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## Prymnesium Parvum and Fish Kills in a Southern Nevada Man-made Reservoir

Tara Gregg  
*University of Nevada, Las Vegas*

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*PRYMNESIUM PARVUM* AND FISH KILLS IN A SOUTHERN  
NEVADA MAN-MADE RESERVOIR

By

Tara Gregg

Bachelor of Science  
University of Nevada, Reno  
2004

A thesis submitted in partial fulfillment  
of the requirements for the

Master of Public Health

School of Community Health Sciences  
Division of Health Sciences  
The Graduate College

University of Nevada, Las Vegas  
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## THE GRADUATE COLLEGE

We recommend the thesis prepared under our supervision by

**Tara Gregg**

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Shawn Gerstenberger, Ph.D., Committee Chair

Steve Weber, Ph.D., Committee Member

Jeff Janik, Ph.D., Committee Member

Penny Amy, Ph.D., Graduate College Representative

Kathryn Hausbeck Korgan, Ph.D., Interim Dean of the Graduate College

**May 2014**

## ABSTRACT

### *Prymnesium parvum* and Fish Kills in a Southern Nevada Man-made Reservoir

By  
Tara Gregg

Dr. Shawn Gerstenberger, Examination Committee Chair  
Interim Dean of the Community Health Sciences  
University of Nevada, Las Vegas

The water chemistry of Lake Las Vegas (LLV), a Southern Nevada man-made reservoir, is influenced by both anthropogenic and natural sources. These sources affect the reservoir's water chemistry, which may promote harmful algal blooms (HABs) leading to massive fish kill events. Fish kills, caused by the golden algae *Prymnesium parvum* (*P. parvum*), continue to pose a threat in the reservoir. However, no effective treatments to control *P. parvum* in large reservoirs such as LLV have been determined. This cross-sectional study evaluated important variables that may affect *P. parvum* HABs in LLV, including non-*P. parvum* biomass ( $\text{mg}/\text{m}^3$ ), total zooplankton abundance ( $\text{\#}/\text{m}^3$ ), temperature, TDS (salinity), mineral concentration (Ca, Mg, K, Na), total nitrogen (TN), total phosphorous (TP), and N:P ratios. Using secondary analysis from Water Quality Monitoring Reports, mid-month data was collected from December 2009 through December 2012 ( $N=38$ ). This time frame was selected due to an algal bloom occurrence in December 2009, which led to the first fish kill event approximately thirty days thereafter. Univariate and multivariate regression analysis were performed using a 30 day lag in order to traverse appropriate date ranges to determine variable significance for hypotheses testing. Statistical analyses found temperature, TDS (salinity), mineral

concentration (Ca, Mg, K, Na), and TN to be significant predictor variables ( $p \leq 0.1$ ) for *P. parvum* bloom formation. Observational analysis of interactions between independent variables on *P. parvum* bloom formation was also assessed, which may be key to making profound research discoveries. This study supports the findings in the literature; however, because *P. parvum* blooms in LLV are unique compared to other strains, different treatments for mitigation may be needed. Further studies are needed to elucidate the mechanisms of golden algae biosynthesis, its biology and ecology, as well as its associated toxicity, in order to better manage blooms leading to fish kill events in LLV.

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## CHAPTER 1

### INTRODUCTION

#### **Introduction to *P. parvum***

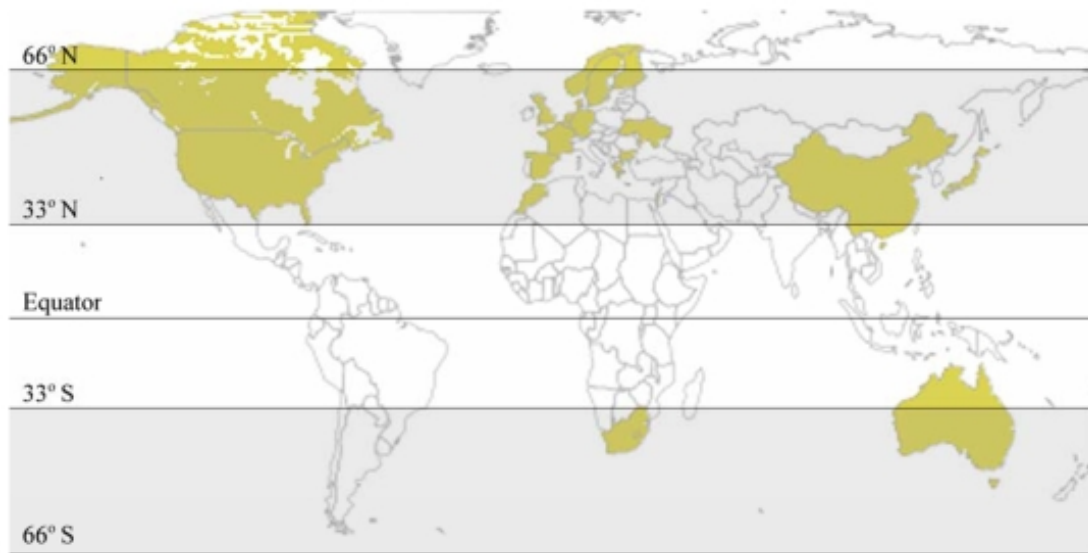
Over the past century, seasonal fish kills associated with golden-alga blooms of *Prymnesium parvum* (*P. parvum*) have devastated aquaculture and gill-breathing organisms worldwide (Manning and La Claire 2010). This organism is known to produce highly potent exotoxins and is responsible for harmful algal blooms (HAB), causing substantial problems to marine and freshwater resources (Granéli and Johansson 2003).

A rapid increase in the density of algae in an aquatic system is known as an algal bloom. Confirmed *P. parvum* blooms typically exceed  $10 \times 10^6$  cells L<sup>-1</sup>, feature golden-colored surface foam and surface waters, and generally occur in brackish estuarine and marine waters worldwide (Brooks, Grover, & Roelke 2011). However, within recent decades, *P. parvum* has become an invasive species in freshwater systems as well (Manning and La Claire 2010). *P. parvum* blooms tend to grow in subtropical and temperate zones during winter and spring months (Figure 1) due to environmental conditions (cooler temperatures, limited nutrients) that are not favorable to other algae (Roelke et al. 2010). A particularly serious issue associated with these environments is invasion and subsequent proliferation of non-native submersed plant species (Roelke et al., 2011).

The first confirmed *P. parvum* HAB in North America was identified in Texas in 1985 on the Pecos River (Roelke et al. 2011). Since then, fish kills caused by *P. parvum* have resulted in the death of an estimated thirty million fish and tens of

millions of dollars in lost revenue with invasions in reservoirs and rivers in fifteen other states. These include Alabama, Arizona, California, Florida, Hawaii, Nevada, New Mexico, Washington, and others (Roelke et al. 2011). In Las Vegas, Nevada, toxic golden algae blooms have led to fish kills in Lake Las Vegas (LLV) (Weber and Janik 2010). The brackish waters of LLV provide a unique environment for *P. parvum* bloom formation, however this alga has been reported to occur in low salinity coastal and mainland waters (Weber and Janik 2010).

Figure 1. *P. parvum* global distribution, mainly occurring in subtropical and temperate zones (Manning & La Claire 2010).



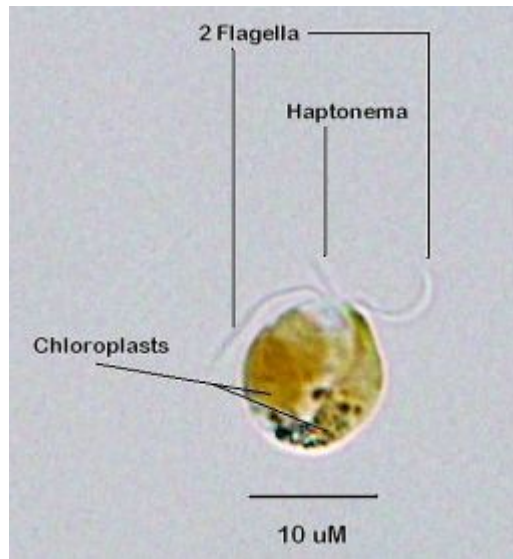
## CHAPTER 2

### LITERATURE REVIEW

#### ***P. parvum* Ecology and Diagnostic Features**

*Prymnesium parvum* is a microalga in the division Haptophyta, the class Prymnesiophyceae, and is a common member of the marine phytoplankton (Brooks, Grover & Roelke 2011). This primary producer is a euryhaline flagellate, having two flagella, is a unicellular eukaryotic organism, ranging in size from 8-15µm in length by 4-10 µm wide (Manning & La Claire 2010). The oblong cells contain two large yellow-green chloroplasts, containing the photosynthetic apparatus, situated on either side of its centrally-located nucleus (Figure 2) (Sager, Fries, Singhurst & Southard 2007). A threadlike organelle, the haptonema, projects between the flagella, allowing a swimming motion of spinning on its longitudinal axis while moving forward (Sager et al. 2007). Proposed functions of the haptonema include its acting as a sensor, attaching to substrates or prey capture for *P. parvum* (Sager et al. 2007). Additionally, *P. parvum* can obtain energy through several modes of nutrition by taking advantage of different environmental conditions. These characteristics of *P. parvum* are discussed in the following sections.

Figure 2. Light micrograph of a *P. parvum* cell. The haptonema is shown projecting between the two flagella (Manning & La Claire 2010).



### **Toxic Metabolites of *P. parvum***

Dangerous exotoxins of *P. parvum*, released to subdue potential prey, are recognized as a part of a group of compounds known as prymnesins. To date, prymnesin-1 and -2 are the only identified exotoxins released by *P. parvum* that are responsible for aquatic toxicity, yet there may be presence of other exotoxins (Baker et al. 2007). Given the presence of *P. parvum* in water samples, it is postulated that prymnesins could be expelled into the environment as a result of cell lysis and death, however the precise mechanisms of how these substances are released is unknown (Carvalho and Granéli 2010).

Prymnesins are complex in that they contain many toxins, allowing them to completely dominate the plankton and aquatic community. Prymnesins include an ichthyotoxin (fish toxin); a neurotoxin (a toxin that damages nerve tissues); a cytotoxin (a toxin that damages cells); and a hemolysin (a protein that causes red

blood cell destruction) (Manning and Claire 2010; Ulitzer and Shilo 1970). The effects of toxins appear to depend on changes in the cell membrane permeability, which causes the membranes to become permselective (Johansson and Granéli 1999). The released toxic compounds act by punching holes in the cell membrane of the organism/cells, through a process called “dasmothrophy”.

The ichthyotoxin component of prymnesins adversely affects gill-breathing organisms such as fish, bivalves, crayfish, gilled amphibians, and some species of plankton. Since these toxins are believed to increase membrane permeability by interrelating with plasma membranes and resulting in ion leakage, they are thought to compromise the reliability of the gills (Manning and Claire 2010). This makes the organism susceptible to any other toxins that are present, including the *P. parvum* toxin itself (Fistarol et al. 2003). Therefore, gills become inefficient and lose their ability to absorb oxygen through water exchange, leading to internal bleeding and resulting in death by asphyxiation (Olli and Trunov 2007). The ichthyotoxic effects are by far the most noticeable, due to the frequency of blooms and distress caused by massive fish kills.

The neurotoxic components of prymnesins attack the central nervous system by blocking postsynaptic membranes as well as contracting muscles (Ulitzer and Shilo 1970). Cytolytic abilities of prymnesins involve water uptake and swelling, leading to cell lysis. This occurs when the osmotic balance of ions (sodium and potassium) and molecules become unstable, due to temperature and pH variations (from higher levels to lower levels), resulting in cell permeability damage of the cell membrane (Dafni et al. 1966). Prymnesin’s hemolytic abilities cause red blood cell destruction

by attaching to a receptor site of the blood cell surface resulting in cell lysis and death (Ulitzer and Shilo 1970). Granéli and Salomon (2010) note that the effects of the toxins are usually measured by hemolytic activity (blood cell lysis) or mortality of co-occurring algal or animal cells. The precise mechanisms of how these toxins are released into the environment is not presently known, but it is postulated that prymnesins could be secreted, excreted or released into the environment as a result of cell lysis and death (Manning and La Claire 2010).

### **Environmental Parameters for *P. parvum***

It is important to understand bloom formation versus toxicity because there are different points throughout the *P. parvum* lifecycle when allelopathy increases. Additionally, bloom formation may be used as a measure of toxicity when hemolytic activity (red blood cell destruction) cannot be measured. This section explains the *P. parvum* lifecycle, including toxicity and different modes of nutrition when nutrients are deplete.

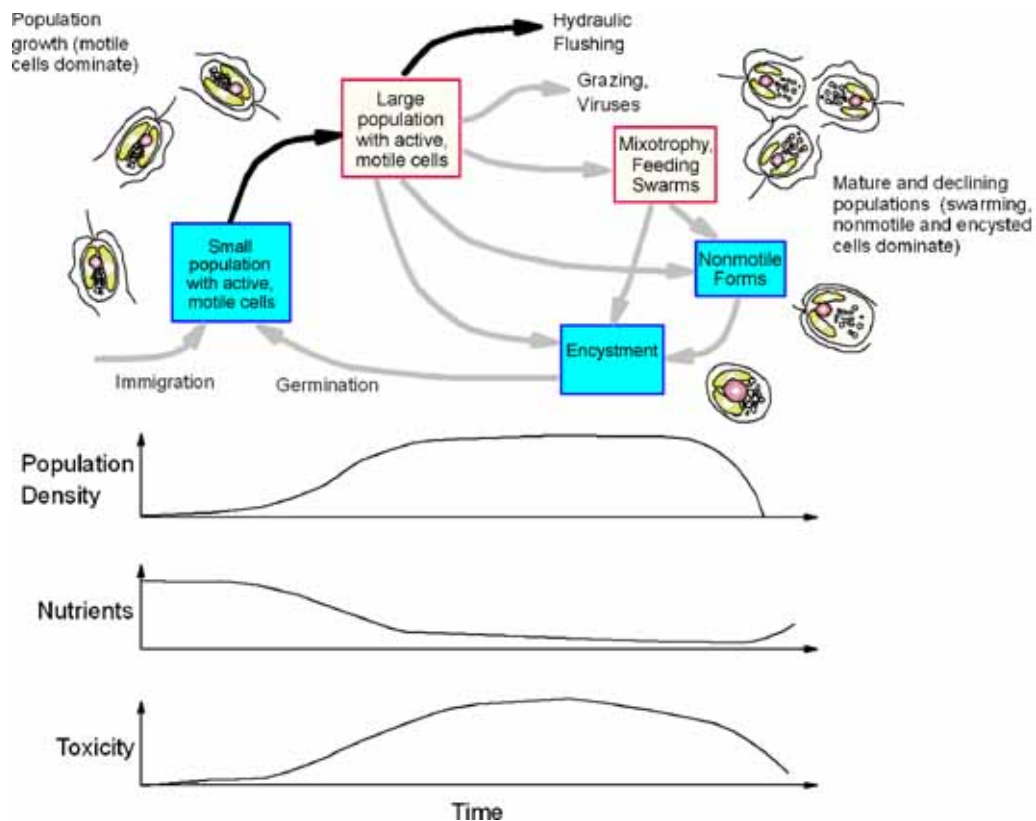
### **Bloom Formation vs. Toxicity**

Bloom formation is a complex process affected by numerous physical, chemical, and biological interactions. The presence of HAB does not necessarily mean the algae will produce and secrete prymnesins into the water. However, as *P. parvum* bloom densities increase, toxicity often increases (Brooks et al. 2011). Motile cells typical of growing populations can express various behaviors and become mixotrophic, non-motile, or form resting cysts. Non-motile cells appear towards the end of HABs, and are related to bloom termination as non-motile cells reduce toxin

production before forming resting cysts in the sediment (Brooks et al. 2011), either in senescence or laying dormant.

