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Substrate Monitoring, Contaminant Monitoring, and Educational Outreach on Quagga Mussels (Dreissena bugensis) in Lake Mead, Nevada

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SUBSTRATE MONITORING, CONTAMINANT MONITORING, AND EDUCATIONAL OUTREACH ON QUAGGA MUSSELS (*Dreissena bugensis*) IN LAKE MEAD, NEVADA

by

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ABSTRACT

Substrate Monitoring, Contaminant Monitoring and Educational Outreach on Quagga Mussels (*Dreissena bugensis*) in Lake Mead, Nevada

by

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The invasive species, the quagga mussel, *Dreissena bugensis*, was found in Lake Mead, Nevada-Arizona, USA on January 6, 2007. Since then, researchers have been attempting to quantify the amount of damage these mussels will cause to the lower Colorado River basin. Three projects were implemented in this thesis to research the quagga mussel in Lake Mead. First, a study to determine which types of substrates quagga mussels will grow on preferentially was conducted. Six different substrates, Acrylonitrile Butadiene Styrene (ABS) plastic, High Density Polyethylene (HDPE) plastic, Concrete Underlayment Board (CUB), aluminum, stainless steel and fiberglass, were placed in the Boulder Basin of Lake Mead for approximately one year in a modified randomized block design. Half of the substrates were removed and replaced every two months, and the other half remained in the water for the duration of the study (one year). Mussels had no preference in substrate type, but settlement was limited by depth. Mussel settlement on substrates at depths from 6-28 m was significantly greater than on substrates from 32-54 m. This divergence in depth preference is likely due to the

different water quality characteristics at these depths. The second study was conducted to determine concentrations of mercury in quagga mussel soft tissue from Lakes Mead and Mohave. The range of mercury concentrations in mussel tissues was 0.017 -0.074 μg/g dry weight. The final project was designed to educate the public and determine certain characteristics of boaters that utilize Lake Mead. Boaters were asked questions about where their next boating destination would be, if they were aware of quagga mussels and if they cleaned their boat between launchings. Of 236 people interviewed, 81% were aware of quagga mussels, but this number needs to increase. To prevent the spread of mussels to other bodies of water, boater education and awareness is vitally important.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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Second, I want to thank my sources of funding. A grant from the National Park Service and the Southern Nevada Water Authority funded the substrate project and a grant from the USFWS funded the survey project.

Third, those individuals who have helped me finish my projects deserve MANY thanks. Briefly, the park service employees who helped me design, implement, and complete my substrate project: Melissa Cheung, Mitch Urban, Rigby Ough, Art Gunzel, Bryan Moore, Jessie Rinella, Emily Lehman and Tom Culler; Alan Sims at SNWA for use of his wonderful microscope; Bryan Moore at the NPS and Carieen Ulepic at the Bureau of Reclamation in Boulder City for collecting mussels on their dives; Wen Baldwin for taking me on my first quagga mussel hunt and helping me with just about everything for the past two years; Ron Veley of USGS for the water quality data; and Lanisa Pechacek, Chris Rendina, Joanna Kramer, Courtney Coughenour and Ashley Phipps for their help in the lab or at the lake.

CHAPTER 1

INTRODUCTION

Lake Mead National Recreation Area, located in Nevada and Arizona, was the result of taming the Colorado River through the construction of Hoover Dam in the 1930s. The lake has increased in area since 1935 to become the largest reservoir by volume in the United States (LaBounty 2008). This body of water provides the Las Vegas valley and the other lower Colorado River basin states (Arizona, California and Nevada) with drinking and irrigation water. In January 2007, the quagga mussel (*Dreissena bugensis*), a notoriously invasive species, was found in Las Vegas Boat Harbor in the Boulder Basin of Lake Mead (LaBounty and Roefer 2007). This mollusk has caused severe ecological and economical damages to every body of water it inhabits. In Lake Erie, phytoplankton populations have declined as a result of over filtering of the water by both zebra (*D. polymorpha*) and quagga mussels (May and Marsden 1992). Lake Huron's natural food web has been seriously altered as a result of species decline from changes in the system as a result of the mussel invasion. Salmon, alewife and zooplankton populations have declined causing an energy shift from pelagic to benthic zones and this has resulted in a $19 million/yr decrease in revenue for sport fisheries in Lake Huron (Michigan DNR 2008).

Quagga mussels may have the ability to severely alter Lake Mead's ecosystem by increasing water clarity, causing toxic algal blooms, making contaminants readily

bioavailable or shifting the energetics of the system from pelagic to benthic zones. Scientists need to closely monitor quagga mussels in Lake Mead to predict when or where one of these alterations in the ecosystem may occur.

Several local, state and federal agencies are working on the establishment of a long-term Lake Mead quagga mussel monitoring plan to appropriately manage the lake. In addition to proper management of the lake, boaters and other citizens should be educated on the damage these organisms may have on the environment. Education may also help prevent the further spread of mussels to uninhabited regions of the United States. This thesis will provide guidance in making a substrate monitoring plan for Lake Mead, determine contaminant concentrations in quagga mussels, and discuss efforts to educate Lake Mead boaters of the damages quagga mussels can have on their boats and on the Colorado River's ecosystem.

CHAPTER 2

LITERATURE REVIEW

Quagga Mussel Biology

Life History

Since the invasion of Dreissenid species into Europe, a vast amount of research has studied these invasive species. The majority of research focuses on the zebra mussel (*D. polymorpha*), but can often be extrapolated to describe quagga mussels (*D. bugensis*) because of their similar genetic signatures (Gelembiuk et al. 2006). Therefore, this literature review will focus on zebra mussel biology with as much quagga mussel information as is currently available. Understanding the basic biology of the quagga mussel is an essential part of trying to manage and control populations within aquatic systems.

The exact origin of zebra mussels is unknown, but the quagga mussels were once confined to the Dnieper and Bug River drainage systems in the Ukraine (Marsden et al. 1996). This drainage system was subject to instability, changing water levels and other climactic events that made the Dressenid species go through numerous bottlenecks resulting in a highly evolutionarily fit species ready to invade new waters (Gelembiuk et al. 2006). The zebra mussel arrived in United States in Lake St. Clair of the Laurentian Great Lakes in 1986 and spread eastward, southward and westward with relative ease (May and Marsden 1992; Mills et al. 1996; Figure 1). During a study in 1991 comparing

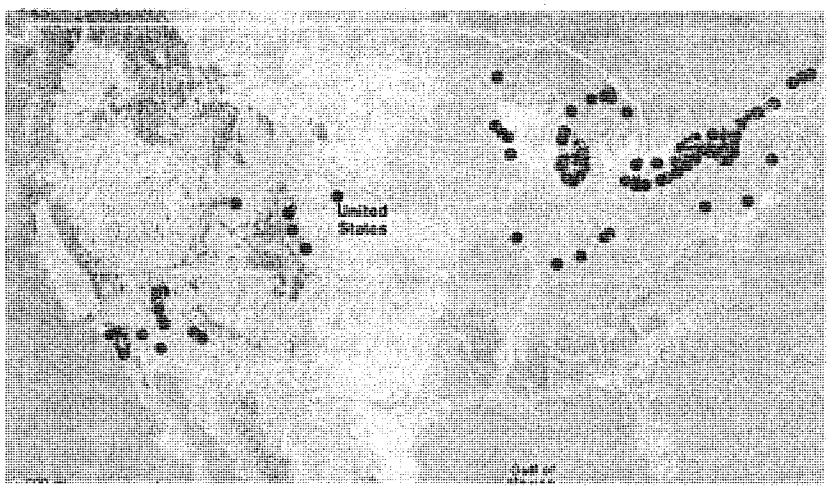
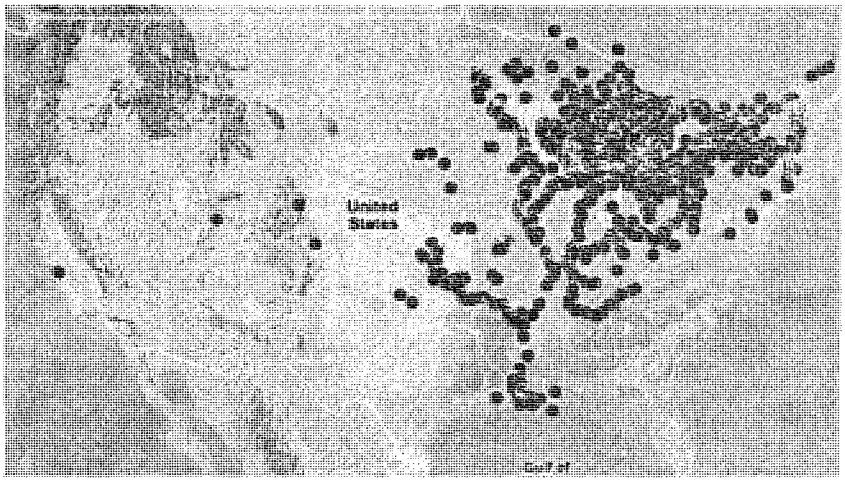


Figure 1. Dreissenid Distributions in North America
a) Quagga Mussel Distribution April 2009



b) Zebra Mussel Distribution April 2009

Images from Benson, A. J. 2009. Zebra mussel sightings distribution. Retrieved [01 Apr 09] from http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/zebramusseldistribution.htm.

the genetics of zebra mussel populations throughout North America, a genetically and morphologically different Dreissenid species was discovered in Lake Ontario (May and Marsden 1992). This species was given the common name "quagga mussel" because the "quagga" is an extinct relative of the zebra with stripes only on the head and shoulders

(May and Marsden 1992). The scientific name was determined to be *Dreissena bugensis*, (Andrusov 1897) based on a thorough literature review (Rosenberg and Ludyanskiy 1994). A search for this new Dreissenid species was conducted after the initial discovery and biologists found populations of the quagga in other areas of Lake Ontario, the Niagara River, and the upper St. Lawrence River (May and Marsden 1992). Since 1991, the quagga mussel has spread over 2,000 miles to the southwest United States (Figure 1).

The quagga mussel is morphologically distinct from the zebra mussel because the zebra mussel has a small, more pronounced angle between the ventral and dorsal surfaces of the shell and the quagga mussel has a more rounded angle between these two surfaces (May and Marsden 1992). To the trained eye, there are differences in the shell patterns of the zebra and quagga mussels, but this difference is slight and the best way to determine if a specimen is a zebra or quagga mussel is to have it genotyped (May and Marsden 1992). Genetically, the zebra mussel has extremely high levels of genetic variability (27-43.5%) and the quagga mussel has low levels of genetic variability (9.7-14.5%) (Marsden et al. 1996). Researchers found a Nei's genetic distance of 1.69 based on allozyme variation between zebra and quagga mussels, indicating completely separate species, but still within the same genera (Spidle et al. 1994).

There are three main life cycles in mollusks that are divided into larval, juvenile and adult stages (Crosier and Molloy 2001). Life begins with external fertilization of gametes. Fertilized gametes, larvae, are found free floating in the water column where they remain until the juvenile stage when they settle on and attach to a substrate (Ackerman et al. 1994). The larval stage is further divided into four stages: trochophore, straight-

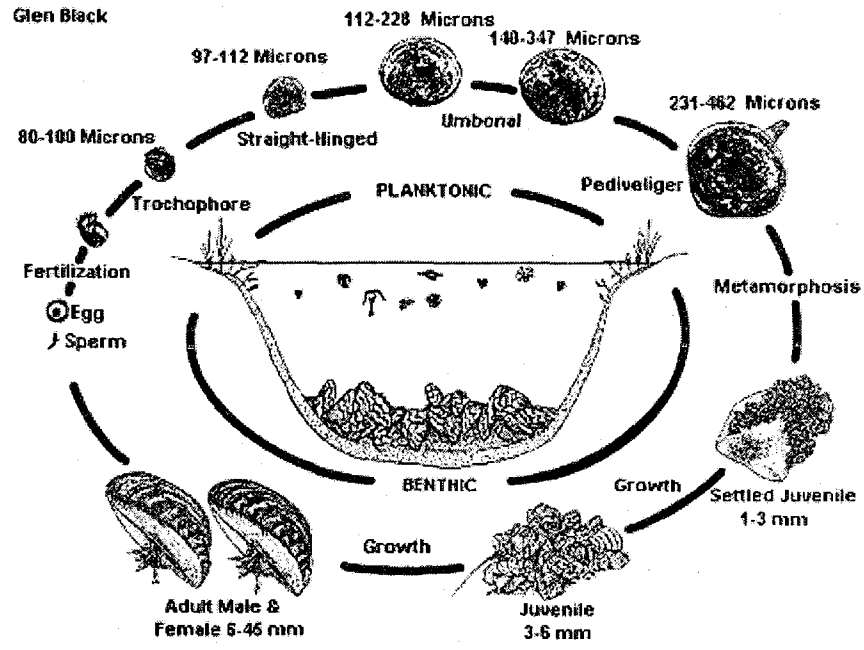


Figure 2. Zebra mussel life cycle. Source: Crosier and Molloy 2001.

hinged veilger (also called D-shaped veliger), umbonal veliger and pediveliger (Figure 2) (Ackerman et al. 1994; Crosier and Molloy 2001). The trochophore stage (lasting 8-48 h) directly follows fertilization. The organism is circular and 57-121 μm in diameter (Ackerman et al. 1994). During this stage, a ciliated organelle called the velum develops allowing the organism to feed and to move freely (Ackerman et al. 1994; Crosier and Molloy 2001). Two to nine days after fertilization, the larvae secrete a D-shaped or straight-hinged shell. D-shaped veligers feed on small algae (Crosier and Molloy 2001). The umbonal stage starts seven to nine days after fertilization when the mantle tissue secretes an ornamented shell on the umbonal region of the mussel (near the hinges) (Figure 3) (Ackerman et al. 1994; Crosier and Molloy 2001). The final larval stage, pediveliger, begins with the development of the foot and ends when the pediveliger settles and attaches to a substrate. Settlement may occur eighteen to 90 days after

fertilization depending on the water conditions (Ackerman et al. 1994; Crosier and Molloy 2001).

The period between the larval and juvenile stages is called the plantigrade stage. This stage triggers the mussel to feed with gills instead of the velum, use the foot instead of cilia for movement, form labial palps around the mouth and use byssal threads to attach to substrates (Ackerman et al. 1994; Crosier and Molloy 2001). During the transformation from plantigrade to juvenile, the plantigrade's shell becomes more triangular and less D-shaped. Juveniles grow at a rate of about 1mm/week (Claudi and Mackie 1994) and can crawl up to 3.8 cm/hr for several days before attaching to a substrate (Marsden 1992). Adult mussels are not restricted to spend their entire lives in one location; they can translocate using their foot (Ackerman et al. 1994). Growth rates of quagga mussels (0.57-0.78 per day) exceed rates of zebra mussels (0.03-0.17 per day) four to nineteen times with the greatest difference observed at low food levels (Baldwin et al. 2002).

Morphology

Figure 3 provides an overview of Dreissenid morphology. The zebra mussel has a more pronounced angle between the ventral and dorsal surfaces than the quagga which is more rounded (May and Marsden 1992). The flat (in zebra mussels) or slightly convex (in quagga mussels) ventral surface allows mussels to attach closely to the substrate using their byssal threads, making predator removal difficult (Claudi and Mackie 1994). Both mussel types have black and cream-colored striped patterns on the shell that vary greatly between individuals.

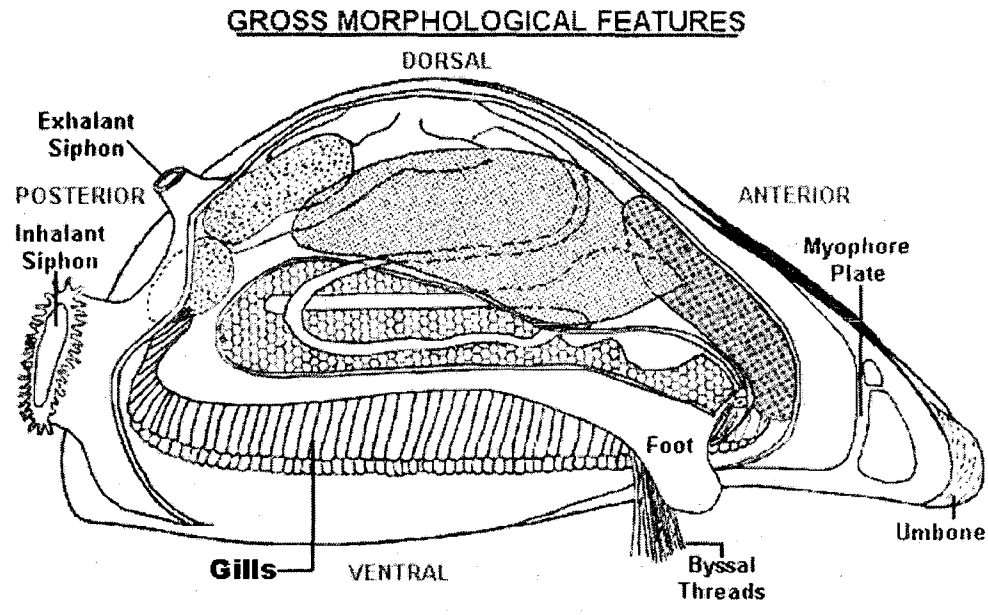


Figure 3. Zebra mussel morphology. Source: Crosier and Molloy 2001.

Reproduction

An adult mussel is one that has reached sexual maturity. Within the first twelve months of life or when mussels reach eight to ten mm in length, mussels are able to reproduce (Claudi and Mackie 1994). One male adult mussel can release ten billion sperm per year and one female mussel can release one millions eggs per year (Claudi and Mackie 1994). A study in Lake Erie on zebra mussel spawning found mussels to undergo spermatogenesis and gametogenesis one time per year in the spring (Garton and Haag 1993). This period of spawning correlated with veliger abundance found in the water. The highest veliger abundance was found from July to August and there were no veligers found in the water from October to April (Garton and Haag 1993). For spawning to occur in zebra mussels, the water temperature must be at least 12°C (Sprung 1989). In

the Capsian and Black Seas, the temperature is warmer than 12°C year round and researchers have found veligers in the water all year (Garton and Haag 1993). It has been proposed that quagga mussels will have multiple spawning cycles in Lake Mead because the average water temperature is greater than 12°C. Quagga mussel sperm is morphologically different than zebra mussel sperm (Denson and Wang 1994). Cross-breeding may occur, but this reproductive effort does not produce viable offspring because hybridization will not occur (Spidle et al. 1995).

