



# Lower Colorado River Multi-Species Conservation Program

*Balancing Resource Use and Conservation*

## Efficacy of Potassium Chloride and Formalin for Removing Quagga Mussel Veligers from Transport Tanks at Willow Beach National Fish Hatchery



November 2009

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National Park Service  
Bureau of Land Management  
Bureau of Indian Affairs  
Western Area Power Administration

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Arizona Game and Fish Department  
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Lower Colorado River RC&D Area, Inc.  
The Nature Conservancy



# **Lower Colorado River Multi-Species Conservation Program**

## **Efficacy of Potassium Chloride and Formalin for Removing Quagga Mussel Veligers from Transport Tanks at Willow Beach National Fish Hatchery**

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**November 2009**

## **ABSTRACT**

Willow Beach National Fish Hatchery (WBNFH) rears 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, operations at WBNFH were modified to prevent further movement of quagga mussels by implementing a fish transport treatment protocol. The standard treatment for removal of zebra mussel veligers from fish transport water is 750 mg/L potassium chloride for 1 h followed by 25 mg/L formalin for 2 h. We tested the efficacy of this treatment on quagga mussel veligers under the water conditions found at WBNFH. No mortality was observed in veligers exposed to the standard treatment without aeration and only 12% mortality occurred in the treatment with aeration. Additional chemical concentrations ranging up to 4250 mg/L potassium chloride and 100 mg/L formalin were tested (without aeration) with 100% recovery observed after veligers were placed in fresh water. Our results show that potassium chloride and formalin are not an effective treatment for quagga mussel veligers under the unique water conditions at WBNFH. Alternative treatments need to be developed that will consistently produce 100% mortality in quagga mussel veligers.

## **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 Bubbling Ponds State 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.

Quagga mussels have a planktonic larval life stage ranging in size from 80 microns to around 400 microns. It is this microscopic veliger 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 protocol accepted for removing zebra mussel veligers (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. The ability to detect and tolerate acute toxin exposure varies greatly among bivalve species (Fisher et al. 1991; Waller et al. 1993; Bidwell et al. 1995) as well as the response even within a sub-species under varying water quality conditions (Wildridge et al 1998; Edwards et al. 2000). To ensure veligers are not passively transferred with fish to bodies of water that are free of quagga mussels, the efficacy of this treatment must be verified for quagga mussels under the unique conditions at individual stations, such as water hardness and temperature. The objective of this study is to determine the efficacy under water conditions at WBNFH of standard KCl/formalin protocols to assure non-transmittal of motile life stages of quagga mussels during fish transport and stocking activities of the LCR MSCP Fish Augmentation Program. This report presents the findings of the research activities conducted under Work Task C30 of the LCR MSCP.

## **MATERIALS AND METHODS**

*Test Organisms* - Various sampling techniques were tested for collecting veligers from hatchery raceways at WBNFH. The most efficient method developed was placing a 35- $\mu$ m plankton net fitted with a 1-L capacity code end jar under the inflow at the head of the raceways and allowing the water to flow through the net for a minimum of 10 minutes for each collection period (Figure 1). Due to the large amount of algae present in the water supply the content of the collection jar was poured through two stainless steel sieves (250- $\mu$ m and 212- $\mu$ m) to remove the larger algae and a 35- $\mu$ m mesh net to collect the veligers (Figure 2). The mesh net was then rinsed into 250 mL sample jars. Minimal mortality was observed in veligers collected with this method. Test organisms were used within five hours of collection.

*Staining technique* - A staining technique developed by Horvath and Lamberti (1999) using neutral red dye to distinguish live and dead mussel veligers was tested. Two 1-L beakers were filled with 500 mL of water with veligers present. One beaker was heated to 50°C to kill

the veligers while the other beaker was kept at ambient temperature. After the heated sample was allowed to cool for 1 h the dye was added to both beakers. In the heated sample all veligers were dead and none absorbed the dye. However, absorption of the dye by live veligers in the unheated sample was highly variable and considered too ambiguous to be used as a clear determination of live or dead veligers (Figure 3). The decision was made not to use the technique.