Bloom formation may occur from small in-situ populations, germination of encysted stages or immigration from other locations via advection (Figure 3) (Brooks et al. 2011). The red boxes designate toxic stages, while the blue boxes indicate stages suspected or known to be non-toxic. The black arrows demonstrate phases known to be critical in toxic bloom formation, while gray arrows signify periods where significance to bloom formation is less known. Graphs below the lifecycle depict typical influences of population density, nutrients, and toxic activity during HABs (Brooks et al. 2011).

Figure 3. Life history and behavior of *P. parvum* (Brooks et al. 2011).



Mixotrophic forms occur when cells feed heterotrophically and cluster in “feeding swarms,” where they encircle and consume prey by phagotrophy. This usually occurs late in a bloom cycle as a strategy to obtain nutrients under depleted conditions (Roelke et al., 2011). Encysted stages occur when *P. parvum* sink to the sediment. This takes place when the golden algae act heterotrophically, during light removal, and form resting cysts. The non-flagellated resting cyst is described as a non-active life stage, which generally forms during unfavorable conditions (e.g., low nutrients) and may be able to revive itself when conditions improve (Roelke et al., 2011). However, very little is known about resting cysts of *P. parvum*, including their ability to produce toxins and survival conditions in nature of the benthic environments.

It is known that exotoxin production occurs when *P. parvum* cells experience stress, for not having enough of a limiting nutrient for cell division. Ideal environments for bloom formation include salinity levels of approximately 22,000 mg/L, TDS ranging from 500-1,000 mg/L, temperate between 25-35°C, and non-limited nutrients with an N:P ratio >16:1 (Table 1) (Baker et al. 2007; Baker et al. 2009; Carvalho and Granéli 2010; Fistarol et al. 2003; Johansson and Granéli 1999; Renner 2009; Prosser et al. 2012). Ideal environments for toxin formation are also noted, where overlap between ranges for salinity, total dissolved solids (TDS) and temperature values can be seen. However, it has been suggested that the most important factor influencing HABs is the relative amount of nitrogen (N) and phosphorous (P) present with toxicity increasing when both of these nutrients are limited (Johansson and Granéli, 1999).

Table 1. Ideal environmental parameters for exotoxin production and bloom formation of *P. parvum*.

Parameter	Ideal environment for exotoxin production of <i>P. parvum</i>	Ideal environment for bloom formation of <i>P. parvum</i>	Synergistic effects	Additional information	Reference
Salinity	Max toxicity at 7,500 and 35,000 mg/L	~22,000 mg/L		Some <i>P. parvum</i> strains are known to bloom at the low salinity edge of niche	Baker et al. 2009
TDS/Minerals (Ca, Mg, K, Na)		500 to 1,000 mg/L		TDS component ions determine the degree and severity of toxic effects	Renner 2009
Temperature	Range: 5-35°C (41-95°F) Acute toxicity increasing at conditions suboptimal for growth and abundance	Unimodal over 5°C - 30°C Rapid growth occurring between 25-30°C		Inhibition of growth occurring at <5°C and >30°C (50°F-86°F)	Baker et al. 2007
Nutrients TN & TP N:P = 16:1	Nutrient limited or N:P ratio unbalanced (most toxic when few nutrients are available)	High nutrient availability and unbalanced N:P ratio	Feeding of <i>P. parvum</i> increases ~ 50% through phagotrophy during N & P limitation	Bloom growth leads to depletion of nutrients, triggering toxin production Toxins are increased up to 10-20 times in P limited environments Carbon is attained through photosynthesis	Brooks et al. 2011; Johansson and Granéli 1999; Carvalho and Granéli 2010
Plankton Non- <i>P. parvum</i> & total zooplankton	<i>P. parvum</i> is a slow growing alga (0.94 day <sup>-1</sup> ) → turns to allelopathy to inhibit growth of competitors	Warding off of competitors and grazers via toxins allows for bloom formation to emerge	Allelopathic effects of plankton caused by prymnesins weaken 2-3 days after exposure	<i>P. parvum</i> has been shown to negatively affect the behavior, feeding and survival of copepods, which seem to be the dominant zooplankton according to its feeding habits	Baker et al. 2007

### Competitive Niche of *P. parvum*

*P. parvum* uses many strategies for growth including the use of inhibitory effects and mixotrophic behaviors. These characteristics enable *P. parvum* to outcompete other species for rapid growth. This section discusses these strategies in further detail.

#### Allelopathy

The use of allelopathy, or inhibitory effects, is a common strategy utilized by many phytoplankton types, and is not unique to *P. parvum*. Allelopathy occurs when

nutrients become reduced or unbalanced, leading to stress and toxin production in order to decrease population losses by inhibiting growth of competing species (Granéli and Salomon 2010). The toxins released by *P. parvum* cells are secondary metabolites released in the water with the ability to punch holes in the cell membrane of the competing organism (osmothrophy) leading to cell lysis and release of nutrients for utilization by *P. parvum* (Fistarol et al. 2003). Not only does *P. parvum* benefit from the nutrients released from lysed cells, but also additional nutrients available in the water. Therefore, inhibitory effects lead to *P. parvum* biomass accumulation, as a result of the competitive advantage, while competing species become suppressed.

A study conducted by Fistarol et al. (2003) describes effects inflicted on bacteria, phytoplankton and ciliates by *P. parvum*. The study demonstrated that allelopathic compounds are used by some toxic phytoplankton species to achieve dominance in the environment. They concluded that *P. parvum* releases compounds that are hemolytic, toxic to ciliates, and allelopathic to bacteria and other phytoplankton. The allelopathic compounds negatively affect the phytoplankton species in a natural community by reducing their growth and production, and therefore *P. parvum* may dominate other phytoplankton, with allelopathic effects caused by prymnesins weakening 2-3 days after exposure (Fistarol et al. 2003).

Additional factors that may affect allelopathy include temperature, light, and the presence of metabolizing/degrading enzymes. In the study, *P. parvum*'s allelopathic effect was reflected in a decrease of chlorophyll *a* concentration and carbon (C) uptake, due to the death of diatoms or suppression of dinoflagellates and

cyanobacteria. Being that these compounds eradicate some of the competing phytoplankton groups and keep the biomass of other groups at lower levels, *P. parvum* is at a competitive advantage (Fistarol et al. 2003).

### **Mixotrophy**

The *P. parvum* cells' capacity to form blooms may also be explained by alternative energy and nutrient sources, such as multiple modes of nutrition, termed mixotrophy. Therefore, even though *P. parvum* is considered predominately phototrophic (using photosynthesis to obtain inorganic carbon and energy), it is also able to act as a heterotrophic organism and uptake dissolved organic substances (using dissolved oxygen to fuel nighttime growth when sunlight is not available for photosynthesis) (Burkholder et al. 2008) *P. parvum* may also feed heterotrophically using phagotrophy to ingest particulate organic carbon (Carvalho and Granéli 2010). Phagotrophy, uptake of particulate food or grazers/prey, refers to absorption of organic substances where digestion occurs in phagocytic (food) vacuoles (Burkholder et al. 2008). Ingestion of organic particles through phagotrophy can significantly supplement carbon, nitrogen, and phosphorous supplies, which ultimately increases biomass accumulation. Phagotrophy is triggered when *P. parvum* becomes stressed during particular environmental conditions, such as limiting nitrogen or phosphorous, in order to keep its constant metabolic activity (Carvalho and Granéli 2010).

Mixotrophy can lead to a competitive advantage over obligate phototrophs and heterotrophs, leading to increases in growth efficiency due to reduced nutrient losses, while additionally reducing competition for food (Lindehoff et. al. 2009).

These advantages give mixotrophic algae a greater chance of survival under adverse conditions.

### **Ecological Factors Influencing Bloom Formation and Toxicity of *P. parvum***

The amount of exotoxins that *P. parvum* produces can be increased under stressful environments while bloom formation can be increased when environmental parameters are sufficient. The following sections describe potential environmental factors that may influence bloom formation and toxicity of *P. parvum*.

#### **Plankton Interaction**

Algal competition and secession are very important factors contributing to lake ecology. Some toxins produced by algal blooms may be harmful to other algae and their associated grazers. Because the maximum growth rate of *P. parvum* is slow ( $0.94 \text{ day}^{-1}$ ) under optimal conditions, when the growth rate becomes reduced, *P. parvum* becomes dependent on toxin production to gain dominance over faster growing phytoplankton, such as cyanobacteria and diatoms (Baker et al. 2007). Even though some cyanobacteria species may produce potent toxins (cyanotoxins), prymnesins released by *P. parvum* are more toxic to fish and plankton than are cyanotoxins. This competitive advantage allows for *P. parvum* bloom formation to emerge. Table 2 lists maximum specific growth rates for some freshwater species of phytoplankton, some being present in LLV.

Table 2. Maximum specific growth rates ( $\text{day}^{-1}$ ) reported for some freshwater species of phytoplankton.

Phylum	Species	$\text{day}^{-1}$
Cyanobacteria	<i>Synechococcus sp.*</i>	1.72
	<i>Planktothrix agardhii*</i>	0.86
	<i>Pseudanabaena limnetica*</i>	0.55
	<i>Anabaena flos-aquae</i>	0.78
	<i>Aphanizomenon flos-aquae</i>	0.98
Chlorophyta	<i>Chlorella Strain 221</i>	1.84
	<i>Ankistrodesmus braunii</i>	1.59
	<i>Eudorina unicocca</i>	0.62
	<i>Volvox aureus</i>	0.46
	<i>Cryptomonas ovata</i>	0.81
Cryptophyta	<i>Monodus subterraneus</i>	0.64
Eustigmatophyta	<i>Dinobryon divergens</i>	1.00
Chrysophyta	<i>Stephanodiscus hantzschii</i>	1.18
Bacillariophyta	<i>Asterionella formosa</i>	1.78
	<i>Fragilaria crotonensis</i>	1.37
	<i>Prymnesium parvum*</i>	0.94
Haptophyta		
* Algal species present in Lake Las Vegas (Reynolds 2006)		

Three main groups of zooplankton (primary consumers) that inhabit the limnetic zone of inland waters and compete for common food sources are cladocerans, copepods, and rotifers. Herbivorous cladocerans, including gill-breathing groups such as *Daphnia*, are considered a principle component in the food webs of lakes because of their ability to exert top-down control on phytoplankton (Rommel et al. 2011). In fact, these primary consumers graze on bacteria and phytoplankton (primary producers), and occupy the role of the primary consumer in the aquatic food web. A study by Rommel et al. (2011) determined a negative relationship between *P. parvum*, cladoceran and the total crustacean biomass. The experiment was conducted with *P. parvum* bloom densities of  $\leq 31,000 \text{ cells mL}^{-1}$ , and marked reductions were observed in *Daphnia* survival and growth rates at *P. parvum* concentrations as low as  $7,750 \text{ cells mL}^{-1}$ , which suggests *P. parvum* may be toxic to zooplankton at relatively low densities (Rommel et al. 2011).

Rotifers and copepods also compete for common food sources in inland waters. Unlike the herbivorous cladocerans, copepods are both herbivorous and carnivorous, which give them a competitive niche over other plankton (Zdenek 2005). During later developmental stages, most copepods become efficient omnivore predators and exhibit various predatory techniques, which enable them to graze on a wide range of zooplankton from protozoans to small cladocerans, with rotifers being the most preferred prey (Zdenek 2005). Although in most inland waters, copepods seem to be the dominant zooplankton according to their feeding habits, *P. parvum* has been shown to negatively affect the behavior, feeding and survival of copepods (Zdenek 2005). Therefore, the entire lake food web may be subject to negative effects when *P. parvum* toxicity is present.

### **Temperature**

Growth of *P. parvum* has been shown to occur as a function of temperature, independent of light or salinity (Baker et al. 2007). Optimal temperatures for growth and toxicity vary greatly depending on the geographic isolates of the alga. Generally, optimal growth rates are unimodal with growth occurring between 25-30°C, and inhibition of growth occurring at <5°C and >30°C. Baker et al. (2007) determined that acute toxicity increases at conditions suboptimal for growth and abundance, within the range of 5-35°C, where warmer temperatures yielded optimal growth conditions reducing the need for nutrients. According to previous studies, most *P. parvum* blooms occur during the winter and spring months when cooler temperatures and limited nutrients are unfavorable to other algae, which appears to give *P. parvum* an advantage. Given the broad temperature range of *P.*

*parvum* bloom tolerances, there are no defining correlations between toxicity and specific temperatures.

**Stratification.** Lake surface temperatures vary depending on the time of the year and stratification of the lake. During the winter seasons, in north temperate lakes, the coldest layer composed of ice is at the surface with warmer water uniformly distributed to the bottom. This arrangement is known as winter stratification (Hoffman, A. D., Tramutt, P. R., & Heller, F. C. 1967). With the advent of spring, solar radiation and wind causes the surface waters to sink towards the warmer less dense water, which leads to mixing of the lake. Along with wind, the lake completely circulates and is resupplied with organic material and nutrients brought up off the lake bottom. This period is known as spring overturn (Hoffman et al. 1967).

During the summer, the increased amount of solar radiation causes increases in temperatures and density gradients. As the lake becomes more resistant to mixing, the lake becomes stratified into three layers: the hypolimnion (bottom layer, thermocline (metalimnion), and the epilimnion (top layer) (Wetzel 1975), with warmer less dense water nearest the surface. The stratification process is often reversed in fall when cooler atmospheric temperatures reduce the surface temperatures of the reservoir. Differences in the hypolimnion and epilimnion temperatures and associated densities diminish allowing wind produced currents to mix the whole reservoir, a process known as turnover (Wetzel 1975).

Lake Las Vegas does not follow the usual cycling pattern seen in temperate dimictic lakes. This reservoir is a warm monomictic lake, meaning that it mixes from top to bottom during only one mixing period each year (Weber & Janik 2010). With

its temperature never reaching below 4°, it freely circulates in the winter, and is directly stratified in only in the summer months (Hoffman et al. 1967; Weber and Janik 2010). Having one long mixing period, nutrients are brought up off the bottom of the lake during fall turnover, supplying *P. parvum* the required nutrients for growth during the cooler time of year.

### **Total Dissolved Solids (Salinity) and Minerals**

Many researchers have examined the effects of salinity on *P. parvum* growth due to the algae's recurring presence in various saline environments. Salinity is generally defined as the sum of all ion concentrations in a given unit volume of water. An established salinity value of 3,000 mg/L is considered the upper limit for healthy freshwater ecosystems (Weber 2006).

High toxicity levels are observed far from optimal growth conditions. Maximum toxicity of *P. parvum* has been observed at 7,500 mg/L and 35,000 mg/L, where intermediate salinities, when algal blooms form, are shown to be less toxic. Growth rates for many strains of *P. parvum* occur when salinity and temperature are ~22,000 mg/L and between 25-30°C respectively (Baker et al. 2009, Roelke et al. 2011). Though these ranges are considered to be typical growth conditions, other strains of *P. parvum* are known to bloom at the low-salinity edge of its niche. A study performed by Baker et al. (2007), for example, yielded results demonstrating growth of *P. parvum* within salinity levels from 2,000 to 3,000 mg/L, and temperatures estimated around 27°C. Results suggest that temperature for growth declines as salinity decreases, indicating that temperature and salinity are statistically significant both independently and in combination (Baker et al. 2007).

