Waller 1995

United States Department of the Interior

NATIONAL BIOLOGICAL SURVEY

MEMORANDUM

Date: March 29, 1995

To: Dick Neves, Unit Leader, Virginia Fisheries and Wildlife Cooperative Research Unit

From: Diane Waller, Upper Mississippi Science Center, La Crosse, Wisconsin

Subject: Unionid treatments for zebra mussels

Dick,
I am attaching a rough (very) draft of a report on the work we completed last year on the unionid treatments. We have some additional testing we need to complete this year and I would really like to attempt some of the more benign treatment possibilities in a field study. Our time schedule on all of this year's work is undetermined so far. Call if you have any questions or SUGGESTIONS. Thanks.

Diane

Diane Waller

PREVENTION OF ZEBRA MUSSEL DISPERSAL DURING TRANSPORT OF UNIONID MUSSELS

Preliminary Report

To:

U.S. Fish and Wildlife Service
Endangered species field office
Asheville, NC

By:

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Introduction

The infestation of native unionid mussels by the exotic zebra mussel *Dreissena polymorpha*, has been well documented (Mackie 1991, 1993; and Schloesser and Kovalak 1991) and is presently recognized as the greatest ecological threat of this invader. The zebra mussel threatens to greatly accelerate species' extinctions, particularly of rare species that are limited to small localized populations in one watershed. In response to the zebra mussel threat, various state, federal, and private agencies are proposing to relocate unionid mussels to sanctuaries. Some "rescue" and research efforts have already been initiated. However, infested unionids are a potential agent for dispersal of the zebra mussel into uninfested waters. In past rescue projects, zebra mussels were removed from unionids by physically scraping or brushing the shells from unionid mussels or quaranting mussels in isolation ponds for several weeks. Cleaning the shells is effective for removing adult zebra mussels, but it does not guarantee removal of microscopic larvae and juveniles that may be undetected in shell ridges, along the mantle edge, or inside the shell. Quarantine ponds may not be available, particularly for large numbers of mussels, and assume that microscopic stages will grow to macroscopic size during the quarantine period.

We evaluated the effectiveness of selected chemical and thermal treatments for preventing transport of zebra mussel larvae and juveniles with unionid mussels. Chemical treatment levels were based on previous studies with fish and two unionid species (Waller et al. 1993; Waller and Fisher 1994). Thermal treatments were based on acclimation equation of Iwanyzki and McCauley (1993).

Methods

The main treatment strategies we are investigating including thermal shock and chemical treatment.

Chemical treatment

The chemicals and treatment levels (Table 1) chosen for evaluation were previously shown to be effective on veliger and early juvenile stages of the zebra mussel (Waller et al. 1995). We eliminated chemicals that required prolonged exposure periods (>24 h), were harmful to the applicator, or were relatively expensive. Static tests were conducted following procedures of the Committee on Methods for Acute Toxicity Testing with Aquatic Organisms (1975). Dissolved oxygen, pH, and temperature were measured daily in each test vessel. Total alkalinity and hardness of the exposure water were measured at the beginning of each test. Tests were rejected if oxygen levels fell below 60% saturation or the control mortality exceeded 10%.

Adult and juvenile zebra mussels were collected from Lake Michigan, near Racine, Wisconsin and transported to the Upper Mississippi Science Center, La Crosse, in chilled insulated coolers. Mussels were held in flowing well water at 10°C and fed live *Ankistrodesmus sp.* daily. Prior to testing, mussels were removed from the stock culture, acclimated to test temperature, and allowed to attach to petri plates of glass dip jars. The petri plate of dip jar with attached mussels was placed into glass jars containing 15 L test water (pH 8.2 $\pm$ 0.5, alkalinity 100 $\pm$ 10mg/L as $CaCO_3$, hardness 140 $\pm$ 10 mg/L as $CaCO_3$) at 12°C or 17°C in a constant temperature water bath.

Three replicates per chemical concentration were tested with 10 mussels per replicate. Mortality was scored after a 48-h postexposure period in untreated water and was defined as failure of a gaping shell to respond to a blunt probe, or failure of a closed shell to resist being pried open and its subsequent failure to reclose.

