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*Zebra Mussel Research Program*

# **Use of Emersion as a Zebra Mussel Control Method**

by *Robert F. McMahon, Thomas A. Ussery, Michael Clarke*  
*University of Texas at Arlington*

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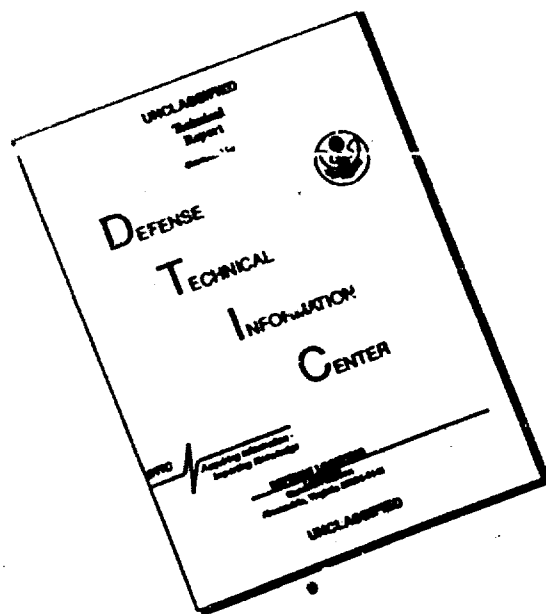
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# **Use of Emersion as a Zebra Mussel Control Method**

by **Robert F. McMahon, Thomas A. Ussery, Michael Clarke**

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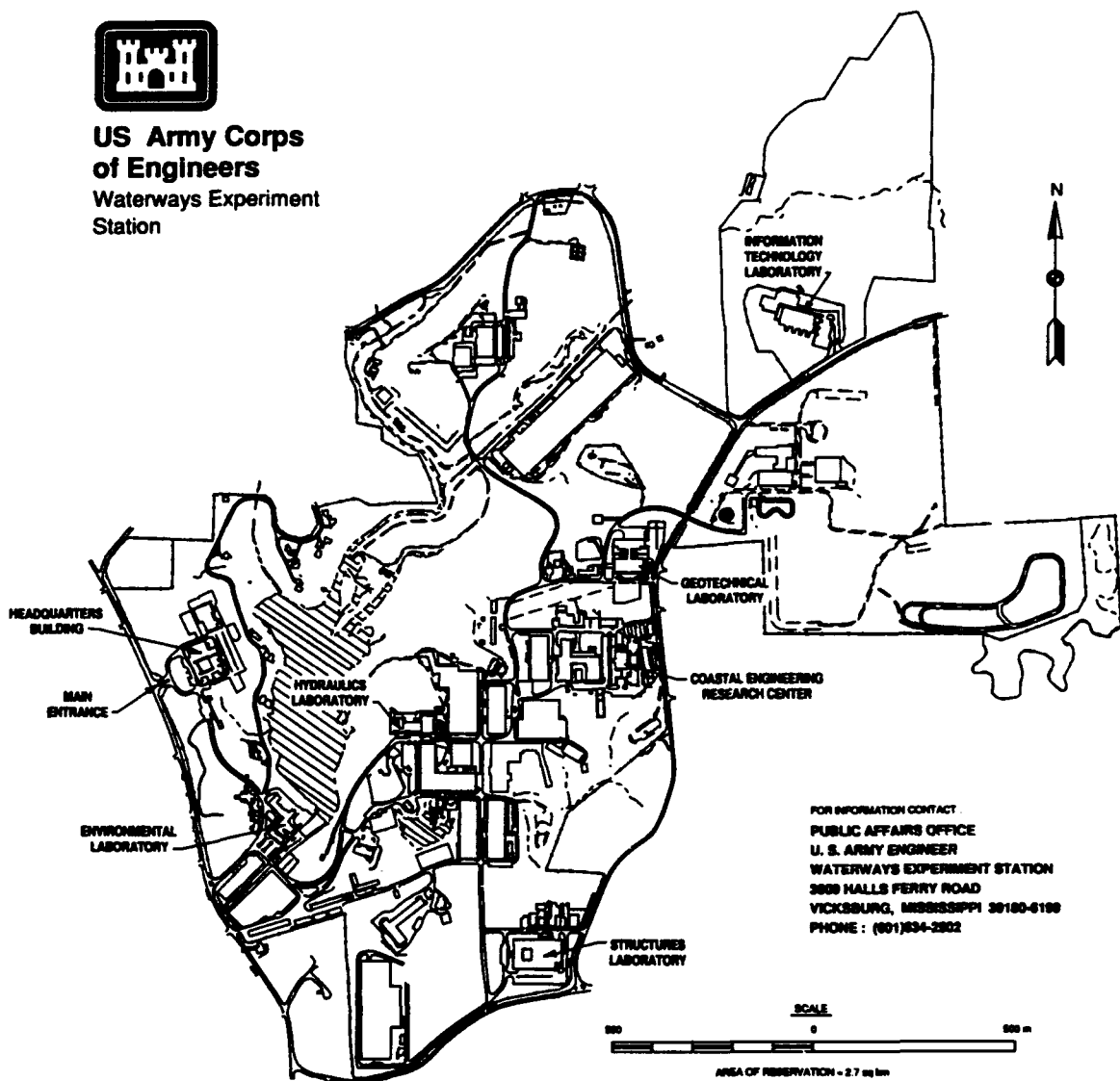
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# Preface

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The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 specified that the Assistant Secretary of the Army, Civil Works, would develop a program of research and technology development for the environmentally sound control of zebra mussels (*Dreissena polymorpha*). As a result of this legislation, the U.S. Army Engineer Waterways Experiment Station (WES) initiated a 4-year program to develop control strategies for this species.

The report was prepared by Dr. Robert F. McMahon, Mr. Thomas A. Ussery, and Mr. Michael Clarke of the Center for Biological Macrofouling Research, Department of Biology, University of Texas at Arlington, Arlington, TX. Research for this report was funded through Broad Agency Announcement #63492 with WES. Dr. Barry S. Payne and Dr. Andrew C. Miller, WES, managed the contract for this work. Dr. Edwin Theriot, WES, is manager of the Zebra Mussel Research Program.

During the conduct of this study, Dr. Edwin Theriot was Chief, Aquatic Habitat Group; Dr. C. J. Kirby was Chief, Environmental Resources Division; and Dr. John Harrison was Director, Environmental Laboratory, at WES.

Dr. Robert W. Whalin was Director of WES at the time of publication of this report. COL Leonard G. Hassell, EN, was Commander.

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# 1 Biology and Ecology of the Zebra Mussel

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## Introduction

The exotic freshwater bivalve *Dreissena polymorpha*, the “zebra mussel,” was released into the Lake St. Clair and Detroit River region of the Great Lakes in 1986, apparently by release of ship ballast water, in which mussel larvae were transported from a freshwater European port (Hebert, Muncaster, and Mackie 1989; Mackie et al. 1989). The zebra mussel’s planktonic larval stage, the “veliger,” has allowed the mussel to spread rapidly downstream throughout the shallow nearshore waters of Lake Erie, Lake Ontario, and the St. Lawrence River (Mackie et al. 1989; Griffiths, Kovalak, and Schloesser 1989). It has also dispersed into most of southern Lake Michigan with isolated populations in Lakes Huron and Superior. In 1989, the zebra mussel entered the Erie Barge Canal from Lake Erie and has since spread eastward into the Seneca, Mohawk, and Hudson Rivers, as well as into Lakes Oneida, Seneca, and Cayuga in upstate New York. The zebra mussel is now reported in the upper Susquehanna River in southern New York. Zebra mussels entered the Mississippi River near St. Louis during 1990 by dispersing from Lake Michigan downstream in the Illinois River (the mussel is now well established in the Illinois River) into its confluence with the Mississippi River. It was reported upstream of St. Louis in the Mississippi River at La Crosse, Wisconsin, in the fall of 1991. Isolated populations were also reported in 1991 in the lower Ohio River near its confluence with the Mississippi River, in the lower Tennessee River in Kentucky Lake, and at several points in the lower portion of the Cumberland River. It has also invaded several smaller inland lakes directly north and south of Lakes Erie and Ontario (McMahon 1992, Great Lakes Sea Grant Network 1991).

The planktonic veliger larvae and juveniles of zebra mussels are entrained with intake water and settle in low-flow areas of raw water systems. Once there, they attach to hard surfaces by production of a holdfast formed from proteinaceous “byssal” threads secreted by a gland at the base of the mussel’s foot. Once settled, the veliger larvae grow to sizes and accumulate in numbers that reduce or block flow (Mackie et al. 1989;

Griffiths, Kovalak, and Schloesser 1989; Clarke 1952; Shtegman 1986; McMahon 1990, 1992; McMahon and Tsou 1990; Claudi and Ackerman 1992). The byssal holdfast prevents mussels from being dislodged even in high-velocity flows. Veligers metamorphose into "postveliger" larvae prior to settlement. Once settled, postveligers quickly transform into juvenile mussels. Postveligers can settle in such great numbers, and juveniles have such rapid postsettlement growth rates, that mussels can rapidly form dense mats many shells thick (4 to 12 in.<sup>1</sup> thick) (Mackie et al. 1989; Griffiths, Kovalak, and Schloesser 1989; McMahon 1990, 1992; McMahon and Tsou 1990; Greenshields and Ridley 1957; Jenner 1983). Rapid buildup of mussel encrustations can reduce maximum-sustainable flow rates even in large-diameter piping (Clarke 1952, Lyakhov 1986). Recent experiences with zebra mussel infestations in power stations, potable water treatment plants, and industrial facilities drawing water from Lake Erie suggest that zebra mussel fouling may develop more rapidly and become more severe in North American raw water facilities than has generally been reported to be the case in Europe (Griffiths, Kovalak, and Schloesser 1989; LePage 1989; Great Lakes Sea Grant Network 1991; Electric Power Research Institute 1991; Ontario Hydro 1992).

