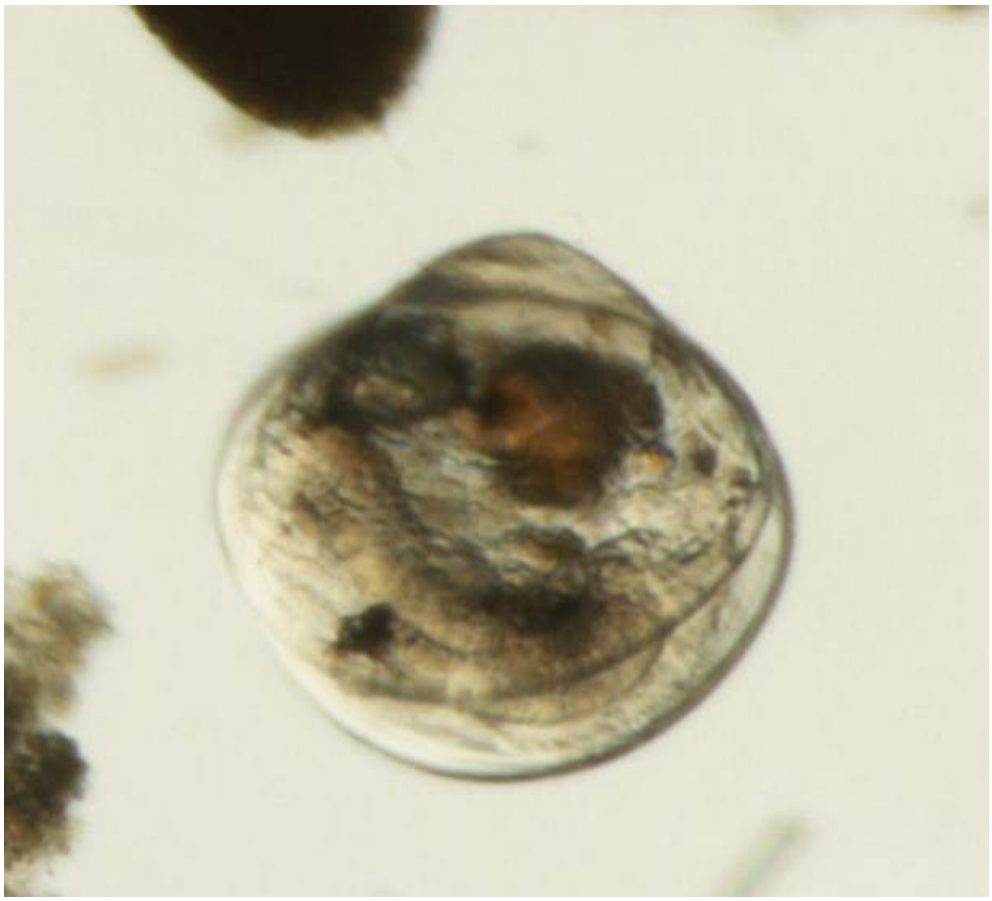
Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Development of an Efficient Method for Removal of Quagga Mussel Veligers from Transport Tanks at Willow Beach National Fish Hatchery



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October 2010

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Lower Colorado River
Multi-Species Conservation Program

Development of an Efficient Method for Removal of Quagga Mussel Veligers from Transport Tanks at Willow Beach National Fish Hatchery

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Multi-Species Conservation Program
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EXECUTIVE SUMMARY

Willow Beach National Fish Hatchery (WBNFH) rears endangered razorback sucker and bonytail for distribution to other fish facilities and repatriation to their native habitat. Upon discovery of quagga mussels at the hatchery in 2007, a treatment protocol using potassium chloride and formalin to remove quagga mussel veligers from fish transport tanks was recommended. Dexter research staff tested the protocol at WBNFH in 2009 and determined that potassium chloride and formalin are not an effective treatment for quagga mussel veligers under the unique water conditions at WBNFH. In 2010, year two of the study, a literature search was conducted to review potential chemicals for testing as alternative treatments. The three chemicals chosen for toxicity tests at WBNFH were Cutrine®-Ultra (copper), Peraclean® 15 (peracetic acid), and Spectrus™ CT1300 (quaternary ammonium compound). Lethality tests were conducted using a time frame (6-7 h) that reflects an average hauling period for stocking fish. Quagga mussel veligers exhibited resistance to most of the concentrations of each chemical tested in the 6-7 h time frame allotted. Mortality in 100% of the veligers was observed only in the two highest concentrations of peracetic acid (30 and 50 mg/L). However, acute toxicity tests were conducted on larval bonytail with 15 mg/L peracetic acid and all fish died within 30 min. The mid-range concentration of Spectrus™ CT1300 tested (25 mg/L) was lethal to bonytail in 5 min even though veliger mortality was only 80% after 6 h. Based on our findings, Cutrine®-Ultra, Peraclean® 15, and Spectrus™ CT1300 are not viable treatment options under the specific protocols used in this study. Veligers withstand most short-term treatments by closing their shells when chemicals are present. We recommend future research focus on developing a pretreatment that will relax the veligers and prevent them from closing their shells and resisting subsequent chemical treatments.