Veligers were produced by inducing reproductively ripe adult mussels to spawn into filtered lake water (Stoeckel and Garton 1993). Sperm and ova were combined in 1-L glass beakers containing 500 to 800 mL of Lake Erie water. Veligers were used for toxicity tests at 3 d of age. Field collected veligers were not available for testing during the present study. Toxicity tests with veligers were conducted in 10-mL glass beakers containing hard standard reference water (SRW); (pH 8.4±0.2, alkalinity 150±10 mg/L as $CaCO_3$, hardness 180±10 mg/L as $CaCO_3$) at 12°C or 17°C. Test concentrations and a control with 10 replicates per concentration were tested for each chemical. An estimated density of veligers was transferred by automatic pipet into the test beaker containing the test chemical; a minimum of 10 veligers was added to each beaker. Beakers were placed in an environmental chamber at 12°C or 17°C on a photoperiod of 14:10, light:dark for the duration of the exposure. Mortality, defined as cessation of ciliary beating, was counted at the end of the exposure period. The postexposure period was excluded from the veliger testing due to the difficulty separating and transferring exposed veligers to clean water.

Settlers were collected from Lake Erie on 2 cm x 8 cm glass slides. Size ranges of the settlers were as follows: 0.21-0.42 mm (17%), 0.46-1.67 mm (63%), and 1.71-2.79 mm (20%). Excess animals were removed from each slide to obtain 100 to 200 mussels per slide. Mussels were examined under a dissecting microscope to obtain a

ratio of live:dead before exposure. The number dead before exposure was subtracted from the final mortality count to obtain the number dead due to treatment. Toxicity tests were conducted in 1-L glass beakers containing 0.5 L hard SRW at 12°C or 17°C on a photoperiod of 14:10, light:dark. Test concentrations and a control were tested in triplicate for each chemical. Mortality was scored after a 24-h postexposure period in untreated water, and was defined as failure of mussels with gaping shells to respond to the touch of a probe (Waller *et al.* 1993) and lack of ciliary activity.

Unionid mussels (Table 1) were collected from navigation pools 5, 8, 9, and 10 and Lock 7 of the upper Mississippi River. Mussels were maintained in the laboratory under the same conditions as zebra mussels, except that water temperature in unionid tanks was 12°C. Animals were acclimated to the test temperature by increasing water temperature 3°C in a 24-h period. Mortality was recorded after 48 h in untreated water

Thermal Shock

Adult and juvenile zebra mussels were collected from Lake Michigan, near Racine, Wisconsin and transported to the Upper Mississippi Science Center, La Crosse, in chilled insulated coolers. Mussels were held in flowing well water at 10°C and fed live *Ankistrodesmus sp.* daily. Veligers were not tested in the thermal shock treatments.

Fusconaia flava, the Wabash pigtoe, were collected from navigation pools 8 and 9 of the upper Mississippi River. Mussels were maintained in the laboratory under the same conditions as zebra mussels, except that water temperature in unionid tanks was 12°C.

Thermal shock treatments included acute exposure to cold and heat or heat only for various lengths of time (Figure 1). Heat shock was tested at both 35°C and 40°C. Both cold- and warm-acclimated zebra mussels were tested. Additionally, we tested zebra mussels in clusters and separated. We first tested a relatively tolerant unionid species, *F. flava*, to thermal shock to determine if further evaluation with more sensitive species was warranted (Figure 1).

Results

Chemical treatment

Our evaluation of chemical treatments was focused on the three chloride salts because these chemicals were the safest and least expensive treatment alternative of those chemicals that we evaluated. We found that all three salts were effective against veliger and early juvenile mussels at various treatment levels (Table 2). However, we did not find a single treatment that was 100% safe to all of the unionid mussel species that we tested. Furthermore, the toxicity of the salts to unionid mussels varied among species. Generally, thinner-shelled and smaller mussels were more sensitive to salt treatment. The most sensitive species overall were *O. reflexa* and *L. fragilis*. *Pyganodon grandis* also showed significant mortality (22.2%) in 2,5000 mg/L KCl after 24 h, but was not available for testing in other treatments.

Preliminary testing of benzalkonium chloride, formalin, and hydrogen peroxide was completed on juvenile zebra mussels and *F. flava* and *O. reflexa* mussels.

Benzalkonium chloride was very toxic to zebra mussels at 500 mg/L for 15 min, but, *O. reflexa* experienced significant mortality in this chemical. Although *F. flava* mussels survived the exposure period, the mussels showed signs of stress including copious mucus production and abortion of glochidia.