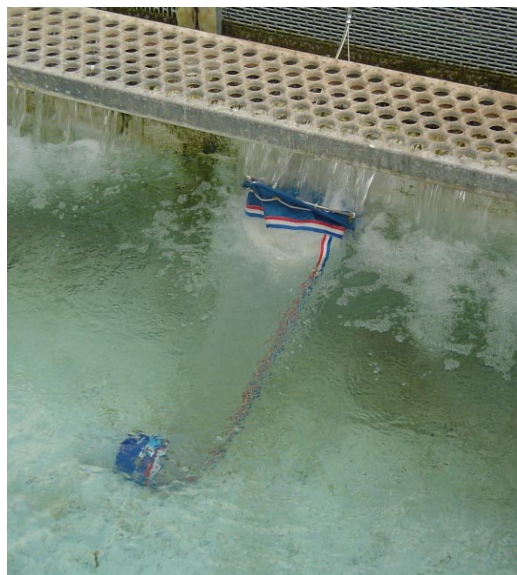
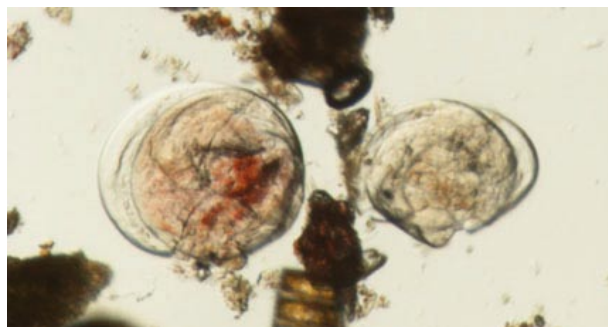


Figure 1. Collecting veligers from hatchery raceways using a 35- $\mu$ m plankton net.



Figure 2. Removal of larger algae from collection sample using a 250- $\mu$ m and a 212- $\mu$ m sieve set above a 35- $\mu$ m mesh net.

Figure 3. Variation in absorption of red dye in two live veligers.



*Testing of treatment protocol on veligers* – In June 2009, preliminary tests with the standard treatment of 750 mg/L KCl for 1 h and 25 mg/L formalin for 2 h were conducted in well water used to fill fish transport trucks at WBNFH. Water quality measurements for

WBNFH river water and well water are provided in Table 1. Additional tests were also conducted using higher levels of KCl and formalin: 1500 mg/L KCl with 25 mg/L formalin; 1500 mg/L KCl with 50 mg/L formalin; 2000 mg/L KCl with 25 mg/L formalin; 2000 mg/L KCl with 50 mg/L formalin. Each treatment was performed with two replicates.

Table 1. Water quality of river water and well water at Willow Beach NFH measured in June 2009.

Water Source	DO	Temperature °C	pH	Conductivity $\mu\text{S}/\text{cm}$	Alkalinity mg/L as $\text{CaCO}_3$	Hardness mg/L as $\text{CaCO}_3$
River	7.89	15.6	8.1	1000	128	310
Well	8.71	19.5	7.9	1404	182	380

Potassium chloride concentrations were based on the active ingredient present in the formulation (99.8%, Cargill Salt, MN). The tests were conducted without aeration in 1000 mL beakers with 500 mL of solution. The KCl was dissolved in 400 mL well water and added to the test beakers. Veligers were collected as previously described, concentrated into 100 mL aliquots and added to the test beakers to bring the solution volume to 500 mL. The test beakers were kept at ambient temperature ( $\sim 22 \pm 1^\circ\text{C}$ ) during the experiments. Controls were run simultaneously with veligers transferred to well water but not exposed to the chemicals to determine if handling or water quality differences between well water and river water causes mortality. At the end of the 1 h KCl treatment, the test solution was poured through a 35- $\mu\text{m}$  mesh net and the veligers transferred to test beakers with 25 mg/L formalin for 2 h. At the end of the 2 h formalin treatment veligers were siphoned into petri dishes and examined under a dissecting microscope. Veligers were recorded as dead if no motion in the velum cilia or internal organs was observed and there was no response to physical touch (Edwards et al. 2000).

