Dallas 1995 return

United States Department of the Interior

NATIONAL BIOLOGICAL SURVEY

MAY 15 1995

May 9, 1995 Rec

USFWS
ASHEVILLE, NC
BPC NAM
5/16 RGB JAR
RDE NC
6/1 DAF LR
JEN SE
VGH

Mr. Richard Biggins
U.S. Fish and Wildlife Service
Endangered Species Field Office
Asheville, NC

Dear Dick:

Attached is a preliminary report of the research we conducted last year to find a control method for zebra mussels on unionid mussels. I included only results of work that showed some potential for further evaluation. We did evaluate several other options, but none were feasible. This summer we plan to pursue several of the items that are listed under future research. Please let me know if you have any suggestions or questions on the report.

Sincerely,

Diane Waller

Diane Waller
Research biologist

Sheri
Please send copies of this report to.
Jackson
Jacksonville
TN + VA Coop Units
Williams in Gainesville FL
Steve Ahlstedt @ TVA.
+ John Alderman, NCWRC

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:

Diane L. Waller
National Biological Service
Upper Mississippi Science Center
La Crosse, WI

1994

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 perhaps 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 federal, state, 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 quarantining 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 success of this procedure depends on the assumption that microscopic stages will grow to macroscopic size during the quarantine period. Additionally, quarantine procedures require moving mussels twice, add to the efforts through maintenance of cultures, and theoretically would not eliminate larvae produced during the quarantine.

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 the acclimation equation of Iwanyzki and McCauley (1993).

Methods

The control treatments that we investigated for eliminating zebra mussels from unionid mussel shells included chemical treatment and thermal shock.

Chemical treatment

The chemicals (Table 1) and treatment levels 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), posed undue risk 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%.

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%

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. Zebra mussels were held in flowing well water at 10°±2°C and fed live *Ankistrodesmus* sp. daily. Prior to testing, zebra mussels were removed from the stock culture, acclimated to test temperature, and allowed to attach to petri plates or glass dip jars. Plates or dip jars with attached mussels were placed into glass jars containing 15 L of test water (pH 8.2 ± 0.5, alkalinity 100 ± 10mg/L as $CaCO_3$, hardness 140 ± 10 mg/L as $CaCO_3$) at 12°±0.5°C or 17°±0.5°C in a constant temperature water bath. Three replicates per chemical concentration were tested with 10 mussels per replicate. Mortality was determined 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°±0.5°C or 17°±0.5°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.

Early juveniles (settlers) were collected from Lake Erie on 2 cm x 8 cm glass slides. Size ranges of early juveniles 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. Juveniles 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 2) 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°$\pm$1°C. Animals were acclimated to the test temperature by increasing water temperature 3°C in a 24-h period. Tests were conducted in glass jars containing 30 L of test water (pH 8.2 $\pm$ 0.5, alkalinity 100 $\pm$ 10 mg/L as $CaCO_3$, hardness 140 $\pm$ 10 mg/L as $CaCO_3$) at 12°C of 17°C. Three replicates of each chemical concentration were tested; the number of mussels in each replicate varied among species and test and was dependent on the number of animals available. Mortality was recorded after 48 h in untreated water and was defined as failure of mussels with gaping shells to respond to the touch of a probe or failure of a closed shell to resist being pried open and its subsequent failure to reclose.

