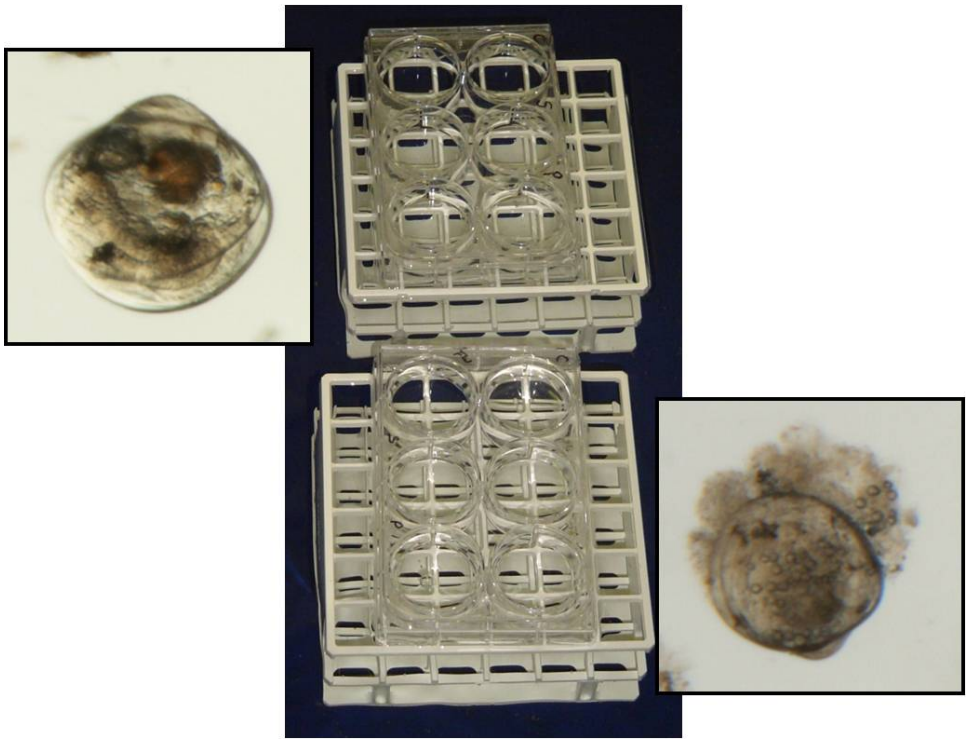
Lower Colorado River Multi-Species Conservation Program

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 – Interim Report



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Multi-Species Conservation Program

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

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Lower Colorado River
Multi-Species Conservation Program
Bureau of Reclamation
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The goal of this study is to develop a treatment protocol for transport tanks moving fish from mussel-positive waters that will ensure 100% mortality of quagga mussel veligers while having a minimal impact on native fish species. The following are specific project objectives to accomplish this goal:

1. Perform a literature search on physiological stress responses of mussel species to chemical treatments.
2. Develop alternative treatment protocols and conduct tests using those protocols in a laboratory setting on quagga mussel veligers at WBNFH to determine lethality.
3. Test protocols found to produce 100% mortality of veligers from Objective 2 on endangered fish species to determine the effect of the treatment on fish.
4. Based on the results from Objective 3, conduct tests to evaluate lethality of treatment protocols under normal transport conditions with fish present.
5. Make management recommendations for hauling protocols to agencies transporting fish from quagga-positive waters.

Objectives 1, 2, and part of 3 were accomplished in FY2011. An extensive literature search was conducted on physiological responses of bivalves to chemical treatments. The search focused on responses of shell gaping, sedation, and intoxication to compile a list of potential pretreatments aimed at preventing veligers from closing their shells when a molluscicide is added to the water. The results from that literature search are presented in Table 1. A second literature search was conducted on the chemicals listed in Table 1 to determine their use and effect on fish species (Table 2). Most of the chemicals found in the literature searches are not regulated for use in the U.S. or were already reported as having higher toxicity levels to fish species than bivalves. From that list the six chemicals chosen to be tested on quagga mussel veligers based on use with fish species, availability of the chemical, and human health hazards were benzocaine, clove oil, magnesium chloride, menthol, a clove oil/menthol mix, and propylene phenoxytol.

