowledge and insight regarding the causes and effects of the various factors, scientists aim to limit, minimize, and predict the negative consequences of ecosystem change while maximizing the benefits of such change. Examples of factors that have the ability to change the overall functioning of an aquatic ecosystem include climate change, eutrophication, nutrient loading, biological invasions, and a plethora of anthropogenic activities. Many of these factors cause negative impacts and can heavily influence system change, and therefore they are the focal point of many scientific research studies aimed at investigating aquatic ecosystem functioning.

The effects and impacts of these drivers of ecosystem change can be seen through alterations of community structure, food web dynamics, nutrient cycling, and optimal habitats for current residents. It should be noted that these factors mentioned are not independent of one another; the occurrence of one could likely cause the occurrence of another. For example, a change in nutrient loading, likely through anthropogenic related activities, can cause eutrophication which then alters the food web and could provide a situation where an opportunistic invader species could out-compete native resident species due to the ecosystem change (Dorgham, 2014). Global climate change can also influence and/or cause many changes and presents a risk for virtually all biotic components in aquatic ecosystems (Doney et al. 2012). Overall, these factors present multiple hazards to aquatic ecosystems and their effects differ in time and across different systems. This, along with the interdependence of the factors makes studying their causes and effects even more difficult. This study deals with many of these factors including eutrophication, global climate change, and invasive species, all of which will be covered in more detail throughout. By analyzing the relationship between a type of mass-occurring cyanobacteria and an invasive species of copepod, this study has multiple ecological and environmental implications.

Aquatic ecosystems:

Aquatic ecosystems play an essential role in many crucial environmental functions such as nutrient recycling, water purification, attenuating floods, recharging vital groundwater, and providing habitats for wildlife. In addition to being vital contributors to biodiversity and ecological productivity, aquatic systems provide for a plethora of recreational uses and a stimulation of the tourism industry in many coastal regions. As mentioned before, global climate changes can alter many factors within an aquatic ecosystem, the biggest contributor being temperature change and its associated effects. It is essential to study and understand aquatic ecosystems on a small scale as well as a global scale, especially with the recent attention given to global climate changes.

The recent changes in global climate, with regards to temperature in particular, have been shown to affect physical and biological processes that regulate energy flow among trophic levels differently, making ecosystems’ responses to climate change difficult to forecast (Walther et al., 2002; Winder & Schindler, 2004). Given these difficulties, it is imperative that we use smaller scale investigations and scientific experimentation to gain a better understanding of the effects of aquatic ecosystems and how they respond. A better understanding of the interactions between the biotic and abiotic factors within an aquatic ecosystem and their response to global climate change will aid in predicting the future ramifications of such events.

Aquatic systems, mainly lakes, have generally been classified based on productivity levels using a trophic state index, labeling the systems as oligotrophic, mesotrophic, or eutrophic (Carlson, 1977). Oligotrophic refers to a system with low primary productivity resulting in a low nutrient content. Mesotrophic systems are those with an intermediate level of productivity and nutrients. Finally, a eutrophic body of water has very high biological productivity due to excess nutrients and autotrophic productivity.

Biotic and Abiotic Characteristics in Aquatic Ecosystems:

An aquatic ecosystem is comprised of biotic communities that are structured by biological interactions and abiotic environmental factors, including temperature increases induced by global climate change. It is important to keep in mind that these two types of factors, living and non-living, are far from independent from one another. Therefore, the regulation of existing communities is controlled by both biotic and abiotic factors (Andrewartha and Birch 1954; Power et al., 1988; Jackson et al., 2001).

Various abiotic factors of importance to an aquatic community include temperature, amount of sunlight, substrate type, water depth, nutrient levels (especially nitrogen, phosphorous, & carbon), pH, water flow & mixing (Loeb, 1994; Keddy, 2010). The amount of dissolved oxygen is of high importance, providing the possibility of life beyond the primary producers in an aquatic setting. While the negative effects of hypoxia (low levels of oxygen) are quite obvious, hyperoxia (too much oxygen) within an aquatic environment can result in harmful effects as well, such as changes in respiration rates and nutrient composition (Burnett, 1997; Nikinmaa, 2002; Olsvik et al., 2006). Nutrient levels, with special regard to total nitrogen (N) and phosphorous (P) ratios, are also of high importance in an aquatic community. Nutrient levels are especially important in regulating and controlling the abundance and diversity of many species of phytoplankton as well as the eutrophication process (Smith et al., 1999; Conley et al., 2009). The phytoplankton community assemblage and species abundance are vital components of any aquatic ecosystem since the phytoplankton are the primary producers and form the base and foundation of the food web. Hebert (2008) stated that N is a crucial nutrient in determining a lake's trophic status while P is one of the most common growth-limiting elements for phytoplankton. The existence of phytoplankton within an aquatic ecosystem is crucial, but over-abundance can cause declines in fish populations and hypoxic conditions leading to "dead zones" (Vallentyne, 1974; Turner & Rabalais, 2003). Temperature is another abiotic factor that can have a large impact on an aquatic ecosystem. Temperature can cause many alterations involving organisms' metabolic rates, nutrient availability/solubility, and even the toxicity of various chemicals (Cairns et al., 1975; Regier et al., 1990).

The biotic characteristics of an aquatic ecosystem are determined by the organisms that inhabit or occur there. These organisms include many kinds of bacteria, plants, phytoplankton, zooplankton, insects, and fish. Together these organisms make up the food web and play an important role in nutrient and resource cycling (Lindeman, 1942; DeAngelis et al., 1989). Out of this large range of organisms that exist in aquatic habitats, each one has unique adaptations, metabolic and physiological needs, diets, habitat preferences, etc. These characteristics that are unique to each organism have evolved, and are constantly evolving in an attempt to reach optimal functionality within the given environment.

All of the organisms that inhabit aquatic systems can be placed into a category of autotrophic or heterotrophic. Autotrophs are the primary producers that generate biomass from the sun and carbon dioxide (CO_2). Heterotrophs on the other hand, are organisms that rely on other organisms as sources of energy, mainly through direct consumption. Therefore, in aquatic ecosystems, there is a high degree of interdependence between all of its inhabiting life forms. Other biotic characteristics of interest include competition, mutualism, co-existence, and predation among the ecosystems' inhabitants. The independent functions as well as dependent interactions of the abiotic and biotic factors are of great interest to aquatic researchers all over the world. Understanding more about these factors and their responses/effects within aquatic ecosystems will aid in providing researchers with predictive and regulatory power. This deeper level of understanding and predictive power will then allow for the control and management of the negative impacts to aquatic systems. The vital importance of these factors is undeniable. However, due to the presence of complicated feedback loops, the peculiarity of water as a growth medium, and the variation in responses by aquatic organisms and communities, studying the specific effects of these factors and generalizing them can be challenging (Kononen, 2001; Anderson et al., 2002; Keddy 2010).

Climate change: Impacts on Aquatic Ecosystems:

Climate change is currently causing significant alterations in physical, chemical, and biological aspects of aquatic systems all around the world. In particular, aspects that may be affected include biogeochemical processes, carbon dynamics, food web structure, biodiversity, primary and secondary production, as well as changes in organism range, distribution, and habitat quality/quantity (Wrona et al., 2006). Although the effects of global warming on the chemical and physical properties of lakes have been documented, biotic and ecosystem-scale responses to climate change have been only estimated or predicted by manipulations and models (O'Reilly et al., 2003). A meta-analysis of the effects of climate change by Daufrense et al. (2009) suggests that there are three universal ecological responses to global warming within aquatic ecosystems. These responses consist of: (*1*) an increase in the proportion of small-sized species and young age classes; (*2*) a shift in species ranges towards higher altitudes and latitiudes; (*3*) seasonal shifts in the life cycle events of aquatic organisms. Wrona and colleagues (2006) also pointed out that while the effects of global climate change are of extreme importance, estimating and generalizing the specific effects can be difficult given that the magnitude, extent, and duration of the impacts and responses will vary and be both system- and location-dependent.

Poff et al. (2002) provide a very informative and in-depth assessment of the impacts of global climate change on aquatic ecosystems. The summary of their work highlights some main points of their extensive study. They stated that aquatic and wetland ecosystems are extremely vulnerable to climate change and the effects can be more substantial than in a terrestrial setting. The metabolic rates of organisms and the overall productivity, distribution, and diversity of these ecosystems are directly regulated by temperature and climate. Temperature increases will also cause a shift in the thermal suitability of aquatic habitats for native, non-native, and novel species. Changes in precipitation and runoff can modify the quality of the habitat and may cause significant negative effects on these ecosystems (nutrient or pollutant loading). Overall, these conclusions made by Poff and colleagues (2002) indicate climate change as a significant threat to the species composition and the functionality of aquatic ecosystems around the world.

There exist critical uncertainties in regards to the manner in which specific species and ecosystems as a whole will respond to climate change. These uncertainties arise both from how regional climate will change and how the complex ecological systems themselves will respond. Therefore, as climate change causes alterations in ecosystem productivity and species composition, there are many unforeseen ecological changes expected that may threaten the functionality of these ecosystems and the services they provide to humans. Finally, the manner in which humans adapt to a changing climate will greatly influence the future status of inland freshwater and coastal wetland ecosystems.

In many cases, changing composition in aquatic ecosystems and rising temperatures have been connected to the spread of invasive species by influencing the likelihood of new species becoming established. Establishment of invasives can be through the elimination of colder temperatures or winter hypoxia that prevent many non-native species from surviving in certain habitats (Rahel & Olden, 2008). The introduction of these novel species will modify the ecological impacts of invasive species by enhancing their competitive and predatory effects on native species and disease virulence (Rahel & Olden, 2008). Overall, Rahel & Olden’s (2008) findings highlight the complex interactions that exist between climate change and invasive species that will influence how aquatic ecosystems and their biota will respond to novel environmental conditions.

The increase in global temperature has also contributed to the growing problem of the occurrence of harmful algal blooms (HABs) and, in particular, cyanobacteria blooms (Paerl & Otten, 2013). These cyanobacteria blooms and their effects on aquatic ecosystems are a main focus of the study at hand and will be referred to repeatedly. The optimal growth conditions for cyanobacterial blooms include increased nutrients and temperatures, both of which result from the on-going climate changes (Paerl & Huisman, 2008; Dionysiou, 2010; Paerl & Otten, 2013). The occurrences, conditions and effects of these HABs will be covered in more detail later in this study.

Aquatic Ecosystems & Invasive Species:

Aquatic systems, like terrestrial systems, are susceptible to the effects of invasive organisms. According to the U.S. Fish and Wildlife service, aquatic invasive species (sometimes referred to as AIS, exotic, nonindigenous or non-native) are aquatic organisms that invade ecosystems beyond their natural, historic range. Humans, either intentionally or unintentionally, have assisted in the spread of invasive species around the globe. Intentional introductions consist of a deliberate transfer possibly for biological control methods (various insects are introduced to control invasive plants), fish stocking, or for other reasons. On the other hand, unintentional introductions are accidental in nature. Examples of how invasive species can be spread in aquatic ecosystems include by ships (ballast water), boats (hull fouling), aquaculture, aquatic recreation, and connected waterways, just to name a few. As humans have facilitated the dispersal of plants and animals in aquatic ecosystems by breaking down various barriers, invasive alien species have increasingly altered the composition and functioning of such ecosystems (Dukes & Mooney, 2004).

The presence of invasive species can have many impacts on an ecosystem including causing harm to native ecosystems and organisms. These impacts then affect commercial, agricultural, and recreational activities that are dependent on these ecosystems. Due to these possible negative implications, invasive species are one of the largest threats to aquatic ecosystems. According to the Environmental Protection Agency, invasive species represent the second leading cause of species extinction and loss of biodiversity in aquatic ecosystems worldwide. Loss of biodiversity and extinctions are often a result of high levels of predation (by the invasive species) or by means of competition where the newly introduced invasive species out-compete the native species. McCormick and colleagues (2010) state that the physical and biological disruptions of aquatic ecosystems caused by invasive species can alter water quantity and water quality through multiple mechanisms, including alterations in nutrient cycling and the food web. Chandra and Gerhardt (2008) point out that the spread and establishment of invasive species are occurring at an accelerated rate and that their introductions are of increasing global concern. Ecologists have recognized for some time that these biological invasions, besides the previously mentioned ramifications, affect various levels of ecological organization and structure (Elton, 1958). It is important to note that though these negative impacts mentioned apply to most species of invasives, their effects on ecosystems are variable and not every introduction will inevitably lead to profound negative impacts. Given the range of potential environmental impacts researchers now recognize biological invasions as an important element of global change (Dukes & Mooney, 1999). Some examples of various invaders in aquatic ecosystems include zebra mussels (*Dreissena polymorpha*), Eurasian Watermilfoil (*Myriophyllum spicatum*), sea lamprey (*Petromyzon* *marinus*), Asian carp (many species of heavy-bodied cyprinid fish), and various types of snails, zooplankton, and phytoplankton.

Bottom-Up and Top-Down Controls:

Populations within an ecosystem have the ability to be controlled or regulated by various factors. There are two general mechanisms that contribute to this control and they are referred to as top-down and bottom-up controls. In aquatic ecosystems these control mechanisms are most studied in terms of their effects on phytoplankton and zooplankton. Bottom-up control concerns the factors that affect growth including light, temperature, and nutrients. The effects of bottom up control work from the bottom of the food web to the top, hence the name. On the other hand, top-down controls refer to elements including predation and defensive mechanisms and therefore work from the top of the food web to the bottom. It is important to keep in mind that these regulatory mechanisms related to the food web vary in time and space and there are many complex interacting components that go into each.

These two concepts have been the focus of numerous studies concerned with investigating food web dynamics and community structure. It has been demonstrated that grazers can be very effective in removing prey biomass and heavily influencing community structure (Feminella and Hawkins 1995,Steinman 1996). However, bottom-up factors including light and nutrient supply can also have effects on various aspects including algal biomass, nutrient composition (type and concentration), productivity, and species composition (Rosemond et al., 1993; Hill et al. 1995; Francoeur 2001; Hillebrand, 2002). It is very important to the proper analysis of these two mechanisms to realize that they are not mutually exclusive and that a strict and distinct separation of the effects on communities is unrealistic (Stewart 1987, Leibold et al. 1997).

Phytoplankton: The Foundation of Aquatic Ecosystems:

Phytoplankton play a vital role in any aquatic ecosystem by providing the autotrophic and photosynthetic foundation to such an environment. Phytoplankton are labeled as the primary producers within aquatic systems, synthesizing organic compounds from carbon dioxide dissolved in water and energy from the sun. Being the primary producers in aquatic ecosystems, phytoplankton are also the primary food source and form the base of the aquatic food web and nutrient cycling pattern. Therefore, phytoplankton effects are distributed throughout the aquatic food web within their given ecosystem (White, 1981; Carpenter et al., 1987; Turner and Tester, 1997; Engström-Öst, 2002).

Phytoplankton productivity is reliant on adequate nutrient supplies, especially nitrates and phosphates. However, the rapidly increasing rates of nutrient supply, much of which is through manmade processes, fuels accelerating primary production or eutrophication (Paerl et al., 2001). One ramification of eutrophication can be formation of HABs or great increases in the amount of phytoplankton in a water body as a response to increased levels of nutrients (Anderson, 2002). That study explains that the negative environmental effects of eutrophication include hypoxia, the depletion of oxygen in the water, which causes a reduction in specific fish and other animals. Water movement and the movement of various physical factors allows for the existence of microhabitats and the coexistence of many populations of phytoplankton within a small area. Such physical factors affecting the community composition of phytoplankton (coexistence) may include nutrient inflows, water mixing and upwelling, convergence or divergence, changes in stratification, or vertical migration (Kononen, 2001).

Included in the classification of phytoplankton are some types of bacteria and protists. However, the majority of phytoplankton are single-celled plants. Among the common kinds are cyanobacteria, diatoms, dinoflagellates, green algae, and coccolithophores. There is a relatively high level of competition between co-existing phytoplankton in an aquatic community. This competition is mainly dependent on the nutrient availability and the phytoplankton's efficiency of nutrient intake, both of which vary on a temporal scale (Sakshaug & Olsen, 1986; Egge & Aksnes, 1992; Litchman et al., 2004). Therefore, all of these types of phytoplankton have their own unique qualities, traits, adaptations, and habitat preferences.

Cyanobacteria as a Food Source:

Cyanobacteria are a plentiful, diverse, and common type of phytoplankton found generally in the pelagic community. Due to the cyanobacteria's blue-green pigment (phycocyanin), they were formerly classified as blue-green algae and belong to a specific phylum of bacteria (Mclean & Sinclair, 2013). Cyanobacteria are typically characterized as a low quality food source for its consumers. The low food quality has mainly been associated with the cyanobacteria's difficult-to-handle morphology, low nutritional quality, and toxin content (Porter and Orcutt, 1980; Lampert, 1987; Kirk & Gilbert, 1992). These toxins have been classified in five broad categories that include more than 200 specific forms (Boyer & Dyble, 2008). Boyer & Dyble (2008) demonstrated that one of the most common types of toxins produced are cyclic hepatotoxins (peptides). Examples include microcystin which is named after its producer, *Microcystis*, as well as nodularin which is produced by *Nodularia* (Brittain et al. 2000), a close relative of *Microcystis*. These toxins have been shown to be harmful and affect zooplankton diversity, grazing, and mortality (Koski et al., 1999; Enström-Öst 2002; Engström-Öst et al., 2002). These effects can accumulate in the food web via bioaccumulation and negatively impact higher trophic levels as well (Kotak et al., 1996; Magalhaes et al., 2003; Xie et al., 2005; Hansson et al., 2007; Karjalainen et al., 2007). Toxins like microcystin have been shown to inhibit protein phosphatases and are therefore dangerous to other living organisms (Kozlowsky-Suzuki et al. 2003). There is a plethora of possible negative effects stemming from these toxins. It should be kept in mind that if a strain of phytoplankton is classified as a cyanobacterium or even as *Microcystis*, this does not necessarily mean that it produces toxins (there exist non-toxic strains as well).

Besides using toxin as a defense, some cyanobacteria have evolved the ability to have a filamentous or colonial morphology, forming aggregates and making them less appealing as a food source due to mechanical difficulties with feeding (Webster & Peters, 1978). As mentioned previously, cyanobacteria have been classified as a low quality food source. This lack of nutritional quality seems to be linked to the cyanobacteria lacking essential compounds such as polyunsaturated fatty acids (Holm & Shapiro, 1984; Demott, 1986; Muller-Navarra et al., 2000). Studying these techniques to avoid predation, their effects, as well as their evolution through time is difficult and is the main focus of many ongoing biological and chemical studies.

Despite the known negative effects of cyanobacteria, there exists some evidence that these phytoplankton can have some positive effects on an aquatic community as a whole (Engström-Öst, 2002). For one, cyanobacteria have the ability to turn light energy into useable biological energy through photosynthesis that can then be transferred throughout the food web. Some types of cyanobacteria are nitrogen fixers, which can have a positive impact on nutrient cycling and dynamics (Mitsui et al. 1986; Kivi et al., 1993). Cyanobacteria can also provide a food source for specialized consumers and therefore allow the specialist to be successful in a community dominated by cyanobacteria (Meyer-Harms et al., 1999). Compared to the actively growing cyanobacteria, the decomposition of this type of phytoplankton (eventually degrading into recycled detritus) may provide a better food source to consumers. This is due to the decrease in toxins during senescence and the attached bacteria associated with the decomposition providing additional nutrients (Repka et al., 1998; Kankaanpaa et al., 2001). Therefore, it seems as though the overall effects of cyanobacteria (positive or negative) may depend on various environmental factors and/or details of the community assemblage (phytoplankton and consumers) and are likely to vary by individual organisms, species, and ecosystems.

Good Food Sources and Selective Feeding:

There are numerous interactions and levels of co-dependence between the phytoplankton and zooplankton populations within any aquatic ecosystem. The means by which food quality is typically determined is by providing zooplankton with different types of phytoplankton and analyzing the effects on the organisms' survival, growth rate and reproductive rate. Herbivorous zooplankton production and success is constrained by the zooplankton's ability to ingest and digest phytoplankton (Brett & Muller-Navarra, 1997). There are various characteristics possessed by phytoplankton that are associated with a high quality source of food for zooplankton grazers. Cummins & Klug (1979) suggest that the natural growth patterns of most zooplankton involve the interaction of temperature and food quality. Food quality for grazers has been related to mineral (C, N, P) and biochemical (amino acids, fatty acids) constituents (Ahlgren & Hyenstrand, 2003). The lipid composition itself has been suggested as a probable factor determining the nutritional quality of the algae, with higher levels possessing more nutritional value (Ahlgren et al., 1990). Aquaculture studies provide some direct evidence of the importance of the long-chained polyunsaturated fatty acids (PUFA) for zooplankton. PUFAs are almost exclusively synthesized by plants and are essential for higher organisms (Brett & Muller-Navarra, 1997).

Phosphorous, C:P ratios, the presence of PUFA and eicosapentaenoic acid (EPA) in phytoplankton are all reported to affect the growth rates of zooplankton significantly (Gulati & Demott, 1997; Breteler et al., 1999). Structural and morphological changes in P-limited cells most likely reduce their digestibility and may be a highly efficient strategy of P-limited algae to resist heavy grazing pressure (Van Donk & Hessen, 1993). Diatoms and flagellates are generally considered as high-quality foods because of their high EPA content. On the contrary, cyanobacteria are low-quality food, having both low EPA and P content. Brett & Muller-Navarra (1997) state that highly unsaturated fatty acids (HUFA), a subset of PUFA, have been found to be critical for maintaining high growth, survival, reproductive rates and high food conversion efficiencies for a wide variety of marine and freshwater organisms. HUFA may be key nutritional constituents of zooplankton diets, and may determine energetic efficiency across the plant–animal interface, secondary production and the strength of trophic coupling in aquatic pelagic food webs (Brett & Muller-Navarra, 1997).

