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EFFECT OF THE TOXIGENIC ALGA *PRYMNESIUM PARVUM* ON A NATURAL ZOOPLANKTON COMMUNITY

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EFFECT OF THE TOXIGENIC ALGA *PRYMNESIUM PARVUM* ON A NATURAL ZOOPLANKTON COMMUNITY

A THESIS APPROVED FOR THE
DEPARTMENT OF BIOLOGY

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DEDICATION

I would like to dedicate this thesis to my father, Chester Eugene Allison, and my grandparents, William Lee Bledsoe and Opal Margaret Bledsoe, who are not here to see my successes, but whose love and commitment to me has always carried on, and who would be immeasurably proud of who I have become and what I have accomplished.

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ABSTRACT

Harmful algae blooms (HABs) have the ability to produce profound changes to aquatic communities through a variety of factors, most commonly their propensity to produce toxins that can harm co-existing organisms. Many zooplankton species are particularly susceptible due to their close trophic relationship as grazers and shifts in zooplankton community structure may result due to species-specific responses such as selective feeding. I hypothesized that selective feeding would alter zooplankton community structure, with overall zooplankton densities decreasing and non-selective grazers dropping out of the community. My study investigated how the toxigenic haptophyte alga *Prymnesium parvum* affects a natural zooplankton community under controlled laboratory conditions. I found that species responses varied, with significant reductions in two taxa known to be non-selective feeders, *D. menodotae* and *Keratella* sp. A predatory species, *Asplanchna* sp., also experienced a significant reduction, possibly due to reductions in prey sources. These reductions drove an overall shift in community structure. In addition, *Prymnesium* cell density had a significant effect on the abundances of all three zooplankton taxa, although this was the result of reductions in specific species and not overall reductions. My study resolves the more intricate relationships related to selective feeding among zooplankton species and provides solid evidence that *Prymnesium* blooms can have detrimental effects on zooplankton community composition and may lead to long-term community changes that can have a profound influence on the affected ecosystem.

INTRODUCTION

Invasive species have been thrust to the forefront of ecological study due to the explosive rate at which exotics have spread worldwide. Human activities, including direct movement of species and changes to ecological landscapes, such as habitat destruction and anthropogenic inputs of nutrients, have facilitated the ability of non-native species to invade new habitats with increasing success (Lockwood 2013). Once established, invaders can have wide-ranging effects on the co-existing organisms in the newly invaded range through both direct and indirect means. Native communities can be disassembled, caused by reductions in species abundances, species richness, and competitive exclusion that shifts the spatial organization of species, all driven by a variety of mechanisms including niche exclusion, predation, or chemical cues (Sanders et al. 2003; Mack et al. 2000). Invaders that are superior competitors can displace native species by overtaking their functional rolls, while other invaders possess toxins that can directly eliminate natives (Mooney & Cleland 2001; Landsburg 2002). However, while not every invasion event leads to ecological trauma, invaders often induce trophic changes that can disrupt the function of an ecosystem (Gallardo et al. 2015).

Harmful algae blooms (HABs), some of which are produced by invasive algal species, have produced devastating consequences worldwide by their ability to dominate pelagic phytoplankton via a variety of hypothesized mechanisms including toxicity, low nutritional value for grazers, increased competitive abilities

following environmental change, and subsequent ability to produce strong bottom-up effects in the afflicted community (Landsburg 2002; Gilbert et al. 2005). HABs can produce direct deleterious effects on a wide range of aquatic organisms at all levels of the food web, including fish, mussels, other microbial species, and zooplankton grazers, thereby effectively changing the structure of the community (Remmel et al. 2011; Zamor et al. 2014; Acosta et al. 2015; Liu et al. 2015). As anthropogenic additions of nutrients to the environment increase through both point and non-point pollution, so does the threat of increased frequency of blooms and the negative ecological effects that follow (Anderson et al. 2008).

At the front line of interaction with harmful algae (HA) are zooplankton, many of which are filter feeders that directly consume algae and other suspended matter. Zooplankton are an important functional group in aquatic food webs, serving as the nexus of primary production (in the form of phytoplankton) and higher-level consumers, such as fish. Much is known with regard to the physiological responses of zooplankton species exposed to various HA, with observed reductions in ingestion, growth rates, fecundity, and survivorship (Colin & Dam 2002; Remmel et al. 2011). However, these effects vary among zooplankton and algal species, with some zooplankton species seemingly unaffected by toxic HA (Turner & Tester 1997). A meta-analysis by Tillmanns et al. (2008) presented the results of 376 treatment-control paired experiments investigating the effects of cyanobacteria on zooplankton population growth rates amongst a variety of cladoceran and rotifer species. They found wide variations in response among

different species within the same taxa, supporting the hypothesis of interspecific variation in zooplankton responses to HABs. This imbalance in species responses can ultimately lead to changes in overall community structure by altering either the total abundance of grazers, abundance of a particular species, or complete removal of species (Hambright et al. 2010). Leonard & Paerl (2005) found that if the toxic cyanobacteria *Cylindrospermopsis raciborskii* was present in low levels within a river system, the zooplankton community was diverse. However as the concentrations of this alga increased the abundance of larger zooplankton decreased, thought to be the result of competitive exclusion by smaller species that would readily feed on the toxic alga.