The chemicals were tested at Willow Beach National Fish Hatchery (WBNFH) during August 2011. Veligers were collected according to the protocol developed at WBNFH by Sykes (2009) and the toxicity tests were conducted following protocols modified from Sykes (2010). Bioassays were conducted at the current river water temperature of 17 ± 1°C with the test plates maintained at that temperature in a water bath. Life stages of veligers used in the assays ranged from straight-hinged to pediveliger larvae. All toxicity tests were conducted in 10 mL six-well

plastic tissue culture plates (Corning Inc., Corning, NY) and run in either duplicate or triplicate on a plate with an average of 10 veligers per well. All chemicals were tested using multiple time frames and concentrations with formalin chosen as the molluscicide to be tested in combination with the chemicals. Determining veliger mortality during a chemical treatment is not possible because of the resiliency of quagga mussel veligers. Even when they appear to be disintegrating they have the ability to recover when placed in fresh water. Therefore, the toxicity tests were designed with multiple times and concentrations and percent mortality was determined after a minimum of 24 hours recovery in fresh water.

None of the chemicals appeared to have an anesthetic effect, but veliger mortality was observed at varying degrees in some of the concentrations of all six chemicals with and without the addition of formalin (Table 3). For the purpose of determining the minimum chemical exposure required for veliger mortality, if 100% mortality appeared to have been reached within the specified time frame of the pretreatment then formalin was not added and the veligers were moved to fresh water to be monitored for recovery. Four of the six chemicals (menthol, clove oil/menthol mix, magnesium chloride, and propylene phenoxytol) produced 100% mortality in quagga mussel veligers but only at relatively high concentrations within the specified time frames of each test. However, a high degree of physical deterioration was observed among the surviving veligers in the lower concentrations of $MgCl_2$.

Preliminary acute toxicity tests with those four chemicals were then conducted at Dexter National Fish Hatchery and Technology Center (Dexter) on juvenile humpback chub. The tests were run without replication due to a limited number of test animals. Menthol, the clove oil/menthol mix, and propylene phenoxytol were found to be highly toxic, killing all the fish in less than 30 minutes in the concentrations needed to kill mussel veligers. For the $MgCl_2$ tests, the fish were exposed to 5 and 10 g/L even though in the initial veliger toxicity test 100% mortality was observed only in the $\geq$ 20 g/L $MgCl_2$ concentrations. The fish exposed to 10 g/L $MgCl_2$ were visibly stressed within 4 hours, lost equilibrium by 7 hours, and the first mortality occurred at 8 hours; however, the fish were able to tolerate 5 g/L $MgCl_2$ for 72 hours without visible signs of stress.

A second round of veliger toxicity tests was conducted at WBNFH in September using 10 mL tissue culture plates as previously described with each chemical tested in duplicate. Additional tests were run with $MgCl_2$ and the clove oil/menthol mix to determine the treatment time required to achieve 100% mortality at lower concentrations. Chloramine-T, Dimilin

(diflubenzuron), malachite green/formalin solution (7.6 g malachite green in 1 gal formalin), and praziquantel were also tested based on their use in aquaculture as biocides. The last chemical, Catch and Release® (Sure-Life Laboratories™ Corp, Seguin, TX), was tested in response to a communication from the Oregon BASS Club concerning the product's claim to kill quagga and zebra veligers. In addition to the chemicals trials, one test plate with 8-15 veligers in each of six wells containing untreated river water was maintained for 96 h as a control for handling and environmental stress. Low veliger mortality (4.6%) was observed in the control plate over the 96 h.

