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Effects of Environmental Variables on the Reproduction of Quagga Mussels (Dreissena rostriformis bugensis) in Lake Mead, NV/AZ

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EFFECTS OF ENVIRONMENTAL VARIABLES ON THE REPRODUCTION OF QUAGGA MUSSELS (*DREISSENA ROSTRIFORMIS BUGENSIS*) IN LAKE MEAD, NV/AZ

by

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ABSTRACT

Effects of Environmental Variables on the Reproduction of Quagga Mussels (*Dreissena rostriformis bugensis*) in Lake Mead, NV/AZ

by

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In 2007, quagga mussels (*Dreissena rostriformis bugensis*) were found in Lake Mead and have spread downstream on the Colorado River and to other lakes and reservoirs in the Southwestern United States. The quagga mussel's extremely quick colonization of Lake Mead and annual veliger abundance trends provide evidence that the quagga mussels in the Southwest have different reproductive behavior than in previous habitats. This study is one of the first to specifically examine quagga mussel reproduction in the Southwest and examines how quagga mussel reproduction varies at sites known to have different temperatures and other environmental variables resulting from the input of Las Vegas Wash, and at different depths at each of these sites. Mussels were collected monthly over the course of a year at four different sites from Las Vegas Bay to Boulder Basin at depths of 20ft, 40ft and 70ft. A histological analysis of the gonads of these mussels was conducted to determine the reproductive development status in the form of a maturity index. This maturity index was then compared to environmental variables

including water temperature, depth, water salinity, and dissolved oxygen. Out of these factors, temperature was the only environmental variable which showed a significant effect upon quagga mussel reproduction. Also the seasonal pattern of maturity indices was found to differ between the deepest and shallowest depths. This study contributes to the understanding of the reproductive biology of quagga mussels living in the southwestern United States.

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This research was made possible through the help and guidance of my advisor Dr. David Wong. He has been extremely helpful through the process of conducting this research, and even after leaving the University of Nevada, Las Vegas, to continue research of dreissenid mussels at the State University of New York in Oneonta, has continued to be a great help in finishing my thesis.

I would also like to thank Dr. Gerstenberger for his help not only with my thesis but through the entire process of being a graduate assistant. He has helped me immensely with all of my research at the University of Nevada Las Vegas and to ensure that I continue to do my best work.

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the water diving. Thanks to Jen Berger and Holly Priest for coming to help out with work on the boat as well, and also to Marija Minić for diving for me in September and October when I was recovering from an injury and unable to do so myself.

Lastly, thanks to the Southern Nevada Water Authority and the Bureau of Reclamation for the field data used in this study. Also thanks to Warren Turkett for helping me to access the data and navigate the SNWA database.

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CHAPTER 1

INTRODUCTION

Dreissena rostriformis bugensis, or quagga mussels, are a freshwater bivalve mollusk that is indigenous to the Dneiper River watershed of Ukraine. Along with another dreissenid species *Dreissena polymorpha* (zebra mussels) are possibly one of the most ecologically and economically damaging invasive species in the country if not the world. In North America, the quagga mussel was first noticed in Lake Erie in 1991 (Mills et al. 1996) and spread throughout the Great Lakes. From there it was transported along with private watercraft across the country into Lake Mead and the lower Colorado River, first appearing in January of 2007 (LaBounty and Roefer 2007; Wong et al. 2012).

Quagga mussels have proved to be extremely effective at biofouling both boats and water infrastructure due to their ability to quickly colonize hard substrates (Comeau et al. 2011). The colonization of boats not only damages them, but also presents major issues and expenses in the containment of the further spread of quagga mussels to other freshwater ecosystems in the West. The colonization of water infrastructure forces hydroelectric dams and water treatment facilities to develop methods to prevent the mussels from clogging pipes and screens. Due to the size of the quagga mussel veligers which can be less than 40µm wide (Anderson and Taylor 2011), the use of filters to prevent colonization is expensive and sometimes unfeasible.

Water treatment plants are often left with chemical treatment (such as chlorine) as the only feasible option for preventing quagga mussel infestation. Increasing chemical treatment in order to control for quagga mussels is one of the major ways in which the mussels present a public health dilemma. The Southern Nevada Water Authority

(SNWA), charged with delivering drinking water to the millions of people living in the cities of Las Vegas, North Las Vegas, Henderson and Boulder City, was forced to greatly increase chlorine treatment after the arrival of quagga mussels in 2007. Chlorine reacts with organic compounds to form toxic, carcinogenic compounds called trihalomethanes (THMs) (Lourencetti et al. 2012). By 2011, the amount of THMs in Las Vegas tap water rose above the EPA maximum level of 80ppm (SNWA 2011). To rectify this problem, SNWA began treating for the mussels with chloramines, which do not produce THMs but are known to leach lead out of piping in older houses (Nguyen et al. 2011). While increased chemical treatment of water due to invasive quagga mussels is one way in which the species presents public health problems, there is potential for others.

Along with affecting boating and water infrastructure, quagga mussels can have major impacts upon freshwater ecosystems in which they invade to the point that they are considered ecosystem engineers. Quagga mussels are filter feeders that can reproduce prolifically and remove large amounts of phytoplankton and suspended particles from a body water of water (Beaver et al. 2010). Wong et al. (2012) found a significant increase in water clarity after the invasion of quagga mussels into Boulder Basin. The enhanced clarity of the water can increase the amount of aquatic plants seen in a system. Quagga mussels can also quickly take over the substrate of a body of water particularly in rocky areas. In this way they compete for habitat for other native benthic species, while potentially providing habitat in the form of the shells for other species (DeVanna et al. 2011). The mussels can compete with native fish populations (Hoyle et al. 2012) and result in a large range of other ecosystem changes. Mussels can also remove nutrients from the water column transporting them into the substrate. Toxic algal blooms have

also been linked to the presence of dreissenid mussels (Sarnelle et al. 2012) which makes a clear case for potential negative effects on water quality.

With so many environmental factors, it is extremely difficult, if not impossible, to predict how quagga mussels will behave and then affect a lake or reservoir ecosystem. These ecosystems play a crucial role in maintaining the water quality of bodies of water that are eventually used for residential, agricultural and commercial purposes. If quagga mussels affected an ecosystem in such a way as to cause a toxic algal bloom, it could have a dramatic effect on water quality and pose a significant public health hazard.

In order to develop effective management plans for a body of water where quagga mussels are present, it is important to have a comprehensive understanding of their life cycle and ecosystem functioning. Research is still needed on the reproductive cycle of quagga mussels, particularly in their newfound habitat of reservoirs in the desert Southwest. To date, little research has been done directly examining the spawning habits of quagga mussels in Lake Mead. Research in this area could provide valuable information on when and under what conditions the spawning of quagga veligers would occur. This would in turn affect the amount of veligers pulled into water intakes in Hoover Dam and in the SNWA water treatment facility, as well as the number of veligers that would be available to settle onto and make their way into boating equipment at different times of the year.

Apart from season, two environmental factors that have been shown to affect dreissenid mussel spawning behavior are temperature (Fong et al. 1995) and depth (Mantecca et al. 2003). Both of these factors are influenced by reservoir management. The level of Lake Mead often varies significantly throughout each year depending on the

level of snowpack in the Colorado River drainage basin and resulting fluctuations in the release of water from Lake Powell down the Grand Canyon. Also, unusual management decisions such as the intentional flooding of the Grand Canyon in 2008 can bring warmer water into Lake Mead. A suggested plan of installing a temperature control device in Glen Canyon Dam in order to warm water in the Colorado River to help endangered species (Peterson and Paukert 2011) could raise the water temperature of Lake Mead.

For these reasons, it is important to understand how changes in depth and water temperature affect quagga mussels in this environment. In Lake Mead, the input of warm water from Las Vegas Wash as well as the existence of quagga mussels along steep slopes of varying depths allows for the sampling of quagga mussels at sites of different temperature, with multiple depths at each site.

Purpose of the Study

In order to properly manage a body water such as Lake Mead that is infected with invasive dreissenid mussels it is crucial to understand the life cycle and behavior of these mussels. This study focuses on investigating potential differences in reproductive behavior of quagga mussels based on variations in physical and chemical factor using temperature, depth, salinity and dissolved oxygen as covariates and maturity index as the outcome variable. Maturity index is an ordinal descriptive measure of an individual mussel's reproductive state.

Research Questions

- Is quagga mussel reproduction enhanced by warmer water temperatures in Las Vegas Bay?
- Do yearly reproductive cycles of quagga mussels at vary by depth?

Hypotheses

H_1: There will be no significant association between maturity indices of mussels collected at three different depths over the course of one year in Lake Mead.

H_2: The maturity indices of quagga mussels will correlate positively with water temperature.

H_3: The maturity indices of quagga mussels will correlate negatively with salinity.

H_4: The maturity indices of quagga mussels will correlate positively with dissolved oxygen.

CHAPTER 2

BACKGROUND

Dreissenid Life Cycle

Quagga mussels typically live in populations mixed with females and males where they commonly reproduce in mass spawning events by releasing gametes into the water, though some populations of dreissenid mussels have been found to reproduce continuously. Fertilization occurs externally and soon after eggs hatch into trochophores, the first phase of the free floating larval form of mussels known as veligers (Wittman et al. 2012).

After four to five days as trochophores the veligers developed d-shaped valves and are then considered d-shaped veligers. The D-shaped veligers eventually develop a portion of their shell called the umbone and are then referred to as umbonal veligers. Finally they develop foot shaped protrusion at which point they are called pediveligers, which allows them to attach to substrate and develop into juvenile mussels. Dreissenid mussels typically exist as veligers for around a month before settling (Wittman et al. 2010).

Larval, juvenile and adult quagga mussels are all filter feeders and consume mainly phytoplankton and zooplankton. Juvenile mussels settle and grow, and females are generally able to reproduce in their second year. Female dreissenids can produce over 40,000 eggs in a single reproductive cycle (Nichols 1996) and quagga mussels in Lake Mead may be able to produce twice or more in a single year. The typical life span of a dreissenid mussel is 4-5 years (Mackie et al 1991). One factor that seems to affect

dreissenid reproduction is water temperature, and it is believed that increases in water temperature may initiate quagga mussel spawning (Claudi and Mackie 1994).

Water Temperature

The water coming into Las Vegas Bay from Las Vegas wash is in many ways vastly different than the water in the rest Lake Mead. The water coming into Lake Mead from Las Vegas Wash is significantly warmer than the water in Lake Mead, particularly during the winter and spring when water temperatures in the lake are lowest. Below the mouth of Las Vegas Wash in Las Vegas Bay, winter and spring water temperatures can be 1-2 °C warmer than in Boulder Basin far from Las Vegas Wash as shown in Figure 1. This significant water temperature variation could cause differences in the health status and reproductive maturity of quagga mussels.

Fong et al. (1995) examined spawning capability of zebra mussels at different temperatures. Zebra mussels were collected from Lake Erie and placed in aquariums at constant temperatures of 12°, 20° and 27°C. Suspensions of eggs and sperm were taken from female and male quagga mussels for analysis. While zebra mussels were able to spawn at all temperatures, the proportion of zebra mussels found to be spawning was highest at 20°C. At the relatively high temperature of 27°C mussels were still able to reproduce. These results show that it is possible that zebra mussels are in fact better equipped to reproduce and colonize an area with higher temperatures than those found in the bodies of water where they are native. Assuming that this holds true for quagga mussels as well, it would help explain how the invasion of quagga mussel into the Lower Colorado River occurred so quickly.