Predicting freshwater zooplankton community responses to HABs can be difficult, largely due to the complex nature of aquatic systems. While the primary driver of the response may be assumed to be the HA, additional environmental factors such as seasonal trophic level shifts or unknown anthropogenic inputs may enhance or mitigate the effects of the HA. However, some predictions can be made based on grazing selectivity and feeding strategies. *Daphnia* sp. and some groups of rotifers, such as *Keratella*, *Polyarthra*, and *Synchaeta*, are known to feed indiscriminately, using only mechanical cues for food selection, while other groups such as *Bosmina* and copepods employ chemical cues to differentiate between nutritionally high-quality items and those which may be less nutritious or toxic (DeMott 1990). Extensive studies have looked at responses of zooplankton to the toxic cyanobacteria *Microcystis aeruginosa* and *Daphnia* have consistently been

shown to be susceptible, exhibiting no reduction in feeding (Lampert 1981; Fulton & Paerl 1987), while copepods avoided consumption (Fulton & Paerl 1987). Hansson et al. (2007) found similar results, with *Daphnia* abundances negatively correlated to bloom events. However, there was interspecific variation in copepod behavioral responses, with larger calanoid species being negatively affected, but smaller cyclopoid species showing avoidance similarly to smaller cladocerans such as *Bosmina*. Research with other toxic algal species has supported the trend of selective feeding by calanoid copepods. In the presence of the toxic haptophyte species *Prymnesium patelliferum, Acartia clausi*, a calanoid copepod, exhibited reduced feeding and subsequently showed no reduction in survival rates at cell densities above those seen during blooms, although egg production was reduced (Nejstgaard & Solberg 1996). Some copepods also have an additional advantage over non-selective feeders in that they are omnivorous and can exploit other food resources (Dodson 1974) therefore enhancing their ability to withstand the negative affects of blooms. Furthermore, resistance to algal toxins by some zooplankton species provides an additional competitive advantage, although resistance can be an adaptation that evolves over time and can be difficult to predict (Fulton & Paerl 1987; Roelke et al. 2015). The culmination of both feeding selectivity and adaptation is likely to play an important role in how zooplankton communities respond during large HAB events. It has also been suggested that these community structural changes can subsequently serve as a positive feedback mechanism, wherein the reduction of non-selective feeders can exacerbate the severity of a bloom, allowing it to proliferate with no form of natural control

(Sunda et al. 2006). Often, HAB events follow blooms of non-HAB species due to nutrient limitation, which favors the development of mechanistic deterrents, such as toxicity, in the HAB species (Anderson et al. 2002). Subsequently, this un-palatability reduces grazing, thus reducing nutrient regeneration and further enhancing nutrient stress allowing the bloom to proliferate (Mitra & Flynn 2006).

The toxic, haptophyte alga *Prymnesium parvum* is an established nuisance species in freshwaters, having acclimated from its marine origins (Edvardsen & Paasche 1998). The first known appearance of *Prymnesium* in the U.S. occurred in the Pecos River, TX in 1985 and it has since spread to 23 other states (Roelke et al. 2016). The environmental conditions conducive to blooms of *Prymnesium* have been well studied and it has been shown that a salinity of 1.67 parts per thousand (ppt) as well as cooler water temperatures are generally a requirement for bloom formation, reflecting its origins in marine systems, and thereby limiting widespread blooms (Hambright et al. 2015). Despite the limited nature of these blooms, the consequences have been devastating. *Prymnesium* produces a suite of toxins that can have cytolytic, hemolytic, and ichthyotoxic affects and can thereby lead to the massive fish kills that have been observed during blooms (Parnas 1963). While the exact mechanism of toxin delivery is unknown, it has been shown that cell-to-cell contact, possibly aided via an appendage called the haptonema, is necessary to produce toxicity to fish (Remmel & Hambright 2012). However, in addition to this physical delivery of toxins, zooplankton likely incur increased effects via ingestion while filter feeding. Remmel et al. (2011) found that *Daphnia*

fed live *Prymnesium* experienced reduced fecundity, shifts in reproductive strategies, and reduced survivorship. In addition, it was found that during lethal concentration bioassays (LC_{50}) utilizing extracted *Prymnesium* toxins, *Daphnia* were much more susceptible to the toxins than copepods (Remmel unpublished). Sopanen et al. (2006) demonstrated that the copepod *Eurytemora affinis* became inactive in the presence of *Prymnesium* resulting in only sublethal effects of reduced fecundity but no immediate mortality. In-situ monitoring of a reservoir during a *Prymnesium* bloom resulted in the absence of cladocerans and also showed a strong reduction in rotifer abundance as cell densities increased (Roelke et al. 2010). However, in a similar reservoir comparison, comparably lower rotifer losses in a reservoir that had only experienced low *Prymnesium* densities versus extensive blooms suggests the possibility that rotifer populations possess the ability to acquire toxin resistance (Davis et al. 2015). These studies suggest that selective feeding or toxin resistance may produce differential survivorship during a *Prymnesium* bloom and may affect zooplankton assemblage structure.