INTRODUCTION

Willow Beach National Fish Hatchery (WBNFH) in Arizona rears razorback sucker, bonytail and rainbow trout, which are transported to other hatcheries for grow-out and stocked into lakes and rivers in the Southwest region. The native fish are reared and stocked as part of the Fish Augmentation Program of the Lower Colorado River Multi-Species Conservation Program (LCR MSCP, Work Task B2). The Fish Augmentation Program also calls for capture of wild razorback sucker larvae from Lake Mohave with subsequent distribution to WBNFH,

Dexter National Fish Hatchery and Technology Center (DNFHTC), and Arizona State Bubbling Ponds Fish Hatchery (BPSH). In 2007 quagga mussels were discovered in Lake Mohave and outdoor raceways at WBNFH. The movement of fish from Lake Mohave and WBNFH is now restricted to waters within the lower Colorado River basin where the presence of quagga mussels is already confirmed. Although this has severely impacted the delivery of fish as prescribed by the LCR MSCP, preventing further movement or transfer of quagga mussels is a priority for all State, Federal and Tribal partners.

Zebra and quagga mussel larvae are planktonic and divided into four basic developmental stages identified by morphology and behavior: trochophore (40-100 µm in diameter), straight-hinged or D-stage (97-112 µm), umbonal (112-347 µm), and pediveliger (231-462 µm). It is these microscopic life stages, collectively referred to as veligers, that can be easily moved with fish that are transferred from mussel-positive waters. Treating transport water with potassium chloride (KCl) at 750 mg/L for 1 h, followed by formalin at 25 mg/L for 2 h, is the standard treatment for zebra mussel veliger larvae (Edwards et al. 2002). Britton and Waclawczyk (2007) included this treatment in their report on quagga mussel control options for WBNFH with the caveat that the efficacy of this treatment on quagga mussels had not yet been determined. Research was conducted by Dexter staff at WBNFH during 2009 to test the efficacy of the KCl/formalin treatment. The results demonstrated that, under the water conditions at WBNFH, quagga mussels are resistant to KCl/formalin treatments at chemical levels that may be toxic to native fish species.

In light of those results, our research for 2010 was redirected to testing alternative chemical treatments. There are a multitude of chemicals listed as effective molluscicides; however, most are used over an extended period of time and are highly toxic to fish (Sprecher and Getsinger 2000). Most acute toxicity studies published to date have been conducted with zebra mussels using standard 24 to 96 hour lethality tests (LC_{50}). Published data on quagga mussel toxicity research is sparse. Addressing the quagga mussel problem at WBNFH requires very specific research goals to ensure endangered fish species can be moved safely and in a timely manner from waters that are positive for quagga mussels. At this time, no facilities are available to hold fish for an extended treatment period. Therefore, our research has been focused on developing a treatment that will remove quagga mussel veligers within a time frame that coincides with an average hauling trip for moving fish from one location to another (6-7 hours).

The objective of the second year of this study was to test alternative treatments for removal of quagga mussel veligers from fish transport tanks with the goal of developing a treatment that will ensure 100% mortality of quagga mussel veligers while having a minimal impact on native fish species. This report presents the findings of the research activities conducted under Work Task C30 of the LCR MSCP.

MATERIALS AND METHODS

Test Organisms – Veligers were collected according to the protocol developed at WBNFH by Sykes (2009). In brief, a 35-µm plankton net fitted with a 1-L capacity code end jar was placed under the inflow at the head of the raceways allowing the water to flow through the net for a minimum of 15 minutes for each collection period. To remove large pieces of algae present in the water supply the content of the collection jar was poured through two stainless steel sieves (250-µm and 212-µm) placed over a 35-µm mesh net, which collected the veligers. The mesh net was then rinsed into a 250 mL sample jar. Veligers were maintained in a 20 ± 1°C water bath until counted into test plates.