In September 2009, another series of KCl and formalin tests were conducted at WBNFH. All tests in this series were conducted in 100 mL beakers with 50 mL of solution with a few modifications to the treatment protocol previously described. The test beakers were placed in an indoor raceway which served as a water bath to maintain a water temperature of approximately  $18^\circ\text{C}$ . Veligers were collected using the same method as before. The sample was poured into petri dishes and veligers individually siphoned out with glass pipets under a dissecting microscope to reduce the amount of organic material present in the treatments. Ten to 15

veligers were counted into 10 mL of water and then added to 40 mL of test solution for each treatment. In this series of tests the veligers were not transferred from the KCl treatment to a formalin treatment. The 25 mg/L formalin was added directly to the KCl treatment which follows the protocol used by WBNFH when treating fish transported from the hatchery. The first test series was without aeration or a recovery period. The second test series was also without aeration but a recovery period was included. The third test series was with aeration and a recovery period. At the end of each treatment veligers were poured into petri dishes and examined under a dissecting microscope to determine mortality. For the second and third test series with recovery periods, veligers recorded as dead were removed from the treatment solution by glass pipette and placed in fresh well water for a minimum of 2 h. The veligers were examined again by the same process after the recovery period. The chemical concentrations, test parameters and results from the first, second and third test series are provided in Table 2. Concentrations of KCl were verified by measuring the conductivity of the solution with a Symphony SP90M5 meter and a four cell conductivity electrode (VWR International, Inc., Brisbane, CA) and interpolating the KCl values from a standard curve calculated from a series of traceable conductivity standards (66.1, 664, and 6637 mg/L KCl).

## **RESULTS AND DISCUSSION**

In the June collections the straight-hinged and umbonal larval stages had the greatest representation in the collection but a few pediveliger larvae were also present. Straight-hinged larvae are D-shaped, range in size from 39-71  $\mu\text{m}$  in shell length, and swim in a circular motion. The umbonal larval stage has a more rounded shape and ranges in size from 39-221  $\mu\text{m}$ . The presence of the velum is also first observed in the umbonal stage. Pediveliger larvae, ranging in size from 150-228  $\mu\text{m}$ , appear more clam-shaped and the foot is present at this stage. In September the pediveliger stage was the most dominant stage with fewer umbonal larvae and no straight-hinged larvae observed. However, the last day of sampling followed an intense wind storm and drop in ambient temperature and the number of larger pediveligers collected was greatly reduced. Smaller umbonal larvae became the dominant stage but in much lower numbers.

In the tests conducted in June all veligers exposed to the 750 mg/L KCl and 25 mg/L formalin survived. The test was repeated a second time with identical results of no mortality. In



Table 2. Chemical treatments and 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 with 10-15 veligers.

Test Series	Chemical (mg/L)	Aeration N=no, Y=yes	Mortality	Recovery	<i>N</i> <sup>a</sup>
			(no movement) %	%	
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

<sup>a</sup> – sample numbers varied because not all animals were recovered during transfer from test beakers to petri dishes.

addition there were no mortalities in the 1500 mg/L KCl tests with either 25 or 50 mg/L formalin and only one mortality in each of the 2000 mg/L KCl tests with 25 and 50 mg/L formalin.

For the September studies chemical concentrations were chosen to fill in gaps from the June study as well as to test variations in the KCl and formalin combinations. In addition, a test was conducted with the standard treatment of 750 mg/L KCl and 25 mg/L formalin using well water from DNFHTC to determine if an unknown substance might be present in well water from WBNFH that may be reducing the toxicity of KCl and formalin to quagga veligers. A different lot of KCl and formalin was also used. However, no mortalities were observed in the DNFHTC well water with the new chemicals demonstrating that the lack of treatment effect was not due to a specific component of WBNFH water quality or test chemical quality.

Across the range of tests conducted none produced 100% mortality in quagga mussel veligers (Table 2). In the first test series the 2250 mg/L KCl and 100 mg/L formalin treatment was recorded as producing 100% mortality; however, after observing the results from the recovery period in the second and third test series it is doubtful that the first test series protocol provided an accurate assessment of lethality. The recovery period was included after a physical response difference was noted in the observed mortalities from the first test series. Some of the veligers recorded as dead were gaping with their velum exposed while others remained tightly closed but exhibited no cilia beating or movement of visible organs (Figure 4). After the inclusion of a recovery period, we observed the veligers recorded as dead that were gaping with an exposed velum did not recover when placed in fresh water. However, almost 100% of those initially recorded as dead with shells tightly closed recovered after being transferred to fresh water.

