

RECLAMATION

Managing Water in the West

Habitat Suitability Parameters for Quagga Mussels in the Lower Colorado River System and at Reclamation Managed Facilities

Research and Development Office
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Mission Statements

The U.S. Department of the Interior protects America's natural resources and heritage, honors our cultures and tribal communities, and supplies the energy to power our future.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

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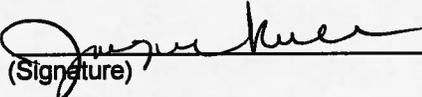
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Executive Summary

Dreissenid mussels can cause biofouling issues that are of specific concern to Reclamation facility managers. Dreissenid mussels have been found to cause physical obstruction of flow in water conveyance systems, ranging from roughening to complete blockage. This invasive species has the capability to seriously impact Reclamation operations, potentially resulting in the interruption of hydropower and water delivery at significant economic costs. The majority of the body of literature that describes the current understanding of the water quality parameters associated with dreissenid mussel habitat suitability is related to studies and distributional records of zebra mussels (*Dreissena polymorpha*). However, quagga mussels (*Dreissena bugensis*) are the dominant species infesting Western water bodies, and there is evidence that this species has different tolerances for certain environmental conditions than zebra mussels.

Accurate information regarding the level of suitability of Western water bodies to support invasive mussel populations is highly useful to Reclamation managers in preparing for and preventing or minimizing the potential for infestation. Quagga mussel populations have been found to be variable in the Colorado River system; this variability provides an opportunity to gain additional insight into mussel habitat requirements. Dense infestations of mussels occur at every major reservoir along the Lower Colorado River. However, similar infestations downriver of Parker Dam have not been reported. The objectives of this project were to investigate what factors downstream of Parker Dam are prohibiting large-scale and dense infestations of mussels and to determine if these data could be useful to help predict suitability in other water bodies. An additional objective was to identify a water quality parameter that could be manipulated in small-scale systems at Reclamation facilities to reduce mussel settlement.

Samples of quagga mussel larvae, adults, and water quality were collected at sites between Lake Mohave and the United States-Mexico border over a three year period. Monthly samples provided information about how mussel populations correlated with water quality overtime. Controlled manipulation tests of water quality were conducted at Davis Dam to determine if a certain water quality parameter could impact mussel settlement.

The results of this study indicate that the increased temperature and total suspended solids (turbidity) at downstream sites are likely contributing to reduced mussel settlement and survival. Conductivity also increases at Southern sites, and experiments conducted at Davis Dam indicate that increased conductivity levels similar to those observed at Imperial Dam can lead to a slight decrease in settlement, but adult mortality is not likely. Additionally, findings indicate that nutrient parameters may not be predictive of veliger survival and abundance in river systems. Utilizing one or a few parameters for habitat suitability may not provide a clear understanding of waterbody suitability for mussel colonization.

Therefore a complete assessment of site specific water quality, hydrology and geology will hopefully provide a better determination of risk.

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Introduction

Invasive dreissenid mussels are spreading throughout the Western United States. Mussel populations are of specific concern to Reclamation facility managers as they can cause physical obstruction of flow in water conveyance systems, ranging from roughening to complete blockage. Intake structures such as pipes and screens can become clogged, reducing delivery capacities, pumping capabilities, and hydropower generation functions. Invasive mussels affect all submerged components, conduits and other structures such as trashracks, fish screens, raw water distribution systems for turbine cooling, fire suppression systems, water intakes (service, domestic, and irrigation), irrigation canals, gauging stations, weirs, gates, diffuser gratings, drains, and virtually all types of instrumentation in contact with raw water. Chemical degradation (corrosion) of infrastructure is also resulting from mussel fouling of metallic structures and equipment. Based on population explosions at Hoover, Davis and Parker Dams, it is likely that the mussels will continue to spread and colonize other Western waters, and threaten to seriously impact Reclamation operations potentially resulting in the interruption of hydropower and water delivery at significant economic costs.

The majority of the body of literature that describes the current understanding of the water quality parameters associated with dreissenid mussel habitat suitability is related to studies and distributional records of zebra mussels (*Dreissena polymorpha*). However, quagga mussels (*Dreissena bugensis*) are the dominant species infesting Western water bodies, and there is evidence that this species has different tolerances for certain environmental conditions than zebra mussels. In addition, the relatively sparse information specific to quagga mussels has some uncertainty due to detections of mussels in water bodies with water quality characteristics outside of the published parameters. As an example, the literature suggests that adult quagga mussels cannot survive at calcium levels (considered the most critical variable for dreissenid survival and population density) below 10 mg/L, and there is little potential for larval development at 10-12 mg/L. However, quagga larvae (veligers) have been found in three Colorado waters with calcium below these thresholds (3.5-11 mg/L Ca). While veliger detections in these water bodies are not necessarily indicators of a sustainable adult population, they do emphasize the notion that the current understanding of water quality parameters associated with invasive mussel habitat is limited or that local adaptations may be occurring.

Accurate information regarding the level of suitability for water bodies to support invasive mussel populations is highly useful to Reclamation managers in preparing for and preventing or minimizing the potential for infestation. Certainty in the knowledge of suitability levels would allow prioritization of budget allocation and decision processes for water bodies that are at high risk for problematic infestations.

In addition, an accurate understanding of habitat suitability could be used as a management tool in small-scale systems (e.g. cooling lines of hydropower

generators). Water quality parameters could be artificially altered to be outside of the acceptable limits within these systems to prevent mussel settlement with minimal environmental impacts. Studies regarding manipulation of pH have shown some promise in this area, and this study may reveal other potential variables that could be used as management tools.

Dense infestations of mussels occur at every major reservoir along the Lower Colorado River. However, similar infestations downriver of Parker Dam have not been reported. A visit to Imperial Dam in February 2012 found several adult mussels attached to substrates, but nowhere near the population densities seen at Lake Mead, Mojave, or Havasu (~1 mussel per 3 square meters vs. ~20,000 mussels per square meter). Similar low-density infestations have been noted in the Central Arizona Project. The presence of numerous larval mussels in the water column of the Lower Colorado River as far south as Senator Wash has been documented, but large colonies of adult mussels have not been established.

The objectives of this project were to investigate what factors downstream of Parker Dam are prohibiting large-scale and dense infestations of mussels and to determine if these data could be useful to help predict suitability in other water bodies. An additional objective was to identify a water quality parameter that could be manipulated in small-scale systems at Reclamation facilities to reduce mussel settlement.

Literature Review: Habitat Suitability Parameters

The following list of parameters has been correlated with dreissenid mussel survival and density, and has been commonly used to estimate quagga and zebra mussel distribution (Mackie and Claudi 2010).

- calcium
- pH
- alkalinity
- hardness
- dissolved oxygen
- chlorophyll a
- total phosphorous
- total nitrogen
- Secchi depth
- mean summer temperature
- conductivity and/or salinity
- total dissolved solids
- turbidity
- total suspended solids

Mackie and Claudi (2010) prepared tables of these parameters, based on the existing literature for both quagga and zebra mussels. These tables provide the range of values for each parameter that correlate with the following four categories: no potential for adult survival, little potential for larval development, moderate potential for nuisance infestations, and high potential for massive infestations.

Calcium concentration is considered the most critical environmental variable for dreissenid survival and density (Claudi and Prescott 2011; Cohen and Weinstein 2001). Multiple authors have used calcium as the primary parameter to rank water susceptibility to dreissenid infestation (Neary and Leach 1992; Cohen and Weinstein 1998; Utah Division of Water Resources 2007; Whittier *et al.* 2008; Colorado Division of Wildlife 2009; Idaho Department of Agriculture 2009; Wells *et al.* 2010; Claudi and Prescott 2011). Low calcium levels can limit the survival of introduced adults and can prevent veligers from developing and reproducing. Dreissenid mussels require calcium for the formation of their shells, which develop shortly after fertilization. The shell is comprised of approximately 40% (dry weight) calcium (Secor *et al.* 1993). Dreissenids have higher calcium requirements than most fresh water mollusks (Claudi and Prescott 2011).

Quagga and zebra mussels have variable tolerances for calcium concentrations. A wide range of minimum calcium requirements for zebra mussels have been reported, ranging from 12-28 mg/L (Cohen and Weinstein 2001). Strayer (1991) examined 70 European lakes and found zebra mussels to be absent in lakes with calcium levels less than 20 mg/L. But in North America, zebra mussels have been found at sites with calcium levels as low as 12-19 mg/L (Mellina and Rasmussen 1994; Jones and Ricciardi 2005). Fewer studies have examined quagga mussel calcium requirements, but in locations where quagga and zebra mussels are cohabitating, quagga mussels are generally found at sites with higher calcium concentrations (Jones and Ricciardi 2005; Zhulidov *et al.* 2004). However, quagga mussels have been found in Colorado water bodies with calcium concentrations as low as 3.5 mg/L. McMahon (1996) suggests that mussel populations become dense at calcium concentrations greater than or equal to 21 mg Ca₂₊/L. Overall researchers have recommended the minimum calcium concentrations of 12-15 mg/L to assess potential dreissenid distribution (Neary and Leach 1991; Baker *et al.* 1993; Claudi and Mackie 1994; McMahon 1996).

When using calcium levels to assess environmental suitability for dreissenid mussels, it is also important to consider pH. The calcium present in dreissenid shells is primarily in the form of calcium carbonate (Secor *et al.* 1993), and calcium carbonate solubility increases as pH decreases. Therefore regardless of calcium availability, if pH is too low or high mussel shells will begin to thin and erode as they lose calcium to the external environment (Hincks and Mackie 1997; McMahon 1996; Claudi and Prescott 2011). Dreissenid mussels have a narrow pH tolerance range from 6.5-7.5 to 9.0-9.5 (Cohen 2005). Veliger development is thought to occur at pH between 7.4 and 9.4 (Sprung 1993). Ramcharan *et al.*

(1992) found an absence of zebra mussels in European lakes with a pH below 7.3, and Smythe *et al.* (1998) found significant mortalities at pH of 5, even with adequate calcium levels. Dreissenid veligers are found in North America at pH between 7.4 and 9, and 8.4 is considered optimal (McMahon 1996). Claudi *et al.* (2012) conducted a study to determine if pH adjustment of calcium rich (41 mg/L) water could prevent settlement of veligers and eliminate adult mussels. The analysis showed that adult shells experienced significant loss of calcium at a pH of 7.1, and at 6.9 the loss of calcium resulted in 40% mortality of adults. Claudi *et al.* (2010) hypothesized that the effect of low pH on mussels may be more profound with lower background calcium levels.

While calcium and pH values may be the most predictive parameters for potential dreissenid mussel distribution, other parameters such as total phosphorous, turbidity (Secchi depth), dissolved oxygen content, mean summer temperature, and conductivity (or salinity) can also limit establishment and abundance. Total phosphorous, turbidity, and dissolved oxygen are parameters that indicate nutrient availability. When total phosphorous, dissolved oxygen and turbidity values are high the biomass of algae is often high. Since mussels feed on algae these indicators can be helpful in predicting dreissenid densities (Claudi and Prescott 2009).

Dissolved oxygen availability is also a limiting factor for mussel establishment. Dreissenid mussels are aerobic organisms that have a metabolic requirement for dissolved oxygen. Studies have suggested that zebra mussels have limited populations below 2-6 mg/L dissolved oxygen levels (Baker *et al.* 1993; Kraft 1994; Doll 1997; Sorba and Williamson 1997; Cohen and Weinstein 1998). McMahon (1996) suggested that quagga mussels may be more tolerant to hypoxic conditions than zebra mussels because they have been found in hypolimnetic waters. But because dissolved oxygen levels are stratified, mussels may be able to move to more habitable locations throughout the year.

Water temperature can influence mussel survival, growth and reproduction rates. Summer temperatures exceeding 30-32°C are considered to be the mussels' upper thermal limit, based on long-term lethal temperature effects (Stanczkowska 1977; Strayer 1991; Baker *et al.* 1993; Armistead 1995; Mills *et al.* 1996; Cohen 2005). Water temperatures above 6-12°C are needed to support adult growth and spawning during the summer (Morton 1969; Stanczykowska 1977; Baker *et al.* 1993; Borcharding 1991; Neumann *et al.* 1993; Sprung 1993; Nichols 1996; McMahon 1996; Roe and MacIsaac 1997). Overall, mussels can survive at a wide range of temperatures, and because water bodies stratify during the summer months, mussels would likely be able to survive and spawn at lower depths even if the surface temperatures reached lethal levels (Cohen 2007).

Conductivity and salinity levels are dependent upon temperature and can constrain the distribution of mussels. Mussels typically have higher salinity tolerance at lower temperatures (Baker *et al.* 1993).

The following study was designed to better understand the limiting habitat factors present in the lower Colorado River, and may provide greater insight in what parameters can reduce dreissenid mussel survivability in highly managed Western river systems.

Methods

This study was conducted on the Lower Colorado River from Davis Dam, AZ to Imperial Dam, CA as well as the All American Canal and within Senator Wash (Figures 1 and 2 and Tables 1 and 2). Monitoring was conducted over a three year period, on a monthly basis at 13 locations from January 2013 through February 2014, and at 8 locations from March 2014 through August 2015. Monitoring sites were located at USGS stream gages or near dams. In 2014, sampling locations were reduced from 13 to 8 because data from the 2013 sampling season indicated that several sites were producing data similar to nearby sites.

