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Impacts to Phytoplankton After The Establishment of Quagga Mussels in Lake Mead, Nevada

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IMPACTS TO PHYTOPLANKTON AFTER THE ESTABLISHMENT OF QUAGGA MUSSELS IN LAKE MEAD, NEVADA

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ABSTRACT

Quagga mussels (*Dreissena rostriformis bugensis*) are an invasive species that were discovered in Lake Mead in January of 2007, and rapidly spread throughout the lake. Quagga mussels are about the size of a fingernail, but are known to clog pipes, damage infrastructure and alter ecosystems. In large numbers, mussels can have a dramatic impact on an ecosystem by reducing the phytoplankton and potentially increasing toxin producing cyanobacteria. This research analyzed data collected from three separate basins in Lake Mead to determine if water quality characteristics that are commonly impacted by invasive mussels have changed. Transparency, chlorophyll-a, phytoplankton and zooplankton samples were collected before (2004-2006) and after (2009-2011) quagga mussel establishment and tested for differences using a Wilcoxon Signed Rank Test. The results indicate that Lake Mead has not had drastic lake wide changes similar to other ecosystems at these three locations. Transparency, chlorophyll-a, and phytoplankton biomass were significantly different in the Boulder Basin, but these results are confounded by long term reductions in phosphorus loading to the Boulder Basin. Although not significant at every location, there was a 23-26% reduction in phytoplankton cell numbers and a 17-68% reduction in phytoplankton biomass between means of the two time periods. Toxin producing cyanobacteria like *Microcystis, Oscillatoria, Cylindrospermopsis* and *Anabaena* did not have a significant change in frequency of detections or cell numbers at these locations. Quagga mussels may have less of an impact in Lake Mead due to some fundamental differences compared to water bodies in the Eastern United States. The large volume of water, deep depths, thermal stratification, and limited food supply may limit quagga mussel populations from changing water quality characteristics similar to other ecosystems. Additional monitoring will be required to understand the long term impacts of quagga mussels in Lake Mead.

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LIST OF ACRONYMS AND ABBREVIATIONS

EPA – the Environmental Protection Agency

mg/L - milligrams per liter

NPS – the National Park Service

Pre-quagga – Sampling from 2004 to 2006 that represents conditions before quagga mussel establishment.

Post-quagga – Sampling from 2009 to 2011 that represents conditions after quagga mussel establishment.

Reclamation – United States Bureau of Reclamation

SNWA - the Southern Nevada Water Authority

WHO – the World Health Organization

µg/L – Micrograms per liter

CHAPTER 1 INTRODUCTION

Zebra mussels (*Dreissena polymorpha*) were discovered in the Great Lakes in 1988 and quagga mussels (*Dreissena rostriformis bugensis*) were discovered a year later (Herbert, Muncaster, & Mackie, 1989). Zebra and quagga mussels are invasive bivalves that are native to Eastern Europe and are believed to have been transported to the Great Lakes via ballast water from oceanic transport ships. After introduction into the Great Lakes, they quickly spread to other regional lakes and rivers causing ecological and environmental damage. In the Great Lakes, invasive mussels have caused economic damages of nearly four billion dollars to infrastructure and sport fisheries over a ten year period (Roberts, 1990). Some ecosystems have increased harmful cyanobacteria like *Microcystis* after mussel invasions (Vanderploeg et al., 2001). *Microcystis* blooms can release toxins that are harmful to skin and can cause liver damage or death if consumed (konst, McKercher, Gorham, Robertson, & Howell 1965).

Mussels feed by drawing water into their shell through a siphon where cilia on their gill structures sort edible particles while discarding non-edible particles back to the water. In large numbers, mussels can have a dramatic impact on an ecosystem by reducing phytoplankton in the water column (Mackie, 1991). High filtration rates combined with large numbers can allow a mussel population to filter 10-100% of the water column per day in some lakes (Strayer, 1999). During filtration, the removal of available particles can cause declines in organisms that rely on phytoplankton as a food source. The actual impact an invasive bivalve population can have on an ecosystem depends on many factors specific to the water body. Type and quantities of phytoplankton, organic material, nutrients, sedimentation processes, thermal processes, and downstream evection all determine the possible impacts to an ecosystem (James et al., 2000).

Bivalves can impact an ecosystem through several major pathways. Filtration capacity is the most important factor in determining the impact to an ecosystem (Strayer, 1999). During filtration, phytoplankton, small zooplankton and inorganic particles are removed from the water column. Rejected particles not utilized by the mussel are released back into the environment as pseudofeces which can alter energy dynamics and increase water clarity. Mussels can also impact an ecosystem by reducing consumers that rely on phytoplankton. Organisms like zooplankton, invertebrates and fish depend on phytoplankton as a food base that supports the entire trophic food chain. In addition, changes to water clarity can alter the amount of light penetrating the water which affects the types and amounts of rooted plants growing (Strayer, 1999).

The scientific literature on ecosystem transformation by invasive mussels varies depending on the water body, but it is commonly reported that 35-78% of the phytoplankton biomass and 40-75% of the zooplankton biomass are reduced after invasions (Higgins & Zanden, 2010). Generally, there is a shift in resources from surface waters to deeper benthic regions that are dominated by mussels. Research has indicated that toxin producing cyanobacteria could be selectively rejected and returned back to the water column causing an increase in cyanobacteria (Vanderploeg et al., 2001). There have been conflicting studies published regarding whether *Microcystis* is a preferred food source compared to other phytoplankton. Research by Silverman, Achberger, Lynn, & Dietz (1995) noticed *Microcystis* being preferentially sorted for ingestion while Baker, Levinton, Kurdziel, & Shumway (1998) documented *Microcystis* being sorted for a path of rejection. Although results differ, some areas that had not previously had *Microcystis* started to experience frequent blooms following mussel invasion.

Research in the Saginaw Bay of Lake Huron indicates that selective rejection of *Microcystis* has helped promote toxic *Microcystis* blooms. *Microcystis* blooms were not abundant two years prior to zebra mussel establishment, but after invasion *Microcystis* was as high as 40% of the phytoplankton biomass (Vanderploeg et al., 2001). A survey of thirty-nine inland lakes in Michigan showed that lakes with zebra mussels had 3.6 times more *Microcystis* biomass compared to lakes without mussels (Knoll et al., 2008). *Microcystis* concentrations have also increased in Lake Erie and Lake Ontario after invasive mussels were introduced (Conroy et al., 2005; Nicholls, Heintsch, & Carney, 2002).

In 2007, quagga mussels were discovered in Lake Mead, and rapidly spread throughout the lake by the end of the year (Wong et al., 2011). Lake Mead is one of America's most popular recreation areas with about nine million visitors each year for boating, swimming and fishing ($USBR_a$, 2015). It is imperative to understand the impacts of quagga mussels to Lake Mead and to minimize the ecological damage and public health concerns that could occur following their introduction.

Microcystis is detected in Lake Mead, but there have not been any documented cyanobacterial blooms similar to the Great Lakes. It is unclear what the long term impacts of the introduction of quagga mussels will be to Lake Mead and if it will increase the amount of *Microcystis* similar to other ecosystems. To date there have not been any published research indicating negative ecological impacts from quagga mussels in Lake Mead (Holdren & Turner, 2010).

This research is important because data from three separate basins were analyzed in Lake Mead to understand if water quality characteristics that are commonly impacted by invasive mussels have changed. Previous research has focused on the Boulder Basin which is the location

of the Las Vegas drinking water supply, but also heavily influenced by the Las Vegas Wash inflow. This research analyzed data from three separate basins in Lake Mead over a six year period to understand if quagga mussels are having lake wide impacts. Chlorophyll-a , transparency, phytoplankton, zooplankton, and toxin producing cyanobacteria data were analyzed for differences since quagga mussel establishment. The results will be valuable in understanding quagga mussel impacts in Western United States reservoirs and to assist lake managers in preventing public health concerns that could occur from harmful algae blooms.

Research Questions and Hypotheses

The following research questions and hypotheses were designed to evaluate if water quality characteristics in Lake Mead are changing similar to other locations after dreissenid mussel invasions. The following hypotheses test for differences in water samples collected on Lake Mead before and after quagga mussel establishment.

Research question 1) Have water quality characteristics in Lake Mead changed similar to other ecosystems since the establishment of *Dreissena polymorpha*?

Hypothesis 1) There will be differences in Lake Mead water characteristics between pre-quagga (2004-2006) and post-quagga establishment (2009-2011); specifically:

a.) Lake transparency has changed since quagga mussel establishment.

b.) Chlorophyll-a concentrations have changed since quagga mussel establishment.

c.) The total count of phytoplankton organisms have changed since quagga mussel establishment.

d.) Total phytoplankton biomass has changed since quagga mussel establishment.

e.) The total count of zooplankton organisms has changed since quagga mussel establishment.

Research Question 2) Has phytoplankton at the division and genus level changed in Lake Mead since the establishment of *Dreissena polymorpha*?

Hypothesis 2) There will be differences in Lake Mead phytoplankton samples between pre-quagga (2004-2006) and post-quagga (2009-2011); specifically:

a.) Chlorophyta (green) division has changed relative frequency (count of chlorophyta organisms in sample/ total count of sample) since quagga mussel establishment.

b.) Cyanophyta (blue-green) division has changed relative frequency (count of cyanophyta organisms in sample/ total count of sample) since quagga mussel establishment.

c.) Phytoplankton diversity has changed since quagga mussel establishment.

Research Question 3) Has there been an increase in the frequency of toxin producing cyanobacteria in Lake Mead since the establishment of *Dreissena polymorpha*?

Hypothesis 3) Toxin producing cyanobacteria like *Microcystis*, *Oscillatoria*, *Cylindrospermopsis* and *Anabaena* have changed frequency since quagga mussel establishment.

CHAPTER 2 REVIEW OF SCIENTIFIC LITERATURE

Quagga mussels are an invasive species native to the Dnieper River drainage system in Ukraine. Quagga mussels are a serious threat because they modify ecosystems, crash food chains and cause severe economic damages to infrastructure. Invasive mussels are possibly one of the most serious North American biofouling pests (LaBounty & Roefer, 2007). Dreissenid mussels were first discovered in the Great Lakes in the late 80s and have spread to over 700 lakes in North America as of March 25, 2015 ($USGS_a$, 2015). Once in America, quagga and zebra mussels have been transported to new locations by recreational boaters. Quagga mussels were first discovered at Lake Mead on January 6, 2007, and within 2 years had spread to every major basin (LaBounty & Roefer, 2007; Wittman et al., 2010). Lake Mead has suitable environmental conditions with favorable calcium, pH, salinity and dissolved oxygen (Whittier, Ringold, Herlihy, & Pierson, 2008; Claudi & Mackie, 1993). Quagga mussels have since been discovered in Lake Powell, Lake Mojave, Lake Havasu and numerous other locations along the Colorado River System (Figure 1)($USGS_b$, 2015). Quagga mussels will probably continue to spread to other water bodies in the Western United States even with boater education campaigns and mussel decontamination programs.

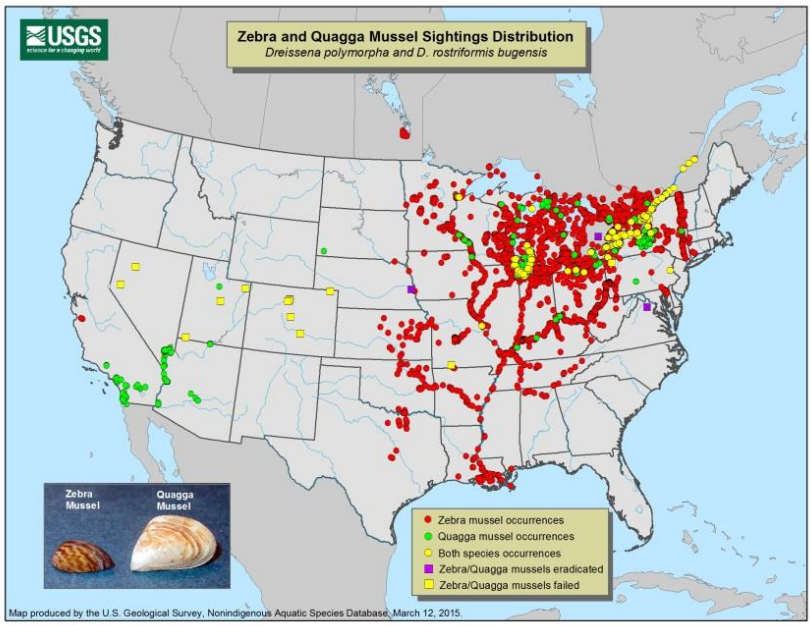


Figure 1 Distribution of invasive mussels in the U.S. ($USGS_b$)

Mussels are efficient filter feeders reducing particles in the water column like chlorophyll, phytoplankton, organic matter and zooplankton. Large mussel populations have had major impacts to the Saginaw Bay, Lake Erie and the Seneca River. Mussel invasions have also been associated with increasing toxin producing cyanobacteria like *Microcystis*. *Microcystis* is thought to increase due to selective filtration of mussels (Vanderploeg et al., 2001). *Microcystis* produces a toxin called Microcystin that can have adverse health effects on humans and animals who drink or come in contact with the water. Research by Knoll et al. (2008) documented that Microcystin concentrations were 3.3 times higher in lakes with zebra mussels compared to lakes without. If changes occurred in Lake Mead similar to other locations there could be public health concerns for millions of visitors that come to the lake to ski, swim, boat and fish.

Dreissenid Ecology

Classification

When quagga mussels were first discovered in North America there was confusion among biologists as to the taxonomic identity (Mills et al., 1996). Rosenberg & Ludyanskiy (1994) determined that quagga mussels corresponded to the original description by Andrusov described in 1897. Taxonomic classifications have been debated over the years as new information has been discovered. The current taxonomic classification for quagga mussel is Kingdom: Animalia, Phylum: Mollusca, Class: Bivalvia, Order: Veneroida, Superfamily: Dressenoidea, Family: Dreissenidae, Genus: *Dreissena*, Species: *Dreissena rostriformis bugensis* (Global Biodiversity Information Facility, 2016). Genetic analysis confirmed the taxonomic classification.

Habitat

Dreissenids can inhabit a wide range of water quality characteristics allowing them to thrive in lakes, streams, estuaries and reservoirs. Calcium, pH, temperature and salinity can limit the distribution of zebra and quagga mussels (Mackie & Schloesser, 1996). Distribution of quagga mussels in their native environment are mostly affected by water salinity and research has shown that quagga mussels can become acclimated to higher or lower salinities depending on environmental conditions (Grigoryev, 1968; Antonov & Shkorbatov, 1983). Salinity limits for *Dreissena polymorpha* have been measured from 2 to 12 parts per trillion in inland seas of

Eurasia, but as low as .5 parts per trillion in estuaries in the Netherlands (Strayer & Smith, 1993). North American quagga mussel populations in laboratory tests were not able to survive salinities greater than 5 parts per trillion (Spindle, May, & Mills, 1995).

Temperature can affect the distribution of mussels in water bodies and determine the depth mussels can survive and reproduce. Strayer (1991) developed a risk model based on temperature and water hardness that predicted most of North America could be inhabitable to zebra mussels, but found that Lake Mead would not be ideal based on warmer temperatures. The upper temperature tolerance for quagga mussels may be as low as 25 °C with less tolerance to heat than zebra mussels (Spindle, May, & Mills, 1995). Claude & Mackie (1993) estimate the temperature range of good growth for zebra mussels is between 21-24 °C, and the optimal growth potential is at temperatures between 18 to 20 °C.

Calcium and pH content of a water body can affect mussel growth and survival. A minimum of 12 mg/L of calcium is required for quagga mussel survival (Neary & Leach, 1992). Jones & Ricciardi (2005) sampled mussel populations in the St. Lawrence River and discovered that zebra mussels were not found in areas below 8 mg/L calcium and quagga mussels were not found in areas below 12 mg/L. Claudi & Mackie (1993) estimate the best growth for zebra mussels is at a pH greater than 8 and with a salinity less than 110 µS. A pH level above 7.1 is required for adult survival and a pH range of 7.4 to 7.5 is required for veliger survival (Mackie & Kilgour, 1995). Whittier & Ringold (2008) developed a water body risk assessment based on calcium concentrations that indicate Southern California and Nevada are at a high risk for mussel invasions.

Dreissenid mussels are able to survive at a variety of depths and on almost any type of substrate. Quagga mussels appear to be able to survive at deeper depths compared to zebra

mussels. In Lake Ontario, quagga and zebra mussels have been recorded at depths of greater than 100 m with only quagga mussel at 130 m (Mills et al., 1999). Quagga mussels outnumbered zebra mussels 14 to 1 in deeper offshore waters (Dermott & Munawar, 1993). In Lake Mead, quagga mussels have been detected on hard and soft substrates at most depths. Mueting & Gerstenberger (2011) discovered that settlement rates were significantly higher at depths of 6-28 m than at depths 32-54 m with no preference to substrate type.

The invasion of mussels to the Western U.S. came as a surprise to many. Analysis by Bossenbroek, Johnson, Peters, & Lodge (2007) concluded that a mussel invasion to the Western United States would be a low probability, but potentially high impact. Strayer (1991) developed a North American habitable range limit for *Dreissena polymorpha* that excluded Lake Mead based on extreme temperatures. Following the invasion of Lake Mead, the spread to other water bodies in western states has continued.

Geographical Distribution

Quagga mussels are native to the Dnieper River drainage of Ukraine, originally discovered near Nikolaev in the bug portion of the Dnieper-Bug Estuary (Mills et al., 1996). Quagga mussels spread since the 1940s throughout the Dnieper River drainage areas and into regions that were inhabited by zebra mussels. Quagga mussels have spread approximately 500 km northward and have been detected in almost every medium and large Dnieper reservoir of Eastern and Southern Ukraine (Mills et al., 1996). The expansion of quagga mussels within their native territory is attributed to human-mediated dispersal mechanisms (Kinzelback, 1992).

Quagga mussels have successfully spread throughout North America as an invasive species due to habitable environments and few natural predators. First discovered in North America around 1985 at Lake St. Clair, within a few years they had spread to all five of the Great Lakes (Hebert, Muncaster, and Mackie, 1989; USGS$_a$, 2015). It is thought that zebra and quagga mussels were transported by oceanic ships through discharged ballast water. Large ships take on ballast water to balance their weight during travel. Larval or young adults could have been introduced when ballast water was released into the Great Lakes. The invasive species have spread quickly throughout the United States by attaching to recreational boats and commercial barges. In many locations, quagga mussels have displaced zebra mussels as the dominant species (Stoeckmann, 2003). In the Eastern United States, zebra and quagga mussels have spread through the Great Lakes and most of the Mississippi River Drainage System. In 2007, quagga mussels were found at Lake Mead National Recreational Area and quickly spread to every basin within 2 years (LaBounty & Roefer, 2007). Quagga mussels have since been discovered in Lake Powell, Lake Mojave, Lake Havasu and numerous other locations along the Colorado River System (USGS$_a$, 2015). Zebra and quagga mussels have been detected in 700 lakes in North America (USGS$_b$, 2015).

Life Cycle and Reproduction

Dreissenid mussels have similar life cycles to marine bivalves with a planktonic and benthic life form. In the Great Lakes, dreissenids reproduce once a year based on temperature limits, but in the warmer Western United States quagga mussels spawn continuously with veliger larva stages being detected year-round in Lake Mead (Gerstenberger, Mueting, &Wong, 2011).

The dreissenid life cycle consists of egg fertilization, larval development and mussel stages (Ackerman, Sim, Nichols, & Claudi, 1994). The life cycle begins with external fertilization of gametes that eventually develop into a swimming trochophore larva (Figure 2). The trochophore becomes a veliger with the development of a velum, which is used for feeding and locomotion (Ackerman, Sim, Nichols, & Claudi, 1994). The trochophore larva will develop a D-shape shell within 2-9 days after fertilization, eventually forming a hinged clam-like shell in the veliconcha stage. The veliconcha stage is the last free swimming larval form. The next stage called the pediveliger is characterized by the development of new organs and a foot which causes changes in the larva's movement. All the larval stages take about 4 weeks to complete (Mackie, 1991). The pediveliger will begin the metamorphosis process where the mussel loses the velum and settlement on substrate occurs, considered the plantigrade stage. After attachment, the mussel will develop into a juvenile adult with gill and mouth structures. The juvenile will grow larger in size and reproduce. Adults attach to substrates using byssal threads which are strong fibers used to hold the mussel in place. Research has indicated that adults have the ability to move locations measured at 7 cm/ night (Ackerman et al., 1994). The life cycle of *Dreissena polymorpha* is 3-5 years in most Polish Lakes, but in the United States an average life span is 1.5 to 2 years (Mackie & Schloesser, 1996). Dreissenids have a very high reproductive potential with the ability to produce 30,000 eggs per female within the first year (Mackie & Schloesser, 1996).

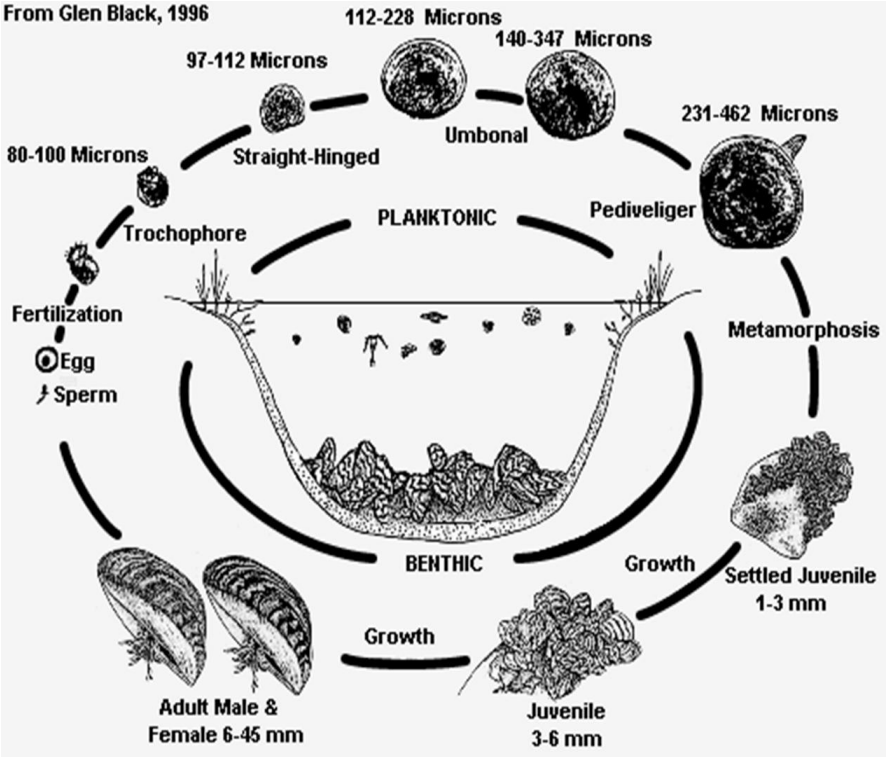


Figure 2 Life cycle of dreissenid mussel (Black, 1996)

The factors that affect reproductive success are poorly understood. Depending on environmental variables an egg can take anywhere from 8 to 240 days to develop into a mussel (Nichols 1996). Temperature is thought to be important in the development of gametes and timing of spawning activities (Nichols, 1996). In Lake Mead, quagga mussel veligers are present year round, which is likely due to the warm water (above 12 °C) found in Lake Mead. Gerstenberger et al. (2011) found the highest veliger densities in metalimnion temperatures (17-22 °C) and lower densities in the epilimnion and hypolimnion temperatures. These results agree with those of Sprung (1987) showing the most favorable spawning would be for temperatures around 18 °C. Environmental factors affecting settling were studied in Lake Mead with the results indicating that water temperature, flow velocity and dissolved oxygen were significant in

veliger settling (Chen, Gerstenberger, Mueting, & Wong, 2011). Total organic carbon, conductivity and pH had little to no effect on substrate settling in Lake Mead.

Dispersal Mechanisms

Dreissenid mussels have numerous potential dispersal mechanisms that can help them spread to new locations. Both adult and larval stages can be transported to new locations through natural processes like water currents or from birds or animals. Planktonic veligers can travel great distances through water currents via streams or rivers. Human mediated transportation has led to a dramatic increase in the distance that mussels can travel. Some human mediated transportation examples are artificial waterways, ships, fishing activities, amphibious planes and recreational equipment (Mackie, Gibbons, Muncaster, & Gray, 1989). Mussel exposure thresholds suggest it could be possible for adult mussels to survive on hulls and anchors during oceanic voyages (McMahon, 1996). As mentioned previously, quagga and zebra mussel larva are thought to have traveled to North America in ballast water that is used by large transport ships for stabilization (Mackie et al, 1989). Veliger's can survive five days in warmer temperatures and up to 27 days in cooler temperatures (Choi, Gerstenberger, McMahon, & Wong, 2013). Recreational boaters need to clean, drain and dry their boats to prevent the spread of mussels.

Mussel Feeding and Filtration

Mussels feed by drawing water into the body and ingesting particles that are edible and rejecting undesirable particles as pseudofeces. In dense numbers, filtering can have dramatic impacts on an ecosystem by reducing the phytoplankton in the water column. Invasive mussels are far superior to native mussels in their filtering abilities due in part to their anatomy and morphology. In most regions, native mussels have been displaced by invasive species because of their abilities to clear particles at higher capacities and exploit resources better than the native species (Baker & Levinton, 2003). Large populations can change an ecosystem by decreasing phytoplankton, crash food chains, and increasing cyanobacteria (Strayer, 1999). Mussel filtration is affected by the size of particles, temperature, particle concentration and food type (Lei, Payne, & Wang, 1996; Baker & Levinton, 2003; Winkel & Davids, 1982). The following sections will discuss some of the relevant literature on filtration for zebra and quagga mussels.

Anatomy and Morphology

The mussel shell provides a hard protective outer layer for the soft internal organs. The shell can open and close depending on environmental conditions and food availability. The internal compartment of the shell is called the mantle cavity (Figure 3). Mussels ingest food suspended in the water column using filtration. Mussels take in water and food particles through the inhalant siphon. The inhalant siphon is ringed with 80 to 100 cilia which are used in food transport and selection (Claudi & Mackie (1993). Dorsal to the inhalant siphon is the smaller exhalent siphon which removes particles from the mussel. Small cilia on the gills propel water

into the incurrent siphon and across the gills. Food and other solid particles are removed by the cilia as they pass over the gills and are sorted for digestion or rejection. Digestible food particles are transported to the mouth for ingestion by labial palps which secrete mucus that bind the suspended food (Riisgard, Egede, & Saavedra, 2011). Undesirable particles are coated in mucus and removed through the exhalent siphon as pseudofeces (Beninger, Veniot, & Poussart, 1999). The labial palps have been shown to play an important part in selectively sorting edible particles from non-edible particles (Morton, 1969). Particle transport along the gills is accomplished through an assortment of cilia and gill structures. Early studies on bivalve feeding classified the gill structures based on the type of cilia and the tract the particles traveled (Atkins, 1936). The gills are comprised of filaments called the inner and outer demibranches that are used to facilitate water and particle transport (Jorgensen, 1990). Particles are mechanically sorted on the food groove of the inner demibranch of zebra mussels (Baker, Levington, & Ward, 2000). Desirable particles are transported inside the marginal food groove to enter the digestive tract. Undesirable particles travel superficially near the food groove in mucus before being rejected (Baker et al., 2000).

Cilia on the gill structures play an important part in directing and sorting particles entering the mussel. Fine cilia are located near the food groove which assists in directing desirable sized particles for digestion. Larger cilia on the demibranches sort larger particles that are directed for rejection (Jorgensen, 1990).Water velocity around the filtration structures of a zebra mussel were measured using endoscopy video images during mussel feeding. The particle velocities ranged from 90-156 μm/s indicating particle transport is mucociliary facilitated instead of hydrodynamicaly (Baker et al., 2000). The ciliary motions cause particles to enter the inhalant siphon and aid in transportation and sorting. Frontal cilia propel water and particles using a

power and recovery stroke. Shafts on the gills called laterofrontal cirri contain rows of cilia that separate suspended particles from water creating a capture net (Riisgard et al., 2011). The latero-frontal cirri consist of about 40 pairs of cilia on two fused ciliary plates (Silverman, Lynn, & Dietz, 1996). The cilia move in unison from a relaxed position extending into the interfilament space to a flexed position that brings the particle over the front of the gill filament. During motion, the cilia have been observed trapping particles less than 1 µm in diameter (Silverman et al., 1996). Comparisons between species of bivalves suggest that clearance rate is directly related to the size and number of cirri per gill surface area (Silverman, Achberger, Lynn, & Dietz, 1995).

