Waller 1997

United States Department of the Interior
U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Mississippi Science Center
Box 818
La Crosse, WI 54602-0818
Ph. 608-783-6451
Fax: 608-783-6066

RECEIVED
JAN 28 1997
USFWS - ASHEVILLE NC

ROUTING

	RRC	NAM	
1/27	RGB	JAR	
	RRC	HV	
	JAF	NC	
	JLN	LR	
	VGH	G SMNP	
2-18			

Richard Biggins
U.S. Fish and Wildlife Service
330 Ridgefield Court
Asheville, NC 28806

January 6, 1997

Dear Mr. Biggins: Dick

Enclosed please find the final report of the research that we conducted to evaluate methods for cleaning zebra mussels from unionid mussels during rescue and relocation projects. I apologize for the delay in sending this information. I had hoped that I could use some base funds to do some further evaluations of other methods and applications. However, our zebra mussel funding was redirected last year and I was forced to terminate the work after the Quick Response Funds were spent. This remains a critical area of research for unionid mussels, as you well know. I would like to continue to evaluate other cleaning methods if funds become available in the future. Please do not hesitate to call should you have any questions concerning this report.

Sincerely,

Diane L. Waller

Diane L. Waller
Fishery biologist

PREVENTION OF ZEBRA MUSSEL DISPERSAL DURING TRANSPORT OF UNIONID MUSSELS

Final Report

To:

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

By:

D.L. Waller
U.S. Geological Survey
Biological Resources Division
Upper Mississippi Science Center
La Crosse, WI

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 unionid species' extinctions, particularly of rare species that are limited to small localized populations in one watershed. In response to the zebra mussel threat, federal, state, and private agencies have initiated rescue and relocation efforts for unionid mussels. However, infested unionids are a potential agent for dispersal of the zebra mussel into uninfested waters. In past relocation projects, zebra mussels were removed from unionids by physically scraping or brushing the shells from unionid mussels and 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 the success of this procedure depends on the assumption that microscopic stages will grow to macroscopic size during the quarantine period and that additional larvae will not be produced. Additionally, quarantine procedures require maintenance of unionids for at least 1 month.

We evaluated methods for removing zebra mussels from unionid shells that were quick, could be applied to large numbers of mussels, and required minimal labor. Chemical treatments were chosen because previous studies indicated that zebra mussels, particularly early life stages, are more sensitive than some unionid mussels to toxicants (Waller et al.

1993; Waller et al. 1996). We also investigated the use of thermal treatments for killing attached zebra mussels. Hot water has been recommended as a nontoxicant method to control zebra mussels in commercial settings (e.g., boat cleaning, industrial pipe). Some unionid mussels can survive brief exposure (e.g., 15 min) to warm air temperatures (40°C; Cope Bartsch, and Waller 1996); moreover, the thicker shell of unionids may provide more insulation from temperature changes than the thin shell of zebra mussels.

Methods

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. 1996). We eliminated chemicals that required prolonged exposure periods (>24 h), posed undue risk to the applicator, and were relatively expensive. The chemicals tested included: benzalkonium chloride, calcium chloride, formalin, hydrogen peroxide, potassium chloride, and sodium chloride.

Zebra Mussel Adults and Juveniles

Static toxicity tests were conducted following procedures of the Committee on Methods for Acute Toxicity Testing with Aquatic Organisms (1975). Adult and juvenile zebra mussels were collected from Lake Michigan, near Racine, Wisconsin, and transported to the Upper Mississippi Science Center in La Crosse, Wisconsin in chilled insulated coolers. Zebra mussels were held in flowing well water at 10±2°C and fed live *Ankistrodesmus* sp. daily.

Zebra mussels were tested within 30 d of collection. Before testing, zebra mussels were removed from the stock culture, acclimated to the test temperature at a rate of 2°C/day, 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 zebra mussels per replicate. The exposure period was 6 or 24 h, depending on the test chemical (Table 2). 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.

Zebra Mussel Veligers

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. 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. Ten replicates of each concentration and the control were tested for each chemical. Veliger density was estimated by counting the number of organisms in 10 1-ml samples of culture water. Veligers were 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 h light:10 h dark for the duration of the exposure (6 or

24 h). 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.