A new method was tested for transferring veligers from collection jars to test plates and then to recovery plates. Sample water containing veligers was poured into a petri dish, and individual veligers were collected under a dissecting microscope by using a 20-µL volumetric pipettor. They were then expelled into culture plates containing 8 mL of water. After one hour the veligers were examined under a dissecting microscope for signs of handling stress, loss of equilibrium, damaged shell, or mortality. The same veligers were then handled a second time by transferring them by pipettor to a new culture plate and, after one hour, were checked again for injury. After transferring veligers twice by pipetting, no injuries or mortalities were apparent. The use of pipettors allow for accurate control of the amount of water transferred with the veligers into the bioassay test wells.

Toxicity Testing – Bioassays were conducted in a water bath maintaining a constant temperature of 20 ± 1°C. Life stages of veligers used in the assays ranged from straight-hinged to pediveliger larvae. All toxicity tests were conducted in six-well plastic tissue culture plates (Corning Inc., Corning, NY) containing a volume of 10 mL in each well. Each test was run with six replicates on a plate. Ten veligers were transferred individually with a pipettor in 0.002 mL of water to each well in a culture plate containing 1.98 mL of water, bringing the well volume to

2 mL. After adding veligers to plates, 8 mL of a test chemical was added to each well for a total volume of 10 mL. Culture plates were placed on test tube racks in the water bath for the duration of each test.

Potassium Chloride/Formalin – Toxicity tests were performed to evaluate the effectiveness of the standard treatment protocol of 1 h exposure to 750 mg/L potassium chloride (KCl) followed by 2 h exposure to 25 mg/L formalin in three different levels of water hardness. Undiluted river water (hardness 284 mg/L as CaCO3) was tested against river water diluted with reverse osmosis water to reduce total hardness by 50% (142 mg/L as $CaCO_3$) and 75% (79 mg/L as $CaCO_3$). Veliger mortalities (as based on no movement or response to stimuli) were recorded at the end of the treatment, after which all veligers were moved to fresh water. Veligers were checked again for mortalities at the end of a 1 h recovery period.

Alternative Test Chemicals – A review was made of molluscicides listed in the Zebra Mussel Chemical Control Guide (Sprecher and Getsinger 2000) to determine potential chemicals for toxicity testing on quagga mussel veligers at WBNFH. Three chemicals were chosen and an additional literature search was conducted on their applications: Cutrine®-Ultra (Applied Biochemists, Germantown, WI), an algaecide based on a 9% copper-ethanolamine complex (Kennedy et al. 2006); Peraclean® 15 (Evonik Industries, Parsippany, NJ), a biocide based on 15% peracetic acid (de Lafontaine et al. 2008; Fuchs and de Wilde 2004; Verween et al. 2009); and Spectrus™ CT1300 (GE Betz, Trevose, PA), a molluscicide based on 50% N-Alkyl dimethylbenzyl ammonium chloride (Waller et al. 1993, Fisher et al. 1994). Cutrine®-Ultra concentrations tested were 0.5, 6.25, 15, and 20 mg/L; Peraclean® 15 concentrations were 10, 20, 35, and 50 mg/L; and Spectrus™ CT1300 concentrations were 20, 25, 30, and 37.5 mg/L. All chemical concentrations, expressed as mg/L, were based on percent active ingredient. A recovery period in fresh water for veligers was included for each chemical tested. Mortality data presented in this report were recorded after veligers were allowed a minimum of 1 h recovery in fresh water.

RESULTS AND DISCUSSION

Research conducted in 2009 (results summarized in Table 1) showed that KCl and formalin were ineffective in killing quagga mussel veligers at concentrations tested at WBNFH (Sykes 2009). To test whether water hardness played a role in the efficacy of the treatment,

additional bioassays were conducted in diluted river water with reduced water hardness. In the 50% diluted water (hardness 142 mg/L as $CaCO_3$), 26% of the veligers showed no movement at the end of the treatment. In the 75% diluted water (79 mg/L as $CaCO_3$), 40% showed no movement. However, when veligers were transferred to fresh water and checked after 1 h, 100% of veligers were fully recovered and swimming freely. Therefore, water hardness does not appear to play a significant role in the efficacy of the KCl and formalin treatment on quagga mussel veligers at WBNFH.

