Filtration and growth rate of Lake Mead quagga mussels (Dreissena bugensis) in laboratory studies and analyses of bioaccumulation

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FILTRATION AND GROWTH RATE OF LAKE MEAD QUAGGA MUSSELS

(Dreissena bugensis) IN LABORATORY STUDIES AND
ANALYSES OF BIOACCUMULATION

by

Carolyn Louise Link

Bachelor of Science
Northern Arizona University
2003

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science Degree in Water Resources Management
Department of Water Resources Management
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ABSTRACT
Filtration and Growth Rate of Lake Mead Quagga Mussels (*Dreissena bugensis*) in Laboratory Studies and Analyses of Bioaccumulation

by

Carolyn Louise Link

Dr. Charalambos Papelis, Examination Committee Chair
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In January of 2007, Quagga Mussels (*Dreissena bugensis*) were identified in Lake Mead, Nevada. An aquatic invasive species, these mussels can significantly alter ecosystems. This study sought to quantify three ecological traits of the species through a series of laboratory experiments and analyses, providing information both for comparison with *Dreissena* in other locations, as well as for limnologic management decisions. Filtration rate of quagga mussels was quantified using algal strains and natural seston. Two strains of green algae, *Nannochloris* and *Scenedesmus* were used to determine mussel filtration rates with a spectrophotometer. Quagga filtration rates of collected Lake Mead seston were determined with a turbidity meter. All clearance rate studies included both a large and small mussel size class, with maximum filtration rates for small mussels from 1167 mL/g dry mussel/hr for algae, and 496 mL/g dry mussel/hr for large mussels filtering algae. Ecologically related to the clearance rate, the growth rate of quagga mussels was also quantified during a 32 day trial in Lake Mead (Lake) water and also in lake water supplemented with the above mentioned algae strains, illustrating potentially higher growth rates than currently
occur in Lake Mead could be possible depending on lake algae levels. Mussel growth was 0.35 day\(^{-1}\) in natural seston, and 1.42 day\(^{-1}\) in supplemented lake water. Implications of mussel growth and filtration led to the study of quagga mussels’ potential for concentration of trace elements of concern in the tissue, and feces and pseudofeces of mussels. The contaminant levels in these biological samples were compared to sediment samples from both Boulder Beach at Lake Mead and from the Las Vegas Wash, a potential source of contaminants that flows into Lake Mead. All samples were processed via EPA Method 3050B and elemental analysis was completed with ICP-OES. While a large spectrum of elements were investigated, elements of concern in which statistically higher levels were observed in mussel tissue or pseudofeces and feces over sediment from the adjacent location included arsenic, molybdenum, lead, and selenium. Based on the results, it can be concluded that quagga mussels filter more when smaller in size and still growing, and they filter more when exposed to lower quality foods, and could potentially grow and expand their population impact if lake parameters change. Contaminant data illustrated that for selenium and arsenic bioaccumulation is appearing in mussel tissue, while for lead and molybdenum bioaccumulation is occurring in mussel excretions, potentially leading to changes in benthic composition. Future research studying assimilation, particle filtration, and the impact of seasonal and climate changes on filtration and concentration rates would be valuable for determining the mass balance impact of this species on aquatic environments in the Southwest.
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CHAPTER 1
INTRODUCTION

Research of aquatic ecosystems provides twofold benefits. With the increasing human population around the planet, and high growth occurring in areas such as the desert Southwestern United States, the importance of protecting clean freshwater and the supplying infrastructure for human civilization is emphasized. Second, understanding these same aquatic environments is valued as these limnologic habitats provide imperative vitality for the intricate biotic world of the Mohave and Sonoran deserts.

One of the largest threats to aquatic ecosystems is that posed by invasive species (Mack et al. 2000); species not native to a habitat; transported by natural or anthropogenic means, capable of moving into a biological niche with minimized population restrictions such as predators and strong competitors, where their populations can then grow exponentially. The invasive population’s unchecked growth impacts the native food web by shifting food and nutrient sources (Thayer et al. 1997, Ward and Ricciardi 2007), and also, in the case of many invasive aquatic species, their new physical presence often impacts aquatic infrastructure necessary for developed areas (Leung et al. 2002).

Lake Mead National Recreation Area is one such impacted area, having recently become inhabited by another invasive aquatic species. In January of 2007, living quagga mussels were identified in Lake Mead (LaBounty 2007). At capacity, capable of retaining over 28 million acre feet, Lake Mead is the largest volume reservoir in the United States, storing and supplying freshwater for Las
Vegas, Nevada; Los Angeles, California; Phoenix, Arizona, and the large surrounding agricultural areas including the Imperial Valley and Coachella.

Quagga mussels, small bivalves native to the Dnieper/Bug River Drainages of Eastern Europe (Marsden et al. 1996), have been slowly infiltrating the world’s freshwater environments through transport in commercial ship ballast waters. With the opening of the St. Lawrence shipping channel, these mussels, like many invasive species, received a gateway to the Great Lakes of North America (Johnson and Carlton 1996), and have since spread to other US waters including those in the southwest US along the Colorado River, likely by attachment to recreational boating or in bilge and live well waters (Stokstad 2007).

Quagga mussels are prolific on substrates from rocky to silty benthos throughout bodies of water, colonizing boat engines and water pumping structures for municipal water supplies and hydroelectric dams that can lead to millions of dollars in damage (Burlakova et al. 2000).

Measurement of quagga mussel filtration rate, growth rate, and any elevated toxicant concentration associated with the mussels’ tissue or waste are all imperative for understanding their biology in Lake Mead and their impact on the ecosystem. Mussels filter water for food, removing any small particles impacting the entire base of the food web by limiting or transferring the food supply for zooplankton, and all subsequent higher species in the food web. Growth rates of quagga mussels, especially under various conditions, give insight to how future populations may increase or decrease based on lake
conditions. Monitoring of contaminant concentration in mussel tissue and excretions in mussels from Lake Mead is important because of the close proximity to Las Vegas; a source of runoff contaminants and usage/return of waters in the Lake. Quagga mussel colonization has spread downriver throughout the lower Colorado River, indicating a potentially seismic impact that the associated lake and reclamation managers will have to deal with.

The studies presented in this thesis examine filtration rates and growth rates of the mussels in Colorado River waters with natural seston as well as in waters supplemented with algae cultures (comparable to past studies), as well as the extent to which quagga mussels in Lake Mead are bioaccumulating certain elements in their tissue, feces, and pseudofeces.

Predicting difference based on size of mussels, size of the food particles, and the quality of the food with respect to carbon / algae content, a series of trials will measure the filtration rates of mussels in static chambers in a laboratory. Similar studies will measure the growth of small mussels in lake water with seston (naturally suspended particles) and also in the same waters with additional algae added, with the prediction that the increased food will increase the growth of the mussels. Finally, sediment samples from the lake and nearby wash, mussel tissue, and mussel waste, –which for this study will be inclusive of settled particles excreted from the mussels within 24 hours of removal from the lake due to the difficulty associated with separating feces and pseudofeces, will be analyzed for toxicant concentration.

The impact of these invasive aquatic species and research on their biology
and impact are of great scientific and economic importance. Decisions and policies based on scientific knowledge can aid protection of freshwater habitats. Characterization of the ecological traits of quagga mussels in the southwest will provide information towards understanding any potential impact they may be having to Colorado River ecosystems.

Separately and together, these three studies contribute not only to the wider body of knowledge about Dreissenid species, but they also lend themselves to larger bioenergetics models useful for understanding long term impacts of the invasion, as well as for making decisions that assist with management, control, and prevention of further spread.
CHAPTER 2
LITERATURE REVIEW

Quagga Mussel Ecology

The quagga mussel (*Dreissena bugensis*), close relative of the zebra mussel (*Dreissena polymorpha*), originated in the Dnieper River drainage of the Ukraine. *Dreissena* spp. have a free swimming larval veliger stage capable traveling through the water column, allowing it to easily be transported by currents throughout a lake, down a river, or inside the ballast of a ship (Ackerman et al. 1994). Classified as an R-strategy species, the mussels have high reproduction rates, reaching maturity within a year, with females having the ability to produce over a million eggs a year (Mills et al. 1996, Vanderploeg et al. 2002). Once settled, the mussels grow densely, reaching concentrations of ~100 - 10,000/m² with an individual adult size of up to 4cm each (Strayer 1999). Preferred settlement on hard strata in non-native habitats has led to fouling of boats and occlusion of pipes causing extensive economical damage (Leung et al. 2002).

Initially found only in the native waters of the Bug / Dnieper Rivers, Dreissenid mussels began spreading throughout waters in Western Europe in the 1960’s (Walz 1978a) bringing attention to the need for research into their ecological impact. In the 1970’s early studies into quantifying ecological traits focused only on the zebra mussels, but did give initial insight to mussel characteristics such as filtration, assimilation, pseudofeces production rates, and overall energy budgets (Walz 1978a, Walz 1978b, Walz 1978c, Walz 1978d, Walz 1978d,
Continuing their spread through waterways, by the mid 1980’s, zebra, and then quagga mussels were discovered to have spread across the Atlantic Ocean into the Great Lakes of North America, ballast water in cargo ships being the predicted mode of transport (Hebert et al. 1989, May and Marsden 1992). Early populations were noted in various locations around the Great Lakes, as is visible in the below distribution map from 1989 (Figure 1).


With the increased distribution of the mussels, came increased importance of ecological research. Research began on how to specifically study mussels in laboratory settings; uncovering important details to their study such as zebra...
mussels have depressed filtration for 24 hr after collection and transport to a laboratory, (Reeders et al. 1989). Additionally, zebra studies began to focus more on specific traits that could be used when quantifying mussel impact. With high correlation, filtration rates of zebra mussels was associated with the dry weight of the mussel soft tissue ($R^2=.90$) (Kryger and Riisgard 1988). Additional studies monitoring filtration efficiency of various sized particles in the 0.7 – 30 µm range, showed clearance efficiency plateauing for particles above 5 µm in size (Sprung and Rose 1988).

By the early 1990’s the significant ecological impact of Dreissenids had been directly associated with their dominance in areas in which they colonize, due in part to their efficient filtration of plankton from the water column (Reeders and Devaate 1992). Other species such as zooplankton and larval fish depend on phytoplankton for sustenance, and subsequently suffer declines in population post mussel invasion (Caraco et al. 1997). More focused studies on filtration rates were undertaken, although still focused solely on zebra mussels. Size of mussels was determined to affect the food particle size and concentration preference of the mussels. Small zebra mussels (10-15mm) were observed filtering at a higher rate at low concentrations (<3.71 µg/mL) of *P. morum* (8-17 µm unicellular, 20-250 µm colonies) while large mussels (20-25mm zebra musels) filtered *C. reinhartii* (~10µm diameter) best at higher concentrations (>2.93µg/mL) (Berg et al. 1996). This same study, noting the importance of not just the mussels’ filtration, quantified the pseudofeces production; a substance that consists of any particles filtered by the mussels, but not consumed. Instead
the material is coated in mucus and ejected undigested, effectively removing it from the pelagic column and shifting it to benthically available nutrients.

Related to these more specific filtration studies, growth studies also began to appear in publications, giving insight into how the impact of individual mussels could be used to extrapolate ecological impacts over time (Jantz and Neumann 1998, Macisaac 1994b).

Since their initial discovery in the United States in the 1980s, the species continued to spread throughout the United States into the Mississippi River Drainage (O’Neill 1997) as is evidenced by distributional mapping in 1999 (see Figure 2).

Figure 2. Zebra Mussel Distribution into Mississippi River, 1999
In the mid 1990’s research began focusing on the distinctions between the two Dreissenid species (Mills et al. 1996). In consideration of this significantly larger body of research available in reference to zebra mussels, quagga mussel studies are often based on these studies without further investigation of potential differences. In reality, temperature and depth tolerances of the two species have been noted to be different (Spidle et al. 1995). Additionally, quagga mussels are capable of colonizing in deeper waters, while still acting as superior biological competitor at shallower depths, replacing zebra mussels in littoral zones both in the native drainages, and in North American invaded lakes (May and Marsden 1992, Stoeckmann 2003).

In 2002, Baldwin et al. completed a study that compared both growth and filtration rates of quagga and zebra mussels in waters from the North Eastern United States. This study showed that while the filtration rates of the two species were not significantly different when food and temperature were held constant; their growth rates were significantly different, with quagga mussels growing faster than zebra mussels many times over.

