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Evaluation of the Tagelus® TA 100D Sand Filter for Removing Quagga Mussel Veligers (Dreissena rostriformis bugensis) From Lake Water and the Effectiveness of the Safeguard Ultraviolet Radiation System as a Biocide Against Veligers

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EVALUATION OF THE TAGELUS® TA 100D SAND FILTER FOR REMOVING QUAGGA MUSSEL VELIGERS (*DREISSENA ROSTRIFORMIS BUGENSIS)* FROM LAKE WATER AND THE EFFECTIVENESS OF THE SAFEGUARD ULTRAVIOLET RADIATION SYSTEM AS A BIOCIDE AGAINST VELIGERS

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Abstract

Evaluation of the Tagelus ®TA 100D sand filter for removing quagga mussel veligers (*Dreissena rostriforms bugensis)* from lake water and the effectiveness of the SafeGUARD ultraviolet radiation system as a biocide against veligers

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The Lake Mead National Recreational Area was created by the construction of the Hoover Dam during the years 1931-1936. In January 2007, the quagga mussel (*Dreissena rostriformis bugensis*), was found in Lake Mead. This became the first known *Dreissenid* species in the southwest and the only time a large water system was first infested by the quagga mussel and not the zebra mussel (*Dreissena polymorpha).* This invasive species has quickly spread to Lake Mohave and further down the lower Colorado River drainage. The microscopic size (70 µm or larger) of the veliger life stage makes it impossible to see with the unaided eye and difficult to remove from water delivery pipes and fish stocking trucks. This invasive mussel has affected the stocking abilities of the United States Bureau of Reclamation Lower Colorado River Multi-Species Conservation Program Fish Augmentation Plan. One purpose of this study is to determine if quagga veligers can be completely removed from lake water by a combination of sand, zeolite, and paper filtration. Results for the filtration experiment show that the relative risk of transferring quagga mussels to Willow Beach National Fish

Hatchery during a night of larval collections is low. Filtered lake water provides a significant reduction of veligers present in the water compared to the unfiltered lake water ($p=.009$). The other purpose of this study is to determine if exposure to different doses of ultraviolet radiation can damage or kill veligers. The UV exposure doses were 1, 3, 6, and 12 times through the SafeGUARD UV system. After exposure, 50 veligers were observed at time 0, 24, 48, 72, and 96 hours. Results from the UV study show that at an exposure of 12 times through UV at an observation time of 96 hours there was 100% mortality of veligers observed. It also shows that there is a significant difference in mortality of veligers between cycle 1 and multiple cycles ($p< 0.05$) while there is no statistical difference between cycles 3, 6, and 12 ($p> 0.05$). 3:6 ($p=.5322$), 3:12 ($p=.5071$), or 6:12 ($p=.9688$).

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CHAPTER 1

INTRODUCTION

The Lake Mead National Recreational Area (LMNRA) was created by the construction of the Hoover Dam during the years 1931-1936. Located 35 miles outside of Las Vegas, Nevada, it covers about 1.5 million acres and includes Lake Mead and Lake Mohave. This recreational area is important for the development of the southwest, supplying drinking water to the Las Vegas area, electricity to the southwest, recreational activities for visitors, and water irrigation to farmlands (Holdren & Turner, 2010). Lake Mead extends from Glen Canyon Dam to Hoover Dam and is the largest reservoir by volume (3.5×10^{10} m^3) in the United States (LaBounty & Burns, 2005). Lake Mohave, smaller than Lake Mead, was created in 1951 following the completion of Davis Dam near Laughlin, Nevada (NPS, 2010). Lake Mohave begins at the Hoover Dam following the original river channel approximately 67 miles to Davis Dam. It covers approximately 30,000 surface acres and has a maximum depth of 120 feet (NPS, 2010).

In January 2007, the quagga mussel (*Dreissena rostriformis bugensis*), was found in Lake Mead. This became the first known *Dreissenid* species in the southwest and the only time a large system was first infested by the quagga mussel and not the zebra mussel (*Dreissena polymorpha)* (Gerstenberger, Mueting & Wong, 2011a). This invasive species has quickly spread to Lake Mohave and further down the lower Colorado River drainage. LaBounty and Roefer (2007) state that the zebra/quagga mussel has become the most serious non-native biofouling pest introduced into North American freshwater systems. In a short amount of time, this species has caused severe economic, ecological,

and human health impacts to the southwest. *Dreissenid* mussels are very efficient filter feeders that are capable of filtering large volumes of water in a very short amount of time (Karatayev, Burlakova & Padilla, 1997). Through filtering the water, they have the ability to reduce the biomass and change the structure of phytoplankton and zooplankton communities (Wong, Gerstenberger, Miller, Palmer & Moore, 2011). This increases the water clarity and reduces the amount of suspended solids and oxygen in the water column, allowing aquatic plants to grow more rapidly (Wong et al., 2011). *Dreissenid* mussels have a rapid filtration rate, a planktonic veliger stage, high fecundity, and the ability to attach easily to surfaces, which has allowed them to spread easily throughout North America (Gerstenberger et al., 2011a; Hebert, Muncaster & Mackie, 1989; Wong et al., 2011). These mussels have the ability to attach to surfaces using their strong byssal threads, allowing them to clog water pipes, damage boat motors, and destroy recreational equipment. The Metropolitan Water District of Southern California is spending $10-15 million a year to deal with quagga mussel damage caused to the 390 km Colorado River aqueduct and reservoir system (Fonseca, 2009; Gerstenberger et al., 2011a). It is estimated that one billion dollars are spent annually in the Great Lakes region and throughout other areas of North America to monitor and control *Dreissenid* populations (Pimentel, Zuniga & Morrison, 2005; Wong et al., 2011).

The microscopic size (70 μm or larger) of the veliger life stage makes it impossible to see with the unaided eye and difficult to remove from water delivery pipes and fish stocking trucks. This invasive mussel has affected the stocking abilities of the United States Bureau of Reclamation (Reclamation) Lower Colorado River Multi-Species

Conservation Program (LCR MSCP) Fish Augmentation Plan. The LCR MSCP is a multi-stakeholder Federal and non-Federal partnership, responding to the need to balance the use of the LCR water resources and the conservation of native species and their habitats in compliance with the Endangered Species Act (ESA) (LCR MSCP, 2006). The MSCP is a 50-year plan to conserve at least 26 species along the LCR from Lake Mead to the Southerly International Boundary with Mexico through the implementation of the Habitat Conservation Plan (HCP) (LCR MSCP, 2006). Most of the species covered by the MSCP are State and/or Federally-listed as special status species meaning they are rare, threatened, or endangered and require special consideration and/or protection. Reclamation is entirely responsible for implementing the LCR MSCP over the 50-year life of the program (LCR MSCP, 2006). The fish augmentation plan requires the stocking of 660,000 native, endangered razorback sucker (*Xyrauchen texanus)* and 620,000 native, endangered bonytail (*Gila elegans*) into the LCR and its connective channels (LCR MSCP, 2004; LCR MSCP 2006). Of these numbers, the LCR MSCP is committed to stock at least 270,000 razorback sucker and 200,000 bonytail into reach four (Parker Dam to USBR Cibola Gage) and reach five (USBR Cibola Gage to Imperial Dam) (Figure 1) (CDFG, 2005).

The razorback sucker is endemic to the Colorado River drainage. One of the four main-stem big river fishes found within the Colorado River basin, it was Federally-listed as endangered by the US Fish and Wildlife Service on October 23, 1991 (http://ecos.fws.gov/speciesprofile). Historically, population abundance in Lake Mohave was estimated to exceed 100,000 fish but, the population has declined over the years to around 44,000 in 1991, to fewer than 3,000 in 2001, to a current population of 2,577 in

2012 (Marsh, Pacey & Kesner, 2003; Pacey, written com., 2012). Collections of wild-born razorback sucker larvae on Lake Mohave began in 1994 to help rebuild and maintain a genetically diverse adult population (LCR MSCP, 2010). To meet the goals of the fish augmentation plan, wild larvae are reared in captivity at Willow Beach National Fish Hatchery (WBNFH) and Bubbling Ponds Hatchery (BPH) and are eventually repatriated back into the system (LCR MSCP, 2010). Bubbling Ponds Hatchery (Page Springs, AZ) is supplied from a freshwater spring that is not infested with quagga mussels. Reclamation is no longer allowed to supply larval fish to BPH because there is no way to insure the delivery water or larval fish are veliger free. The hatchery now receives larval fish from razorback sucker brood stock held at Dexter National Fish Hatchery and Technology Center (DNFHTC) (Dexter, NM), which has increased the production and labor costs to Reclamation.

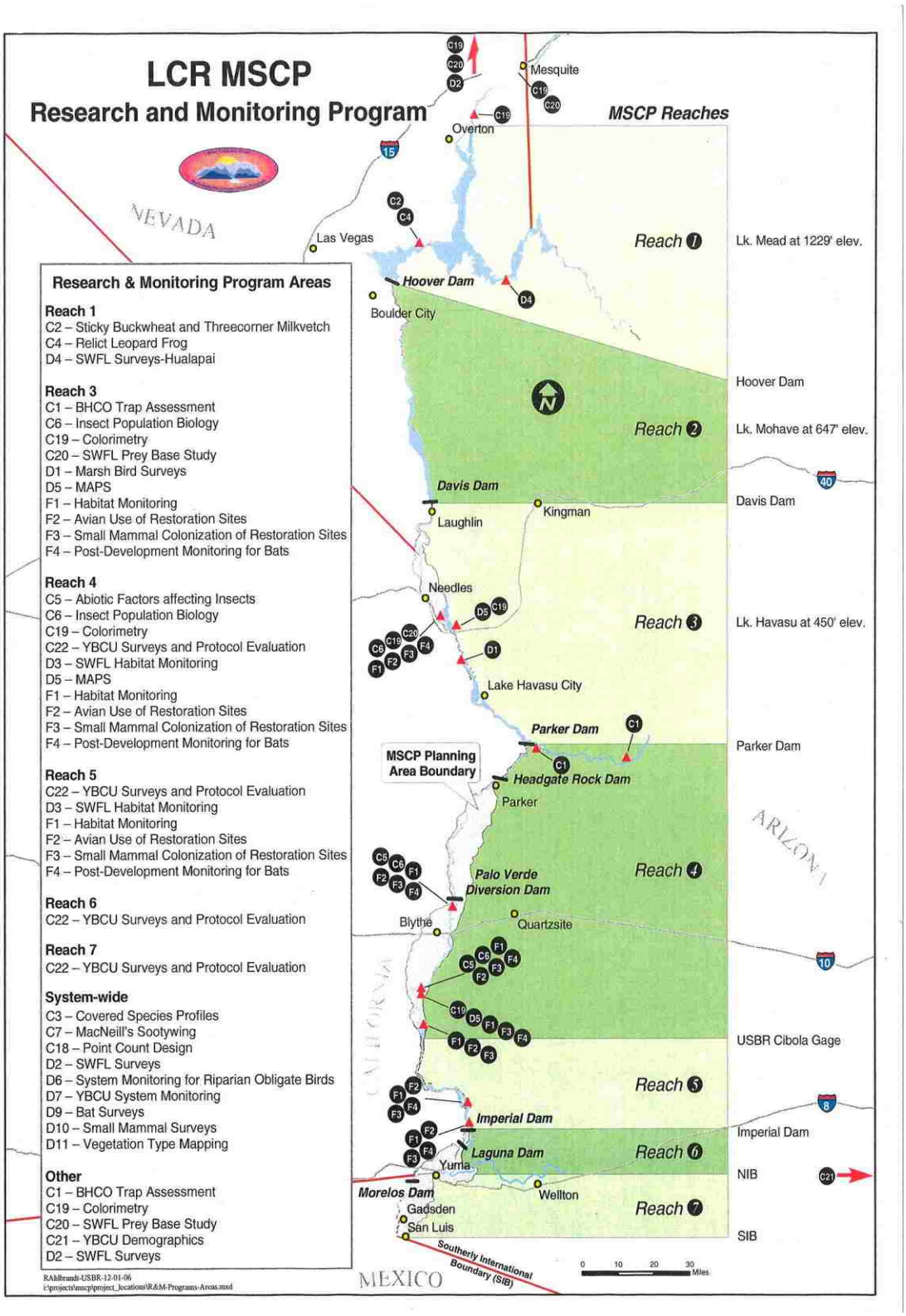


Figure 1. Map of the reach divisions of the Lower Colorado River Multi-Species Conservation Program: Research and Monitoring Program. Map created by Ray Ahlbrandt 12/01/06

Purpose of the Study

The purpose of this study is to determine if quagga veligers can be completely removed via filtration and the biocidal effectiveness of exposure of veligers to UV radiation in a water system. First, by examining the transfer rate of veligers onto a 6“ nylon aquarium dip net used for razorback sucker larval collections, estimates of the number of veligers present in a bucket that is transported to WBNFH can be made. From these estimates, the potential number of veligers per 10 gal aquarium can be used by hatchery staff to evaluate the effectiveness of veliger removal methods. This study will also examine the ability of a common pool filtration system, the Tagelus® TA 100D sand and zeolite filter (Pentair, Inc. Minneapolis, MN) and Big Bubba® paper filter (Watts Water Technologies, Inc., North Andover, MA), at removing veliger mussels from lake water. The results would give WBNFH staff a better idea of the amount of time water in a stocking truck would need to be cycled through the filtration system to remove any veligers present. The final purpose of the study is to determine if the SafeGUARD UV radiation system (Emperor Aquatics, Inc., Pottstown, PA) can damage or kill veligers in a recirculating water system. This would allow the veliger removal process to begin at the initial fish rearing stage and reduce the potential number of veligers present on a fish stocking truck. The results of this study will be used as a baseline for Reclamation along with other state and Federal agencies, to determine if Lake Mohave razorback suckers can be transported and stocked from areas where quagga mussels are present to areas that currently are not infested with the invasive quagga mussel.