Thermal Shock

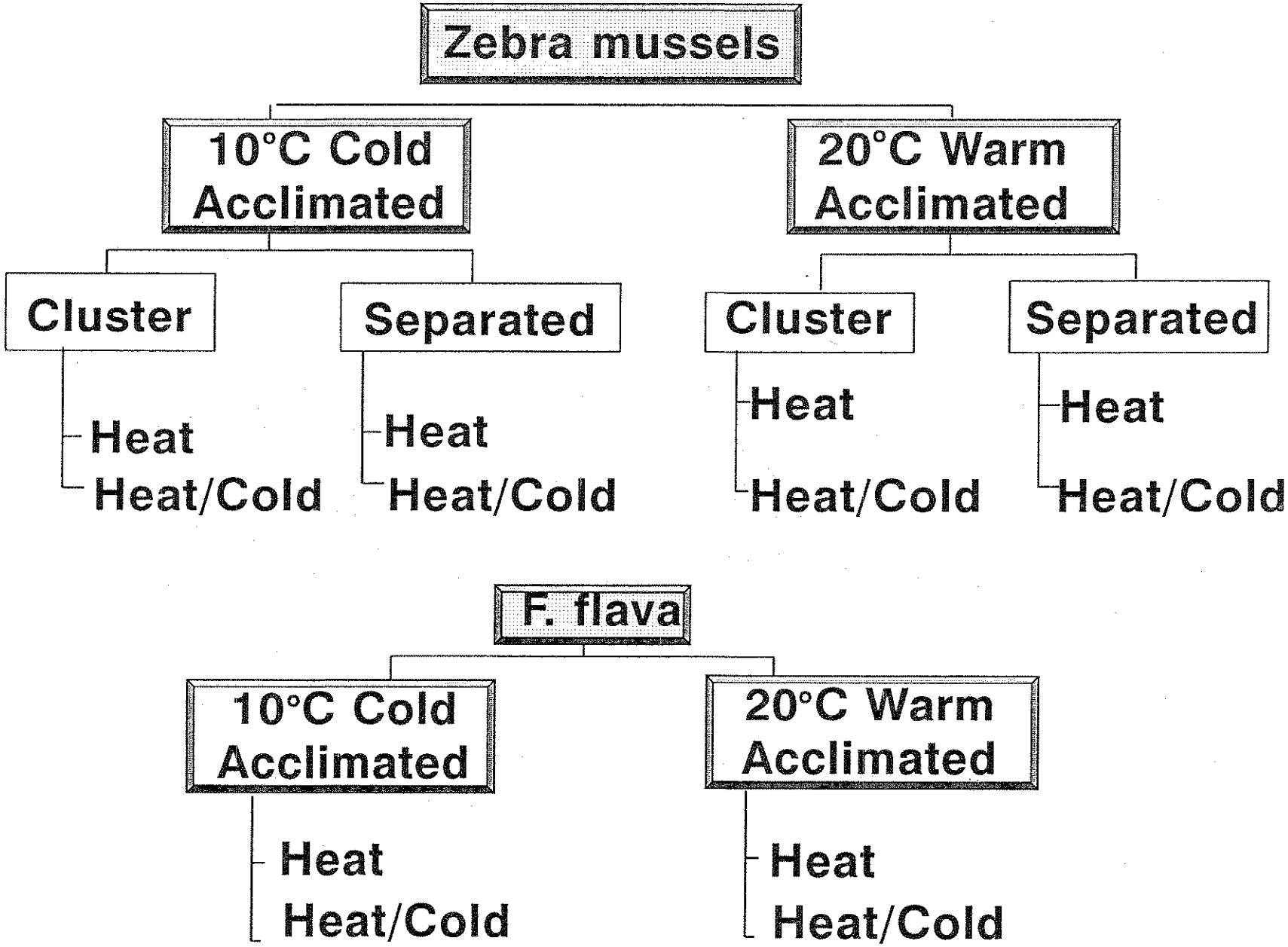
Adult and juvenile zebra mussels were collected and maintained as described previously for the chemical treatment portion of the study. Veligers were not tested in the thermal shock treatments.

Fusconaia flava, the Wabash pigtoe, were collected from navigation pools 8 and 9 of the upper Mississippi River. Unionid 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 heat and cold or heat only for various lengths of time (Figure 1). Heat shock was tested at water temperatures of 35°C and 40°C. Both cold- and warm-acclimated zebra mussels were tested. Additionally, we tested zebra mussels in clumps and as separated individuals to determine if clumping provided more protection from extreme temperature changes. A relatively tolerant unionid species, *F. flava*, was tested in thermal shock treatments to determine if further evaluation with more sensitive species was warranted (Figure 1).