In addition to their impact on other aquatic species, the capacity of Dreissenid mussels to filter large volumes of water allows them to bioaccumulate toxicants that may otherwise be at trace or non-detectable levels (Mills et al. 1993). Trace metals have been found in the tissue and shell of mussels in concentrations 300,000 times the level present in the environment (Snyder 1997). Some pollutants are also known to bioaccumulate in the feces and pseudofeces of these mussels (Klerks et al. 1997).
Recent studies examined the calcium content of waters across the United States in relation to the likelihood of success of Dreissenid mussels if introduced. The waters of the southwestern United States, classified as high (>28mg/L) are considered to be at high risk for invasion if not already invaded, and if invaded, the mussels are likely to do well as the high levels of calcium are ideal to the Dreissenids, for whom calcium is a vital element for mussel development and shell building (Whittier et al. 2008).

By 2009, 20 years after initial Dreissena invasion discovery in North America, both zebra and quagga mussels had spread west of the 100th Meridian, in the Western United States; including the calcium rich waters of the Colorado River Drainage (see Figure 3).

Reviewing research into the recently colonized Lake Mead, found along the Colorado River between Nevada and Arizona, illustrates how amicable the Lake is for mussel colonization. Temperatures and pH levels throughout Lake Mead (LaBounty and Burns 2005) fall well within the tolerance ranges for quagga mussels (McMahon 1996, Mills et al. 1996, Spidle et al. 1995). Calcium levels in the above mentioned research (Whittier et al. 2008) give the range of concentration throughout the Lake to be between 69.1 - 87 mg/L, well above minimum quagga requirements of ~12mg/L (Jones and Ricciardi 2005).

Need for Research

While many filtration, growth, and metal concentration research studies focused on zebra mussels, little data exists specifically on quagga mussels. This study focused on answering similar questions that have previously been asked about zebra mussels, for quagga mussels in the Southwest. These studies will provide pertinent data that can be used and applied for aiding scientists and water resource managers with understanding of the quagga ecology. Just as understanding the impact of the mussels in the desert Southwest has become of significant importance since their invasion, research to quantify their ecological traits in the Southwest is imperative to scientists and water resource managers for understanding the effect of this new population.
Applications for scientific mussel collection and possession were completed, and permits were obtained from the Nevada Department of Wildlife. (see Appendix 5 for more information). Initially, two 20 liter aquaria were set-up with secondary containment vessels (restaurant bus tubs) per containment protocols. The tanks were filled with Lake Mead water that had been filtered to 30 µm to remove macro-invertebrates. Tank water was left at ambient laboratory temperature, approximately 20°C. For biological filtration, large sponge filters powered by air pumps were connected into each tank, with floating bioballs added to increase available surface area for nitrifying bacteria (See Figure 4).
Fluorescent freshwater aquatic lighting was added with light timers to maintain 12 hr light / dark cycles unless otherwise noted. Water changes were dictated by the bio-load of the system, which was monitored with a La Motte freshwater chemistry test kit. Detectable levels of ammonia and/or nitrite (above 1 ppm), or pH levels below 8, necessitated water changes of 50%. Disposing of all water containing, or that had come into contact with mussels, (including unused raw lake water) required first screening to 200 micron to remove large veligers and small mussels, then chlorinating for a minimum of 30 minutes (5000 ppm) and then de-chlorinating with sodium thiosulfate before disposal. All particles from filtering wastewater, detritus, and dead mussels were heated to 100°C for 5 minutes before disposal.

Clorox® bleach (6.15% sodium hypochlorite) in a 1:10 dilution ratio with tap water (5000 ppm available chlorine) and Proline© Dechlorinator (sodium thiosulfate) in 5 gallon dip buckets were kept near all sinks, and any laboratory equipment or sampling tools were soaked in each for 30 minute minimum before further cleaning.

Laboratory algae culturing was instated prior to experimentation for the strains Scenedesmus and Nannochloris. Algae culture seeding samples were obtained from Carolina Biological Supply. All algae culture media was made by dilution of Florida AquaFarms AlgaeGro© with Nanopure water to approximate f/2 media (Guillard and Ryther 1962), and was subsequently autoclaved and then adjusted to a pH between 7.5 and 8.5 with sodium hydroxide.
Site Selection

Two sites were selected for all lake collection. Lake Mead NRA contains Lake Mead, a large reservoir behind Hoover Dam located along the Colorado River, approximately 45 minutes from Las Vegas, Nevada (see Figure 5).

Figure 5. Lake Mead Overview Map for Sampling Site Selection
Site One (see Figures 5 and 6) was located nearshore along Boulder Beach, within Boulder Basin, a public beach near camp grounds on Lake Mead where littoral benthos consists mostly of small cobble (up to 20 cm in diameter) and sandy substrate. Site One was used for all water collection, mussel collection, and portions of the sediment sampling. This site was selected because of the high traffic of people and the associated potential for human interaction with mussels and sediment, and also for ease of access, as it allows cars to drive within 10m of the water, an important feature when collecting and hauling raw lake water in 20 L carboys, and 5 gallon buckets full of mussels and water.

Figure 6. Boulder Beach Site for Mussel, Sediment and Water Sampling
Site Two was selected approximately 0.4km downstream from the closed Las Vegas Bay launch ramp, along Las Vegas Wash (see Figures 5 and 7). The location was approximately 0.5km upstream from the lake confluence when Lake Mead’s water line was at an elevation of 1093ft (Nov. 2009).

![Sampling Site 2](image)

**Figure 7. Las Vegas Wash Site for Sediment Sampling**

**Mussel Collection**

Collection of mussels for clearance and filtration studies began with divers shore diving to depths between 2 and 10m for mussel collection. Rocks with visibly siphoning mussels attached were collected, transported in lake water in containers, in coolers, to the laboratory, where the mussels were inspected for damage. Mussels were collected monthly, with the specific collections for growth rate studies occurring in July 2009 and the mussels used for clearance rate studies in November 2009. Once transported to the lab, un-crushed mussels
were removed from the substrate with a scalpel, with attention being paid to minimize bysal threads being detached from the mussel. Mussels were then rinsed in deionized water for approximately 5 minutes and gently scrubbed to remove residual sediment and any other externally attached organisms with nylon brushes. The mussels were then placed in the 20L laboratory aquaria at a density of 10 large (15-20mm) mussels per liter, or 100 small (5-10mm) mussels per liter. Mussels were acclimated to laboratory ambient temperatures and lighting for two weeks before experimentation. After daily 50% water changes for the first 3 days, water changes were reduced to 3 times a week, and then eventually weekly, once ammonia and nitrate levels were below detection (1ppm). Daily algae supplementation began after the third day of acclimation, with 250mL frozen cubes of $3 \times 10^6$ cell/mL Nannochloris culture being placed in funnels suspended 5 cm above the tank. The funnels were placed with the neck wedged in between the glass cover and the lip of the tank, with the bottom of the funnel positioned over the area of the tank where the outflow current from the sponge filter would disperse the algae throughout the tank. The frozen algae cubes would defrost and drip feed algae into the tank over the course of approximately 12 hours.

Water Collection

All water used in this project was raw water collected from Lake Mead at Sampling Site One at Boulder Beach (see Figure 6). Nalgene® 20L carboys were carried or swam at least 10m into the lake from the shoreline. Carboys
were triple rinsed, then submerged until the mouth of the carboy was 10cm below the water’s surface to minimize collection of floating debris, and allowed to fill themselves. After collection, carboys were immediately transported back to the laboratory, where the water was then poured through a 120µm Nitex mesh filter, which was made by placing Nitex mesh in between a threaded 2 in PVC bushing and a threaded ring cap. Filtered water was stored in 5 gallon buckets with lids; with a small hole in the lid through which rigid air line tubing was placed to aerate the water. Water was collected as needed and was used within 7 days from collection.

Equipment Decontamination

In between sampling events, all sampling equipment including tools, buckets, water carboys, sample transport containers, and coolers were decontaminated by first removing any large visible debris, then soaking equipment in a bleach dip (5000 ppm available chlorine) for a minimum of 1 hour, and then washing in 60°C soapy water, rinsing in 60°C water, then rinsing with DI water and air drying for a minimum of 24 hours. All diving equipment was decontaminated per the protocols specified in the NDOW permit application, as is described in Appendix 5.

Statistics

All statistical analyses were run using JMP 5.0.1 (SAS Institute, Cary, North Carolina) with a \( p = 0.05 \), equivalent to a 95% confidence level. A one-way
ANOVA (analysis of variance) was used to determine if statistical differences existed between filtration rates for different sizes of mussels in different media, between growth rates in different food concentrations, and between element concentrations in mussel tissue, pseudofeces and feces, and sediments from the lake and nearby wash. If ANOVA resulted in a statistical difference, Tukey HSD (honestly significant difference) pairwise test was used to show grouping of similar and different data sets. The Results of the Tukey HSD is illustrated in figures as letters above the columns, with same letters indicating no significant difference, and different letters meaning a significant difference was detected.
CHAPTER 4
FILTRATION RATE TRIALS
Questions, Objectives, Hypothesis

Question
What are the filtration rates of quagga mussels for different algae strains and natural seston from Lake Mead?

Objective
Determine filtration rates of adult and juvenile mussels in both natural seston and laboratory cultured *Nannochloris* and *Scenedesmus*, as well as a mixture of the two strains.

Hypothesis
Filtration rates of mussels will vary based on size of mussels, quality of material (carbon content), and size of particles.

Methods
Algae Preparation
The day of experimentation, algae cultures were filtered through a 20 micron Nitex mesh for removal of larger particles and stationary growth clumping. The two cultures selected for this experiment included a *Scenedesmus spp.*, a green sickle shaped flagellate algae approximately 7-10µm in length, with a tendency to grow in clumps of 2-8 cells. The second strain selected was *Nannochloris spp.*, a smaller round green algae of approximately 1-3µm in cell diameter, with small to minimal clumping. After filtration for size separation,
algae were then centrifuged for 10 minutes at 3220 rcf (4,000 rpm setting on an Eppendorf 5810 R centrifuge), to concentrate and remove any nutrient rich growth media. Algae were then re-suspended into <20 µm filtered lake water.

**Cell Density Determination**

Cell density of the algae cultures was then determined by counting cell density of 30 samples with a hemocytometer. Once density of the culture was determined, the dilutions needed to reach the trials starting density was estimated, and dilutions were made to 1, 5, 10, 20, 50, 100, 200% of the initial concentration to be used in the filtration rate trial. These diluted samples were measured for optical intensity of the green associated with the chlorophyll in the algae with a Shimadzu UV-Vis spectrophotometer measuring at wavelengths 664 and 750nm. The cell densities of the diluted samples were regressed to the optical intensities at the two separate wavelengths, and then these two regression equations were averaged to correlate cell density of a sample during the filtration trial with the intensity of light absorbed at the two wavelengths in the spectrophotometer (Moed and Hallegraeff 1978). This method facilitated cell density measurements throughout the filtration study to be completed in a more timely manner than counting cell densities of individual samples with the hemocytometer. While running the dilution samples for regressions, the selected trial initial cell concentration of each type of media was confirmed or adjusted to equal ten times the limit of detection of the UV Vis spectrophotometer.

**Seston Preparation**

Natural Seston was collected from Lake Mead at the Boulder Beach
location by artificially recreating turbid waters as collection was completed on a winter day when it was not windy and the water was clear. A sampler waded out approximately 10 meters from the shore, then returned to the shoreline, and repeated this action three times, thus creating turbid waters with material that could be easily suspended by minimal agitation. This turbid water was collected into carboys, then transported back to the laboratory, and filtered to 120 µm to remove large detritus and zooplankton. Turbid water was then aerated for 1 hr, to allow for equilibration.