Feeding Physiology

Adult Dressenids are filter feeders. Cilia on the internal gills create a current that pulls water through the inhalant siphon and into the inner organs (Claudi and Mackie 1994; Crosier and Molloy 2001). Particles are filtered, sorted and transported to the mouth through the gills and palps. Digestible particles between 5-35 μm in diameter (Sprung and Rose 1988) including micro-algae, micro-invertebrates, bacteria, and detritus (Crosier and Molloy 2001) are digested and excreted as feces. Indigestible materials are excreted through the exhalant siphon as pseudofeces (Claudi and Mackie 1994). These materials are called pseudofeces because they have not been digested by the organism. The filtration rate of quagga mussels is up to 37% greater than that of zebra mussels (Diggins 2001). Baldwin et al. (2002) compared zebra and quagga mussel feeding efficiency and found that in epilimnetic waters, the quagga can survive, grow and feed more efficiently than zebra mussels.

Quagga Mussel Influence on Lake Mead

Lake Mead Characteristics

As the largest reservoir by volume in the United States, Lake Mead holds over 9 trillion gallons (36.7×10^9 m^3) of water, spreads 106 km (65.87 mi) in width, supplies 80% of Las Vegas' domestic water needs, provides 3.3 billion kWh of power via hydroelectric generation from Hoover dam, and provides a valuable sport fishery to millions of visitors and residents every year (LaBounty and Burns 2005; LaBounty and Roefer 2007). There are five main basins in Lake Mead: Boulder, Virgin, Temple, Overton and Gregg (Figure 4). Lake Mead is mostly mesotrophic and usually monomictic; meaning the lake is in between eutrophism and oligotrophism and is stratified only every other year for 9 months at a time (LaBounty 2008).

The limnological characteristics of Lake Mead make it an extremely suitable habitat for mussel colonization (Table 1). The lower temperature limit of reproduction and survival of quagga mussels is from 2-5°C (Mills et al. 1996) and the upper limit is 30°C (Spidle 1994). Lake Mead's average yearly temperatures range from 12-12.5°C in the hypolimnion, 12-18°C in the metalimnion and 12-27°C in the epilimnion (LaBounty and Burns 2005). When Lake Mead is stratified, typically during the summer, the epilimnion is often 15 m thick, the metalimnion is 20 m thick and the hypolimnion is up to 120 m thick (LaBounty 2008). Quagga mussels need at least 12 mg/l of calcium (Jones and Ricciardi 2005) to survive and Lake Mead provides that with median values of calcium at 69.1-87 mg/l (Whittier et al. 2008). At salinity concentrations higher than 5 ppt, mussels cannot survive (Spidle 1994); Lake Mead has <1ppt salinity (LaBounty and Burns 2005). One of the final limits to quagga mussel survival is water pH. At pH 6.5 or below,

mussels cannot survive (McMahon 1996) and Lake Mead has an average pH of 8.3 (LaBounty and Burns 2005).

Table 1. Limnological characteristics of Lake Mead in relation to quagga mussel tolerances.

Limnological Parameter	Tolerance Limit	Conditions in Lake Mead
Temperature	Minimum: 2-5°C (Mills et al. 1996); Maximum: 30°C (Spidle 1994)	Epilimnion: 12-27°C; Metalimnion: 12-18°C; Hypolimnion: 12-12.5°C (LaBounty and Burns 2005)
Salinity	Maximum: 5 ppt (Spidle 1994)	<1 ppt (LaBounty and Burns 2005)
Calcium	Minimum: 12 mg/L (Jones and Ricciardi 2005)	69.1-87.0 mg/L (Whittier et al. 2008)
pH	Minimum: 6.5 (McMahon 1996)	8.3 (LaBounty and Burns 2005)

Trophic Interactions

The recent invasion of quagga mussels into Lake Mead raises concerns about the trophic dynamics of the system. Trophic dynamics include all the different nutritional levels in which organisms feed within a niche or ecosystem. For example, the trophic level of quagga mussels is quite different from the level of the piscivorous bird, the cormorant. The mussels' capability to filter massive quantities of water may lead to decreased phytoplankton and zooplankton populations in Lake Mead. Mussel feces and pseudofeces concentrations will increase in the water column and in the benthos. This will cause an energy transfer from pelagic to benthic zones. Now that the basic biology and ecology of mussels has been explained, it is easy to see how much a quagga mussel

invasion can change the normal ecosystem of a lake. Monitoring quagga mussels is an essential research tool to predict how mussels can change the ecosystem.

Need for Research on Lake Mead

A standardized monitoring protocol has been requested to be established in Lake Mead by various federal, state and local agencies in the Las Vegas Valley to predict ecological changes that will occur since the introduction of the quagga mussel. A long term structured and standardized monitoring plan is the ultimate goal to be able to compare data in the present to data collected throughout the future. This monitoring plan should not only have a quagga mussel population monitoring plan, but also include contaminant monitoring on the mussels and their predators. Baseline contaminant concentrations need to be determined. Boaters that recreate on Lake Mead need to be aware of the water they utilize. Educational outreach is a vital part of boater awareness. The 100th Meridian project, through the use of surveys, offers research into boater awareness and can be used as an educational tool. Boater education should continue well into the future to protect Lake Mead and other non-infested waters from more invasive species.

CHAPTER 3

SUBSTRATE MONITORING

Monitoring Protocols

Researchers have monitored veliger, juvenile and adult zebra and quagga mussel population densities in water bodies all over the United States and Europe. There are two types of study techniques typically employed: active sampling and passive sampling. Active sampling involves collecting mussels from natural materials found in the water such as rocks, silt, and sediment. Passive sampling involves placing an artificial substrate in water and examining mussel density after a specified period of time. The following information will focus on reviewing juvenile and adult population monitoring studies using passive techniques. Studies have found that mussel settlement on an artificial substrate depends on orientation, substrate type and texture, depth, intensity of light reaching the substrate, proximity to other mussels or adjacent surfaces and ionic concentrations of surrounding waters (Marsden 1992). Substrate monitoring is one of the most useful techniques in early detection of dreissenid mussels. Determining mussel settling preferences on different substrates will assist researchers and resource managers decide how to mitigate the colonization on architectural structures, public infrastructures and sensitive environmental areas.

Primary settling of mussels occurs when a pediveilger becomes too heavy to remain in the water column and the first settlement is stochastic. After the first initial contact

with a substrate, a mussel will search for, and attach to, a more suitable substrate (Marsden and Lansky 2000). Daily settlement rates have been found to correlate with veliger concentrations in the water ($r=0.93-0.98$; $p<0.001$) (Martel et al. 1994). Mussels search for a suitable substrate based on the texture, chemical composition, orientation of the substrate in water column, and environmental cues such as the amount of light penetration and the presence of a microscopic biofilm (Kavouras and Maki 2003). These factors are reviewed in depth below.

Substrate Materials

Experiments previously conducted show that mussels will grow on almost any shape of material from tube to plate (Kilgour and Mackie 1993; Kobak 2004; Marsden and Lansky 2000). The quantification of mussels on a plate is a more accurate and simple process than counting them on all surfaces of a tube. Different types of plate materials have been used in zebra and quagga mussel adult and juvenile monitoring in the literature including: acrylic, aluminum, asbestos, black Pelxiglas, black steel, brass, clear Plexiglas, copper, fiberglass, galvanized iron, galvanized steel, glass, limestone, pine, polypropylene, PVC (polyvinyl chloride), raw steel, resocart, rubber, stainless steel, Teflon, vinyl, wood and zinc (Czarnoleski et al. 2004; Kilgour and Mackie 1993; Kobak 2004; Marsden and Lansky 2000). In these studies, settlement rates were determined based on densities of mussels currently attached to the substrate. Mussels did not settle on copper plates because of its toxic properties toward mussels (Table 2) (Kilgour and Mackie 1993). The highest densities after 3 months were found on wood (618,558 individuals/ m^2), concrete (469,712 individuals/ m^2) and limestone (401,250 individuals/

Table 2. Mussel densities on artificial substrates.

Substrate	Mean mussels/m²	Time in water	Plate size	Location	Species	Reference
Acrylic	6896	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Aluminum	296,635	3 months	15 X 15 cm	Michigan City, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Aluminum	88,558	3 months	15 X 15 cm	Port of Indiana, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Aluminum	5825	4 months	10 X 10 cm	Wlocalawek Dam Reservoir, Poland	*D. polymorpha*	Kobak 2004
Aluminum	2324	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Asbestos	21,333	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Black Steel	15,420	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Brass	75	4 months	10 X 10 cm	Wlocalawek Dam Reservoir, Poland	*D. polymorpha*	Kobak 2004
Concrete	469,712	3 months	15 X 15 cm	Michigan City, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Concrete	140,288	3 months	15 X 15 cm	Port of Indiana, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Copper	0	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Fiberglass	297,885	3 months	15 X 15 cm	Michigan City, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Fiberglass	139,615	3 months	15 X 15 cm	Port of Indiana, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Galvanized Iron	548	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Glass	6875	4 months	10 X 10 cm	Wlocalawek Dam Reservoir, Poland	*D. polymorpha*	Kobak 2004
Limestone	401,250	3 months	15 X 15 cm	Michigan City, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Limestone	72,981	3 months	15 X 15 cm	Port of Indiana, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Pine	16,117	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993

Plexiglass	5625	4 months	10 X 10 cm	Wlocalawek Dam Reservoir, Poland	*D. polymorpha*	Kobak 2004
Polypropylene	17,554	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Pressure-treated wood	15,255	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
PVC	7471	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
PVC	4200	4 months	10 X 10 cm	Wlocalawek Dam Reservoir, Poland	*D. polymorpha*	Kobak 2004
Resocart	7300	4 months	10 X 10 cm	Wlocalawek Dam Reservoir, Poland	*D. polymorpha*	Kobak 2004
Rubber	1075	4 months	10 X 10 cm	Wlocalawek Dam Reservoir, Poland	*D. polymorpha*	Kobak 2004
Steel (galvanized)	10,769	3 months	15 X 15 cm	Michigan City, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Steel (galvanized)	0	3 months	15 X 15 cm	Port of Indiana, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Steel (raw)	351,635	3 months	15 X 15 cm	Michigan City, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Steel (raw)	70,192	3 months	15 X 15 cm	Port of Indiana, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Steel (stainless)	21,812	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Teflon	8593	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Vinyl	12,068	2.5 months	12.7 X 7.7 cm	Lake St. Clair near Puce, Ontario	*D. polymorpha*	Kilgor and Mackie 1993
Wood	618,558	3 months	15 X 15 cm	Michigan City, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Wood	36,058	3 months	15 X 15 cm	Port of Indiana, Lake Michigan	*D. polymorpha*	Marsden and Lansky 2000
Zinc	575	4 months	10 X 10 cm	Wlocalawek Dam Reservoir, Poland	*D. polymorpha*	Kobak 2004

m^2). Based on these studies, the two main factors that regulate mussel attachment to a substrate appear to be texture and chemical composition of the substrate.

Texture

Marsden and Lansky (2000) and Czarnoleski et al. (2004) found that mussels attached to substrates with the greatest amount of texture preferentially over smooth substrates. This is likely due to the increased surface area that provides more potential attachment sites. This concept is supported by the fact that byssal thread attachment may be more secure on a material with more texture than a smooth material (Ackerman et al. 1996).

Chemical Composition

Ackerman et al. (1996) found that mussels attached with greater strength to natural substrates (plywood, concrete, limestone) than metallic substrates (aluminum, steel) and with the least strength to polymeric substrates (epoxy coated steel, plexiglass, acrylic, Teflon and PVC). Materials containing zinc or copper, like galvanized steel, are toxic to mussels, and usually have lower settling rates than other materials (Marsden and Lansky 2000).

Orientation

The top of horizontally orientated plates were found to have lower densities than the bottom of horizontally orientated plates in Marsden and Lansky (2000) and Yankovich and Haffner (1993). However, Kobak (2005) found no significant difference between densities at either location. Horizontally orientated plates have higher colonization rates than vertically orientated plates (Marsden and Lansky 2000). Orientation of the substrate will have a little effect on colonization if there is too much light penetration on the

substrate. Light penetration can increase the temperature of the water making it unsuitable for mussel inhabitation (Marsden and Lansky 2000).

Light

Mussels tend to avoid living in direct sunlight. Marsden and Lansky (2000) found that mussels grew 7.7 times more frequently on shaded substrates than on substrates in direct sunlight. The mussels' avoidance of light may be due to a natural instinct to avoid exposure to predators, avoid wave damage or because of an increased water temperature caused by sunlight (Marsden and Lansky 2000). If light penetration is high, it may also negatively affect the growth of microscopic organisms that form a biofilm on a substrate, another important characteristic in mussel settlement.

Biofilms

A biofilm initially forms from a gathering of glycoproteinacious film on a substrate and then the film is colonized by bacteria, diatoms, and protozoa (Wainman et al. 1996). Veligers typically do not settle, or settle at a reduced rate (10-20%), on substrates that have no microscopic biofilm (Marsden 1992; Kavouras and Maki 2003; Wainman et al. 1996). Biofilms may increase the surface area of the substrate, by providing more attachment sites or may alter the surface chemistry to become more favorable to mussels (Kavouras and Maki 2003). Using the knowledge from this literature review, an experiment was designed to determine which types of substrates quagga mussels in Lake Mead would grow preferentially on in order to design a quagga mussel monitoring plan.

Questions, Objectives and Hypotheses

Questions

- Do mussels settle and grow preferentially on different substrate materials?
- Do mussels settle and grow preferentially at different depths?

Objectives

- This study will determine which type of substrate, if any, mussels grow preferentially on in Lake Mead.
- This study will determine at which depths quagga mussels in Lake Mead grow at the highest densities.

Hypotheses

- Quagga mussels will settle and grow on substrates with the most texture preferentially, concrete underlayment board and fiberglass.
- Quagga mussels will settle and grow at depths suitable for their reproduction and survival, below the photic zone and within the nutrient zone (5-15m).

Methodology

Experimental Design

A team of researchers from UNLV and the National Park Service (NPS) surveyed Boulder Basin and the Callville Bay area for an appropriate sampling site by measuring depth and temperature of certain habitat areas previously known to contain mussels. The chosen sampling site was located in an area of easy access to researchers, had a depth of at least 30 m, and had a suitable flow rate for mussel colonization. Appropriate water flow can facilitate the attachment of mussel larvae to a substrate (Navarro et al. 2006).

The identified area was located between Sentinel Island and the Boulder Islands in the Boulder Basin (Figure 4). This project was approved by the NPS and awarded the study number LAME-00139 and the protocol number LAME-2008-SCI-0008.

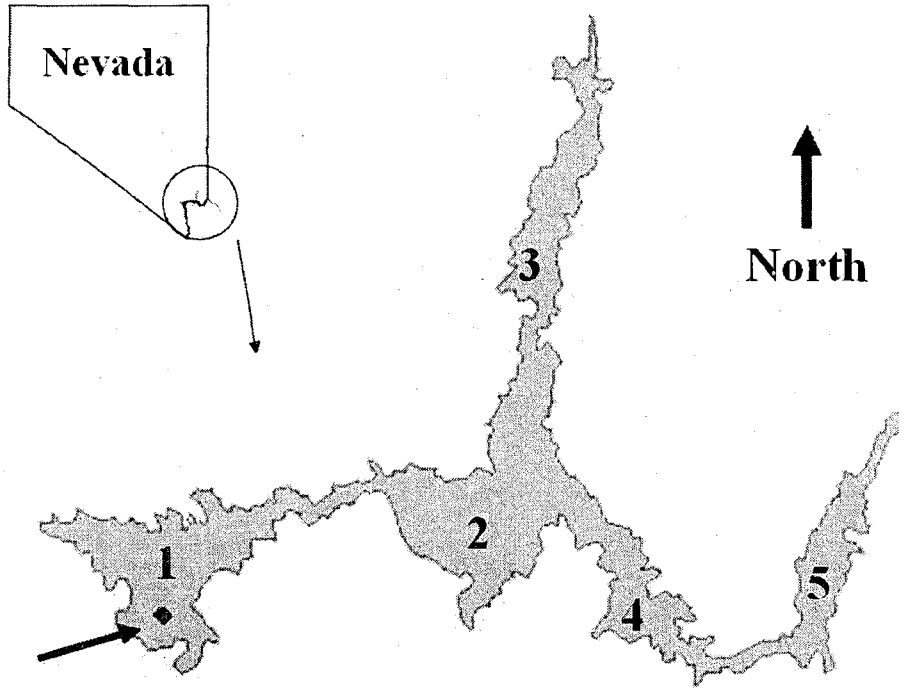


Figure 4. Location of substrate experiment in Lake Mead indicated by dot with arrow. 1=Boulder Basin, 2=Virgin Basin, 3= Overton Arm, 4= Temple Basin, 5= Gregg Basin. Image by Dr. Chad Cross at UNLV.

There were six different substrates tested: concrete underlayment board (CUB), Acrylonitrile Butadiene Styrene (ABS) plastic, fiberglass, aluminum, stainless steel, and high density polyethylene (HDPE) on four separate samplers. The CUB, fiberglass and one side of the ABS plate had a rough, textured surface based on visual assessments, but

the other substrates had smooth surfaces. Aluminum, concrete, fiberglass and steel were previously confirmed to allow zebra and quagga mussel colonization (Kilgour and Mackie 1993; Kobak 2004; Marsden and Lansky 2000). This experiment was the first to test mussel settlement on ABS and HDPE plastic. The substrates were cut into 10.16 cm (4 in) squares and connected with stainless steel screws, washers and stop nuts to a 15.2 cm (6 in) piece of conduit pipe. These substrate units were secured in polypropylene rope with a knot referred to as a Russian splice at evenly spaced depths.

The depth at the experiment location was approximately 63 m (208 ft). The buoy needed to be submerged at least 1.2 m (4 ft) below the surface of the water on each sampler to prevent mishandling by boaters, and the buoy was connected to the rope with the substrates attached with approximately 3 m (10 ft) of chain. The anchor was connected to the rope with approximately 7.6 m (25 ft) of chain. This left 50 m (166 ft) of space for the substrates on the rope, and when this is divided by 12 (the number of substrates on each sampler), the result is 4.4 m (14.5 ft). Substrates were placed in the rope with 4.4 m (14.5 ft) between each substrate (Figure 5).