Research questions

- To determine the transfer rate of quagga mussel veligers (*Dreissena rostriformis bugensis)* from Lake Mohave to a 6" nylon aquarium dip net (Blue Ribbon Pet Products©, Commack, NY).
- To determine if the Tagelus® TA 100D sand and zeolite filter (Pentair Inc., Minneapolis, MN) along with the Big Bubba® paper filter (Watts Water Technologies, Inc., North Andover, MA) can produce quagga mussel veliger (*Dreissena rostriformis bugensis)* free water.
- To determine the number of cycles water needs to pass through the SafeGUARD ultraviolent radiation system (Emperor Aquatics, Inc., Pottstown, PA) to damage or kill quagga mussel veligers (*Dreissena rostriformis bugensis).*

Significance of the study

The outcome of this study will be used to determine if razorback sucker stockings can be resumed by WBNFH into areas where quagga mussels currently are not present. If the filtration system removes veligers from the water, then BPH can receive fish from WBNFH. This would help maintain the genetic diversity of Lake Mohave razorback suckers, along with reducing some of the labor and production cost associated with producing, growing, and transporting fish from Dexter, NM. Because few studies have been conducted on the ability of UV radiation to damage or kill adult *Dreissenid* mussels (Chalker-Scott, Scott, Carnevale & Smith, 1994; Chalk-Scott, Scalia & Titus 1994; Seaver, Ferguson, Gehrmann & Misamore, 2009) this study will fill gaps in

the research pertaining to quagga veliger mussels. It will also give hatchery staff a better understanding of the number of cycles water needs to be exposed to UV radiation to kill veligers.

CHAPTER 2

REVIEW OF THE RELATED LITERATURE

Dreissenid mussel biology

Spread of *Dreissenid* Mussels

The zebra mussel (*Dreissena polymorpha),* was first discovered and identified in the Ural River in 1771 by the Russian naturalist Peter Pallas (Ludyanskiy, McDonald, MacNeil, 1993). The zebra mussel is endemic to the Black, Caspian, and Azov Seas. The quagga mussel (*Dreissena rostriformis bugensis)* is indigenous to the Dnieper River in the Ukraine (Karatayev et al., 1997; Mills et al., 1996). During the 1800's, these mussels began to spread rapidly throughout Europe. This expansion in population is due to the free-swimming veliger larval life stage and to the high fecundity of females (>30,000 eggs/female) (Hebert et al., 1989). Studies suggest these invasive mussels spread by both natural processes and human transport (Strayer, 2009). These invasive species continued to spread to North America, most likely in the ballast water discharged from commercial vessels (Herbert et al., 1989). *Dreissenid* mussels were first detected on natural gas wellheads and well markers in the western and eastern basins of Lake Erie, Ontario, Canada between April and November 1986 (Carlton, 2008). This species continued to spread through the Laurentian Great Lakes in the United States and were first detected in Lake Michigan near East Chicago in May of 1988 (Carlton, 2008). Shipping canals from Lake Michigan that join the Des Plaines River in Illinois and continue to flow into the Mississippi River have caused this invasive species to spread

throughout the Mississippi River and other water systems on the east side of the 100th Meridian (100˚ W longitude) (Gerstenberger et al., 2011a) (Figure 2).

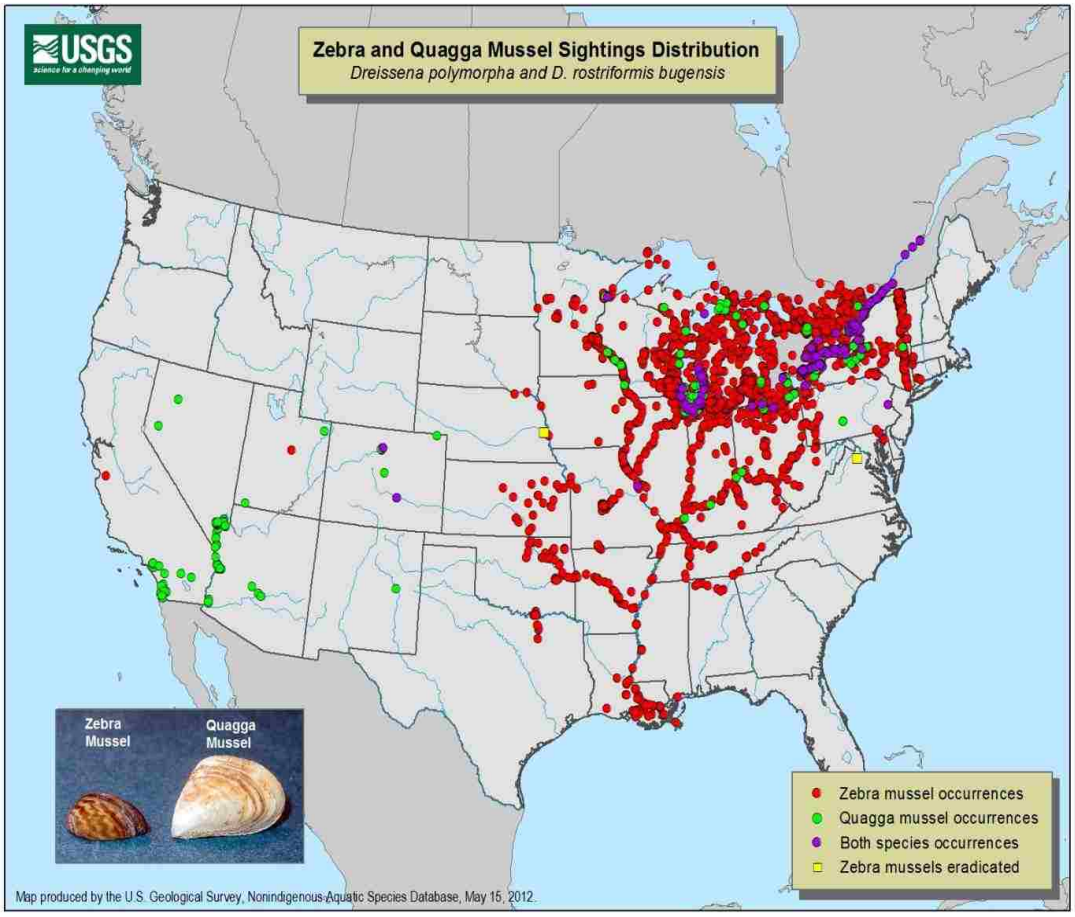


Figure 2. Zebra and Quagga mussel distribution in the United States as of May 2012. Image obtained from the US Geological Survey (USGS), Nonindigenous Aquatic Species (NAS) Database. Retrieved on June 19, 2012 from http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/maps/current_zm_quag_mp.jpg

Researchers suggested extreme ambient and water temperatures (both warm and cold) and low concentrations of calcium in the water (Strayer, 1991; Drake & Bossenbroek, 2004) would restrict the *Dreissenid* mussels range. Until recently, both species have only been detected in the Great Lakes region and the Mississippi River near

St. Louis (Missouri, USA) (Gerstenberger et al., 2011a). However, on January 6, 2007 this species was detected in the Boulder Basin of Lake Mead (Nevada, USA) (Gerstenberger et al., 2011a). Most likely, it was transported here by a visitor from the Great Lakes region, in the wheel wells of a boat trailer, the live well of the boat, or within the cooling system of the boat engine. This invasive species was able to establish itself rather easily in the lower Colorado River system because both lakes have high calcium concentrations and the average water temperatures for Lake Mead and Lake Mohave are 23^{o} C and 15^{o} C, respectively. Since the initial discovery in Lake Mead, Nevada, this species has expanded its range into California, Arizona, Utah, and Colorado river systems (Figure 3).

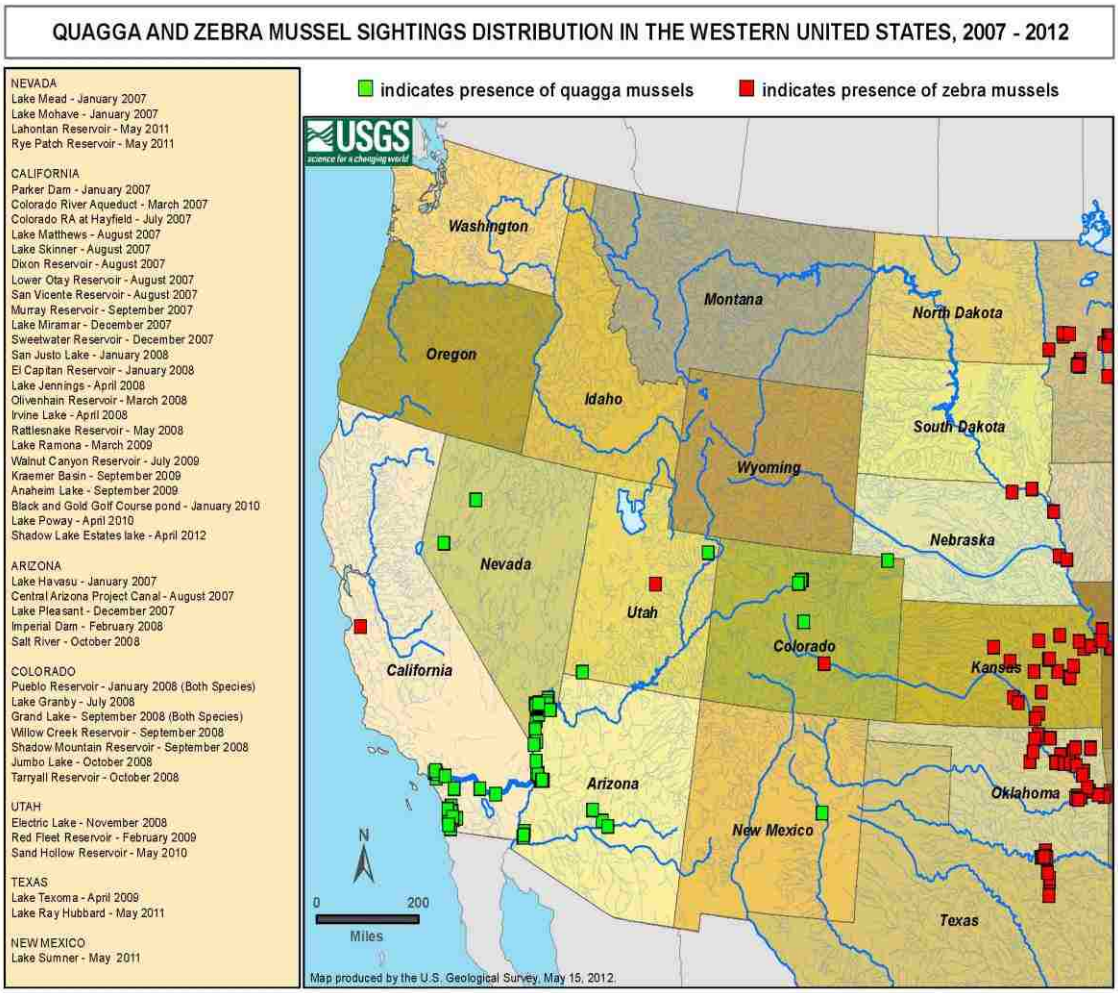


Figure 3. Quagga and Zebra mussel distribution in the Western United States, 2007-2012. Image obtained from the US Geological Survey (USGS), Nonindigenous Aquatic Species (NAS) Database. Retrieved on June 19, 2012 from http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/maps/southwest_quagga.pdf

Morphological differences between the species of *Dreissenid* mussels

The two *Dreissenid* species, the zebra mussel and quagga mussel, are morphologically and genetically distinct species (Mills et al., 1996). One way to distinguish between the two species is by the shape and size of their outer shell (Figure 4). The zebra mussel (*D. polymorpha*) has a flat or concave ventral margin with a pronounced carina, so the ventral edge of the shell is perpendicular to the lateral (Mills et al., 1996). This allows the zebra mussel to stay upright when placed on a flat surface

(Mills et al., 1996). In contrast, the quagga mussel (*D. rostriformis bugensis*) has a distinctive shell with a convex ventral margin (Rosenberg & Ludyanskiy, 1994; Mills et al., 1996). It does not have a carina between the ventral and lateral shell surfaces, so a cross-section of the shell looks round (Rosenberg & Ludyanskiy, 1994; Mills et al., 1996). Studies have found that natural populations of quagga mussels have longer shell lengths than the zebra mussel; this increases the longevity and growth rates for that species (Mills et al., 1996; Baldwin et al., 2002). Zebra mussels have evolved into a keeled shape that allows them to attach tightly to hard substrates using their byssal threads. Quagga mussels lack this shape and cannot attach as firmly, so they prefer a softer substrate (Mills et al., 1996). Zebra mussel shells are usually triangular and tend to have a uniform stripped pattern on their shell, whereas, quagga mussel shells are rounder in shape and do not have a uniform pattern on the outside of the shell. The quagga mussel shells also tend to be lighter in color and have finer line markings than zebra mussel shells.

