Limits to tolerance of temperature and salinity in the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*Dreissena polymorpha*)[1]

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Abstract: The quagga mussel (*Dreissena bugensis*) and the zebra mussel (*Dreissena polymorpha*) were exposed to varied levels of salinity and temperature in the laboratory to compare the tolerance of each species to environmental stress. The zebra mussel could tolerate 30°C for extended periods and higher temperatures (<39°C) for a period of hours depending on the acclimation temperature and the rate of temperature change. The upper thermal limit of the quagga mussel may be as low as 25°C. Mussels of both species acclimated to 5°C were less able to survive at high temperatures (30–39°C) than mussels acclimated to 15 or 20°C. The reduced upper temperature limit of the quagga mussel implies that it will not be able to expand as far south in North America as has the zebra mussel. Both *D. bugensis* and *D. polymorpha* were exposed to three concentrations of NaCl (5, 10, and 20‰) to test salinity tolerance. No individuals of either species survived beyond 18 days in salinities of 5‰ or higher. No interspecific difference occurred in salinity-induced mortality rate.

Résumé : La moule couagga (*Dreissena bugensis*) et la moule zébrée (*Dreissena polymorpha*) ont été exposées à divers niveaux de salinité et de température en laboratoire dans le but de comparer la tolérance des deux espèces à un stress environnemental. La moule zébrée peut tolérer une température de 30°C pendant de longues périodes et même des températures supérieures (<39°C) pendant quelques heures, selon la température d'acclimatement et la vitesse de variation de la température. La limite supérieure de température tolérée par la moule couagga ne semble pas dépasser 25°C. Les moules des deux espèces acclimatées à 5°C sont moins aptes à survivre à de hautes températures (de 30 à 39°C) que les moules acclimatées à des températures de 15 ou 20°C. Étant donné le bas niveau de la limite supérieure de température tolérée par la moule couagga, celle-ci ne peut pas, en Amérique du Nord, se répandre aussi loin vers le sud que la moule zébrée. *Dreissena bugensis* et *D. polymorpha* ont été exposées à trois concentrations de NaCl (5, 10 et 20‰) afin d'évaluer leur tolérance à la salinité. Aucun spécimen des deux espèces n'a survécu plus de 18 jours à des salinités de 5‰ et plus. Aucune différence interspécifique n'a été observée dans le taux de mortalité imputable à la salinité.

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Introduction

The first public response to the invasion of an exotic species such as the quagga mussel (*Dreissena bugensis*) or the zebra mussel (*Dreissena polymorpha*) is to predict its potential distribution in the new habitat. Research to determine the effect of the introduced species on its new habitat and to find control mechanisms can then follow. Tolerance to salinity and temperature needs to be assessed experimentally for both species of *Dreissena* to determine if their environmental limits overlap, and what their final distributions in North America might be. *Dreissena bugensis* has been observed to displace *D. polymorpha* in many parts of their overlapping habitat in Ukraine (Pligin 1984). Whether this pattern will be repeated in North America is unclear.

Substantial genetic differentiation has been documented between *D. bugensis* and *D. polymorpha* (May and Marsden 1992; Rosenberg and Ludyanskiy 1994; Spidle et al. 1994). The large genetic distance between the quagga and zebra mussels suggests that these two species will not respond identically to their new habitat. Ecological evidence suggests that the two North American species of *Dreissena* behave differently in lakes Erie and Ontario, where the quagga

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mussel occurs disproportionately or exclusively in deeper areas where temperatures seldom exceed 5–10°C (Dermott and Munawar 1993; Mills et al. 1993). Models in the current literature may not be able to predict the distribution of the quagga mussel with the same accuracy they do for the zebra mussel (McMahon 1990; Strayer 1991; Ramcharan et al. 1992*a*, 1992*b*; Schneider 1992).

If the quagga mussel's tolerance to high temperatures is less than that of the zebra mussel, as suggested by previous research (Dermott and Munawar 1993; Mills et al. 1993), then *D. bugensis* may not be able to colonize the southern United States, where *D. polymorpha* currently infests areas in which midsummer water temperatures regularly reach 30°C. Additionally, the temperature of the hot water used to back-flush intake pipes, an important control mechanism for industrial facilities drawing cooling water from *Dreissena*-infested waterways, must be chosen on the basis of the temperature tolerance of the species involved. If the quagga mussel's thermal maximum is the same as or lower than that of the zebra mussel, *D. bugensis* may not pose an additional threat to commercial enterprises.

To date, three studies using three different techniques have tested the tolerance of *D. polymorpha* to temperature increase. McMahon et al. (1993) tested animals by gradually increasing the temperature from acclimation temperature to test temperature, after methods previously used to evaluate the vertical distribution of littoral gastropods (Broekhuysen 1940; Stirling 1982). Iwanyzki and McCauley (1993) exposed animals to lethal temperature without gradual change from acclimation temperature, to avoid the confounding factor of time spent in changing temperatures, after methods developed by Fraenkel (1960, 1968) for testing the vertical distribution of intertidal snails. A temperature of 30°C was estimated to be 100% fatal to zebra mussels across acclimation temperatures ranging from 2.5 to 25°C by one research group (Iwanyzki and McCauley 1993), while other reports suggest that zebra mussels are capable of tolerating 30°C indefinitely, with temperatures of 31°C or higher being lethal (McMahon et al. 1993, 1994). This result is perhaps anomalous, as Stirling (1982) demonstrated that the method used by McMahon et al. (1993) would, if anything, overestimate the lethal temperature for a given species of gastropod mollusc relative to the method of Iwanyzki and McCauley (1993). An additional study using the method of Iwanyzki and McCauley (1993) comparing the responses of quagga and zebra mussels to lethal temperatures indicated that the quagga mussel dies sooner at temperatures lethal to both species (Domm et al. 1993).

The range of salinity levels inferred to limit *D. polymorpha* in Eurasia is quite wide, from 2 to 12‰ in inland seas, but only 0.5‰ in estuaries on the Atlantic coast of the Netherlands (for review see Strayer and Smith 1993). However, the composition of the salinity in these areas is variable. The salinity of the inland seas is more rich in minor divalent ions (Ca^{+2}, Mg^{+2}, and SO_4^{-2}) than marine salinity (Strayer and Smith 1993). The presence of minor ions may reduce the effect of Cl^-, which is suggested to be the ion most limiting to the distribution of *D. polymorpha* (Strayer and Smith 1993; see also Zhadin 1952). Mg^{+2} in particular is essential for osmoregulation in *D. polymorpha* (Dietz et al. 1994).