**Media Characterization**

Three samples of each media type were filtered onto pre-ashed Whatman GF/F filters held in VWR aluminum tins, then dried in a VWR drying oven at 60°C for 24-48 hours until constant weight was measured at ambient temperature. Samples were cooled in a desiccator before weighing. This weight, minus the pre-ashed weight of the unused filter and tin, provided data for dry weight algae concentration. These dry filter samples were then ashed in a muffle furnace at 500°C for 60 minutes, then cooled and weighed. This weight, minus the unused filter and tin weight provided the ash free dry weight (AFDW) of the sample, which is equivalent to the concentration of non-carbon material in the sample. Subtracting the ashed sample weight from the dry sample weight provided concentration of carbon in the sample. Three samples of each media type were also filtered and processed for chlorophyll analysis with a fluorometer by EPA Method 445.0 (Arar and Collins 1997). All quantified characteristics of media used are compiled in Table 1.
Table 1. Filtration Rate Media Characteristics

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Particle Size</th>
<th>Algae Cell Density (10^6/L)</th>
<th>Media Dry Weight Concentration (mg/L)</th>
<th>Media Carbon Density (mg/L)</th>
<th>Media Chlorophyll α Density (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenedesmus</td>
<td>&lt;20 µm (6-10 individual)</td>
<td>140</td>
<td>22.24</td>
<td>21.48</td>
<td>2.73</td>
</tr>
<tr>
<td>Nannochloris</td>
<td>&lt;20 µm (1-3 individual)</td>
<td>300</td>
<td>6.51</td>
<td>6.15</td>
<td>4.78</td>
</tr>
<tr>
<td>Mixed</td>
<td>mixed</td>
<td>100</td>
<td>14.28</td>
<td>15.18</td>
<td>1.64</td>
</tr>
<tr>
<td>Scenedesmus</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nannochloris</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seston</td>
<td>&lt;120 µm</td>
<td>74.74</td>
<td>11.76</td>
<td>1.58E-06</td>
<td></td>
</tr>
</tbody>
</table>

Mussel Preparation

Mussels were not fed for 24 hr pre-experiment to minimize feces production during the trial. The morning of the trial, mussels were placed in newly collected water filtered to 30 µm with Nitex mesh. After half an hour of acclimation, mussels were selected for experiments if they were observed siphoning. Small mussels used for clearance rate trials were selected in the 6.0-9.0 mm range (n=5 for each algae replicate, n=10 for seston replicates, with 5 replicates for each media), while large mussels were selected in the 15.0 -18.0 mm range (n=2 for each media replicate, with 5 replicates for each media). For algae filtration trials with small mussels, 5 small mussels were selected per container, with 5 replicate containers per treatment. For large mussel algae clearance trials, 2 large mussels were selected per container, again with 5 replicate containers per treatment. Mussels were measured for shell length along the hinge, with calipers to the nearest 0.1 mm, wet weighed before the trial and processed for dry mass and carbon content after the trial by drying and ashing in aluminum tins in a 60°C drying oven, weighing, then ashing in a 500°C muffle furnace for 60 minutes and weighing again.
Controls

Controls consisted of 3 containers of media (algae or seston) without mussels to correct for settling and/or algae growth during the experiment. See Table 2 below for the experimental matrix.

Table 2. Experimental Matrix for Filtration Rate Studies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 replicates per treatment</th>
<th>Vessels including 3 per trial for control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>small mussels</td>
<td>large mussels</td>
</tr>
<tr>
<td>Scenedesmus</td>
<td>5(5)</td>
<td>5(2)</td>
</tr>
<tr>
<td>Nannochloris</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Mixed algae</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Seston</td>
<td>5(10)</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>40</td>
</tr>
</tbody>
</table>

Treatment Vessels

Treatments were administered in 1.5 L containers with lids fitted with capped holes that can be opened to allow for airline introduction (see Figure 8). Each treatment vessel contained 1L media. During the trials, all vessels were aerated with rigid air lines placed to 1/2 depth to allow for current and to maintain suspension without disturbing the mussels.
Filtration Measurements

Spectrophotometer measurements were collected at time zero, at 0.5 hr, 1.0 hr, and at varying 1-12 hour increments thereafter, until readings approached the limit of detection determined during the cell density regression creation. For each measurement, 3mL of sample was removed from each container at 10cm depth from the surface, which had been determined to be the deepest the 10mL pipette tip could be inserted into the sample for collection close to the mussels without disturbing filtration. The 3mL sample was placed in a 1cm disposable cuvette, analyzed in the spectrophotometer, and was then returned to the treatment vessel after analysis to maintain experiment volume. Measuring cell density required less than one minute for each sample. All treatment vessels were kept on a cart allowing for transport from the laboratory where the
spectrophotometer was located to a room where all lighting could be turned off to reduce algae growth during the trial, and where treatment containers could be connected to aeration in between sampling. While in the spectrophotometry laboratory, vessels were covered with aluminum foil to minimize lighting.

Seston readings were taken at time 0.0 and at every 0.5 hour after for four hours with a La Motte turbidity meter. The turbidity meter was calibrated with a 0 NTU solution at the beginning of collection of each sampling time. Mussel selection followed the same criteria as for algae clearance trial, with the one difference of 10 small mussels were used per small treatment as opposed to 5 (see Table 2). All filtration rate data were weight adjusted to a per mussel, or per g dry weight mussel tissue basis during data analysis, so the different number of mussels in each replicate did not impact the comparability of the data.

Post Trial Mussel Characterization

Post trial, mussels were transferred to pre weighed, pre ashed aluminum weigh boats, then weighed for wet mass on a Mettler analytic electronic balance to the nearest 0.01 mg. Dissection was deemed unnecessary for filtration rate trials, as the data is used for comparison with wet weights or dry weights of mussels in field conditions. Samples were then dried in a convection drying oven at 60°C until constant weight, approximately 48 hours, then cooled in desiccators, weighed for dry weight, then ashed in a VWR muffle furnace at 500°C for 60 minutes, allowed to cool, and reweighed for determination of carbon content (AFDM).
Results

Figures 9 through 12 below illustrate the densities and concentrations of media over time during the filtration rate trials. Measurements for replicates were averaged with one standard deviation shown in error bars. These measurements were then used to determine average filtration rates by mussel size and media.

*Nannochloris*

![Figure 9. Nannochloris Density over Time in 1L Vessels with Large and Small Quagga Mussels](image)

*Nannochloris* density decreased from $3 \times 10^5$ to $2 \times 10^5$ cell/ mL during the 240 minutes of this filtration rate trial. The density decreased from $3 \times 10^5$ cell/ mL to approximately $9 \times 10^4$ cell/ mL in the replicates containing 5 small mussels. The density in containers containing 2 large mussels each decreased from $3 \times 10^5$ cell/ mL, again the initial density for all trial replicates, to $5 \times 10^4$ cell/ mL during the course of the trial.
Scenedesmus density decreased from the initial density for all replicates of $1.4 \times 10^5$ cell/mL to $5 \times 10^4$ cell/mL in the control replicates over the course of the 1080 minute trial. The algae density decreased in the small mussel replicates also from $1.4 \times 10^5$ cell/mL to $5 \times 10^4$ cell/mL, but at a different rate. The cell density in containers containing 2 large mussels each decreased from $1.4 \times 10^5$ cell/mL, again the initial density for all trial replicates, to $2 \times 10^4$ cell/mL during the course of the trial.
The mixed algae density did not decrease from the average initial density for all replicates of $1.1 \times 10^5$ cell/ mL in the control replicates over the course of the 180 minute trial. The algae density decreased in the small mussel replicates from $1.1 \times 10^5$ cell/ mL to $8 \times 10^4$ cell/ mL. The cell density in containers containing 2 large mussels each decreased from $1.1 \times 10^5$ cell/ mL, again the initial density for all trial replicates, to $8 \times 10^4$ cell/ mL during the course of the trial.
Seston concentration decreased from 74 mg/L to 24 mg/L during the 240 minutes of this filtration rate trial. The concentration decreased from the same initial concentration to approximately 0.6 mg/L in the replicates containing 10 small mussels. The concentration in containers containing 2 large mussels each decreased from 74 mg/L, again the initial concentration for all trial replicates, to 1.4 mg/L during the course of the trial.
Filtration Rate Calculations

Filtration rate was calculated using the following equation (Coughlan 1969).

\[
FR = \frac{\text{Vol.}}{n \cdot t} \left( (\ln \text{Conc}_0 - \ln \text{Conc}_t) - (\ln \text{Conc}_0' - \ln \text{Conc}_t') \right)
\]

\[
FR = \text{Filtration Rate (L/mussel/hr)}
\]

\[
\text{Vol.} = \text{Suspension Volume (L)}
\]

\[
n = \text{Number of Mussels (mussels)}
\]

\[
t = \text{Length of Time Between Measurements (hr)}
\]

\[
\text{Conc}_0 = \text{Initial Concentration (cells/mL)}
\]

\[
\text{Conc}_t = \text{Final Concentration (cells/mL)}
\]

\[
\text{Conc}_0' = \text{Control Concentration (cells/mL)}
\]

Below are the resulting average filtration rates for both size classes of mussels and for the four different media tested.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Filtration Rate Lg Mussels (mL/per mussel/hr)</th>
<th>Lg Mussels (mL/per g dry weight mussel/hr)</th>
<th>Filtration Rate Sm Mussels (mL/per g dry weight mussel/hr)</th>
<th>Sm Mussels (mL/per g dry weight mussel/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenedesmus</td>
<td>22.96</td>
<td>125.43</td>
<td>3.08</td>
<td>194.14</td>
</tr>
<tr>
<td>Nannochloris</td>
<td>88.06</td>
<td>496.44</td>
<td>19.24</td>
<td>1166.51</td>
</tr>
<tr>
<td>Mixed</td>
<td>34.10</td>
<td>172.53</td>
<td>10.43</td>
<td>630.15</td>
</tr>
<tr>
<td>Scenedesmus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nannochloris</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seston</td>
<td>48.57</td>
<td>254.13</td>
<td>23.42</td>
<td>1202.76</td>
</tr>
</tbody>
</table>

Mussel filtration rate calculations for *Scenedesmus* media resulted in approximately 23mL/mussel/hr for large mussels, which was equivalent to 125mL/g dry weight of mussel/hr for the same mussels. For small mussels in the same algae, filtration rates were approximately 3mL/mussel/hr, which was
equivalent to 194 mL/g dry weight of mussel/hr for the same small mussels.

Mussel filtration rate calculations for *Nannochloris* media resulted in approximately 88mL/mussel/hr for large mussels, which was equivalent to 496 mL/g dry weight of mussel/hr for the same mussels. For small mussels in the same algae, filtration rates were approximately 19ml/mussel/hr, which was equivalent to 1167 mL/g dry weight of mussel/hr for the same small mussels.

Mussel filtration rate calculations for the mixed algae media resulted in approximately 34mL/mussel/hr for large mussels, which was equivalent to 173 mL/g dry weight of mussel/hr for the same mussels. For small mussels in the same algae, filtration rates were approximately 10mL/mussel/hr, which was equivalent to 630 mL/g dry weight of mussel/hr for the same small mussels.

Mussel filtration rate calculations for seston resulted in approximately 49mL/mussel/hr for large mussels, which was equivalent to 254 mL/g dry weight of mussel/hr for the same mussels. For small mussels in the same algae, filtration rates were approximately 23ml/mussel/hr, which was equivalent to 1203 mL/g dry weight of mussel/hr for the same small mussels.

Discussion

The hypothesis that the filtration rate of mussels would vary by media type was supported by the results. Additionally, the data supported filtration rate differences between mussel sizes when compared side by side as grams of dry weight mussel.

*Nannochloris* density decreased at a faster rate in the small mussel
replicates (middle line, Figure 8) than in the control replicates (top line, Figure 9). The cell density decreased fastest in the large mussel replicates (bottom line, Figure 9). Average measurements of both treatments and the control samples correlated well ($R^2 > 0.99$) to natural log exponential relationships.

*Scenedesmus* cell density decreased at a faster rate in both the small mussel replicates and large mussel replicates (bottom two lines, Figure 10) than in the control replicates (top line, Figure 10). Despite large margins of error, the trend shows initial higher filtration rate of small mussels over large mussels, and then later higher rate of filtration for large mussels. Throughout most of the *Scenedesmus* trial, the algae density decreased fastest in the large mussel replicates (bottom line, Figure 10). Average measurements of both treatments correlated well ($R^2 > 0.90$) to natural log exponential relationships, as did the control samples ($R^2 > 0.80$).

The mixed algae cell density decreased at a faster rate in both the small mussel replicates and large mussel replicates (bottom two lines, Figure 11) than in the control replicates (top line, Figure 11). The trend shows initial higher clearance rate of small mussels over large mussels, and then later higher rate of filtration for large mussels. The trend lines of the average measurements of both treatments correlated well ($R^2 > 0.50$) to natural log exponential relationships, but the control measurements more closely trended toward a constant cell density.

Seston concentration decreased at a faster rate in the large mussel replicates (middle line, Figure 12) than in the control replicates (top line, Figure 12). The concentration decreased fastest in the small mussel replicates (bottom
Average measurements of both treatments and the control samples correlated well ($R^2 > 0.98$) to natural log exponential relationships. Error bars within the filtration graphs were significant, but it should be considered that these data had not yet been weight adjusted, so variation in filtration was to be expected.

Highest mussel filtration rates were observed in seston for both size classes. While per mussel filtration rates were higher for large mussels, when the rates were adjusted for mussel size, small mussels exhibited higher filtration rates per gram dry weight.