Each type of substrate alternated depths on each of the four samplers (Table 3). For example, on sampler four, the CUB plate was near the surface, but on sampler two, the CUB plate was at 30 m, and on sampler three, it was at 50 m and so on for each type of substrate employing a modified randomized block design. There was a permanent substrate attached to the line, as well as a removable substrate of the same type that was easily removed for bi-monthly analysis and then replaced with a new substrate. The plates were placed in the water horizontally. This design was similar to the Portland

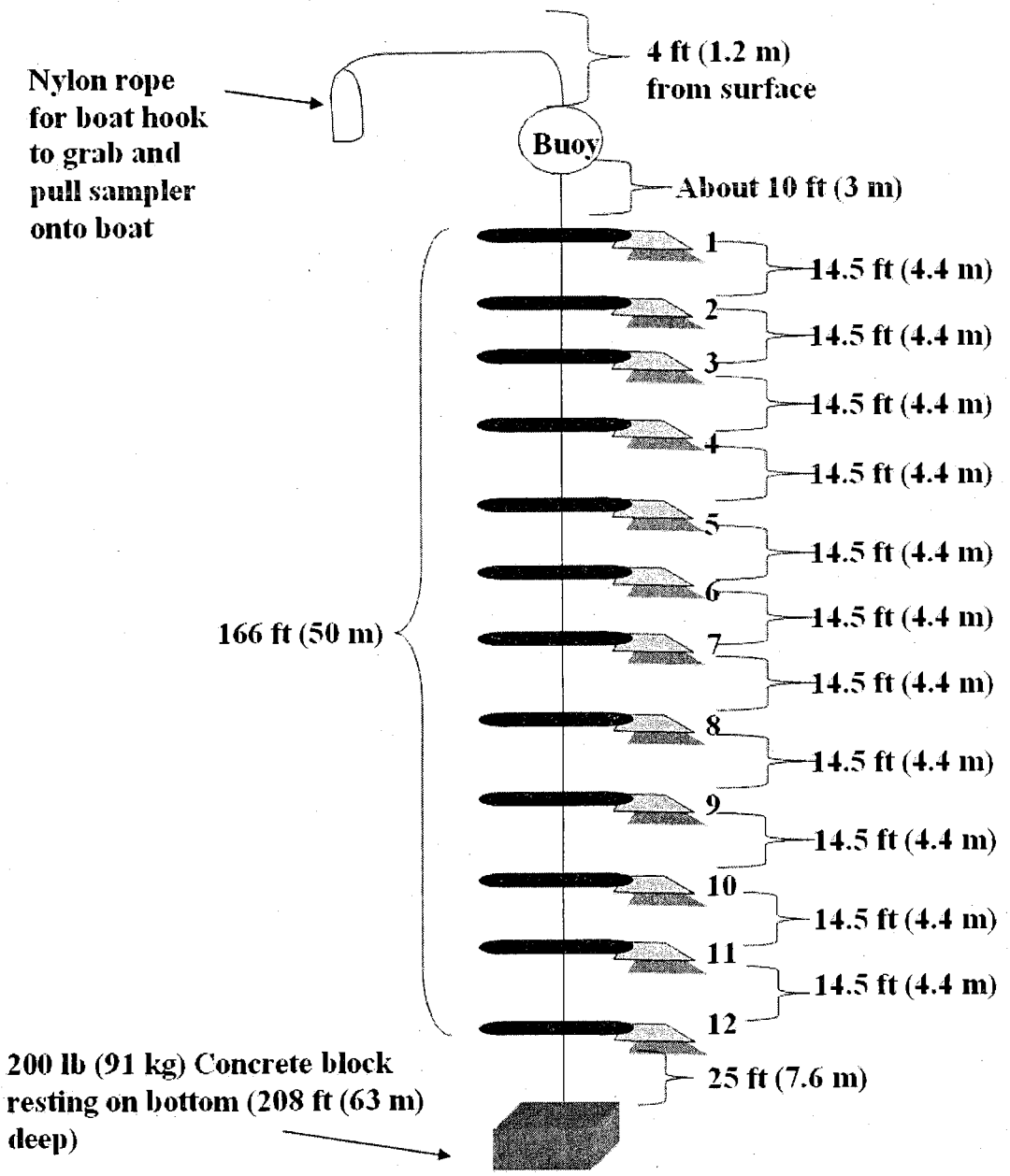


Figure 5. Substrate sampler field design.

Table 3. Substrate sampler experimental design.

Depth (ft)	Depth (m)	Sampler 1	Sampler 2	Sampler 3	Sampler 4
19.00	5.79	Al-P1	F-P2	ABS-P3	CUB-P4
33.50	10.21	Al-R1	F-R2	ABS-R3	CUB-R4
48.00	14.63	F-P1	ABS-P2	HDPE-P3	Steel-P4
62.50	19.05	F-R1	ABS-R2	HDPE-R3	Steel-R4
77.00	23.47	ABS-P1	HDPE-P2	F-P3	Al-P4
91.50	27.89	ABS-R1	HDPE-R2	F-R3	Al-R4
106.00	32.31	HDPE-P1	CUB-P2	Steel-P3	F-P4
120.50	36.73	HDPE-R1	CUB-R2	Steel-R3	F-R4
135.00	41.15	CUB-P1	Steel-P2	Al-P3	HDPE-P4
149.50	45.57	CUB-R1	Steel-R2	Al-R3	HDPE-R4
164.00	49.99	Steel-P1	Al-P2	CUB-P3	ABS-P4
178.50	54.41	Steel-R1	Al-R2	CUB-R3	ABS-R4

Al= aluminum, F= fiberglass, ABS= Acrylonitrile butadiene styrene plastic, HDPE= high density polyethylene plastic, CUB= concrete underlayment board, Steel= stainless steel; P=permanent substrate that remained in the water for one year, R=removable substrate that was removed and replaced every two months.

sampler commonly used in mussel monitoring studies (NPS 2007). The four substrate samplers were placed about five meters away from each other to prevent line tangling, but placed in the same general area. All sampling units were located in the same area general following the guidance of Marsden and Lansky (2000) who recommended focusing monitoring experiments on the number of units and not on the number of replication sites in a well-mixed body of water such as Lake Mead. The location was marked by an emerged yellow buoy with "Government Property Restricted Access" written on the side. GPS coordinates of the yellow buoy were N 36.0537 W 114.75. Lake levels were monitored daily on the Bureau of Reclamation's website (http://www.usbr.gov/lc/riverops.html) to ensure that the buoys remained submerged or would be within reach at the next sampling event. The samplers remained in the water for a period of twelve months.

Before placing the samplers in the water, the following measurements were taken with a Hydrolab DS5 (Hach Environmental, Loveland, CO) multi-parameter water quality probe: depth, water temperature, pH, dissolved oxygen, and conductivity. In addition to water quality characteristics taken with the UNLV Hydrolab DS5, water quality characteristics were measured every six hours for the duration of the study at a United States Geological Survey (USGS) water quality monitoring station located within a few hundred feet of the experiment site (GPS coordinates: N 36.0524 W 114.7509). Data from this station were provided to the author by Ronald Veley of USGS, Henderson, NV. Characteristics measured by the USGS were depth, water temperature, pH, dissolved oxygen and conductivity.

Every two months, the samplers were removed from the water column, laid out on the deck of the boat, and the substrates were placed in a bucket of lake water to reduce stress on the mussels. The two month period was determined as adequate time for a biofilm to form on the substrate (more than 2 weeks) and for mussels to attach (Kavouras and Maki 2003). During the sampling event, each side of the permanent and removable substrates was photographed on a grid laden platform, and the removable substrate was detached and placed in a Ziploc bag on ice in a cooler then replaced with a new plate of the same type. After the photographs, the permanent substrates were placed back in the bucket of water until returned to the water column.

Each month, starting in August 2008, a vertical plankton tow was conducted to enumerate the number of quagga mussel veligers present in the water near the area of the experiment. A 64 μm plankton net with a 15 cm diameter opening was used following the protocol set up by the Lower Colorado River Bureau of Reclamation Fisheries group

(Appendix I). Veligers were quantified by the author using a cross polarizing microscope according to the protocol written by the Bureau of Reclamation at Hoover Dam (Appendix I).

Laboratory Work

Substrate plates were taken to the Environmental and Occupational Health laboratory at UNLV and stored in a 0°C freezer until further analysis. Mussels were carefully removed by scraping or picking from the upper and lower side of the plate and placed in a 9x9 cm gridded petri dish with 1.3 cm square grids. Plates were then examined using a Zeiss Discovery.V8 stereomicroscope (Carl Zeiss, Inc., Peabody, MA) at 20 X power to quantify any mussels that did not get scraped into the petri dish. Mussels in the gridded petri dishes were then counted using 6.4 to 20 X power, depending on the number of mussels to be counted. When all mussels were counted on both sides of the plate and in the gridded petri dish, the total number of mussels was divided by 0.01032256 to determine the number of mussels per m^2 (hereafter reported as m^{-2}). The mussel densities on some plates were so great that counting each individual mussel would have been extremely time-consuming. In this case, the mussels in a 2.7 cm square area were counted from three random locations on the plate, averaged and then extrapolated to mussels/m^2 by dividing the average number of mussels counted in this area by 0.000027. Mussels kept in the laboratory were in accordance with Nevada Department of Wildlife (NDOW) possession permit #S30712.

Statistics

The four sampler locations were tested for spatial independence using Moran's I. GPS coordinates of each sampler were compared with mussel densities using a weighted

correlation coefficient. The null hypothesis of this test was that the samplers were spatially independent allowing for the proper use of a randomized block design ANOVA. The alternate hypothesis was that the samplers were autocorrelated, and hence not suitable for an ANOVA. Data were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests on SPSS version 16.0 (SPSS Inc., Chicago). If data were determined to be normal, a univariate ANOVA was conducted with number of mussels as the dependent variable, substrate type as the treatment and depth as the block. A randomized block design assumes that within each block the experimental conditions are homogeneous. In this experiment, a randomized block design permitted the assumption that all substrates at depth one were exposed to the same environmental conditions and so on for each depth. If the block was insignificant, this would indicate that there were no differences between depths; all substrates were exposed to the same environmental conditions. In this case, the previous ANOVA would be discarded and another ANOVA would be conducted with the number of mussels as the dependent variable and substrate type as the treatment.

If data were non-normal, a Friedman's non-parametric test for randomized block designs was conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC). This test accounts for non-normal data by ranking the dependent variables within the blocks. The null hypothesis was the same for parametric and non-parametric methods: all substrate types had the same amount of mussel settlement. The alternate hypothesis was that at least one type of substrate had significantly different levels of mussel settlement. Bonferroni post hoc tests using SPSS were conducted if data were found to be significantly different.

Veliger concentrations in number per L and the average number of settled mussels for each date were compared with the omnibus test that compares the two distributions, Kolmogorov-Smirnov, using SAS version 9.1 (SAS Institute Inc., Cary, NC). Kolmogrov-Smirnov tests the alternate hypothesis that the distribution of veliger concentrations throughout the year is different from the distribution of mussels on substrates throughout the year. If the null hypothesis of this test is accepted, this implies that the two distributions are similar indicating a relationship between veliger concentrations in the water and mussel densities on the substrates.

To determine if there were any relationships between water quality characteristics and mussel settlement at depth, a non-parametric Spearman correlation was conducted using SPSS. The data collected by the USGS were averaged over the two month sampling periods and compared with overall averages of mussel settlement at each depth. All statistical analyses utilized α=0.05.

Results

Samplers were deployed on 27 March 08. Substrates were pulled out of the water approximately every 60 days. Collection dates were 27 May 08, 30 July 08, 24 Sept 08, 20 Nov 08, 10 Jan 08 and the final date was 10 Mar 08. The results of the Moran's I test indicated to accept the null hypothesis stating that the samplers were independent of each other (no autocorrelation) making an ANOVA an appropriate statistical analysis (I values ranged from -0.59 to 0.53 with p values ranging from 0.322 to 0.495). Unfortunately, a few substrates were lost either during field work or in the laboratory due to mishandling or the substrates were missing when pulled out of the water.

Removable Substrates

For the removable substrates, exactly 5% of the data were missing so these data points were simply removed from analysis as recommended by Tabachnick and Fidel (2007). Some substrates were missing when pulled out of the water, and others were lost during counting procedures. A final total of 136 removable substrates were counted: 22 fiberglass, 23 aluminum, 24 ABS, 24 HDPE, 20 CUB and 23 stainless steel. Raw mussel counts are shown in Figure 6. Data were non-normal due to a wide range of variances and accordingly, non-parametric statistical methodologies were employed. Friedman's test found that the block (depth) was significant ($F=5.54$; $p<0.001$), but there were no differences in mussel settlement between substrate types (Friedman's $\chi^2= 2.7485$; $p=0.739$). A post hoc test (Bonferroni) was conducted to elucidate the differences between settlement and depth and settlement and date on the ranked data. Average numbers of mussels counted by substrate, by depth and by date are shown in Figures 7, 8 and 9, respectively.

Although not significant, removable substrate preference followed the order: ABS (342,483 m^{-2}) > aluminum (293,282 m^{-2}) > fiberglass (188,528 m^{-2}) > HDPE (150,427 m^{-2}) > CUB (121,645 m^{-2}) > steel (70,121 m^{-2}). Depths from 10-28 m were significantly different from the lower depths from 37-54 m. Overall mussel settlement increased by date from March 2008 to January 2009, and decreased significantly from January 2009 to March 2009. Figure 6 demonstrates that at some depths, settlement remained constant throughout the study.

To determine if there were differences in substrate preference during individual sampling events, each sampling event was analyzed separately by date for the removable

substrates with the Friedman's χ^2 with the following results: 27 May 08 χ^2=5.99, p=0.306; 30 July 08 χ^2=6.56, p=0.255; 24 Sept 08 χ^2=4.49, p=0.48; 20 Nov 08 χ^2=5.89, p=0.317; 20 Jan 09 χ^2=6.01, p=0.305; 10 Mar 09 χ^2=6.71, p=0.243. There was no substrate preference in any of the individual sampling dates.

Although the depth component of the overall experiment was significant, when individual removable substrate types were analyzed for depth differences, only three were found to be significant. Settlement on ABS (student's t =5.366; p<0.001), aluminum (student's t=2.301; p=.03) and HDPE (student's t=2.536; p=.02) was dependent on depth. Mussels settled at significantly greater densities at shallower depths on these substrates.

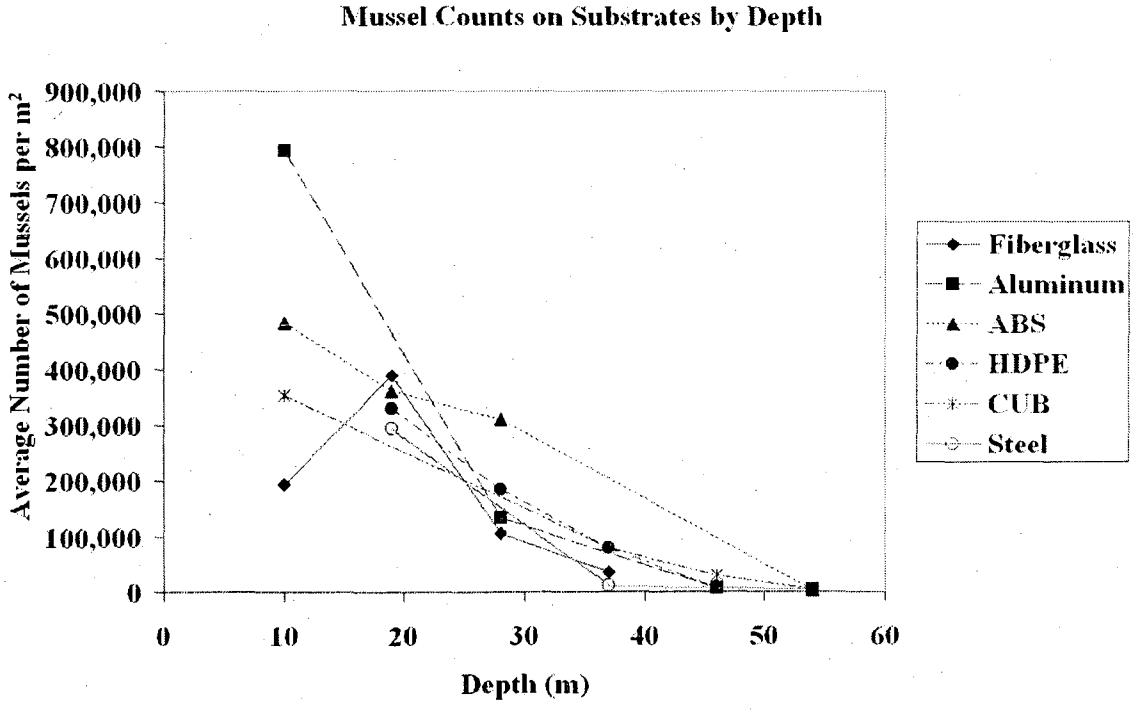


Figure 6. Mussel settlement on removable substrates by type and depth. Averages were taken for each two month sampling period and reported on this graph. Note similar patterns of decline for all substrate types after 30 m.