Harmful Algal Blooms: Conditions, Causes, Effects, and Management:

It is important to understand the conditions that provide for the formation and maintenance of HABs within an aquatic ecosystem when trying to control and limit the negative effects of these blooms. The basis of the success of these blooms is based on out-competing other phytoplankton species co-existing in an environment. Numerous freshwater phytoplankton are capable of forming blooms; however, cyanobacteria are the most notorious bloom formers. This is especially true for harmful toxic, surface-dwelling, scum-forming species (e.g., *Anabaena, Aphanizomenon, Nodularia, Microcystis*) and some subsurface species (*Cylindrospermopsis, Oscillatoria*) that are efficient at exploiting nutrient-enriched conditions (Paerl et al., 2001). Many harmful species are tolerant of extreme environmental conditions, including very high light levels (UV), high temperatures, various degrees of desiccation, periodic nutrient deprivation and overload (Paul, 2008). Blooms are a prime agent of water quality deterioration, including foul odors and tastes, deoxygenation of bottom waters (hypoxia and anoxia), toxicity, fish kills, and food web alterations.

The physiological strategies by which different groups of species obtain their nutrients have been better understood recently, and alternate modes of nutrition such as heterotrophy and mixotrophy are now recognized as occurring substantially among HAB species (Anderson et al., 2002). Some of the most noxious cyanobacterial bloom genera (e.g., *Anabaena, Aphanizomenon, Cylindrospermopsis, Nodularia*) are able to fix atmospheric nitrogen (N_2) to fulfill their N-related needs. This ability makes it possible for these cyanobacteria to out-compete other species of phytoplankton and periodically dominate under nitrogen-limited conditions (Paerl et al., 2001).

Blooms can be attributed to two primary factors: natural processes such as circulation, upwelling relaxation (decrease in water movement) and river flow; and anthropogenic loadings leading to eutrophication (Sellner et al., 2003). Eutrophication and increases in the nutrient load are mechanisms by which harmful algae appear to be increasing in extent and duration in many locations. In waters susceptible to blooms, human activities in water- and airsheds have been associated with the extent and magnitudes of the blooms. Nutrient enrichment has been strongly linked to stimulation of some harmful species, but for others it has not been an apparent contributing factor (Anderson et al., 2002). This shows that similar nutrient loads do not have the same impact in different environments or in the same environment at different points in time. The overall effect of nutrient over-enrichment on harmful algal species is clearly species-specific. The types and amount of nutrient input constraints depend on hydrologic, climatic, geographic, and geologic factors, which interact with anthropogenic and natural nutrient input regimes. Recall that temperature can also play an important role in the promotion of HABs since increases in temperature tend to lead to higher nutrient levels, more effective toxins, strengthening of vertical stratification (lowering mixing), and longer optimal growth periods (Paerl & Huisman, 2008). In general the study by Paerl & Huisman points out that cyanobacteria grow better at higher temperatures (often above 25°) than do other species of diatoms and green algae. Low CO_2 availability, high pH, and low grazing rates may also contribute to the increased growth of cyanobacteria in HABs (Lehman et al., 2008).

The degraded water quality from increased nutrient pollution by humans can promote the development and persistence of many HABs. These effects are dependent on the composition, not just the total quantity, of the nutrient pool (Heisler et al., 2008). The sources of nutrients potentially stimulating algal blooms include sewage, atmospheric deposition, groundwater flow, as well as agricultural and aquaculture runoff and discharge (Anderson et al., 2002). These authors also stated that the increases in nutrient loading from these sources typically leads to, as mentioned previously, anoxia and even toxic or harmful impacts on fisheries resources, ecosystems, and human health and recreation. Many of the regions affected by cyanobacterial blooms have witnessed reductions in phytoplankton biomass (as Chlorophyll *a*) or HAB incidence when nutrient controls were put in place. Shifts in species and community composition have often been attributed to alterations in the nutrient supply ratios, primarily N:P or N:Silica. Recently, important factors have been expanded to include organic forms of nutrients, and that an increase in the dissolved organic carbon to dissolved organic nitrogen ratio (DOC:DON) has been observed during periods of HABs (Anderson et al., 2002).

Boyer and Dyble (2008) provided an extensive analysis of the effects that various environmental (chemical and physical) factors have on HABs gathered from other studies (primarily from Paerl, 1996). Some of these factors outlined by these researchers may be viable controls or regulatory mechanisms for HABs. The introduction of new water, flushing of water, and shortening water residence time can all serve as removal mechanisms for blooms. Vertical mixing (usually on a large-scale) can disrupt near surface accumulations of buoyant bloom populations. Shading might serve as a means of altering the phytoplankton community composition and negatively affecting cyanbacterial surface bloom taxa. Since temperatures in excess of 20°C (or increases in overall temperature), stratification and high nutrient loading can promote blooms, avoiding these circumstances would be ideal. Modifications in pH levels can alter community composition as well; low pH (under 6) has been shown to favor eukaryotes while high pH (above 8) favors cyanobacteria. Nutrient (N and P) input reductions (long-term) are frequently effective in reducing cyanobacteria algal bloom potentials. In particular, P levels should be the center of attention for regulation since low N:P ratios (<20), often caused by excessive P, can increase bloom prevalence. Restricted availability of iron may also promote cyanobacteria (and HAB) growth since cyanobacteria are able to compete effectively for low levels of iron compared to other species of phytoplankton. Salinity in excess of a few percentages can be an effective barrier to development and persistence of some cyanobacteria species

Management of these HABs and their negative impacts is an important topic to environmental groups around the world and the Environmental Protection Agency is the main advocate in the United States. Some control mechanisms have been previously mentioned in the preceding paragraphs with regards to the summarization of the findings of Paerl, 1996 (from Boyle and Dyble, 2008). Recently emerging and newly developed tools and techniques are improving the detection and onset of HABs and their toxins. Researchers are rapidly advancing toward being able to have strong predictive power when it comes to the formation of HABs. Experimental studies are crucial to further the knowledge and understanding about the role various factors (especially nutrients) play in HABs expression (Heisler et al. 2008).

Overall, the main avenue for the control and management of cyanobacterial and other phytoplankton blooms includes nutrient input constraints, most often focused on nitrogen (N) and/or phosphorus (P). Single nutrient input constraints have been shown to be effective in some water bodies, but it is the dual N and P input reductions that are usually required for effective long-term control and management of HABs. In some systems where hydrologic manipulations are possible, enhanced flushing and artificial mixing (in conjunction with nutrient input constraints) can be particularly effective alternatives (Paerl et al., 2001).

Phytoplankton Used in This Study: The Green and the Blue-Green:

In this study two types of phytoplankton were used to investigate the effects that the toxin-producing cyanobacteria have on the feeding and reproduction ecology of a particular species of invasive calanoid copepod. The “good” food source, known to be nutritionally adequate, is a type of green algae named *Scenedesmus quadricauda* (Ahlgren et al., 1990). The toxin-producing cyanobacteria species under investigation is *Microcystis aeruginosa* and the name of the toxin it produces is microcystin, a cyclic hepatopeptide toxin (Brittian et al., 2000) (Figure 2). The general qualities and characteristics of cyanobacteria covered previously are applicable to the species *Microcystis aeruginosa.* The use of *Scenedesmus* and *Microcystis* together as contrasting food sources has been employed in a variety of other studies (Bringmann & Kuhn, 1978; Lampert, 1981; Hairston et al., 1999; Kuwata & Miyazaki, 2000 Lurling, 2003; Dwyer, 2013).

Microcystis aeruginosa is a non-nitogen fixing colonial cyanobacteria species and the toxin that it produces has been the focus of many studies investigating the effects of toxins on aquatic ecosystems (Kozlowsky-Suzuki et al., 2003; Juhel et al., 2006; Jang et al., 2007; Karjalainen et al., 2007). *Microcystis aeruginosa* is characterized by small cells that are only a few micrometers in diameter and lack individual sheaths (Figure 1).

Microcystis has also been known to form cellular aggregations which, in conjunction with the toxins, can also act as a defensive mechanism impairing ingestion by zooplankton (Shen et al., 2011; Wang et al., 2011).

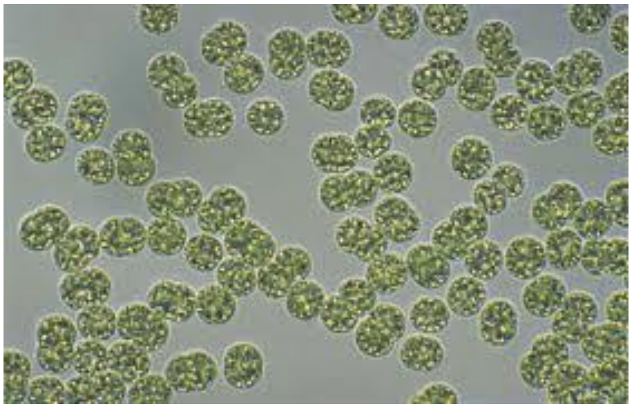

Figure 1-*Microcystis aeruginosa*.
Source: www.recetox.muni.cz

Various factors affecting the growth rate of *M. aeruginosa* have been well documented (Zehnder & Gorham, 1960). The specific effects of nitrogen, phosphorous, ammonia, trace metals, light, pH and temperature have all been investigated (Van der Westhuizen & Eloff, 1983; Lukac & Aegerter, 1993; Bury et al., 1995; Long et al., 2001; Downing et al., 2005). Biotic and abiotic factors previously discussed in the cyanobacteria and HAB sections apply also to the species of interest, *Microcystis aeruginosa*. Van der Westhuizen & Eloff (1983) propose that a pH of 9 is the optimal level for growth of *M. aeruginosa*. In most cases, many of these biotic and abiotic factors can also influence the toxicity of the *M. aeruginosa* cell.

The toxin produced by *Microcystis* is appropriately named microcystin (Figure 2). Once again, this particular strain of cyanobacteria is known to produce harmful algal blooms which are a topic of interest in this study (Wilson et al., 2005). Besides by means of direct ingestion, the microcystin toxin has a hydrophobic component, a specific amino group that can provide additional mechanisms for transfer throughout an ecosystem by physically attaching itself to another surface, like another organism (Harada, 1996; Yuan and Carmichael, 2004). *Microcystis* has also been shown to have the ability to regulate buoyancy via gas vesicles, allowing for movement of the cell as well as its toxins throughout the water column (Thomas & Walsby, 1985). The same study also provided evidence that the formation and regulation of these gas vesicles seems to be dependent on various environmental factors including light, temperature, and nutrient availability. Reynolds et al. (1981) gives an informative review of the annual cycle exhibited by *Microcystis aeruginosa*.

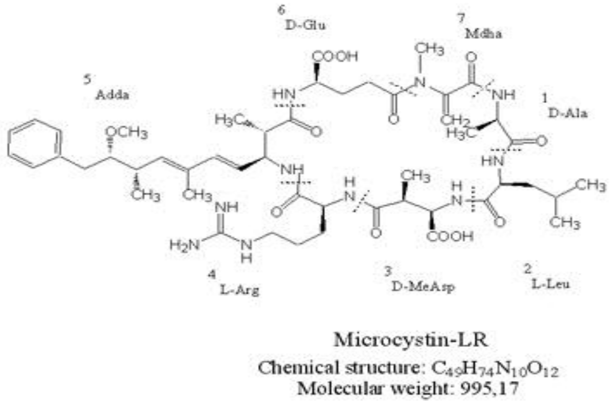

Fig. 2- Structure of microcystin –LR produced by *Microcystis aeruginosa* (according to J. Nawrocki et al., 2000).

Scenedesmus, which is one of the most common freshwater genera, exhibits extremely diverse morphologies among species. This makes them a difficult group to understand and identify (Lurling & Beekman, 1999). Species of *Scenedesmus* are of the class Chlorophyceae and are colonial and non-motile. In general, the morphology of *S. quadricauda* can be explained as an elongated oval (Figure 3). It has been documented that many species of *Scenedesmus*, including *quadricauda*, have defenses that include colony and spine formation and are likely induced by the presence of grazing predators, light availability and temperature (Lurling & Van Donk, 2000; Mayeli et al., 2005). The importance of ionic metals like iron, copper and zinc on the growth and development of *S. quadricauda* have been suggested and documented (Peterson, 1982). Phosphates and ammonium have also been shown to be important factors influencing growth rates and nutrient intake abilities of *Scenedesmus* species (Rhee, 1973; Kuwata & Miyazaki, 2000). It has been suggested through experimentation that the growth rate of *S. quadricauda* has an optimal pH of 6.5 (Ostilrlind, 1950).

Figure 3- *Scenedesmus quadricauda*
Source: CCALA- www2.butbn.cas.cz

Zooplankton:

Zooplankton are a morphologically and taxonomically diverse group of aquatic organisms. This categorization includes unicellular, colonial, and multicellular forms, protozoa, protists, invertebrates as well as vertebrates. Sizes of zooplankton span many orders of magnitude, from few-micron-sized flagellates to meter-sized gelatinous forms. Zooplankton are very important to overall ecosystem functioning and are responsible for transferring energy from the primary producers (phytoplankton) to the rest of the food web. Although most zooplankton species have an herbivorous diet, there are also many species that exhibit predatory behavior and feed on other small zooplankton. Zooplankton may be found in the open ocean (marine) habitats, lentic freshwater, as well as lotic waterways. Zooplankton are primarily transported by ambient water currents, but many have the ability of self-induced locomotion (diel vertical migration) that can be used to avoid predators and or increase prey encounter rate (Folt & Burns, 1999; Saiz, 2009).

Despite being a very diverse group, all zooplankton are faced with the common problem of finding and capturing food in a dilute and usually viscous environment. There are two components to food acquisition: the first is to find or encounter the food, the second is capturing it. Kiorboe (2011) points out that one aspect of finding food includes behaviors that utilize a patchy distribution of food, which is a common form of food distribution in aquatic ecosystems. It is well documented that many zooplankters either migrate to the surface, which is where the phytoplankton is concentrated, or have motile behaviors that allow them to congregate in patches of food, essentially by modifying their motility in response to the ambient food concentration (McLaren, 1963; Tiselius, 1992; Fenchel & Blackburn, 1999). There are numerous strategies or mechanisms of feeding across the species of zooplankton. Kiorboe (2011) provides an overview of zooplankton feeding mechanisms and suggests that one can distinguish between four major feeding types in zooplankton: (*1*) passive ambush feeders that passively encounter and intercept prey due to the motility of the prey; (*2*) active ambush feeders that passively encounter (perceive) motile prey and capture these by active attacks; (*3*) feeders that generate a feeding current and retrieve prey either by directly intercepting it, by straining/filtering the prey out of the feeding current, or by perceiving and capturing individual prey that arrive in the feeding current; and (*4*) cruise feeders that move through the water and capture individual prey, typically after having perceived the prey remotely. Food quality/preference, clearance rate and ingestion rates are very important aspects of zooplankton feeding and can differ between individuals and species (Vargas & Madin, 2004).

Besides competition, food availability and the ability to obtain the food (phytoplankton and small zooplankton) are the main factors affecting growth rates of zooplankton on both a community and individual scale (Huntley & Boyd, 1984). This same study also generally concluded that marine, herbivorous zooplankton are less likely to experience food-limited growth in coastal areas than in the open ocean. Temperature is also an important component directly related to zooplankton growth and food availability and its effects differ between organisms and ecosystems (McLaren, 1963; Vidal, 1980). Vidal (1980) suggests that the final body size of small species of copepods may be determined primarily by temperature, whereas final body size of large species may actually be more dependent on food concentration than on temperature. This study by Vidal also suggests that species of zooplankton seem to be geographically and vertically distributed in relation to body size and food availability in order to optimize growth rates at various stages of their life cycles.

Food availability is not the only component affecting zooplankton community assemblage. Competition (for resources) and predation are critical regulatory components in zooplankton survival and community structure (Dodson, 1974; Lynch, 1977; DeMott, 1989). Resource competition is an indirect interaction, affecting the competitors via the exploitation of common food sources. DeMott (1989) states that in regards to competition within a community, there have been two broad alternatives considered. First, organisms constantly face food limitation and coexist via niche partitioning. In contrast, nonequilibrium models emphasize the role of changing conditions (environmental, predatory, exploitative competition) in stabilizing species coexistence since a shift in conditions could likely cause shifts in competitive ability. In another case, Brooks and Dodson (1965) proposed a two-part hypothesis that incorporates zooplankton size, competition, predator presence, food size and food availability. The first part of this hypothesis was that size-selective predation by vertebrate predators (fish) can exclude large species of zooplankton from an aquatic ecosystem and leave only small species. The other part of this hypothesis deals with size-efficiency feeding which explains the absence of small species in associations of large species. The large species are thought to discourage the smaller species via competition for food by feeding in a more efficient manner and the ability to digest larger food sources. In another study, Rothhaupt (1990) suggests that the concepts of threshold food concentrations, of resource partitioning due to different feeding modes, and of the effects of resource variance are regarded to be the most promising approaches to understanding zooplankton competition.

Study environment: Green Bay, Lake Michigan:

The Green Bay, Little Sturgeon Bay, and Lake Michigan freshwater environments are the aquatic ecosystems of interest in this study (Figure 4a & 4b). Much of the following information about the Green Bay water system comes from a report by Bertrand et al. (1976) and related articles. Green Bay is best characterized as an estuary since it functions as a nutrient trap, has exceptionally high biological productivity, and because of the thermal and chemical differences between the water of its tributaries and that of Lake Michigan. Green Bay is the largest freshwater estuary in the world. There exists a strong trophic gradient between lower and middle bay areas of Green Bay (De Stasio et al., 2008). An embayment protruding from the northwest edge of Lake

Michigan, it is orientated in a northeast-southwest orientation. The south end is located at 44° 31’ N, and the north end is located at 45° 54’N. The length of this body of water is about 193 km, with an average width of 37 km and covers an area of 4120 square km. The complete watershed, which consists of one third of all land that drains into Lake Michigan, is about 40,000 square km. There are eleven rivers and streams that drain into Green Bay, the largest being the Fox River. The southern bay can be more than 7° C warmer than the northern bay, and 12° C warmer than deep lake water. The overall average depth of Green Bay is 20m, but on the southern end the average depth is much less than 10 m (2-3m on the extreme south end). As a result of this relatively shallow water depth, there is no persistent stratification in the lower bay region (Qualls et al., 2007). The depth increases to the north of the bay and this greater depth prevents the entire water column from successfully mixing during the summer, allowing for stratification and a decrease in algal blooms compared to the southern region. The southern bay is also directly exposed to an increased flow of nutrients coming from the lower Fox River resulting in considerable bloom productivity throughout the summer months (Stoermer, 1978). Therefore, this southern region of the bay is classified as hyper-eutrophic, whereas the northern region is has more meso-oligotrophic qualities (Sager & Richman, 1991).

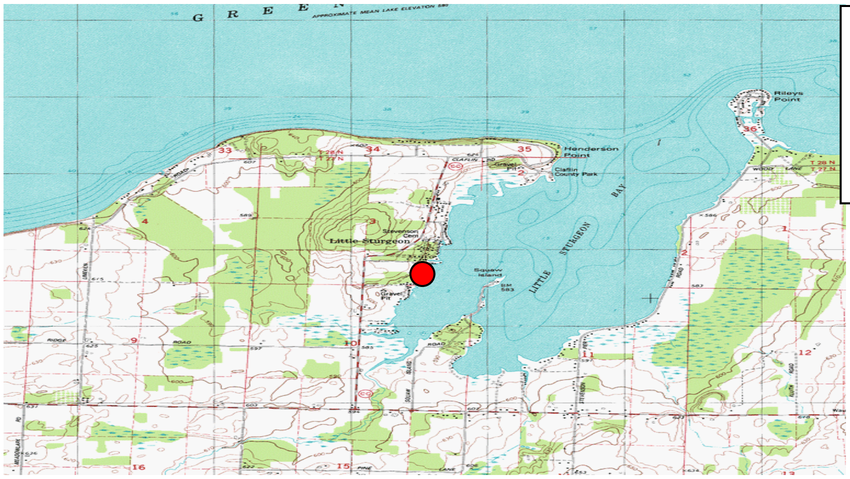

Figure 4a- A map of Little Sturgeon Bay. The red dot indicates the approximate location of the dock where the *E. affinis* were sampled.

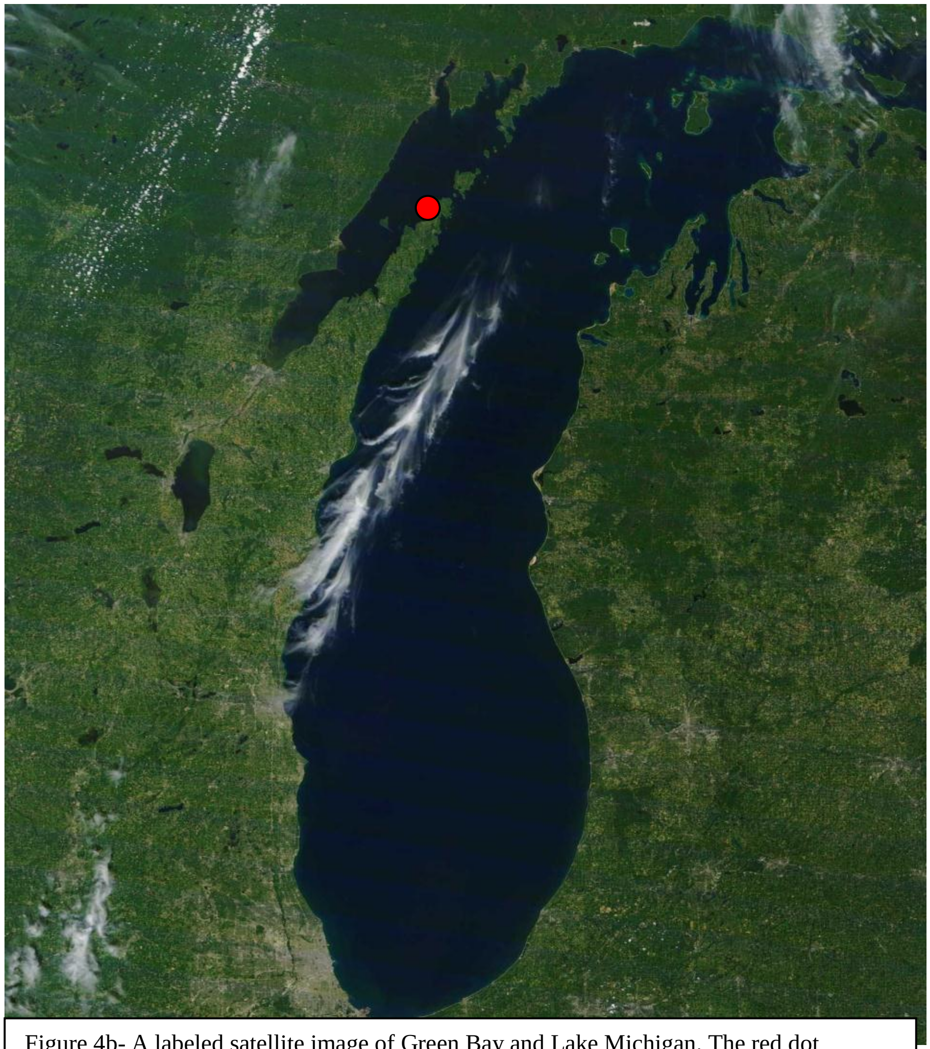

Figure 4b- A labeled satellite image of Green Bay and Lake Michigan. The red dot indicates approximate location of Little Sturgeon Bay. This is where the *Eurytemora affinis* used in this study were sampled.