Highest filtration rates for the varying algae strains was observed for *Nannochloris*, with the mix of *Nannochloris* and *Scenedesmus* exhibiting second highest filtration rates, and the monoculture of *Scenedesmus* having the lowest filtration rate for both size classes in this study.

Higher filtration rates for monoculture *Nannochloris* over *Scenedesmus* (and the mixed media as well) could be due to the particle size and shape of the algae. Even though both cultures were filtered to the same size, below 20 µm, *Scenedesmus* has both individually larger particles, ~7 µm, and also tends to grow in clumps, (2-6 per clump), while *Nannochloris* cells are approximately 1-3 µm in size and do not tend to clump as much as *Scenedesmus*.

The high filtration rate in seston is potentially due to the mussels’ attempt to increase consumption of actual food, as the seston exhibited the lowest concentration of carbon per gram of media. These results potentially suggest that quagga mussel filtration rate may be higher when there is low food quality.
Higher filtration rates were observed for small mussels when compared to larger mussels per gram, indicative that the smaller mussels potentially consume more as they are still at an early stage of their growth.

Filtration rate determination studies should be kept within the context from which they were collected. While attention was paid to try replicate natural settings as much as possible, i.e. mussels were selected based on health and were acclimated before studies, other variables could not be optimized. Optimal feeding temperatures have been published to be closer to 12.5°C (Baldwin et al. 2002), as opposed to the 20°C used in this study; however no appropriate chilling device is possessed by the laboratories used for these studies. Additionally, natural variables should also be considered when extrapolating filtration rates at different times of the year, --seasonal effects on filtration should be considered. Highest filtration by mussels correlates to spring; the time when body condition and reproduction in the mussels is highest (Vanderploeg et al. 2009). Summer studies noted poorer quality seston present, inclusive of higher turbidity, and cyanobacteria. Previous studies by the same authors observed lowered feeding rates in the presence of *Microcystis* strains, with subsequent high concentrations in the mussel pseudofeces, indicating selective avoidance of ingestion of the toxic bacterial strains (Vanderploeg et al. 2001).

Additionally, the filtration rates of these studies should not be used for predicting energy budgets of mussels, for that, assimilation studies would be more appropriate. Graphing of parallel studies that compare the filtration rate of zebra mussels experiencing various concentrations of algae and also the
pseudofeces production during the differing concentration treatments results in an incipient limiting point for mussels given a specific food; a point at which assimilation of food is highest, without a reduction in filtration due to fouling / blocking of the cilia and gills. For example, this limit can be as low as 0.60 µg/mL for adult (25-30mm) zebra mussels when fed the algal strain *C. reinhardtii*, but may vary by strain (Sprung and Rose 1988). This point is also dependent on the size of mussels, as well as particle type.

These studies were conducted with static chambers as opposed to flow through chambers. Studies with the highest measured filtration rates to date, 574mL/hr/g wet mass, were conducted with an industrial flume, in which zebra mussel filtration was measured in a flow through environment (Elliott et al. 2008). Neither those trials nor the ones completed for this study were complete representations of the natural environment, and this should be considered when interpreting filtration rates.

Significantly effecting the extrapolation of our study’s filtration rate and growth rate determinations are the mussel densities at which these rates were measured. In open water settings, as has previously been covered in this paper, mussels grow at high density, while in our study all measurements were made either of individual mussels or of groups of 2 to 10 mussels. Studies on densely colonized mussels found mussels at the surface ingested 75% more than the bottom mussels, likely due to reduced flow rate and refiltration (Tuchman et al. 2004). This study indicates that individual mussel filtration should be adjusted dependent on density of mussels in a given setting.
Overall the results of our study, combined with recommendations and indications of other studies, provides direction for scientists and managers when comprehending and predicting the impacts of mussel filtration.
CHAPTER 5
GROWTH RATE TRIAL
Questions, Objectives, Hypothesis

**Question:**
What are the growth rates of mussels in both natural seston and in water supplemented with algae?

**Objectives:**
Determine if there is a growth rate difference between mussels in Lake Mead natural seston from mussels grown in the same waters supplemented with algae.

**Hypothesis:**
Growth rates of mussels in supplemented waters will be higher than mussels grown in natural concentrations of seston due to increased food.

**Methods**
Replication of natural growth rates in a laboratory setting can be difficult. The closest approximations can be obtained by providing the maximum ingestion rate of food continuously to mussels. While this cannot be replicated without a flow through system, our study attempted to replicate it as well as possible by maintaining a low mussel to volume ratio, and by replenishing food supplies every 2\textsuperscript{nd} day throughout a 32 day (4-8 day ‘weeks’) growth trial.

**Mussel Selection**
Mussels were collected in accordance with laboratory collection protocols explained above. Mussels underwent a two week acclimation to ambient room
temperature, (approximately 20°C). After the two week acclimation period, mussels observed siphoning were selected for growth studies. For each replicate one small mussel (7.0 - 9.0 mm hinge-side length) was selected. Experimental parameters were set to 19 replicates of each treatment, plus 5 control replicates for a total sample group of n=43 (see Table 4).

Table 4. Experimental Matrix for Quagga Growth Rate Studies

<table>
<thead>
<tr>
<th></th>
<th>Number of Replicates</th>
<th>Water Required (L)</th>
<th>Supplements Required (in 10mL cubes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Seston</td>
<td>Supplemented</td>
</tr>
<tr>
<td>Week One</td>
<td>5</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Week Two</td>
<td>5</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Week Three</td>
<td>5</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Week Four</td>
<td>5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Water Preparation

Water collection for the study took place every 5 days, from the Site One location on Boulder Beach, at different times of the day, to ensure the mussels in the laboratory would experience as similar as possible conditions to mussels in a natural environment. After water was collected, it was immediately transported to the laboratory where it was filtered through 120 µm Nitex mesh to remove any zooplankton and large detritus. Water was stored in 5 gallon buckets, lightly covered, and gently aerated until use. On the day water was collected, it was analyzed for chlorophyll content, dry weight, and carbon concentration. Throughout the experiment, the lake water ranged from 0.0013 to 0.0022 mg/L Chlorophyll α, 0.15 to 3.98mg/L dry weight content, and 0 to 1.22 mg/L for carbon content (see Table 5). This water was directly used for all seston treated
mussels. Mussels receiving the algae supplemented water, received the same water, with the addition of algae. Control mussels also received water from the same water supply, but it was filtered through 0.7 µm glass fiber filters to remove all particulate sources of food first.

Table 5. Media Characteristics for Quagga Growth Rate Studies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chl α (mg/L)</th>
<th>Dry Density (mg/L)</th>
<th>Carbon Density (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae Supplement</td>
<td>0.0103</td>
<td>0.63</td>
<td>0.64</td>
</tr>
<tr>
<td>Naturally Collected Seston Water</td>
<td>0.0013 - 0.0022</td>
<td>0.15 - 3.98</td>
<td>0 - 1.22</td>
</tr>
</tbody>
</table>

**Supplement Preparation**

Before the study commenced, cultures of *Scenedesmus* and *Nannochloris* were prepared for supplementation treatments for growth studies. To eliminate the variability introduced by using algae from a growing culture (varying daily concentrations), the two cultures were analyzed for content, then combined in a 50 / 50 ratio and frozen into 10 mL aliquots (5mL of each culture) and stored in a freezer. During the growth study, every other day prior to a water change, the number of treatments receiving supplementation was determined (see Table 4), and the pre-frozen aliquots were defrosted and added to the appropriate amount of Lake Water, thoroughly mixed in a 20 L bucket, and allowed to equilibrate to room temperature.

**Water Changes**

Throughout the experiment, all supplemented treatments received identical supplements every two days. Water changes occurred every two days,
with 95% of their lake water removed and replenished so as to replace as much as possible without removing mussels from water. Waste water was disposed of (per permit protocols). If mussels were attached to the vessel walls near the surface of the water, they were gently detached so as to prevent any accidental desiccation.

**Experimental Conditions**

Mussels were kept in an incubator during the experiment to minimize variability in environmental parameters, and containers were rotated from front to back of the shelves to equalize any lighting differences. All treatment containers were clear food grade plastic. The light cycle during the experiment was set to 14 hr light / 10 hr dark as opposed to a 12 / 12 cycle to more closely replicate the natural summertime lighting occurring during the experiment (August 2009). Temperature in the incubator was set to a constant 20°C (+/- 1°C). to simulate the ambient laboratory temperatures the mussels had acclimated to over the previous two weeks. Aeration was provided through rigid air lines placed at full depth of the treatment vessel to maintain dissolved oxygen levels and water homogeneity.

**Growth Monitoring**

Measurements of the mussels for growth rate analysis included wet weighing; blotting live mussels dry with a paper towel and then weighing in a weigh boat in a Mettler Analytic balance to 0.01mg, and also measuring the length of the mussels along the hinge side of the mussel with vernier calipers to 0.1mm. These measurements were collected at initiation of the experiment, and
every 8 days until the conclusion of the trial.

**Body Condition Monitoring**

In addition to monitoring the externally measurable growth of the mussels, mass and length, body condition was also monitored by sacrificing 10 mussels from the experimental population on day 0, and 3 mussels from each of the two treatments every week (8 days) thereafter. Dissection allowed for mantle tissue to be weighed and body condition, or mantle weight to length ratio to be monitored throughout the experiment as well. All remaining mussels at the end of the trial were also dissected and added to body condition monitoring data.

**Results**

Figures 13 through 15 below illustrate the growth of mussels over time by various measurements. Body condition of dissected mussels over the growth rate experiment are described first. Length and mass growth rates of mussels were measured for mussels in the lake seston water and for mussels in the same water with algae supplementation. The control treatment for this experiment was lake water filtered to be devoid of particulate food. Growth was monitored both by length and weight. These measurements were then used with previously published equations to determine average growth rates by treatment. Measurements for replicates were averaged with one standard deviation shown in error bars.
Body condition of mussels in algae supplemented lake waters shows increased Dry Weight Tissue Mass to Length ratio during the course of the 32 day experiment from approximately 0.11 to 0.32 mg/mm. Body condition of mussels grown in natural level Lake Seston increased from 0.11 to 0.17 during the experiment. Body condition in the control replicates remained constant from 0.11 to 0.11 mg/mm from initial measurements to final.
Average mussel length increased in mussels grown in algae supplemented Lake Waters from 8.2 to 9.2mm during the four weeks of the trial. Mussel length increased from 7.8 to 8.0mm during the four week trial in the seston only media when average between replicates. Mussels grown in the control prepared waters had no detectable change in length throughout the experiment, beginning and finishing at an average length of 7.6mm.
Average mussel mass increased in mussels grown in algae supplemented Lake Waters from 53.2 to 84.1mg during the four weeks of the trial. Mussel length increased from 44.7 to 48.9mg during the four week trial when average between replicates. Mussels grown in the control prepared waters increased in mass during the growth trial from 39.1 to 39.7mg on average.
Growth Rate

Growth rate was determined as the instantaneous rate of mass/length change using the following equation (Baldwin 2002).

\[ G = \frac{100(\ln W_2 - W_1)}{T} \]

\( G \) = instantaneous rate of mass change (day\(^{-1}\))

\( W_1 \) = Initial Weight (mg)

\( W_2 \) = Final Weight (mg)

\( T \) = Time (days between measurements)

This equation was also used for determination of change in length.

\[ G = \frac{100(\ln L_2 - \ln L_1)}{T} \]

\( G \) = instantaneous rate of length change (day\(^{-1}\))

\( L_1 \) = Initial Length (mm)

\( L_2 \) = Final Length (mm)

\( T \) = Time (days between measurements)

Table 6. Growth Rates of Quagga Mussels in Different Media

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average Growth Rate Based on Length (day(^{-1}))</th>
<th>Average Growth Rate Based on Mass (day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Seston</td>
<td>0.06</td>
<td>0.35</td>
</tr>
<tr>
<td>Algae Supplemented</td>
<td>0.35</td>
<td>1.42</td>
</tr>
</tbody>
</table>

Close to zero growth rate was detected for the control mussels, with 0.01/day change in length, and 0.00/day change in mass. The algae supplemented mussels exhibited average growth rates of 0.35/day change in length, and 1.42
/ day change in mass. The mussels grown in lake seston had average growth of 0.06 / day change in length, and 0.35 / day change in mass.

Discussion

The hypothesis that growth rate would vary dependent on food was supported by this experiment. Zero growth rates in the control mussels were to be expected, as all particulate food was removed from the water. The small detectable level of mass growth may be the effect of the mussel's ability to absorb dissolved organic carbon from the water, as has been shown in other research (Baines et al. 2007). The growth rates for juvenile mussels both per mussel and per gram mussel were significantly higher for mussels in algae supplemented water than for natural seston lake levels. The algae supplemented mussels exhibited roughly 4 times higher growth in mass and over 5 times higher growth in length than the mussels in Lake Mead Seston Water.

