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Comparison of the Effects of Toxic Cyanobacteria on the Reproductive Success of Eurytemora affinis Populations in the Baltic Sea and Green Bay, Wisconsin

Amanda Dwyer
Lawrence University

Comparison of the Effects of Toxic Cyanobacteria on the Reproductive Success of *Eurytemora affinis* Populations in the Baltic Sea and Green Bay, Wisconsin

by Amanda Dwyer

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Introduction

Climate change is an important phenomenon currently altering various physical, chemical and biological systems across the globe. Aquatic systems are considered to have less separation between trophic levels as seen in terrestrial systems, as there are minimal physical boundaries (Kononen, 2001). This makes it difficult to determine not only specific changes in an area, but also the potential ramifications of these changes as there are few ways to actually control experiments in the field. Nonetheless, it is important to continue to develop as much knowledge as possible on these systems. While the progression for trophic-scale changes is generally a slow process, it is important to both distinguish a baseline to monitor present changes, and to develop predictions for the rate of future changes and their potential consequences. An important component of climate change is the increase in global temperature, which can lead to the alteration or elimination of a suitable environment for specific organisms (Winder & Schindler, 2004).

A specific example of an ecosystem change in an aquatic environment related to increasing temperature is cyanobacteria blooms. These blooms are the result of increased growth of cyanobacteria, a specific phylum of bacteria formerly classified as blue-green algae due to their blue-green pigment, phycocyanin (McLean & Sinclair, 2013). Good growth conditions for cyanobacteria result from increased nutrients and temperatures (Zurawell et al, 2005). Cyanobacteria become the dominant phytoplankton in prime bloom conditions, and therefore are the potential main food source for zooplankton. This coincides with the depletion of their natural food source, threatening zooplankton's survival. This threat sparks from the decreased nutritional value of cyanobacteria compared to a good algal food source. Additionally, the exposure to toxins produced by cyanobacteria can lead to decreased growth rates and reproductive success of zooplankton**.** The subsequent decrease, or disappearance, of other phytoplankton can potentially create a trophic cascade if zooplankton are not able to live and reproduce off of the less nutritional cyanobacteria. The lack of adequate prey can lead to a

zooplankton population decrease, which will limit the food for small fish and upward through the food chain (Casini et al, 2008). Therefore, it is important to continue to learn more about the underlying causes and relationships in ecosystems to best predict how to prevent a complete collapse of a functioning ecosystem.

Phytoplankton in Aquatic Ecosystems

Phytoplankton are the primary source of energy for the rest of the ecosystem since they are able to transform light energy from the sun into biologically useable energy, which is then passed throughout the ecosystem. In aquatic systems, the water movement allows for changes in specific conditions resulting in different phytoplankton species surviving in close proximity to each other. This co-existence of competing species is not common in most other natural environments, but often occurs as a 'patchy' distribution within a single aquatic ecosystem. This is largely due to a combination of physical factors affecting unique microhabitats, allowing for the coexistence of many populations in a small area. These physical factors include exposure to upwelling, convergence/divergence zones, changes in stratification and/or vertical migration, and nutrient inflows (Kononen et al, 1996 in Kononen, 2001).

Varying nutrient levels, a problem in many environments, actually creates the opportunity for species to coexist based on the different nutrient uptake abilities (Kononen & Leppänen, 1996 in Kononen, 2001). It has been seen that smaller phytoplankton often are able to successfully use nutrients at low concentrations while many other species would not be able to survive. Larger phytoplankton usually have higher uptake potentials, which gives them an advantage in areas with fluctuating nutrient concentrations. When there is a high input of nutrients to an area, certain types of phytoplankton quickly deplete the resource, providing an advantage to phytoplankton that are more efficient at using the resource at low concentrations. This situation will continue until an additional input of nutrients occurs (Capblancq J., 1990, in Kononen, 2001).

Two possible mechanisms exist in an ecosystem to control phytoplankton populations: top-down and bottom-up controls. These terms refer to mechanisms that affect survival and reproduction of phytoplankton and therefore limit energy flow through an ecosystem. Top-down controls are mechanisms in which the population is depleted, usually due to grazing by zooplankton (Buskey, 2008). The grazing on phytoplankton of zooplankton is dependent on a variety of issues. The feeding rates of zooplankton are dependent on the surrounding environment and the concentration of available food. This also depends on the specific characteristics of the available food source as many zooplankton are limited mechanically by the size or shape of the algae they graze on. Certain zooplankton are even able to sense whether or not the nearby food is beneficial in terms of nutritional quality and therefore can save energy by not wasting it on feeding on algae that will not result in a positive energy gain. Therefore top-down controls are variable within an ecosystem depending on the variability and mixture of the various zooplankton and phytoplankton coexisting.

When toxic algae blooms are introduced and become the dominant phytoplankton source, this often creates a large disruption in the ecosystem as many zooplankton will either shut down feeding ,or if they don't they will likely be affected by the toxins produced. A 'bottom-up' control refers to a factor that limits the growth potential of phytoplankton populations. These are usually physical or chemical factors that may result in a change of environment conditions for the species, or may affect the abundance or availability of necessary nutrient sources. For example, a few species, including *Nodularia* and *Microcystis,* are able to use gas vacuoles to change their buoyancy to be able to change their position in the water column (Zurawell et al, 2005). This opportunity for vertical migration allows a better opportunity to be in an environment of ideal PAR levels and CO_2 levels (Paerl et al 1985 in Zurawell et al, 2005).

Top-Down Controls

Many grazing experiments have been carried out in various environments to investigate the role of top-down controls in aquatic ecosystems. Studies have looked into the relationship between copepod grazers and cyanobacteria presence, showing some copepods are an important factor in controlling toxic cyanobacteria blooms while others completely avoid feeding in the presence of toxic algae (Turner and Tester, 1997 in Buskey, 2008). Additionally, phytoplankton have developed mechanisms to help protect themselves from grazing. Some of these are evolutionary traits such as increasing appendages, developing mucus or growing in colonies to increase feeding difficulty for grazers (Kononen, 2001). Additionally, cyanobacteria have the ability to produce toxins, which in some settings does act as a grazing inhibitor (Kierstead & Slobodkin, 1953 in Kononen, 2001). While a small amount of grazing pressure on phytoplankton results from protozoans, the main effects come from crustacean zooplankton.

Unfortunately, laboratory results cannot always be applied directly to field studies due to the complex interactions in nature that cannot be easily controlled. In laboratory situations, controls are set up to look at the specific effect of the research question, while holding other parameters constant. While this is beneficial to gain a greater understanding of specific dynamics, there are little occurrences in marine systems where any relationship can be completely isolated from other surrounding factors. Therefore, any laboratory experiment results need to be considered how changing a multitude of outside factors will affect the results.

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laboratory experiment results need to be considered how changing a multitude of outside factors will affect the results.

Bottom Up Controls

The growth of phytoplankton is largely based on numerous limiting factors. These can be categorized as either physiological or functional. The major physiological limitation is the capacity for uptake and intracellular storage of nutrients. Phosphorous can be stored more easily than nitrogen. Some phytoplankton species are able to store phosphorous, up to increasing their biomass by 70%, but very minimal concentrations of nitrogen (Kivi et al 1993). A species that is able to successfully store large amounts of phosphorus when it is available will likely be able to outcompete other species with a lower storage capability. Many cyanobacteria are nitrogen-fixers, so if they have a high storage capability of phosphorus, they will be more successful in many nitrogen limited environments, than phytoplankton that cannot fix nitrogen **(**Andersen et al, 1991 in Kivi et al, 1993).

Additionally, light can be a functional limitation. Phytoplankton need to stay in the photic zone to obtain light energy and conduct photosynthesis to survive, produce food for themselves, and provide energy throughout the ecosystem. However, being in the photic zone increases their vulnerability to grazing by zooplankton. They have little control over their movement which is mainly controlled through the movement of the water in their environment. Phytoplankton also may be subjected to sinking through the photic zone, and while this may be beneficial to avoid zooplankton, it is difficult to return to the shallower depths to receive energy for photosynthesis (Turner and Tester, 1997 in Buskey, 2008).

Favorable Bloom Conditions

In addition to specific factors that contribute to the success of a phytoplankton population surviving in a particular environment, there are also specific factors that lead to a cyanobacteria

bloom. During blooms the population of cyanobacteria grows and can out-compete other phytoplankton species. Increased nutrients and temperature are the major drivers of this situation, which is important, as these are both anthropogenic effects. The pH level of the environment is another driving factor for bloom conditions (Mogelhog, 2006). Cyanobacteria are able to tolerate much higher pH levels than other phytoplankton, allowing them to outcompete under these conditions. Additionally, low CO_2 availability along with low grazing rates allow for increased growth of cyanobacteria (Zurawell et al, 2005). Oxygen concentrations also play an important role in bloom production. The de-nitrification process can only occur in anoxic environments, or those that have very low concentrations of oxygen. This creates an environment which is nitrate limited and therefore an ideal environment for cyanobacteria to outcompete other species as they undergo nitrogen fixation (Karlson, 2005 in Karlson et al 2007). The level of mixing also contributes, as a bloom will be more likely to occur in a stable water column. While it is possible for blooms to occur in any location when the conditions are favorable, the most common location for blooms are in tropical or sub-tropical areas (Kotak et al 1995 in Zurawell et al, 2005)

The benthic macrofauna and organisms also play a role in creating an environment supporting cyanobacteria blooms. An increase in amphipods allows for increased mineralization which can increase nitrogen availability depending on the season. This leads to increased primary production based on the amount of organic material available for re-mineralization in the benthic community (Lehtonen, 1995 in Karlson et al, 2007). All of these factors are found to be true in both freshwater and marine environments (Sellner, 1997).

Cyanobacteria Interactions throughout an Ecosystem

One of the largest changes in an ecosystem experiencing a bloom is the shift in the phytoplankton community composition as the cyanobacteria become the dominant phytoplankton species. Additionally, this can pose a change to the system as some strains of

cyanobacteria produce toxins. This change could lead to potential negative effects for zooplankton and/or fish populations. Certain types of cyanobacteria are nitrogen fixers and are able to uptake naturally occurring 15N from the environment , which is an isotope that can easily be tracked through the food web and is labeled as $\delta^{15}N$**.** This specific form, found in pure cyanobacteria has been shown to move through the food web either by direct consumption of cyanobacteria or from exposure after being released from the cells into the environment (Kozlowsky-Suzuki et al, 2003).

The blooms also have a negative effect on the ecosystem when the cyanobacteria begin to die. The death of blooms is often hard to track its exact effect on the environment, because as the cells begin to die and release dissolved organic matter (DOM) into the water, marine microbes can quickly take it up in even very limited concentrations due to their large surface to volume ratio and small overall size. The decomposition of the dead algae by the bacteria leads to more hypoxic conditions for the environment due to the increased respiration. Additionally, the energy from the release of the dying blooms is slowly passed through the food chain by flagellates that may feed on cyanobacteria and microzooplankton which feed on these flagellates (Azam, 1983). The large mass of decomposing algae leads to a large decrease in oxygen and an increase in the concentration of ammonia. This creates poor environmental conditions for fish, leading to increased fish mortality (Paerl 1988, in Zurawell et al 2005). Additionally, dying cyanobacteria cells are more permeable. This allows intracellular toxins to be released into the environment at increased concentrations (Jones and Orr, 1994).

The relationship between phytoplankton and zooplankton also depends heavily on environmental conditions. Oligotrophic aquatic systems as well as high nutrient-low chlorophyll (HNLC) areas are environments that will often exhibit a well-balanced interaction between phytoplankton production and zooplankton grazing. However, temperate areas, especially around coasts, will be areas that have greater potential increases in phytoplankton abundance (Buskey, 2008). Fortunately, in addition to grazing by zooplankton and natural senescence,

blooms also can be eliminated partially through microbial degradation. The rates of this process strongly depend on the environmental history of the area and the previous exposure to blooms and optimal bloom conditions. Bacterial communities with high degradation rates for cyanobacteria are more likely to exist in areas that have previously been exposed to blooms for a longer period. In some cases, this degradation can happen in just over a week, a very beneficial event for an ecosystem experiencing a bloom (Christoffersen et al 2002, in Zurawell et al 2005).

Microcystis & Nodularia

Two closely related groups of cyanobacteria that produce toxins are *Microcystis* and *Nodularia*. *Microcystis* is a non-nitrogen fixing colonial cyanobacteria genus that produces the cyclic hepatopeptide toxin microcystin (Brittain et al, 2000). *Nodularia* is a nitrogen-fixing filamentous cyanobacteria producing the hepatotoxin nodularin, which is related to microcystin (Brittain et. al, 2000). However, both of these cyanobacteria also include non-toxic strains. Both microcystin and nodularin can be dangerous to other living organisms as they inhibit protein phosphatases (Ward et al, 1998 in Kozlowsky-Suzuki et al 2003). Previous studies have found both toxins are endotoxins, meaning the toxin continues to exist in the cell throughout its growth and is not normally excreted or released from the cell(Zurawell et al, 2005). Additionally, they are considered secondary metabolites because they are not created to benefit the algae directly in terms of cell division or growth (Carmichael, 1992 in Zurawell et al 2005). The main difference between these two toxins is in their chemical structure. While they are both cyclic hepatotoxins, they vary in their number of amino acids. The hydrophobic component, a specific amino group, of both nodularin and microcystin creates an important additional mechanism for transfer throughout the ecosystem (Yuan and Carmichael, 2004). When the dissolved toxin is in the water, if possible it will try to pull out and may attach itself to zooplankton. This therefore will not have the same effect as when the toxin is ingested and then is a part of the tissue, but it can

have effects by the physical interactions, as indicated in our negative effects from the filtrate treatments. Different strains of the toxins can be produced by the same cyanobacteria species, and these are categorized based on the specific amino acid groups of the molecules. Microcystin has seven amino acids and pairs 2&4 and 3&7 are the main determinant of the specific toxin strain (Zurawell et al, 2005; Figure 1). In comparison, nodularin only has five amino acids (Figure 2).

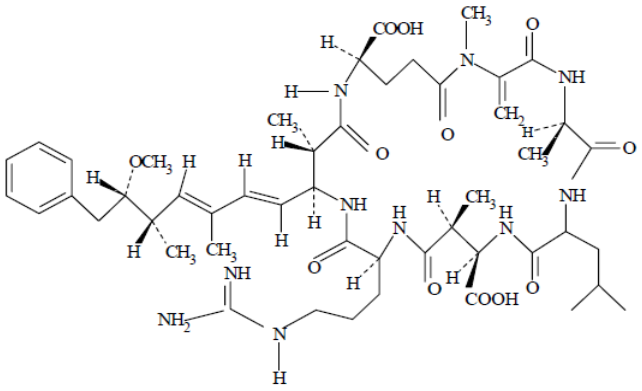


Fig. 1. Structural pattern of microcystin -LR produced by *Microcystis aeruginosa* (according to J. Nawrocki *et al.*, 2000)

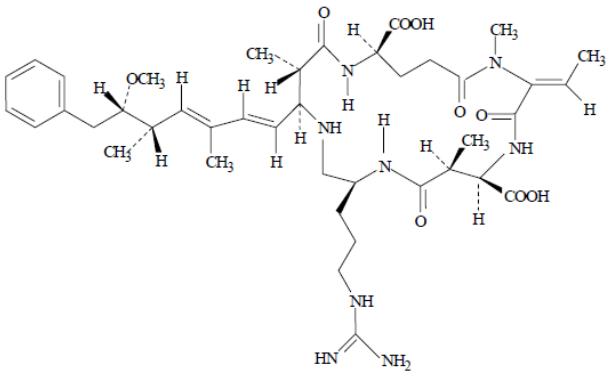


Fig. 2. Structural pattern of nodularin produced by *Nodularia spumigena* (according to Nawrocki *et al.*, 2000)

The risks associated with the amount of toxin production in a natural area are difficult to predict because it is hard to know the combination of toxin producing cells and non-toxin producing cells in a specific environment. Additionally, the strength and abundance of these toxins produced can vary between strains. The production of these toxins is currently under investigation, both in terms of what regulates the production in specific strains as well as the overall effects of various organisms in the ecosystem. Based on recent studies, there are

currently thought to be three main levels of microcystin product regulation. The first is the genetic level, which looks into the toxicity of individual strains (Zurawell et al 2005). The second is the cellular level, which indicates that environmental factors play a role in regulating toxin production. The range of toxin production at this level has not exceeded a difference of ten-fold (Sivonen and Jones 1999 in Zurawell et al 2005). Finally, at the population, level the toxicity is considered in terms of both toxic and nontoxic strains in a cyanobacteria bloom community. At this level, it is possible for the toxicity of various strains to differ by 1000-fold (Zurawell et al, 2005).

Various environmental factors play an important role in toxin production. A positive relationship has been found between cell growth rate and microcystin production rate. However, in non-limiting environmental conditions, non-toxin producing strains of *Microcystis* have better conditions. This suggests there is a possibility that the toxic strains also produce metabolites that inhibit the non-toxic strains around them, which are more vulnerable in limited conditions. (Briand et. al, 2008)

The pH of the environment also affects toxin production. In a pH range of 6-9, there is a limitation of the toxin's ability to diffuse into the water (de Maagd et al 1999, in Zurawell et al, 2005). Water temperature and light intensity both play a role in toxin production, but the actual relationship is specific to individual species and sometimes will differ between individual strains of the same species (Zurawell et al 2005). Additionally, cyanobacteria strains that produce toxins appear to have a higher requirement of nitrogen and phosphorous. This is likely in due to the need for extra energy to be able to undergo the synthesis of toxin production (Vezie et al 2002, in Zurawell et al 2005). Field experiments on toxin producing cyanobacteria have indicated three important factors that affect the magnitude of toxin production, and its effect on the ecosystem. The first is the composition and diversity of the phytoplankton in the area, especially considering the biomass that accounts for toxin producing cyanobacteria species. The second is the change in presence of distinct toxic and nontoxic strains. The final factor is

the combination of environmental variable effects that contribute to toxin production mentioned earlier (Carmichael & Gorham 1981 in Zurawell et al 2005).