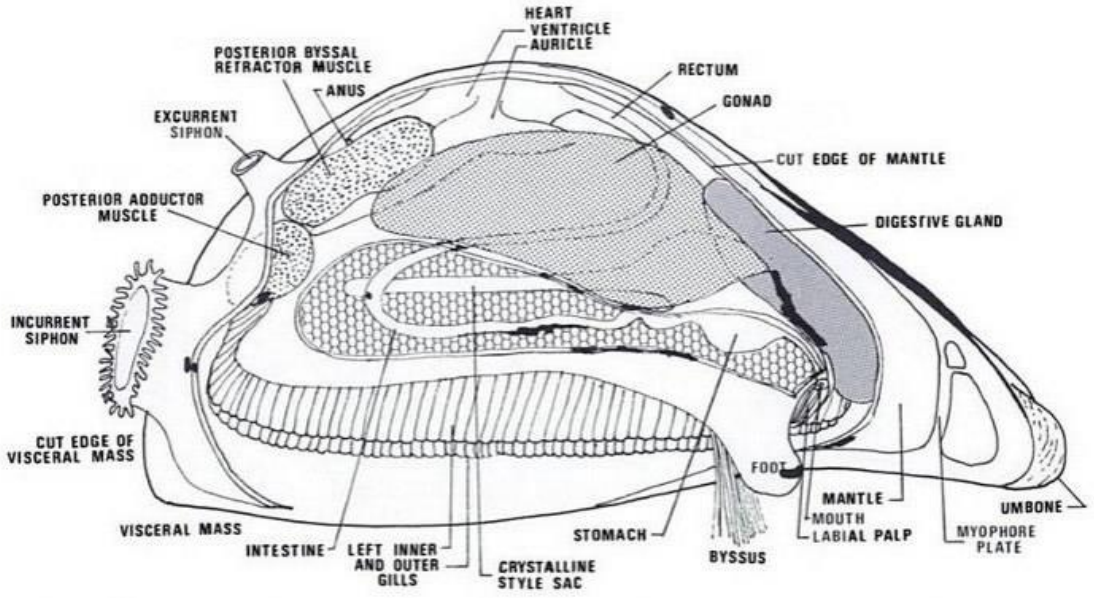


Figure 3 Anatomy of dreissenid mussel (Claudi, R., & Mackie, G. L., 1993)

Filtration Rate

Mussel populations can cause drastic changes to ecosystems due to the high individual clearance rate of each mussel combined with a large population. Filtration rate is the measure of

particles captured by a mussel per unit time, and clearance rate is the volume of water that passes through the gills per unit time. Dreissenids have a maximum filtration rate when food particles are relatively low and a maximum ingestion rate when food concentration is high (Sprung & Rose, 1988). The filtration rate is equivalent to the ingestion rate when no pseudofeces are produced (Lei et al., 1996). High particle concentrations reduce the pumping rate, and after the maximum ingestion rate is reached pseudofeces are expelled through the exhalent siphon (Sprung & Rose, 1988).

The filtering capacity of a population is determined by the population size, available phytoplankton and the filtration rate of the mussels. Filtration rate of a population is calculated by the volume of water cleared of particles and the percentage of mussels filtering (Horgan & Mills, 1997). Silverman et al. (1995) determined that the rate of bivalve filtration is related to the amount and size of cirri structures on the gills. Dreissenid gills have 138 times more cirri than *Corbicula fluminea*, resulting in a clearance rate 30 times larger (Silverman et al., 1995). Mussels have sessions of filtering activity with occasional non-filtering with their valve closed and siphon withdrawn (Horgan & Mills, 1997). Mussel filtering activity was found to be 6-9% higher at night than during the day (Horgan & Mills, 1997).

Filtration rates have been calculated by several authors. Mackie (1991) used tank experiments with activated sewage sludge to determine filtration rates in zebra mussels. The experiments showed zebra mussels were able to completely filter all suspended sludge from tanks within 96 hours. The filtration rate of zebra mussels was determined to be 10 to 100 ml/individual/h (Mackie, 1991). Fanslow, Nalepa, & Lang (1995) calculated filtration rates between 4.0 to 40.7 mL/mg/h using natural seston in Saginaw Bay.

The type of food particle affects clearance rates in zebra mussels. A preferred food source like *Paraphysomonas* had clearance rates between 5.1 and 13.6 Ash Free Dry Weight/hour and a non-preferred food source like *Microcystis aeruginosa* had a clearance rate of 0 Ash Free Dry Weight/hour (Lavrentyev, Gardner, Cavaletto, & Beaver, 1995). Vanderploeg et al. (2001) confirmed similar results when feeding rates decreased as zebra mussels were fed less desirable particles like *Microcystis* along with a desirable food source like *Cryptomonas*. Berg, Fisher, & Landrum (1996) observed that pseudofeces increased when zebra mussels were fed less desirable algal particles. Contrary to other results Fanslow et al. (1995) observed that filtration rates were not impacted by seston composition after different ratios of chlorophyll and total suspended solids were evaluated.

Temperature affects the filtration rates of zebra and quagga mussels. Fanslow et al. (1995) calculated the highest filtration rates between 10 and 20 °C. Maximum filtration rates occurred in the spring and the lowest were in late summer. Lei et al. (1996) measured the optimum filtration rate between temperatures of 14 and 26 °C in *Dreissena polymorpha*.

Baldwin et al. (2002) found no significant difference between clearance rates or feeding behaviors between zebra and quagga mussels. Baldwin et al. (2002) noted that quagga mussels could selectively reject inorganic material at lower seston levels giving them an advantage over zebra mussels. Clearance rates for zebra and quagga mussels were reduced as clay levels increased during feeding (Baldwin et al., 2002).

Particle Size

Particle size can affect the filtration rate and efficiency in mussels. Several authors have used microscopy to identify particle ranges that mussels can effectively digest. Sprung & Rose (1988) observed zebra mussels removing particles as small as .7 µm by cilia, and determined the maximum retention efficiency is for particles from 5 to 35 µm in diameter. Silverman et al. (1996) observed zebra mussels trapping particles less than 1 µm in diameter with latero-frontal cirri during sorting, and Jorgensen, Kiorboe, Mohlenberg, & Riisgard (1984) observed zebra mussels filtering particles from .7 to 450 µm in diameter. Winkel & Davids (1982) examined the content of zebra mussels' stomachs and mid gut to determine that particles 15-40 µm are preferred for ingestion.

Experiments by Lei et al. (1996) showed that small particle sizes can affect filtration rates. Zebra mussel filtration rates increased 4.8 times as the diameter of microsphere increased from .5 to 1.0 µm. Maximum filtration rate was measured at 1.5 µm and did not vary significantly from 1.5 to 5.1 µm particle diameter in size. Microspheres larger than 1.5 µm in diameter had nearly 100% retention during the experiment (Lei et al., 1996).

Contrary to other studies, Horgan & Mills (1997) fed zebra mussels with 6 different sized particles ranging from 10 to >150 µm and found no significant differences between clearance rates between size ranges. Phytoplankton taxa of *Chlamydomonas, Mallomonas, Cryptomonas, pusilla, Cryptomonas ovate, Melosira and Chroococcus* were fed to Zebra mussels with no significant clearance rate difference among taxa (Horgan & Mills, 1997).

Mussels have been observed clearing very large particles, but most are rejected as pseudofeces. Horgan & Mills (1997) observed larger mussels (9-21 mm) in length were observed

clearing particles 900 to 1200 µm in size, which is much larger than previous estimates of 750 µm reported by Winkel & Davids (1982). Direct observations indicated zebra mussels could clear unicells, filaments and globular shaped colonies (Horgan & Mills, 1997). The results of these experiments indicate that mussels effectively sort particles between 15-40 µm, but can filter smaller and larger particles with less efficiency.

Particle Concentration

Particle concentration can affect a mussel's ability to efficiently filter and sort particles. Mussel filtration rate is normally at maximum rate under optimal conditions, but in very high particle concentrations the filtering rate is reduced. Fanslow et al. (1995) measured zebra mussels filtration rates in the Saginaw Bay to discover the lowest rates were in areas with high concentration of chlorophyll, particulate organic carbon and total suspended solids. Reducing the filtration rate during high concentrations reduces the amount of particles on the gills to prevent clogging.

Riisgard et al. (2011) fed algal particles to the bivalve *Mytilus edulis* to understand feeding behavior in mussels. Algal concentrations were slowly increased and monitored to determine that 5,000 to 8,000 cells/mL or the equivalent of 6.3 to 10 µg chl-a/L was the limit before the filtering rate was decreased. The threshold before pseudofeces production began was 12,000 cells/mL or a 15.0 µg chl-a/L (Riisgard et al., 2001).

Filtration rate has been determined to increase as a function of particle concentration (Lei et al., 1996). As particle concentration increased from 7 to 27 mg/L the filtration rate increased by a factor of three, but after 27 mg/L there were no significant increases in filtration (Lei et al.,

1996). Particle concentrations less than 27 mg/L had maximum clearance rates, and particle concentrations between 27 to 84 mg/L had decreased clearance rates by 62% (Lei et al., 1996). These results agree with other authors who concluded that a mussel's filtration rate decreases when food concentrations exceed the incipient limiting level (Sprung & Rose, 1988; Berg et al., 1996).

Effects of inorganic Particles on Filtration

Inorganic particles like silt, clay or sand can impact filtration processes in bivalves. Inorganic particles have little nutritional value for an organism, so discarding inorganic particles and ingesting organic particles with the highest nutritional content is critical for survival. Effects of inorganic particles on mussel filtration have been studied by several authors with conflicting results.

Experiments on the suspension feeding bivalve *Mytilus edulis*, showed that the addition of an inorganic silt kaolinite at low levels caused no effect on filtration, but pseudofeces concentrations were high in silt (Riisgard et al., 2011). When silt levels were increased to 40 mg dry weight/L the filtration rate was reduced. This study indicates that suspension feeding bivalves can efficiently remove non-nutritious particles as pseudofeces.

Fanslow et al. (1995) experimented with zebra mussels using natural seston to determine that filtration rate was not related to the composition of particles. Seston compositions of varying ratios of particulate organic carbon and total suspended solids, or chlorophyll and total suspended solids did not significantly impact filtration rates.

Results obtained by Baldwin et al. (2002) contradicted the findings of Fanslow et al. (1995). Baldwin et al. (2002) found that inorganic particles can decrease filtration rates in both zebra and quagga mussels. When food particles were combined with increasing clay levels from 0 to 100 mg/L the mean clearance rate decreased from 0.210 to 0.072 L/mussel/h for zebra mussels and from 0.187 to 0.061 L/mussel/h for quaggas. The experiment indicated that zebra and quagga mussels have similar responses for increasing clay content during filtration, and that filtration is decreased in the presence of non-nutritious particles.

Pseudofeces

Filter feeders sort non-nutritious inorganic particles from edible particles to maximize survival rates. Mussels remove undesirable particles by expelling pseudofeces, which is a mucus coated particle ball. Long cilia in the rejection tract of mussels are used to move particles superficially in mucus and eventually rejected through the exhalent siphon (Beninger et al., 1999).

The production of pseudofeces in zebra and quagga mussels is directly related to food quality and indirectly related to food quantity (Balwin et al., 2002). Zebra mussels have been observed selectively feeding on higher nutritional content particles. Higher quality food resulted in lower pseudofeces production while low quality food had the largest rate of pseudofeces production (Baldwin et al., 2002).

Mussels produce two types of feces depending on the amount of digestion that occurs. When food quantity is plentiful, intestinal feces are produced and are generally the majority of feces produced (Riisgard et al., 2011). Intestinal feces bypass the digestive diverticula so the

food is less digested. The other type of feces produced by mussels are glandular feces which is food that is processed by the digestive diverticula and is more digested (Riisgard et al., 2011).

A laboratory feeding experiment on the freshwater mussel *Mytilus edulis* indicated that pseudofeces production starts at 12,000 cells/mL which is equivalent to a 15.0 µg chlorophyll-a concentration. Videography recordings observed that the threshold concentration for reduction is around 5,000 to 8,000 cells/mL which is around 6.3 to 10 µg chl-a/L (Riisgard et al., 2001). At this concentration the mussel starts to reduce the filtration rate and if the threshold is exceeded pseudofeces production occurs. Inspected pseudofeces showed undigested algal cells indicating an overload of the digestive system.

Baker, Levinton, Kurdziel, & Shumway, (1998) studied the contents of pseudofeces using flow cytometry to determine the fate of different particles released as pseudofeces. Large green cells ejected as pseudofeces were in compact balls that remained intact for long periods of time. Clay particles and diatoms were ejected as loose diffuse particles that easily broke up and re-suspended back into the water column (Baker et al., 1998).

Phytoplankton Filtering

Phytoplankton type and size affect grazing by mussels. Phytoplankton that swim or are buoyant could reduce grazing by mussels. Flagellated phytoplankton or species with gas vacuoles like cyanobacteria may provide additional protection from mussel filtration. Phytoplankton that relies on water currents for motion could be more susceptible to predation (Horgan & Mills, 1997). Phytoplankton in large colonies that are larger than the mussel's incurrent siphon would have an advantage of not being ingested by the mussel.

Dreissenid Preference for *Microcystis*

Microcystis has been debated in the literature as a preferred food source compared to other phytoplankton. Some studies have observed *Microcystis* being preferentially sorted for ingestion while others have observed *Microcystis* being sorted for a path of rejection. Although results differ on mussel preference of *Microcystis*, there are a number of locations where *Microcystis* blooms continue to occur with large populations of mussels. The following are the findings from different experiments involving mussels and *Microcystis*.

Baker et al. (1988) studied Zebra mussel ingestion using different food types and observed that *Microcystis* is a preferred food particle in the Hudson River. Laboratory experiments and flow cytometry analysis of pseudofeces determined that *Microcystis* was preferentially ingested compared to the control suspension. The results of this study are consistent with the changes in the cyanobacteria composition in the Hudson River. It is hypothesized by the authors that the difference in *Microcystis* being ingested or rejected is dependent on the size, strain and toxicity of the *Microcystis* present in the system (Baker et al., 1988). In another study supporting *Microcystis* as a preferred food source, feeding experiments using zebra mussels indicated clearance rates higher for *Microcystis* compared to five other types of phytoplankton (Baker et al., 2003). The filtration rate was increased in the presence of *Microcystis* even when less nutritious particles were present (Baker et al., 2003). The authors believe that the preference for *Microcystis* has reduced their numbers in the Hudson Bay and shifted the dominant species to Diatoms.

Sarnelle, Wilson, Hamiliton, Knoll, & Raikow, (2005) studied the interaction between zebra mussels and *Microcystis aeruginosa* with lake enclosure experiments. The results showed

differing relationships between years depending on the amount of phosphorus present. In 2000, when total phosphorus averaged 3 µg/L the biomass of *Microcystis aeruginosa* had a negative correlation across all mussel densities studied. In 2001, when total phosphorus averaged 9 µg/L there was a positive association between mussel densities and *Microcystis aeruginosa* across all mussel densities (Sarnelle et al., 2005). The results suggest that the positive effects of zebra mussels on *Microcystis aeruginosa* can be larger than the rate of consumption in certain conditions. One possible explanation given by the author is that *Microcystis* colonies were larger in size when the phosphorus concentrations were higher. Larger colonies could reduce grazing by mussels which would cause the positive effect. This research also indicated phosphorus concentrations influence *Microcystis* abundance.

In the Saginaw Bay of Lake Huron and Lake Erie, selective rejection of *Microcystis* has helped promote toxic *Microcystis* blooms. *Microcystis* blooms were not abundant two years prior to zebra mussel establishment, but blooms were discovered 3-5 years after invasion. After invasion in 1992, 1994 and 1995 *Microcystis* was as high as 40% of the phytoplankton biomass (Vanderploeg et al., 2001). Laboratory experiments indicated mussel filtering rates were increased in the presence of *Microcystis*, but large quantities of pseudofeces were produced in unconsolidated colonies that were easily broken down after being expelled as pseudofeces. The mussel response to *Microcystis* was determined to be strain and size dependent through laboratory experiments. *Microcystis* in size ranging from 53-153 or > 153 µm were found to be readily filtered. Larger colonies greatly reduced filtering speed and very large colonies could impede filtering. The authors' findings suggest that the strain and colony type in an ecosystem could determine if *Microcystis* will be a preferred food source.

The results of a meta-analysis of 39 inland Michigan lakes indicated that cyanobacterial toxins were positively associated with zebra mussel invasions. Lakes infested with zebra mussels had 3.6 times larger *Microcystis* biomass than lakes without zebra mussels (Knoll, et al., 2008).

Although there are conflicting results regarding if *Microcystis* is a preferred food source for dreissenids compared to other phytoplankton, there are locations like the Saginaw Bay that had not previously had *Microcystis* blooms, but after mussel invasion, started to see shifts in phytoplankton compositions to *Microcystis* as the dominant organism. Results observed by Baker et al. (1988) indicate that the size, strain and toxicity of the *Microcystis* present in the system will determine if *Microcystis* is a preferred food source. Laboratory experiments by Vanderploeg (2001) indicate that *Microcystis* could be a preferred food source and then later rejected as pseudofeces, resulting in larger populations in the system. It is likely that other available food sources, mussel population size, water quality, limnology and other factors could also play a role in determining the fate of *Microcystis* in an ecosystem. If *Microcystis* does increase in Lake Mead similar to the Saginaw Bay, public health concerns could be an issue for the millions of visitors that the area receives each year.

Differences between *Dreissena polymorpha* and *Dreissena bugensis*

Baldwin et al. (2002) examined the differences in growth and feeding rates between zebra and quagga mussels in laboratory conditions using natural and cultured food particles. Quagga mussels were found to grow 4 to 19 times faster than zebra mussels with the largest difference during low food concentrations. No significant differences between zebra and quagga mussels were found for clearance rates, functional responses or feeding behavior when fed

Chlamydomonas, *Nannochloris*, or a mixture of *Nannochloris* and clay. No differences in feeding behavior during day and night or the effects of inorganic particles were observed between the two species. When natural seston was used for food particles, quagga mussels had high assimilation efficiencies (81%) compared to zebra mussels (61%) at low concentrations (Baldwin et al, 2002). The authors hypothesized that quagga mussels have a lower requirement for growth than zebra mussels and can support growth with less available food sources compared to zebra mussels. This may account for only quagga mussel populations being present at greater depths when both species are present.

Water Quality Impacts

Bivalves can have a profound impact on ecosystem dynamics. During filtration, edible small particles are retained while other inorganic or less desirable food particles are deposited back into the environment. The actual impact an invasive bivalve population can have on an ecosystem depends on many factors specific to the water body. Type and quantities of available phytoplankton, organic material, nutrients, sedimentation processes, thermal processes and downstream evection all determine the possible impacts on an ecosystem (Strayer, 1999).

The largest direct impact a mussel has to an ecosystem is through filtration of the water column. During filtration, phytoplankton, small zooplankton and inorganic particles are removed from the water column. As these particles are removed, the water can become clearer from the reduction of particles in the water column. The mussel's ability to filter the water column is controlled by the vertical and horizontal heterogeneity of the water column, the filtration capacity of the population, plankton population characteristics, particles in column, and growth

rates and controls of plankton (Strayer, 1999). Impacts from Invasive mussels have been documented in the Hudson River with an 85% decline in phytoplankton biomass, Lake Erie with 100% higher transparency, and in the Seneca River with 16 fold reduction in chlorophyll (Caraco et al., 1997; Holland, 1993; Effler, 2004).

Mussels can impact the consumers that use phytoplankton as a food source. Organisms like zooplankton, invertebrates, and fish depend on phytoplankton as a food base that supports the entire trophic food chain. Removal of the food base can reduce or alter fish populations that rely on phytoplankton for survival. Examples of reductions in consumers by mussels have been documented in the Hudson River and Lake Erie (Pace, Strayer, Fischer, & Malcom, 2010; MacIsaac, 1996). Literature documenting ecological impacts after zebra and quagga mussel invasions is shown in Appendix A.

Ecosystem Impacts in the Saginaw Bay of Lake Huron

The Saginaw Bay and the Hudson River are two ecosystems that have been studied extensively after mussel invasions, and both ecosystems have been modified after mussel establishment. Zebra mussels starting colonizing the Saginaw Bay of Lake Huron in 1991 and within three years had reduced chlorophyll by 59% and increased transparency by 60% (Vanderploeg et al., 2001; Fahnenstiel et al., 1995). The inner bay of Saginaw Bay averages around 5 meters and the outer bay averages 14 meters in depth. The inner bay has about 30% of the volume of the outer bay, and the Saginaw Bay is well mixed especially in the shallow areas like the inner bay (Lavrentyev et al., 1995). The inner bay has a higher density of mussels than the outer bay which likely explains why the most significant changes have been observed in the

inner bay. The filtration capacity of the mussels were estimated to take less than a day to filter the entire inner bay water column (Bridgeman et al., 1995; Fanslow et al., 1995). Before mussel establishment, the Saginaw Bay phytoplankton was dominated by light sensitive species like *Oscillatoria redekki* and chlorophyta divisions. After mussel establishment, phytoplankton were replaced by *Cyclotella* diatoms and *Microcystis* (Fishman et al., 1995). It is thought that diatoms increased because the large sizes are less efficient to filtrate, and *Microcystis* increased from selective rejection (Stoermer and Theriot, 1985; Fishman et al., 1995).

Ecosystem Impacts in the Hudson River Estuary

Zebra mussels were first discovered in the Hudson River estuary in 1991 and within one year phytoplankton biomass was reduced to 90% of pre-invasion values (Caraco et al., 1997). Zooplankton biomass was reduced more than 70% after invasion (Pace et al., 1998). The Hudson River Estuary is a freshwater tidal estuary that spans 247 kilometers. The estuary averages about 9.5 meters in depth, is well mixed, and has high nutrient concentrations. The high densities of mussels were calculated to filter the estuary every 2-3 days (Caraco et al., 1997). Phytoplankton shifts were observed from cyanobacteria to diatoms after invasion. Contrary to the observed results in the Saginaw Bay, cyanobacteria were decreased after invasion. Cyanobacteria dominated by *Microcystis* were almost eliminated two years after invasion of zebra mussels (Fernard, et al., 2007). These results were consistent with laboratory tests which showed zebra mussel could filter cyanobacteria and *Microcystis* (Bastviken, et al., 1996). The Saginaw Bay and the Hudson River estuary both had relatively quick ecosystem changes after mussel establishment.

Quagga mussels in Lake Mead

Quagga mussels were discovered in Lake Mead in January of 2007, causing concern among state and federal agencies (LaBounty & Roefer, 2007). Lake Mead was the first documented infestation west of the 100th meridian in North America and the first time a large ecosystem was infested by quagga mussels without a previous zebra mussel infestation (LaBounty & Roefer, 2007). The Great Lakes were infested by dreissenid mussels in the late 80s and it is thought that mussels were transported to Lake Mead via recreational boaters. Mussels probably established in 2003 or 2004 to have the population size that was sampled two months after discovery (McMahon, 2011). The rate of spread to other locations has been rapid compared to the Eastern United States. Mussels have been detected in five bodies of water in California, eight in Arizona, three in Colorado and two in New Mexico (USGS$_a$, 2015). Quagga mussels are in the head waters of the Colorado River and will likely spread to other western locations in the coming years. Dreissenid mussels in the Great Lakes have caused severe ecological and economic damages to many of the areas they inhabit, so lake managers in Southern Nevada were concerned regarding the possible impacts that may occur in Lake Mead. Lake Mead is of particular concern because of the large number of recreational visitors that it receives and because it is used as the primary drinking water source for over 1.3 million people in the Las Vegas Metropolitan area.

To minimize the impacts of quagga mussels in Lake Mead, local, federal and regional agencies have responded with educational, decontamination and monitoring programs. The National Park Service has started a “Clean, Drain and Dry” campaign to educate boaters of the possible spread of aquatic species and started boat decontamination centers to inspect and clean recreational boats before leaving. The Metropolitan Water District of California and the Southern

Nevada Water Authority (SNWA) installed chemical feed lines that release chloramines to prevent mussel attachment on the inside of intake pipes and water treatment facilities. However, mechanical removal is required periodically for intake structures and trash racks that are exposed to lake water.

Research projects and sampling programs were created to monitor, prevent and control quagga mussels. An Interagency Monitoring Plan (I-MAP) was developed by the National Park Service that described each agency's objectives, and documented monitoring and research efforts with the goal of providing a long term cost effective monitoring program (Turner, Wong, Gerstenberger, & Miller, 2011). The Bureau of Reclamation (Reclamation) office in Denver developed an early detection program to monitor bodies of water for the presence of quagga mussels which uses analytical procedures like Polymerase Chain Reaction, scanning electron microscopy, cross polarized light microscopy and flow cell cytometry (Hosler, 2001). Reclamation staff at the Lower Colorado River dams have tested technologies to prevent settlement including ultra violet light, screen filtering technologies and coating materials for critical infrastructure (Wong & Gerstenberger, 2011). The Southern Nevada Water Authority has had an ongoing water quality program that samples water quality characteristics along with zooplankton and phytoplankton samples.

Current research is shared through interagency meetings like the Quagga Mussel Interagency Group and the Lake Mead Water Quality Forum. A special issue of the Aquatic Invasions journal was devoted to Lake Mead quagga mussel research in 2011. The mobilization of stakeholders improved the coordinated effort to learn and prevent mussel damages to Lake Mead and other locations in the Western United States. The exact dollar amount spent to date on Lake Mead quagga controls has not been quantified, but the economic costs have been severe to

prevent damages to critical infrastructure (Turner et al., 2011). These costs will continue indefinitely into the future to minimize quagga impacts.

Veliger settlement and population structure have been researched by several authors. Whittmann et al. (2010) studied early population structure in Lake Mead to determine that quagga mussel densities are limited by the type of substrate and basin, but not by depth or water temperature (Wittmann et al., 2010). The largest densities of adult mussels were found on hard or coarse substrates, but densities found on silt and fine sand indicate the ability to colonize any substrate in Lake Mead (Wittmann et al., 2010). It is likely that fine sediment can interfere with filtering processes and decrease densities. Mueting & Gerstenberger (2010) found no preference of settlement on artificial substrates, but determined settlement was limited by depth. Settlement on artificial substrates at depths of 6-28 meters was significantly greater than at depths of 32-54 meters (Mueting & Gerstenberger, 2010). Reduced settlement with increased depth could be the result of water quality characteristics like colder temperature and lower food availability that may decrease veliger growth success. Environmental factors like flow velocity, water temperature and dissolved oxygen were determined to affect mussel settlement on substrates, while total organic carbon, conductivity and pH had little to no effect on veliger settlement (Chen et al., 2010). The carrying capacity of quagga mussel populations in the Boulder Basin of Lake Mead was estimated at 1.02×10^{13} which is a population size that would reduce chlorophyll-a levels to a level at the threshold of survival (Cross, Wong, & Che, 2011). Veligers are present year-round in Lake Mead with the highest percentage of competent veligers peaking between November and January (Gerstenberger et al., 2011). Settlement rates on acrylonitrile butadiene styrene pipes in Lake Mead were found to be highest between October 20 and December 19 which correlates with the highest percent of competent veligers (Wong,

Gerstenberger, Baldwin, & Moore, 2011). Concentrations of veligers peaked in June and September which correlated to the temperature of the metalimnion (Gerstenberger et al., 2011). The results of these studies confirm that a temperature of 17-22 °C is optimal for spawning and settlement (McMahon, 1996).

Several authors have investigated the impacts of quagga mussels in Lake Mead. Beaver et al. (2010) studied the zooplankton *Daphnia* in three basins of Lake Mead and found the zooplankton community remained comparable to past historic patterns. Loomis, Sjoberg, Wong, & Gerstenberger (2011) analyzed the abundance of a prey species fish called threadfin shad and found no significant changes since the introduction of quagga mussels to Lake Mead. Wong et al. (2010) analyzed differences in chlorophyll-a, water clarity and zooplankton abundance in the Boulder Basin of Lake Mead. The results of their study indicated no significant changes in the Boulder Basin since mussel establishment (Wong et al., 2010).

Lake Mead Phosphorus Removal

Water used in the Las Vegas Valley is treated by the Clark County Water Reclamation, City of Henderson, and North Las Vegas Waste Water Treatment Facilities and returned to Lake Mead via the Las Vegas Wash. The effluent has high concentrations of nitrogen and phosphorus that affect the water quality characteristics of the Boulder Basin. In 2001, a large algal bloom formed that covered the entire Boulder Basin. Starting in 2002, treatment plants began year round phosphorus removal to reduce the amount of phosphorus reaching Lake Mead (Figure 4). In 2005, plant optimization further decreased total phosphorus to pre-1956 levels (Drury, 2013). After 2005, there were steady increases in the lbs/day phosphorus loading released into the Las

Vegas Wash (Figure 4). In the summer of 2011, the City of North Las Vegas started releasing effluent to the Las Vegas Wash causing an increase in phosphorus to the Boulder Basin. The treatment facility optimizations were the lowest during the pre-quagga time frame (2004 to 2006) and phosphorus loading increased orthophosphate in the Boulder Basin during the post-quagga time frame (2009-2011).