Salinity is often expressed as a measure of the water's electrical conductance (EC) or measured in units of total dissolved solids (TDS), as TDS in water mainly consists of ionic constituents that conduct electricity. EC is widely used as a surrogate measure of salinity because it is much simpler to measure EC of water compared to measuring TDS. TDS concentrations that are required to support *P. parvum* blooms are estimated to be between 500 and 1000 mg/L (Renner 2009), however, TDS concentrations for irrigation purposes must remain below the annual average of 2,000 mg/L.

TDS refers to the fraction of solids in water that will pass through a 1.2  $\mu\text{m}$  filter and that will remain suspended on a dish when a water sample is dried at a specific temperature. It is simply an analysis of water purity by measuring non-organic contaminant weight. It does not test organic products, such as pesticides and biological impurities that will additionally pass through the filter (Manning and La Claire 2010). The majority of TDS in most ambient waters are ionic non-organic minerals (component ions or salts) such as calcium, magnesium, sodium, potassium, carbonate, bicarbonate, sulfate, chloride, bromide, and nitrate.

In order to “activate” prymnesins, TDS minerals (component ions or salts) are required, which is why prymnesins and ichthyotoxicity are not necessarily linked (Manning and La Claire 2010). Moreover, the type of ion determines the degree and severity of toxic effects of *P. parvum* cells. The presence of minerals, as well as antibiotics (e.g., streptomycin), greatly enhances toxicity to fish (Manning and La Claire 2010). Ulitzer and Shilo (1964) determined that neomycin, spermine and other polyamines play a role in activating ichthyotoxicity. They concluded that, *P.*

*parvum* toxicity depends on the specific activity of each mineral present and their relative concentrations in the surrounding waters. It was further discovered that high ichthyotoxic activity of *P.parvum* is dependent cofactors such as calcium and magnesium, however, the reasons for this are unknown, and more research is needed in order to understand these mechanisms.

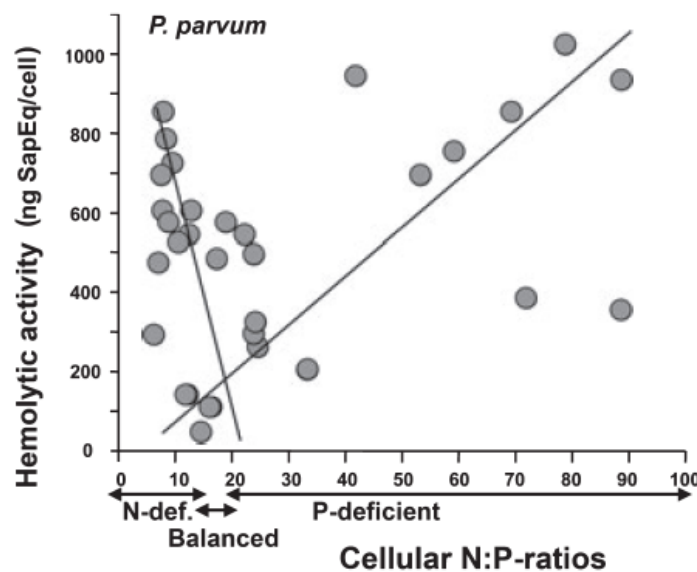
### **Water Nutrients**

*P. parvum* can thrive in a wide range of conditions, but availability of nitrogen and phosphorous has been shown to greatly influence HAB and toxin formation. These nutrients may become unbalanced through eutrophication, the increased input of N and P into aquatic environments (e.g. fertilizer input from storm events and run-off), resulting from excessive amounts of nutrients available for primary producers. Eutrophication results in a deviation of the Redfield ratio, i.e., the *P. parvum* cellular carbon to nitrogen to phosphorus ratio, C:N:P=106:16:1 (Granéli and Salomon 2010). The use of this ratio has become universal in freshwater and marine studies (Ekholm 2008). Comparisons of nutrient concentrations in lakes have suggested that a mass N:P ratio >17 indicates P limitation, a ratio <10 indicates N limitation, and values <17 and >10 indicate that either N or P may be limiting (Ekholm 2008).

When the N:P ratio is either unbalanced or when nutrients are limited, *P. parvum* toxicity levels have been shown to increase drastically, with levels found to be most toxic when few nutrients are available (Figure 4) (Errera et al. 2008; Johansson and Granéli 1999; Granéli and Salomon 2010). The N:P ratio has also been suggested to be a predictor of faster growing phytoplankton, such as cyanobacteria, with N:P

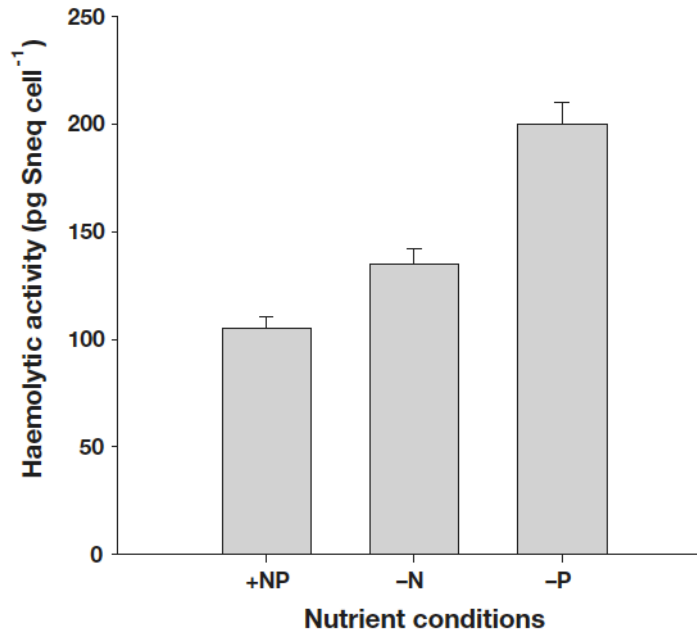
ratios >30 (by weight) projected as a threshold to reduce blooms (Schindler and Vallentyne 2008). Other studies suggest that N:P ratios of ~75 (by weight) may reduce cyanobacteria bloom volumes, suggesting that increasing the N:P ratio may offer reductions in phytoplankton (Graham et al. 2004).

Figure 4. *P. parvum* hemolytic activity (toxicity) as a function of their intracellular N:P ratios (Granéli and Salomon 2010).



In a study performed by Barreiro et al. (2005), hemolytic activity, measured by cell densities of *P. parvum*, was evaluated with each nutrient condition: balanced nitrogen and phosphorous (+NP), nitrogen depleted (-N), and phosphorous depleted (-P). Results showed the highest hemolytic activity to be from those cells grown in -P limited environments, while the lowest cell lysis was seen in cells grown in +NP conditions (Figure 5) (Barreiro et al. 2005). Therefore, altering algal composition through nutrient manipulation (e.g. pond fertilization) suppress *P. parvum* blooms (Kurten et al. 2001).

Figure 5. *P. parvum* hemolytic activity in the -P, -N and +NP cultures. Values are mean  $\pm$ SE (Barreiro et al. 2005).



When nutrients become limited, *P. parvum* cells may use an alternate mechanism for growth. Granéli and Johansson (2003) have suggested that when *P. parvum* cells are stressed, under unbalanced N and P conditions, the production of allelopathic compounds is stimulated. By killing cells of the target species, *P. parvum* is able to not only utilize the limiting available N or P from the surrounding water but also the N and P released by lysed target cells of the co-occurring target species. A study by Carvalho and Granéli (2010) describes an alternative method of receiving nutrients with the use of phagotropic behaviors. The study suggested that feeding of *P. parvum* was increased close to 50% through phagocytic ingestion during N and P limitation. Even when nutrients were available, *P. parvum* cells still expressed phagotropic behaviors as soon as prey became available. Adaptive properties such

as alternate modes of nutrition (mixotrophy) provide numerous benefits to *P. parvum* populations.

**Phosphorous.** Phosphorous is a critical growth-limiting nutrient due to the fact that the only biological form of phosphorous that is available is in the form of soluble phosphate ( $\text{PO}_4$ ). Most phosphorous is unavailable to biota because it tends to strongly absorb to particles in the water, however a literature review by Watson (2001) reported that extracellular alkaline phosphate activity was found to be highest in *P. parvum* when compared to other algae species. It was discovered that an increase in the Redfield ratio led to an increase in phosphate uptake and enzyme activity, and concluded that this would give *P. parvum* a competitive advantage when phosphate is limited. Phosphate-limited environments cause a restriction in *P. parvum* membrane synthesis that may lead to toxin leakage of intracellular molecules with findings showing increases in toxicity levels of up to 10-20 times (Johansson and Granéli 1999). This is a plausible explanation of why toxicity levels are increased when phosphate is limited.

**Nitrogen.** Nitrogen availability is also an important factor related to prymnesin toxicity. Nitrogen in aquatic systems may exist as dissolved nitrogen gas ( $\text{N}_2$ ), organic nitrogen incorporated into organic matter, or ionized ammonia ( $\text{NH}_4^+$ ), nitrite ion ( $\text{NO}_2^-$ ), and nitrate ion ( $\text{NO}_3^-$ ). One study suggests that nitrogen limiting environments are indicative of conditions favoring nitrogen-fixing cyanobacteria ('blue-green algae') in lakes and estuaries, enabling cyanobacteria to thrive (Weber 2006).

## **Fish Physiology**

Understanding fish physiology and how *P. parvum* can effect the permeability of the gill cells is important when attempting to resolve the amount of fish kill events. This section discusses fish metabolism and fish kill causal factors and effects, including oxygen depletion and fish intoxication by prymnesins.

### **Fish Metabolism and Gas Exchange**

The oxygen requirements of fish depends on factors such as temperature, the CO<sub>2</sub> level of water, and the metabolic rate of the fish (Svobodová et al. 2003). The metabolic rate of fish is closely correlated to the water temperature in which they live (e.g., an increase in water temperature from 10 to 20°C at least doubles the oxygen requirements); fish that live in warm water have a greater metabolism than fish that live in cold water, and therefore require more oxygen for survival (Svobodová et al. 2003).

In order for fish to obtain oxygen, they must breathe seawater, freshwater, air or some combination thereof. The gills are the primary site designed for gas exchange between water and blood. When oxygen is brought into close contact with the gills, through the flow of water, oxygen diffuses across the gills into the blood. At a gradient of between 40 and 100 mm Hg (millimeters of mercury) P<sub>O2</sub> (oxygen partial pressure), oxygen reaches the capillaries and diffuses across the capillary walls into the tissues (Shukla 2009). Gas partial pressures (tensions) are estimated to be in the range of 1-15 mm Hg P<sub>O2</sub> and 3-15 mm Hg P<sub>CO2</sub> (carbon dioxide partial pressure) (Shukla 2009). The partial pressure of oxygen dissipates as oxygen moves from the water into the tissues via the capillaries.

The oxygen diffusion into the bloodstream of fish is positively correlated to partial pressure variations between the fish's blood and the surrounding water. That is, a drop in the  $P_{O_2}$  in the water reduces the  $P_{O_2}$  in the blood (Shukla 2009). Thus, in warm waters, fish's partial pressure decreases slightly due to increased molecular activity, but a fish in warm water must pass more water over their gills to deliver the same amount of oxygen to the bloodstream per unit time due to their greater metabolism (Shukla 2009). Subsequently, fish living in warm water are exposed to more toxins due to the greater amount of water needed to pass over their gills per unit time for survival.

### **Fish Kill Causal Factors and Effects**

**Oxygen depletion.** Oxygen in water diffuses from the air, mainly where the water surface is turbulent, and also comes from photosynthesis of aquatic plants and algae, such as *P. parvum* (Svobodová et al. 2003). Oxygen may be removed from water through decomposition of fish and plankton biomass from mortality by *P. parvum* toxins, nutrient loading, respiration of aquatic organisms, and by aerobic degradation of algal detritus at night by bacteria (Svobodová et al. 2003). Bacteria either integrate the algal detritus into their biomass or re-mineralize organic nutrients, thereby making inorganic nutrients available. In the process of decomposition of detritus, nutrients are again formed with large amounts of dissolved oxygen being utilized in the biochemical process (Misra et al. 2011). Therefore, we can see how algal blooms, including *P. parvum*, negatively influence oxygen volumes for aquatic species.

Oxygen depletion causes fish sensitivity that can vary depending on species, life stage, and oxygen exposure (Townsend et al. 1992). However, a recommended dissolved oxygen (DO) concentration for ideal fish health is generally about  $\geq 5$  mg/L (Francis-Floyd 2012). Most species of fish become distressed when DO levels fall to between 2-4 mg/L with mortality occurring around concentrations  $<2$  mg/L (Francis-Floyd 2012). Yet, some fish can survive for several days in waters with dissolved oxygen levels between 0.5 and 1.0 mg/L (Townsend et al. 1992).

**Fish intoxication by prymnesins.** Fish that are exposed to prymnesins tend to have damaged permeability of gill cells, compromising reliability by permitting ion leakage, and therefore rendering the fish unable to obtain oxygen. The source of oxygen depletion in this case begins within the fish from algal-produced ichthyotoxins, as opposed to the processes occurring in the water itself (Ultizer and Shilo 2007). Fish affected by prymnesins tend to behave erratically. They may accumulate in the shallows, swim slowly just below the surface, or show no normal avoidance of humans or other disturbances (Olli and Trunov 2007). If clean water flows into a body of water that is affected, fish will often accumulate around the fresh, incoming water.

## CHAPTER 3

### RISK ASSESSMENT

#### **Lake Las Vegas and Fish Kills**

Lake Las Vegas (LLV) is a 320 acre man-made reservoir at the center of a 3,592 acre combined resort, with three golf courses, and approximately 1,900 residential homes, and was conceptualized in the 1960's. This reservoir is located in the City of Henderson, Clark County, Nevada and lies about seventeen miles southeast of the Las Vegas Strip (Figure 6) (MWH Global, Inc. 2012). *P. parvum* was first identified in Lake Las Vegas in December 2009 (Weber and Janik 2010). On February 27, 2010 the first dead fish was discovered with approximately 4,937 dead fish collected over a fifty-eight day period thereafter. The common carp appeared to be the primary species affected making up over eighty percent of the fish kill. In addition, largemouth bass (11%), channel catfish (5%), and bluegills (4%) were also affected (Weber and Janik 2010). The City of Henderson reported that fish kills caused by *P. parvum* continue to pose a problem in this reservoir, as approximately 50 to 200 fish die per day when algae blooms occur.

Figure 6. Location of Lake Las Vegas, Clark County, Nevada.



The Lake Las Vegas Master Association stated that some treatments have been effective on small reservoirs, but there are no effective treatments to control *P. parvum* in larger reservoirs such as LLV (Lake Las Vegas Special Report, 2010). Increased operating costs associated with lake monitoring and testing are necessary in order to help prevent future blooms, but discovering the source of HABs could prevent these measures in the first place. Further studies are needed to unravel the mechanisms of golden algae biosynthesis, biology and ecology, as well as its associated toxicity, in order to better manage its blooms and prevent future fish kill events. The need for more accurate methods to detect and identify possible indicators of *P. parvum*, before HABs occur, is a necessary step towards a solution.

### Questions and Hypotheses

The following research questions and hypotheses are aimed at assisting in identifying contributing factors in *P. parvum* biology and HAB assemblages in Lake

Las Vegas in order to reduce the number of fish kill occurrences in the reservoir.

### Question 1

Does non-*P. parvum* biomass ( $\text{mg}/\text{m}^3$ ) and zooplankton abundance ( $\#/\text{m}^3$ ) predict *P. parvum* bloom formation (biomass,  $\text{mg}/\text{m}^3$ ) in Lake Las Vegas?