Error bars within the weekly growth of the mussels express distribution of mussels throughout the size range (6-9mm) and were expected to be present throughout the trial. This variation was corrected for when the growth rates were calculated and length and mass change were considered.

Body condition of the mussels increased throughout the experiment for mussels grown in algae supplemented waters, indicating that growth in mass was not restricted to shell development, and was not the result of external growth of algae on the mussel shells.

Following growth of the mussels’ length throughout the experiment shows
higher growth for the mussels in waters supplemented by algae (top line, Figure 14). Mussels in waters comparable to Lake Mead (middle line, Figure 14) showed higher growth than the mussels in the prepared control waters (bottom line, Figure 14). Trend lines for both natural seston mussels and algae supplemented mussels showed high correlation to natural log exponential equations (0.89 and 0.94 respectively).

Following growth of the mussels' weight throughout the experiment shows higher growth for the mussels in waters supplemented by algae (top line, Figure 15). Mussels in waters comparable to Lake Mead (middle line, Figure 15) showed higher growth than the mussels in the prepared control waters (bottom line, Figure 15). Trend lines for both natural seston mussels and algae supplemented mussels showed high correlation to natural log exponential equations (0.90 and 0.97 respectively).

Our results suggest that given higher food conditions, the mussel population in Lake Mead could grow at a significantly faster rate if more algae were present.

Our results are comparable to growth rate by mass of seston mussels in other studies; Baldwin et al.'s study (2002) included mussels within the same size class at similar temperature (23°C) as our study. Like the Baldwin study, our quagga growth rates are higher than those they observed for zebra mussels (0.17 day\(^{-1}\) for zebras vs. 0.35 day\(^{-1}\) for our quagga results). While the Baldwin study has comparably higher growth rates for quagga mussels in their seston versus quagga growth in our seston (0.71 day\(^{-1}\) vs. 0.35 day\(^{-1}\)), the seston used
in their experiment contains between 2 and 4 times the chlorophyll concentrations at 4.4µg/L of the seston we used in this study (Baldwin et al. 2002).

Other studies have investigated growth rates of quagga mussels in-situ and have concluded higher growth rates than ours (Macisaac 1994a), however that study again had significantly higher levels of chlorophyll in the seston (~5µg/L) and had constant replenishment of seston in the flow through cages.

While our results do have limitations to their applications, these growth rates provide insight into future population dynamics for Lake Mead by indicating that with increased algae present, mussel growth would be significantly greater. Our results also provide a baseline of growth rates to be compared to throughout the differing seasons and throughout the various waters of the Southwestern United States.
CHAPTER 6
CONTAMINANT BIOACCUMULATION STUDIES

Questions, Objectives, Hypothesis

**Question**

Are concentrations of elements of concern (As, Se, Mo, Pb) bioaccumulating as a result of quagga mussels.

**Objectives**

Determine if mussels are bioaccumulating harmful elements in their tissue or mussel feces / pseudofeces.

**Hypothesis**

Mussels are accumulating elements in their tissue and pseudofeces.

**Elements of Interest**

Bioaccumulation of trace metals in Dreissenid mussel tissue has been well documented in the past (Ravera et al. 2003, Richman and Somers 2005, Secor et al. 1993). Study of mussel filtration and growth aids in connecting the population to the impact; one of these impacts being the concentration of elements that may present negative health impacts at higher concentrations.

Lead is a metal present throughout the environment that at high levels can cause problems to the human nervous system. It is primarily introduced into humans through exposure to lead based paints as well as consumption of food or water, which could have naturally occurring lead, or could have elevated concentrations from leaching of lead pipes or pottery or paint or any number of lead containing substances. Common lead levels in the surface waters around
the U.S. range from 5 to 30 µg/L (ATSDR 2007b)

Molybdenum, Mo, a trace metal is necessary at a biological level as it is used in nitrogen fixation. Also like other trace metals, it is toxic at higher concentrations (Goldhaber 2003), and thus environmental concentrations should be monitored. Studies have also shown potential for Mo to be present in higher concentrations in biosolids (sewage sludge) which is used for pasture application, offering a pathway for introduction to cattle which has been shown to cause copper deficiency (O’Connor et al. 2001).

Another trace element of concern in the study area is selenium, Se. While Selenium is important to living organisms for its role in selenoproteins, at higher concentrations selenium can cause selenosis, a condition that causes medical problems with symptoms such as hair loss and garlic halitosis (Goldhaber 2003).

Arsenic is a trace element not vital to humans, although some studies have indicated it may be useful to other animals (Uthus 1994). Arsenic is present in both organic, and inorganic forms, inorganic arsenic, As (III) or As(V) is absorbed through water and diet, with the highest food sources being fish (Dabeka et al. 1993), although usually organic arsenic is not as toxic as inorganic arsenic (ATSDR 2007a). Acute effects from arsenic consumption at or above 10 mg/kg/day can lead to brain dysfunction (Civantos et al. 1995).

Both arsenic and selenium are elements of interest due to their naturally higher occurrence in soils and waters in the Southwest (Cizdziel and Zhou 2005, Walker and Fosbury 2009). Molybdenum is of interest as it has in the past been analyzed for potential mining near the Colorado River, and also due to the
potential of runoff introduction to Lake Mead from the Las Vegas Wash. Due to its significant potential health impact, Lead concentrations were also analyzed.

Methods

Sediment Sampling

Samples were prepared via EPA method an adaptation of EPA Method 3050B (see Appendices for detailed methods). Sediment samples were collected from each site. Soil samples were collected from 3 sub-sites at each location, all within 10m of one another, with equivalent distances from the water (approximately 0.5m). Three sub-samples were collected from each sub-site, for a total of nine soil samples collected from each site. The top 1cm of sediment was collected at approximately 1m into the lake from the shore, which was usually at a depth of approximately 0.3m, with each sample filling a 1 L container, for an approximate total sample area of 900cm², and transported in containers in a cooler with ice packs to the laboratory where it was then dried in glass pans in a convection drying oven at 60°C for 48 hours, when constant weight was obtained.

Pseudofeces and Feces Collection

Mussel samples were collected from the Boulder Beach Site for pseudofeces collection. Water was first collected and filtered at the site to <20µm. This water was placed in an acid washed 20L carboy. Mussels attached to cobble were then collected by skin snorkel diving in the littoral zone (<5m depth) and collected into a sampling bucket, then swam back to the shore for rough cleaning of attached detritus. Mussels, while still attached to rocks, were
gently scrubbed with a nylon brush to remove all external detritus and sediment. No crushed mussels were observed. Mussels attached to rocks could be carefully handled by only holding the bottom side of the rocks, as they were not colonized. After scrubbing, rocks were held and swished in buckets of clean water until no material was observed to be displacing anymore. If material was observed, mussels on rocks were returned to the scrub station for further cleaning. Once clean, mussels and rocks were placed in clean 5 gallon buckets filled with the previously filtered lake water. These containers were then transported immediately back to the laboratory where they were held in an incubator set to present Lake temperature, (~15°C). Buckets were gently aerated overnight, and after 24 hours, mussels were removed, and water was decanted until ~100mL of sample and pseudofeces / feces could be collected from settlement on the bottom of the bucket. This was then dried in glass beakers in the laboratory convection oven for 48 hours at 60°C.

**Mussel Soft Tissue Collection**

After removal from pseudofeces collection buckets, mussels were then detached from the rocks and dissected for tissue collection. Tissue from three different rocks was sorted into three separate containers. Mussels were equally selected for dissection, without selection for size or location of attachment. Mussel tissue samples were then placed in wide beakers and dried in the convection drying oven until constant mass (~48 hrs). Tissue samples were then homogenized with a mortar and pestle, until uniform in composition. In between sampling, mortar and pestle were cleaned with triplicate rinsing with ethanol. All
samples; sediment, pseudofeces, and pulverized tissue and pseudofeces were screen sorted to particles below 190µm, so as to digest similar samples, and also to obtain a particle fraction that in the environment might be likely to be re-suspended in water or be ingested by detrital feeders.

**Sample Digestion**

One gram samples were weighed into Pyrex glassware that had been previously acid washed. The one gram samples were then digested with Trace Metal Grade Nitric Acid and 30% Hydrogen Peroxide in series. Samples were allowed to flux and digest in a Hot Water Bath set to 95°C +/-0.5°C. After digestion was complete, samples were cooled and reconstituted with Nanopure water to approximately 100mL. Samples were transferred to centrifuge tubes and centrifuged at 3220rcf for 10 min to settle particles, and then supernatant was transferred to separate sterile disposable centrifuge tubes for transport.

**Sample Analysis**

Samples were refrigerated until shipped. Samples were sent for analysis to the Arizona State University Goldwater Environmental Laboratory, where they were analyzed on a Thermo iCAP 6300 Inductively Coupled Plasma Optical Emission Spectrometer.

**Results**

**QA/QC Samples**

All sediment samples were collected in triplicate and then sub-sampled in triplicate for analysis. No samples were analyzed in duplicate due to budget
restraints. Analysis completed at the ASU Goldwater Environmental Laboratory included analysis of Quality Control Reference Standards after every tenth sample. These QC samples are premade to contain 1ppm of all elements analyzed in the spectrum analysis (see Appendix 3 for listing of elements and associated limits of detection), except for Ca, K, Mg, and Na, which were made to 10ppm in the standard. All elements were analyzed at two wavelengths, for comparison and optional interference avoidance. Data selected for each element were dependent on whichever dataset (from the two wavelengths) had the best QC data throughout the trial, with no data being selected for elements in which QC measurements exceeded 5% drift.

Elemental Concentrations

Below can be found the average concentrations for elements of interest in the before described sample groups, along with Sample ID abbreviations that are used in subsequent graphing. Additional concentration averages of other elements analyzed can be found in Appendix 3.

Table 7. Parts per Million Element Concentration in Samples

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Sample Type</th>
<th>Sample ID</th>
<th>As1890</th>
<th>Mo2816</th>
<th>Pb2169</th>
<th>Se2039</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boulder Beach</td>
<td>Boulder Beach Sediment</td>
<td>BB</td>
<td>8.33</td>
<td>6.90</td>
<td>9.25</td>
<td>2.17</td>
</tr>
<tr>
<td>Las Vegas Wash</td>
<td>Las Vegas Wash Sediment</td>
<td>LVW</td>
<td>11.58</td>
<td>11.30</td>
<td>29.39</td>
<td>2.52</td>
</tr>
<tr>
<td>Boulder Beach</td>
<td>Boulder Beach Pseudofeces</td>
<td>PS</td>
<td>9.42</td>
<td>11.87</td>
<td>21.47</td>
<td>3.35</td>
</tr>
<tr>
<td>Boulder Beach</td>
<td>Boulder Beach Mussel Tissue</td>
<td>TS</td>
<td>14.68</td>
<td>3.39</td>
<td>4.16</td>
<td>12.57</td>
</tr>
<tr>
<td>Boulder Beach</td>
<td>Boulder Beach Water</td>
<td>BBWA</td>
<td>0.0043</td>
<td>0.0081</td>
<td>0.0029</td>
<td>0.0065</td>
</tr>
</tbody>
</table>
Figure 16. Arsenic Concentration by Sample Type. Arsenic concentrations (mean ±SE, with n=9 for BB and LVW, and n=3 for PS and TSA) for four sample types with letters above bars indicating significant differences for groupings in pairwise comparisons (Tukey HSD, p<0.001).

Pair wise comparison using Tukey HSD shows highly statistically significant difference between the arsenic concentrations in samples from both Boulder Beach (8.3ppm) and Pseudofeces (9.4ppm) samples when compared to either the Las Vegas Wash Samples (11.6ppm), or the Mussel Tissue (14.7ppm) samples (p<0.001).
Figure 17. Selenium Concentration by Sample Type. Selenium concentrations (mean ±SE, with n=9 for BB and LVW, and n=3 for PS and TSA) for four sample types with letters above bars indicating significant differences for groupings in pairwise comparisons (Tukey HSD, p<0.001).

Pairwise comparison with Tukey HSD resulted in a statistically significant difference between selenium concentration in Boulder Beach Mussel Tissue Samples (12.6ppm) compared to other samples (2.2 - 3.4ppm) (p<0.001).
Figure 18. Molybdenum Concentration by Sample Type. Molybdenum concentrations (mean ±SE, with n=9 for BB and LVW, and n=3 for PS and TSA) for four sample types with letters above bars indicating significant differences for groupings in pairwise comparisons (Tukey HSD, p<0.001).