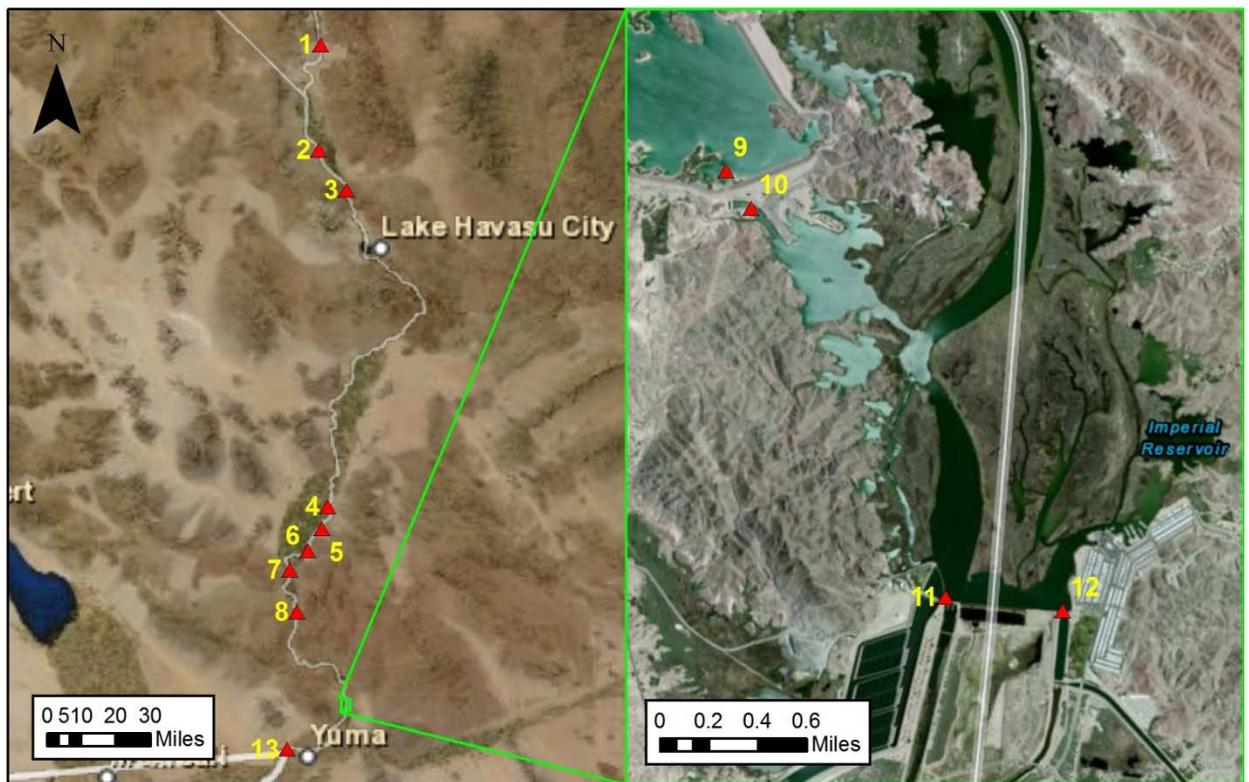


Figure 1. Map of lower Colorado River sites, sampled January 2013-February 2014. Close-up at right: Detail of sampling locations near Imperial Dam.

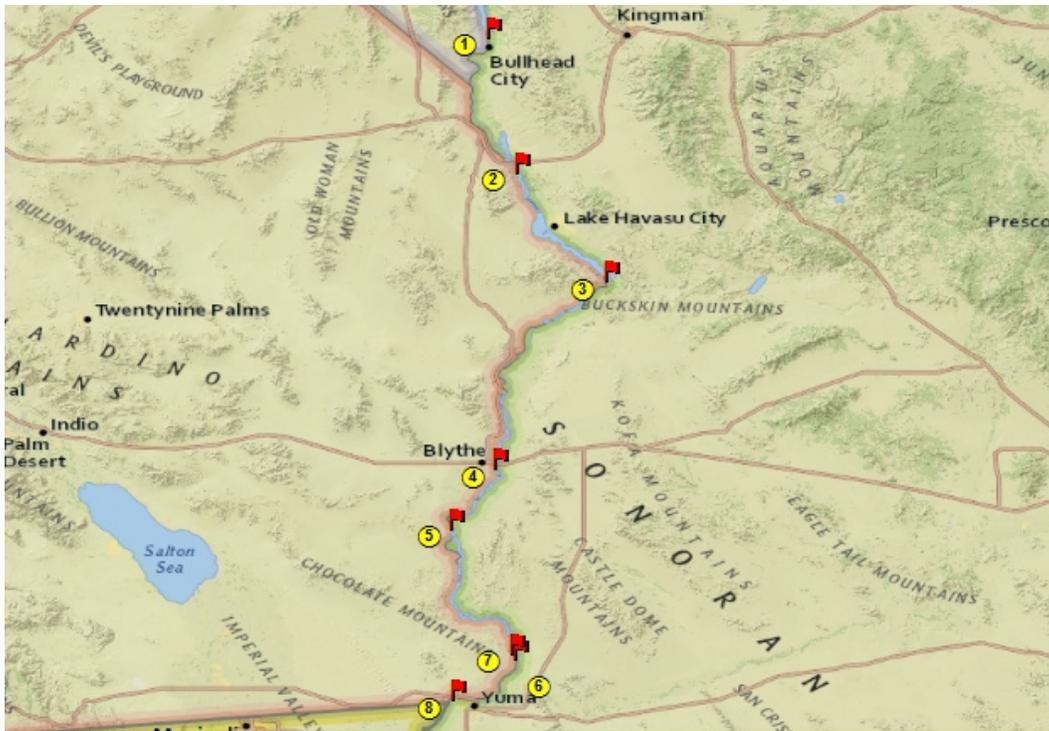


Figure 2. Map of lower Colorado River sites, sampled March 2014-August 2015.

Table 1. Descriptions of lower Colorado River sites, sampled January 2013-February 2014

Site #	River Mile	Description	Latitude	Longitude
1	275	Davis Dam Gage	35° 11.286'	-114° 34.251'
2	244	Needles Bridge Gage	34° 49.504'	-114° 34.870'
3	231	River Section 41 Gage	34° 41.255'	-114° 27.759'
4	120	I-10 Bridge Gage	33° 35.362'	-114° 32.559'
5	113	McIntyre Gage	33° 30.659'	-114° 34.090'
6	106	Taylor Ferry Gage	33° 26.063'	-114° 37.567'
7	98.5	Oxbow Bridge Gage	33° 22.060'	-114° 42.195'
8	87	Cibola Gage	33° 13.256'	-114° 40.354'
9	50.2	Senator Wash Buoy Line	32° 54.276'	-114° 28.845'
10	50.1	Senator Wash Pump Intake	32° 54.164'	-114° 28.741'
11	49.21	Imperial Dam, AZ	32° 52.980'	-114° 27.700'
12	49.2	Imperial Dam, CA	32° 53.005'	-114° 28.058'
13	24.2	All American Canal, Pilot Knob	32° 44.004'	-114° 42.000'

Table 2. Descriptions of lower Colorado River sites, sampled March 2014-August 2015

Site #	River Mile	Description	Latitude	Longitude
1	275	Davis Dam Gage	35° 11.286'	-114° 34.251'
2	231	River Section 41 Gage	34° 41.255'	-114° 27.759'

3	191.75	Below Parker Dam	34° 17.009'	-114° 08.056'
4	120	I-10 Bridge Gage	33° 35.362'	-114° 32.559'
5	98.5	Oxbow Bridge Gage	33° 22.060'	-114° 42.195'
6	49.2	Imperial Dam, CA	32° 53.005'	-114° 28.058'
7	50.2	Senator Wash Buoy Line	32° 54.276'	-114° 28.845'
8	24.2	All American Canal, Pilot Knob	32° 44.004'	-114° 42.000'

Samples were initially collected at both the stream gage and at the approximate thalweg perpendicular to the gage to examine differences in water quality and veliger numbers. Little difference was found, so later sampling included only near-shore sampling at the gages and dam faces. Samples were collected from a boat or from the top of the gage.

Water Quality Samples

Basic water quality readings were collected with a hand-held YSI data logger and multi-probe at the water surface (Figure 3). These parameters included pH, dissolved oxygen (DO), conductivity, total dissolved solids, and temperature. Secchi depth readings were collected to obtain quick measures of transparency or turbidity (Figure 4). In order to collect Secchi readings in significant current, the boat motored up in line with the stream gage and was then put in neutral to float downstream at the same rate as the river. This assured the Secchi disk sank straight down to the river bottom and that readings were not affected by the water current.

Additional water quality parameters were determined by collecting surface water samples for independent laboratory analysis. Laboratories sent pre-labeled and pre-preserved sample bottles prior to each sampling event. Tests included quantification of nitrate/nitrite, silica, total phosphate, ortho-phosphate, TDS, TSS, chlorophyll-*a*, cations (Na, K, Ca, Mg) anions (CO₃, HCO₃, CL, SO₄), and fluoride. Samples collected to detect contaminants of emerging concern (CEC, see Appendix A for full list) were only collected at river miles 275, 120, 50.2, and 24.2. Ortho-phosphate samples were filtered immediately after collection through a 0.45- μ m, 47-mm filter as per laboratory standards. Chlorophyll-*a* samples were filtered through 47-mm glass microfiber filters and were preserved with dry-ice. All other samples were stored and shipped on ice within 24 hours after collection.



Figure 3. Collecting water quality data with YSI multi-probe and data logger.



Figure 4. Collecting Secchi disk readings to determine water clarity.

Dreissenid Veliger Samples

Dreissenid veliger samples were collected monthly at each site using 64- μm plankton tow nets, with a 29.5 cm diameter opening. When veliger samples were collected from the boat at high flow locations a net with an integrated flow meter was used to collect a horizontal tow at the water's surface (Figures 5a and 5b). After approximately 1,000 units had been counted on the flow meter (~27 meter tow), the plankton tow net was brought up and rinsed into a sample bottle. At low flow locations, and when sampling from the gauge instead of the boat, a net without a flow meter was used to collect multiple vertical tows (Figure 5c). In all cases, the amount of water sampled was recorded so the veliger count could be normalized to total veligers per cubic foot (cu. ft.). Veliger samples were preserved with baking soda and isopropyl alcohol per the Reclamation field sampling standard operating procedure (SOP) (Carmon and Hosler 2013a) and were sent to the Reclamation Detection Laboratory for Exotic Species (RDLES) for quantification. Veliger samples were analyzed per the RDLES Lab SOP, using cross-polarized light microscopy (Carmon and Hosler 2013b).



5a)



5b)



5c)

Figures 5a) veliger sample collection via horizontal plankton tow from boat; 5b) plankton tow net with integrated flow meter; 5c) veliger sample collection via vertical plankton tow from gauge

Dreissenid Settlement Samples

Settlement substrates were deployed to monitor yearly mussel settlement and growth at Davis Dam and Imperial Dam, and at gauging stations along the river. In February, 2013 two ceramic brick substrates (surface area= 625.5 cm² each) were deployed at each sample site on the river (Figure 6). The bricks were anchored to the gage ladder and suspended just off the streambed. The bricks

were analyzed after one year to estimate settlement density at different locations within the river. There were multiple variables to consider at each gauge location, specifically flow rate, depth, and brick orientation. This variability made it difficult to compare settlement between sites; therefore the bricks were not re-deployed after settlement was analyzed in 2014.



Figure 6. Brick settlement substrates deployed in Colorado River at gauge

In February 2013, settlement plates were also deployed in the forebay at Davis Dam and at two locations at Imperial Dam (on the California side, next to the trash rack, and on the Arizona side near the Gila head-works). Gray plastic plates (15.2 cm x 15.2 cm = 462.08 cm²) were strung onto plastic coated wire at 1-meter distances. A metal weight was secured to the end to keep the string of plates vertical in the water column. Six strings of plates were installed at each location. At Davis Dam each string held 9 plates from 1-9 meters in depth (Figure 7). At the Imperial Dam, CA site the water depth is 7 meters and 6 plates per string were deployed at 1-6 meters in depth. At the Imperial Dam, AZ site the water is only 2 meters deep so all 6 plates were pushed together in the 2m distance. During each sampling trip, water quality parameters (temperature, conductivity, TDS, pH, and dissolved oxygen) were collected with a YSI at the depth of each settlement substrate.



Figure 7. String of settlement plates at Davis Dam

Settlement substrates at Davis and Imperial Dams were analyzed in February 2014 and 2015. In 2014, mussels were collected by scrapping the entire surface area of the substrate with a razor blade. In 2015 a sub-sampling method was used to collect mussels from Davis Dam plates because of the high settlement density observed in the previous year. A wire template was used to collect a 25 cm² sub-sample (5cm x 5cm= 25cm²). Mussels were scraped into a collection tray with a razor blade (Figure 8). All adult mussel samples were placed in sample bottles and preserved with denatured alcohol.



Figure 8. Collection of settled quagga mussels from settlement plates

In 2014, after settlement was analyzed, the plates at Davis and Imperial Dam, CA were re-deployed and the plates on 3 of 6 strings were replaced with black, PVC plates (14.7 cm X 14.7cm= 432.18cm²) (one textured side and one smooth side). Half of the plates were replaced with these new plates to determine if substrate type significantly impacted mussel settlement. Plates were not re-deployed at Imperial Dam, AZ after the first year because of the shallow water depth. A single

settlement plate was deployed at Senator Wash at 6 meters in 2014 to help quantify settlement at this unique site. This depth was selected because water depth in Senator Wash is variable and did not get lower than 6 meters in the previous year. Senator Wash is a storage reservoir, and was unique in comparison to the other river and reservoir sites.