Zebra Mussel Settlers

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 zebra 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

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 ± 1°C. Animals were acclimated to the test temperature at a rate of 2°C/day. Tests were conducted in stainless steel tanks containing 30 L of test water (pH 8.2 ± 0.5, alkalinity

100±10 mg/L as $CaCO_3$, hardness 140±10 mg/L as $CaCO_3$) at 12±0.5°C or 17±0.5°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 determined 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. Dissolved oxygen, pH, and temperature were measured in each test vessel once a day. Tests were invalidated and repeated if dissolved oxygen levels fell below 60% saturation.

Thermal Shock

The unionid species, *Fusconaia flava*, was used in thermal shock treatments because it is relatively tolerant and abundant in the UMR. If *F. flava* experienced significant mortality in initial thermal tests, no further testing of sensitive and less common species would be warranted. *Fusconaia flava* mussels were collected from Navigation pools 8 and 9 of the Upper Mississippi River. Zebra mussels and *F. flava* mussels were maintained as described previously for toxicity tests. Veligers were not tested in the thermal shock treatments because we assumed that they are more sensitive to thermal stress than juvenile mussels; thus, treatments effective on juvenile and adult zebra mussels will also kill veliger mussels.

The effectiveness of thermal shock was tested in two separate trials. In the first trial, we evaluated the effect of clumping (zebra mussels attached to one another) on the lethality of hot and cold shock to adult and juvenile zebra mussels. In the second trial, only juvenile

zebra mussels were tested (<5 mm length); lethal combinations of water temperature and exposure time were determined for zebra mussels that were acclimated to different temperatures. The effects of temperature shock were also evaluated on *F. flava* in both trials.

In trial 1, cold acclimation was designated at 10°C and warm acclimation was designated at 20°C (Figure 1). Zebra mussels and unionids were held at one of the designated acclimation temperatures for a minimum of 3 weeks before testing. A clump or druss of zebra mussels was randomly assigned to test groups of "clumped" or "separated" individuals. One replicate consisted of one to two drusses (about 25 mussels/druss;clumped) or about 50 individual mussels (separated). Mussels were separated from drusses by severing the byssal thread with a scalpel. Zebra mussels were placed into 50 L glass jars that had 1 mm mesh screen bottoms; *Fusconaia flava* mussels (n=5 per replicate) were placed into wire baskets. Treatments were tested in duplicate with zebra mussels and *F. flava*. Shell length of zebra mussels was measured with a vernier caliper at the conclusion of the test. Mean shell length was 9.1 ± 3.0 mm (range 2.0 to 23.0).

Shock treatments included (1) immersion in hot water (35°C and 40°C) or (2) immersion in hot water followed by immersion in cold water (4°C; Figure 1). The duration of immersion was 10, 20, or 30 min. in each water temperature bath (e.g., 10 min at 35°C, followed by 10 min at 4°C). Water baths were prepared by filling stainless steel tanks (30 L) with well water. Hot water baths were adjusted with an in-line mixing valve. Cold water baths were adjusted by adding crushed ice to the water. Water temperature was measured with a mercury thermometer at the beginning of the exposure and every 10 min thereafter. Temperature was readjusted after each test replicate. Immediately after immersion, mussels

were returned to holding tanks at the designated acclimation temperature (10°C or 20°C). Mortality was recorded at 24 and 48 h after thermal treatment.

In trial 2, juvenile zebra mussels (2 to 5 mm) were separated from the stock culture and placed into 37 L aquaria for acclimation to 15°C, 20°C, or 25°C. Three days before testing, 15 mussels were placed into test vials (2 cm wide x 6 cm height glass tubing with 600 μm mesh bottoms) and then returned to holding aquaria for 3 d to allow attachment.

Thermal treatments included immersion in water at 34, 36, 38, 40 and 42°C for 5, 10, 15, or 20 min. Vials were immersed in a Precision water bath shaker. The water temperature was measured with two thermocouple probes place inside three separate vials and the average of all readings was recorded. Vials were immediately returned to aquaria after the designated immersion period. Mortality of mussels was measured 24 h after thermal treatment.

Mortality among treatments was compared by analysis of variance. General linear model was used to compare mean shell length among treatment groups and between dead and live mussels within a treatment. Differences were defined as significant if $p \leq 0.05$.