Cold-acclimated zebra mussels were held at 10°C for a minimum of 3 weeks and maintained as previously described for mussels in chemical tests. Zebra mussels and unionid mussels were acclimated to warm water by increasing water temperature to 20°C at a maximum rate of 3°C per day. All warm-acclimated mussels were held at 20°C for 3 weeks prior to treatments.

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



Zebra mussels were placed into dip jars for the temperature shock treatments. One to two clumps of mussels were placed into each dip jar for treatment replicates of clumped zebra mussels. The byssal threads of all mussels in a clump were severed and mussels were individually placed into the jar for treatments with separated mussels. The number of mussels per replicate varied from 15 to 50 and was dependent on the number of mussels in a clump. Each temperature treatment was duplicated for clumped and separated zebra mussels. Fusconaia flava mussels were removed from the holding tank and placed in wire baskets for the temperature shock treatments. Treatments were duplicated with five mussels in each replicate.

Temperature treatments consisted of hot water only treatments and hot water/cold water treatments. Hot water treatments included immersing mussels in hot water (35°C or 40°) for 10, 20, or 30 min. and immediately returning animals to the holding tanks (10°C or 20°). In hot and cold water treatments, mussels were immersed in cold water (4°C) immediately after hot water immersion for an equivalent amount of time. After cold water immersion, mussels were returned to the holding tanks. Mortality was recorded daily, and a final mortality was measured after 48 h.

Results

Chemical treatment

Our evaluation of chemical treatments emphasized the three chloride salts because these were the safest and least expensive 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 experienced high 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.

Thermal Shock

Heat treatment was effective for killing juvenile and adult zebra mussels (Figure 2). We 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 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.

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 veliger and early juvenile zebra mussels and sensitive species of unionid mussels.

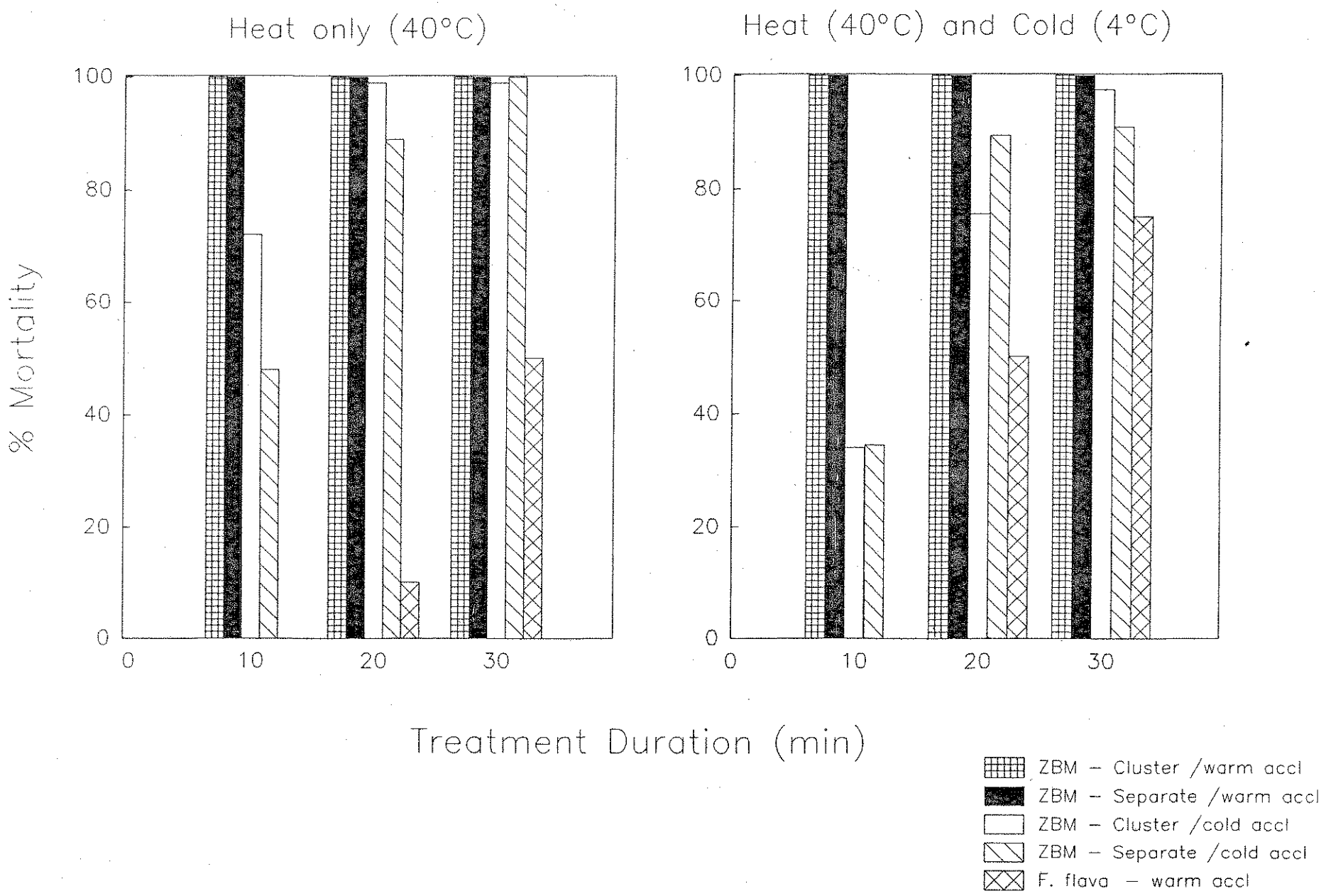
Table 2. Toxicity of chloride salts to veliger and early juvenile zebra mussels and unionid mussels at 17°C.