As an alternative to the ion ratio model of salinity tolerance, Strayer and Smith (1993) also pointed out that the Caspian and Aral seas, with *D. polymorpha* populations found at 8 and 12‰, respectively, do not undergo such wide swings in salinity as the coastal areas do. Thus, the zebra mussel may exhibit tolerance to moderate salinity when it is constant (8–12‰), but to lower salinities (0.5‰) if the salinity undergoes frequent changes, as in tidal estuaries. Exposure to 1.6‰ NaCl for a week was fatal to zebra mussels in one set of laboratory experiments (Horohov et al. 1992), while other tests showed perturbations in respiration, but no mortality, resulting from increasing salinity from 0 to 10‰ over a period of 10 days (Karpevich 1947).

We report the results of tests to experimentally determine the relative susceptibility to mortality from salinity and (or) temperature of *D. bugensis* and *D. polymorpha* under the following conditions: (*i*) chronic temperature stress such as may occur during range expansion (immersion in 30°C water for up to 14 days); (*ii*) acute temperature stress caused by increasing the temperature at varying rates and then returning to ambient levels, as would occur during the use of thermal mitigation techniques in industrial cooling systems, following the methods of McMahon et al. (1993); and (*iii*) different constant levels of salinity in combination with different constant (sublethal) levels of temperature.

Methods

Sample collection and maintenance

Quagga and zebra mussels were collected by bottom trawling in Lake Ontario offshore from Olcott, New York, in April 1993 and in Lake Erie near Dunkirk, New York, in July 1993, at depths of 25–30 m. The mussels were immediately transported to the Cornell Biological Field Station in Bridgeport, New York, where they were divided into three groups that were acclimated to temperatures of 5, 15, and 20°C. Temperatures were changed from ambient at a rate of 1°C/h. Animals were considered acclimated after being left undisturbed at a constant temperature (±0.5°C) for a minimum of 14 days, at which point most of the animals had bysally attached to each other and (or) surfaces in their containers. The process was intended to allow recovery from the stress of collection and transport and to simulate seasonal variation in response to environmental stress. Mussels acclimated to 5°C were held in 800-L fiberglass tanks with temperatures maintained by circulating chillers. Mussels acclimated to 15 and 20°C were held in 75-L glass tanks insulated by 5 cm of Styrofoam with temperatures maintained by circulating heaters. Two attempts to acclimate zebra and quagga mussels to 25°C failed because sufficient numbers of quagga mussels could not be kept alive at that temperature to complete the experimental designs. Zebra mussels in the same aquarium suffered no mortality. Filtered and aerated Oneida Lake (New York) water was used in all holding tanks and all test baths.

The mussels were not fed and were used within 4 weeks if held at elevated temperatures and within 8 weeks if held at 5°C (after Horohov et al. 1992; McMahon et al. 1993). The mussels collected at Olcott were maintained at 5°C for 14 days and then adjusted to their final acclimation

temperatures. The mussels collected at Dunkirk were immediately divided into acclimation groups. The Olcott mussels were used in temperature experiments while the Dunkirk mussels were used in salinity experiments.

All treatments called for either 10 or 12 mussels per experimental unit. When possible, mussels that were bysally attached to each other were used, attachment being used as evidence of lack of stress. When extra mussels could not be removed from a clump without disrupting the remainder of mussels, they were included in the test, and when there were not enough small clumps to sum to the total required in a treatment, then available attached mussels were used. Each experimental unit was always within two mussels of the designated size, with preference given to exceeding rather than falling short of the designed sample size. Individual mussels greater than 20 or less than 10 mm in length were not used unless they were inextricably attached to a test clump of mussels.

Response to chronic temperature stress

To test the differential response of the *Dreissena* species to a constant elevated temperature, 50 animals of each species from each of the three temperature acclimation groups (5, 15, and 20°C; 300 animals total) were maintained at temperatures of 30 and 35°C (increase to test temperature from acclimation temperature was at a rate of 1°C/h) for a maximum of 14 days. These temperatures were chosen because they are among the highest temperatures that these mussels might encounter in the northeast United States but that may or may not cause rapid mortality in zebra mussels (Iwanyzki and McCauley 1993; McMahon et al. 1993). Mussels of each species from each acclimation group were divided and placed into five labeled glass dishes (50 mm high × 80 mm in diameter) covered with a 1-mm nylon mesh to prevent migration. The daily mortality of the zebra and quagga mussels was recorded for each dish. Gaping individuals that did not respond to stimulus with a soft-bristle brush were tested with a dissecting probe. If this elicited no response, the animal was considered dead. Dead animals were measured for length and discarded. This definition of death was used in all three experiments discussed in this paper. At the conclusion of the experiment the length of all survivors was measured. This experiment was run twice at 30°C and once at 35°C.

Response to acute temperature stress

Experiments were designed to test the tolerance of each species of *Dreissena* to different regimes of acute temperature change ranging from a quick pulse of heat to prolonged exposure. The response to acute temperature stress was examined using a fully factorial design of quagga and zebra mussels from each of three acclimation temperatures (5, 15, and 20°C) at four different rates of temperature increase (1°C per 5, 15, 30, or 60 min), for a total of 24 treatments (2 species × 3 acclimation temperatures × 4 rates of temperature increase). One hundred mussels were used in each treatment, for a total of 2400 animals. Each treatment was tested once. Our procedure follows that of McMahon et al. (1993).

The two species were tested simultaneously in the same water bath for a given combination of acclimation temperature and rate of temperature increase. For each treatment the mussels were divided into groups of approximately 10 animals that were placed into glass dishes (10 dishes for each species in a given trial) covered with a 1-mm nylon mesh to prevent escape as in the chronic temperature stress experiment. Each dish was an experimental unit for analytical purposes. No treatments were replicated. The 20 dishes were placed into an insulated water bath (60-L cooler) held at the appropriate acclimation temperature for those treatments. After a 2-h waiting period to minimize additional stress from transfer to the test bath, the temperature was raised from the initial acclimation temperature at the test rate of increase: 1°C every 5, 15, 30, or 60 min. One dish of each species was removed from the heated bath when the temperature reached 30°C, well before mortality. The nylon mesh was removed and the warm water in the dish was allowed to equilibrate to room temperature (13–18°C). A new pair of dishes was removed from the bath as each temperature degree was reached (from 30 to 39°C) and allowed to equilibrate to room temperature until all dishes had been removed.