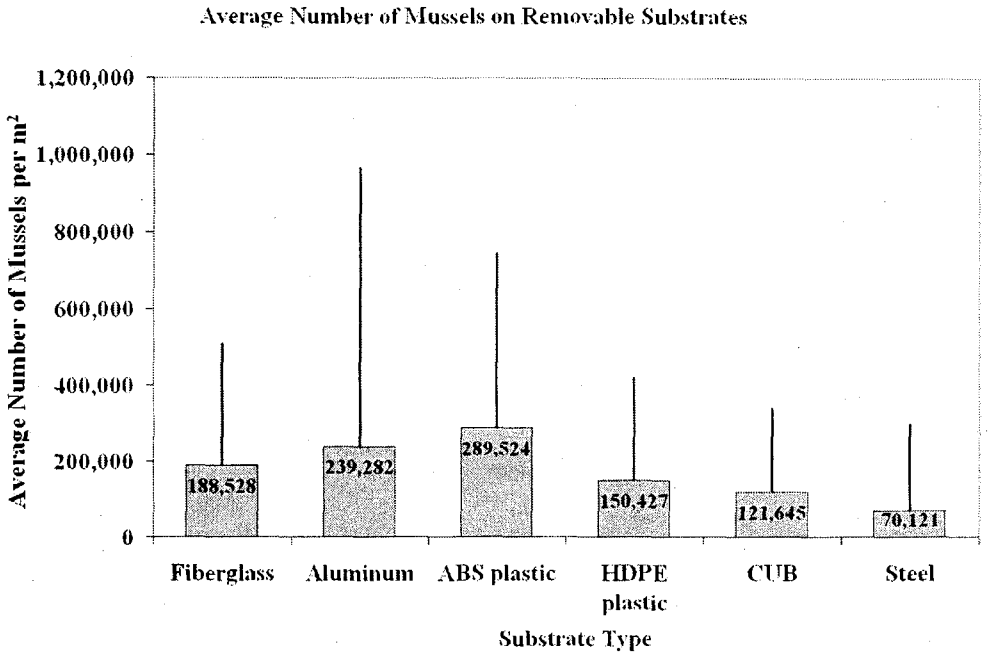


Figure 7. Average number of mussels by type on removable substrates. Error bars indicate +1 SD. There were no statistically significant differences between substrates.

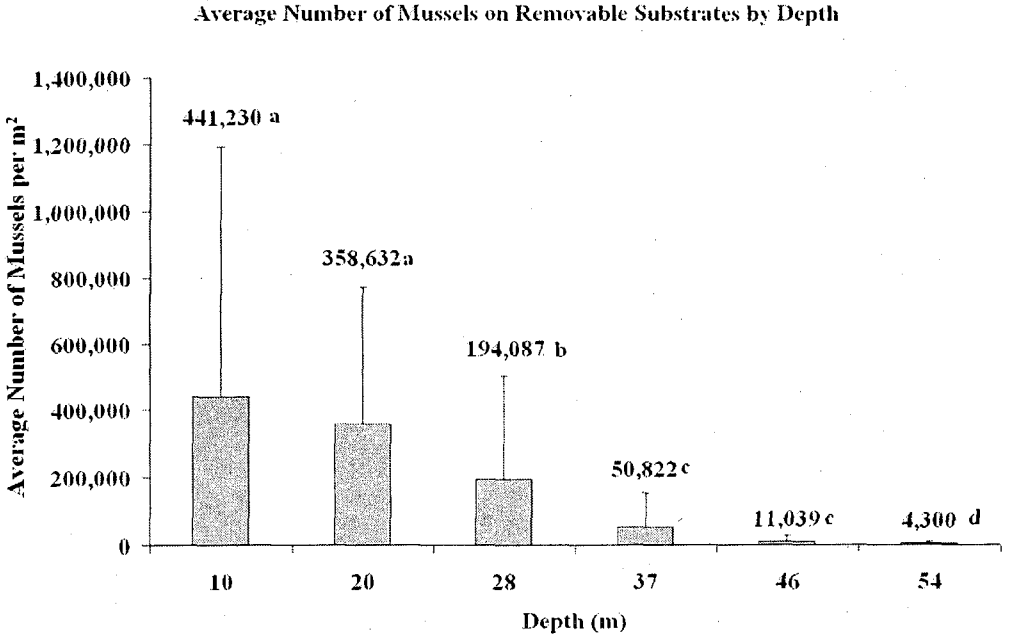


Figure 8. Average number of mussels by depth on removable substrates. Depths labeled "a" were significantly different from "c" and "d" ($p=0.017$ and $p<0.001$ respectively). Depth "b" was significantly different from depth "d" ($p=0.002$). Error bars indicate +1 SD.

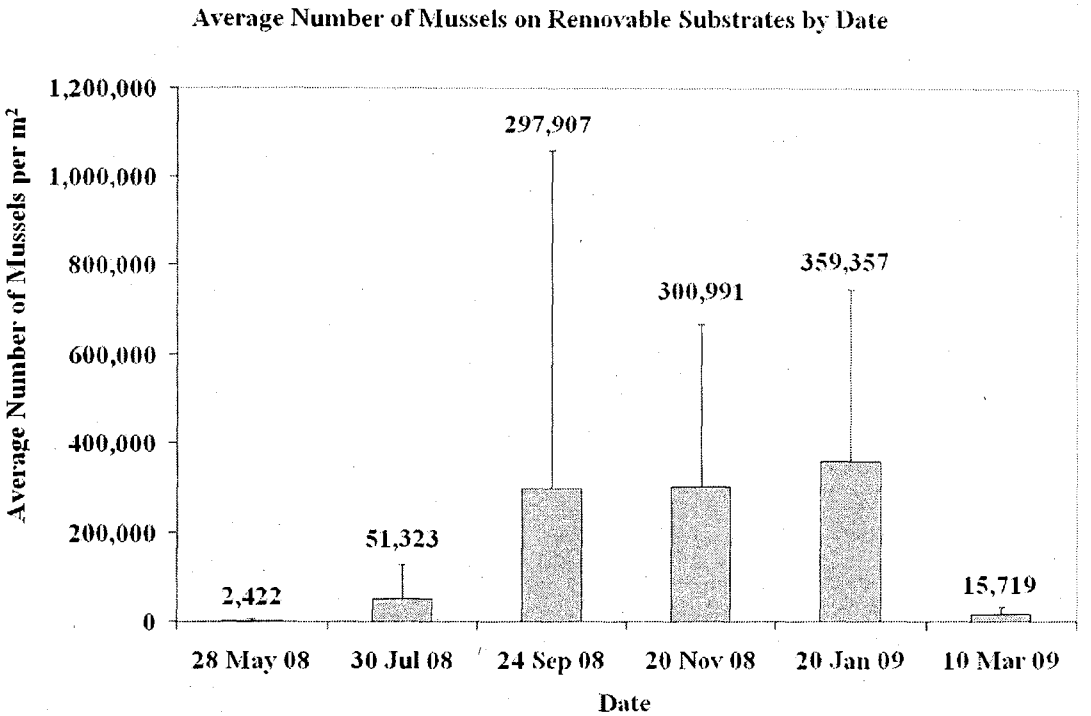


Figure 9. Average number of mussels by date on removable substrates. Error bars indicate +1 SD.

Permanent Substrates

There were a total of 4 permanent plates that were not present on the sampler when it was brought onto the boat. All of these were CUB. CUB on sampler 4 was missing and replaced on 27 May 08, CUB on sampler 2 and 3 were replaced on 30 July 08, CUB on sampler 3 was replaced again on 20 Nov 08, and CUB on sampler 1 was missing on the final collection date. The hard water conditions of Lake Mead may have weakened the concrete and during the process of pulling the sampler out of the water, the substrate may have broken off. These concrete substrates were weighted and the final number of mussels per m^2 was determined from the weighted value based on a prorated year. All of

the other substrates remained on the sampler for the duration of the study (approximately one year).

The pictures taken at each sampling date are located in the supplemental Appendix. It was suggested to calculate mussel density and growth rates via pictures, but the quality of the pictures was low and the rough weather conditions that the pictures had to be taken in (i.e. rocking boat) did not allow for precise measurements. The pictures are useful as a visual tool to estimate mussel colonization rates, however.

As with the removable data, non-parametric methods were used due to unequal variances with the permanent substrate data. There was no significant difference in settlement between substrate types (Friedman's χ^2= 6.2165; p=0.2857) (Figure 10), but

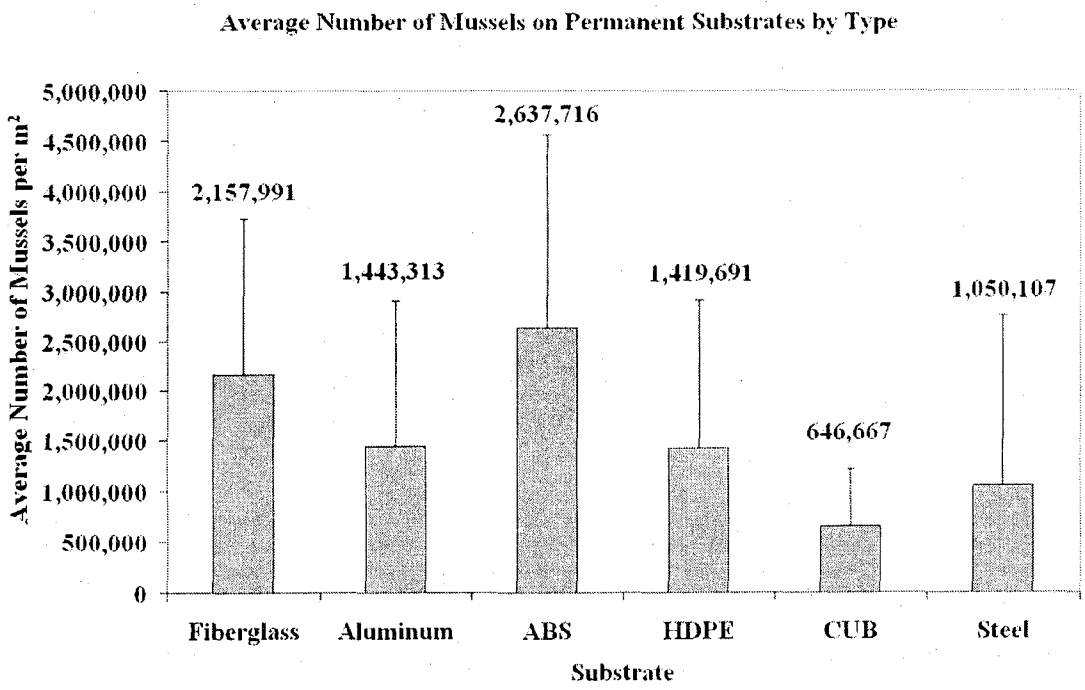


Figure 10. Mussel settlement on permanent substrate by type. There were no significant differences between types.

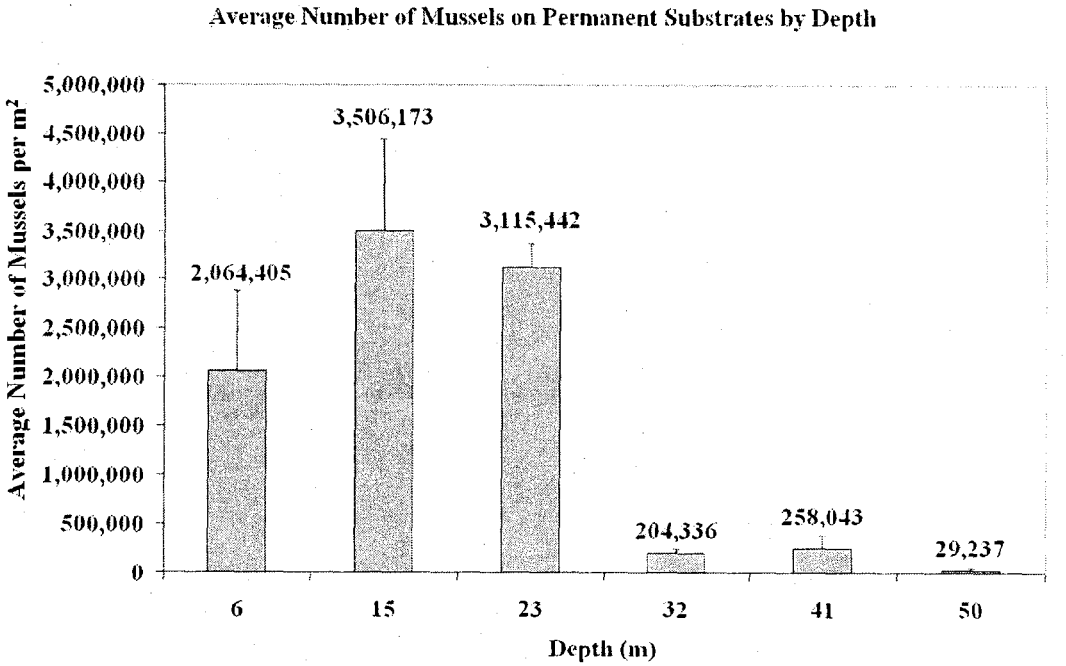


Figure 11. Mussel settlement on permanent substrates by depth. Depths 6, 15, and 23 were significantly different from depths 32, 41 and 50.

there was a significant difference between depth and settlement ($F= 35.04$; $p<0.001$) (Figure 11). The substrate with the highest number of mussels was the same as with the removable substrates: ABS plastic with an average of 2,637,716 m^{-2}. There were slight differences in overall mussel settlement from the removable substrates, however. Permanent substrate densities are listed in decreasing order: ABS plastic (2,637, 716 m^{-2}) > Fiberglass (2,157,991 m^{-2}) > Aluminum (1,443,313 m^{-2}) > HDPE plastic (1,419,691 m^{-2}) > steel (1,050,107 m^{-2}) > CUB (646,667 m^{-2}).

Veligers

The distributions of veliger abundance and mussel abundance were significantly different (Figure 12) (KS=0.5, p=0.002 indicating to reject the null hypothesis that the

two distributions were the same). From a visual assessment of the Figure 12, there is a lag phase occurring between veliger concentrations in the water column and pediveligers and juveniles that have settled on a substrate. A continuation of data collection may result in significantly similar distributions. Although Martel et al. (1994) reported a correlation between daily settlement rates and larvae concentrations in the water, Sprung (1989) reported that finding correlations between larvae collected in the water column and newly settled mussels is difficult due to the high mortality rates of larvae. The daily

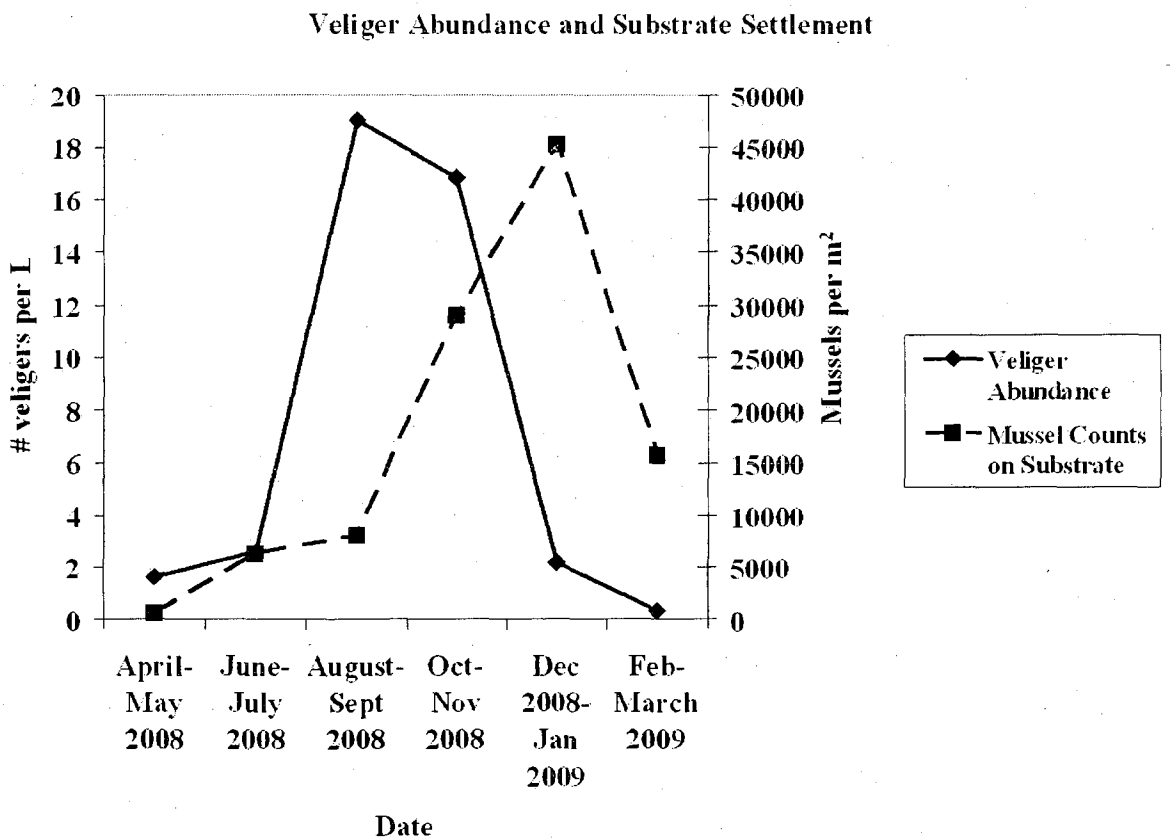


Figure 12. Veliger abundance in the water column with number of mussels on the substrate plates. Data from March-July 2008 are from Denise Hosler, Bureau of Reclamation, Denver, CO. Data from August 2008-March 2009 were collected by the author.

collection of larvae could provide an advantage in finding a correlation between larvae in the water column and settled mussels due to the ability to collect larvae before the high mortality rates take effect.

Discussion

Substrate Preference

The hypothesis that mussels would settle preferentially on substrates with the most texture, CUB and fiberglass, was not supported by this experiment. There was no statistically significant difference in mussel settlement between substrate types. Mussels may not have had an overall substrate preference due to the stochastic nature of the first settling event as a veliger. In the post-veliger stage, settlement is dictated more by preference because of their ability to detach byssal threads (Marsden and Lansky 2000). All of the substrates in this experiment potentially provided a suitable place for the mussels to attach and grow. The chemical composition and texture of the plates did not seem to have a significant effect on mussel settlement.

For the removable and permanent substrates, settlement was highest on ABS plastic. This could be due to the textured surface on one side of the plate. This, however, does not explain why the concrete substrates had some of the lowest mussel concentrations. The concrete was textured on both sides, but there may have been confounding factors on the concrete plates such as an unknown chemical coating. The aluminum plates had the 2nd highest densities on the removable substrates (293,282 m^{-2}) and the 3rd highest densities on the permanent substrates (1,443,313 m^{-2}). These results are contrary to Kilgour and Mackie (1993), but in agreement with Marsden and Lansky (2000) and

Kobak (2004) (Table 2). Kilgour and Mackie (1993) reasoned that lower densities on aluminum were due to known toxic effects of aluminum on Unionid mussels. Dreissenid mussels may not be as susceptible to the toxic effects of aluminum as Unionid mussels. The results of this experiment indicate that substrate preference is based more on the location of the substrate in the water column than the texture or chemical composition of the substrate.