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 2. Thermal Shock Treatments of Zebra Mussels and Fusconaia flava



Future research

Additional research is needed on the methods of control that were evaluated in the first year of this research. Future research is planned in the following areas:

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

2) 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 in a solution.

In addition, other methods or control agents also need to be investigated. These may include the following:

(1) Evaluation of the toxicity of acute shifts in pH to veliger and juvenile mussels.

(2) Evaluation of the toxicity of other chemicals to zebra mussels and unionid mussels.

(3) Evaluation of combination treatments for killing zebra mussels such as two chemicals (e.g., formalin and salt), thermal and chemical treatment, and hypoxia and thermal treatment.

References

Committee on Methods for Acute Toxicity Testing with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecological Research Series. EPA-660/3-7-009. Corvallis, OR.

Iwanyzki, S. and R. W. McCauley. 1993. Upper lethal temperatures of adult zebra mussels (*Dreissena polymorpha)*. Pages 667-676, In Zebra Mussels: Biology, impact and control. T. Nalepa and D. Schloesser, eds. Boca Raton, FL.

Mackie. G. L. 1991. Biology of exotic zebra mussel, *Dreissena polymorpha*, in relation to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia 219:251-268.

Mackie. G. L. 1993. Biology of the zebra mussel (*Dreissena polymorph*a) and observations of mussel colonization on unionid bivalves in Lake St. Clair of the Great Lakes. Pages 153-165 in T.F. Nalepa and D. W. Schloesser, eds. *Zebra Mussels: Biology, Impacts, and Control*. Lewis Publs., Chelsea, MI.

Schloesser, D. W., and W. P. Kovalak. 1991. Infestation of native unionids by *Dreissena polymorpha* in a power plant intake canal in Lake Erie. J. Shellfish Res. 10:355-359.

Stoeckel, J. A. and D. W. Garton. 1993. Techniques for mass-spawning and culture of *Dreissena polymorpha* larvae. Third International Zebra mussel research conference, Electric Power Research Institute (EPRI), Toronto, Ontario, Canada.

Waller, D. L., S. W. Fisher, H. Dabrowska, J. J. Rach, L. L. Marking, and W. G. Cope. 1993. Toxicity of candidate chemicals for zebra mussel control to selected nontarget organisms. Journal of Great Lakes Research. 19:695-702.

Waller, D.L., S. W. Fisher, and H. Dabrowska. 1995. Prevention of zebra mussel infestation and dispersal during aquaculture operations. North American Journal of Fisheries Management. Submitted.