Intermittent blooms of *Prymnesium* have occurred in Lake Texoma (OK-TX) since 2001. These occurrences tend to be restricted to coves within the reservoir due to a strong salinity gradient produced by inflow from the highly saline Red River in the west and freshwater input from the Washita River in the east (Hambright et al. 2010). Large and devastating fish kills have resulted from these blooms, but population recovery has been swift, likely due to the small scale of blooms and subsequent immigration (Zamor et al. 2014). However, the fate of

affected zooplankton communities is not as well studied, although evidence of small-scale shifts does exist (Hambright et al. 2010). The aim of this study was to understand the impacts of *Prymnesium* on zooplankton community structure. I used an environmentally controlled microcosm study and varying *Prymnesium* cell densities to test whether selective feeding or possible toxin resistance would produce differential survivorship within the community. I hypothesized that selective feeding would alter the zooplankton community structure, with overall zooplankton densities decreasing and non-selective grazers dropping out of the community.

MATERIALS AND METHODS

Study site and zooplankton collection

Lake Texoma (OK-TX) has a history of *Prymnesium* blooms, with the first reported bloom occurring in 2004 (Hambright et al. 2010). Blooms have historically been isolated to the western sides of the lake where salinity remains higher due to inflow from the Red River. Long term monitoring of *Prymnesium* cell densities across the lake, both littoral and pelagic, has provided a clear picture of the seasonal dynamics of blooms, offering the opportunity to study zooplankton communities based on prior exposure (Hambright et al. 2010). Because adaptation may lead to toxin resistance as the result of long term exposure and could misrepresent the immediate effects to a zooplankton community during a bloom, I chose to study a community that has not experienced high bloom densities, yet has

the potential to encounter a *Prymnesium* bloom in the future. Zooplankton were collected from the Soldier Creek Inlet, an area of the lake for which cell densities to date have never exceeded 3000 cells mL^{-1}, thus reducing the chances of exposure-mitigated toxin resistance. Zooplankton were collected using both 63*μm* and 350*μm* Wisconsin nets to ensure a complete collection of the community, inclusive of all size ranges. In addition, water was collected at ~1m depth using a Van Dorn sampler and filtered through 20-*μm* Nitex netting to remove all zooplankton while still maintaining the natural algal community. This provided a semi-natural environment inclusive of the natural assemblage of phytoplankton (but not containing *Prymnesium*), as well as the ability to control the abundance of zooplankton in the experiment. Salinity and water temperature readings were taken at the time of collection and were recorded at 0.91 ppt and 23.8°C respectively.

Community composition

To evaluate the effects of *Prymnesium* on the zooplankton community, experiments were conducted in 2.5-liter Nalgene bottles with addition of lab-cultured *Prymnesium*. Batch cultures of *Prymnesium* (University of Texas Culture Collection of Algae, UTEX; culture LB 2797; originally isolated from the Colorado River, Texas) were maintained in COMBO medium (Kilham 1998) with a molar N:P of 16:1 (800 μmol L^{-1} nitrate N: 50 μmol L^{-1} phosphate P) and salinity of 6 g L^{-1} (6 ppt) of Instant Ocean® (Hambright et al. 2014). Approximately one month prior to experimentation, fresh cultures were inoculated under the same culture conditions

and were maintained on a 12-h light:12-h dark cycle at 20°C, with constant aeration by bubbling with filtered ambient air to enhance optimal growth. Because *Prymnesium* blooms in Lake Texoma are capable of producing fish kills at ranges of 25,000 to 200,000 cells mL^{-1} of *Prymnesium*, we chose to examine densities of 50,000, 100,000, and 200,000 cells mL^{-1} in order to determine whether cell densities were an important factor in responses. *Prymnesium* cultures were diluted with 6 ppt 16:1 N:P COMBO to acquire the desired cell densities for each treatment (verified using microscopic counts, Utermöhl 1958). Six replicate bottles were randomly created for each treatment, containing 2.13 L^{-1} of filtered lake water, 0.17 L^{-1} of the respective *Prymnesium* dilution, and 0.2 L^{-1} of the zooplankton suspension. Average salinity increases due to the addition of *Prymnesium* culture were 0.35 ppt and the average nutrient increase was 0.00001 ug L^{-1} P, based on the volume of COMBO addition, with no measurable addition of N. To account for these additions, and ensure no significant resulting effects to the community, six replicates of both nutrient and salinity controls were established using 0 ppt 16:1 N:P COMBO and 6 ppt no nutrient COMBO, respectively, in equivalent amounts to those in *Prymnesium* treatments. Six replicate bottles representing initial community composition were filled with 2.3 L^{-1} of lake water and 0.20 L^{-1} of the collected zooplankton mixture (additional lake water accounted for the earlier additions of *Prymnesium* or control mixtures) (Figure 1). A 2-mL sample was taken from each bottle to measure chlorophyll using a Trilogy® Laboratory Fluorometer and a 10-mL subsample was also taken and preserved in 1% Lugol's solution. Bottles were placed on a laboratory bottle roller at 0.5 rotations min^{-1} to create

gentle mixing and prevent settling of particles. Incubation was maintained on a 12-h light:12-hr dark cycle (GE© F40 plant and aquarium florescent bulbs; ~μmol m^{-2} s^{-1}) at a temperature of ~23°C, representing current lake conditions at the time of zooplankton collection. After 48-hr, bottles were removed and each was sampled for chlorophyll. A 10-mL sample was preserved in Lugol's for *Prymnesium* counts. The remainder of each bottle was filtered through 20-*μm* plankton mesh to collect all zooplankton, which were preserved in a 70% ethanol-5% glycerol solution. Identification and enumeration of adult crustacean zooplankton was completed using dissection microscopy (50-100× magnification). Due to the high abundance of copepod nauplii and rotifers, inverted microscopy (200× magnification) was used for identification and enumeration in subsamples representing 20% of the total sample and results were multiplied by 5 to estimate overall abundance.