Pairwise comparison using Tukey HSD shows highly statistically significant differences in molybdenum concentrations between the Boulder Beach Sediment (6.9ppm), the group of Boulder Beach Pseudofeces (11.3ppm) and Las Vegas Wash Sediment (11.9ppm), and the Boulder Beach Mussel Tissue (3.4ppm) samples (p<0.001).
Figure 19. Lead Concentration by Sample Type. Lead concentrations (mean ±SE, with n=9 for BB and LVW, and n=3 for PS and TSA) for four sample types with letters above bars indicating significant differences for groupings in pairwise comparisons (Tukey HSD, p<0.001).

Pair wise comparison using Tukey HSD shows highly statistically significant difference in lead concentrations between the Boulder Beach Sediment (9.3ppm) grouped with Boulder Beach Tissue Samples (4.2ppm), compared to both Las Vegas Wash Sediment (29.4ppm) and Boulder Beach Pseudofeces (21.5ppm) (p<0.001).
Discussion

The hypothesis that certain elements would concentrate to a significant extent in mussel tissues or pseudofeces was supported by the results.

When comparing concentrations of arsenic in the analyzed samples, mussel tissue shows significantly higher levels that that found in the sediments collected adjacent at Boulder Beach, indicating bioaccumulation of arsenic in the tissue. No significant bioaccumulation of arsenic was detected in the pseudofeces of the mussels.

Arsenic concentrations occur at higher levels in sediments from Las Vegas Wash than in sediments from Boulder Beach, indicating that if mussels were to colonize in this area; their tissue concentration could potentially be higher than that observed in the Boulder Beach Mussels.

Selenium concentrations in the mussel tissue samples analyzed are significantly higher than in the Boulder Beach sediment, Las Vegas Wash sediment, or in the pseudofeces samples, indicating bioaccumulation of selenium in the tissue. No significant bioaccumulation of selenium was detected in the pseudofeces of the mussels.

Concentrations of molybdenum in the pseudofeces analyzed were significantly higher than the levels observed in the Boulder Beach sediments or in the mussel tissue samples, indicating bioaccumulation of molybdenum may be occurring in the mussel excretions.

Significantly higher levels of molybdenum were also noted in the Las Vegas Wash sediment samples compared to the Boulder Beach sediment
samples, indicating that were mussels to colonize these waters, higher bioaccumulation could be expected.

Lead concentrations in Boulder Beach mussel pseudofeces samples were significantly higher than the concentrations found in the sediment samples collected at Boulder Beach, indicating bioaccumulation in the pseudofeces of the waste of the mussels. Significantly lower levels of lead were found in the mussel tissue samples, indicating the mussels are not bioaccumulating lead compared to the Boulder Beach sediments.

The highest concentration of lead found in any sample group was found in the sediment samples collected in the Las Vegas Wash. These significantly higher levels indicate that if mussels were located here, higher levels of lead would be expected to be found in their pseudofeces than what was found in the analyses of the samples from mussels collected at Boulder Beach.

Although in comparison with other analysis of Lake Mead sediments our concentrations are considerably lower (Rosen and Van Metre 2009) despite similar analysis technique (ICP-AES), the samples in this experiment were processed without the aid of microwave digestion, potentially explaining the lower element concentrations as due to a comparably lower degree of total digestion. Despite this, our results are still pertinent and useful as the increased levels / levels of interest are comparable to the other samples within this study that were processed in the same manner, thus still supporting our conclusion that mussels are bioaccumulating elements.

Similarly, the only other study found that analyzed
pseudofeces/biodeposition of zebra mussels for elemental analysis used microwave digestion in the methodology. This study also analyzed only nickel, copper and zinc (Klerks and Fraleigh 1997).

This paucity of comparable data was found repeatedly; Richman and Somers (2005) analyzed both zebra and quagga mussels for lead concentration, resulting in comparable levels of non-detectable up to 14ppm, compared to this studies average lead concentration of tissue of 4.2ppm, however, the Richman and Somers study did not analyze pseudofeces, which in our study contained over five times that concentration at 21.5ppm. One interesting point that Richman and Somers (2005) presented was that the zebra mussels analyzed alongside the quagga mussels in this study had statistically higher concentrations of the elements analyzed; leading the results of our study to indicate that even higher levels of bioaccumulation and bioaccumulation should be expected if Lake Mead were to become infested with zebra mussels in addition to quagga.
CHAPTER 7

CONCLUSIONS

As the quagga mussel continues to impact native habitats and reservoirs, research on the species will become of greater and greater importance. Filtration and growth rate can be used not only to understand the impact they have on the ecology and food web of a system, but also of the mussels’ impact on physical traits of bodies of water.

The clearance rate experiments completed in this study indicated the quagga mussels in Lake Mead filter at higher rates per mass when still in an early growth stage, and that they filter smaller algae particles faster than larger ones. The highest filtration rates were observed in the waters with high sediment and low nutrient concentration, an important indication mussel impact. Waters in lakes and rivers that experience high turbidity, or times of high turbidity, may potentially see a larger impact in water clarity and clearance by the mussels.

Our growth study results suggest that quagga mussel growth in Lake Mead may be food limited at this time, and it would be reasonable to predict significant increases in their growth at times during the year when algae concentration is greater, inclusive of summertime increases in lake productivity.

Future studies to determine if mussel clearance impacts the algae concentration in the lake, either positively by allowing for more light penetration and nutrient concentration, or negatively through mussel grazing, would give lake managers further guidance for decisions concerning mussel impact.

Though there are no natural predators of the Quagga mussel present in Lake Mead at this time, quagga mussels filtering in Lake Mead appear to be
bioaccumulating and bioconcentrating elements. There have been recorded instances of fish and birds developing an affinity for the mussel in other areas where the mussel has been introduced (Custer and Custer 2000, Ward and Ricciardi 2007). A popular destination for fishermen (and fisherwomen), Lake Mead has large populations of largemouth bass, striped bass, and channel catfish, offering not only an opportunity for the toxicants to enter the food chain, but also to cause a hazard via direct prolonged human contact with the sediment pseudofeces. In addition, the lake has known standing populations of two protected native fish species, the razorback sucker, and the bonytail chub, both of which spawn in benthic areas that may contain mussels and pseudofeces.

Considering these points, further studies of contaminants in the mussel tissue and their contribution to the benthos through their feces and pseudofeces should be undertaken at a larger scale.

This study is not only sound valuable ecological information about the quagga species, comparable with data worldwide; results from this research also identify a potential for the quagga mussel to pose a threat to wildlife at Lake Mead, as well as potential risk for environmental exposure of certain elements at higher levels.
APPENDIX 1

Adapted EPA Method 3050B

1) Set water bath to 95 °C
2) Weigh 1 g +/- 0.0005g of sample
3) Transfer sample to a 400 mL beaker (use 10 mL Nano to rinse sample into beaker)
4) Pour Trace Metal Grade concentrated nitric acid into a dispensing beaker
5) Add 10 mL nitric acid to sample beakers and cover loosely with Petri dish
6) Then add 20 mL nitric acid to beaker and cover loosely
7) Put covered beaker into water bath
8) Heat the sample to 95 °C and reflux for 10 to 15 minutes without boiling
9) Allow the sample to cool, add 5 mL of nitric acid, replace the cover, and reflux for 30 minutes
   [If brown fumes are generated, indicating oxidation of the sample by nitric acid, repeat this step (addition of 5 mL of conc. nitric acid) over until no brown fumes are given off by the sample indicating the complete reaction with nitric acid]
10) Either allow the solution to evaporate to approximately 5 mL without boiling or heat at 95 °C without boiling for two hours
    [Maintain a covering of solution over the bottom of the vessel at all times]
11) After the sample has cooled, add 2 mL of water and 3 mL of 30% hydrogen peroxide. Cover the vessel and return the covered vessel to water bath to start the peroxide reaction
[Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence]

12) Heat until effervescence subsides and cool the vessels

13) Continue to add 30% hydrogen peroxide in 1 mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged. Do not add more than a total of 10 mL 30% hydrogen peroxide

14) Cover the sample and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 mL or heat at 95 °C without boiling for two hours

   [Maintain a covering of solution over the bottom of the vessel at all times]

15) After cooling, dilute to 100 mL with water

16) Particulates in the digestate should then be removed by centrifugation at 3220rcf for 10 minutes

17) Decant supernatant and store for analysis.
APPENDIX 2

Algae Culturing

Protocol Courtesy of Florida Seagrant

1. Use sterile technique to maintain cultures effectively. Be fastidious.

2. Culture initial stocks in an incubator at 23°C, 18 h light/6 h dark, according to Provasolli-Guillard protocols, with progression from the 15 mL stock delivery vessel to covered 150 mL flasks to 1.5 L Fernbach flasks as culture densities allow.

3. Swirl flasks daily to maintain stocks in suspension.

4. Transfer to larger vessels as a function of cell density and culture age.

5. Transfer any cultures exceeding 500,000 cells/mL to the next larger vessel.

6. Transfer any cultures ten days old, irrespective of cell density, if its color remains healthy.

7. Fill all incubator vessels to working depth and microwave to 85°C for sanitation (Keller et al, 1988). Cool to 23°C, inoculate vessels with F/2 (0.15 mL/L each of F/2 stocks A and B) and algae stock under a laminar flow hood to minimize contamination.
8. Scrub all carboys (20L) inside and outside with a brush, and rinse thoroughly with hot water. Acid-wash all carboys with muriatic acid, outdoors.

9. Fill all carboys completely to the top with 1 µm filtered, UV-sterilized water.

10. Add 10% hypochlorite at 0.2 mL /L and treat vessels for 24 h

11. Dechlorinate vessels with thiosulfate stock solution at 0.2 mL /L, and allow to stand for 4-6 h.

12. Filter all air to 0.2 µm with Gelman Acro50 air filters, to minimize contamination during culture.

13. Sterilize your hands with 70% isopropyl alcohol. Wipe the mouth and neck of the carboy with alcohol prior to inoculation.

14. Decant carboys to 15 L working depth.

15. Inoculate carboys with F/2 stocks A&B at 0.15 mL /L each, 6 g sodium bicarbonate, NaHCO₃ (3 millimole, final concentration), and swirl to dissolve the bicarbonate.

16. Inoculate carboys with 750 mL algae culture from incubator Fernbach vessels and label for species and starting date.

17. Start moderate aeration immediately.

18. Swirl carboys twice daily to maintain algae in the water column.
19. After 5 days, add additional F/2 A&B stock and slightly increase aeration.

20. When the pH elevates above 9, bubble CO$_2$ to further increase cell density. If CO$_2$ is unavailable, add a little more NaHCO$_3$ to buffer the solution.

21. When cultures are 5-7 d old, cell densities should exceed 1,000,000 cells/ml.

22. Maintain the culture as long as cell density continues to increase. Nutritive values of algae are greatest during the log-phase of growth. Feed to copepods, or use as an inoculant for larger scale algae culture.

Discard cultures when cell density ceases to rise. The algae culture has reached senescence and its nutritive value plummets. Use as feed for copepods only in emergencies.

**For each species of algae:**

Feed concentration x liters of copepods cultured = Daily Production Demand

Algae concentration x liters of algae cultured = Daily Production Capacity

This demand specifies how many liters of a given species will be required daily (plus extra volume to maintain a production safety buffer).
## APPENDIX 3

### Additional Elemental Concentrations in Contaminant Analyses

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Ag3382</th>
<th>As1890</th>
<th>Be2348</th>
<th>Ca3179</th>
<th>Cd2144</th>
<th>Co2286</th>
<th>Cr2677</th>
<th>Fe2599</th>
<th>K_7664</th>
<th>Li6707</th>
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<tbody>
<tr>
<td>BB1A</td>
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<td>8.6890244</td>
<td>0.3988</td>
<td>46133</td>
<td>0.1045</td>
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Detection Limit (mg/L) 0.0006 0.002 9E-05 5E-05 0.0001 0.0002 0.0002 0.0001 0.0003

### Water Elemental Concentration ppm (mg element/L sample)

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Detection Limit (mg/L) 0.0001 0.0005 0.0005 0.0005 0.0001 0.0002 0.0004 5E-05 0.0005 0.0005

### Water Elemental Concentration ppm (mg element/L sample)
**APPENDIX 4**

**Hazardous Assessment Management Plan and Dive Plan**

**Concurrence Signature Page**

Project Title: Quagga Mussel Ecology  
Budget Number: _____________________

HAMP Author: Carolyn Link  
Division: DHS

Rev. Number: _____________________

### Concurrences

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<tr>
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<td>(702) 862 – 5453 and (702) 862 – 5371</td>
<td><a href="mailto:lambis.papelis@dri.edu">lambis.papelis@dri.edu</a>, <a href="mailto:Kumud.Acharya@dri.edu">Kumud.Acharya@dri.edu</a></td>
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**Location of Field Research**

- **Country:** USA
- **Geographical Site:** Lake Mead NRA
- **Nearest City:** Boulder City, NV
- **Nearest Hospital:** Boulder City Hospital  
  901 Adams Blvd  
  Boulder City NV 89005-2299  
  (702) 293-4111  
  see attached directions

**Field Research (Nature of Work):** (Brief description of field work)

Mussel, water and substrate collection within Lake Mead NRA. Shore collection, skin diving and SCUBA diving from shore and also from boat.