Results

Chemical treatment

The three salts (calcium chloride, potassium chloride, and sodium chloride) produced almost 100% mortality to veliger and juvenile zebra mussels at various treatment levels (Table 2). However, no single treatment 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, thin-shelled and small mussels were most sensitive to salt treatment. The

most sensitive species overall were *O. reflexa* and *L. fragilis*. *Pyganodon grandis* also experienced high mortality (22.2%) in 2,500 mg/L KCl after 24 h, but was not available for testing in other treatments.

Benzalkonium chloride produced 89% mortality to juvenile zebra mussels at 500 mg/L for 60 min, but was also toxic to *O. reflexa* (30% mortality after 15 min exposure). Although benzalkonium chloride did not cause acute mortality of *F. flava,* the chemical elicited sublethal responses in mussels including copious mucus production and abortion of glochidia.

Hydrogen peroxide was ineffective against juvenile zebra mussels (23% mortality) at the highest concentration tested (500 mg/L for 1 h), and not caused 0% mortality to *F. flava* at this treatment level. Formalin produced 100% and 86% mortality of veliger and settled zebra mussels, respectively, at the highest treatment level (1667 mg/L for 15 min); however, formalin caused $<1\%$ mortality to juvenile and adult zebra mussels. Formalin killed 0% of *F. flava* and 10% of *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 (Figures 2 and 3). However, in trial 1, we found that water temperatures of 40°C were necessary to produce $>90\%$ mortality in treatments <30 min. At 35°C, mortality was 0% after immersion for 20 min. The mean percent mortality in treatments at 40°C was not statistically different between 20 min and 30 min immersion periods and ranged from about 80 to 100%.

Mortality was not significantly different between heat only and combined heat and cold shock. Additionally, there was no difference in mortality of clumped and separated zebra mussels. However, warm acclimated mussels had a higher percent mortality than cold acclimated mussels in 10 and 20 min treatments of hot water. Mean shell length did not vary significantly among treatment groups and there was no difference in mean shell length of dead and live mussels within a treatment.

Fusconaia flava mussels experienced no mortality at 35°C for 10 min (Figure 2). At 40°C, only 50% of the mussels survived a combined heat/cold treatment for 20 min. However, there was $<10\%$ mortality of *F. flava* in a heat only treatment for 20 min at the higher temperature.

In trial 2, heat treatments >38°C were lethal to juvenile zebra mussels at immersion periods >5 min (Figure 3). Immersion for 5 min at 42°C caused 100% mortality in all treatment groups, whereas at least 10 min was necessary for $>70\%$ mortality in 38 and 40°C water. The minimum heat treatments to produce $>90\%$ mortality of juvenile zebra mussels, regardless of acclimation temperature, were 5 min in 42°C water or 15 min in 38°C water (Figure 3).

Acclimation temperature significantly affected mortality only at the lower temperatures (34 and 36°C). Generally, mussels that were acclimated to 25°C experienced less mortality than mussels acclimated to 15°C in the same treatments. These results contrast those in trial 1 in which mortality of 20°C acclimated mussels was greater than that of 10°C acclimated mussels.

Conclusions

No single treatment investigated in this study produced 100% mortality to zebra mussels and 0% mortality to the unionid mussel species that were tested. Heat treatments (e.g. 38°C for 15 min) were effective against zebra mussels, but based on tests with *F. flava*, thermal shock is more toxic to unionids than many of the chemical treatments. Additional tests of thermal treatments would be necessary on different unionid species before recommending any treatment regime with hot water. Thermal shock is also a less feasible option in the field than chemical treatment because of the unavailability of heated water and inability to regulate 1 to 2°C changes in water temperature. Hot water treatments are probably only feasible if applied as a spray onto unionid valves.

Limited application of specific chemical treatments (e.g., 20,000 mg/L NaCl for 6 h) may be feasible for more tolerant unionids, such as *F. flava* and *E. dilatata*. However, preliminary testing is necessary to determine toxicity to each species before treatment. Alternative application methods, such as spraying or scrubbing shells with a toxicant, need to be evaluated. Spraying or scrubbing shells with a chemical is probably less harmful to unionid mussels, but could effectively reach veliger and settled zebra mussels that occupy the mantle edge or cavity. More toxic chemicals, such as benzalkonium chloride, could then be used if the area of application on the unionid valve was limited. A combination of physical methods, such as high powered spraying or scrubbing, and chemical treatment may be necessary to remove externally attached zebra mussels and to kill microscopic stages that reside in the mantle cavity.