Statistical analysis

I found that one of the replicates within the nutrient treatment was distinctively far removed from the mean. It was determined to be an outlier based on the criterion of Leys et al. (2013) using the absolute deviation around the median at a very conservative value (threshold of 3). I tested for differences in total zooplankton abundance between treatments using one-way ANOVA and Tukey post-hoc tests following confirmation of normal distributions (Shapiro-Wilk test, Table 1.1) and homogeneity of variances (Levene test, Table 1.2) (SPSS version 19.0). A Non-Metric Multidimensional Scaling (NMDS) analysis was used to examine the community composition of each treatment, using individual species

abundances based on Bray-Curtis similarity indices (PAST version 2.0). Species that did not occur in ≥ 10% of samples were removed to avoid skewing based on rarity. I then ran a PERMANOVA (9999 permutations) to determine whether significant differences existed between communities using sequential Bonferroni significance corrections (PAST version 2.0) (Anderson 2001). An indicator species analysis (ISA) to determine which species were driving any seen differences allowed me to assess whether feeding selectivity played a part in the community composition (R version 3.2.1) (Dufrêne & Legendre 1997). Lastly, I used linear regression (SPSS version 19.0) at both the species and taxon level to determine if the cell density of *Prymnesium* had an effect on total abundances. A Bonferroni correction for multiple comparisons was used based on an initial alpha of 0.05 and 17 independent samples.

RESULTS

Identification and enumeration of the zooplankton resulted in a community composed of 12 taxa within the cladocerans, copepods, and rotifers (Figure 6). For the nutrient treatment, the calculation for absolute deviation from the median determined that any replicates with total zooplankton abundance above 300 or below 129 individuals L^{-1} should be considered outliers; therefore one replicate was removed from all statistical analysis (n=6, *n=5 nutrient treatment). I attribute this to either a mistake during the initial additions of zooplankton or during the final sampling. I found that the overall abundances of zooplankton were

significantly different between the *Prymnesium* treatments and the controls ($F= 46.15$; $p < 0.0001$, Table 2). Mean abundances of zooplankton ranged from 216 to 223 individuals L^{-1} in the control treatments as compared to 90 to 123 individuals L^{-1} in the *Prymnesium* treatments (Figure 2). Although there were no statistically significant differences in abundances between the three *Prymnesium* treatments, a visible trend can be seen with an overall reduction in the mean from the 50,000 to 200,000 cell mL^{-1} treatment (Figure 3, Table 3). Community composition was significantly different between the *Prymnesium* treatments and controls as shown through NMDS (stress value= 0.08, Figure 3) and significance of this trend was verified via PERMANOVA ($p < 0.001$). However, there were no significant differences between the two controls or between the three *Prymnesium* treatments (Table 4). The composition differences seen between the controls and *Prymnesium* treatments were the result of reductions in the abundances of three specific species groups, as the Indicator Species Analysis determined that *D. mendotae*, *Keratella* sp., and *Asplanchna* sp. were the drivers of change (Table 5). This was due to significant reductions in population numbers in *Prymnesium* treatments versus controls (Table 6). The regression analysis showed that *Prymnesium* cell density had a significant effect on cladocerans, copepods, and rotifers, but only select taxa within those groups were significantly affected (Table 6). *D. menodotae*, one of the indicator species, was the only cladoceran significantly affected by cell density, as was *Keratella* sp. for the rotifers. Within the copepods, *Diaptomus* sp. and nauplii were both significantly affected, although neither was determined to be implicit in driving the distinctive assemblage changes.

DISCUSSION

As the frequency and duration of HABs in freshwater ecosystems increases, so does the possibility of extensive changes to the preexisting aquatic communities that are exposed to HABs, including zooplankton that serve as an important link between algal producers and planktivorous fish (Hallegraeff 1993). I hypothesized that selective feeding would alter zooplankton community structure, with overall zooplankton densities decreasing and non-selective grazers dropping out of the community. I conclude, based on my results, that zooplankton assemblages (in Lake Texoma) are vulnerable to blooms of *Prymnesium* and that overall abundance is reduced along with shifts in the community structure. I found that abundances were reduced by as much as 60% in the 200,000 cell mL^{-1} treatment, although reductions were apparent at all densities. A contradictory study looking at the related toxic species *Prymnesium polylepsi* found no reduction in overall abundance following a bloom (Gorokhova et al. 2014), however this community was initially dominated by copepods, the taxa for which I showed a lesser negative response. Copepod exposure to *Prymnesium patelliferum* has been shown to result in depressed feeding and minimal mortality, although reductions in fecundity were observed (Nejstgaard & Solberg 1996). Additionally, Sopanen et al. (2008) investigated the effect of *Prymnesium* on the copepod *E. affinis* and, while it was shown that copepods can succumb to the presence of *Prymnesium*, at high densities, ingestion was reduced and survival was high, particularly in mixed feeding suspensions where high-quality food was available. Additional high-quality

food may explain the higher copepod survival rate seen in my study, as alternative food sources were available from the native phytoplankton community included in the microcosms. Alternatively, predation on other zooplankton species may have provided a competitive advantage for cyclopoid copepods over other non-predatory species and thus exacerbated the declines seen in *Daphnia* and rotifer species. As my regression analysis showed, some copepods were significantly affected by *Prymnesium* cell density, however these effects were not strong enough to cause any to be indicator species and therefore be a driver in the assemblage changes.