The overall abundance and composition of the phytoplankton species in Green Bay and Lake Michigan has changed a great deal in the last few decades. It is likely that this change can be contributed to multiple factors (and their interactions), including dynamic shifts in nutrient loading, increased temperatures, and the arrival of certain invasive species. The invasive species with the largest impact on the phytoplankton community is likely to be the zebra mussel (*Dreissena polymorpha*), which invaded this system in 1992 (Qualls et al., 2007). Related to the changes in phytoplankton abundance and composition, this invasion caused a decrease in Chlorophyll *a* concentrations throughout the Green Bay system. Pre-invasion, this aquatic system was dominated by diatoms and had a few species of cyanobacteria in the fall (Sager & Richman, 1991). The cyanobacteria would usually increase in composition during the summer due to the increased temperatures leading to increased environmental suitability. The main species during this time consisted of *Aphanizomenon, Oscillatoria, Anabaena,* and *Microcystis*. Post-invasion, overall Chlorophyll *a* increased and phytoplankton biovolume was significantly higher following invasion in middle bay (De Stasio et al., 2008). Along with these increases in abundance, there was a significant shift to higher and more frequent dominance of the phytoplankton community involving cyanobacteria, especially *Microcystis*. The increased phytoplankton abundance during the post-invasion period is likely explained by selective filter feeding and increased nutrient recycling by the rapidly growing zebra mussel populations that experience high turbidity conditions in this highly productive embayment of Lake Michigan (Qualls et al., 2007; De Stasio et al., 2008). There has also been an observed decrease in the zooplankton abundance as well, which is likely resulting from more abundant but less nutritional food sources available (cyanobacteria). The decrease in zooplankton populations may also be due, in part, to increased rates of predation by fish (De Stasio et al., 2008).

Study Organism: *Eurytemora affinis:*

Our study was interested in investigating the reproduction and feeding ecology of a copepod species that is invasive to the Great Lakes, *Eurytemora affinis* (Poppe, 1880) (Figure 5). This species is native to the Ponto-Caspian region, the North American Atlantic coast including the Gulf of Mexico, the North American Pacific coast, the western European coast, and parts of Asia, generally in brackish and saltwater regions (Torke, 2001). Average body length for this species has been documented as 1.20-1.26mm by a report by Czaika (1982). *Eurytemora affinis* has extended caudal rami, each with 5 caudal setae that are three times longer than their width. The long caudal rami combined with the presence of large metasomal wings on the females makes for relatively simple identification and sexing of this species (Kipp et al., 2013). With regards to its life cycle, which has been documented to reach adulthood in no more than a month at 25°C, this species passes through 6 naupliar stages and 5 copepodite stages before it is considered mature **(**Czaika 1982). The effects of temperature and salinity on *Eurytemora* reproduction and development are extensive and have been well documented (Devreker et al., 2004; Beyrend-Dur et al., 2009; Devreker et al., 2009).

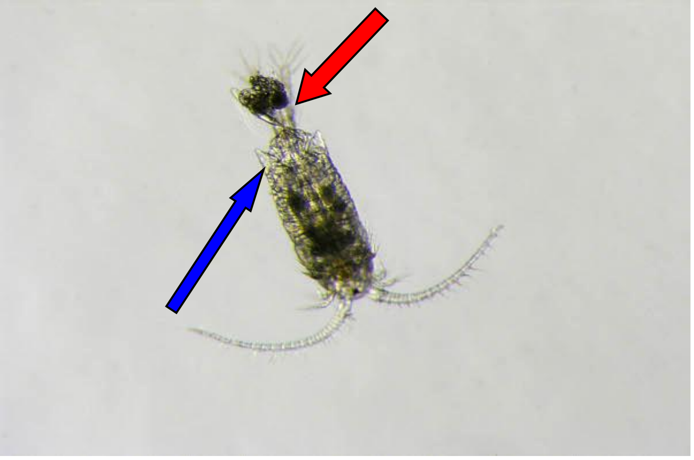

Figure 5- *Eurytemora affinis* female carrying eggs. Note the presence of metasomal wings (blue arrow) and the long caudal setae (red arrow).

Eurytemora affinis was first documented in Lake Michigan by Robertson (1966). It is commonly found during late summer through fall but is typically difficult to find during the winter and spring months (Torke, 2001; Kipp et al., 2013). This implies that *E. affinis* has a late seasonal developmental pattern, and therefore becomes established later in the year. The introduction of *E. affinis* to North American freshwater systems follows a trend of multiple species increasing their ability to adapt to the large salinity gradient during the transition from marine or brackish waters (Lee & Bell, 1999). This versatility allows *E. affinis* to exist and be commonly found in coastal areas as well as estuaries and marshes. The ability for local adaptation has led to differentiation within the *E. affinis* populations over time. Before the year 2000, there was documentation of six divergent clades of this calanoid copepod, four in America, one in Europe, and one in Asia (Lee, 2000). Winkler et al. (2008) conducted a population genetic analysis of sympatric invasive and non-invasive clades of *E. affinis* that provides more information on its evolution. It has been suggested that the ideal environment for *E. affinis* success and dominance is a brackish, coastal habitat within an oligotrophic system (McNaught et al., 1980).

Calanoid copepods, like *E. affinis*, have a unique, selective feeding strategy that can allow them to out-compete other co-existing zooplankton species (DeMott, 1986; Haney, 1987). Generally, calanoid copepods seem best adapted to utilizing large, colonizing cyanobacteria (Haney, 1987; Koski et al., 2002). *Eurytemora affinis* is a suspension feeder (in top of water column with cyanobacteria) and is considered relatively less selective when compared to other calanoid copepod genera (i.e. *Acartia*) (Jonsson & Tiselius, 1990; Gasparini & Castelt, 1997). The specific effects of cyanobacteria on the feeding of *E. affinis* have been shown to vary in different experiments (Dwyer, 2013). Some studies have shown increased feeding rates in the presence of cyanobacteria, like *Nodularia* (Koski et al., 2002). On the other hand, in the presence of cyanobacteria like *Microcystis*, these organisms have shown, in some cases, decreased feeding rates (Dwyer, 2013). Overall it appears that calanoid copepods in general exhibit increased feeding (uptake) in high concentration of cyanabacteria as a way to compensate for the lower food quality of the cyanobacteria (Koski et al., 2002; Kozlowksky-Suzuki et al., 2003). The effects of the toxic, low-quality food source of cyanobacteria can also have implications that reach to reproduction and the development of offspring, possibly affecting fitness and evolution over time. Adverse effects could be decreased growth and maturation rates and a decrease in egg production and/or viability. Previous studies have shown that the number of eggs produced by *E.affinis* females did not differ between those exposed to the cyanobacteria and those that were not (Koski et al., 1999; Dwyer, 2013). *Eurytemora affinis* has the ability to produce diapausing (resting) eggs which could result in a hatching event of multiple evolutionary stages in one area within a good environmental condition (Lee & Frost, 2002).

Significance and Importance of this Study:

Much research is still needed to understand the effects of the HABs that are occurring at higher rates around the world. To do so, researchers must continue analyzing work within a laboratory setting as well as in the field. It is important to understand these effects in order to manage the negative impacts of the blooms and make estimates and predictions about future occurrences. The occurrence of these blooms has recently become a regular part of the Green Bay, Lake Michigan ecosystem in a relatively short period of time. The management of these blooms is important to the entire Great Lakes system. Although the occurrence of HABs is unfortunate and affects a plethora of aquatic ecosystems, it does create an opportunity for important studies and research. Using a well-studied organism such as *Eurytemora affinis* to study the effects of HABs is also very important since much evolutionary, behavioral, and physiological knowledge about

E. affinis exists. However, there is a limited number of studies that investigate the effects on the fitness of *E. affinis* with regards to the evolutionary and habitat changes experienced by this organism. Fitness is a large component in ecological studies and provides for many intriguing evolutionary implications regarding the future existence of the respective organism and species.

The previous research suggesting that *E. affinis* can survive and successfully reproduce in an environment dominated by/consisting of cyanobacteria with low levels of nutrition (HABs) is an important discovery. It seems as though as long as there is a mixture of phytoplankton composition available for consumption, this species of calanoid copepod can survive with moderate levels of fitness. Another important feature is that *E. affinis* can continue to actively graze during a bloom and could help prevent an even larger bloom from forming and providing for a natural management mechanism. *Eurytemra affinis* has been shown to have decreased egg production when exposed to a toxic cyanobacterial food source (Koski et al., 1999; Karjalainen et al., 2007; Dwyer, 2013). These studies stress the importance of studying and investing the long-term effects of exposing *E. affinis* to toxin producing (extracellular and intracellular) and lower nutritional value algae. In these cases, if the adults are able to survive, but their egg production is impaired, the cyanobacteria are still having a large negative impact (long-term) on *E. affninis* despite the evidence for short-term success.

This study aims to investigate the effects of toxin producing cyanobacteria, with separate emphasis on intra- and extracellular toxins, on a particular *E. affinis* population in Green Bay, Lake Michigan. It is important to mention that this study utilizes preliminary experiments as well as focal experiments, which are made distinct in the methods and results sections. This work will aid in increasing the knowledge of how the toxins and food availability are specifically interacting with the copepods and give a higher level of insight into their direct effect. By exposing the copepods to situations where there is an availability of both good and bad food sources, we can see how phytoplankton community composition can affect their feeding and ultimately their fitness (short and long-term implications). This study will also aim to analyze whether there is an effect of actively ingesting the cyanobacterial cells and/or whether exposure to extracellular toxins will have a similar effect or not. In particular, we examine survivorship, grazing rates (filtering and ingestion), and egg production by the *E. affinis* population from Green Bay, Lake Michigan. This will provide greater insight into the implications of the interactions between environmental factors, algal composition, and zooplankton feeding as well as provide for interesting analysis of the effects higher in the aquatic food web (i.e. higher level consumers such as fish). It is hypothesized that feeding and egg production will be negatively affected by the presence of the toxic cyanobacteria (*Microcystis aeruginosa*) and that these negative effects will be strongest when the cells themselves are offered as food (intra- and extracellular toxins) as opposed to the filtrate (extracellular toxin), which will produce intermediate impacts on *E. affinis*.

MATERIALS AND METHODS

Water acquisition:

For this experiment we used water collected directly from the sampling area, Little Sturgeon Bay. Using the water directly from the area of animal collection helps eliminate various confounding factors associated with using other sources of water (i.e. aged tap water, artificial pond water, etc.) in our experiment. The use of other types of water for experimentation could provide for unfit or less suitable conditions for the copepods and therefore could affect our experimental data. Given the fact that the captured organisms are adapted to and live in this water, it seemed like a viable choice to use the Little Sturgeon Bay water throughout the experimental process.

On 9/21/13, 20 L of Little Sturgeon Bay water was collected using a carboy. It was then necessary to filter all of the large particles and algae out of this collected sample to ensure that the only food available to the copepods is that of the added green algae and cyanobacteria (*Scenedesmus quadricauda* & *Microcystis aeruginosa*). This filtering of the Little Sturgeon Bay water was done by using a pump to double filter the water into another carboy. The first level of filtering involved a larger pore Whatman #1 qualitative paper filter (15cm diameter) to remove the larger particles. The second level of filtration was done using a Whatman GF/C filter paper to remove the smaller particles that passed through the first level of filtration. The resulting water after double filtration was referred to as Filtered Little Sturgeon Bay (FLSB) water. This water was stored at room temperature within the laboratory during the experiments (approximately 21^{o} C).

Animal acquisition and sorting:

The copepods used in these experiments were also collected from Little Sturgeon Bay. Based on a study performed during summer of 2013 examining the spatial distribution of *Eurytemora affinis* within Little Sturgeon Bay, the municipal dock boat landing was where the highest density of these copepods was observed (A. Poli, N. Barrett, & B. DeStasio, unpublished). Therefore, we chose to carry out our animal collection by walking along the dock area using a zooplankton towing net. The zooplankton net used has an opening diameter of 0.5m and a mesh size of 250 micrometers. These net tows (animal collections) were performed twice and were done in the late Fall between 10/9/13 and 10/20/13. Tows were done at night after it was observed that there were higher densities of *Eurytemora affinis* within the water column (probably due to a diel vertical migration behavior). The animals collected were transferred to 6 liter buckets of Little Sturgeon Bay water, stored on ice, and transported back to the Lawrence University laboratory. The samples were kept in the aquarium room at a temperature of 17° C until they were sorted.

Sorting of these animals was performed as quickly after collection as possible, usually the next morning. Sorting consisted of identifying and capturing *Eurytemora affinis* individuals based on maturity level and sex (adult males and gravid females). Using a small pipette and dissecting microscope, animals were sorted based on the number of males and females needed. Typically, the individual copepods were kept in beakers with FLSB water and a good green algal food source until further use. Once beakers of strictly *Eurytemora affinis* adults were obtained, further sorting into the treatments was performed based on number of individuals needed in each replicate.

Food Sources: Green Algae and Cyanobacteria:

The green alga *Scenedesmus quadricauda,* and the cyanobacterium *Microcystis aeruginosa*, are the food sources that were used throughout the experiments. The *S. quadricauda* was obtained from Carolina Biological Supply Company (Burlington, NC). The toxic *M. aeruginosa* used was strain number PCC7820. Both of the cultures used were initiated from a larger lab culture on 8/23/13 when nutrient media were added. The *S. quadricauda* received a Bristols solution (125ml/L of Bristols) to optimize growth, while the *M. aeruginosa* received Cyanobacteria BG-11 Freshwater Solution (C3061) from Sigma (St. Louis, MO) at 20ml/L. The *S. quadricauda* culture was kept in direct sunlight near a window and was lightly bubbled. The *M. aeruginosa* was kept in moderate indirect sunlight and stirred gently on a shaker table.

Preliminary Experiments:

Survivorship in Varying Conductivities

After observing high mortality in previously attempted experiments, various parameters of the treatment water were measured to find possible causes for the high mortality. Using a Hydro Lab D55 multisonde unit, conductivity (SpC) was found to be very high, especially in the treatments of cyanobacteria. This high conductivity seemed to be caused by the BG-11 cyanobacterial growth medium, resulting in conductivity values that were more than double the values measured in the field. Previous studies have stressed the importance of salt concentrations on the survival of *E. affinis*, salinity being a main component of the conductivity level (Lee & Petersen, 2003). Survivorship experiments were performed to evaluate the effect that high conductivity may have on the survivorship of *E.affinis*. Three treatments were created with varying conductivities: aged aerated tap water (SpC= 275 microsiemens/cm), recommended concentration (20ml/L) of BG-11 growth medium (SpC=1320 microsiemens/cm), and half of the recommended solution of BG-11 growth medium (SpC=693 microsiemens/cm). Twelve females were placed in each treatment, one per well in 12-well tissue culture plates at room temperature with 4.5ml of treatment water and no food added. Survivorship was measured twice a day for 5 days. The results prompted us to create a method of adding the correct amount of cyanobacterial toxins to the treatments without increasing the conductivity to a high degree by the addition of the BG-11 growth medium.

Water Conditioning

Two different methods of conditioning the water with *M. aeruginosa* (and extracellular toxins) were performed in order to address the problems associated with high conductivity and *E. affinis* survivorship. First, two 5ml samples of the *M. aeruginosa* culture were filtered down on to GFC filter paper (2cm diameter). These filter papers including the *M aeruginosa* cells were placed in to 25ml beakers of FLSB water. A time course of toxin analysis was performed at times of 0, 24, and 48 hours. A sample of the filtered *Microcystis* culture, FLSB water, and Milli-Q water were used as positive and negative controls in this experiment. The concentrations of toxins were analyzed using the standard assay protocol and the Quantiplate Kit Microcystin ELISA from Envirologix (Portland, ME).

The second technique used for conditioning the treatment water with *M. aeruginosa* toxins without adding the BG-11 growth medium involved centrifugation. A 200ml sample of the *M. aeruginosa* culture was spun in a SORVAL centrifuge for 30 min. at 3,000 rpm using an SS34 rotor. After centrifugation, the liquid was decanted and the remaining pellet of the cyanobacteria was resuspended in about 600 ml of FLSB water. Conductivity measured after resuspension was 385 microsiemens/cm. 5 ml samples were taken at 0, 24, and 48 hours. A sample of FLSB water was used as a negative control. Once again, the concentrations of toxins were analyzed using the standard assay protocol and the Quantiplate Kit Microcystin ELISA from Envirologix (Portland, ME).

Survivorship with Varying Levels of Microcystis

In order to help us determine what concentrations of *M. aeruginosa* (relative to the *Scenedesmus*) to use in the grazing and reproduction experiments, we conducted a survivorship experiment using varying levels of *Microcystis*. *Microcystis* was added at 0%, 10%, 20%, and 30% of the total carbon supplied as *S. quadricauda*, which was held constant at 500 µgC/L. Survivorship of animals was assessed by holding *E. affinis in* two 12-well tissue culture plates for each food treatment concentration. The percent of individual *E. affinis* surviving in each treatment and replicate wells was recorded roughly every 24 hours for 5 days.

Survivorship in Differing Temperatures

In order to help us determine appropriate laboratory conditions for the experimental organisms, we conducted an experiment involving female *E.affinis* with and without egg sacs held under two different environmental conditions. The conditions analyzed consisted of room temperature (21°C) and relatively indirect sunlight as well as a 15°C and dark condition. For each condition temperature we employed two 12-well culture plates, one containing 10 females with eggs sacs, and the other containing 10 females without egg sacs. An adequate supply of *S. quadricauda* was added to each well at a concentration of 500 µgC/L. The percent alive was recorded roughly every 24 hours for 7 days. The data were assessed with regards to the temperature and lighting conditions alone as well as whether the female organisms were carrying egg sacs or not.

Focal Experiments:

Grazing Experiments

The grazing experiments consisted of three different treatment conditions, one with only *S. quadricauda* as the food source (500 µgC/L), another with *S. quadricauda* and 10% *M. aeruginosa* (500 µgC/L & 50 µgC/L), and one with the good food source (500 ugC/L) and the filtrate from the same volume of *M. aeruginosa* added to the other treatment. The treatments will be referred to as GF (good food), GF/Cyan, and GF/ Filt respectively. To determine the amount of food that needed to be added, the cell density of the algal cultures was measured with a hemocytometer counting chamber and converted into µgC/L. The food and FLSB water were mixed in large 3L buckets and then dispersed throughout the treatment beakers to ensure adequate mixture and dispersal in to the beakers.

There were three treatment conditions; each condition consisted of 3 replicate beakers, as well as corresponding controls, making for 18 total beakers (all in 250 ml beakers). The number of individuals (*E. affinis* females) added to each condition and replicate did not differ by more than 3 between individual beakers. There were 35 individuals added to the GF beakers (12, 12, 11); 37 individuals added to the GF/Cyan beakers (13, 12, 12) and 41 individuals added to the GF/Filt beakers (14, 14, 13). The beakers were kept within a 15°C incubator with no light source and covered with a sheet of plastic to reduce evaporation. The organisms were allowed to acclimate to the experimental conditions for a period of 41 hours. During the grazing experiments survivorship was recorded and all three treatments had survival rates of at least 90%. The experimental grazing period was about 22 hours for each treatment condition. At the end of the grazing experiment, all copepods were removed, counted, and preserved in Formalin. This process was done by gently filtering the beakers through a mesh cup 4 times (128 um mesh net). Initial and final samples were taken using a pipette in order to assess cell densities (10ml), extracellular toxins (20ml), and total toxins (10ml).

In order to determine the filtering (ml/copepod/hr) and ingestion rates (µgC/copepod/hr), calculations were used that converted the number or density of cells present (taking into consideration the cell volumes) in the experimental and control beakers at the start and end of the grazing period. The calculations used were obtained from Frost (1972).

Egg Production Experiments

The experimental food treatments (GF, GF/Cyan, GF/Filt) were made up in the same way as they were for the grazing experiment with the same concentrations of *Scenedesmus* and *Microcystis.* A total of 50 gravid female *E. affinis* were placed in beakers containing the three experimental food conditions. These individuals were allowed to acclimate to the food conditions for at least 40 hours. This acclimation period also allowed any of the 50 female *E. affinis* added to each condition that were carrying egg sacs to drop them before addition of male *E. affinis.* It was important that the females used in the reproduction experiment were not carrying eggs to ensure that the egg sacs produced during the experiment were actually produced within the treatment conditions. The animals in the egg production experiment were kept in a 15°C incubator in constant dark.

At the end of the acclimation period those females that were gravid and not carrying egg sacs were left in the experimental conditions while those still carrying egg sacs were isolated in separate beakers (containing the same treatment conditions). This experiment used only one large beaker for each treatment condition since each female could be treated as an individual replicate. A total of 15 females were added to a beaker of the GF treatment. In the GF/Cyan treatment 24 females were added to the beaker. Finally, 30 females were added to the GF/Filt treatment condition. To signal the start of the egg production experiments, males were placed in the beakers with the gravid females. A total of 5 males were placed in the GF condition, 8 males in the GF/Cyano treatment, and 9 males to the GF/Filt treatment beaker. The females that had not dropped their egg sacs previously were added to the beakers with the males after they had dropped their egg sacs, about 30 hours after the initial addition.