Mussel samples (Figure 9) were analyzed by counting the total number of mussels, and measuring mussel shell length with calipers. If mussel numbers were high, only 20 mussels were measured.



Figure 9. Mussel settlement samples

Biobox Conductivity Study at Davis Dam

During the 2013 and 2014 sampling seasons conductivity was found to be greater at sites downstream of Davis and Parker Dams, where quagga mussel settlement was not as dense. In January and April 2015, biobox studies were set up at Davis Dam to determine the impact of increased conductivity on quagga mussel settlement. The tests were conducted at Davis Dam because of the large mussel population. Two existing, flow-through, bioboxes installed on the cooling line of power generator Unit 4 were retrofitted with a flowmeter and conductivity loggers. Each biobox received cooling water, containing mussel larvae, from the generator cooling system.

The water flow rate into the bioboxes was set to 2 gallons/ min and was logged hourly, along with conductivity and temperature. The control biobox received unaltered water. The treated biobox received a continuous drip of concentrated salt solution to increase conductivity to downstream levels. The concentrated salt solution consisted of 446.27 grams of magnesium sulfate ($MgSO_4$) and 472.21grams of sodium chloride ($NaCl$) per gallon of water. A peristaltic pump was used to pump the concentrated salt solution from a holding tank into the biobox at 5 ml/ min (Figure 10). Ten PVC settlement plates (14.7 cm X 14.7cm= 432.18cm²) were placed in each biobox to monitor settlement. The duration of

each test was one month. At the end of a test, settlement plates were scraped and the collected sample was observed under a microscope to determine the total number of mussels collected from the plate. The water quality in each biobox was monitored weekly using a YSI multi-probe. Additional water samples were collected from each box and sent to the Lower Colorado Regional Lab for analysis of conductivity, pH, TDS, cations and anions.



Figure 10. Conductivity study set-up at Davis Dam, including bioboxes, flow meter, salt tank and peristaltic pump

The effect of increased conductivity on adult mussels was also observed. Adult mussels from the Davis Dam forbay were collected and transported into the Dam. Mussels were placed in mesh bags. Two bags, each containing 10 mussels, were placed in each bio-box (Figure 11). Adults were observed in a tray of water before they were selected for the test to make sure they were alive. Mussels were considered alive when they opened and began to siphon. At the end of the month long exposure, adult mussels were examined in the same way to determine mortality.



Figure 11. Adult quagga mussels in mesh bags placed in bioboxes to determine conductivity related mortality

Data Analysis

Ordination techniques were used to examine patterns in the veliger data, and to identify environmental variables that were most closely associated with veliger distributions. Primer (Plymouth Routines in Multivariate Ecological Research) statistical software was used to run metric multi-dimensional scaling (MDS) ordination analysis of bootstrap averages, based on a Bray-Curtis similarity matrix of quagga mussel veliger abundance to examine similarity over time and between sites. Bootstrap averages were run for the entire data set (all months during 2013-2015) and for data collected during spring and summer months (May-September during 2013-2015), when veliger numbers tend to peak.

Veliger data were square root transformed prior to bootstrap averaging analysis.

An analysis of similarity test (ANOSIM) was run to test the null hypothesis that there were no differences in veliger abundance between river miles, followed by pairwise testing between river mile sites. Principal components analysis (PCA) graphs were created to identify the environmental variable gradients for samples collected. Environmental variables were log transformed (if required) and normalized. Variables that were found to be highly correlated were combined. Conductivity was highly correlated with TDS, NA, CA, hardness, and alkalinity, and these variable were removed from the analysis. Primer software was also used to determine which environmental variables were best-fitting (BEST test) to veliger abundance between sites.

Results and Discussion

Dreissenid Veliger Samples

Veligers were found at all locations sampled in the lower Colorado River (Figures 12-14). In general, veliger numbers decreased at Southern sites. Veliger numbers were variable throughout the year and between years at all sites. Veliger numbers were lowest in the winter months and highest between the spring and fall.

Extreme veliger peaks were observed at river mile 275 in July and August 2013 (Figure 12) and August and September 2014 (Figure 13), river mile 191.75 in August 2014 (Figure 13), and river mile 50.2 in May 2013 (Figure 12) and April and May 2014 (Figure 13). Similar peaks were not observed during the 2015 sampling season. Veliger numbers were greatest throughout the year at river mile 275 below Davis Dam. Veliger numbers began to decline at river mile 120 below the 1-10 bridge in Blythe, CA.

Senator Wash (river mile 50.2) is an off-river storage reservoir just north of Imperial Dam and the veliger populations at this site behaved differently than at other sites within the river. Average numbers at Senator Wash were typically between 2-19 veligers per cu. ft. June through February, veliger numbers began to increase in March to an average of 83 veligers per cu. ft. Veliger numbers dramatically increased to close to 2,000 veligers per cu. ft. in April 2014 and May 2013 and 2014 (Figures 12 and 13). In 2015, veliger numbers at Senator Wash did not increase to the levels seen in 2013 and 2014. Imperial Dam is a few miles downstream of Senator Wash and veliger numbers were relatively low at this site with numbers increasing between March and June to 14-59 veligers per cu. ft. Although veliger numbers peaked at Senator Wash in April and May in 2013 and 2014 these large peaks were not observed at Imperial Dam. Lowest veliger numbers, throughout the year, were observed at the southernmost site, river mile 24.2 at the Pilot Knob Drop Structure.

2013 Veliger Numbers per Site

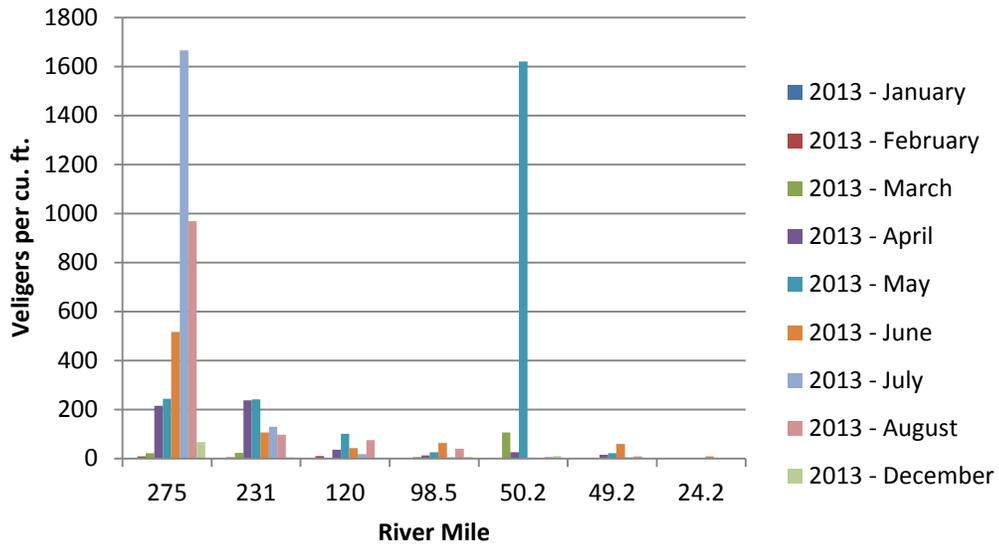


Figure 12. Monthly veligers numbers per cubic foot, at each site along the lower Colorado River during the 2013 sampling season.

2014 Veliger Numbers per Site

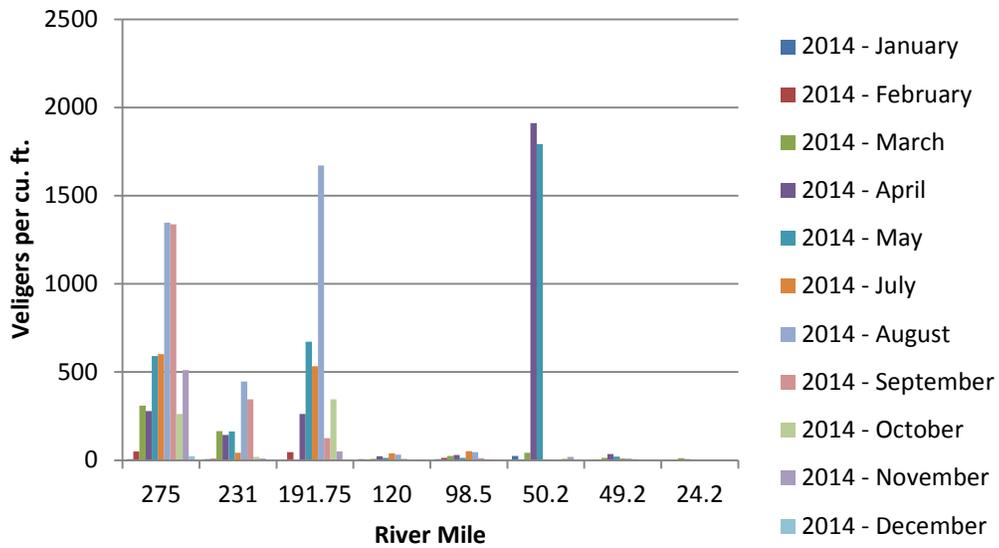


Figure 13. Monthly veligers numbers, per cubic foot, at each site along the lower Colorado River during the 2014 sampling season.

2015 Veliger Numbers per Site

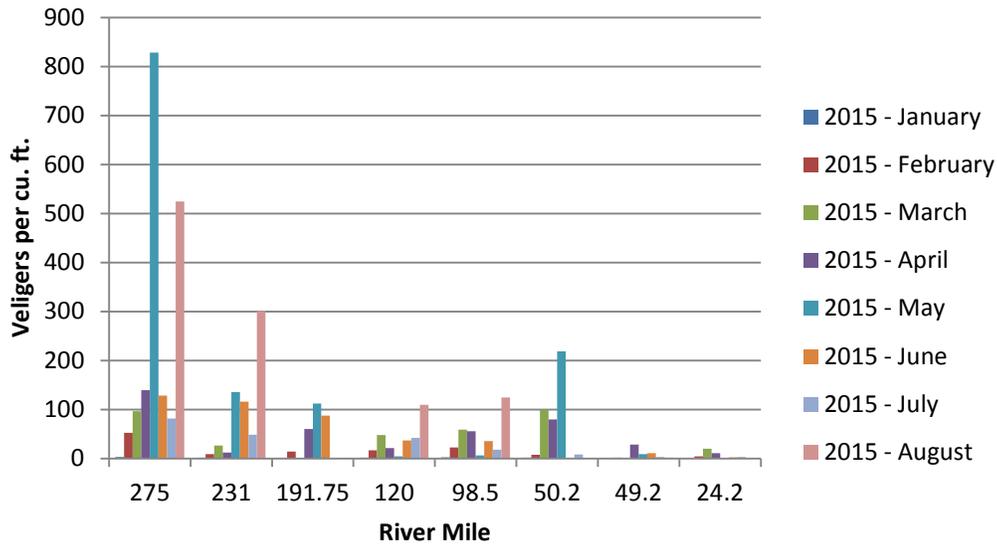


Figure 14. Monthly veligers numbers, per cubic foot, at each site along the lower Colorado River during the 2015 sampling season.

Water Quality Samples

Calcium, pH, Alkalinity, Total Hardness

Average calcium, alkalinity, and total hardness values across study locations and dates were relatively high with low variability and tended to increase downstream (Figures 15, 16, 17, 18). Mackie and Claudi (2010) suggest that waters with calcium levels between 30-120 mg/L and alkalinity and total hardness values between 100-420 mg/L are suitable for massive quagga mussel infestation.

Average calcium, alkalinity, and total hardness values at all sites and dates were within this range, indicating that calcium is abundant and available and is not likely limiting mussel survival and growth at any location within the system. The average pH at each sample location was between 8 and 8.3 (Figure 18) indicating that pH levels should be adequate for calcium availability.