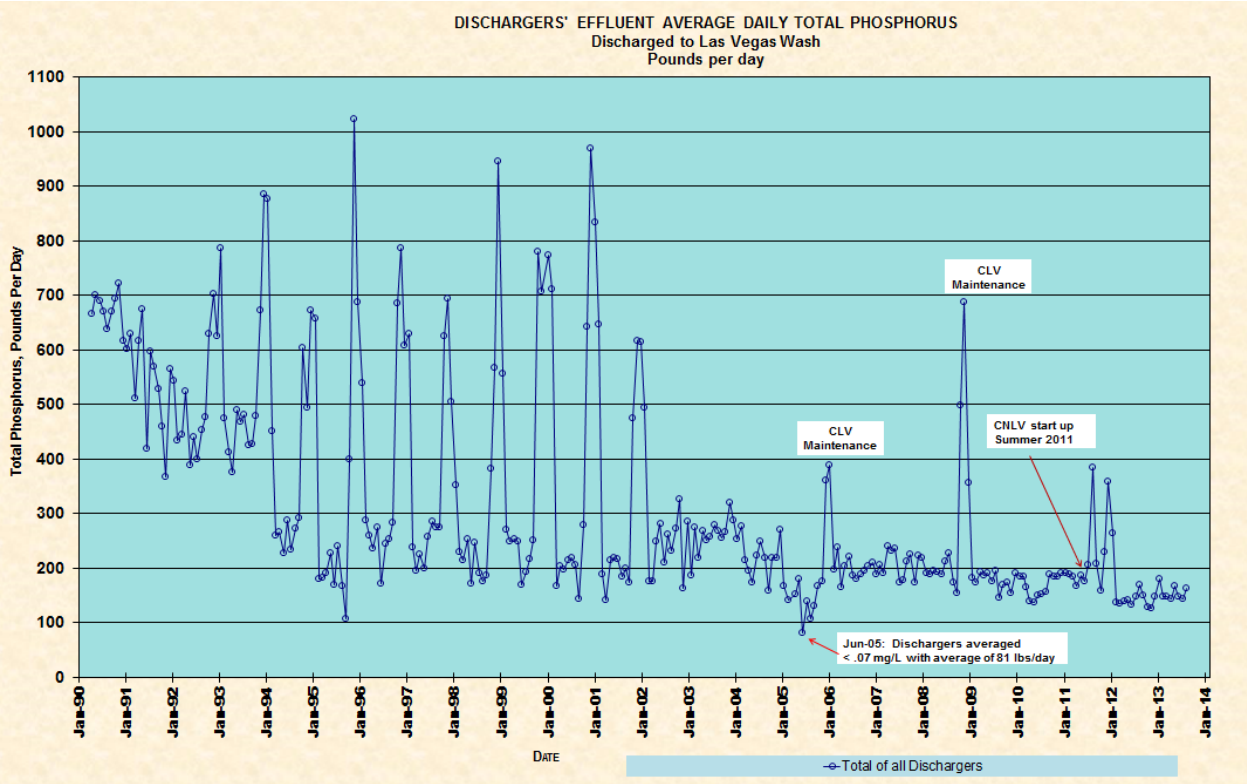


Figure 4 Phosphorus discharge from waste water treatment facilities (Drury, 2013)

Lake Mead Elevation

Lake Mead has experienced significant drought conditions in the Upper Colorado River Basin which has impacted the level of storage in Lakes Mead and Powell. Lake Mead storage increases with releases from Lake Powell and decreases from Hoover Dam releases to lower

basin water users in California, Arizona and Mexico. The extended drought since 2000 has decreased Lake Mead elevation by 41.3 meters (136 feet) and to 38% of capacity ($USBR_b$, 2015). The change in elevation has impacted water quality characteristics in Lake Mead. Total dissolved solids and specific conductance decreased significantly as Colorado River inflows were more prominent with less volume in Lake Mead (Holdren, Horn, Lieberman, 2011). Figure 5 shows the elevation change from 2000 to 2015. In 2011, there was an above average runoff in the upper basin triggering larger releases from Lake Powell. Since 2011, Lake Mead has continued to decline from lower than average snow accumulation in the upper basin.

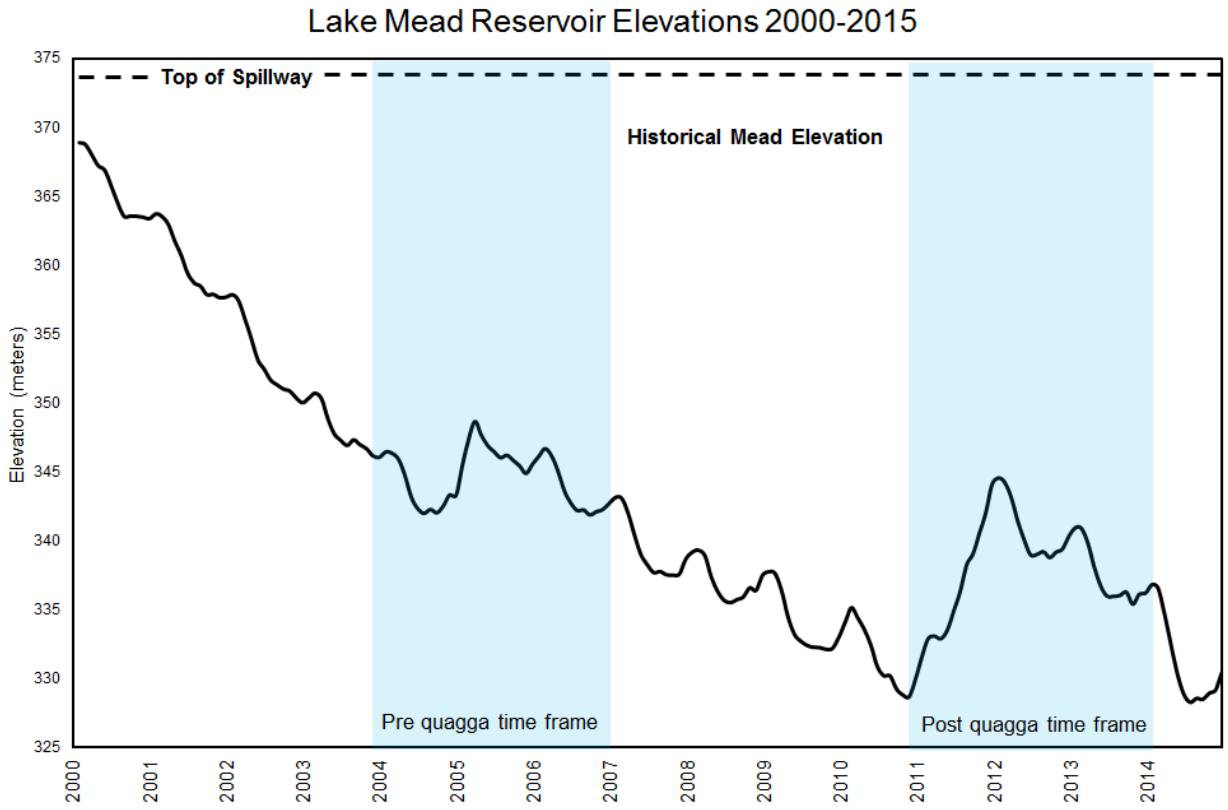


Figure 5 Lake Mead elevations from 2000 to 2014

Toxin Producing Cyanobacteria

Microcystis is a type of blue-green algae (cyanobacteria) that is the most common fresh water harmful algae found around the world (Zaccaroni & Scaravelli, 2008). Some types of cyanobacteria, like *Microcystis* can release toxins under certain circumstances. *Microcystis* can be found in lakes, estuaries and rivers in colonies or clusters of photosynthetic cells. When *Microcystis* cells reach high numbers from favorable nutrient and temperature conditions, the cells can form a bloom that can be toxic. When large numbers of cells accumulate on the water surface from water currents or wind movements large poisonous surface scum can appear. Toxins released by cyanobacteria are not regulated by the Clean Drinking Water Act and left for state regulation. Nevada does not have cyanobacterial toxin regulations for drinking water or recreational contact.

Cyanobacteria Toxins

There are many different species of blue-green algae or cyanobacteria that can cause public health concerns and adverse environmental conditions. The primary toxin producing species of cyanobacteria are *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, *Nodularia*, and *Oscillatoria*. Toxic algae form two main types of toxins called neurotoxins and hepatoxins. Neurotoxins act like anticholinesterase inhibitors and hepatoxins damage liver tissue causing liver damage or failure. The most common form of hepatoxin produced by *Anabaena*, *Microcystis*, and *Planktothrix*, is called microcystin-LR. Algal toxins can range in size and chemical structures depending on the species. There are as many as 60 versions of microcystin

with slight variations of methylation, acetylation and sulfated variants (Backer, 2002). Microcystin toxins are classified as cyclical peptides with a molecular weight between 800-1100 daltons (Zaccaroni & Scaravelli, 2008). Microcystin toxin is a very stable structure that can take 2 days to 3 weeks for natural environmental degradation to occur (Zaccaroni & Scaravelli, 2008). Contact with microcystin toxins can irritate the skin, and ingestion can cause liver damage and death.

Regulations Pertaining to Toxins

Currently, there are no federal regulations or guidelines controlling toxins or cyanobacteria within the Clean Water Act or the Safe Drinking Water Act. The Safe Drinking Water Act requires the Environmental Protection Agency (EPA) to publish a list of unregulated contaminates that are of public health concern called Contaminant Candidate Lists. Cyanobacteria and cyanotoxins have been listed by the EPA in 1998, 2005, and 2009. The lack of certified standards, standardized analytical methods and the large amount of compound variants have made establishing guidelines difficult for drinking water. Data are currently being collected under the Unregulated Contaminant Monitoring Rule which requires large drinking water providers to sample and monitor unregulated contaminants. Data collected from the public water systems could aid in future drinking water regulations.

In 2014, The Harmful Algal Bloom and Hypoxia Research and Control Amendments Act tasked the National Oceanic and Atmospheric Administration to conduct research, monitor, detect and forecast algal blooms within the United States. Annual funding of $20.5 million for

years 2014 through 2018 was authorized to fund additional studies to improve forecasting, health and ecological impacts of blooms.

The EPA's Secondary Maximum Contaminant Levels for drinking water are established as guidelines and are not enforceable. The Secondary Maximum Contaminant Levels do not contain any guidelines for cyanobacteria, but include recommended measures for odor and color which could be related to the amount of algae in the water.

A large number of states in the U.S. have developed guidelines for toxin exposure for recreational contact and drinking water standards. Drinking water guidance for Minnesota, Ohio and Oregon are listed in Table 1. Some states use the recreational guidelines to post advisories or shut down hazardous bodies of water. Recreational guidelines that have been adopted by states are shown in Appendix B. Recreational contact to toxins in water varies by state and ranges between .8 µg/L to >20 µg/L. Cell counts are monitored by states to determine the recommended action depending on the severity of the public risk. Nevada has not adopted any state guidelines for recreational contact or drinking water standards for cyanobacteria or algal toxins.

Table 1 State drinking water guidance (Graham, Loftin, &Kamman, 2009)

State	Drinking Water Guidance/Action Level
Minnesota	Microcystin-LR: 0.04 µg/L
Ohio	Microcystin: 1 µg/L Anatoxin-a: 20 µg/L Cylindrospermopsin: 1 µg/L Saxitoxin: 0.2 µg/L
Oregon	Microcystin-LR: 1 µg/L Anatoxin-a: 3 µg/L Cylindrospermopsin: 1 µg/L Saxitoxin: 3 µg/L

The World Health Organization (WHO) has adopted a provisional value of 1 µg/L for microcystin-LR in drinking water based on toxicity studies in mice (World Health Organization, 1998). However, the WHO was unable to develop guidelines for other cyanotoxins due to insufficient data. Recreational guidelines were developed by the WHO for cyanobacteria, microcystin-LR and chlorophyll-a. The WHO recreational guidelines are listed in Table 2.

Table 2 Guidelines for recreational waters (World Health Organization, 2003)

Relative Probability of Acute Health Effects	Cyanobacteria (cells/mL)	Microcystin-LR (µg/L)	Chlorophyll-a (µg/L)
Low	< 20,000	<10	<10
Moderate	20,000-100,000	10-20	10-50
High	100,000-10,000,000	20-2,000	50-5,000
Very High	> 10,000,000	>2,000	>5,000

Other countries have developed drinking water and recreational guidelines to reduce public health risk. Australia has a drinking water standard of 1.3 µg/L for microcystin, and New Zealand has a provisional value of 1 µg/L for microcystin-LR (Donohue et al., 2008). New Zealand also has provisional values for other toxins like anatoxins, *cylindrospermopsin* and nodularin. Australia uses conditional based guidelines for closing recreational areas based on concentration of cells. The United Kingdom does not regulate algal toxins, but monitors for cyanobacterial cells or toxins when a risk is present. Many other countries have drinking water guidelines and recreational exposure based on the WHO provisional guidelines for Microcystins.

Health Concerns from Cyanobacteria

Exposure Routes

Harmful algae can increase in water bodies through eutrophication, which is a result of increased concentrations of nutrients in the water. Eutrophication can occur through farm runoff, urban runoff and wastewater entering a water body. Cyanobacteria can float in the water column using gas vesicles to keep buoyant and can become dangerous when they accumulate in large numbers near shores through wind and wave processes. As the cells disintegrate, toxins are released creating potentially harmful areas for humans and animals. Accumulations of toxins can affect animals through several exposure routes.

The most common form of exposure is through direct contact with water where blooms or toxic scum has formed. Swimming and boating in lakes can expose people to harmful toxins. Dermal contact can also occur if raw or treated water contains algal toxins. Ingestion is another exposure route of toxins. Accidental ingestion of algal toxins during recreational activities or drinking water where toxins were not appropriately removed can cause harm. Municipalities need to treat raw water containing toxic algae appropriately to prevent cell lysis which can release toxins into the water. Consumption of food containing algal toxins can occur through ingestion of shellfish or nutritional products containing algae (Codd et al., 1999). A less common form of exposure is inhalation of toxins through water sports or showering in water containing toxins (Codd et al., 1999). Injection of toxins is the last major exposure route of algal toxins. Exposure by injection can occur if toxins in water are used during dialysis and trigger the most lethal response in humans (Codd et al., 1999).

Adverse Health Effects

Microcystis toxins have been documented to cause death and sickness in mammals, fish, waterfowl and humans (Gorham, 1960). Microcystins are one of the most frequent algal toxins found in the environment (Zaccaroni & Scaravelli, 2008). Laboratory experiments giving lethal doses of the toxins have been administered to better understand the effects of toxins on rat, rabbit, calf and chicken tissues. A lethal oral dose causes symptoms of increased frequency and depth of breath, convulsions and eventually death within hours. Pathology of animals suggests microcystin affects the peripheral circulatory system with the largest changes to the liver (Konst et al., 1965). Microcystins act by blocking protein phosphatases 1 and 2a causing hepatic damage and possible death (Zaccaroni & Scaravelli, 2008). Microcystin toxins enter the liver through the bile acid transport of hepatic and intestinal cells causing accumulation and damage in the liver.

Exposure to toxins on the skin can cause a variety of different adverse health effects, but with less severity than ingestion. Conjunctivitis, earache, swollen lips and allergic dermatitis can occur after coming in contact with toxins (Backer, 2002). Reports of symptoms by recreationalists have been documented after exposure to cyanobacterial blooms (Pilotto et al., 1997). Ingestion of toxin causes symptoms of weakness, vomiting, urinary bleeding, cold extremities and diarrhea. Ingestion can lead to hepatitis, renal or intestinal failure (Zaccaroni & Scaravelli, 2008). In 1931, thousands of people were reported to have symptoms after drinking water from the Ohio River during a cyanobacterial bloom (Backer, 2002). An example of severe intoxication occurred after ten Army recruits ingested microcystin toxins from a blooming lake during canoeing. The recruits had symptoms of blistering mouths, vomiting, diarrhea, abdominal pain, and showed signs of liver toxicity (Turner, Gammie, Hollinrake, & Codd, 1990). Ingestion

of low concentrations over a long period of time has been documented to damage liver tissue. A cross sectional investigation of chronic exposure of Microcystin revealed childhood liver damage when exposed to toxins in drinking waters over long periods of time (Li, 2011). In 1996, 55 patients died from liver hemorrhage and failure after being infused with Microcystin toxins during dialysis treatment (Jochimsen et al., 1998). Since algal blooms form near shores they can create a hazard for wildlife and animals when they drink water. In a severe example, over 10,000 livestock were killed after a cyanobacteria outbreak in the Darling River in Australia (Falconer, 2001).

The impacts of invasive mussels have been documented in many ecosystems. Phytoplankton reductions are common after mussel establishment because phytoplankton is their main food source. Reductions in phytoplankton can cause some of the impacts observed in ecosystems like reduced chlorophyll-a and increasing transparency. The methods section explains the sampling locations and statistical procedures that were used to test multiple hypotheses about the possible impacts of quagga mussels in Lake Mead based on current literature.

CHAPTER 3 METHODS

The research design incorporated monthly sampling collected at 3 locations in Lake Mead, each representative of a major basin. The sampling stations were located in the Boulder Basin, Virgin Basin and Temple Basin areas of Lake Mead (Figure 6). Data were sampled from two time frames collected during 2004-2006 (pre-quagga) and 2009-2011 (post-quagga). This research tested multiple hypotheses about potential changes that may have occurred from the establishment of a large mussel population in Lake Mead. The first hypothesis evaluated changes in transparency, chlorophyll-a, phytoplankton, and zooplankton. The second hypothesis evaluated specific changes at the division and genus level of phytoplankton between the two time periods. The third hypothesis tested if the frequency of toxin producing cyanobacteria increased after the establishment of quagga mussels in Lake Mead.

Study Area

Lake Mead is located in the Mohave Desert on the Arizona-Nevada border. Lake Mead was created by the impoundment of water after the construction of the Hoover Dam in 1935. Lake Mead is the largest reservoir in the United States by volume (36.7×10^9 m^3) and is the second largest reservoir in the United States by surface area (660 km^2) (Holdren & Turner, 2010). When full, Lake Mead will reach an elevation of 374 m above mean sea level and span 106 km (67.87 miles) from the Hoover Dam to Pierce Ferry (LaBounty & Burns, 2005). Lake Mead can be divided into 4 main sub basins which were the lowest areas of topography when the lake was filled. The three basins sampled in this research are the Virgin, Temple, and Boulder Basin (Figure 6).

Figure 6 Sampling locations in Lake Mead (White dots indicate sampling locations)

The Colorado River contributes about 97% of the annual flow into Lake Mead (LaBounty & Burns, 2005). The Virgin and Muddy River tributaries account for about 2% of Lake Mead annual inflow, and the remainder 1% of inflow comes from the Las Vegas Wash which consists of highly treated effluent from the City of Henderson, Clark County Water Reclamation District and the City of North Las Vegas Treatment Facilities. The Las Vegas Wash also receives city storm flows, urban runoff and ground water flows that enter the lake. Each basin in Lake Mead is unique due to the inflows that influence the water quality characteristics. The Southern Nevada Water Authority has studied the Boulder Basin extensively because the city's two drinking water

intakes are located in the Boulder Basin at depths of 304.8 and 320 meters (LaBounty & Burns, 2005). Water is released through Hoover Dam for water deliveries downstream to Arizona, California and Mexico. Lake Mead has a residence time between 1 and 3 years because of constant inflow from the Colorado River and releases from the Hoover Dam (LaBounty & Burns, 2005).

Sampling Stations

Three sampling locations (n=201) were used to represent Lake Mead. Each location is a deep water station representative of each major basin in Lake Mead. Figure 6 shows the Boulder Basin, Virgin Basin, and Temple Basin sampling locations. Descriptions of each sampling location are shown below:

a.) Boulder Basin (n=71)
Location: 36E 03' 39.47"N, 114E 44' 19.50" W
Description: Deep station in Boulder Basin located between Sentinel Island and Fortification Hill in the thalweg of the Colorado River.

b.) Virgin Basin (n=63)
Location: 36E 09' 41.01" N, 114E 25' 08.83" W
Description: Within main channel of the Virgin River by the north side of the Virgin Basin.

c.) Temple Basin (n=67)
Location: 36E 02' 48.30" N, 114E 16' 24.09" W
Description: Within main channel of Colorado River near area where Boulder Canyon begins to widen in Temple Basin.

Time Frame

Two time frames were analyzed for differences in water quality characteristics that are commonly impacted by dreissenid mussel invasions. Samples collected in 2004-2006 represent lake conditions before dreissenid mussel establishment, and samples collected in 2009-2011 represent lake conditions after dreissenid population establishment. Table 3 shows the number of samples collected at each location.

Table 3 Sample size at each sampling location

Year	CR346.4 Boulder Basin	VR2.0 Virgin Basin	CR380.0 Temple Basin
2004 (pre-quagga)	11	4	8
2005 (pre-quagga)	12	11	11
2006 (pre-quagga)	12	12	12
2009 (post-quagga)	12	12	12
2010 (post-quagga)	12	12	12
2011 (post-quagga)	12	12	12
Total	71	63	67

Sampling Methods

Water Transparency

Water transparency was measured using a standard Secchi disk with white and black markings. A metal cord marked with meters was used to measure the depth of the disk in the water column. The measurement is taken with the unaided eye, and depth is measured when the disk is no longer visible in the water (Preisendorfer, 1986).

Chlorophyll-a

Chlorophyll-a composite samples were collected from surface to five meters using a flexible swimming pool hose. The hose was rinsed using composite water before collecting each sample. Samples were poured into one liter bottles and placed on ice until returning to the laboratory. A volume of 650 ml was filtered through a Whatman glass microfiber filter grade GF/C (Whatman, New Jersey) using a Millipore manifold (EMD Millipore Corporation, Missouri) and vacuum pump apparatus. Filters were suction dried and frozen until analysis, which usually occurred within two weeks (Holdren et al., 2011). The extraction method followed procedures described by Holm-Hansen and Reimann (1978) using acetone as the extracting agent (Holdren et al., 2011). Absorbance was measured using a Varian Cary Model 100 UV-Vis spectrophotometer (Varian, California). Chlorophyll-a concentrations were calculated following the spectrophotometric determination of chlorophyll-a method described in standard methods 10200 H (2) (APHA, 2005). Chlorophyll-a sampling and analysis were performed by Reclamation staff in Boulder City, Nevada.

Phytoplankton

Phytoplankton samples were collected using the same composite sampling method used for chlorophyll-a. Composite sample water was collected from surface to five meters using a flexible swimming pool hose. The hose was rinsed before each composite sample. Samples were placed on ice and preserved with a 1% glutaraldehyde solution. Phytoplankton algal slides were

created using the HPMA (2-hydroxypropyl methacrylate) method described by Crumpton (1987) and identification procedures follow Standard Method 10200 F (APHA, 2005; St. Amand, 1990). Phytoplankton identification and enumeration were performed by Dr. Ann St. Amand at PhycoTech Incorporated (St. Joseph, Michigan). Samples were analyzed in triplicate, and original samples were retained. Samples were analyzed using an "an Olympus BHT, research-grade compound microscope equipped with Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (400x), epifluorescence (blue, green and UV Excitation), and a trinocular head for photography" (PhycoTech Inc, 2016). Counting was done at multiple magnifications to enumerate taxa from different sizes. Additional information on equipment and methodology used by PhycoTech Incorporated for identification and enumeration are provided in Appendix C. Phytoplankton samples were identified by division (Table 4) and genus level of organism.

Table 4 Phytoplankton divisions

Division Group	Division Name
Blue-Green	Cyanophyta or cyanobacteria
Green	Chlorophyta
Diatoms	Bacillariophyceae
Golden-Brown	Chrysophyta
Euglenoids	Euglenophyta
Dinoflagellates	Pyrrhophyta
Cryptomonads	Cryptophyta
Miscellaneous	Microflagellates, cysts and Gonyostomum

Zooplankton

Zooplankton samples were collected using a plankton net that was 15 cm diameter with a 64 µm mesh. Samples were collected from a depth of 30 meters to surface of the water column. After collection, samples were preserved with Lugol's solution and placed on ice. Dr. John

Beaver with BSA Environmental Services (Beachwood, Ohio) performed the analysis. Zooplankton identification followed methods developed by Stemberger (1979), Edmundson (1959), Ruttner-Kolisko (1974), and Pennak (1989). A minimum of 200 individuals were counted at a 100x magnification using a Wilovert inverted microscope. Identification of *Dreissena* veligers was conducted at a 100x magnification following the Zebra Mussel Information System developed by the U.S. Army Corps of Engineers (USACE, 2000). Zooplankton was grouped by major taxa for presentation: cladocerans, ostracods, rotifers, copepods, and veligers. Total number of zooplankton organisms were analyzed with and without veligers included.

Water Quality Probes

Multi-parameter probes were used to measure the temperature, dissolved oxygen, specific conductance and pH during each sampling. A Hydrolab 5 multi-probe instrument (Hydrolab Corporation, Indiana) was used to record data with a Surveyor 4 Data Collector (Hydrolab Corporation, Indiana). Temperature, dissolved oxygen, pH, specific conductance, and turbidity probes were attached to the Hydrolab instrument. Water quality data were collected each meter from surface to 5 meters, which correspond to the phytoplankton composite sample depth. Probe maintenance was followed per Hydrolab maintenance protocols (Hydrolab Corporation, User Manual Edition 3, 2006). The Clark cell, dissolved oxygen probe membrane, was replaced the night before each sampling event. The pH reference solution was replaced weekly. Probes were cleaned with soapy toothbrushes weekly to remove debris. The morning of sampling pH and specific conductance were calibrated using a two-point calibration method with known reference solutions. The dissolved oxygen probe was calibrated using saturated water. More details on

instrument maintenance and calibration protocol used in sampling can be located in the Hydrolab 5 user's manual (Hydrolab Corporation, User Manual Edition 3, 2006). Repeatability of instrument measurements are within 0.2% (Labounty and Burns, 2005). The probe type and reference method used by the Hydrolab instrument are shown in Table 5.

Table 5 Hydrolab instrument probe type and method (APHA, 2005)

Probe Type	Method	Reference
Temperature	Thermistor	2550 B
Dissolved Oxygen	Membrane Electrode	4500-OG
Specific Conductance	Conductivity Cell	2510 B
pH	Electrometric	4500-H B
Turbidity	Nephelometric	2130 B

Nutrients

Nutrient samples were collected from a grab sample at the surface of the water column. Water samples were placed into 250 ml bottles and placed on ice until laboratory analysis which was always less than 6 hours. Ortho-phosphate samples were filtered using a .45 µm pore size and refrigerated until analysis. Total phosphate, ammonia, and nitrate samples were preserved using 1 ml of 10% sulfuric acid in each 250 ml bottle and refrigerated until analysis. During the sampling time frame different analysis methods were used to improve detection limits. The Southern Nevada Water Authority, Henderson, Nevada, Lower Colorado Reclamation Laboratory (LCRL), Boulder City, Nevada, High Sierra Water Laboratory (HSWL), Truckee, California and Weck Laboratories (Weck), City of Industry, California, were used to analyze

nutrient samples. The Standard Method laboratory procedures used to analyze nutrient samples are summarized in Table 6 for each year of analysis.

Table 6 Nutrient sample methods (APHA, 2005; EPA, 2000)

Analysis Type	Anaysis Year	Referenced Method	Detection Limit (mg/L)
Ortho Phosphate (PO4-P)	2004 (LCRL[a])	4500-P E	0.002
	2005 (LCRL)	4500-P E, EPA 365.1	0.002, 0.001
	2006 (SNWA[b])	EPA 365.1	0.001
	2007 (HSWL[c], SNWA)	4500-P F	0.001
	2008 (HSWL, SNWA)	4500-P F	0.001
	2009 (HSWL, SNWA)	4500-P F	0.001
Total Phosphorus (TP)	2004 (LCRL)	4500-P E	0.002
	2005 (LCRL)	4500-P E, EPA 365.1	0.002, 0.003
	2006 (HSWL)	EPA 365.1	0.003
	2009 (HSWL, SNWA)	4500-P E	0.001
	2010 (HSWL, SNWA)	4500-P E	0.001
	2011 (HSWL, SNWA)	4500-P E	0.001
Ammonia Nitrogen (NH3-N)	2004 (LCRL)	4500-NH3 C	0.04
	2005 (LCRL)	4500-NH3 C, EPA 350.1	0.03, 0.003
	2006 (Weck[d])	4500-NH3 H	0.02
	2009 (Weck)	4500-NH3 H, EPA 350.1	0.02
	2010 (Weck)	4500-NH3 H, EPA 350.1	0.02
	2011 (Weck)	4500-NH3 H, EPA 350.1	0.02
Nitrate Nitrogen (NO3-N)	2004 (LCRL)	4500-NO3 B	0.052
	2005 (LCRL)	4500-NO3 B, EPA 353.2	0.052
	2006 (Weck)	4500-NO3 F	0.052
	2009 (Weck)	4500-NO3 F	0.052
	2010 (Weck)	4500-NO3 F	0.052
	2011 (Weck)	4500-NO3 F, EPA 353.2	0.052

[a]Lower Colorado Reclamation Laboratory, Boulder City, Nevada (LCRL)

[b]Southern Nevada Water Authority, Henderson, Nevada (SNWA)

[c]High Sierra Water Laboratory, Truckee, California (HSWL)

[d]Weck Laboratories, City of Industry, California (Weck)

Data Sources

The data used in this study were collected as part of the SNWA water quality monitoring program which is used to monitor water quality conditions in Lake Mead. The SNWA contracted

Bureau of Reclamation, Denver staff to sample water quality conditions in Lake Mead. During sampling, transparency, chlorophyll-a, physical data (temperature, conductivity, dissolved oxygen and pH), nutrients, zooplankton and phytoplankton samples were collected. The sample data were downloaded from the SNWA Regional Water Quality Database (SNWA, 2014).

Software

Microsoft Excel (Microsoft Corporation, Washington) was used to maintain data and create time series graphs. Statistical tests were performed using SPSS (IBM, New York) statistical software Version 22.

Data Quality Assurance

Data collected by the SNWA is checked for quality assurance through the data collection and analysis methods used. Field samplers calibrate probes on Hydrolab instruments per manufacturer's instructions using known standards to check instruments' accuracy. Laboratory methods are checked for accuracy using reference and duplicate samples. A summary and explanation of quality assurance methods used for data collected during these time frames are listed in Table 7.

Table 7 Quality assurance procedures

Procedures	Quality Assurance
Field Instrument Calibration	Field instruments are calibrated using 2 point calibrations and verified with known solutions.
Field Instrument Cleaning	Instruments are cleaned weekly to manufacturer's recommendations.
Reference Samples	Laboratory analysis procedures are tested through the use of known reference samples.
Standard Methods	Best common practices in water and wastewater for sampling and laboratory procedures.
Duplicate Sample	Duplicate samples are collected during sampling to evaluate precision.
Standard Operating Procedures	Laboratory and field sampling methods at Reclamation and the SNWA follow standard operating procedures.

Statistical Methods

General Data Analysis

SPSS was used to explore the distribution of data. Descriptive analyses of the data were performed to understand the distribution of data. Sample data were compiled using pivot tables to create summary statistics. Time series graphs were generated for each water quality characteristic, phytoplankton divisions, and genera using Microsoft Excel. The Shapiro-Wilk normality test was run in SPSS statistical software before determining which statistical tests would be needed. The majority of data tested revealed that nonparametric statistical tests would be required for the population distributions.