The mussels were surveyed for mortality 1 and 12 h after removal from the test bath. The number of dead mussels out of the total number in a dish was determined for the 10 dishes of each species of mussel in a trial. Because some recovery was observed between 1- and 12-h observations, only the latter were used in the analysis. The temperature at which 100% sample mortality was observed to occur (SM_{100}) was recorded. At the conclusion of each experiment the length of all animals was measured.

A logistic regression model was used (logit analysis) to describe the effect of species, acclimation temperature, rate of temperature increase, instantaneous test temperature of observation (30–39°C), degree-minutes (a measure of the combined effect of temperature and time), and all interactions on survival of temperature stress by *Dreissena*. Length was used as a covariate. Length and instantaneous test temperature were coded continuously, but the other predictors were coded categorically because there were not enough levels of observation to justify continuous coding of acclimation temperature or rate of temperature increase. Nonsignificant terms were omitted from the final model ($\alpha = 0.05$). The experimental unit for analysis was the group of mussels within a given dish, coded as number dead out of total number. Logit models were also used to predict the LT_{50} and LT_{100} (temperature causing 50 or 99.9% mortality) for each species in each treatment. This analysis was carried out using SYSTAT LOGIT (Steinberg and Colla 1991).

Regression coefficients in the multivariate logit model represent the natural logarithm of the odds of response (death) given a particular level of a predictor (probability of death for each level of predictor divided by probability of death at a baseline level of predictor). The baseline model against which all predictors in this study were compared is a zebra mussel acclimated to 5°C with temperature increased by 1°C per 60 min. If a coefficient is negative, the interpretation is that the given level of the predictor is less likely than the baseline level to cause a mussel to die. Positive coefficients indicate that a given level of the predictor is more likely to cause mortality. The coefficients

were exponentiated (antilog of the regression coefficient) to give an odds ratio for survival at each level of each predictor relative to the baseline (regression coefficients were generated for species = quagga, acclimation temperature = 15 or 20°C, and rate of temperature change = 1°C per 5, 15, or 30 min). See Hosmer and Lemeshow (1989) for further discussion of logistic regression.

Response to salinity

Salinity experiments were designed to include the effect of temperature on the mussels' ability to tolerate different concentrations of Na^+ and Cl^-, the principal ions in seawater. Mussels were maintained at four constant concentrations of NaCl for a maximum of 18 days (0, 5, 10, and 20‰). Samples of about 24 mussels of each species from each acclimation temperature (5, 15, and 20°C) were tested in a fully factorial design at each of three test temperatures (5, 15, and 20°C). Each sample was divided into two replicates of 12 mussels each. As in the temperature tolerance experiments, mussels from each species – acclimation temperature combination were divided and placed into individually labeled glass dishes covered with 1-mm nylon mesh. Each treatment was replicated once (two dishes per treatment).

All mussels began in fresh water (0‰). Jars of mussels were randomly assigned to treatments before being exposed to salinity. On day 1 of the test, 24 h after temperatures had been adjusted from acclimation to test temperature (at a rate of 1°C/h) all test mussels were moved into water with 5‰ NaCl. Control mussels (temperature manipulation only) remained in fresh water. After a further 24 h mussels to be tested at elevated salinities were moved from the 5‰ baths into water with 10‰ NaCl. After a final 24 h, mussels to be tested at 20‰ were moved into baths of that salinity from the 10‰ baths. The daily mortality of zebra and quagga mussels was recorded for each dish and dead animals were measured and removed from that dish. At the end of the experiment the length of all survivors was measured.

The results were examined using analysis of variance. The experimental unit was the glass dish containing approximately 12 mussels. There were, therefore, two observations for each treatment (combination of species, test temperature, acclimation temperature, and salinity). The response variable, mean survival time of the mussels in each glass dish, was transformed by taking the natural logarithm to stabilize variance in the model. Mussels that died before reaching the designated test salinity (mussels acclimated at 20‰ that died in the 5‰ bath, for instance) were coded as having died on day 0. All other mussels were coded as having died on day X, where X is the number of 24-h periods (from 1 to 18) after each mussel reached its designated test salinity until death occurred.

Candidate predictors included temperature of prior acclimation, level of salinity, test temperature (temperature at which the animals were exposed to salinity), and all interactions. Length was used as a covariate. All predictors but the length covariate were coded categorically. Nonsignificant terms were omitted from the final model ($\alpha = 0.05$). Post-ANOVA comparisons to determine significant differences in mean survival time of all treatments were calculated using Fisher's least significant difference procedure (LSD) to correct for multiple simultaneous tests (overall $\alpha = 0.05$; Fisher 1966; see also Ott 1988). This analysis was carried out using SYSTAT for Windows 5.02 (Wilkinson 1992).

Table 1. Percent survival through time of *D. bugensis*, acclimated to three different temperatures (5, 15, and 20°C) and exposed to 30°C.

	5°C		15°C		20°C	
Day	*a*	*b*	*a*	*b*	*a*	*b*
1	18.37	48.98	100.00	98.00	83.33	92.00
2	0.00	2.04	75.51	92.00	81.25	88.00
3	—	2.04	59.18	88.00	58.33	84.00
4	—	2.04	24.49	72.00	37.50	74.00
5	—	0.00	12.24	60.00	25.00	64.00
6	—	—	4.08	60.00	20.83	56.00
7	—	—	2.04	58.00	4.17	48.00
8	—	—	0.00	54.00	2.08	44.00
9	—	—	—	48.00	2.08	42.00
10	—	—	—	48.00	2.08	38.00
11	—	—	—	+42.00	2.08	+36.00
12	—	—	—	—	2.08	—
13	—	—	—	—	2.08	—
14	—	—	—	—	0.00	—

Note: The experiment was replicated once. Trial *a* lasted 14 days. Trial *b* ended after day 11 because of mechanical failure; + indicates the percent surviving mussels in the last assay before failure occurred. *Dreissena polymorpha* was tested in the same aquarium as *D. bugensis* for each trial, in different sample dishes, and experienced no mortality.