Average Calcium

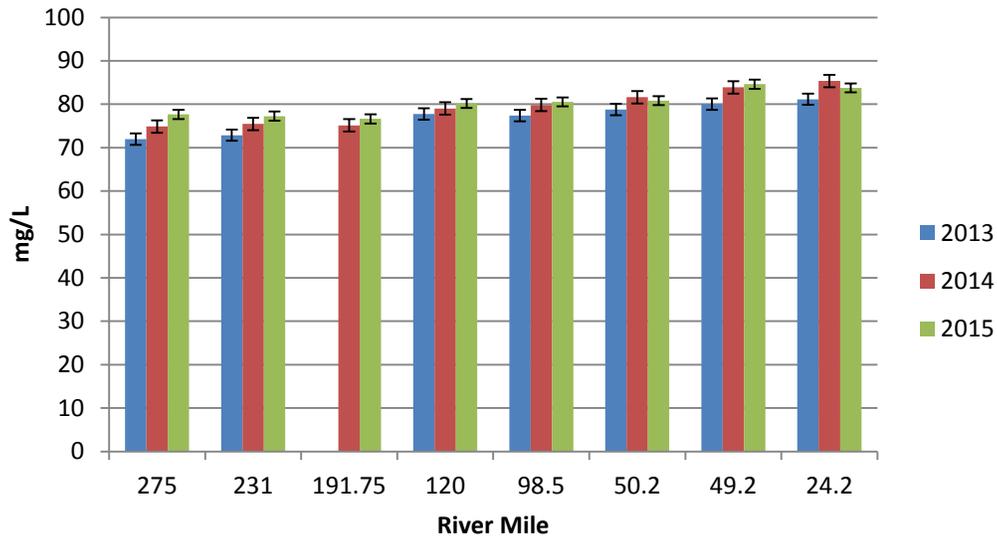


Figure 15. Average calcium (mg/L) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Average Alkalinity (as CaCO₃)

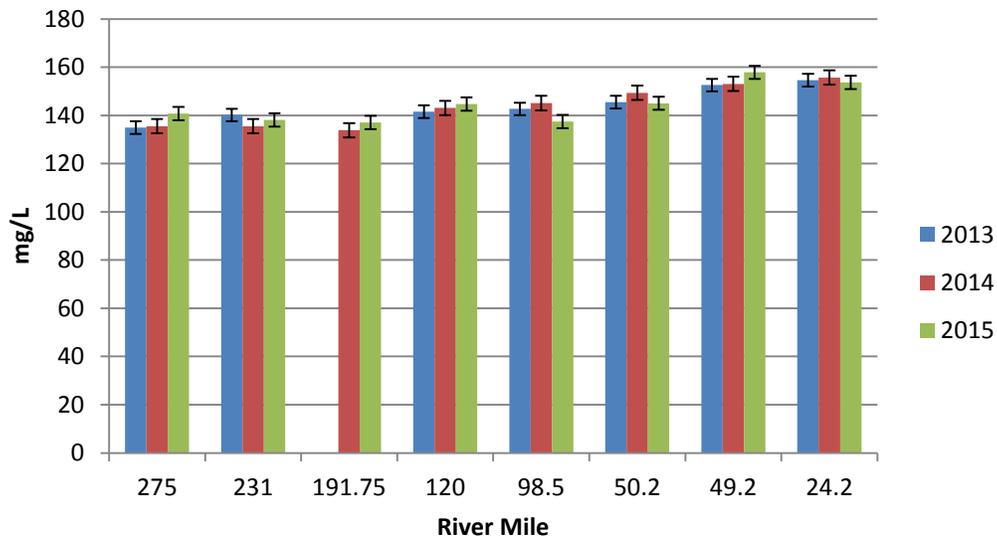


Figure 16. Average alkalinity (as CaCO₃) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Average Total Hardness (CaCO₃ mg/L)

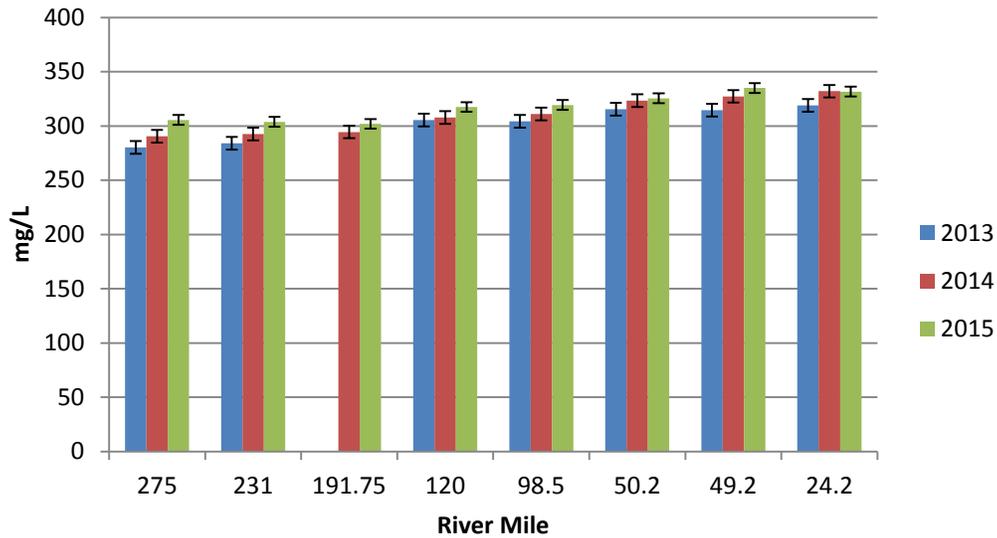


Figure 17. Average total hardness (as CaCO₃ mg/L) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Average pH

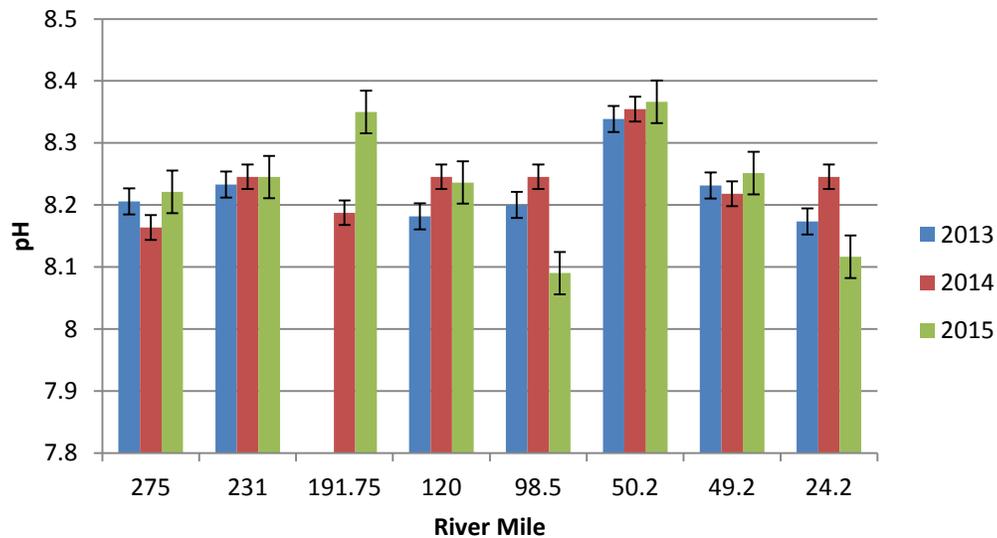


Figure 18. Average pH (standard units) and standard error at lower Colorado River mile sites during the 2013-2015 sampling seasons.

Trophic Indicators:

Total phosphorus would be used to indicate nutrient availability in the lower Colorado River system (as opposed to total nitrogen) because it is the limiting nutrient, according to the N_t: P_t ratio. Increases in phosphorous levels did not correlate with increases in veliger numbers. Average total phosphorus values

generally increased downstream and were greatest at river mile 49.2 (Imperial Dam; Figure 19). Mackie and Claudi (2010) indicate that phosphorus values below 0.005 mg/L indicate no potential for adult survival. Interestingly, average phosphorus values were below 0.005 mg/L at sites where mussel populations were greatest.

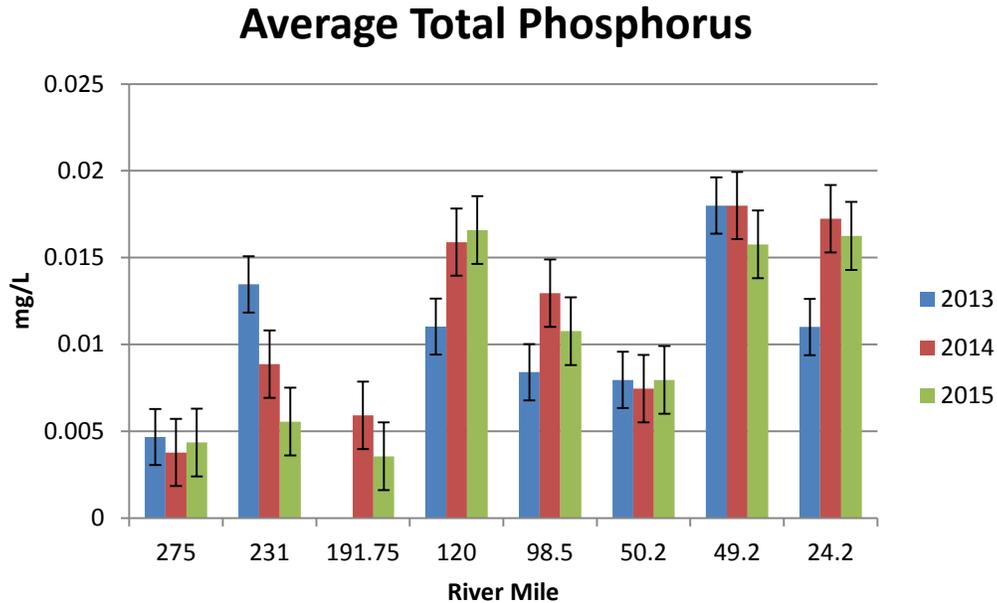


Figure 19. Average total phosphorus (mg/L) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Although there have been no studies relating the presence and abundance of quagga mussels to chlorophyll *a* levels, chlorophyll *a* values can indicate the levels of algae present for veliger consumption. Average chlorophyll *a* values were regularly below 2 mg/m³, which Mackie and Claudi (2010) suggest would indicate no potential for adult survival (Figure 20). Average chlorophyll *a* values slightly increased at river miles 50.2 (Senator Wash) and 49.2 (Imperial Dam). At each site, monthly increases in chlorophyll *a* did not correlate to increases in veliger numbers.

Average Pheophytin-Corrected Chlorophyll *a*

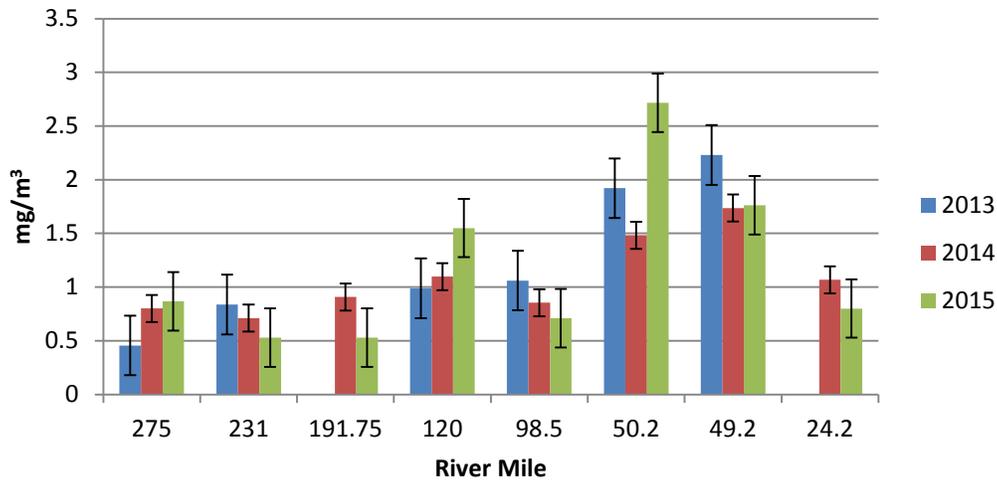


Figure 20. Average pheophytin-corrected chlorophyll *a* (mg/m³) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Secchi depth is a measure of water clarity. Water clarity is affected by algae, sediment particles and other materials suspended in the water column. Deeper readings indicate greater water clarity, while shallow readings can indicate greater algae abundance or high turbidity. Secchi readings can be compared to chlorophyll *a* and total suspended solids values to help determine if shallow readings are a result of algae or suspended sediments. Mackie and Claudi (2010) use Secchi depth values to indicate algae abundance, with readings below 1 m or above 8 m indicating no potential for adult survival, and values between 2-4 m indicating potential for massive infestations.

Average Secchi readings at river miles 275 (below Davis Dam) and 231 (River Section 41) were between 2-4 m, and slightly higher at 191.75 (Figure 21). Veliger numbers were also greatest at these sites. Of all sites sampled, water clarity is greatest at river mile 275, below Davis Dam, where it is possible to see the bottom of the river. Secchi depth values from river sites may be more difficult to interpret, because many river sites are shallow.

Secchi depth decreased below 2 meters at river mile 120 and 98.5. The shallower Secchi depths at these sites were likely indicating greater turbidity as a result of suspended sediments because total suspended solids also increased at these sites. Average Secchi depth increases at river mile 50.2 (Senator Wash) as does chlorophyll *a* (Figure 20), while the total suspended solids decrease (Figure 22). Secchi depth readings at this site likely indicate an increase in algae versus suspended sediment. Secchi depths decrease again at river miles 49.2 (Imperial Dam) and 24.2 (Pilot Knob Drop Structure), where both chlorophyll *a* and total suspended solids increase.