Three days after the start of the reproduction experiment, females that were carrying egg sacs were placed in 35mm capped petri dishes with 5ml of treatment food condition water. The treatment water used was newly made to ensure appropriate food and filtrate concentrations. Those females that were not carrying eggs were left in the original egg production beakers along with the males. At the end of the egg production experiment a total of 10 females in the GF treatment, 7 in the GF/Cyano treatment, and 18 in the GF/Filt treatment had successfully produced eggs and were used in analysis.

Individual females were assessed for the number of eggs she was carrying and then were checked daily for the number of hatched nauplii. Once the eggs were hatched and the nauplii seen in the petri dish, the sample was preserved with Lugol’s solution for later analysis. Some of the females that produced egg sacs did not successfully produce nauplii. If the sample began to decompose and the eggs were not viable to hatch, Lugol's was added to the sample. There were 6 females in the GF treatment, 4 females in the Cyano treatment, and 5 in the GF/Filt treatment that had produced nauplii that were used for the nauplii measurements. Nauplii measurements were done using a microscope with a 20x occular lens and a 50x objective. A stage and eyepiece micrometer was used to determine the size of each nauplius.

Statistical Analysis

In order to perform the correct statistical analysis of the results, test of normality were performed on the data. Using the PAST program it was determined that the data met the assumptions of normality and did not need to be transformed for statistical analysis. Analysis of variance (ANOVA) followed by multiple comparison two tailed T-tests were used to analyze the data with EXCEL and PAST programs. To assess the survivorship of individual organisms, a PetoPeto survivorship analysis was performed on the data. This test adjusted for the fact that survivorship data can only stay the same or decrease in value. Regressional analysis was also performed on a small subset of the data to investigate the degree of correlation present between variables. Both standard error and standard deviation values were used for analyzing the variance within the data set and represented graphically as error bars.

RESULTS

Preliminary Results: ***Survivorship in Varying Conductivities***

There was a gradual decline in survivorship of *E. affinis* at increasing levels of conductivity (Figure 6). By the end of the experiment it was assumed that survivorship was being heavily affected by starvation because no food was provided in this experiment. The biggest difference in survivorship was seen around the 36-48 hour range where the highest conductivity had the lowest survivorship and the lowest conductivity had the highest. At the 40 hour mark the treatment with the highest conductivity had a survivorship of about 32%, the middle conductivity treatment had survivorship of about 42%, and the treatment with the lowest conductivity had a survivorship of about 75%. Although these absolute survivorship levels were different at specific time points, there was no overall statistically significant difference in survivorship rates between the three treatment conditions using a Peto & Peto survivorship analysis test (df= 2, Chi-square= 2.01, p>.05).

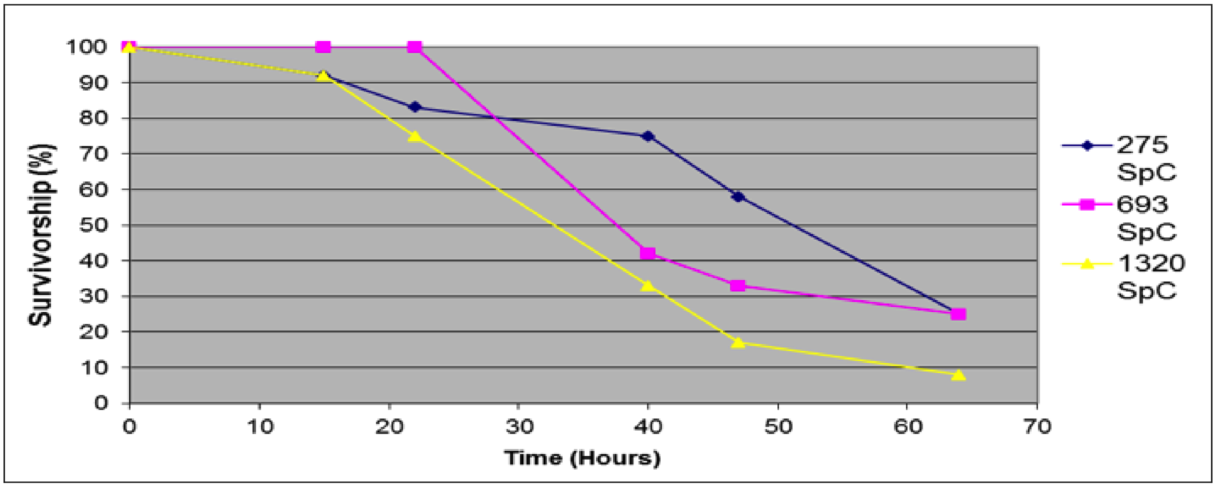

Figure 6- Survivorship of *Eurytemora. affinis* adults over time (hours) when exposed to environments with varying levels of conductivity (SpC or microsiemens/cm).

Water Conditioning: Centrifugation Technique

Of the two techniques attempted for conditioning the treatment water with *M. aeruginosa* (and it's toxins) without increasing the conductivity of the water by adding the BG-11 growth medium, the technique involving centrifugation proved to be the most reliable and efficient. The results of the ELISA assay performed to analyze the level of extracellular microcystin present in the given sample indicated a rapid increase in microcystin levels over time (Figure 7). The FLSB water (negative control) had very low levels of microcystin present. Levels of microcystin present in the sample at 0, 24, and 48 hr were much higher than that of the FLSB. The water was therefore almost immediately conditioned with the extracellular toxins of *M. aeruginosa* upon resuspension. There was very little increase in the level of extracellular toxins present in the water at each successive time interval measured. An ANOVA indicated a statistically significant difference in the level of microcystin present between the samples ($F_{(3,4)}$ =569.36, p=0.00001). Using a two-tailed T-test, there was a significant difference between the level of microcystin in the FLSB control and the conditioned water at every time period. At the 0 hour mark p=0.013, at 24 hours p= 0.024, and at 48 hours p= 0.0034. There was

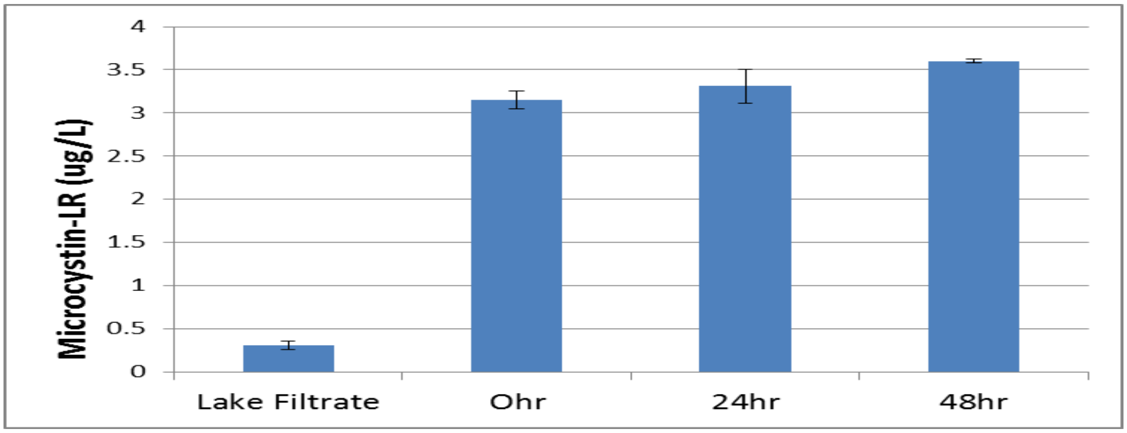

Figure 7- Concentration of microcystin-LR (µg/L) over time following centrifugation and re-suspension of *Microcystis aeruginosa*. The lake filtrate served as a control. Error bars represent +/- 1 standard error.

no significant difference in the levels of microcystin present between the 0, 24, or 48 hour time periods ($p>0.05$).

Survivorship with Varying Levels of Microcystis

The survivorship of *E. affninis* individuals varied with respect to the concentration of *M. aeruginosa* in the treatment (Figure 8). The treatments of 30% and 10% *M. aeruginosa* followed very similar trends throughout the experimental time frame. Initially, the treatments of 0% and 20% *M. aeruginosa* had relatively similar levels of survivorship, each lower than the other two treatments. It wasn't until between the fourth and fifth sampling times that there was a large decrease in survivorship in the 20% *M. aeruginosa* treatment. The other three treatment concentrations followed similar patterns of slowly decreasing survivorship throughout the experiment.

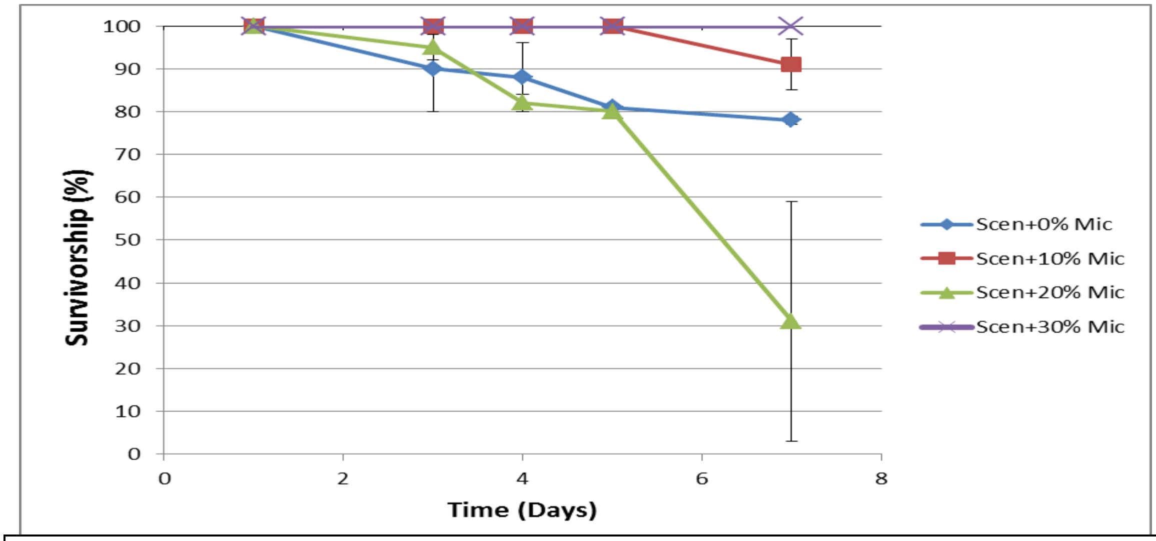

Figure 8- Survivorship of *Eurytemora affinis* adults over time (days) when exposed to environments comprised of various mixtures of *Scenedesmus quadricauda* and *Microcystis aeruginosa*. The error bars represent +/- 1 standard error.

There was a significant difference between the 0% and 20% *M. aeruginosa*, with the 0% treatment having higher survivorship (Peto & Peto test; df= 2, Chi-square = 4.23, $p<0.05$). There was also a significant difference between the 0% and 30% *M. aeruginosa*, with the 0% treatment having lower survivorship (Peto & Peto test; df= 2, Chi-square= 5.31, p<0.05). Comparing the treatments with 10% and 20 % *M. aeruginosa* also produced a statistically significant difference in survivorship, with the 10% condition having higher survivorship (Peto & Peto test; df=2, Chi-square= 12.28, p<0.05). Finally, there was a significant difference between the 20% and 30% *M aeruginosa* treatments, with the condition containing 30% having a higher survivorship (Peto & Peto test; df=2, Chi-square= 13.80, p<0.05). Interestingly, the condition containing the most toxic *M. aeruginosa* (30%) had the highest survivorship over the experimental time period. Overall, there existed no clear trend in survivorship in relation to the level of *M. aeruginosa* added based on this experiment.

Survivorship at Differing Temperatures

The results of the experiment regarding the survivorship of females with and without eggs at two different temperatures indicated a higher survivorship at lower temperature (Figure 9a, 9b & 9c). There seemed to be no clear trend in survivorship over the 7-day span with respect to whether the females were carrying egg sacs or not in each condition. The females without eggs in both conditions showed similar rates of reduction in survivorship over the experimental period. The females with eggs in the dark 15° C condition had the highest survivorship while the females with eggs in the light room temperature condition had the lowest survivorship. The females with egg sacs that were in the dark 15°C condition had significantly higher survivorship than the females with egg sacs in the light and room temperature condition (Peto & Peto; df=2, Chi-squared= 11.91, p<.05). When only analyzed with respect to the condition, survivorship was significantly higher in the dark 15°C condition throughout the 7 days (Peto & Peto test;

df=2, Chi-square= 8.10, p<.05).

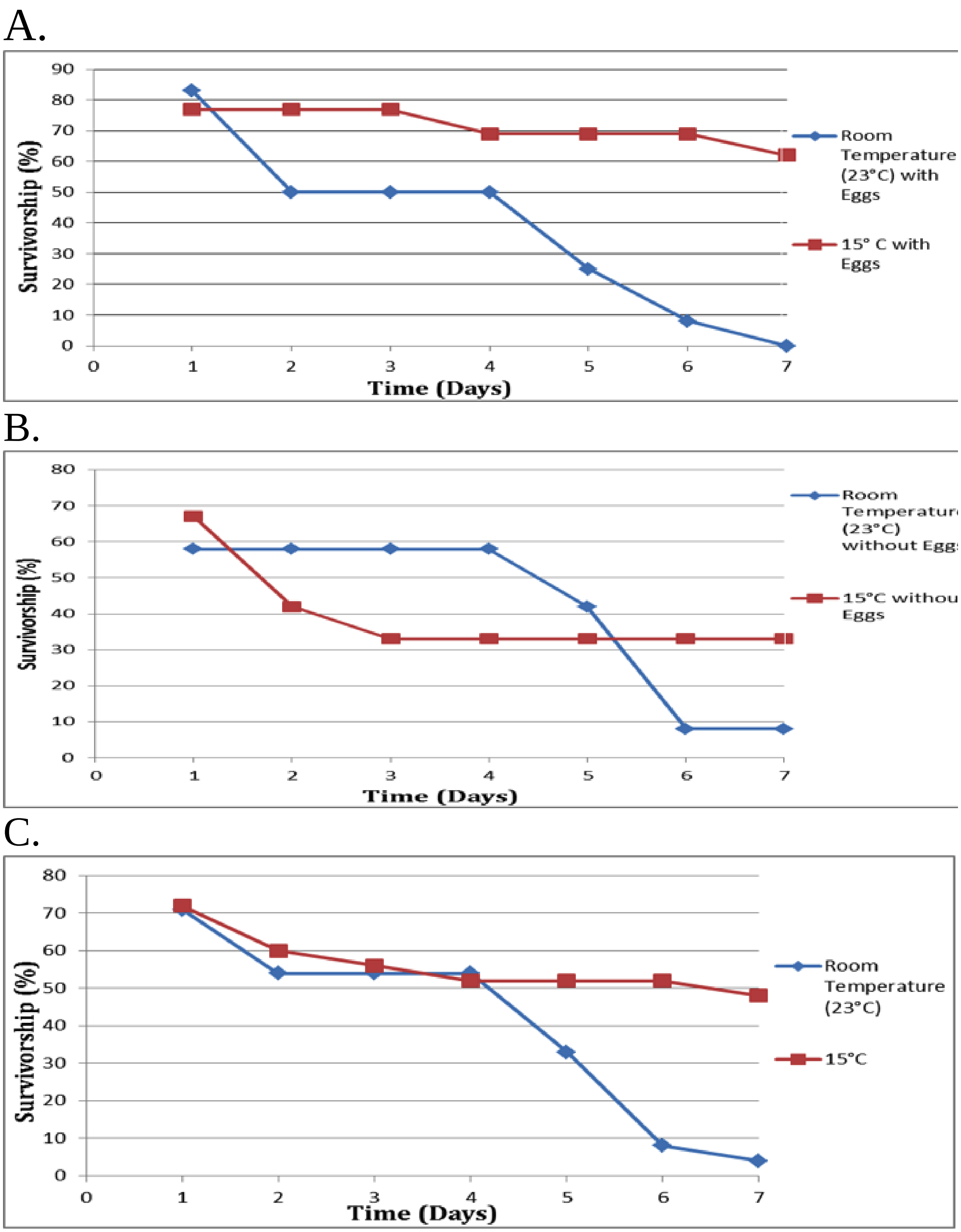

Figure 9- Survivorship of female *Eurytemora affinis* adults over time (days) when exposed to room temperature (about 23°C) and 15°C environments. The room temperature condition also included daylight, while the 15°C environment was dark. The survivorship of females with eggs (A), without eggs (B), and the overall (combined) survivorship (C) was analyzed.

Recall that the results from these preliminary studies aided in planning and carrying out the procedures in the focal experiments.

Focal Results:

Grazing Experiments

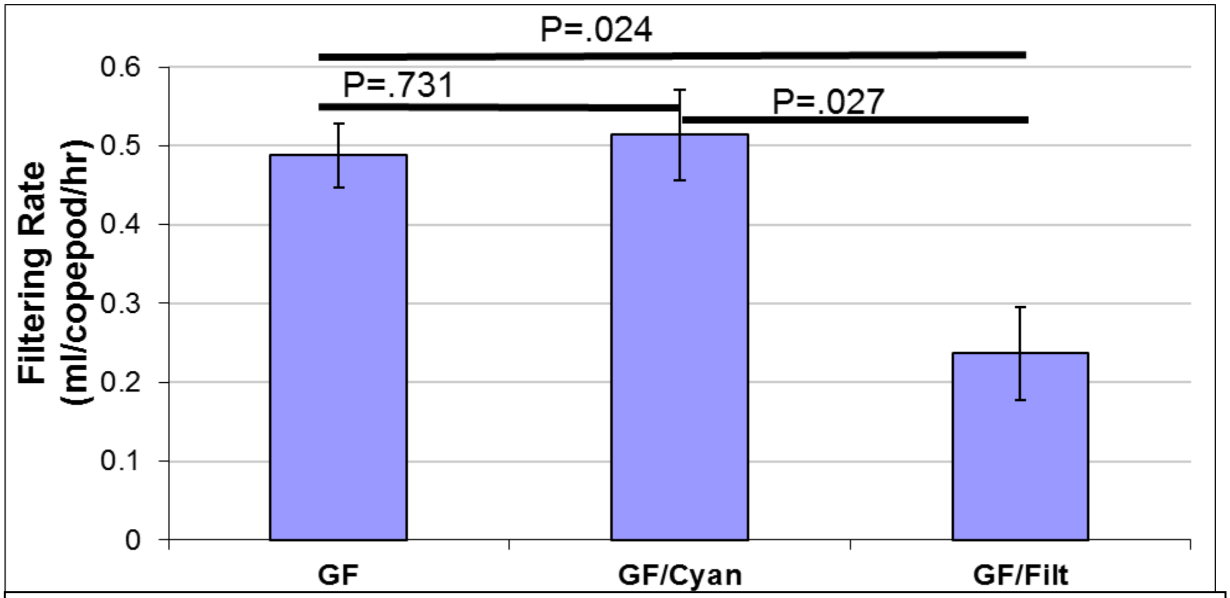

Figure 10- Filtering Rate (ml/copepod/hr) of adult *Eurytemora affinis* when exposed to various treatments including different compositions of food available. GF is only *Scenedesmus quadricauda*, GF/Cyan is *S. quadricauda* and *Microcystis aeruginosa*, and GF/Filt is *S.quadricauda* and *M. aeruginosa* filtrate. Calculations used come from Frost, 1972. P-values shown with connecting bars corresponding to compared data sets. Error bars represent +/-1 standard error.

Table 1- Summary of ANOVA results concerning the filtering rate data set. ANOVA was done using Microsoft Excel.

Filtering Rate ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.140378	2	0.070189	8.412893	0.018163	5.143253
Within Groups	0.050058	6	0.008343			
Total	0.190436	8				

The average filtering rate (ml/copepod/hr) exhibited by the *E. affinis* was the lowest in the GF/Filt treatment condition (Figure 10). The copepods exposed to the GF and GF/Cyan treatments had filtering rates that were comparable, around 0.5 ml/copepod/hr, while the copepods in the GF/Filt treatment had a filtering rate of about 0.25 ml/copepod/hr. The ANOVA results indicated a significant difference in filtering rate between the three treatment conditions (F(2,6)= 8.413, p=.018; Table 1). Two-tailed T-tests were then performed to analyze the degree of difference between the individual treatments. The GF and GF/Cyan treatments were not significantly different from each other in regards to the filtering rate of the copepods (p=0.731). Copepods in the GF/Filt treatment had significantly lower filtering rates when compared to both the GF and GF/Cyan treatments (p=0.024 and p=0.027 respectively).

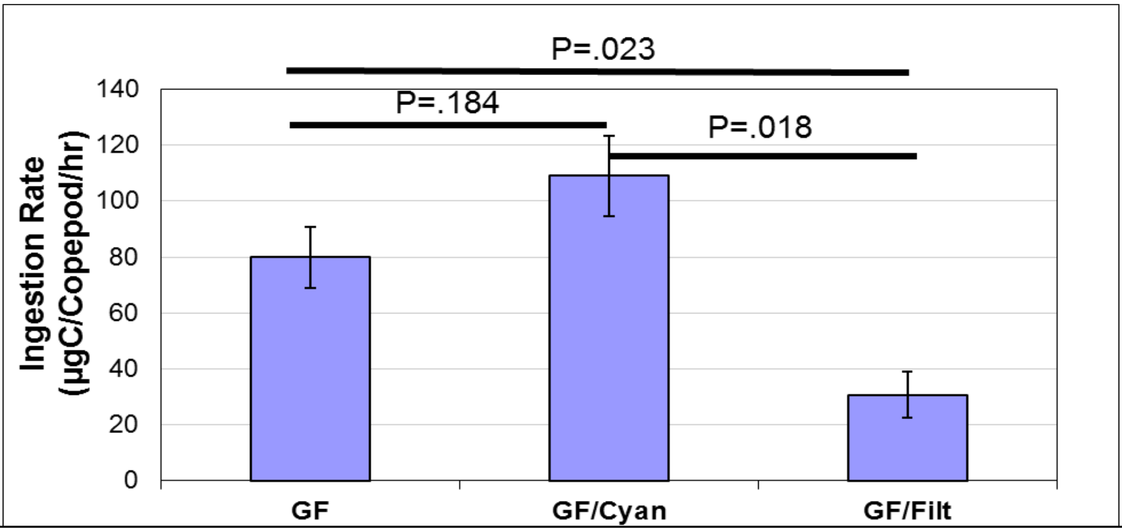

Figure 11- Ingestion rate (µgC/copepod/hr) of adult *Eurytemora affinis* when exposed to various treatments including different compositions of food available. GF is only *Scenedesmus quadricauda*, GF/Cyan is *S. quadricauda* and *Microcystis aeruginosa*, and GF/Filt is *S.quadricauda* and *M. aeruginosa* filtrate. Calculations used come from Frost, 1972. P-values shown with connecting bars corresponding to compared data sets. Error bars represent +/-1 standard error.