Results

Response to chronic temperature stress

Experiments designed to test the response of quagga and zebra mussels to chronic temperature stress indicate that the zebra mussel is much more tolerant of elevated temperatures than the quagga mussel. In the first of two replicates, only one quagga mussel survived longer than 7 days at 30°C, and it died on the 14th day. No zebra mussel mortality occurred in 14 days of observation. The second replicate was disrupted after day 11 when the thermostat malfunctioned and the temperature was raised to 37°C, killing all remaining mussels of both species. In this second trial, the mortality rates for the quagga mussel appeared to be different from those in the first trial (Table 1). The gross result, of extreme quagga mortality and no zebra mussel mortality at 30°C, was the same, however.

When tested at 35°C, all animals of both species (n = 300) had died by the first observation period, 24 h after the test temperature was reached. No mortality occurred in the control treatments in which quagga and zebra mussels from each acclimation temperature were adjusted to sublethal temperatures (5, 15, and 20°C).

Response to acute temperature stress

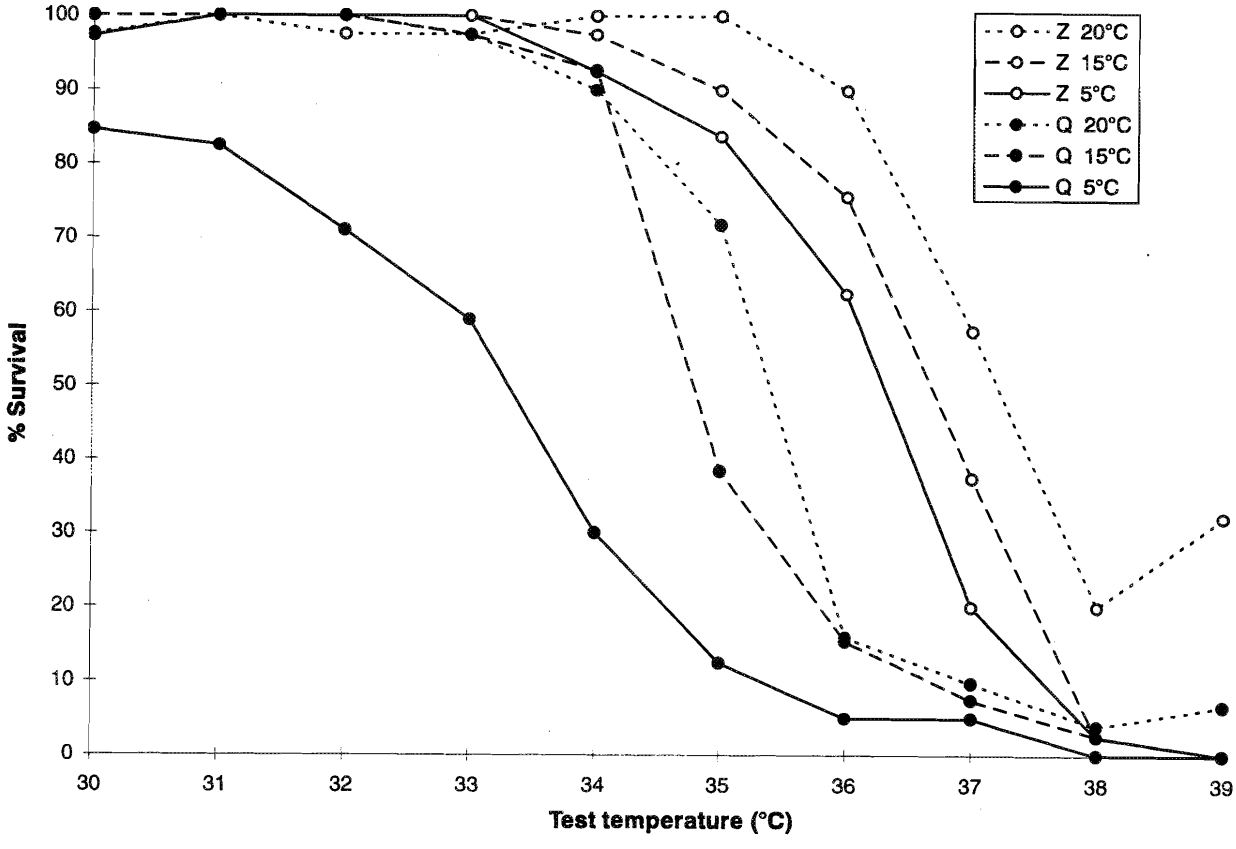
When a logistic regression model was fit to the data, each of the main effects except degree-minutes and the covariate

Table 2. Response of zebra and quagga mussels to increasing temperature.

Predictor	Regression coefficient			Odds ratio		
	Estimate	SE	p	95% CI lower bound	Estimate	95% CI upper bound
Constant	−45.578	2.017	<0.001	—	—	—
Rate = 30	−0.445	0.210	0.034	0.425	0.641	0.967
Rate = 15	−1.204	0.218	<0.001	0.196	0.300	0.460
Rate = 5	−2.483	0.235	<0.001	0.053	0.083	0.132
Acclimation = 15	−1.838	0.199	<0.001	0.108	0.159	0.235
Acclimation = 20	−2.660	0.215	<0.001	0.046	0.070	0.107
Temperature	1.317	0.058	<0.001	3.332	3.733	4.183
Species = quagga	2.602	0.184	<0.001	9.410	13.496	19.358