Asian clam (*Corbicula fluminea*) macrofouling has been projected to cost the U.S. power industry alone over \$1 billion annually (Isom 1986), and zebra mussel macrofouling has been estimated to cost \$2 billion over the next decade in the Great Lakes alone (Roberts 1990). Thus, the spread of zebra mussels throughout the inland waterways of the United States and Canada will eventually lead to greatly increased macrofouling costs unless efficacious, environmentally acceptable, and cost-effective control technologies are developed and implemented in the immediate future.

## Biological Basis for Zebra Mussel Macrofouling

Zebra mussels rarely exceed 5 cm in shell length, most specimens in North American fresh waters being less than 3 cm long (Griffiths et al. 1991). Newly settled juveniles are 0.2 to 0.3 mm in length (Mackie et al. 1989). Retention of a byssal holdfast in adults and the planktonic veliger larva make zebra mussels a major macrofouling species. The byssal holdfast allows mussels to attach and grow on the walls of piping, tube sheets, embayments, or any other hard-surfaced components in raw water systems (McMahon 1990; McMahon and Tsou 1990). For settlement of the postveliger (length = 160 to 290  $\mu$ m), flow rates must be below 1.5 to 2.0 m/sec (Jenner 1983; Lyakhov 1986), but once attached, individuals can tolerate considerably higher velocities. Thus, if postveligers settle during low-flow conditions (e.g., in redundant systems, during off-line or

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<sup>1</sup> To convert inches to meters, multiply by 0.0254.

low-flow periods), they are unlikely to be dislodged by later exposure to higher flows. North American and European experience has been that mussels can make byssal attachment to any firm-surfaced material including metal, concrete, stone, wood, cloth, nylon, plastic, fiberglass, vinyl, glass, and the shells of other bivalves (Mackie et al. 1989, Ontario Ministry for the Environment 1989).

Zebra mussels have separate sexes with external fertilization. The temperature range for embryonic development and hatching is 12 to 24 °C (54 to 75 °F). The veliger larva hatches from the egg (Mackie et al. 1989) having a thin, bivalved shell (protoconch), a ciliated "velum" for swimming and feeding, and a rudimentary foot. It is 0.04 to 0.07 mm in diameter at hatching and reaches 0.16 to 0.29 mm as a settlement-competent postveliger. The veliger shell has a straight hinge (i.e., shell valves do not project above the shell hinge line) while the shell valves of the postveliger are characterized by the presence of distinct "umbos" (i.e., a dorsal portion of the shell valves projecting above the hinge line). Only the postveliger is capable of settlement. After settlement and initial byssal attachment, the umbonal postveliger shell rapidly transforms into the typically anteriorly pointed, mussel-shaped shell of the adult (marked by reduction of the anterior end of the shell) (Hopkins 1989).

Zebra mussel growth rates and life span are dependent on environmental conditions. Greatest growth occurs in habitats with elevated temperatures and high food levels (i.e., suspended algae and bacteria) (Griffiths et al. 1991, Morton 1969, Mikheev 1986). Growth rate decreases with water depth. Growth rate is stimulated at water velocities of 0.5 to 0.8 m/sec, but it is reduced where flow exceeds 1.5 m/sec (Mikheev 1986). Studies of North American zebra mussel populations suggest that annual mortality rates are high, with few individuals surviving beyond 3 years of life. Thus, the majority of individuals in natural and fouling populations are small and less than 2 years old (Griffiths et al. 1991).

The high reproductive rates (30,000 to 40,000 eggs per female per year) and growth rates of zebra mussels allow populations to rapidly form thick encrustations in natural habitats and raw water systems. Natural population densities of 5,000 to 30,000 mussels/m<sup>2</sup> are not uncommon (Mackie et al. 1989) with 114,000 mussels/m<sup>2</sup> being reported in a lagoon pond (Wiktor 1963). In raw water systems, where continuous flow and abundance of hard substrata provide mussels with optimal conditions for settlement and growth, even greater densities have been reported. Densities of 700,000 individuals/m<sup>2</sup> occurred in the intake canal of a power station on Lake Erie (Griffiths, Kovalak, and Schloesser 1989), indicating that raw water systems may be particularly susceptible to massive zebra mussel infestations.

## Characteristics of Zebra Mussel Macrofouling

Zebra mussel veliger densities in intake water can range from 70 to 400,000 veligers/m<sup>3</sup> or 0.27 to 1,516 veligers/gal<sup>1</sup> (Mackie et al. 1989), leading to extremely high entrainment and fouling rates. Extensive entrainment of postveligers allows mussel infestations to rapidly develop. Up to 45,000 to 700,000 mussels/m<sup>2</sup> have been reported to settle in raw water systems within a single spawning season (Ontario Ministry of the Environment 1989; Szlauer 1974; Griffiths, Kovalak, and Schloesser 1989). The presence of microbial films and surface corrosion stimulate postveliger settlement (Jenner 1983, Jenner and Janssen-Mommen 1992, Lyakhov 1986) by reducing flow at the substratum surface to levels that induce postveliger settlement even in systems with flow velocities above those generally reported to inhibit settlement (Mackie et al. 1989, Lyakhov 1986).

Mussel fouling in raw water systems can occur on any hard surface (including embayment walls, stationary trash racks, pump intake housings, and other exposed surfaces) receiving adequate flow and oxygen concentration and not exposed to temperatures greater than 30 °C (86 °F) (McMahon 1990). Mussels may even attach to traveling screens and not be removed by high pressure spray wash systems, particularly if screens periodically stand idle for periods greater than 24 hr. Mussel fouling has also been reported to have nearly completely occluded flow across stationary primary screens (Kovalak 1990). Mussels infesting intake structures can reach sizes or be sloughed off as clusters of shells (bound together by byssus threads) which are large enough to foul small-diameter downstream components such as condenser and heat exchanger tubes, small-diameter piping, and fire protection systems (McMahon 1990, McMahon and Tsou 1990). Such blockage reduces efficiency and aggravates corrosion downstream of blockage points. Blockage can also result from large mussel shells becoming lodged lengthwise across tube inlet openings, from young mussels attaching directly to tube walls, and from byssally bound clusters of mussel shells becoming lodged in tube inlets (McMahon 1990, McMahon and Tsou 1990). Tube blockage by clusters of individuals makes the openings of even relatively large-diameter tubing (>1 in.) prone to mussel fouling (McMahon 1990, McMahon and Tsou 1990). In addition to flow restriction or blockage, accumulation of sediments and reduction of oxygen concentrations within thick encrustations of mussels can create conditions exacerbating corrosion of metallic piping (McMahon 1990).

Zebra mussels tend to accumulate in any area where discontinuities in water flow make conditions optimal for settlement. Thus, even in high-flow systems (velocity >1.5 to 2 m/sec) not generally susceptible to mussel

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<sup>1</sup> To convert gallons to liters, multiply by 3.785.

fouling, postveliger settlement will occur in the immediate vicinity of pipe joints, valve seats and flanges, and joints of unequal pipe diameter where low-flow refugia develop (Jenner and Janssen-Mommen 1992). It is this capacity to settle on almost any available hard surface where flow conditions are appropriate that makes the zebra mussel a major macrofouling organism within its natural European range and that is likely to make it the most costly and damaging aquatic species ever introduced into North American fresh waters (Roberts 1990).

## **2 Investigation of the Efficacy of Emersion as a Zebra Mussel Macrofouling Control Technology**

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Dewatering of mussel-infested structures and subsequent lethal exposure of mussels to air could be a highly efficacious zebra mussel control strategy, particularly in raw water systems such as navigation locks and water-intake structures that are designed to be periodically dewatered for maintenance. Such structures can often *sustain relatively heavy mussel infestations*. Thus, the structures could remain operational for extended periods before needing to be dewatered to eradicate mussel fouling. Utilization of dewatering strategies to mitigate zebra mussel fouling in such structures has the obvious advantage of eliminating chemical treatment, thus minimizing environmental impact (this may be a major consideration for control of mussel fouling in navigation locks) while being relatively cost-effective. It would be cost-effective because it would require no retrofitting of these systems and because dewatering treatment of mussel infestations could be integrated into routine maintenance schedules.

In spite of the potential efficacy of dewatering and emersion as a means to control zebra mussel macrofouling, only a minimal amount of literature exists regarding the mussel's emersion tolerance. Indeed, only relatively anecdotal information regarding its response to emersion has been reported in European literature (Alyakrinskaya 1978) without extensive effort to quantify the effects of either temperature or relative humidity on emersion tolerance and/or evaporative water-loss rate. There, also, have not been any reports on the tolerance of this species to freezing temperatures while emersed.

Based on literature reports (reviewed by McMahon 1991), available evidence indicates that zebra mussels are much less tolerant of emersion than are the vast majority of freshwater bivalves, suggesting that dewatering

could be utilized as a zebra mussel control strategy. In order to provide a basis for the evaluation of dewatering as a zebra mussel control technology, the tolerance of zebra mussels to emersion under a wide range of ambient temperatures and relative humidities was investigated. Temperatures tested ranged from subfreezing to above the mussel's upper lethal limit. Tolerance of emersion at temperatures above freezing was determined over a relative humidity range of <5 percent to >95 percent. Water-loss rates were determined throughout tolerated emersion periods, and quantitative models allowing prediction of emersion tolerance under specific combinations of temperature and relative humidity were developed.