Figure 4. The difference in zebra and quagga mussel shape and uniformity of pattern. Image obtained from the US Geological Survey (USGS), Nonindigenous Aquatic Species (NAS). Retrieved on June 19, 2012 from http://nas.er.usgs.gov/taxgroup/mollusks/images/zebra&quagga2.gif

Life cycle and reproduction behavior of quagga mussels

Quagga mussels settle, grow, and spawn over a larger temperature range and at greater depths than the zebra mussel (Baldwin et al., 2002). Baldwin et al. (2002), found by exposing these two species to the same laboratory conditions, the quagga mussels grow up to 19 times faster than zebra mussels. They also suggest that quagga mussels grow better than zebra mussels when food levels are naturally low or declining. From these results, Baldwin et al. (2002) concluded that quagga mussels can filter food and water at higher rates and for longer periods of time than zebra mussel can.

Quagga mussels have two distinct life stages: the first, a planktonic stage, is the free-swimming larval life form; the second, a benthic stage, occurs when the larvae

develop into adults and attach to substrates on the lake bottom (Ackerman, et al., 1994; Gerstenberger et al., 2011a). During reproduction, a mature egg (40-96 µm) and sperm (4-9 µm) perform external fertilization in the water column; the fertilized egg then divides by mitosis (Gerstenberger et al., 2011a). The quagga mussel life cycle consists of three life stages: larval veliger, juvenile, and adult stages (Figure 5) (Ackerman et al., 1994; Gerstenberger et al., 2011a).

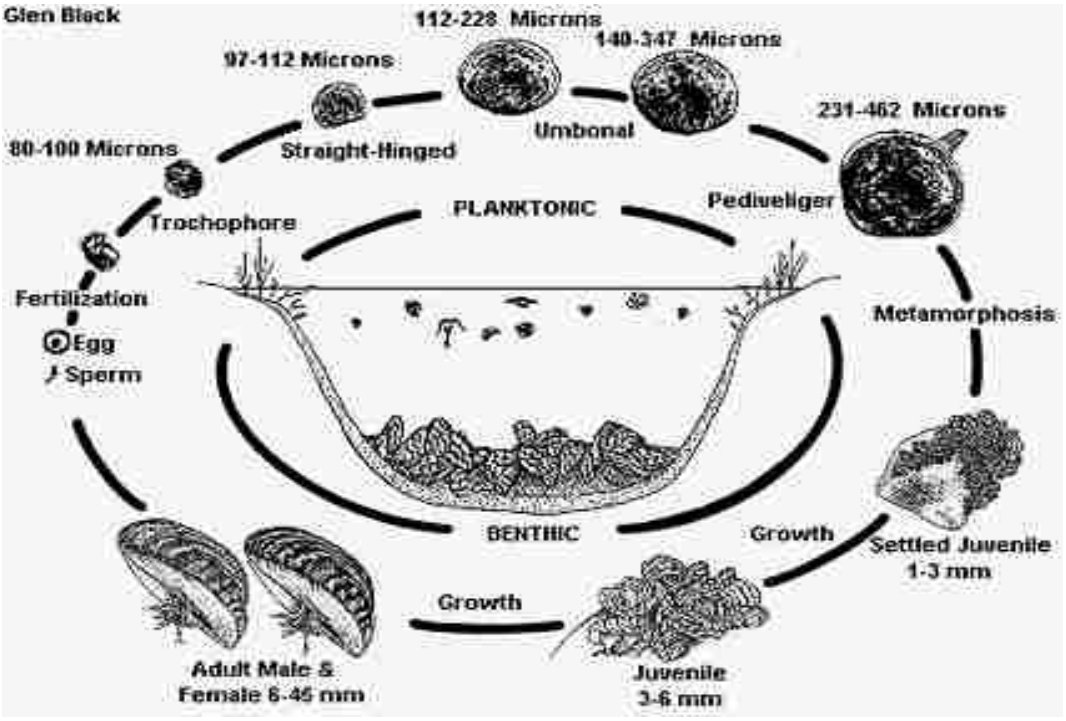


Figure 5. Life cycle of *Dreissenid* mussels. Image obtained from the US Army Corps of Engineers. Retrieved on June 20, 2012 from http://el.erdc.usace.army.mil/zebra/zmis/zmishelp4/life_cycle.htm

The four initial stages of larval development are trochophore (80-100 µm), straight-hinged veliger or D-shaped veliger (97-112 µm), umbonal or veliconcha veliger (112-347 µm), and pediveliger (231-462 µm). Distinctions among the four larval stages are important to determine the recruitment ability (Ackerman et al., 1994). Distinctions

should be based on the morphology of the shell shape and the presence of a foot, not on size because some of the larval stage sizes overlap one another (Ackerman et al., 1994). In the trochophore stage (80-100 µm) the velum, a ciliated feeding and swimming organelle, begins to develop therefore it is considered a veliger (Figure 6) (USACE, 2012).

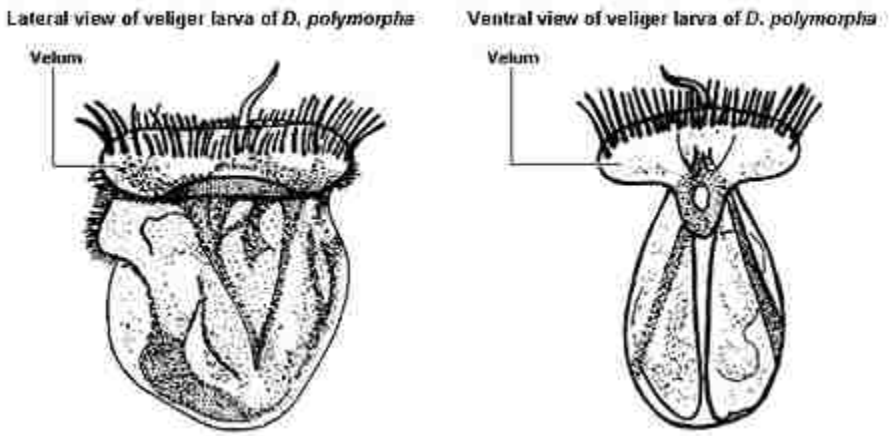


Figure 6. Lateral and ventral view of velum on *Dreissenid* veliger larvae. Obtained from US Army Corps of Engineers. Retrieved on June 20, 2012 from http://el.erdc.usace.army.mil/zebra/zmis/zmishelp4/veliger_stages.htm

About 2-9 days after fertilization, larvae are referred to as D-shaped or straight-hinged veligers (97-112 µm) because an unornamented D-shaped shell is exuded from the shell gland (Figure 7). On the side of the hinge, the shell becomes straight and the open valve side becomes rounded (http://www.usace.army.mil/zebra/zmis/zmishelp4/life_cycle). The next stage of development, umbonal veliger, usually occurs 7-9 days after fertilization and is the last larval stage that is completely planktonic (Figure 7) (http://www.usace.army.mil/zebra/zmis). At this time, the shell has a defined bump

(umbone) that covers the hinge and the shell shape appears more rounded in the profile (http://www.usace.army.mil/zebra/zmis). The final larval stage, the pediveliger, occurs 18-90 days after fertilization (http://www.usace.army.mil/zebra/zmis). The pediveliger uses its velum to swim, or the foot to crawl on to the surface of substrates. It receives a cue to attach its byssal threads and settles for further transformation (Figure 7) (Ackerman et al., 1994; http://www.usace.army.mil/zebra/zmis).

Figure 7. Images of straight-hinged, umbonal, and pediveliger larval stages for *Dreissenid* mussel larvae. Obtained from US Army Corps of Engineers. Retrieved on June 20, 2012 from http://el.erdc.usace.army.mil/zebra/zmis/zmishelp4/veliger_stages.htm

The time required for a fertilized gamete to become a developed juvenile is 8-240 days, depending on the temperature, food quality and quantity, and the available substrates (Nichols, 1996; Gerstenberger et al., 2011a). After the pediveliger stage, larvae descend to the lake bottom and transform into postveligers (juveniles) which begin to transition into the adult bivalve mussel (Herbert et al., 1989).

Filtration Systems

Types of filtration systems

Three different filtration systems are used to filter particles from water. Sand filters are the easiest to operate and require a minimal amount of maintenance. Water is pushed through a bed of filter material, usually #20 silica sand, which traps particles 20-100 µm and removes them from the water. To remove the lodged particles and prevent channeling, the system needs to be backflushed periodically. To backflush, water is pumped backward through the system to flush out the particles and to redistribute the sand; this avoids channels from forming within the sand. The cartridge is another economic and low maintenance filter that is typically used in swimming pools. Water passes through the filter material, which captures the debris. To remove the debris, the cartridge is removed and the debris is washed off using a hose. Cartridge filters are designed to run at a lower pressure than sand filters and do not need to be cleaned as often. Cartridges typically filter out material that is > 20 µm in size. Diatomaceous Earth (DE), the third type of filter, is more expensive and requires more maintenance than the other two filtration methods. The DE material is made up of fossilized exoskeletons of tiny diatoms that coat the filter housing and act as tiny sieves to remove debris. This material is very small, which allows it to filter material that is as small as 5 µm. To clean the debris from the system, the internal grid assembly must be removed and cleaned periodically. This type of filter runs at higher pressures than cartridge filters which can lead to some inefficiency and flow loss.

Filtration technology is an ideal, clean technology for a number of reasons. Unlike chlorine or other oxidants, filtration systems can provide chemical-free protection against quagga mussels. Filtration systems removing particles 40 µm in diameter have been effective at controlling zebra and quagga mussel populations (Lauria, 2009). The Gerald Andrus Station of the Mississippi Power and Light Company in Greenville, MS used a 40 µm self-cleaning screen filter; they found no viable life forms of *Dreissenid* mussels and the small proportion of eggs and veligers that made it through the filter were torn, compressed/deflated, or dead/dying (Lauria, 2009). In addition, these types of filtration systems require low filter maintenance. The sand filter use less than one percent of the water flow to backflush the system. The energy requirements for these systems are minimal because only a small motor is needed to pump the water through the filtration system. The benefit of the system to remove or damage veligers is far greater than the cost of the filtration system. Since no chemicals are added to the water, this system can be used in areas where there are sensitive species or concerned water users.

Design of the filtration system

Imperial Catfish Farm (Imperial, CA) designed and built a water filtration system to prevent the spread of quagga mussels during their channel catfish stocking activities. The design of the system uses all three types of filtration methods: sand, DE, and cartridge. The filtration system uses a Tagelus® TA 100D sand filter (Pentair Inc., Minneapolis, MN) that is comprised of #20 silica sand and zeolite; it can filter particles

down to sizes of 20-100 µm and 3 µm, respectively. In addition to the sand and DE, one to three Big Bubba® paper filters (Watts Water Technologies, Inc., North Andover, MA) can be added, having a filtration particle size of 20 µm. Zeolite, a naturally occurring mineral, has void spaces as small as 3 µm and will to crush, cut, or tear material in the water. This material works more efficiently than sand because it creates a surface area 100 times greater than sand and can remove smaller particles from the water. The filtration system uses a Honda© water pump WB30X (Honda Motor Co., Alpharetta, GS) to bring water into the sand filter. Water enters at the top of the sand filter, it trickles down through the sand and then through the zeolite material. Next, it passes through the paper filter and is released through the outflow hose (Figure 8).