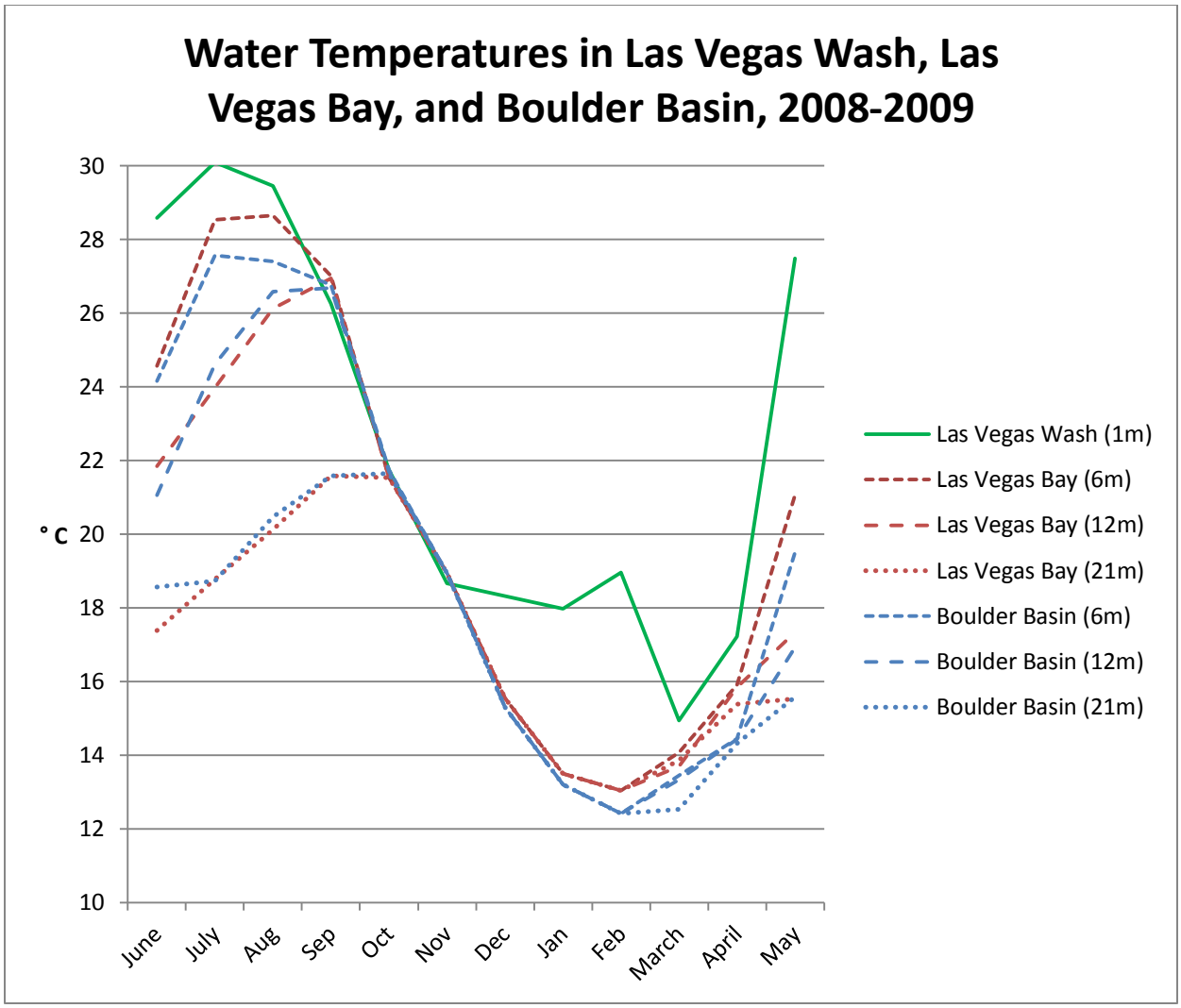

Figure 1 Water temperatures at Las Vegas Wash before confluence, LVB 4.95 in Las Vegas Bay, and CR346.4 in Boulder Basin from 2008 to 2009. The temperature trends show warmer temperatures in Las Vegas Wash during the winter resulting in warmer temperatures in Las Vegas Bay relative to Boulder Basin in the Spring. Temperature also varies by depth throughout the year. Data used are from the SNWA Water Data Base.

Water temperatures in Lake Mead are typically closer to 12°C than 20°C in the spring and winter months. With temperatures slightly warmer in Las Vegas Bay, it could be expected that mussels begin to spawn earlier, or perhaps spawn more frequently due to the increased water temperature. If either case were true, the overall average maturity index of the mussels would likely be greater.

Depth

Quagga mussel behavior and activity in Lake Mead is likely affected to some extent by depth. Quagga mussels will settle at shallow depths just below the surface, though they have a tendency to settle deeper as shallow water is disturbed more by wind and currents which make settlement difficult (Chen et al. 2011.) While zebra mussels exist up to and deeper than 25 meters, quagga mussels are commonly found at extremely deep depths, and mussels living 55 meters below the surface have been discovered to be reproductively active, though whether or not fertilization is successful is unknown (Roe and MacIsaac 1997).

A study in Italy of zebra mussel reproduction at different depths showed a significant variation between populations at 2 and 25 meters (Mantecca et al. 2003). Zebra mussels are known to have a high seasonality in reproduction, often having one large spawning event, sometimes considered to be triggered by an increase in temperatures. While the mussels at 2 meters showed this pattern clearly, there was a loss of seasonality in the deep water mussels. Suggested reasons for this were the lack of temperature variation, darkness, and low food availability, most likely water temperature as this appears to trigger the normal spawning events. The study also found an increased occurrence of degenerating oocytes at these depths, indicating that the lack of a clear reproductive event is potentially disadvantageous. This study should be able to determine if similar depth effects can be found in Lake Mead.

Other Variables

Along with being unseasonably warm, the water that enters Las Vegas Bay from Las Vegas Wash can carry with it large amounts of sediments, minerals and pollution from the city of Las Vegas. This means that the same temperature gradient found from the mouth of Las Vegas Wash across Las Vegas Bay may parallel trends with other factors which could affect mussel health and reproduction. Inorganic sediment has shown a negative correlation with dreissenid mussel health (Madon et al. 1997) and could therefore also negatively affect reproduction. Salinity has shown to be negatively correlated with spawning activity in dreissenid mussels (Spidle et al. 1995) as well as simply in their health (Horohov et al. 1992, Fong et al. 1995). Las Vegas bay is also subject to eutrophication. Quagga mussels prefer oligotrophic conditions and it would not be surprising if a lower dissolved oxygen concentration caused lower spawning activity. However, increases in algal content may prove beneficial as it would create an increase in food availability. It will be important to take into account that temperature is not the only variable that differs between collection sites.

CHAPTER 3

METHODOLOGY

Mussel Collection

Quagga mussels were sampled from hard substrate (rocks) each month for a year from four sites from Las Vegas Wash to Boulder Basin beginning in May of 2011. The outflow of Las Vegas Wash results in the closest sites having higher water temperatures. Sites were selected based upon proximity to existing water quality sites LVB 3.5, LVB 4.95 and LVB 7.3 in Las Vegas Bay, and at Sentinel Island in Boulder Basin.

Figure 2 Map of Sampling Locations in Boulder Basin and Las Vegas Bay of Lake Mead, NV/AZ. Las Vegas Wash enters Las Vegas Bay West of site LVB 3.5 (Image from Google Earth satellite imagery) Mussels were sampled monthly from May 2011 through April 2012.

Three depths were sampled from at each site. Mantecca (2003) found with *Dreissena polymorpha* in Lake Iseo in northern Italy, that mussels at 2 meters depth (above the summer thermocline) had a different reproduction strategy than mussels sampled at 25 meters (below the summer thermocline). Specifically, mussels in the epilimnion spawned synchronously following an annual pattern, while the mussels in the hypolimnion reproduced all year round.

Over the course of the study the surface elevation of Lake Mead ranged from 334m to 346m above sea level. For the initial collection in May, mussels were be collected at 6m within the epilimnion, 12m within the metalimnion and 21m in the hypolimnion at each site, except for the furthest site up Las Vegas Bay, which was only deep enough to collect at 6m. Because of increase in reservoir level, the sampling locations did not remain within their intended water layers and by the winter season, the shallowest depth was located in the metalimnion and the deeper two depths in the hypolimnion. To continue sampling mussels from the same location, sampling was based on elevation from sea level.

Table 1: Sampling Site Elevations from Sea Level. Sites were sampled at consistent elevations rather than depths to adjust for changing reservoir levels.

Site name:	LVB 3.5	LVB 4.95	LVB 7.3	BB 8
Depth 1	328m	328m	328m	328m
Depth 2	NA	322m	322m	322m
Depth 3	NA	313m	313m	313m

Rocks with quagga mussels were removed randomly by divers by hand from each site. Rocks were opportunistically selected, based on the presence of mussels that appeared to be greater than 15mm in length. Rocks were also selected based upon being an appropriate size to bring to the surface and were typically around 10 to 15 cm in diameter. The sites of collection were kept as consistent as possible by using above and below water land marks, mainly rocks in the forms of cliffs, large boulders and underwater reefs.

Due to the deep depths that were required and that there are four sites that divers needed to reach on each collection date, procedure was kept as simple as possible. Divers descended to each specific site. Within the immediate area one diver reached out and removed one rock by hand, approximately 6 inches in diameter that had a healthy population of mussels with some greater than 15mm in size. The rock was placed into a 40x80 cm spat bag and carried by divers to the next site. Upon collection of one rock for each of the three depths at a sampling location, the spat bags were be attached to a float bag and returned to the surface with divers. Dive computers were used to track time at depth and multiple safety stops were taken on each ascent. The dive team consisted of a researcher trained with the Professional Diving Association (PADI) Advanced Open Water certification, and a PADI certified diving instructor.

From each rock collected, 6-10 mussels greater than 15mm (Ron and MacIsaac 1997) in length were removed by hand (to prevent breaking) and placed into 10% neutral buffered formalin (Ortiz-Zarragoitia and Cajaraville 2006) for fixation for a period of approximately one month. Next they were removed from the 10% neutral buffered

formalin with tweezers and processed histologically or preserved in a 50% ethanol solution for later processing.

Histology

Five of the fixed and preserved mussels from each site, depth and date were taken for histological analysis. Each mussel was measured, and then dissected to remove the gonad, which was placed in a 1" by 1.5" labeled tissue cassette. Tissue cassettes were labeled by engraving on the front with a scalpel as any marker would wash off when exposed to xylene. Larger mussels (20-25mm) were preferred as they were considerably easier to analyze than ones shorter than 20mm in length, however this did not allow for the size of the mussels to be controlled in this study. The gonads were then dehydrated in the ascending alcohol series described in Table 2. The tissues were transferred simultaneously between 500mL beakers containing each solution with the use of tweezers.

Table 2: Series of solutions, times and number of changes for gonadal tissues transferred through for dehydration in histological processing. Tissues were moved in tissue cassettes through beakers containing each of the following solutions.

Solution:	Time:	# of Changes:
70% ethanol	12 hours	1
80% ethanol	1 hour	1
95% ethanol	1 hour	1
100% ethanol	1 hour	2
Xylene (for clearing)	1.5 hours	2

After being cleared in Xylene the tissue cassettes were placed into 500mL beakers containing paraffin wax heated in an oven at 56°C for 1.5 hours. This process was repeated twice for paraffin to become imbedded in the tissue. Next the tissue cassettes were removed and the paraffinized gonads were taken from the tissue cassettes by hand and placed on 1" by 1.5" paraffin mounting blocks. Excess paraffin was poured onto the mounting blocks to embed the tissue.

Once the paraffin dried, the tissue was sectioned. Paraffin blocks were trimmed down to the appropriate size to fit into a hand microtome. 5 sections of approximately 10µm thick were cut from the gonadal tissue and placed into water. The tissue was then rehydrated using 500mL beakers of solutions using the series described in Table 3.

Table 3: Series of solutions, times and number of changes for gonadal tissues to be transferred through for dehydration. Tissues were moved through beakers containing the following solutions in order to clear paraffin wax and rehydrate the tissues prior to staining.

Solution:	Time:	# of Changes:
Xylene	10 minutes	2
100% ethanol	3 minutes	2
95% ethanol	3 minutes	2
Distilled water	Rinse	-

Next the rehydrated tissue was stained with hematoxylin and eosin (Ortiz-Zarragoitia and Cajaraville 2006), dehydrated and mounted using the series described in Table 4. In this process, cassettes were moved in groups of 4 in between 150mL beakers containing the solutions described using tweezers.

Table 4: Series of solutions, times and number of changes for gonadal tissues transferred through for hematoxylin and eosin staining. Hematoxylin stains the nuclei of gametes blue, acid alcohol removes excess hematoxylin, and eosin stains cytoplasm red.