Average Secchi Depth

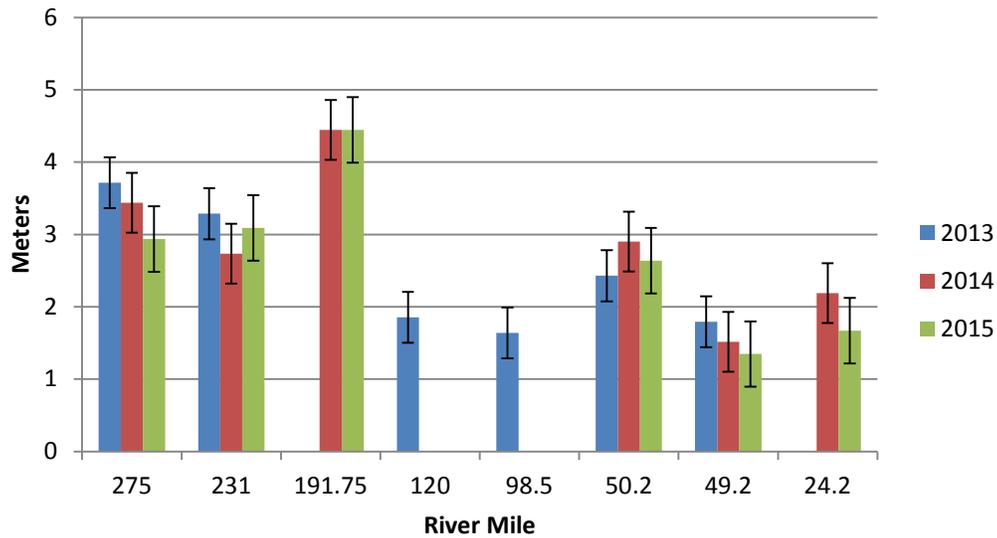


Figure 21. Average Secchi depth (meters) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Total Suspended Solids

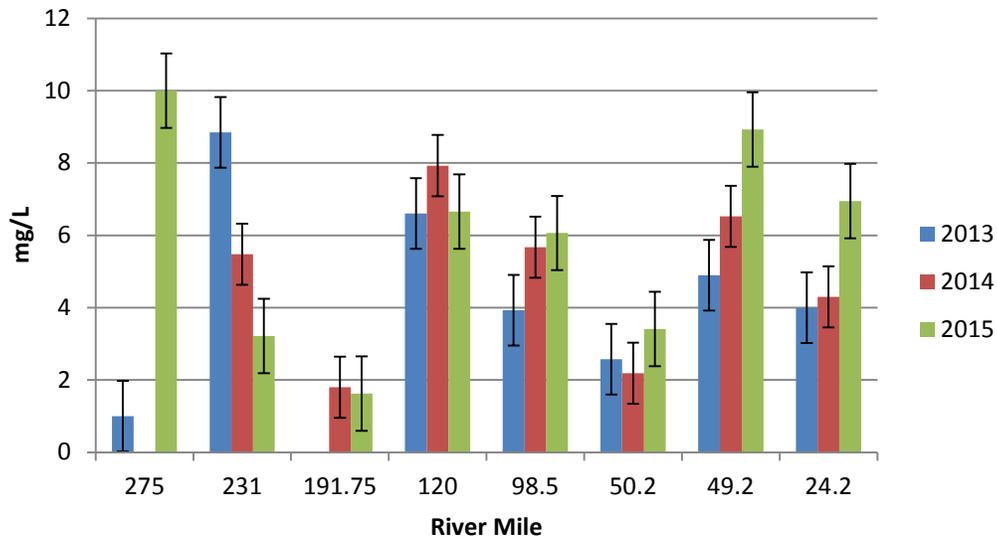


Figure 22. Average total suspended solids (mg/L) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Surface dissolved oxygen levels were relatively stable throughout the year and were similar at all sites (Figure 23). Dissolved oxygen levels above 8 mg/L are thought to indicate high potential for massive infestation. Average surface dissolved oxygen levels were above 8 mg/L at all sites.

Average Dissolved Oxygen

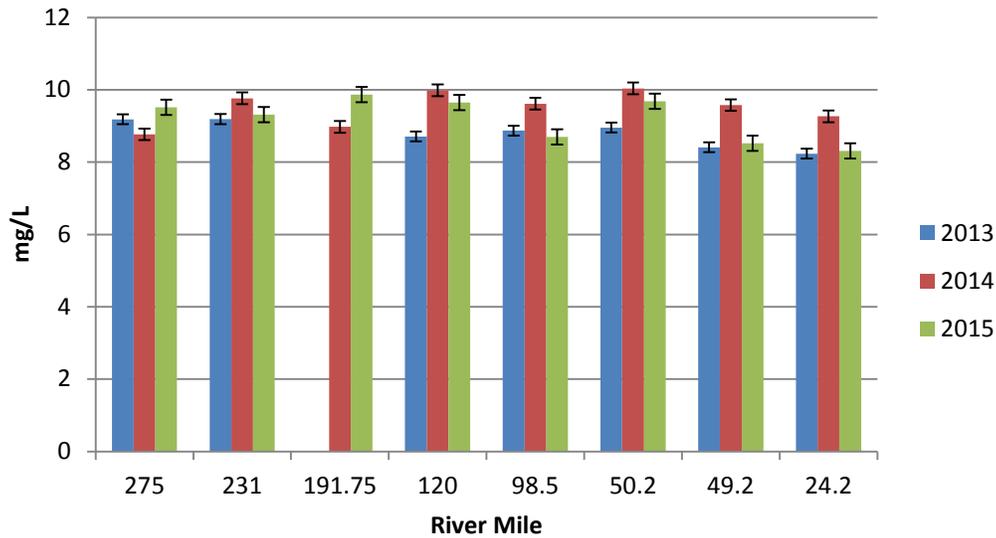


Figure 23. Average, surface, dissolved oxygen (mg/L) and standard error at lower Colorado River mile sites during the 2013-2015 sampling seasons.

Physical Variables:

Surface water temperature was found to gradually increase from upstream to downstream sites (Figure 24), which correlates with veliger numbers. Temperatures at river miles 275 and 191.75 stayed cooler throughout the summer months because they are both sites below dams, where water is continuously mixed. The site at river mile 231 is located in a canyon with more shade than the other river sites, and temperatures were cooler at this site. Throughout most of the year, surface temperatures were within a range that would support moderate-high potential for infestation.

Maximum surface temperatures at mid and downstream sites (starting at river mile 120) reached 29-30°C during the summer months, which is nearing the mussel's upper thermal limit (28-30°C) (Figure 25). It is possible that mussel populations at some Southern sites along the Colorado River are greatly impacted by these extreme temperatures. This is especially true at sites within the river and at Senator Wash and in the Imperial Dam forebay because there is no thermal stratification. Bottom temperatures collected at Senator Wash and Imperial Dam were the same as surface temperatures during each sampling event.

Average Surface Temperature

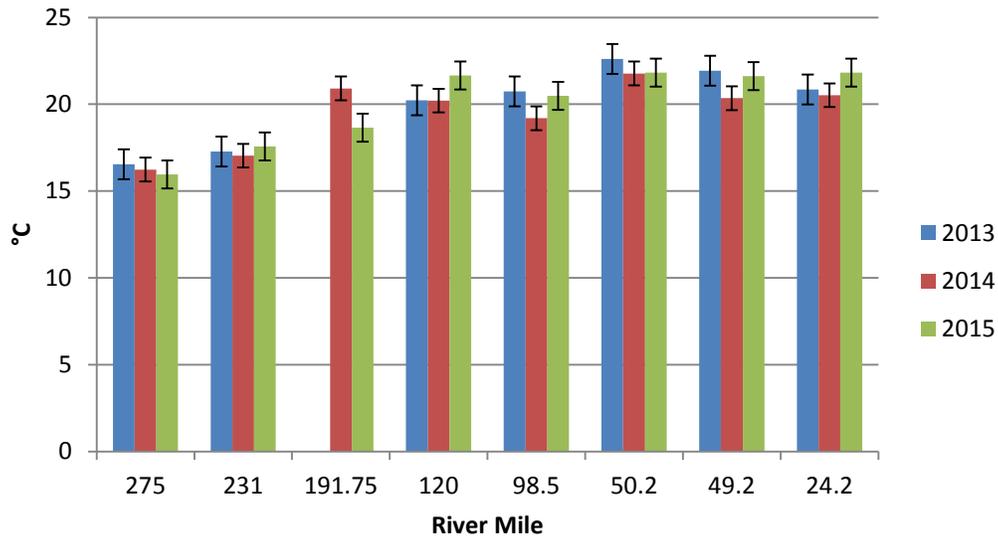


Figure 24. Average surface temperature (°C) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Max Surface Temperature

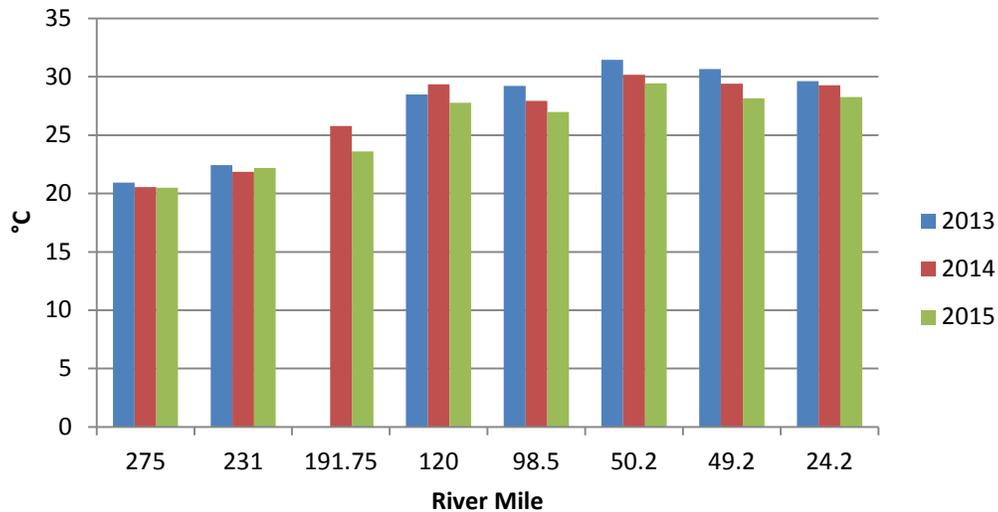


Figure 25. Maximum surface temperature (°C) at lower Colorado River sites during the 2013-2015 sampling seasons.

Minimal conductivity threshold is an important criterion for mussel survival. The recorded conductivity levels throughout the system were well above 110 $\mu\text{s}/\text{cm}$ required for high potential of infestation (Figure 26). Conductivity and other salt values (TSS, sodium, calcium, magnesium, bicarbonate, chlorine, and sulfate) including total dissolved solid (TDS) levels increased downstream. TDS levels

throughout the system were well above 70 mg/L, indicating high potential for infestation (Figure 27).

Average Specific Conductance

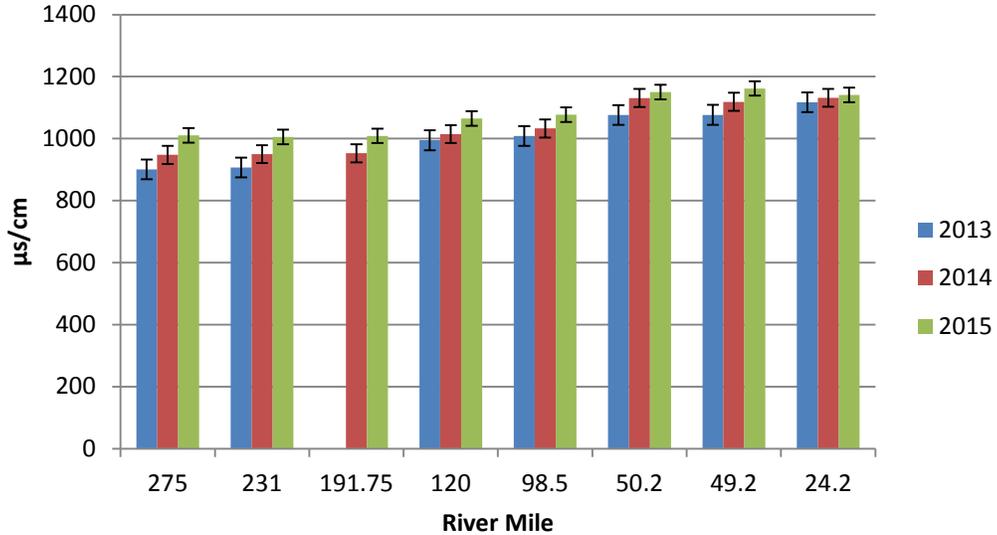


Figure 26. Average specific conductance (µs/cm) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Average Total Dissolved Solids

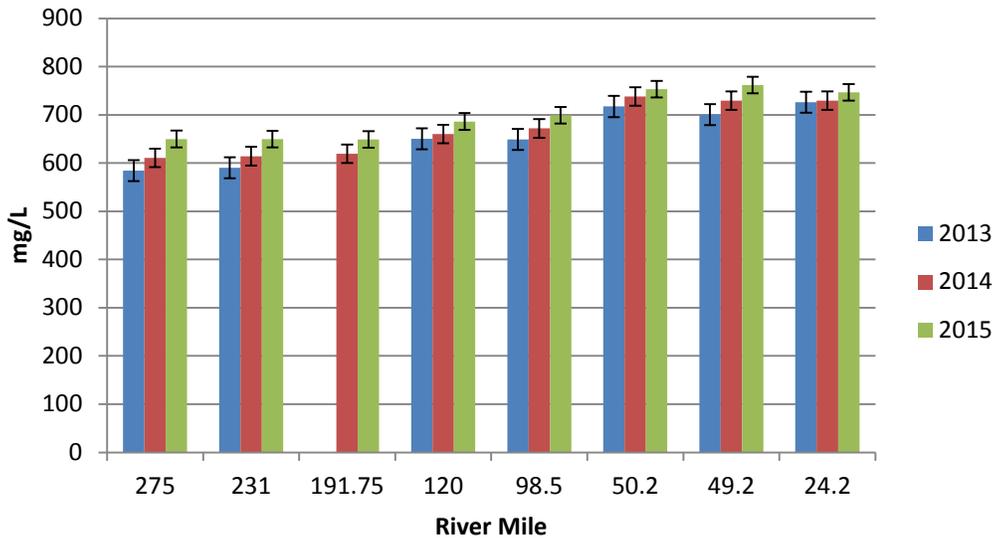


Figure 27. Average total dissolved solids (mg/L) and standard error at lower Colorado River sites during the 2013-2015 sampling seasons.