Background of Phytoplankton in Green Bay

The dynamics of phytoplankton abundance and composition of species has changed over the past few decades. This is partly due to increasing temperatures, but is thought to be more strongly related to the zebra mussel invasion in 1992 (Qualls et al, 2007). The phytoplankton composition before the invasion included few cyanobacteria and was dominated by diatoms. During the summer, from June to September, the cyanobacteria become the dominant phytoplankton. Prior to the invasion by zebra mussels the main species of cyanobacteria were *Aphanizomenon, Oscillatoria, Anabaena,* and *Microcystis.* During the fall, the composition typically returned to diatoms (Sager et al, 1991). After the invasion, the phytoplankton community changed. There was an overall decrease in the bio-volume of Chlorophyta and a large increase in cyanobacteria dominance, especially *Microcystis*. (De Stasio et al 2008,2010).

Background of Phytoplankton in Northern Baltic

In the Northern Baltic, various monitoring studies have begun assessing the phytoplankton composition throughout the year. Kuosa & Kivi (1989), documented that the spring blooms may start underneath ice cover in early spring, with this study marking the spring season starting in early April based off of primary productivity peaks and thermocline dynamics. It is common, and now more or less expected, for late summer cyanobacterial blooms to be dominated by *Aphanizomenon* and *Nodularia spumigena* (Kononen 2001). *Nodularia* became more common after the 1960s (Poutanen & Nikkila, 2001). However, geological records indicate that cyanobacteria blooms have been a common occurrence in the Baltic Sea for the past 7,000 years (Bianchi, 2000). This has created a hypothesis that certain species may have been able

to adapt to the consequences of these blooms (Reinikainen et al, 2002). These two algae are able to co-exist due to the fact that they have different optimal temperatures and phosphorous uptake abilities, different functional needs which allow the populations to occur together as previously mentioned. *Aphanizomenon* does well in an environment with changing nutrient levels whereas *Nodularia spumigena* blooms are less dependent on nutrient availability, but rather need to be in a shallow, upper mixed layer of the water column. This is a warm environment due to heat from solar radiation, which increases the subsurface temperatures. *Nodularia* also benefits from calm weather conditions that reduce vertical mixing of water(Ostenfield, 1931 in Finni et al, 2001).

Zooplankton in Aquatic Ecosystems

Zooplankton help transfer energy, produced by phytoplankton through the food chain, which is vital for ecosystem function. The growth rate of zooplankton is thought to rely on both temperature limitation as well as food availability. The food availability constraint for marine zooplankton plays a larger role in open ocean environments rather than coastal, but each environment has a ‘’critical concentration’ level, where the growth rate remains constant when food concentrations exceed this level (Huntley & Boyd, 1984). This also will lead to a decrease in the clearance rate of copepods when there is this abundance of food. The grazing of zooplankton on toxic algae varies among populations due to previous exposure. There has been evidence that toxic algae does reduce growth and reproductive success in various zooplankton groups, including copepods, but it is difficult to accurately distinguish if this is specifically due to the toxins. This is an important area for further investigation because if certain copepods are able to select between toxic and non-toxic strains of algae, this could actually result in a stimulation of the bloom. However, if copepods are only eliminating non-toxic strains, this may strengthen toxic strains as they will have more nutrients available to them in addition to decreased copepod grazing (Zurawell et al, 2005).

Another possibility is that other metabolites produced in addition to the toxins or the lower nutritional value of the cyanobacteria causes a decreased growth and reproductive success of zooplankton (Colin and Dam, 2002; in Buskey, 2008). Additionally, over time zooplankton are able to develop their own defenses to overcome those of the phytoplankton, allowing them to continue successful grazing. This dynamic is typically termed an “evolutionary arms race”, where each species develops new traits to outcompete its competitor. If certain zooplankton populations have increased exposure to toxic algae over time, this may lead to the development of a resistance to the toxin while species with less exposure are not able to develop this resistance (Colin and Dam, 2003). Furthermore, the cyanobacteria may then be able to create stronger toxins to overcome the new defenses of the copepod as this cycle continues. This “evolutionary arms race” depends specifically on the direct relationship between copepods in a specific area. This means various populations of the same species may diverge over time and be classified as unique groups.

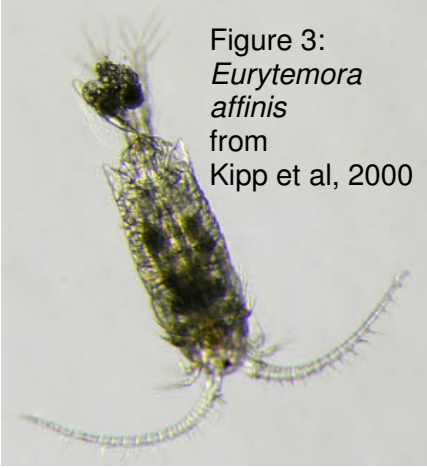


Figure 3: *Eurytemora affinis* from Kipp et al, 2000

Calanoid copepods have a unique and more selective feeding strategy than other types of zooplankton which results in a generally lower overall ingestion rate compared to other zooplankton of a similar size. However, the food selected for ingestion is typically of higher nutrition and energy is not wasted on food with lower nutritional value. Additionally, this feeding method is beneficial because it works in conditions with both high and low food concentrations. Therefore, it has been found that calanoid copepods become the dominant zooplankton species in systems that have a high abundance of low quality food or that have either extremely high or low food abundance (Richman & Dodson, 1983).

Background of the Calanoid Copepod *Eurytemora affinis*

The defining characteristics of *Eurytemora affinis* are the extended caudal rami with five caudle setae that are three times longer than their width. The fifth setae is slightly more separate and higher along the rami in comparison to the distance between the remaining setae (Figure 3). Average body length for this species is 1.20-1.26mm. *Eurytemora affinis* can be separated by gender quite easily as the females possess metasomal wings (Kipp et al, 2013). In a laboratory culture, it was observed that the life cycle reaches adulthood in not more than a month at a temperature of 25℃. During this period, it transforms through six naupliar stages and five copepodite stages (Czaika 1982; Williamson 1991; Souissi & Ban 2001 in Kipp et al, 2013).

Eurytemora affinis has adapted to many different environments. This versatility allows *E. affinis* to be commonly found in coastal areas as well as estuaries and marshes. Their typical habitat is variable throughout the year and even seasonally in terms of salinity and temperature. These fluctuations result in many changes in the ecosystem characteristics in terms of abundance of various other species. (Lee & Frost, 2002) This ability for local adaptation has also led to differentiation within *E. affinis* over time. For example, before the year 2000, there were thought to be six divergent clades of the calanoid copepod *E. affinis*. There were four found in America and one in Europe and one in Asia (Lee, 2000). There has been less investigation on the European clade to determine possible divergence in populations from different geographic areas. This research is important before considering other factors about *E. affinis* in western Europe, because one should know the breadth of applying the findings (Winkler et al, 2011).

More research was done over the past decade to discover that the American clades were very different from each other. This was based on genetic divergence, life history traits and salinity tolerance (Beyrend-Dur et al 2009 in Winkler et al, 2011). The interesting findings on the

divergence of the American clades are that they appear to be divergent enough to be reproductively isolated although their population distributions still overlap. This is likely due to speciation events occurring before the recent distributions of the various populations became more established (Lee, 2000). The patchiness existing in a single ecosystem may be responsible for this overlap in populations that have diverged enough to be reproductively independent. Additionally, the developmental success and survivorship also are dependent on the microhabitats in terms of salinity concentrations and food availability (Dodson et al 2010 in Winkler et al, 2011). However, it is common for individuals to have a wide tolerance range for varying salinity overall. This is clear as this species expanded from high salinity habitats in the marine environments and invaded freshwater habitats of the Laurentian Great Lakes. This being said, *E. affinis* does favor the more brackish environments associated with the coast rather than offshore areas with larger plankton communities. In oligotrophic systems, calanoid copepods are found to be the dominant zooplankton species (McNaught 1975 in Richman and Dodson, 1983).

The specific effect of cyanobacteria on the feeding of *E. affinis* has been shown to vary in different studies. The exposure to *Nodularia spumigena* initially indicated poor grazing conditions for the copepod (Sellner, et al 1996). However, recent studies show that *E. affinis* do actively and successfully feed on *N. spumigena* (Kozlowsky-Suzuki et al 2003; Koski et al 2002). Overall, it appears that calanoids in general show increased feeding in high concentrations of cyanobacteria (Koski et al 2002). This increased feeding on the harmful cyanobacteria is thought to be a form of compensation for the lower food quality of cyanobacteria in comparison to the other phytoplankton (Kozlowksky-Suzuki et al 2003).

The lower nutritional quality of cyanobacteria, compared to other phytoplankton, is an important factor to examine because *E. affinis* can successfully feed on cyanobacteria when exposed to high concentrations. This lower nutritional value may be having adverse effects on the copepods besides affecting mortality, such as decreased growth rates or female egg

production rates. Previous studies have shown that the number of eggs produced by *E. affinis* females did not differ between those exposed to *N. spumigena* and water without cyanobacteria. Mating cues for *E. affinis* are olfactory, as they do not have an eye that allows them to see images (Katona, 1973 in Lee & Frost, 2002). Therefore, when tracking sexual selection between various populations, it is important to look at the evolution of chemical signals produced. Additionally, *E. affinis* are able to produce diapausing eggs. This could result in a hatching event in good environmental conditions of multiple evolutionary stages of *E. affinis* in one area (Ellner & Hairston, 1994 in Lee & Frost 2002).

E. affinis in Lake Michigan, Wisconsin

The introduction of *E. affinis* to North American freshwater systems coincides with a trend of multiple species increasing their ability to adapt to the large salinity gradient during the movement from marine or brackish waters (Lee and Bell 1999 in Lee 1999). *Eurytemora affinis* was first documented in North America in 1880. Over the past 40 years, it has been found in Green Bay in both littoral areas as well as within the offshore plankton communities. Within the past 30 years it has commonly been found in Lake Michigan in coastal regions, especially near Indiana. It spread quickly to be a common species in all of the Laurentian Great Lakes. Its first appearance in Lake Michigan was documented by Robertson in 1966. It is commonly found during late summer through fall, approximately July through November. It is difficult to find during the winter and spring months (Kipp et al, 2013; Torke, 2001).

Eurytemora affinis in Northern Baltic

Investigation into the European populations of *E. affinis* is an on-going process. Since these populations were native species and already established in their habitat in Europe, there was less initial investigation on these organisms (Lee, 1999). Additionally, there are fewer calanoid copepods species in this area. The only other main copepod is *Acartia.* These two

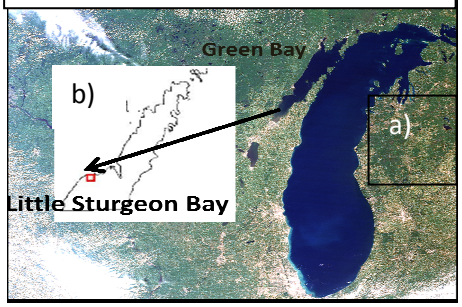
species have begun to be studied heavily to investigate the effects of cyanobacteria blooms on copepods (Engström et al, 2000). There have been three distinct lineages found for European *E. affinis,* all associated with a specific geographic region (Winkler, 2011). This study estimates the divergence occurring around 1.9 million years ago.

Study Environments

Green Bay/Lake Michigan

Green Bay is a large body of water, stretching 191.5 kilometers long with an average width of 37 kilometers. The complete watershed equals approximately one third of the area of Lake Michigan at 40,000 square kilometers. The southern end has a depth that is less than 10 meters on average, leading to stratification in the summer season, which is not typical for most larger bodies of water (Bertrand et al, 1976 in Qualls et al., 2007). As a result there is no persistent stratification in the lower bay region (Qualls et al., 2007). The depth increases to the North up the bay. This increase in depth prevents the entire water column from successfully mixing during summer months, which results in stratification and a decrease in algal blooms compared to the southern region (Bertrand et al, 1976 in Qualls et al, 2007). Southern Green Bay also is directly exposed to an increased flow of nutrients coming in from the lower Fox River. While this loading of nutrients has been decreased due to law enforcement causing businesses to decrease their waste production, the nutrients remaining in the system are able to be mixed, resulting in considerable bloom productivity throughout the summer months. These mixing conditions allow for greater variability across seasons, as well as among years, based on the nutrient flux dynamics. This is in contrast to systems that stratify and have a more consistent exposure to nutrients (Stoermer, 1978). The northern part of Green Bay is found to be more meso-

Figure 4. a) Satellite Image of Green Bay & Lake Michigan
b) Little Sturgeon Bay Map



oligotrophic, compared to the hyper-eutrophic southern region (Sager, 1991). Little Sturgeon Bay is the specific area of the current study, and is located in the southeastern part of Green Bay.

The Laurentian Great Lakes have been exposed to the zebra mussel invasion, which has caused serious changes in ecosystem dynamics. Documentation of the zebra mussel invasion began in Lake Michigan in 1992. Prior to the invasion, this system had a strong trophic gradient in the lower portion of Green Bay as a result of the nutrient inputs coming from the Fox River. A study monitoring the system for four years after the invasion reports an increase in phytoplankton and Chlorophyll a concentration (De Stasio et al 2008, 2010). This is the opposite trend compared to that experienced by many other systems experiencing zebra mussel invasions, which instead have increased water clarity due to decreased phytoplankton (Barbiero & Tuchman 2004; Idrisi et al 2001 in DeStasio et al 2008). More important than a simple increase in phytoplankton was the large shift in the composition of various phytoplankton species. The Chlorphyta populations decreased while the cyanobacteria and diatom populations increased, culminating in heavy blooms. The presence of zebra mussels could potentially be contributing to a higher recycling rate of nutrients, which would increase the phytoplankton population (Arnott & Vanni 1996 in DeStasio et al 2008).

This increase in phytoplankton populations is associated with the observed decrease in zooplankton abundance as well. Chlorophyta are a good food source for both zebra mussels and zooplankton. The decrease in Chlorophyta is likely attributed to the increased predation from the zebra mussels, and results in overall decreased grazing by zooplankton on the less nutritional, but more abundant phytoplankton concentrations. Increased fish feeding also could be a source of the decreased zooplankton population (DeStasio et al 2008).

Northern Baltic

The Northern Baltic, including the area around the Gulf of Finland, has some unique characteristics separating it from that of the Baltic Proper. In terms of chemical characteristics, it is shown to have generally higher de-nitrification rates and few, if any, macrofauna in the benthic environment. The de-nitrification rates are variable and may shift in relation to other parts of the Baltic throughout the year based on other seasonal changes (Karlson et al 2007). The chemical composition of the Northern Baltic is predominantly nitrogen limiting during the productive season (spring and late summer), which allows blooms to occur with nitrogen-fixing *Aphanizonmenon* and *Nodularia.* In between these two seasons, in the early summer, the phytoplankton growth conditions are limited by both nitrogen and phosphorous. The transition into early fall switches over to a more physical limitation based on light and/or temperature, which results in the depletion of the blooms (Kivi et al 1993).

Figure 5. Satelite Image of Tvarminne Zoological Station, Hangö, Finland
(A) Baltic Sea Study Site

The physical characteristics of the Northern Sea also distinguish this as a unique environment. There is a steep environmental gradient in this area (HELCOM, 2006 in Karlson et al 2007). The basin is semi-enclosed and therefore a non-tidal environment. This creates a system with a water renewal turnover period of several decades. This results in an environment of lower disturbance than commonly found in larger bodies of water due to the lack of wave disturbance or the shift of high and low tide exposures. The Intermediate Disturbance Hypothesis states that the highest diversity occurs in an environment that has an intermediate level of disturbance. Therefore, an environment with lower disturbance is expected to have slightly lower diversity. The disturbance found in the Northern Baltic comes from strong winds, which are often associated with strong storms. These occur a few times per week, much less than on a tidal daily basis (Sommer, U., 1995, in Kononen 2001). However, in some ways the

lack of disturbance is beneficial for this ecosystem as it is still considered to be recovering from the latest glaciation event.

The physical make-up of the Northern Baltic also creates unique dissolved oxygen, temperature and salinity concentrations. There are large areas of hypoxia and anoxia in this area, allowing for the conditions to occur that support large cyanobacteria blooms. Eutrophication is a term used to indicate the introduction of increased nutrients, particularly nitrogen and phosphorous in an aquatic system (McLean & Sinclair, 2013). The eutrophication leads to creating an anoxic environment and the increased nutrients decrease the de-nitrification process. The lack of oxygen in the water also allows more phosphorus to be released from the sediment into the environment, therefore reducing both nitrogen and phosphorous limitation (Kuparinen & Tuominen, 2001 in Karlson et al, 2007).

There is limited vertical mixing due to the lack of water movement, which creates horizontal gradients of salinity and temperature. The water movement that does occur is based mostly out of the Danish Straights. However, this input and mixture is highly variable in amount and in regularity. When this water movement does occur, it allows for more vertical mixing, which increases oxygen in deeper areas, therefore creating a change in the overall dynamics and conditions for blooms. Additionally, such water movements will often decrease the salinity stratification, which is another important component for the growth and sustainability of phytoplankton, as well as for zooplankton (HELCOM, 2002 in Karlson et al 2007).

In the Baltic Sea, previous studies have shown that the main limitation for ecosystem energy transfer is through bottom-up controls. This was shown by field experiments in mesocosms, where the removal of meta-zooplankton resulted in little observed differences. This indicates that grazing may not be controlling phytoplankton production. However, it was noted that while the large grazers were removed, the protozoans may have had an impact on decreasing the phytoplankton population. During the early spring, it was clear that little bottom-up (i.e. nutrient limitation) and top-down controls (i.e. grazing control) were present, allowing for

bloom conditions to occur. During this period there are lots of nutrients and low grazing although the main limiting factor that would have any control over the bloom would be light (Kononen 2001).