The tests with Catch and Release®, chloramine-T, clove oil/menthol mix, Dimilin, malachite green/formalin solution, and praziquantel were ended at the time point when all veligers appeared to be dead, at which point the veligers were moved to fresh water. If recovery of veligers moved to fresh water takes place, it most often occurs within the first 24 hours. However, veligers exposed to the malachite green/formalin treatment exhibited greater physical deterioration as compared to veligers from the other chemical treatments. To ensure a more accurate assessment of veliger mortality, the recovery period was extended to a minimum of 48 h. Only the 22 h treatment of 2 g/L Catch and Release® combined with 100 mg/L formalin produced 100% mortality after the recovery period (Table 4). Due to the difficulty of accurately determining mortality during the treatments, the $MgCl_2$ tests were set up with predetermined time periods using varying concentrations of $MgCl_2$ either alone or with formalin added. The lowest concentration of $MgCl_2$ (with or without formalin) that produced 100% veliger mortality was 5 g/L (Table 5). Some of the $MgCl_2$ treatment recovery periods were also extended to 48 h, but the recovery periods for the 10 to 15 h treatments were ended at 18 h due to time constraints. Considering the extremely deteriorated condition of all the veligers within those treatments it is highly unlikely that any would have recovered; however, those tests should be repeated with longer recovery periods to confirm the results. One observation noted in the $MgCl_2$ tests (Table 5), as well as other chemical treatments (Table 3), was the variable results in the treatments with formalin added. These results suggest formalin may have an antagonistic effect in the presence of other chemicals.

In summary, based on these results and the preliminary fish toxicity tests, $MgCl_2$ has shown the most promise to date for a potential treatment to be used for quagga mussel veligers. In FY2012, further research to define the lowest concentration of $MgCl_2$ required to produce 100% veliger mortality needs to be conducted as well as testing other chemical additives in place

of formalin for potential synergistic effects. Another area for consideration is monitoring the survivability of veligers after exposure to $MgCl_2$. Although 100% mortality was not observed at the immediate conclusion of the 1 and 3 mg/L $MgCl_2$ tests, the deteriorated state of the veligers warrants further investigation into their survival over time. In conjunction with continued veliger testing, research on the acute toxicity of $MgCl_2$ to endangered larval fish species needs to be expanded as well as measuring the chronic effects on larval development. In the interest of facilities that need to consider a treatment for fish transport tanks hauling sport fish, Colorado Division of Wildlife (CDOW) is providing assistance in the investigation of regulations on the use of $MgCl_2$ to treat water containing fish intended for consumption.

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Table 1. Results from literature search for anesthetics that have been tested on bivalves.

Chemical	Species	Citation
2-phenoxyethanol	queen conch	Acosta-Salmon and Davis (2007)
	abalone	Bilbao et al. (2010)
	prawns	Coyle et al. (2005)
	oysters	Mamangkey et al. (2009)
	oysters	Norton et al. (1996)
	abalone	White et al. (1996)
Aqui-S	prawns	Coyle et al. (2005)
aspirin	scallops	Heasman et al. (1995)
benzocaine	queen conch	Acosta-Salmon and Davis (2007)
	oysters	Acosta-Salmon et al. (2005)
	abalone	Aquilina and Roberts (2000)
	scallops	Heasman et al. (1995)
	abalone	Hooper et al. (2011)
	oysters	Mamangkey et al. (2009)
	muricids	Noble et al. (2009)
	oysters	Norton et al. (1996)
	oysters	Suquet et al. (2009)
chloral hydrate	oysters	Culloty and Mulcahy (1992)
	scallops	Heasman et al. (1995)
	oysters	Norton et al. (1996)
clove oil/eugenol	abalone	Bilbao et al. (2010)
	prawns	Coyle et al. (2005)
	oysters	Mamangkey et al. (2009)
	oysters	Norton et al. (1996)
	prawns	Saydmohammed and Pal (2009)
	oysters	Suquet et al. (2009)
EDTA	abalone	White et al. (1996)
ethanol	scallops	Heasman et al. (1995)
	muricids (sea snail)	Noble et al. (2009)

Table 1. (continued)