Table 1. Summary of results from research conducted in 2009 for chemical treatments, observed mortality and recovery rates of quagga mussel veligers. Mortality rates represent animals recorded with no observable motion at end of treatment. Recovery rates represent the percent of animals with no observable motion that revived when placed in fresh water. Sample size (*N*) based on two replicate beakers.

Test Series	Chemical (mg/L)		Aeration N=no, Y=yes	Mortality (no movement) %	Recovery %	*N*
1	KCl (2250)	Formalin (25)	N	27	na	15
1	KCl (2250)	Formalin (50)	N	25	na	12
1	KCl (2250)	Formalin (100)	N	100	na	17
1	KCl (3500)	Formalin (25)	N	20	na	5
1	KCl (3500)	Formalin (50)	N	13	na	16
1	KCl (3500)	Formalin (100)	N	60	na	15
2	Controls (no chemicals)		N	0	na	20
2	KCl (1500)	Formalin (100)	N	72	na	22
2	KCl (4250)	Formalin (25)	N	39	100	18
2	KCl (4250)	Formalin (50)	N	93	100	15
2	KCl (4250)	Formalin (100)	N	50	100	22
3	Controls (no chemicals)		Y	2	na	20
3	KCl (750)	Formalin (25)	Y	76	38	25
3	KCl (750)	Formalin (200)	N	100	50	10

na - recovery period not included in test

The first alternative chemical tested was Cutrine®-Ultra, a copper-based algaecide with an added emulsifier, surfactant and solvent and was selected because of possible increased toxicity to veligers from the additives. Copper toxicity is water hardness dependent but

monoethanolamine and triethanolamine in Cutrine®-Ultra prevent the precipitation of copper in hard water, allowing the copper to remain active as a biological control. Kennedy et al. (2006) reported a 24 h LC_{50} of 0.012 mg Cu/L for 72-h old zebra mussel larvae (preveliger stage before shell begins forming). Generally, sensitivity of mussels to chemicals decreases with maturing life stages (Fisher et al. 1994). We chose a higher concentration of 0.5 mg Cu/L for preliminary testing on the veliger life stages collected from the raceways and set an initial time limit of 4 h to observe if veligers were stressed. At the end of 4 h there was no sign of physiological stress in any of the veligers. In an attempt to find a concentration that would produce mortality within a 6 h time frame, copper concentrations were increased to 6.25, 15, and 20 mg/L. At 6 h of exposure and after the recovery period, 50% mortality was recorded in the 6.25 mg/L, 80% in the 15 mg/L, and 84% in the 20 mg/L. No higher concentrations were tested because even the 6.25 mg Cu/L is a concentration that exceeds what can be tolerated by native fish species. Hamilton and Buhl (1997) reported a 24 h LC_{50} of 0.305 mg/L copper for larval Colorado pikeminnow *Ptychocheilus lucius* and 0.408 mg/L for larval razorback sucker *Xyrauchen texanus.* As a time-limited treatment, copper does not appear to be an option for treating water in fish hauling tanks.

The second chemical tested was Peraclean® 15 (peracetic acid) and has been used as a disinfectant to control micro-organisms in sewage treatment plants. It easily hydrolyzes into acetic acid and hydrogen peroxide, which are biodegradable by-products (Christiani 2005). Peracetic acid also has undergone extensive testing and has been found effective as a treatment for controlling aquatic species introduced through ship ballast waters in both salt water and fresh water (de Lafontaine et al. 2008; Fuchs and de Wilde 2004). Verween et al. (2009) tested the toxicity of peracetic acid on 4-h old zebra mussel larvae and reported 95% mortality with a 15 min exposure to 3 mg/L. Our initial test included concentrations of 1.25, 2.5, 5, and 10 mg/L peracetic acid for 7 h. After the recovery period, we recorded 11.4% mortality at 1.25 mg/L, 22.7% at 2.5 mg/L, 50% at 5 mg/L, and 70.2% at 10 mg/L. A second test was conducted with 35 and 50 mg/L peracetic acid to determine the length of time to 100% mortality. At 35 mg/L 100% mortality was observed after 4 h of exposure and confirmed with 0% recovery observed after veligers were moved to fresh water. After 2 h of exposure to 50 mg/L, all veligers were dead with no recovery observed in fresh water. Some veligers were affected earlier than the times mentioned above and appeared to be disintegrating from the acid (Figure 1). However, after being placed in fresh water they began showing signs of recovery through cilia movement

(Figure 2) and erratic swimming, another indicator of the importance of including recovery periods with all toxicity work involving quagga mussel veligers.