The abundance reductions in my study resulted from a shift in the makeup of the community, with a significant difference seen between the *Prymnesium* treatments and the controls. Further, I concluded that three taxa were responsible for driving these community changes: *D. mendotae*, *Keratella* sp., both of which are known non-selective feeders (DeMott 1991), and *Asplanchna* sp., which are predatory rotifers (Sarma 1993) and may have experienced declines due to reductions in available prey. Studies on the toxic cyanobacteria *Microcystis* have shown similar susceptibilities by *Daphnia* sp. (Hansson et al.2007), corroborating the idea that feeding selectivity plays an important role in how zooplankton community structure changes following a toxic algal bloom. Yet, other studies have demonstrated toxin resistance and reduced feeding for *Daphnia* sp. fed *Microcystis*, which could mitigate species reductions, further complicating the story as to how and why species respond differently (DeMott 1991). It has been shown that

zooplankton populations exposed to toxins have higher survival than those with no prior exposure (Dam 2013). Gustafsson et al. (2005) showed that not only can individuals develop tolerance to toxins after exposure, but that this can also be passed on to offspring. However, resistance to toxins is likely influenced by a multitude of factors, including species-specific responses and levels of exposure; therefore it is difficult to infer when or why resistance may arise. The community in my study had historically limited exposure to *Prymnesium,* although genetic influx from other areas of the reservoir with a history of blooms introduces the possibility that resistance may have played a factor in my results. However, the species specific responses in my study in regards to known feeding strategies leads me to conclude that selective feeding is the mechanism driving the change in species abundances.

Individual species responses to a wide range of harmful algae have been well studied and allude to the possibilities of larger scale assemblage shifts, however minimal studies have looked directly at entire community responses. One such study looking at the in situ response of zooplankton to a *Prymnesium* bloom garnered similar results to mine, with cladocerans completely disappearing and rotifers being drastically reduced (Michaloudi et al. 2009). The diversity of species in this study was low, with 5 of the 7 identified species being rotifers, therefore removing some of the more intricate interactions, such as predation, that can influence assemblage dynamics. An increase in the study of entire assemblages

may lead to a better consensus on how *Prymnesium* blooms can alter zooplankton assemblage organization under varying composition conditions.

A multitude of trophic effects may result from changes to zooplankton communities, affecting both lower and higher levels of the food web. Some studies have suggested that zooplankton trophic level shifts may produce a positive feedback loop, which can enhance toxic algae blooms. Inhibited feeding by zooplankton can reduce nutrient recycling within the system, producing stressed conditions which enhance the formation of toxic blooms, subsequently reducing the availability of quality grazing sources, and further intensifying the ratio of selective to non-selective grazers (Mitra & Flynn 2006; Sunda et al. 2006). Further, bottom-up effects can be predicted based on knowledge of zooplankton-fish interactions. Overall reductions in zooplankton abundance may be especially troubling for fish communities should these overlap with the hatching season of juvenile fish that rely on zooplankton as a primary food source. A study looking at prey selection in juvenile yellow perch showed that newly hatched perch selected copepods but a shift in prey selection towards cladocerans occurred in larger juveniles as capture efficiency of copepods decreased (Graeb & Dettmers 2004). Furthermore, open niches left by removal of species may leave systems vulnerable to additional species invasions. The cladoceran *Daphnia lumholtzi* has become a prominent invader in freshwater reservoirs (Havel et al. 1995) with the ability to outcompete other species, but also possessing distinctively enhanced head spines that can impede predation by young-of-year fish (Engel & Tollrian 2009). Reduced

predation pressure on this species may allow it to maintain great enough population numbers to circumvent displacement during a toxic bloom and subsequently maintain a competitive advantage over other zooplankton. A multifaceted danger consisting of reductions in prey availability coupled with direct toxicity from *Prymnesium* could lead to prominent reductions in fish stock, leading to further trophic changes.

Although the threat of large-scale change exists, it is possible that a community response may be localized and short term. In large reservoirs, such as Lake Texoma, restriction of blooms to localized areas may allow recovery of the zooplankton community once the bloom has collapsed. In studies looking at fish community response to *Prymnesium*, the ability for populations to recover may be not only relative to the frequency and duration of blooms, but might also be impacted by the physical attributes of the system. VanLandeghem et al. (2013) described differential recovery of fish populations between two Texas river basins that have experienced repeated blooms of *Prymnesium* over an extended period of time. Although both systems experienced short-term impacts, the reestablishment of fish communities varied by species as well as by system, indicating that multiple factors, including hydrological differences, may influence community responses. In a similar study, Zamor et al. (2014) found that fish populations within an affected cove of a large reservoir experienced a rapid rebound within 6 months of a toxic bloom, suggesting that immigration from non-affected areas was sufficient to overcome the initial losses. Similar re-establishment of zooplankton communities

is probable but is likely subject to the same specificities of the given system. Chronic exposure to *Prymnesium* has been shown to cause reductions in growth, fecundity, and survivorship in zooplankton (Remmel et al. 2011) and could lessen the ability of populations to rebound if blooms increase in frequency. In addition, the speed at which zooplankton immigration can occur is highly dependent on environmental factors such as water currents and may lead to an inability of communities to reestablish before exposure to another disruptive bloom (Seebens et al. 2012; Reichwald et al. 2013).