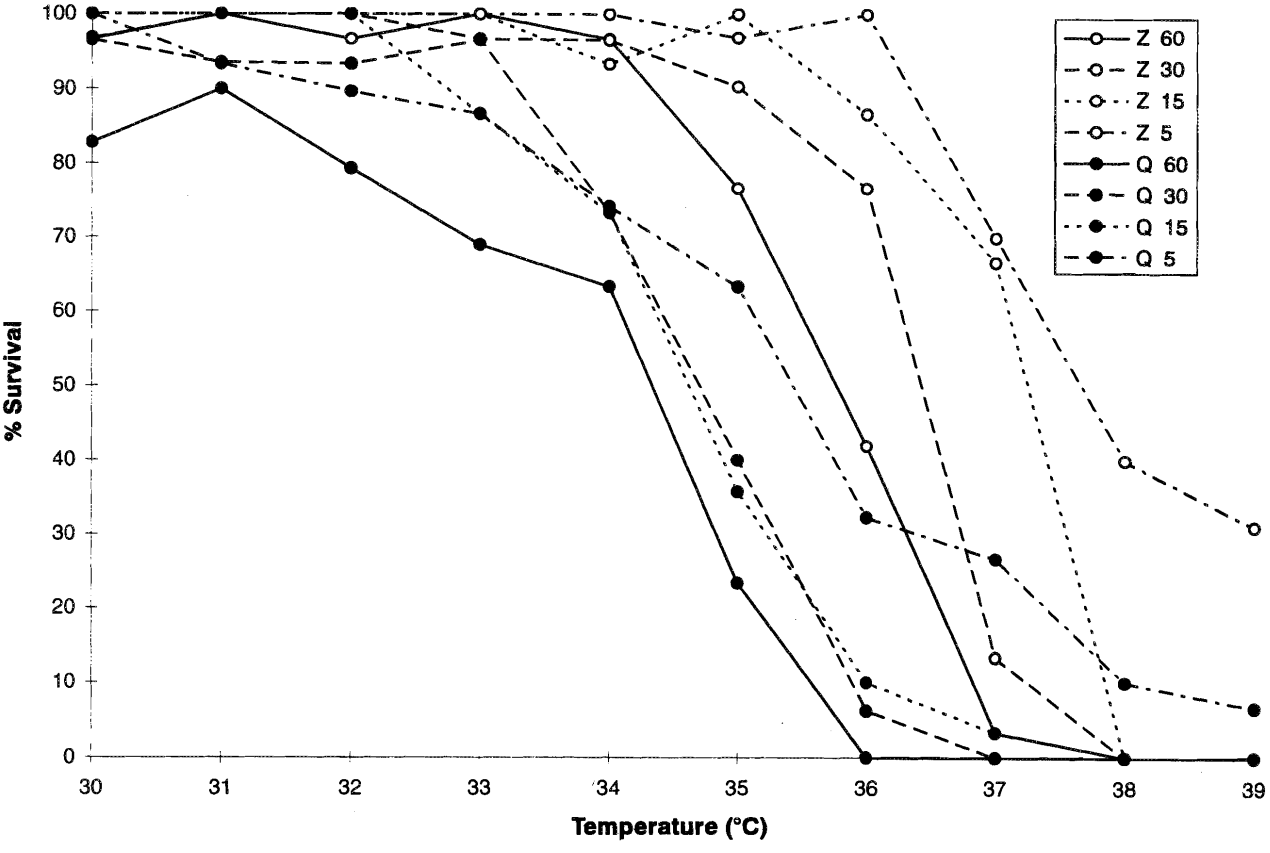
Note: The baseline model is a zebra mussel acclimated to 5°C in a bath with the temperature increased by 1°C each 60 min. The odds ratio indicates the change in risk for an individual mussel at each level of the predictor relative to the baseline. If the odds ratio is greater than one, that predictor is a positive risk, and increasing it increases the risk to the individual: if the odds ratio is less than one, the predictor is a negative risk and increasing it reduces the risk to the individual.

Fig. 1. Percent survival of quagga (●) and zebra (○) mussels, combined across four rates of temperature increase, for each of three acclimation temperatures (5, 15, and 20°C) and 10 test temperatures from 30 to 39°C.



of length was significant ($p < 0.05$) and retained in the final model (Table 2). No interaction term was found to have a significant effect ($p < 0.05$) on the likelihood of survival for a given mussel and all were dropped from the final model. Species was the predictor found to have the greatest effect on survival. In any treatment the quagga mussel had a 10- to 20-fold greater chance of dying than the zebra mussel at a given instantaneous temperature of observation. Instantaneous temperature level followed species as a significant positive risk. Each degree of temperature increase, within the range of observation, was found to raise the risk of death by 3–4 times (Table 2).

Fig. 2. Percent survival of quagga (●) and zebra (○) mussels, combined across three acclimation temperatures, for each of four rates of temperature increase and 10 test temperatures from 30 to 39°C. Rate is given as minutes per degree Celcius.



Increasing acclimation temperature improved the mussels' likelihood of survival relative to be baseline model (zebra mussel acclimated to 5°C with temperature raised 1°C per 60 min; see Fig. 1). Mussels of each species acclimated to 15 or 20°C were less likely to die from acute stress due to increased temperature than those acclimated to 5°C ($p < 0.01$). The two higher acclimation temperatures were slightly different from each other ($p = 0.05$; see Table 2 for confidence intervals around the predicted odds ratios).

The rate of temperature increase was also a negative risk to survival (Fig. 2). The effects of the two intermediate rates (1°C increase per 30 or 15 min) increased the likelihood of survival relative to the baseline (1°C increase per 60 min) by 2–5 times but were not significantly different from each other ($p > 0.05$). The fastest rate of temperature increase (1°C per 5 min) significantly increased the likelihood of survival for each species relative to the two intermediate rates ($p < 0.05$) by 2–10 times and by 10–20 times over the baseline rate of 1°C per 60 min. This result suggests a correlation between survival and time exposed to an elevated temperature, since mussels exposed to slower rates of temperature increase spent more time in temperature baths regardless of the final temperature reached.

The LT_{50} of the quagga mussel ranged from 1 to 5°C below that of the zebra mussel in every treatment but one (20°C acclimation: temperature increased by 1°C per 30 min; $p > 0.05$; see Table 3). The LT_{100} values for each species were significantly different in only one treatment (20°C acclimation; temperature increased by 1°C per 30 min; $p < 0.05$; see Table 3). Predicted LT_{100} values were substantially different from the observed SM_{100} values. However, LT_{100} and SM_{100} values did follow trends similar to those of LT_{50} as determined by treatment levels. Complete mortality did not occur in the trials for either species acclimated to 20°C when the temperature was raised by 1°C per 5 min. The quagga mussels experienced 80% mortality at 38 and 39°C, while the zebra mussels experienced no mortality at all in this trial.

Response to salinity

Quagga and zebra mussels exposed to salinity (≥5‰) exhibited 100% mortality within 18 days (Table 4). No significant mortality occurred in the control treatments, where temperatures were varied but no salt was added ($p < 0.001$). Analysis of variance (response variable was mean survival time for each experimental unit (approximately 10 mussels in each glass dish) from the salinity >0‰ treatments) indicates that test temperature, salinity, and an interaction between test temperature and salinity were significant predictors of mortality rate (which was transformed by taking the natural logarithm to stabilize variance). Species, prior acclimation, and the covariate of length did not affect mortality rate of *Dreissena* spp. exposed to salinity ($p > 0.05$); therefore, these predictors were dropped from the final

Table 3. LT_{50} and LT_{100} estimated from logit models, and SM_{100} observed for each treatment in the acute temperature stress experiment for quagga (QM) and zebra (ZM) mussels.