Depth

The hypothesis that mussels will settle at depths suitable for reproduction and survival was supported by data from this experiment, however the depths are slightly different from what was predicted. Depths from 6- 28 m had significantly greater mussel settlement on both removable and permanent substrates than depths from 32- 54 m. There was a 15 fold increase in the average number of mussels on the removable substrates from 10- 28m (336,969 m^{-2}) than from mussels on removable substrates at 37- 54 m (21,894 m^{-2}). For the permanent substrates, there was a 19 fold increase in average number of mussels from 6- 23 m (2,884,160 m^{-2}) to those from 32- 50 m (154,815 m^{-2}). Lower temperatures at deeper depths can result in reduced availability of food, lowering the survivability of mussels at these depths and can delay release and maturation of gametes (French et al. 2007; Wacker and von Elert 2003).

Comparison to Other Bodies of Water

Previous literature has indicated that mussel settlement at different depths is dependent on the unique characteristics of individual water bodies. In Lakes Erie, Michigan and Ontario, zebra mussels had previously established populations before the quagga mussel also invaded (French III et al. 2007; Mills et al. 1993a; Patterson et al.

2005; Roe and MacIsaac 1997). These studies found that, in general, quagga mussel densities increased with increasing depth and zebra mussel densities decreased with increasing depth. This could be explained by the fact that quagga mussels are more evolutionarily fit than zebra mussels and thereby, more able to survive and reproduce in colder temperatures. In Lake Ontario, both quagga and zebra mussels were found living at depths from 8-110 m, but at 130 m only quagga mussels were found (Mills et al. 1993a). Another explanation for increased quagga mussel densities with depth is that these deeper depths were not previously colonized by zebra mussels and the quagga mussels simply settled where there were no other mussels currently residing.

The current study is in contrast to these findings in the Great Lakes due to the observation that as depth increased, quagga mussel density decreased. A benthic study based on ponar dredges in Lake Mead found the highest quagga mussel densities on muddy and sandy substrates at depths from 12-42 m (Chandra et al. 2009). In a reservoir in Spain, Navarro et al. (2006) found the highest densities of zebra mussels in the hypolimnion, but the biomass of the individuals was much less than mussels found in the epilimnion. The current study and Wacker and von Elert (2003) both found the lowest densities of mussels in the hypolimnion and the highest densities in the epilimnion.

Date

A significant increase in colonization rates by date was due to multiple factors (Figure 9). There was heavy mussel settlement on the rope connecting the substrates on the sampler. The mussels on the rope may have detached from the rope and then attached to the substrates, artificially inflating the settlement numbers. Mussels were removed from the rope before placing them back in the water during the sampling events to try and

prevent this occurrence. Over the course of the year long experiment, the laboratory methodologies changed slightly to become more efficient. At the beginning of the experiment, it was attempted to remove mussels with forceps and place them in the gridded petri dish. This process may have resulted in an underrepresentation of mussel settlement. Later methods included gently scraping the mussels off the substrate directly into the gridded petri dish and subsequently examining substrates under a stereomicroscope at 20 X power to find and count mussels that may have been missed in the scraping process.

A natural explanation for the increase in mussel settlement from March 2008 to January 2009 is the changes in water quality over this time period. As stated above, the lake stratifies approximately one time per year (monomictic) making the temperatures within the epi- and metalimnion fluctuate throughout the year (Figure 13). A Spearman correlation was conducted to determine if there were any relationships between mussel settlement throughout the year and four water quality characteristics: temperature (°C), conductivity (μS/cm), pH and dissolved oxygen (mg/L) (Table 4). Temperature was the only characteristic that had a significant correlation with mussel settlement. The low

Table 4. Non-parametric correlations between mussel settlement and water quality characteristics. Only temperature and mussel density were significantly correlated.

		Temperature	Conductivity	pH	Dissolved Oxygen
Average	Spearman's rho	0.705	0.038	0.161	-0.106
# Mussels	Signifance	<.001	0.814	0.308	0.504

temperatures of Lake Mead in the hypolimnion were still within the threshold for mussel survival (minimum temperature 2-5 °C (Mills et al. 1996)), but they may have been low enough to alter growth and reproductive behavior.

High mussel densities in the lower depths (28-37 m) for the 10 Mar 09 sampling date may be an artifact of lake stratification (Figure 13). An event entitled down-welling occurs when warmer water is forced deeper into the lake when the lake mixes to become destratified (the same temperature from the surface to the bottom) (Kavouras and Maki 2003). This process forces veligers currently suspended in the water column to settle at depths that they previously would not have settled. A veliger's velum, which allows veligers to feed and swim, begins to degenerate during the growth process and if they are forced into deeper water without a velum, they must settle on a substrate (Kavouras and Maki 2003).

Limitations of the Study

There were many processes in this experiment in which mussels could have been lost. The process of dragging the sampler from the lake to the shoreline may have resulted in mussel detachment due to a slight shaking of the line as the anchor was dragged along the bottom of the lake and an increase in water flow over the substrates. Removing the substrate from the sampler line was a difficult task and as it was often wet and slippery, it was easy to accidently knock mussels off the substrates. Once in the laboratory, mussels could have been lost during the scraping process.

The substrates used in this experiment were not all of the same texture and color. Rubbing the substrates with sandpaper to equalize the texture and soaking them in filtered lake water before the experiment would have been beneficial. Another issue with

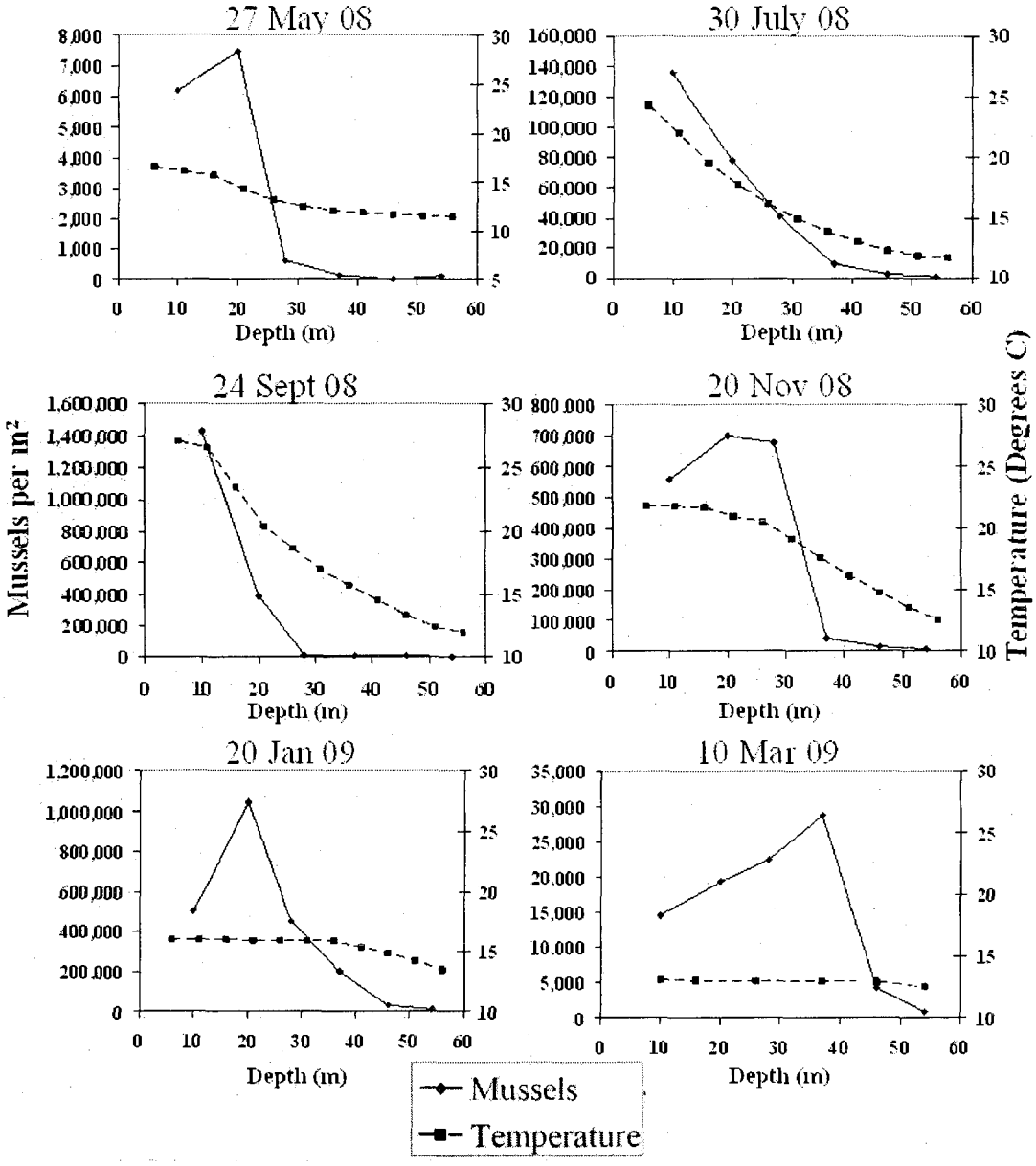


Figure 13. Mussel settlement on removable substrates by date compared with water temperatures at depth. The lake is stratified from May 2008 to Nov 2008 and becomes destratified between Jan 09 and March 09. Down-welling occurred in Mar 09.

the experimental design was that not all of the substrates were at all of the different depths. Some substrate types had three above the metalimnion, and only one below; and others had the opposite or had equal numbers above and below. This could have artificially inflated or deflated averages increasing the likelihood for types 1 or 2 error (finding significance when there is none and not finding significance when there is significance, respectively).

To gain an increased understanding of the interactions quagga mussels have with the various water quality characteristics in the lake, an experiment lasting longer than one year should be conducted. This experiment only lasted one year, so extrapolating this data to make accurate predictions about the future must be done with extreme caution. Lake conditions vary between years and an average of multiple years would yield more statistical power, and reduce the variance by conducting a multiple linear regression.

Recommendations for Future Research

To further understand why most mussels are settling at reduced densities at depths greater than 32 m, researchers should measure food and calcium levels at these depths. A laboratory experiment in which water from depths greater than 32 m and less than 32 m was collected separately and used to rear mussels simultaneously might determine if the reason why mussels do no survive at greater depths is due to the chemical composition of the water.

The amount of effort that went into each sampling event in this experiment was incredible. A team of at least five people were assembled and the use of a behemoth NPS maintenance boat was necessary. Toward the halfway point in the experiment, it was recommended that instead of suspending the sampling lines on four separate buoy/anchor

lines, simply install a small platform and suspend the lines from the platform. This was not implemented in the current experiment due to the fact that half of the experiment had already elapsed, but it would have required much less effort in the field. While setting up a platform would necessitate tedious work and permit filing, it would be worth it to have the freedom to check on the mussels at any time, in any weather conditions and with a team of only two people.

Suggestions for Lake Managers and Users

Based on the data from this year long experiment, the low colonization rates on substrates from January to March 2009 indicate that if an industry needs to place any type of material in Lake Mead, this would be the time to do so, or alternatively, whenever the lake is destratified. It is important to note that when the lake becomes destratified, veligers will settle on substrates at depths deeper than normal because of down-welling.

As documented by Ackerman et al. (1996), it was observed in this experiment that mussels had the strongest attachment to natural surfaces (concrete), a fairly strong attachment to metallic surfaces (aluminum and steel), and an even weaker attachment to polymeric surfaces (ABS and HDPE). HDPE plates required the least amount of work to remove mussels. This could have implications for maintenance of equipment in Lake Mead. If materials placed in the lake become colonized, it is easiest to clean materials of a polymeric nature.

Previous literature stressed the importance of a microscopic biofilm to stimulate mussel settlement (Kavouras and Maki 2003; Marsden 1992; Wainman et al. 1996). Based on a visual assessment at 20 X power using a stereomicroscope, a biofilm was not always present on the substrates in this experiment that had mussel attached. Two

months in Lake Mead may not be enough time for an appropriate biofilm to form, but mussel settlement did occur after this period of time without a biofilm on the substrate. Based on this experiment, quagga mussels in Lake Mead may not be as dependent on the presence of a biofilm as mussels in other bodies of water.

Several federal, state and local agencies are collaborating in the Las Vegas Valley to build the Systems Conveyance and Operations Program (SCOP) which will divert treated effluent to a lake diffuser in Lake Mead (BOR 2008). If these agencies want to avoid the nuisance of quagga mussel colonization during construction of the pipeline, the pipe should only be in the water (especially at the surface) from late January to early April, or when the lake is destratified. The SCOP project will use black colored HDPE as the pipe material, and it is important to note that while the HDPE in this study did not have high colonization rates, it was a white plastic. The black colored plastic in this study had the highest densities of mussels in both removable and permanent substrates. It is unknown whether mussels preferred this plastic because of its chemical composition or because of the color.

A material that is placed in the water past 32 m deep will have much lower colonization rates than materials placed above this point. If an agency wanted to test different types of substrates or coatings that would not allow mussels to settle, the most efficient depths to conduct this would be from the surface to 32 m. The most important finding from this study was that mussels will settle and grow on all surfaces tested if it is located above a depth of 32 m. This means that most substrates at these depths, with the exception of copper, or other toxic materials, experiencing a water flow rate of less than 1.5 m/s (4.9 ft/s) (Claudi and Mackie 1994) have the potential to become colonized,

especially if it is in the water during the autumn season when veliger counts are the highest and the lake is stratified.

CHAPTER 4

CONTAMINANT MONITORING

Review of Contaminants in Quagga Mussels

An important part of understanding the impact quagga mussels may have on the environment is to assess certain characteristics of the environment and see if they have changed since the introduction of mussels. The quagga mussels' ability to filter large quantities of water allows them to bioconcentrate toxicants found in water. This ability to bioconcentrate toxicants makes mussels a useful biomonitor and accordingly, can be used to estimate overall environmental health. Biomonitoring organisms are an effective way to estimate contaminant concentrations in water when concentrations are too low to measure using conventional water sampling methodologies (Richman and Somers 2005). For most studies conducted world-wide, concentrations of contaminants in a wide variety of mussel species' soft tissue is reflective of concentrations in the environment (Chiu et al. 2000; Marvin et al. 1994; Metcalfe and Charlton 1990; Muncaster et al. 1990; Peven et al. 1996; Rainbow et al. 2000). Similar findings have been reported for Dreissenid species in North America (Bruner and Fisher 1994; Mills et al. 1993b; Rutzke et al. 2000; Secor et al. 1993).

While monitoring quagga mussel population size and density, researchers can easily monitor the lake for contaminants through harvesting mussels. Once a sample of mussels is retrieved, counted and weighed, contaminant analysis is not complicated with an

appropriate mercury analyzer such as a Leco AMA-254 or a Perkin-Elmer FIMS 100. For an accurate prediction of ecological changes the quagga mussel is causing, they must be monitored and analyzed for criteria contaminants.

Mercury

Mercury (Hg) is an element that has become a ubiquitous component of aquatic environments from overuse of pesticides, antiseptics and preservatives, the burning of fossil fuels and natural weathering processes (Clarkson et al. 2003). The most dangerous form of mercury, methyl mercury, bioaccumulates up the food web and may have adverse health affects on consumers (Williams et al. 2000). Mercury's ability to cause severe health problems, especially for pregnant women and children, caused the FDA and EPA to determine when a fish or other animal should not be consumed because of high mercury concentrations. The FDA states that a fish should not be consumed if it contains greater than 1.0 ppm (1.0 μg/g) mercury (Gutenmann and Lisk 1991). The EPA's limit is more conservative at greater than 0.3 ppm (0.3 μg/g) (Ball 2002).

Mercury has been found in fish tissue, (Cizdziel et al. 2003; Cizdziel et al. 2002) surface water, sediment and groundwater from Lake Mead (Cizdziel and Zhou 2005). Concentrations in fish mussel tissue ranged from 0.0084 to 0.309 ppm (μg/g), depending on the species (Cizdziel et al. 2003). Mercury concentrations in surface and groundwater were under 10^{-6} ppm (μg/g) and sediment concentrations averaged 0.034 ppm (μg/g) (Cizdziel and Zhou 2005). Quagga and zebra mussels easily bioaccumulate organic mercury from the water column, suspended particles, sediment and interstitial water because they must filter massive amounts of water on a daily basis to get enough nutrients to survive (Table 5). The invasion of quagga mussels will allow more

Table 5. Mercury concentrations in quagga and zebra mussels cited in the literature.

Species	Hg µg/g dw	Location	Reference
D. bugensis	0.09-0.28	Lake Ontario	Mills et al. 1993b
D. bugensis	ND-0.15	Niagara River, NY	Richman and Somers 2005
D. bugensis	0.11	Lake Ontario	Rutzke et al. 2000
D. bugensis	0.15-0.22	Lake Erie	Rutzke et al. 2000
D. polymorpha	0.049-0.158	Insubria region, N. Italy	Camusso et al. 2001
D. polymorpha	0.02-0.05	Upper Mississippi River	Cope et al. 1999
D. polymorpha	0.109-0.22	St. Lawrence River, Canada	Kwan et al. 2003
D. polymorpha	0.04-1.37	Lake Ontario, Erie and Niagara River, NY	Lowe and Day 2002
D. polymorpha	0.1-0.25	Lake Ontario	Mills et al. 1993b
D. polymorpha	ND-0.12	Niagara River, NY	Richman and Somers 2005
D. polymorpha	0.1	Lake Ontario	Rutzke et al. 2000
D. polymorpha	0.26	Lake Erie	Rutzke et al. 2000
D. polymorpha	0.05-0.38	Upper New York State	Secor et al. 1993
D. polymorpha	0.817-0.102	Kleines Haff, Germany	Wiesner et al. 2001

dw= dry weight; ww= wet weight; *D. bugensis*= quagga mussel; *D. polymorpha*= zebra mussel; ND= none detected

contaminants to be bioavailable and travel up the food chain at higher concentrations. Mussels provide an easy way to monitor aquatic contaminants because of their ease of collection, sedentary lifestyle and relatively wide distribution (Kwan et al 2003). No research on contaminant concentrations in Lake Mead quagga mussels has been conducted prior to this study.