Ordination Analysis

Analysis of similarity tests (ANOSIM) indicate that veliger abundances are significantly different between sites (R statistic= 0.213, p= 0.001). Bootstrap average analysis comparing veliger abundance between sites is illustrated in the metric multi-dimensional scaling (mMDS) configuration in Figure 28. mMDS ordination ranks similarities, and the associated configuration can be interpreted in terms of relative similarities of samples to each other. Sample sites closer to one another on the mMDS ordination have more similar veliger abundance throughout the year. The 2-dimensional mMDS ordination analysis used a Bray-Curtis similarity matrix of veliger abundance data. Stress is the measure of distortion in the configuration. A stress factor of <0.5 gives an excellent representation; the mMDS 2-dimensional configuration of veliger abundance similarity data had a stress factor of 0.03. Pairwise ANOSIM tests of river mile sites found no significant veliger abundance differences between river miles 49.2 and 50.2 (R statistic= 0.006, P= 0.301), 50.2 and 120 (R statistic= 0.028, P= 0.117), 98.5 and 120 (R statistic= -0.011, P= 0.549), 191.75 and 231 (R statistic= 0.024, P= 0.313), and 191.75 and 275 (R statistic= - 0.060, P= 0.837).

*Bootstrap Averages of Veliger Abundance by CO River Mile (All Months)
Metric MDS*

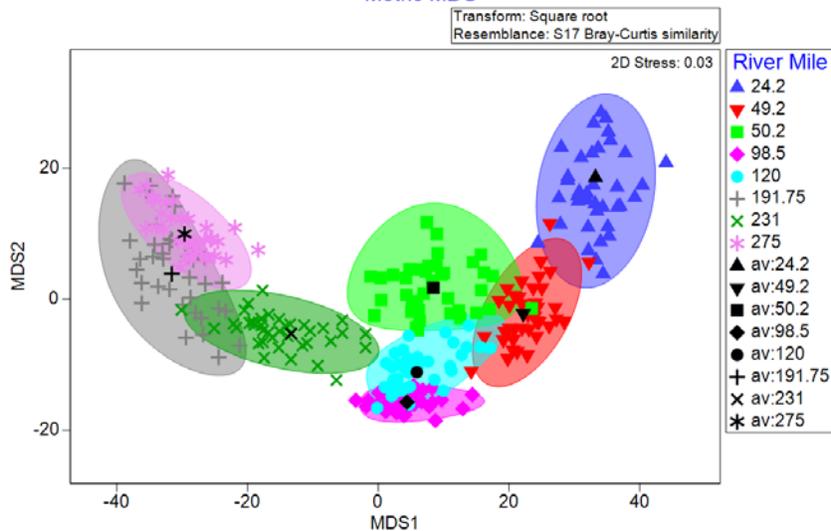


Figure 28. mMDS ordination of bootstrap average veliger abundance by Colorado River site based on square root transformed data and Bray-Curtis similarities (stress=0.03).

ANOSIM tests of data collected May-September, during peak veliger production, indicate even greater differences between sites (R statistic= 0.466, P= 0.001). Bootstrap average analysis comparing veliger abundance between sites during May- September is illustrated in the mMDS configuration in Figure 29. Sample sites closer to one another on the mMDS ordination have more similar veliger abundance during May-September. Pairwise ANOSIM tests of river mile sites found no significant veliger abundance differences during May-September between river miles 24.2 and 50.2 (R statistic= 0.088, P= 0.078), 98.5 and 120 (R statistic= -0.059, P= 0.9.9), 191.75 and 231 (R statistic= 0.120, P=0.120), and 191.75 and 275 (R statistic= 0.061, P= 0.203).

*Bootstrap Averages of Veliger Abundance by CO River Mile (May-Sept.)
Metric MDS*

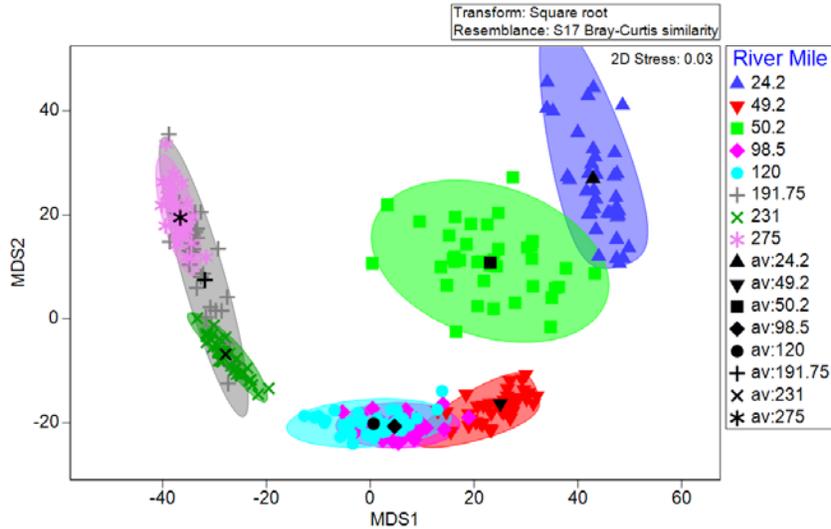


Figure 29. mMDS ordination of bootstrap average veliger abundance by Colorado River site, during May-September, based on square root transformed data and Bray-Curtis similarities (stress=0.03).

The PCA graph in Figure 30 shows the gradient of environmental variables (where the center of the graph is 0 and values increase outward) and where the average of the samples fell in those gradients. The best-fitting environmental variables and the abundance (square root transformed) of veligers that contributed to overall (dis)similarity at Colorado River sites are also included. Conductivity, and total suspended solids (TSS) were found to be best-fitting with veliger distribution throughout the year (correlation coefficient= 0.319, $p= 0.001$). The PCA graph in Figure 31 shows the best-fitting environmental variables and the abundance (square root transformed) of veligers that contributed to overall (dis)similarity at Colorado River sites during May-September. Temperature, conductivity, and dissolved oxygen (DO) were found to be best-fitting with veliger distribution during May-September (correlation coefficient= 0.403, $p= 0.001$).

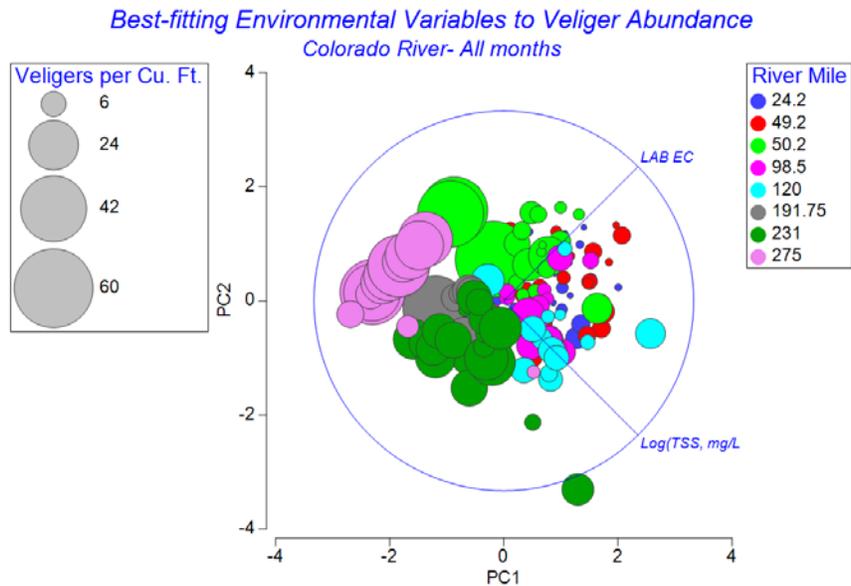


Figure 30. Principal components analysis graph showing best-fitting environmental variables and general abundance of veligers (square root transformed data) per Colorado River site.

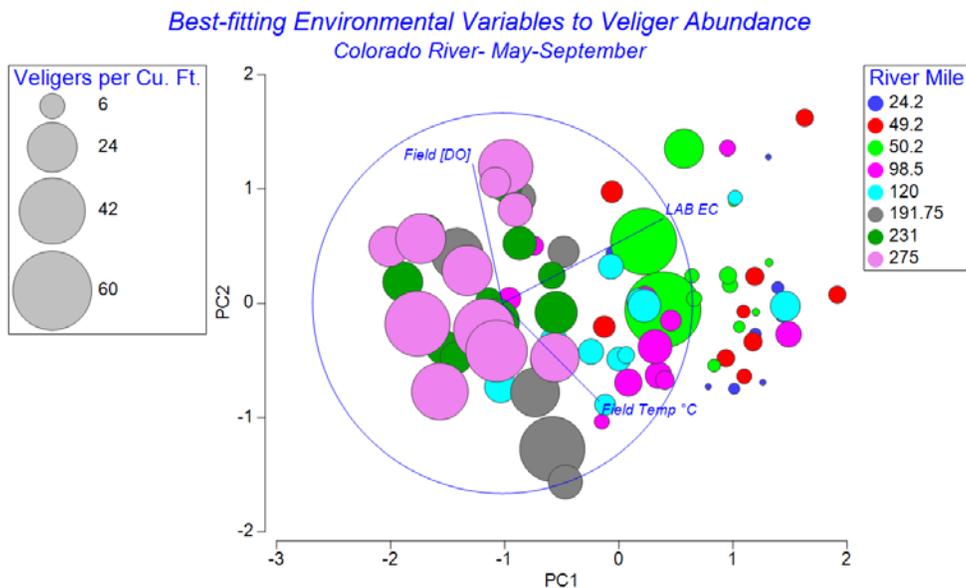


Figure 31. Principal components analysis graph showing best-fitting environmental variables and general abundance of veligers (square root transformed data) per Colorado River site during May-September.

Contaminants of Emerging Concern (CEC):

CEC samples were collected at river miles 275, 120, 50.2, and 49.2. These sites provide a representation of how CEC concentrations change within the river from Davis Dam to Imperial Dam. Samples were analyzed for 94 CEC's (Appendix A). Table 3 lists the 46 contaminants that were detected at the four sites sampled, and the highest concentration detected during the three year sampling period. Of the 46 contaminants detected, 19 were found at all four sites at different levels. The

site with the greatest concentration of each contaminant is highlighted in Table 3. The greatest CEC concentrations were generally found at sites south of river mile 275 (below Davis Dam). There were also 16 contaminants (pesticides, herbicides, pharmaceuticals, and food additives) that were only found at sites south of river mile 275. No CEC's were found at river mile 275 that were not also found at downriver sites. CEC's are present throughout the lower Colorado River system. Although more CEC's and generally greater concentrations were detected at downstream sites where quagga mussel numbers are decreased it was not possible to detect a single CEC that may have impacted quagga mussel survival.

Table 3. List of Contaminants of Emerging Concern (CEC) that were detected at Colorado River sites (river miles 275, 120, 50.2, and 49.2). The maximum concentration found at each site during the 2013-2015 sampling period is listed, and the site with the greatest concentration is highlighted in yellow. Contaminants highlighted in gray were not detected at the northernmost site below Davis Dam (river mile 275).

CEC	River Mile				Description
	275	120	50.2	49.2	
Acesulfame-K	290	260	160	270	Food additive/ human waste
DEA	220	200	170	210	PPCP shampoo, cosmetics
Iohexal	54	16	47	52	Medical testing
Sucralose	1400	1200	900	950	Food additive/ human waste
Triclocarban	11	5.4	5.7	5.8	EDC
Carisoprodol	7.7	9.1	<5	5.6	Pharmaceutical
DEET	25	290	72	65	Pesticide
Diuron	<5	310	52	250	Herbicide
TDCPP	170	360	200	120	Flame retardant, pesticide, plasticizer
Methylparaben	<20	170	270	410	Food additive/ human waste
Primidone	<5	<5	6.4	<5	Pharmaceutical
TCEP	<10	13	30	<10	Flame retardant, furniture
Sulfamethoxazole	15	15	28	28	Pharmaceutical
Albuterol	24	35	31	43	Pharmaceutical
Caffeine	12	170	28	210	Food additive/ human waste
Iopromide	29	29	5.9	95	Medical testing
Propylparaben	9.9	59	20	210	Food additive/ human waste
Quinoline	18	26	20	30	Cigarettes, other
Theobromine	11	63	30	170	Food-chocolate
Fluoxetine	29	16	-	<10	Pharmaceutical
Cotinine	22	26	13	-	Cigarettes, other
Estrone	<5	8.9	-	<5	Hormone-estrogen, EDC
2,4-D	-	14	6.5	11	Herbicide
Oxolinic Acid	-	31	60	28	Pharmaceutical
Sulfachloropyridazine	5.6	-	55	-	Pharmaceutical
Azithromycin	-	-	340	69	Pharmaceutical
Flumequine	-	22	96	-	Pharmaceutical
Carbamazapine	7.5	6.6	-	11	Pharmaceutical
Diclofenac	14	6.5	-	7.4	Pharmaceutical
1,7-Dimethylxanthine	<10	<10	-	15	Pharmaceutical
Dehydronifedipine	5.4	<5	-	13	Pharmaceutical
Theophylline	<20	51	-	73	Pharmaceutical
Atenolol	-	5	5.3	7.1	Pharmaceutical
Cyanazine	-	-	7.5	12	Pesticide
Ibuprofen	<10	<10	-	<10	Pharmaceutical
Lidocaine	<5	<5	-	<5	Pharmaceutical

Pentoxifylline	-	8.3	-	-	Pharmaceutical
Cimetidine	-	16	-	-	Pharmaceutical
Ethylparaben	-	21	-	-	Food additive/ human waste
Sulfadimethoxine	-	-	14	-	Pharmaceutical
Simazine	-	-	5	-	Herbicide
Acetaminophen	-	-	200	-	Pharmaceutical
Bromacil	-	-	15	-	Herbicide
Nifedipine	-	-	23	-	Pharmaceutical
BPA	-	-	-	10	Plastic additive
Dilantin	-	-	-	22	Pharmaceutical

Quagga Mussel Settlement:

Settlement was analyzed on plates hanging at 1-meter depths in the Davis Dam and Imperial Dam forebays after one year. Quagga mussel settlement was significantly greater at Davis Dam (Figure 32) compared to Imperial Dam (Figure 33). At Davis Dam settlement plates were deployed at 1-meter depths up to 9 meters (not to bottom). Settlement began to increase at 3 meters and was greatest at 9 meters (169,600 mussels per m²). At Imperial Dam settlement plates were deployed at 1-meter depths up to 6 meters (bottom). At this site mussel settlement was greatest at 6 meters (88.31 mussels per m²).