Wilcoxon Signed Rank Test

The Wilcoxon Signed Rank test is a non-parametric test to compare means of two paired samples, and is an alternative to the two-sample t test (Lehman, D'abrera, & Lehmann, 1975). The Wilcoxon Signed Rank Test has greater efficiency than the t-test on non-normal distributions and nearly the same efficiency as the t-test on normal distributions when samples are large (>100) (Marusteri & Bacarea, 2010). Analysis of smaller sample sets (<15) are not powerful using nonparametric tests. The Wilcoxon Signed Rank test is based on ranks which improves the results if the dataset has outliers present. The EPA recommends using this statistical procedure when identifying changes from a compliance sample to a baseline or to identify differences in time (EPA, 2009). This test was used to evaluate changes in transparency, chlorophyll-a, number of phytoplankton organisms, phytoplankton biomass, frequency of chlorophyta division, frequency of cyanophyta division and phytoplankton diversity.

The test hypothesis:

Ho: The distributions of water quality characteristics and phytoplankton are the same for both populations.

Ha: The distributions of water quality characteristics and phytoplankton are not the same for both populations.

Assumptions that must be met:

1.) Samples are paired.

2.) Ordinal data are collected to determine which value is larger.

3.) There is symmetry between the two populations so that each value has an equal likelihood of being sampled from each population.

4.) The groups must be two categorical, related, or matched pairs.

Time Series Graphs

Time series graphs were created to visually represent the data that were tested in each hypothesis. Graphs were created for water quality characteristics and phytoplankton. Time series graphs have the sampled data on the Y axis and date on the X axis. Time series graphs were used to provide a visual representation of the data collected over time.

Simpson Diversity Index

The Simpson Diversity Index is a measure of diversity when individuals from a population are classified into groups (Simpson, 1949). The Simpson Index is used in ecology to determine the biodiversity within an ecosystem by taking into account the richness and the evenness of the population samples. Enumeration to the species level is not possible or economically feasible in some cases. Several studies have determined that higher ranked taxa like family and genus are very good indicators of species diversity (Lee, 1996; Bertrand, Pleijel, Rouse, 2006). The Simpson Diversity Index is a very robust diversity measurement that calculates the variance of species abundance distribution (Magurran, 2004). The Simpson Index is a non-parametric approach which does not require assumptions about the population distribution. The Simpson Diversity Index is calculated with equation 1:

$$D = \frac{\sum n_i(n_i - 1)}{N(N-1)}$$

Equation 1, Where: n_i = Total number of organisms of each genera
N = Total number of organisms of all genera

Values of the Simpson Diversity Index can range from 0 to 1. A value of 0 represents the highest diversity and a value of 1 represents the lowest possible diversity. A larger score suggests the environment is stressful with a relatively small number of species within the ecosystem. Lower scores suggest an environment with more organisms and complex food webs. This test was used to determine if phytoplankton diversity in the Boulder, Temple, and Virgin Basin has changed since the quagga mussel invasion.

Hypotheses and Methods

The literature suggests that quagga and zebra mussels can have a profound impact to water quality characteristics after establishment. The following hypotheses investigate potential impacts to water quality characteristics, changes to phytoplankton, and if toxin producing algae has changed frequency after quagga mussel establishment.

Changes in Water Quality Characteristics

Research question 1) Have water quality characteristics in Lake Mead changed similar to other ecosystems since the establishment of *Dreissena polymorpha*?

Hypothesis 1) There will be differences in Lake Mead water characteristics between pre-quagga (2004-2006) and post-quagga establishment (2009-2011); specifically:

a.) Lake transparency has changed since quagga mussel establishment.

b.) Chlorophyll-a concentrations have changed since quagga mussel establishment.

c.) The total count of phytoplankton organisms have changed since quagga mussel establishment.

d.) Total phytoplankton biomass has changed since quagga mussel establishment.

e.) The total count of zooplankton organisms has changed since quagga mussel establishment.

Methods

Hypothesis 1_{a-e}) Wilcoxon Signed Rank Test

The Wilcoxon Signed Rank Test was used to test for differences between transparency, chlorophyll-a, total number of phytoplankton organisms, total phytoplankton biomass, and total number of zooplankton organisms after quagga mussel invasion. The pre and post means, % mean change between time periods, Z statistic, and the significance for a two tailed test are provided in the results section. p=0.05

Hypothesis 1_{a-e}) Time series graphs

Time series graphs were created for transparency, chlorophyll-a, total number of phytoplankton organisms, total biomass, and total number of zooplankton organisms.

Changes in Phytoplankton

Research Question 2.) Has phytoplankton at the division and genus level changed in Lake Mead since the establishment of *Dreissena polymorpha*?

Hypothesis 2.) There will be differences in Lake Mead phytoplankton between pre-quagga (2004-2006) and post-quagga (2009-2011); specifically:

a.) Chlorophyta (green) division has changed relative frequency (count of chlorophyta organisms in sample/ total count of sample) since quagga mussel establishment.

b.) Cyanophyta (blue-green) division has changed relative frequency (count of cyanophyta organisms in sample/ total count of sample) since quagga mussel establishment.

c.) Phytoplankton diversity has changed since quagga mussel establishment.

Methods

Hypothesis 2_{a-c}) Wilcoxon Signed Rank Test

The Wilcoxon Signed Rank Test was used to test for changes in relative frequency of chlorophyta, cyanophyta, and Simpson Diversity Index calculations after quagga mussel establishment. The pre and post means, % mean change between time periods, Z statistic, and the significance for a two tailed test are provided in the results section. $p=0.05$

Hypothesis 2_c) Simpson Diversity Index

The Simpson Diversity Index was used to calculate phytoplankton diversity for each sample date. The calculated values of the Simpson Diversity Index for each basin were tested for

differences using a Wilcoxon Signed Rank Test. The pre and post means, % mean change between time periods, Z statistic, and the significance for a two tailed test are provided in the results section. p=0.05

Hypothesis 2_{a-c}) Time series graphs

Time series graphs were created for the relative frequency of chlorophyta, relative frequency of cyanophyta, and for the Simpson Diversity Index calculations.

Changes in Toxin Producing Cyanobacteria

Research Question 3.) Has there been an increase in the frequency of toxin producing cyanobacteria in Lake Mead since the establishment of *Dreissena polymorpha*?

Hypothesis 3.) Toxin producing cyanobacteria like *Microcystis, Oscillatoria, Cylindrospermopsis* and *Anabaena* have changed frequency since quagga mussel establishment.

Methods

Hypothesis 3) Wilcoxon Signed Rank Test

The Wilcoxon Signed Rank Test was used to test for changes in frequency of *Microcystis, Oscillatoria, Cylindrospermopsis* and *Anabaena* following quagga mussel establishment. The pre and post means, % mean change between time periods, Z statistic, and the significance for a two tailed test are provided in the results section. p=0.05

Hypothesis 3) Time series graphs

Time series graphs were created for *Microcystis, Oscillatoria, Cylindrospermopsis* and *Anabaena* samples for each basin.

CHAPTER 4 RESULTS

Temple Basin Results

Water characteristics were collected and analyzed at Temple Basin for differences after quagga mussel establishment. The results indicate that there were no significant changes at Temple Basin to water characteristics commonly impacted by mussels. Transparency, chlorophyll-a, total phytoplankton, and phytoplankton biomass were not significantly different between the two time periods (Table 8). When the means were compared between the two time periods, total phytoplankton was reduced by 23% and phytoplankton biomass was reduced by 17%. Total zooplankton populations were significantly different between the two time periods, but contrary to the expected results, total zooplankton increased. Further analysis revealed the zooplankton counts were increased in post samples due to the large number of veligers. The veligers were removed, and total zooplankton was evaluated again at Temple Basin with significant differences between the two time periods. Total zooplankton means without veligers increased 77% in post samples.

Changes in phytoplankton were tested between pre and post quagga establishment at Temple Basin. The relative frequency of cyanophyta was significantly different with a 3% decrease between the two time periods. The relative frequency of chlorophyta and phytoplankton diversity did not show significant differences between the two time periods (Table 8).

Table 8 Differences between two time periods at Temple Basin

Temple Basin		Wilcoxon Signed Rank Test				
Category	units	Pre mean	Post mean	% change	Z	p (2 tailed)
Transparency	m	7.96	7.48	-6%	-0.998	0.318
Chlorophyll-a Surface	µg/L	1.20	1.23	2%	-0.862	0.389
Chlorophyll-a Composite	µg/L	1.28	1.36	7%	-1.117	0.264
Phytoplankton Total	Cells/mL	27,540	21,339	-23%	-1.080	0.280
Phytoplankton Biomass	μm^3	14,937	12,361	-17%	-1.306	0.192
Zooplankton Total	#/L	8.6	22.2	157%	-4.409	**0.000***
Zooplankton Total no veliger	#/L	8.6	15.2	77%	-3.106	**0.002***
Chlorophyta frequency	%	0.42%	0.48%	13%	-0.967	0.334
Cyanophyta frequency	%	97.22%	94.24%	-3%	-2.829	**0.005***
Simpson Diversity Index	-	0.477	0.452	-5%	-0.956	0.339

Asterisk indicates significant difference (P<0.05).

Toxin producing algae taxa between the two time periods were analyzed at Temple Basin for changes after quagga mussel establishment. *Anabaena, Cylindrospermopsis, Microcystis* and *Oscillatoria* were analyzed, but there were not enough data collected to perform statistical testing between the two time periods (Table 9). During the time frames, there were less than four detections of any taxa. The average numbers of cells were below 5 cells/ml which would not be considered a public health concern. Toxin producing algae did not appear to be increasing at Temple Basin during the time frames sampled.

Table 9 Toxin producing cyanobacteria at Temple Basin

Temple Basin	Number of Detections			Average Number of Cells		
	total	pre	post	avg total	avg pre	avg post
Anabaena	4	4	0	2.04	2.04	0.00
Cylindrospermopsis	1	1	0	4.54	4.54	0.00
Microcystis	4	0	4	5.33	0.00	5.33
Oscillatoria	1	1	0	2.02	2.02	0.00

Phytoplankton analysis was performed using a Wilcoxon Signed Rank Test on cell concentration and biomass for each major phytoplankton division between the two time frames. There was a significant increase in bacillariophyta and cryptophyta divisions for number of cells. There were no significant differences in division biovolumes noted (Table 10). Analysis of means between the two time periods show a 23% reduction in total phytoplankton cell numbers and a 17% reduction in total phytoplankton biovolume.

Table 10 Phytoplankton division analysis at Temple Basin

Temple Basin		Wilcoxon Signed Rank Test				
Category	units	Pre mean	Post mean	% change	Z	p (2 tailed)
Phytoplankton bacillariophyta	cells/mL	105	316	201%	-3.548	**0.000***
Phytoplankton Chlorophyta	cells/mL	117	106	-9%	-0.442	0.658
Phytoplankton Chrysophyta	cells/mL	326	543	67%	-1.594	0.111
Phytoplankton Cryptophyta	cells/mL	101	182	80%	-1.975	**0.048***
Phytoplankton Cyanophyta	cells/mL	26,910	20,231	-25%	-1.286	0.199
Phytoplankton Pyrrhophyta	cells/mL	5	6	21%	-0.200	0.841
Phytoplankton Total	cells/mL	27,540	21,339	-23%	-1.080	0.280
Phytoplankton bacillariophyta	μm^3	3,597	3,762	5%	-0.998	0.318
Phytoplankton Chlorophyta	μm^3	2,457	1,486	-40%	-1.553	0.120
Phytoplankton Chrysophyta	μm^3	861	554	-36%	-0.817	0.414
Phytoplankton Cryptophyta	μm^3	396	519	31%	-1.388	0.165
Phytoplankton Cyanophyta	μm^3	1,885	1,486	-21%	-1.697	0.090
Phytoplankton Pyrrhophyta	μm^3	5,470	4,500	-18%	-0.821	0.411
Phytoplankton Total	μm^3	14,937	12,361	-17%	-1.306	0.192

Asterisk indicates significant difference (P<0.05).

Descriptive analysis was performed on the largest changes in genera between the two time periods at Temple Basin. Table 11 lists the average phytoplankton cell composition and the average difference in cells between the two time periods. The largest genera change in average

cell concentration between the two time periods was a decrease in *Synechococcus* (cyanophyta) which accounts for 44% of phytoplankton cell composition. *Synechococcus* was decreased in post samples by an average of 3,351 cells. The next largest change was the reduction in the family Chroococcaceae (cyanophyta). Chroococcaceae was unidentified to the genus level, but were less than 1 µm and spherical in shape. Chroococcaceae accounted for an average of 43% of phytoplankton cell composition and decreased by 2,270 cells in post time period samples at Temple Basin. *Cyanogranis* (cyanophyta) was the next largest contribution of total phytoplankton cell composition at 4% of total cells and had an average increase of 1,693 cells in post samples.

Table 11 Largest change in cell concentrations at Temple Basin

Temple Basin, Largest change in cell concentrations

Division	Genus	average #	average %	average pre #	average pre %	average post #	average post %	difference
Cyanophyta	*Synechococcus*	10,822	43.512%	12,626	45.042%	9,276	42.237%	-3,351
Cyanophyta	*Unidentified (Chroococcaceae)*	9,671	42.937%	10,909	42.986%	8,640	42.895%	-2,270
Cyanophyta	*Cyanogranis*	4,283	4.142%	3,741	4.837%	5,434	3.563%	1,693
Cyanophyta	*Synechocystis*	706	3.699%	508	1.910%	866	5.190%	358
Cyanophyta	*Aphanocapsa*	352	0.779%	433	1.180%	236	0.445%	-197
Chrysophyta	*Erkenia*	265	1.274%	179	0.916%	329	1.572%	150
Cyanophyta	*Aphanothece*	252	0.379%	326	0.699%	89	0.113%	-237
Chrysophyta	*Dinobryon*	211	0.693%	187	0.570%	238	0.795%	51
Cyanophyta	*Rhabdoderma*	258	0.210%	383	0.398%	43	0.054%	-340
Cryptophyta	*Rhodomonas*	113	0.670%	56	0.335%	159	0.949%	103
Bacillariophyta	*Cyclotella*	203	0.872%	102	0.331%	289	1.323%	187
Chlorophyta	*Unidentified (Chlorococcaceae)*	33	0.115%	41	0.143%	25	0.093%	-16
Cyanophyta	*Merismopedia*	100	0.163%	55	0.118%	185	0.201%	129
Chlorophyta	*Oocystis*	25	0.060%	27	0.085%	23	0.039%	-4
Miscellaneous	*Unidentified (Misc)*	36	0.029%	45	0.057%	5	0.006%	-40
Chlorophyta	*Pyramichlamys*	19	0.047%	22	0.052%	17	0.043%	-4
Cryptophyta	*Cryptomonas*	13	0.078%	12	0.051%	13	0.100%	1

Water quality samples analyzed at Temple Basin between showed that there were differences between the two time periods. There were significant changes in dissolved oxygen, specific conductance and total dissolved solids (Table 12). Total phosphate significantly increased, and nitrate, ammonia, and total inorganic nitrogen significantly decreased.

Table 12 Water quality samples at Temple Basin

Temple Basin		Wilcoxon Signed Rank Test				
Category	units	Pre mean	Post mean	% change	Z	p (2 tailed)
Temperature	°C	20.6	20.1	-2%	-1.901	0.057
Dissolved Oxygen	mg/L	8.6	9.0	5%	-3.548	**0.000***
Dissolved Oxygen	% Sat	103.7	103.0	-1%	-0.648	0.517
pH	s.u.	8.3	8.3	-1%	-1.911	0.056
Specific Conductance	µS/cm	978	866	-12%	-4.860	**0.000***
Total Dissolved Solids	g/L	0.626	0.557	-11%	-4.861	**0.000***
Ortho-phosphate	µg/L	0.814	0.600	-26%	-0.682	0.495
Total Phosphate	µg/L	4.208	7.610	81%	-3.672	**0.000***
Nitrate	mg/L	0.288	0.189	-34%	-3.957	**0.000***
Ammonia	mg/L	0.007	0.001	-86%	-2.924	**0.003***
Total Inorganic Nitrogen	mg/L	0.295	0.197	-33%	-3.871	**0.000***

Asterisk indicates significant difference (P<0.05).

Virgin Basin Results

Water quality samples were collected and analyzed at Virgin Basin for differences after quagga mussel establishment. The results indicate that there were no significant changes at Virgin Basin to water characteristics commonly impacted by mussels. There were no significant differences in transparency measurements or chlorophyll-a concentrations between the two time periods (Table 13). Total phytoplankton cell concentrations had a significant difference at Virgin

Basin with a 24% reduction in organisms in post-quagga establishment. Total phytoplankton biomass did not show a significant difference, but there was a 36% decrease in means between the two time periods. Total zooplankton with veligers removed had a significant difference with a 69% increase during the post quagga time period.

Changes in phytoplankton at the division and genus level were compared between pre and post quagga establishment at Virgin Basin. There were no significant differences noted in relative frequency of chlorophyta, relative frequency of cyanophyta, or Simpson Diversity Index calculations (Table 13).

Table 13 Differences between two time periods at Virgin Basin

Virgin Basin		Wilcoxon Signed Rank Test				
Category	units	Pre mean	Post mean	% change	Z	p (2 tailed)
Transparency	m	8.61	8.04	-7%	-1.238	0.216
Chlorophyll-a Surface	µg/L	0.96	0.97	1%	-0.216	0.829
Chlorophyll-a Composite	µg/L	1.29	1.26	-3%	-0.072	0.943
Phytoplankton Total	Cells/mL	26,025	19,696	-24%	-2.354	**0.019***
Phytoplankton Biomass	μm^3	11,906	7,678	-36%	-1.511	0.131
Zooplankton Total	#/L	7.9	21.1	169%	-3.518	**0.000***
Zooplankton Total no veliger	#/L	7.9	13.3	69%	-2.438	**0.015***
Chlorophyta frequency	%	0.37%	0.38%	4%	-0.316	0.752
Cyanophyta frequency	%	96.80%	95.23%	-2%	-1.754	0.079
Simpson Diversity Index	-	0.494	0.457	-7%	-0.793	0.428

Asterisk indicates significant difference (P<0.05).

Toxin producing algae taxa between the two time periods were analyzed at Virgin Basin for changes after quagga mussel establishment. *Anabaena*, *Cylindrospermopsis*, *Microcystis* and *Oscillatoria* were analyzed, but there were not enough detections to perform statistical testing between the two time periods (Table 14). During the time frames, there were less than four detections of any taxa. The average numbers of cells were below 6 cells/ml which would not be

considered a public health concern. Toxin producing algae did not appear to be increasing at Virgin Basin during the time frames sampled.

Table 14 Toxin producing cyanobacteria at Virgin Basin

Virgin Basin	Number of Detections			Average Number of Cells		
	total	pre	post	avg total	avg pre	avg post
Anabaena	2	0	2	5.64	0.00	5.64
Cylindrospermopsis	0	0	0	0.00	0.00	0.00
Microcystis	1	0	1	2.19	0.00	2.19
Oscillatoria	4	4	0	4.87	4.87	0.00

Phytoplankton division analysis was performed using a Wilcoxon Signed Rank Test on cell concentration and biomass for each major phytoplankton division between the two time frames. The Virgin Basin had significant differences in cell concentrations for cyanophyta and total phytoplankton (Table 15). There was a 25% reduction in means between time periods for cyanophyta and a 24% reduction in means for total phytoplankton cell concentrations in the post-quagga time period. Phytoplankton biomass had a significant difference for the pyrrhophyta division.

Table 15 Phytoplankton division analysis at Virgin Basin

Virgin Basin		Wilcoxon Signed Rank Test				
Category	units	Pre mean	Post mean	% change	Z	p (2 tailed)
Phytoplankton bacillariophyta	cells/mL	132	217	65%	-1.802	0.072
Phytoplankton Chlorophyta	cells/mL	92	78	-16%	-1.156	0.248
Phytoplankton Chrysophyta	cells/mL	448	448	0%	-0.048	0.962
Phytoplankton Cryptophyta	cells/mL	61	100	63%	-1.490	0.136
Phytoplankton Cyanophyta	cells/mL	25,281	18,848	-25%	-2.475	**0.013***
Phytoplankton Pyrrhophyta	cells/mL	4	3	-25%	-0.867	0.386
Phytoplankton Total	cells/mL	26,025	19,696	-24%	-2.354	**0.019***
Phytoplankton bacillariophyta	μm^3	3,359	2,639	-21%	-0.597	0.551
Phytoplankton Chlorophyta	μm^3	2,614	2,766	6%	-1.156	0.248
Phytoplankton Chrysophyta	μm^3	758	703	-7%	-0.800	0.424
Phytoplankton Cryptophyta	μm^3	369	487	32%	-0.794	0.427
Phytoplankton Cyanophyta	μm^3	993	880	-11%	-0.597	0.551
Phytoplankton Pyrrhophyta	μm^3	3,255	142	-96%	-2.577	**0.010***
Phytoplankton Total	μm^3	11,907	12,391	4%	-0.902	0.367

Asterisk indicates significant difference (P<0.05).

Descriptive analysis was performed on the largest changes in genera between the two time periods at Virgin Basin. Table 16 lists the average phytoplankton cell composition and the average difference in cells between the two time periods. The largest genera change between the two time periods was a decrease in *Synechococcus* (cyanophyta) which accounts for an average of 46% of cell phytoplankton. *Synechococcus* was decreased in post samples by an average of 3,340 cells. The next largest change in cyanophyta was the reduction in the family Chroococcaceae. Chroococcaceae was unidentified to the genus level, but were less than 1 µm and spherical in shape. Chroococcaceae accounted for an average of 41% of total cells counted, and decreased by 3,180 cells in the post quagga time period samples. *Cyanogranis* (cyanophyta) was the next largest contributor of total cell count at an average of 4%, and had an average increase of 1,702 cells in post samples.

Table 16 Largest change in cell concentrations at Virgin Basin

Virgin Basin, Largest change in cell concentrations

Division	Genus	average #	average %	average pre #	average pre %	average post #	average post %	difference
Cyanophyta	*Synechococcus*	10,505	46.487%	12,414	46.067%	9,074	46.802%	-3,340
Cyanophyta	*Unidentified (Chroococcaceae)*	8,715	41.061%	10,533	42.873%	7,353	39.703%	-3,180
Cyanophyta	*Cyanogranis*	3,016	4.377%	2,408	4.935%	4,110	3.958%	1,702
Cyanophyta	*Synechocystis*	600	3.344%	420	1.663%	733	4.605%	313
Chrysophyta	*Erkenia*	232	1.203%	264	1.164%	208	1.232%	-56
Chrysophyta	*Dinobryon*	227	0.694%	254	0.720%	205	0.673%	-49
Cyanophyta	*Aphanocapsa*	213	0.545%	223	0.568%	202	0.528%	-21
Cyanophyta	*Aphanothece*	141	0.267%	168	0.433%	97	0.143%	-70
Bacillariophyta	*Cyclotella*	162	0.704%	118	0.430%	197	0.910%	79
Cryptophyta	*Rhodomonas*	82	0.545%	71	0.355%	90	0.687%	20
Cyanophyta	*Merismopedia*	120	0.127%	100	0.154%	144	0.107%	44
Chlorophyta	*Oocystis*	18	0.068%	20	0.085%	16	0.056%	-4
Bacillariophyta	*Anomoeneis*	55	0.031%	55	0.072%	0	0.000%	-55
Cyanophyta	*Rhabdoderma*	107	0.109%	55	0.070%	159	0.138%	105
Chlorophyta	*Unidentified (Chlorococcaceae)*	22	0.081%	22	0.067%	21	0.091%	-1
Chlorophyta	*Tetraedron*	25	0.023%	30	0.048%	12	0.004%	-18
Chlorophyta	*Stichococcus*	30	0.030%	32	0.045%	26	0.019%	-6
Chlorophyta	*Pyramichlamys*	12	0.030%	12	0.033%	13	0.028%	1

Water quality samples were analyzed at Virgin Basin between the two time periods using a Wilcoxon Signed Rank Test. There were significant differences between the two time periods in the Virgin Basin. Temperature significantly increased while dissolved oxygen, pH, specific conductance, and total dissolved solids were significantly reduced post quagga time period (Table 17). Total phosphate was significantly increased, and nitrate and total inorganic nitrogen were significantly decreased post quagga time frame. There was insufficient data collected for ammonia to perform statistical analysis.

Table 17 Water quality samples at Virgin Basin

Virgin Basin		Wilcoxon Signed Rank Test				
Category	units	Pre mean	Post mean	% change	Z	p (2 tailed)
Temperature	°C	20.8	20.2	-3%	-2.234	**0.025***
Dissolved Oxygen mg/L	mg/L	8.4	8.8	4%	-2.667	**0.008***
Dissolved Oxygen % Sat	% Sat	102.7	102.1	-1%	-0.288	0.773
pH	s.u.	8.3	8.2	-1%	-2.007	**0.045***
Specific Conductance	µS/cm	1,017	896	-12%	-4.541	**0.000***
Total Dissolved Solids	g/L	0.651	0.577	-11%	-4.542	**0.000***
Ortho-phosphate	µg/L	0.550	1.659	202%	-1.721	0.085
Total Phosphate	µg/L	2.525	6.993	177%	-3.653	**0.000***
Nitrate	mg/L	0.287	0.212	-26%	-3.436	**0.001***
Ammonia	mg/L	-	-	-	-	-
Total Inorganic Nitrogen	mg/L	0.291	0.218	-25%	-3.400	**0.001***

Asterisk indicates significant difference (P<0.05).

Boulder Basin Results

Water characteristics were collected and analyzed at Boulder Basin for differences after quagga mussel establishment. The Boulder Basin had more changes in water quality

characteristics expected after a quagga mussel invasion than the other basins included in this study. Transparency and composite chlorophyll-a concentrations were significantly different between the two time periods (Table 18). Transparency means increased 13% and composite chlorophyll-a concentrations were reduced 31% between the two time periods. Phytoplankton biomass was significantly different with a 68% reduction between means. Zooplankton totals without veligers were significantly increased in the post quagga time period.

Table 18 Differences between two time periods at Boulder Basin

Boulder Basin		Wilcoxon Signed Rank Test				
Category	units	Pre mean	Post mean	% change	Z	p (2 tailed)
Transparency	m	8.78	9.96	13%	-2.685	**0.007***
Chlorophyll-a Surface	µg/L	1.20	0.87	-28%	-1.818	0.069
Chlorophyll-a Composite	µg/L	1.49	1.02	-31%	-2.236	**0.020***
Phytoplankton Total	Cells/mL	23,622	17,591	-26%	-0.710	0.478
Phytoplankton Biomass	μm^3	19,655	6,249	-68%	-3.029	**0.002***
Zooplankton Total	#/L	7.1	13.8	93%	-3.538	**0.000***
Zooplankton Total no veliger	#/L	7.1	9.3	31%	-2.858	**0.004***
Chlorophyta frequency	%	0.55%	0.48%	-11%	-1.718	0.086
Cyanophyta frequency	%	95.28%	93.89%	-1%	-0.556	0.578
Simpson Diversity Index	-	0.497	0.485	-2%	-0.573	0.567

Asterisk indicates significant difference (P<0.05).

Toxin producing algae taxa between the two time periods were analyzed at Boulder Basin for changes after quagga mussel establishment. *Anabaena, Cylindrospermopsis, Microcystis* and *Oscillatoria* were analyzed, but there were not enough data collected to perform statistical testing between the two time periods (Table 19). During the time frames, there were less than seven detections of any taxa. The average numbers of cells were below 11 cells/ml which would not be

considered a public health concern. Toxin producing algae did not appear to be increasing in the Boulder Basin during the time frames sampled.

Table 19 Toxin producing cyanobacteria at Boulder Basin

Boulder Basin	Number of Detections			Average Number of Cells		
	total	pre	post	avg total	avg pre	avg post
Anabaena	6	4	2	2.39	0.55	6.06
Cylindrospermopsis	0	0	0	0.00	0.00	0.00
Microcystis	4	3	1	11.03	14.34	1.10
Oscillatoria	7	7	0	9.71	9.71	0.00

Phytoplankton analysis was performed using a Wilcoxon Signed Rank Test on cell concentration and biomass for each major phytoplankton division between the two time frames. Concentrations of cells for chlorophyta were significantly reduced post quagga time period (Table 20). Cell concentrations of pyrrhophyta were significantly different between the two time periods, but there were a very small number of cells present. Phytoplankton biomass was significantly increased for cryptophyta and significantly reduced for chrysophyta, cyanophyta, and total phytoplankton biomass. The largest change in total biomass was from the division cyanophyta.

Table 20 Phytoplankton division analysis at Boulder Basin

Boulder Basin		Wilcoxon Signed Rank Test				
Category	units	Pre mean	Post mean	% change	Z	p (2 tailed)
Phytoplankton bacillariophyta	cells/mL	269	231	-14%	-0.556	0.578
Phytoplankton Chlorophyta	cells/mL	126	69	-45%	-2.146	**0.032***
Phytoplankton Chrysophyta	cells/mL	447	401	-10%	-9.949	0.343
Phytoplankton Cryptophyta	cells/mL	65	92	42%	-1.633	0.103
Phytoplankton Cyanophyta	cells/mL	22,675	16,791	-26%	-0.658	0.510
Phytoplankton Pyrrhophyta	cells/mL	7	3	-52%	-2.336	**0.020***
Phytoplankton Total	cells/mL	23,622	17,591	-26%	-0.710	0.478
Phytoplankton bacillariophyta	μm^3	5,114	2,091	-59%	-1.914	0.056
Phytoplankton Chlorophyta	μm^3	3,325	2,088	-37%	-1.143	0.253
Phytoplankton Chrysophyta	μm^3	1,700	533	-69%	-2.589	**0.010***
Phytoplankton Cryptophyta	μm^3	443	736	66%	-2.829	**0.005***
Phytoplankton Cyanophyta	μm^3	2,876	221	-92%	-4.029	**0.000***
Phytoplankton Pyrrhophyta	μm^3	5,862	499	-91%	-1.456	0.145
Phytoplankton Total	μm^3	19,655	6,249	-68%	-3.029	**0.002***

Asterisk indicates significant difference ($P<0.05$).