H<sub>a1</sub>:

Increases in non-*P. parvum* biomass and total zooplankton abundance predict drops in *P. parvum* biomass in Lake Las Vegas, ( $\beta_1 \neq 0$  and/or  $\beta_2 \neq 0$ )

H<sub>01</sub>:

Increases in non-*P. parvum* biomass and total zooplankton abundance do not predict drops in *P. parvum* biomass in Lake Las Vegas, ( $\beta_1 = \beta_2 = 0$ )

### Method 1

Simple linear regression analysis will be used to identify relationships (positive correlation, negative correlation, or no correlation) between *P. parvum* biomass ( $\text{mg}/\text{m}^3$ ) and non-*P. parvum* biomass and total zooplankton abundance ( $\#/\text{m}^3$ ). The correlation ( $R^2$ ) will be used to determine what variability of zooplankton biomass can be explained by *P. parvum* biomass. If the p value is  $\leq 0.1$ , it can be said that an increase in *P. parvum* biomass is a predictor of zooplankton counts; reject the null hypothesis ( $H_0$ ).

### Question 2

Do specific temperatures predict *P. parvum* bloom formation?

H<sub>a2</sub>:

Temperatures between 5°C and 30°C predict the highest *P. parvum* bloom formation, ( $\beta_3 \neq 0$ )

H<sub>02</sub>:

Temperatures between 5°C and 30°C do not predict the highest *P. parvum* bloom formation, ( $\beta_3=0$ )

### Method 2

Stepwise multiple regression analysis will be used to assess the relationships between *P. parvum* biomass (dependent variable) and TDS, mineral speciation, temperature, nitrogen (TN), phosphorous (TP), and N:P (independent variables). Using stepwise multiple regression we can determine what the best combination of independent variables (highest correlation:  $R^2$ ) would be to predict *P. parvum* bloom formation; however not all independent variables may end up in the equation if they do not yield significant *t*-tests. Additionally, variables with a p value >0.1 not considered statistically significant and are also restricted from entering the regression equation. However, if the p value is  $\leq 0.1$ , it can be said water temperatures between 5°C and 30°C are a predictor of *P. parvum* bloom formations; reject the null hypothesis (H<sub>0</sub>).

### Question 3

Do increased TDS and mineral concentrations predict *P. parvum* bloom formation (biomass, mg/m<sup>3</sup>)?

H<sub>a3a</sub>:

Increased TDS concentrations predict and increase in *P. parvum* bloom formation, ( $\beta_4 \neq 0$ )

H<sub>a3b</sub>:

Increased mineral concentrations predict an increase in *P. parvum* bloom formation ( $\beta_5 \neq 0$ )

H<sub>03</sub>:

Increased TDS and mineral concentrations do not predict an increase in *P. parvum* bloom formation ( $\beta_4 = \beta_5 = 0$ )

### Method 3

Stepwise multiple regression analysis will be used to assess the relationships between *P. parvum* biomass (dependent variable) and TDS, mineral speciation, temperature, nitrogen (TN), phosphorous (TP), and N:P (independent variables). Using stepwise multiple regression we can determine what the best combination of independent variables (highest correlation:  $R^2$ ) would be to predict *P. parvum* bloom formation; however not all independent variables may end up in the equation if they do not yield significant *t*-tests. Additionally, variables with a *p* value  $> 0.1$  are not considered statistically significant and are also restricted from entering the regression equation. However, if the *p* values are  $\leq 0.1$ , it can be said that increased TDS and mineral concentrations are a predictor of *P. parvum* bloom formations; reject the null hypothesis (H<sub>0</sub>).

### Question 4

Does total nitrogen (TN) and total phosphorous (TP) individually and together predict *P. parvum* bloom formation?

H<sub>a4a</sub>:

Non-limited TN or TP predict increases in *P. parvum* bloom formation, ( $\beta_6 \neq 0$  and/or  $\beta_7 \neq 0$ )

H<sub>a4b</sub>:

Unbalanced N:P ratios predict increases in *P. parvum* bloom formation, ( $\beta_8 \neq 0$ )

H<sub>04</sub>:

Non-limited TN or TP, and unbalanced N:P ratios do not predict increases in *P. parvum* bloom formation, ( $\beta_6 = \beta_7 = \beta_8 = 0$ )

#### Method 4

Stepwise multiple regression analysis will be used to assess the relationships between *P. parvum* biomass (dependent variable) and TDS, mineral speciation, temperature, nitrogen (TN), phosphorous (TP), and N:P (independent variables). Using stepwise multiple regression we can determine what the best combination of independent variables (highest correlation:  $R^2$ ) would be to predict *P. parvum* bloom formation; however not all independent variables may end up in the equation if they do not yield significant *t*-tests. Additionally, variables with a *p* value  $>0.1$  and are not considered statistically significant and are also restricted from entering the regression equation. However, if the *p* values are  $\leq 0.1$ , it can be said that sufficient TN and TP and balanced N:P ratios are a predictor of *P. parvum* bloom formations; reject the null hypothesis (H<sub>0</sub>).

## CHAPTER 4

### PROJECT AND METHODOLOGY

This section covers the project description and data collection while explaining the chosen research method, site selection criterion, field measurements, as well as data and statistical analysis.

#### **Project Description**

At an elevation of 1,405.9 feet (ft), LLV has a maximum volume of nearly 10,000 acre-feet (AF), consisting of two-miles in length, one-mile in width, and nearly 12 miles of shoreline (Weber 2006). The lake is constructed over the Las Vegas Wash and water from the wash runs underneath LLV through two 84-inch diameter pipes (Lake Las Vegas Special Report, 2010). To meet the requirements of irrigation, seepage and evaporation losses of LLV, an average of 7,000 AF of fill water must be placed into the lake yearly from Lake Mead, sold by the City of Henderson (Weber 2006); however, some water received comes from overflow storm water.

#### **Data Collection**

The Clark County 208 Water Management Plan, approved in 1988, required a water quality monitoring program to be developed for LLV to ensure that the reservoir's water quality was maintained based on standards established for Lake Mead per the Nevada Administrative Code (NAC) as part of County 208 Amendment (Weber 2006). In 1991, a long-term water quality program was implemented where annual Water Quality Monitoring Reports, prepared by MWH, were submitted to the Nevada Division of Environmental Protection for review.

Data used to compile the Water Quality Monitoring Reports were put into Microsoft Access databases. Files were organized for each category of collected data; physical measurements, chemical and biological measurements, and plankton. Data were compiled and analyzed using Microsoft Access 2000, Microsoft Excel 2000, Microsoft Word 2000, SPSS Statistics 7.0 for Windows, SAS version 8 statistical software, Sigma Stat version 3.0, and Sigma Plot version 9.0.1 (Weber 2006).

### **Research Method**

An analytical cross-sectional study design was used with data ranging from December 2009 to December 2012 at one point in time. Data was collected using secondary analysis from the Water Quality Monitoring Reports prepared by MWH, as discussed above. IBM SPSS Statistics 21 and Microsoft Excel 2000 were used for data analysis.

With the use of the analytical cross-sectional study design, it enabled me to grasp the overall characteristics of possibly why *P. parvum* bloom occurred, while looking at water quality characteristics and the effects of *P. parvum* on targeted biota. *P. parvum* toxicity levels were measured using bloom formation quantities (*P. parvum* biomass (mg/m<sup>3</sup>)), for toxicity could not be accurately assessed based on available data. Recall, effects of the toxins are usually measured by hemolytic activity or mortality of algal cells or animals; hemolytic activity and mortality of algal cells were not provided, and even though fish kill data was available within these reports, total population counts were not, therefore toxicity levels could not be presumed. Non-*P. parvum* biomass (mg/m<sup>3</sup>), total zooplankton abundance (#/m<sup>3</sup>), temperature, TDS, mineral concentrations, and nutrients (TN, TP, N:P

Ratio), provided in the reports, were analyzed against *P. parvum* biomass (mg/m<sup>3</sup>), using SPSS Statistics 21 to determine variable significance.

### Site Selection

The Nevada Division of Environmental Protection originally required four sites at LLV to be sampled monthly. In 2008, sampling locations were reduced from four sites to two sites: LLV-1A and LLV-3A. In 2010, the water sampling protocol was provisionally revised from monthly to biweekly sampling at site LLV-1A (Figure 7) to better monitor water quality and changes in plankton populations due to a *P. parvum* bloom that first occurred in December 2009. Site LLV-3 was only sampled from January to March, and therefore the 2010 annual water quality report only addressed water quality at site LLV-1A. Sampling frequencies at LLV-1A remained constant through December 2012, and therefore data from the LLV-1A sampling location were used for analysis.

Figure 7. Lake Las Vegas water quality monitoring location.



## Field Measurements

In accordance with the water quality monitoring protocol, vertical sampling was performed at 0 meters (m), 5m, 10m, and 20m depths. Lake Las Vegas Water Quality Monitoring variables, including the type of measurements, constituents, depths of measurements and methods used for this study, can be seen in Table 3. Physical measurements, including temperature, pH, and EC, were measured throughout the water column using a Hydrolab Surveyor Model 4; results were recorded from the instrument. Chemical and biological measurements were also taken at determined depths from 0-2.5 meters for TN, TP, and TDS. Additional depth samples were collected using a Van Dorn sampler (Figure 8) at 5 meters, 10 meters, and 20 meters at site LLV-1A for major anions/cations (minerals), including calcium, bicarbonate, sodium, magnesium, potassium, chloride, and sulfate. Samples were transferred to Nalgene preserved sampling containers, labeled appropriately, and shipped on ice to a State of Nevada certified laboratory for analysis, using methods approved by the Environmental Protection Agency (EPA). Duplicate field measurements were made on approximately 10 percent of the samples for quality control purposes.

An 80µm Wisconsin plankton net was used to collect zooplankton (cladocerans, copepods, and rotifers) samples in a vertical tow from 0-15 m. Bi-weekly surface (0-2.5m) samples of phytoplankton (*P. parvum*) and were collected from the sampling location, and were identified and counted to the level of species when possible by Janik, Inc. of Davis, California.

Table 3. Lake Las Vegas Water Quality Monitoring variables including type of measurements, constituents, depths of measurements, and methods used.

Analytical Characteristics for Lake Las Vegas			
Measurement Type	Constituent	Depth (m)	Method
Physical	Temperature (°C)	2.0 meter intervals	Electronic multimeter and sonde
	pH (std. units)	surface to 1.0 m	
	Conductivity (µS/m)	above bottom	
Chemical	Total Nitrogen (TN) (µg/L)	Integrated 0-2.5 m	APHA (1995)
	Total Phosphorus (TP) (µg/L)		EPA 365.2
	Total Dissolved Solids (mg/L)		EPA 160.2
	Major Anions/Cations (mg/L)	Integrated 5-20 m	EPA 200.7
Biological	Phytoplankton (mg/m <sup>3</sup> )	Integrated 0-2.5 m	APHA (1995)
	Zooplankton (#/m <sup>3</sup> )	0-15 m Vertical Tow	APHA (1995)

Figure 8. Use of a Van Dorn sampler for additional depth sampling performed at 0 meters (m), 5m, 10m, and 20m depths.



### Data Analysis

Using mid-month data from the 2009 and 2012 Water Quality Monitoring Reports, statistical analysis, by means of Microsoft Excel 2000 and IBM SPSS Statistics 21 for Windows, were carried out to determine if independent variables

were significant for *P. parvum* bloom formation (biomass mg/m<sup>3</sup>), and if the null hypothesis ( $H_0$ ) should be accepted or rejected.

First, data sets were compared for unusual trends or data outliers, and were evaluated for explanations of the results. Next, simple linear regression was used to assess the relationships between non-*P. parvum*, biomass and total zooplankton abundance variables on *P. parvum* bloom formation (dependent variable), and if a positive or negative relationship could be determined. The correlation ( $R^2$ ) was used to determine what variability of non-*P. parvum* biomass (mg/m<sup>3</sup>) and total zooplankton abundance (#/m<sup>3</sup>) could be explained by *P. parvum* biomass (mg/m<sup>3</sup>). If the  $p$  value was  $\leq 0.1$  with a slope ( $\beta$ )  $\neq 0$ , it was projected that an increase in *P. parvum* biomass was a predictor of decreased non-*P. parvum* biomass and zooplankton abundance, and the null hypothesis ( $H_0$ ) was rejected. A broader inclusion criterion ( $p \leq 0.1$ ) was selected to avoid oversight of contributing variables significant for *P. parvum* bloom formation.

Stepwise multiple regression analysis was additionally used to determine if correlations existed between *P. parvum* biomass and temperature, TDS, mineral speciation, total nitrogen (TN), total phosphorous (TP), and N:P ratios, since these variables have all been shown to be significant for *P. parvum* bloom formation. By using stepwise multiple regression analysis, the best combination of independent variables (highest  $R^2$ ), to predict *P. parvum* bloom formation, could be determined. Variables with a  $p$ -value  $> 0.1$  were not considered statistically significant and were restricted from the equation. However, if the results did meet the selection criteria, it was projected that the independent variable was a predictor of *P. parvum* bloom

formation, and the null hypothesis ( $H_0$ ) was rejected. The stepwise multiple regression method was used due to its ability to eliminate many autoregression lags and then sequentially remove autoregressive parameters until all remaining independent variables yield significant  $t$ -tests (Weber 2006).

Visual analysis of initial results demonstrated a 30 day lag between independent variable and *P. parvum* biomass fluctuations. Therefore, regressions were performed using a 30 day lag in order to traverse appropriate date ranges to determine variable significance, otherwise independent variables that were indeed significant may not have demonstrated their strength of association. This lag was observed with Weber and Janik's (2010) work, where fish mortality was observed approximately 30 days after the first occurrence of *P. parvum* in December 2009.

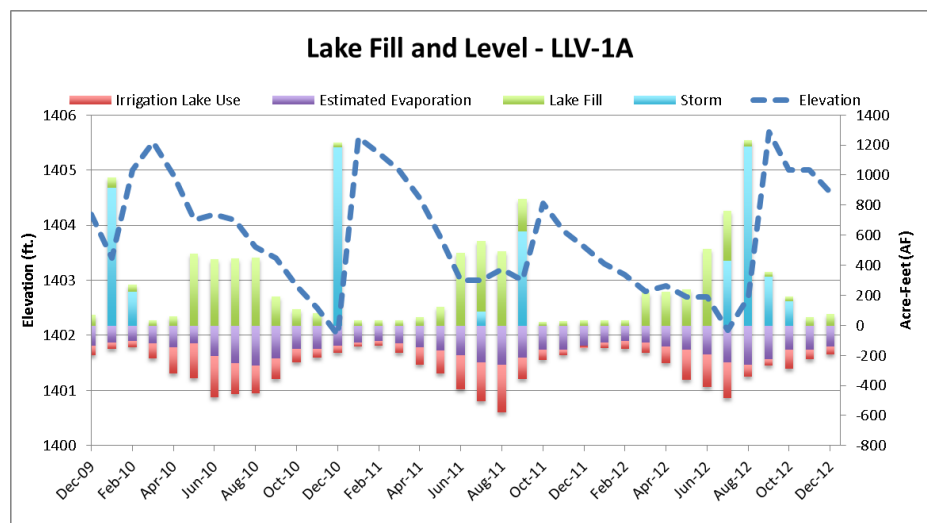
## CHAPTER 5

### RESULTS

#### Lake Fill and Level

To meet the requirements of irrigation, seepage and evaporation losses of LLV, an average of 7,000 AF of fill water must be placed into the lake yearly from Lake Mead, sold by the City of Henderson (Weber 2006); however, some water received comes from overflow storm water. Figure 9 demonstrates the sequence and timing of storm events, lake fill, estimated evaporation, irrigation lake use and lake elevation. Lake elevation and water acquisition is an important factor to consider when assessing the lake's plankton and water characteristics on *P. parvum* bloom formation.

Figure 9. Reservoir lake fill and level for Lake Las Vegas, Clark County, Nevada for the study period of 2009-2012.