Cyanobacteria blooms have occurred in the Northern Baltic for centuries. However, there was still considerably less *Nodularia* present in the early 20th century than seen today. An important part of this difference is the human impact has allowed for warming to occur, allowing open ocean to become sources of blooms in addition to just coastal areas as was documented in earlier years (Finni et al, 2001). These various factors continue to occur leading to the yearly expectation of summer cyanobacteria blooms, whereas although there are record of blooms throughout the century, they were not a consistent occurrence as has become the case over the past few decades (Poutanen and Nikkilä, 2001). Presently, numerous studies are investigating the amount of responsibility humans have had for the increase in these cyanobacteria blooms.

Importance and Purpose of Study

The two environments addressed in this study are similar in their ability to be sources for large cyanobacteria blooms. It is still unclear what the effects of blooms will be, and it is important to continue analyzing work in the field and in the laboratory to reach reasonable estimates of the future changes coming to these environments. The Baltic Sea and Green Bay area of Lake Michigan are both similar as they have experienced extremely large cyanobacteria blooms that have become a regular part of their ecosystem in a very short amount of time. These areas are also more sheltered areas, one of the possible explanations for why the blooms do so well in these locations. While it is unfortunate for these ecosystems to be experiencing these blooms, it does create an opportunity for an important study. Previous studies have investigated the evolutionary divergence of *E. affinis* from Europe and Asia to North America (Lee, 2000). However, few other studies have been done, to see if these evolutionary and habitat changes have significantly affected the major components of *E. affinis*

fitness. The European populations of *E.* affinis have been used to gain a better understanding of the ecosystem dynamics and changes that may be occurring. These studies also document the environment and interactions in order to have a better understanding of changes that may be present in the very near future.

Previous research has shown various effects of feeding by the Baltic *E. affinis* population when exposed to *N. spumigena.* The overall conclusion is that they are able to feed on this algae when it is mixed in with an algae considered to be a good food source. This is advantageous as numerous other copepods in the area cease feeding completely when exposed to *N. spumigena* no matter what other food is available. The opportunity for *E. affinis* to continue feeding on the toxic algae is an important discovery in two ways. The first is that it likely won't starve as long as there is some mixture of phytoplankton composition in its habitat. Second is that *E. affinis* can continue to actively graze during a bloom to help prevent an even larger bloom from forming. *Eurytemora affinis* show decreased egg production when exposed to a diet consisting of toxic cyanobacteria (Karjalainen et al, 2007). These studies indicate the need to investigate the long-term effects of exposing *E. affinis* to the toxin producing and lower nutritional value algae as well as exposure to extracellular toxins. If the adults are able to survive, but have reduced reproductive success, the cyanobacteria are still having a large negative impact on *E. affinis* fitness.

This study investigates the effects that intracellular and extracellular toxins produced by the natural cyanobacteria have on two separate *E. affinis* populations; one in Green Bay, Lake Michigan and the other in the Northern Baltic in the Gulf of Finland. This work will help to increase knowledge of how the toxin is specifically interacting with the copepods and give greater insight on their direct effect. More specifically the aim is to see if it is consumption or simply exposure to toxins that have a negative effect on the survivorship, grazing rates and egg production by the two *E. affinis* populations. The ability to conduct the same laboratory study on the two separate populations, based on the toxin produced by natural cyanobacteria in their

food supply, allows greater insight as to how much divergence has actually occurred after centuries of separation. It is expected that feeding and egg production will be comparable between the two populations. There is an expected difference in egg production when intracellular and extracellular toxins are delivered by exposure rather than ingestion.

Methods

Baltic Experiments

Friday August 3, 2012 at 10:30am, 80L of water were collected at five meters depth in the Baltic Sea at the Tvärminne Zoological Station in Hangö, Finland, using a Limnos water bottle sampler. The water at the collection station and depth was 15℃ with a pH of 8.06, dissolved oxygen of 9.33mg/L and 106.5% saturation of oxygen and a salinity of 5.7. Four vertical zooplankton tows with 200µm mesh to a depth of 25 meters were also taken at that time and location. Five treatment solutions were made from lab cultures of *Nodularia spumigena (*strain AV1, provided by Prof. Sivonen, University of Helsinki, Finland) and *Rhodomonas sp.* (strain 07B6, provided by Dr. Anke Kremp, Finnish Environment Institute). The pH of the *Rhodomonas* lab culture was 9.25 and the pH of the *Nodularia* lab culture was 10.21. The salinity of the nutrient broth was 9.8, so 200mL of Milli-Q water was added to 300mL of the culture medium to correct the salinity of the Z8 salt nutrient broth used to culture the culture of *Nodularia*. Due to a limited volume of cultured algae, 500 µgC/L was determined to be the minimum concentration of food in each treatment to ensure enough food for survival. The treatments were: a) Nod50 (250µgC/L *Rhodomonas, 2*50µgC/L *Nodularia*), R+ N *(*500µgC/L *Rhodomonas* 100µgC/L *Nodularia),* R+Filt (500µgC/L *Rhodomonas,* filtrate at a volume that would have provided 100µgC/L of *Nodularia*), Rhod (500µgC/L), and R+Nut (500µgC/L *Rhodomonas,* volume of medium that would have provided 100µgC/L of*Nodularia* from the nutrient broth of the *Nodularia* lab culture. This treatment served as a secondary control to ensure the nutrient broth used to culture the *Nodularia* was not having an effect on the copepods that could otherwise be mistaken as an effect of the toxic algae. Except for the high

concentration of *Nodularia* in the Nod50 treatment the baseline 500µgC/L of *Rhodomonas* was kept constant to eliminate variation of lower food quality from the cyanobacteria rather than the effect of the toxin. The calculations to add the correct volume of algae to add to the treatments were developed through cell counts of each algae culture. Each treatment had five experimental replicates with *E affinis,* and five control replicates without animals, carried out in 1.2L glass flasks with a screw cap. Replicates were incubated in a climate chamber kept at 17℃. The replicates with *E. affinis* contained 12 females and three males.

Immediately after collection, the field sample was filtered (0.2µm pore size, Sartobran 300 filters; Sartorius Stedim Biotech GmbH. Göttingen, Germany) in the laboratory and put into the treatment bottles. This eliminated any bacteria or other small particles that came with the field water that could have been an additional food source. After filtration, the pH rose to 8.09, 9.35 mg/L of dissolved oxygen and 106.6% saturation of oxygen, whereas the salinity did not change. During this time, the zooplankton tow sample was sorted and 12 female *E. affinis* with egg sacs were placed into the filtered sea water in each treatment bottle. In the evening of Aug 3, three additional zooplankton tows were collected to ensure enough female copepods were obtained to have 12 females with eggs in each treatment bottle. At 8pm, the correct algal concentrations were added to treatment bottles containing the filtered water and female copepods with egg sacs. The females were left to acclimate for 60 hours.

On Mon. Aug 6, another collection was made for water and zooplankton following the same procedure. Again, immediately after collection the water was filtered in the laboratory and the zooplankton were sorted, this time for male *E. affinis.* Meanwhile, each treatment bottle was filtered and fresh treatment solutions were made. Females that had not yet dropped their egg sacs that had been produced in the field before collection were placed into a small beaker of the same treatment water to be used as replicates later if needed. The number of dead females was recorded. Females surviving acclimation and that had dropped their egg sacs from the field were placed along with three males into a treatment bottle containing a fresh solution of the

same treatment to which they were previously exposed. The time the copepods were added to the treatment bottle was recorded, marking the beginning of the grazing experiment (high mortality in the Nod 50 treatment led to the exclusion of the grazing experiment in this treatment only and surviving females without egg sacs were placed into a well with an individual male). Initial samples were collected before addition of the copepods to be used as starting samples for the grazing experiment. Samples (20mL for Chlorophyll *a*; 1mL extracellular toxins) were collected each for Chlorophyll *a*, cell counts and toxin analysis (ELISA). Similar samples were taken also after 24 & 48 hours. After 48 hours, the grazing experiment was complete and the containers were filtered to check for survivorship and number of females carrying egg sacs.

Females without an egg sac at the end of the grazing experiment were placed in a beaker of the same treatment solution with the females that still had egg sacs from the field after acclimation and with males from the grazing experiment. Females with egg sacs after the completion of the grazing experiment were moved into individual wells of 12 well-plates in the same treatment solution. There was one tray for each replicate treatment bottle. Once all of the egg sacs hatched from one tray, acid Lugol's solution was added to each well to preserve the nauplii for later counting under a dissecting microscope and measurement with an inverted microscope. Ten nauplii from each treatment were measured under an inverted microscope at 10x magnification to assess size of offspring produced.

Green Bay Experiments

This experiment was conducted on a smaller scale, with modifications based on the results of the Baltic Sea experiments. The algal cultures used in these experiments were new strains of the Chlorophyta alga *Scendesmus quadracauta* begun in August 2012 and the *Microcystis aeruginosa* strain PCC 7820. The nutrient addition treatment was excluded from this experiment based on the results of the Baltic Sea study, and only one treatment including a combination of toxic algae and good algae was employed. A preliminary survivorship

experiment was run to determine the percentage of *Microcystis* to use in the single *Microcystis* and *Scenedesmus* treatments.

In the initial survivorship experiment the good food source treatment (*Scenedesmus)* was kept at 500µgC/L, with treatments consisting of the addition of 50%, 20%, 10% and 0% of *Microcystis.* The treatment solutions were made up with artificial pond water, which had been used for previous experiments to eliminate any possible background cyanobacteria toxin presence in the solutions. These experiments were run in the 12-well tissue culture plates. Each well was checked once a day and the number of copepods alive and dead were recorded. High mortality seen within the first 24 hours resulted in creating new treatments using filtered aged tap water, instead of artificial pond water. The aged tap water was kept in the aquarium room at Lawrence University and then was filtered under low vacuum pump and Millepore filters (0.45µm pore size)**.** The survivorship experiment was restarted with these solutions on Friday September 15.

On Wednesday September 12, 2012, three zooplankton tows were done using a net with 73mm mesh by walking alongside the dock at the Little Sturgeon Bay municipal boat ramp. This was one of the few locations searched in the Green Bay area to have a large enough population of *E. affinis* to provide sufficient animals for the experiments. On Sunday September 17, 12 tows were conducted in the same manner on the inside of the Little Sturgeon Bay dock. The surface temperature of the water was 20℃. Sorting for female copepods began that evening and eight females with egg sacs were placed in individual wells of 12-well trays for each treatment. Sorting for female *E. affinis* continued the next day. These individuals were left to acclimate until September 20th. Fresh treatment solutions were created and added to 500mL beakers as the experimental containers. Initial samples were taken in the same manner as during the Baltic Sea experiment and used as the starting samples for the grazing rate experiment. The grazing experiment began at 1130a.m. There were 19 (6,6,7) females added to the S+M10%, 22 (6,8,8) to the MF and 23 (8,8,7) to S100% treatment. Due to poor

survivorship during the acclimation period, only one male (instead of three) was added to each beaker during this experiment. The grazing experiment followed the same protocol as the Baltic Sea experiment with samples taken at 24 hours. On September 22, the beakers were filtered to check for survivorship and number of females that produced egg sacs. The first treatment had 48hr samples taken but unfortunately not many females produced egg sacs, so at 6pm 4 more tows were done at Little Sturgeon Bay dock and one more male was added to each beaker at 6am on September 23rd. Additional females were sorted from those tows and kept in a beaker of filtered aged tap water with *Scenedesmus* as a food source. The next day each beaker was sorted and counted for survivorship and females with egg sacs. There were only enough surviving females with egg sacs to have one tray per treatment for the hatching part of the experiment. Each following day, the trays were checked and it was recorded which copepods were alive or dead. Few females were staying alive let alone producing eggs so we decided to separate the grazing experiment from the reproduction experiment.

On October 6, new treatments were created. The *Scenedesmus* culture was counted again and the cells were measured, and it was discovered to have a large difference from the measurements done during the summer. As a result, our calculations for determining the amount of culture to add to achieve 500µgC/L were inaccurate, resulting in lower concentrations of algae than intended. This was corrected for with the remaining copepods that were still alive when transferred into this solution. Additionally on Oct. 7th, 11 tows were done on the inside of the dock at Little Sturgeon Bay, and then females without egg sacs were added to the trays. On October 15, it was realized that the reason no nauplii were being observed was that they were disintegrating in less than 24 hours. We then restarted the experiment in new wells, and tracked when an egg sac appeared and disappeared in a single well. When an egg sac disappeared, even if no nauplii were visible, this was counted as successful reproduction and an estimate of the number of eggs was recorded when the egg sac first appeared.

Cell Counts

Cell counts for the initial culture concentration measurements in the Baltic Sea experiments were performed on an inverted microscope using the transect method with a micrometer eyepiece. One transect was positioned across the vertical diameter of the field of view in a grid eyepiece. The *Nodularia* filaments were all measured for length to provide a multiplier to convert number of filaments into the concentration of cells, while for the other three algae species we simply used individual cell counts. The total transect area was determined by using the diameter of the chamber multiplied by the conversion of the grid length. The biovolume was calculated using the volume of the cell multiplied by the carbon conversion factor, divided by the sample area to determine the concentration in μgC/L to create the treatment solutions. The treatment solutions were kept at the intended concentration, while various amounts of algae were added to each treatment bottle by calculating the appropriate amount of filtered seawater to add into each treatment.

The Green Bay grazing experiment cell counts were done using settling chambers using methods from Witzel (2001). Each settling chamber was tested to make sure it held water for one hour without leaking. The entire 20mL sample was then poured into the chamber with a glass slide greased over the top to prevent evaporation. The sample was placed in a drawer to remain in the dark to settle for at least ten hours**.** The slides were counted using the transect method with the diameter of the slide being one transect. The cell counts were converted from cells/mL into mgC/L using the biovolume calculation and carbon conversion equations above for each algal species (Reynolds, 1984).

Chlorophyll a

In both experiments absorbance values from the samples were used to calculate Chlorophyll *a* content of the samples. Samples were analyzed using standard chlorophyll extraction procedures, employing spectrophotometers for both studies. In the Green Bay study

chlorophyll was measured using the standard acetone extraction protocol. Samples were filtered on to Millepore filters (0.45 um pore size), were extracted with buffered acetone and sonified to disrupt cells before a 24 hr dark extraction period in the freezer. Absorbance was measured on with a spectrophotometer at 663 nm and 750 nm. These Chlorophyll *a* values were then converted into filtering and ingestion rates using equations from Frost (1972). The filtering and ingestion rates were converted into mgC/L to be comparable to the cell count measurements (Reynolds, 1984; Lind, 1985).

ELISA

The ELISA procedure for toxin analysis was run for both the Baltic and Green Bay experiments using the Envirologix Quantiplate kit for microcystins. For the Green Bay samples, after an initial run, sample microcystin concentrations were found to be below the standard detection limit of 0.16 µg/L of microcystin LR. The samples were then run under an ELISA using the increased sensitivity protocol, achieving a detection limit of 0.05 ug/L.

Analysis

Survivorship data were calculated using the number of females found alive divided by the total number of females accounted for at the end of the grazing experiment. The males were not included, and for a few bottles, not all of the females recorded going into the grazing experiment were accounted for at the end, so they were eliminated from the results. Additionally, the egg production experiment calculated the percentage of eggs produced in reference to the average number of eggs produced in the Rhod/S100% treatment respectively to allow for better comparison between the two population results.

The statistical analysis of the results was done by using SPSS & PAST to first determine if the results were normally distributed using a Shapiro-Wilks test ($p<0.05$ considered significant and indicating non-normal distributions). For results that were not normally distributed, the

Mann-Whitney ranking significance test was run in addition to a 2-sample T-test, with the not assuming equal variance P-value employed. For both tests significance was determined by $P<0.05$. For the egg production statistics, the individuals that produced zero eggs are not included in the statistics, but they are shown in the distribution of the range of eggs produced per female (brood size).

Results:

Baltic Sea

Treatment Conditions

The initial starting concentrations of algal abundance varied between treatments (Table 1). All of the treatments were above the intended starting concentration of 500µgC/L, as determined by Chlorophyll *a* analysis. The treatments with either filtrate or nutrient solution added to the *Rhodomonas* cultures had the lowest concentrations of algae, which were still above the intended levels. Treatments with *Nodularia* added contained three to five times as much carbon per liter as anticipated.

Toxin levels varied among treatments as expected based on food manipulations (Table 2). Levels of toxin in treatments with only *Rhodomonas* added were below detection limits; only R+N, Nod50, and R+Filt treatments resulted in a detectable level of toxin (Table 2). The starting toxin levels for these treatments varied with the lowest being in R+Filt at 0.6 ppm. The R+N and Nod50 had larger values of 1.1 ppm and 2.5 ppm respectively. The toxin levels in the R+N treatment remained constant during the course of the experiment in the control bottles, at around 0.75 ppm. The toxin level almost doubled in the experimental bottles significantly increasing ($P=0.01$) to an average value of 1.13 ppm. The toxin level was lower than the starting concentration in

the R+Filt experimental bottle and the control bottle significantly decreased (P=0.02) but both remained in the detectable range, indicating the presence of extracellular toxin in the filtrate from the *Nodularia* culture.