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.

Cope, W.G., M. R.Bartsch, D. L. Waller, and J.A. Luoma. 1996. Variation in emersion and thermal tolerances of selected freshwater unionid mussels. 17th Annual Meeting of the Society of Toxicology and Chemistry, Washington, D.C., November 17-21, 1996.

Iwanyzki, S. and R. W. McCauley. 1993. Upper lethal temperatures of adult zebra musséls (*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 polymorpha*) 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. 1996. Prevention of zebra mussel infestation and dispersal during aquaculture operations. Progresseive Fish-Culturist 58:77-84.

Table 1. Chemicals tested in toxicity test with 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 juvenile zebra mussels and unionid mussels at 17°C. Mortality (standard deviation) is reported as average percent.

Test Chemical (mg/L)	Exposure (h)	Organism Tested	Life Stage	Number of Replicates	Mussels per Replicate	Average Mortality	S.D.
$CaCl_2$ (10,000)	6[a]	*Dreissena polymorpha*	veliger	10	10[b]	100	-
			juvenile	3	100-200	99.5	0.4
		Fusconaia flava	adult	3	9	0	-
		Obliquaria reflexa	adult	3	10	93.3	9.4
		Leptodea fragilis	adult	3	3	0	-
		Potamilus alatus	adult	3	3	11.1	15.7
		Lampsilis cardium	adult	3	3	0	-
		Lamspsilis radiata	adult	3	3	11.1	15.7
KCl (2,500)	24	*Dreissena polymorpha*	veliger	10	10[b]	100	-
			juvenile	3	100-200	95.9	1.8
		Fusconaia flava	adult	3	10[d]	0	-
		Elliptio dilatata	adult	3	3	0	-
		Pyganodon grandis	adult	3	3	22.2	15.7
		Lasmigona complanata	adult	3	2	0	-
		Obliquaria reflexa	adult	3	5	6.7	9.4
		Leptodea fragilis	adult	3	3	52.8	17.1
		Lampsilis cardium	adult	3	6	0	-
		Lampsilis radiata s.	adult	3	3	0	-
KCl (10,000)	6	*Dreissena polymorpha*	juvenile	3	100-200	97.0	1.3
		Fusconaia flava	adult	3	10[d]	0	-
		Elliptio dilatata	adult	3	3	0	-
		Lasmigona complanata	adult	3	2	0	-
		Obliquaria reflexa	adult	3	10[d]	0	-
		Leptodea fragilis	adult	3	3	47.2	17.1
		Potamilus alatus	adult	3	3	22.2	15.7
		Lampsilis cardium	adult	3	6	8.3	11.8
		Lampsilis radiata s.	adult	3	3	33.3	27.2

Table 2. Continued

Test Chemical (mg/L)	Exposure (h)	Organism Tested	Life Stage	Number of Replicates	Mussels per Replicate	Average Mortality	S.D.
NaCl (10,000)	24	*Dreissena polymorpha*	veliger	10	10[b]	100[c]	-
			juvenile	3	100-200	98.1[c]	1.3
		Fusconaia flava	adult	3	8	29.2	5.9
NaCl (20,000)	6	*Dreissena polymorpha*	veliger	10	10[b]	100	-
			juvenile	3	100-200	99.2	0.6
		Fusconaia flava	adult	3	4	0	-
		Elliptio dilatata	adult	3	3	0	-
		Lasmigona complanata	adult	3	2	0	-
		Obliquaria reflexa	adult	3	5	26.7	18.9
		Leptodea fragilis	adult	3	3	0	-
		Potamilus alatus	adult	3	3	22.2	31.4
		Lampsilis cardium	adult	3	6	0	-
		Lampsilis radiata s.	adult	3	3	0	-

[a] At 3 h exposure, veliger mortality was 100% and juvenile mortality was 94.5% (4.8).

[b] Minimum of 10 organisms/replicate

[c] Tested at 12°C.

[d] Two tests were conducted with a similar number of replicates and number of mussels/replicate.

Figure 1. Thermal shock treatments of zebra mussels and *F. flava* in trial one.