Settlement substrates were only deployed at sites within the river for one year. The greatest settlement (42,175 mussels per m²) was observed at river mile 275 (below Davis Dam) and settlement declined at downstream sites (Figure 34). Settlement at Senator Wash was greater in 2014 compared to 2013. Although settlement was greatest at upstream locations, average mussel shell length increased at downstream locations (Figure 35). Summer temperature may impact mussel survival and settlement at Imperial Dam. Figure 36 shows the average temperature over the three year study at the surface and at the depth of the deepest plate at Davis (9m) and Imperial Dams (6m). The surface and 6m depth temperatures are the same throughout the year at Imperial Dam and average summer temperatures (June-September) are close to 30°C, which is reaching what is thought to be the mussel’s upper thermal limit. Surface temperatures are about 5° cooler at Davis Dam compared to Imperial Dam. Temperatures at Davis Dam are cooler at deeper depths in the summer, compared to the surface, allowing mussel veligers to escape the warmer temps.

Freshwater sponge growth and sponge gemmules (dormant stage) were detected on settlement plates at Imperial Dam. In February 2015 a sponge sample along with sponge gemmules were collected from plates at Imperial Dam and were sent to BSA Environmental Services Inc. for taxonomic identification. The sample was identified as *Trochospongilla leidii*. It is possible that the sponge interfered with mussel settlement in some way (mechanical or chemical), because sponges and mussels can be in competition for similar resources.

Mussel Settlement after 1 year (2014) at Davis Dam

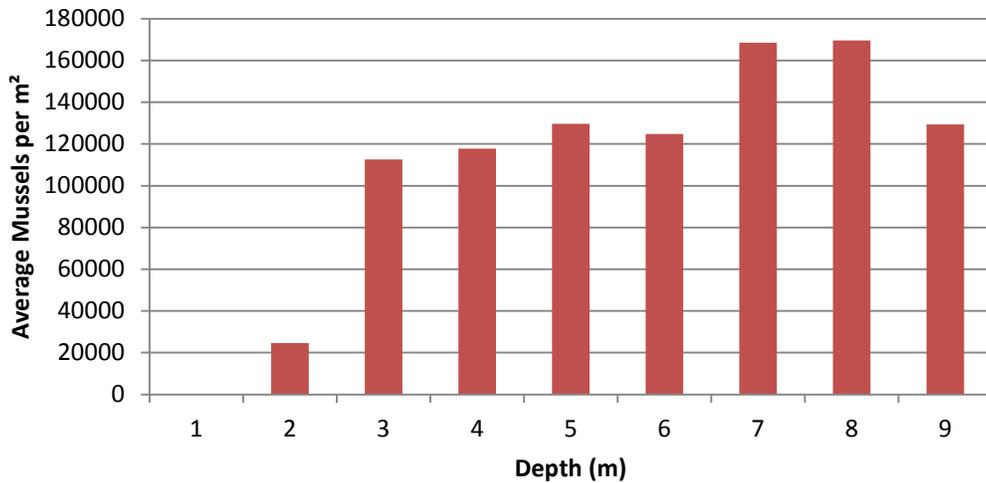


Figure 32. Average mussel settlement per m² on settlement plates at 1-9 meters in depth in the Davis Dam forebay (Lake Mohave NV/AZ). Settlement was analyzed after plates were deployed for one year, from February 2013-February 2014.

Mussel Settlement after 1 year at Imperial Dam

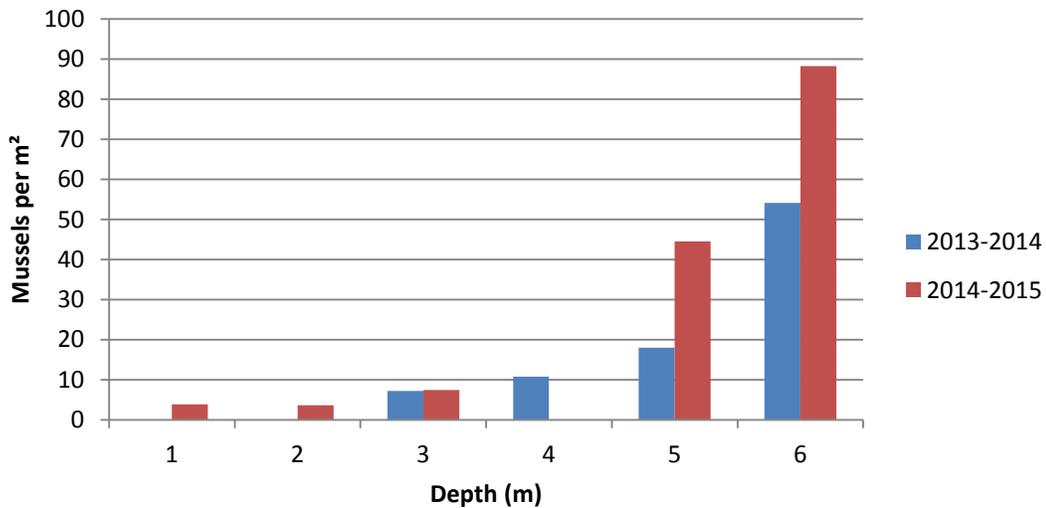


Figure 33. Average mussel settlement per m² on settlement plates at 1-6 meters in depth in the Imperial Dam forebay (CA/AZ). Settlement was analyzed after plates were deployed for one year (February 2013-February 2014 and February 2014-February 2015).

Quagga Mussel Settlement after 1 year in CO River

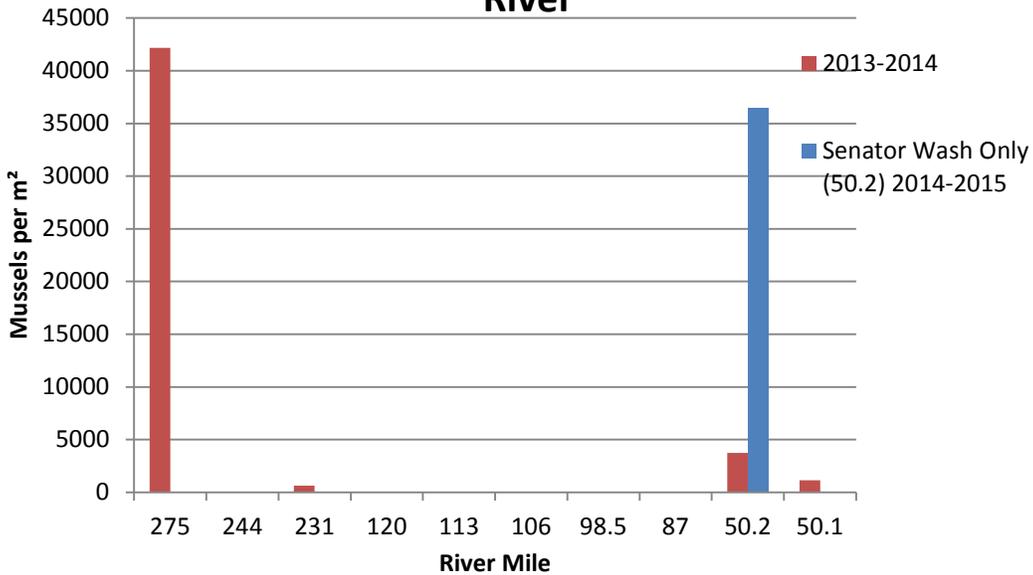


Figure 34. Average mussel settlement per m² on settlement substrates deployed in the lower Colorado River from river mile 275-87 and at Senator Wash (50.2) and Senator Wash pump intake (50.1). Settlement was analyzed after substrates were deployed for one year (February 2013-February 2014). Settlement at Senator Wash was also analyzed after an additional year (February 2014-February 2015).

Settlement Substrates: Mussel Size

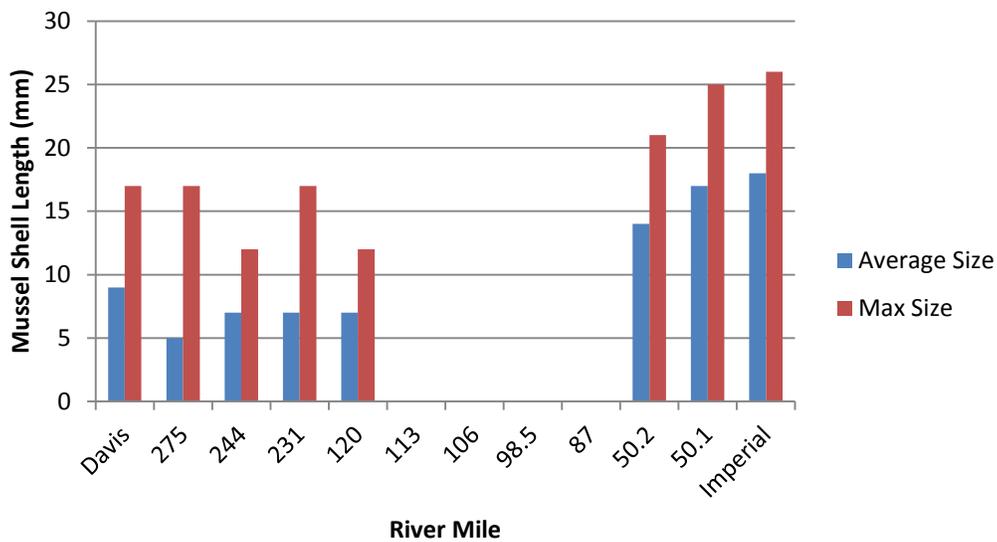


Figure 35. Average and maximum mussel shell length (mm) on settlement substrates deployed for one year in the Lower Colorado River.

Average Monthly Temperature at Davis and Imperial Dams (surface, 6m, and 9m)

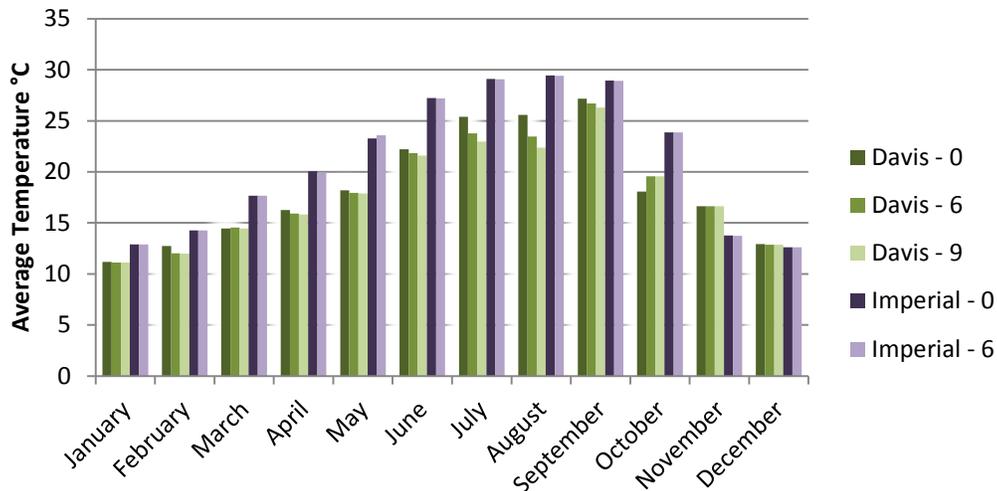


Figure 36. Average monthly temperature in the forebay at Davis and Imperial Dams during 2013, 2014, and 2015. The average surface (0) temperature is shown for both sites during each month. Average temperature is also shown at the depths of the deepest settlement plate (Imperial= 6 meters and Davis= 9 meters) to show how temperature changes with depth at each site.

Biobox Conductivity Study at Davis Dam:

The goal of this study was to increase conductivity values at Davis Dam to simulate values recorded at Imperial Dam to determine if higher conductivity impacts adult mussel survival and settlement. Dripping a salt solution into a biobox at Davis Dam increased the conductivity to 1.178 ms/cm during test 1 (Figure 37) and 1.200 ms/cm during test 2 (Figure 38). The conductivity levels achieved during these two tests were comparable to conductivity observed at Imperial Dam (Figure 26). Average conductivity in the control biobox during test 1 was 0.992 ms/cm (Figure 37) and 1.002 ms/cm during test 2 (Figure 38). Other water quality parameters stayed consistent between the control and treated bioboxes throughout the duration of the studies. Mussel settlement was reduced by 22% in the first test and 30% in the second test (Table 4). During each test, 20 adult mussels, per biobox were observed for mortality. No adult mortality was observed in either control or treated biobox at the end of each month-long test.