## Descriptive Analysis

Descriptive frequencies for categorical plankton and lake water characteristic variables vary due to natural and anthropogenic sources, as seen in Table 4.

Variables include the dependent variable, *P. parvum* (mg/m<sup>3</sup>), along with independent variables: non-*P. parvum* biomass (mg/m<sup>3</sup>), total zooplankton abundance (#/m<sup>3</sup>), temperature, TDS, mineral concentrations, TN, TP, and N:P ratio. The number of sampling events (n), mean ( $\bar{x}$ ), standard deviation (SD), and range were reported on an annual basis.

Table 4. Descriptive frequencies for plankton and lake water characteristics collected for site Lake Las Vegas -1A, Clark County, Nevada.

Variable	Year	(n)	( $\bar{x}$ )	SD	Range
<b><i>P. parvum</i> biomass (mg/m<sup>3</sup>)<sup>a</sup></b>	2009	2	0	0	[0, 0]
	2010	12	5173.88	7747.54	[0, 21553.5]
	2011	12	2213.0	3556.5	[7.0, 10103.0]
	2012	13	511.77	675.37	[0, 1785.3]
<b>Non-<i>P. parvum</i> biomass (mg/m<sup>3</sup>)<sup>b</sup></b>	2009	2	1093.4	420.30	[796.2, 1390.6]
	2010	12	485.66	446.69	[82.3, 1597.0]
	2011	12	593.55	428.39	[116.6, 1312.0]
	2012	12	9001.59	12241.14	[213.6, 44134.5]
<b>Tot. Zooplankton abundance (#/m<sup>3</sup>)<sup>b</sup></b>	2009	2	12623.50	13090.67	[3367, 21880]
	2010	12	19827.66	27917.65	[345, 89613]
	2011	12	74433.83	98063.54	[0, 287483]
	2012	13	51732.69	66232.31	[0, 166185]
<b>Temp °C (0 depth)<sup>b</sup></b>	2009	2	17.05	3.75	[14.40, 19.70]
	2010	12	19.33	7.45	[8.20, 30.10]
	2011	12	18.70	7.17	[8.2, 28.8]
	2012	12	18.73	7.76	[8.1, 30.70]
<b>TDS (mg/L)<sup>b</sup></b>	2009	2	1913.0	94.75	[1846, 1980]
	2010	12	1821.25	207.73	[1434, 2090]
	2011	12	1662.67	154.6	[1370, 2007]
	2012	12	1804.46	295.28	[1308, 2084]
<b>Mineral Concentration (mg/L)<sup>b</sup></b>	2009	2	727.0	26.87	[708, 746]
	2010	12	555.25	72.59	[446, 694]
	2011	12	549.67	79.7	[414, 751]
	2012	12	551.15	100.25	[402, 668]
<b>TN (µg/L)<sup>b</sup></b>	2009	2	885.0	304.06	[670, 1100]
	2010	12	1155.83	493.12	[660, 2130]
	2011	12	1153.3	681.23	[355, 3035]
	2012	12	1019.31	528.31	[245, 2305]
<b>TP (µg/L)<sup>b</sup></b>	2009	2	25.0	28.28	[5, 45]
	2010	12	20.25	10.58	[5, 39]
	2011	12	23.83	12.17	[7, 46]
	2012	12	37.46	23.16	[13, 82]
<b>N:P Ratio (µg/L)<sup>b</sup></b>	2009	2	117.0	145.66	[14, 220]
	2010	12	64.83	31.64	[36, 158]
	2011	12	55.25	32.28	[22, 137]
	2012	12	32.69	19.0	[9, 63]
a. Dependent Variable: <i>P. parvum</i> biomass (mg/m <sup>3</sup> ) b. Independent Variables: Non- <i>P. parvum</i> biomass (mg/m <sup>3</sup> ), Total Zooplankton abundance (#/m <sup>3</sup> ), Temperature, TDS, Total Minerals, TN, TP, N:P Ratio,					

## Correlation Analysis

Regression analyses for appropriate data from the December 2009 to December 2012 study period were performed using SPSS Statistics 21 software. Results were evaluated to better understand the correlations between non-*P. parvum* biomass (mg/m<sup>3</sup>) and total zooplankton abundance (#/m<sup>3</sup>), temperature, TDS, mineral concentration, total nitrogen (TN), total phosphorous (TP), and N:P ratio on predicting *P. parvum* bloom formation.

### Simple Linear Regression Analysis

#### Plankton analysis.

- H<sub>a1</sub>: Increases in non-*P. parvum* biomass and total zooplankton abundance predict drops in *P. parvum* biomass in Lake Las Vegas, ( $\beta_1 \neq 0$  and/or  $\beta_2 \neq 0$ )
- H<sub>01</sub>: Increases in non-*P. parvum* biomass and total zooplankton abundance do not predict drops in *P. parvum* biomass in Lake Las Vegas, ( $\beta_1 = \beta_2 = 0$ )

The regression yielded an R<sup>2</sup> of .061, indicating only 6% of the variability in *P. parvum* biomass could be explained in the regression by non-*P. parvum* biomass and total zooplankton combined, and 94% of *P. parvum* biomass was explained by other factors other than those analyzed in this study (Table 5). The standard error of measurement for both independent variables was low (SE =0.103 and 0.012), indicating a high reliability of the test and more precision. The line of best-fit designated an inverse relationship, and it was predicted as non-*P. parvum* biomass and total zooplankton abundance decreased, *P. parvum* biomass increased ( $\beta_1 \neq 0$ ,  $\beta_2 \neq 0$ ) (Appendix III). According to selection criterion ( $p \leq 0.1$ ), non-*P. parvum* biomass and total zooplankton abundance were non-significant variables for

predicting *P. parvum* bloom formation ( $p = 0.337$ ) and increases in these measures did not predict drops in *P. parvum* biomass in LLV. Therefore, it can be said that non-*P. parvum* biomass and total zooplankton abundance were not good predictors of *P. parvum* biomass.

Table 5. Regression analysis data for plankton collected at Lake Las Vegas -1A, Clark County, Nevada.

Sampling Events	Analysis	Variables	Model Sum <sup>b</sup>	Coefficients <sup>a</sup>		t	p-value
				B ‡	Std. Error		
N=38	Linear Regression	<i>P. parvum</i> biomass (mg/m <sup>3</sup> ) <sup>a</sup> Non- <i>P. parvum</i> biomass (mg/m <sup>3</sup> ) <sup>b</sup> Tot. Zooplankton abundance(#/m <sup>3</sup> ) <sup>b</sup>	R <sup>2</sup> = 0.061	B <sub>1</sub> ‡ = -0.182  B <sub>2</sub> ‡ = -0.160	0.103  0.012	-0.974  -1.113	0.337
a. Dependent Variable: <i>P. parvum</i> biomass (mg/m <sup>3</sup> )							
b. Predictors: non- <i>P. parvum</i> biomass (mg/m <sup>3</sup> ), Total Zooplankton abundance (#/m <sup>3</sup> )							
* R <sup>2</sup> indicating % of the variability in <i>P. parvum</i> biomass is explained by independent variables (x)							
‡ β <sub>1,2,...</sub> = intercept							
‡ <i>p</i> ≤0.1, reject the H <sub>0</sub> and use H <sub>a</sub>							

### Stepwise Multiple Regression Analysis

For the remaining independent variables, stepwise multiple regression was used determine what the best combination of independent variables (highest R<sup>2</sup>) would be to predict *P. parvum* bloom formation. TN and TP are not independent of N:P ratio, therefore they were permissible for the same regression. However, since TDS is considered a function of mineral concentration, two separate stepwise regression analyses were carried out in order to attain accurate significance levels of the two variables. As shown in Table 6, temperature and TDS appeared to be significant independent variables in predicting *P. parvum* bloom formation ( $p \leq 0.1$ ), while the remaining variables were excluded from the stepwise regressions models

( $p > 0.1$ ). Similarly, when mineral concentration took place of TDS in the regression (Table 7), mineral concentration, temperature, and TN appeared to be significant variables ( $p \leq 0.1$ ) in predicting *P. parvum* bloom formation.

#### **Temperature analysis.**

- H<sub>a2</sub>: Temperatures between 5°C and 30°C predict the highest *P. parvum* bloom formation, ( $\beta_3 \neq 0$ )
- H<sub>02</sub>: Temperatures between 5°C and 30°C do not predict the highest *P. parvum* bloom formation, ( $\beta_3 = 0$ )

Stepwise regression results from Table 6 and Table 7 show temperature alone was a statistically significant variable in predicting *P. parvum* bloom formation (model 1:  $R^2 = .151$ ,  $p = 0.016$ ). Model 1 results concluded that 15% of the variability in *P. parvum* biomass was explained in the regression by temperature, and 85% of *P. parvum* biomass was explained by other factors. The standard error of measurement was high (model 1: SE= 109.11), indicating a low reliability of the test and less precision within the models. The line of best-fit designated an inverse relationship, and it was predicted that as temperature decreased, *P. parvum* biomass increased ( $\beta_3 \neq 0$ ) (Appendix III). Temperature results from model 2 and model 3 are discussed in further detail in the TDS/mineral section below. According to selection criterion ( $p \leq 0.1$ ), temperature alone was significant in predicting *P. parvum* bloom formation (model 1:  $p = 0.016$ ), and was considered a good predictor of *P. parvum* bloom formation, with the highest bloom formation occurring between 8.1°C and 17.3°C.

### **TDS and mineral concentration analysis.**

- H<sub>a3a</sub>: Increased TDS concentrations predict *P. parvum* bloom formation, ( $\beta_4 \neq 0$ )
- H<sub>a3b</sub>: Increased mineral concentrations predict *P. parvum* bloom formation, ( $\beta_5 \neq 0$ )
- H<sub>03</sub>: Increased TDS and mineral concentrations do not predict an increase in *P. parvum* bloom formation, ( $\beta_4 = \beta_5 = 0$ )

Stepwise regression results in Table 6 show TDS, along with temperature in model 2, are statistically significant variables in predicting *P. parvum* biomass. TDS and temperature together yielded an R<sup>2</sup> of .259. This concluded that 26% of the variability in *P. parvum* biomass was explained in the regression by minerals and temperature, and 74% of *P. parvum* biomass was explained by other factors not analyzed in this regression. The standard error of measurement was low (SE=3.29), indicating a high reliability of the test and more precision within the model for TDS. The line of best-fit designated an inverse correlation, and it could be predicted that as TDS decreased, *P. parvum* biomass increased ( $\beta_3 \neq 0, \beta_4 \neq 0$ ) (Appendix III). According to selection criterion ( $p \leq 0.1$ ), temperature and TDS were significant in predicting *P. parvum* bloom formation ( $p = 0.005$ ). Although findings do not support the hypotheses, TDS was a strong predictor of *P. parvum* biomass.

Likewise, regression results shown in Table 7 reveal mineral concentration, along with temperature in model 2, were statistically significant variables in predicting *P. parvum* bloom formation. Mineral concentration and temperature together yielded an R<sup>2</sup> of .284. This concluded that 28% of the variability in *P.*

*parvum* biomass was explained in the regression by minerals and temperature, and 72% of *P. parvum* biomass was explained by other factors not assessed in the regression. The standard error of measurement was low (SE=8.43), indicating a high reliability of the test and more precision within the model for minerals. The line of best-fit designated inverse correlation, and it could be predicted that as mineral concentrations decreased, *P. parvum* biomass increased ( $\beta_3 \neq 0, \beta_5 \neq 0$ ) (Appendix III). According to selection criterion ( $p \leq 0.1$ ), temperature and mineral concentrations were significant in predicting *P. parvum* bloom formation ( $p = 0.003$ ). Although studies do not support the hypotheses, mineral concentration appeared to be a strong predictor of *P. parvum* biomass.

#### **Nutrient analysis.**

- H<sub>a4a</sub>: Non-limited TN and TP predict increases in *P. parvum* bloom formation, ( $\beta_6 \neq 0$  and/or  $\beta_7 \neq 0$ )
- H<sub>a4b</sub>: Unbalanced N:P ratios predict increases in *P. parvum* bloom formation, ( $\beta_8 \neq 0$ )
- H<sub>04</sub>: Non-limited TN and TP, and unbalanced N:P ratios do not predict increases in *P. parvum* bloom formation, ( $\beta_6 = \beta_7 = \beta_8 = 0$ )

Regression results shown in Table 7 concluded that TN, was significant in predicting *P. parvum* bloom formation while TP and N:P ratio were non-significant. Model 3 demonstrates TN, temperature and mineral concentration together yielded an R<sup>2</sup> of .341. This concluded that 34% of the variability in *P. parvum* biomass was explained in the regression by TN, temperature and minerals, while 66% of *P. parvum* biomass was explained by other factors not assessed in the regression. The

standard error of measurement was low ( $SE=1.31$ ), indicating a high reliability of the test and more precision within the model for TN. The lines of best-fit demonstrated positive correlations for TP and TN, and it could be predicted as TN and TP increased, *P. parvum* biomass also increased ( $\beta_6 \neq 0$ ,  $\beta_7 \neq 0$ ). The line of best-fit for N:P ratio revealed a slight inverse correlation, and it could be predicted as N:P ratio decreased, *P. parvum* biomass increased ( $\beta_8 \neq 0$ )(Appendix III). According to selection criterion ( $p \leq 0.1$ ), TN, temperature, and mineral concentrations in model 3 were significant in predicting *P. parvum* bloom formation ( $p = 0.002$ ). Findings support the hypothesis for TN only, and an increase in TN was a predictor of *P. parvum* biomass, while TP and N:P ratio were non-significant in predicting *P. parvum* bloom formation.

Table 6. Regression analysis for *P. parvum* and lake water characteristics (mineral concentration excluded) collected at Lake Las Vegas -1A, Clark County, Nevada.

Sampling Events	Analysis	Variables	Model Sum <sup>b</sup>	Coefficients <sup>a</sup>		t	p-value
				B <sub>‡</sub>	Std. Error		
N= 38	Stepwise Model 1	<i>P. parvum</i> biomass (mg/m <sup>3</sup> ) <sup>a</sup>	R <sup>2</sup> =				
		Temp °C <sup>b</sup>	0.151*	B <sub>3</sub> ‡ = -0.389	109.11	-2.53	0.016 ‡
	Stepwise Model 2	<i>P. parvum</i> biomass (mg/m <sup>3</sup> ) <sup>a</sup>	R <sup>2</sup> =	B <sub>3</sub> ‡ = -0.394	103.41	-2.71	
		Temp °C <sup>b</sup>	0.259*	B <sub>4</sub> ‡ = -0.328	3.29	-2.26	
		TDS (mg/L) <sup>b</sup>					0.005 ‡
	Excluded Variables (Model 1)	TDS (mg/L) <sup>b</sup>	n/a	n/a		-2.26	0.03
		TN (µg/L) <sup>b</sup>				1.97	0.057
		TP (µg/L) <sup>b</sup>				.78	0.444
		N:P Ratio (µg/L) <sup>b</sup>				-.144	0.887
	Excluded Variables (Model 2)	TN (µg/L) <sup>b</sup>	n/a	n/a		1.167	0.251
		TP (µg/L) <sup>b</sup>				-.327	0.745
		N:P Ratio (µg/L) <sup>b</sup>				.182	0.857
a. Dependent Variable: <i>P. parvum</i> biomass (mg/m <sup>3</sup> )							
b. Predictors: Temperature, TDS, TN, TP, N:P Ratio							
* R <sup>2</sup> indicating % of the variability in <i>P. parvum</i> biomass is explained by independent variables (x)							
‡ β <sub>1,2,...</sub> = intercept							
‡ P≤ 0.1, reject the H <sub>0</sub> and use H <sub>a</sub>							

Table 7. Regression analysis data for *P. parvum* and lake water characteristics (TDS excluded) collected at Lake Las Vegas -1A, Clark County, Nevada.