## Methods

Zebra mussels were collected from the intake of a power station drawing water from Lake Erie, shipped overnight in air, and held in insulated, cooled containers while transported to the University of Texas at Arlington. They were held in 75 gal of continually aerated, dechlorinated, City of Arlington tap water in a refrigerated "living stream" holding tank at a constant water temperature of  $5\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$  (41 °F).

Prior to experimental determination of emersion tolerance, adult individuals were randomly selected (shell length range = 11 to 30 mm), marked by painting an identifying number on each shell, weighed wet to the nearest 0.1 mg, and reimmersed for 24 hr. After 24 hr reimmersion, they were emersed under varying conditions of temperature and relative humidity. Samples of 60 mussels each were exposed to one of 20 temperature and relative humidity combinations in plastic desiccator chambers. Relative humidity (RH) was maintained in the desiccators by saturated salt solutions. Mussels were held above these solutions on stages of 0.5-cm wire mesh covered with 1-mm nylon mesh. The RH levels tested were < 5 percent over silica gel desiccant, 33 percent RH over  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 53 percent over  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , 75 percent over NaCl, and >95 percent over distilled water (Byrne, McMahon, and Deitz 1988). Test temperatures for each RH treatment were 5, 15, 25, and 35 °C (41, 59, 77, and 95 °F) maintained at  $\pm 0.2\text{ }^{\circ}\text{C}$  in a refrigerated incubator.

Periodically, subsamples of six individuals were removed from each desiccator, reweighed, and their viability tested by rehydration in dechlorinated City of Arlington tap water for 12 hr at room temperature (22 to 24 °C; 72 to 75 °F). Frequency of subsample removal was designed to include emersion durations ranging from 100 percent subsample survival to 100 percent subsample mortality. Lethal emersion times were estimated as  $LT_{50}$  (estimated time for 50 percent sample mortality) and  $LT_{100}$  values (estimated time for 99.9 percent sample mortality) by probit analysis (Bliss 1936) and time to first observation of 100 percent subsample mortality. The natural logarithms of lethal emersion time values were fitted to a least squares multiple linear regressions against temperature and RH as independent variables.

At the end of the recovery period, all subsampled individuals were dried to constant weight at 90 °C (194 °F). Subtraction of dry-weight values from initial-weight values yielded the total water content (total water weight = corporal + extracorporal water (i.e., mantle cavity water weight)). Subtraction of the wet weight after emersion from the initial wet weight yielded the weight of water lost during the emersion period. Water loss was expressed as a percentage of the total water weight in fully hydrated individuals just prior to emersion (Byrne, McMahon, and Dietz 1988). Computation of mean percent total water loss values for subsamples of individuals removed at different periods over the course of emersion at any one RH and temperature combination allowed evaluation of cumulative water loss over the entire tolerated emersion period. In all cases, water-loss values were computed only for individuals surviving a particular emersion period.

Tolerance to freezing was determined for adult mussels held at 5 °C (41 °F) prior to experimentation under conditions similar to those described above for emersion tolerance determinations. Subsamples of 10 adult mussels ranging in shell length from 8.5 to 34.0 mm were placed in jacketed, 400-ml glass beakers in which subzero degree Celsius temperatures were maintained ( $\pm 0.1$  °C) by circulation of water containing anti-freeze from a Lauda K-2/R refrigerated constant-temperature circulator whose cooling capacity was increased by insertion of a refrigerated cold-probe (Forma Scientific, model 8366). The bottom of the freezing chamber was covered with two layers of 1-mm nylon mesh to prevent freezing of mussels' shells to the vessel's walls. Specimens were placed in the bottom of the chamber either as 10 separated individuals or as clusters of 10 individuals bound together in 1-mm nylon mesh held closed with a rubber band. The chamber opening was closed with a rubber stopper penetrated by a thermometer to record chamber temperature. The chamber temperature was first stabilized at experimental temperatures of either 0, -1.5, -3, -5, -7.5, or -10 °C (32, 29, 27, 23, 19, and 14 °C). The sample of mussels (separated or clustered) was then quickly placed in the chamber, and the chamber restoppered. After a predetermined exposure period, mussels were removed from the chamber and allowed to recover for 12 hr in dechlorinated, City of Arlington tap water held at 5 °C (41 °F) in a refrigerated incubator. Duration of exposure of successive subsamples at any one test temperature was increased until 100 percent sample mortality was achieved in at least two consecutive subsamples or for a total 48-hr exposure if 100 percent sample mortality did not occur.

In both emersion tolerance and freezing tolerance experiments, the viability of individuals in subsamples was estimated after recovery and rehydration by gently touching the posterior mantle edge and siphons of individuals with the bristles of a small, fine brush. If this gentle, tactile stimulation did not induce valve closure, mantle edges and siphons were more strongly stimulated with a dissecting needle. If either gentle or strong stimulation resulted in valve closure, the individual was considered recovered. If strong stimulation did not elicit valve closure, the individual was considered dead.



## Results

At test temperatures within the ambient temperature range of 5 to 25 °C (41 to 77 °F), water-loss rates were clearly correlated with both temperature and RH. Water-loss rates increased with increasing temperature within any RH treatment and decreased with increasing RH within any one temperature treatment (Figure 1). At 15 °C (59 °F) and 25 °C (77 °F), water loss was continuous throughout tolerated emersion; however, at 5 °C, water-loss rates were greatly reduced after an initial 24-hr period in which 35 to 40 percent of total water was lost. This initial high rate of water loss appeared to be associated with expulsion of extracorporeal mantle cavity water due to the extensive valve-gaping behavior displayed by specimens emersed at 5 °C in all tested RH. Tendency to gape was less pronounced in specimens emersed at 15 and 25 °C, reducing the relative rate of water loss in the early stages of emersion compared with individuals emersed at 5 °C. In all RH treatments at the lethal temperature of 35 °C (95 °F), water-loss rate was initially high ( $\approx$ 40 percent of total water) within the first hour of emersion, again due to extensive valve gaping. Thereafter, water-loss rates at 35 °C slowed at all RH, being slower at 75 and >95 percent RH and faster at 53, 33, and <5 percent RH (Figure 2).

From <5 to 75 percent RH, mean total water lost just prior to death at 5, 15, and 25 °C (41, 59, and 77 °F) ranged between 58 and 71 percent. But at >95 percent RH, death occurred at lower levels of water loss (range = 25 to 45 percent) in all three temperatures (Figure 3). Whether determined as  $LT_{50}$ ,  $LT_{100}$ , or time to first 100 percent sample mortality values, the effects of temperature and RH on emersion tolerance within the tolerated ambient temperature range of 5 to 25 °C were essentially similar (Figure 4). All three measures of emersion tolerance generally increased with increasing RH within any one temperature treatment and decreased with increasing temperature within any one RH treatment (Figure 4). Maximum  $LT_{50}$  values occurred at >95 percent RH and ranged from 70 hr at 25 °C to 560 hr at 5 °C; corresponding  $LT_{100}$  and time to first 100 percent sample mortality ranges were 97 to 1,124 hr and 96 to 1,152 hr, respectively. Minimal  $LT_{50}$  values occurred at <5 percent RH and ranged from 42 hr at 25 °C to 170 hr at 5 °C, corresponding to  $LT_{100}$  and time to first 100 percent sample mortality ranges of 70 to 363 hr and 72 to 312 hr, respectively. The effects of relative humidity on emersion tolerance became much more pronounced at lower temperatures (Figure 4).

The  $LT_{50}$ ,  $LT_{100}$ , and time to 100 percent sample mortality values were transformed into natural logarithms and fitted to least squares multiple linear regression equations against temperature and RH as independent variables. The resulting regression equations were highly significant ( $P < 0.00001$ ) allowing tight prediction of emersion tolerance under specific temperature-relative humidity conditions. The regression equations computed were:

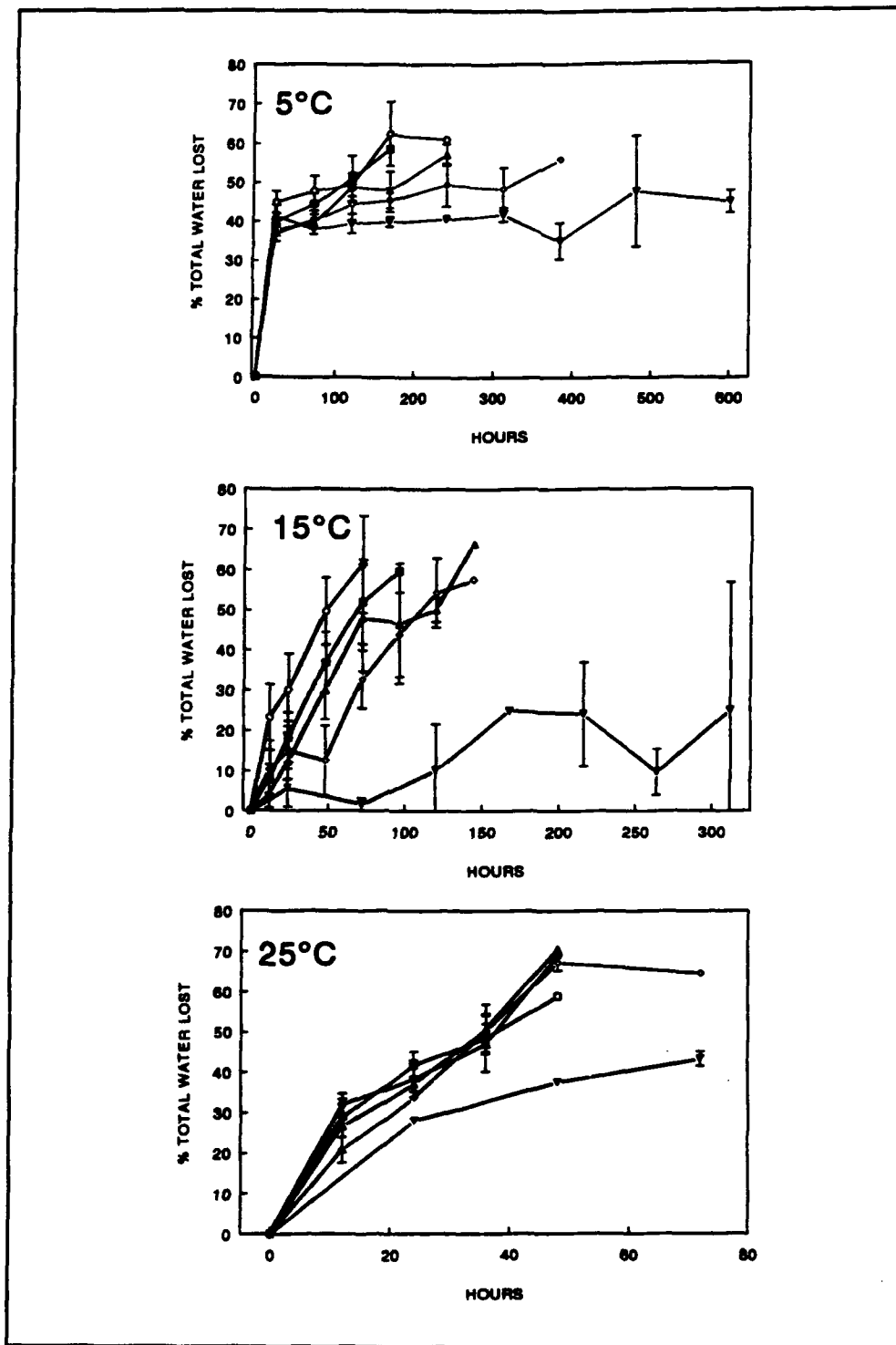


Figure 1. Cumulative percent of total water (corporal + extracorporal) lost over the duration of tolerated emersion (horizontal axis) by zebra mussels, *Dreissena polymorpha*, emersed at 5 °C (41 °F), 15 °C (59 °F), and 25 °C (77 °F) under relative humidities of <math><5\%</math> (<math>\circ</math>), <math>33\%</math> (<math>\square</math>), <math>53\%</math> (<math>\Delta</math>), <math>75\%</math> (<math>\diamond</math>), and <math>>95\%</math> (<math>\nabla</math>)

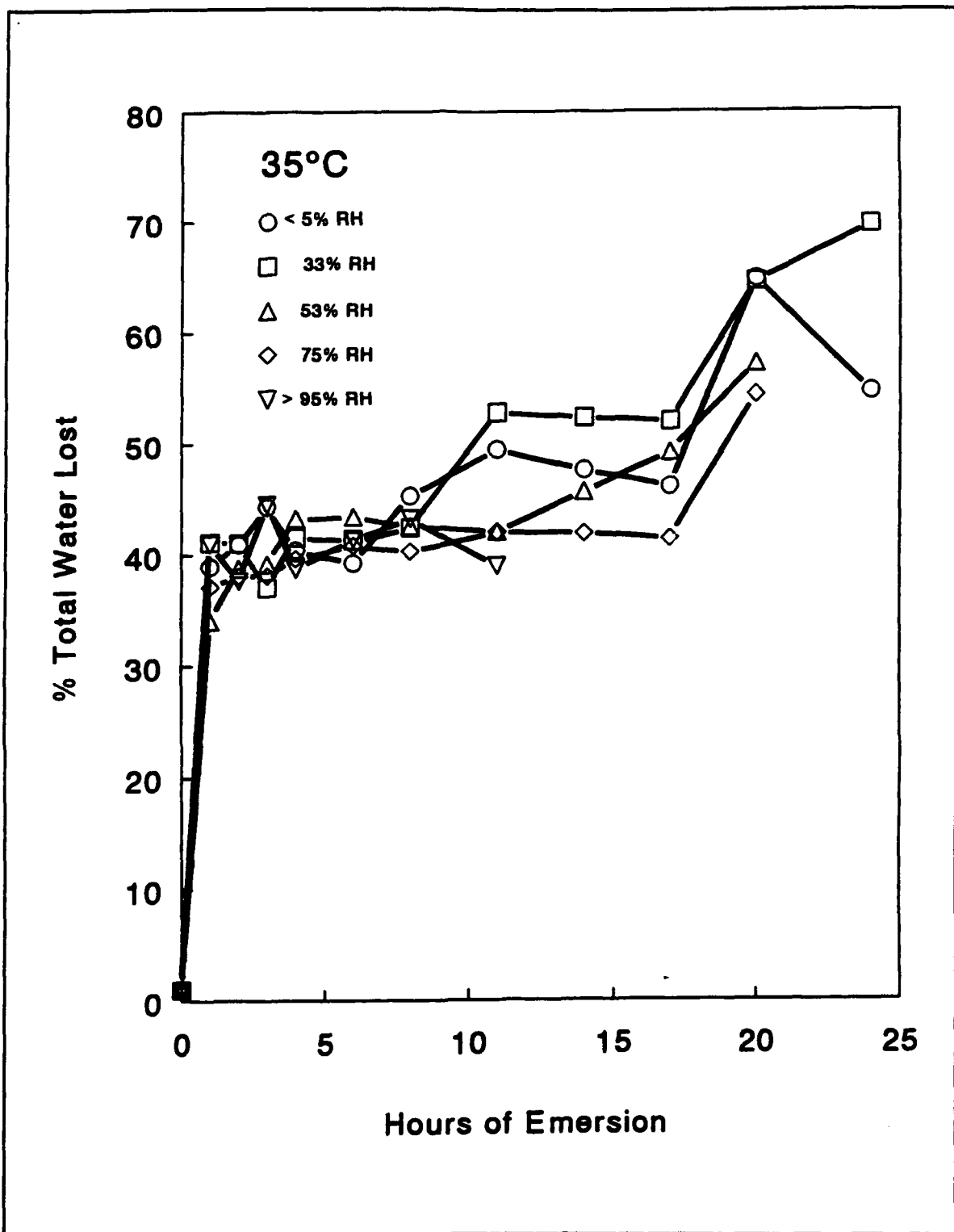


Figure 2. Cumulative percent of total water (corporal + extracorporal) lost over the duration of tolerated emersion (horizontal axis) by zebra mussels, *Dreissena polymorpha*, emersed at a lethal temperature of 35 °C (95 °F) under relative humidities of <5, 33, 53, 75, and >95%

