FREEZING TOLERANCE IN *MYTILUS EDULIS*

Donald A. McCrimmon[1,2] and Jennifer Rock[3]
[1]MDI Biological Laboratory, Salsbury Cove, ME 04672
[2]Present Address: Department of Biological Sciences,
Oakland University, Rochester, MI 48309
[3]College of the Atlantic, Bar Harbor, ME 04609

On Mount Desert Island, tidal range averages 3.2 m. Sessile blue mussels, *Mytilus edulis,* living in the upper regions of the intertidal zone must endure longer periods of exposure (6-8 h) to air than their counterparts in lower intertidal zones. A challenge which all *Mytilus* face is exposure to winter ambient air temperatures which can drop as low as -30 °C for prolonged periods. Under such circumstances, exposed *Mytilus* repeatedly freeze, yet survive. Mechanisms postulated to account for the tolerance of marine invertebrates to these harsh conditions include differential movement of water, increased blood cation concentrations (Murphy, D. and Pierce, S., J. Exp. Zool. 193:313-22, 1975) and increases in the concentrations of certain amino acids, including strombine and taurine (Loomis, S., *et al.* Biochemica et Biophysica Acta 943:113-118, 1988). A shift from aerobic respiration to anaerobic metabolism is associated with these physicochemical changes (Murphy, D., J. Exp. Biol. 69:1-12, 1977a; J. Exp. Biol. 69:13-21, 1977b).

We examined the relationships between exposure to different degrees of cold, intertidal location, and duration of forced anaerobic metabolism to freezing tolerance in *Mytilus*. We also gathered data on the extent of seasonal acclimation to sub-freezing temperatures in this species. All specimens were collected from shoreline of the Mount Desert Island Biological Laboratory in Salsbury Cove, Maine.

To establish a basis for tolerance to cold-exposure, in April, 1990, we collected *Mytilus* from high intertidal (HIT) or sub-tidal (SUB) locations and exposed experimental groups (n = 30 each) for 12 h in a Freas 815 low temperature incubator to test temperatures ranging from -17 to -1 °C. The mussels next were placed for at least one hour in Living Stream units (LSU's) recirculating 7 °C natural sea water. Specimens were then removed from the LSU and allowed to sit undisturbed in air for up to 3 minutes, sufficient time for natural valve closure. The posterior adductor muscle is especially susceptible to freezing injury; its failure to contract leaves the valves gaping. Mussels whose valves sealed spontaneously or which closed and remained closed after being forced shut by hand were classed as survivors. Animals whose shells did not close naturally after removal from the LSU or upon several forced closures were considered dead. The results, expressed as the percentage of each group surviving, are presented in Figure 1. HIT *Mytilus* always exhibited greater survivorship, compared to SUB (Wilcoxon signed ranks test, $p < 0.03$). The greatest difference in percent survival between HIT and SUB experimental groups occurred at test temperatures of -10 °C, the temperature at which a log-linear regression also estimated 50% survival between animals from the two locations.

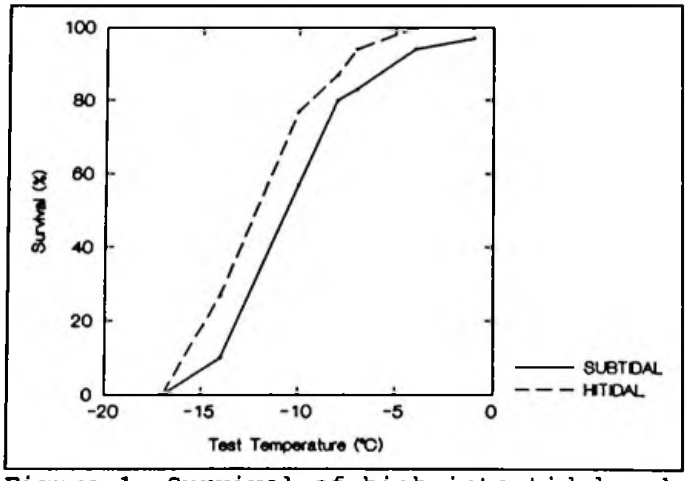

Figure 1. Survival of high intertidal and sub tidal *Mytilus* samples exposed to 12 h of sub-freezing temperatures in a low temperature incubator.