Sampling Events	Analysis	Variables	Model Sum <sup>b</sup>	Coefficients <sup>a</sup>		t	p-value
				B ‡	Std. Error		
N= 38	Stepwise Model 1	<i>P. parvum</i> biomass (mg/m <sup>3</sup> ) <sup>a</sup> Temp °C <sup>b</sup>	R <sup>2</sup> = 0.151*	B <sub>3</sub> ‡ = -0.389	109.11	-2.53	0.016 ‡
	Stepwise Model 2	<i>P. parvum</i> biomass (mg/m <sup>3</sup> ) <sup>a</sup> Temp °C <sup>b</sup> Mineral Conc. (mg/L) <sup>b</sup>	R <sup>2</sup> = 0.284*	B <sub>3</sub> ‡ = -0.453 B <sub>5</sub> ‡ = -0.371	103.15 8.43	-3.12 -2.55	0.003 ‡
	Stepwise Model 3	<i>P. parvum</i> biomass (mg/m <sup>3</sup> ) <sup>a</sup> Temp °C <sup>b</sup> Mineral Conc. (mg/L) <sup>b</sup> TN (µg/L) <sup>b</sup>	R <sup>2</sup> = 0.341*	B <sub>3</sub> ‡ = -0.399 B <sub>5</sub> ‡ = -0.334 B <sub>6</sub> ‡ = 0.246	102.86 8.309 1.31	-2.755 -2.334 1.712	.002 ‡
	Excluded Variables (Model 1)	Mineral Conc. (mg/L) <sup>b</sup> TN (µg/L) <sup>b</sup> TP (µg/L) <sup>b</sup> N:P Ratio (µg/L) <sup>b</sup>	n/a	n/a		-2.55	0.015
						1.97	0.057
						.78	0.444
						-1.44	0.887
	Excluded Variables (Model 2)	TN (µg/L) <sup>b</sup> TP (µg/L) <sup>b</sup> N:P Ratio (µg/L) <sup>b</sup>	n/a	n/a		1.71	0.941
						-4.99	0.621
						1.169	0.251
	Excluded Variables (Model 3)	TP (µg/L) <sup>b</sup> N:P Ratio (µg/L) <sup>b</sup>	n/a	n/a			
						-1.308	0.200
					.638	0.528	
a. Dependent Variable: <i>P. parvum</i> biomass (mg/m <sup>3</sup> )							
b. Predictors: Temperature, Total Minerals, TN, TP, N:P Ratio							
* R <sup>2</sup> indicating % of the variability in <i>P. parvum</i> biomass is explained by independent variables (x)							
‡ β <sub>1,2,...</sub> = intercept							
‡ P≤ 0.1, reject the H <sub>0</sub> and use H <sub>a</sub>							

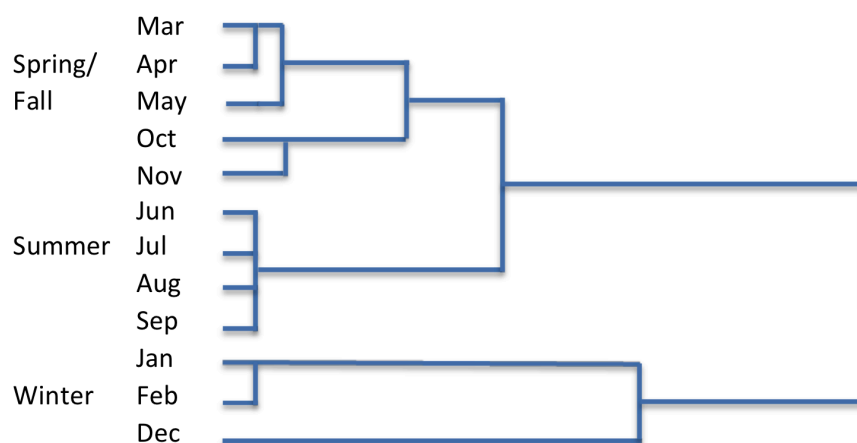
## Observational Analysis

Solely based on regression inclusion criteria, data does not reflect the mere importance of variables assessed. However, observation of interactions between independent variables on *P. parvum* bloom formation may be key to making profound discoveries, and may lead to more awareness and a better understanding of the research.

## Seasonality Analysis

In the years following the 2009 bloom, fish-killing *P. parvum* blooms were recurrent winter and spring phenomena. With *P. parvum* blooms' tendency to grow during winter and spring, seasonal groupings were used to help exemplify the seasonal water quality and plankton characteristics on *P. parvum*. In this study, the lake water surface temperature (0 meters) was used as the LLV-1A variable to cluster, due to relatively consistent yearly seasonal temperatures. Figure 10 illustrates the seasonal groupings based on months that share similar surface temperatures at zero meters. The three major seasonal groupings include: spring/fall (March, April, May, October), summer (June, July, August, September), and winter (December, January, February) (Weber 2006).

Figure 10. Seasonal groupings of *P. parvum* designated by ordered clusters, with surface temperature (0 meters) as the independent variable (Weber 2006).



The seasonal groupings of the three planktonic groups (*P. parvum* biomass, non-*P. parvum* biomass and total zooplankton) were evaluated (Figure 11). *P. parvum* was seen dominating the reservoir during the winter and spring/fall months, generally seen in December through May ( $\bar{x}$ : 2,622 mg/m<sup>3</sup>), whereas total zooplankton thrived in the reservoir during the summer, seen in June through September ( $\bar{x}$ : 39,654 #/m<sup>3</sup>) due to less competition and favorable growth environments. Total zooplankton composition predominately consisted of the herbivorous and carnivorous copepods during all three seasonal groupings, which may have been primarily due to their feeding habits (Figure 12).

Figure 11. Plankton seasonal grouping averages during the study period of 2009-2012 at site Lake Las Vegas -1A, Clark County, Nevada.

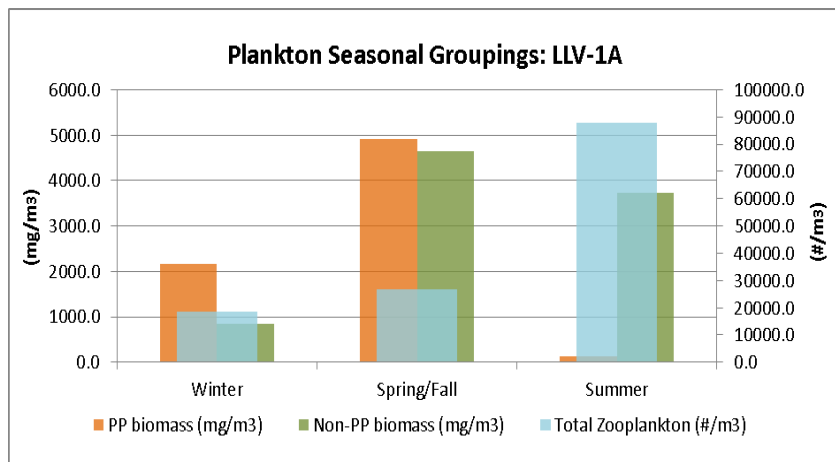
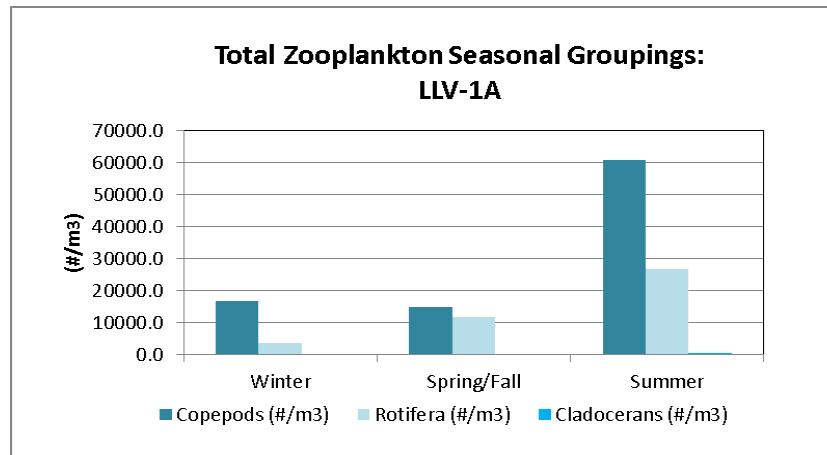


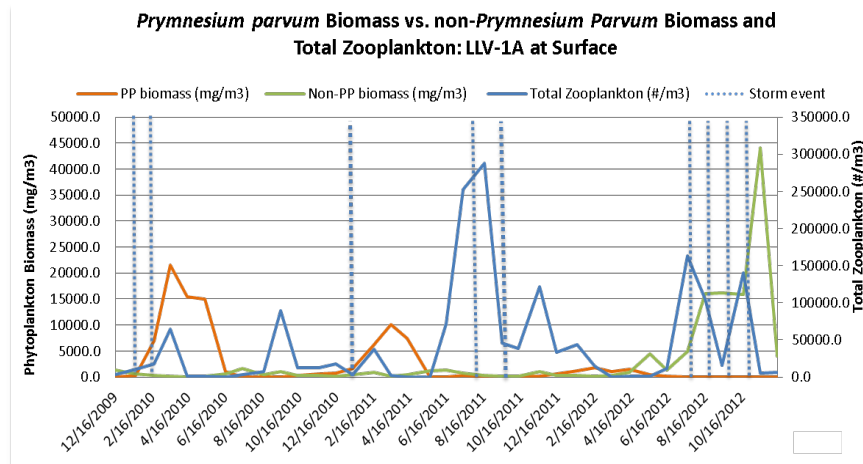
Figure 12. Total zooplankton seasonal grouping averages during the study period of 2009 - 2012 at site Lake Las Vegas- 1A, Clark County, Nevada.



## Biotic Factors

***P. parvum* and plankton interaction.** Algal competition and secession are important to consider. It is evident that phytoplankton and zooplankton compete for resources in LLV (Figure 13). During the first bloom cycle of 2009-2010, *P. parvum* gained dominance over competitive species, mainly copepods and cladocerans, with its ability to take advantage of environmental conditions that were less favorable to other species. During times of stress (i.e. limited nutrients) the use of *P. parvum*'s allelopathic effects may have provided means to attain ample nutrients needed for cell division and bloom formation; by releasing hemolytic compounds, phytoplankton communities became reduced allowing *P. parvum* to flourish. After this bloom the native species *Daphnia* disappeared and did not reoccur during the next bloom cycles.

Figure 13. *P. parvum*, non-*P. parvum* biomass and total zooplankton during the study period of 2009-2012 at site Lake Las Vegas -1A, Clark County, Nevada.



During the 2010-2011 and 2011-2012 blooms, *P. parvum* was gradually reduced, where it was outcompeted mainly by total zooplankton (primary consumers) consisting mainly of copepods and rotifers, while non-*P. parvum* communities remained low (Appendix I). However, the dynamics of LLV changed with zooplankton and non-*P. parvum* thriving in the reservoir during these bloom cycles. After the 2010-2011 bloom, a significant increase in zooplankton appeared, which may be explained by the sensitivity levels of zooplankton to prymnesins. One study performed by Schwierzke et al. (2010) found the rotifer, *Notholca*, was less sensitive to prymnesins and grazing reduced *P. parvum* abundance. This may also explain the decreases in *P. parvum* abundance during the last semi-bloom cycle of 2011-2012.

Increases in non- *P. parvum* biomass during late 2012 consisted primarily of the cyanobacterium *Pseudanabaena limnetica*. Having a high surface/volume ratio, this cyanobacterium can multiply and reproduce very quickly. This species along with N-fixing cyanobacteria present in the lake, such as *Cylindrospermopsis sp.*, may have

also contributed to increases in non-*P. parvum* biomass since it was at this time that N was limiting in the reservoir. However, additional factors may have contributed *P. parvum* bloom suppression.

### **Abiotic Factors**

Physical and chemical factors may play an important role in *P. parvum* bloom secession. Variables observed include temperature, TDS (mg/L), mineral concentration (mg/L), TN ( $\mu\text{g/L}$ ), TP ( $\mu\text{g/L}$ ), and N:P ratio. Parameters for *P. parvum* growth in LLV are discussed below.

**Optimal growth conditions for *P. parvum*.** Parameters for *P. parvum* optimal growth conditions in LLV are documented in Table 8. *P. parvum* was found to grow within the listed parameters, when temperature was between 8.1-17.3°C, with TDS  $\leq 2,058$  mg/L and mineral concentrations  $\leq 750$  mg/L. Nutrients also appeared significant for *P. parvum* bloom formation, with blooms occurring when TN ranged from 670 -3,035  $\mu\text{g/L}$  and TP levels between 5-57  $\mu\text{g/L}$ . The N:P ratio (range: 22-158 by concentration) appeared to influence *P. parvum* biomass, with concentrations <22 possibly mitigating blooms. Decreases in *P. parvum* during the 2011-2012 bloom may have enabled other phytoplankton and zooplankton to ensue with increasing temperatures.

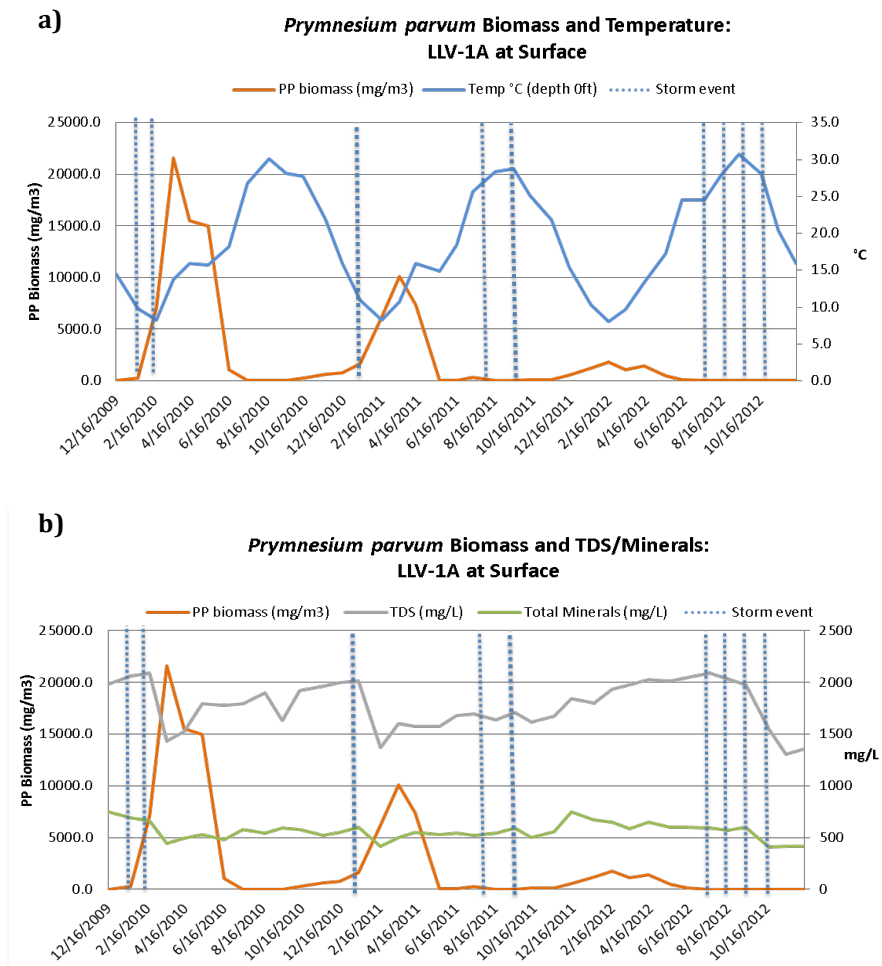
Table 8. *P. parvum* bloom formation and relevant abiotic parameters during the study period of 2009-2012 at site Lake Las Vegas -1A, Clark County, Nevada.