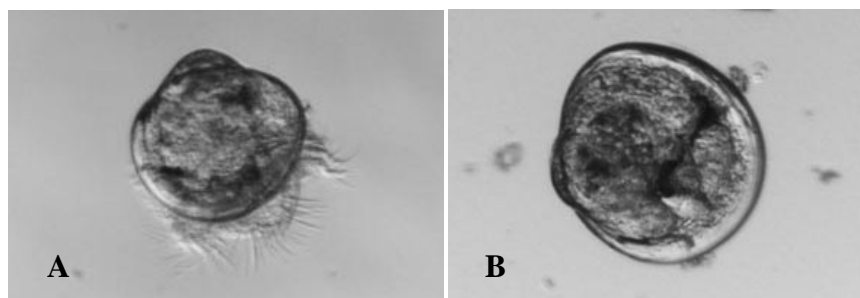


Figure 4. A – Veliger recorded as dead gaping with velum exposed. B – Veliger recorded as dead with no observable motion but shell tightly closed.

Bivalves have sensitive chemoreceptors that allow them to detect chemicals in the water and adults may keep their shells closed for up to two weeks to avoid exposure. Therefore, a recovery period appears to be a critical component of any toxicological research involving bivalves. Wildridge et al. (1998) observed in their study of  $K^+$  acute toxicity to adult zebra mussels that mortality could not be assessed by lack of movement or response to tactile stimulation. They reported a five-fold lower estimate of lethality after a 96 h recovery period. In both KCl/formalin studies performed by Edwards et al. (2000, 2002) on zebra mussel veligers a recovery period was not included. Although no specific studies have addressed this issue in mussel larvae, our study demonstrates that quagga mussel umbonal and pediveliger larvae are capable of withstanding exposure to potassium and formalin for a short period of time. Adding

aeration to the treatment does appear to make the veligers more vulnerable to the chemicals. No mortalities were observed in the 750 mg/L KCl:25 mg/L formalin treatment without aeration while 12% confirmed mortality after the recovery period was observed in the same treatment with aeration. However, even KCl concentrations almost six times greater (4250 mg/L) and formalin concentrations four times greater (100 mg/L) than those identified in the standard protocol written for WBNFH (Britton and Waclawczyk 2007) did not achieve the desired 100% mortality. In addition, KCl acute toxicity tests conducted at DNFHTC on razorback suckers and bonytail demonstrated that concentrations at that level may have negative impacts on the physiology of several life stages of these fish. Treatment protocols to be adopted at WBNFH need to eliminate 100% of the quagga mussel veligers but this was not observed with KCl and formalin under the test conditions at WBNFH.

Based on these findings we recommend that no fish be moved from WBNFH to quagga-free waters or facilities until additional research can be completed. The initial focus of this research project in year two was to test the efficacy of the KCl/formalin protocol under actual stocking conditions with fish in a transport truck. In light of the current results, we suggest that the study be redirected to developing alternative treatment methods to establish a protocol that is effective at killing quagga mussel veligers at WBNFH while having minimal impact on the native fish species being cultured. Development of a treatment method capable of consistently removing 100% of mussel veligers is vital in order to maintain the effectiveness of the LCR MSCP Fish Augmentation Program.

### **ACKNOWLEDGEMENTS**

Funding for this project was provided by the U.S. Bureau of Reclamation in partial fulfillment of a cooperative agreement with the Lower Colorado River Multi-Species Conservation Plan under Work Task C30. Sincere thanks goes to all the staff at Willow Beach National Fish Hatchery for their support and assistance, and to Renee Martin and Wade Wilson for their technical assistance with the research and Mike Childs for his background research on the subject.

## EXPENDITURES – FY2009

Salary	\$22,653.60
Travel	1528.36
Supplies/Equipment	8917.44
Utilities	518.55
Total	\$33,617.95

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