Microcosm studies, such as the one I present, only provide a snapshot of acute effects of the toxic alga *Prymnesium* and do not provide a full picture of the complex ecosystem interactions that may enhance or mitigate negative effects over extended time periods. Long-term environmental monitoring within invaded systems can more accurately demonstrate how communities respond, however these efforts can be tireless and costly to maintain. My study resolves the more intricate relationships related to selective feeding amongst zooplankton species and provides solid evidence that *Prymnesium* blooms can have detrimental structural effects on zooplankton assemblages. I have shown that differential survival amongst species can drive changes in abundance and is also influenced by the severity of bloom conditions. This may lead to long-term community changes that can have a profound influence on the affected ecosystem.

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APPENDIX

Table 1 Results from Shapiro-Wilk test for normal distribution and the Levene test for homogeneity of variances. Both P-values were above the significance value (α = 0.05); therefore the assumptions of normal distribution and homogeneity of variances were met for use of parametric statistics.

Treatment	Shapiro-Wilk			Levene			
	Statistic	df	*P*	Statistic	df1	df2	*P*
Nutrient	0.97	5	0.84	1.08	4	24	0.39
Salinity	0.97	6	0.79				
50	0.96	6	0.78				
100	0.91	6	0.42				
200	0.94	6	0.65				

Table 2: ANOVA results for test of significance between groups for total abundance. Significant differences between treatment groups are shown (α = 0.05).

	Sum of squares	df	Mean square	*F*	*P*
Between Groups	88771	4	22192	46.15	< .0001
Within Groups	11540	24	481		
Total	100312	28			

Table 3. Tukey post-hoc results for ANOVA test of significance of total abundance between all treatment groups (α = 0.05). Results denoted by asterisk (*) represent groups that show significant differences in abundance. Control groups are not significantly different, however comparisons between each *Prymnesium* treatment and controls all show significance.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	*P*	95% Confidence Interval	
					Lower Bound	Upper Bound
Nutrient	Salinity	7.41	13.3	0.98	-31.7	46.5
	50	101*	13.3	0.00	61.6	140
	100	114*	13.3	0.00	75.1	153
	200	133*	13.3	0.00	93.8	172
Salinity	Nutrient	-7.41	13.3	0.98	-46.5	31.7
	50	93.3*	12.7	0.00	56.0	131
	100	107*	12.7	0.00	69.5	144
	200	125*	12.7	0.00	88.1	163
50	Nutrient	-101*	13.3	0.00	-140	-61.6
	Salinity	-93.3*	12.7	0.00	-131	-56.0
	100	13.5	12.7	0.82	-23.8	50.8
	200	32.1	12.7	0.12	-5.17	69.4
100	Nutrient	-114*	13.3	0.00	-153	-75.1
	Salinity	-107*	12.7	0.00	-144	-69.5
	50	-13.5	12.7	0.82	-50.8	23.8
	200	18.7	12.7	0.59	-18.6	56.0
200	Nutrient	-133*	13.3	0.00	-172	-93.8
	Salinity	-125*	12.7	0.00	-163	-88.2
	50	-32.1	12.7	0.12	-69.4	5.17
	100	-18.7	12.7	0.59	-56.0	18.6

Table 4. Results of the PERMANOVA comparing community composition differences between treatments based on Bray-Curtis similarity indices (9999 permutations). Values are calculated using sequential Bonferroni correction (α = 0.05).

	Sum of squares	Within group sum of squares		*F*	*P*
	1.335	.4307		12.6	< .0001
	Nutrient	Salinity	50	100	200
Nutrient	------	.796	.003	.002	.002
Salinity	.796	------	.002	.002	.002
50	.003	.002	------	.399	.029
50	.002	.003	.399	------	.135
200	.002	.002	.029	.135	------

Table 5: Results of ISA to identify the species driving assemblage changes (α = 0.05). The three species shown represent those responsible for driving the seen changes in the zooplankton assemblage.

	Cluster	Indicator value	*P*
Asplanchna sp.	1	0.436	0.019
Keratella sp.	1	0.243	0.005
D. mendotae	5	0.306	0.003

Table 6: Results of regression analyses to test the effect of *Prymneisum* cell density on the abundance of each taxa. Asterisks (*) represent groups for which the p-value is below the level of significance (α = 0.003). A Bonferroni correction for multiple tests was used based on an initial α = 0.05 and 17 independent samples.