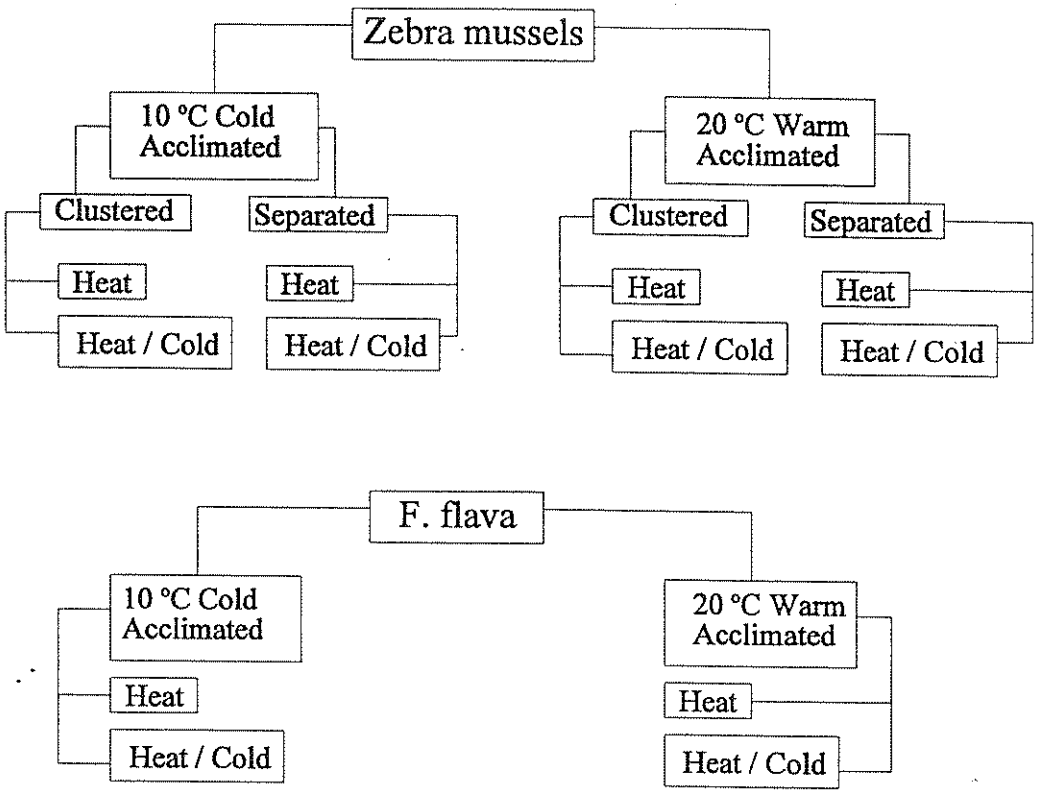
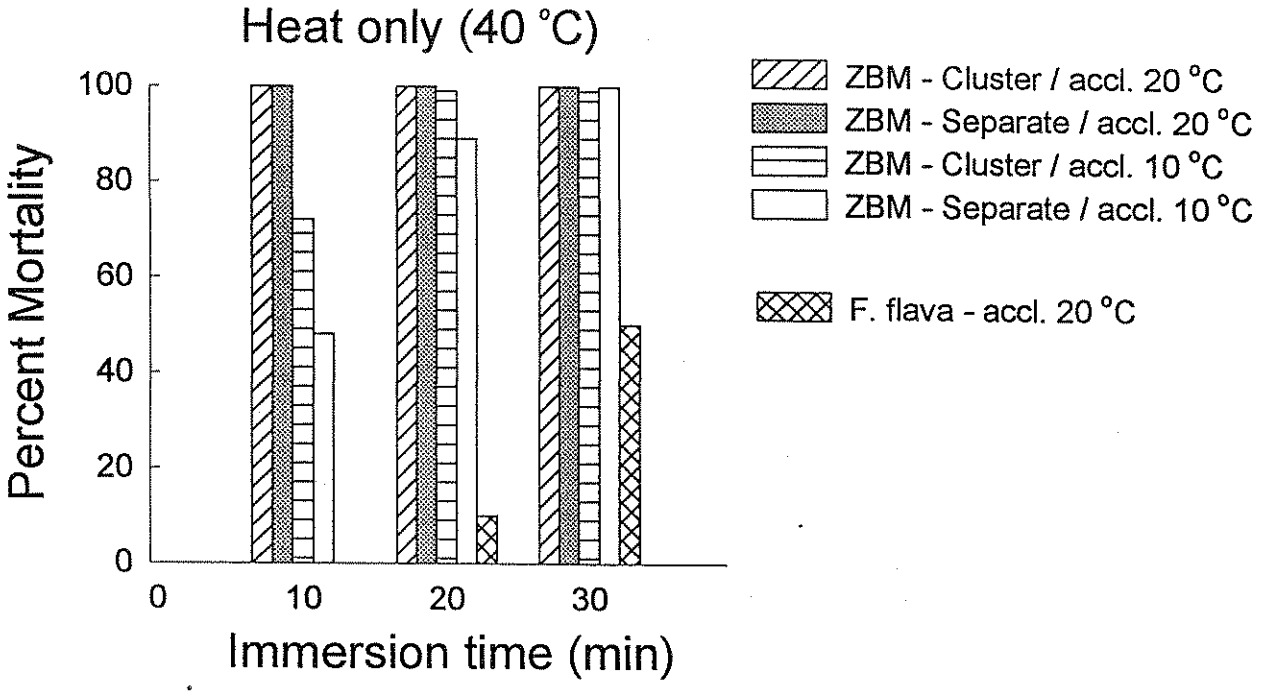