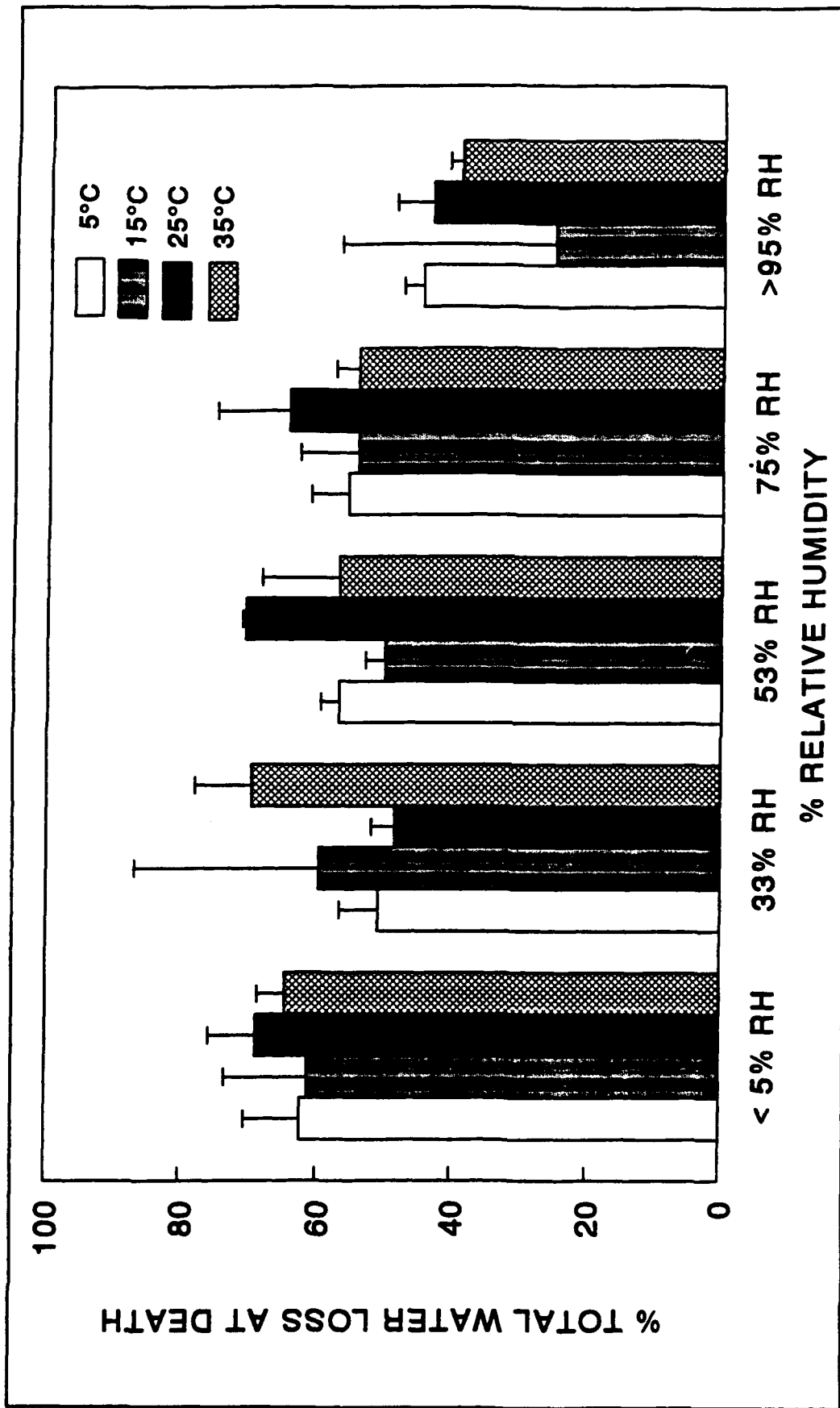


Figure 3. Mean percent of total water (corporal + extracorporal) lost just prior to death in zebra mussels, *Dreissena polymorpha*, emersed under different relative humidities at temperatures of 5 °C (41 °F), 15 °C (59 °F), 25 °C (77 °F), and 35 °C (95 °F). Vertical bars at top of each histogram represent 95 percent confidence limits of the mean

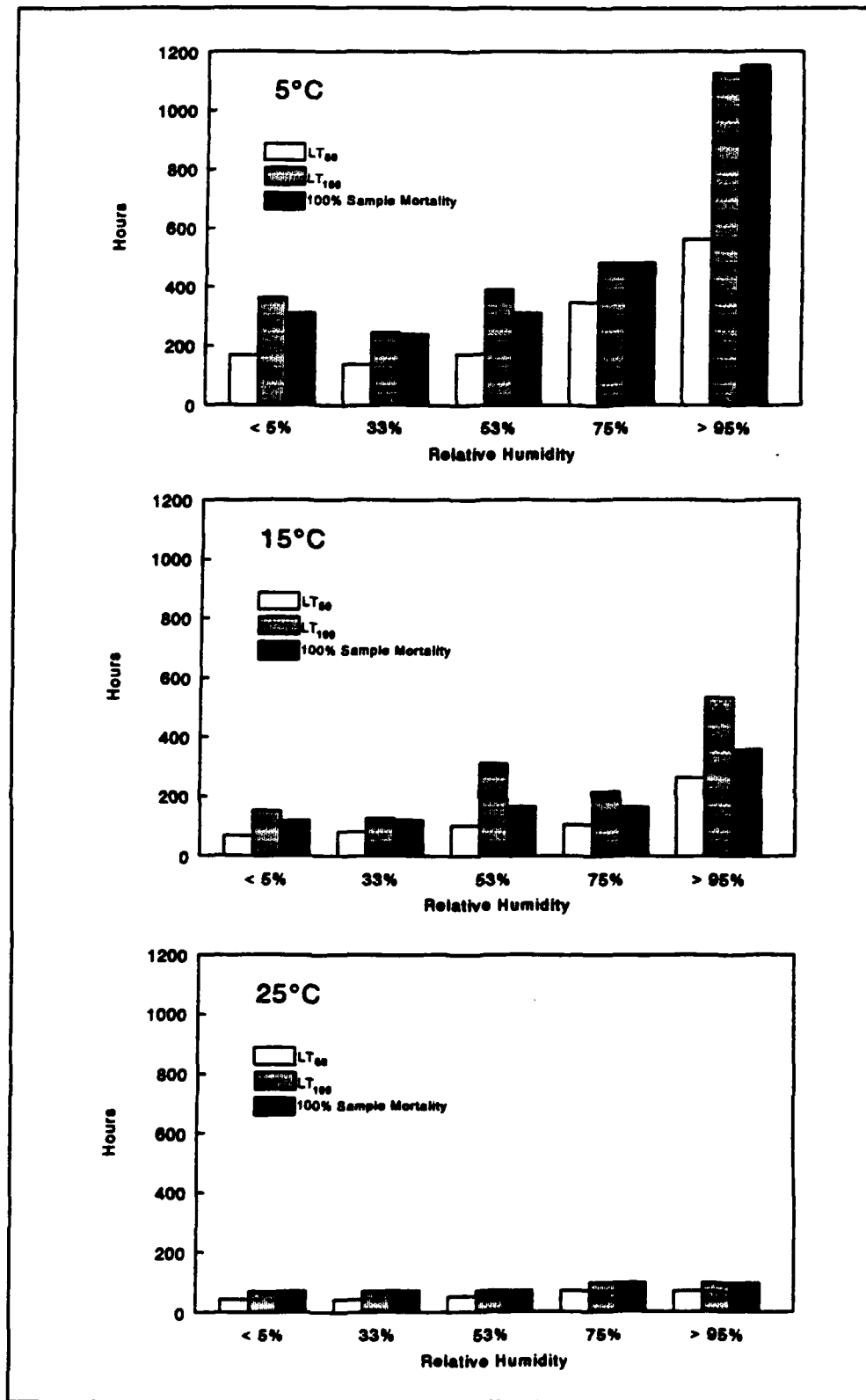


Figure 4. Emersion tolerance times of zebra mussels, *Dreissena polymorpha*, under varying conditions of temperature and relative humidity

$$\ln LT_{50} = 5.243 - 0.074(^{\circ}\text{C}) + 0.011 (\% \text{ RH})$$

(r = 0.94, n = 15, F = 56.6)

$$\ln LT_{100} = 6.091 - 0.087(^{\circ}\text{C}) + 0.010 (\% \text{ RH})$$

(r = 0.92, n = 15, F = 39.8)

$$\ln \text{Time of First 100\% Mortality} = 5.917 - 0.082(^{\circ}\text{C}) + 0.010 (\% \text{ RH})$$

(r = 0.93, n = 15, F = 47.2)

where

r = correlation coefficient

n = number of individuals

F = F Test

The effects, predicted by these equations, of temperature and RH on the emersion tolerance of zebra mussels are displayed in Figure 5. This figure indicates that RH has a small impact on emersion tolerance at temperatures above 25 °C (77 °F); however, it has an increasingly greater impact on tolerance time as temperatures decline below 25 °C. The tolerated period of emersion becomes greatly extended by elevated RH at temperatures below 10 °C (50 °F).

The effects of RH on the pattern of zebra mussel emersion tolerance at a lethal temperature of 35 °C (95 °F) (Jenner 1983, Jenner and Janssen-Mommen 1992) was reversed compared with that recorded within the normal ambient temperature range (5 to 25 °C or 41 to 77 °F). Instead of increasing with increased RH as occurred within the normal ambient temperature range, LT<sub>50</sub>, LT<sub>100</sub>, and time to first 100 percent sample mortality values increased with decreasing RH being 16.9, 41.0, and 30.0 hr at <5 percent RH, respectively, and decreasing to 9.6, 14.9, 14.0 hr, respectively, at >95 percent RH (Figure 6).

Zebra mussels were not greatly tolerant of freezing temperatures. When exposed as separate individuals, 100 percent mortality was recorded at all test temperatures except 0 °C (32 °F) within 48 hr; LT<sub>50</sub>, LT<sub>100</sub>, and time to first 100 percent sample mortality values were 13.5, 15.1, and 15.0 hr, respectively, at -1.5 °C (29 °F). All three values declined to less than 2 hr at -10 °C (14 °F) (Figure 7). Somewhat surprisingly, tolerance of freezing temperatures increased at all test temperatures in clustered mussels. When clustered, mussels exposed to 0 and -1.5 °C displayed no mortality over the maximal 48-hr exposure duration. At -3.0 °C (27 °F), LT<sub>50</sub>, LT<sub>100</sub>, and time to first 100 percent sample mortality values were 3.2, 24.0, and 7.0 hr, respectively, declining to 1.1, 3.7, and 2.0 hr, respectively, at -10 °C (Figure 7).