Step	Solution	Time
1	Hematoxylin stain	30 seconds
2	Water	Rinse
3	.3% acid alcohol	Dip twice
4	Water	Rinse
5	Eosin Stain	20 seconds
6	Water	Rinse
7	70% ethanol	3 minutes
8	90% ethanol	3 minutes
9	100% ethanol	3 minutes
10	Xylene	5 minutes
11	Xylene	5 minutes
12	Water	Rinse

After rinsing in water, each tissue segment was placed on a 3” by 1” glass slide, to be viewed microscopically.

Maturity Index

A SteREO Discovery V8 stereo microscope was used at 8X magnification. The maturity index was determined and a picture was taken and saved for each sample for future reference. Maturity index is a descriptive measure commonly used in the histological evaluation of dreissenid and other bivalve gonads (Mantecca et al. 2003, Ortiz-Zarragoitia and Cajaraville 2006, Smaoui-Damak et al. 2006). It is an evaluation of the mussel's progression through a reproductive cycle which provides an ordinal variable that can be used for statistical analysis.

Maturity index was determined based on the qualifications listed in Table 5, and are based upon the study on dreissenid gonads by Mantecca et al. (2003.) An acinus is a cluster of cells, in which female quagga oocytes form. When formed around the edge of the acinus the cells are considered developing. When they fill the acinus the gonad is considered in the pre spawn phase, and when only a few large oocytes remain the gonad is considered in the post spawn phase.

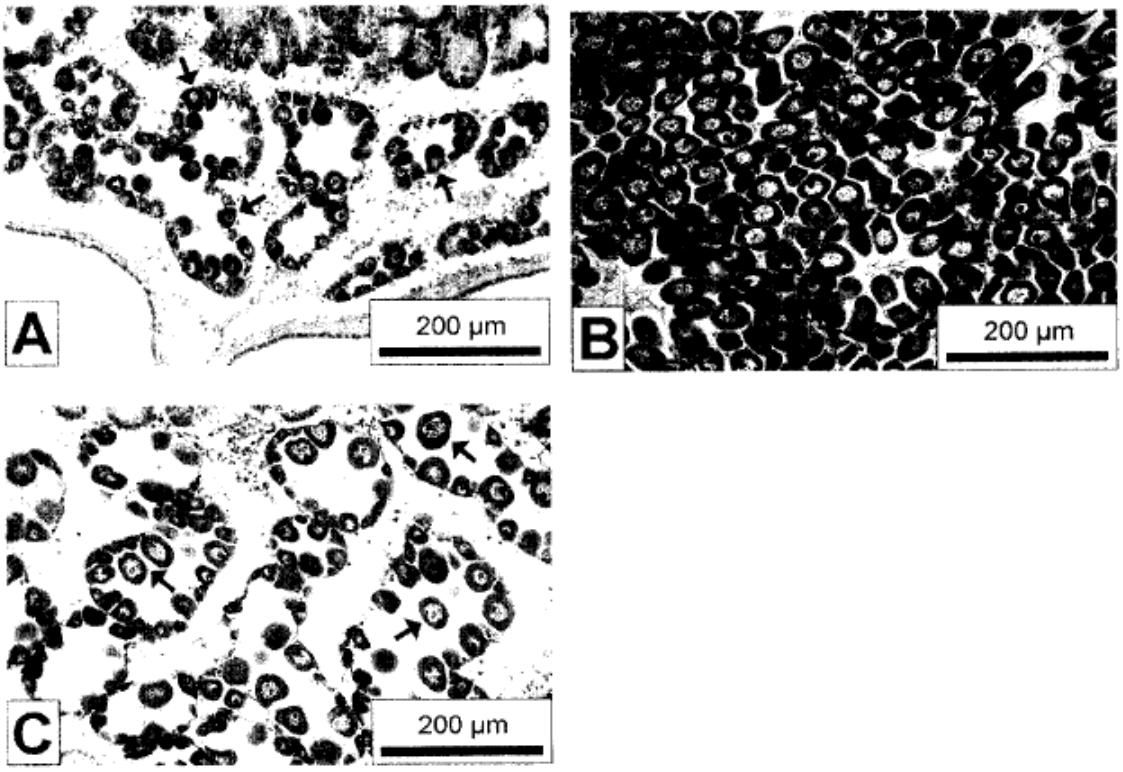

Figure 3: Development stages of female *Dreissena polymorpha*: **A:** Developing (stage 1), **B:** Pre-spawn (stage 2), **C:** Post-spawn (stage 3) (Mantecca et al. 2003)

For male gonads, the presence of spermatocytes in an undifferentiated pattern is indicative of a developing gonad. The spermatozoa eventually begin arranging with tails pointed towards the center of the lumen at which point the gonad is considered pre spawn. Afterwards, degenerating follicles and detached spermatazoa free in the lumen indicate a post spawn stage (Mantecca et al. 2003).

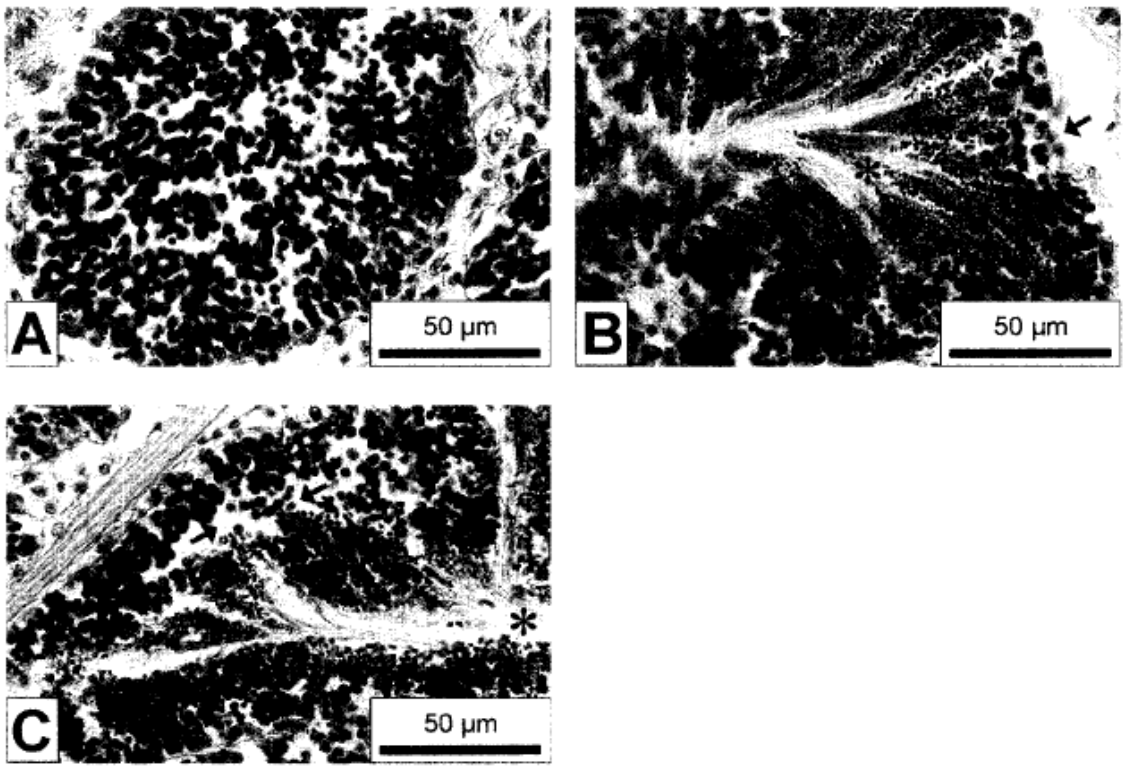

Figure 4: Development stages of male *Dreissena polymorpha*: **A:** Developing (stage 1), **B:** Pre-spawn (stage 2), **C:** Post-spawn (stage 3) (Mantecca et al. 2003)

Table 5: Qualifications used for the determination of Maturity Index for male and female gonads.

	Male	Female
Stage 0: Inactive	Follicles are empty	Ovaries are empty
Stage 1: Developing	Presence of spermatocytes, undifferentiated	Developing gametes attached to walls of acini
Stage 2: Pre spawn	Spermatazoa with tails point towards the center of the lumen	Acini filled with gametes
Stage 3: Post spawn	Follicles degenerate	Follicles degenerate, some residual gametes

Environmental Variables

The depth at which each mussel sample is collected was recorded based on dive computers and corresponded with the combination of site elevation and the level of Lake Mead. Water temperature data was retrieved from the Southern Nevada Water Authority's water database for statistical analysis. Data measuring other available variables (salinity and dissolved oxygen) was also extracted from the SNWA water quality database. As sampling dates were based off of the availability of a boat and divers, along with weather conditions, they did not coincide with the dates water samples were collected by the SNWA and were offset sometimes by up to two weeks. The Las Vegas Bay samples were all collected on the same dates, but collection dates for the Colorado River site near Sentinel Island differed. Also, the water data is based on a column of water below the site, where mussel samples were taken off of the lake bottom and therefore varied in location. Exact coordinates for collection sites were not able to be

determined, and may have been located up to approximately 50 meters away from the water sampling locations. Because of these variations, the water quality data is an approximation of the actual water quality at each site.

Table 6: Dates of Mussel and Water Quality Data Collection
*Data were collected by the Bureau of Reclamation for SNWA on these dates

Mussel Collection Date	LVB Sites	CR 346.4
5/19/2011	5/18/2011	5/17/2011
6/14/2011	6/13/2011	6/15/2011
7/25/2011	7/11/2011	7/26/2011
8/29/2011	8/24/2011	8/23/2011
9/25/2011	9/12/2011	9/20/2011
10/28/2011	10/16/2011	10/24/2011
12/23/2011	12/12/2011*	12/13/2011
2/13/2012	2/14/2012*	2/14/2012
3/19/2012	3/12/2012*	3/8/2012
4/20/2012	4/16/2012*	4/3/2012

Statistical Analysis

Linear Mixed Model

Average maturity index in correlation to temperature and depth was analyzed using a linear mixed effect model in order to address hypotheses 2 through 3. Specifically the Laird-Ware form of the model (Chen et al. 2011; Laird and Ware 1982):

$$y_{ij} = \alpha + \beta_1 x_{1ij} + \cdots + \beta_6 x_{6ij} + b_i z_{ij} + \varepsilon_{ij} \quad (1)$$

where y_{ii} is the value of the average maturity index for observation j out of n_i observations in group i (where there are 4 groups, 1 for each site). β is the slope of x, an independent variable such as temperature or dissolved oxygen. b_i is the random effect coefficient and is assumed to be normally distributed. z_{ij} is the random effect variable, which in this case is water depth. ε_{ij} is the error for observation j in group i which is also assumed to be normally distributed.

As the dependent variable, maturity index is an ordinal variable, and linear models are built to model continuous variable, we transformed the ordinal variable into a continuous variable by taking the average of the maturity indexes for the five mussels sampled from each site and depth (Mantecca et al. 2003; Cooper 2010). This average provided a continuous variable between 0 and 3 that was used for the linear mixed model.

Fixed effects included temperature in degrees Celsius as well as other variables likely to affect reproduction including salinity and dissolved oxygen (DO). Prior to being used in the model specified, each of these variables was adjusted to a common scale in order to allow a comparison in the effect size of the different fixed effects.

$$x_i = x_{i0} / x_{max}$$

Where x_{io} is the original variable, x_{max} is the maximum value of x among observations and x_i is the value used for this linear mixed effects model. This allowed us to determine the relative importance of different fixed effects in the model.

Spearman's Rank Correlation

The first hypothesis is that maturity indices between different depths will lack association throughout the year. In other terms, the reproductive cycles will differ significantly. In order to test this we used a Spearman's rank Correlation (Mantecca et al. 2003). The average of maturity indexes of samples from the same substrate of each of three depths was used. The correlation between the upper (6-17 meters) and middle (12-23 meters), middle and lower (21-32 meters), and upper and lower depths were compared individually using Spearman's rank correlation. Las Vegas Bay 3.5 samples will be exempted from this test as only one depth was sampled.

Analysis of Variance

Finally, an analysis of variance was used to test for variability of maturity index between sites. This could be used to show if the input of Las Vegas's effluent at Las Vegas wash alone creates enough change in water chemistry to alter the reproductive activity of quagga mussels. The average maturity index will be used again as an output variable for the ANOVA with site as the input.

CHAPTER 4

RESULTS

The study revealed mussels to be capable of producing during each of 10 sampling periods. Mussels were found of each reproductive state. Spawning appeared to peak twice in the months of June and September with a smaller third peak in December at all but the deepest collection depths.