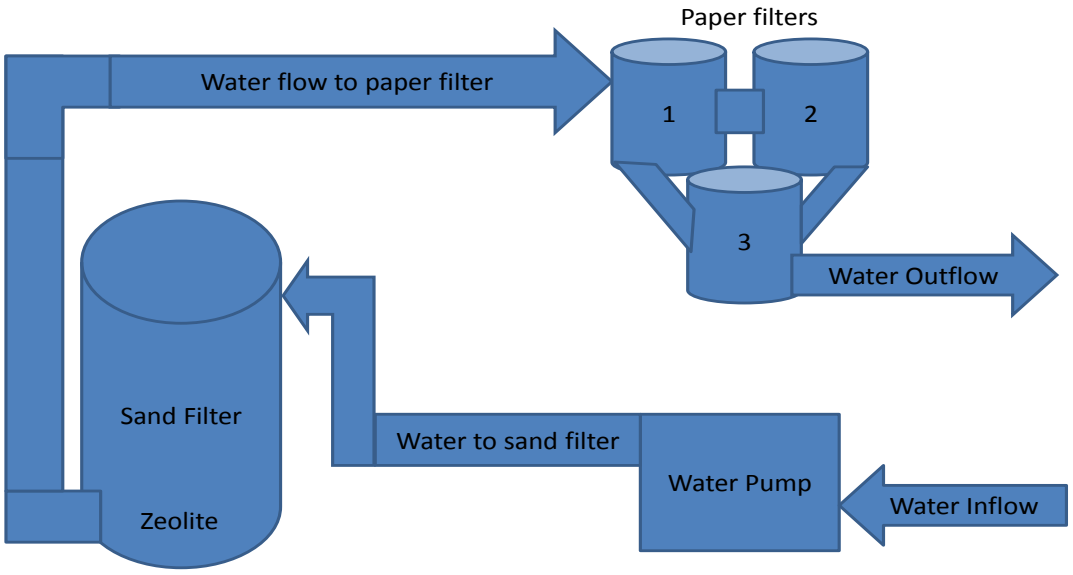


Figure 8. Design of the water filtration system created by Imperial Catfish Farm. Water pump is a Honda© WB30X motor (Honda Motor Co., Alpharetta, GA), sand filter is a Tagelus® TA 100D (Pentair Inc., Minneapolis, MN), paper filters are Big Bubba® paper filters (Watts Water Technologies, Inc., North Andover, MA), and zeolite, naturally occurring volcanic mineral.

Ultraviolet Radiation

Types of ultraviolet rays

The sun is a natural and major source of ultraviolet radiation (UVR), but it can also be emitted by manufactured lamps. According to the Natural Science Foundation, UVR is high in energy; therefore, it has the ability to change the chemical structure of a DNA molecule and causes mutations in the genetic code. This change in the chemical structure can cause cell damage and deformities in living organisms. UVR is divided into three categories that are based on the wavelength band, the amount of energy it contains, and the effects it has on biological material. The shortest wavelength band,

UV-C wavelength (200-280 nm), is the most energetic of the three, but the least harmful, because the radiation is absorbed by the ozone layer and does not hit the Earth. Man-made lamps can emit UV-C radiation, but most of the rays are absorbed by the water, so only the aquatic organisms in the immediate area of absorption are effected (Chalker-Scott et al., 1994a). Exposure to UV-C rays has been linked to major human health hazards in occupational settings, such as welders (Chalker-Scott et al., 1994a; http://uv.biosphereical.com, 2012). The second type, UV-B (280-320 nm), rays are able to pass through the ozone layer and reach the Earth's surface. Studies have shown this type is the most damaging to biological systems under natural conditions. *D. polymorpha* veligers have shown sensitivity to mid-range ultraviolet radiation (UV-B) with 100% mortality but, mortality decreases with increasing larval age (Chalker-Scott et al., 1994a). Researchers have also found that UV-C radiation has the ability to change veliger behavior and increased mortality (Chalker-Scott et al., 1994b). Radiation from the longest wavelength band, UV-A (320-400 nm), has enough energy to reach the Earth's surface and depending on the cloud cover, up to 95% of the rays can penetrate the Earth's surface. However, most of the rays penetrating through the ozone layer are unfiltered (http://uv.biosphereical.com, 2012). Black lights and florescence lights are a manufactured ways of producing UV-A rays. UV-A does not damage DNA directly, but it produces chemicals such as hydroxyl and oxygen radicals that can cause damage to an organisms DNA.

Design of the SafeGUARD ultraviolet system

The SafeGUARD Ultraviolet Radiation system (Emperor Aquatics, Inc., Pottstown, PA) currently in place at Willow Beach National Fish Hatchery (WBNFH) Willow Beach, AZ, will be used to determine the number of cycles veligers need to be exposed to UV radiation to cause damage or death (Figure 9). The UV system contains three 80 watt UV lights that are encased in a metal vessel and are arranged to maximize the output potential. The quartz sleeve, made from transparent hard quartz glass, thermally protects each lamp, which allows the highest UV transmittance to ensure maximum UV energy output (Emperor Aquatics Inc., 2008). The spectral power distribution (SPD) for the unit is 180,000 $\mu Ws/cm^2$, with a suggested flow rate of 6 GPM. The rays emitted are UV-C, which have been found to cause damage to veliger DNA along with increased mortality and behavior changes (Chalker-Scott et al., 1994a & 1994b). The owner's manual states that the low pressure, mercury arc germicidal lamp produces about 90% of its radiation energy at 253.7 nm, which is close to the most lethal wavelength to microorganisms (265 nm) (Emperor Aquatics Inc., 2008).

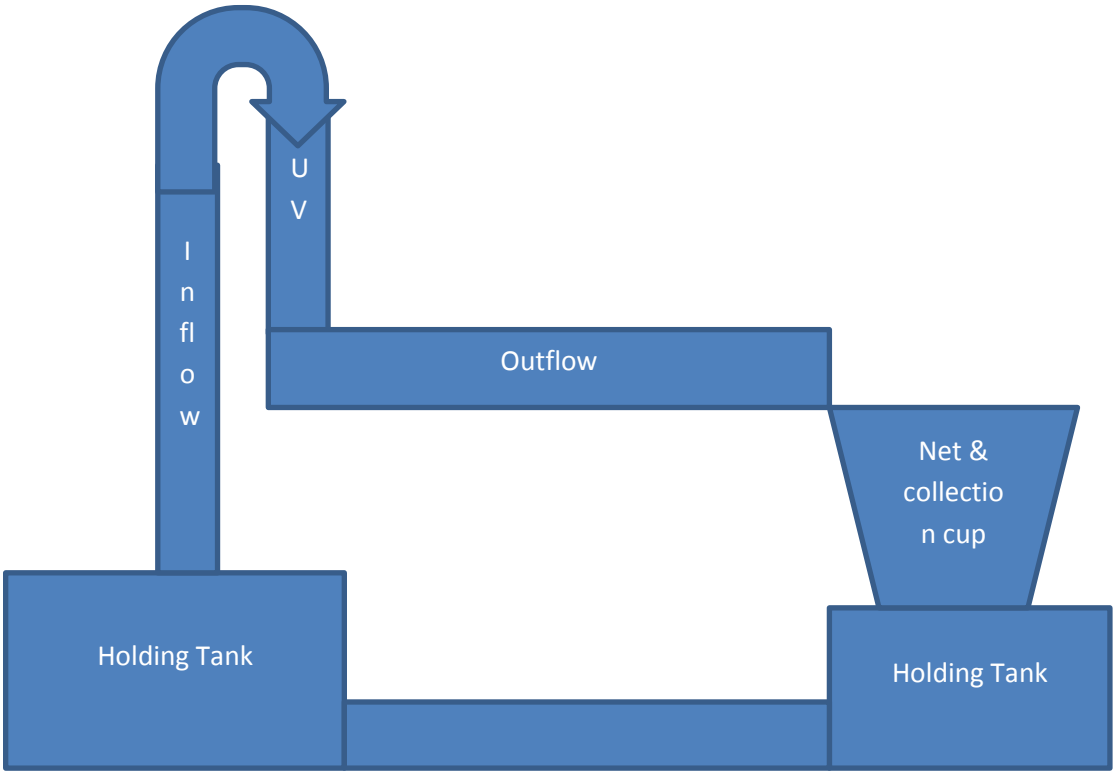


Figure 9. Design for SafeGUARD UV radiation system at Willow Beach National Fish Hatchery to observe damage caused to veliger mussels

CHAPTER 3

MATERIAL AND METHODS

Protocol for sampling veligers with plankton net

The protocol for veliger collection was adapted from the Bureau of Reclamation Technical Service Center in Denver, CO, and is a standard protocol for veliger monitoring in the Lower Colorado River Basin (Wong et al., 2011). For a detailed description of the sampling protocol, refer to Appendix A. A 64 µm plankton net was gently lowered into the water at a rate of approximately 1 m/sec using a steady and unhurried hand-over-hand motion (Gerstenberger et al., 2011a; Wong et al., 2011). The net was raised at a similar speed because pulling it up too fast can cause a wave of pressure to build up in front of the net, pushing the water and plankton away from the mouth of the net and affecting the amount of water that is filtered. Once the net is pulled out of the lake, distilled water is used to rinse the outside of the net and the screens on the collection cup to concentrate the veligers into a 250 ml plastic bottle. To preserve the sample, laboratory grade ethanol (190 proof) was added to the sample to obtain a final concentration of 25% ethanol. The bottle was labeled with the date, location, and depth, and was placed on ice for transport. The samples were stored at 4˚C until veliger enumeration was conducted. The net was disinfected by placing it in a 5% acetic acid (white vinegar) bath for one hour. Before the next sampling period, the plankton net was thoroughly rinsed with DI water.

Determining the transfer rate of veligers on a 6" aquarium dip net

To determine the transfer rate of quagga mussel veligers from Lake Mohave with a 6" nylon aquarium dip net, the following method was used. Three gallons of surface water from Lake Mohave were placed in a five 5 gallon buckets. Using a new 6" aquarium dip net, a scoop was made through the water at the surface. The net was turned inside out and dipped into one of the buckets to wash the veligers off. This simulated the normal larval collection process. This method was repeated 250 times across the surface of the water for each of the five buckets (Figure 10). When the sampling was completed for the bucket, the water was filtered through a 64 µm plankton net to concentrate the veligers. The bucket was rinsed with DI water to remove any veligers that may have adhered to the sides of the bucket. This water was also poured through the plankton net. The sample was transferred to a 250 ml bottle and the inside of the plankton net was rinsed with DI water to remove any veligers that may have attached to the plankton net and the collection basket. The sample was placed on ice until returning to the laboratory. To preserve the sample, 190 proof laboratory grade ethanol (Decon Laboratories, King of Prussia, PA) was added to obtain a final concentration of 25% ethanol. The sample was kept at 4° C until analysis was performed. Between sampling, the plankton net was disinfected by placing it in 5% acetic acid (white vinegar) overnight. In the laboratory, the samples were added to Imhoff settling cones and allowed to settle for a minimum of 24 hours (Gerstenberger et. al., 2011a). From a well-mixed sample, five aliquots of 1 ml were placed onto a gridded Sedgewick rafter 1 mm^2 counting slide. To count the number of veligers present,

the slide was placed under an Olympus BX41 stereoscope (Olympus, Valley Center, PA) that was fitted with a cross polarized lens (Olympus, Valley Center, PA) and the veligers were counted (Gerstenberger et al., 2011a). Five aliquots of 1 ml each were counted from each of the five 250 ml sample bottles. After enumeration was completed, an average number of veligers/L was calculated. The following calculation was used to determine the potential number of veligers transferred in a 5 gal bucket to WBNFH during a night of larval collection.

Average number of veligers= Total number of veligers/ 5ml

The calculation to obtain the final concentration of veligers/L is: C X V'/ V" X V"'

C= average number of veligers counted per ml

V'= volume of the concentrated sample (50 ml)

V"= volume of counted (since this is the average of 5 1ml counts, this is 1 ml)

V"'=volume of total sample in L

These results will be used to determine the potential number of veligers per bucket transferred to WBNFH during a normal night of razorback sucker larval collections.