Test 1: Biobox Conductivity

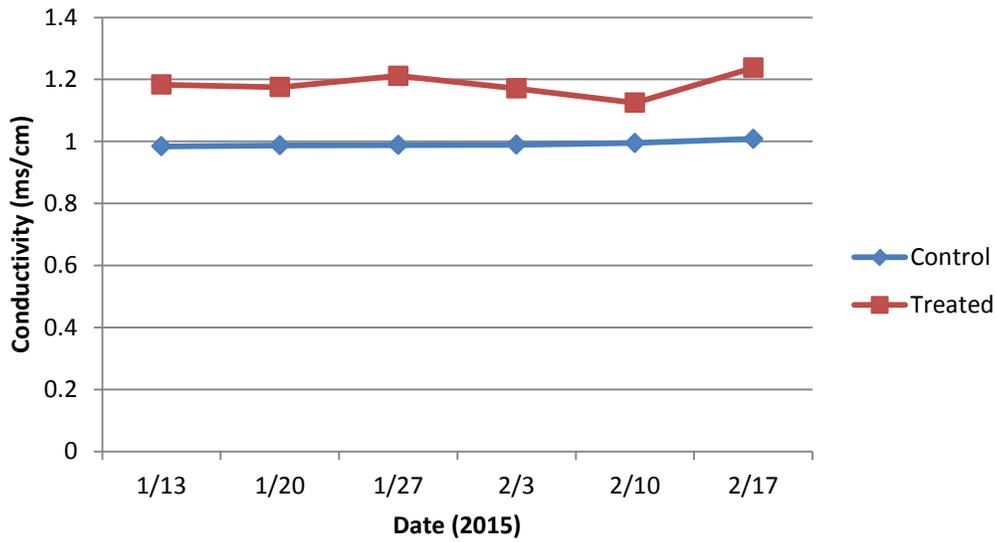


Figure 37. Control and salt treated biobox conductivity during the first settlement test (January-February 2015) at Davis Dam.

Test 2: Biobox Conductivity

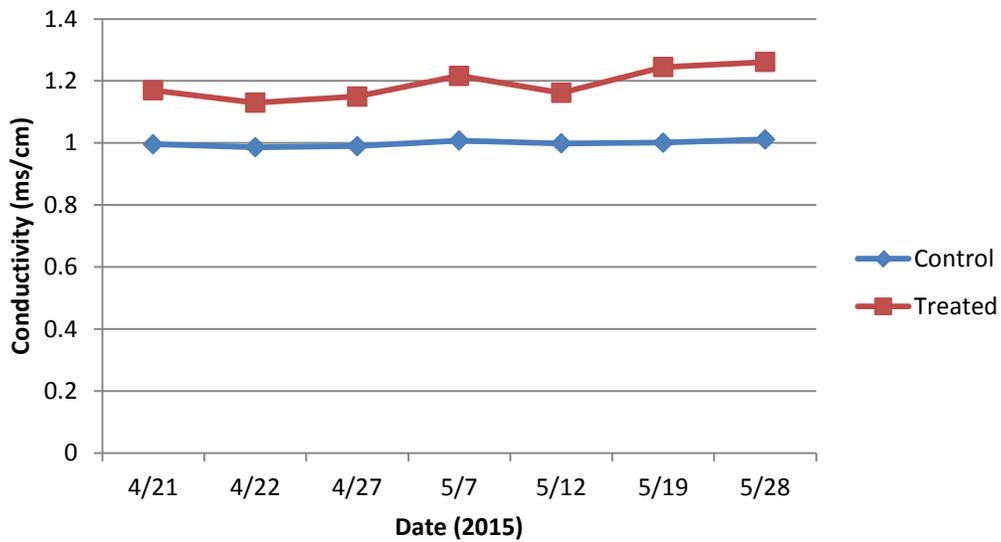


Figure 38. Control and salt treated biobox conductivity during the second settlement test (April-May 2015) at Davis Dam.

Table 4. Total mussel settlement in control and treated (increased conductivity) bioboxes during two, one month settlement tests conducted at Davis Dam.

	Total Mussels	% Reduction	Test Dates
Test 1			Jan-Feb 2015
Control	58	22%	
Treatment	45		
Test 2			April-May 2015
Control	10,265	30%	
Treatment	7,208		

Conclusions

Quagga mussel veliger abundance in the Colorado River was greatest at Northern sites, and progressively decreased at Southern sites. Throughout the year, quagga mussel veliger counts were consistently greater at sites below Davis Dam (river mile 275) to below Parker Dam (river mile 191.75). Mussels were able to successfully settle and grow within the river at these sites. Veliger abundance decreased, and was found to be similar at “mid-river” sites below the 1-10 bridge (river mile 120) in Blythe and just downstream near the Oxbow bridge (river mile 98.5). Very little settlement was observed at these sites.

It is difficult to directly compare Senator Wash, Imperial Dam, and Pilot Knob, drop structure sites to other sites because they are more static than river sites. In theory, these static sites would provide good opportunity for mussel settlement and reproduction, which should correspond to higher veliger numbers. However, these sites generally had the lowest monthly veliger abundances. Veliger numbers were low at Senator Wash throughout most of the year with major peaks in the early spring (March, April and May). The veliger peaks at Senator Wash do not correspond to peaks at upstream Reservoirs (Lake Mead, Mohave, and Havasu) in the late Summer, suggesting that there is an established breeding population in Senator Wash. Water temperatures at Senator Wash begins to increase early in the Spring and is near the mussels upper thermal limits, according to Mackie and Claudi 2010, throughout the Summer, which may limit reproduction. The depth of Senator Wash is variable throughout the year and the temperature remains relatively constant throughout the water column from top to bottom. Mussels at Senator Wash appear to have a peak breeding period in the early spring due to depth fluctuations and warmer temperatures throughout the water column. Although veliger numbers are low throughout the rest of the year, it appears settled mussels are able to survive and grow due to the dense settlement and large shell size found on settlement substrates.

Mussel settlement at Imperial Dam was significantly less dense than at Senator Wash and Davis Dam forebay. Although veliger numbers are low at this site, there is a constant replenishment of veligers from upstream sites as it flows past the dam. The reduced settlement at Imperial Dam indicates that either the water quality is less suitable for mussel survival and growth, mussels entering the Imperial Dam forebay have experienced high mortality due to upstream factors

like turbulence, or the highly managed water conveyance and associated flow rates are impacting the mussel's ability to settle. Interestingly, mussels that are able to settle are significantly larger than mussels found at upstream sites. This size difference may be due to reduced competition for resources like nutrients.

Calcium, pH, alkalinity, and total hardness are considered critical "chalk" habitat parameters for mussel survival and growth. Calcium is required for shell production, alkalinity indicates availability of calcium (total alkalinity consists of bicarbonate alkalinity (HCO_3) and carbonate alkalinity (CO_3)), and pH determines the forms of carbon dioxide and the carbonates available. pH values below 8.2 indicate that calcium is available in HCO_3 form, and pH values above 8.2 indicate calcium is available in CO_3 form. Calcium, pH, alkalinity, and total hardness were similar at all sites and do not appear to be limiting mussel settlement at downstream sites.

Mussels feed on planktonic algae, therefore trophic indicators like nutrient parameters (total phosphorous and total nitrogen), chlorophyll *a*, Secchi depth, and dissolved oxygen content can be used as a criteria for predicting dreissenid mussel presence and abundance (Mackie and Claudi 2010). High nutrient, chlorophyll *a*, and surface dissolved oxygen values and low Secchi depth values typically indicate greater algae biomass availability for mussels. Nutrient parameters do not appear to be predictive of veliger population increase at the Colorado River sites observed in this study. The total phosphorous and chlorophyll *a* values observed at sites with significant mussel populations are at levels that, according to Mackie and Claudi (2010), would not support adult survival. Downstream sites appear to have a greater algae biomass available for mussel consumption, but veliger numbers generally decrease at these sites. Nutrient values may also be difficult to interpret in river systems because unlike lakes and reservoirs, rivers are constantly flowing and nutrients are continuously recharged. A single water quality sample may not capture the total amount of nutrients that the mussel is exposed to over time.

Secchi depth values appear to be more predictive of quagga mussel numbers in the LCR, especially when chlorophyll *a* and total suspended solid values are considered. Secchi depth decreases at most downstream sites, and total suspended solids increases. Although decreased Secchi depth can indicate greater algae biomass is can also indicate greater turbidity, which can decrease light penetration, impacting algae growth. Greater turbidity and total suspended solid values can indicate that there is an increased amount of suspended sediment, which has been found to disrupt mussel feeding and can increase mussel mortality due to damage of internal organs (Steele and Wong 2015). It is possible that veligers and settled mussels at downstream sites are greatly impacted by turbidity.

Additionally, mussels have an upper and lower lethal thermal limit, and water temperature directly impacts their physiological activity, including spawning, development, and growth. Quagga mussel survival is thought to be limited above

28°C and below 10°C (Mackie and Claudi 2010). Lakes and reservoirs typically stratify throughout the year, and if surface temperatures reach the mussels upper thermal limit, veligers can survive at deeper depths where temperatures are cooler. Seasonal and diurnal temperatures in river systems follow atmospheric temperatures more closely than lakes and reservoirs. Rivers are usually in a proportional equilibrium with mean monthly air temperature. Because rivers are shallow and turbulent, thermal stratification is not generally an attribute. Atmospheric temperatures did increase at Southern sites and the temperature was always constant throughout the water column. Temperatures at these sites did reach the upper thermal limit, which may have caused mussel mortality.

The results of this study indicate that the increased temperature and total suspended solids (turbidity) at downstream sites are likely contributing to reduced mussel settlement and survival. Conductivity also increases at Southern sites and the biobox experiments conducted at Davis Dam do indicate that increased conductivity levels similar to those observed at Imperial Dam can lead to a slight decrease in settlement, but adult mortality is not likely. Additionally, the amounts and types of contaminants of emerging concern (CEC) generally increased at Southern sites. Although this is an interesting trend, the effects of CEC on mussel settlement and mortality are currently not well understood.

Ordination techniques found that conductivity and TSS were the best fitting environmental variables that corresponded to veliger abundance between sites when considering all months of the year. Conductivity, temperature and DO showed a better fit to trends seen in May- September. DO is likely found to be a significant variable because DO can be correlated with temperature and conductivity. DO decreases in warmer water and with higher conductivity.

There are many variables that need to be considered when attempting to determine habitat suitability for dreissenid mussels. The parameters set out by Mackie and Claudi (2010) provides a good background for making habitat suitability predictions, but cannot be consistently applied to every water system, especially rivers. The results of this study indicate that nutrient parameters may not be predictive of veliger survival and abundance in river systems and that turbidity may significantly reduce survival, even in otherwise ideal habitat conditions. Utilizing one or a few parameters for habitat suitability may not provide a clear understanding of waterbody suitability for mussel colonization. For example, calcium concentration is commonly used as a key indicator of mussel survival and growth, and it was recently discovered that quagga mussel veligers can survive and grow in low calcium (12 ppm) waters (Davis et al. 2015). This finding provides insights into the uncertainty of using a single parameter in assigning establishment risk (Davis et al. 2015). Therefore a complete assessment of site specific water quality, hydrology and geology will hopefully provide a better determination of risk. This case study provides a potential explanation of reduced mussel settlement in the Colorado River near the United States-Mexico border, and while the results are site specific they may help guide assessments at

other locations, while highlighting the importance of considering a variety of habitat variables.

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Appendix

Appendix A: List of contaminants of emerging concern (CEC) monitored in water samples from the lower Colorado River

1,7-Dimethylxanthine	Norethisterone	Ethinyl Estradiol –
Acetaminophen	Sulfameturon,methyl	17- alpha
Albuterol	Oxolinic acid	Ethylparaben
Amoxicillin	Pentoxifylline	Gemfibrozil
Androstenedione	Phenazone	Ibuprofen
Atenolol	Primidone	Iohexal
Atrazine	Progesterone	Iopromide
Bezafibrate	Propazine	Isobutylparaben
Bromacil	Quinoline	Methylparaben
Caffeine	Simazine	Naproxen
Carbadox	Sulfachloropyridazine	Propylparaben
Carbamazepine	Sulfadiazine	Sucralose
Carisoprodol	Sulfadimethoxine	Triclocarban
Chloridazon	Sulfamerazine	Triclosan
Chlorotoluron	Sulfamethazine	Warfarin
Cimetidine	Sulfamethizole	
Cotinine	Sulfamethoxazole	
Cyanazine	Sulfathiazole	
DACT	TCEP	
DEA	T CPP	
DEET	TDCPP	
Dehydronifedipine	Testosterone	
DIA	Theobromine	
Diazepam	Theophylline	
Dilantin	Thiabendazole	
Diltiazem	Trimethoprim	
Diuron Erythromycin	2,4-D	
Flumequine	4-nonylphenol	
Fluoxetine	4-tert-Octylphenol	
Isoproturon	Acesulfame-K	
Ketoprofen	Bendroflumethiazide	
Ketorolac	BPA	
Lidocaine	Butalbital	
Lincomycin	Butylparaben	
Linuron	Chloramphenicol	
Lopressor	Clofibric Acid	
Meclofenamic Acid	Diclofenac	
Meprobamate	Estradiol	
Metazachlor	Estriol	
Nifedipine	Estrone	

Data Sets that support the final report

Share Drive folder name and path where data are stored:

Team (T): ENGR LAB: HYD LAB: RDLES: VELIGER RESEARCH projects:
LC Veliger Study

Point of Contact name, email and phone:

Sherri Pucherelli, spucherelli@usbr.gov, 303-445-2015

Keywords:

quagga mussels, dreissenid mussels, habitat suitability, water quality,
Colorado River

Approximate total size of all files: folder size= 4.45 GB