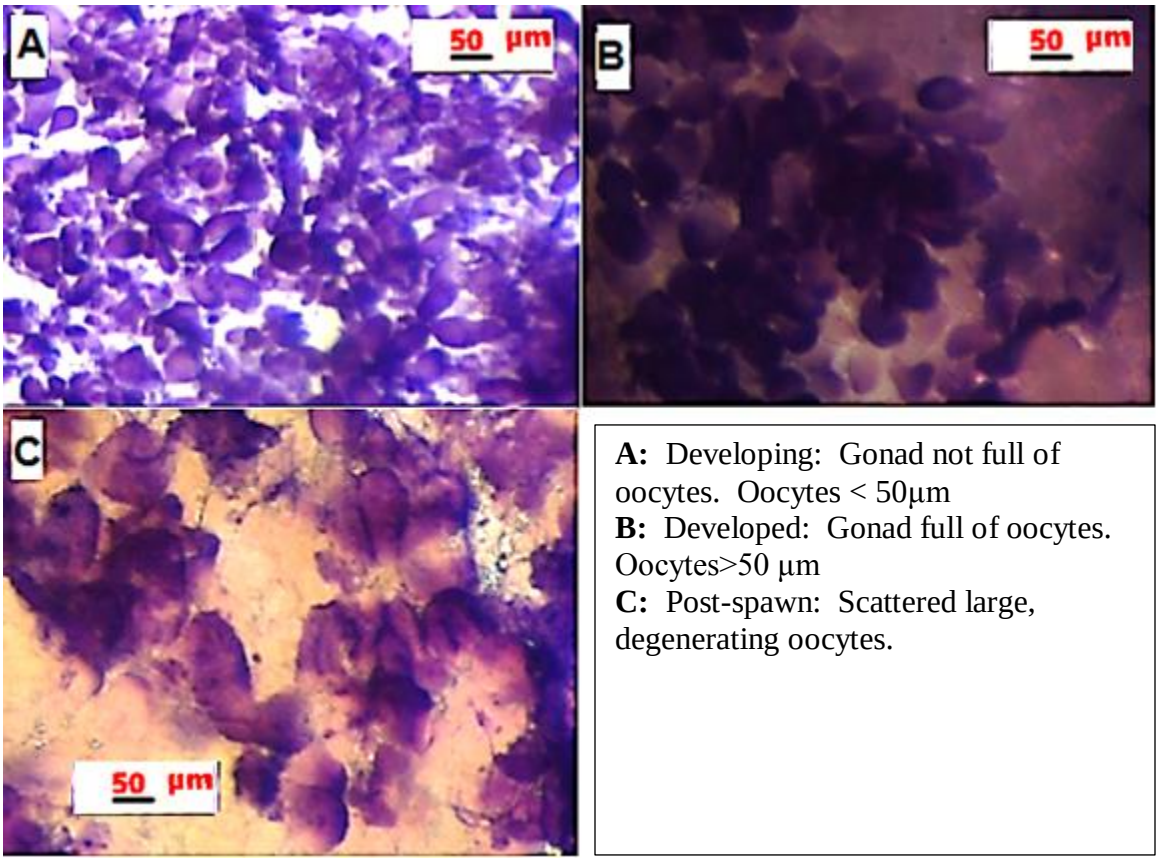

Figure 5 Reproductive State of Female Quagga Mussels from Lake Mead. Viewed microscopically after histological processing. Tissues cut into 10um sections and stained with hematoxylin and eosin, then viewed with a SteREO Discovery V8 stereo microscope was used at 8X magnification.

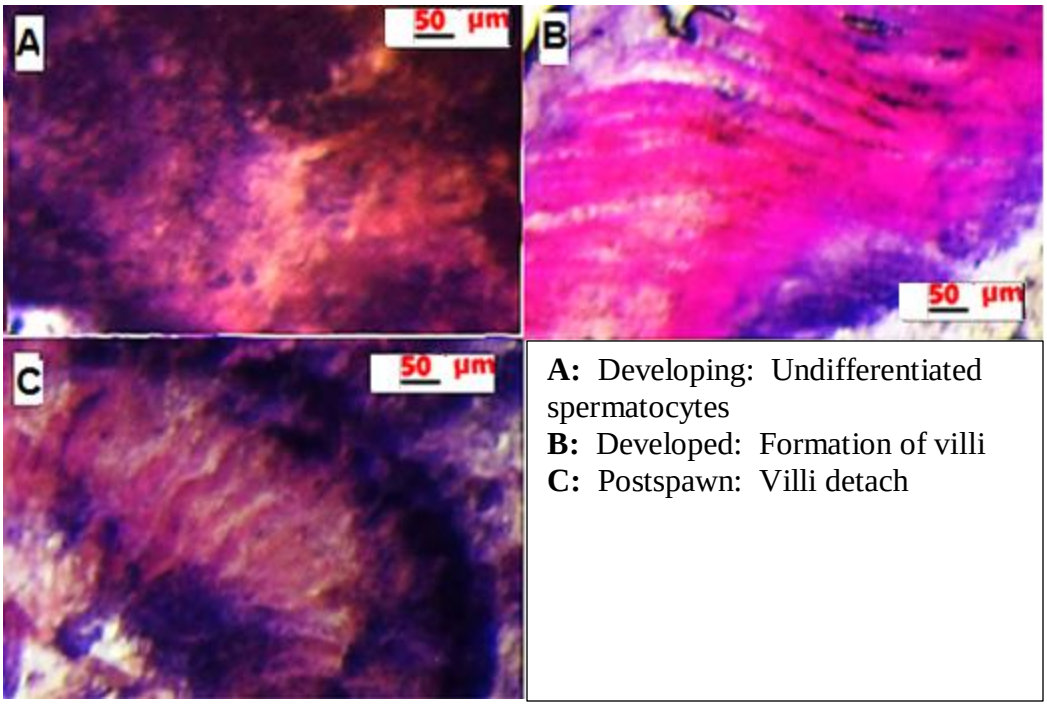

Figure 6 Reproductive States of Male Quagga Mussels from Lake Mead. Viewed microscopically after histological processing. Tissues cut into 10um sections and stained with hematoxylin and eosin, then viewed with a SteREO Discovery V8 stereo microscope was used at 8X magnification.

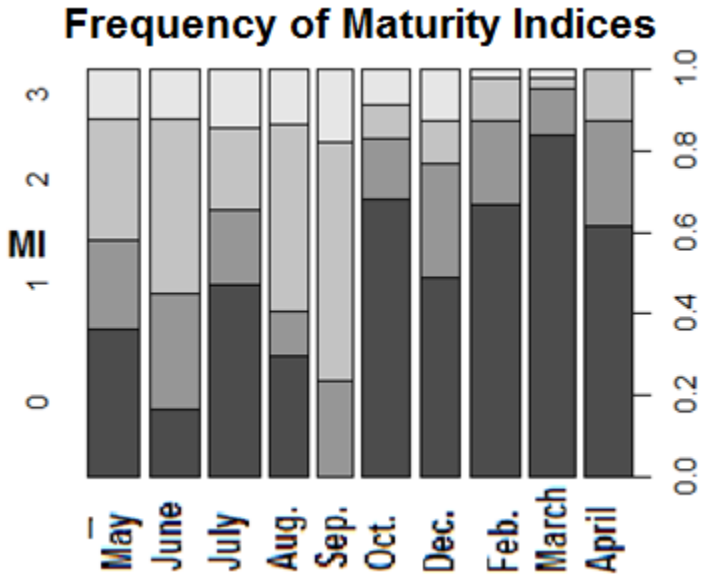

Figure 7 Frequency of Maturity Indices. 0= inactive, 1= developing, 2= pre-spawn, 3=post-spawn (n=445)

Linear Mixed Model

Neither dissolved oxygen, conductivity nor depth showed a significant effect on the average maturity index with $p>0.05$. Temperature did show a significant effect with $p<0.05$.

Table 7: Linear Mixed Model Results

	Value	Std. Error	Degrees of Freedom	t-value	p-value
Temperature	1.915	1.347	65	2.413	0.019 *
Dissolved Oxygen	0.198	1.066	65	0.186	0.853
Conductivity	0.149	0.958	65	0.156	0.877
Depth	-0.061	0.541	28	-0.113	0.911

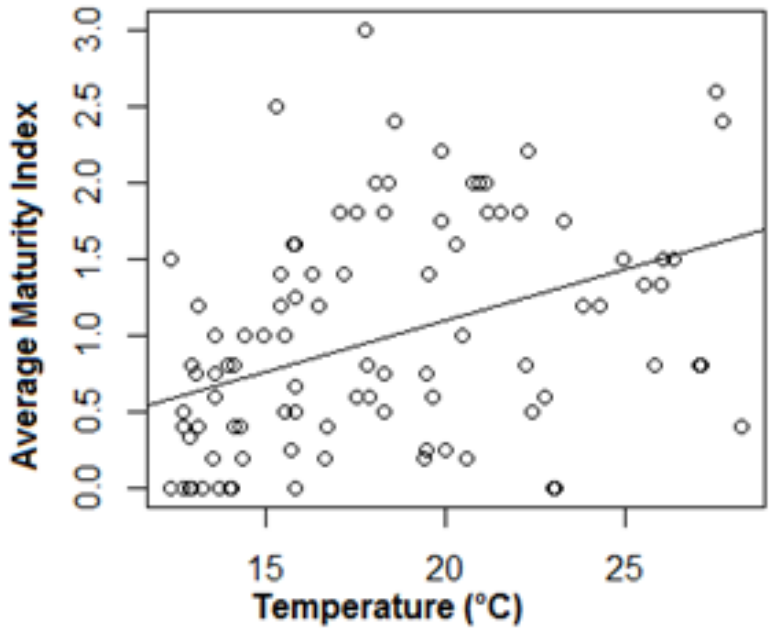

Figure 8 Average Maturity Index as a Function of Temperature. (n=98)

Spearman’s Rank Correlation

Spearman’s rank correlation between the upper and middle, and middle and lower depths showed significant correlation with rho values of 0.49 and 0.43 respectively and $p<0.05$ indicating that maturity indexes between these sites showed significant correlation throughout the year. However between upper and lower depths, rho was only .15 with $p>0.05$ indicating that between these depths maturity indexes did not show significant correlation.

Table 8: Spearman's Rank Correlation between depths results

Depth Range X	Depth Range Y	rho	P value
Upper: 6-17 meters	Middle: 12-23 meters	0.491	0.007*
Middle: 12-23 meters	Lower: 21-32 meters	0.429	0.020*
Upper: 6-17 meters	Lower: 21-32 meters	0.149	0.447