Descriptive analysis was performed on composition of cells and the largest changes in genera between the two time periods at Boulder Basin. Table 21 lists the average phytoplankton cell composition and the average difference in cells between the two time periods. The largest contribution of total cells in the Boulder Basin was in the classification Unidentified *(Chroococcaceae)* (cyanophyta) which accounted for an average of 45% of total cell composition. The largest change in cell numbers in the Boulder Basin was from a reduction of Unidentified *(Chroococcaceae)* which decreased in post samples by an average of 3,449 cells. The next largest change was the reduction in *Synechococcus* (cyanophyta) which accounted for an average of 41% of total cells counted. *Synechococcus* decreased by an average of 1,170 cells in post time period samples. *Cyanogranis* (cyanophyta) was the next largest contributor of total cell count at an average of 2%, and had an average decrease of 215 cells in post samples

Table 21 Largest change in cell concentrations at Boulder Basin

Boulder Basin, Largest change in cell concentrations

Division	Genus	average #	average %	average pre #	average pre %	average post #	average post %	difference
Cyanophyta	*Unidentified (Chroococcaceae)*	8,458	45.377%	10,109	49.031%	6,660	41.513%	-3,449
Cyanophyta	*Synechococcus*	9,421	41.872%	9,981	40.772%	8,811	43.035%	-1,170
Cyanophyta	*Cyanogranis*	3,242	2.252%	3,304	2.420%	3,090	2.076%	-215
Bacillariophyta	*Cyclotella*	269	1.470%	321	1.667%	214	1.262%	-107
Chrysophyta	*Erkenia*	287	2.054%	294	1.562%	280	2.573%	-14
Cyanophyta	*Synechocystis*	612	2.942%	355	0.762%	780	5.247%	425
Cyanophyta	*Aphanocapsa*	236	0.667%	286	0.739%	154	0.591%	-132
Chrysophyta	*Dinobryon*	193	0.621%	232	0.718%	149	0.518%	-84
Cyanophyta	*Aphanothece*	220	0.561%	202	0.612%	259	0.508%	57
Cryptophyta	*Rhodomonas*	81	0.613%	74	0.423%	88	0.815%	14
Cyanophyta	*Rhabdoderma*	714	0.536%	211	0.205%	1,432	0.887%	1,220
Chlorophyta	*Stichococcus*	111	0.125%	167	0.164%	50	0.085%	-118
Chlorophyta	*Oocystis*	21	0.082%	25	0.125%	13	0.038%	-13
Miscellaneous	*Unidentified (Misc)*	63	0.066%	90	0.102%	14	0.028%	-75
Cyanophyta	*Merismopedia*	48	0.113%	41	0.084%	65	0.144%	24
Bacillariophyta	*Anomoeneis*	57	0.033%	57	0.064%	0	0.000%	-57
Cryptophyta	*Cryptomonas*	8	0.064%	10	0.061%	7	0.066%	-3
Chlorophyta	*Planctonema*	24	0.040%	30	0.060%	15	0.019%	-16

Water quality samples were analyzed at Boulder Basin and there were significant differences noted between the two time periods. Specific conductance and total dissolved solids were significantly reduced in post quagga time period (Table 22). Total phosphate, nitrate, ammonia, and total inorganic nitrogen were significantly increased in post-quagga time frame.

Table 22 Water quality samples at Boulder Basin

Boulder Basin		Wilcoxon Signed Rank Test				
Category	units	Pre mean	Post mean	% change	Z	p (2 tailed)
Temperature	°C	19.9	19.5	-2%	-1.286	0.199
Dissolved Oxygen mg/L	mg/L	8.6	8.8	3%	-1.933	0.053
Dissolved Oxygen % Sat	% Sat	102.6	101.2	-1%	-1.097	0.272
pH	s.u.	8.3	8.3	0%	-0.860	0.390
Specific Conductance	µS/cm	1,071	970	-9%	-4.963	**0.000***
Total Dissolved Solids	g/L	0.685	0.623	-9%	-4.964	**0.000***
Ortho-phosphate	µg/L	0.684	1.203	76%	-1.913	0.056
Total Phosphate	µg/L	3.234	7.283	125%	-3.559	**0.000***
Nitrate	mg/L	0.522	0.693	33%	-3.904	**0.000***
Ammonia	mg/L	0.004	0.024	500%	-4.254	**0.000***
Total Inorganic Nitrogen	mg/L	0.527	0.717	36%	-4.226	**0.000***

Asterisk indicates significant difference (P<0.05).

CHAPTER 5 DISCUSSION AND CONCLUSION

Changes in Water Quality Characteristics

Water quality characteristics were evaluated for differences between pre-quagga (2004-2006) and post-quagga (2009-2011) time periods to determine if changes are occurring in Lake Mead similar to other locations with invasive mussels. It was expected that transparency would increase, and chlorophyll-a, total phytoplankton cells, phytoplankton biomass, and total zooplankton would decrease. The results indicate that there have not been lake wide changes to water quality characteristics in Lake Mead similar to other locations. The Boulder Basin is the only location that had significant differences for transparency, chlorophyll-a, and phytoplankton biomass. The results in the Boulder Basin are confounded by waste water facilities optimizing phosphorus removal during the same time period as quagga mussel establishment. There is a strong correlation to transparency and phosphorus concentrations in the Boulder Basin, and Labounty (2008) reported a decreasing average transparency in the outer basin of 3 meters from 1994 to 2007 as phosphorus concentrations were reduced. It is interesting to note that orthophosphate concentrations at the Boulder Basin sampling location were higher during the post-quagga time period (2009-2011) than they were in the pre-quagga time period (2004-2006), but the higher concentrations did not result in an increase to chlorophyll and phytoplankton. With higher phosphorus concentrations in the Boulder Basin there were still significant decreases in chlorophyll-a and phytoplankton biomass and an increase in transparency measurements between the two time periods.

The Virgin Basin is the only station to have a significant difference in phytoplankton cell numbers between the two time periods. Although Temple Basin and Boulder Basin did not have

significant differences, all three locations had average cell concentrations decreased 23 to 26% and phytoplankton biomass decreased 17 to 68% between the two time periods. It is not possible to determine if the reduction in phytoplankton cell count and biomass are attributed to quagga mussels, but it is an expected result after a mussel invasion (Strayer, 1999). Locations like the Saginaw Bay and Lake Erie have observed drastic ecosystem changes in phytoplankton numbers and composition within a few years of mussel invasion, but the size and volume of water in Lake Mead may take a mussel population longer to impose ecosystem changes.

The lake wide results indicate that quagga mussels have not had dramatic ecosystem impacts in Lake Mead as they have in other locations during the time periods analyzed. Lake Mead has some fundamental differences compared to the ecosystems in the Eastern United States that could contribute to having less of an impact. Lake Mead is thermally stratified for a large portion of the year. The stratification limits mixing and creates a barrier that keeps phytoplankton in the upper portion of the water column. Lake Mead is a very deep reservoir with depths of 118 meters in January of 2009 in the Boulder Basin. As mussels colonized the optimal depths other mussels would be required to compete for space or settle in deeper less suitable areas. The thermal stratification could limit the available food sources of quagga mussels located at deeper depths and could prevent portions of the quagga mussel population from consuming phytoplankton at their optimal ingestion rates. Both the Saginaw Bay and the Hudson River estuary are very shallow and are evenly mixed for the majority of the year allowing mussel populations access to the entire water column. It is likely thermal stratification prevents quagga mussels from causing larger impacts to Lake Mead.

Lake Mead is the largest reservoir by volume in the western United States with a capacity of over 26 million acre feet at full capacity ($USBR_b$, 2014). Even at less than half capacity the

volume of water would take much longer for a mussel population to filter than smaller shallow areas. The inner Saginaw Bay mussel population was estimated to filter the inner bay in less than a day (Bridgeman et al., 1995; Fanslow et al., 1995). In the Hudson Bay estuary, the mussel population was estimated to filter the entire water column every 2-3 days (Caraco et al., 1997). By comparison, Wong et al., 2010 estimated that the Lake Mead mussel population in the Boulder Basin would filter the water column every 169 days. Areas with larger volumes have had less dramatic impacts from mussels compared to shallow areas. The deeper waters of the Saginaw Bay did not have significant differences after mussel establishment likely due to the larger volume of water in the outer bay (Fahnenstiel et al., 1995). The large volume of water in Lake Mead may prevent mussels from filtering the water quick enough to cause ecosystem changes.

Another factor that may prevent mussels in Lake Mead from impacting water quality characteristics is the limited food supply, especially in the open deep water locations. In all three basins of Lake Mead there was an average chlorophyll concentration between .87 and 1.36 µg/L. In contrast, the Saginaw Bay had chlorophyll concentrations of 18-22 µg/L before mussel establishment (Fahnenstiel et al., 1995). Quagga growth rates were evaluated on substrates in Lake Mead with results indicating higher growth rates in areas with higher nutritional inputs like the Las Vegas Bay (Wong et al., 2012). Quagga mussel shell sizes studied in the Boulder Basin showed decreases in median shell length and decreasing population densities from March 2008 to March 2009 suggesting competition for food or location may be limiting population sizes (Wittman et al., 2010). Low food availability probably limits the population size in Lake Mead and may prevent water quality characteristics in Lake Mead from being impacted similar to other locations due to lower population densities.

Lake Mead was created through the impoundment of the Colorado River after the construction of Hoover Dam. Water filling tall canyons created deep areas in the reservoir. Deep depths may prevent mussels from colonizing, reproducing, and growing as efficiently as other areas. The deeper depths are colder with the hypolimnion ranging from 12-12.5 ºC, which is above the spawning threshold minimum (Gerstenberger et al., 2011). Veliger's reach high densities when temperatures are between 17-18 ºC, meaning the warmer water found in the metalimnion are significant to veliger development (Gerstenberger et al., 2011). The hypolimnion depths are usually below optimal larval temperature development indicating that settlement of these areas may be from mature veligers from the metalimnion. Monitoring of quagga growth in Lake Mead has indicated significantly greater mussel settlement at depths of 6-28 meters than from depths of 32-54 meters with no difference from substrate type (Mueting et al., 2010). Quagga mussel enumeration in the Boulder Basin and Overton Arm of Lake Mead showed mussels were primarily in the top 40 meters, with smaller populations down to 100 meters (Wittmann et al., 2010). There are no indications of the health of the mussels at lower depths, but the abundance at higher elevations suggests that food availability or temperature is controlling mussel population growth. The temperatures at deeper portions of the lake may prevent development of larva stages that are better at settlement and this could limit quagga population sizes at lower depths.

Changes in Phytoplankton

Phytoplankton at the division and genus taxa were evaluated between pre-quagga (2004-2006) and post-quagga (2009-2011) time periods for differences. The relative frequency of

chlorophyta was expected to be reduced and the relative frequency of cyanophyta was expected to increase, but none of the basins had an expected change in relative frequency. Descriptive phytoplankton analysis revealed the division cyanophyta accounts for about 94% of total phytoplankton at each location. Similar to the Hudson River estuary, Lake Mead had declines in cyanobacteria. It is likely that quagga mussels in Lake Mead are feeding on the most abundant phytoplankton. Some areas such as the Saginaw Bay and Hudson River estuary observed increases in diatoms as a result of mussel's inability to filter the shapes and sizes as efficiently as other phytoplankton. The bacillariophyta division had a significant increase in average number of cells in the Temple and Virgin Basin, but not in the Boulder Basin. The results may indicate that quagga mussels have difficulty filtering and ingesting diatoms in Lake Mead similar to other locations. Phytoplankton diversity was expected to be reduced after quagga establishment, but the diversity did not have a significant difference in any of the basins indicating that the changes to phytoplankton have not impacted the richness or evenness of the phytoplankton community.

The reduction in cyanophyta division accounted for the largest change in total phytoplankton. Unidentified *Chroococcaceae* (<1 µm in size) and *Synechococcus* accounted for 86 to 87% of phytoplankton cells counted in total phytoplankton and were the largest contributors to decreasing phytoplankton in Lake Mead. Feeding experiments by Winkel et al. (1982) determined that the optimal particle size is 15-40 µm, but Sprung et al. (1988) observed zebra mussels feeding on particles as small as .7 µm. The results observed in Lake Mead indicate that mussels may be consuming and ingesting the most abundant particles which are < 5 µm in diameter, causing a reduction in the two most abundant types of cyanophyta.

Changes in Toxin Producing Cyanobacteria

During the sampling dates there was not enough data collected to perform Wilcoxon Rank Sum Tests on toxin producing genera. All three locations had less than seven detections during the six years of the study. The number of toxin producing cells averaged below 11 cells/ml, and no algal toxins were detected during the sampling. The results indicate that there have not been large increases in toxin producing algae in the three basins similar to other locations after mussel establishment. Lake Mead phytoplankton is dominated by cyanophyta species, but toxin producing species are rare. There is conflicting literature regarding *Microcystis* as a preferred food source for mussels. Baker et al. (1988) indicated that *Microcystis* was preferentially ingested over control suspensions while Vanderploeg et al. (2001) observed *Microcystis* being selectively rejected as pseudofeces. With the limited amount of *Microcystis* data collected, it is difficult to determine which is more plausible. If *Microcystis* was being selectively rejected, detections should become more frequent and this was not the case at any of the sampling locations. This study chose to use deep sampling locations that would be more representative of Lake Mead than shallow stations that have changed considerably from elevation decline during the drought. Accumulations of *Microcystis* are commonly found near shorelines after wind blows buoyant cells into clumps. The National Park Service had a swimming advisory for Lake Mead and Lake Havasu on March, 13 2015, warning visitors to not swim in or drink waters that appear to have surface scum growth with yellow or green streaks (National Park Service, 2015). Although this study did not quantify an increase in toxin producing algae, if selective rejection of *Microcystis* is occurring, the most likely location would be near shorelines, which the sampling done in this study would not have captured. With blooms

of *Microcystis* in Lake Havasu and Lake Mead becoming more frequent, it is important to monitor shorelines for potential blooms to prevent public health concerns and to continue monitoring phytoplankton for long term impacts from quagga mussels.

Discussion of Zooplankton Results

Contrary to expected results, zooplankton increased significantly during the study at every location. Additional analysis indicated that cladocerans and copepods contributed most to the increase in total zooplankton. The increase in zooplankton indicates that Lake Mead zooplankton populations have not been negatively impacted from the introduction of quagga mussels at these three deep water locations. Veligers were first identified in 2007 and continued to increase in post samples. At each sampling location veligers were identified year round confirming previous results by Gerstenberger, Mueting, &Wong (2011) that quagga mussels reproduce year round in Lake Mead. Beaver et al. (2010) reported that Lake Mead zooplankton Daphnia has persisted following quagga mussel introductions due to the influence of nutrients from the Las Vegas Wash. It is likely that the large volume of water in Lake Mead, thermal stratification, and deep depths have prevented quagga mussels from decreasing zooplankton similar to other areas. Zooplankton should continue to be sampled so that trends can be used to evaluate the long term impacts of mussels in Lake Mead.

Confounding Factors and Limitations

Lake Mead has several possible confounding factors during this study. One of the most noticeable changes during the research is the decline in reservoir elevations. The reduction in reservoir volume has made the Colorado River inflow more prominent causing decreases in salinity and total dissolved solids during the sampling. These water quality changes could favor species that prefer the new conditions.

A confounding factor in the Boulder Basin of Lake Mead is the reduction of phosphorus loading from the waste water treatment facilities. The waste water treatment facilities have improved technologies and optimized operations which have reduced the amount of phosphorus entering the Boulder Basin. Since 1994, the reduction in phosphorus has decreased phytoplankton and increased Secchi depth measurements in the Boulder Basin (LaBounty, 2008). The phosphorus reductions in the Boulder Basin and the introduction of mussels make it difficult to determine the individual impacts of each. After 2005, phosphorus loading increased and in 2011 the City of North Las Vegas started discharging effluent which increased phosphorus concentrations in the Boulder Basin. Water quality samples of phosphorus were higher in the Boulder Basin in post-quagga than pre-quagga time frames. The other sampling locations in the Virgin and Temple Basin are not impacted by the Las Vegas Wash because of their location.

Quagga mussels were originally discovered in 2007, but were likely introduced in 2004 based on early population structure (McMahon, 2007). In 2007, National Park Service divers detected some mussels under rocks and within crevices (below 15 meters) of the Boulder Basin, but mussels were not detected in the other basins (Brian Moore, personal communications). The

mussel population in 2007 had low densities in the Boulder Basin and had not colonized the shallow areas. The 2004 to 2006 time frame used in this study assumes that mussels in the Boulder Basin had not reached a sufficiently large population to modify water quality characteristics. By 2009, large populations in the Boulder Basin were observed, and mussels were established in the other basins (Wittmann et al., 2010). Limited mussel density data were available during these time frames making it difficult to estimate population sizes in each basin.

Conclusions and Recommendations for Further Study

Samples were evaluated before and after mussel establishment in Lake Mead to determine if there have been changes similar to other locations with established mussel populations. The results indicate that Lake Mead is not changing similar to other ecosystems at these three locations during the time frames sampled. Transparency, chlorophyll-a, and phytoplankton biomass were significantly different in the Boulder Basin between the two time periods, but these changes are confounded by improvements in waste water treatment operations that decreased phosphorus loading to the Boulder Basin. Phytoplankton cell numbers and biomass did not have significant changes lake wide, but there was a 23-26% reduction in cell numbers and a 17-68% reduction in biomass between the two time periods. It is difficult to attribute the changes to quagga mussels, but a reduction in phytoplankton is an expected result. The largest change in phytoplankton between the two time periods was a reduction of the cyanophyta division which accounts for 94 to 96% of all phytoplankton in Lake Mead. Zooplankton totals were not significantly lower indicating that zooplankton were not negatively impacted from quagga mussels at these locations. The relative frequency of chlorophyta, relative frequency of

cyanophyta, and phytoplankton diversity did not have significant lake wide changes. Analysis of toxin producing taxa like *Microcystis, Oscillatoria, Cylindrospermopsis* and *Anabaena* indicated that there have not been significant changes in the number of detections or cell counts of toxin producing cyanobacteria during the two time periods.

Lake Mead has not observed significant changes similar to other ecosystems with established mussel populations. Some fundamental differences at Lake Mead may prevent quagga mussels from drastically altering the ecosystem. Lake Mead has a large volume of water that takes longer (169 days) for the mussel population to filter compared to other ecosystems like the Saginaw Bay (< 1 day) or the Hudson River Estuary (2-3 days) (Bridgeman et al., 1995) (Fanslow et al., 1995; Wong et al., 2010). Lake Mead is thermally stratified for most of the year preventing mixing and causing a barrier for available food. Mussels located below the stratification may be limited on food availability causing more mussels to be concentrated in depths above 40 meters (Wittmann et al., 2010). Lake Mead hypolimnion temperatures are above spawning temperatures, but may not be adequate for proper veliger development. Veligers at these depths may have limited settling capabilities, and lower depths may be colonized from veligers from higher depths. Lake Mead has a relatively low food supply with chlorophyll concentrations averaging < 1.36 µg/L in open waters, which may be a limiting factor in sustaining a large population.

Although this study did not observe drastic changes in Lake Mead after quagga mussel establishment, it is important to continue collecting phytoplankton data since it is the food source of quagga mussels. The sampling data used in this study are relatively recent after invasion, and additional data are required to understand the long term impacts of quagga mussels in Lake Mead. Population estimates of quagga mussels in each of the basins would greatly improve the

understanding of quagga establishment and population growth in Lake Mead. With the increase sighting of *Microcystis* in Lake Mead and Lake Mojave it is important to continue monitoring for toxin producing cyanobacteria near shorelines that swimmers and boaters may come in contact with. With the limited amount of toxin producing cyanobacteria data collected in this study it is not possible to determine if quagga mussels are able to selectively reject *Microcystis*, but long term sampling needs to be conducted to understand if a public health concern could develop.

APPENDIX A. LITERATURE ON DREISSENID INVASIONS

Characteristic	Location	Impact	Reference
Phytoplankton	Hudson River	85% decline in phytoplankton biomass, and chlorophyll reduced from average of 30 mg/m3 to <5 mg/m3 after invasion.	Caraco et al., 1997
Phytoplankton	Lake Michigan	Loss of spring phytoplankton bloom.	Fahnenstiel et al., 2010
Phytoplankton	Hatchery Bay, Lake Erie	Transparency 100% higher in post zebra mussel samples, and 86-92% lower planktonic diatoms.	Holland, 1993
Phytoplankton	Seneca River	16 fold reduction in chlorophyll concentrations and 2.5 fold increase in secchi disk transparency since invasion.	Effler, 2004
Phytoplankton	Saginaw Bay, Lake Huron	Colonies of blue-green algae reduced by 50%. Mussels reduced protozoa numbers 70-80% and reduced species richness by 30-50%.	Lavrentyev et al., 1995
Phytoplankton, cyanobacteria and Microcystis	Saginaw Bay, Lake Huron	Change in phytoplankton composition from cyanobacteria to diatoms. Microcystis dominated assemblages after invasion.	Fishman, Alderstein, Vanderploeg, Fahnenstiel, & Scavia, 2010
Phytoplankton	Enclosure experiment	Change in the concentrations of suspended particles, chlorophyll and algal biomass in laboratory.	Heath, Fahnenstiel, Gardner, Cavaletto, & Hwang, 1995
Phytoplankton	Lake Erken	Zebra mussels expelled chlorophytes and cyanobacteria or anything larger than >50 µm and < 7µm.	Naddafi, Pettersson, & Eklov, 2007
Phytoplankton and cyanobacteria	Hudson River	Decline in Cyanobacteria and increased diatom composition.	Baker et al., 1998

Characteristic	Location	Impact	Reference
Phytoplankton and cyanobacteria	Hudson River, New York	Average phytoplankton cell density decreased 17 fold from pre-invasion. Cyanobacteria decreased after invasion with increase of diatoms. Reduction of seasonal variation in composition after invasion.	Smith, Stevenson, Caraco & Cole, 1998
Phytoplankton biomass	Lake Erie Modeling	Phosphorus reductions decrease algal biomass 55-60%, and filter feeding mussel reduced algal biomass 25-30%.	Boegman et al., 2008
Primary production	Saginaw Bay, Lake Huron	45% reduction in primary production.	Fahnenstiel, Lang, Nalepa, & Johengen, 1995
Cyanobacteria and chlorophyll	Lake Erie	Cyanobacteria biomass increased, and chlorophyll-a concentrations decreased during spring and summer after invasion.	Conroy, Kane, Dolan, & Edwards, 2005
Cyanobacteria	Laboratory experiment	Zebra mussels capable of removing colonial or filamentous cyanobacteria. Toxic and non-toxic strains were both cleared.	Pires, Bontes, Van Donk, & Ibelings, 2005
Cyanobacteria	Lake Erie	Bacillariophyta, total phytoplankton and chlorophyll-a concentrations were reduced after invasion. Spring Cyanobacteria biomass increased.	Makarewicz, Lewis, & Bertram, 1999
Cyanobacteria	Saginaw Bay, Lake Huron	Zebra mussels impact phytoplankton composition through selective rejection	Fishman et al.,, 2010
Cyanobacteria	39 inland lakes, Southern Michigan	Lakes with Zebra mussels had 3.3 times higher microcystin concentration and 3.6 times higher Microcystis biomass than lakes without mussels.	Knoll et al., 2008

Characteristic	Location	Impact	Reference
Chlorophyll	27 Northern temperate lakes	Chlorophyll declined 40-45% after Dreissenid invasions.	Higgins, Vander Zanden, Joppa, & Vadeboncoeur, 2011
Chlorophyll	Lower Green Bay, Lake Michigan	Chlorophyll-a was reduced 33% in zone 1 and 2 and 3 were reduced by 40% post zebra mussel.	Quals et al., 2007
Chlorophyll	25 U.S. Lakes	Dreissenid invasions are frequently associated with chlorophyll declines after invasion.	Cha, Stow, & Bernhardt, 2013
Chlorophyll	Saginaw Bay, Lake Huron	Average chlorophyll reduced 59%, and 60% increase in secchi disk measurements in post invasion data.	Fahnenstiel, Bridgeman, Lang, McCormick, & Nalepa, 1995
Chlorophyll and secchi	Seneca River, New York	Chlorophyll reduction of 47 µg/L to 3.4 µg/L after zebra mussels. Transparency increase from .75 to 2.1 m.	Effler et al., 1996
Chlorophyll-a	Lake St. Clair	63% reduction in chlorophyll-a values after zebra mussel invasion.	Vanderploeg et al., 2002
Water clarity	Laboratory Study	Zebra mussels restored clarity to tank after 6.5 hours after introduction of 1.5% activated sewage sludge.	Mackie, 1991
Zooplankton	Hudson River	Zooplankton biomass declined 50% after initial invasion, but eventually recovered.	Pace, Strayer, Fischer, & Malcom, 2010
Zooplankton	Saginaw Bay, Lake Huron	Zooplankton biomass reduced 40% in inner bay and 70% outer bay.	Bridgeman, Fahnenstiel, Lang, & Nalepa, 1995
Zooplankton	Hudson River	Zooplankton biomass decreased by more than 70% after invasion. Size dependent reductions in ciliates, rotifers and copepods declined.	Pace, Findlay, & Fischer, 1998

Characteristic	Location	Impact	Reference
Zooplankton	Lake Erie	Most zooplankton taxa declined with rotifers declining worst after mussel invasion.	MacIsaac, 1996
Ciliate biovolume	Mesocosm experiment	Reduction of ciliate biovolume by 71%.	Wilson, 2003
Microcystis/ phytoplankton	Microcosm experiments	Zebra mussels reduced green algae and increased abundance of Microcystis relative to tanks without mussels.	Bykova, Laursen, Bostan, Bautista, & McCarthy, 2006
Microcystis	Saginaw Bay, Lake Huron	Microcystis not abundant prior to invasion, but became abundant 3-5 summers after establishment.	Vanderploeg et al., 2001
Microcystis	Bay of Quinte, Lake Ontario	13 fold increase of Microcystis biomass after establishment of Zebra mussel.	Nicholls, Heintsch, & Carney, 2002
Various	Meta analysis of 200 published articles and databases	35-78% reduction in phytoplankton biomass, and 40-77% zooplankton biomass reductions depending on habitat.	Higgins & Zanden, 2010
Various	Lough Doon, Ireland	48% lower concentration total suspended solids, 41% lower chlorophyll, 33% lower phytoplankton biomass and 70% higher secchi transparency measurements.	Higgins et al., 2008

APPENDIX B. STATE GUIDANCE FOR RECREATIONAL WATER

(Graham et al., 2009)

State	Recreational Water Guidance/Action Level	Recommended Action
California	Microcystin: 0.8 µg/L Anatoxin-a: 90 µg/L Cylindrospermopsin: 4 µg/L	Advisory if value met or exceeded.
Connecticut	-Visual Rank Category 1: Visible Material is not likely cyanobacteria or water is generally clear.	No Action
	-Visual Rank Category 2: Cyanobacteria present in low numbers. There are visible small accumulations but water is generally clear.	No Action
	-Visual Rank Category 3: Cyanobacteria present in high numbers. Scums may or may not be present. Water is discolored throughout. Large areas affected. Color assists to rule out sediment and other algae.	-Visual Rank Category 3, or blue-green algae cells > 100,000/ml: POSTED BEACH CLOSURE (If public has beach access, alert water users that a blue-green algae bloom is present), POSTED ADVISORY (At other impacted access points)
Illinois	Microcystin-LR concentration results approach or exceed 10 µg/L	Reporter of harmful algae bloom event and the local lake management entity will be informed immediately.
Indiana	Level 1: very low/no risk < 4 µg/L microcystin-LR	Level 1: use common sense practices
	Level 2: low to moderate risk 4 to 20 µg/L microcystin-LR	Level 2: reduce recreational contact with water
	Level 3: serious risk > 20 µg/L microcystin-LR Warning Level: Cylindrospermopsin: 5 ppb	Level 3: consider avoiding contact with water until levels of toxin decrease
Iowa	Microcystin ≥ 20 µg/L	Caution - bloom present no toxin data available
		Warning - when toxin levels exceed 20 µg/L
Kansas	Advisory: >4 µg/L to <20 µg/L for microcystin or > 20,000 cell/mL to <100,000 cell/mL cyanobacteria cell counts	Public Health Advisory (PHA): avoid contact

State	Recreational Water Guidance/Action Level	Recommended Action
	Warning: > 20 µg/L or > 100,000 cell/mL cyanobacterial cell counts and visible scum present	Public Health Warning (PHW): all contact with water is restricted
Kentucky	Advisory: >20,000 cells/mL of cyanobacteria cell counts	Advisory: contact discourage, water may be unsafe
(Louisville District)	Caution: > 100,000 cells/mL of cyanobacteria cell counts	Caution: Closure, contact prohibited
Massachusetts	14 µg/L for microcystin-LR and ≥ 70,000 cells/mL for cyanobacteria cell counts	Advisory - Avoid contact with water
Nebraska	Microcystin ≥ 20 µg/L	Health Alert
New Hampshire	>50% of cell counts from toxigenic cyanobacteria	Public Health Advisory
North Carolina	Visible discoloration of the water or a surface scum may be considered for microcystin testing	Advisory/Closure
Ohio	Microcystin-LR: PHA: 6 µg/L; NCA: 20 µg/L	Values for Public Health Advisosory and no contact advisory listed for each toxin in left column. Public Health Advisory (PHA) - swimming and wading are not recommended, water should not be swallowed and surface scum should be avoided. No Contact Advisory (NCA) -recommend the public avoid all contact with the water
	Anatoxin-a: PHA: 80 µg/L; NCA: 300 µg/L	
	Saxitoxin: PHA: 0.8 µg/L; NCA: 3 µg/L	
	Cylindrospermopsin: PHA: 5 µg/L; NCA: 20 µg/L	
Oklahoma	100,000 cells/mL of cyanobacteria cell counts and > 20µg/L for microcystin	Blue-Green Algae Awareness Level Advisory
Oregon	Option 1: Visible scum and cell count or toxicity	Public Health Advisory if Option 1-4 met
	Option 2: Toxigenic species >100,000 cells/mL	
	Option 3: Microcystis or Planktothrix > 40,000 cells/mL	
	Option 4: Toxin Testing Microcystin: 10µg/L Anatoxin-a: 20 µg/L Cylindrospermopsin: 6µg/L Saxitoxin: 100 µg/L	

State	Recreational Water Guidance/Action Level	Recommended Action
Rhode Island	Visible cyanobacteria scum or mat and/or cyanobacteria cell count > 70,000 cells/mL and/or ≥14 µg/L of microcystin-LR	Health Advisories
Texas	>100,000 cell/mL of cyanobacteria cell counts and >20µg/L microcystin	Blue-Green Algae Awareness Level Advisory
Vermont	4,000 cells/mL cyanobacteria cell counts or ≥ 6µg/L microcystin-LR and the visible presence of cyanobacterial scum	Beach Closure if either level exceeded
	Anatoxin-a ≥ 10 µg/L	
Virginia	5,000 to <20,000 Microcystis cells/mL	Local agency notification; initiate bi-weekly water sampling
	20,000 to 100,000 Microcystis cells/mL	Public notification indicating a harmful algal bloom is present in recreational water; initiate weekly sampling
	> 100,000 Microcystis cells /mL, or > 6 µg/L microcystin concentration, or Blue-green algal "scum" or "mats" on water surface	Immediate public notification to avoid all recreational water contact where bloom is present; continue weekly sampling
Washington	Microcystin-LR: 6 µg/L	Tier 1. Caution: when a bloom is forming or bloom scum is visible (toxic algae may be present)
	Anatoxin-a: 1 µg/L	Tier 2. Warning: Toxic algae present
	Cylindrospermopsin: 4.5 µg/L	Tier 3. Danger: Lake closed if criteria exceeded
	Saxitoxin: 75 µg/L	
Wisconsin	> 100,000 cells/mL or scum layer	Advisory/Closure

APPENDIX C. PHYCOTECH IDENTIFICATION METHODOLOGY

http://www.phycotech.com/technical.html
Retrieved 12-20-2014

PhycoTech, Inc.
620 Broad Street, Suite 100
St. Joseph, MI 49085, USA
Tel: 1-269-983-3654
Fax: 1-866-728-5579 / 1-269-983-3653
Email: info@phycotech.com

Taxonomic Accuracy
Dr. Ann St. Amand, a senior level phycologist and taxonomic expert, will perform all phytoplankton and periphyton identifications and biovolume measurements. Dr. St. Amand has published extensively in the area of algal ecology and has processed over 23,000 algal and bacterial samples and is qualified to analyze zooplankton and macroinvertebrates. Outside taxonomists will be utilized for taxonomic verifications when necessary.
All samples are initially test mounted for counting density before final mounting. Any major questionable IDs are noted in the database during counting, and indicated on the report as uncertain for taxonomic clarity. If enough sample is present, samples are sent out to other taxonomists for taxonomic confirmation. Distribution is checked on approximately every tenth sample, during the counting process. All biovolume calculations have been verified by comparing with current literature, and by comparing calculations using outside mathematical consultations.