Table 1. BalticStarting Algal Concentrations

	Average Starting Algal Conc. (mgC/L)	Standard Deviation
Rhod	1.256	0.09019
R+Filt	0.751	0.05606
R+N	1.533	0.0732
R+Nut	0.555	0.08049
Nod50	2.5	.12728

Table 2. Baltic Toxin Concentrations

Treatment	Start EC Toxin Levels (ppm)	Standard Deviation	Control End EC Toxin Levels (ppm)	Standard Deviation	*Eurytemora* End EC Toxin Levels (ppm)	Standard Deviation
Rhod	BDL	n/a	---	---	---	---
R+Filt	0.770	0.105783	0.518	0.32447	0.6210	0.04823
R+N	0.753	0.111436	0.752	0.03535	1.13	0.109077
R+Nut	BDL	n/a	---	---	---	---
Nod50	2.471	0.512611	---	---	---	---

Survivorship

Survivorship varied among the five treatments after the initial 60-hour acclimation period (Figure 6). The highest survivorship was in the R+Nut treatment closely followed by the Rhod treatment, both having survivorship greater than 80%. Both the R+N and R+Filt treatments had between 60-70% survivorship after acclimation. The Nod50 culture had significantly lower survivorship (P=0.01) at the completion of the acclimation period at just below 20%. The surviving females in the Nod50 treatment were transferred immediately into 12-well plates with males and are not included in any of the grazing experiment data.

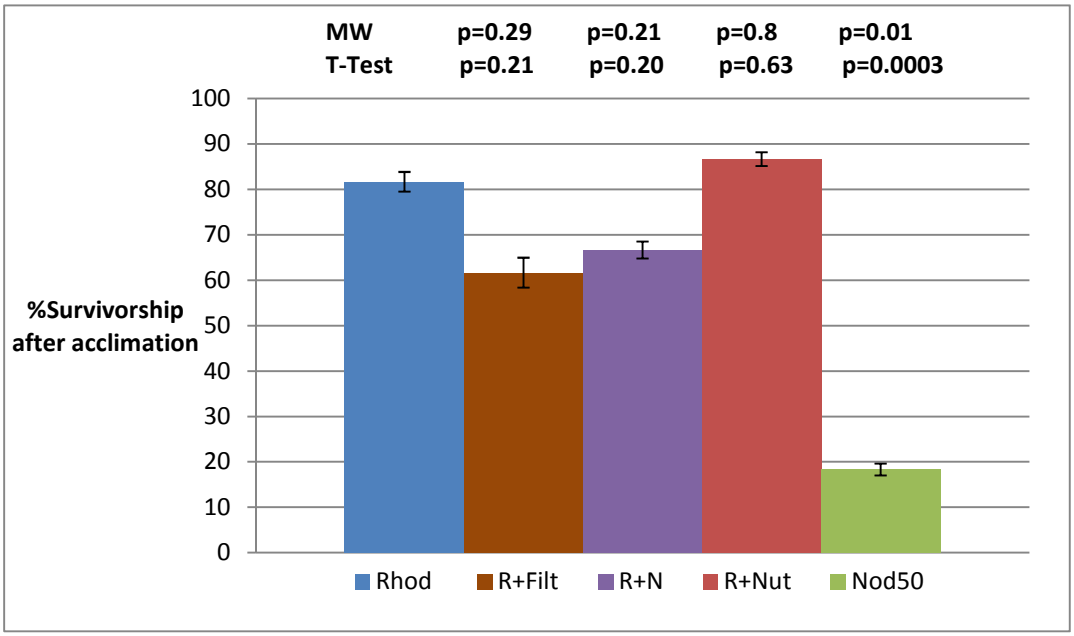


Figure 6. Baltic Survivorship Percentages after Acclimation Period (60hrs) (mean +/- 1 SEM) per treatment. Results of Mann-Whitney and Two-Sample T-Tests comparisons with Rhod treatment are indicated.

The survivorship percentages and pattern shifted after the grazing experiment in comparison to the acclimation survivorship results (Figure 7). There was not a significant difference between survivorship in the Rhod, R+N or R+Nut treatments. The

Rhod and R+N treatments showed an increase to around 90% survivorship compared to survivorship in the acclimation period, and survivorship in the R+Nut treatment was around 80%. The R+Filt treatment resulted insignificantly lower survivorship , approximately 40% of that observed for the Rhod control group (P=0.03) to 40% survivorship.

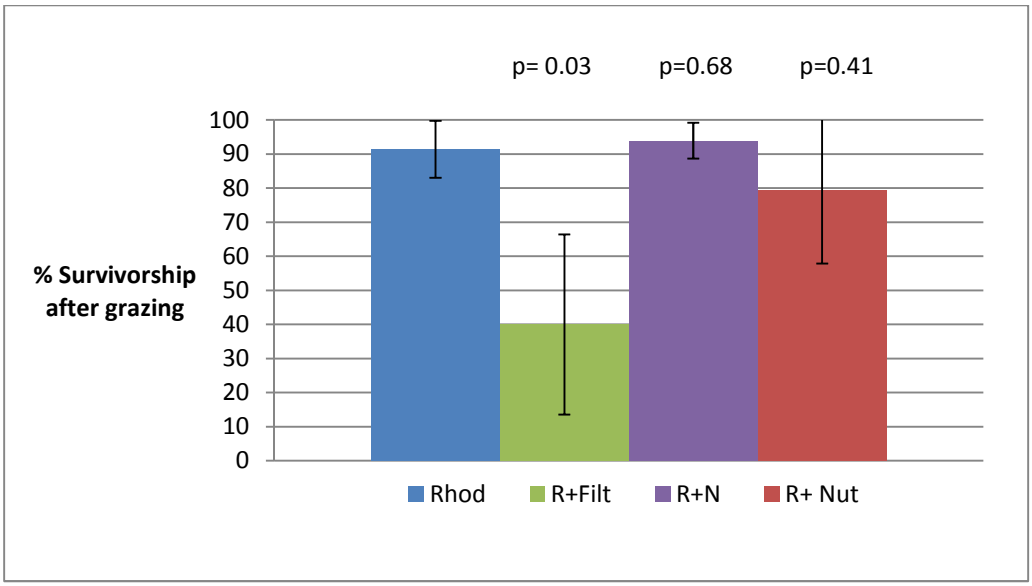


Figure 7. Baltic Survivorship Percentages after Grazing Experiment (48hrs) (mean +/- 1 SEM) per treatment. Results of Mann-Whitney and Two-Sample T-Tests comparisons with Rhod treatment are indicated.

Grazing

The algal concentrations measured through Chlorophyll *a* determination (mgC/L) were variable between treatments. However, all treatments indicate both the control and experimental bottles increased in algal concentration from their start concentration (Figure 8). The Rhod treatment, R+N and R+Nut treatment had about a 0.5mgC/L increase in the control bottles after 24 hours. The concentration of algae in the experimental bottles containing *E. affinis* was generally lower than in the control bottles at the end the experiment, indicating that animals had grazed on the algae. Animals in

the R+Filt treatment did not appear to graze appreciably because both control and experimental animal bottles increased approximately the same amount.

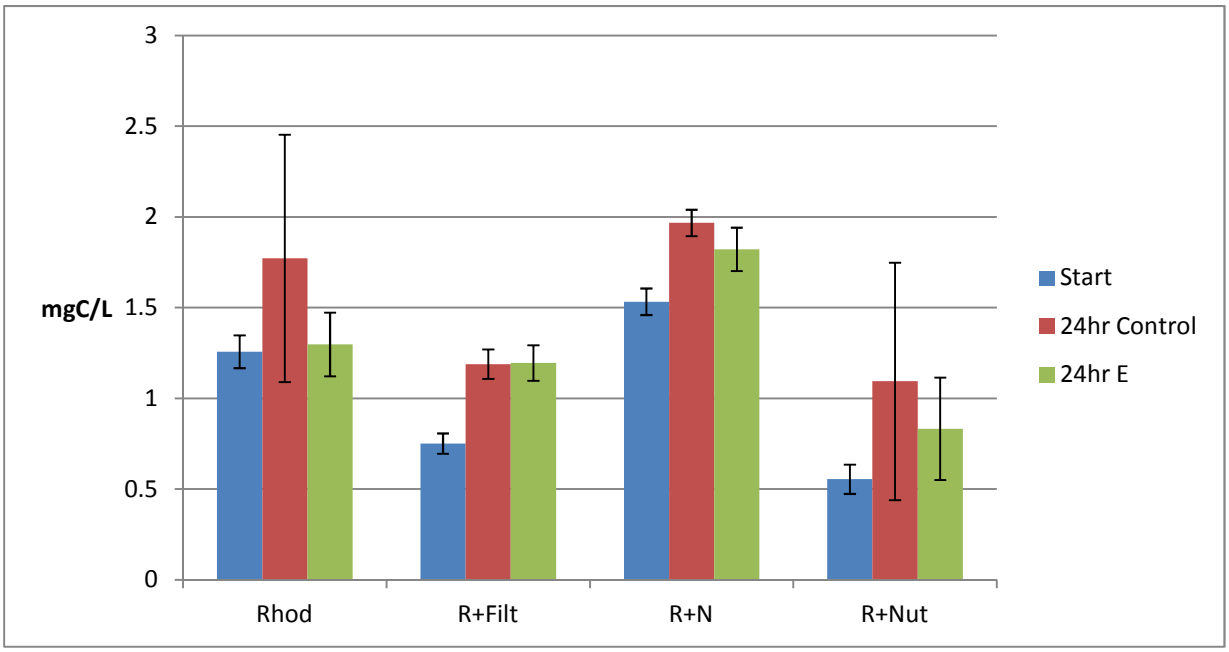


Figure 8. Baltic Grazing Experiment Cell Concentrations Measured by Chlorophyll *a* (mean +/- 1 SEM) per treatment.

There was a wide range of filtering rates for Baltic *E. affinis* among the four treatments (Figure 9). The highest filtering rates by *E. affinis* were recorded in the treatments with only *Rhodomonas* as a food supply. Filtering rates in the Rhod treatment was just below 0.4 mL/copepod/hr, while the rate in the Rho+ Nut treatment was just above 0.3mL/copepod/hr. Filtering rates by animals exposed to either the *Nodularia* cells or the filtrate were decreased. The Rho + Nod treatment had filtering rate at just above 0.1mL/copepod/hr, the filtration rate for R+Filt treatment was slightly negative, meaning it was undetectable by our methods.

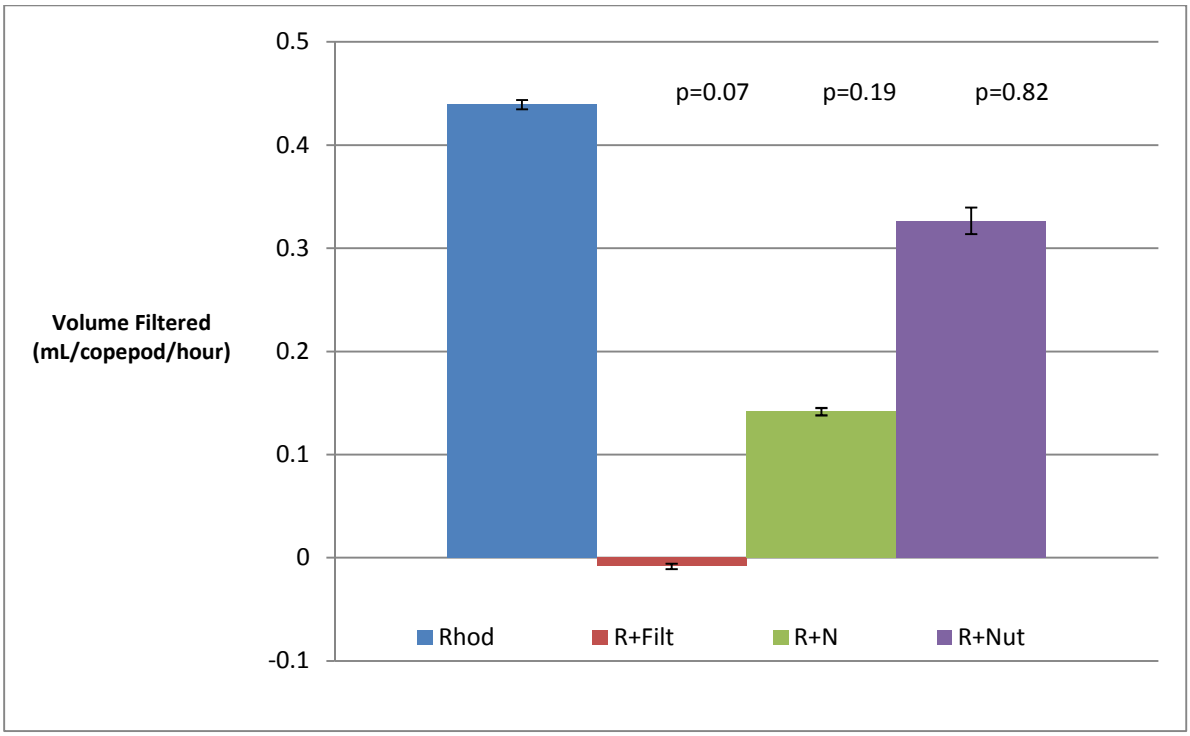


Figure 9. Baltic Grazing Experiment Filtration Rate (mL/copepod/hr) (mean +/- 1 SEM) per treatment. Results of Two-Sampled-T-Tests comparison with Rhod

The ingestion rates for Baltic *E. affinis* had a slightly different pattern than the filtering rates (Figure 10). The Rhod treatment still had the highest rate at just above 0.5mgC/mL/copepod/hr. The R+N treatment had the second highest ingestion rate, followed by the *Rhodomonas* plus nutrient culture. The R+Filt treatment had a significantly lower (and negative) filtration rate (P=0.05).

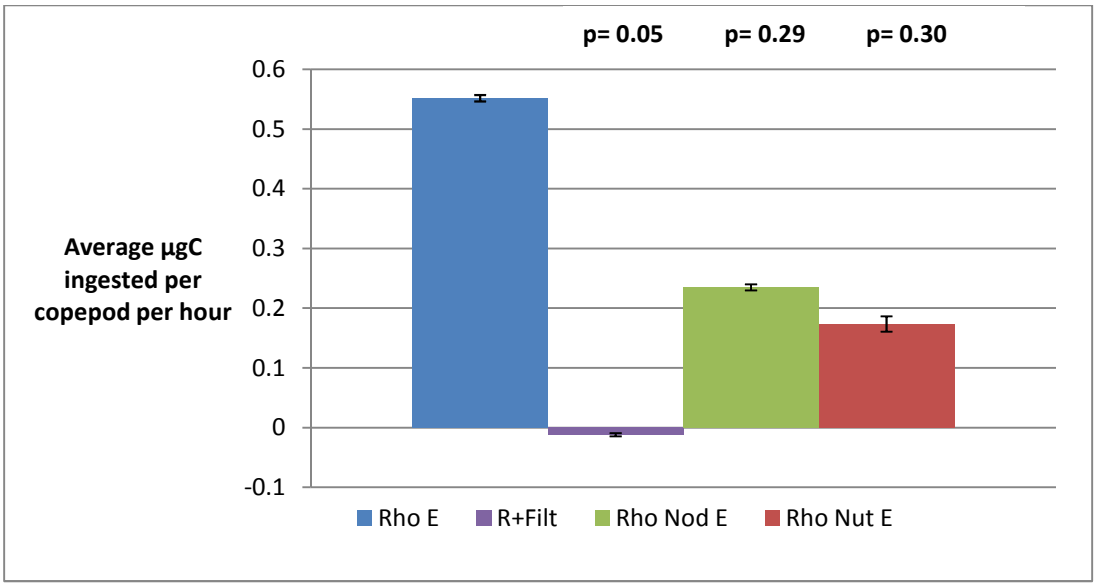


Figure 10. Baltic Grazing Experiment Ingestion Rate (µgC/copepod/hr) (mean +/- 1 SEM) per treatment. Results of Two-Sampled-T-Tests comparison with Rhod treatment are indicated.

Egg Production Experiment

The egg production between the various treatments was not normally distributed, but no individual values were considered outliers in any of the treatments (Figure 11). The largest difference between the treatments is that the lowest average clutch size for the R+Nut treatment is comparable to the highest clutch size in the Nod50 treatment. The R+N treatment had the lowest range of clutch size.

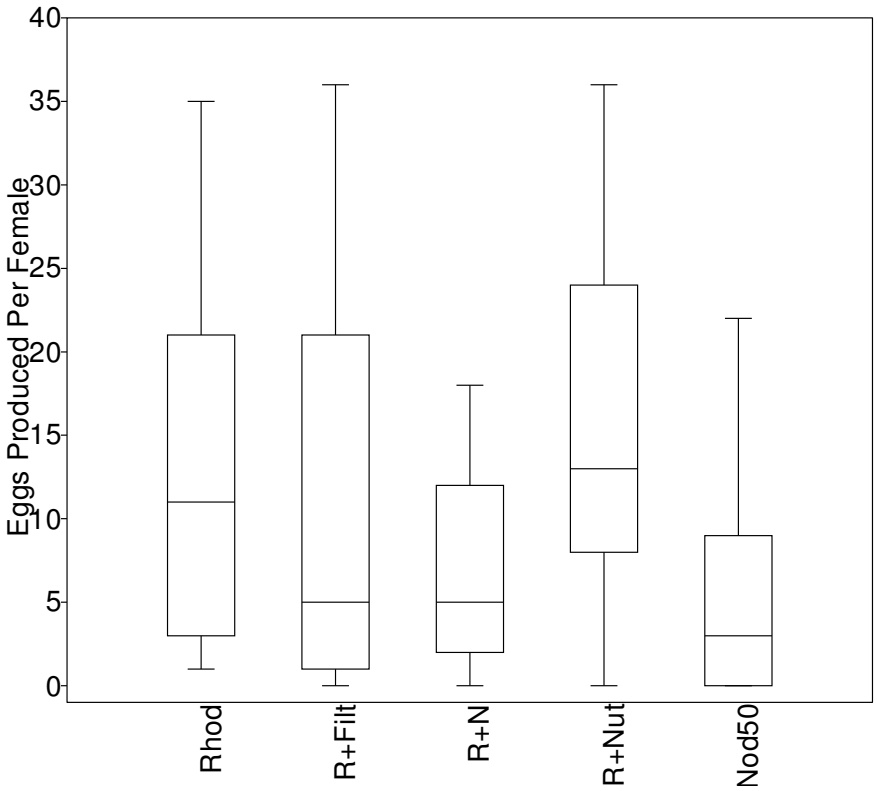


Figure 11. Baltic Box Plot of Average Eggs Produced per Female by Treatment

The Rhod treatment had an even distribution of females producing between 1-25 eggs, with a couple individuals producing 30-40 eggs (Figure 12a). The R+Filt treatment exhibited greater variability in its egg production (Figure 12b). The most common clutch size was between 1-5 eggs. A few copepods produced 6-30 eggs and a single individual produced a clutch of 36 eggs. The R+N treatment also had the most common clutch size of 1-5 eggs, in addition to 11-15 (Figure 12c). No individuals in this treatment produced a clutch size of greater than 20 eggs. The R+Nut treatment had the largest variation in clutch sizes (Figure 12d). The most common clutch sizes were 6-10 and 16-20 eggs and a single copepod had a clutch of 35 egg. The eggs per female in the 0 bin in these four treatments indicate females that produced eggs, but which

disintegrated before an accurate count could be measured. The Nod50 treatment resulted in much lower egg production per female (Figure 12e). There were five individuals that did not produce eggs, which was the most common group in this treatment. These 0s do indicate females that did not produce eggs as this treatment skipped the grazing experiment however they are still not included in the statistical analysis. Of those that did reproduce most produced less than 10 eggs per females but a few individuals did produce between 11-25 eggs.