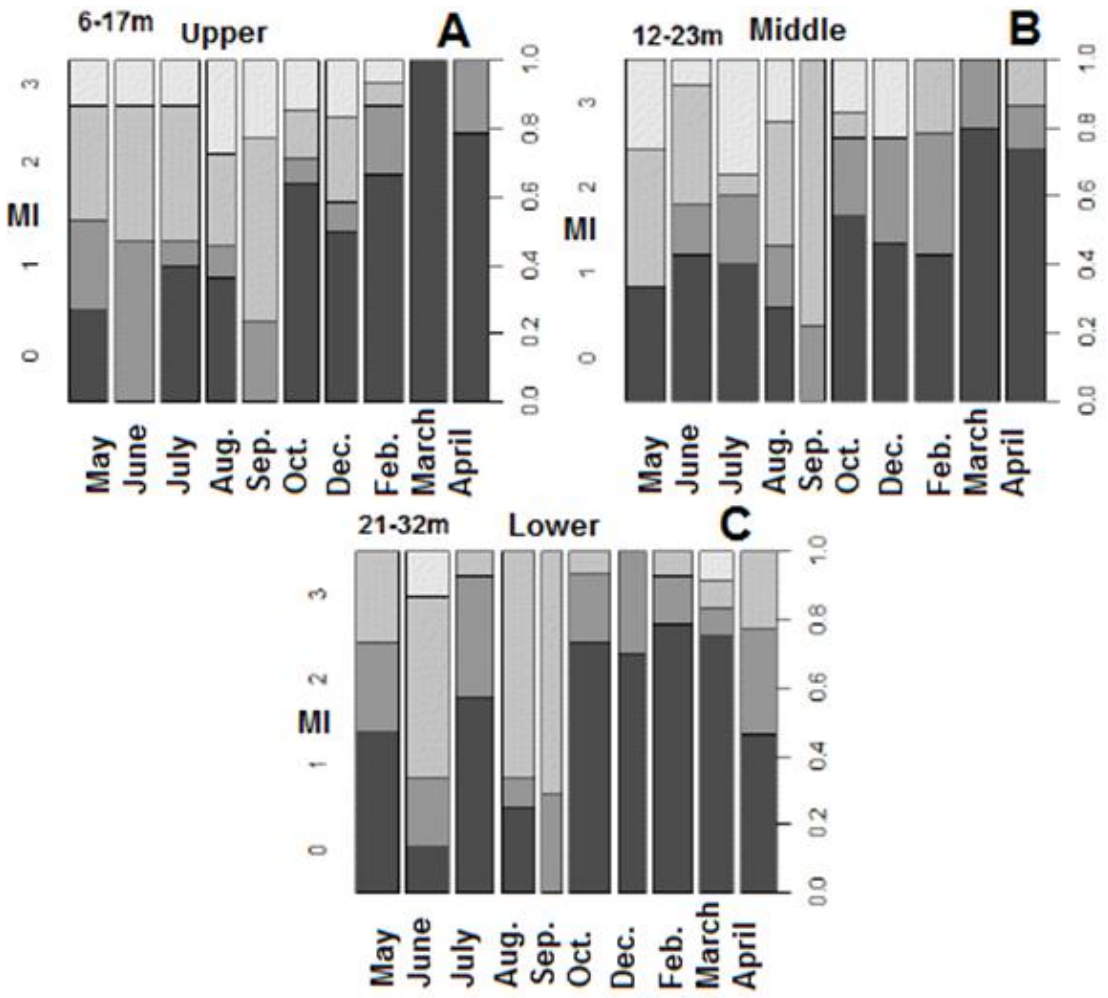

Figure 9 Frequency of Maturity Indices by Depth. 0= inactive, 1= developing, 2= pre-spawn, 3=post-spawn (n=445)

Analysis of Variance

The analysis of variance of average maturity index between sites provided a p value of .22 ($p>.05$) showing no significant difference in maturity index between sites, as alpha was set at $\alpha=.05$.

Table 9: Analysis of variance of maturity index between sites

Degrees of Freedom	Sum of Squares	Mean Square	F value	P value
4	3.161	0.790	1.462	.220

CHAPTER 5

DISCUSSION

Water Temperature

The result that temperature significantly affects the maturity index of quagga mussel gonads is not a particularly surprising one. Quagga mussels are known to have reproductive peaks in the summer months when temperatures are highest (Gerstenberger et al. 2011). It is important to note that despite temperature's effect on reproduction, mussels were found reproductively active at the highest (28.2°C) and lowest (12.7°C) temperatures indicating that during the year mussels were sampled, temperatures did not exceed an acceptable range for quagga mussel reproduction in Lake Mead. Based on the relation between average maturity index and temperature (Figure **6**), reproductive activity increased the most between 12 and 18°C. These data appear similar to that found on Zebra mussels in Fong et al. (1995) where reproductive activity was highest at 20°C, slightly lower at 27°C and lowest at 12°C. With fewer samples taken above 25°C it is possible though not necessarily apparent that these high temperatures inhibit reproduction.

It has been suggested that a temperature 12°C initiates spawning in zebra mussels (Mantecca et al. 2003.) If this were true for quagga mussels as well, then this event may not occur with temperatures consistently above 12°C. These constantly high temperatures allowed mussels to spawn year round, allowing two or three reproductive cycle within one year, while in many locations such as the Great Lakes there is typically only one. However the temperature still has a moderating effect on reproduction as exhibited by the data. Instead of affecting the ability of a mussel to spawn or the number of times spawning occurred in over the year, it seems that the temperature affected the frequency at which quagga mussels spawn in Lake Mead.

Depth and Seasonal Reproduction

The overall seasonal reproductive pattern between combined revealed two major peaks in reproductive activity as shown by the frequency of the different maturity indices (Figure 5). These appeared in the months April leading into June, and then in August leading into September. The existence of these two peaks is supported by existing annual veliger count data (Gerstenberger et al. 2011). There also seems to be a smaller peak in maturity index in December, which did not show up in the trend of overall veliger abundance.

The results of the three Spearman's rank correlation tests indicate that while the seasonal reproductive pattern is significantly similar between the upper (Figure 6A) and middle depths (Figure 6B), and the middle and lower depths (Figure 6C). However, the upper (6A) and lower (6C) depths were not shown to be significantly similar. These data indicate a gradual change of reproductive pattern with depth. Mantecca et al. (2003) demonstrated a difference in reproduction between shallow and deep depths, but as only two depths were examined, did not reveal whether or not this variation was continuous or discrete.

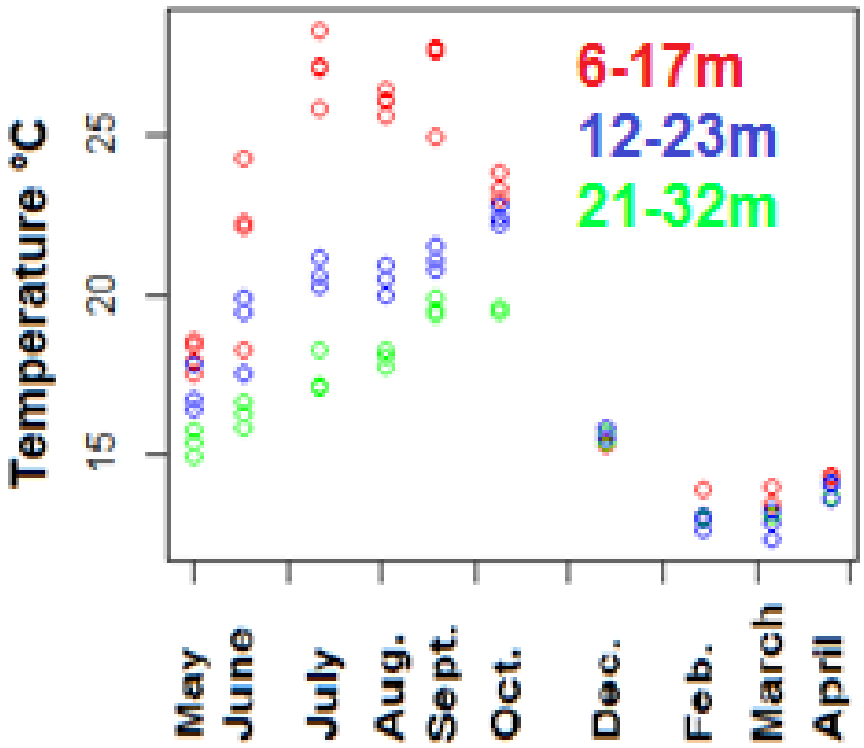

Figure 10 Temperature of Sites by Depth over the Sampling Period. (n=100)

This smaller peak in reproduction may partially explain the result of Spearman's rank correlation between the upper and lower collection depths. When looking at individual depths, the upper (Figure 6A) and middle (Figure 6B) depths clearly show the December increase in reproduction. However, at lower (Figure XC) depths in December, there were no mussels in the spawning or post spawned staged. A second difference between upper and lower depths appears to be the reproductive states during the months of March and April. In this case, mussels with developed gonads were found at lower

depths in March and April with developed gonads, where none were found at the upper depths in these months, and none were found at the middle depth in March.

These differences support the concept that temperature affects the frequency of spawning in quagga mussels. The mussels at the upper and middle depth for instance, had approximately a 3 month interval between the June and September Spawning events. Temperatures in September and October were warmer and these mussels appeared to have another spawning peak again in December. However at the lower depths sampled water temperatures were lower and spawning appeared to lag behind, perhaps not spawning until as late as February, March and April, which would explain the higher incidence of spawning at the deepest depths in these months.

Dissolved Oxygen, Salinity and Site

The analysis of variance between sites showed no significant variation in maturity indexes between sites. This indicates that the input of Las Vegas's effluent at Las Vegas Wash did not significantly affect the reproduction of the mussels, and that environmental conditions at all sites were within the bounds of what quagga mussels require for reproduction. This aligns with the results of the linear mixed effects model which failed to find both dissolved oxygen and conductivity (as a measure of salinity) as significant variables in the prediction of maturity index.

Salinity (derived from temperature and conductivity, UNESCO 1983) ranged between values of 0.449 and 0.926 psu (practical salinity units). The lack of difference here was not surprising as even the highest value of .926 psu is not particularly high. Fong et al. (1995) showed zebra mussels were able to reproduce at all salinities measured ranging from 0 to 7 psu.

Dissolved oxygen concentrations ranged between 2.7 and 12.38 mg/L. Even though the lower range of these dissolved oxygen rates is considered hypoxic, mussels were still found with developing or developed gonads. Quagga mussels are known to inhabit waters in Lake Mead of over 100 meters. This result indicates that mussels living in these dark hypoxic conditions may indeed still be able to reproduce.

The lack of variation between sites is perhaps surprising considering the large amount of variables that are introduced by the input of effluent water by Las Vegas Wash, but considering the confounding nature of all of these environmental variables, it is still difficult to tell if this is the case. Specifically, the warmer temperatures found closer to Las Vegas Wash may have a positive effect upon spawning in these locations, while other environmental conditions and pollutants may have a negative effect, leading to insignificant net change in reproduction.

Study Implications

The exhibited lack of effect of variables such as salinity, dissolved oxygen, and small (non-seasonal) variations in temperature has many implications in reservoir and aquatic nuisance species management. Within Lake Mead itself, these results indicate that typical changes in water temperature and water chemistry twill not significantly affect quagga mussel reproduction. Even changes in depth which can vary greatly over the course of a year, likely do not have huge impacts on quagga mussel reproduction, excepting if water level lowered dramatically to cause desiccation of large mussel populations.

In regards to invasive species management, this study supports what the establishment of quagga mussels in the Lower Colorado River already suggested: that

quagga mussels are a highly adaptable species that can not only inhabit, but thrive in a large variety of different lake and reservoir ecosystems. Quagga mussels appear to be able to reproduce at least two to three times each year. Those managing bodies of water not already affected by quagga mussels should not quickly ignore the possibility of a quagga mussel infestation, even if the unaffected body of water has conditions that are expected to lie outside of the realms of what quagga mussels would likely inhabit.

Lake and reservoir managers should not only consider the potential changes in water quality and threats to recreation, but also the public health effects of quagga mussel infestations, including additional chemical treatment of drinking water, and the possibility of toxic algal blooms.

Study Limitations

There were multiple limitations to the way in which this study was conducted. One major limitation was that mussels could only be sampled on a monthly basis. Monthly sampling lacks the resolution required to closely follow the reproduction cycle. Exact times of spawning events and small changes in spawning behavior may have been missed due to the low resolution of monthly sampling. Also, heavy winds prevented samples from being taken in the months of November and January decreasing the sampling resolution further.

Also, when removing mussels from substrates to be processed histologically, the largest mussels from each site were taken for ease of processing. Ideally, shell length would have been controlled between samples. For instance, instead of removing the largest mussels, one mussel of 15, 16, 17, 18, and 19mm could have been removed and studied from each site. Jantz et al. (1993) displayed that with zebra mussels the number of oocytes found in a mature gonad changes with mussel length but did not show that the length of adult mussels altered their reproductive cycle. In fact, in this study zebra mussels that settled in the month of May were found to grow to 9mm and spawn synchronously with already mature mussels as soon as three months afterwards in August. This provides some evidence that the lack of control for shell size may not have significantly affected the time of gonad maturation and spawning.

Water analysis at the site of mussel collection may provide more accurate analysis. SNWA sampling was done from a boat, and would have occurred vertically, whereas there were horizontal differences in sampling of mussels to obtain different depths, so measurements may not have been exact. Also, if more variables could be included, such

as suspended solids, chlorophyll-a, or levels of certain endocrine disruptors, a more comprehensive view of environmental effects on quagga mussel spawning could have been identified.

The use of a hand microtome during histological processing made it difficult to cut uniform 10μm tissue sections, and also did not allow for serial sectioning of the gonadal tissue. Using a rotary microtome, a cryotome, or other more advanced methods would likely provide clearer microscopic images of the gonadal tissues. This should allow easier identification of male and female mussels for samples with undeveloped gonads.