**DRI Contact:**

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**Local (field) Contact:**

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<td>Carolyn Link</td>
<td>(702) 372 - 5452</td>
</tr>
</tbody>
</table>

**Field Work Team Members**

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Category (check all that apply)</th>
<th>Train first aider</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambis Papelis</td>
<td>DRI – DHS</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Kumud Acharya</td>
<td>DRI – DHS</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Carolyn Link</td>
<td>DRI – DHS</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>DRI GA field research assistant</td>
<td>DRI</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>UNLV/ DRI student research assistant</td>
<td>UNLV - DRI</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

**Emergency Procedures:** (Include detailed plans for the field location including evacuation and emergency communications.) Use a separate sheet of paper if necessary.
Evacuate from collection sites and proceed to the NRA Park Gates. Contact P.I.s or 911 as appropriate. In case of fire on boat, follow boating safety procedures, and in case of SCUBA emergency, follow appropriate dive emergency plans.

### Hazards Inherent to the Project Site

a. **Hazards inherent to the project site**, such as

- High Altitude
- Extreme Cold/Heat
- Excess exposure to sun, wind, blowing sand, etc.
- Work in Confined Spaces (natural or man-made)
- Work Over/Under Water
- Falling Objects (avalanches, rock falls, etc.)
- Remote Location
- Rough Terrain
- Wild Animal/Plant Hazards
- Potential for Adverse Weather
- Flash Flood Potential
- Long Distance to Medical Services
- Difficult Communications with the outside world
- Climbing/Strenuous Hiking required
- Crossing High Water required
- Travel on Primitive Roads or cross county required.
- Towing
- Work along roadway shoulders (Attach traffic control plan and permit, if required)
- Other ____________________________

b. **Additional hazards** that might be present

- Cut hazards, such as those associated with working with metal, sharp edges on equipment, etc.
- Mechanical/Moving Parts
- Trenching/Excavating
- Heavy Equipment Operations
- Overhead Hazards, including, but not limited to power and other utility lines
- Slip/Trip/Fall Hazards
- Falls (from height)
- Use of Ladders/Scaffolding
- Work at Night/in Poor Lighting
- Long Drive to work site
- Manual Lifting > 50 lb
- Noise Generated > 85 dBA
- Dust/other Airborne Hazard generated by work
- Potential for Oxygen Deficient or other hazardous atmospheres generated by work
- Fire issues related to hot work, ignition sources, flammable materials use, etc.
- Potential for Hazardous Material Spill
- Waste Generation
- Lack of Potable Water
- Lack of Sanitary Facilities
- Transportation of Hazardous Materials to/from work site
- Storage of Hazardous Materials on site

### Major Equipment Required to Conduct the Fieldwork:

c. **Use of heavy equipment**, such as:

- Forklift
- Backhoe
- Excavator
- Crane/hoist/man lift
- Dump Truck
- Loader
- Scraper
- Steam Cleaner
- High Pressure Washer
- Jack Hammer/Concrete Saw
- Hydraulic Ram
- Vacuum Truck
- Water Truck
- Snowmobile/ATV
- Airplane/helicopter
- Drill Rig
- Dumpster/Roll-Off Container
- Other _______________________

### d. Use of other equipment/materials that might pose a safety hazard or require safety training, for example:
- Generator
- Pump
- Compressor
- Towers
- Chemicals
- Biologicals
- Radioactive Materials Class 3b or 4 lasers/laser systems
- High Energy Sources
  - Boats/Kayaks, Canoes
  - Pressurized/Vacuum Systems
  - Fire Extinguishers
  - Personal Protective Equipment

**Chemicals and other Hazardous Materials Used on this Project:**

**none**
### Personal Protective Equipment Required:

List here the minimal PPE required for the field work and note any additional PPE requirements based on risks listed as part of the control measures noted in the table below.

- All jobs require basic Level D including sturdy work clothing; work gloves (leather/cotton); safety shoes/boots, and safety glasses
- Gloves -- specify type(s)
- Face Shields
- Hearing Protection
- Impervious Boots
- Cotton Coveralls
- Eye Wash
- Extraction Equipment (confined space)

- Goggles
- ANSI approved hardhat
- Respirator -- specify type(s) and cartridge type (if APR)
- Disposable Work Boot Covers
- Rain Gear
- Disposable Coveralls
- Moisture Resistant Disposable Coveralls
- Emergency Shower
- Fall Protection

### Risk Assessment:

List identified risks associated with the field activity or physical environment (such as extreme heat/cold, wild animals, endemic disease, etc.). For each identified risk list the appropriate measures to take to eliminate or reduce the risk. **Use additional sheets if necessary.**

<table>
<thead>
<tr>
<th>Identified Risk</th>
<th>Control Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun / heat exposure</td>
<td>Review of DRI Heat Stress Fact Sheet, Recommended hydration, sunscreen, appropriate clothing, breaks</td>
</tr>
<tr>
<td>Work over / under water</td>
<td>Review of DRI Working on or over water Safety Policy</td>
</tr>
<tr>
<td>Cut hazard posed by mussel shells</td>
<td>Gloves required when handling mussels or substrate samples</td>
</tr>
<tr>
<td>Boat usage</td>
<td>Review of DRI Safe Boating Guidelines</td>
</tr>
<tr>
<td>SCUBA diving</td>
<td>SCUBA certification required, compliance with DRI Diving Safety Program</td>
</tr>
</tbody>
</table>
**Travel Immunizations:** (List any required immunizations/prophylaxis required for this field study)

None needed, although a tetanus shot within the past 10 years is recommended.

**Safety Training Required:**

<table>
<thead>
<tr>
<th>Section 1—General Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>X HAMP Orientation and dive plan review</td>
</tr>
<tr>
<td>X First Aid/CPR</td>
</tr>
<tr>
<td>X Emergency Action and Preparedness</td>
</tr>
<tr>
<td>X Fire Extinguisher Use</td>
</tr>
<tr>
<td>□ Ergonomics, includes back safety, lifting, manual material movement</td>
</tr>
<tr>
<td>□ Hazard Communication (general chemical safety)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Section 2—Project Specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ OSHA Carcinogens</td>
</tr>
<tr>
<td>□ Compressed Gases and Cryogenic Liquids</td>
</tr>
<tr>
<td>□ Project specific Hazard Communication (specific to chemical hazards)</td>
</tr>
<tr>
<td>□ Dangerous Goods/Hazardous Materials Shipping</td>
</tr>
<tr>
<td>□ Hazardous Waste Generator Training</td>
</tr>
<tr>
<td>□ HAZWOPER Training</td>
</tr>
<tr>
<td>□ First responder awareness level</td>
</tr>
<tr>
<td>□ Hazardous Waste Operations level</td>
</tr>
<tr>
<td>□ Storm water Awareness Training</td>
</tr>
<tr>
<td>□ Energy Control (Lockout/Tagout)</td>
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<tr>
<td>□ Electrical Safety</td>
</tr>
<tr>
<td>□ Biosafety (infectious agents)</td>
</tr>
<tr>
<td>□ Bloodborne Pathogens</td>
</tr>
<tr>
<td>□ Radiation Safety</td>
</tr>
<tr>
<td>□ Laser Safety</td>
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<tr>
<td>□ Personal Protective Equipment</td>
</tr>
<tr>
<td>□ Respiratory Protection</td>
</tr>
<tr>
<td>□ Hearing Conservation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Section 3—Other (ex. project specific SOPs, equipment operation, etc.) List All</th>
</tr>
</thead>
<tbody>
<tr>
<td>X SCUBA certification</td>
</tr>
</tbody>
</table>
Dive Plans

Dives should be planned around the competency of the least experienced diver. Before conducting any diving operations under the auspices of the organizational member, the lead diver for a proposed operation must formulate a dive plan that should include the following:

Dive Plan for: Quagga Mussel Ecology Research Project

Prepared by: Carolyn Link      Date: 6/22/2009

a) Divers qualifications, and the type of certificate or certification held by each diver.

Carolyn Link: PADI Certified Advance Open Water Diver

Diver No: 0703084111

Lambis Papelis: PADI Instructor Development Course Staff Instructor

Diver No: 171766

b) Emergency plan (Appendix 7):

General Procedures

Depending on and according to the nature of the diving accident:
1. Make appropriate contact with victim or rescue as required.
2. Establish (A)irway, (B)reathing, (C)irculation as required.
3. Stabilize the victim
4. Administer 100% oxygen, if appropriate (in cases of Decompression Illness, or Near Drowning).
5. Call local Emergency Medical System (EMS) for transport to nearest medical treatment facility. Explain the circumstances of the dive incident to the evacuation teams, medics and physicians.
   Do not assume that they understand why 100% oxygen may be required for the diving accident victim or that recompression treatment may be necessary.
6. Call appropriate Diving Accident Coordinator for contact with diving physician and decompression chamber. etc.
7. Notify DSO or designee according to the Emergency Action Plan of the organizational member.
8. Complete and submit Incident Report Form (www.aaus.org) to the DCB of the organization and the AAUS (Section 2.70 Required Incident Reporting).

1. Name, telephone number, and relationship of person to be contacted for each diver in the event of an emergency.
Carolyn Link: Ken Link : Father: (928) 646-6940

Lambis Papelis:

2. Nearest operational decompression chamber.

NOTE: Contact Diver Alert Network at 1-919-684-4326 first for confirmation of nearest functioning decompression chamber.

Valley Hospital Medical Center: Wound Healing & Hyperbaric Center
2020 Goldring Avenue
Las Vegas, NV 89106
(702) 671-8660
see attached directions


Boulder City Hospital
901 Adams Blvd
Boulder City NV 89005-2299
(702) 293-4111
see attached directions


Vehicle used to reach sampling sites.
c) Approximate number of proposed dives.

Potential for average of 1 dive per month, until completion of project. Estimated Latest Project Completion: May 2010. (total 10 dives)

d) Location(s) of proposed dives.

Nevada areas of Lake Mead National Recreation Area.

e) Estimated depth(s) and bottom time(s) anticipated.

100ft Estimated max depth. Bottom times calculated appropriately pre-dive as part of standard pre-dive protocol; dependent of depth of individual dive.

f) Decompression status and repetitive dive plans, if required.

No repetitive dives planned

g) Proposed work, equipment, and boats to be employed.

Collection of mussels and lake water to be completed either from shore or from DRI boat located currently in storage at DRI’s facility in Boulder City.

h) Any hazardous conditions anticipated.

None anticipated
Directions to Boulder City Hospital from Collection Area

901 Adams Blvd, Boulder City, NV 89005-2299 - (702) 293-4111
7.1 mi – about 13 mins

Lakeshore Dr/NV-166

Show: Text only | Map | Street View

1. Head southeast on Lakeshore Rd/NV-166/State 146 toward Lake Mead National Rec Area
   Continue to follow Lakeshore Rd/NV-166
   About 5 mins

   go 2.8 mi
total 2.8 mi
Show: Text only | Map | Street View

2. Slight right at US-93
About 6 mins
go 3.8 mi
total 6.5 mi
Show: Text only | Map | Street View

3. Continue on Buchanan Blvd
About 1 min
go 0.4 mi
total 6.9 mi
Show: Text only | Map | Street View

4. Turn left at Adams Blvd
go 0.1 mi
total 7.1 mi
Show: Text only | Map | Street View

Boulder City Hospital
901 Adams Blvd, Boulder City, NV 89005-2299 - (702) 293-4111
Quagga Mussel Diver Decontamination Protocols

After inspecting bodies of water known or suspected to contain live quagga mussels at any life stage, all field equipment that was in some way in contact with the water should be thoroughly cleaned before moving to another site. If sampling is being performed to determine whether quagga mussels are present at a given site, assume that they are present and thoroughly clean all diving and sampling equipment before moving to another site.