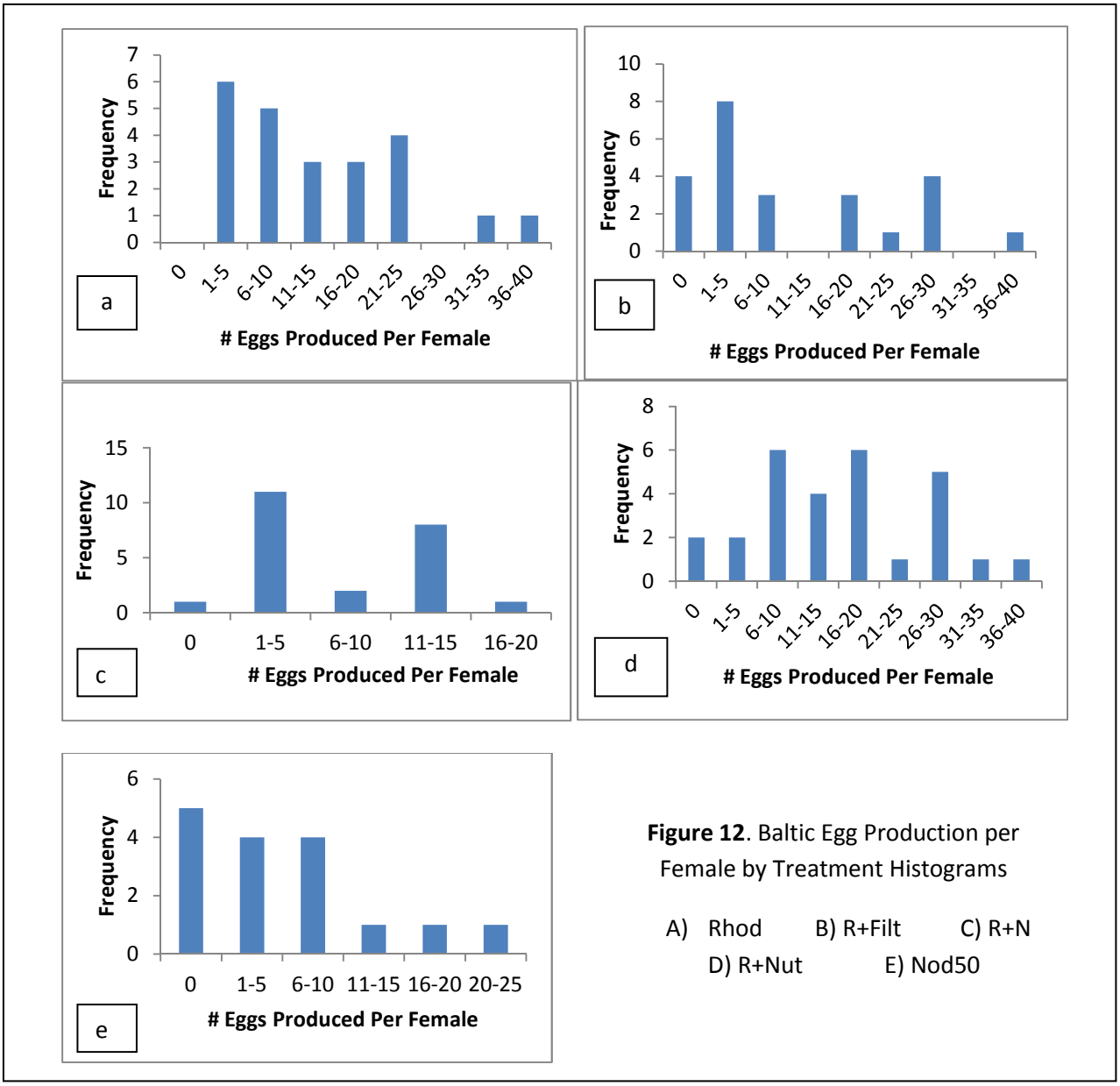


Figure 12. Baltic Egg Production per Female by Treatment Histograms

A) Rhod B) R+Filt C) R+N
D) R+Nut E) Nod50

The average number of eggs produced per female varied between treatments, with highest values seen in the treatments with only *Rhodomonas* as the food (Figure 13). The R+Nut treatment showed a 120% increase from the Rhod treatment egg production. The remaining three treatments had decreased average egg production. R+Filt had the highest of these three treatments. The R+N treatment produced 60% (P=0.04) of that of the Rhod treatment. The R+Filt treatment produced 85% of eggs compared to the Rhod treatment. Finally, the Nod50 treatment, with an average of five eggs per female, was about 40% of the Rhod control treatment.

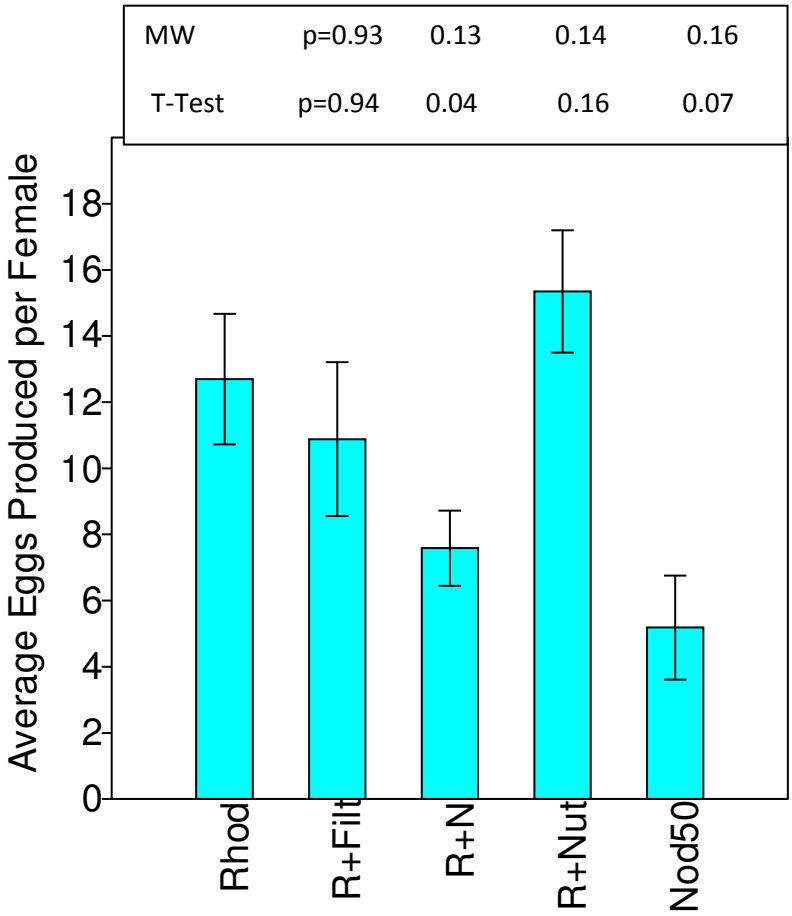


Figure 13. Baltic Average Egg Production per Female (mean +/- 1 SEM) per treatment. Results of Mann-Whitney and Two-Sample T-Tests comparison with Rhod Treatment

Nauplii Size

The size of the nauplii for the Baltic *E. affinis* showed a different pattern than the egg production and included a large number of outliers (Figure 14). The Rhod treatment had the largest offspring by size and all three other treatments were found to be significantly smaller. The mixed R+N treatment was the highest of the three other treatments (P=0.04) followed by the Rhod and nutrient treatment (P=0.01). The R+Filt had the smallest nauplii by size (P<0.0001). The average length of nauplii produced by each treatment shows a significant difference in comparison to the Rhod treatment with the exception of the Nod50 treatment (Figure 15). The Rhod treatment produced the longest average nauplii followed closely by the Nod50 treatment. The R+N treatment produced slightly longer nauplii on average than the R+Nut treatment. The R+Filt treatment had the smallest average nauplii lengths.

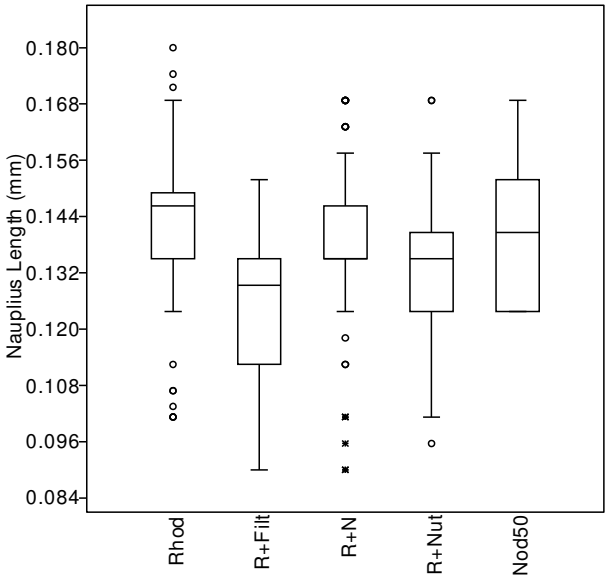


Figure 14. Baltic Nauplii Length Box Plot (mean +/- 1 SEM) per treatment

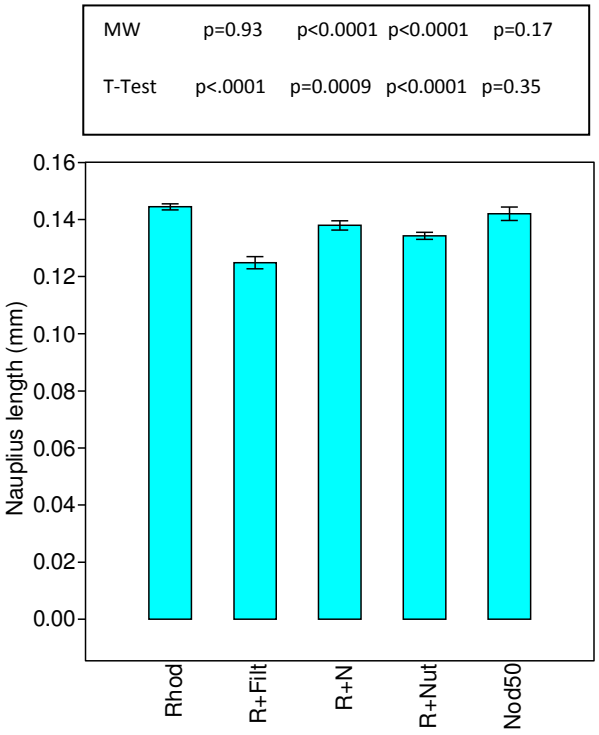


Figure 15. Baltic Average Nauplii Length(mean +/- 1 SEM) per treatment. Results of Mann-Whitney and Two-Sampled-T-Tests in comparison with Rhod Treatment are indicated.

Green Bay

Treatment Conditions

The starting algal concentrations in the Green Bay experiment were lower than the intended concentration of 500µgC/L (Table 3). These starting calculations are based off field concentrations and calculated through cell counts converted into mgC/L (Andreas Brutemark, pers.comm). There was no toxin detected in the S100% or MF treatments, but the treatment with *Microcystis* added contained approximately 0.3 µg/L of the toxin Microcystin LR (Table 3).

Table 3. Green Bay Grazing Experiment Treatment Conditions

	Starting Algal Concentration (mgC/L)	Toxin Concentration (MCYST µg/L)	Standard Deviation
S100%	0.0725	BDL	n/a
MF	0.0535	BDL	n/a
S+M10%	0.0535	0.306	0.066

Grazing

The start and end algal concentrations show an interesting pattern between treatments based on of the Chlorophyll *a* analysis (Figure 16). The S100% treatment shows that the algal concentration decreases in the control, while the algal concentration in the experimental bottle slightly increases from the starting concentration. The MF treatment shows the algal concentration of the control and experimental bottles decreasing from the starting concentration. The S+ M10% treatment shows an increase in algal concentration in the control and a decrease in the

experimental bottle. Since we have data from both Chlorophyll *a* analysis and cell counts, the grazing experiment will be based off of the cell count calculations, due to the larger error associated with the chlorophyll measurement procedures.

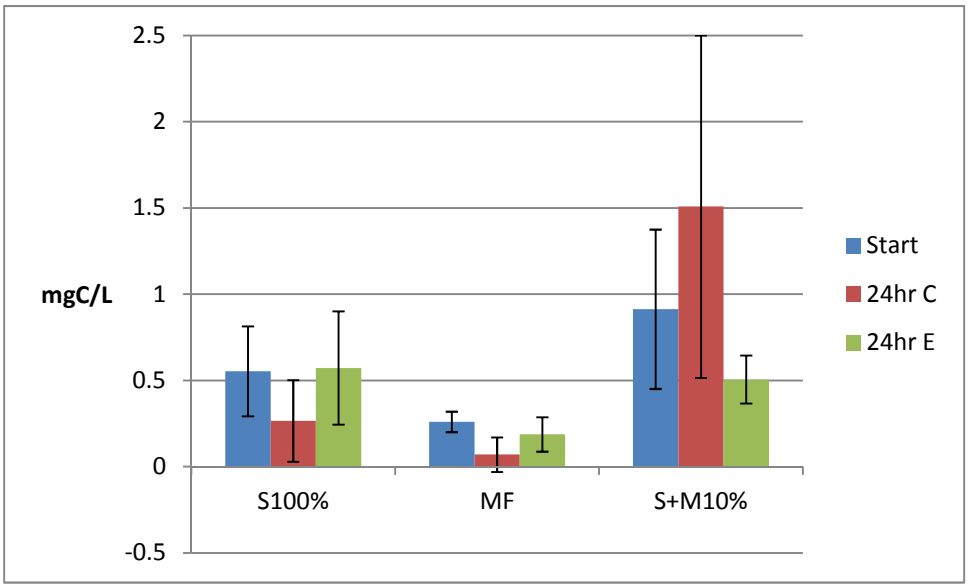


Figure 16. Green Bay Cell Concentrations during Grazing Experiment measured by Chlorophyll *a* (mean +/- 1 SEM) per treatment.

The cell counts from the grazing experiment show a different pattern between the treatments than shown from the Chlorophyll *a* analysis (Figure 17). Cell counts were lower at the end of the experiment than at the beginning in all treatments containing animals. The S100% treatment follows the expected grazing rate pattern with the control bottle concentrations increasing and the experimental concentrations decreasing. However, both of the other treatments had both the control and experimental bottles at lower concentrations than at the start.

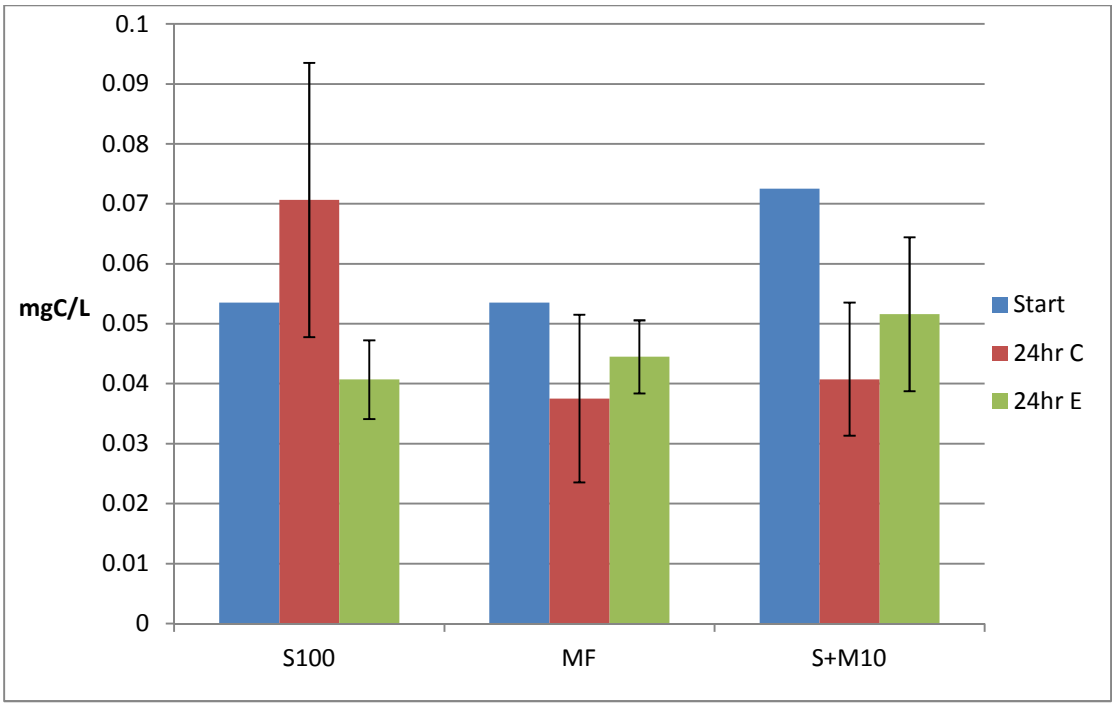


Figure 17. Green Bay Cell Concentrations during Grazing Experiment measured by cell counts (mgC/L)(mean +/- 1 SEM) per treatment.