Trophic Transfer of Contaminants

Bioaccumulation is a phenomenon in which pesticides, mercury or other contaminants increase in concentration in individuals as one goes up the food web (Figure 14). Small invertebrates are commonly found at the base of the food chain. Diving ducks and certain fish species may ingest these small invertebrates (like mollusks), then humans may eat these ducks or fish and ingest the mercury and selenium

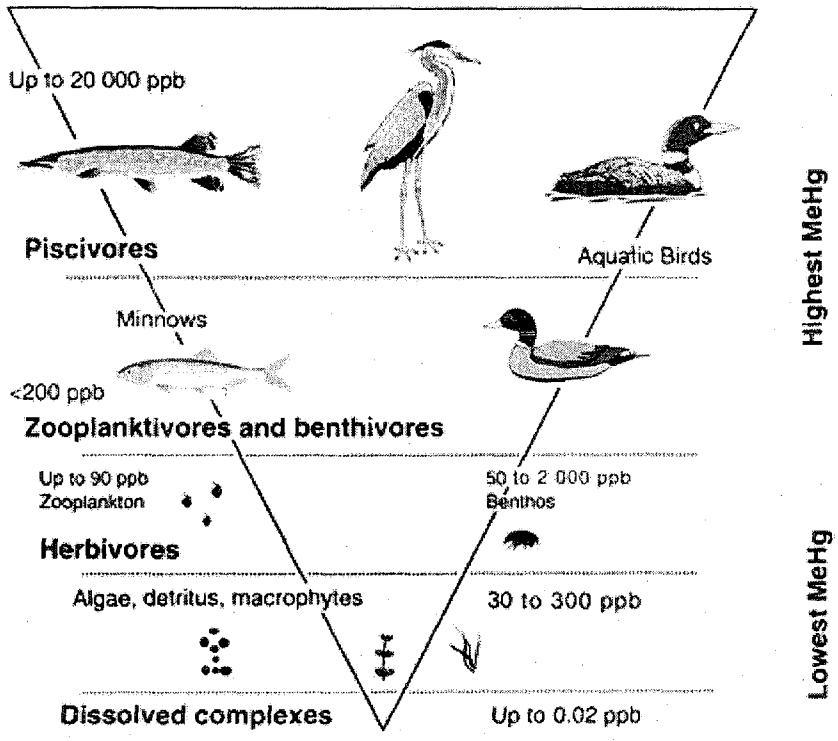


Figure 14. Bioaccumulation of mercury up food webs. Image from http://www.ec.gc.ca/ceqg-rcqe/English/html/mercury/images/fig4.gif

those organisms may have accumulated in their bodies. Waterfowl and other small omnivores, depending on their diet, may consume quagga mussels or consume an animal that has consumed quagga mussels as a part of complex food webs. The potential for bioaccumulation of mercury up the food chain in Lake Mead is increased for species that ingest quagga mussels. The following study was designed to evaluate concentrations of mercury in quagga mussels from Lakes Mead and Mohave.

Question, Objective, and Hypothesis

Question

- Do mussels bioaccumulate mercury in their soft tissue?

Objective

- Determine mercury concentrations in the soft tissues of quagga mussels from Lake Mead.

Hypothesis

- Quagga mussels will bioaccumulate toxicants such as mercury in their soft tissues because they are filter feeders.

Methodology

Collection of Specimens

Samples were collected from August 2007 to November 2008. Mussels were collected by National Park Service and Bureau of Reclamation SCUBA divers at various locations throughout Lakes Mead and Mohave (Figure 15). Other mussels were collected by scraping the bottom of docks, or collecting rocks by UNLV employees. All mussels were collected in accordance with NDOW collection and possession permit #S30712.

Mercury Analysis of Quagga Mussels

Mussels were separated into size classes based on shell length. Size classes included: 10-14.9 mm, and >15 mm. The soft tissue was removed from the shell and then a composite sample of all soft tissue from each size class was made and weighed in grams. Composite soft tissue was homogenized and then lyophilized for at least 24 h at -55°C in a Bench Top 4K Freeze Dryer (SP Industries, New York). After lyophilization, if a

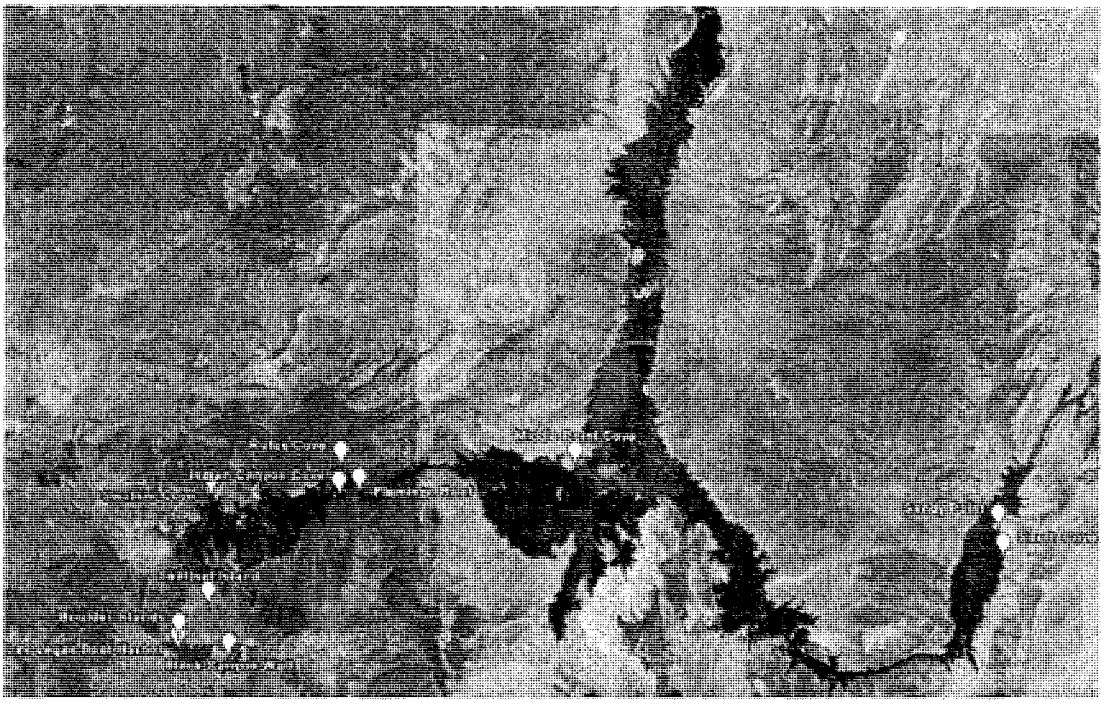
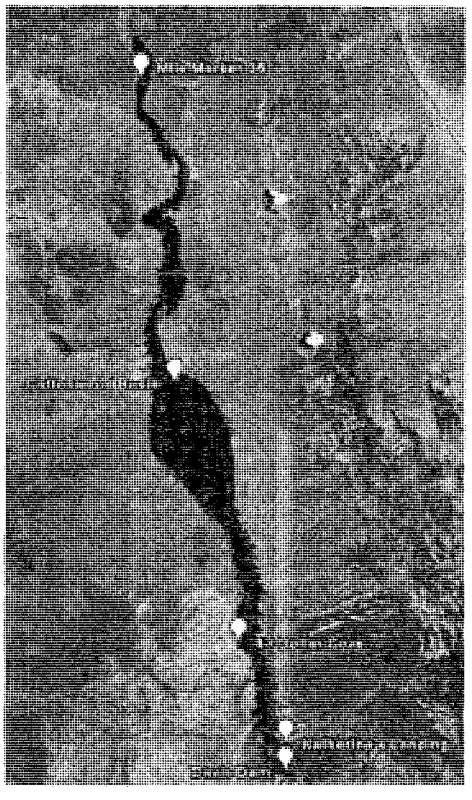


Figure 15. Locations of collection sites for mercury analysis of quagga mussels in

a) Lake Mead and



b) Lake Mohave

particular location did not contain more than 1.0 g of dry tissue for each size class, then the size classes were combined. A sample of 0.75-1.0 g dry tissue was digested in an Anton-Parr Multiwave 3000 microwave digestion system using the Fish Tissue Digestion Procedure for Cold Vapor Mercury Analysis (Appendix I). Total mercury was analyzed in accordance with EPA Method 245.6 using a Perkin-Elmer FIMS 100 equipped with an AS-91 autosampler employing the flow-injection mercury cold-vapor technique (PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA). The instrument detection limit was reported to be 0.2 parts per billion (ppb). The method detection limit was calculated to be 0.010 ppm.

For analysis, a 4 mL aliquot of this raw digested material was transferred into a separate clean and labeled centrifuge tube containing 4 mL 3% HCl. This 1:2 solution was used in most cases for analysis using the FIMS 100. Each sample was diluted with additional 3% HCl as necessary for the concentration of mercury in the sample to fall within the range of the calibration curve.

Quality Assurance/ Quality Control is a necessary research tool and was conducted by performing a calibration blank each day prior to analysis. If the FIMS had a 0.995 or higher correlation coefficient it was considered acceptable for the calibration curve. Standard reference materials (SRMs) were digested and analyzed with each quagga mussel analysis. The reference materials were National Research Council Canada dogfish muscle, DORM-3 (Ontario, Canada), and National Institute of Standards and Technology 1946 Lake Superior Fish Tissue (Gaithersburg, MD). SRMs had to be within 80-120% of their listed mercury concentrations. Due to the small amount of sample for some locations, only one analysis on the tissue was conducted. Usually a

sample is analyzed in triplicate to avoid within sample error, but in this case there was not enough tissue, therefore no inferential statistics were conducted.

Results and Discussion

A total of nine composite samples were analyzed from different locations. Eight other locations contained enough tissue to conduct mercury analysis on both size classes. Results are reported in Table 6. The overall mean of mercury in quagga mussel soft tissue was 0.035±0.015 μg/g dry weight. Mussels from Lake Mead (n=11) had an average of 0.036±0.015 μg/g dry weight (dw) and Lake Mohave mussels (n=5) had 0.033±0.007 μg/g dw. Mercury concentrations ranged from 0.017 μg/g dw at the Black Canyon Wall location to 0.074 μg/g dw at the Boulder Islands location. There were no significant differences between shell length and mercury accumulation.

Comparison to Other Data

The concentrations of mercury found in this study were low when compared to other studies. Only Richman and Somers (2005) found concentrations of mercury in quagga mussels to contain the range of concentrations in the present study (Table 5). The range of mercury concentrations in this study was well below the range of concentrations found in the other studies (Mills et al. 1993b; Rutzke et al. 2000). When data from the present study were compared to contaminant concentrations in zebra mussels, similar findings as with quagga mussels occur. Using quagga mussels in Lake Mead as a biomonitor is feasible based on the data from the current study. Sediment and water concentrations of mercury are very low and difficult to detect, but mussel tissue concentrations are within the range for simple analytical analyses. Cope et al. (1999) reported methylmercury

Table 6. Mercury concentrations in quagga mussels from Lakes Mead and Mohave.

Date Collected	Location	Mussel Size	Hg µg/g (ppm*) dw**
8/22/2007	Indian Canyon Cove	>15 mm	0.048
8/22/2007	Indian Canyon Cove	10-14.9 mm	0.044
8/29/2007	Boulder Islands	composite sample***	0.027
9/6/2007	Flamingo Reef	composite sample***	0.043
3/27/2008	Swallow Cove	composite sample***	0.020
4/17/2008	Middle Point Cove	composite sample***	0.043
5/2/2008	Cottonwood Basin	composite sample***	0.040
5/6/2008	Mile Marker 36	>15 mm	0.039
5/6/2008	Mile Marker 36	10-14.9 mm	0.039
5/7/2008	83 Dollar Cove	>15 mm	0.026
5/7/2008	83 Dollar Cove	10-14.9 mm	0.030
5/21/2008	Las Vegas Boat Harbor	>15 mm	0.036
5/21/2008	Las Vegas Boat Harbor	10-14.9 mm	0.036
6/25/2008	Rufus Cove	composite sample***	0.028
7/9/2008	Black Canyon Wall	>15 mm	0.024
7/9/2008	Black Canyon Wall	10-14.9 mm	0.017
7/16/2008	Davis Dam	composite sample***	0.034
8/5/2008	Boulder Islands	>15 mm	0.060
8/5/2008	Boulder Islands	10-14.9 mm	0.074
8/6/2008	Sentinel Island	composite sample***	0.028
9/17/2008	Sandy Point	composite sample***	0.022
9/17/2008	South Cove	>15 mm	0.026
9/17/2008	South Cove	10-14.9 mm	0.028
11/17/2008	Katherine's Landing	>15 mm	0.022
11/17/2008	Katherine's Landing	10-14.9 mm	0.032

*= mercury in parts per million; **= dry weight; ***=combination of all size classes

concentrations in zebra mussels to comprise 30-70% (average of 50%) of the total Hg. The potential for bioaccumulation in Lakes Mead and Mohave are evident. The overall average of total Hg in fish tissue from Lake Mead was found to be 0.119±0.104 µg/g wet weight (Kramer 2009). This is a three fold increase in mercury from quagga mussels.

Limitations to the Study and Recommendations For Future Research

Due to the fact that this was the first contaminant analysis on quagga mussels in Lakes Mead and Mohave, numerous lessons were learned. First, it was unknown prior to this study exactly how many mussels would be necessary to conduct contaminant analysis. From this study, it can be determined that more than a 0.5 L bottle full of mussels is prudent. The amount of mussels collected for contaminant analysis should be at least 2 -1 L sized bottles full of mussels. Second, once mussels were homogenized and lyophilized, they became extremely staticky. This made the weighing and transferring of samples difficult. The use of a pulverizer in a ball mill to make the mussel tissue into a powder would have been beneficial in this project.

Mussels were collected in a non-random manner. The divers from the NPS and BOR had official business at these locations, and it was convenient to collect mussels while they were doing their work. A random, independent sampling technique with multiple samples collected would allow the use of inferential statistical analysis. Analysis of quagga mussel tissues for other contaminants, such as other heavy metals, metalloids, organochlorine pesticides, or PCBs on a yearly basis in predetermined, permanent locations would be logical strategy for biomonitoring Lake Mead with quagga mussels. It will also be important to monitor other trophic levels for contaminants. Fish from all trophic levels and diving ducks should be sampled for contaminant concentrations to gain a better understanding of the complex web of contaminant transfer in Lake Mead. In conclusion, these preliminary data for mercury in quagga mussels from Lakes Mead and Mohave provide a baseline of data for future research. There is potential to use quagga mussels as a biomonitor of overall lake health in Lakes Mead and Mohave.

CHAPTER 5

PUBLIC OUTREACH ON QUAGGA MUSSELS

History of the 100th Meridian Initiative

Zebra and quagga mussels and other ANS can easily attach to trailered boats and consequently, be introduced into new environments making boater education an imperative part of preventing the spread of aquatic nuisance species (ANS). Boater surveys are a form of boater education and can also reveal boater travel patterns to predict the location of the next zebra/quagga invasion (Britton and McMahon 2005). The United States Aquatic Nuisance Species Task Force, comprised of individuals from multiple governmental agencies such as the United States Fish and Wildlife Service (USFWS), the National Park Service (NPS) and numerous state fish and wildlife departments, began an initiative in 1997 to prevent the spread of zebra mussels and other ANS to waters west of the 100th meridian (100° west longitude) (Britton and McMahon 2005). The 100th meridian runs through Texas, Oklahoma, Kansas, Nebraska, South Dakota and North Dakota. The initiative was entitled the 100th Meridian Initiative and is a part of the United States National Invasive Species Act of 1996 (Public Law 101-636). Members of this initiative formed a collaborative organization including federal, tribal, regional and local agencies of the United States and Canada.

The Western Regional Panel within the USFWS took the lead on the 100th Meridian project and began administering three types of surveys to boaters in twelve states in 1998.

Contact surveys were conducted by interviewers with a standard form including questions that asked: 1) where boaters were from and where they had previously launched their boat; 2) where they were launching their boat next; 3) if they clean their boat between launchings; and 4) if they were aware of zebra or quagga mussels or other ANS (Appendix II). Mail-in surveys were placed on vehicle windshields (Appendix II). The last portion of the 100th Meridian survey project included trailer counts (Appendix II). The trailer count involves researchers counting and recording the state of origin of trailers at boat ramps and in marina parking lots.

Review of 2003 Project

As part of the nationwide initiative, the aforementioned surveys were conducted by employees of the University of Nevada Las Vegas from 01 September 2002 to 31 March 2003 at Lake Mead. Boat launch ramps at Hemenway Harbor, Lake Mead Marina, Las Vegas Bay, Callville Bay, Echo Bay, and Overton Beach were identified as survey locations (McCoy 2003). During the study period, researchers interviewed 246 boaters with the contact survey, left 3,005 mail-in surveys on vehicle windshields and counted 6,799 trailers. Only 1.2% of boaters interviewed came from zebra mussel infested states, 78.6% of boaters cleaned their boat between launchings, and awareness of zebra mussel was low (34.2%) (Gerstenberger et al. 2003). Of the 3,005 mail-in surveys distributed 132 (4.2%) were returned. The self-surveyed boaters were more aware of zebra mussels (48.1%) and cleaned their boats less (76.3%). Only 45 trailers of the 6,799 counted were from states infested with zebra mussels (Gerstenberger et al. 2003). When this study was conducted, there were no quagga mussels in Lake Mead. The discovery of the quagga mussel in Lake Mead in January 2007 sparked a new interest in studying boater

behaviors. The following questions, objectives and hypotheses describe the study conducted in 2007 and 2008 that closely mimic the 2003 study.

Questions, Objectives and Hypotheses

Questions

- Do boaters that utilize Lake Mead National Recreation Area (LMNRA) clean their boats after each time they launch?
- Are boaters aware of quagga mussels or other aquatic invasive species?
- Are different types of boat owners (ex. angling boat vs. pleasure boat) more likely to be aware of quagga mussels?
- Do boaters at LMNRA travel to bodies of water that are currently non-infested with quagga mussels?
- Do survey data from 2007-2008 differ from data collected in 2003?