Sample Custody
The chain-of-custody requirements for all laboratory operations for each sample (broadly interpreted to include procedures for the preparation of reagents or supplies which become an integral part of the sample, record keeping associated with sample acquisition, documentation of sample preservation, sample labeling, sample tracking to establish chain-of-custody, and shipping and packing) and laboratory analysis (i.e., laboratory coding, storage, check-out, and documentation of sample movement) will be fully documented in our data management software. Each sample received will be assigned an individual tracking number. The sample bottle, chain-of-custody, and sample log sheet which accompanies each sample sent are then used in conjunction with one another, to enter the samples individual tracking number and all available sample information, into our sample database, ASA System. The database allows for quick and accurate tracking of each sample received by PhycoTech. Dated and initialed entries by appropriate personnel on all worksheets and in the log database are required for data validation. All information entered into ASA System is fully QA/QC'd for content and accuracy. Sample receipt is confirmed with each customer.

Microscope:
There are two microscopes used to process algal samples: an Olympus BHT, research-grade compound microscope equipped with Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (400x), epifluorescence (blue, green and UV Excitation), and a trinocular head for

photography, with a Ricoh Camera Back attached using traditional slide and print film and 2) an Olympus BX60, research-grade compound microscope equipped with Nomarski optics (40x, 100x, 200x, 400x, and 1000x), Phase Optics (400x, 1000x), a 1.25-2X multiplier, epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Microfire digital camera attached. For larger material PhycoTech also has a dissecting microscope. The BX60 is the primary microscope used for algal and zooplankton identification.

Data Entry:
Samples are enumerated within ASA System directly. ASA System is a database driven program with an integrated virtual TallyMeter module. Up to 400 taxa can be enumerated within any one sample, and the entire database currently contains almost 33,000 taxa. All calculations are completed within ASA System, including concentrations, biovolumes and diversity indices. Data files are also generated by ASA System and saved in Excel format, while reports are formatted and saved to pdf format utilizing Microsoft Access, including summary graphics on a per sample basis. PhycoTech can then format data files in any format required by the customer. QA/QC on counting is a recount done on approximately every 10th sample. ASA System produces a QA/QC report comparing the original sample and the recount sample (quantitatively and qualitatively), including the distribution check. Samples pass that are within 10% of the QA/QC recount, quantitatively. Percent similarity may vary up to 20% on exceptionally diverse or sparse samples.

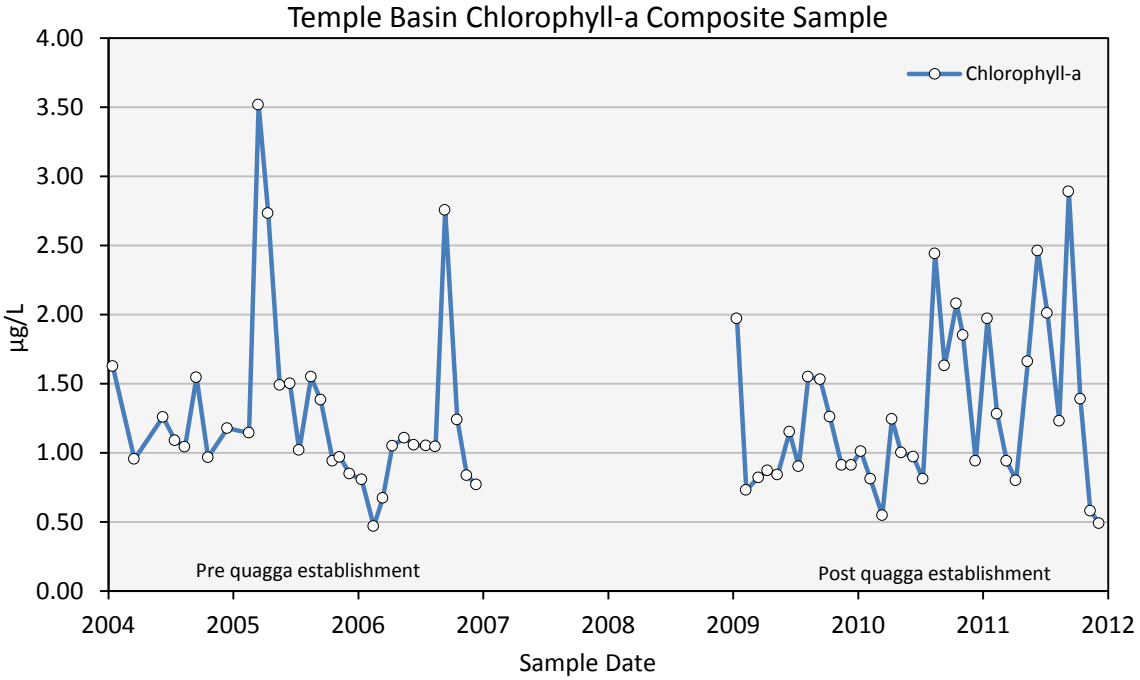
Identification:
The magnification used will depend on the size of the dominant taxa and the size and number of particulates. The goal is to count at multiple magnifications in order to correctly enumerate and identify taxa present that may vary by several orders of magnitude in size. If the sample is dominated by cells below 10-20 µm or the cells are fragile and difficult to identify, the majority of counting will be completed at 400x-1000x. Measuring for biovolume includes measuring GALD and additional measurements including length, width and depth of different aspects of the colony or cell. ASA System allows up to 28 separate measurements per taxa. Cell and colony shapes are approximated to a geometric figure and or figures and the appropriate calculations made. Currently, ASA System has over 44 different shapes defined. From 10 up to a total of 30 natural units (sometimes higher on exceptionally variable taxa) are measured for each taxa depending on variability and number encountered.

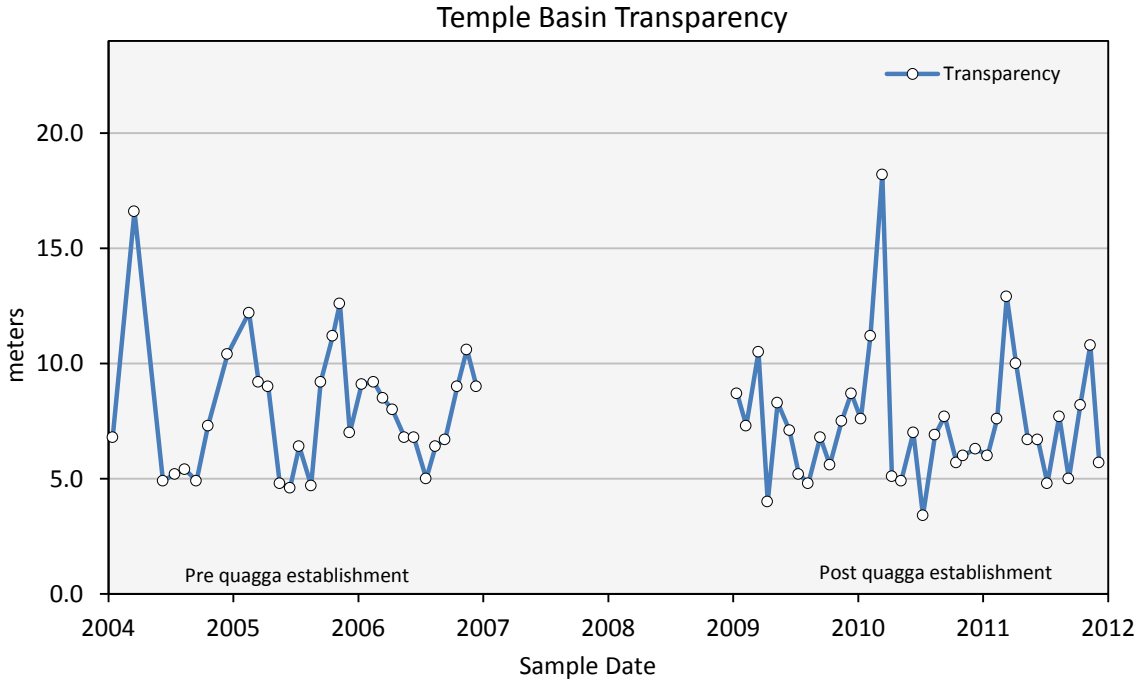
1. Use ONE of the following methods depending on sample composition:
    A. **DOMINATED BY SOFT ALGAE:** If the sample is dominated by soft algae greater than 10-20 µm in GALD, count a minimum of 300 natural units and 15 fields at 200x (when possible, maximum of 100 fields). In addition, count taxa below 10 µm or fragile, difficult to identify taxa at 400x (minimum of 100 natural units and 10 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

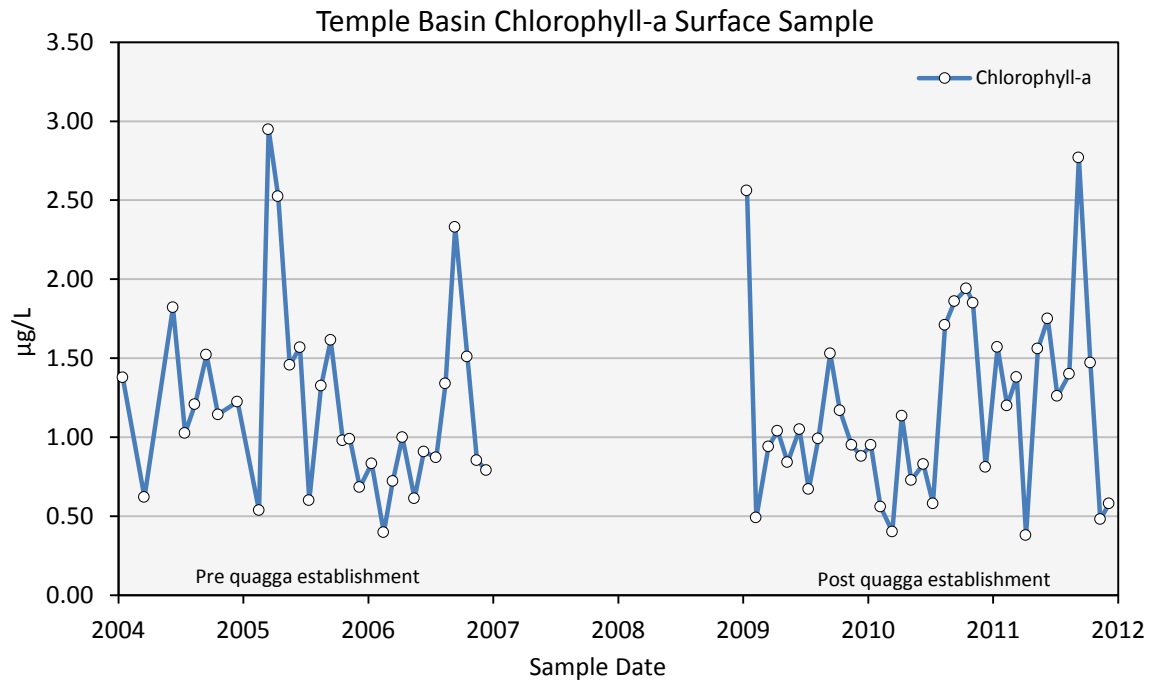
B. **DOMINATED BY SOFT ALGAE:** If the sample is dominated by soft algae less than 10-20 µm in GALD or is dominated by fragile, difficult to identify taxa, count a minimum of 400 natural units and 15 fields at 400x (when possible, maximum of 100 fields). In addition, count taxa above 20-30 µm in GALD at 200x (minimum of 15 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

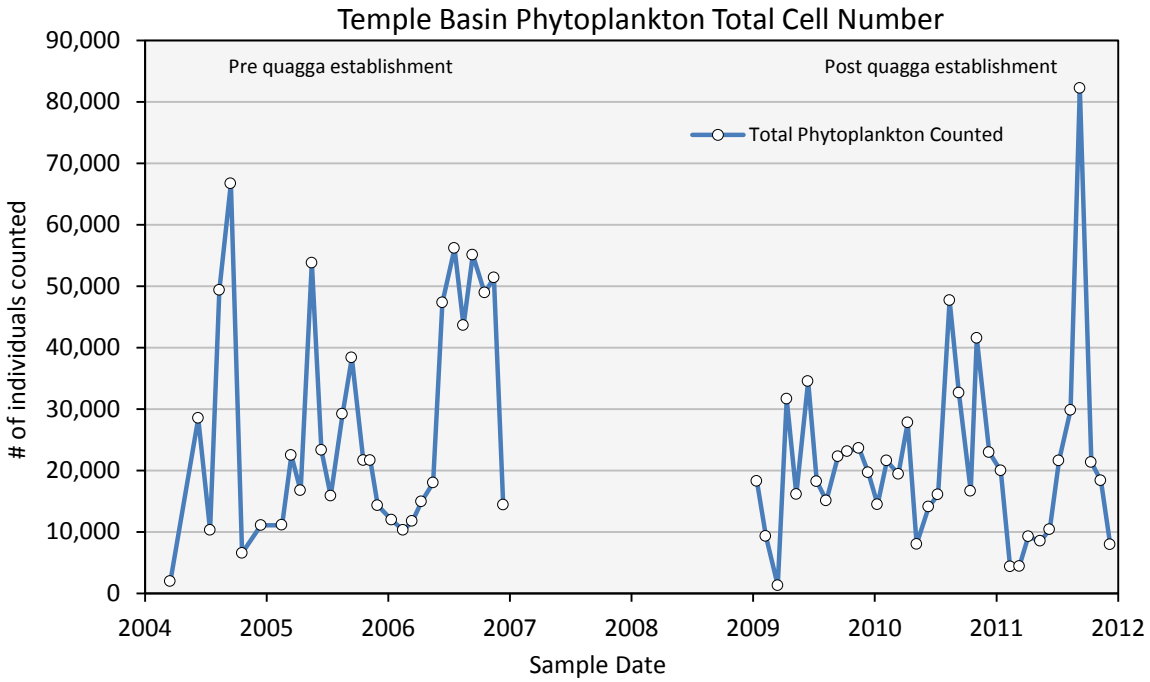
C. **DOMINATED BY DIATOMS:** If the sample is dominated by diatoms other than large, easily identified taxa (e.g. Asterionella), count a minimum of 15 fields at 1000x, and a minimum of 400 natural units total (when possible, maximum of 100 fields). In addition, count soft algae according to size distribution (see A or B above) for a minimum of 15 fields at either 200x or 400x. Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

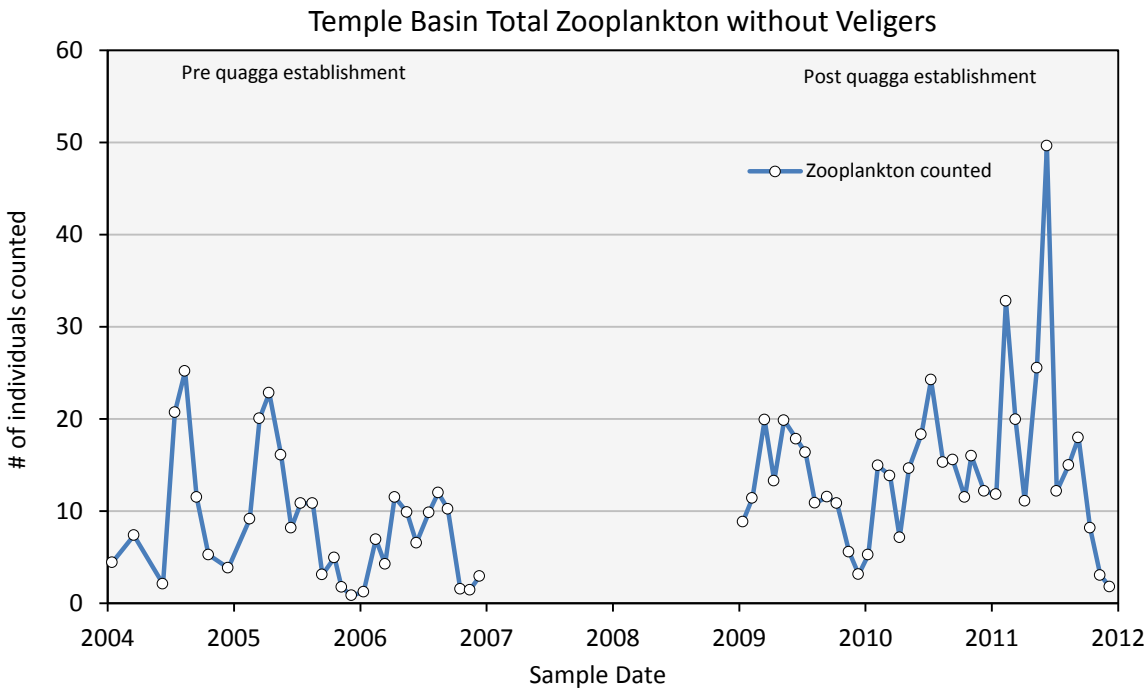
APPENDIX D. TEMPLE BASIN FIGURES

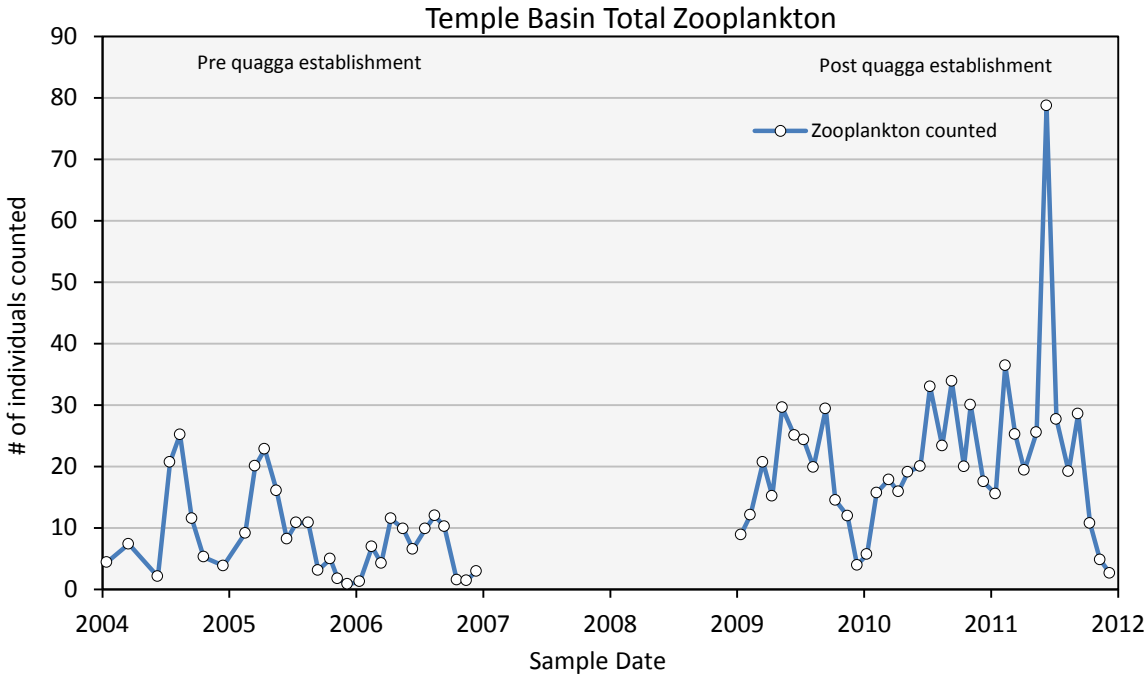


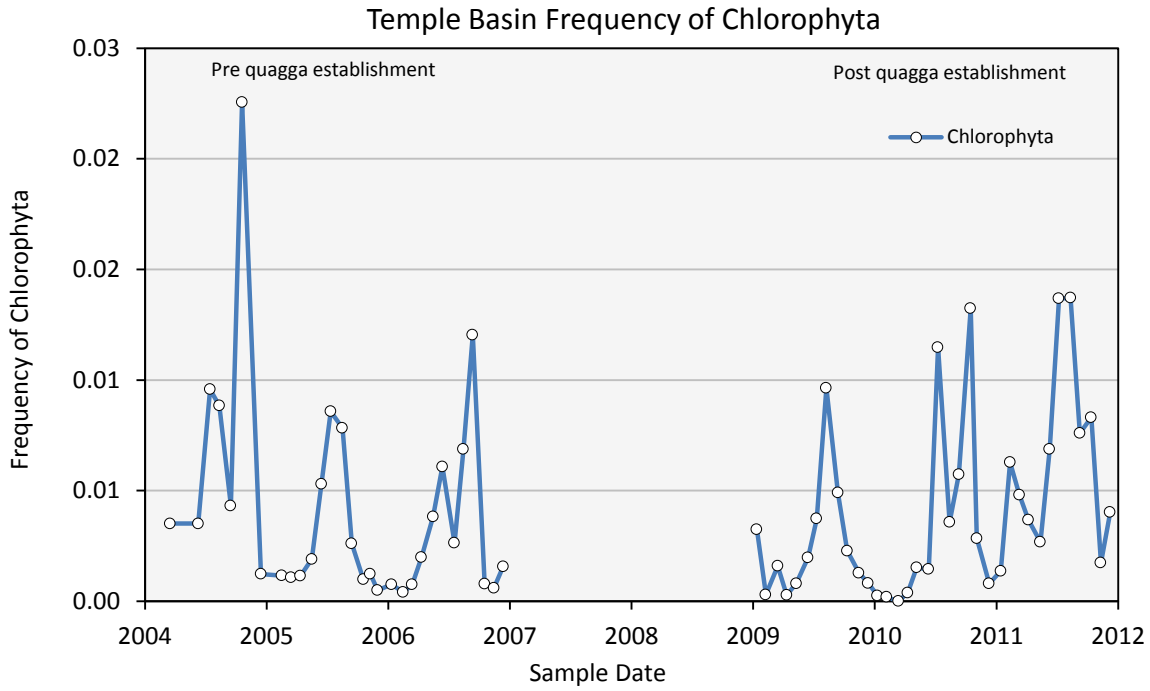


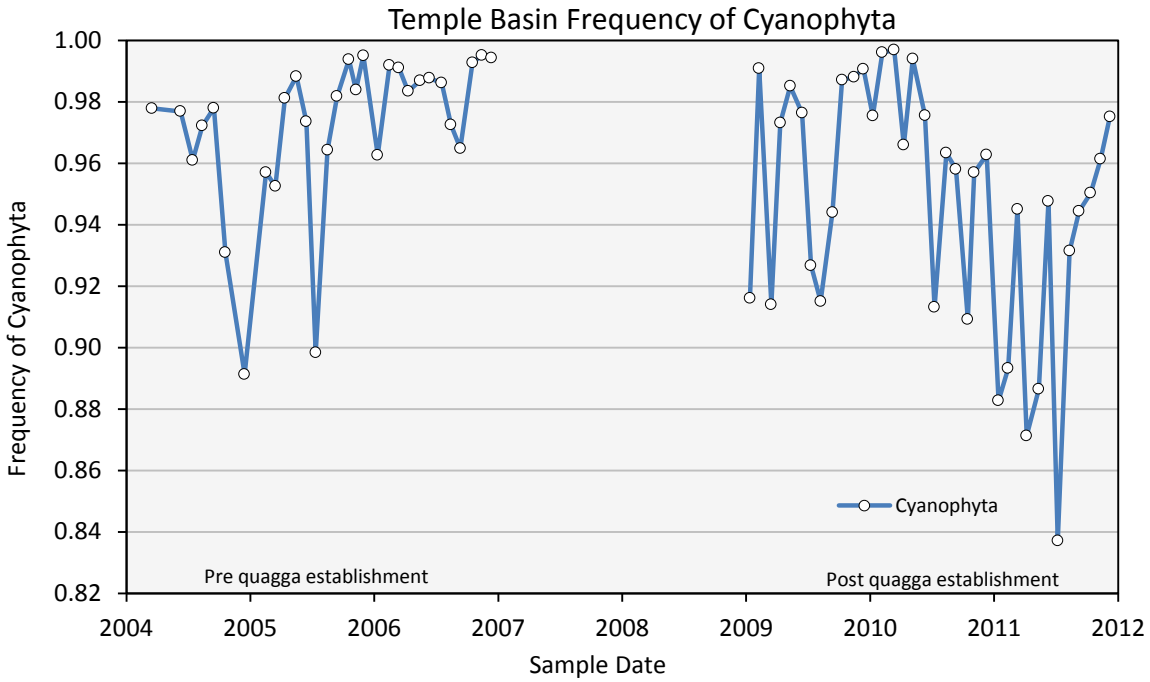

Temple Basin Chlorophyll-a Surface Sample
Chlorophyll-a
µg/L
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pre quagga establishment
Post quagga establishment
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

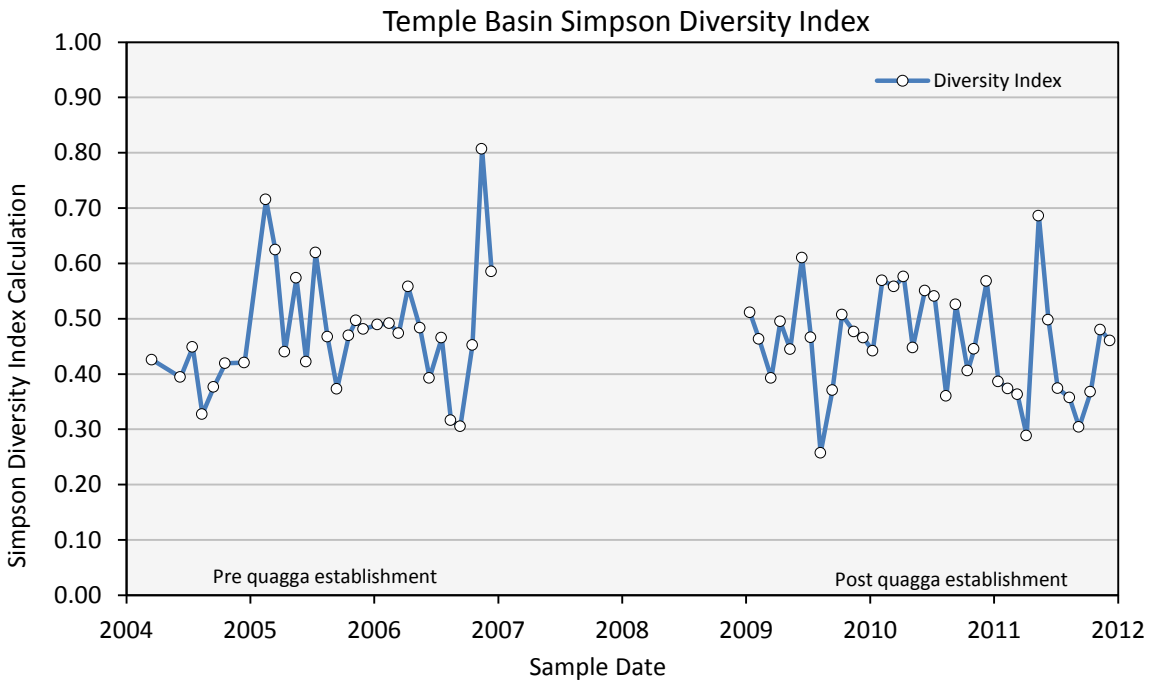

Temple Basin Phytoplankton Total Cell Number
Pre quagga establishment
Post quagga establishment
Total Phytoplankton Counted
# of individuals counted
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