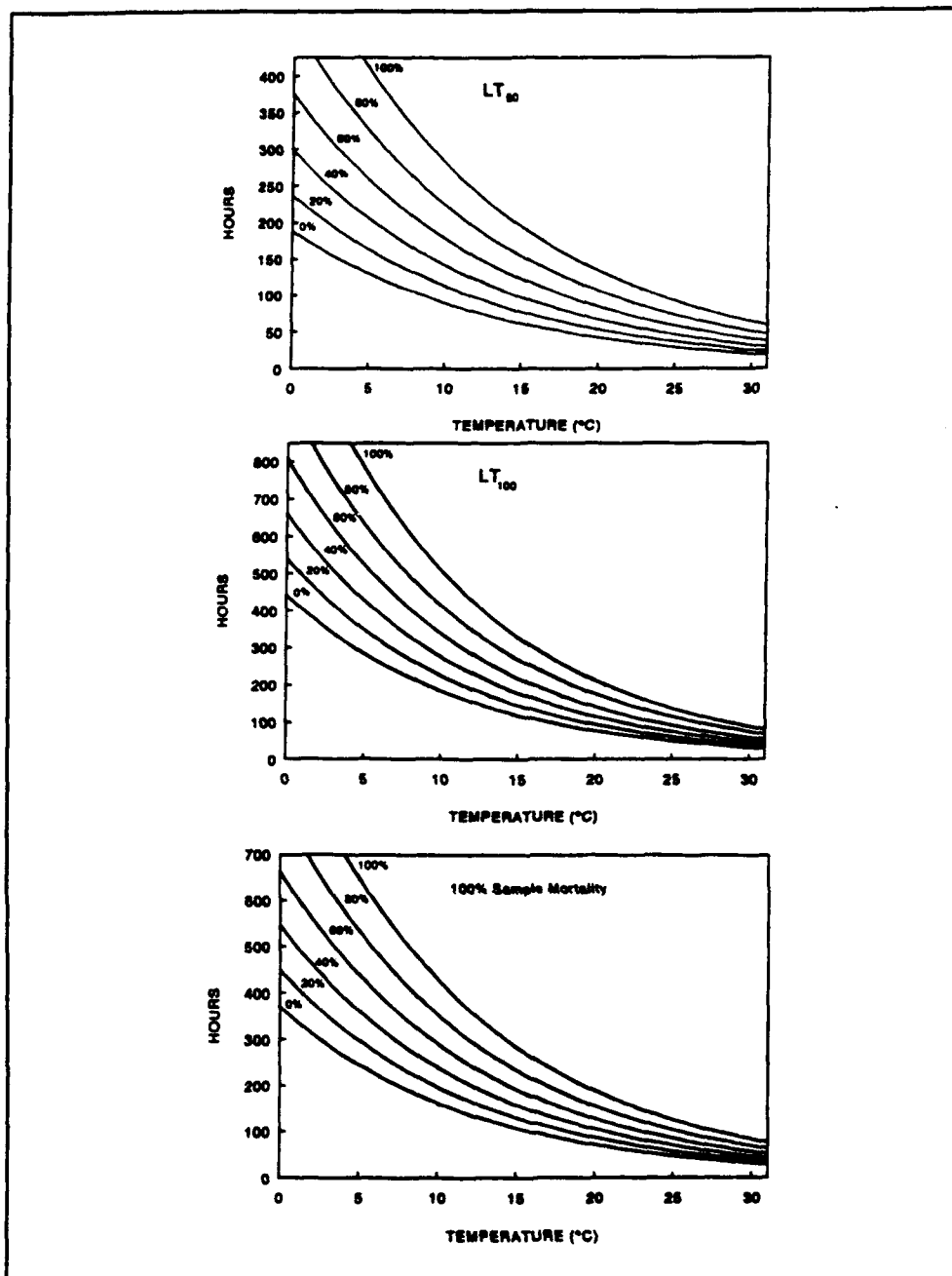


Figure 5. Effects of temperature and relative humidity on the emersion tolerance of zebra mussels, *Dreissena polymorpha*. Emersion tolerance times estimated as time to 50% sample mortality ( $LT_{50}$ ), to 100% sample mortality ( $LT_{100}$ ), and to the first observation of 100% sample mortality (100% sample mortality) over the tolerated ambient temperature range of 0 to 30 °C (32 to 86 °F) at various indicated relative humidities. See text for the multiple linear regression equations relating the natural logarithm of emersion tolerance time values to temperature and relative humidity on which the data presented in this figure are based

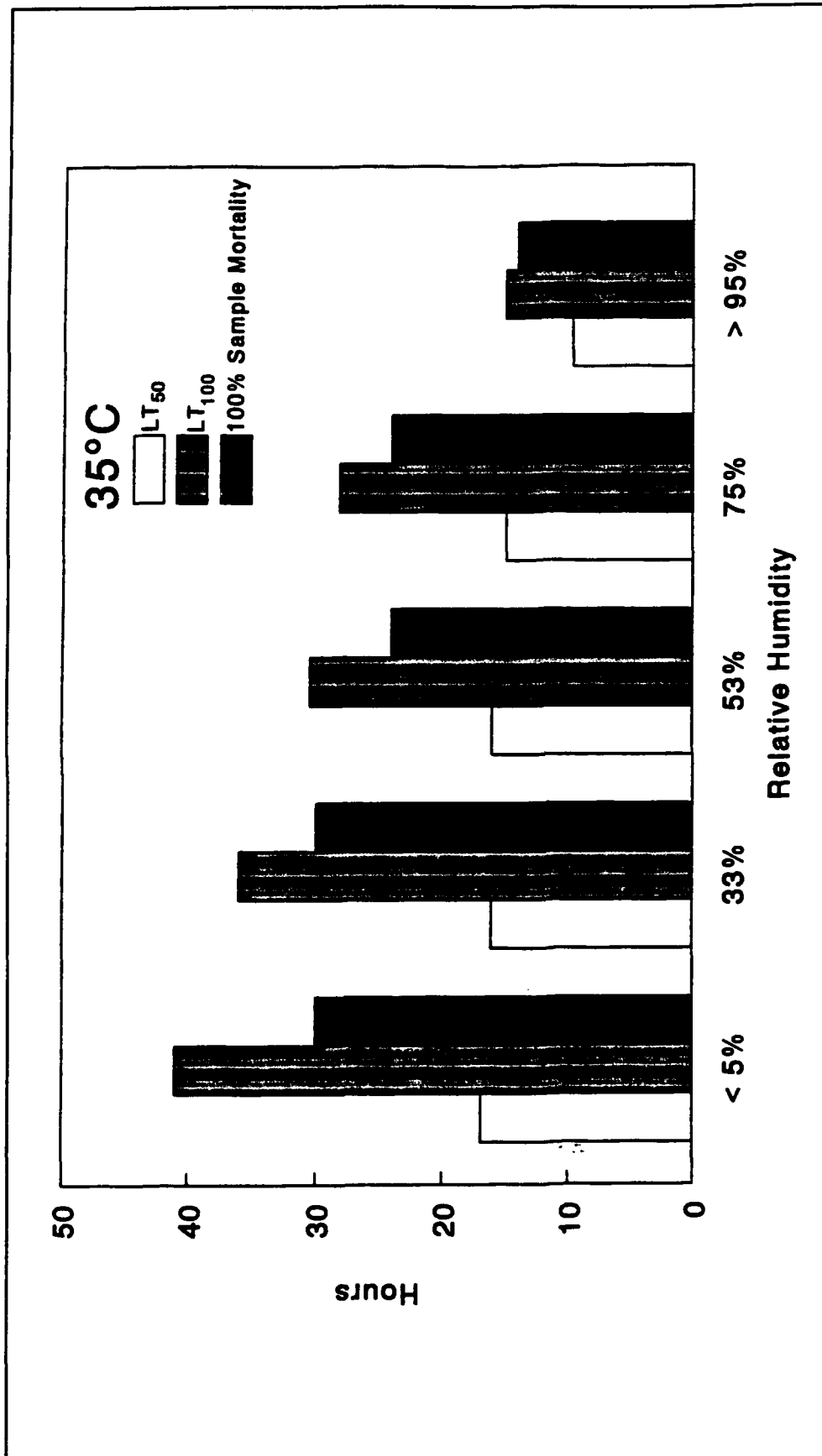


Figure 6. Emersion tolerance times of zebra mussels, *Dreissena polymorpha*, under varying relative humidities at a lethal temperature of 35 °C (95 °F). Emersion tolerances were estimated as times for 50% sample mortality (LT<sub>50</sub>), for 100% sample mortality (LT<sub>100</sub>), and for the first observation of 100% sample mortality (100% sample mortality) at relative humidities of <5, 33, 53, 75, and >95%