Abundance (mean ± SD)

Treatments *P. parvum* cells mL^{-1}	Control	50,000	100,000	200,000			
	Zooplankton abundances, mean ± SD				Regression Slope	R^2	*P*
Total Zooplankton	548 ±68.2	306 ±42.1	272 ±43.5	226 ±40.4	−0.478	0.06	0.245
D. mendotae *	37.4 ±10.2	9.2 ±4.36	3.2 ±1.83	2.2 ±0.98	−0.177	0.597	<0.001
Bosmina sp.	22.5 ±7.9	22.7 ±10.5	22.2 ±6.8	23.2 ±12.4	0.005	0.002	0.805
Chydorus sp.	1 ±1.18	0 ±0	0.33 ±52	0.83 ±.41	−0.001	0.005	0.715
Cladocerans *	60.8 ±11.1	31.8 ±13.2	25.7 ±8	26.2 ±12.4	−0.182	0.494	<.0001
E. affinis	17.2 ±7.2	17.5 ±2.9	16 ±1.4	8.7 ±6.9	−0.041	0.242	0.007
Diaptomus sp. *	45.2 ±10.6	36.3 ±9.3	34.8 ±11.8	28.3 ±10.9	−0.084	0.277	0.003
Cyclopoids	48 ±10.8	48.3 ±9.4	40.8 ±9.2	42.7 ±7.8	−0.031	0.06	0.201
Harpacticoids	0.55 ±.82	1.5 ±1.52	1.17 ±1.17	1 ±1.26	0.002	0.012	0.572
Nauplii *	151 ±40.2	113 ±25.4	101 ±17.4	86.7 ±38.7	−0.316	0.347	0.001
Copepods *	261 ±48.5	216 ±30.4	194 ±27.9	167 ±41.7	−0.424	0.409	0.001
Keratella sp. *	206 ±53.2	58.3 ±16	53.3 ±15.4	30.8 ±13.6	−0.856	0.580	<0.0001
Lecane sp.	2.73 ±4.67	0 ±0	0 ±0	1.67 ±2.58	−0.006	0.017	0.504
Asplanchna sp.	15 ±17.5	0 ±0	0 ±0	0 ±0	−0.073	0.191	0.018
Polyarthra sp.	1.36 ±2.34	0 ±0	0 ±0	0 ±0	−0.007	0.108	0.082
Brachionus sp.	0.91 ±3.02	0 ±0	0 ±0	0 ±0	−0.004	0.033	0.343
Rotifers *	226 ±67.5	58.3 ±16	53.3 ±15.4	32.5 ±13.3	−0.997	0.584	<.0001

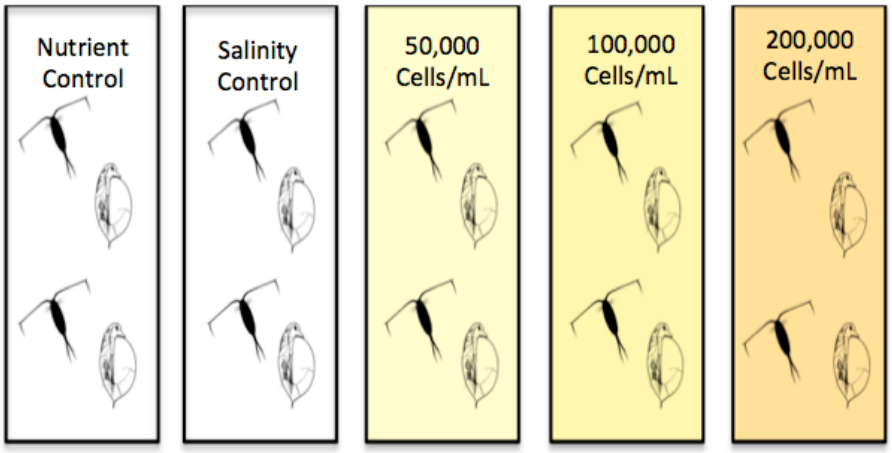

Figure 1: Experimental design for bottle roller rack. Four different treatment levels of *Prymnesium* cell density (0, 50,000, 100,000, and 200,000 cells mL^{-1}) were added to 2.5L bottles along with lake water and a mixed zooplankton community (n=6). To account for the additions of salinity and nutrients in lab grown *P. parvum* cultures, 0 *P. parvum* cell mL^{-1} controls received either nutrients or Instant Ocean at levels equivalent to the other *P. parvum* treatments (n=6, *n=5 nutrient control).