Rate (min/°C increase)	Acclimation temperature	LT_{50}		SM_{100}		LT_{100}	
		QM	ZM	QM	ZM	ZM	ZM
60	5	30.869	35.003	35	37	39.166	39.822
60	15	34.056	35.669	35	37	34.335	38.755
60	20	35.063	36.201	36	38	35.332	40.276
30	5	33.057	36.014	36	38	39.451	39.717
30	15	34.815	36.099	37	37	36.931	38.940
30	20	35.479[a]	36.444[a]	37	38	36.961[b]	42.276[b]
30	5	34.099	36.306	37	38	38.869	41.005
15	15	34.949	37.192	39	39	39.410	39.348
15	20	34.634	37.085	37	38	39.793	37.652
5	5	33.491	37.001	38	39	42.767	39.261
5	15	36.102	37.203	39	39	40.223	40.489
5	20	36.442	—[c]	na[d]	na	45.195	—[c]

[a]These LT_{50} values were not significantly different. All other values of LT_{50} were significantly different between quagga and zebra mussels at $p < 0.05$.

[b]These LT_{100} values were significantly different. No other values of LT_{100} were significantly different between quagga and zebra mussels at $p < 0.05$.

[c]No mortality occurred during the period of observation.

[d]na, 100% mortality was not reached.

model describing the response of the zebra and quagga mussels to salinity (Table 5). The mean survival time for each of the nine test treatments in the final model was compared (corrected by Fisher's LSD procedure to maintain an overall error rate of $\alpha = 0.05$; Table 6).

Increasing the test temperature from 5 to 15 to 20°C greatly increased the mortality rate of the quagga and zebra mussels in the presence of salinity ($p < 0.001$; Fig. 3; note natural logarithm transformation of survival time). Averaged over each test temperature, the two lower salinity levels were statistically equivalent in their effect on survival of *Dreissena* spp. ($p < 0.05$); when tested at 20‰, however, there was a marked increase in mortality rate ($p < 0.05$; Fig. 4; note natural logarithm transformation of survival time). There was a significant interaction between test temperature and salinity level ($p < 0.001$). This was due to a deviation from the linear temperature response at low temperatures. The mean survival time of mussels tested at 5°C did not vary with salinity, although the mussels tested at 5°C and 10‰ salinity did survive longer than those tested at 5°C and 20‰ or 15°C and 5‰ (Table 6).

Discussion

The current distribution of the quagga mussel in North America is much more limited than that of the zebra mussel. The quagga mussel is found in large numbers only in the St. Lawrence River and lakes Erie and Ontario (Dermott and Munawar 1993; Mills et al. 1993), while the zebra mussel occurs in all of the Great Lakes and many waterways east of the Mississippi River (Nalepa and Schloesser 1993). Where the two species coexist in lakes Erie and Ontario, their populations exhibit marked stratification by depth. The correlation between water temperature and the proportion of quagga mussels suggests that the quagga mussel may be more tolerant of the cold temperatures (<10°C) in the deeper waters of lakes Erie and Ontario than of the warmer temperatures found in shallower waters (Mills et al. 1993). The current study does not shed light on the relative performance of the two North American species of *Dreissena* at low temperature, but it does indicate that the quagga mussel was at a marked disadvantage to the zebra mussel when the temperature was raised past 20°C in these experiments.

Tests of the tolerance to elevated temperatures of both North American species of *Dreissena* showed that the quagga mussel could not survive in warm water (30°C), as opposed to the zebra mussel, which suffered no mortality at all in our experiments. These results correspond with those of McMahon et al. (1994) who reported that zebra mussels can be maintained indefinitely at 30°C. Our results showing 100% mortality of each species when kept at 35°C also correspond with those of McMahon et al. (1994) who demonstrated that zebra mussels cannot survive temperatures above 31°C for more than a week. These results correspond with observations of the zebra mussel's distribution through the southern Mississippi River, into areas where summer surface temperatures approach 30°C (McMahon et al. 1994). Subsurface temperatures experienced by the mussels are presumably lower.

Laboratory measurements of LT_{50} from exposure to acute increases in temperature support the results of Domm et al. (1993) indicating that the quagga mussel is more vulnerable than the zebra mussel under most combinations of acclimation temperature and rate of temperature increase (Table 3). The predicted LT_{100} values are much higher than the observed SM_{100} values because of the use of multiplicative rather than additive variance in logistic regression,

Table 4. Mean survival time (maximum survival time in parentheses) for both species of *Dreissena* in each combination of salinity and test and acclimation temperatures.

Test temperature (°C)	Salinity (‰)	Acclimation temperature (°C)	Mean survival time (days)	
			Quagga mussel	Zebra mussel
5	5	5	4.8 (13)	4.9 (18)
5	5	15	7.4 (13)	5.3 (18)
5	5	20	6.8 (12)	3.7 (16)
5	10	5	6.1 (10)	8.0 (12)
5	10	15	6.4 (10)	6.8 (12)
5	10	20	5.9 (10)	3.8 (11)
5	20	5	3.4 (7)	6.0 (9)
5	20	15	4.5 (7)	5.3 (9)
5	20	20	4.7 (8)	3.1 (9)
15	5	5	4.4 (9)	4.2 (9)
15	5	15	4.4 (8)	4.7 (9)
15	5	20	4.9 (9)	4.7 (9)
15	10	5	3.4 (5)	2.9 (6)
15	10	15	3.5 (6)	4.1 (7)
15	10	20	4.2 (6)	3.6 (7)
15	20	5	2.1 (4)	2.6 (6)
15	20	15	2.6 (4)	3.2 (5)
15	20	20	2.7 (4)	3.4 (6)
20	5	5	3.1 (5)	2.2 (6)
20	5	15	3.4 (5)	2.7 (6)
20	5	20	3.2 (5)	3.2 (6)
20	10	5	2.0 (3)	1.5 (4)
20	10	15	1.8 (3)	2.3 (4)
20	10	20	2.8 (4)	2.9 (4)
20	20	5	0.9 (2)	1.1 (3)
20	20	15	1.3 (2)	1.5 (3)
20	20	20	1.2 (2)	1.6 (3)

Table 5. ANOVA model to describe the mortality rate (natural log transformed) of *Dreissena* species in salinity.