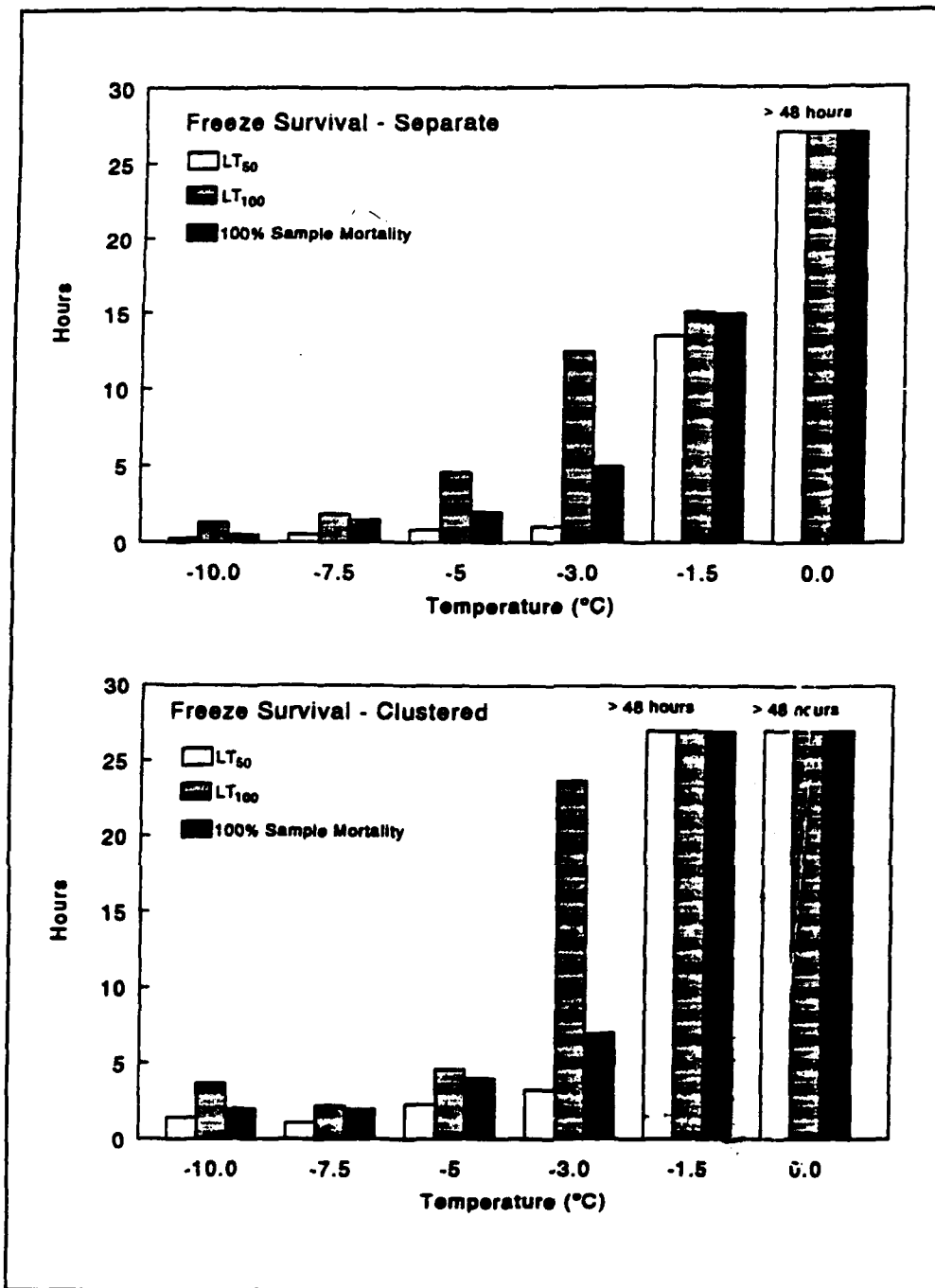


Figure 7. Tolerance of emersion in subfreezing temperatures by zebra mussels, *Dreissena polymorpha*, as separated individuals or clustered groups. Freeze tolerances were estimated as times for 50% sample mortality (LT<sub>50</sub>), for 100% sample mortality (LT<sub>100</sub>), and for first observation of 100% sample mortality (100% sample mortality) at temperatures of 0, -1.5, -3, -5, -7.5, and -10 °C (32, 29, 27, 23, 18.5, and 14 °F). The notation ">48 hours" above sets of histograms in both panels indicates that mussels emersed at those temperatures survived the entire 48-hr exposure period

# 3 Discussion and Conclusions

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## Discussion

Of the presently available nonchemical options for mitigation and/or control of zebra mussel macrofouling, thermal treatment, nontoxic foul-resistant coatings, disposable substrata, and manual removal techniques have been most fully developed and implemented in Europe and North America (Mackie et al. 1989, McMahon 1990, Jenner 1983, Jenner and Janssen-Mommen 1992). In contrast, mitigation and control technologies centered on dewatering and exposure of mussel infestations to desiccation or freezing temperatures have received no experimental attention. The only information previously available regarding the desiccation resistance of zebra mussels resulted from studies of their physiological capacity to buffer the hemolymph (i.e., blood) pH while emersed (Alyakrinskaya 1978). In this work, mussels were exposed to air at room temperature (20 to 22 °C, 68 to 72 °F) without controlling RH. Under these conditions, they survived no longer than 4 days.

Based on the limited data of Alyakrinskaya (1978), it has been recommended that before transportation between bodies of water, recreational vessels be held out of water for at least 2 to 5 days in a hot, dry, sunny location in order to kill any zebra mussels attached to the hull. It was believed that this strategy could prevent inadvertent mussel dispersal into new drainage systems (Ontario Ministry of Natural Resources 1990, 1991a, 1991b). The data presented in this paper suggest that even at near zero RH at 25 °C (77 °F), an exposure duration of at least 3 days would be required to kill 100 percent of adult mussels attached to a boat hull. At 25 °C, RH has little effect on lethal emersion times with 4 to 5 days required for 100 percent mortality, which is a level of emersion tolerance similar to that reported by Alyakrinskaya (1978). However, as temperature declines below 25 °C, survival times of emersed mussels increase exponentially. Thus, emersion durations at temperatures below 15 °C (59 °F), particularly at higher RH, may become too extended (>10 days) for practical control of mussel dispersal on trailered boats. Indeed, prolonged emersion tolerance of zebra mussels at temperatures  $\leq 15$  °C suggests

that dewatering may not be a practical method for controlling mussels in power stations, waterways structures, and industrial facilities at any other time than midsummer because of the extensive downtime required for mitigation by dewatering at lower air temperatures. At lower temperatures, dewatering would be a practical method for mitigation of zebra mussel fouling only in redundant systems where redundant components could be alternately dewatered for periods long enough to achieve high mussel mortalities without affecting system operation.

It has been hypothesized that zebra mussels were introduced to the Great Lakes by the dumping of ship ballast water containing veliger larvae or juveniles carried across the Atlantic Ocean from a European freshwater port (Hebert, Muncaster, and Mackie 1989; Mackie et al. 1989). The exponential extension of emersion tolerance in zebra mussels with decreasing temperature and increasing RH suggests a second potential mode of transoceanic transport. Zebra mussels are highly mobile. They readily detach from the byssal holdfast and disperse away from areas of high density to colonize fresh substrata (McMahon 1990). This behavior could result in mussels rapidly colonizing the anchors and anchor chains of vessels moored in a freshwater European port harboring an extensive zebra mussel population. Under the cool, moist conditions prevalent in the northern Atlantic ocean, mussels attached to an anchor chain could readily survive emersion long enough to be transported into the Great Lakes. Once the vessel moored in the Great Lakes, mussels could disperse from the anchor chain onto the surrounding substratum, forming a reproductive, founding population.

At 35 °C (95 °F), a temperature only 5 °C (9 °F) higher than the maximum tolerated temperature of 30 °C (86 °F) (Jenner and Janssen-Mommen 1992), zebra mussels had an  $LT_{100}$  of less than 2 days regardless of RH. Thus, even mild heating of air in dewatered structures could induce rapid zebra mussel kills, making it a potent mussel-mitigation technology. Kills of zebra mussels in dewatered pipes could also be greatly accelerated by forcing hot air through them.

Zebra mussels appear to be very intolerant of prolonged emersion relative to other freshwater bivalve species. At 15 °C (59 °F) from <5 percent to 75 percent RH, Asian clams (*Corbicula fluminea*) tolerated emersion more than twice as long as zebra mussels (*Dreissna polymorpha*). However, at 25 °C (77 °F) from <5 percent to >95 percent RH, the emersion tolerance of the two species was relatively similar ( $LT_{50}$  for *C. fluminea* = 71.4 to 78.2 hr (Byrne, McMahon, and Dietz 1988),  $LT_{50}$  for *D. polymorpha* = 42 to 70 hr). As occurred with zebra mussels in this study, increased RH had little effect on the emersion tolerance of Asian clams above 25 °C, but greatly extended emersion tolerance at lower temperatures (Byrne, McMahon, and Dietz 1988). Both freshwater unionacean and sphraeiid bivalves appear to be much more tolerant of emersion than zebra mussels. Riverine and pond species of these two bivalve groups can survive many months of emersion when exposed by receding water levels during droughts and dry seasons (McMahon 1991). The very reduced

emersion tolerance of zebra mussels relative to unionacean and sphraeiid bivalves suggests that they, like the emersion-intolerant *C. fluminea*, are only recent invaders of fresh waters (McMahon 1991). The frequency and duration of emersion experienced by freshwater bivalves are less predictable and more extended than those experienced by intertidal species. Neither zebra mussels nor Asian clams appear to have had a long enough evolutionary history in fresh waters to have fully evolved the high levels of emersion tolerance characteristic of most unionacean and sphraeiid species which entered fresh waters in the Triassic and Cretaceous periods, respectively (McMahon 1991).

Percent total water loss at death in zebra mussels ranged from 56 to 71 percent at <5 percent to 75 percent RH at all four test temperatures. This range is similar to that of specimens of *C. fluminea* emerged under similar conditions ( $\approx$ 50 to 80 percent) (Byrne, McMahon, and Dietz 1988) and falls within that reported for three species of freshwater unionaceans more tolerant of emersion than zebra mussels (Holland 1991). The similarity of percent water-loss-at-death values for zebra mussels emersed in 5, 15, 25, and 35 °C (41, 59, 77, and 95 °F) over <5 percent to 75 percent RH suggests that mussels emersed under these conditions died as a result of lethal tissue desiccation. However, at >95 percent in all four test temperatures, percent water loss was considerably below that recorded at lower RH values. The occurrence of mortality at lower than tolerated levels of desiccation suggests that death of emersed specimens at >95 percent RH was not due to lethal tissue desiccation, but, rather, to some other emersion-induced stress such as disruption of hemolymph acid-base balance, ammonia toxicity, or exhaustion of organic energy stores (McMahon 1991, Byrne and McMahon 1993).