Divers:

1. Check all gear that could potentially hide any veligers (include regulators, BCDs, wetsuits, masks, snorkels and any other dive gear),
2. Thoroughly clean all regulators, BCDs, wetsuits, masks, snorkels and any other dive gear, making sure to clean both the inside and outside of the BCD to ensure that no mud or organic matter is present – use a brush if necessary.
3. After cleaning, rinse your suit, equipment and inside of BCD with hot (<40° C or 104°F) or salt (1/2 cup salt/gallon) water. Note, if you use the salt-water solution, it is very important to thoroughly rinse the equipment in freshwater after your cleaning because the salt crystals can harm your equipment.
4. Allow gear, suit and other equipment to dry before diving in different waters. Veligers can survive for a period of time on wet scuba gear;
5. Consider using two sets of gear if applicable, alternating between set every other day.
6. If feasible, consider freezing your equipment overnight to kill any veligers.

Sampling equipment (nets, waders, boots, buckets, etc.):

1. All field equipment needs to be visually inspected and all visible mussels removed and killed.
2. All field equipment must be cleaned by soaking, dipping in, or scrubbing with a salt water solution. If one of these approaches is not possible, the equipment should ideally be pressure-washed or at least rinsed with water (hot and/or high pressure if possible) and allowed to dry completely before next use.
3. Particular attention should be given to places where the mussels could be accidentally trapped, such as the treads of boots and waders, hinges of benthic grabs, etc.
4. If feasible, consider freezing your equipment overnight to kill any veligers.


### APPENDIX 5

**N.D.O.W. Scientific Collection Permit**

---

**APPLICATION**

**SCIENTIFIC COLLECTION/POSSESSION/BANDING PERMIT**

Fee [Check one]: ☑ $50.00 – 1 Year Permit (permit class 22.85)

☐ $100.00 – 2 Year Permit (permit class 22.92)

Please PRINT all information except for your signature. Incomplete or illegible applications will be returned.

PROCESSING TIME: All applications will be routed for review and approval, which can take up to 6 weeks, depending on complexity and bureau recommendations.

**PURPOSE:** (check one) ☑ Scientific ☐ Educational

I hereby make application for:

☐ New application: Complete the entire applicant information block and all sections. Sign and date the application. Do not send fee until notified of approval.

☐ Renewal of last year’s PERMITTED projects with changes or new projects: Complete the entire applicant information block and then complete all other sections in the application where changes are being requested. CLEARLY DESCRIBE CHANGES. Sign and date the application. Do not send fee until notified of approval.

☐ Renewal of last year’s PERMITTED projects without changes: Complete the entire applicant information block. Sign and date the application. Do not send fee until notified of approval.

---

**APPLICANT INFORMATION**

<table>
<thead>
<tr>
<th>NAME [LAST]</th>
<th>LING</th>
<th>[FIRST]</th>
<th>CAROLYN</th>
<th>[MIDDLE]</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHYSICAL ADDRESS: DESERT RESEARCH INSTITUTE (DRI)</td>
<td>755 E. FLAMINGO RD.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CITY:</td>
<td>LAS VEGAS</td>
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<tr>
<td>STATE:</td>
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<td>WORK PHONE:</td>
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</tr>
<tr>
<td>EMAIL ADDRESS:</td>
<td><a href="mailto:carolyn.ling@dri.edu">carolyn.ling@dri.edu</a></td>
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<td></td>
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</tr>
<tr>
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<tr>
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<tr>
<td>DRIVER’S LICENSE NUMBER:</td>
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<td>STATE:</td>
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<tr>
<td>DATE ISSUED:</td>
<td>7/27/05</td>
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<tr>
<td>OCCUPATION:</td>
<td>UNLV Graduate Student</td>
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<tr>
<td>EMPLOYER:</td>
<td>UNLV / DRI</td>
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<tr>
<td>WORK ADDRESS:</td>
<td>SAME AS ABOVE</td>
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</tbody>
</table>

INSTITUTION OR COMPANY YOU ARE REPRESENTING, IF NOT YOURSELF OR THE EMPLOYER LISTED ABOVE (NAME/ADDRESS/PHONE):

Desert Research Institute
- see above for address

---

STATE OF NEVADA – Department of Wildlife  SLAP 22.85/22.92  Rev. 1/8/2008  Page 1 of 3

RECEIVED

JUN 25 2008

NEVADA DIV. OF WILDLIFE
1. In the table below, list the species and number of each that you intend to capture and possess, kill, band and release, etc. by each specific capture situation. Do not combine several capture situations with a single number; e.g. “200 – a, b, c, d.” Provide a specific number with each capture situation/method. (See example below in table.)

<table>
<thead>
<tr>
<th>Species (common &amp; scientific names)</th>
<th>#/Site/Year</th>
<th>C.S.</th>
<th>Species (common &amp; scientific names)</th>
<th>#/Site/Year</th>
<th>C.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Example): Pharynx Killfish</td>
<td>10</td>
<td>b</td>
<td>Quagga Mussel</td>
<td>1000</td>
<td>b</td>
</tr>
<tr>
<td>(Emphrithys latos)</td>
<td>15</td>
<td>c</td>
<td>Dreissena bugensis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Give dates and locations of sampling or educational activity. Provide your best estimate of the specific location(s) (e.g. lake, river, stream, drainage, etc.) include county as part of the location whenever possible with the dates of the proposed trapping/collection/sampling or educational activity.


Lake Mead, Clark County
July 2008 (pending permit) - July 2010

3. Provide the purpose and justification for this request. Attach a synopsis, not exceeding 5 pages, of the research or educational project being proposed, including methods of capture and the names of additional collectors/agents. Also, describe your qualifications.

See attached

4. Disposition: Name and address of the public, scientific, or educational institution(s) to which all specimens will be transferred.

Desert Research Institute
755 E. Flamingo Rd.
Las Vegas, NV 89119

5. Federal Permits: Attach a copy of your federal permit, issued by the U.S. Fish and Wildlife Service, which is valid for Nevada (required for threatened or endangered wildlife and migratory birds unless specifically exempted by the Service).

Not Applicable
I, the signator, in signing this application, hereby state that I am entitled to this permit under the laws of the State of Nevada and that no false information or false statement has been made by me to obtain this license.

Signature of Applicant       Date

Submit your completed application to:

Nevada Department of Wildlife
License Office – Scientific Collection
4600 Kietzke Lane D-135
Reno, NV 89502

Department: ____________________________

Date Received: ________________________

Date Approved: ________________________ Date Returned for Additional Information: ________________________

Date Disapproved: _____________________ Department Representative: ______________________

REASON FOR DISAPPROVAL:

__________________________

STATE OF NEVADA – Department of Wildlife    SLAP 22 85727 427    Rev. 1/1/2008    Page 3 of 3
Question 3:

Purpose / Synopsis of Research

The primary focus of the research will be on the biological and ecological traits of Quagga mussels present in Lake Mead, and their impact on biodiversity and water quality in Lake Mead. Research goals include contributing to current knowledge on the species to assist in future containment and management strategies. Studies will pertain to physiological ecology, autecology, phylogenetics, and bioaccumulation of the mussels. Specific projects will study the effects on mussels and their tolerance of manipulations to their environment in a controlled laboratory setting, and also the geographical, genetic, and chemical traits of the Lake Mead specific population.

Methods of Capture / Containment / Disposal Protocols

Methods of capture will include shore collection, collection from deeper waters with the aid of S.C.U.B.A. equipment, Ponor/Elkman collection by boat, and filtration collection of veligers from water with mesh screening and netting.

With reference to the protocols of the Zebra-Mussel-Specific Containment Protocols by Reid et al. (1999), the EPA disposal requirements followed at DRI for biological waste, the Protocol for Quagga Mussel and Corbicula Transport and Containment by Dr. Sudeep Chandra from University of Nevada Reno, and the Quagga Mussel Diver Decontamination Protocols provided online by the California Department of Fish and Game, (see attached source information) every measure will be made to ensure all equipment and lab practice is properly monitored to prohibit mussel introduction outside of the confines of the study. This will include appropriate training of all staff in contact with the lab, cleaning and sterilization of equipment and secondary and tertiary containment vessels when needed.

Additional Collectors / Agents / Qualifications

Additional collectors/agents will include Principle Investigators Dr. Kumud Acharya, Dr. Lambis Papelis, and Dr. Mark Stone, as well as research assistants (future hires). Dr. Kumud Acharya has extensive publications in peer reviewed journals about limnology and physiological ecology, including work on zebra mussel studies during his post-doctoral tenure at The University of Louisville. Dr. Lambis Papelis has over 25 years of experience in aquatic chemistry and water quality related research including projects at Lake Mead. He is SCUBA certified as a Professional PADI Course Instructor and for Deep Diving up to 140ft (40m). Both Dr. Acharya and Dr. Papelis have well equipped biological and chemical laboratories at DRI with advanced microscopic and spectroscopic facilities. As the master’s student researcher, Carolyn Link has research assistance experience in aquatic ecology and environmental chemistry (Northern Arizona University). Current coursework towards a M.S. in Water Resource Management (University of Nevada Las Vegas) and work experience in a public aquarium add skills in tank maintenance. animal husbandry, and Association of Zoos and
NEVADA DEPARTMENT OF WILDLIFE
SPECIAL LICENSE/PERMIT

Date Issued: 7/25/2008    License Type: Scientific Collection Permit

Name of Licensee/Permittee: Link Carolyn
SSN: 601-50-0813

Mailing Address: Desert Research Institute, 755 E Flamingo Rd

Street Address: Same

City: Las Vegas    State: NV    ZIP: 89119

License Class: 22.92    Agent No: 1950    Issued by: jgm    Fee $100.00

License/Permit Valid Form: July 25, 2008 - June 30, 2010

FORM 220X
S 31134

— Special Conditions —
All applicable sections set forth in the Nevada Administrative Code (NAC) and Title 45 of the Nevada Revised Statutes (NRS) shall apply.

- Authorizations and Conditions Attached –
- Period of Collection Activities: See Condition #4–
- Activity Report(s) Due: 07/30/2009; 07/30/2010 –
specimens of each species taken; species name; the habitat type where each specimen was taken; numeric breakdown of sex whenever possible; and a description of the location where each specimen was taken, by the following methods: (Don't use common geographic names)  
- UTM Coordinates, NAD 83, Zone 11, rounded to the nearest meter;  
- Coords. of longitude & latitude, WGS 84, in decimal-degrees to 4 places (117.2456°);  
- Township, Range and 1/4 Section.

The records must be submitted to the Nevada Department of Wildlife License Office – Scientific Collection Report, 4600 Kietzke Ln D-135, Reno, NV 89502, by 7/30/08 for 2008/2009 "take" activities; and 7/30/10 for 2009/2010 "take" activities. Digital reports in Excel spreadsheet (preferred) or Quattro Pro are accepted (please follow column sequence as outlined in the Department report form, 22.85-5.)

7. A copy of all pertinent research or technical papers must be submitted to the Department.

8. Wildlife collected under the authority of this permit shall not be sold, bartered, traded or converted to personal use. Except as provided in Condition #5, wildlife may only be permanently transferred with the written approval of the Department.

9. No fee may be charged to the public for the privilege to view wildlife which is held under the authority of this permit.

10. Permit Cancellation: A violation of a condition or stipulation is cause for the cancellation of the permit.

11. Additional Authorized Collectors: Dr. Kumud Acharya, Dr. Lambris Papelis, Dr. Mark Stone and authorized research assistants under the direction of the Permitee.

Julie Meadows  
Program Officer I

jgm  
enclosure
<table>
<thead>
<tr>
<th>Date of Capture/ Collection</th>
<th>Species Name</th>
<th>Number Taken by method</th>
<th>Location of &quot;take&quot;: longitude and latitude</th>
<th>Habitat</th>
<th>Number by sex</th>
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</table>

Habitat key:
19: other: underwater benthic sampling
REFERENCES


Snyder, F. L., M. B. Hilgendorf, and D. W. Garton. 1997. Zebra Mussels in North America: The invasion and its implications! *Ohio Sea Grant, Ohio State University, Columbus, OH.*


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Thesis Title:
Filtration and Growth Rate of Lake Mead Quagga Mussels (Dreissena bugensis) in Laboratory Studies and Analyses of Bioaccumulation

Thesis Examination Committee:
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Committee Co-Chair: Dr. Kumud Acharya, Ph.D.
Committee Member: Dr. Shawn Gerstenberger, Ph.D.
Graduate Faculty Representative: Dr. Carl Reiber, Ph.D.