Hydrogen peroxide was ineffective against zebra mussel juveniles at the highest concentration tested (500 mg/L for 1 h), but was safe to *F. flava* at this treatment level. Formalin was effective against zebra mussel veligers and juveniles at the highest treatment level (1667 mg/L for 15 min) and caused minimal mortality to *O. reflexa*. Additional data is needed on formalin toxicity to both zebra mussels and unionid mussels to determine its usefulness as a zebra mussel control chemical.

Table 1. Chemicals tested against zebra mussels and unionid mussels

Compound	Formulation
Benzalkonium chloride	50%
Calcium chloride ($CaCl_2$)	77% powder
Formalin	37% formaldehyde
Hydrogen peroxide	35%
Potassium chloride (KCl)	100%
Sodium chloride (NaCl)	100%

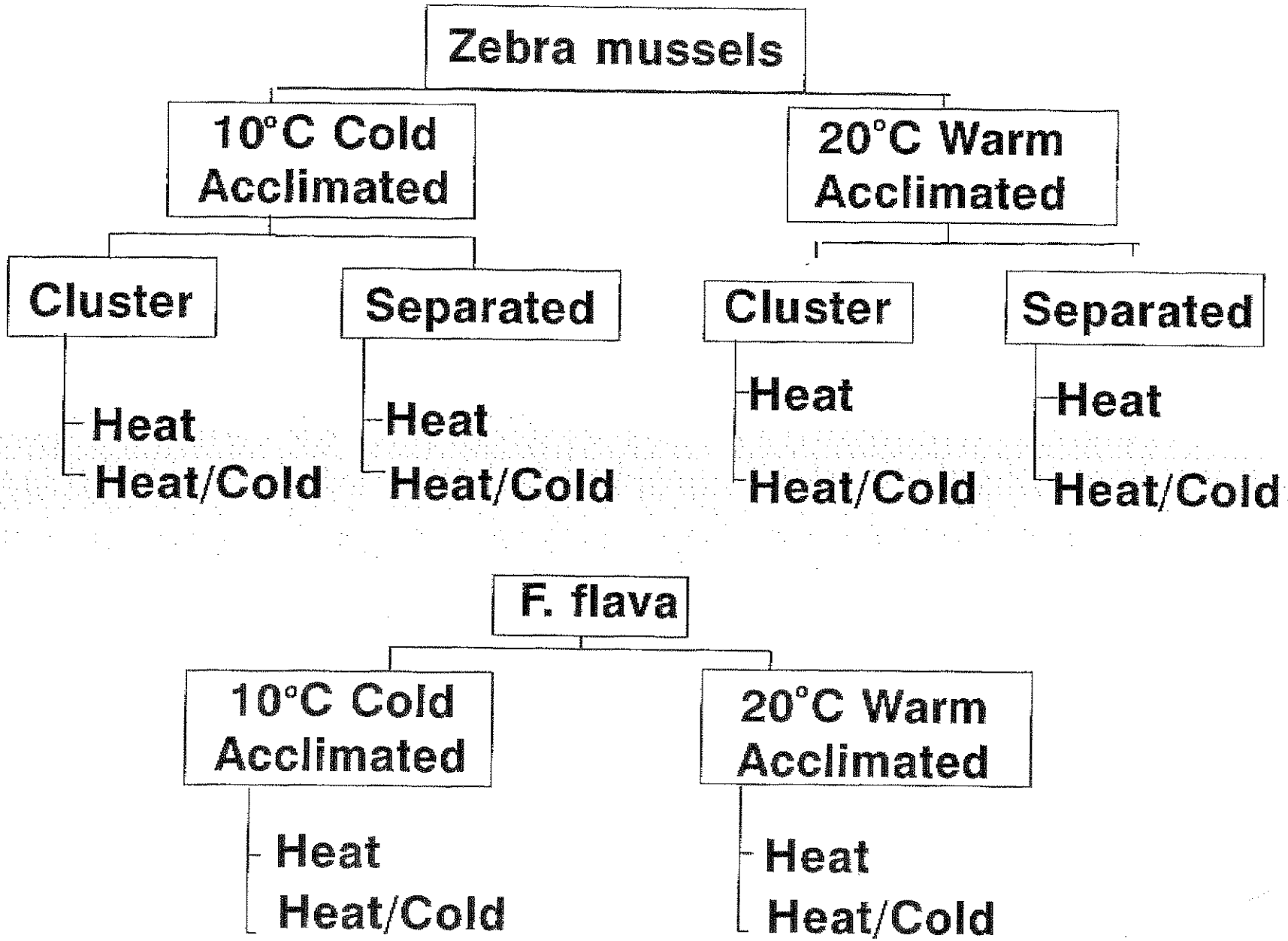
Table 2. Toxicity of chloride salts to veliger and early juvenile zebra mussels and unionid mussels at 17°C.