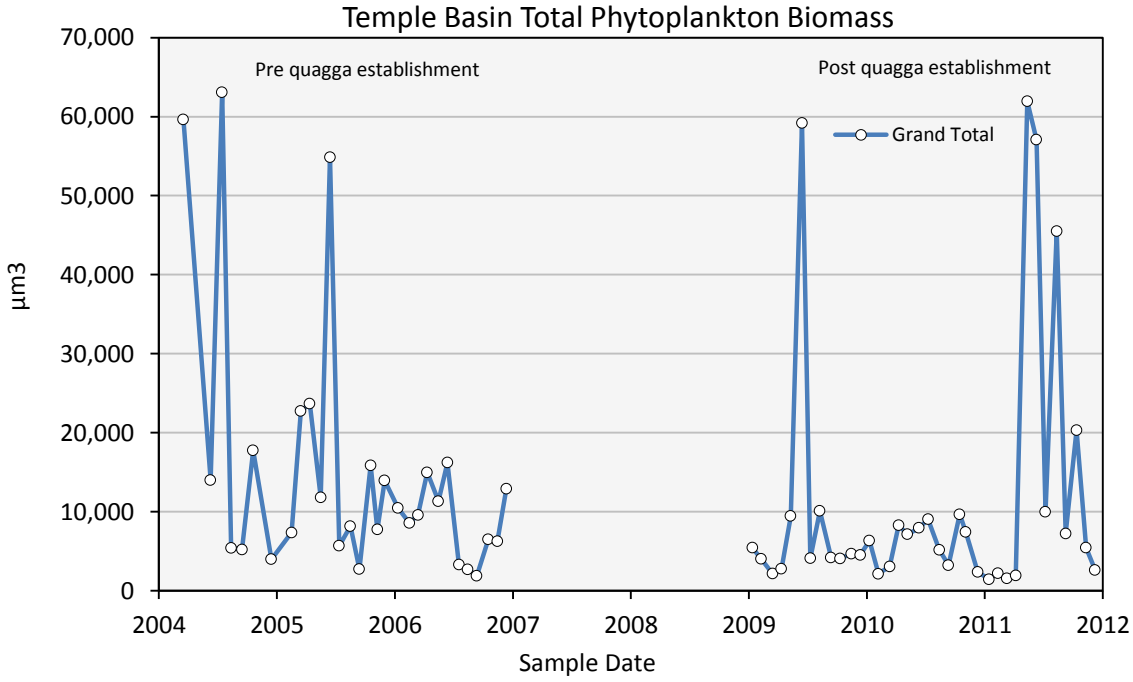

Temple Basin Total Zooplankton without Veligers
Pre quagga establishment
Post quagga establishment
Zooplankton counted
# of individuals counted
Sample Date
0
10
20
30
40
50
60
2004
2005
2006
2007
2008
2009
2010
2011
2012


Temple Basin Total Zooplankton
Pre quagga establishment
Post quagga establishment
Zooplankton counted
# of individuals counted
Sample Date
0
10
20
30
40
50
60
70
80
90
2004
2005
2006
2007
2008
2009
2010
2011
2012

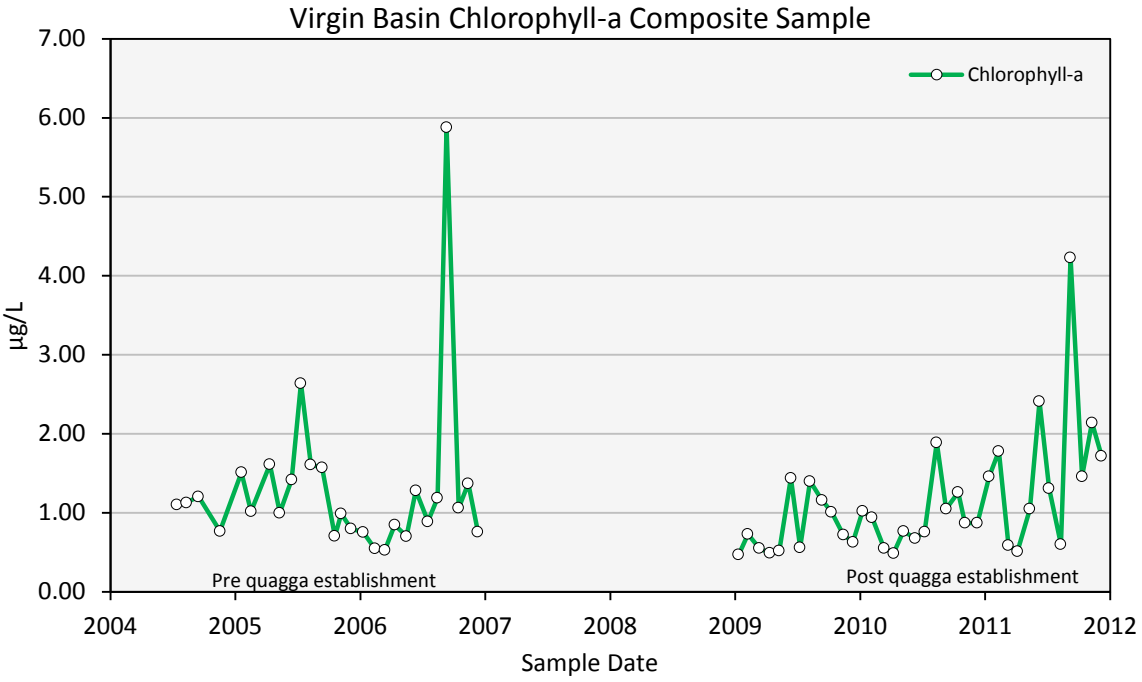

Temple Basin Frequency of Chlorophyta
Pre quagga establishment
Post quagga establishment
Chlorophyta
Frequency of Chlorophyta
0.03
0.02
0.02
0.01
0.01
0.00
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

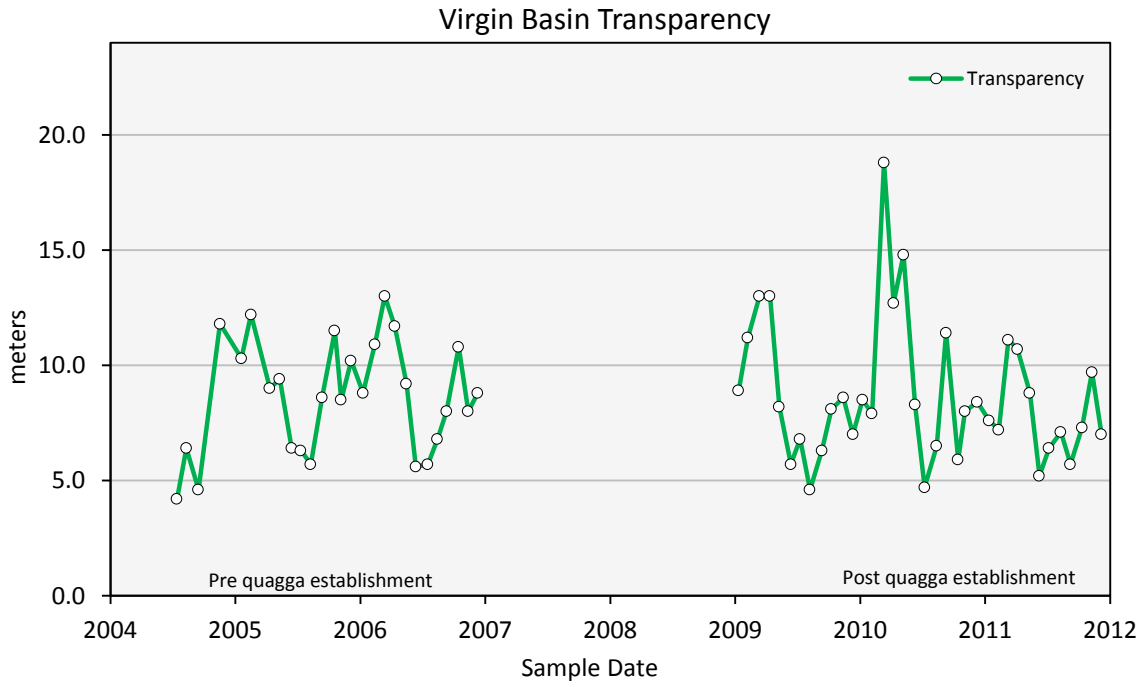

Temple Basin Frequency of Cyanophyta
Frequency of Cyanophyta
1.00
0.98
0.96
0.94
0.92
0.90
0.88
0.86
0.84
0.82
Cyanophyta
Pre quagga establishment
Post quagga establishment
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

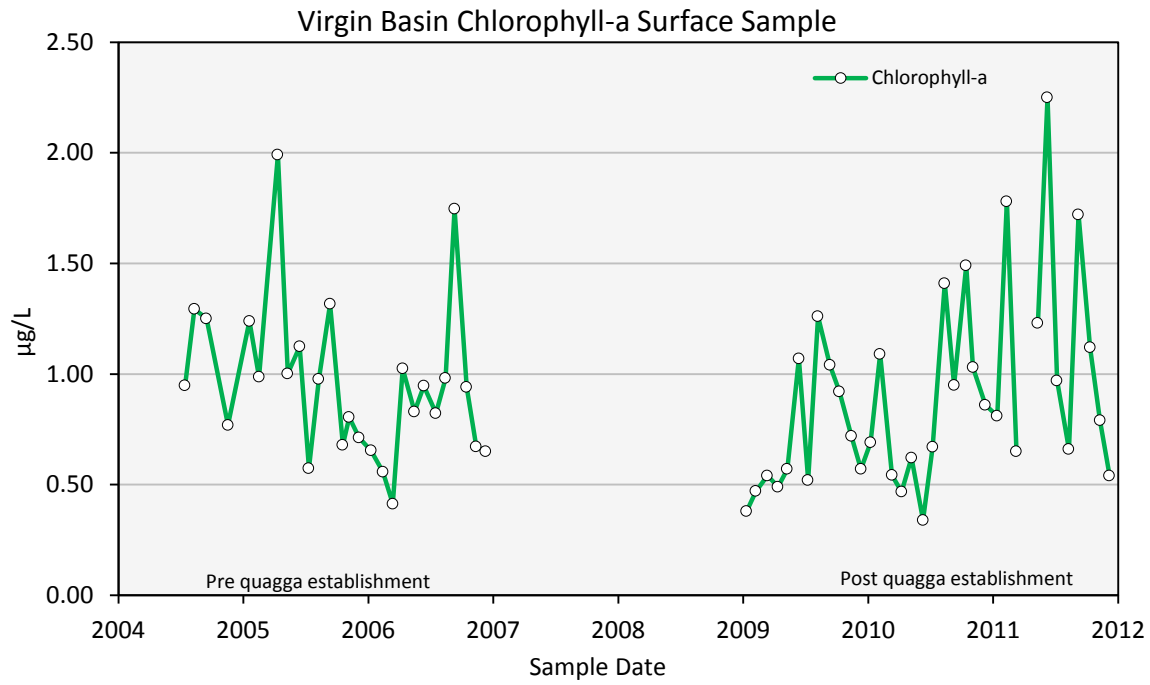

Temple Basin Simpson Diversity Index
Diversity Index
Simpson Diversity Index Calculation
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Pre quagga establishment
Post quagga establishment
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

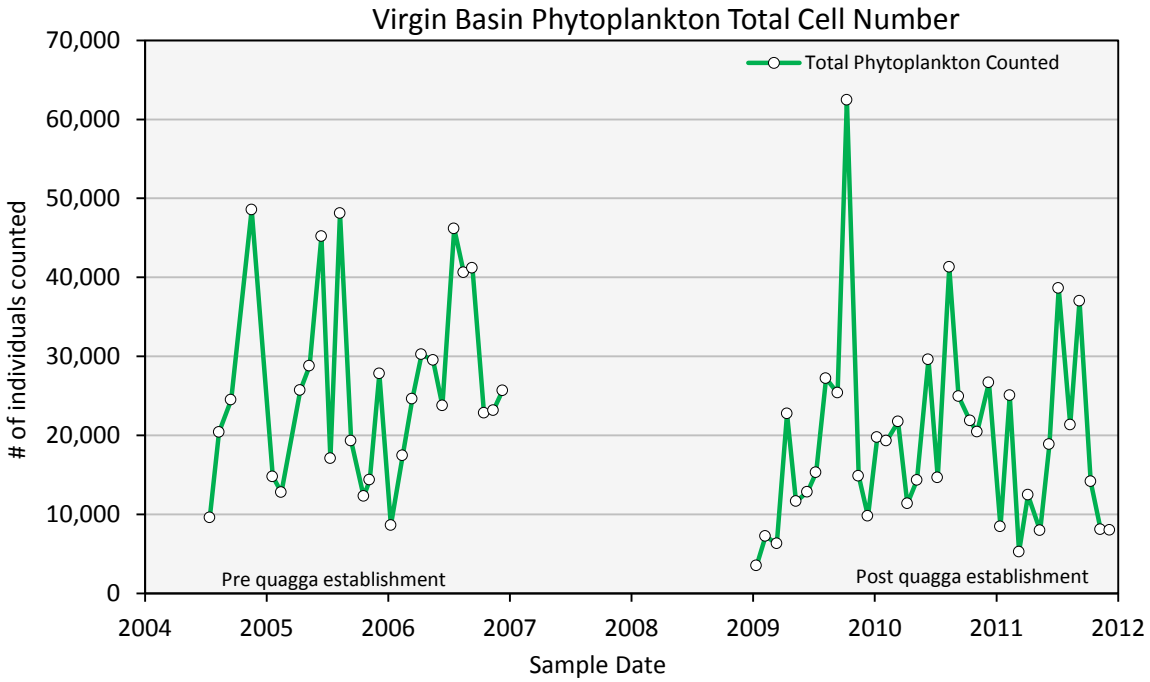

Temple Basin Total Phytoplankton Biomass
Pre quagga establishment
Post quagga establishment
Grand Total
µm3
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

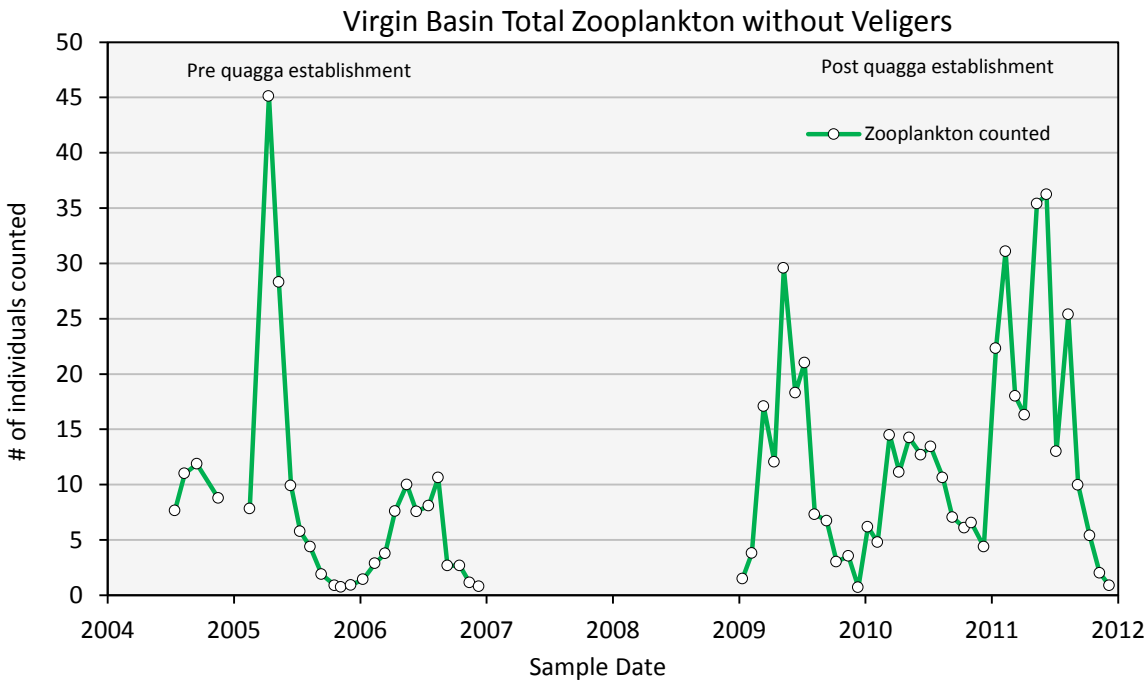
APPENDIX E. VIRGIN BASIN FIGURES

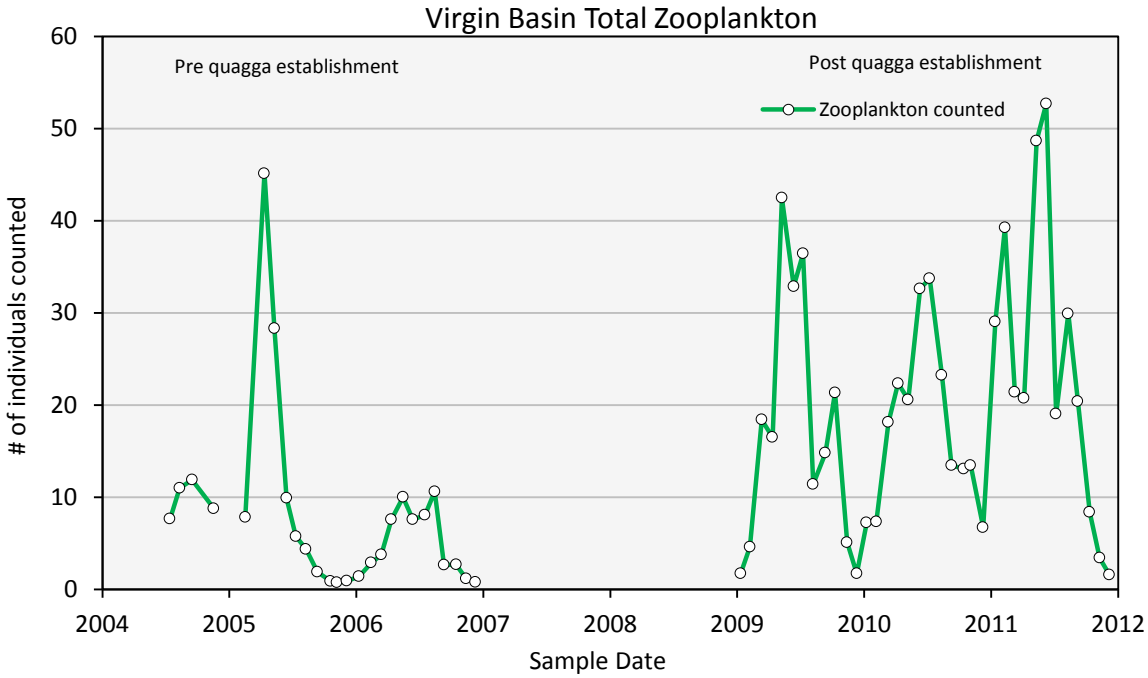


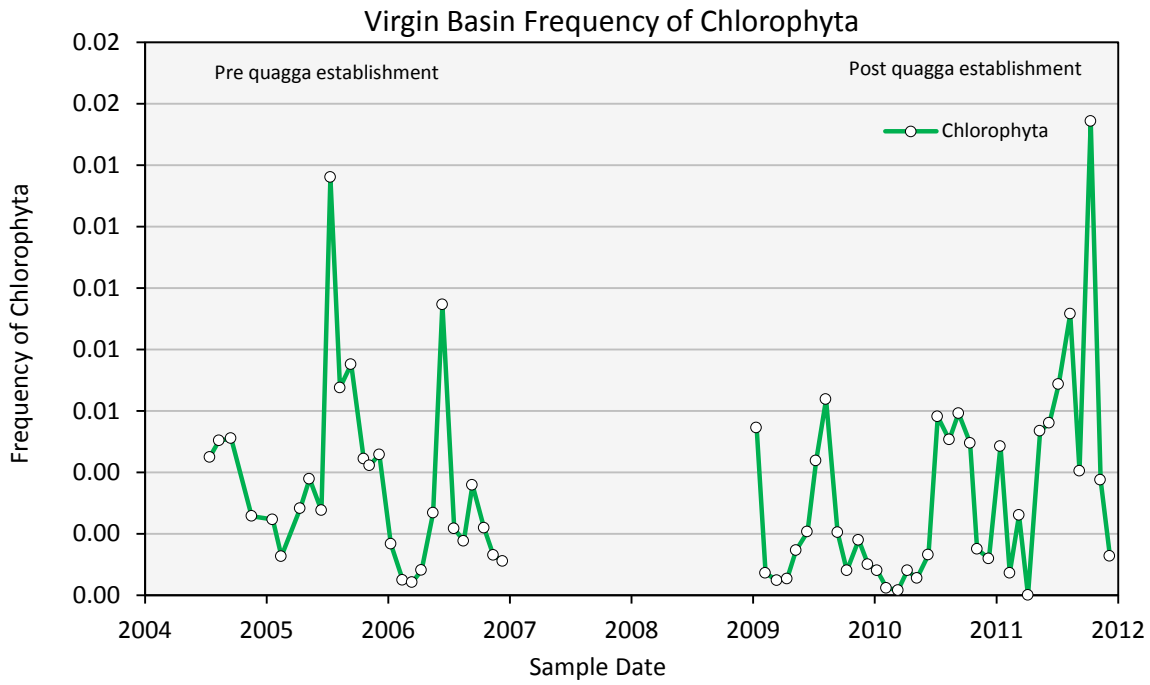


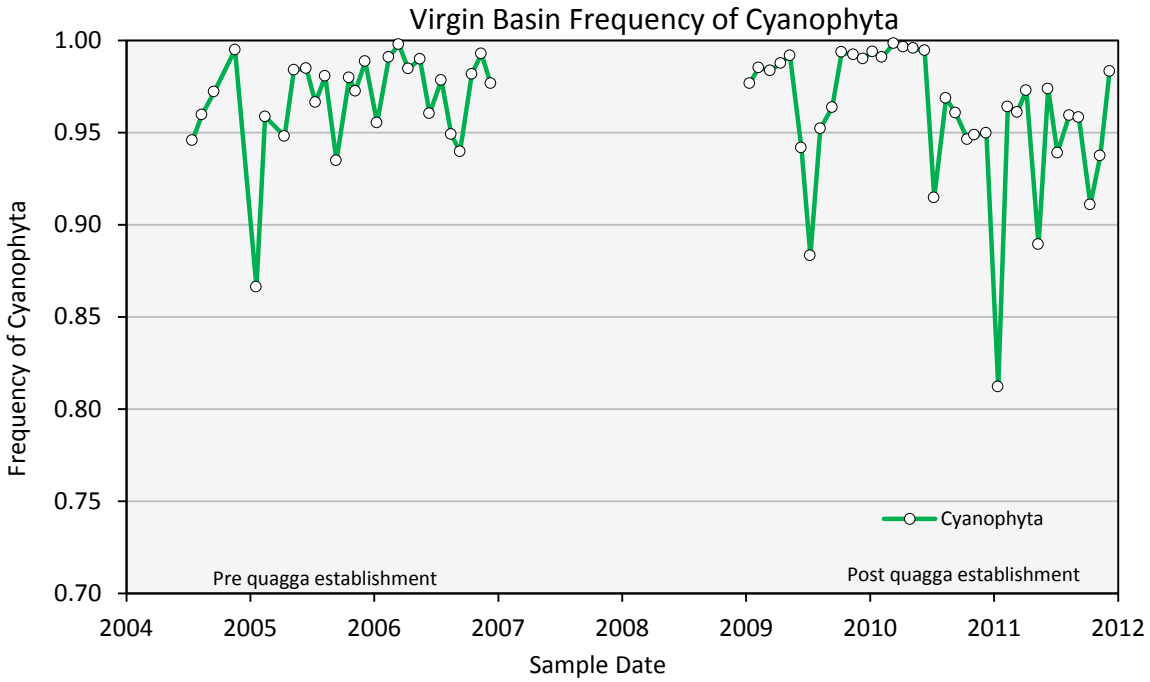

Virgin Basin Chlorophyll-a Surface Sample
Chlorophyll-a
µg/L
2.50
2.00
1.50
1.00
0.50
0.00
Pre quagga establishment
Post quagga establishment
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

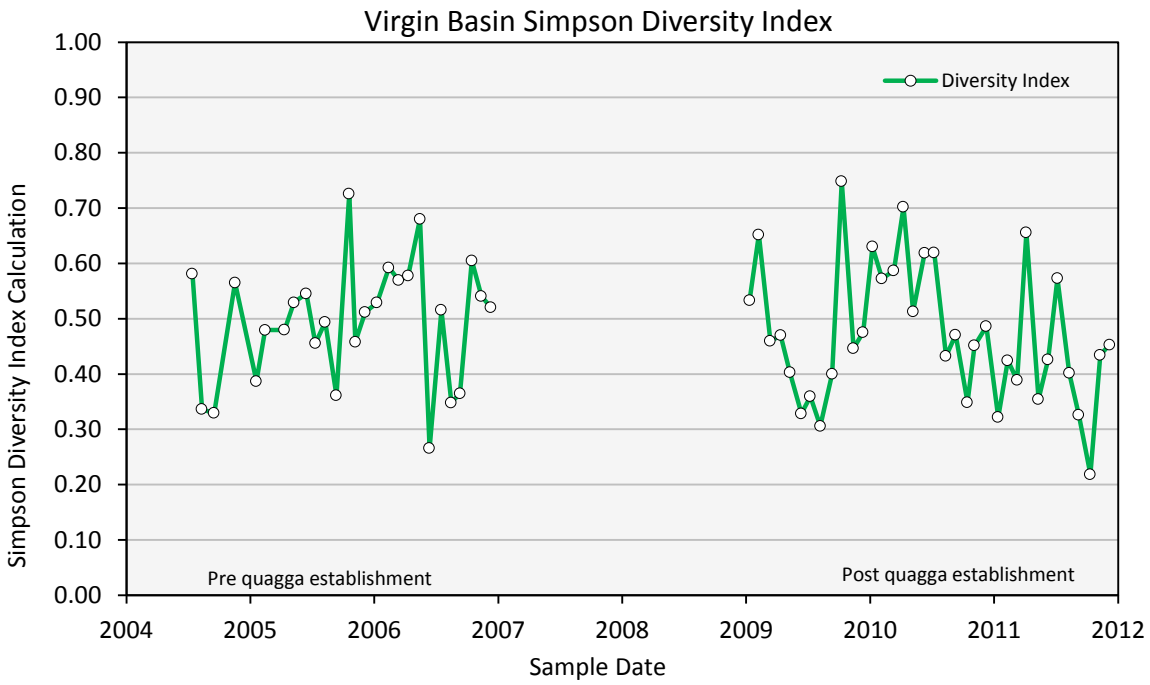

Virgin Basin Phytoplankton Total Cell Number
Total Phytoplankton Counted
# of individuals counted
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
Pre quagga establishment
Post quagga establishment
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

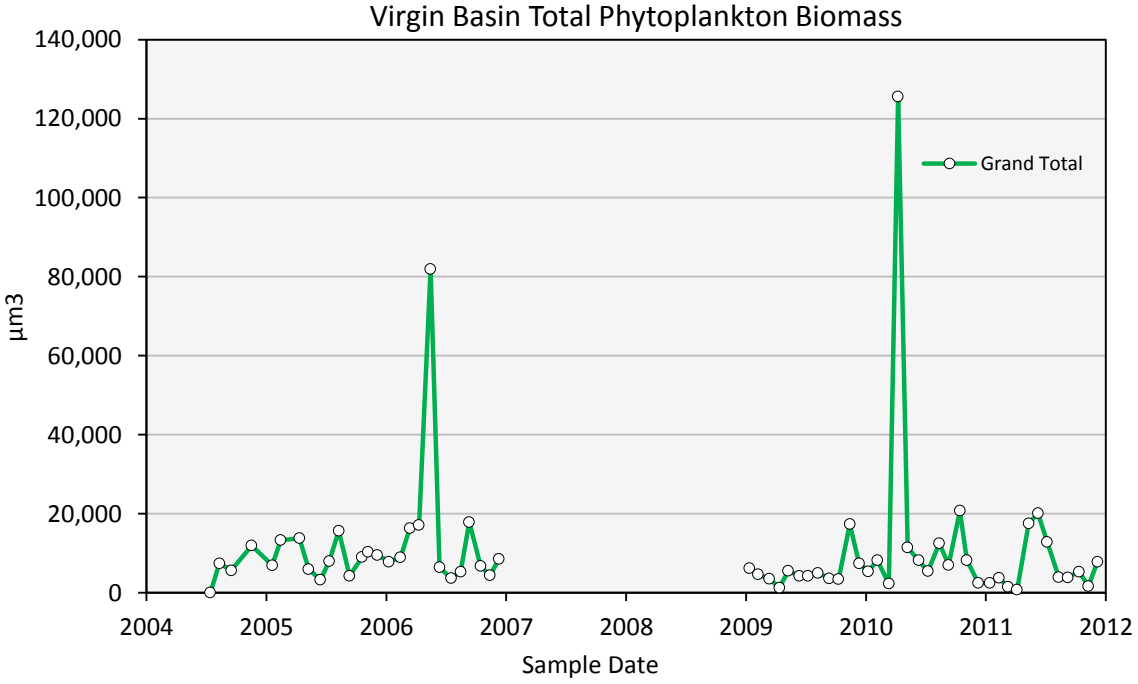

Virgin Basin Total Zooplankton without Veligers
Pre quagga establishment
Post quagga establishment
Zooplankton counted
# of individuals counted
Sample Date
0
5
10
15
20
25
30
35
40
45
50
2004
2005
2006
2007
2008
2009
2010
2011
2012