Objectives

- The survey data will determine if boaters that use LMNRA clean their boats and are aware of quagga mussels.
- Survey data will determine what bodies of water are at the highest risk for invasion due to boaters traveling there after they have launched at LMNRA.
- Comparing 2007-2008 data to 2003 data will determine any differences between boater behaviors in the different study periods.

Hypotheses

Boater Cleaning Habits

- The majority of boaters (>66%) interviewed will clean their boats after every launch.

Quagga Mussel Awareness

- Boat owners with an angling boat will be more aware of quagga mussels than other types of boaters because they are typically more involved in learning about the aquatic ecosystem they are utilizing.
- Boaters interviewed in 2007-2008 will be more aware of quagga mussels than boaters interview in 2003 due to increased advertising and more information available than in 2003.

Boater Traveling Habits

- Most boaters (>66%) that use LMNRA will only use LMNRA; they will not travel to other bodies of water due to long travel times and distances to get to other water bodies.

Methodology

Contact Surveys

Surveys were conducted at LMNRA from October 2007 to September 2008. All survey administrators received the proper collaborative institutional training in accordance with UNLV's Institutional Review Board (IRB). The protocol for research was approved on 03 July 2007 by UNLV and was awarded the number 0706-2391. The National Park Service also approved the project and awarded the study number LAME-00063 and the protocol number LAME-2007-SCI-0020. Researchers went to launch

ramps at Lake Mead National Recreation Area (n=4: Echo Bay, Callville Bay, Boulder Harbor, and Hemenway Harbor) (Figure 16) to administer the contact surveys. Boaters were approached by survey administrators and asked to participate in a short survey (Appendix II). If a boater agreed, they read the informed consent form and verified it

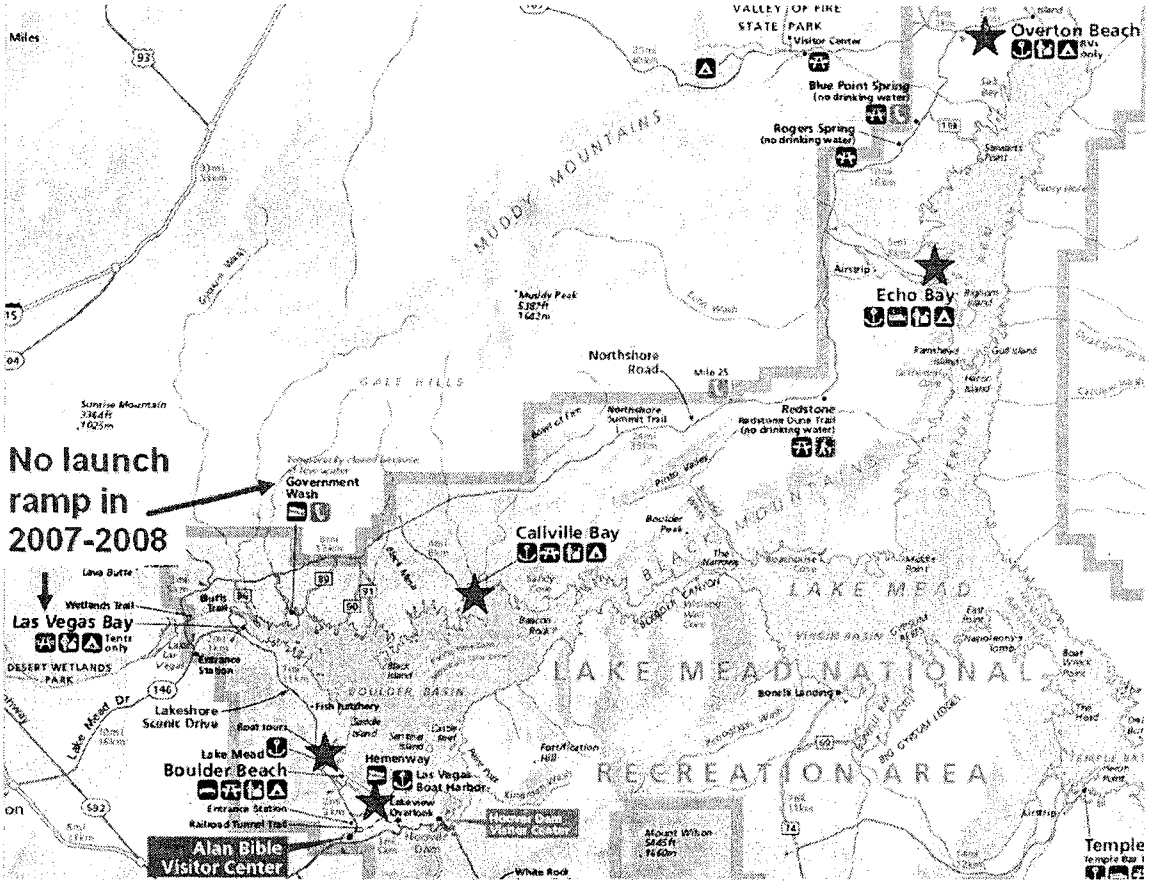


Figure 16. Locations of 100th Meridian Surveys at Lake Mead 2007-2008. Interview surveys were conducted at locations indicated by stars: Hemenway Harbor, Boulder Beach, Callville Bay and Echo Bay. Mail-in surveys were distributed and trailer license plate states were documented at these locations plus Overton Beach.
Map source: http://www.nps.gov/lame/planyourvisit/maps.htm.

with their signature. After this, one of the administrators would ask the boater the questions on the official 100th Meridian survey obtained from the 100th Meridian website at www.100thmeridian.org (Appendix II). If boaters were not aware of the threats of quagga mussels to the lake and to their boats, administrators would inform them and give them a Zap the Zebra brochure (Appendix II) for further information.

Mail-in Surveys

At the same launch ramps and marinas where contact surveys were conducted plus the Overton Beach launch ramp (n=5), mail-in surveys were placed on the windshield of vehicles with a boat trailer attached (Appendix II). If a boater chose to participate, they would fill in the survey then deposit it in the nearest mail box with prepaid postage to UNLV.

Trailer Counts

In the parking lots of the launch ramps and marinas (n=5), researchers documented the state of origin of boat or Personal Water Craft (PWC) trailers and recorded the counts on the 100th meridian official sheet (Appendix II). A review of boater destinations after their time at LMNRA was used to determine if currently non-infested waters are at high risk for contamination.

Statistics

Contact survey data were used to create contingency tables to further understand the relationships between boater types and cleaning habits and boater types and quagga mussel awareness. Contingency tables were also created to compare expected and observed trends of awareness and cleaning habits in 2003 vs. 2007-2008. Chi-square analyses were conducted on these contingency tables using SPSS version 16.0 to

compare the observed and expected frequencies at the significance level $p \leq 0.05$. In the event of a cell in the contingency table containing an expected count less than 5, the likelihood ratio statistic (G^2) was reported; otherwise the chi-square statistic (χ^2) was reported.

Results

Contact Surveys

Contact surveys began on 25 Oct 07 and continued through 01 Sept 08 culminating in a total of 236 surveys were completed, while 31 people declined to participate. The surveys were administered at launch ramps at Echo Bay (n=23), Callville Bay (n=60), Boulder Harbor (n=109) and Hemenway Harbor (n=44). Most of the boaters interviewed owned pleasure boats (69%), others had angling boats (21%), some had PWCs (7%) and 3% owned a craft classified as "other." The boaters were primarily from Nevada (n=192), but there were some from out-of-state: CA (n=22), UT (n=7), AZ (n=6), WY (n=2), WA (n=2), LA (n=1), IL (n=1), MI (n=1), OK (n=1) and PA (n=1). Most boaters (86.4%) said they clean their boats between launchings and 18% of boaters had no awareness of zebra or quagga mussels or any other aquatic nuisance species. Approximately 61% (n=145) of boaters interviewed said they only launch in Lake Mead, and had no plans to launch in any other body of water.

When comparing mussel awareness between boater types, no significant difference was found (G^2=1.028, p=0.794). All boat owners had the same level of quagga and zebra mussel knowledge. The cleaning habits of different types of boat owners was significantly different (G^2=13.120, p=0.004). Boat owners with a craft classified as other

cleaned at a significantly lower frequency than boat owners with an angling boat, pleasure boat or PWC.

Significantly more boaters interviewed in 2007 or 2008 always launched their boats in Lake Mead than boaters interviewed in 2003 (χ^2=18.668, p<0.001). The chi-square analysis determined that boater cleaning habits did not change between the study years (χ^2=0.949, p=0.330). Mussel awareness increased significantly overall from 2003 to 2007 and 2008 (χ^2=106.5, p<0.001).

Mail-in Surveys

Mail-in surveys were placed on windshields of vehicles with trailers attached from 10 Nov 07 through 14 Feb 09 at five launch ramps throughout LMNRA. Of the 888 surveys distributed, 57 were returned for a 6.4% return rate. The majority of people that returned the survey were from Nevada (62.5%; n=35), but others were from CA (n=10), UT (n=4), ID (n=2), AZ (n=2), WI (n=1), ND (n=1) and MT (n=1). Pleasure boats were the most common type of boat owned (54.8%; n=34), followed by angling (35.4%; n=22) and PWC and other were both at 4.8%. Only two (3.5%) participants had no prior knowledge of zebra or quagga mussels; 81% of boaters cleaned their boat between launchings; and 56% only launch their boats in Lake Mead.

Trailer Counts

A total of 1864 trailer license plate state of origins were recorded from 12 Nov 07 to 28 Feb 09. Figure 17 gives detailed geographic distributions of boaters found at Lake Mead. Briefly, 97% of states documented were Nevada (64.9%), California (26.1%), Utah (3.1%) or Arizona (3.0%).

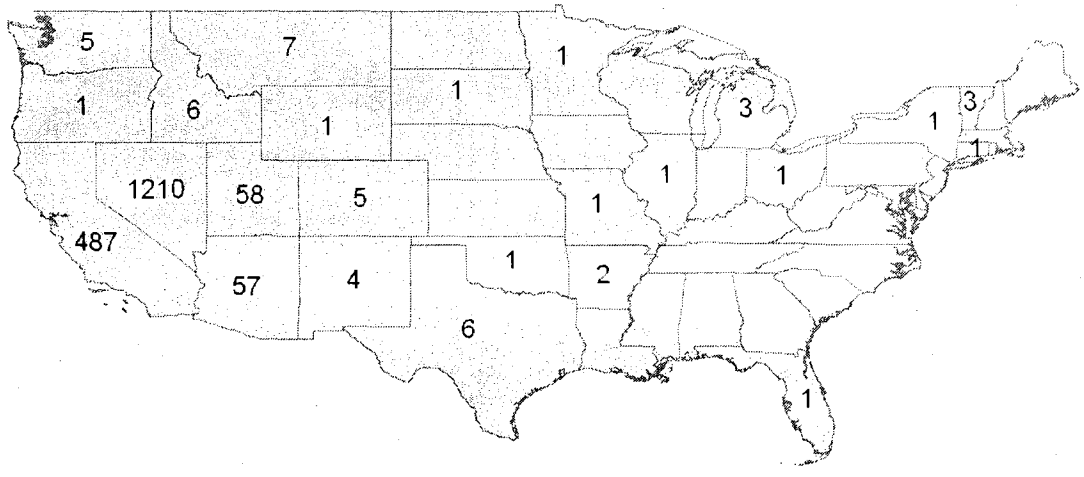


Figure 17. Trailer count distribution. All but seven of these states currently have a quagga or zebra mussel infestation (ID, MT, NM, OR, TX, WA, WY).

Discussion

It is of the utmost importance for people to clean, drain and dry their boats after each launch into a body of water due to the fact that mussels have been found to survive out of water for more than 10 days at less than 15°C and high humidity (Britton and McMahon 2005). This may allow live, adult mussels to be transferred to non-infested bodies of water and dominate the aquatic system. Theoretically, it only takes two mussels to produce millions of veligers leading to exponential growth and colonization in a new environment. Boater surveys and boater education may assist in the prevention of invasion and the prediction of where the next infested body of water may be.

This study found that a large percentage of boaters (81%) clean their boats after each launch, and this may contribute to the prevention of the spread of aquatic invasive

species. Different boaters may have different perspectives on what "clean" exactly means. An additional question was added to the survey to gain an improved understanding of a boater's definition of "clean." If a boater answered that they cleaned their boat after each use, survey administrators then asked how they cleaned their boat. There were a wide variety of answers, but the most common answers were: wipe down or dry boat after use (25%), use soap and water (19%), use a vinegar and water solution to wash boat (16%) or use a pressure wash (15%). Other answers included: rinsing off boat with a hose, taking the boat to a carwash, using bleach, or spraying down boat with Pink Stuff, Windex or Simple Green. According to www.ProtectYourWaters.net, an informative boater website ran by the USFWS, a boat should be cleaned using the following procedure: 1) remove all visible mud, plants, and fish or animals from the boat, trailer and all equipment; 2) eliminate water from all equipment; and 3) clean and dry anything that came into contact with the water with a 100% vinegar solution or a salt solution or with water that is at least 104°F (40°C) (USFWS 2009).

The hypothesis that angling boaters have an enhanced awareness of mussels than other types of boaters was rejected. All types of boaters were found to have the same amount of prior knowledge about zebra and quagga mussels. A large portion of boaters (61%) interviewed only launch in Lake Mead, but this is slightly lower than the hypothesized value of 66%. The long distances to other bodies of water in the southwestern United States may prevent people from traveling to other waters.

Between the study in 2003 and the current study, awareness increased from 35% to 82%. The increase in boater awareness in this study from the one in 2003 may be explained by an increase in press about mussels. There are signs stating "Don't Move a

Mussel Clean, Drain and Dry Your Equipment" and "Stop Aquatic Hitchhikers" at each entrance to the park and at every launch ramp. Numerous newspaper articles and television interviews have been conducted on the serious problems quagga mussels have on the lake since the discovery of the quagga mussel in Lake Mead in January 2007.

The most popular destination for boaters after launching in Lake Mead was Lake Mohave (n=29). Lake Havasu was second (n=25) and Lake Powell in Utah was third (n=8) according to the boater surveys. In Appendix II, all future destination replies are listed. Lakes Mohave and Havasu already have quagga mussel infestations, but Lake Powell does not. Officials at Lake Powell should be ready for an influx of boaters coming from Lake Mead and be prepared to inspect boats for aquatic invasive species to protect Lake Powell. Based on the trailer counts, most boaters are coming to Lake Mead from Nevada, California, Utah and Arizona, all of which currently contain bodies of water infested with quagga mussels. These bodies of water were not infested at the beginning of this study. It is possible that boaters from Lake Mead went back to their home state and infected untainted waters.

Increased boater awareness will help prevent the spread of aquatic invasive species and the 100th Meridian Initiative is an excellent way to not only educate boaters, but also to collect relevant data on future mussel invasions. The results from this study will assist lake managers and operators in deciding the course of preventative action they need to take to defend their lake against invaders. Due to 19% of people interviewed not aware of zebra or quagga mussels, this education/research initiative should be continued to ensure the protection of other lakes from the damages of aquatic invasive species. The study conducted by Britton and McMahon (2005) identified "high-risk" bodies of water

for mussel invasion, informed lake officials, and helped to prepare lakes for invasion. This preparation saved the park money and raised invasive species awareness. The preservation of natural waters is vital for the conservation of native species and the prevention of quagga mussel invasions will assist in this preservation.

CHAPTER 6

CONCLUSIONS

Since the discovery of the quagga mussels in Lake Mead on January 6, 2007, researchers all over the world are looking to this region for advice on how to quell the ecological and economic impacts of the mussel. These projects attempted to evaluate some of the impacts the quagga mussel will have on the lake ecosystem. Quagga mussels will grow at high densities on all of the substrates tested from depths between 6 and 30 m. All equipment, machinery and vehicles in the lake at these depths that do not have an antifoulant coating have the potential to become colonized by quagga mussels. This may cause thousands of dollars in damage, maintenance and repairs.

The mercury study in quagga mussels demonstrated that, although mercury concentrations were low compared to other water bodies in North America, there is potential for bioaccumulation up the food chain in Lake Mead. There may be potential in using quagga mussels as a biomonitoring species. Mussels are known to accumulate contaminants and are abundant in the lake therefore determining contaminant concentrations in their tissues can reflect the overall health of the ecosystem. Future studies may use this baseline data to discover if contaminant concentrations in mussel tissue increase or decrease over time. Mussels were collected anywhere from eight months to almost two years after the discovery of the mussel in the lower Colorado River. The pseudofeces and feces that mussels produce as waste often contain contaminants

(Klerks et al. 1997). After a few more years of mussel colonization in Lake Mead, there may be increased levels of contaminants across all trophic levels of the Lake due to the build up of pseudofeces in the benthic regions. Monitoring contaminants should continue in the future, not only on quagga mussels, but on other trophic levels such as fish and diving ducks.

Finally, the boater surveys provided valuable information such as future destinations of boaters after leaving Lake Mead and the level of awareness of quagga mussels. Although the percentage of boaters aware of quagga mussels has increased significantly since 2003, it is still below desirable levels. To help prevent the spread of quagga mussels to non-infested waters, it is vital to educate boaters on the importance of cleaning their boat properly after leaving Lake Mead, or other infested waters. The quagga mussel has become a permanent addition to Lake Mead. These projects will serve as a baseline for substrate and contaminant research that should continue in order to understand and predict changes in the ecosystem caused by quagga mussels.

APPENDIX I

PROTOCOLS

BOR Veliger Sampling Protocol

The Lower Colorado Region Bureau of Reclamation Fisheries group is currently conducting monthly sampling for Quagga mussel veligers *Dreissena burgensis* on Lake Mohave. Samples are being collected following guidelines put forth by Kevin Kelly and Fred Nibling of the Bureau of Reclamation Technical Service Center in Denver, Colorado. Credit for this protocol should go to them as it is an adaptation of their original work. In addition to collecting water samples for analysis, water quality data is also being recorded. The following summarizes equipment needs as well as sampling, storage, and shipping methods.