Source	Sum of squares	df	Mean square	F	p
Test temperature	10.845	2	5.422	100.543	<0.001
Salinity	4.440	2	2.220	41.168	<0.001
Test temperature × salinity	1.761	4	0.440	8.161	<0.001
Error	5.339	99	0.054		

Note: Acclimation temperature and species were not significant predictors ($p > 0.05$) and were not included in this final model.

suggesting that the effect of extreme temperatures cannot be predicted reliably from these models. SM_{100}, which reflects observed mortality, seems to be preferable to the predicted LT_{100} value, which underestimates the mortality rate of *Dreissena* species in temperatures that will cause rapid mortality (Table 3).

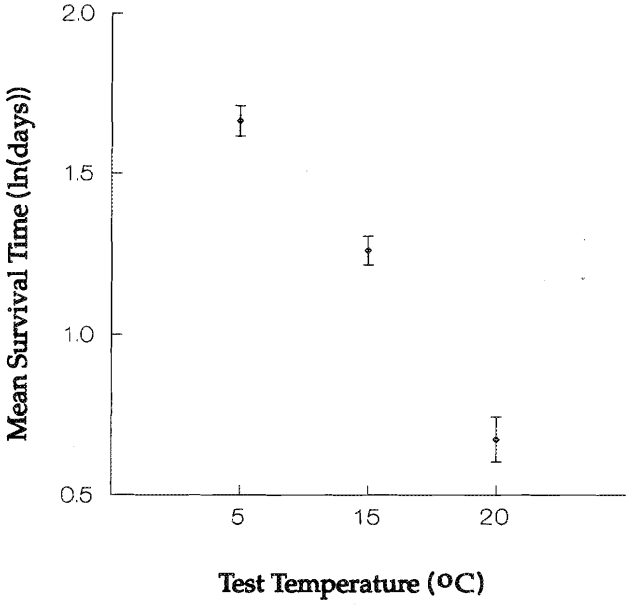
In three treatments (quagga mussels acclimated to 15°C with temperature increased by 1°C each 60 min, quagga mussels acclimated to 20°C with temperature increased by 1°C each 60 min, and zebra mussels acclimated to 20°C with temperature increased by 1°C each 15 min) the SM_{100} is very close to the predicted LT_{100} (Table 3). This is because there was no gradual transition between 100% survival and 100% mortality at two observation points (no mortality observed at 34°C and complete mortality observed at 35°C, for instance). This phenomenon, termed complete or quasicomplete sample separation, occurs when the predictors perfectly fit the data (temperature of observation exactly predicts when 100% mortality will occur in these three cases). Quasicomplete sample separation resulted from the small sample size (10 mussels in each observation) and lack of replication (Bryson and Johnson 1981; see

Table 6. Matrix of differences between mean survival times of *D. bugensis* and *D. polymorpha* (corrected by Fisher's LSD to maintain overall $\alpha = 0.05$) of each salinity treatment.

Treatment (°C–‰)	Mean survival time (days)	Treatment (°C–‰)							
		5–5	5–10	5–20	15–5	15–10	15–20	20–5	20–10
5–5	5.3	x	—	—	—	—	—	—	—
5–10	5.9	ns	x	—	—	—	—	—	—
5–20	4.7	ns	*	x	—	—	—	—	—
15–5	4.4	ns	**	ns	x	—	—	—	—
15–10	3.6	**	**	**	*	x	—	—	—
15–20	2.7	**	**	**	**	**	x	—	—
20–5	2.8	**	**	**	**	**	ns	x	—
20–10	2.1	**	**	**	**	**	**	**	x
20–20	1.2	**	**	**	**	**	**	**	**

Note: Because there was no significant difference between species, both species have been pooled within temperature × salinity treatments here. ns, no significant difference in survival times ($p > 0.05$); *, significant difference between treatments ($0.01 < p < 0.05$); **, highly significant difference between treatments ($p < 0.01$).

Fig. 3. Effect of test temperature on natural log transformed mean survival time per treatment of both *Dreissena* species (pooled because there is not significant difference in response between the species; $p > 0.05$) in salinity. Error bars are ±1 SE.



also Hosmer and Lemeshow 1989). Because the predicted LT_{50} values for those cases follow the trend observed in the other 21 cases (quagga mussels reasonably lower than zebra mussels), these estimates still seem reasonable.

Zebra mussel LT_{50} values predicted using the current model (Table 2) are within a degree of that predicted from the models of McMahon et al. (1993) in all cases but two. First, the predicted LT_{50} values of zebra mussels acclimated to 5°C were as much as 2°C higher in the current model than in that of McMahon et al. (see Table 3). This result is still well within the range of variation one might expect from comparisons across laboratories of animals collected in different places at different times. Second, no mortality was observed in this study for zebra mussels acclimated to 20°C tested with the temperature increasing 1°C every 5 min in the range 30–39°C, while McMahon et al. (1993) reported complete mortality within that temperature range. The quagga mussels tested under these conditions also followed the observed trend, never reaching 100% mortality (Table 3). Our two other tests of zebra mussels at temperature increases of 1°C every 5 min produced very similar results to those of McMahon et al. (1993), as did our three other tests of zebra mussels acclimated to 20°C.

The response of the zebra mussel to acute temperature change reported here follows similar trends to those reported previously, with increasing acclimation temperature resulting in increased survival time at lethal temperature levels (Iwanyzki and McCauley 1993). The sole exception to this trend occurred when Iwanyzki and McCauley (1993) found that mussels acclimated to 2.5°C and exposed to 30°C water had the longest mean survival time. This exception suggests that acclimation temperature does not have a linear effect on tolerance to thermal stress in *Dreissena* spp. Our results differ from those of Iwanyzki and McCauley (1993) in that they observed complete mortality in zebra mussels previously acclimated to 5, 15, and 20°C maintained at 30°C for 14 days and we observed none at all. A possible explanation for this difference is that in our experiments the temperature was raised gradually (1°C/h) to the test temperature whereas Iwanyzki and McCauley (1993) transferred the mussels directly from the acclimation to the test baths, resulting in instantaneous temperature change. The current results do correspond with those of McMahon et al. (1994) who also found that zebra mussels could be indefinitely maintained at 30°C regardless of acclimation temperature.