Future Research

This study is one of the first on quagga mussel reproduction in Lake Mead and in the Southwestern United States and there is still a great deal to be learned. Only a few environmental parameters were addressed, and only a small portion of Lake Mead was examined. For instance, examining phytoplankton and zooplankton availability as a way to compare potential food abundance with reproductive activity would provide a greater understanding of the trophic role of quagga mussels in ecosystems.

Quagga mussels were only taken from depths of up to 32 meters within Lake Mead for this study, however they exist at depths over 100 meters. Determining if there is in fact a maximum depth at which quagga mussels are reproductively viable could beneficial in understanding how well quagga mussels can withstand hypoxic, extremely low light conditions. The results of this study already indicate that quagga mussels might be reproductively viable down to extreme depths.

Quagga mussels have been established in several reservoirs in the Southwestern United States. Mussels exist downstream of Lake Mead at Lake Mohave and Lake Havasu, where chemical, physical and biological conditions including shallower depths, and higher temperatures. The reproductive cycle of quagga mussels could very likely vary in between other reservoirs within the same region.

Conclusions

The invasion of quagga mussels in Lake Mead and elsewhere has proved to be a serious economic, environmental and public health issue, due to their ability to attach to and foul boats and water infrastructure, their alteration of ecosystems through heavy consumption of zooplankton and phytoplankton within bodies of water, and their effect on water quality through their presence or treatment of their presence. Their extremely high populations caused by rapid reproduction allow all of these problems to escalate into serious issues within a few years of them colonizing a body of water. It is therefore critically important to understand this process of reproduction and what variables do or do not affect it.

The results of this study showed that within Lake Mead, several environmental variables had no significant effect on quagga mussel reproduction, and that at different sites and depths, the spawning varied over the course of the year independent of these other inputs. This provides further evidence for the high affectivity of quagga mussels as invasive species in the Southwestern United States and likely elsewhere.

Appendix

Table 10: Raw data of Maturity Index, Length and Sex of each mussel. Sex was indeterminate in reproductively inactive mussels. Data are from the individual measurement of mussels sampled and the results of the following histological processing.

Date	Site	length (mm)	Sex	Maturity Index
5/19/2011	LVB 3.5	13	m	2
5/19/2011	LVB 3.5	14	f	2
5/19/2011	LVB 3.5	12	m	2
5/19/2011	LVB 3.5	13	m	3
5/19/2011	LVB 3.5	13	f	3
5/19/2011	LVB 4.95	26	na	0
5/19/2011	LVB 4.95	24	f	2
5/19/2011	LVB 4.95	23	f	3
5/19/2011	LVB 4.95	22	f	2
5/19/2011	LVB 4.95	19	m	3
5/19/2011	LVB 4.95	23	m	2
5/19/2011	LVB 4.95	22	f	2
5/19/2011	LVB 4.95	19	na	0
5/19/2011	LVB 4.95	17	m	2
5/19/2011	LVB 4.95	16	na	0
5/19/2011	LVB 4.95	21	f	2
5/19/2011	LVB 4.95	27	na	0
5/19/2011	LVB 4.95	24	na	0
5/19/2011	LVB 4.95	19	m	3
5/19/2011	LVB 4.95	22	f	3
5/19/2011	LVB 7.3	24	na	0
5/19/2011	LVB 7.3	23	m	1
5/19/2011	LVB 7.3	21	m	1
5/19/2011	LVB 7.3	20	f	1
5/19/2011	LVB 7.3	20	na	0
5/19/2011	LVB 7.3	22	f	1
5/19/2011	LVB 7.3	21	na	0
5/19/2011	LVB 7.3	20	na	0
5/19/2011	LVB 7.3	24	m	1
5/19/2011	LVB 7.3	16	na	0
5/19/2011	LVB 7.3	17	f	2
5/19/2011	LVB 7.3	18	na	0
5/19/2011	LVB 7.3	15	f	1
5/19/2011	LVB 7.3	16	f	1
5/19/2011	LVB 7.3	15	m	2
5/19/2011	CR 346.4	17	na	0
5/19/2011	CR 346.4	15	m	1
5/19/2011	CR 346.4	16	f	1
5/19/2011	CR 346.4	13	na	0
5/19/2011	CR 346.4	14	m	1

5/19/2011	CR 346.4	15	na	0
5/19/2011	CR 346.4	15	f	2
5/19/2011	CR 346.4	14	f	2
5/19/2011	CR 346.4	15	na	0
5/19/2011	CR 346.4	15	na	0
5/19/2011	CR 346.4	22	na	0
5/19/2011	CR 346.4	16	na	2
5/19/2011	CR 346.4	18	na	0
5/19/2011	CR 346.4	20	m	2
5/19/2011	CR 346.4	10	f	1
6/14/2011	LVB 3.5	24	f	1
6/14/2011	LVB 3.5	19	m	1
6/14/2011	LVB 3.5	20	m	1
6/14/2011	LVB 3.5	25	m	1
6/14/2011	LVB 3.5	17	f	2
6/14/2011	LVB 4.95	19	m	2
6/14/2011	LVB 4.95	20	f	2
6/14/2011	LVB 4.95	22	m	2
6/14/2011	LVB 4.95	23	m	3
6/14/2011	LVB 4.95	20	m	2
6/14/2011	LVB 4.95	22	f	2
6/14/2011	LVB 4.95	21	m	1
6/14/2011	LVB 4.95	23	na	0
6/14/2011	LVB 4.95	17	na	na
6/14/2011	LVB 4.95	17	na	0
6/14/2011	LVB 4.95	17	na	0
6/14/2011	LVB 4.95	23	na	0
6/14/2011	LVB 4.95	19	f	1
6/14/2011	LVB 4.95	23	na	0
6/14/2011	LVB 4.95	18	na	0
6/14/2011	LVB 7.3	21	f	2
6/14/2011	LVB 7.3	20	f	3
6/14/2011	LVB 7.3	15	f	2
6/14/2011	LVB 7.3	16	f	1
6/14/2011	LVB 7.3	20	m	1
6/14/2011	LVB 7.3	20	f	3
6/14/2011	LVB 7.3	21	f	2
6/14/2011	LVB 7.3	24	m	2
6/14/2011	LVB 7.3	21	m	1
6/14/2011	LVB 7.3	20	f	3
6/14/2011	LVB 7.3	24	m	1
6/14/2011	LVB 7.3	18	f	2
6/14/2011	LVB 7.3	19	m	1
6/14/2011	LVB 7.3	20	m	2
6/14/2011	LVB 7.3	22	m	2
6/14/2011	CR 346.4	16	f	3

6/14/2011	CR 346.4	15	f	2
6/14/2011	CR 346.4	16	f	3
6/14/2011	CR 346.4	15	na	0
6/14/2011	CR 346.4	14	f	1
6/14/2011	CR 346.4	19	f	2
6/14/2011	CR 346.4	18	f	2
6/14/2011	CR 346.4	15	m	2
6/14/2011	CR 346.4	19	m	2
6/14/2011	CR 346.4	20	m	1
6/14/2011	CR 346.4	15	f	2
6/14/2011	CR 346.4	16	m	1
6/14/2011	CR 346.4	18	na	0
6/14/2011	CR 346.4	19	m	2
6/14/2011	CR 346.4	20	f	2
7/25/2011	LVB 3.5	24	na	0
7/25/2011	LVB 3.5	24	na	0
7/25/2011	LVB 3.5	22	na	0
7/25/2011	LVB 3.5	19	na	0
7/25/2011	LVB 3.5	18	f	2
7/25/2011	LVB 4.95	25	na	0
7/25/2011	LVB 4.95	26	f	1
7/25/2011	LVB 4.95	22	f	3
7/25/2011	LVB 4.95	24	na	0
7/25/2011	LVB 4.95	20	na	0
7/25/2011	LVB 4.95	21	m	1
7/25/2011	LVB 4.95	23	f	2
7/25/2011	LVB 4.95	23	f	3
7/25/2011	LVB 4.95	24	na	0
7/25/2011	LVB 4.95	24	f	3
7/25/2011	LVB 4.95	16	f	1
7/25/2011	LVB 4.95	17	na	0
7/25/2011	LVB 4.95	23	f	3
7/25/2011	LVB 4.95	22	f	3
7/25/2011	LVB 4.95	24	na	0
7/25/2011	LVB 7.3	23	na	0
7/25/2011	LVB 7.3	24	na	0
7/25/2011	LVB 7.3	23	m	2
7/25/2011	LVB 7.3	20	m	2
7/25/2011	LVB 7.3	14	na	0
7/25/2011	LVB 7.3	20	m	2
7/25/2011	LVB 7.3	20	m	1
7/25/2011	LVB 7.3	17	f	2
7/25/2011	LVB 7.3	15	f	3
7/25/2011	LVB 7.3	12	na	0
7/25/2011	LVB 7.3	20	f	2
7/25/2011	LVB 7.3	21	na	0

7/25/2011	LVB 7.3	20	f	2
7/25/2011	LVB 7.3	16	m	2
7/25/2011	LVB 7.3	13	f	3
7/25/2011	CR 346.4	17	na	0
7/25/2011	CR 346.4	14	f	1
7/25/2011	CR 346.4	14	na	0
7/25/2011	CR 346.4	12	m	1
7/25/2011	CR 346.4	NA	m	2
7/25/2011	CR 346.4	14	na	0
7/25/2011	CR 346.4	13	f	1
7/25/2011	CR 346.4	13	na	0
7/25/2011	CR 346.4	12	na	0
7/25/2011	CR 346.4	NA	na	0
7/25/2011	CR 346.4	16	m	1
7/25/2011	CR 346.4	16	na	0
7/25/2011	CR 346.4	15	na	0
7/25/2011	CR 346.4	15	f	1
7/25/2011	CR 346.4	13	na	na
8/29/2011	LVB 3.5	18	m	2
8/29/2011	LVB 3.5	19	na	0
8/29/2011	LVB 3.5	19	f	2
8/29/2011	LVB 3.5	16	m	2
8/29/2011	LVB 3.5	17	na	na
8/29/2011	LVB 4.95	18	na	na
8/29/2011	LVB 4.95	17	f	2
8/29/2011	LVB 4.95	16	f	2
8/29/2011	LVB 4.95	NA	na	na
8/29/2011	LVB 4.95	15	na	0
8/29/2011	LVB 4.95	17	f	2
8/29/2011	LVB 4.95	19	na	na
8/29/2011	LVB 4.95	17	na	0
8/29/2011	LVB 4.95	18	f	2
8/29/2011	LVB 4.95	16	na	0
8/29/2011	LVB 4.95	18	m	3
8/29/2011	LVB 4.95	16	na	na
8/29/2011	LVB 4.95	20	f	1
8/29/2011	LVB 4.95	20	f	3
8/29/2011	LVB 4.95	19	f	1
8/29/2011	LVB 7.3	20	na	0
8/29/2011	LVB 7.3	17	f	2
8/29/2011	LVB 7.3	20	na	na
8/29/2011	LVB 7.3	19	m	2
8/29/2011	LVB 7.3	20	f	na
8/29/2011	LVB 7.3	21	na	0
8/29/2011	LVB 7.3	24	na	na
8/29/2011	LVB 7.3	23	na	0