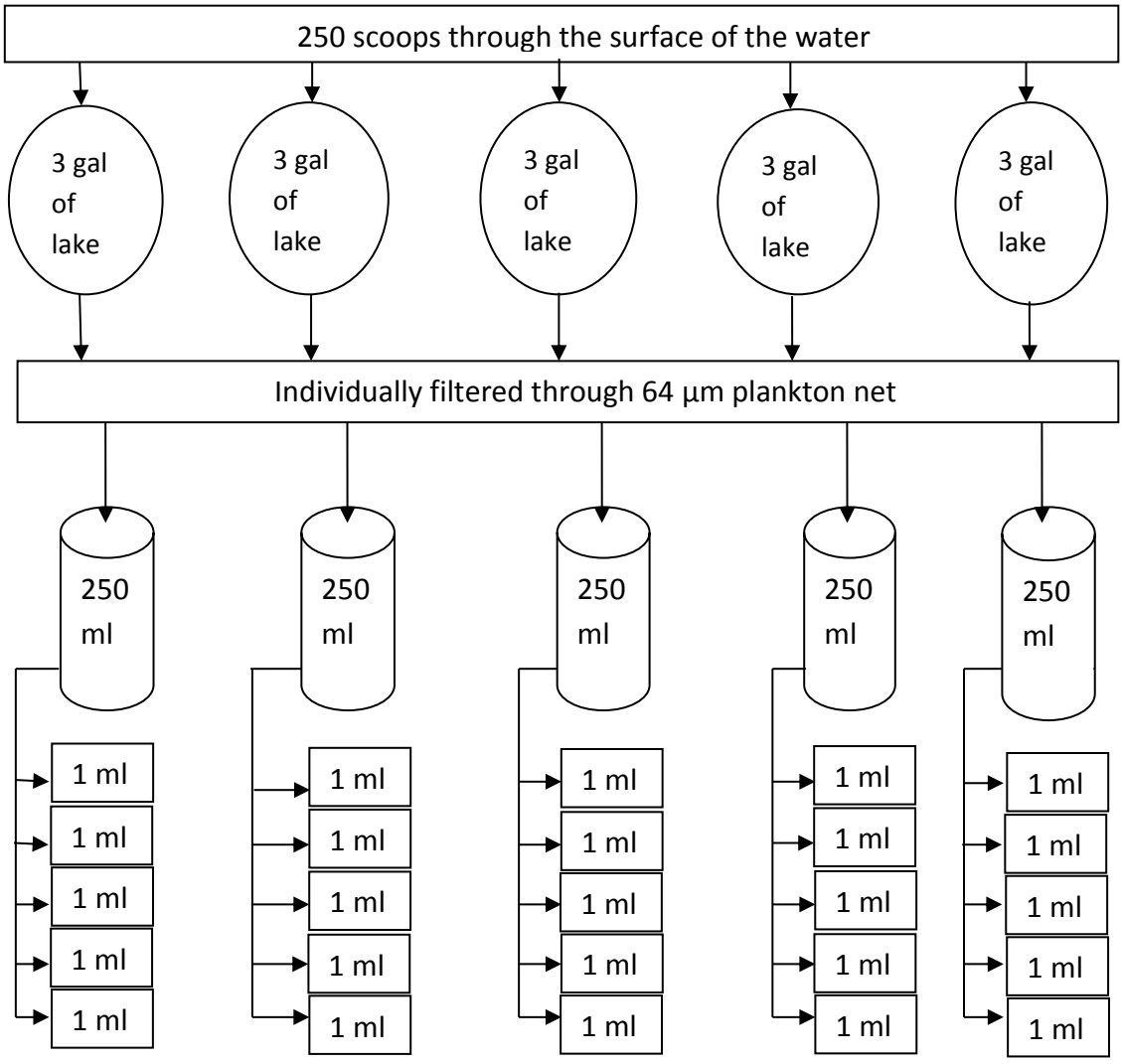


Figure 10. Sampling design for determining the transfer rate of quagga veligers on a 6" aquarium dip net.

Selection of sampling sites

To determine the transfer rate of quagga veliger mussels onto a 6" aquarium dip net, sampling was done at Yuma Cove, Lake Mohave, AZ. This is a location where razorback sucker larval collections are normally conducted. The water filtration test was done at Cottonwood Cove Marina, NV so the desired depth could be reached. The UV radiation test was conducted at Willow Beach National Fish Hatchery, Willow Beach, AZ

because this facility rears razorback suckers from larvae to adult stages and uses the same UV system being tested.

Testing the water filtration system

Veliger samples can be collected by either towing a net through the water or pumping water through a hose from the water source and draining it into the net (Wong et al., 2011). Pumping allows sampling from a known depth, sampling water that is too shallow to conduct a net tow, and allows for the avoidance of algal blooms or disturbed sediment that may clog the net (Wong et al., 2011). At the sampling location, six samples were collected to be used as controls. Each sample contained three net tows taken at 30 ft. From these controls, verification that veligers were present in the water column was made and the number of veligers/L was estimated. To test the efficiency of the Tagelus® TA 100D sand/zeolite filter and one Big Bubba® paper filter, raw lake water was pumped through the filtration system using a 2' trash pump powered by a GX 160 Honda© engine (Honda Motor Co., Alpharetta, GA, WB30X GX 160). The discharge capacity of the trash pump is 275 GPM, but the discharge capacity of the sand filter is 100 GPM. Therefore, the system was operated at a maximum speed of 50 GPM. The flow rate was monitored using a Midwest Instruments & Controls in-line flow meter Model 9002 (Midwest Instruments & Controls, Rice Lake, WI). The inflow hose was placed into the lake at a minimum depth of 20 ft. near the same location the plankton net tows were taken. The pump was turned on and given time to prime. Once the water was flowing out of the outflow hose, the pump was considered primed. An in-line

programmable paddle wheel flow meter with totalizers was placed on the outflow hose to determine the desired 50 GPM rate was reached and stayed constant. For each of the six samples, a 64 µm plankton net was placed underneath the outflow hose until 200 gals were filtered through the system. The samples were placed into a 250 ml plastic bottles and the collection cup and plankton net was rinsed with DI water (Figure 11). The samples were placed on ice until returning to the laboratory. To preserve the samples, 190 proof laboratory grade ethanol (Decon Laboratories, King of Prussia, PA) was added until a final concentration of 25% ethanol was obtained. The sample was stored at 4˚C until analysis was performed. Six samples of 200 gal of filtered water were analyzed for presence or absence of quagga veliger mussels because if one veliger was found after filtration, the system did not work. A paired t-test was performed to determine if the risk of veligers present in the filtered water was reduced.

In the laboratory, the samples were added to Imhoff settling cones and allowed to settle for a minimum of 24 hours (Gerstenberger et al., 2011a). From a well-mixed sample, five aliquots of 1 ml were placed on a gridded Sedgewick rafter 1 mm^2 counting slide. The slide was placed under an Olympus BX41 stereoscope (Olympus, Valley Center, PA) fitted with a cross polarized lens (Olympus, Valley Center, PA). Samples were analyzed to determine the presences or absence of veligers. Veligers present in the sample were counted and the life stage was recorded (Gerstenberger et al., 2011a). The calculation for determining the volume of water that was filtered during each net tow is

h=30 ft.=9.144 meters

9.144 X 3 net tows= 27.432 meters

27.432 X 100 cm= 2743.2 cm

Volume of the cone=πr^2 X h

3.14 $(7.5\ cm)^2$ X 2743.2/ 1000ml= 484.52 L

The calculation for determining the number of veligers present in a net tow is:

$$C \times V' / V'' \times V'''$$

These data were used to determine the effectiveness of the filtration system. From the data of veligers found in the samples, a paired t-test was performed to determine if there is a significant statistical difference between the numbers of veligers/L present in the raw lake water to the numbers of veliger/L present in the filtered sample.

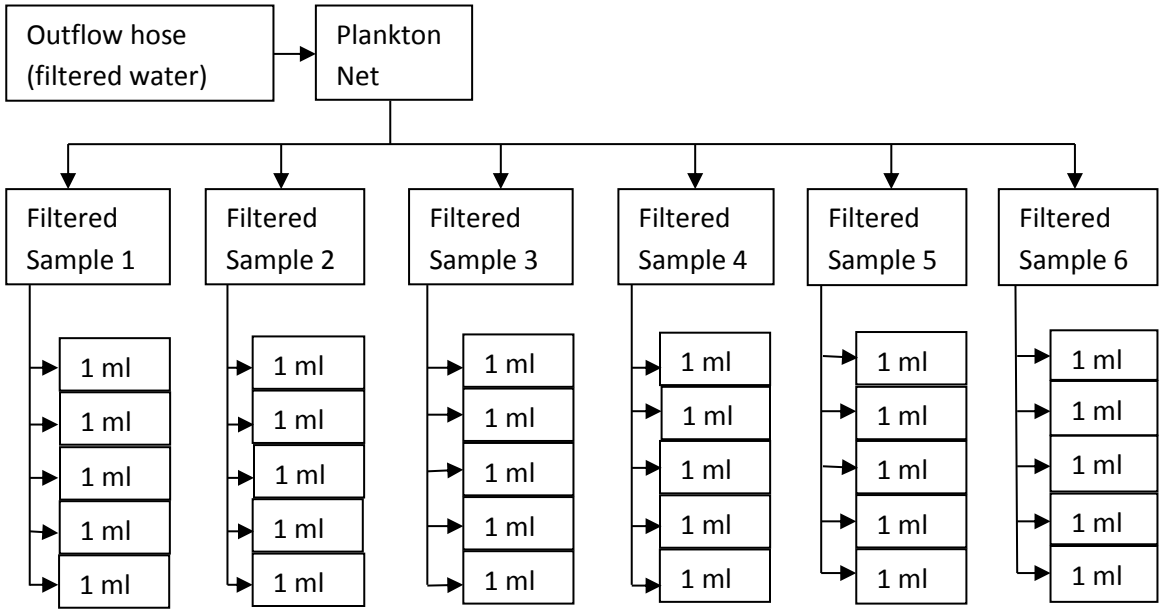


Figure 11. Sampling design for the Tagelus® TA 100D sand/zeolite filter and Big Bubba® paper filter.

Testing the SafeGUARD UV System

On the day of sampling, veligers were collected from B8 raceway at WBNFH. This was done by placing a plankton net under the water flowing out of the headbox. After a 20 minute collection time, the sample was placed in a 300 ml beaker and the plankton net and collection cup were rinsed with well water. Using a pipette and 64 µm sieve, water was decanted from the sample until a volume of 50 ml was reached. From a well-mixed sample, 5 ml were removed and placed in a glass petri dish. The 5 ml sample was observed under an Olympus SZX7 dissecting scope (Olympus, Valley Center, PA) and the number of veligers was counted. The 5 ml was returned to the sample and the petri dish was rinsed with well water to remove any veligers that may have adhered to the sides of the petri dish. The sample was then added to 60 gal of well water and pumped through the system. The manufacturer suggests a flow rate of 6-8 GPM, so the system was run at 6 GPM. After the sample had been cycled through the SafeGUARD UV system the desired number of times, a 64 µm plankton net was place on the outflow pipe and the sample was collected in a 300 ml beaker. The plankton net and collection cup were rinsed using well water and then placed in a 5% acetic acid bath. Using a pipette and 64 µm sieve, the water was decanted off until a volume of 50 ml was reached. After thoroughly mixing the sample, 5 ml were removed and place in a glass petri dish for a second enumeration under the dissecting microscope (Figure 12). This is done to all the samples (control or UV) the first time they are run through the system to ensure veligers are not getting trapped or lost within the pump or the UV system.

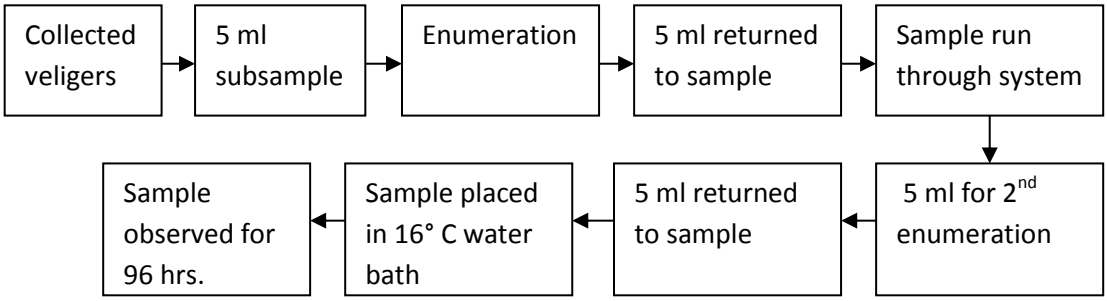


Figure 12. Sample design to ensure veligers are not getting lost within the pump or the UV system.