At 5 (41), 15 (59), and 25 °C (77 °F), emersion tolerance decreased progressively with decreasing RH. In contrast, at 35 °C (95 °F), emersion tolerance increased progressively with decreasing RH. At all test temperatures, the rate of evaporative water loss increased with decreasing RH, suggesting that the more rapid mortalities recorded at lower RH at test temperatures of 5, 15, and 25 °C were due to lethal desiccation. Thus, the increase in tolerance in specimens emersed in lower RH at 35 °C initially appeared somewhat anomalous. However, as 35 °C was marginally above the mussels' upper lethal temperature limit, emersion under low RH appeared to allow mussels to maintain tissue temperatures below the long-term upper lethal temperature limit of 31 °C (88 °F) (Jenner 1983, Jenner and Janssen-Mommen 1992) by evaporative cooling. In contrast, at >95 percent RH, capacity for evaporative cooling appeared to be greatly reduced as indicated by the near zero water-loss rates of emersed individuals after an initial rapid loss of mantle cavity water due to valve gaping (Figure 3). Inability to evaporatively cool tissues at >95 percent RH appeared to result in very rapid mortality ( $LT_{50} < 10$  hr). However, the  $LT_{100}$  for mussels emersed at 35 °C and >95 percent RH was still approximately 75 times greater than that recorded in submerged individuals held at that temperature (Jenner 1983, Jenner and Janssen-Mommen 1992),

suggesting that even under near 100 percent RH, evaporative cooling can increase the tolerance of lethal temperatures in emersed individuals.

The rapid kills resulting when emersed mussels were exposed to air temperatures only a few degrees above the species' upper lethal temperature limit suggest that injection of heated air into dewatered structures could greatly increase the effectiveness of dewatering as a zebra mussel macrofouling control technology. Generation of heated air requires far less energy than heated water, and the equipment for producing heated air is readily available, highly mobile, and easily scaled to almost any size raw water structure. Use of heated air would not require excessive piping as would use of heated water or steam. In addition, venting of warmed air into the atmosphere would have negligible environmental impact. Heated air treatment may be particularly effective in small structures that are readily dewatered such as pipe segments, heat exchanges, and gauges.

There have been no previous studies of the freezing tolerance of zebra mussels or other freshwater bivalve species. Northern temperate intertidal gastropods and bivalves, which withstand regular tidal emersion in freezing temperatures, tolerate air temperatures as low as  $-22\text{ }^{\circ}\text{C}$  ( $-8\text{ }^{\circ}\text{F}$ ) and tissue temperatures as low as  $-10$  to  $-6\text{ }^{\circ}\text{C}$  ( $14$  to  $21\text{ }^{\circ}\text{F}$ ) (Newell 1979). In contrast, zebra mussels showed little tolerance of emersion in freezing temperatures, being intolerant of emersion at temperatures  $\leq -1.5\text{ }^{\circ}\text{C}$  ( $29\text{ }^{\circ}\text{F}$ ) when exposed as separated individuals and intolerant of  $\leq -3.0\text{ }^{\circ}\text{C}$  ( $27\text{ }^{\circ}\text{F}$ ) when emersed as clusters of individuals. The rapid mortality resulting from emersion at temperatures  $\leq -3\text{ }^{\circ}\text{C}$  ( $LT_{100}$  at  $-3\text{ }^{\circ}\text{C} < 24\text{ hr}$  and at  $-10\text{ }^{\circ}\text{C} < 4\text{ hr}$ ) indicates that dewatering of mussel-infested structures during periods of freezing air temperatures could be a very efficacious, cost-effective, environmentally acceptable mitigation technology in northern North America where freezing air temperatures are common during winter months. Mitigation of zebra mussels by dewatering during freezing conditions may be a particularly effective control strategy in facilities such as navigation locks which are not in service or have little operational demand when the waterways on which they are located are frozen over. This mitigation technique could also be utilized in raw water systems with redundant components which could be alternately dewatered during freezing periods without affecting operations.

The increased freezing tolerance of zebra mussels when clustered suggests that longer periods of emersion in freezing temperatures will be required to achieve 100 percent mussel mortality in structures infested with dense mussel encrustations many shells thick. The mechanism causing increased freezing tolerance in clustered mussels is unclear. It is unlikely to result from differences in tissue temperatures or rate of tissue freezing in clustered versus separated individuals because exposure times were many times greater than required for tissue temperatures to equilibrate to test temperatures. Research is presently under way to determine the actual tissue freezing temperatures of clustered versus separated mussels.

The results of this research indicate that reservoir drawdown could be a potentially efficacious method to control mussels in source water habitats, particularly if carried out in midsummer when elevated temperatures greatly reduce the time required to achieve mortality or in midwinter when emersed mussels could be exposed to lethal subfreezing temperatures. Natural zebra mussel populations generally occur above the thermocline (Mackie et al. 1989), limiting them to shallow, nearshore habitats. Limitation to shallow waters would allow a high proportion of a zebra mussel population to be eradicated by relatively small reductions in water level. Indeed, the tendency for juvenile mussels settling in shallow water (<1 m in depth) to rapidly migrate to depths >1 m may be an adaptive emersion-avoidance behavior in a species with little tolerance of aerial exposure or freezing temperatures.

## Conclusions

There are a wide variety of options presently available for mitigation and control of zebra mussel macrofouling. These options include chemical and nonchemical approaches and off-line or on-line application strategies. Among chemical control options, chlorination and a nonoxidizing biocide formulated as a combination of dimethylbenzyl ammonium chloride and dodecylguanidine hydrochloride are most commonly used in North American raw water facilities. However, a number of other molluscicides are undergoing testing and field demonstration for efficacy against zebra mussels. This will increase the arsenal of chemical treatments that will be available to control fouling by this species in the future. Toxic paints and coatings, particularly those impregnated with copper or zinc salts, also appear to have efficacy in preventing zebra mussel settlement.

Among nonchemical zebra mussel control technologies, manual removal, line pigs, and thermal treatments have been most extensively utilized in North America. Promising nonchemical zebra mussel control technologies that require future research and development include robotic cleaning devices, dewatering and desiccation, nontoxic foul-release coatings, mechanical strainers, infiltration systems, exposure to anoxia or hypoxia, and disposable substrata.

The most efficacious strategy for mitigation and control of zebra mussels at a specific raw water installation will depend on the design and operational requirements of that facility; the quality, hydrography, and physical characteristics of its source waters; and the environmental and other regulatory constraints under which it operates. In any one system, the treatment strategy utilized is likely to involve a combination of several technologies designed to be efficacious, cost-effective, and environmentally acceptable under the specific conditions and regulatory restraints of that system. Certainly, the rapid spread of zebra mussels in North America is providing impetus for extensive research and development of improved bivalve macrofouling controls, including appropriation of funding specifically

for development of efficacious control of zebra mussel fouling in United States inland waterways under the newly enacted Federal Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (U.S. Congress 1990). Increasing environmental concern over the quality of North American surface waters is likely to increase regulatory pressure for development and implementation of nonchemical zebra mussel control technologies over the next decade.

Among emerging nonchemical zebra mussel control technologies receiving scant experimental attention in either Europe or North America, is one that concerns dewatering of fouled structures or source water impoundments to expose zebra mussels to lethal desiccation or freezing. The evidence presented in this report strongly suggests that dewatering could be an efficacious, cost-effective, and environmentally acceptable control technology for zebra mussels. Even though zebra mussels appear to be among the most emersion intolerant of all freshwater bivalve species, their capacity to survive emersion periods of more than several days below temperatures of 25 °C (77 °F), particularly at high relative humidities, suggests that dewatering and emersion at ambient air temperature would be an efficacious control strategy only during warm periods or in structures where operations would allow dewatering of fouled structures for 1 to 3 weeks at temperatures below 20 °C (68 °F).

However, at lower ambient temperatures, very rapid mitigation of mussel infestations in dewatered structures could be accomplished by exposure to air heated above the species' upper thermal limit. Exposure to moist, heated air inhibits evaporative tissue cooling, producing more rapid kills than exposure to dry, heated air. Therefore, an increase in the moisture content of injected heated air by evaporation of residual water in dewatered systems will reduce the time required to achieve 100 percent mussel mortality. In contrast, when emersed within its tolerated ambient temperature range, increased relative humidity increases the emersion tolerance of zebra mussels by reducing their evaporative water-loss rates and, thus, the time required to achieve a lethal level of tissue desiccation.

Dewatering of fouled structures during winter months to expose mussel infestations to freezing air temperatures could be an efficacious control technology as zebra mussels are intolerant of even minimally subzero temperatures ( $LT_{100} \leq 24$  hr at temperatures  $\leq -3$  °C (27 °F)). Thus, winter dewatering of structures such as navigation locks and water intakes under freezing conditions for periods  $\leq 1$  day could effectively kill all emersed individuals even if thick encrustations of mussels had formed.

This research also indicated that planned drawdown of artificial impoundment water levels during warm periods in summer months, or under freezing conditions during winter, could result in 100 percent kills of emersed, natural mussel populations within relatively short time periods. Thus, water level drawdown appears to be a potentially efficacious zebra mussel control strategy in source-water habitats.

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# REPORT DOCUMENTATION PAGE

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<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for public release; distribution is unlimited.		<b>12b. DISTRIBUTION CODE</b>	
<b>13. ABSTRACT (Maximum 200 words)</b> Data are presented indicating that dewatering of fouled structures or source water drawdown is potentially efficacious for zebra mussel control. These strategies can expose mussels to lethal desiccation or freezing. Zebra mussels survive emersion < 3 days at temperatures ≥25 °C (77 °F) regardless of relative humidity (RH). However, as emersion tolerance increases exponentially with decreasing temperature and increasing RH, dewatering or drawdown is best applied during warm, dry summer months. Emersion tolerance was greatly reduced (LT <sub>100</sub> < 10-40 hr) (estimated time for 99.9 percent sample mortality) at a lethal temperature of 35 °C (95 °F), which suggested that injection of heated air into dewatered structures would increase the mortality rate among emersed mussels. Freezing temperatures ≥-3 °C (27 °F) caused rapid mussel mortality (LT <sub>100</sub> ≥ 24 hr); thus, dewatering of mussel fouled structures when air temperatures are subfreezing shows promise as a mussel-fouling control strategy.			
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