The average ingestion rate (µgC/copepod/hr) exhibited by *E. affinis* was the lowest in the GF/ Filt treatment, similar to the filtering rate (Figure 11). The copepods exposed to the GF/Cyan treatment exhibited the highest average ingestion rate with about 110 µgC eaten/copepod/hr. The individuals in the GF/Filt treatment had the lowest average ingestion rate of about 30 µgC eaten/copepod/hr. The ANOVA analysis performed on this set of data indicated that there was a significant effect of treatment condition on the ingestion rate of the copepods ($F_{(2,6)}$= 11.89, p= 0.008; Table 2). There was no significant difference in the ingestion rate of the copepods between the GF and GF/Cyan treatments (two-tailed t-test: p=0.18). Copepods in the GF/Filt treatment had a

Table 2- Summary of ANOVA results for ingestion rate data set. ANOVA performed using Microsoft Excel.

Ingestion Rate ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9386.4	2	4693.2	11.89033	0.008178	5.143253
Within Groups	2368.244	6	394.7073			
Total	11754.64	8				

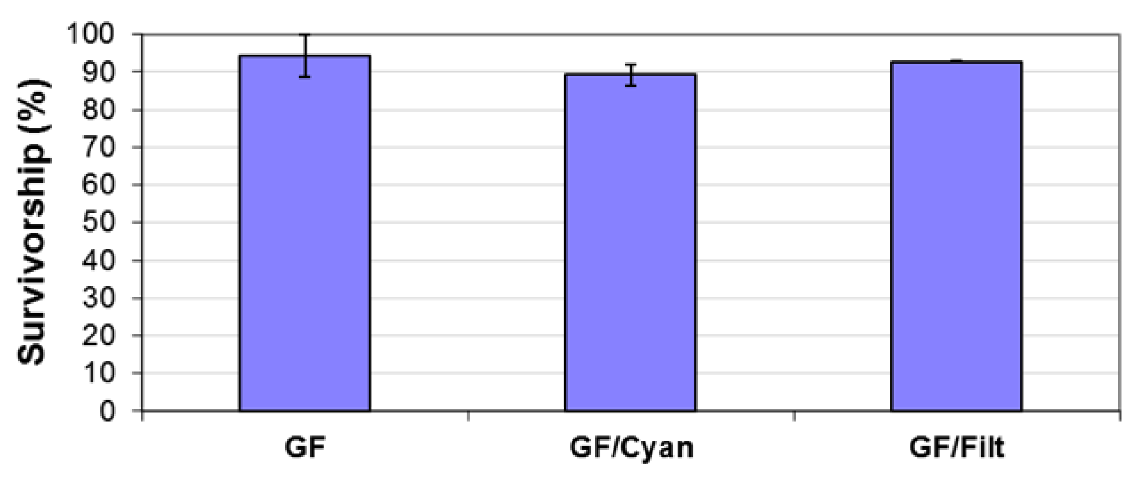

Figure 12- Survivorship of adult *Eurytemora affinis* during the grazing experiment. GF is only *Scenedesmus quadricauda*, GF/Cyan is *S. quadricauda* and *Microcystis aeruginosa*, and GF/Filt is *S .quadricauda* and *M. aeruginosa* filtrate. Error bars represent +/-1 standard error.

significantly lower ingestion rate than individuals in both the GF and GF/Cyan treatment (p=0.023 & p=0.018 respectively).

It is also important to mention that given the problems of high mortality of *E. affinis* in previous experiments, survivorship in the grazing experiments was measured (Figure 12). This was also a check on the presence of any potential confounding factors between the treatments. All of the treatments had survivorship rates around 90% or above. An ANOVA indicated that there was no significant difference in survivorship between the treatments during the grazing period ($F_{(2,6)}$ =0.331, p>.05).

Egg Production Experiments

The average number of eggs produced per female *E.affinis* was the highest in the GF/Cyan treatment (Figure 13). The females in the GF/Cyan treatment averaged about 15 eggs per female, but also had the most variation. The GF and GF/Filt treatments had similar values regarding the number of eggs produced per female, around 11. An

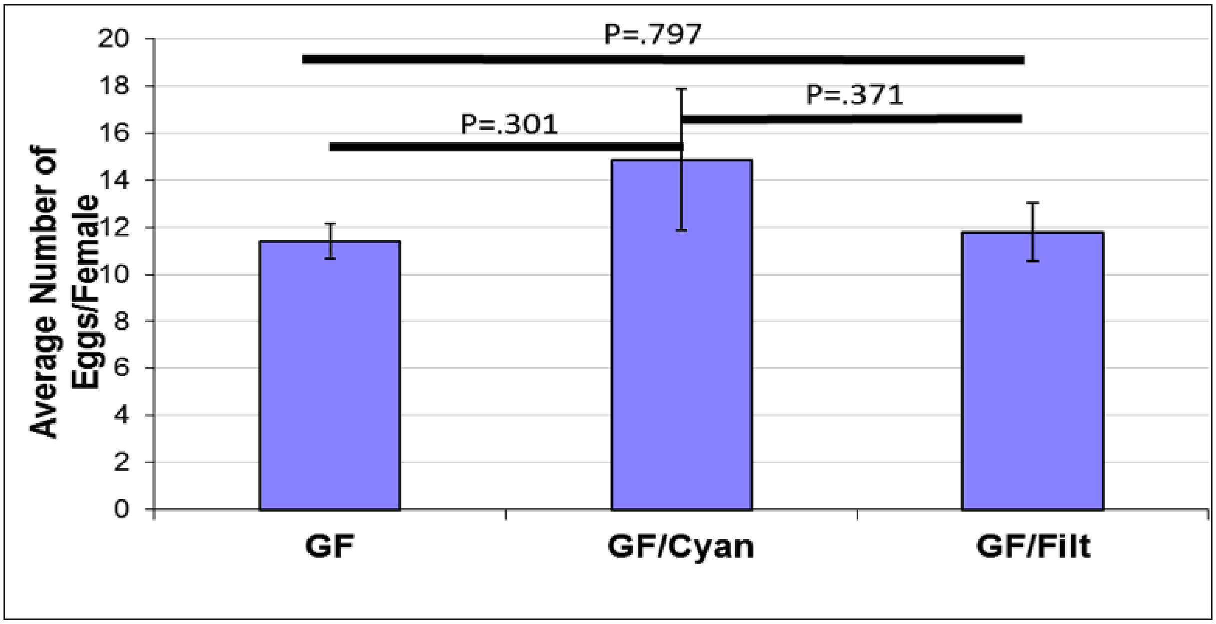

Figure 13- Average number of eggs per *Eurytemora affinis* after exposure to the grazing experiment. GF is only *Scenedesmus quadricauda*, GF/Cyan is *S. quadricauda* and *Microcystis aeruginosa*, and GF/Filt is *S.quadricauda* and *M. aeruginosa* filtrate. P-values shown with connecting bars corresponding to compared data sets. Error bars represent +/-1 standard error.

ANOVA Indicated that there was no statistically significant difference between the three treatments in the number of eggs produced per female ($F_{(2,32)}$ =1.047, p= .36; Table 3). T-tests also showed that there was no significant difference between any of the two treatments.

Table 3- Summary of ANOVA results for average number of eggs per female data set. ANOVA was done using Microsoft Excel.

Egg Production ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	58.7746	2	29.3873	1.04678	0.362779	3.294537
Within Groups	898.3683	32	28.07401			
Total	957.1429	34				

The average nauplius size produced did not differ to a high degree between the three treatment conditions (Figure 14). The females in the GF/Cyan treatment produced the largest nauplii, followed by the GF/Filt treatment and the GF treatment, with considerable amounts of variation within each treatment. An ANOVA was performed in order to identify significant difference between the three data sets. There was not a statistically significant difference between the three treatment conditions ($F_{(2,11)}$=.377, p=.694; Table 4). T-tests also indicated that there was not a significant difference between any two of the data sets regarding the size of the nauplii produced.

Table 4- Summary of ANOVA results concerning the average nauplius size data set. ANOVA was done using Microsoft Excel.

Nauplius Size ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.31E-05	2	1.16E-05	0.377494	0.694125	3.982298
Within Groups	0.000337	11	3.06E-05			
Total	0.00036	13				

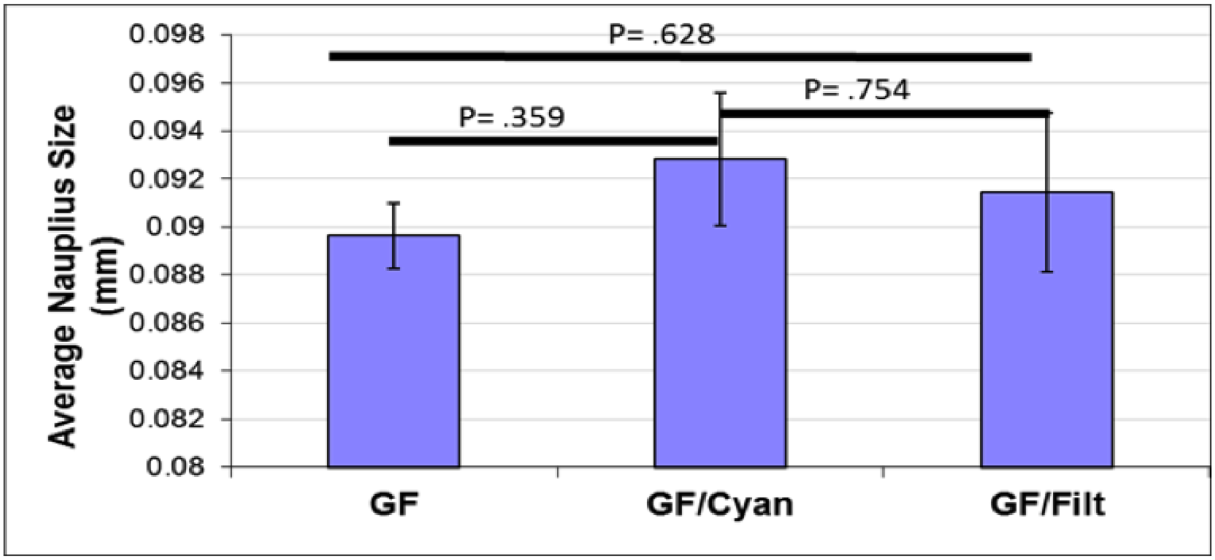

Figure 14- Average nauplius size of *Eurytemora affinis* after exposure to the grazing experiment. GF is only *Scenedesmus quadricauda*, GF/Cyan is *S. quadricauda* and *Microcystis aeruginosa*, and GF/Filt is *S.quadricauda* and *M. aeruginosa* filtrate. P-values shown with connecting bars corresponding to compared data sets. Error bars represent +/-1 standard error.

Average nauplius size (mm) and the number of eggs produced per female are negatively correlated in each treatment, with the strongest correlations being seen in the GF/Cyan and GF/Filt treatments (Figure 15). Females in the GF/Filt treatment showed the largest variation in average nauplius size, while females in the GF/Cyan treatment had the largest variation in number of eggs produced. The females in the GF treatment had a narrow range of nauplius size as well as number of eggs produced relative to the females in the other two treatment conditions. Regression analysis was performed in order to assess the degree of correlation between the number of eggs a female produces and the size of the offspring. There was the highest/strongest degree of correlation seen in the GF/Cyan treatment (R^2= .85) followed by the GF/Filt treatment (R^2= .70) and finally by the GF treatment which exhibited very little correlation between these two variables (R^2= .06).

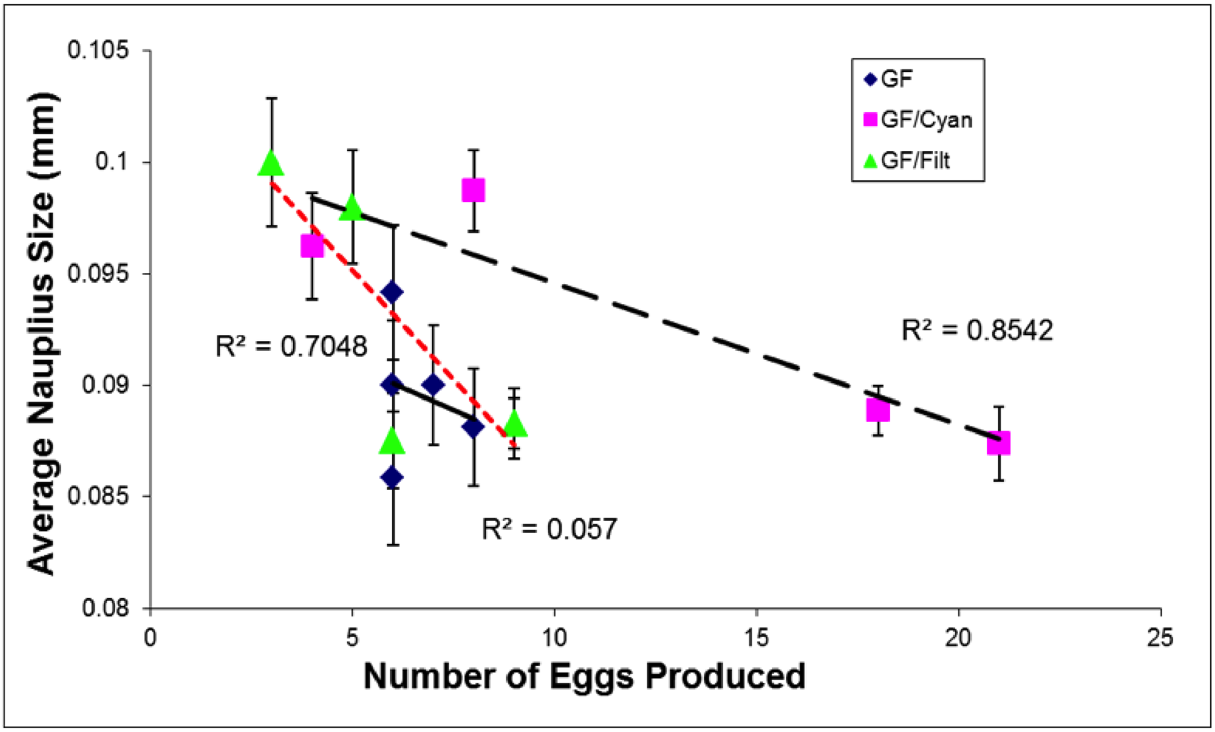

Figure 15- Average nauplius size of *Eurytemora affinis* after exposure to the grazing experiment as a function of how many eggs were produced. GF is only *Scenedesmus quadricauda*, GF/Cyan is *S. quadricauda* and *Microcystis aeruginosa*, and GF/Filt is *S.quadricauda* and *M. aeruginosa* filtrate. R^2 values shown for regression analysis. Error bars represent +/-1 standard error.

DISCUSSION

Points of Emphasis:

Overall, this study provided interesting and compelling results concerning the grazing and reproduction ecology of *Eurytemora affinis* in relation to a toxic strain of cyanobacteria, *Microcystis aeruginosa*, which has been known to be a main contributor to HABs. After numerous preliminary experiments aided in the fine tuning of the focal experiments, the survivorship of these organisms in an experimental setting improved, which was a major caveat and area of concern in previous experiments. An increase in the conductivity of the experimental water caused by the algal growth medium was suggested as a possible factor affecting *E.affinis* survivorship based on the results obtained. This problem was taken care of using a centrifugation-resuspension technique with the cells about 24 hours before experimental set-up and start. The temperature of storage also had an effect on copepod survivorship, and the organisms were kept in an environment that supported an optimum level of survivorship.

As a brief recap of the results, using the three treatment mixtures for comparison, some data sets produced differences while others did not. Filtering rates as well as ingestion rates of the copepods were similar in the GF and GF/Cyan treatments but significantly lower in the GF/Filt treatment where there were not any *Microcystis aeruginosa* cells available for ingestion (only extracellular toxins and substances in the filtrate). On the other hand, there was not any significant difference in the number of eggs produced or the size of the nauplii across the three treatments. The high degree of variability as well as individual differences in resource allocation and resource availability are discussed as possible reasons for the lack of significant differences in egg number or offspring size between the treatments.

As a final component, the existence of a trade-off was analyzed between the number of eggs and the size of the offspring. There was strong evidence suggesting that this trade-off is most prominent or evident in the GF/Cyan treatment, while not being much of a factor in the GF treatment. Given a finite amount of resources possessed by the mother *Eurytemora affinis*, it is reasonable to assume the presence of some sort of strategy regarding the allocation of these resources. The trade-off is essentially between producing a higher number of smaller offspring or a lower number of large offspring. This trade-off can change over time and space and is likely mainly influenced by the current environmental conditions (i.e. food availability, predator presence, environmental stress, etc.). The most optimal, most effective, and most efficient strategy of resource allocation will be one that increases the fitness of both the mother and future generations to the highest degree.

Grazing Experiment and Related Topics

Using data from a study involving the effects of size and concentration of food on the feeding of *Calanus pacificus*, a popular marine copepod, it was demonstrated that copepods display an ingestion rate which is directly dependent on concentration and size of the cell at low food concentrations (Frost, 1972). This feeding rate is constant and apparently independent of concentration, size, and type at high concentrations of food. The feeding rate typically increases until a saturation point is reached, which can vary over time and space and between species. A study by Barthel (1983) that investigated the optimal uptake by *Eurytemora affinis* predicted that copepods fed at a concentration of 340 µgC/L will grow and feed optimally.

Originally, the intent was to have 500 µgC/L of *Scenedesmus quadricauda* and 10% *Microcystis aeruginosa*. This would provide for a food concentration that is more than enough for optimal feeding and growth. Analysis of food concentration in the experimental beakers through cell counts and calculations indicated that the food concentrations used during grazing were actually about 300 µgC/L for *S. quadricauda* and about 30 µgC/L for *M. aeruginosa.* This error was likely due to errors in food concentration calculations and additions. Despite this error, the provided amounts of food, according to past research, should have been adequate enough to achieve close to a saturation point of feeding and to support optimal growth of the copepods (Barthel 1983).

Survivorship of *Eurytemora affinis* in all treatments after the grazing experiment was around 90%, indicating that the presence and consumption of *Microcystis aeruginosa* did not affect copepod survival during this short term experiment. Koski et al. (2002) also demonstrated that *E. affinis* is able to survive and reproduce in a planktonic community that is dominated by toxic cyanobacteria (during HAB). This study was done using populations from the Baltic Sea and the toxic cyanobacteria *Nodularia* sp., a typical pairing of organisms due to their abundance in the Baltic. In contrast to the results obtained in this study, another study that used the pairing of *Eurytemora affinis* from Little Sturgeon Bay and *Microcystis aeruginosa* in one set of experiments and *E.affinis* from the Baltic Sea and *Nodularia spumigena* found negative effects on survivorship, egg production and offspring size as well as reduced grazing rates in both of these populations (Dwyer, 2013).

The results of the grazing experiments suggest that *Eurytemora affinis* has the ability to control and regulate its feeding. In the GF and GF/Cyan treatments the copepods exhibited similar grazing rates, but in the GF/Filt treatment condition, the copepods drastically reduced both filtering rates and ingestion rates. These results are supported by the findings of a study by Dwyer (2013), where the lowest grazing rate demonstrated by *E.affinis* was observed in the treatment involving a good food source and *Microcystis aeruginosa* filtrate. In both the GF/Cyan and GF/Filt treatments there is the presence of toxins (microcystin), in the extra-cellular form in the GF/Filt and both intra- and extracullular form in the GF/Cyan treatment. The main factor differing between these two treatments is the presence of the actual *Microcystis aeruginosa* cells themselves. In the GF and GF/Filt treatments the main factor differing is the presence of extracellular microcystin (and possibly other substances) in the GF/Filt treatment. This suggests that the copepods are sensing the presence of the toxin, and depending on whether or not the cell is present, they will either repress grazing if the cells are not present or continue grazing at a standard rate.

A previous study by DeMott & Moxter (1991) stated that the inhibition of feeding is either an adaptive behavior to avoid eating toxic cells or a direct ramification of a weakened condition due to poisoning. If the copepods have the option of benefitting from physically ingesting the nutrients provided by the cell, it seems as though they will endure/tolerate the toxin exposure and ingestion. On the other hand, if the nutrients of the cell are not present and not available for uptake by *Eurytemora affinis*, there is no benefit to grazing at a standard rate, and the grazing rate is reduced. Therefore, the nutrients provided by *M. aeruginosa* may act as a buffer to the effects of the toxin it produces when being fed on by *E. affinis*.

While other studies have shown that *Eurytemora affinis* has the ability to demonstrate adaptive behavior by decreasing feeding rates when exposed to toxic strain of cyanobacteria (Engström-Öst, 2002), the data presented here suggests that this is only true if the cyanobacteria cells themselves are not available for ingestion. In other words, the alteration of grazing rate is contingent upon the toxins/cyanobacteria cells actually being available for feeding and ingestion; if they are present, grazing rate is not decreased. It has been suggested that cyanobacteria may possess various elements that are essential for grazers (Engström-Öst, 2002). It would be favorable for *E. affinis* to evolve the ability to utilize the nutrients of cyanobacterial cells and could serve as a key driver in its ability to outcompete other copepod species that are not capable or not efficient at utilizing the cyanobacteria as a potentially beneficial/quality food item. The study by Engström-Öst (2002) points out that a cyanobacterial bloom and its related elements could provide as a diverse and highly useful food source for the dominant species of copepods in the Baltic Sea. This ability to feed and efficiently utilize the nutrients of the cyanobacterial cell may also be a main component influencing the successful invasions of freshwater systems by *E. affinis*.