Treatment	Duration of Exposure (h)	Mean Percent Mortality (S.D.)		Unionid Species	N	Mean Percent Mortality (S.D.)
		Veliger	Early juvenile			
$CaCl_2$ 10,000 mg/L	6[a]	100	99.5 (0.4)	Fusconaia flava	27	0
				Obliquaria reflexa	30	93.3 (9.4)
				Leptodea fragilis	10	0
				Potamilus alatus	10	11.1 (15.7)
				Lampsilis cardium	10	0
				Lamspsilis radiata	9	11.1 (15.7)
KCl 2,500 mg/L	24	100	95.9 (1.8)	Fusconaia flava	58	0
				Elliptio dilatata	10	0
				Pyganodon grandis	10	22.2 (15.7)
				Lasmigona complanata	7	0
				Obliquaria reflexa	15	6.7 (9.4)
				Leptodea fragilis	9	52.8 (17.1)
				Lampsilis cardium	20	0
				Lampsilis radiata s.	10	0
KCl 10,000 mg/L	6		97.0 (1.3)	Fusconaia flava	76	0
				Elliptio dilatata	10	0
				Lasmigona complanata	8	0
				Obliquaria reflexa	45	0
				Leptodea fragilis	11	47.2 (17.1)
				Potamilus alatus	10	22.2 (15.7)
				Lampsilis cardium	20	8.3 (11.8)
				Lampsilis radiata s.	9	33.3 (27.2)
NaCl 10,000 mg/L	24	100[b]	98.1[b] (1.3)	Fusconaia flava	24	29.2 (5.9)
NaCl 20,000 mg/L	6	100	99.2 (0.6)	Fusconaia flava	14	0
				Elliptio dilatata	10	0
				Lasmigona complanata	7	0
				Obliquaria reflexa	15	26.7 (18.9)
				Leptodea fragilis	10	0
				Potamilus alatus	10	22.2 (31.4)
				Lampsilis cardium	20	0
				Lampsilis radiata s.	10	0

[a]At 3 h exposure, the percent mortality of veligers was 100 and of early juveniles was 94.5 (4.8).

[b]Tested at 12°C.

Figure 1. Thermal shock treatments of zebra mussels and F. flava.



found that water temperatures of 40°C were necessary to produce acceptable levels of mortality in treatments < 30 min. Additionally, a combined heat and cold shock did not consistently change the incidence of mortality to the mussels. There was no significant difference between mortality of clumped or separated zebra mussels. We did, however, note that warm acclimated mussels had a higher percent mortality than cold acclimated mussels. This may be an artifact of laboratory holding conditions.

Fusconaia flava mussels experienced no mortality at 35°C for 20 min. At 40°C, only 50% of the mussels survived a combined hot/cold treatment for 20 min. However, there was no mortality of *F. flava* in a hot only treatment for 20 min.

Further work is needed to determine the effect of short (<10 min) thermal treatments on both veliger and early juvenile zebra mussels and sensitive species of unionid mussels.

Future research

Further research is planned to on the following areas:

1) Determination of minimal heat treatments needed to kill veliger and juvenile zebra mussels. This includes minimal water temperature and minimal duration of exposure.

2) Evaluation of the toxicity of formalin treatments of 1667 mg/L for 15 min to sensitive unionid species.

3) Evaluation of various delivery methods for treating unionid mussels infested with zebra mussels. This may include spraying a salt solution or brushing a disinfectant such as benzalkonium chloride on the outside of the unionid rather than immersing the mussel into a solution.

4) Field validation of methods in a relocation or rescue operation of unionid mussels.

Thermal Shock Treatment Effectiveness

Zebra Mussels Vs. Hot(40 °C), Cold(0 °C), and Hot then Cold Treatments