The number of cycles veliger samples were pumped through the SafeGUARD UV system was 1, 3, 6, and 12. After the desired number of cycles, a 64 µm plankton net was placed under the outflow pipe and the sample was collected. Two plankton nets were used, one for controls and one for tested samples. Immediately after each sampling period, 5 ml of the sample was examined under an Olympus SZX7 dissecting microscope. From each 5 ml sample, 50 veligers were observed for any movement or structural damage and the data was recorded. The 5 ml subsample was added back into the sample along with fresh well water to a volume of 300 ml. The sample was placed in a 16° C water bath until the next observation time. All samples were observed at 0, 24, 48, 72, and 96 hours (Figure 13). Controls were passed through the system without the UV lights turned on and the tested samples were passed through the system with the UV lights turned on.

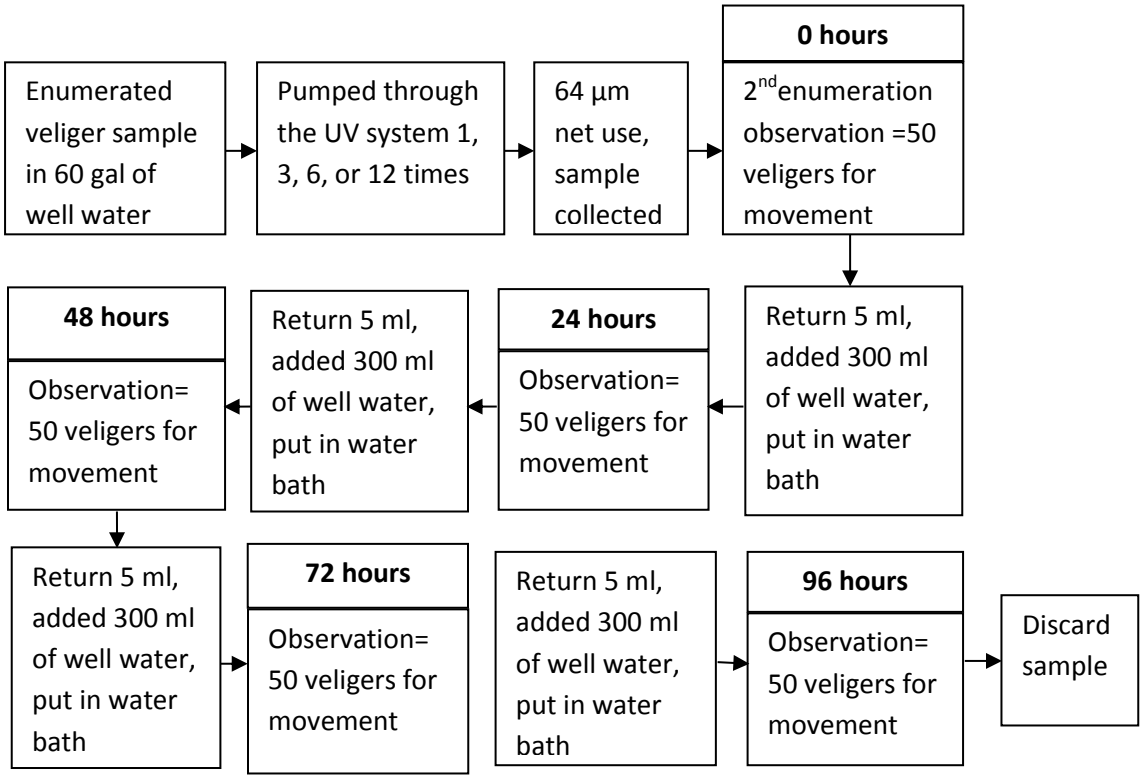


Figure 13. Sampling design for UV system. Controls=no UV light, Tested=UV light. Cycles trough system are 1, 3, 6, and 12 times. Fifty veligers were observed at time 0, 24, 48, 72, and 96 hours.

Between sampling events, the plankton net was disinfected by placing it in 5% acetic acid for a 24 hr. period. The UV system and holding tanks were rinsed with well water, drained, and dried before the next sampling period began. In addition, a sample of veligers were collected, enumerated, and placed in 300 ml of well water. This was to ensure the chemistry of the well water was not killing them. This sample was placed in a 16° C water bath and observed at 0, 24, 48, 72, and 96 hrs. For the complete parameters of the well water before sampling began and after sampling was conducted refer to Appendix C. To determine if there was a significant difference between the numbers of UV exposures, an analysis of covariance (ANCOVA) was performed. Before the UV testing began, well water was collected and observed under stereoscope to ensure no

veligers were present in the water. Veligers were not found in the well water samples. A sample of veligers was placed in the well water and held in a 16°C water bath for 96 hours. This sample was observed at the same time intervals as the controls and treatment group. After 96 hours, all life stages were observed and majority of the veligers were actively swimming and feeding. From this, it can be concluded that the well water at WBNFH does not kill veligers after a 96 hr. period. At each time interval, 0, 24, 48, 72, and 96 hours, 50 veligers were observed for movement or no movement. After each UV experiment was conducted, water samples were collected to determine the UV transmittance percentage. The %UVT is the total amount of UV light energy available to treat the water. The higher the percent value the greater the UV dose will be. The %UVT readings were determined by sending two 100 ml water samples to Emperor Aquatics (Pottstown, PA) to be analyzed. Before treatments, the source water was analyzed and determined to have a %UVT reading of 93%. UVT readings for the various cycles examined ranged from 94%-96%. At a flow rate of 6 GPM and a 95% UVT reading with a 10% safety factor included, Emperor Aquatics determined the fluence (UV dose) to be 700.11 mJ/cm^2. For the values used to determine the fluence refer to Appendix D. The fluence calculation is proprietary information; therefore the dose at 94% and 96% UVT can only be estimated to be 700.11 mJ/cm^2.

CHAPTER 4

RESULTS

Determining the transfer rate of veligers with a 6" aquarium dip net

The transfer rate of veligers onto a 6" aquarium dip net was conducted during July, when numbers of veligers/L tends to be higher than when larval collections take place from January to May (Gerstenberger et al., 2001b). Table 1 shows that the potential for transferring veligers to WBNFH during a night of larval collections is very low. Sample 1 had the highest number at 2.64 veligers/L and Sample 2 and 5 had the lowest number at 0.00 veligers/L. Because the sampling took place in July when veliger populations are at their highest peak (Appendix B), the estimate of three veligers per bucket is higher than what would be found from January to May when larval collections regularly take place (Gerstenberger et al., 2011b).

Table 1. Veliger counts for determining the potential transfer rate of veligers with a 6" aquarium dip net at Yuma Cove, Lake Mohave, AZ.

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**
1 ml	0	0	2	1	0
1 ml	0	0	0	0	0
1 ml	1	0	0	0	0
1 ml	0	0	0	0	0
1 ml	2	0	0	0	0
Total (5 ml)	**3**	**0**	**2**	**1**	**0**
Veligers/L	**2.64**	**0.00**	**1.76**	**0.88**	**0.00**

Testing the water filtration system

Table 2 shows the risk of transferring veligers in filtered water is reduced by 99.9% when comparing it to raw lake water and statistical analysis indicates that

reduction is highly significant (df=5, t=4.123, p=0.009). Even though the risk of transferring veligers in filtered water is greatly reduced, the pump is considered to have failed because veligers are still found in the filtered water (0.01 veligers/L). When trying to remove veligers, no veligers may be found in the final product to consider it effective. In addition to veligers being present after the lake water had been filtered, three species of zooplankton (*Rotifer, Copepoda,* and *Cladocera*) were also found in large numbers (3-33 organisms) in all of the samples. These zooplankton have size ranges that are greater than the zeolite 5 µm filtration size, which further justifies that the pump failed to filter out material properly.

Table 2. Veliger counts from the plankton net tows and the Tagelus® TA 100D and Big Bubba® filtration pump, conducted at Cottonwood Cove Marina, Cottonwood, NV.

	Net Tow						**Pump Test**					
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
1 ml	54	5	119	120	4	8	0	0	1	1	0	0
1 ml	26	19	14	16	11	13	0	0	0	0	0	0
1 ml	9	21	17	16	3	24	0	0	0	0	0	0
1 ml	8	18	3	4	1	6	0	0	0	0	0	0
1 ml	6	22	6	12	1	2	0	0	0	0	0	0
Total (5 ml)	**103**	**85**	**159**	**168**	**20**	**53**	**0**	**0**	**1**	**1**	**0**	**0**
Veligers/L	2.12	1.75	3.28	3.47	0.41	1.09	0	0	0.01	0.01	0	0

Testing the SafeGUARD UV system

For the controls, it can be determined that passing veligers through the system multiple times without the UV lights on did not damage or kill them at time 0 (Table 3 and 4).

Table 3. Control and SafeGUARD UV treatment data for number of cycles (1, 3, 6, and 12) through the system. Fifty veligers were observed at each time interval (0, 24, 48, 72, and 96 hours) for movement or no movement. UVT readings were determined from Emperor Aquatics. M=movement and N=no movement.

	Time (Hr.)	0		24		48		72		96	
		M	N	M	N	M	N	M	N	M	N
Control	# of cycles										
	1	50	0	48	2	50	0	49	1	47	1
	3	50	0	47	3	49	1	49	1	45	5
	6	50	0	47	3	48	2	44	6	43	7
	12	50	0	49	1	39	11	18	32	17	33
UV	# of cycles										
	1	17	33	40	10	39	11	6	44	2	48
	3	7	43	15	35	7	43	5	45	5	45
	6	6	44	2	48	7	43	7	43	1	49
	12	0	50	12	38	7	43	3	47	0	50

After being exposed to UV radiation, veligers initially showed higher percentages of no movement (Table 3 and 4). As the UV exposure cycles increased so did the number of veligers that appeared not to be moving. Veligers observed at 24 hours showed signs of recovery but as the observation times increased, so did the number of veligers not moving. After 96 hours of observation, all UV treatments had an increase in the percentage of veligers not moving (Table 4). With a treatment of 12 times through the UV system at a period of 96 hours, 100% of the veligers observed were not moving. Under the same conditions without the UV lights on, there was a 66% chance of veligers

not moving. Therefore, UV increased the likelihood of killing veligers and the more times they are exposed to UV the greater the chance they will die.

Table 4. Veliger percent of no movement in controls and after UV exposure at time 0, 24, 48, 72, and 96 hours in the four treatment cycles, 1, 3, 6, and 12.

	Control					**UV Exposure**				
	0	**24**	**48**	**72**	**96**	**0**	**24**	**48**	**72**	**96**
1	0%	4%	0%	2%	2%	66%	20%	22%	88%	96%
3	0%	6%	2%	2%	10%	86%	70%	86%	90%	90%
6	0%	6%	4%	12%	14%	88%	96%	86%	86%	98%
12	0%	2%	22%	64%	66%	100%	76%	86%	94%	100%

From the ANCOVA differences of least square means, it can be determined that there is a statistically significant difference between veligers being exposed once to UV compared to the other treatment cycles. The more veligers are exposed to UV radiation the more significant the difference between the cycles becomes, 1:3 p=.0153, 1:6 p=.0032, and 1:12 p=.0029. When comparing 3:6 (p=.5322), 3:12 (p=.5071), and 6:12 (p=.9688) there is not a significant difference between the cycles. To get the highest % mortality of veligers, the maximum number of exposure cycles should be used (Figure 14 and 15). The longer the exposure to UV radiation, the more damaging it is to veligers.

Figure 14. Using the control samples, the number of veligers not moving at time (h) for the SafeGUARD UV radiation system.