Chemical	Species	Citation
isobutynol	scallops	Heasman et al. (1995)
ketamine	clam	Jamieson and Lander (1984)
	snails	Martins-Sousa et al. (2001)
magnesium chloride	queen conch	Acosta-Salmon and Davis (2007)
	rock oyster	Butt et al. (2008)
	oysters	Culloty and Mulcahy (1992)
	sea urchin	Hagen cited in Acosta-Salmon and Davis (2007)
	scallops	Heasman et al. (1995)
	muricids (sea snail)	Noble et al. (2009)
	oysters	Norton et al. (1996)
	oysters	Suquet et al. (2009)
magnesium sulphate	oysters	Culloty and Mulcahy (1992)
	scallops	Heasman et al. (1995)
	abalone	White et al. (1996)
menthol	queen conch	Acosta-Salmon and Davis (2007)
	oysters	Mamangkey et al. (2009)
	oysters	Norton et al. (1996)
	prawns	Saydmohammed and Pal (2009)
metomidate	scallops	Heasman et al. (1995)
MS222	queen conch	Acosta-Salmon and Davis (2007)
	abalone	Aquilina and Roberts (2000)
	scallops	Heasman et al. (1995)
	oysters	Norton et al. (1996)
phenoxy ethanol	scallops	Heasman et al. (1995)
procaine hydrochloride	abalone	White et al. (1996)
propylene phenoxetol	oysters	Acosta-Salmon et al. (2005)
	abalone	Aquilina and Roberts (2000)
	oysters	Mamangkey et al. (2009)
	oysters	Norton et al. (1996)

Table 1. (continued)

Chemical	Species	Citation
quinaldine	prawns	Coyle et al. (2005)
	scallops	Heasman et al. (1995)
serotonin (5-HT)	zebra mussel adults	Fong (1998)
	zebra mussel adults	Kennedy et al. (2006)
	zebra mussel adults	Ram et al. (1999)
sodium bicarbonate	oysters	Norton et al. (1996)
sodium pentobarbital	abalone	Aquilina and Roberts (2000)
	oysters	Culloty and Mulcahy (1992)
	scallops	Heasman et al. (1995)
	snails	Martins-Sousa et al. (2001)
	muricids (sea snail)	Noble et al. (2009)
	oysters	Norton et al. (1996)
	abalone	Sharma et al. (2003)
tertiary amyl alcohol	scallops	Heasman et al. (1995)
valium	scallops	Heasman et al. (1995)

Table 2. Results from literature search on effects of anesthetics on fish species.

Chemical	Species	Citation
2-phenoxyethanol	sea bass	Basaran et al. (2007)
	dusky kob	Bernatzeder et al. (2008)
	carp	Dziaman et al. (2010)
	rainbow trout	Gilderhus and Marking (1987)
	sea bass	King et al. (2005)
	sea bream	Tsantilas et al. (2006)
	trout	Ucar and Atamanalp (2010)
	trout	Velisek et al. (2011)
	perch	Velisek et al. (2009)
	sole	Weber et al. (2009)
	cod	Zahl et al. (2009)
Aqui-S	striped bass	Davis and Griffin (2004)
	atlantic salmon	Iversen et al. (2003)
	channel catfish	Small (2004)
	channel catfish	Small and Chatakondi (2005)
	striped bass	Woods III et al. (2008)
benzocaine	codling	Bolasina (2006)
	rainbow trout	Cotter and Rodnick (2006)
	rainbow trout	Gilderhus and Marking (1987)
	carp, rohu	Hasan and Bart (2007)
	catfish	Hayton et al. (1996)
	carp	Heo and Shin (2010)
	salmon	Iversen et al. (2003)
	atlantic salmon	Kiessling et al. (2009)
	rainbow trout	Stehly et al. (1998)
	cod	Zahl et al. (2009)
	halibut	Zahl et al. (2010)
chloral hydrate	mullet fry	Durve (1975)
	tilapia	Lanzing (1971)
clove oil/isoeugenol	largemouth bass	Cooke et al. (2004)
	rainbow trout	Cotter and Rodnick (2006)
	reef fishes	Cunha and Rosa (2006)
	striped bass	Davis and Griffin (2004)
	caspian salmon	Ghazilou et al. (2010)

Table 2. (continued)