The filtration rates for all three treatments are normally distributed and are considered significantly different from the Rhod treatment (Figure 18). The S100% treatment filtration rate is extremely close to zero, but is positive in comparison to the two negative filtration rates associated with the MF & S+M10% treatments. The S+M10% is a lower negative rate compared to the MF treatment. The ingestion rates for this experiment follow the same pattern as the filtration rates with positive ingestion rates only detected in the S100 treatment (Figure 19).

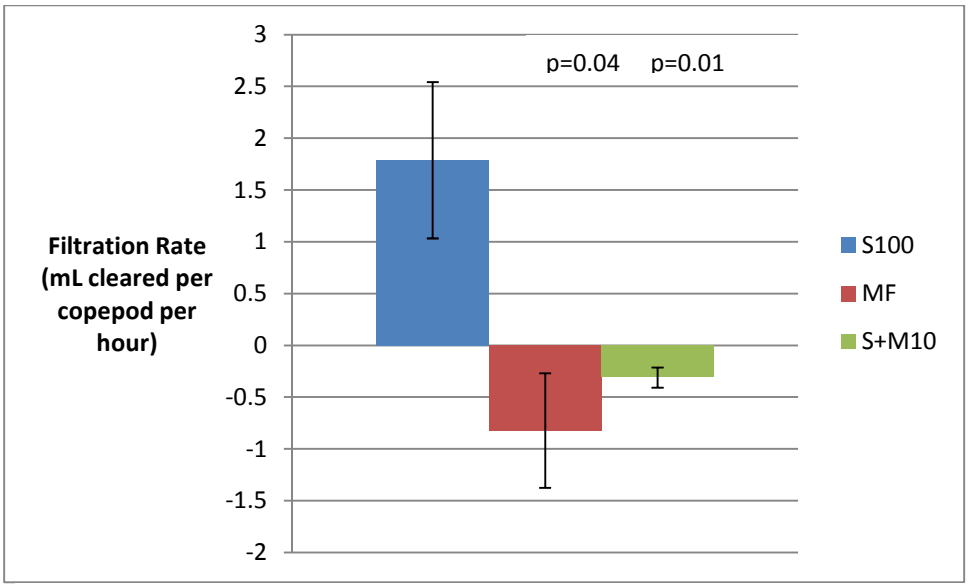


Figure 18. Green Bay Filtration Rates (mL cleared/hr)(mean +/- 1 SEM) per treatment. Results of Two-Sample T-Tests in comparison with Rhod Treatment are indicated.

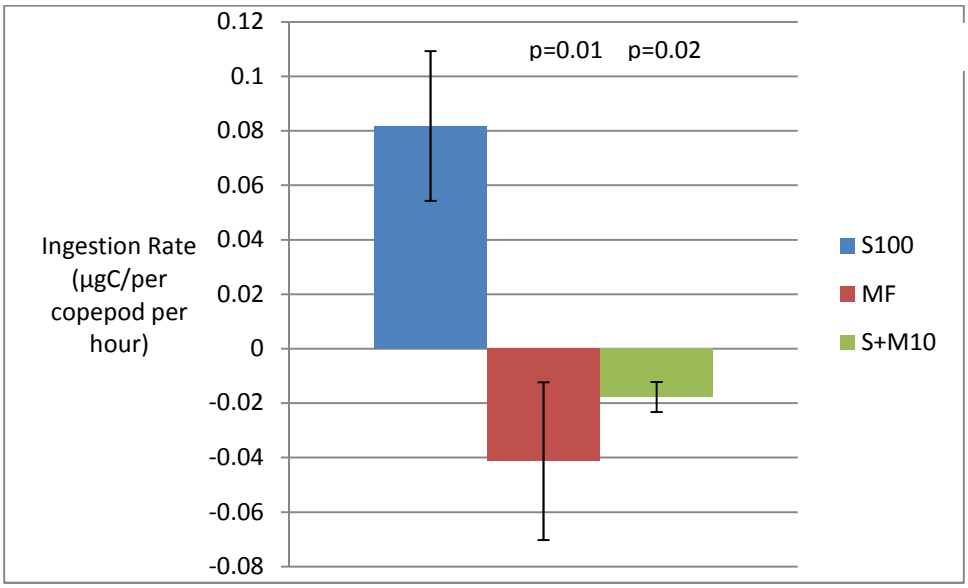


Figure 19. Green Bay Ingestion Rates (µgC/copepod/hr) (mean +/- 1 SEM) per treatment. Results of Two-Sample T-Tests in comparison with Rhod Treatment are indicated.

The filtration and ingestion rates were also calculated without the controls factored in for the MF and S+M10% treatments. This was done because the controls are built into the experiment to account for the additional algal growth during the experiment when calculating the grazing rate, and there was no increase in algal

concentration in these two treatments during this grazing experiment. The elimination of these two control bottle results in positive filtration rates (Figure 20). Animals in the S100% treatment had the highest filtration rate, followed by the S+M10% treatment rate just above 0.0015L, and then followed by the MF treatment at less than 0.001 L/copepod/hr. The ingestion rate without the control component also follows the same pattern of the filtration rate without the control (Figure 21). In terms of carbon consumed, the largest ingestion rate is seen in the S+M10% treatment. The ingestion rates for S100% was slightly lower, followed by the MF treatment with approximately 0.00004mgC/L/copepod/hour.

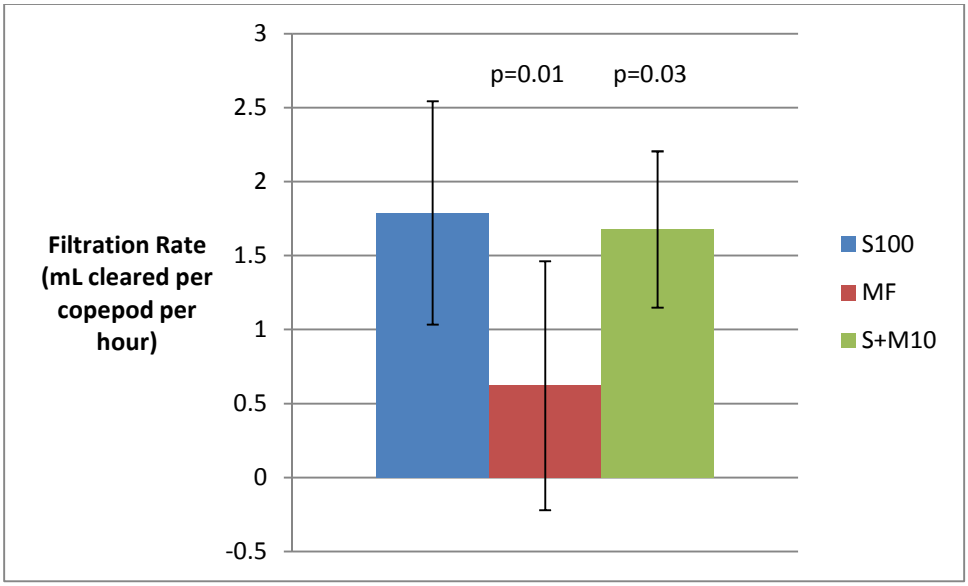


Figure 20. Green Bay Filtration Rates eliminating MF and S+M10 controls (mL cleared/copepod/hr) (mean +/- 1 SEM) per treatment. Results of Two-Sampled-T-Tests in comparison with Rhod Treatment are indicated.

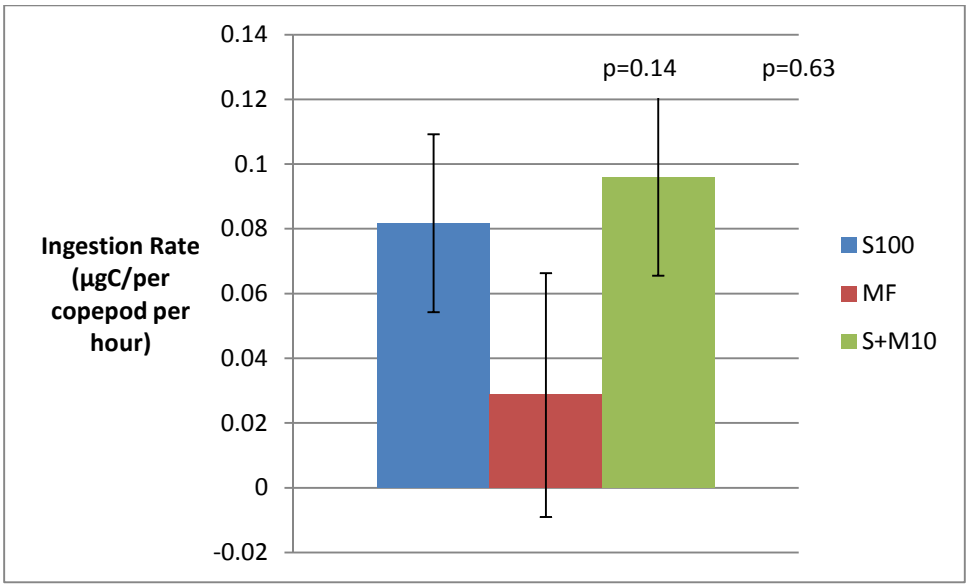


Figure 21. Green Bay Ingestion Rates eliminating MF and S+M10 controls (mgC/copepod/hr) (mean +/- 1 SEM) per treatment. Results of Two-Sampled-T-Tests in comparison with Rhod Treatment are indicated.

Egg Production

The three treatments varied in their range of egg production but all were found to have distributions that were not significantly different than a normal distribution of eggs produced per individual females (Figure 22). Egg production was slightly more skewed towards higher values in the MF treatment than in the others.

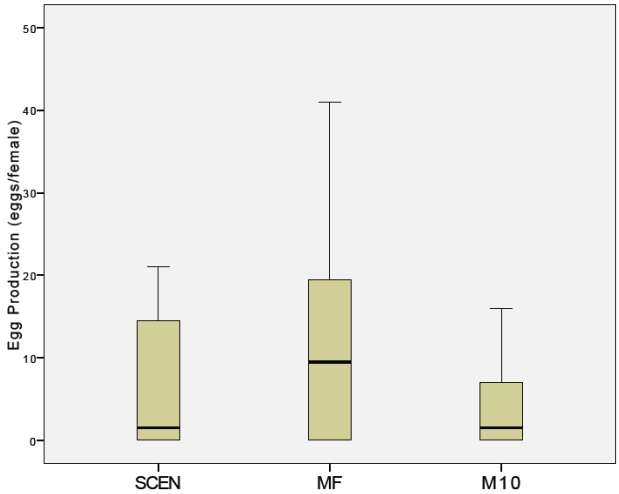
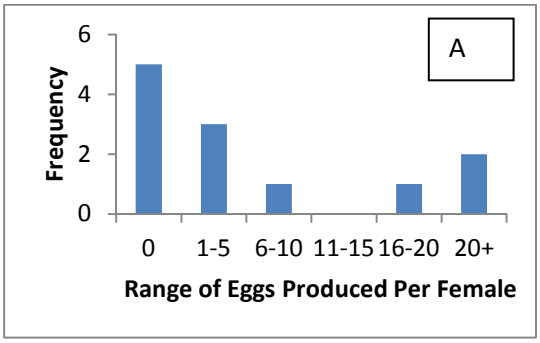
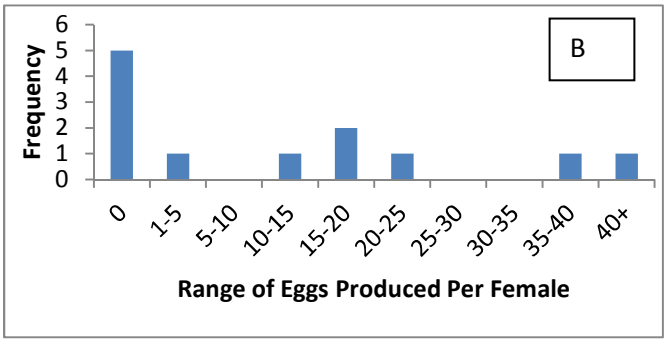


Figure 22. Green Bay Egg Production per female by Treatment Box Plot

There also was large variance between individual copepods within the same treatment (Figure 23). All of the females producing 0 eggs indicates females that never formed an egg sac. These individuals are excluded from the statistical analysis, but still included in the presentation of the distributions. Females in the MF treatment had nearly double the egg production of females in the S100 treatment. The S+M10% treatment had the lowest egg production. The S100 treatment had the largest distribution of females not producing any eggs. Two females produced between 5-15 eggs and three individuals produced between 20-25 eggs. (Figure 23a). The MF treatment also had the most individual females produce 0 eggs. One individual produced less than 5 eggs and 4 individuals produced 15-25 eggs. One individual produced over 40 eggs. (Figure 23b) The S+M10% treatment also had the highest number of individuals not produce any eggs. Two individuals produced between 1-5 and then four individuals produced between 5 and 20 eggs. (Figure 23c)





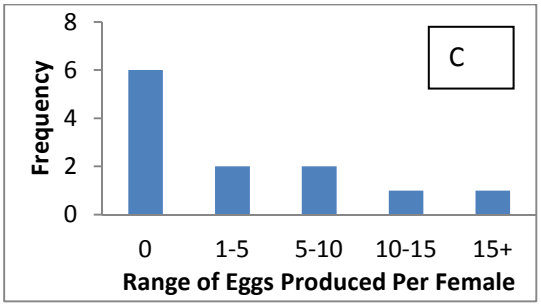


Figure 23. Green Bay Egg Production per Female by Treatment Histograms

A) S100 B) MF C) S+M10

The average egg production per female varied between each treatment (Figure 24). The MF treatment showed the highest overall average egg production with 202% of the eggs on average compared to the S100 treatment. The S+M10% treatment produced the fewest eggs on average and was only 74% compared to the S100% treatment. The S100 treatment produced 11 eggs on average per female.

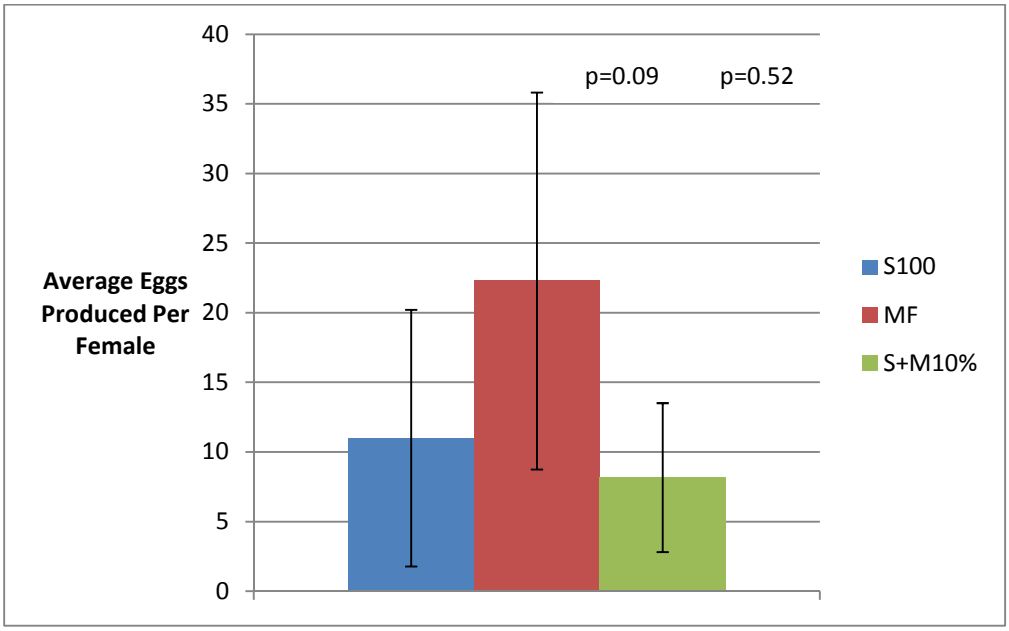


Figure 24. Green Bay Average Egg Production per female. (mean +/- 1 SEM) per treatment. Results of Two-Sample T-Tests in comparison with Rhod Treatment are indicated.

Discussion

Baltic Sea

Nodularia appears to have a negative effect on *E. affinis* in the Baltic Sea, both when exposed as whole cells or as filtrate from cell cultures, but these two types of exposure result in varying negative impacts on the copepods. This follows the hypothesis of blooms creating a negative impact on the ecosystem around them. In our study, the starting concentrations for all five treatments were planned to consist of a concentration of 500µgC/L, but all the treatments

showed elevated concentrations. This increase is likely because the cell counts for the calculation of the biovolume of the culture were done at the start of the acclimation period and not recounted before making the grazing treatments. As we only have the chlorophyll data as a measure of algal concentration, we are not able to determine the relative concentrations of the two algae to know if the *Nodularia* has a larger presence than accounted for. The R+Filt and R+Nut treatment have starting values closer to the expected starting value suggesting those additions may have an effect on the growth rate of *Nodularia* (Table 1). The Nod50 treatment had a starting concentration of over three times that of the R+Filt (Table 2). This indicates that the *Nodularia* is likely a driving force of the increased carbon concentrations rather than the *Rhodomonas*. The Nod50 also had three times as much toxin as the R+Filt treatment. Interestingly, the R+Filt had about half the carbon concentration as the R+N treatment, but they both had comparable starting concentrations of toxins, as would be expected if the filtrate contained toxin but no cell-bound carbon.