Equipment

- 64 μm Plankton Tow Net (15 cm diameter opening)
- Water Quality probe (In-Situ Troll 9500 for recording date, time, Lat/Long, UTM and measuring temp, SpC, DO, pH, depth, turbidity, and TDS)
- 1 L spray bottle
- (4) Sample bottles (500 mL Nalgene HDPE bottles)
- Ethyl Alcohol (200 proof, preservative)
- Disposable diapers
- Plastic electrical tape

- 1 gallon Ziploc bags
- Waterproof markers
- Data sheet on waterproof paper
- Ice chest with ice
- 2 gal. white vinegar (5% acetic acid, for plankton net decontamination)
- (2) 5 gal. buckets (one used as a decontamination container, one for WQ probe)
- Secchi disk (10.5 in.)
- 26 in. Aquavue scope (for use with the Secchi disk)

Sample Collection

In order to obtain the minimum sample volume of 1000 L for analysis, plankton nets are lowered and towed for a total of 60 meters. In actuality none of our four sites are 60m deep, so we instead use multiple tows at the same location until the plankton net has passed through 60 total meters. As an example, the max depth at the Katherine Landing site is 31-33m so we simply do two 30m tows to obtain our 60m sample. With the exception of Willow Beach Marina, all plankton net tows are vertical. At Willow Beach the current is too strong to allow for vertical tows so horizontal tows are taken. This is achieved by anchoring the boat, determining the flow rate (m/s), and holding the plankton net stationary below the surface for the appropriate duration.

After each tow a 1 L spay bottle is used to wash the net top to bottom from the outside to rinse veligers into the collection cup. The collection cup side screens are also washed top to bottom and then emptied into a 500mL Nalgene bottle. The collection cup is rinsed twice more with small amounts of water and emptied into the same 500mL bottle. Sample bottles are marked at the 375 mL line prior to each trip using a waterproof

marker. This line is labeled level 1. By marking them before each trip we can ensure our samples are near the desire volume of 375 mL. The bottle is also labeled with the date, location, and sample depth. Sample bottles are kept on ice while in the field and then refrigerated until they are shipped.

Once sampling at any site is complete the plankton net must be decontaminated before it can be used at the next site. The treatment recommended by Kelly and Nibling is to rinse the net with clean water to remove any remaining veligers and then completely immerse the net in white vinegar. We use two gallons of white vinegar in a five gallon bucket for decontamination. The plankton net is soaked for approximately 45 minutes between samples and the same vinegar bath is used following all samples. Plankton nets are thoroughly rinsed with clean water after each soaking and before collecting the next sample.

Water quality data is also being recorded at each sample site. Current parameters include temp (C°), depth (m), pH, SpC (µs/cm), DO (mg/L), turbidity (NTU), and TDS (mg/L). Secchi disk depth readings (with and without Aquavue scope) are also being taken at each site and are recorded in meters. For Secchi readings, the disk is lowered in the water until it is not visible by the naked eye and then it is slowly brought up to where it can be seen. This process is repeated in the same manner using the Aquavue scope. Other data taken at each site includes date, time, location name, air temp, wind speed/direction, and GPS coordinates (we are currently reporting data using both Lat/Long and UTM).

Storage and Shipping

Once sample bottles are back in our office they are taken out of the cooler and preserved using ethyl alcohol (200 proof). The ethyl alcohol is added until it is 25% of the final sample volume. After the alcohol has been added, the sample level on the bottle is marked with a short line and labeled level after alcohol. Samples are refrigerated until they are ready to be analyzed.

Protocol courtesy of Jim Stolberg, Bureau of Reclamation, Boulder City, NV.

BOR Protocol for Analyzing Plankton Tows, Pumped Samples, and Shallow Water Samples for *Dreissena* spp. Veliger Density

Scope and Application

This is a Reclamation method that was developed using the Standard Method 10200 G Zooplankton Counting Techniques, Standard Operating Procedure for Zooplankton Analysis and the US. Army Corps of Engineers (USACE) method for calculating *Dreissena* spp. veliger densities in water samples collected with a 63 μm plankton net.

Summary of Method

To avoid transporting live veligers in the sample, preserve each sample with 25% ethanol while in the field. Record the total volume of the sample (tow volume) and the volume of ethanol added to the concentrated sample. In the laboratory, the sample is added to an Imhoff settling cone with a venoset delivery system. The veligers are allowed to settle in the Imhoff cone for a minimum of 24 hours. Veligers are identified at the laboratory using cross-polarized light microscopy where they appear as a distinctive, bright "iron cross" among the other, darker planktonic material. Enumeration of veligers is performed with a Sedgwick-Rafter counting cell. The Sedgwick-Rafter counting cell chamber is divided lengthwise into three compartments and each compartment is counted separately, and then added together to determine the total number of veligers in 1mL of sample. Count five 1-mL aliquots from the same sample, record the number of veligers, and calculate the mean of the five counts. . When the veliger concentration is very high, samples may be split with a Folsom plankton splitter

or diluted with ultrapure deionized water (UPDI). It is possible to confuse veligers with Ostracods which also appear as a similar-shaped, bright "iron cross." However, ostracods are kidney bean-shaped, and veligers are either round or D-shaped. Recount the cell to verify the veliger count.

Apparatus and Reagents

Dissecting microscope (10x-50x magnification) with cross polarized light filters

1-mL syringes or pipettes

Imhoff Cones set into a ringstand, with a venoset apparatus attached to the bottom

Sedgwick-Rafter counting cell (cover glass optional)

Small sieves with 45-μm mesh

50- and 500-mL beakers

15 mL Calibrated test tubes

UPDI

Isopropyl or ethyl alcohol

5% acetic acid solution

Analytical Procedure and Enumeration

1. All samples should be kept on ice or refrigerated from the time of collection. Record the total volume of the tow or the total volume of the watered filtered through the net into the sample cup (total volume sampled). Record the volume of ethanol that was added to preserve the sample or mark the levels on the sample bottle so that the discrete volumes can be recorded back in the lab.
2. Shake sample well and immediately pour into Imhoff cone with the venoset attachment. If the sample contains a large amount of debris, filter through a net as

you pour the sample into the cone. Rinse the net contents thoroughly into cone with a wash bottle containing distilled water.

3. Allow to settle in the Imhoff cone for at least twenty four hours and up to 48 hours to allow veligers to settle.
4. Collect the first 15 mLs in a calibrated tube cover with parafilm and number it 1, collect the second 15 mls in a calibrated tube and cover with parafilm and number it 2. If there is still sediment remaining, continue collecting 15mLs at a time and number the tubes as they come off the cone. Note: the venoset may become clogged if the larger debris is not removed. If the smaller debris gets clogged, the flow is easily recovered by moving the clamp and squeezing the tube to move the constricting materials.

   Note: Generally it will not be necessary to examine the second 15 mLs under the microscope. However, the second collection may be used to verify that all of the veligers were collected in the first 15 mLs.
5. Pipette a 1-mL aliquot from a well-mixed sample and dispense into a Sedgwick-Rafter counting cell. If desired, a cover glass may be used.
6. Place the filled Sedgwick-Rafter cell under a dissecting microscope using cross polarized light. Examination of the counting cell is simplified by counting the cells by each compartment. Split or dilute the sample as needed to maintain a single layer of organisms, taking care to record dilutions or concentrations and factor them into the final count.

7. If needed, a drop of detergent in the Sedgwick-Rater cell will sink the microorganisms and reduce motion; however, veligers will sink fairly rapidly on their own.
8. Examine the contents of the cell and record the number of veligers present.
9. Repeat with same sample, using 1 mL aliquot for five counts, taking care to shake the sample container to keep the sample well mixed and the veligers suspended.
10. The mean of the five rafter cell counts is used to obtain the mean number of veligers per milliter in the sample.
11. The final concentration is then: $\frac{C \times V'}{V'' \times V'''}$

Where C= average number of veligers counted per mL

V′ is the volume of the concentrated sample (15 mLs)

V″ is the volume counted (Since this is an average of 5 - 1mL counts, it is 1mL)

V‴ is the volume of the total sample or plankton tow in L

QA/QC

If desired, the standard deviation may also be calculated to determine the frequency distribution and significant differences in the data. It is expected that the counts should not differ by greater then 10%, or all counts should be within 90% of the mean. If they do not, the reasons for the discrepancies should be evaluated and discussed in the data report.

To prevent cross contamination, all lab equipment and tools must be well cleaned. Utilizing a vinegar bath soak for a minimum of one hour to dissolve the veliger shells and prevent cross-contamination of samples. When possible Reclamation uses two sets of equipment, one for water bodies where zebra mussels have not been detected, and one for water bodies where zebra mussels have been detected.

Protocol courtesy of Denise Hosler, Bureau of Reclamation, Denver, CO.

Data Sheet for Quagga Mussel Veliger Enumeration

QUAGGA MUSSEL VELIGER COLLECTION AND BENCH SHEET

Entity: _____

Location: _____ Submission ID: _____

Date Started: _____ Date Finished: _____

Time Started: _____ Time Finished: _____

Collected by: _____ Collected by: _____

Meter Reading Start: _____ Meter Reading End: _____

PHYSICAL PARAMETERS:

Temperature: _____ °C Conductivity: _____ µS/cm

Dissolved Oxygen: _____ mg/L pH: _____ Units

Chlorine Free: _____ mg/L Chlorine Total: _____ mg/L

Turbidity: _____ NTU

ENUMERATION: Workgroup ID:

Total Volume Collected: _____ Gallons Total Volume Collected: _____ Liters

Total Concentrate Volume: _____ Sub-sample Volume: _____

Ethanol Volume Used: _____ Raw Water Concentrate Volume: _____

Volume Filtered: _____ Final Concentrate Volume: _____

Sample: _____ Counted By: _____ Date & Time: _____

Slide #	1	2	3	4	5	6	7	8	9	10	Mean #
Track 1:											
Track 2:											
Track 3:											
Track 4:											
Total:											

*Veliger Count per Liter: _____

Sample: _____ Counted By: _____ Date & Time: _____

Slide #	1	2	3	4	5	6	7	8	9	10	Mean #
Track 1:											
Track 2:											
Track 3:											
Track 4:											
Total:											

*Veliger Count per Liter: _____

Analyst Signature: _____ Date: _____

Supervisor Signature: _____ Date: _____

* Formula for Veliger Count Per Liter:
total mean # of veligers x (total concentrate volume/raw water concentrate volume) x (volume filtered/final concentrate volume) x raw water concentrate volume = total # of veligers/total volume collected in liters = veliger count per liter

Tissue Digestion Procedure for Cold Vapor Mercury Analysis

(Adapted from EPA Method 245.6)

1. Weigh approximately 1 gram of tissue into a clean vessel liner.
2. Add 4 mL trace metal grade nitric acid.
3. Add 4 mL deionized water.
4. Cap and seal liner within digestion vessel.
5. Place 16 digestion vessels in rotor including a reagent blank, a spiked sample, and a standard reference material.
6. Place rotor into the Anton-Parr Multiwave 3000 Microwave Reaction System.
7. Program the system for 5 minutes of increasing power until a temperature of 150 degrees C is reached.
8. Hold samples at this temperature for 25 minutes.
9. After a cooling time of 30 minutes, empty contents of each vessel into a clean, labeled, metal-free centrifuge tube.
10. Add 4 mL amidosulfonic acid to each tube.
11. Dilute samples to 15 mL with dI water.
12. Vortex samples to ensure homogeneity.

APPENDIX II

100th MERIDIAN SURVEY MATERIALS

Contact Survey

Zebra Mussel 

100TH MERIDIAN INITIATIVE TO PREVENT THE WESTWARD EXPANSION OF ZEBRA MUSSELS
Interview Form for Trailered Boat Survey

Interviewer: Last name	First name		
Date:	Time: AM / PM	Survey Type:	◻ Contact ◻ Observation
Water Body:	State:		
Launch Site:			

Where are you from?

Home State:	Zip Code:	Personal ◻ **Type of Transport** Commercial ◻ Other ◻ explain
How many times have you launched in the last year?		
Do you always launch in the same water body? Yes ◻		
Type of Boat: ◻ Angling ◻ Pleasure ◻ Jet Ski ◻ Canoe ◻ Other		explain

Where else have you launched recently?

Water Body:	State:	County:	Date:
1.			
2.			
3.			

Where will you launch next?

Water Body:	State:	County:	Date:
1.			
2.			

Do you clean your boat and trailer between launchings?	◻ Yes	◻ No If so, how?
Is you boat kept on land or in water when not in use?	◻ On Land	◻ In Water
If in water, where is it kept? Water body:	For how long?	State:

Do you know the approved method to clean your vessel?

Information Exchange: ◻ Viewed? ◻ Read? ◻ Both? ◻ Boater asked questions

Boater already aware of threats of... ◻ Zebra Mussels ◻ Any ANS

Boat Inspection Results: Inspection ◻ Rejected

Nothing Found: ◻ Undertaken by: ◻ Party ◻ Interviewer ◻ Both

	Zebra Mussels	Still Alive	Vege-tation	Other Exotics	Describe Other	Action Taken
Boat Deck	◻	◻	◻	◻		
Boat Hull	◻	◻	◻	◻		
Bilge & Bait Wells	◻	◻	◻	◻		
Motor	◻	◻	◻	◻		
Trailer	◻	◻	◻	◻		
Fishing Equipment	◻	◻	◻	◻		
Other	◻	◻	◻	◻		

Comments:

Mail-in Self Survey

LOCATION _____ STATE _____ DATE _____

The Zebra Mussel

100TH MERIDIAN INITIATIVE TO PREVENT THE WESTWARD EXPANSION OF ZEBRA MUSSELS
BOATER SELF-SURVEY

The 100th Meridian Initiative is a multi-agency partnership effort to prevent the westward spread of zebra mussels and other aquatic nuisance species to western North American waters. The U.S. Fish & Wildlife Service is sponsoring and coordinating education outreach and voluntary trailered boat surveys with other agencies in the states on the 100th meridian. Surveys similar to this are being conducted in Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota and the Canadian Province of Manitoba. This survey is now being extended to the Colorado River. You as a boater are being asked to voluntarily inspect your trailer, boat and related equipment for any transported aquatic species, such as the **zebra mussel**, which may be carried accidentally to new locations. Your assistance and participation is appreciated in completing this survey and *returning it in the provided, stamped envelope to the agency that is conducting this survey for the U.S. Fish* and Wildlife Service. Please review the enclosed information on introduced aquatic species and boat and trailer inspections. Be sure to clean your boat, trailer and equipment after hauling-out the boat and before leaving the ramp area. Thanks for your help!

The following instructions will help you complete the survey.

Part One – Where are you from? (Any information provided is voluntary and anonymous.)
Please state the purpose of your visit, and fill in the boxes relating to your boat and home state. Your most recent launches are very important information, so please be as complete as possible.

Part Two – Where are you going?
Please indicate where you will be launching next **after you leave this lake**. Do not list further launchings at this lake. Again, please be as complete as possible in filling out this section.

Part Three – Returning the survey.
That's all there is to it! All you need to do is place this page in the provided, stamped, return envelope, seal it, and drop it in the mail.

SURVEY INFORMATION (Please Print)

PART ONE: Where are you from?	Home State:	Zip Code:

Type of Boat:	☐ Angling	☐ Pleasure	☐ Jet Ski	☐ Canoe	☐ Other	explain

How many times have you launched in the last year?

Do you always launch in the same water body? ☐ Yes ☐ No
If no, please list below where else you have launched recently.

Water Body:	State:	County:	Date:
1.			
2.			
3.			

PART TWO: Where are you going? Please list below where you plan to launch next.

Water Body:	State:	County:	Date:
1.			
2.			

Are you already aware of threats of zebra mussels? ☐ Yes ☐ No
Or any other aquatic nuisance species? ☐ Yes ☐ No

Do you clean your boat and trailer between launchings? ☐ Yes ☐ No

Is you boat kept on land or in water when not in use? ☐ On Land ☐ In Water
If in water, where is it kept? Water body: State:

Any Comments:

Trailer Count Form

Zebra Mussel

THE 100TH MERIDIAN INITIATIVE TO PREVENT THE WESTWARD SPREAD OF ZEBRA MUSSEL
TRAILER COUNTS FOR LAUNCH AREAS & RELATED FACILITIES

Surveyor: last	first	Date:	Time:	am / pm
Location:		State:		

LIST STATES AND NUMBERS OF TRAILERS COUNTED

	(Your State)	States and Numbers of Trailers								
SITE										
TOTALS (by state):										

TOTAL (All):		Your State	TOTAL (from your state)
TOTAL (Out of State):		Percent Out of State:	
Self-Interview Forms Distributed:			

Organisms:

Nothing Found: ☐ Zebra Mussels: ☐ States of Origin: _____
Vegetation: ☐ States of Origin: _____
Other: ☐ States of Origin: _____

If other is checked indicate types of organisms found: _____
_____

100th Meridian Zap the Zebra Brochure Front Side

100th Meridian Zap the Zebra Brochure- Back Side

Invasive Mussels: Expensive Damage

When zebra or quagga mussels invade our local waters, they clog power-plant and public-water intakes and pipes. Routine treatment is necessary and very expensive; this leads to increased utility bills. If you use water and electricity, then you do not want zebra/quagga mussels!

Zebra/Quagga Mussels May Use Your Boat to Invade Additional Waters

If your boat has been in infested waters, it could be carrying invasive mussels. These creatures usually spread to new habitats on boats trailered by the public or by commercial haulers. Zebra and quagga mussels attach to almost anything: boats, aquatic plants, bait buckets, and other aquatic recreational equipment. You could unintentionally transport microscopic mussel larvae in water in your live well or bilge. An adult female zebra mussel can release up to 1,000,000 eggs in a lifetime. Please take the precautions outlined in this brochure to reduce the chance that zebra or quagga mussels will spread to uninfested areas.

Boater Destinations After Leaving Lake Mead

Water Body	State	Number of people
Lake Mohave	AZ	29
Lake Havasu	AZ	25
Lake Powell	UT	8
Clear Lake	CA	2
Lake Alamo	AZ	2
Lake Piru	CA	1
Catalina Island	CA	1
Lake Pleasant	AZ	1
San Vincinta	CA	1
Pangwich	UT	1
Apache Lake	AZ	1
San Halo	UT	1
San Dimas	CA	1
Isabel	CA	1
Minorsville	UT	1
Pittsburg	CA	1
Flaming Gorge	UT	1
Buffalo Bill Reservoir	WY	1
Baja	CA	1
Long Beach	CA	1
Big Bear	CA	1
Puget Sound	WA	1

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