Variables	Approximate parameters for <i>P. parvum</i> growth in LLV	2009- 2010 <i>P. parvum</i> Bloom ( $\bar{x}$ : 9,416 mg/m <sup>3</sup> )	2010- 2011 <i>P. parvum</i> Bloom ( $\bar{x}$ : 4,349 mg/m <sup>3</sup> )	2011- 2012 <i>P. parvum</i> Bloom ( $\bar{x}$ : 1,089 mg/m <sup>3</sup> )	Dec. 2012 <i>P. parvum</i> No Bloom
Temp. °C	8.1 -17.3 °C (winter/spring months)	↓ Temp ( $\bar{x}$ : 12.4 °C)	↓ Temp ( $\bar{x}$ : 12.2 °C)	↓ Temp ( $\bar{x}$ : 12.3 °C)	↑ Temp (16 °C)
TDS	≤ 2,058 mg/L	↓ TDS ( $\bar{x}$ :1,812 mg/L)	↓ TDS ( $\bar{x}$ :1,612 mg/L)	↑ TDS ( $\bar{x}$ :1,929 mg/L)	↓ TDS (1,352 mg/L)
Minerals (Ca, Mg, K, Na)	≤ 750 mg/L	↓ Minerals ( $\bar{x}$ : 595 mg/L)	↓ Minerals ( $\bar{x}$ : 523 mg/L)	↑ Minerals ( $\bar{x}$ : 650 mg/L)	↓ Minerals (416 mg/L)
TN	670 -3,035 µg/L	↑ TN sufficient ( $\bar{x}$ : 1,265 µg/L)	↑ TN sufficient ( $\bar{x}$ : 1,123 µg/L)	↑ TN sufficient ( $\bar{x}$ : 1,334 µg/L)	↑ TN sufficient (1,270 µg/L)
TP	5-57 µg/L	↓ TP limited ( $\bar{x}$ : 28 µg/L)	↓ TP limited ( $\bar{x}$ : 37 µg/L)	↓ TP limited ( $\bar{x}$ : 26 µg/L)	↑ TP sufficient (70 µg/L)
N:P Ratio (conc.)	22-158	↑ High N:P Ratio ( $\bar{x}$ : 45)	↑ High N:P Ratio ( $\bar{x}$ : 30)	↑ High N:P Ratio ( $\bar{x}$ :51 )	↓ Balanced N:P Ratio (18)
↓ Decreases in independent variable were sufficient and within parameters for <i>P. parvum</i> growth in LLV					
↑ Increases in independent variable were sufficient and within parameters for <i>P. parvum</i> growth in LLV					
↓ Independent variable was low for <i>P. parvum</i> growth in LLV, which led to <i>P. parvum</i> growth					
↑ Independent variable was balanced for <i>P. parvum</i> growth in LLV, which led to <i>P. parvum</i> suppression					

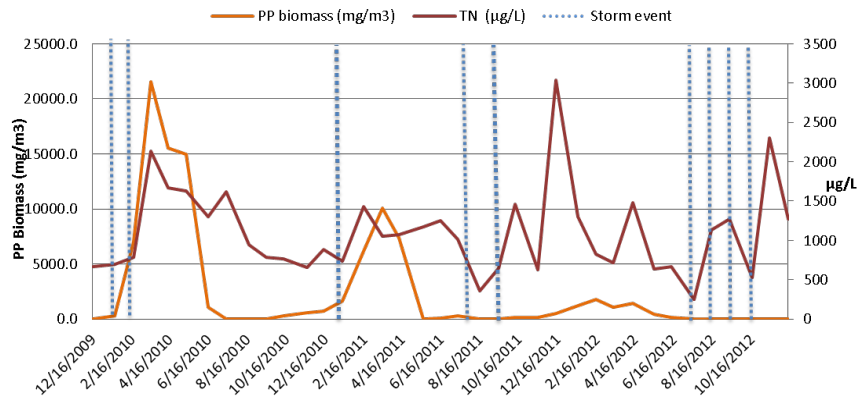
***P. parvum* and temperature.** Surface temperatures in LLV did not vary considerably throughout the study period with measurements averaging 19°C. When observing *P. parvum* biomass and temperature for LLV (Figure 14a), increases in *P. parvum* HAB were seen during the cooler winter and spring months (December through May), where temperatures between 8.1°C and 17.3°C appeared to predict the highest *P. parvum* bloom formation (Table 8). It was this time of year that LLV, lake, was completely circulated and resupplied with organic material and nutrients brought up off the lake bottom due to fall turnover (Hoffman et al. 1967). When the lake became stratified in the summer, fewer nutrients (N and P) were available for *P. parvum* bloom formation, and non-*P. parvum* species along with zooplankton became prominent the reservoir. Notice, however, that during winter 2011 through

spring 2012, *P. parvum* bloom formation only reached a peak of about 1,700 mg/m<sup>3</sup>, indicating that factors other than temperature contribute to *P. parvum* biomass accumulation.

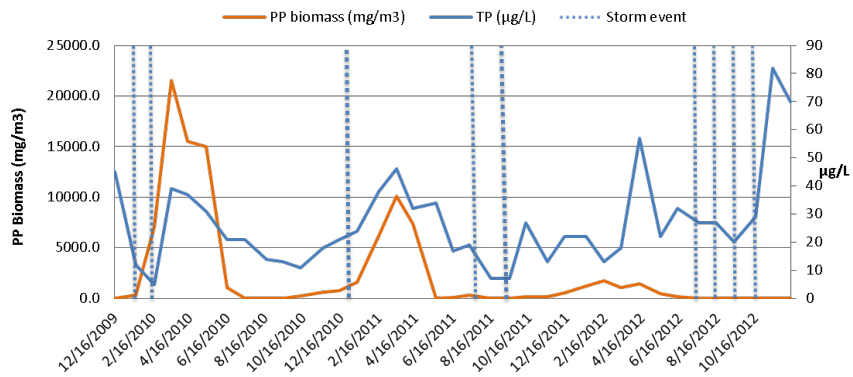
Figure 14. *P. parvum* biomass and temperature (a), TDS and mineral concentration (b), total nitrogen (c), total phosphorous (d) and N:P ratio (e) during the study period of 2009-2012 at site Lake Las Vegas -1A, Clark County, Nevada.



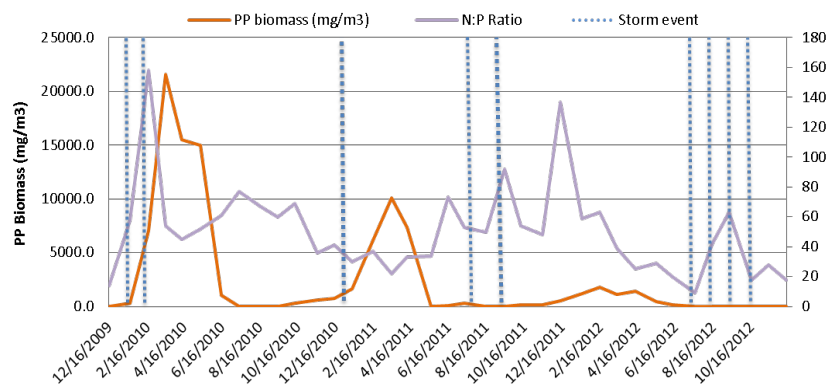
c) *Prymnesium parvum* Biomass and Total Nitrogen: LLV-1A at Surface



d) *Prymnesium parvum* Biomass and Total Phosphorous: LLV-1A at Surface

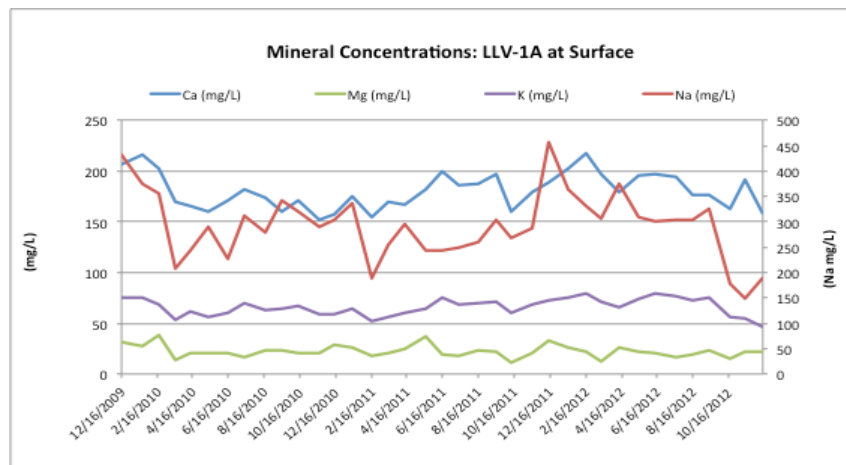


e) *Prymnesium parvum* Biomass and N:P Ratio Concentration: LLV-1A at Surface



***P. parvum* and TDS (salinity)/mineral concentrations.** To “activate” prymnesins, TDS minerals (component ions or salts) must be present (Manning and La Claire 2010). The majority of TDS in most ambient waters are ionic non-organic minerals (component ions or salts) such as calcium, magnesium, sodium, potassium, carbonate, bicarbonate, sulfate, chloride, bromide, and nitrate. Essential mineral concentrations for LLV are shown in Figure 15, where sodium (Na) and calcium (Ca) values were highest during the 2011-2012 semi-bloom (Table 8), and *P. parvum* concentrations were low.

Figure 15. Mineral concentrations during the study period of 2009-2012 at site Lake Las Vegas -1A, Clark County, Nevada.



TDS concentrations required to support *P. parvum* blooms are estimated to be between 500 and 1000 mg/L (Renner 2009), according to previous studies. However, *P. parvum* blooms emerged in LLV with TDS concentrations ranging from 1,370 to 2,090 mg/L. *P. parvum* biomass was highest during the 2009-2010 and 2010-2011 bloom occurrences (Figure 14b). Winter storm events in December

2009 and December 2010 brought in approximately 916AF and 1186AF into LLV (Figure 9). Dilution of the reservoir from the storm events led to decreases in TDS ( $\bar{x}$ :1,812 mg/L,  $\bar{x}$ :1,612 mg/L) and minerals ( $\bar{x}$ : 595 mg/L,  $\bar{x}$ : 523 mg/L) where increases in *P. parvum* of up to 21,553 mg/m<sup>3</sup> ( $\bar{x}$ : 9,416) were observed (Appendix I). It is highly probable that storm event overflows diluted the TDS content of the reservoir if the storm water runoff was less saline than the water of LLV. During the 2011-2012 semi-bloom, however, winter storms did not occur, and evaporation removed distilled water from the reservoir while leaving behind minerals. This led to increases in TDS ( $\bar{x}$ :1,929 mg/L) and mineral concentration ( $\bar{x}$ : 650 mg/L), where *P. parvum* biomass was reduced to an average of 1,089 mg/m<sup>3</sup>. Although TDS and mineral concentrations decreased in August 2012, *P. parvum* was not observed, as the temperature of LLV was not efficient in supporting *P. parvum* blooms in LLV. However, TDS was at decreased levels (1,352 mg/L) when water temperatures became favorable for *P. parvum* growth during December 2012 (16 °C), which should have led to *P. parvum* bloom formation if TDS was in fact a significant predictor variable. Therefore, it appears TDS is not as significant in predicting *P. parvum* bloom formation as the data analysis suggests, and that other factors play a role in its emergence.

***P. parvum* and Nutrients.** Generally, lower TN and TP concentrations at LLV existed in the summer months (Figure 14c, 14d), when nutrients were suppressed in the benthic zone during stratification. At this time *P. parvum* cells were likely non-flagellated resting cysts that were able to revive when nutrients became available during nutrient loading from storm events (Figure 9), recycled nitrogen by

zooplankton, fertilization of golf courses, and mixing of the reservoir in the cooler months. However, mixotrophic characteristics were likely used as a strategy to obtain nutrients under depleted conditions.

TN and TP limitation was determined based on previous studies and observational analysis of N:P ratio concentrations (N:P ratio  $\geq 22$  = P limitation; N:P ratio  $\leq 10$  = N limitation; N:P ratio  $< 22$  and  $> 10$  = balanced). When temperatures for *P. parvum* growth ensued, during the winter and spring months, TP was limiting throughout all three bloom cycles (N:P ratio concentrations:  $\bar{x}$ : 63.5;  $\bar{x}$ : 32;  $\bar{x}$ : 58) (Table 8). It's suggested that LLVs' unique environment allowed for high competitive ability for *P. parvum* growth due to P-limited brackish waters (Baker et al. 2009). While TP was limiting and TN was sufficient, increases in the Redfield ratio were observed. Consequently, a high N:P ratio demonstrated that TP was the primary limiting nutrient allowing *P. parvum* growth. Stress caused by P limitation may have increased phosphate uptake by *P. parvum* and stimulated hemolytic activity, which enabled its dominance over other phytoplankton in the reservoir, leading to fish kills. Notice, during the December 2012 non-bloom occurrence, the N:P ratio was balanced (18), suggesting N:P concentrations  $< 22$  suppressed *P. parvum* bloom formation in LLV at this time. The N:P ratio, along with the amount of TP and TN in the reservoir appeared to be significant predictors of *P. parvum* growth determination leading to fish kill events.

## CHAPTER 6

### DISCUSSION AND IMPLICATIONS

#### **Discussion**

The results of this study demonstrated the dependence of growth of *P. parvum* under conditions of competition and secession with non-*P. parvum* biomass and zooplankton abundance, as well as abiotic factors. Temperature explained 15% ( $R^2 = 0.151$ ) of the variability in *P. parvum* biomass ( $\text{mg}/\text{m}^3$ ). Higher biomass was observed at lower winter and spring temperatures ranging between 8.1°C and 17.3°C, and was not observed during the summer months, when non-*P. parvum* phytoplankton and total zooplankton thrived in the reservoir. Temperature, in combination with TDS ( $R^2 = 0.259$ ) and minerals ( $R^2 = 0.284$ ) appeared to be significant predictors of *P. parvum* bloom formation, however, observational analysis of TDS and mineral concentration challenges these findings.

TN explained about 6% of the variability in *P. parvum* biomass; though, observational analysis revealed that TP was also significant in predicting *P. parvum* bloom formation. Nutrients required for optimal *P. parvum* growth and toxicity were observed at N:P ratio concentrations  $\geq 22$ , when TN was sufficient and TP was limiting (high/unbalanced N:P ratio). Therefore, it could be that bloom emergence led to depletion of nutrients in the process, thus increasing toxicity. Unbalanced N:P ratios were influenced by eutrophication from late winter storm events and possible golf course fertilization. N:P ratio concentrations that were  $< 22$  and  $> 10$  (by concentration) at the end of 2012 were indicative of *P. parvum* suppression, suggesting that *P. parvum* is not an active competitor when nutrients are balanced.

## Implications

A significant amount of nutrients come from the land surrounding LLV, including golf courses, roads, and the build environment. When storm events occur, water flow increases and percolation into the soil is reduced, causing more water to reach the tributaries and other point sources. Point sources deliver nutrients to LLV from runoff in the watershed. Developing a Best Management Practice (BMP) to incorporate ecosystem management strategies to construct wetlands around LLV, or mechanical management strategies involving barrier applications may be significant for nutrient inflow reduction into the lake. Inflow reduction would reduce inputs of nutrients into watersheds, which can control the niche for *P. parvum* (Kurten 2010). By reducing the amounts of N into the reservoir, the N:P ratio may naturally become balanced, and thus attenuate fish-killing blooms.