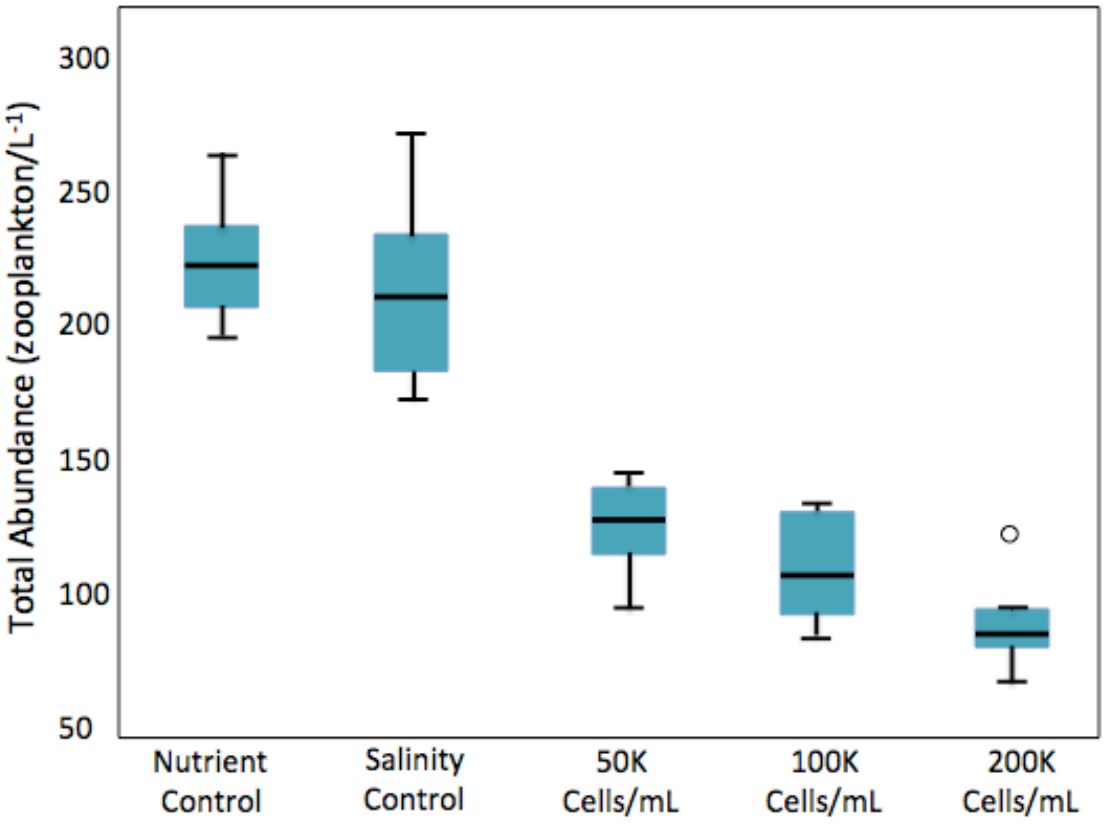

Figure 2: Mean abundance of total zooplankton for each treatment. Distribution of abundance was significantly different between groups ($p<0.001$) as determined by ANOVA. Mean abundance ranged from 216 to 223 individuals L^{-1} in the control treatments as compared to 90 to 123 individuals L^{-1} in the *Prymnesium* treatments.

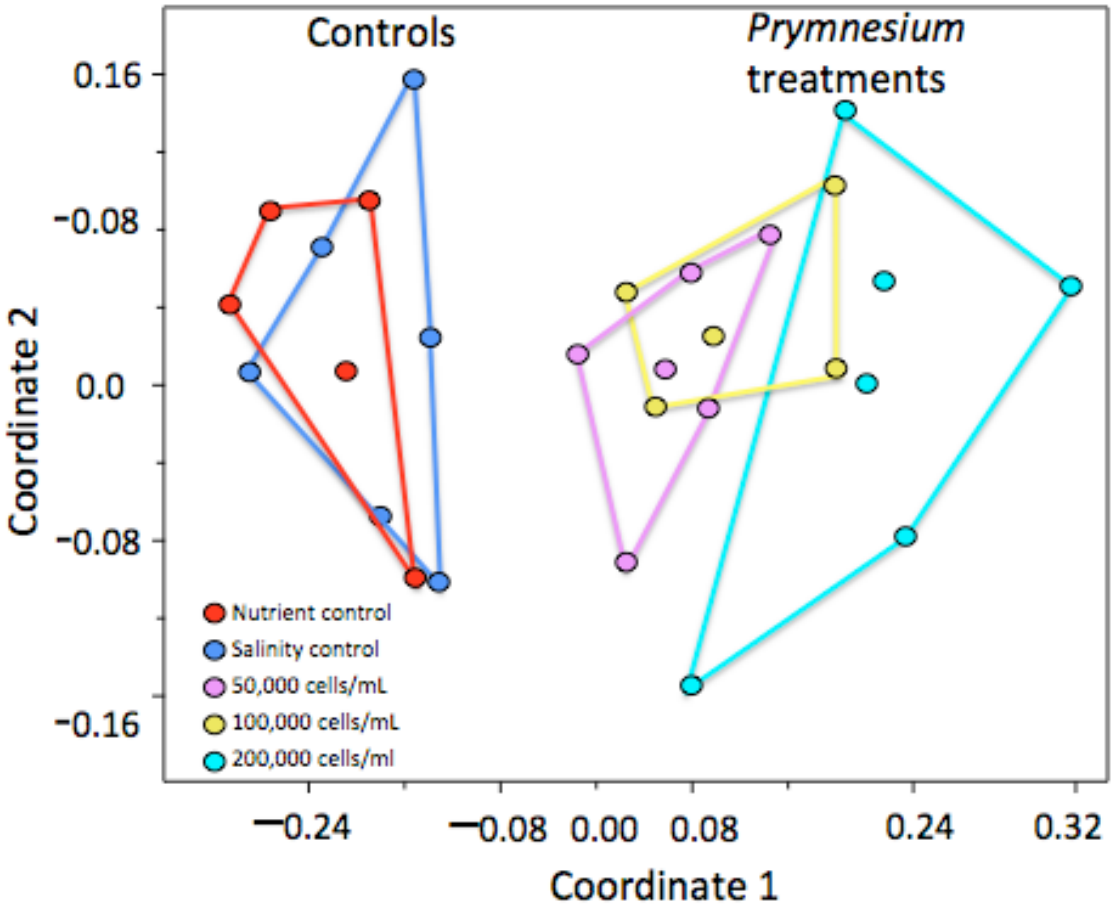

Figure 3: Non-metric multidimensional scaling analysis (NMDS) depicting differences in overall zooplankton community structure between treatments (PAST version 2.0). Colored polygons represent treatment groups with circles represent individual replicates. Distinct differences between the controls and the treatment are evident (stress value = 0.08) and confirmed to be significant using PERMANOVA (Table 4).