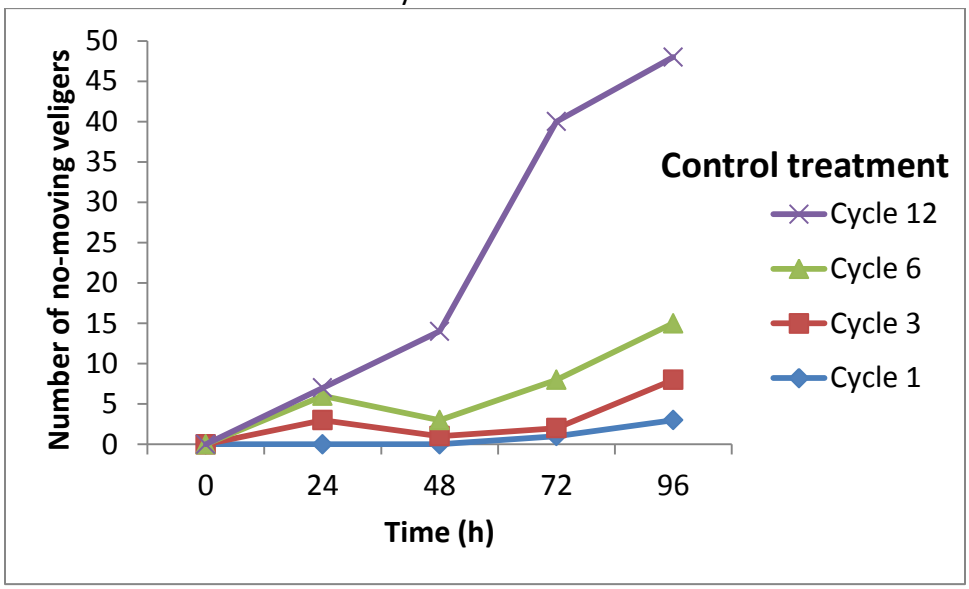
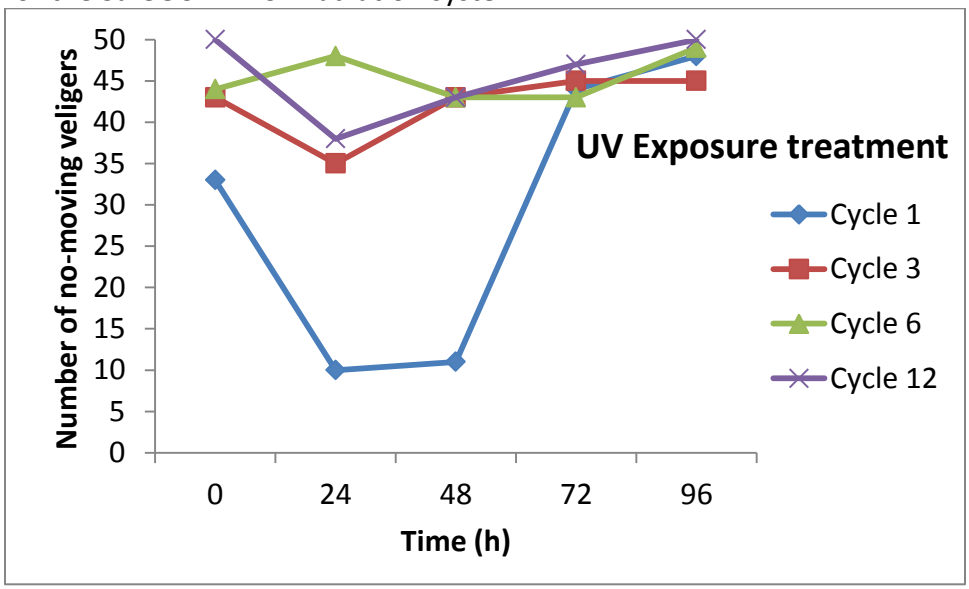


Figure 15. Using the UV exposed samples, the number of veligers not moving at time (h) for the SafeGUARD UV radiation system.



CHAPTER 5

DISCUSSION

Since quagga mussels were found in Lake Mead in January 2007, they quickly spread throughout the Colorado River drainage. They are considered to be the most serious non-native biofouling pest introduced into a large North American freshwater system (LaBounty & Roefer, 2007). Quagga mussels have caused severe economic, ecological, and human health impacts to the southwest. There have been many efforts such as the introduction of an enemy species or the application of toxic chemicals directed toward the eradication and control of this invasive species but, often these efforts result in more ecological harm such as the excessive poisoning of non-target organisms, the transfer of poisons up the food chain, or a population explosion of introduced enemy species (Simberloff, Parker, & Windle, 2005). Research should focus on ways to eradicate this invasive species without causing harm to the aquatic environment. In addition, fish stocking operations need to ensure they are not contributing to the continued spread of quagga mussels. The first step in this process is to reduce the presence of quagga mussels during the initial fish larval collection and rearing process. From the July sampling, it was determined that there would be an average of one veliger per three gallons of water. However, razorback sucker larval collections take place from January to May when water temperatures are low (10-15°C) and veligers/L are at their lowest concentration (Appendix B & Gerstenberger et al., 2011ab). It has been reported that veliger presence and spawning begin when water temperatures are more than 12°C because *Dreissenid* eggs cannot fully develop at

temperatures less than 11°C (Nichols, 1996). It can be concluded that there is a relatively low risk for transporting veligers to WBNFH during razorback sucker larval collections on Lake Mohave.

Veliger samples can be collected by either towing a net through the water or pumping water through a hose from the water source and draining it into the net (Wong et al., 2011). Filtration systems provide chemical-free protection against quagga mussels. When dealing with veligers, there needs to be zero present in the final product. Therefore, it is unacceptable to transfer water as long as there is a risk that veligers maybe present in the water. Precautions such as water filtration and UV radiation need to be taken to ensure there are no veligers in the water when it is being transfer to a new location. It has been found that filtration systems that have the ability to remove particles 40 µm in diameter have been effective at controlling zebra and quagga mussel populations (Lauria, 2009). A study conducted at the Gerald Andrus Station of the Mississippi Power and Light Company in Greenville, MS found that by using a 40 µm self-cleaning filter there were no viable life forms of *Dreissenid* mussels in filtered water (Lauria, 2009). In addition, they found that the small proportion of eggs and planktonic veligers that passed through the filter were torn, compressed/deflated, or dead/dying (Lauria, 2009). However, other studies have found the presence of veligers in samples after filtration has occurred. Pucherelli et al. (2011) determined that flaws in the construction of the filtration material inhibited the complete exclusion of quagga mussels in the samples. The Tagelus® TA 100D and Big Bubba® paper filters have the ability to filter particles smaller than the planktonic stages of veligers and reduces the

number of veligers/L in the raw lake water, which would reduce the risk of transporting veligers during fish stocking activities. However, because the Tagelus® TA 100D and Big Bubba® paper filters were unable to completely remove veligers present in the filtered water, it was considered ineffective.

After the initial exposure to UV radiation, veligers appeared to be dead, but after 24 hours they began to recover. After 1 cycle of UV exposure, it took 72 hours to see increasing rates of mortality (88%) and by 96 hours there was 96% mortality found in the sample. It can be determined that one exposure to UV radiation is not enough to kill quagga veliger mussels immediately and that multiple exposures are needed. After 3 cycles of UV radiation, the percent mortality increased compared it to one exposure cycle. The longer veligers are exposed to UV radiation, the higher the mortality rate became. Under the laboratory conditions at WBNFH, 100% mortality was reached at 12 exposure cycles with an observation time of 96 hours. To ensure increased mortality, veligers should be exposed to UV radiation for a minimum of 3 cycles and held for a minimum of five days. This study confirms the findings by Chalker-Scott et al. (1994) that veligers are sensitive to multiple exposures of UV-C radiation and it has potential effectiveness as a control strategy. It has also been suggested that adult mussels are able to survive higher doses of UV-C radiation (Chalker-Scott et al., 1994), which would explain why 100% mortality was not seen until the highest exposure cycle. Chalker-Scott et al., (1994) state that UV-C rays are absorbed by the water, so only the aquatic organisms in the immediate area of the source are affected by the UV rays. This may explain why % mortality varies among the treatment cycles. To reduce the length of

time it takes to obtain 100% mortality, veligers should be passed under UV radiation multiple times and at a flow rate of 6 GPM or slower.

Limitations

There are a few limitations to the study presented within this thesis. The results potentially overestimated the number of veligers present in a 3 gal bucket because they took place when veliger populations are at their highest concentration. To get a more accurate determination of the veligers/L that could be transferred to WBNFH during a night of larval collections, the sampling should be conducted during the same time period larval fish are being collected, from January to May. For the UV treatment, using UV-C lamps that emit a range of wavelengths at 240-280 nm instead of exactly 264 nm, the wavelength that kills most biological organisms, could have caused a longer time periods for veligers to die. Because these lamps emit a range of wavelengths, there is the chance that the lower end of the wavelength was being emitted and the veligers were not receiving the wavelength that is most damaging to their systems. To optimize the filtration system, a backflush of the system is recommended to redistribute the sand and zeolite material to reduce any channelization that may have been present within the filtration system. In addition, a close inspection of the Big Bubba® paper filters should be conducted to ensure there are not any rips or tears in the material that would allow veligers and zooplankton to pass through the system. The 50 GPM flow rate could be reduced to ensure the pressure of the water flowing through the system does not increase the spaces within the paper filter allowing larger sized particle to pass through the system.

Future Studies

Recommendations for further studies would include performing the transfer rate study during the same time as larval collections are being conducted, along with sampling all sites where larval collections are performed. By adding additional Big Bubba® paper filters to the system and using a smaller mesh size, the relative risk of veligers present in filtered water could be reduced more, with the goal of complete exclusion of veligers and zooplankton species. Other studies could be conducted that reduce the flow rate from 50 GPM to 25 GPM to ensure the water pressure is not compromising the integrity of the filter material. However, at a flow rate of 25 GPM the length of time needed to filter a fish stocking truck would increase considerably therefore, it might not be practical to run the filtration system at this reduced flow rate. The flow rate of 6 GPM through the UV system showed 30% mortality in the control samples, a reduced flow rate should be used to ensure the UV radiations is killing the veligers and not the pressure of the water going through the system. Since the 30 minutes it took to pass veligers through UV radiation system 3 times did not kill them immediately, doing more treatment cycles could give a better idea of how long exposure to UV radiation is needed to kill veligers immediately. Studies could be performed using more UV-C lamps and longer exposure times to determine if the length of hours between exposure and death could be reduced. Samples should be held longer than the 96 hours observation time and rechecked to determine if % mortality increased over time. A recommendation of looking at veligers for longer than the three minute

observation time is made; this would ensure veligers are dead because they have the ability to appear dead when they really are not. In addition, tripling the test cycles for the UV radiation study and increasing the number of veligers per sample observed, would help to clarify differences in % mortality between the treatment cycles and observation times. Testing more numbers of cycles through the UV system would give a more accurate determination of the cycles veligers need to be exposed to UV rays in order to kill them sooner. In conclusion, both the water filtration and UV radiation methods should be used to completely eliminate the presence of veligers in a fish stocking truck. If the combination of these two methods can produce veliger free water in the final product, they can be considered successful. When no veligers are present in the water of a fish stocking truck, the time needed to perform these two methods will be worth it because fish stocking activities could resume in areas where veligers are currently not present in the system.

APPENDIX A-Sampling protocol for veliger collection

Collecting Water Samples For *Dreissena* spp. Veliger PCR Analysis

Bureau of Reclamation
Technical Service Center
Denver, Colorado

Equipment Needed:

- 63-µm Plankton Tow Net (Mesh size is critical). (We use custom Wildco plankton net with a 500 mm-diameter opening, flow meter (optional), and a 2-m length.)
- Spray Bottle – 1-L
- Ethanol (lab grade, 200 proof; or from a local liquor store, e.g., Everclear 190 proof = 95% or Rum 151 proof = 75.5%)
- Sample Bottles (1000-mL Nalgene leak-proof poly (HDPE))
- Disposable Diapers
- Plastic electrical tape
- Ziploc Bags – 1-gal.
- Plastic Garbage Bags (large enough to hold 4 sample bottles)
- Waterproof Markers and Labels
- Data Sheet and Waterproof paper
- Ice chest with cubed/crushed ice or frozen "blue ice"
- Decontamination container for sampling net (e.g., ½ plastic barrel with inside diameter greater than plankton net hoop to permit complete submersion)
- White vinegar (from grocer) or 5% acetic acid solution - 12-16 L (i.e., enough to cover plankton net in decontamination container)

Sample Collection Procedures:

1. Introduction - These procedures are designed to collect the veligers or the free-swimming larval form of zebra and quagga mussels (*Dreissena* spp.) as plankton samples for laboratory detection using polymerase chain reaction (PCR). Step-by-step collection procedures are included below. The volumes of water sampled through the plankton net are needed both for sample size standardization and for calculating the number of veliger density by microscopic methods to confirm the PCR results. Collect a minimum of two replicate plankton samples at each location.

Note: If the plankton net has been contaminated with zebra or quagga mussel veligers from previous collection events, it should be decontaminated with acetic acid (vinegar) and rinsed prior to sample collection. Go to Steps 6-8 for this procedure. Save the final water rinsate sample for laboratory analyses to confirm decontamination. Record and label information about the rinsate (Step 5).

2. There are two methods of acquiring the water sample:
    a. Plankton net tow – Lower the net to the desired, measured depth and slowly tow it for a known recorded distance. The volume of water that is sampled can be determined based on the diameter of the net opening and the distance towed. A minimum sample

volume of 1,000 L is recommended. Record: Depth and distance of the tow. (Caution: To assure accuracy of the sample volume, do not let the retrieval speed exceed the filtration rate of the net.) Remember that veligers from spawning zebra and quagga mussels are more commonly found in deeper water so sample accordingly. Go to Step 3.

b. Pumped source – This may be taken either by a portable pump from a boat or from the raw, untreated water plumbing system of a dam or water treatment plant. Open the flow valve and completely purge the supply line of any stagnant water. If a flow meter is not available on the pipe, use a five gallon bucket and a second timer to determine the flow rate (gallons per minute) through the pipe. Calculate the mean of at least 3 replicate runs for determining the flow rate. Place the plankton tow net under the hose and collect all of the water flowing out of the valve and keep an accurate measure of the volume of water flowing into the net by recording the elapsed time. A minimum of 1,000 L must pass through the net. Record the total volume of filtered water collected per sample and the water depth of the intake of the water source. Go to Step 3.