Results provide evidence that the copepods in the grazing experiment did not selectively feed on either of the strains of food provided. Both *Microcystis aeruginosa* and *Scenedesmus quadricauda* were fed upon with no indication of selectivity by *Eurytemora affinis*. Other studies, in contrast, have demonstrated that *E.affinis* does selectively feed on certain food sources. It has been demonstrated that *E. affinis*, when presented with a choice of toxic or non-toxic cyanobacteria, preference was shown for the non-toxic strain/cells (Engström-Öst, 2002). Other studies have shown that *E. affinis* is a suspension feeder and considered a relatively less selective species of copepod (Jonsson and Tiselius, 1990; Gasparini and Castel, 1997). This could explain the evolution of the adaptive and efficient use of cyanobacteria (toxic and non-toxic) by *E. affinis* given that it is commonly found in the same spatial area as the typically suspended cyanobacteria.

It has been proposed by past studies that zooplankton which exhibit selective feeding to avoid cyanobacteria tend to have lower physiological tolerance of their toxins (DeMott & Moxter, 1991; Kozlowski-Suzuki et al., 2003; Sarnelle and Wilson, 2005). This type of behavior is beneficial to the copepods that are not tolerant of the toxin and has therefore evolved over time. On the other hand, if a copepod is tolerant of the toxins, this type of selective feeding would not be as beneficial because there are no (or very limited) negative side effects of ingesting the toxin. This may suggest that *E. affinis* can tolerate the toxins (specifically microcystin-LR) better than other copepods, and therefore it does not selectively feed and can take advantage of the resources and nutrients provided by the toxin producing cyanobacterial cells. There would be no benefit of feeding on toxic cells, unless the organism was tolerant of this toxin or the nutrients of the ingested cell acted as a buffer to the negative consequences of toxin ingestion. Toxin tolerance and the ability to derive nutrition from the cells is very advantageous during mass-occurrences of cyanobacteria (Engström-Öst, 2002).

There seem to be two ways that a copepod may be able to distinguish between toxic and non-toxic cells: either by recognizing the toxin before ingesting or by learning from prior ingestion of a toxic cell and the following avoidance of future ingestion (Carlsson et al. 1995). The recognition of the toxin would likely be through sensing various extracellular cues released by the toxic cells. Our results suggest this very view, that *E. affinis* has the ability to sense the presence of *Microcycstis aeruginosa* in the environment based on some cue, and can therefore adjust feeding depending on whether or not the actual cells are available for ingestion and nutrition. The apparent ability of *E.affinis* to sense the presence of a toxin (versus non-toxin) in the water has been demonstrated in other studies (Engström et al. 2000).

As mentioned, the results suggest that *Eurytemora affinis* has the ability to sense cues in the water that provide information concerning food availability and toxin presence. It is likely that the sensing of the *Microcystis* cells and the related toxins is related to the chemosensory capacity of the individual copepod and species. Studies have shown that the setae on the first antennae provide copepods with mechano- and chemosensory information that can be used in prey detection, predator detection, and mate recognition (Griffiths and Frost, 1976; Lenz and Yen, 1993)**.** A study by Lenz et al.

(1996) aimed at investigating the sensory specialization along the antennae of a calanoid copepod (*Pleuromamma xiphias*). Their results indicate that there are purely mechanoreceptive setae, purely chemoreceptive setae, and mixed-modality setae. The setae that are capable of chemoreception are distinguished by the presence of apical pores and small sensory dendrites. Using a dye, Lenz and colleagues (1996) demonstrated that the chemoreceptive setae have the ability to absorb external substances in the water. Their results were inferred from both comparative morphology as well as observed behavior. Based on their results, the researchers hypothesized that the function of the mixed-modality setae is "gustatory." This means that the setae can sense particles by tasting (chemoreception) and mechanical contact (mechanoreception) combined. As a result, it was proposed that these setae may be involved in the acceptance and/or rejection of potential food particles which come into physical contact with the first antennae. Our results suggest that the ability to sense substances (toxins, food, etc.) in the environment was utilized by *Eurytemora affinis* during the grazing experiments.

A study by Lehtimaki et al. (1997) demonstrated that the extracellular toxin release by cyanobacteria is affected by temperature, light, salinity, growth stage, and phosphorous concentration. This shows that extracellular cues in the form of toxins are variable, in space and time, and therefore dependence on using extracellular toxins as the primary way of recognition may be unreliable. Given this unreliability, the presence of the cyanobacteria is likely physically/mechanically sensed as well, by cell capture and/or ingestion (Lenz et al., 1996). Subsequent feeding would then be altered or regulated given the physically sensed cues. This means that when the copepod captures the given cell, it has the ability to recognize it by shape and/or taste. This occurrence would then change the potential for subsequent feeding on this type of cell. If the cell was pleasing to the copepod, it would continue eating it in the future. On the other hand, the opposite would be true if the cell was not pleasing to the copepod and therefore the copepod would avoid feeding on this type of cell in the future. This type of behavior is very difficult to establish experimentally and instead needs to be proposed or inferred in many cases.

The combination of mechanoreceptive and chemoreceptive setae present on the copepods and their sensory abilities could account for the results observed in this study. In the GF treatment, no toxins were present and therefore there was no cue received by the receptive setae on the copepod and no change in feeding behavior. On the other hand, in the GF/Cyan and the GF/Filt treatments, there were toxins present and likely sensed by the chemoreceptive setae. In the GF/Cyan treatment, it is possible that the cells themselves were mechanically sensed but in the GF/Filt treatment there is an absence of cells and a lack of availability for ingestion. This could prompt the copepod to continue a normal grazing rate in the GF/Cyan treatment since the cells (and its nutrients/resources) are available for ingestion while significantly decrease grazing in the GF/Filt treatment due to toxin presence and lack of cell availability. Therefore, it may be reasonable to suggest that *Eurytemora affinis* has the ability to use both mechano- and chemoreceptive setae to sense food availability and adjust grazing rate accordingly.

Studies conducted in the San Francisco estuary have focused on the relationship between *Eurytemora affinis* and the toxin, microcystin, produced by *Microcystis* (Ger et al., 2009). Ger and colleagues found that in this environment, it is very rare and unlikely to have microcystin at high enough levels to cause significant depletions in survivorship of *E. affinis*. The results of this study indicate that toxicity from dissolved microcystin is not a direct threat to zooplankton of the San Francisco Estuary, and other mechanisms such as dietary exposure to *Microcystis* constitute a more severe risk. The risk of dietary exposure to *Microcystis* was not supported by the findings of our short term study using *E.affinis* from Lake Michigan.

Another study by Ger et al. (2010) produced some interesting findings concerning the effects of *Microcystis aeruginosa* (and microcystin) on the copepods of the upper San Francisco Bay (including *E.affinis*). This study used strains of *Microcystis* that produced toxins, and strains that did not. Contrary to expectations and findings from other studies, Ger and colleagues found that the strain with the toxin did not result in higher mortality as compared to the strain without the toxin, suggesting that non-microcystin metabolites of *Microcystis* can negatively affect copepods. This finding is interesting since it seems to indicate that negative impacts may not be related to the cellular microcystin concentration at all, and therefore any feeding on *Microcystis* (toxic or non-toxic) cannot be justified. Though *E. affinis* has been shown to feed and survive in the presence of cyanobacteria in other studies, this work by Ger seems to suggest otherwise.

The suggestion of non-microcystin metabolites (released by cyanobacterial cells) possibly having a negative effect on copepods by Ger et al. (2010) brings up an interesting topic. Is there another way, other than relying on toxin presence, that *Eurytemora affinis* could sense the presence of *Micorcystis aeruginosa*? This is a very difficult topic of research given the nature and complexity of the sensory system and the related cues, especially for zooplankton in aquatic ecosystems. Therefore, there does not seem to be much scientific evidence for such recognition abilities. As of now it seems like there may be other ways that *E. affinis* could sense the presence of *M. aeruginosa*, including through sensing various types of metabolites or waste products given off by *M. aeruginosa* cells, but much more work is needed in this field to gain insight into the possibility of various or multiple recognition cues.

As mentioned, *Eurytemora affinis* is invasive to the Great Lakes system, originating in a brackish water environment. It turns out that food concentration was very important in the move to freshwater by *E. affinis*. A recent study conducted by Lee et al. (2013) investigated multiple parameters concerning the move to freshwater from brackish water by the copepod *E. affinis,* a process completed by the populations found in Lake Michigan. In this study by Lee and colleagues, it was observed that high food concentrations significantly increase low-salinity tolerance of *E. affinis*. Therefore, this suggests that ample amounts of food can actually enable freshwater invasions. A common feature of HAB and eutrophication is an increase in phytoplankton, including mass-occurrences of cyanobacteria. North American Great Lakes, including Lake Michigan, tend to be more eutrophic (from anthropogenic nutrient inputs) along the coastlines, which is where the originally brackish copepod, *E. affinis* tends to persist (Lee et al. 2013). Overall, it was demonstrated by Lee et al. (2013) that high food concentrations could lead to extended ranges and condition-specific competition between saline invaders and currently inhabiting freshwater species, serving as a type of equalizer between novel and resident species. Although invasions often lead to high levels of competition over native species, the gradients that are ever present within aquatic ecosystems allows for the co-existence of invaders and current species. The ability to feed on *Microcystis* therefore may have facilitated *E. affinis*'s move from brackish water to the freshwater system of the Great Lakes.

Adding the component of competition is always important when analyzing an ecological interaction between a primary producer and consumer. Field or natural situations where competition is prevalent can be very different than the situations faced in isolated lab experiments. One study investigated the species specific differences in the ingestion of *Microcystis* cells by two species of dominant calanoid copepods (*Eurytemora affinis* and *Pseudodiaptomus forbesi*) from the San Francisco estuary (Ger et al. 2010). Results from this study indicate that *E. affinis* is less efficient at avoiding *Microcystis* than the other copepod used in the experiment. Note that a similar comparison also found that *E. affinis* was relatively inefficient in avoiding *Nodularia* (cyanobacteria) when compared with a raptorial feeding copepod (Engström et al., 2000). The researchers suggest that unlike *E. affinis*, *P. forbesi* may actually promote blooms of *Microcystis* via highly selective feeding on competing phytoplankton species. This high degree of selective feeding can have profound effects on the aquatic community and increase the impacts of HABs. Once again, selective feeding was not exhibited by *E. affinis* in this experiment. If *E. affinis* can effectively utilize the nutrients provided by the cyanobacterial cell, then there would be no valid reason for avoidance of the cell. This may be why *E. affinis* was shown to not avoid the cyanobacterial cells in the previously discussed studies.

In relation to competition with other species of copepod, it has been suggested through experiments involving *Arcatia bifilosa* and *Eurytemora affinis* from the Baltic Sea that when food availability is scarce and/or during bloom conditions, that *A. bifilosa* may starve, whereas *E.affinis* would continue feeding at a low rate (Engström et al. 2000). A study by Richman et al. (1980) which looked at the grazing interactions among four freshwater calanoid copepods found that *E. affinis* demonstrated the most restricted feeding range and rate. According to their findings, *E.affinis* grazed at an optimally efficient level (peak size and bio-volume selection). The presence of varied size-dependent feeding behaviors among calanoid copepods allows for their coexistence in aquatic ecosystems.

Egg Production Experiment and Related Topics

The results from our experiments concerning the egg production of female *Eurytemora affinis* exposed to the various food treatment conditions suggest that there was essentially no difference in the average number of eggs produced per female or the average size of the nauplii. The absence of negative effects on the reproduction of *E.affinis* has been demonstrated and supported by past studies (Engström-Öst, 2002; Koski et al., 2002). On the other hand, there has been evidence of negative effects of toxic cyanobacteria on *E. affinis* reproduction (Dwyer, 2013).

The copepods exposed to the GF/Cyan treatment ingested a relatively high amount of the *M. aeruginosa* cells (and the toxins). The ingestion of these cells and their toxins had essentially no effect on egg production or offspring size according to our results. This means that despite the diet consisting of *Microcystis aeruginosa* in the GF/Cyan treatment or the presence of *M. aeruginosa* filtrate in the GF/Filt treatment, there was not a negative effect on the reproductive capabilities of the copepods. It may be important to mention that the considerable amount of variation within the GF/Cyan and GF/Filt may have led to the lack of statistical significance in both of these categories. This variation within these two treatments could be a sign of differences in toxin tolerance, resource allocation, and/or recognition abilities between individual copepods, a prime component for natural selection and evolution to work on.

The copepods in the GF/Filt treatment drastically shut down their grazing rates. Despite doing so, these copepods were still able to produce a similar amount of eggs and offspring that were similar in size to the individuals exposed to the GF and GF/Cyan treatments, who showed no reduction in feeding rate. This occurrence suggests that *E. affinis* may have the capability to utilized stored materials for use in maintenance and reproduction when food quality is low and or feeding mechanisms are shut down. It is also likely that *E. affinis* would not have evolved to have the ability to shut down feeding if there was not some sort of mechanism to combat the low levels of nutrient ingestion associated with grazing shutdown (utilization of stored products). It is likely that these stored materials are forms of lipids that the copepod can use (if needed, i.e. feeding is shut down) in the maintenance of its own physiological needs or, as seen in this study, in reproduction and egg production.

As previously mentioned, the ability to utilize stored resources would be beneficial for the copepod, especially while reproducing in an environment with low food quality and/or availability. The utilization of stored resources for the purpose of reproduction may have been demonstrated in this study given the results obtained in the GF/Filt treatment. The copepods exposed to this feeding environment drastically shut down their feeding rates and therefore ingestion of nutrients and critical resources used in maintenance and reproduction. Despite this lack of resource ingestion, the females still produced offspring that were similar in size and quantity to those produced in the other two treatments where feeding and ingestion was not shut down. Therefore, the females in the GF/Filt treatment likely allocated similar amounts of resources to offspring/egg production as did the females in the other treatments despite the differences in nutrient and resource ingestion. This may have occurred through the utilization of stored reserves.

The storage of lipids by zooplankton has been shown to have an important role in reproduction (Tessier & Goulden, 1982; Butler, 1994; Brett & Muller-Navarra, 1997; Ackman, 1999; Lee et al., 2006). Much of the time, these stored lipids are in various forms, including triacylglycerols, wax esters and phosopholipids (Lee et al., 2006). It is often noted that there are lipid droplets that can be transferred to developing oocytes. These studies have found that the yolk in the eggs of zooplankton has high levels of lipovitellin, a lipoprotein that females must convert their stored lipids into during reproduction. The developing embryos then use this lipovitellin and the lipid droplets for growth, energy, membranes and hormones until active feeding begins (Lee and Walker, 1995). The availability of more yolk and lipids would be an undeniable benefit for any developing embryo, especially in a less than favorable environment caused by toxin presence, predator presence, or low food. The biochemistry of lipid use and storage is beyond the scope of this experiment but there has been work done in this area involving calanoid copepods (Sargent & Flak-Petersen, 1981).

Lee et al. (2006) suggested that before the onset of a spring phytoplankton bloom (not necessarily an HAB, but it may be included), many species of zooplankton use stored lipids for energy and materials for reproduction. This study by Lee and colleagues also states that the occurrence of these phytoplankton blooms may increase the amount of lipid reserves that a female has. This means that given the cycles of low food and high food, the zooplankton can still reproduce in low food conditions thanks to the ability to store and allocate lipid reserves to developing offspring. This ability may have been demonstrated in our study by the female *Eurytemora affinis* in the GF/Filt treatment who shut down feeding but still produced offspring in comparable numbers and sizes to those females that did not shut down their feeding. Although not tested, it would be reasonable to assume that after the offspring were produced, the females within the GF/Filt treatment would have much lower lipid content in storage than the females in the other treatments. This would be the effect of the GF/Filt females needing to allocate their reserves to the offspring since feeding was shut down. A study by Butler (1994) provides direct support for this, stating that individuals could enhance reproduction during periods of unfavorable food conditions by utilizing energy stores.

The topic of cell content (in terms of quality) with regards to different species of phytoplankton brings up an interesting area of research that is slightly beyond the scope of this experiment, but may be worth mentioning. It seems that cell content may be directly related to the quality of the cell as a food option and the availability of useable and beneficial nutrients. Sterner (1994) who looked at algal nutrient limitation and nutrition of aquatic herbivores stated that grazers with high nutrient demands are not limited by quantity or energy, but by the quality of the food. The quality of this food is directly related to the type and quantity of nutrients and mineral elements within the cell including carbon, nitrogen, phospohrous, etc. There are numerous other trace elements that may differ between phytoplankton cells, making them more or less appealing to various types of herbivore grazers, including copepods. Also, note that a previously mentioned study by Ger et al. (2010) suggested that non-toxin related metabolites of the cyanobacteria might also have an effect on the grazers. The differences in cell content help explain why some types of phytoplankton are more beneficial to grazers than others. Previous studies have shown that it is difficult to determine whether it is specifically the toxin that is having an effect on the copepod or if it was another substance (i.e. metabolites) produced by the cyanobacteria (Suikkanen et al., 2006). The cell content and its nutritional quality may also be related to the sensory capacity of *Eurytemora affinis*, a topic that was previously discussed.

As covered in the introduction of this study, the low food quality has mainly been associated with the cyanobacteria's difficult-to-handle morphology, low nutritional quality, and toxin content (Porter and Orcutt, 1980; Lampert, 1987; Kirk & Gilbert, 1992). This lack of nutritional quality seems to be linked to the cyanobacteria lacking essential compounds such as polyunsaturated fatty acids (Holm & Shapiro, 1984). These findings regarding cyanobacteria and food quality are generalized to the entire class, and it must be kept in mind that these factors and effects are dependent on space, time, and the consumer. Meyer-Harms et al. (1999) suggested that cyanobacteria can provide a valuable food source for specialized consumers and allow for this specialist to be successful in a community dominated by cyanobacteria. This type of specialization could be exhibited by *Eurytemora affinis* in relation to *Microcystis aeruginosa* in this environment (Lake Michigan). This would account for *E. affinis* feeding on the cyanobacterial cells when they were present and the cell's nutrients were available for ingestion and utilization.

As it has been mentioned, this experiment represents a short term study that attempts to gain information about a complex ecological relationship that is likely dependent on time in some way. In a study that looked at a slightly longer time period (5 days) of cyanobacteria exposure, showed that *E. affinis* had increased mortality, declines in egg production and hatching success (Koski et al., 1999). In contrast to other studies, including my own, the study by Koski et al. suggested that *E. affinis* is not able to reproduce on a diet of cyanobacteria (*Nodularia* sp.). Surprisingly, this effect was irrespective of the toxicity of the *Nodularia* cells and therefore, the nutritional quality of this cyanobacteria is not sufficient in maintenance and egg production, although negative effects of nodularin on egg sacs (deformations) was noted in this study. Though they share some similarities, it is important to distinguish the types of cyanobacteria used in the study by Koski et al. (1999) and the type used in this present study. *Nodularia* is a filamentous cyanobacteria while *Microcystis* is not. This difference in form combined with the difference in cell content between the two species may account for the difference in findings.

Trade-off: More or Less?

Given a limited and finite amount of resources to allocate, it is reasonable to assume that a trade-off between the number and size of offspring exists in nature. According to the Lacks competitive clutch hypothesis there is an inverted U-shape for the quantity, survival, and fitness of offspring in relation to clutch size (Stearns, 1992). The Reproductive Effort Model suggests that there are decreasing returns from reproductive investments which leads to intermediate levels of reproductive effort by parents. The evidence available strongly supports that organisms flexibly adjust their reproduction to optimize individual fitness (current and future offspring potential).

An interesting aspect that was analyzed in this study was the potential trade-off present between the number of eggs produced and size of offspring. This trade-off mainly deals with the allocation of resources by the *Eurytemora affinis* to various physiological aspects (growth, reproduction, etc.). It is accepted that the quality and size of offspring produced are positively correlated, so larger offspring represent a better quality (more strong and tolerant) (Stearns, 1992). According to our results there was a strong trade-off present in the GF/Cyan treatment, a slightly weaker correlation in the GF/Filt treatment, and essentially no correlation between offspring size and number of eggs produced in the GF treatment. It seems as though given the presence of a good food source and a quality environment (toxin-free), as in the GF treatment, there is no need for the copepods to allocate high amounts of resources to investment in offspring quantity or quality (size). On the other hand, in the less than favorable environments of the GF/Cyan and GF/Filt, there was a trade-off between quantity and quality. This seems reasonable that the copepods would rather produce a low number of high quality and resilient offspring when in a less than optimal/favorable environment. Instead of investing resources in the quality of the offspring, the individual might also produce a high number of offspring, betting on the chance that at least a few of them would survive. In both cases, the copepod has the interest of maximizing its own fitness and does so through the control of resource allocation. In the GF/Filt treatment especially, there were two individuals that exhibited the lower number but higher quality (larger) offspring approach, whereas the other two individuals had similar number and sizes of offspring as those in the GF treatment.

In the GF/Cyan treatment, much like the trend seen in the GF/Filt treatment, there were two individuals that exhibited one of the resource allocation techniques previously discussed and two that exhibited the other. There was a clear difference in the strategy of resource allocation between the individuals within each treatment. Half of the individuals opted for the production of less but higher quality (larger) offspring approach, while the other half exhibited the more but less quality (smaller) offspring approach. These stark differences exhibited between individuals in relation to resource use and allocation is the exact component that natural selection and evolution work on.