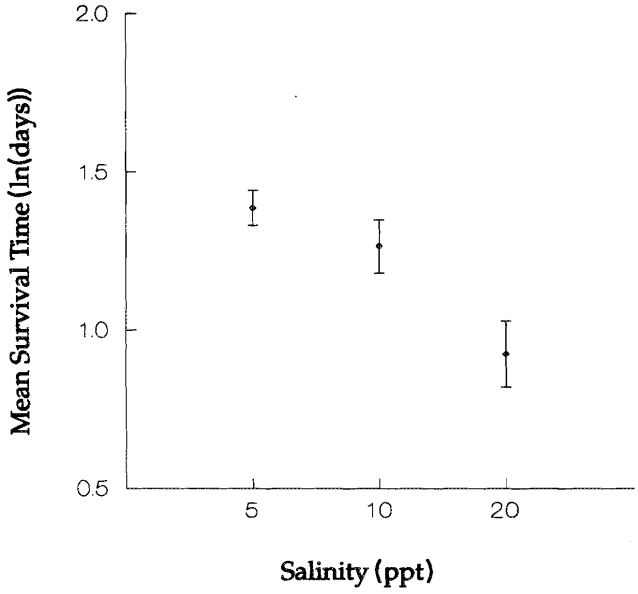
The quagga mussel was found to have lower LT_{50} values than the zebra mussel throughout the range from 30 to 39°C. However, both species can tolerate short-term exposure (a few hours) to temperatures that would be lethal if experienced for an entire day. While the quagga mussel is in general more likely to die from pulses of warm water than the zebra mussel (Table 2), both species are more tolerant of temperature stress if acclimated to higher temperatures (15 or 20°C). Quagga mussels acclimated to 5°C were particularly vulnerable to thermal stress (see Fig. 1). These results suggest that thermal back-flushing treatments which successfully control zebra mussel infestation of water intakes will be equally effective in controlling the quagga mussel.

A brief discussion of the relative merits of different methods of testing upper thermal tolerance in molluscs is appropriate. Stirling (1982) demonstrated that gradually raising the temperature from acclimation levels to lethal levels will overestimate the acute lethal temperature relative to that determined by exposing a mollusc directly to a lethal temperature. Part of this effect could be due to temperature acclimation during temperature increase, as pointed out by Domm et al. (1993), and the remainder is due to the confounding factor of time, as pointed out by Fraenkel (1960, 1968). Our analysis did not discriminate fully between the effect of acute lethal temperature and the cumulative effect of time spent at elevated sublethal but potentially stressful temperatures until the final lethal temperature was reached. Nonetheless, our experimental design does match what mussels would experience in intake pipes or in smaller bodies of water that may change temperature rapidly.

Temperature stress may explain the failure of the quagga mussel to colonize the inland waterways of New York State. The initial discovery of the quagga mussel was based on a single individual from the Eric Canal near Palmyra, New York (May and Marsden 1992). Individual quagga mussels were found in the midst of large zebra mussel populations from several sites in the Erie Canal and the outlet from Onondaga Lake (New York) in 1991 (Mills et al. 1993). However, no quagga mussels have been found outside lakes Erie and Ontario since the fall 1991 field season. While the quagga mussel may be able to hitchhike toward inland waters on boats, it will not survive there unless it finds a thermal refuge that will not reach 30°C. Our inability to maintain quagga mussels at 25°C for further testing (see Methods) suggests that the thermal maximum of the quagga mussel is even lower than could be tested during the experiments reported here.

No significant differences were found between the two species of *Dreissena* in terms of mortality rate in the presence of salinity. Zebra and quagga mussels could not tolerate NaCl alone in concentrations of 5‰ or greater. Prior acclimation to low levels of salinity (<2‰) has been suggested to mitigate the effect of sudden salinity changes, as were implemented in the first 3 days of the current salinity experiment, on respiration in *D. polymorpha* (Karpevich 1947). Karpevich's results also showed that mussels acclimated to fresh water and plunged into a salinity of 5‰, as in the current experiments, experienced no change in respiration. All of the freshwater mussels we plunged into salinities of 5‰ or greater died, a much more extreme response than cutting hourly respiration by 66% as Karpevich observed when plunging the mussels into salinities as high as 20‰ (see Fig. 4 in Strayer and Smith 1993). The extent of difference between the response observed here (complete mortality) and that reported by Karpevich (depressed respiration) suggests a drastically different conditioning regime for the animals that were tested, Karpevich's Caspian populations and ours from Lake Erie.

Fig. 4. Effect of salinity (‰) on natural log transformed mean survival time per treatment of both *Dreissena* species (pooled because there is no significant difference in response between the species; $p > 0.05$). Error bars are ±1 SE.



Whether an increase in the amount of divalent ions would have enabled the mussels to tolerate salinity levels of 5, 10, or 20‰ needs to be examined further (see Strayer and Smith 1993; Dietz et al. 1994). The data reported here do not provide a basis to evaluate the hypothesis that constant salinity is more bearable than variable salinity to the zebra mussel (suggested by Strayer and Smith 1993). The mussels tested here experienced salinity changes of 5‰/day for 1 or 2 days, followed by a further 10‰ if tested at 20‰ (see Methods). Mussels treated with the least variation in salinity (5‰) survived the longest but still experienced complete mortality in spite of evidence that they shouldn't even have experienced respiratory distress (Karpevich 1947). One recent study did show 100% mortality of *D. polymorpha* in <2‰ NaCl (Horohov et al. 1992). Predictions of the range expansion of *D. polymorpha* through tidal estuaries on the basis of a limit of 2‰ salinity appear reasonable (Strayer and Smith 1993). The current results suggest that zebra and quagga mussels certainly cannot be expected to survive in tidal areas with salinity greater than 5‰.

Expansion of the quagga mussel into water bodies beyond the Great Lakes is unlikely where summer temperatures exceed its upper thermal limit, which is <30°C

according to our results. If attempts to acclimate *D. bugensis* to different temperatures are a guide, the upper thermal limit of the quagga mussel may be 25°C. A reasonable prediction is that *D. bugensis* will have a difficult time expanding its range toward inland waters in the absence of thermal refugia. The current results suggest that these two related bivalves are distinct in terms of physiological response to temperature if not salinity. These differences should be taken into account when formulating control mechanisms or predicting the spread of these aquatic nuisances through North America. However, it is important to retain the distinction between upper lethal temperature and predictors of success in the natural environment. Definitive predictions of the final range of *D. bugensis* and *D. polymorpha* await studies of the relative abilities of each species to successfully reproduce and grow through the range of environments they may encounter throughout the Great Lakes basin and the rest of North America. The relative performance (growth and spawning ability), not just mortality, of *D. bugensis* and *D. polymorpha* at low temperatures will be of particular interest.

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