Chemical	Species	Citation
clove oil/isoeugenol (*cont.*)	rockpool fishes	Griffiths (2000)
	atlantic salmon	Iversen et al. (2003)
	atlantic salmon	Kiessling et al. (2009)
	sea bass	King et al. (2005)
	rock bream	Park et al. (2009)
	rainbow trout	Sattari et al. (2009)
	striped bass	Sink and Neal (2009)
	trout	Ucar and Atamanalp (2010)
	trout	Velisek et al. (2011)
	perch	Velisek et al. (2009)
	sole	Weber et al. (2009)
	steelhead fry	Woolsey et al. (2004)
	halibut	Zahl et al. (2010)
dimilin	mosquitofish	Draredja-Beldi and Soltani (2003)
	freshwater organisms	Fischer and Hall (1992)
etomidate	carp	Dziaman et al. (2010)
	rainbow trout	Gilderhus and Marking (1987)
ketamine	carp	Al-Hamdani et al. (2010)
magnesium chloride	daphnia	Dowden and Bennett (1965)
	gambusia	Wallen et al. (1957)
menthol	tambaqui	Facanha and Gomes (2005)
	guppy	Pickering et al. (1983)
	tilapia	Simoes and Gomes (2009)
metomidate	koi	Crosby et al. (2010)
	striped bass	Davis and Griffin (2004)
	rainbow trout	Gilderhus and Marking (1987)
	atlantic salmon	Iversen et al. (2003)
	ornamental fish	Kilgore et al. (2009)
	sea bass	King et al. (2005)
	sole	Weber et al. (2009)
	cod	Zahl et al. (2009)
	halibut	Zahl et al. (2010)

Table 2. (continued)

Chemical	Species	Citation
pentobarbital	goldfish	Greizerstein (1979)
praziquantel	carp/shiners	Mitchell and Hobbs (2007)
propylene phenoxetol	tilapia	Lanzing (1971)
	fish	cited in McKay and Hartzband (1970)
quinaldine	striped bass	Davis and Griffin (2004)
	carp, rohu	Hasan and Bart (2007)
	tilapia	Lanzing (1971)
quinaldine sulphate	striped bass	Davis and Griffin (2004)
	rainbow trout	Gilderhus and Marking (1987)

Table 3. Results from acute toxicity tests conducted August 2011 at WBNFH on quagga mussel veligers. Veligers were pretreated with the specified chemical followed by the addition of formalin. Total treatment time is the combination of pretreatment hours and formalin treatment hours. Percent mortality was recorded after a minimum of 24 hour recovery in fresh water. Asterisks denote samples in which surviving veligers were highly deteriorated.

Chemical	Concentration	Pre-treatment Time (hours)	50 mg/L Formalin Treatment Time (hours)	Total Treatment Time (hours)	Percent Mortality	
Benzocaine	100 mg/L	6	15	21	< 100	
	200 mg/L	3	4	7	0	
	400 mg/L	3	4	7	0	
Clove oil	800 uL/L	1	3	4	0	
	1 mL/L	1.5	3	4.5	0	
	2 mL/L	1.5	2.5	4	0	
	2 mL/L	2	2	4	0	
	3 mL/L	2	2	4	0	
	4 mL/L	2	2	4	56	
	2 mL/L	3	2	5	0	
	3 mL/L	3	2	5	15	
	4 mL/L	3	2	5	35	
	2 mL/L	4	2	6	15	
	3 mL/L	4	2	6	0	
	4 mL/L	4	2	6	60	
Magnesium Chloride	20 g/L	1	n/a	1	100	
	40 g/L	1.5	2	3.5	100	
	60 g/L	1.5	1.5	3	100	
	1 g/L	1	3	4	0	
	3 g/L	1	3	4	0	
	5 g/L	1	3	4	0	*
	1 g/L	2	3	5	0	
	3 g/L	2	3	5	0	
	5 g/L	2	2	4	33	*
	1 g/L	3	4	7	0	
	3 g/L	3	3.5	6.5	0	*
	5 g/L	3	2	5	0	*
	1 g/L	4	2.5	6.5	0	
	3 g/L	4	2	6	5	*
	5 g/L	4	2	6	55	*

Table 3. (continued)