Although the general trend of a lower number of larger or a higher number of smaller offspring is exhibited by the individuals in the GF/Cyan treatment, it is interesting to note the quantity and size in relation to the eggs and offspring produced in the other treatments. Keep in mind that the grazing rates for the individuals in the GF/Filt treatment were much lower than the individuals in the GF/Cyan treatment. The individuals' from the GF/Filt treatment likely had less or a lower quality of resources (likely stored resources) to allocate towards reproduction and egg production. On the other hand, the individuals from the GF/Cyan treatment did not reduce grazing rates and likely had an ample amount of resources to allocate to reproduction and egg production. This will help explain why a few individuals in the GF/Cyan treatment produced many more offspring of the same size as those in the GF/Filt treatment. The size and number of offspring are relatively comparable between GF and GF/Filt while the individuals from the GF/Cyan treatment either produced a higher quantity offspring of the same size as those produced in the other two treatments or a similar quantity but larger in size. This also suggests that the individuals within the GF/Cyan treatment had an ample stock of nutrients and had the ability to allocate more resources to offspring to make them higher in number or larger in size within a toxic environment and increase their chance of survival (increases in fitness).

Van Noordwijk and De Jong (1986) also attempt to explain this trade-off embedded within the reproduction process. They state that if producing one additional offspring reduces future reproduction by more than one individual, then this additional offspring is not selected for. Therefore, though it may seem a bit counter intuitive, an individual can have an increase in fitness from fewer offspring. The variation of resources available to individuals across time and space helps explain why it may be difficult to observe these trade-offs within an entire population. Tessie and Consolatti (1991) analyzed resource quantity and offspring quality and found direct evidence in zooplankton that the adaptive plasticity in reproduction is related to resource availability and environmental circumstances. Our results seem to support this claim given the high degree of plasticity and variation shown in the GF/Cyan treatment condition which had the most food available for consumption and was in an environment with the highest toxin levels.

Difficulties & Future Experiments

As with any scientific experiment, it is always important to realize potential confounding factors or problems with the experiment itself. Bringing these factors and problems into focus and making improvements or controlling for them will allow for more efficient and effective experiments in the future. Throughout the experimental process careful considerations were taken when considering potential factors (those other than the feeding conditions) that may affect the living organism. Some of these factors were worked through using preliminary experiments (i.e. the high conductivity from the algal growth medium). The animals were picked randomly and allocated to the beaker and treatment conditions. The beakers were placed in a similar environment within the lab and placed in no particular order to account for any spatial factors present that may affect the beakers (light, temperature, airflow etc.). These confounding factors were controlled for to the best of our abilities and are likely not a large factor in this experiment.

A few problems did occur throughout this experiment. As mentioned at the start of the grazing discussion section, the food concentrations were lower than intended. We aimed to produce food concentrations of about 500 µgC/L, but in reality when checked, the concentrations were around 300 µgC/L. This was not thought to have been an issue given that previous studies indicated that *Eurytemora affinis* obtain an optimal growth rate at about 340 µgC/L (Barthel, 1983).

Regarding the calculation of the grazing rates, it is typical to use more than one method. In this study we used cell counts to calculate the grazing rates and attempted to use Chl*a* composition to do so as well. Unfortunately, the Chl*a* analysis did not provide reliable data and was not able to be used. This is likely due to an error in the extraction method and/or a lack of sensitivity related to the spectrophotometer or cuvette given the somewhat dilute food concentrations and the size of the sample used for analysis (5-10ml). It would be useful to have two different indications of the grazing rates of the copepods and this problem of sensitivity or Chl*a* extraction procedure should be altered for future experiments.

Although the problem related to survivorship was addressed by using information provided by preliminary experiments, there were still periods of considerable mortality when collecting and storing the organisms for future use. Many of the females produced eggs, but there were numerous females and egg sacs that began to decompose before they were allowed to hatch. As decomposition started, Lugol's solution was added immediately in order to obtain data for the number of eggs produced, but the offspring size was not attainable in some cases. It is unknown what the exact cause of this decomposition and lack of hatching was, but this problem should be addressed in future experiments.

This study also provides a pathway for future experiments. It would always be more interesting, representative, and indicative of real-world situations if experiments be run on a longer term basis. Sometimes there can be stark differences in the short term and long term effects of such ecological factors. It is, without a doubt, more useful to investigate the long term effects of these interactions present in nature given that the process of natural selection and adaptation in general occur over a long time period. Using different combinations of food concentrations and food types would also be interesting. Given the large difference seen in this study between the GF/Cyan and GF/Filt treatments, it may be interesting to add an intermediate feeding condition to the experiment. Food limited situations could also be implemented into future experimental design and could produce different affects compared to a non-food limited environment. The addition of various types of organisms and foods allows for greater comparisons between species and or populations from both similar and different environments.

One could also take an approach that investigates the molecular and biochemical related interactions between the toxins produced by cyanobacteria and its effect on the consumers. This experiment did not go into much detail about this topic so gaining more insight about the details of these types of ecological interactions and their effects on the individual organism from a cellular view could be very beneficial for future experiments.

On the other hand, studies similar to this one may also shift more into the evolutionary view, looking across or between populations over a given amount of time. This could be beneficial for future experiments as well and could provide valuable insight into the future fates of various species and ecological interactions.

Overall, the variability and complex interdependent factors in aquatic ecosystems combined with the differences in response by individuals' leads to complications in analyzing these types of ecological interactions. As seen throughout the introduction and discussion of this experiment, there seems to be no real consensus or generalizable pattern available within the literature regarding the effects of cyanobacteria on zooplankton. This makes conducting and discussing these types of studies difficult at times. It is likely that these interactions are dependent on space, time, as well as the players (predator and prey). Therefore, it is crucial that more work be done in an attempt to gain knowledge and understanding about these specific and complex ecological interactions between producers and consumers.

Implications and Conclusions

There are many implications of this study that investigated the grazing and reproduction ecology of a copepod that is invasive to the Great Lakes region by paring it with a toxin producing cyanobacteria that is known to produce HABs within the aquatic ecosystem of interest. There are behavioral implications indicated by the results of the grazing experiment. These behaviors are adapted over time and essentially shaped by natural selection and evolution. The egg production section of this study gives indication of the possible long term effects of being exposed to a toxic cyanobacterial diet and how they influence an organism's fitness. The fact that the HABs themselves are a product of eutrophication and global climate change make them an environmental concern in many areas. Gaining more knowledge about HABs could have many environmental and management implications. As with any study that investigates an interaction between a primary producer and consumer within an ecosystem, there are numerous implications that can reach higher trophic levels within the food web. Given the fact that the copepods ingested the toxin, microcystin, and that toxin ingestion has been suggested as a vector for spread throughout the food web, this ingestion of the cyanobacteria may affect other organisms that may not be tolerant to the toxin (Lehtinimi et al. 2002). Although, it is important to realize that the overall effects on these organisms may be stronger in nature and over time (through multiple generations), it is important to use scientific studies to gain more insight regarding the ecological, cellular, biochemical, evolutionary, environmental implications of such ecological interactions.

In conclusion, this study provides for interesting parameters of analysis given the results obtained. The results of this study indicate that *Eurytemora affinis* has the ability to adjust its feeding rate in relation to the food environment. *Microcystis aeruginosa* may be a beneficial food source for *E. affinis* as long as the cells themselves are available for ingestion. This may suggest that *E.affinis* is specialized to recognize, (likely through sensory capabilities in the antennae) ingest, and utilize the nutrients provided by the cyanobacteria *M .aeruginosa*. This could provide many for many benefits in certain food conditions and allow for this copepod to invade and outcompete other species that may be negatively affected by the cyanobacteria. This is likely a key component to the invasion of certain aquatic environments by *E. affinis*.

Even though feeding was shut down within the GF/Filt treatment, there was no significant difference in the number or size of offspring produced between the treatments. This could suggest that *E. affinis* could have the ability to utilize stored reserves for egg production even when ingestion is low, another factor that could be important for invasion and outcompeting other species of copepods in less than favorable food conditions. The results also suggest a trade-off present in reproduction regarding the number and size of offspring given finite resources for allocation. This trade-off was very evident in the treatments containing the *M. aeruginosa* cells and filtrate, suggesting that more attention is given to this trade-off and resource allocation in less than favorable environments (toxins and poor or low food availability).

By investigating multiple parameters including feeding behavior, resource allocation, egg production, and offspring production this study provides valuable knowledge concerning the ecological interaction between *Eurytemora affinis* and *Microcystis aeruginosa*. This study has important implications that reach to multiple fields of science.

REFERENCES

Ackman RG (1999) Comparison of lipids in marine and freshwaterorganisms. In: Arts MT, Wainman BC (eds) Lipids in freshwater ecosystems. *Springer, Berlin*, p 263–298.

Ahlgren, G., & Hyenstrand, P. (2003). Nitrogen limitation effects of different nitrogen sources on nutritional quality of two freshwater organisms, *Scenedesmus quadricauda* (Chlorophyceae) and *Synechococcus* sp. (Cyanophyceae). *Journal of Phycology, 39*(5), 906-917.

Ahlgren, G., Lundstedt, L., Brett, M., & Forsberg, C. (1990). Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *Journal of Plankton Research, 12*(4), 809-818. Anderson, D. M., Glibert, P. M., & Burkholder, J. M. (2002). Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries, 25*(4), 704-726.

Andrewartha, H. G., & Birch, L. C. (1954). The distribution and abundance of animals. *University of Chicago Press, Chicago.*

Barthel, K. G. (1983). Food uptake and growth efficiency of Eurytemora affinis (Copepoda: Calanoida). *Marine Biology*, *74*(3), 269-274.

Bertrand, G., Lang, J., & Ross, J. (1976). The Green Bay watershed- Past/Present/Future. *Report Wis-Sg-76-229.*

Beyrend-Dur, D., Souissi, S., Devreker, D., Winkler, G., & Hwang, J. (2009). Life cycle traits of two transatlantic populations of *Eurytemora affinis* (copepoda: Calanoida): Salinity effects. *Journal of Plankton Research, 31*(7), 713-728.

Breteler, W. K., Schogt, N., Baas, M., Schouten, S., & Kraay, G. (1999). Trophic upgrading of food quality by protozoans enhancing copepod growth: Role of essential lipids. *Marine Biology, 135*(1), 191-198.

Brett, M., & Muller-Navarra, D. (1997). The role of highly unsaturated fatty acids in aquatic food web processes. *Freshwater Biology, 38*(3), 483-499.

Bringmann, G., & Kuhn, R. (1978). Grenzwerte der schadwirkung wassergefahrdender stoffe gegen blaualgen (*Microcystis aeruginosa*) und grunalgen (*Scenedesmus quadricaud*a) im zellvermehrungshemmtest. *Vom Wasser, 50*, 45-60.

Brittain, S. M., Wang, J., Babcock-Jackson, L., Carmichael, W. W., Rinehart, K. L., & Culver, D. A. (2000). Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. *Journal of Great Lakes Research, 26*(3), 241-249.

Brooks, J. L., & Dodson, S. I. (1965). Predation, body size, and composition of plankton. *Science (New York, N.Y.), 150*(3692), 28-35.

Burnett, L.E. (*1997)* The challenges of living in hypoxic and hypercapnic aquatic environments. *American Zoologist* ***37****, 633–640.*

Butler, N. M. (1994). Lipid storage in Diaptomus kenai (Copepoda; Calanoida): effects of inter-and intraspecific variation in food quality. In *Limnology of Mountain Lakes* (pp. 9-16). Springer Netherlands.

Bury, N., Eddy, F., & Codd, G. (1995). The effects of the cyanobacterium *Microcystis aeruginosa*, the cyanobacterial hepatotoxin microcystin–LR, and ammonia on growth rate and ionic regulation of brown trout. *Journal of Fish Biology, 46*(6), 1042-1054.

Cairns Jr, J., Heath, A. G., & Parker, B. C. (1975). The effects of temperature upon the toxicity of chemicals to aquatic organisms. *Hydrobiologia*, *47*(1), 135-171.

Carlson, R. E. (1977). A trophic state index for lakes1. *Limnology and Oceanography*, *22*(2), 361-369.

Carlsson, P., Granéli, E., Finenko, G., & Maestrini, S. Y. (1995). Copepod grazing on a phytoplankton community containing the toxic dinoflagellate *Dinophysis acuminata*. *Journal of plankton research*, *17*(10), 1925-1938.

Carpenter, S. R., Kitchell, J. F., Hodgson, J. R., Cochran, P. A., Elser, J. J., Elser, M. M., et al. (1987). Regulation of lake primary productivity by food web structure. *Ecology*, *68*(6), 1863-1876.

Casini, M., Lovgren, J., Hjelm, J., Cardinale, M., Molinero, J. C., & Kornilovs, G. (2008). Multi-level trophic cascades in a heavily exploited open marine ecosystem. *Proceedings.Biological Sciences / the Royal Society*, *275*(1644), 1793-1801.

Chandra, S., & Gerhardt, A. (2008). Invasive species in aquatic ecosystems: Issue of global concern. *Aquatic Invasions*, *3*(1), 1-2.

Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., et al. (2009). Controlling eutrophication: Nitrogen and phosphorus. *Science*, *323*(5917), 1014-1015.

Cummins, K. W., & Klug, M. J. (1979). Feeding ecology of stream invertebrates. *Annual review of ecology and systematics*, 147-172.

Czaika, S. C. (1982). Identification of nauplii N1–N6 and copepodids CI–CVI of the Great Lakes calanoid and cyclopoid copepods (calanoida, cyclopoida, copepoda). *Journal of Great Lakes Research, 8*(3), 439-469.

Daufresne, M., Lengfellner, K., & Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences of the United States of America, 106*(31), 12788-12793.

De Stasio, B. T., Schrimpf, M. B., Beranek, A. E., & Daniels, W. C. (2008). Increased chlorophyll a, phytoplankton abundance, and cyanobacteria occurrence following invasion of Green Bay, Lake Michigan by dreissenid mussels. *Aquatic Invasions, 3*(1), 21-27.

DeAngelis, D. L., Mulholland, P. J., Palumbo, A. V., Steinman, A. D., Huston, M. A., & Elwood, J. W. (1989). Nutrient dynamics and food-web stability. *Annual Review of Ecology and Systematics*, 71-95.

DeMott, W. R. (1986). The role of taste in food selection by freshwater zooplankton. *Oecologia, 69*(3), 334-340.

DeMott, W. R. (1989). The role of competition in zooplankton succession. *Plankton ecology* (pp. 195-252) Springer.

DeMott, W. R., & Moxter, F. (1991). Foraging cyanobacteria by copepods: responses to chemical defense and resource abundance. *Ecology*, 1820-1834.

Devreker, D., Souissi, S., & Seuront, L. (2004). Development and mortality of the first naupliar stages of *Eurytemora affinis* (copepoda, calanoida) under different conditions of salinity and temperature. *Journal of Experimental Marine Biology and Ecology, 303*(1), 31-46.

Devreker, D., Souissi, S., Winkler, G., Forget-Leray, J., & Leboulenger, F. (2009). Effects of salinity, temperature and individual variability on the reproduction of *Eurytemora affinis* (copepoda; calanoida) from the seine estuary: A laboratory study. *Journal of Experimental Marine Biology and Ecology, 368*(2), 113-123.

Dionysiou, D. (2010). Overview: Harmful algal blooms and natural toxins in fresh and marine waters–Exposure, occurrence, detection, toxicity, control, management and policy. *Toxicon*, *55*(5), 907-908.

Dodson, S. I. (1974). Zooplankton competition and predation: An experimental test of the size-efficiency hypothesis. *Ecology.* 605-613.

Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., et al. (2012). Climate change impacts on marine ecosystems. *Marine Science, 4.*

Dorgham, M. M. (2014). Effects of eutrophication. *Eutrophication: Causes, consequences and control* (pp. 29-44) Springer.

Downing, T., Meyer, C., Gehringer, M., & Van de Venter, M. (2005). Microcystin content of *Microcystis aeruginosa* is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. *Environmental Toxicology*, *20*(3), 257-262.

Dukes, J. S., & Mooney, H. A. (1999). Does global change increase the success of biological invaders? *Trends in Ecology & Evolution*, *14*(4), 135-139.

Dukes, J. S., & Mooney, H. A. (2004). Disruption of ecosystem processes in western North America by invasive species. *Revista chilena de historia natural*, *77*(3), 411-437.

Dwyer, A. (2013). Comparison of the effects of toxic cyanobacteria on the reproductive success of *Eurytemora affinis* populations in the Baltic Sea and Green Bay, Wisconsin.

Egge, J., & Aksnes, D. (1992). Silicate as regulating nutrient in phytoplankton competition. *Marine Ecology Progress Series.Oldendorf, 83*(2), 281-289.

Elton, C. S. (1958). The ecology of invasions by plants and animals. *Methuen, London, 18*.

Engström-Öst, J. (2002). *Effects of cyanobacteria on plankton and planktivores* Walter and Andrée de Nottbeck Foundation.

Engström, J., Koski, M., Viitasalo, M., Reinikainen, Repka, S., and Sivonen, K. (2000). Feeding interactions of the copepods *Eurytemora affinis* and *Arcatia bifilosa* with the Cyanobacteria *Nodularia* sp. *Journal of Plankton Research, 22(7),* 1403-1409.

Engström-Öst, J., Koski, M., Schmidt, K., Viitasalo, M., Jonasdottir, S., Kokkonen, M., et al. (2002). Effects of toxic cyanobacteria on plankton assemblage: Community development during decay of *Nodularia spumigena*. *Marine Ecology-Progress Series, 232*, 1-14.

Fenchel, T., & Blackburn, N. (1999). Motile chemosensory behaviour of phagotrophic protists: Mechanisms for and efficiency in congregating at food patches. *Protist, 150*(3), 325-336.

Feminella, J. W., & Hawkins, C. P. (1995). Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. *Journal of the North American Benthological Society*, 465-509.

Folt, C. L., & Burns, C. W. (1999). Biological drivers of zooplankton patchiness. *Trends in Ecology & Evolution, 14*(8), 300-305.

Frost, B. (1972). Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol.Oceanogr, 17*(6), 805-815.

Gasparini, S., & Castelt, J. (1997). Autotrophic and heterotrophic nanoplankton in the diet of the estuarine copepods *Eurytemora affinis* and *Acartia bifilosa*. *Journal of Plankton Research*, *19*(7), 877-890.

Ger, K. A., Arneson, P., Goldman, C. R., & Teh, S. J. (2010). Species specific differences in the ingestion of Microcystis cells by the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*. *Journal of plankton research*, *32*(10), 1479-1484.

Ger, K. A., Teh, S. J., Baxa, D. V., Lesmeister, S., & Goldman, C. R. (2010). The effects of dietary *Microcystis aeruginosa* and microcystin on the copepods of the upper San Francisco Estuary. *Freshwater biology*, *55*(7), 1548-1559.

Ger, K. A., Teh, S. J., & Goldman, C. R. (2009). Microcystin-LR toxicity on dominant copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi* of the upper San Francisco Estuary. *Science of the Total Environment*, *407*(17), 4852-4857.

Griffiths, A. M., & Frost, B. W. (1976). Chemical communication in the marine planktonic copepods Calanus pacificus and Pseudocalanus sp. *Crustaceana*, 1-8.

Gulati, R., & DeMott, W. (1997). The role of food quality for zooplankton: Remarks on the state-of-the-art, perspectives and priorities. *Freshwater Biology, 38*(3), 753-768.

Hairston, N. G., Lampert, W., Cáceres, C. E., Holtmeier, C. L., Weider, L. J., Gaedke, U., et al. (1999). Lake ecosystems: Rapid evolution revealed by dormant eggs. *Nature, 401*(6752), 446-446.

Haney, J. F. (1987). Field studies on zooplankton- cyanobacteria interactions. *New Zealand Journal of Marine and Freshwater Research, 21*(3), 467-475.

Hansson, L. A., Gustafsson, S., Rengefors, K., & Bomark, L. (2007). Cyanobacterial chemical warfare affects zooplankton community composition. *Freshwater Biology*, *52*(7), 1290-1301.

Harada, K. (1996). Chemistry and detection of microcystins. *Toxic Microcystis*, , 103-148.

Hebert, P., & Ontario, B. (2008). Chemical properties of lakes. Retrieved from http://www.eoearth.org/view/article/151077.

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., et al. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae, 8*(1), 3-13.

Hill, W. R., Ryon, M. G., & Schilling, E. M. (1995). Light limitation in a stream ecosystem: responses by primary producers and consumers. *Ecology*, 1297-1309.

Hillebrand, H. (2002). Top-down versus bottom-up control of autotrophic biomass—a meta-analysis on experiments with periphyton. *Journal of the North American Benthological Society*, *21*(3), 349-369.

Francoeur, S. N. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. Journal of the North American Benthological Society 20:358–368.

Holm, N. P., & Shapiro, J. (1984). An examination of lipid reserves and the nutritional status of *Daphnia pulex* fed *Aphanizomenon flos-aquae*. *Limnology and oceanography (USA).*

Huntley, M., & Boyd, C. (1984). Food-limited growth of marine zooplankton. *American Naturalist,* , 455-478.

Jackson, D. A., Peres-Neto, P. R., & Olden, J. D. (2001). What controls who is where in freshwater fish communities the roles of biotic, abiotic, and spatial factors. *Canadian Journal of Fisheries and Aquatic Sciences, 58*(1), 157-170.

Jang, M., Jung, J., & Takamura, N. (2007). Changes in microcystin production in cyanobacteria exposed to zooplankton at different population densities and infochemical concentrations. *Limnology and Oceanography, 52*(4), 1454.

Jonsson, P. R., & Tiselius, P. (1990). Feeding behaviour, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. *Marine ecology progress series. Oldendorf, 60*(1), 35-44.

Juhel, G., Davenport, J., O'halloran, J., Culloty, S., O'riordan, R., James, K., et al. (2006). Impacts of microcystins on the feeding behaviour and energy balance of zebra mussels, *Dreissena polymorpha*: A bioenergetics approach. *Aquatic Toxicology, 79*(4), 391-400.

Kankaanpää, H. T., Sipiä, V. O., Kuparinen, J. S., Ott, J. L., & Carmichael, W. W. (2001). Nodularin analyses and toxicity of a *Nodularia spumigena* (Nostocales, Cyanobacteria) water-bloom in the western Gulf of Finland, Baltic Sea, in August 1999. *Phycologia*, *40*(3), 268-274.