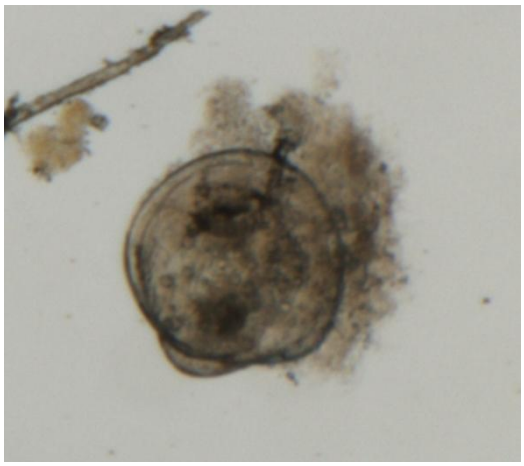

Figure 1. Veliger after exposure to peracetic acid.

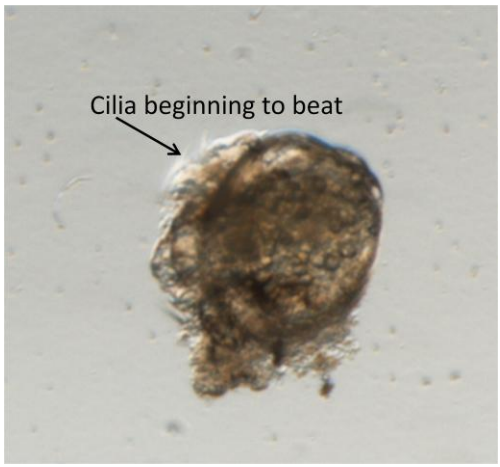

Figure 2. Veliger recovering from exposure to peracetic acid.

To test the lethality of peracetic acid on a native fish species, we conducted a test using 15 mg/L with 30 mm bonytail. All fish were dead within 30 min. Since concentrations below 15 mg/L were not effective in removing all veligers, and fish cannot survive the exposures that will remove veligers, peracetic acid is not a viable option for short-term treatments in fish stocking trucks.

The last chemical tested was Spectrus™ CT1300 (formerly packaged as Clamtrol CT-2), a quaternary ammonium hydrochloride compound (QUAT) approved by the EPA as a mollusk control agent. Toxicity tests were previously conducted by Fisher et al. (1994) and Waller et al. (1993) using Clamtrol CT-1, which contained 8% active ingredient as opposed to 50% in Spectrus™ CT1300 (or Clamtrol CT-2). For zebra mussel, Fisher et al. (1994) reported a 24 h LC_{50} value of 0.175 mg/L QUAT for post-D-stage veligers (composite of umbonal and pediveliger stages) and 8.8 mg/L for plantigrades (early settled juvenile stage). We chose an initial dose of 10 mg/L QUAT for preliminary tests, but observed little adverse response of the veligers to the chemical. Subsequent tests were conducted using 25, 30, and 37.5 mg/L QUAT. At the end of 6 h, 80% mortality was observed in the 25 mg/L, 90% in the 30 mg/L, and 91% in the 37.5 mg/L. In toxicity tests conducted with 30 mm bonytail using 25 mg/L QUAT, all fish

died within 5 min. As observed with the two previous chemicals, Spectrus™ CT1300 should not be considered an effective treatment under the specific testing conditions presented in this study.

In summary, quagga mussel veligers are able to withstand exposure to a copper-based compound, peracetic acid, and QUAT at concentrations that cannot be tolerated by endangered native fish species. Zebra and quagga mussel veligers and adults have sensitive chemoreceptors that allow them to detect chemicals in the water and close their shells to avoid exposure. Finding a chemical that cannot be detected by veligers, is lethal to them in a relatively short time frame, and can be tolerated by fish species is a monumental challenge. Based on the findings of this study, we recommend future research be focused on developing a pretreatment that will relax the veligers and prevent them from closing their shells, subsequently allowing a second chemical to be applied in lethal doses that will not be toxic to fish.

ACKNOWLEDGEMENTS

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EXPENDITURES – FY2010

Salary	$25,716.80
Travel	3,443.73
Supplies/Equipment	5,038.47
17% Indirect Costs	5,813.00
Total	$40,012.00

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