3. Using water, wash down the net from the outside to concentrate veligers into the collection cup. Carefully unscrew the collection cup and pour the sample into a 1000-mL Nalgene leak-proof poly bottle. Thoroughly rinse the collection cup with spray bottle with minimal volume of water and transfer the rinses into the same sample bottle. Take care to keep the wash and/or rinse water away from the opening of the plankton net and wash only along the outside of the plankton net and cup, so that the filtered volume remains unchanged. MARK THE WATER LEVEL ON THE SAMPLE BOTTLE WITH PERMANENT INK (Draw a line on the bottle and label "Level 1).

4. Add an appropriate volume of ethanol to get 25% final concentration in the sample bottle (visually estimate, does not have to be exact). For example, if using lab grade ethanol or 190 proof Everclear, use 3 parts lake water and 1 part etEverclear. Replace bottle cap snugly. (Note: The volume of ethanol will be needed in the calculation of number of veligers per unit volume; therefore be sure that the sample bottle is marked with a second line to indicate total volume (sample + ethanol) so that the lab can also determine the volume of ethanol that was added.) Draw a line on the bottle and label "Level after ETOH". Tape the secured bottle cap with black electrical tape to cover the seam between the cap and bottle to prevent leakage. Wrap the bottle in a disposable diaper and place in a Ziploc bag (push all air out of bag before closing). Put both the replicates from same location into one single plastic garbage bag. Put on ice in cooler for transport.

5. Labeling sample bottles. Use waterproof Sharpie pens for bottle labels and mechanical pencils for data sheets. Be careful to avoid spillage of ethanol – Sharpie ink will run if contacted with ethanol. For backup, record sample bottle information with a mechanical pencil on a piece of waterproof paper and insert paper into the Ziploc bag along with the sample bottle. Record the following information on both sample bottle and data sheet:
   - Sample Date

- Sample Location (GPS if available, otherwise describe location – i.e. near north shore boat dock, etc.)
- Sample depth or intake depth in water column
- Volume of water filtered through the plankton net
- Mark sample poly bottle with two lines of permanent ink, one for level of sample and one for total level of sample + ethanol
- Preservative used (e.g., 25% ethanol)
- Name of person collecting sample with contact information (phone number)

6. Veligers easily stick to the walls of the plankton net. Decontamination (and disinfection) is critical to avoid cross contamination from one sample location or event to another and possibly the spread of mussels to new waters. It is recommended that each sampling location (reservoir) has a dedicated collection net. Each time the net is used at a new sample site, the procedure will require a soak treatment in a 5% v/v acetic acid bath. A 5% acetic acid solution may be purchased as white vinegar, or a 5% solution may be prepared with concentrated (glacial) acetic acid and water. These steps will both denature the DNA for the PCR process and dissolve the veliger shells otherwise visible in microscopic observations.

7. The recommended treatment for the plankton net following sample collection is to first rinse the net with clean water to wash as many veligers from the net as possible, and then totally immerse the net in the 5% acetic acid bath. The ideal soak time is overnight; however, if it is necessary to use the net at the next sampling location during the same day, a one hour soak followed up with a rinse prior to the next sampling should be the minimum. The same acetic acid bath may be used repeatedly for all sample sites. Following the acetic acid soak, rinse the net with a large volume of clean water (e.g., 100 L) allowing the rinse water to drain and collect into the collection cup.

8. Pour the collected rinsate into a sample bottle, preserve with ethanol, and labeled as directed in Steps 4 and 5. The final rinsate from each sample location may be combined at the end of the day and sent as one sample. Ship on ice with the other samples at the address given.

9. Keep samples cool at all times. Samples may be stored under refrigeration for a few days if a delay is necessary to avoid shipping over a weekend.

10. **Ship samples using FedEx Overnight Express (AVOID WEEKEND DELIVERIES!) to:**
Kevin Kelly/Denise Hosler (86-68220)
U.S. Bureau of Reclamation
Denver Federal Center
Corner of 6th Ave. & Kipling
Bldg 67, Room 152
Denver, CO 80225-0007

Contact information:
Kevin Kelly: kkelly@do.usbr.gov
Denise Hosler: Phone: (303) 445-2195; dhosler@do.usbr.gov

APPENDIX B-Data for veliger collections on Lake Mohave from October 2007-October 2010.

Comparison Results for Microscopy Counts (Veligers/Liter)

Sample Location:	Oct-07	Nov-07	Dec-07	Jan-08	Feb-08	Mar-08	Apr-08	May-08	Jun-08	Jul-08	Aug-08	Sep-08
	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L
Bouy line /Willow Beach	1.08	0.17	0.08	0.02	0.12	0.05	0.20	1.17	1.04	0.53	0.30	0.07
Placer Cove	6.8 *	0.12	0.04	0.05	0.03	0.03	0.13	0.66	1.22	3.22	0.15	0.06
Cotton Wood Cove	0.46	0.68	0.03	0.20	7.08	5.30	1.73	3.92	8.16	8.40	7.66	16.44
Katherine Landing	5.71	0.08	0.61	0.35	5.89	6.28	4.30	4.17	12.57	43.08	28.15	19.37

* Calculated value based upon an average number of veligers settled in a final volume of 15 mLs.

mead 1/09 - 12/09
Mohave

Comparison Results for Microscopy Counts (Veligers/Liter)

Sample Location:	Jan-09	Feb-09	Mar-09	Apr-09	May-09	Jun-09	Jul-09	Aug-09	Sep-09	Oct-09	Nov-09	Dec-09
	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L
Sandy Point	3.80	4.49	2.22	9.85	2.31	6.12	2.78	17.33	9.08	32.43	17.23	5.82
Echo Bay	0.98	1.56	0.91	21.00	5.88	5.87	1.95	12.06	15.40	19.44	5.35	11.28
Temple Bar	1.01	1.22	3.69	2.18	8.02	16.35	1.21	10.09	8.40	32.65	1.64	2.64
Hoover Deep Tow	0.59	0.14	0.57	14.86	15.18	8.86	3.51	9.48	18.30	41.18	0.31	2.54
Hoover Shallow Tow	0.11	0.06	0.85	21.39	12.23	0.14	1.25	1.03	1.63	4.71	1.64	0.81
Willow Beach	NC	NC	0.11	0.39	0.19	1.13	0.31	0.10	0.16	1.04	0.40	0.12
Placer Cove	NC	NC	0.02	0.43	0.17	0.28	0.76	3.07	1.06	0.61	0.35	0.28
Cotton Wood Cove	NC	NC	19.33	5.34	4.78	3.58	0.00	18.01	1.19	0.47	0.73	0.53
Katherine Landing	NC	NC	33.84	28.29	14.15	22.35	16.42	7.26	19.32	12.33	7.90	5.29

Calculated value based upon an average number of veligers settled in a final volume of 15 mLs.

Mead → 12/09-10/10
Mohave

Comparison Results for Microscopy Counts (Veligers/Liter)

Sample Location:	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10	May-10	Jun-10	Jul-10	Aug-10	Sep-10	Oct-10
	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L	v/L
Sandy Point	5.82	0.00	1.73	0.81	2.57	8.41	5.20	3.21	5.06	13.33	11.87
Echo Bay	11.28	0.23	3.13	7.63	17.26	9.12	4.50	30.59	27.26	8.13	51.76
Temple Bar	2.64	0.92	1.48	4.23	15.31	8.03	23.34	15.36	3.19	31.36	29.24
Hoover Deep Tow	2.54	0.00	1.66	1.51	10.67	37.24	18.04	37.20	35.91	13.83	17.64
Hoover Shallow Tow	0.81	0.00	0.37	0.53	1.91	16.47	1.64	11.77	2.99	0.41	4.62
Willow Beach	0.12	0.00	0.30	0.37	0.07	0.67	0.56	3.68	2.24	0.00	0.24
Placer Cove	0.28	0.04	0.01	0.02	0.48	0.19	1.21	2.05	0.12	0.88	0.01
Cotton Wood Cove	0.53	0.00	5.74	1.62	9.57	2.10	20.96	6.88	0.94	2.76	4.80
Katherine Landing	5.29	4.35	0.00	14.20	9.12	36.74	32.47	45.59	87.55	22.66	76.11

NOV / DEC

Calculated value based upon an average number of veligers settled in a final volume of 15 mLs.

APPENDIX C. Well water parameters before and after sampling

Date	9/11/12	9/27/12
Temperature (°C)	25.30	22.18
Dissolved Oxygen	0.65	4.04
Total Dissolve Solids (mg/L)	782.5	1022
Conductivity (µs)	1204	1572
pH	7.4	7.52
Turbidity (NTU)	0.2114	0.313

APPENDIX D. Values for determining the fluence of the SafeGUARD UV system manufactured by Emperor Aquatics.

Emperor Aquatics Inc. Model # COM4240HOSS @ X-gpm @ ELL @ 95% UVT

UVCalc Version 2A

Fluence Rate Distribution Results

Lamp Power =	80	Watts	Fluence Rate Distribution Values are listed in units of:
Lamp Efficiency at end of life (9,000-hr) =	29	%	**Fluence Rate Distribution Value Plotted:**
Lamp Length =	73.9	cm	Experimental Medium: water --> Refractive Index:
Reactor Length =	84.3	cm	Lamp Sleeve Medium: Quartz --> Refractive Index:
Lamp Sleeve Diameter =	2.5	cm	Number of Lamps for this calculation:
Maximum Cylinder Diameter =	14.6	cm	Longitudinal Correction Factor:
			Flow rate:
			Reactor Volume:
			Hydraulic Residence Time:
			Average Fluence Rate in Central Plane:
			Average Fluence Rate in Total Reactor:
			Fluence (UV Dose):
			Fluence (UV Dose); with 10% safety factor

mW/cm^2			
95	**%T**		
1.372			
1.516			
3			
0.8772			
6	**Gal/min(US)**		
3.4	Gal(US)		
34	seconds		
26.0812	mW/cm^2		
22.87945	mW/cm^2		
777.9012	**mJ/cm^2**		
700.111	**mJ/cm^2**		

Lamp Number:	**1**	**2**	**3**
X Coordinate:	4.76	-2.38	-2.38
Y Coordinate:	0	4.12	-4.12
Lamp power factor:	1	1	1

<--y \ x -->	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7
-7					22.3256	20.97879	18.8424	16.84628	15.24595						
-6			25.47655	31.45132	32.77643	28.20377	23.26397	19.82591	17.57977	16.01852	14.79533				
-5		23.46833	33.7888			39.13444	28.00599	22.77585	19.9961	18.32711	17.0894	15.88841			
-4	18.97301	25.30519	38.62357			46.34977	30.86053	25.01726	22.35545	21.02699	20.10129	18.91641	17.23164		
-3	19.07919	24.55915	33.85285	49.32102		39.27689	30.37542	26.27292	24.68905	24.4863	24.5787	23.64807	21.18996		
-2	18.59099	22.70194	27.9032	33.07884	34.61593	31.8551	28.62119	26.93082	27.1404	29.31536	32.5415	32.70699	27.68499	21.74966	
-1	18.06672	21.25924	24.61568	27.38366	28.60079	28.23158	27.38001	27.38352	29.47494	35.73194	50.24206		38.71884	25.87787	
0	17.85385	20.74357	23.60793	25.89547	27.07317	27.18262	26.98832	27.58714	30.53924	39.73775				27.98841	
1	18.06672	21.25924	24.61568	27.38366	28.60079	28.23158	27.38001	27.38352	29.47494	35.73194	50.24206		38.71884	25.87787	
2	18.59099	22.70194	27.9032	33.07884	34.61593	31.8551	28.62119	26.93082	27.1404	29.31536	32.5415	32.70699	27.68499	21.74966	
3	19.07919	24.55915	33.85285	49.32102		39.27689	30.37542	26.27292	24.68905	24.4863	24.5787	23.64807	21.18996		
4	18.97301	25.30519	38.62357			46.34977	30.86053	25.01726	22.35545	21.02699	20.10129	18.91641	17.23164		
5		23.46833	33.7888			39.13444	28.00599	22.77585	19.9961	18.32711	17.0894	15.88841			
6			25.47655	31.45132	32.77643	28.20377	23.26397	19.82591	17.57977	16.01852	14.79533				
7					22.3256	20.97879	18.8424	16.84628	15.24595						

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