Predicting the effects of stressors on environmental configuration and *P. parvum* biomass is essential for effective lake management and restoration of water quality. Lake management strategies may involve appropriate fertilization of golf courses and timely lake dredging in order to minimize the risk of fish kills related to *P. parvum* blooms. Golf course fertilization should be completed during the beginning of summer, when the least amount of storms occurred. If done in the winter when more storms occurred, excessive amounts of N from golf course runoff and nutrients may be released which may cause a deviation in the Redfield ratio, leading to *P. parvum* stress, growth, and toxicity. Organic “slow-release” fertilizers may be recommended which are broken down gradually by microbes in the soil, limiting runoff.

When water quality begins to deteriorate and chemical composition of the soil is contaminated, determined by field measurements, spot dredging may be recommended (Herbich 2000). Spot dredging should be conducted around the LLV at site sources incurring the highest amount of inflow. Spot dredging may improve the water quality by reducing nutrient loads from the sediments, thereby reducing *P. parvum* blooms in LLV (Herbich 2000). However, optimal timing of lake dredging is difficult to suggest because the dynamics of LLV are always evolving. Additionally, because the lifecycle of *P. parvum* is unclear, sediment removal could provide prime environmental cues necessary for *P. parvum* germination.

### **Limitations**

Factors predicting growth and toxicity of *P. parvum* are many and diverse, and are difficult to target, as hemolytic activity may be more related to specific strains than to biotic and abiotic factors. The *P. parvum* strain currently present in LLV may be different in toxin composition compared to other strains, where differences appear to be tied to the lake ecology and built environment. Therefore, different growth parameters for the *P. parvum* strain in LLV may allow for bloom formation when compared to growth parameters for strains found in other lakes. Another limitation is the *P. parvum* lifecycle, primarily the resting stage (encysted stage), is unclear including their ability to produce toxins and survival conditions while in benthic environments. Grasping a better understanding of *P. parvum* growth and bloom dynamics could allow for optimal timing for bloom management.

## Conclusion

The importance of understanding *P. parvum* can be seen in the millions of fish kills across the world. The collected data allows for a better understanding of when and why *P. parvum* bloom formation occurs in LLV, and may lead remedies of these fish killing blooms. Statistical analysis of the research may be important for predictors on *P. parvum* bloom formation, however, based solely on regression inclusion criteria, it does not reflect the mere importance of the variables assessed. However, observational analysis may be key to making profound discoveries with the ability to view interactions between predictor variables on *P. parvum* biomass variability. This data suggests that nutrients may be stronger predictors of *P. parvum* biomass that statistical analysis reflects. Yet, when chemical analysis can't explain *P. parvum* bloom formation, species interaction may be a stronger predictor. Predicting the effects of stressors on environmental configuration and *P. parvum* biomass is essential for effective lake management and restoration of water quality. Further studies are needed to elucidate the mechanisms of golden algae biosynthesis, its biology and ecology, as well as its associated toxicity, in order attenuate blooms leading to fish kill events in LLV.

# APPENDIX I

## DATA ANALYSIS FOR PLANKTON CHARACTERISTICS

Data analysis for plankton characteristics collected in Lake Las Vegas, Clark County, Nevada for the period of 2009 through 2012.

Date	<i>P. parvum</i>		ZOOPLANKTON			
	PP biomass (mg/m3)	Non-PP biomass (mg/m3)	Total Copepods (noperm3)	Cladocerans (noperm3)	Rotifera (noperm3)	Total Zooplankton (noperm3)
1/13/2009	0.0	30 day lag				
2/18/2009	0.0	122.5	18483.0	2424.0	0.0	20907.0
3/17/2009	0.0	17.6	13742.0	15407.0	0.0	29149.0
4/14/2009	0.0	70.0	49712.0	22130.0	321.0	72163.0
5/12/2009	0.0	30.4	41257.0	14457.0	0.0	55714.0
6/16/2009	0.0	33.2	22794.0	5699.0	0.0	28493.0
7/14/2009	0.0	65.4	22401.0	2286.0	0.0	24687.0
8/19/2009	0.0	439.6	41322.0	780.0	4678.0	46780.0
9/15/2009	0.0	1066.0	6964.0	0.0	464.0	7428.0
10/13/2009	0.0	1353.0	14353.0	0.0	957.0	15310.0
11/17/2009	0.0	796.2	21620.0	0.0	260.0	21880.0
12/16/2009	0.0	1390.6	3367.0	0.0	0.0	3367.0
1/19/2010	287.0	622.6	10547.0	0.0	0.0	10547.0
2/18/2010	7058.0	297.0	17638.0	0.0	0.0	17638.0
3/17/2010	21553.5	107.0	62842.0	0.0	1533.0	64375.0
4/13/2010	15513.0	82.3	1046.0	0.0	0.0	1046.0
5/12/2010	14975.0	220.0	1128.0	0.0	0.0	1128.0
6/15/2010	1066.0	591.0	345.0	0.0	0.0	345.0
7/14/2010	0.0	1597.0	3298.0	0.0	0.0	3298.0
8/18/2010	0.0	445.0	1562.0	0.0	5663.0	7225.0
9/14/2010	0.0	1084.0	83982.0	0.0	5631.0	89613.0
10/12/2010	283.0	311.0	10.9	0.0	12156.0	12166.9
11/17/2010	596.0	270.0	6778.0	0.0	5874.0	12652.0
12/14/2010	755.0	201.0	10642.0	0.0	7256.0	17898.0
1/12/2011	1633.0	407.0	2555.0	0.0	365.0	2920.0
2/15/2011	6177.0	871.0	34697.0	0.0	2339.0	37036.0
3/16/2011	10103.0	203.0	2117.0	0.0	0.0	2117.0
4/11/2011	7387.2	488.9	0.0	0.0	0.0	0.0
5/19/2011	40.7	1254.0	113.0	0.0	0.0	113.0
6/15/2011	66.0	1312.0	48282.0	0.0	23627.0	71909.0
7/12/2011	312.0	760.0	180481.0	0.0	71562.0	252043.0
8/16/2011	7.0	260.0	201330.0	0.0	86153.0	287483.0
9/15/2011	9.5	195.0	39899.0	448.0	5380.0	45727.0
10/11/2011	137.2	116.6	19116	0	19775	38891.0
11/15/2011	141.7	990.5	64820	0	56976	121796.0
12/13/2011	541.3	264.6	19775	0	13396	33171.0
1/17/2012	1207.2	245.0	40530	0	2732	43262.0
2/15/2012	1785.3	213.6	7440	0	6657	14097.0
3/13/2012	1103.6	379.9	0	0	0	0.0
4/12/2012	1435.3	891.1	1180	0	0	1180.0
5/16/2012	465.7	4447.7	727	0	0	727.0
6/12/2012	144.1	1355.8	6034	0	5229	11263.0
7/17/2012	0.0	4954.6	108896	0	54222	163118.0
8/14/2012	0.0	15994.4	44382	0	60970	105352.0
9/11/2012	0.0	16161.8	11481	0	4074	15555.0
10/17/2012	0.0	15934.8	61476	0	78512	139988.0
11/13/2012	0.0	44134.5	3142	0	2094	5236.0
12/12/2012	0.0	3943.2	6562	0	0	6562.0
		8364.3	166185	0	0	166185.0

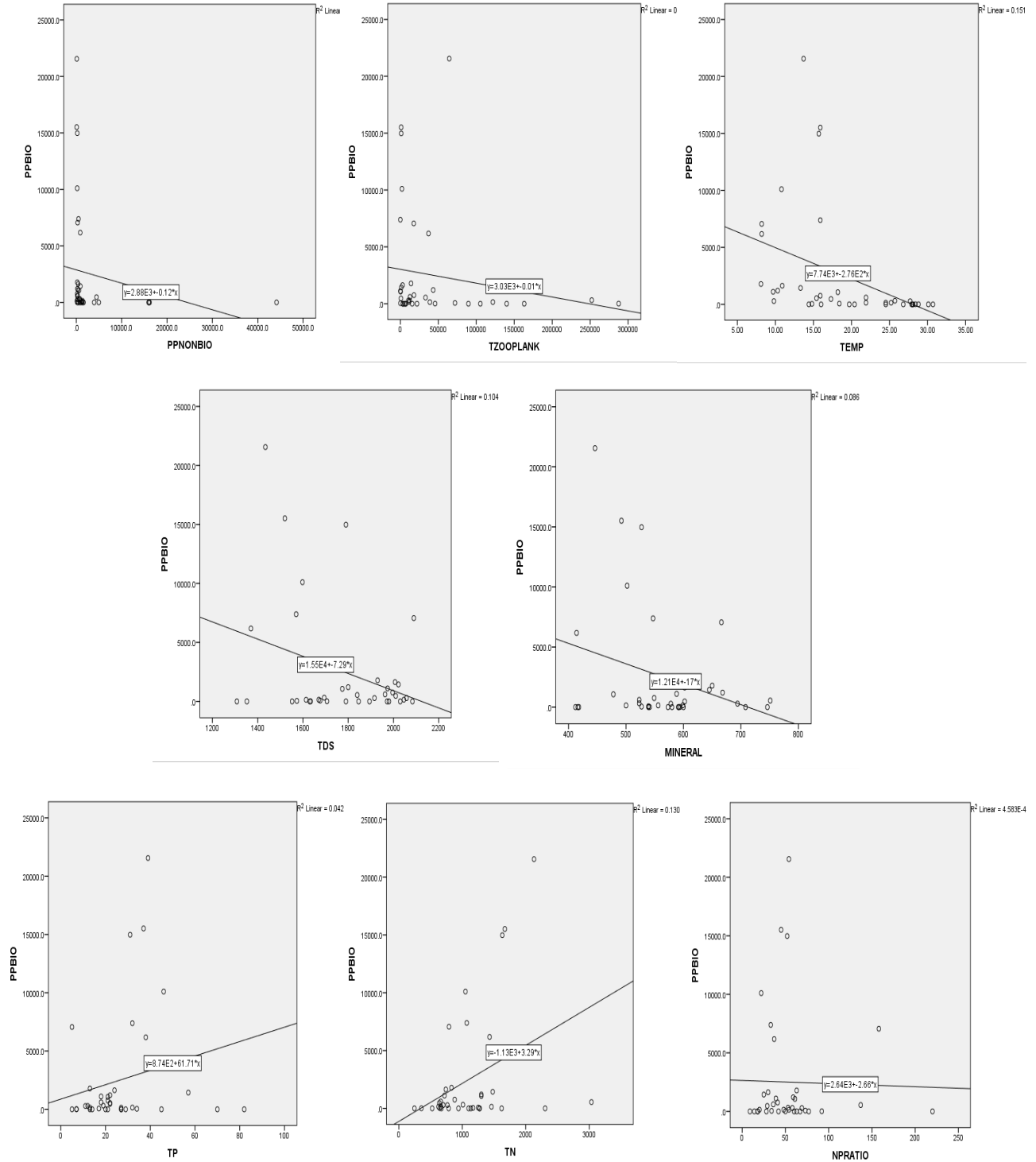
## APPENDIX II: DATA ANALYSIS FOR LAKE WATER CHARACTERISTICS

Data analysis for lake water characteristics collected in Lake Las Vegas, Clark County, Nevada for the period of 2009 through 2012.

Date	BIOLOGICAL /PHYSICAL									
	Temp °C (depth 0ft)	TN (µg/L)	TP (µg/L)	N:P Ratio (µg/L)	TDS (mg/L)	Ca (mg/L)	Na (mg/L)	K (mg/L)	Mg (mg/L)	Total Mineral (mg/L)
	with 30 day lag									
12/16/2009	14.4	670	45	14	1980	207	432	32	74.8	746
1/19/2010	9.8	700.0	12	58	2058	216	375	27.9	74.9	694
2/18/2010	8.2	790	5	158	2090	202	357	38.2	68.9	666
3/17/2010	13.7	2130	39	54	1434	169	209	13.4	54.1	446
4/13/2010	15.9	1670	37	45	1520	166	244	20	62	492
5/12/2010	15.7	1630	31	52	1790	160	290	20.3	56.5	527
6/15/2010	18.2	1300	21	61	1774	171	226	21.2	60.1	478
7/14/2010	26.8	1620	21	77	1790	182	311	16.6	70.4	580
8/18/2010	30.1	940	14	67	1894	174	279	23.8	62.6	539
9/14/2010	28.1	785	13	60	1630	160	343	23.4	64.7	591
10/12/2010	27.7	765	11	69	1916	171	319	21	67	578
11/17/2010	21.9	660	18	36	1963	152	291	20.4	59.3	523
12/14/2010	15.9	880	21	41	1996	157	305	28.2	58.4	549
1/12/2011	10.9	740	24	30	2007	175	337	26	63.8	602
2/15/2011	8.2	1430	38	37	1370	154	190	18.3	51.7	414
3/16/2011	10.8	1050	46	22	1598	170	254	20.8	56.7	502
4/11/2011	15.9	1070	32	33	1570	167	295	25	60.2	547
5/19/2011	14.8	1170	34	34	1573	182	243	37.4	64.3	527
6/15/2011	18.4	1250	17	73	1676	200	245	19.3	75.1	539
7/12/2011	25.7	1010	19	53	1694	186	250	18.5	68.9	523
8/16/2011	28.4	355	7	50	1634	187	261	23.3	70.1	541
9/15/2011	28.8	645	7	92	1706	197	304	21.7	71.2	594
10/11/2011	25.2	1460	27	54	1614	160	268	11.3	60.2	500
11/15/2011	21.9	625	13	48	1670	179	288	20.9	68.2	556
12/13/2011	15.4	3035	22	137	1840	189	457	32.4	72.2	751
1/17/2012	10.3	1300	22	59	1800	202	364	26.2	76	668
2/15/2012	8.1	830	13	63	1930	217	331	22.3	79.2	650
3/13/2012	9.7	719	18	39	1974	197	307	12.2	71.7	588
4/12/2012	13.3	1479	57	25	2022	179	374	26.6	65.7	645
5/16/2012	17.3	638	22	29	2010	195	310	22.3	74.6	602
6/12/2012	24.5	665	32	20	2046	197	302	20.8	78.9	599
7/17/2012	24.5	245	27	9	2084	194	304	16.3	77.2	592
8/14/2012	27.9	1135	27	42	2030	177	305	18.8	72.3	573
9/11/2012	30.7	1275	20	63	1972	176	325	23	76	600
10/17/2012	28.0	525	29	18	1552	163	178	15.5	55.7	412
11/13/2012	20.4	2305	82	28	1308	192	149	21.9	55.2	418
12/12/2012	16.0	1270	70	18	1352	159	188	22.5	46.5	416
	12.8	865	68	12	1378	143	197	25.3	36.9	402

### Appendix III: SCATTERPLOTS

Scatterplots with best-fit line demonstrating correlations between *P. parvum* and independent variables during the study period of 2009-2012 at site Lake Las Vegas - 1A, Clark County, Nevada.



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VITA

Graduate College  
University of Nevada, Las Vegas

Tara Gregg

Local Address:

6625 Virtuoso Court  
Las Vegas, Nevada 89139

Degrees:

Bachelor of Science, Health Ecology, 2004  
University of Nevada, Reno

Thesis Title: *Prymnesium Parvum* and Fish Kills in a Southern Nevada Man-Made Reservoir

Thesis Examination Committee:

Advisory Committee Chair, Shawn Gerstenberger, Ph.D.  
Advisory Committee Member, Steven Weber, Ph.D.  
Advisory Committee Member, Jeff Janik, Ph.D.  
Graduate College Representative, Penny Amy, Ph.D.