Additionally, the different shift in toxin concentration (Table 2) during the grazing experiment between the R+Filt and R+N treatments indicates the filtrate of *Nodularia* is unique from the toxins produced when the cells are present. The larger toxin concentrations in the presence of copepods leads to the speculation there may be a connection between toxin abundance and presence of copepods as both toxin levels were higher in the presence of copepods. A previous study also shows that the introduction of zooplankton to *Nodularia* results in a decrease of intracellular toxin concentration related to an increase in extracellular nodularin, but this study concludes that the extracellular nodularin increase is not likely associated with the direct production of toxin in response to the presence of copepods. Rather it is more likely due to sloppy eating or toxin released by dying cells (Gorokhova & Engström-Öst, 2009). Repka et al. (2004) also found there is no direct connection between the abundance or presence of zooplankton and toxin production (Repka et al., 2004).

Baltic Survivorship

The higher concentration of food and toxin in the Nod50 treatment had a large effect on survivorship as less than 20% of the copepods were alive at the end of the acclimation period (Figure 1). While the concentration of *Nodularia* in this treatment was more excessive than planned, the toxin level (Table 2: 2.471 µg/L) still falls within the range of extracellular concentrations in the Baltic Sea, from 0.1 to 10µg/L (Sivonen and Jones, 1999). While the toxin concentration for the Nod50 treatment in our study is well below the highest concentrations reported in the Baltic Sea, it is also well above the concentrations used in various studies examining the effect of *Nodularia* on zooplankton, usually less than 1 µg/L (Sivonen and Jones, 1999; Gorokhova & Engström-Öst, 2009). Therefore, it is important to examine the thresholds of toxin concentrations to better estimate in the field what levels will start to show immediate negative effects such as declines in survivorship, and what levels may mask the underlying effects of the toxin on the entire system, such as decreased growth or production rates.

The remaining treatments had survivorship rates high enough to continue with the grazing experiment (Figure 1). The R+Filt and R+N treatments showed lower survivorship than the Rhod & R+Nut treatments. Survivorship is expected to be lower during this period due to the stress of changing environments, which is an additional importance of the acclimation period. This allowed more confidence that the significant decrease in survivorship in the R+Filt treatment after the grazing experiment was due to the treatment itself, as Rhod and R+N treatments both produced higher survivorship (Figure 2). The R+Nut treatment, which had the highest survivorship after acclimation, also showed a slightly decreased survivorship with just below 80%. The process of determining the survivorship of females created some problems as not every individual was recovered from the treatment bottle. There was one bottle in each treatment where not every female added in the beginning of the grazing experiment was recovered. This is accounted for in the percentages, and the missing ones were excluded, but if 100% of the females' outcome were determined, this might have resulted in a slight change on

the overall survivorship results. However, there is a distinct difference still seen between the R+Filt treatment having approximately half the survivorship percentage observed in the other three treatments and because all treatments had at least one female missing, these results are considered representative of the overall effect.

Baltic Grazing

The decreases in algal concentrations during the grazing experiments indicate that feeding is occurring in most of the treatments, and that the greatest feeding occurs in the Rhod and R+Nut treatments. The R+N treatment shows decreased feeding compared to the Rhod and R+Nut treatments, and the R+Filt treatment shows the experimental bottle actually has a slightly higher concentration than the control bottle (but not significantly so). This results in a negative filtering rate for the R+Filt treatment. The R+N treatment also shows a lower filtration rate in comparison to the treatments not affected by *Nodularia* as there is less than half the volume filtered in this treatment than that of R+Nut. The Rhod treatment also clearly is a better environment for feeding mechanisms even in comparison to the R+Nut treatment. The ingestion rates are similar to the filtration rates for the Rhod and R+Filt treatments, as the Rhod treatment ingestion rate is more than double that of any other treatment. The R+Filt treatment ingestion rate also is negative, indicating there is no feeding occurring in this treatment. The R+Nut treatment and R+N treatment ingestion rates do not follow the same pattern as indicated with the filtration rates as the R+Nut treatment drops slightly below the R+N treatment.

Eurytemora affinis are suspension feeders, and are less selective than other calanoids, which may contribute to their feeding on the toxic blooms (Engström et al., 2000). The 0.2 μgC/copepod/hour ingestion rate for the R+N treatment is larger than the ingestion rate Engström et al. (2000) found for *E. affinis* exposed to toxic *Nodularia* at 0.003μgC/copepod/hour. The clearance rate of the good food source was comparable to ours.

We had about twice the concentration of *Nodularia* exposed and approximately twice the clearing rate, however that was on *Brachimonas* rather than *Rhodomonas* as in this experiment. Previous studies indicate that grazing rates decrease with the presence of cyanobacteria (Sellner et al, 1996). This study also resulted in a few replicates where feeding decreased on the good food source in the presence of *Nodularia.* However, both the concentration of the good food source and cyanobacteria source affect the grazing rate. Initially, as the concentration of food increases the ingestion rate will also increase (Huntley & Boyd, 1984). The ingestion rate is based on a critical concentration, and once the concentration of food reaches beyond this value, the ingestion rate will remain constant and the filtering rate will decrease. It is thought that the critical concentration is often reached in the open ocean, but is not normally attained in coastal areas that experience greater seasonal change and therefore decreased food supply at various times of the year. Having a better representation than simply Chlorophyll *a* for concentration of carbon analysis would be beneficial so we could see the ratio of the different algae concentrations at beginning and end of the grazing experiment.

The decrease in filtration and ingestion in the R+Nut treatment indicates there may be something in the nutrient broth triggering decreased feeding. This result is important for distinguishing whether or not the nutrient broth is having an effect on the copepod's feeding directly, as it is incorporated in both the R+Filt and R+N treatment and could be affecting the results in addition to the effects of *Nodularia*. Additionally, the lower starting carbon concentrations could be due to an alleopathic effect of the toxin or another metabolite produced by *Nodularia* that is actually reducing the abundance of *Rhodomonas* cells.

Egg Production

The Nod50 and R+N treatments produced the lowest egg production results. The largest brood size in both of these treatments was half the size of the largest brood size in the remaining treatments and was produced by only a couple of individual copepods whereas the

other treatments had the majority of their brood sizes in this range. The R+N and Nod50 treatments both had lower average number of eggs produced per female in comparison to the other treatments. The R+Nut treatment appears to be the best treatment for egg production per female. The Rhod and R+Filt both had a similar average number of eggs produced per female, again suggesting that the extracellular toxins had a negligible effect on the reproductive success of *E. affinis.*

Nauplii Size

The significant decrease in nauplius size for all of the treatments in comparison to the Rhod treatment indicates the *Rhodomonas* is a good supporting food source for *E. affinis* in terms of nauplii fitness. The most noticeable decrease in size is in the R+Filt treatment. This indicates the likelihood that either the extracellular toxins or other metabolites produced are having an effect on the actual production of individual egg quality in *E. affinis.* While animals in the R+Filt treatment produce large brood sizes in comparison to the other treatments (in fact, as large as those in the Rhod treatment), the individual nauplii produced are not as fit as those in the other treatments. This also appears to be true for the R+Nut treatment that had a much higher abundance of eggs produced, but has nauplii smaller than in the Rhod treatment. These results support the conclusion that there is a trade-off in number and quality of offspring produced. The R+N treatment has smaller nauplii than the Rhod treatment, but larger than both the R+Nut and R+Filt, suggesting that the smaller number of eggs are of higher quality.

Overall Analysis of Effect of Nodularia On Eurytemora affinis in the Baltic Sea

These results indicate that *Nodularia* does have a negative effect on *E. affinis* in the Baltic Sea. Extremely high abundances of *Nodularia* appear to have a strong and immediate impact on the survivorship of *E. affinis.* For the small number of individuals that are able to survive under these conditions, they produce fewer eggs per female and these eggs result in

nauplii that are significantly smaller than those produced by individuals not exposed to large concentrations of *Nodularia.* A smaller concentration of *Nodularia* introduced to the diet of *E. affinis* also shows a negative effect on their overall fitness. At the lower concentrations there is no immediate negative effect in terms of survivorship as seen with exposure to a large concentration of *Nodularia*. The survivorship was actually higher for this treatment after the grazing experiment in comparison to the acclimation period survivorship estimates, perhaps suggesting the acclimation period selected copepods that were more tolerant of the feeding conditions. However, *E. affinis* does show decreased grazing rates in the presence of the *Nodularia* in comparison to those in the Rhod treatment. The number of eggs produced per female is similar to those produced in the Nod50 treatment indicating that the concentration of toxin may not be as important as simply presence or absence once it is past a certain concentration.

Another study investigating the effects of *Nodularia* on copepods looked at the effect of both toxic and non-toxic strains of *Nodularia (*Koski et al., 1999). Both of these treatments resulted in egg production results comparable to a control treatment (i.e. starvation treatment) containing just filtered sea water, indicating that *Nodularia* is likely lacking essential nutrients needed for successful egg production in *E. affinis*. This supports our conclusions that nodularin, especially in high concentrations has a strong impact on *E. affinis,* as survivorship decreased with increasing concentrations of *Nodularia.* Additionally, Koski et al. (1999) introduced *Noduaria* into a diet of a good natural food source, *Brachimonas submarina,* and this resulted in decreased mortality and better egg production. They concluded that *E. affinis* are able to avoid consuming the toxic *Nodularia* strains. The non-toxic strains, also appeared to be able to be of high enough nutritional quality, if in high enough abundance, to ensure increased survivorship for the current population. The same study showed multiple deformed egg sacs, in addition to decreased overall brood size. As we did not observe deformed egg sacs *Rhodomonas* may be a better source of nutrition for *E. affinis,* indicating that it is also important to look at the

composition of all the phytoplankton in algal blooms in the field. This would help create a better prediction of the effect of blooms on the diet of Baltic *E. affinis.*

Analysis of the R+Filt treatment results presents the idea that the extracellular toxins, or possibly other metabolites, have a different type of negative effect on *E. affinis* than does just consumption of the toxin. There are various views on this possibility. Suikkanen et al. (2006) investigated the alleopathic effects of three cyanobacteria including *Nodularia,* on the growth of possible competitors. They tested the effects of adding filtrate, which may contain other compounds, in with a *Rhodomonas* treatment. Their results demonstrated inhibition of the *Rhodomonas.* Another study looked into the effect of the purified nodularin toxin and found it had no effect on *Rhodomonas,* whereas the *Nodularia* filtrate did stunt *Rhodomoas* growth (Suikannen et al., 2006). This leads to the conclusion that there is likely a metabolite that is produced by at least certain strains of *Nodualria,* in addition to nodularin, that is preventing the growth of *Rhodomonas.* Therefore, this metabolite is also potentially negatively affecting other phytoplankton, and in turn affecting the feeding availability of surrounding zooplankton in natural blooms. It is also possible this metabolite is having direct negative effects on zooplankton.

The R+Filt treatment had the lowest initial survivorship during acclimation, with the exception of the Nod50 treatment, but it was still within 10% of the R+N treatment. However, after another 48 hours in the treatment during the grazing experiment, it is clear that something in this treatment has led to the shutdown of feeding mechanisms in *E. affinis*. This could simply be due to starvation, and may be related to the toxins or other metabolites causing a complete shutdown of feeding. Additionally, there could be a direct effect between the extracellular toxin or metabolites causing a direct effect on the mortality of the organisms. Interestingly, the R+Filt did not have a negative effect on the reproductive success. This treatment actually had a higher yield of eggs in comparison to the Rhod treatment, but these nauplii were smaller than the nauplii produced in smaller brood sizes. So the decreased feeding could result in the smaller nauplii produced, again not directly affected by toxin, but through decreased feeding

mechanisms that are important for maintaining fitness. Animals in the R+Filt treatment were able to produce eggs at a rate that was 85% of the average egg production by females in the Rhod treatment. However, given only a 40% survivorship of adult females, it can be argued that energy would be better allocated to produce fewer nauplii with higher fitness and an overall better chance of surviving, as seen in the response from the Nod50 & R+N treatments.

A final consideration when analyzing these results are the effects seen in the R+Nut treatment. This was designed to be a secondary control for the possible effects of the medium used to culture the *Nodularia* in the laboratory. While it appears the culture may have a negative effect on the feeding mechanisms of the *E. affinis,* this more likely due to the decreased starting concentration of food availability. The results for the R+Nut treatment are similar to the effects seen with the R+Filt. The filtration rate for the R+Nut treatment was higher than the R+N treatment, but the ingestion rate is lower. This is likely due to the lower concentration at the start in comparison to the other treatments, inhibiting this treatment to reach a comparable ingestion rate. It is likely that this treatment did not produce negative effects, especially as in comparison the Nod50 treatment, which had a higher concentration of available food, but of a lower nutritional value. Therefore the R+Nut treatment still was able to produce more eggs per female on average.

This study, in support of previous studies, show that the Baltic *E. affinis* should be able to survive in mixed blooms, when the concentration of *Nodularia* is fairly low. This is potentially beneficial as many other copepods just shut down feeding completely, which leads to starvation and doesn't help to break down the bloom. Therefore, it is again important to further investigate the effects of these levels and what different thresholds exist in terms of effect on fitness, specifically, survivorship, feeding, and reproduction effects.

Green Bay

Microcystis also results in a negative effect on *E. affinis* in the Green Bay area in a similar pattern to the *E. affinis* affected by *Nodularia* in the Northern Baltic Sea. Although the overall effects were negative, there were differences in the impacts driven by the *Microcystis* as compared to those caused by *Nodularia*. The starting concentrations for the Green Bay grazing experiment turned out to be only about 10% of the concentration we intended to ensure that food abundance was at a saturation level for feeding (500µgC/L). The source of error for this comes from using cell counts of the culture from the previous experiments run during the summer. When the acclimation period and grazing experiment ended, there were not enough surviving and healthy copepods to carry out the egg production experiment with the same group of animals. The grazing experiments therefore employed a separate group of animals that were acclimated to the feeding conditions. We later re-measured the cells of the algal cultures and realized they were smaller than the measurements taken over summer and therefore the biovolume calculation to convert into µgC/L was inaccurate. While the concentrations were enough for some copepods to survive, it was below the saturation feeding limit, meaning that the clearance rate was still potentially affected by food abundance.

The only microcystin toxin level detected in the three treatments was in the S+M10%, found to be 0.306 µg/L at the end of the grazing experiment. The initial sample for this treatment taken at the beginning of the grazing experiment evaporated during storage, so it is impossible to tell if there were changes during the experiment. However, initial analysis of the samples using the standard ELISA protocols showed that all others were below the detection limit (0.16 ug/L). Samples were then reanalyzed employing a higher sensitivity protocol (with the ability to detect 0.05 ug/L). Unfortunately, the MF 24hr treatment samples did not get rerun under higher sensitivity. However, levels measured in the initial run show that they were similar to the values of the S100% and contained no detectable toxin concentrations. We also had

samples from MF treatments after 48hrs and they indicate no detectable toxin concentrations (data not shown).

Due to the large mortality at the end of the grazing experiment, there are no survivorship results presented. We did record the number of females that survived to the end of the experiments in order to calculate the average number of copepods in the treatment bottle, which was needed to calculate the grazing rate. However, the survivorship data are not reported because there are too many factors that could contribute to the high mortality aside from the effect of *Microcystis* cells or dissolved microcystin. The main discrepancy would be the decrease in food availability that could have produced starvation without any relation to the toxin itself.

Grazing Experiment

The Chlorophyll *a* concentrations indicate that there is an irregular pattern occurring in the grazing experiment for the S100% and MF treatments. The control algal concentrations decrease to below the starting concentrations while the experimental concentrations at the end are comparable to the starting concentrations. This leads to the suggestion that there may be intercellular interactions occurring to cause the decrease in the concentration of algae in the control bottles. The S+M10% treatment shows the opposite effect, with the control treatment bottles showing an increase in algal concentration while the experimental bottles decrease, which would generally indicate feeding is occurring. The samples taken from the treatments were small samples, and in addition to the decreased food concentration, there was large variability between the samples, indicating that Chlorophyll *a* may not be the best measurement to analyze the changes in algal abundance at these low concentrations. They expected concentrations appeared to fit within the calibration curve for the procedure but the variability associated with the values are large and another form of measurement would be better to analyze the grazing results. There also is the possibility of error with the spectrophotometer.

Cell counts were also taken from each treatment, and will be the primary measurements used for drawing conclusions from the grazing experiment. While human error is still possible through cell counts, it allows for a higher confidence level because it is based on physical observation of the actual abundance of the two cell types in the sample. The ability to examine the two cell concentrations separately is also an important advantage over the Chlorophyll *a* analysis. The cell counts also show a decreased algal concentration in the control bottles for the MF treatment, as well as for the S+M10% treatment. The cell counts for the S100% treatment show a "regular" grazing rate response, with increased algal concentration in the control bottles and decreased algal concentration in the experimental bottles. These data result in a negative filtration rate for the R+Filt and R+N treatments, meaning such a small filtration rate is too low to detect. The filtering rate of 1.5mL/copepod/hr seen for the S100 treatment in our experiment is higher than other reported values for calanoid copepods feeding on *Scenedesmus,* reported to be approximately 4.1mL/copepod/day (Malovitskaia and Sorokin, 1961 as cited in Wetzel, 2001). However, our results are supported further by findings from Richman et al. (1980) that show *E. affinis* grazing rates similar to ours, but much larger in comparison to other calanoid copepods from Little Sturgeon Bay (same location as our study).