8/29/2011	LVB 7.3	21	na	0
8/29/2011	LVB 7.3	22	m	1
8/29/2011	LVB 7.3	20	na	na
8/29/2011	LVB 7.3	20	f	3
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8/29/2011	CR 346.4	13	f	2
8/29/2011	CR 346.4	10	na	0
8/29/2011	CR 346.4	13	m	2
8/29/2011	CR 346.4	14	f	2
8/29/2011	CR 346.4	13	na	na
8/29/2011	CR 346.4	12	f	2
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8/29/2011	CR 346.4	20	f	1
8/29/2011	CR 346.4	13	m	2
8/29/2011	CR 346.4	18	na	0
8/29/2011	CR 346.4	18	na	0
9/25/2011	LVB 3.5	16	m	3
9/25/2011	LVB 3.5	19	m	2
9/25/2011	LVB 3.5	15	m	2
9/25/2011	LVB 3.5	17	m	3
9/25/2011	LVB 3.5	19	f	3
9/25/2011	LVB 4.95	NA	na	na
9/25/2011	LVB 4.95	NA	na	na
9/25/2011	LVB 4.95	NA	na	na
9/25/2011	LVB 4.95	NA	na	na
9/25/2011	LVB 4.95	NA	na	na
9/25/2011	LVB 4.95	18	f	2
9/25/2011	LVB 4.95	20	m	2
9/25/2011	LVB 4.95	17	m	2
9/25/2011	LVB 4.95	17	m	2
9/25/2011	LVB 4.95	18	m	1
9/25/2011	LVB 4.95	23	f	2
9/25/2011	LVB 4.95	21	m	1
9/25/2011	LVB 4.95	21	f	2
9/25/2011	LVB 4.95	20	m	2
9/25/2011	LVB 4.95	NA	na	na
9/25/2011	LVB 7.3	17	f	3
9/25/2011	LVB 7.3	15	m	2
9/25/2011	LVB 7.3	15	f	3
9/25/2011	LVB 7.3	15	m	3

9/25/2011	LVB 7.3	18	m	1
9/25/2011	LVB 7.3	17	na	na
9/25/2011	LVB 7.3	15	na	na
9/25/2011	LVB 7.3	16	f	2
9/25/2011	LVB 7.3	20	m	2
9/25/2011	LVB 7.3	17	f	2
9/25/2011	LVB 7.3	21	m	2
9/25/2011	LVB 7.3	21	f	1
9/25/2011	LVB 7.3	20	m	2
9/25/2011	LVB 7.3	21	f	1
9/25/2011	LVB 7.3	19	f	1
9/25/2011	CR 346.4	15	m	1
9/25/2011	CR 346.4	13	f	2
9/25/2011	CR 346.4	14	na	na
9/25/2011	CR 346.4	12	f	2
9/25/2011	CR 346.4	13	m	1
9/25/2011	CR 346.4	10	m	2
9/25/2011	CR 346.4	12	m	2
9/25/2011	CR 346.4	11	m	2
9/25/2011	CR 346.4	12	na	na
9/25/2011	CR 346.4	NA	na	na
9/25/2011	CR 346.4	NA	na	na
9/25/2011	CR 346.4	NA	na	na
9/25/2011	CR 346.4	NA	na	na
9/25/2011	CR 346.4	NA	na	na
9/25/2011	CR 346.4	NA	na	na
10/28/2011	LVB 3.5	17	f	3
10/28/2011	LVB 3.5	19	na	0
10/28/2011	LVB 3.5	20	f	1
10/28/2011	LVB 3.5	16	m	2
10/28/2011	LVB 3.5	17	na	0
10/28/2011	LVB 4.95	26	f	1
10/28/2011	LVB 4.95	20	f	1
10/28/2011	LVB 4.95	23	na	na
10/28/2011	LVB 4.95	20	m	2
10/28/2011	LVB 4.95	23	f	3
10/28/2011	LVB 4.95	20	f	3
10/28/2011	LVB 4.95	21	na	0
10/28/2011	LVB 4.95	20	na	0
10/28/2011	LVB 4.95	20	na	0
10/28/2011	LVB 4.95	21	na	0
10/28/2011	LVB 4.95	23	na	0
10/28/2011	LVB 4.95	22	na	na
10/28/2011	LVB 4.95	23	na	0
10/28/2011	LVB 4.95	19	f	1
10/28/2011	LVB 4.95	19	na	0

10/28/2011	LVB 7.3	15	na	0
10/28/2011	LVB 7.3	13	na	0
10/28/2011	LVB 7.3	12	na	0
10/28/2011	LVB 7.3	12	na	0
10/28/2011	LVB 7.3	10	na	0
10/28/2011	LVB 7.3	17	na	0
10/28/2011	LVB 7.3	17	na	0
10/28/2011	LVB 7.3	18	na	0
10/28/2011	LVB 7.3	15	m	2
10/28/2011	LVB 7.3	NA	na	na
10/28/2011	LVB 7.3	21	na	0
10/28/2011	LVB 7.3	20	na	0
10/28/2011	LVB 7.3	24	na	0
10/28/2011	LVB 7.3	20	m	3
10/28/2011	LVB 7.3	20	na	0
10/28/2011	CR 346.4	14	na	0
10/28/2011	CR 346.4	14	na	0
10/28/2011	CR 346.4	14	na	0
10/28/2011	CR 346.4	14	na	0
10/28/2011	CR 346.4	13	na	0
10/28/2011	CR 346.4	15	na	0
10/28/2011	CR 346.4	14	m	1
10/28/2011	CR 346.4	14	na	0
10/28/2011	CR 346.4	13	na	1
10/28/2011	CR 346.4	12	na	2
10/28/2011	CR 346.4	20	na	0
10/28/2011	CR 346.4	19	m	1
10/28/2011	CR 346.4	18	na	0
10/28/2011	CR 346.4	17	na	0
10/28/2011	CR 346.4	17	na	0
12/23/2011	LVB 3.5	20	f	3
12/23/2011	LVB 3.5	19	m	2
12/23/2011	LVB 3.5	23	m	3
12/23/2011	LVB 3.5	22	na	na
12/23/2011	LVB 3.5	21	f	2
12/23/2011	LVB 4.95	19	f	3
12/23/2011	LVB 4.95	18	na	na
12/23/2011	LVB 4.95	21	na	0
12/23/2011	LVB 4.95	18	na	0
12/23/2011	LVB 4.95	20	f	1
12/23/2011	LVB 4.95	20	f	1
12/23/2011	LVB 4.95	18	m	1
12/23/2011	LVB 4.95	20	na	0
12/23/2011	LVB 4.95	18	na	0
12/23/2011	LVB 4.95	22	na	na
12/23/2011	LVB 4.95	22	f	3

12/23/2011	LVB 4.95	20	na	0
12/23/2011	LVB 4.95	19	f	1
12/23/2011	LVB 4.95	19	f	3
12/23/2011	LVB 4.95	18	na	0
12/23/2011	LVB 7.3	20	na	na
12/23/2011	LVB 7.3	20	f	1
12/23/2011	LVB 7.3	20	f	1
12/23/2011	LVB 7.3	18	m	2
12/23/2011	LVB 7.3	19	f	1
12/23/2011	LVB 7.3	20		0
12/23/2011	LVB 7.3	18	na	0
12/23/2011	LVB 7.3	18	na	na
12/23/2011	LVB 7.3	16	m	2
12/23/2011	LVB 7.3	NA	na	0
12/23/2011	LVB 7.3	21	na	0
12/23/2011	LVB 7.3	20	na	na
12/23/2011	LVB 7.3	20	na	0
12/23/2011	LVB 7.3	19	na	0
12/23/2011	LVB 7.3	20	f	1
12/23/2011	CR 346.4	22	na	na
12/23/2011	CR 346.4	18	na	0
12/23/2011	CR 346.4	20	f	1
12/23/2011	CR 346.4	17	na	na
12/23/2011	CR 346.4	16	f	1
12/23/2011	CR 346.4	18	na	0
12/23/2011	CR 346.4	16	na	0
12/23/2011	CR 346.4	15	na	0
12/23/2011	CR 346.4	13	na	0
12/23/2011	CR 346.4	14	na	0
12/23/2011	CR 346.4	18	na	na
12/23/2011	CR 346.4	18	f	1
12/23/2011	CR 346.4	16	na	na
12/23/2011	CR 346.4	15	na	na
12/23/2011	CR 346.4	16	na	0
2/13/2012	LVB 3.5	18	m	1
2/13/2012	LVB 3.5	19	m	3
2/13/2012	LVB 3.5	19	na	0
2/13/2012	LVB 3.5	17	na	0
2/13/2012	LVB 3.5	19	na	0
2/13/2012	LVB 4.95	19	m	1
2/13/2012	LVB 4.95	20	na	0
2/13/2012	LVB 4.95	22	na	0
2/13/2012	LVB 4.95	20	na	0
2/13/2012	LVB 4.95	19	m	1
2/13/2012	LVB 4.95	24	na	0
2/13/2012	LVB 4.95	24	f	1

2/13/2012	LVB 4.95	20	f	1
2/13/2012	LVB 4.95	20	m	2
2/13/2012	LVB 4.95	18	f	2
2/13/2012	LVB 4.95	22	na	na
2/13/2012	LVB 4.95	21	m	2
2/13/2012	LVB 4.95	20	f	1
2/13/2012	LVB 4.95	20	na	0
2/13/2012	LVB 4.95	20	na	0
2/13/2012	LVB 7.3	23	na	0
2/13/2012	LVB 7.3	18	na	0
2/13/2012	LVB 7.3	17	na	0
2/13/2012	LVB 7.3	17	na	0
2/13/2012	LVB 7.3	15	na	0
2/13/2012	LVB 7.3	25	na	0
2/13/2012	LVB 7.3	16	na	0
2/13/2012	LVB 7.3	15	na	0
2/13/2012	LVB 7.3	15	na	0
2/13/2012	LVB 7.3	13	na	0
2/13/2012	LVB 7.3	21	na	0
2/13/2012	LVB 7.3	17	m	1
2/13/2012	LVB 7.3	18	na	0
2/13/2012	LVB 7.3	20	m	1
2/13/2012	LVB 7.3	16	m	2
2/13/2012	CR 346.4	15	na	0
2/13/2012	CR 346.4	14	m	1
2/13/2012	CR 346.4	15	na	0
2/13/2012	CR 346.4	13	f	1
2/13/2012	CR 346.4	15	na	na
2/13/2012	CR 346.4	15	na	0
2/13/2012	CR 346.4	16	na	0
2/13/2012	CR 346.4	16	na	0
2/13/2012	CR 346.4	14	na	0
2/13/2012	CR 346.4	14	na	0
2/13/2012	CR 346.4	16	m	2
2/13/2012	CR 346.4	17	na	0
2/13/2012	CR 346.4	15	na	0
2/13/2012	CR 346.4	14	na	0
2/13/2012	CR 346.4	14	na	0
3/19/2012	LVB 3.5	25	na	0
3/19/2012	LVB 3.5	24	na	0
3/19/2012	LVB 3.5	22	na	0
3/19/2012	LVB 3.5	22	na	0
3/19/2012	LVB 3.5	21	na	0
3/19/2012	LVB 4.95	18	na	0
3/19/2012	LVB 4.95	23	na	0
3/19/2012	LVB 4.95	20	f	1

3/19/2012	LVB 4.95	18	na	0
3/19/2012	LVB 4.95	19	na	0
3/19/2012	LVB 4.95	20	na	0
3/19/2012	LVB 4.95	21	na	0
3/19/2012	LVB 4.95	17	na	0
3/19/2012	LVB 4.95	20	na	0
3/19/2012	LVB 4.95	16	na	0
3/19/2012	LVB 4.95	20	f	1
3/19/2012	LVB 4.95	22	na	0
3/19/2012	LVB 4.95	23	na	0
3/19/2012	LVB 4.95	24	f	1
3/19/2012	LVB 4.95	20	na	0
3/19/2012	LVB 7.3	22	f	1
3/19/2012	LVB 7.3	20	na	0
3/19/2012	LVB 7.3	23	na	0
3/19/2012	LVB 7.3	20	na	na
3/19/2012	LVB 7.3	19	na	na
3/19/2012	LVB 7.3	20	na	0
3/19/2012	LVB 7.3	17	na	0
3/19/2012	LVB 7.3	19	na	na
3/19/2012	LVB 7.3	16	na	0
3/19/2012	LVB 7.3	18	na	0
3/19/2012	LVB 7.3	22	na	0
3/19/2012	LVB 7.3	23	na	0
3/19/2012	LVB 7.3	23	na	0
3/19/2012	LVB 7.3	22	na	0
3/19/2012	LVB 7.3	21	na	0
3/19/2012	CR 346.4	16	na	0
3/19/2012	CR 346.4	18	na	na
3/19/2012	CR 346.4	17	na	0
3/19/2012	CR 346.4	15	na	0
3/19/2012	CR 346.4	14	na	0
3/19/2012	CR 346.4	16	na	0
3/19/2012	CR 346.4	16	na	0
3/19/2012	CR 346.4	16	na	0
3/19/2012	CR 346.4	17	na	0
3/19/2012	CR 346.4	18	na	na
3/19/2012	CR 346.4	15	na	0
3/19/2012	CR 346.4	15	na	1
3/19/2012	CR 346.4	16	na	2
3/19/2012	CR 346.4	16	na	3
3/19/2012	CR 346.4	16	na	na
4/20/2012	LVB 3.5	20	f	1
4/20/2012	LVB 3.5	24	na	0
4/20/2012	LVB 3.5	23	na	0
4/20/2012	LVB 3.5	20	na	0