Karjalainen, M., Reinikainen, M., Spoof, L., Meriluoto, J. A., Sivonen, K., & Viitasalo, M. (2007). Trophic transfer of cyanobacterial toxins from zooplankton to planktivores: Consequences for pike larvae and mysid shrimps. *Environmental Toxicology, 20*(3), 354-362.

Keddy, P. A. (2010). *Wetland ecology: principles and conservation*. Cambridge University Press.

Kiørboe, T. (2011). How zooplankton feed: Mechanisms, traits and trade- offs. *Biological Reviews, 86*(2), 311-339.

Kirk, K. L., & Gilbert, J. J. (1992). Variation in herbivore response to chemical defenses: zooplankton foraging on toxic cyanobacteria. *Ecology*, 2208-2217.

Kivi, K., Kaitala, S., Kuoso, H., Kuparinen, J., Leskinen, E., Lignell, R., Marcussen, and Tamminen, T. (1993). Nutrient limitation and grazing control of the Baltic plankton community during annual succession. *Limnology and Oceanography, 38* (5) 893-905.

Kononen, K. (2001). Eutrophication, harmful algal blooms and species diversity in phytoplankton communities: examples from the Baltic Sea. *AMBIO: A Journal of the Human Environment, 30*(4), 184-189.

Koski, M., Engström, J., Viitasalo, M. (1999). Reproduction and survival of the calanoid copepod *Eurytemora affinis* fed with toxic and non-toxic Cyanobacteria. *Marine Ecology Progress Series 186:*187-189.

Kotak, B. G., Zurawell, R. W., Prepas, E. E., & Holmes, C. F. (1996). Microcystin-LR concentration in aquatic food web compartments from lakes of varying trophic status. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(9), 1974-1985.

Kozlowsky-Suzuki, B., Karjalainen, M., Lehtiniemi, M., Engström-Öst, J., Koski, M., & Carlsson, P. (2003). Feeding, reproduction and toxin accumulation by the copepods *Acartia bifilosa* and *Eurytemora affinis* in the presence of the toxic cyanobacterium Nodularia spumigena. *Marine Ecology Progress Series, 249*, 237-249.

Kuwata, A., & Miyazaki, T. (2000). Effects of ammonium supply rates on competition between *Microcystis novacekii* (cyanobacteria) and *Scenedesmus quadricauda* (chlorophyta): Simulation study. *Ecological Modelling, 135*(1), 81-87.

Lampert, W. (1981). Inhibitory and toxic effects of blue- green algae on daphnia. *Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie, 66*(3), 285-298.

Lee, C. E., & Bell, M. A. (1999). Causes and consequences of recent freshwater invasions by saltwater animals. *Trends in Ecology & Evolution, 14*(7), 284-288.

Lee, C. E. (2000). Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate “populations”. *Evolution*, *54*(6), 2014-2027.

Lee, C. E., & Frost, B. W. (2002). Morphological stasis in the *Eurytemora affinis* species complex (copepoda: Temoridae). *Hydrobiologia, 480*(1-3), 111-128.

Lee, C. E., & Frost, B. W. (2002). Morphological stasis in the *Eurytemora affinis* species complex (copepoda: Temoridae). *Hydrobiologia, 480*(1-3), 111-128.

Lee, C. E., Moss, W. E., Olson, N., Chau, K. F., Chang, Y. M., & Johnson, K. E. (2013). Feasting in fresh water: impacts of food concentration on freshwater tolerance and the evolution of food× salinity response during the expansion from saline into fresh water habitats. *Evolutionary applications*, *6*(4), 673-689.

Lee, C. E., & Petersen, C. H. (2002). Genotype- by- Environment interaction for salinity tolerance in the Freshwater- Invading copepod *Eurytemora affinis*. *Physiological and Biochemical Zoology, 75*(4), 335-344.

Lee, C. E., & Petersen, C. H. (2003). Effects of developmental acclimation on adult salinity tolerance in the Freshwater- Invading copepod *Eurytemora affinis*. *Physiological and Biochemical Zoology, 76*(3), 296-301.

Lee, R. F., Hagen, W., & Kattner, G. (2006). Lipid storage in marine zooplankton. *Marine Ecology Progress Series*, *307*, 273-306.

Lee RF, Walker A. (1995). Lipovitellin and lipid droplet accumulation in oocytes during ovarian maturation in the blue crab, *Callinectes sapidus*. J Exp Zool 272:401–412.

Lehman, P., Boyer, G., Satchwell, M., & Waller, S. (2008). The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in san francisco estuary. *Hydrobiologia, 600*(1), 187-204.

Lehtimaki, J., Moisander, P., Sivonen, K., & Kononen, K. (1997). Growth, nitrogen fixation, and nodularin production by two Baltic Sea cyanobacteria. *Applied and environmental microbiology*, *63*(5), 1647-1656.

Lehtinimi, M., Engström-Öst, J., Karjalainen, M, Kozlowsky-Suzuki, B., Viitasalo, M. (2002). Fate of cyanobacterial toxins in the pelagic food web: transfer to copepods or to fecal pellets? *Marine Ecology, 241*, 13-21.

Leibold, M. A., J. M. Chase, J. B. Shurin, and A. L. Downing. 1997. Species turnover and the regulation of trophic structure. Annual Review of Ecology and Systematics 28:467–494.

Lenz, P. H., & Yen, J. (1993). Distal setal mechanoreceptors of the first antennae of marine copepods. *Bulletin of Marine Science*, *53*(1), 170-179.

Lenz, P. H., Weatherby, T. M., Weber, W., & Wong, K. K. (1996). Sensory specialization along the first antenna of a calanoid copepod, *Pleuromamma xiphias* (Crustacea). *Marine & Freshwater Behaviour & Phy*, *27*(2-3), 213-221.

Litchman, E., Klausmeier, C. A., & Bossard, P. (2004). Phytoplankton nutrient competition under dynamic light regimes. *Limnology and Oceanography, 49*(4), 1457-1462.

Loeb, S. L. (1994). An ecological context for biological monitoring. *Biological monitoring of aquatic systems*, 3-7.

Long, B. M., Jones, G. J., & Orr, P. T. (2001). Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate. *Applied and Environmental Microbiology*, *67*(1), 278-283.

Lukač, M., & Aegerter, R. (1993). Influence of trace metals on growth and toxin production of *Microcystis aeruginosa*. *Toxicon, 31*(3), 293-305.

Lürling, M., & Beekman, W. (1999). Grazer-induced defenses in *Scenedesmus* (chlorococcales; chlorophyceae): Coenobium and spine formation. *Phycologia, 38*(5), 368-376.

Lürling, M. (2003). Daphnia growth on microcystin-producing and microcystin-free *Microcystis aeruginosa* in different mixtures with the green alga *Scenedesmus obliquus*. *Limnology and Oceanography, 48*(6), 2214-2220.

Lürling, M., & Van Donk, E. (2000). Grazer- induced colony formation in *Scenedesmus*: Are there costs to being colonial? *Oikos, 88*(1), 111-118.

Lynch, M. (1977). Zooplankton competition and plankton community structure. *Limnol.Oceanogr, 22*(4), 775-777.

Magalhaes, V. D., Marinho, M. M., Domingos, P., Oliveira, A. C., Costa, S. M., Azevedo, L. O., & Azevedo, S. M. F. O. (2003). Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ). *Toxicon, 42*(3), 289-295.

Mayeli, S., Nandini, S., & Sarma, S. (2005). The efficacy of *Scenedesmus* morphology as a defense mechanism against grazing by selected species of rotifers and cladocerans. *Aquatic Ecology, 38*(4), 515-524.

McCormick, F. H., Contreras, G. C., & Johnson, S. L. (2010). Effects of nonindigenous invasive species on water quality and quantity. *Dix, M.and Britton, K., Eds,* , 2009-2029.

McLaren, I. A. (1963). Effects of temperature on growth of zooplankton, and the adaptive value of vertical migration. *Journal of the Fisheries Board of Canada, 20*(3), 685-727.

Mclean, TI. & Sinclair, GA. (2013). *Environmental Toxicology*. New York: Springer Science

McNaught, D. C., Griesmer, D., & Kennedy, M. (1980). Resource characteristics modifying selective grazing by copepods. *Evolution and Ecology of Zooplankton Communities, ,* 292-298.

Meyer-Harms, B., Irigoien, X., Head, R., & Harris, R. (1999). Selective feeding on natural phytoplankton by *Calanus finmarchicus* before, during, and after the 1997 spring bloom in the Norwegian Sea. *Limnology and Oceanography, 44*(1), 154-165.

Mitsui, A., Kumazawa, S., Takahashi, A., Ikemoto, H., Cao, S., & Arai, T. (1986). Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. *Nature, 323*(6090), 720-722.

Müller-Navarra, D. C., M. T. Brett, A. M. Liston, and C. R. Goldman. 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature* 403:74–77.

Nawrocki, J., Biłozor, S., & Wydawnictwo Naukowe, P. (2000). *Uzdatnianie wody: Procesy chemiczne i biologiczne: Praca zbiorowa* Wydaw. Naukowe PWN.

Nikinmaa, M. (2002). Oxygen-dependent cellular functions—why fishes and their aquatic environment are a prime choice of study. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *133*(1), 1-16.

Olsvik, P. A., Kristensen, T., Waagbo, R., Tollefsen, K. E., Rosseland, B. O., & Toften, H. (2006). Effects of hypo- and hyperoxia on transcription levels of five stress genes and the glutathione system in liver of atlantic cod *Gadus morhua*. *The Journal of Experimental Biology, 209*(Pt 15), 2893-2901.

O'Reilly, C. M., Alin, S. R., Plisnier, P., Cohen, A. S., & McKee, B. A. (2003). Climate change decreases aquatic ecosystem productivity of Lake Tanganyika, Africa. *Nature, 424*(6950), 766-768.

ÖStilrlind, S. (1950). Inorganic carbon sources of green algae. I. growth experiments with *Scenedesmus quadricauda* and *Chlorella pyrenoidosa*. *Physiologia Plantarum, 3*(4), 353-360.

Paerl, H. W. (1996). A comparison of cyanobacterial bloom dynamics in freshwater, estuarine and marine environments. *Phycologia, 35*(6S), 25-35.

Paerl, H. W. (1996). A comparison of cyanobacterial bloom dynamics in freshwater, estuarine and marine environments. *Phycologia, 35*(6S), 25-35.

Paerl, H. W., Fulton, R. S.,3rd, Moisander, P. H., & Dyble, J. (2001). Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *Thescientificworldjournal, 1,* 76-113.

Paerl, H. W., & Huisman, J. (2008). Blooms like it hot. *SCIENCE-NEW YORK THEN WASHINGTON-, 320*(5872), 57.

Paerl, H. W., & Otten, T. G. (2013). Harmful cyanobacterial blooms: causes, consequences, and controls. *Microbial ecology*, *65*(4), 995-1010.

Paul, V. J. (2008). Global warming and cyanobacterial harmful algal blooms. *Cyanobacterial harmful algal blooms: State of the science and research needs* (pp. 239-257) Springer.

Petersen, R. (1982). Influence of copper and zinc on the growth of a freshwater alga, *Scenedesmus quadricauda*: the significance of chemical speciation. *Environmental Science & Technology*, *16*(8), 443-447.

Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the united states. *Ecological Economics*, *52*(3), 273-288.

Poff, N. L., Brinson, M. M., & Day, J. W. (2002). *Aquatic Ecosytems & Global Climate Change: Potential Impacts on Inland Freshwater and Coastal Wetland Ecosystems in the United States* (pp. 1-56). Arlington: Pew Center on Global Climate Change.

Porter, K. G., & Orcutt, J. D. (1980). Nutritional adequacy, manageability, and toxicity as factors that determine the food quality of green and blue-green algae for *Daphnia*. *Evolution and ecology of zooplankton communities. University Press of New England*, 268-281.

Lampert, W. (1987). Laboratory studies on zooplankton- cyanobacteria interactions. *New Zealand Journal of Marine and Freshwater Research*, *21*(3), 483-490.

Power, M. E., Stout, R. J., Cushing, C. E., Harper, P. P., Hauer, F. R., Matthews, W. J., ... & Wais De Badgen, I. R. (1988). Biotic and abiotic controls in river and stream communities. *Journal of the North American Benthological Society*, *7*(4), 456-479.

Qualls, T. M., Dolan, D. M., Reed, T., Zorn, M. E., & Kennedy, J. (2007). Analysis of the impacts of the zebra mussel, *Dreissena polymorpha*, on nutrients, water clarity, and the chlorophyll-phosphorus relationship in lower Green Bay. *Journal of Great Lakes Research*, *33*(3), 617-626.

Regier, H. A., Holmes, J. A., & Pauly, D. (1990). Influence of temperature changes on aquatic ecosystems: an interpretation of empirical data. *Transactions of the American Fisheries Society*, *119*(2), 374-389.

Lindeman, R. L. (1942). The trophic-dynamic aspect of ecology. *Ecology*, *23*(4), 399-417.

Rahel, F. J., & Olden, J. D. (2008). Assessing the effects of climate change on aquatic invasive species. *Conservation Biology*, *22*(3), 521-533.

Repka, S., van der Vlies, M., & Vijverberg, J. (1998). Food quality of detritus derived from the filamentous cyanobacterium *Oscillatoria limnetica* for *Daphnia galeata*. *Journal of plankton research*, *20*(11), 2199-2205.

Reynolds, C. S., Jaworski, G., Cmiech, H., & Leedale, G. (1981). On the annual cycle of the blue-green alga *Microcystis aeruginosa* kutz. emend. elenkin. *Philosophical Transactions of the Royal Society of London.B, Biological Sciences, 293*(1068), 419-477.

Rhee, G. (1973). a continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. 1. *Journal of Phycology, 9*(4), 495-506.

Richman, S., Bohon, S. A., & Robbins, S. E. (1980). Grazing interactions among freshwater calanoid copepods. In *Am. Sot. Limnol. Oceanogr. Spec. Symp* (Vol. 3, pp. 219-233).

Robertson, A. (1966). The distribution of calanoid copepods in the Great Lakes. In *Proc. 9th Conf. Great Lakes Res., Univ. Michigan, Great Lakes Res. Div., Pubi* (No. 15, pp. 129-139).

Rosemond, A. D., Mulholland, P. J., & Elwood, J. W. (1993). Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. *Ecology, 74*(4), 1264-1280.

Rothhaupt, K. (1990). Resource competition of herbivorous zooplankton: A review of approaches and perspectives. *Archiv Für Hydrobiologie, 118*(1), 1-29.

Sager, P. E., & Richman, S. (1991). Functional interaction of phytoplankton and zooplankton along the trophic gradient in Green Bay, Lake Michigan. *Canadian Journal of Fisheries and Aquatic Sciences, 48*(1), 116-122.

Saiz, E. (2009). Swimming dynamics of zooplankton.

Sakshaug, E., & Olsen, Y. (1986). Nutrient status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition. *Canadian Journal of Fisheries and Aquatic Sciences, 43*(2), 389-396.

Sargent, JR, Falk-Petersen, SF. (1981). Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: Lipids and fatty acids in *Meganyctiphanesnorvegia, Thysanoessa raschi,* and *T. inermis* during midwinter. Mar Biol 62:131–137.

Sarnelle, O., & Wilson, A. E. (2005). Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria. *Limnology and oceanography, 50*(5), 1565-1570.

Sellner, K. G., Doucette, G. J., & Kirkpatrick, G. J. (2003). Harmful algal blooms: Causes, impacts and detection. *Journal of Industrial Microbiology and Biotechnology, 30*(7), 383-406.

Shen, H., Niu, Y., Xie, P., Tao, M., & Yang, X. (2011). Morphological and physiological changes in *Microcystis aeruginosa* as a result of interactions with heterotrophic bacteria. *Freshwater Biology, 56*(6), 1065-1080.

Smith, V. H., Tilman, G. D., & Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental pollution*, *100*(1), 179-196.

Stearns, S. C. (1992). *The evolution of life histories* (Vol. 248). Oxford: Oxford University Press.

Steinman, A. D. (1996). Effects of grazers on freshwater benthic algae. *Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, California*, 341-373.

Sterner, R. W. D. 0. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual Review of Ecology and Systematics*, *25*, 1-29.

Stewart, A. J. 1987. Responses of stream algae to grazing minnows and nutrients: a field test for interactions. Oecologia (Berlin) 72:1–7.

Stoermer, E. (1978). Phytoplankton assemblages as indicators of water quality in the Laurentian Great Lakes. *Transactions of the American Microscopical Society*, 2-16.

Suikkanen, S., Engström-Öst, J., Jokela, J., Sivonen, K., & Viitasalo, M. (2006). Allelopathy of Baltic Sea cyanobacteria: no evidence for the role of nodularin. *Journal of plankton research*, *28*(6), 543-550.

Tessie, A. J., & Goulden, C. E. (1982). Estimating food limitation in cladoceran populations. *Limnol. Oceanogr*, *27*(4), 707-717.

Tessier, A. J., & Consolatti, N. L. (1991). Resource quantity and offspring quality in *Daphnia*. *Ecology*, 468-478.

Thomas, R., & Walsby, A. (1985). Buoyancy regulation in a strain of *Microcystis*. *Journal of General Microbiology, 131*(4), 799-809.

Tiselius, P. (1992). Behavior of *Acartia tonsa* in patchy food environments. *Limnology and Oceanography, 37*(8), 1640-1651.

Torke, B. (2001). The distribution of calanoid copepods in the plankton of Wisconsin lakes. *Hydrobiologia, 453*(1), 351-365.

Turner, R. E., & Rabalais, N. N. (2003). Linking landscape and water quality in the Mississippi River Basin for 200 years. *Bioscience*, *53*(6), 563-572.

Turner, J. T., & Tester, P. A. (1997). Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. *Limnology and Oceanography*, *42*(5), 1203-1214.

Vallentyne, J. R. (1974). The Algal Bowl—Lakes and Man. Miscellaneous Special Publication 22. Department of the Environment. *Fisheries and Marine Service, Ottawa, Canada*.

Van der Westhuizen, A., & Eloff, J. (1983). Effect of culture age and pH of culture medium on the growth and toxicity of the blue-green alga *Microcystis aeruginosa*. *Zeitschrift Für Pflanzenphysiologie, 110*(2), 157-163.

Van der Westhuizen, A., & Eloff, J. (1985). Effect of temperature and light on the toxicity and growth of the blue-green alga *Microcystis aeruginosa* (UV-006). *Planta, 163*(1), 55-59.

Van Donk, E., & Hessen, D. O. (1993). Grazing resistance in nutrient-stressed phytoplankton. *Oecologia, 93*(4), 508-511.

Van Noordwijk, A. J., & de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist*, 137-142.

Vargas, C. A., & Madin, L. P. (2004). Zooplankton feeding ecology: Clearance and ingestion rates of the salps *Thalia democratica*, *Cyclosalpa affinis, Salpa* cylindrica on naturally occurring particles in the mid-Atlantic bight. *Journal of Plankton Research, 26*(7), 827-833.

Vidal, J. (1980). Physioecology of zooplankton. I. effects of phytoplankton concentration, temperature, and body size on the growth rate of *Calanus pacificus* and *Pseudocalanus* sp. *Marine Biology, 56*(2), 111-134.

Walther, G., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J., et al. (2002). Ecological responses to recent climate change. *Nature, 416*(6879), 389-395.

Wang, Y., Zhao, J., Li, J., Li, S., Zhang, L., & Wu, M. (2011). Effects of calcium levels on colonial aggregation and buoyancy of *Microcystis aeruginosa*. *Current Microbiology, 62*(2), 679-683.

Ward, G., & Cummins, K. (1979). Effects of Food Quality on Growth of a Stream Detritivore, *Paratendipes albimanus* (Meigen) (Diptera: Chironomidae). *Ecology* Vol. 60, No. 1, pp. 57-64.

Webster.K.E and Peters.R.H. (1978) Some size-dependent inhibitions of larger cladoceran filters in filamentous suspensions. *Limnol. Oceanogr.*. 23.1238-1245.

White, A. W. (1981). Marine zooplankton can accumulate and retain dinoflagellate toxins and cause fish kills. *Limnology & Oceanography.* 26:103-109.

Wilson, A. E., Sarnelle, O., Neilan, B. A., Salmon, T. P., Gehringer, M. M., & Hay, M. E. (2005). Genetic variation of the bloom-forming cyanobacterium *Microcystis aeruginosa* within and among lakes: Implications for harmful algal blooms. *Applied and Environmental Microbiology, 71*(10), 6126-6133.

Winder, M., & Schindler, D. E. (2004). Climate change uncouples trophic interactions in an aquatic ecosystem. *Ecology, 85*(8), 2100-2106.

Winder, M., & Schindler, D. E. (2004). Climatic effects on the phenology of lake processes. *Global Change Biology*, *10*(11), 1844-1856.

Winkler, G., Dodson, J. J., & Lee, C. E. (2008). Heterogeneity within the native range: Population genetic analyses of sympatric invasive and noninvasive clades of the freshwater invading copepod *Eurytemora affinis*. *Molecular Ecology*, *17*(1), 415-430.

Winkler, G., Dodson, J. J., & Lee, C. E. (2008). Heterogeneity within the native range: Population genetic analyses of sympatric invasive and noninvasive clades of the freshwater invading copepod *Eurytemora affinis*. *Molecular Ecology*, *17*(1), 415-430.

Wrona, F. J., Prowse, T. D., Reist, J. D., Hobbie, J. E., Lévesque, L. M., & Vincent, W. F. (2006). Climate change effects on aquatic biota, ecosystem structure and function. *AMBIO: A Journal of the Human Environment, 35*(7), 359-369.

Xie, L., Xie, P., Guo, L., Li, L., Miyabara, Y., & Park, H. D. (2005). Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. *Environmental toxicology, 20*(3), 293-300.

Yuan, M., & Carmichael, W. W. (2004). Detection and analysis of the cyanobacterial peptide hepatotoxins microcystin and nodularin using SELDI-TOF mass spectrometry. *Toxicon, 44*(5), 561-570.

Zehnder, A., & Gorham, P. R. (1960). Factors influencing the growth of *Microcystis aeruginosa* kütz. emend. elenkin. *Canadian Journal of Microbiology, 6*(6), 645-660.