Figure 2. Mean percent mortality of zebra mussels (2-23 mm shell length) and the unionid mussel F. flava to immersion in hot (40 °C) water and hot followed by cold (4 °C) water.



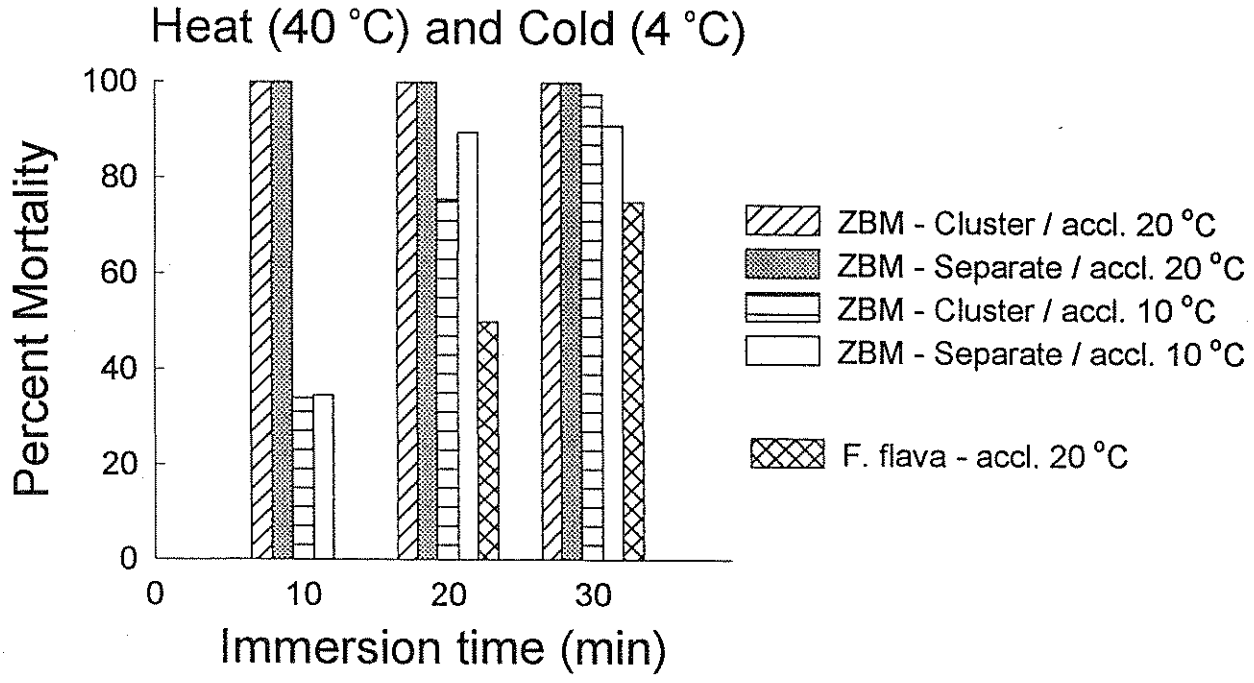


Figure 3. Mean percent mortality of zebra mussels (2-5 mm shell length) after immersion at various treatment temperatures and durations. Mussels were acclimated to 15 °C, 20 °C, or 25 °C before testing.