The negative ingestion rate for the MF and S+M10% treatments do not appear to be a result of starvation due to the error in the amount of food added, as the concentrations are higher than those at the start. Also, the S100% treatment shows successful grazing. In an attempt to investigate the effect of the lack of growth in the control bottles, the calculation was adjusted to examine grazing rates without regard to the control group. This resulted in the filtration rate of the S+M10% becoming comparable to the S100 treatment and the MF treatment increasing to be about 1/3 the rate of the other treatments. The ingestion rates changed so the S+M10% was actually slightly higher than the S100 treatment with the MF treatment about ¼ the rate of the other treatments. However, studies have shown that the cyanobacteria may result in allelopathic effects causing a decrease in algal concentration not related to grazing

(Leao et al., 2009). Therefore, it is important to keep the controls in this analysis to be able to factor in the decrease in the control. If there was little to no change, then it may be appropriate to eliminate the controls in the equation.

The cell counts therefore indicate that feeding rate was significantly reduced due to exposure to either *Microcystis* or its filtrate. Richman et al. (1977) show that *E. affinis* have a restricted feeding rate in that they selectively feed on large particles within a spectrum, and when given a diet of small sized particles, their feeding becomes limited. Therefore, they may select not to feed on *Microcystis* because of its small cell size (less than 5 um cell diameter), however this would not apply to the MF treatment since this contains only *Scenedesmus* cells (diameter greater than 15 m), which the S100 treatment shows they are feeding on (Richman et al., 1980).

Additionally, the cell counts can be used to distinguish specifically the change in algal concentrations of the *Scenedesmus* and *Microcystis* individually among the treatments. In the S+M10% treatment the *Scenedesmus* decreased by about 0.03mgC/L whereas the *Microcystis* concentration decreased by a factor of ten in the control bottle. As there were no grazers in the bottle to deplete the algae, this suggests that allelopathic forces from the *Microcystis* are acting on the *Scenedesmus*. The experimental bottle had about twice the concentration of *Microcystis* as the control bottle. The *Scenedesmus* also decreased in the experimental bottle, but there was a higher concentration than in the control bottle. This suggests that perhaps the presence of grazers and their uptake of toxins, or other metabolites produced, help to protect other phytoplankton from the cyanobacteria. *Microcystis* has been shown to produce other metabolites that negatively affect other zooplankton resistant to microcystin, such as *Daphnia.* The metabolic compound, now termed DTC (*Daphnia*-toxic compound), was extracted from a treatment with *Daphnia* and resulted in decreased survivorship of the animals (Jungmann, 1995).

The S100 treatment, not containing *Microcystis,* shows an increase in carbon concentration in the control bottle, and a decrease in the experimental bottle. The decrease in the experimental bottle is comparable to the decrease in the control bottle of the S+M10%. This is an interesting comparison, as it suggests that *Microcystis* can cause a similar reduction in *Scenedesmus* abundance as the predation effect by *E. affinis.* Of course this would depend on relative concentrations and other parameters existing in the field as well. The MF treatment showed a decrease for both the control bottle and the experimental bottle. While toxin levels were below detection in this treatment, there was still an obvious effect seen, indicating that the *Microcystis* may be producing other metabolites that are having a negative effect on the surrounding phytoplankton. The decrease of *Scenedesmus* in the MF control bottle is similar to the decrease seen in the S+M10% control bottle. This is interesting, as the S+M10% treatment would not be expected to have the same concentration of dissolved toxins as the filtrate, which has all the toxin released into the water.

Egg Production

There is distinguishable variation in the egg production between treatments, but the large number of individuals that did not successfully produce eggs in each of these treatments limits our ability to examine the true distribution of the reproductive success. The lack of fertilization is interesting as the results from the grazing experiment occurred by placing females that had survived but not produced egg sacs during the extended grazing experiment with a male in an individual well of a 12-well plate. This theoretically should have increased the fertilization success with a 1:1 M/F ratio in a small area. The lack of fertilization could be attributed to higher stress levels from being in a small space, or high stress during the two-hour drive from Little Sturgeon Bay back to the laboratory. There also may be individual factors preventing successful fertilization, not attributed to the introduction of toxin. The egg counts also were done to the highest level of accuracy, but it was hard to determine the exact number

of eggs without destroying the egg sac. Lastly, the process of counting the individual nauplii after they hatched did not work because they disintegrated within hours after hatching, and Lugol's preservative was often not added quickly enough to preserve them in a clear enough state for accurate counts.

Individuals in the MF treatment produced brood sizes that were over twice as large as any individual produced in either of the other two treatments. This indicates perhaps the microcystins, or other metabolites released, are potentially beneficial to *E. affinis*, but are counteracted by the presence of the actual *Microcystis* cells. We were not able to obtain information on size of the nauplii as they did not survive long enough to be counted accurately. Reinikainen et al. (2002), found that eggs that hatched and were exposed to *Microcystis* filtrate resulted in higher death rates of nauplii than eggs fertilized in conditions where the adults were exposed to *Microcystis* filtrate. However, when moved to natural water to hatch, there was very low nauplii mortality. This indicates that the *Microcystis* filtrate appears to not be affecting the production of the eggs, but rather has a negative effect upon direct exposure to the eggs.

Our grazing experiment indicates that *E. affinis* shut down feeding in both the MF and S+M10% treatments, but the lack of reaching the saturation concentration of food abundance may misrepresent the actual feeding conditions. Previous studies have determined other calanoid species have been able to feed on *Microcystis* in mixed food treatments (DeMott & Moxter, 1991). This feeding on *Microcystis* could result in a decrease of their nutritional consumption, which may then contribute to decreased egg production.

Overall Analysis of Effect of Microcystis on Eurytemora affinis in Green Bay

These results indicate that the presence of *Microcystis* in the diet of *E. affinis* does result in multiple negative effects. The presence of the actual *Microcystis* cells results in the shutdown of the feeding mechanisms and decreased egg production. The MF treatment indicates that the

filtrate of the *Microcystis* also results in a feeding shut down, but egg production is not compromised and, according to our study, is comparable to that obtained in the S100 treatment. Due to the rapid decomposition of the nauplii after hatching, we were not able to determine the effect of the treatments on the nauplii size from these experiments. Therefore we are not able to draw any conclusions on the quality of the eggs or nauplii produced, except that they did not survive long after hatching. An experiment that is able to create conditions to keep the copepods alive through the entire run of the experiment will be beneficial to gaining greater insight on all of the connections of this experiment. However we were able to draw conclusions on individual components successfully without having a cohort of copepods completing the entire cycle of the experiment.

Comparison of Overall Effect of Toxic Algae on Eurytemora affinis in the Baltic Sea & Green Bay

The populations of *E. affinis* both show negative effects from the presence of toxic algae. The specific negative effects differ between the presence of actual toxin producing cells and released dissolved toxins. The magnitude of the toxin concentrations varied among the treatments in both study sites, but there appears not to be a large difference in the magnitude of the response. The toxins themselves however are known to have different responses when exposed to zooplankton. There are lower levels of nodularin produced in the presence of zooplankton, but in freshwater systems an increase in microcystin production may occur when cells are exposed to grazers. However, it is not yet known if the grazers are a direct trigger for the production increase or decrease, or if there is another mechanism creating this change in toxin abundance associated with the presence or absence of grazers (Sopanen, 2009). Another important connection between the Baltic population and Green Bay population is that both the R+F and MF treatments followed the same patterns throughout the various parts of the experiment that were carried out in both locations. Since the R+Nut followed similar patterns to

the R+F treatment, it would be interesting to see the results of a treatment incorporating the *Microcystis* nutrient broth. The R+Nut did not completely shut down feeding, but had a much lower ingestion rate in comparison to the Rhod treatment, and it did not experience the significant decrease in survivorship during the grazing experiment. However, the egg production and nauplii sizes were comparable to both filtrate treatments. This followed the same general results in comparison to the other treatments as the MF treatment in Green Bay, with a feeding shutdown and increased egg production in comparison to the treatment with the cyanobacteria cells. The magnitude of the increase of eggs differed as the MF treatment actually produced twice as many eggs on average per female than the S100 treatment, whereas the R+F only produced 85% of the average eggs produced by the Rhod treatment. The R+Nut did have higher egg production (120% of Rhod treatment), so again this could be an important component of a further study. In terms of the range of egg production between the filtrate treatments of the two populations, the maximum eggs per female was comparable but there was a lower overall number of females producing eggs in the Green Bay study. This makes it difficult to be able to accurately compare the sampling distributions.

The largest difference between the two populations is that our study shows the Baltic population is able to still feed in the presence of the actual *Nodularia* cells, but the Green Bay population shut down feeding in the presence of *Microcystis,* as seen by the negative filtration and ingestion rates, This is initially surprising as the current environmental conditions during the time of sampling had much larger blooms occurring in Green Bay, and there was no actual bloom at the time of sampling in the Baltic Sea. This would suggest the opposite results to be expected as the Green Bay *E. affinis,* would be expected to have a higher tolerance. This could however, be an important indicator in the evolution of the invasive *E. affinis,* in the freshwater system. Another calanoid copepod, *Acartia tonsa,* was treated with various diet mixtures of the nutritional diatom *Thalassoiosira weissflogii* and gradually increasing concentrations of

Nodularia or *Microcystis (*Schmidt and Jonaasdottir, 1997). This study showed the opposite response to our study as this species did not feed at all on *Nodularia,* but it did feed on *Microcystis.* Additionally, large concentrations of *Microcystis* mixed in with *T. weissflogii* resulted in the same results as our study with decreased egg production and mortality. However, low enough concentrations resulted in what we found with the *Microsystis* filtrate and increased egg production. This study concluded that cyanobacteria are not nutritionally stable enough to be the main part of a diet for zooplankton, but it is possible that they can be beneficial in supplementing the diet. This, with the combination of our data from the filtrate treatments, gives greater reason to investigate more of the other metabolites *Microcystis* produces and their role and abundance in various environments. However, both locations have experienced high toxic algal blooms over the past decades, ensuring that the environment is readily exposed to the toxins.

Additionally, temperature has been shown to play an important role on grazing rate, as another limiting factor besides food availability. Therefore temperate locations have been shown to have higher clearance rates as compared to areas of higher latitude and lower temperature (Huntley & Boyd, 1984). This supports the results of our findings as the Green Bay clearance rates are about three times higher than those from Baltic populations. Our initial temperature for the Green Bay experiments in the summer was 23°C, but we decreased it to 18°C in the fall for the grazing experiments. This is also important to note when applying the laboratory results to the field, as these two populations do experience different seasonal changes at their different latitudes. Again, the egg production is comparable between these two populations, but the Green Bay population has a slightly higher percentage in comparison to the control bottles for the good food source, with 74% compared to R+N's 60%. The Nod50 treatment had an even lower percentage of egg production in comparison to the Rhod treatment, indicating that the concentration of toxin may play a role in affecting egg production, rather than just the presence or absence as indicated

in other parts of this study. The percentages are important for the comparison of these treatments, as it helps to adjust for the smaller sample size in Green Bay due to the higher mortality throughout the experiment. Temperature also is an important factor affecting grazing rate and can affect not only the number of eggs produced, but also may change the time required before a new clutch of eggs can be produced. This may create a problem for the population in the long run (Williamson & Butler, 1987). This same study also noted that feeding rate of copepods may vary within not only the various habitats encountered, but even among the locations selected by the copepods each day according to its vertical migration behavior. There will often be variation in the abundance and composition of food at different depths because the algal concentrations will be higher in the shallower areas in the photic zone. However, this may also result in greater exposure for predation risk, so during the light period the copepods will typically select greater depths to avoid visual predators and consequently be exposed to lower food concentrations.

There are many areas that could have been improved to achieve a more complete set of results from the Green Bay studies. The Baltic portion of this study ran smoothly, with the only real deviation from the plan being the increase in starting algal concentrations, which again had no appreciable effect on the experiment itself. An additional improvement would have been to start with a larger number of animals per treatment, to hopefully ensure a large enough number of animals could survive through to the end of the experiment. A lot of the problems that occurred in the Green Bay experiments will automatically be prevented in subsequent studies, as future studies are improved based on trial and error while discovering appropriate field and laboratory procedures and conditions. At the beginning of this experiment the incubator containing the copepods was started at 23℃, which another study determined could significantly decrease egg production (Dur et al., 2009). However, this was the field temperature from the first collection of copepods in July.

Eurytemora affinis, are only being studied by a few people in the U.S. currently, and there is a lot of progress to be made in this area of research for the freshwater populations. Having an accurate concentration of an actual good food source and running the experiment at a constant and stable temperature for the animals will result in a large reduction of the problems encountered in this experiment. Additionally, there were some issues with mislabeling and losing certain samples. Luckily there were enough samples but a more conscientious effort should be made in sample taking and storing.

Another component of this study that would have been beneficial to monitor more closely in Finland, and which was not monitored at all in Green Bay, was pH. While we measured the pH at the beginning of making each of the treatments, we could have done a better job of monitoring them throughout the experiment. As photosynthesis occurs the amount of DIC in solution can decrease. This in turn can lead to an increase in pH, which may affect some algae. While *Nodularia* has a high tolerance for higher pH conditions, other algae are not as able to survive in these conditions, which could mean that the pH of the nutrient broth becomes a limiting growth factor in treatments with a single algae. The *Nodularia* could be controlling the pH level when present if they are able to photosynthesis appreciably (Mogelhoj et al., 2006). This increase in pH may help explain the negative effects seen on the *E. affinis* through the lack of *Rhodomonas,* rather than the direct effect of toxin on *E. affinis* or specific allelopathic interactions between the two algal species. Previous studies show that it is difficult to determine whether it is specifically the nodularin having the negative effects or if it is another metabolite produced by the cyanobacteria (Suikkanen et al., 2006). In the Baltic experiments the pH actually slightly decreased after the 48hrs of the grazing period, so it appears as though this would not be playing a major role in causing the observed effects. However, these measurements were taken from the large starting culture so it was never exposed to the

presence of *E. affinis.* A preliminary experiment to determine the specific effects of the nutrient broth used would be beneficial in future experiments.

The next logical step will be to do an extensive rerun of this Green Bay experiment. The treatments employed in the Baltic experiment should all be included in future Green Bay studies. . The nutrient broth medium may be playing a role in the results so this will be interesting to see if it follows the pattern of the nutrient broth used in the Baltic experiment, or if its presence shifts the findings in this experiment. If feasible, it may be beneficial to increase the number of treatments to be able to test various concentrations of *Microcystis* to get a better idea of how much the actual concentration of toxin is affecting the *E. affinis,* rather than simply the response to the presence versus absence of the toxin. It will also be beneficial to do as best as possible to actually preserve the nauplii to obtain measurements that would permit a comparison of the quality in terms of size of the nauplii to the number produced per female. Additionally, it would be interesting to do a simple feeding experiment to try to determine the effect of particle size in the grazing rates, as *Nodularia* and *Microcystis* are very different in their cell size and formation of colonies. As the two copepod populations have been introduced to their respective cyanobacteria communities over time, it is likely that they may have adjusted to these food size ranges. With respect to the specific green algae chosen as a good food source, the relative size of the good food compared to the toxic species may play a factor regardless of toxin production or even the overall nutritional quality of the cells.

Given these two populations of copepods are distantly related but now occur in different locations, another study to try would be to expose each copepod population to the natural cyanobacteria from the other location to see if they respond differently. The toxins produced by *Nodularia* and *Microcystis* are related, but the cells themselves are different, so it would be interesting to see if the two populations are able to respond the same to the different

cyanobacteria. This would also give greater insight into the dynamics of the evolution of the *E. affinis* that has separated from the original European population.

Once these experiments are run to develop a stronger baseline of information, a greater focus on the mechanism behind these results should occur. This will include not only analyzing the extracellular and intracellular toxins and their transfer to copepods, and then through the rest of the ecosystem, but also investigating what else may be produced from these cells to have an effect on zooplankton, or other phytoplankton, besides the known nodularin and microsystin. Lehtiniemi et al (2002) investigated the transfer of toxin through the rest of the ecosystem with the conclusion that the ingestion of the toxin is the greater vector for passing it through the ecosystem, rather than it being picked up directly from the water.. As the conditions for blooms continue to become more common with climate change, and water temperatures continue to change, it is important to evaluate how ecosystems are going to respond to the increase in cyanobacteria. This increase in blooms and toxins, which are thought to follow a similar path through the ecosystem as the microbial loop, could potentially result in a large change throughout the entire ecosystem. If the long-term effects start to eliminate key populations, this will create a problem for the functioning of the entire ecosystem. As the copepods are a vector for the toxins, they are also a vector for the energy created by phytoplankton through photosynthesis. If this vector is removed a shift may be made in the ecosystem, but the actual consequences of these shifts need to be carefully examined to ensure no unforeseen detrimental links are destroyed and cause a collapse in the ecosystem that could have been prevented.

This area of science needs to be better investigated to get a better grasp of whether there is a stopping point in the ecosystem of the extracellular toxin, or if it continually flows through the ecosystem by its various vectors. The *E. affinis* are a crucial part of the aquatic ecosystem. Not only are zooplankton essential in passing on the energy generated by the algae, but this specific

species is better able to feed on the toxic algae, which may be beneficial in helping to eliminate blooms. Yet, if this consumption has a greater negative effect in the long term, it is also important to spend enough time looking into the changes that will occur in the ecosystem if the dynamics of the population of *E. affinis* changes, or if the blooms themselves are changing. If dynamics or other species are affected by the presence of the blooms, these could also result in indirect negative effects that need to be better investigated. This is important as the trophic levels in the aquatic systems are closely connected, and depletion of *E. affinis* will greatly change the composition and dynamics of these components of the food web. Additionally, *E. affinis* is currently being utilized as a key parameter for checking water quality with new ecological models; therefore, it is important to understand as much as possible about their life history and reproduction, to be able to better track any changes that are occurring and to determine specific thresholds for proper regulation.

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