4/20/2012	LVB 3.5	20	na	0
4/20/2012	LVB 4.95	19	na	0
4/20/2012	LVB 4.95	18	na	0
4/20/2012	LVB 4.95	25	m	1
4/20/2012	LVB 4.95	18	f	1
4/20/2012	LVB 4.95	22	na	0
4/20/2012	LVB 4.95	23	na	0
4/20/2012	LVB 4.95	20	na	0
4/20/2012	LVB 4.95	18	na	0
4/20/2012	LVB 4.95	18	na	0
4/20/2012	LVB 4.95	16	na	0
4/20/2012	LVB 4.95	18	na	0
4/20/2012	LVB 4.95	22	na	0
4/20/2012	LVB 4.95	22	na	0
4/20/2012	LVB 4.95	18	m	2
4/20/2012	LVB 4.95	18	m	2
4/20/2012	LVB 7.3	25	m	1
4/20/2012	LVB 7.3	23	m	2
4/20/2012	LVB 7.3	21	na	0
4/20/2012	LVB 7.3	20	f	1
4/20/2012	LVB 7.3	21	m	1
4/20/2012	LVB 7.3	19	m	1
4/20/2012	LVB 7.3	21	na	0
4/20/2012	LVB 7.3	19	f	1
4/20/2012	LVB 7.3	24	na	0
4/20/2012	LVB 7.3	22	na	0
4/20/2012	LVB 7.3	21	na	0
4/20/2012	LVB 7.3	21	na	0
4/20/2012	LVB 7.3	19	na	0
4/20/2012	LVB 7.3	19	na	0
4/20/2012	LVB 7.3	17	na	0
4/20/2012	CR 346.4	15	na	0
4/20/2012	CR 346.4	15	m	1
4/20/2012	CR 346.4	16	f	1
4/20/2012	CR 346.4	15	f	1
4/20/2012	CR 346.4	15	na	0
4/20/2012	CR 346.4	15	na	na
4/20/2012	CR 346.4	15	f	2
4/20/2012	CR 346.4	15	na	0
4/20/2012	CR 346.4	15	na	0
4/20/2012	CR 346.4	15	m	1
4/20/2012	CR 346.4	17	na	0
4/20/2012	CR 346.4	17	f	2
4/20/2012	CR 346.4	16	m	2
4/20/2012	CR 346.4	16	na	0
4/20/2012	CR 346.4	15	na	na

Table 11: Water Quality Data from the Southern Nevada Water Authority Water Quality Database. Date of water quality collection and date of mussel collection included. *Water quality data collected for SNWA by the Bureau of Reclamation.

Mussel Sample Data	Water Quality Date	Site	Depth (m)	Temperature °C	Conductivity uS/cm	Dissolved Oxygen mg/L
5/19/2011	5/18/2011	LVB 3.5	6.00	18.6	1594	7.79
5/19/2011	5/18/2011	LVB 4.95	6.00	18.4	1116	8.5
5/19/2011	5/18/2011	LVB 4.95	12.00	16.45	957.5	9.04
5/19/2011	5/18/2011	LVB 4.95	21.00	15.75	1015	9.09
5/19/2011	5/18/2011	LVB 7.3	6.00	17.54	966.5	9.63
5/19/2011	5/18/2011	LVB 7.3	12.00	16.73	946	9.75
5/19/2011	5/18/2011	LVB 7.3	21.00	15.4	944.2	9.6
5/19/2011	5/17/2011	CR 346.4	6.00	17.88	950	9.65
5/19/2011	5/17/2011	CR 346.4	12.00	17.83	954	9.72
5/19/2011	5/17/2011	CR 346.4	21.00	14.95	939	9.83
6/14/2011	6/13/2011	LVB 3.5	7.37	24.27	1172	8.15
6/14/2011	6/13/2011	LVB 4.95	7.37	22.27	1082	8.36
6/14/2011	6/13/2011	LVB 4.95	13.37	19.47	1090	7.77
6/14/2011	6/13/2011	LVB 4.95	22.37	16.64	1006	7.35
6/14/2011	6/13/2011	LVB 7.3	7.37	22.08	976.5	8.33
6/14/2011	6/13/2011	LVB 7.3	13.37	19.87	953.3	8.89
6/14/2011	6/13/2011	LVB 7.3	22.37	15.82	945	7.8
6/14/2011	6/15/2011	CR 346.4	7.37	18.3	975.9	9.68
6/14/2011	6/15/2011	CR 346.4	13.37	17.52	972.7	9.7
6/14/2011	6/15/2011	CR 346.4	22.37	16.29	961.2	9.52
7/25/2011	7/11/2011	LVB 3.5	8.80	28.24	1741	3.42
7/25/2011	7/11/2011	LVB 4.95	8.80	27.02	1301	5.71
7/25/2011	7/11/2011	LVB 4.95	14.80	21.16	1083	6.51
7/25/2011	7/11/2011	LVB 4.95	23.80	17.17	974	6.22
7/25/2011	7/11/2011	LVB 7.3	8.80	27.1	981	7.53
7/25/2011	7/11/2011	LVB 7.3	14.80	20.29	0	21.65
7/25/2011	7/11/2011	LVB 7.3	23.80	17.06	934	7.35
7/25/2011	7/26/2011	CR 346.4	8.80	25.8	1008	8.86
7/25/2011	7/26/2011	CR 346.4	14.80	20.58	947.7	10.3
7/25/2011	7/26/2011	CR 346.4	23.80	18.28	914.8	9.19
8/29/2011	8/24/2011	LVB 3.5	10.74	26.07	1369	3.29
8/29/2011	8/24/2011	LVB 4.95	10.74	25.98	1019	7.04
8/29/2011	8/24/2011	LVB 4.95	16.74	20.49	927	4.93
8/29/2011	8/24/2011	LVB 4.95	25.74	18.04	954	5.2
8/29/2011	8/24/2011	LVB 7.3	10.74	25.54	958	8.8
8/29/2011	8/24/2011	LVB 7.3	16.74	19.99	883	7.94
8/29/2011	8/24/2011	LVB 7.3	25.74	17.75	901	7.09
8/29/2011	8/23/2011	CR 346.4	10.74	26.35	1004	8.54
8/29/2011	8/23/2011	CR 346.4	16.74	20.96	874.4	8.64
8/29/2011	8/23/2011	CR 346.4	25.74	18.3	908.3	7.7

9/25/2011	9/12/2011	LVB 3.5	11.53	27.53	1352	8.2
9/25/2011	9/12/2011	LVB 4.95	11.53	27.63	1140	7.57
9/25/2011	9/12/2011	LVB 4.95	17.53	21.54	931	3.95
9/25/2011	9/12/2011	LVB 4.95	26.53	19.88	937	2.7
9/25/2011	9/12/2011	LVB 7.3	11.53	27.71	1021	7.77
9/25/2011	9/12/2011	LVB 7.3	17.53	21.1	841	7.63
9/25/2011	9/12/2011	LVB 7.3	26.53	19.55	856	6.31
9/25/2011	9/20/2011	CR 346.4	11.53	24.91	949.5	7.68
9/25/2011	9/20/2011	CR 346.4	17.53	20.75	851.2	7.41
9/25/2011	9/20/2011	CR 346.4	26.53	19.4	870.1	6.79
10/28/2011	10/16/2011	LVB 3.5	13.04	23.84	1348	7.63
10/28/2011	10/16/2011	LVB 4.95	13.04	23.27	1075	7.94
10/28/2011	10/16/2011	LVB 4.95	19.04	22.75	1255	5.35
10/28/2011	10/16/2011	LVB 4.95	28.04	19.48	875	4.07
10/28/2011	10/16/2011	LVB 7.3	13.04	23.03	983	7.97
10/28/2011	10/16/2011	LVB 7.3	19.04	22.4	1093	6
10/28/2011	10/16/2011	LVB 7.3	28.04	19.62	838	5.65
10/28/2011	10/24/2011	CR 346.4	13.04	22.97	954	8.02
10/28/2011	10/24/2011	CR 346.4	19.04	22.21	888.4	7.1
10/28/2011	10/24/2011	CR 346.4	28.04	19.44	827.7	5.91
12/23/2011	12/12/2011*	LVB 3.5	16.65	15.3	916	9.25
12/23/2011	12/12/2011*	LVB 4.95	16.65	15.56	913	9.04
12/23/2011	12/12/2011*	LVB 4.95	22.65	15.53	913	9.06
12/23/2011	12/12/2011*	LVB 4.95	31.65	15.4	916	9.19
12/23/2011	12/12/2011*	LVB 7.3	16.65	15.83	903	8.88
12/23/2011	12/12/2011*	LVB 7.3	22.65	15.81	904	8.99
12/23/2011	12/12/2011*	LVB 7.3	31.65	15.69	910	9.02
12/23/2011	12/13/2011	CR 346.4	16.65	15.81	930	8.36
12/23/2011	12/13/2011	CR 346.4	22.65	15.81	930.1	8.36
12/23/2011	12/13/2011	CR 346.4	31.65	15.81	929.9	8.32
2/13/2012	2/14/2012*	LVB 3.5	16.72	13.93	1136	9.17
2/13/2012	2/14/2012*	LVB 4.95	16.72	13.12	908	9.49
2/13/2012	2/14/2012*	LVB 4.95	22.72	13.12	907	9.46
2/13/2012	2/14/2012*	LVB 4.95	31.72	13.07	915	9.17
2/13/2012	2/14/2012*	LVB 7.3	16.72	12.96	904	9.34
2/13/2012	2/14/2012*	LVB 7.3	22.72	12.96	904	9.34
2/13/2012	2/14/2012*	LVB 7.3	31.72	12.95	905	9.3
2/13/2012	2/14/2012	CR 346.4	16.72	12.7	918.2	9.65
2/13/2012	2/14/2012	CR 346.4	22.72	12.7	918.6	9.66
2/13/2012	2/14/2012	CR 346.4	31.72	12.7	917.5	9.65
3/19/2012	3/12/2012*	LVB 3.5	15.60	14.01	1015	10.37
3/19/2012	3/12/2012*	LVB 4.95	15.60	13.52	955	10.39
3/19/2012	3/12/2012*	LVB 4.95	21.60	13.23	939	10.29
3/19/2012	3/12/2012*	LVB 4.95	30.60	13.13	962	10.21
3/19/2012	3/12/2012*	LVB 7.3	15.60	12.87	917	10.06
3/19/2012	3/12/2012*	LVB 7.3	21.60	12.87	917	10.06

3/19/2012	3/12/2012*	LVB 7.3	30.60	12.87	920	10.1
3/19/2012	3/8/2012	CR 346.4	15.60	12.38	911.4	12.38
3/19/2012	3/8/2012	CR 346.4	21.60	12.38	911.1	9.58
3/19/2012	3/8/2012	CR 346.4	30.60	12.37	911	9.63
4/20/2012	4/16/2012*	LVB 3.5	13.93	14.38	969	9.59
4/20/2012	4/16/2012*	LVB 4.95	13.93	14.31	917	9.85
4/20/2012	4/16/2012*	LVB 4.95	19.93	14.09	920	9.74
4/20/2012	4/16/2012*	LVB 4.95	28.93	14.13	993	9.14
4/20/2012	4/16/2012*	LVB 7.3	13.93	14.4	927	9.88
4/20/2012	4/16/2012*	LVB 7.3	19.93	14.13	910	9.87
4/20/2012	4/16/2012*	LVB 7.3	28.93	13.69	928	9.19
4/20/2012	4/3/2012	CR 346.4	13.93	13.63	919.2	9.78
4/20/2012	4/3/2012	CR 346.4	19.93	13.63	919.1	9.78
4/20/2012	4/3/2012	CR 346.4	28.93	13.63	919.4	9.77

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Thesis Title: Effects of Environmental Variables on the Reproduction of Quagga Mussels (*Dreissena rostriformis bugensis*) in Lake Mead, NV/AZ

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