Virgin Basin Total Zooplankton
Pre quagga establishment
Post quagga establishment
Zooplankton counted
# of individuals counted
Sample Date
0
10
20
30
40
50
60
2004
2005
2006
2007
2008
2009
2010
2011
2012

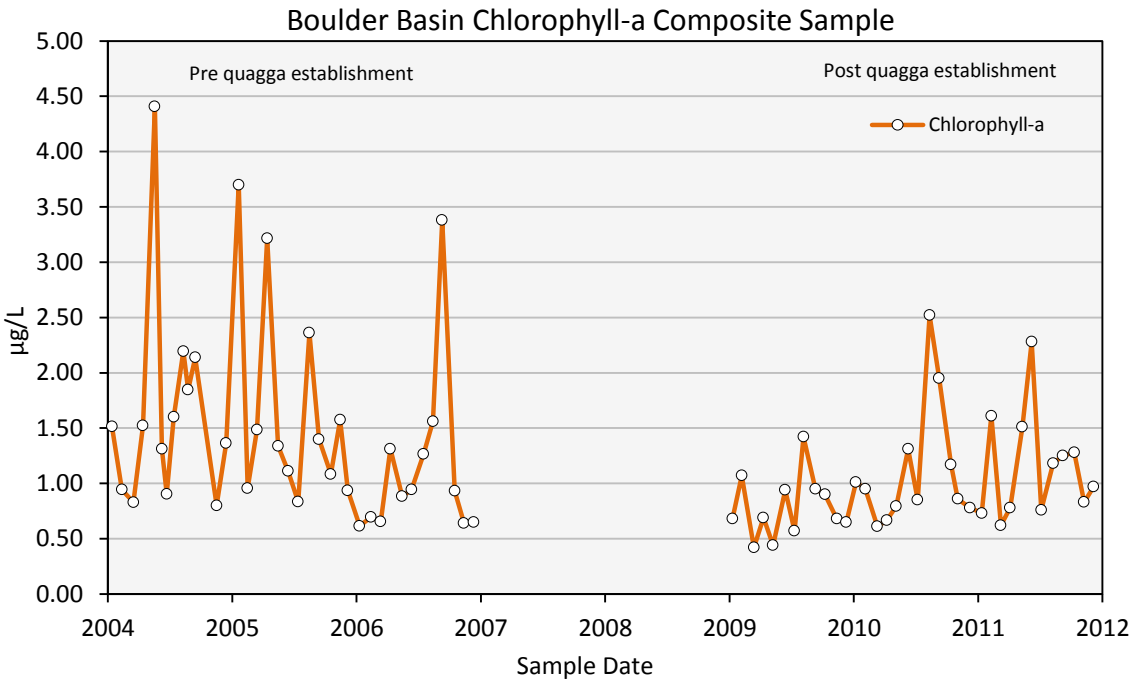

Virgin Basin Frequency of Chlorophyta
Pre quagga establishment
Post quagga establishment
Chlorophyta
Frequency of Chlorophyta
0.02
0.02
0.01
0.01
0.01
0.01
0.01
0.00
0.00
0.00
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

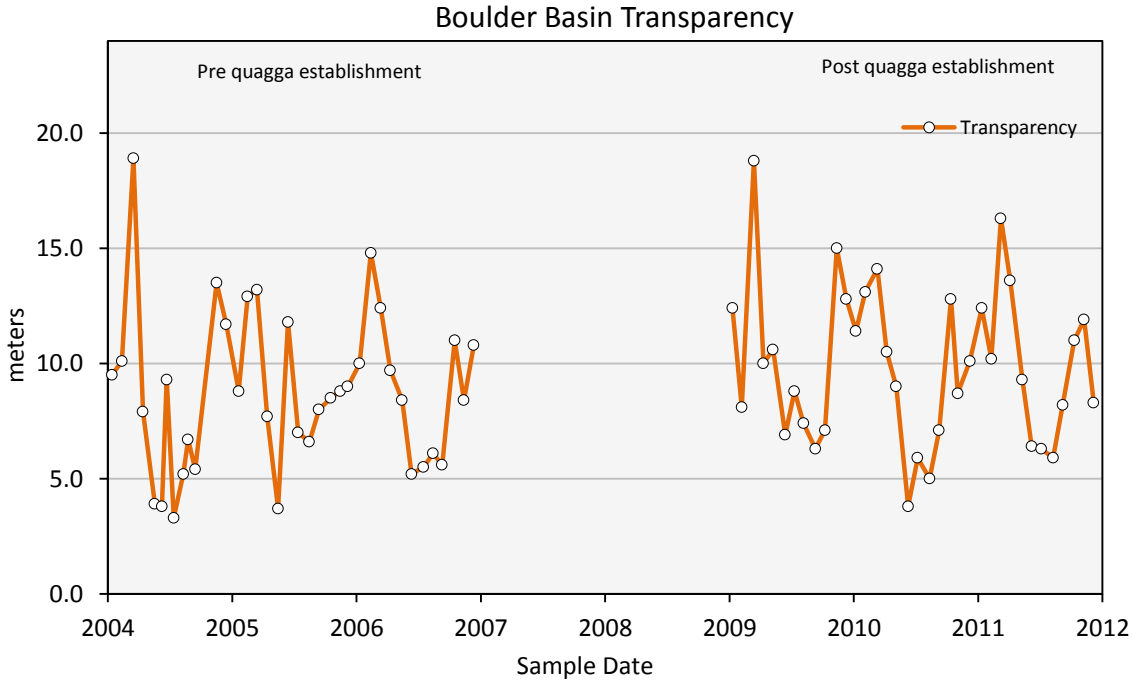

Virgin Basin Frequency of Cyanophyta
Frequency of Cyanophyta
1.00
0.95
0.90
0.85
0.80
0.75
0.70
Cyanophyta
Pre quagga establishment
Post quagga establishment
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

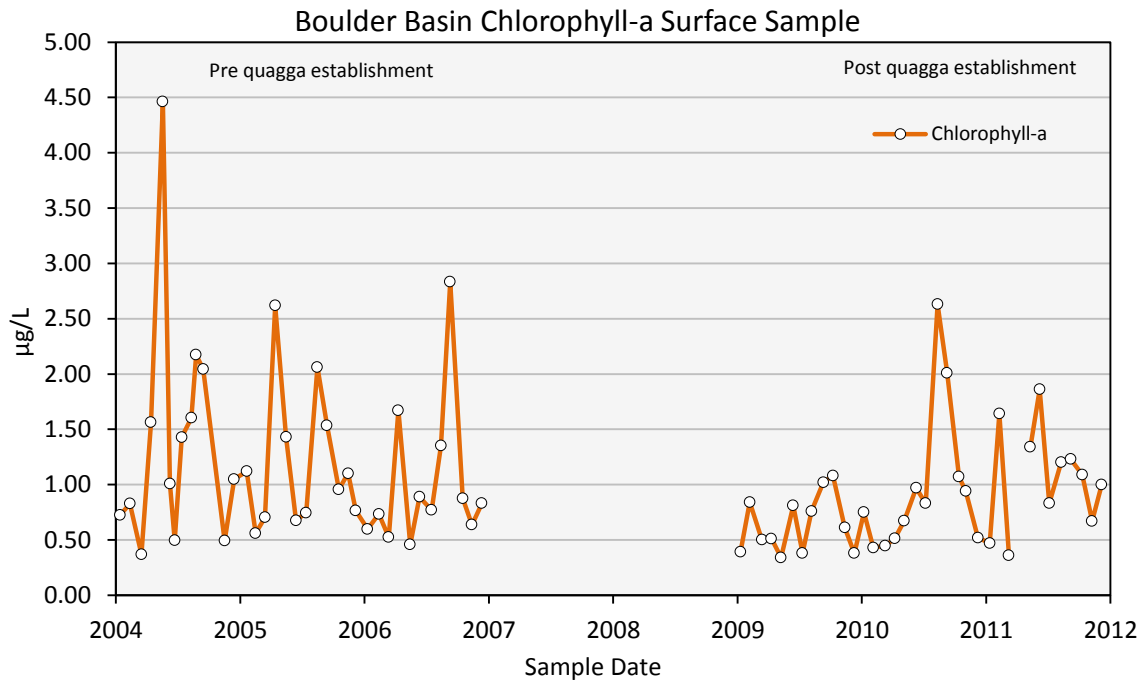

Virgin Basin Simpson Diversity Index
Simpson Diversity Index Calculation
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Diversity Index
Pre quagga establishment
Post quagga establishment
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

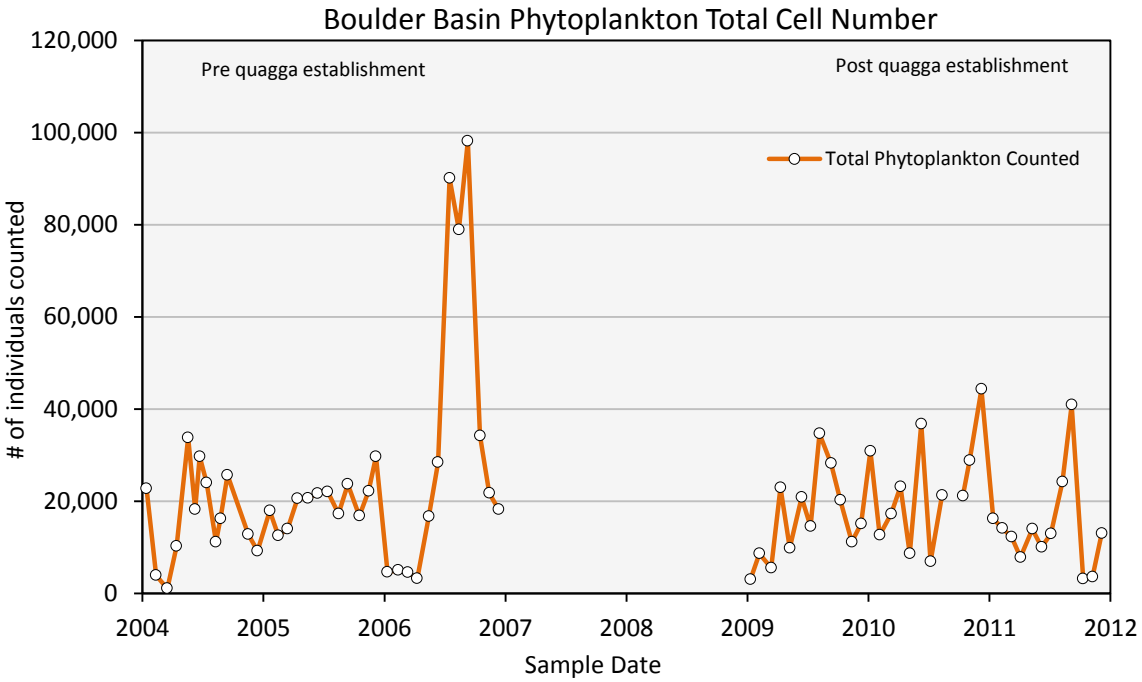

Virgin Basin Total Phytoplankton Biomass
µm3
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
Grand Total
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

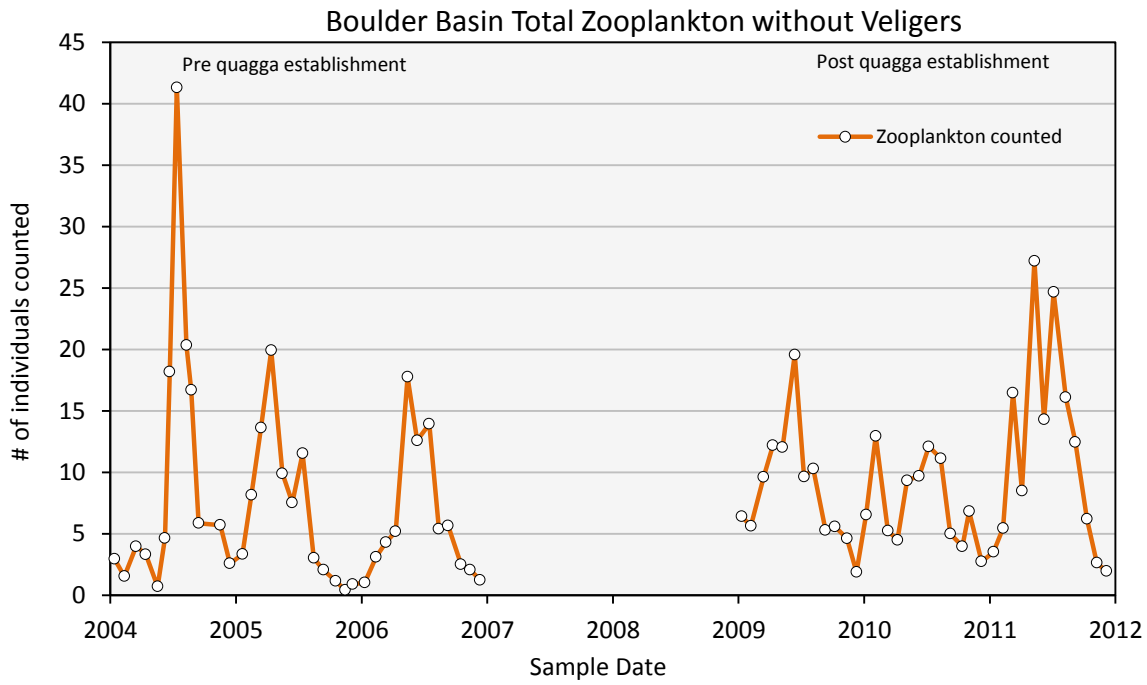
APPENDIX F. BOULDER BASIN FIGURES

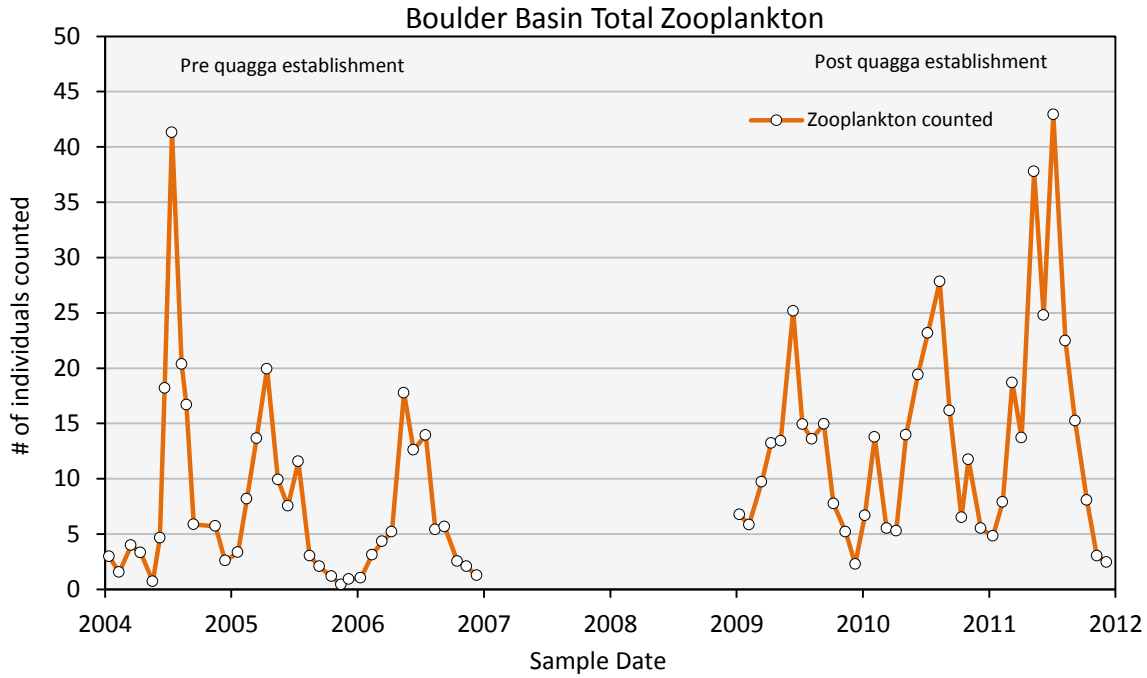


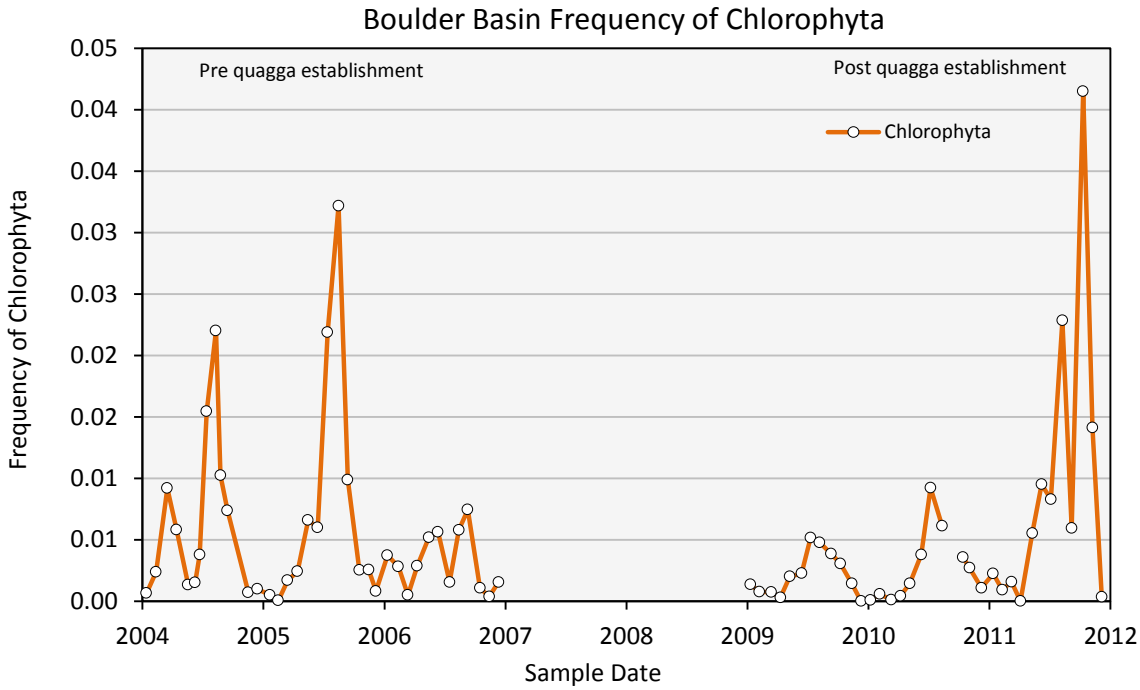


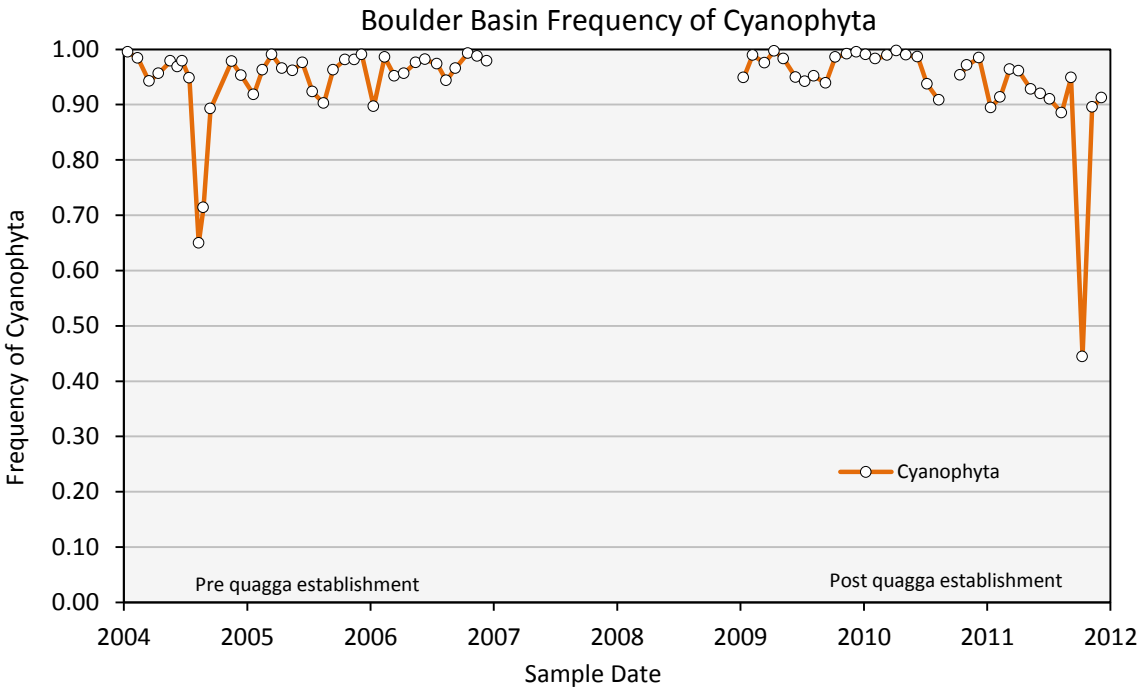

Boulder Basin Chlorophyll-a Surface Sample
Pre quagga establishment
Post quagga establishment
Chlorophyll-a
µg/L
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

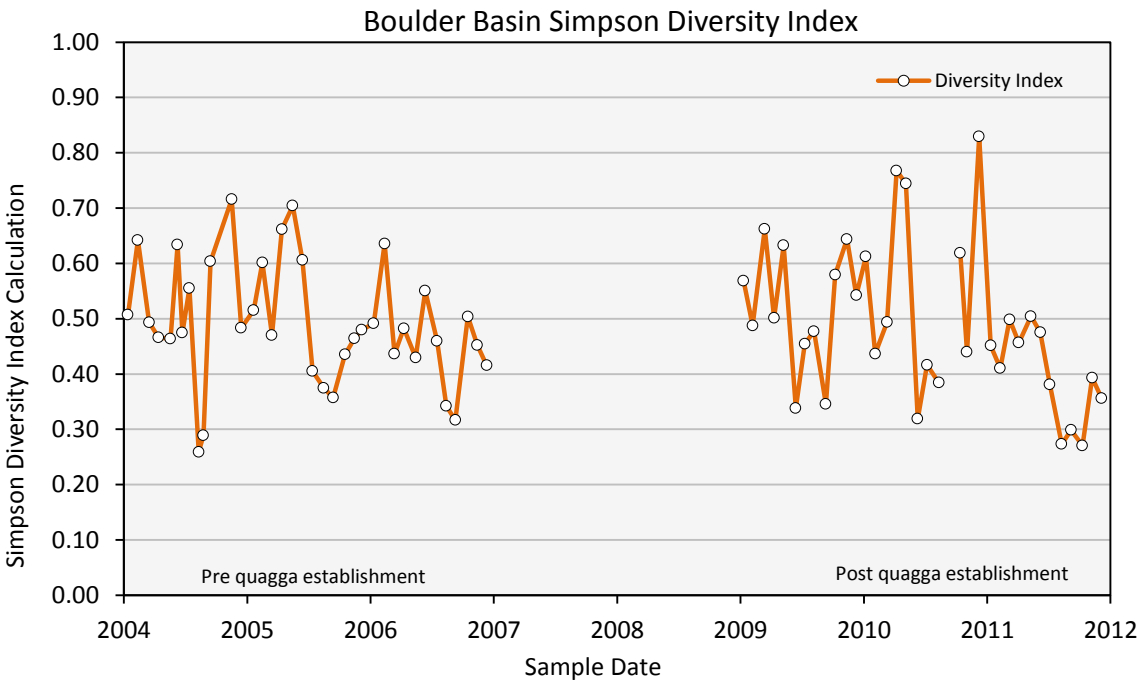

Boulder Basin Phytoplankton Total Cell Number
Pre quagga establishment
Post quagga establishment
Total Phytoplankton Counted
# of individuals counted
120,000
100,000
80,000
60,000
40,000
20,000
0
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

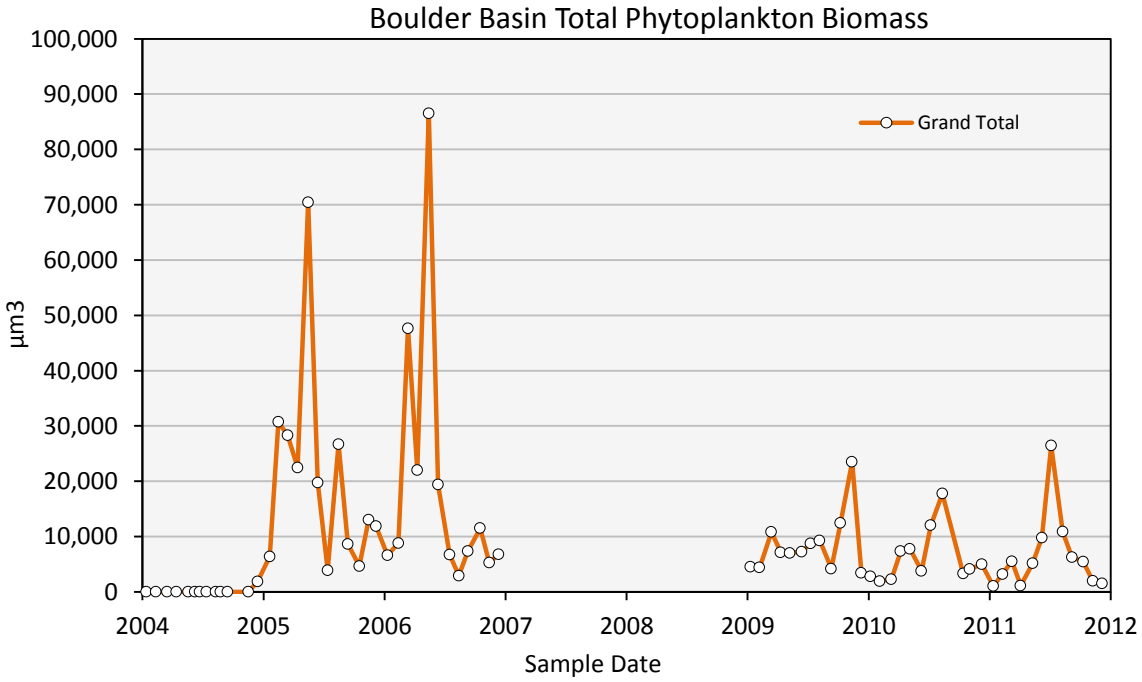

Boulder Basin Total Zooplankton without Veligers
Pre quagga establishment
Post quagga establishment
Zooplankton counted
# of individuals counted
Sample Date
0
5
10
15
20
25
30
35
40
45
2004
2005
2006
2007
2008
2009
2010
2011
2012


Boulder Basin Total Zooplankton
Pre quagga establishment
Post quagga establishment
Zooplankton counted
# of individuals counted
Sample Date
0
5
10
15
20
25
30
35
40
45
50
2004
2005
2006
2007
2008
2009
2010
2011
2012


Boulder Basin Frequency of Chlorophyta
Pre quagga establishment
Post quagga establishment
Chlorophyta
Frequency of Chlorophyta
0.05
0.04
0.04
0.03
0.03
0.02
0.02
0.01
0.01
0.00
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date


Boulder Basin Frequency of Cyanophyta
Frequency of Cyanophyta
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Cyanophyta
Pre quagga establishment
Post quagga establishment
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date


Boulder Basin Simpson Diversity Index
Simpson Diversity Index Calculation
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Diversity Index
Pre quagga establishment
Post quagga establishment
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date


Boulder Basin Total Phytoplankton Biomass
µm3
100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
Grand Total
2004
2005
2006
2007
2008
2009
2010
2011
2012
Sample Date

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CURRICULUM VITAE

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EDUCATION

University of Nevada, Las Vegas
Doctor of Philosophy, Environmental Health (Graduated 2016)

University of Nevada, Las Vegas
Master of Science, Water Resource Management (Graduated 2009)

Wright State University, Dayton
Bachelor of Science, Biology (Graduated 1997)

RESEARCH INTERESTS

Current research interests are water quality and ecosystem impacts of invasive mussels and water use imbalance and drought in the Colorado River Basin.

PROFESSIONAL EXPERIENCE

Colorado River Commission (03/2013 – current) – Natural Resource Analyst
Natural Resources Group, Water Division, Las Vegas

Consultant (07/2011 – 02/2013) – Resource Analyst
Southern Nevada Water Authority - Regional Water Quality Department, Las Vegas

Southern Nevada Water Authority (05/08 – 07/2011) – Environmental Biologist
Regional Water Quality Division - Las Vegas

Southern Nevada Water Authority (5/06 – 05/08) – Graduate Intern
Environmental Monitoring and Management, Las Vegas

PRESENTATIONS

Hydrologic Update, status on the hydrologic conditions, drought, and climate of the Colorado River Basin.
Colorado River Commission, monthly since 6/13

Using the Lower Colorado River Water Quality Database to Share and Exchange Data Between Agencies and Researchers Along the Lower Colorado River
National Water Quality Monitoring Council, poster 2012

Evaluation of Water Quality Monitoring Instruments with Interagency Sampling Events in Lake Mead
Lake Mead Science Symposium, 2009

Evaluation of Water Quality Monitoring Instruments with Interagency Sampling Events in Lake Mead
Interagency and Sampling Coordination Committee, 2009

AFFILIATIONS

Basin States Technical Committee, since 2013

Colorado River Water Users Association, since 2013

Inadvertent Overrun and Payback Policy Technical Workgroup, since 2013

Nevada Water Resources Association, since 2013

Salinity Control Forum, Workgroup and Advisory Council member, since 2013

Southern Nevada Drought Taskforce, since 2013

Water Education Foundation, since 2013