Chemical	Concentration	Pre-treatment Time (hours)	50 mg/L Formalin Treatment Time (hours)	Total Treatment Time (hours)	Percent Mortality	
Magnesium Chloride	1 g/L	5	1.5	6.5	0	
(cont.)	3 g/L	5	2	7	0	*
	5 g/L	5	2	7	0	*
	1 g/L	6	2	8	0	
	3 g/L	6	2	8	65	
	5 g/L	6	n/a	6	53	
Menthol	0.05 g/L	6	2	8	0	
	0.1 g/L	6	2	8	0	
	0.25 g/L	6	2	8	0	
	0.5 g/L	6	2	8	0	
	0.75 g/L	6	n/a	6	74	
	1 g/L	1.5	3	4.5	33	
	1 g/L	2	2.5	4.5	100	
	1 g/L	3	n/a	3	100	
	1 g/L	4	n/a	3	100	
	1 g/L	6	n/a	6	100	
Menthol/Clove oil mix	800 uL/L	1.5	2	3.5	100	
Propylene phenoxytol	1.75 mL/L	2.5	3.5	6	0	
	2.5 mL/L	2.5	2	4.5	0	
	2.5 mL/L	5	2.5	7.5	0	
	3 mL/L	2	2	4	60	
	4 mL/L	2	n/a	2	95	
	5 mL/L	2	n/a	2	100	
	3 mL/L	3	2	5	59	
	4 mL/L	3	n/a	3	100	
	5 mL/L	3	n/a	3	100	
	3 mL/L	4	2	6	6	*
	4 mL/L	4	n/a	4	100	
	5 mL/L	4	n/a	4	100	
	3 mL/L	5	2	7	79	
	3 mL/L	6	n/a	6	82	

Table 4. Results from acute toxicity tests conducted September 2011 at WBNFH on quagga mussel veligers. Percent mortality was recorded after a minimum of 48 hour recovery in fresh water. Formalin was present the full treatment time for each of the tests in which it was added.

Chemical	Concentration	Treatment Time (hours)	Percent Mortality
Catch & Release only	1 g/L	20	0
with 50 mg/L formalin	1 g/L	8	60
with 50 mg/L formalin	2 g/L	22	64
with 100 mg/L formalin	2 g/L	22	100
Chloramine-T	20 mg/L	1	0
	20 mg/L	2.5	80
Clove oil mix	50 uL/L	19	0
	100 uL/L	19	<100 [a]
Dimilin (22% formula)	1.25 mg/L	15	0
	2.5 mg/L	15	0
	5 mg/L	15	0
Dimilin (0.10% formula)	100 mg/L	22	0
Malachite Green/Formalin	26 uL/L	20	78
Praziquantel only	20 mg/L	16.5	0
with 50 mg/L formalin	20 mg/L	16.5	0
with 100 mg/L formalin	20 mg/L	2	0

[a] at least one veliger was still alive at 19 hours so test was ended without a recovery period.

Table 5. Results from acute toxicity tests conducted with magnesium chloride ($MgCl_2$) on quagga mussel veligers. Percent mortality was recorded after a minimum of 48 h recovery in fresh water (asterisks denote tests ended at 18 h recovery due to time constraints).

$MgCl_2$ Concentration		**Total Treatment Time (hours)**	**Percent Mortality**	
	without formalin			
5 g/L		10	100	*
5 g/L		12	100	*
5 g/L		13	100	*
5 g/L		15	100	
6 g/L		15	100	
7 g/L		15	100	
	formalin - added at end			
5 g/L	50 mg/L, 2 hours	10	100	*
5 g/L	100 mg/L, 1 hour	10	100	*
5 g/L	200 mg/L, 1 hour	10	100	*
5 g/L	50 mg/L, 2 hours	12	100	*
5 g/L	100 mg/L, 1 hour	12	100	*
5 g/L	200 mg/L, 1 hour	12	100	*
	formalin - for full treatment			
5 g/L	50 mg/L formalin	6	91	
5 g/L	75 mg/L formalin	6	92	
5 g/L	100 mg/L formalin	6	86	
5 g/L	50 mg/L formalin	7	100	
5 g/L	75 mg/L formalin	7	96	
5 g/L	100 mg/L formalin	7	91	
6 g/L	100 mg/L formalin	7.5	100	
7 g/L	100 mg/L formalin	7.5	100	
8 g/L	100 mg/L formalin	7.5	96	
5 g/L	50 mg/L formalin	8	100	
5 g/L	75 mg/L formalin	8	96	
5 g/L	100 mg/L formalin	8	95	