A second series of experiments investigated in detail the process of

acclimation. HIT and SUB Mytilus were placed in a LSU in which the temperature was held at a constant 13 °C for two weeks. This temperature approximated within 2 °C of the ambient seawater temperature at the time the experimental animals were first collected. Following this initial period, samples (n= 20 each) of both HIT and SUB Mytilus were removed and their freezing tolerance tested by exposure to -10 °C for 12 hours. The water temperature for the remaining mussels in the LSU was then lowered to 10 °C, where it remained for two additional weeks. Second HIT and SUB samples (n = 20 each group) were then removed and tested. The remaining mussels in the LSU were permitted to acclimate to 5 °C for a final two week period and subsequently tested. During the entire experimental period, fresh pre-cooled sea water was introduced frequently to help insure that the mussels had access to planktonic food.

For comparison with the laboratory experiments, samples of HIT and SUB Mytilus were taken also in November and December, 1990 directly from the natural environment and tested as ambient sea temperatures declined during early winter. The range of temperature variation for experimental animals was 8 °C, within 0.5 °C of that for animals acclimating at the shore. Results, expressed as percent surviving standard test conditions of -10 °C temperature exposure for 12 h, are presented in Table 1.

Table 1. Effects of 12 h exposure to -10 °C on survival of Mytilus acclimated to varying natural and experimental temperatures.

		ENVIRONMENT			LSU		
Acclimation Temp (C)		11	7.5	3.5	13	10	5
% survivorship	HIT	85	60	95	90	80	55
	SUB	40	35	85	50	45	45

Kruskal-Wallis ANOVA revealed no statistically consistent directional differences in acclimation among environmental HIT and SUB samples (χ^2 = 1.77, $p > 0.18$). In comparison, as LSU acclimation temperatures declined, there was a significant reduction in survivorship among HIT Mytilus, while SUB animals consistently demonstrated poorer freezing tolerance, showing virtually no acclimation (χ^2 = 3.97, $p < 0.05$). Of considerable interest to us, LSU HIT animals demonstrated apparently paradoxical acclimation. Although these HIT animals had higher overall survival, extended time in the LSU was associated with a loss of acclimation ability to decreasing temperatures. The periodic replenishment in the LSU's of fresh sea water should have eliminated nutritional stress. Therefore, we hypothesized that a lack of periodic reversion to anaerobic metabolism, which intertidal-dwelling Mytilus normally undergo during low tide, was responsible for the observed decline in freezing tolerance.

To evaluate this hypothesis, we compared survival of Mytilus held for different lengths of time under conditions requiring either aerobic or anaerobic metabolism. To avoid the potentially extreme acclimation histories of either SUB or HIT organisms, animals from mid-tidal locations were used in these experiments. Mussels were collected in April, 1991 from 3 °C sea water and divided in two experimental groups. Animals to be tested following periods of aerobic respiration were placed in a LSU circulating 3 °C aerated sea water. For comparison, animals to be tested following anaerobic metabolism were clamped shut with rubber bands, wrapped in several layers of parafilm and placed in a low temperature incubator at 3 °C. The use of parafilm and rubber bands prevented gaping and, thus, aerobic respiration during exposure to air, a possibility suggested for Mytilus by Helm and Trueman (Comp. Biochem. Physiol. 21:171-77, 1967). Samples (n = 20 each) were tested for freezing tolerance after 10, 20 and 30 h of either aerobiosis or anaerobiosis.

The significantly different (Likelihood Ratio χ^2 = 19.01, $p < 0.001$) distributions of freezing tolerance are presented in Figure 2 and corroborate Theede et al.'s (Mar. Biol. 15:160-91, 1972) finding that Mytilus' exposure to anaerobic conditions increases freezing resistance. Our results further demonstrate that although aerobically respiring Mytilus show greater freeze tolerance after 10 h in the LSU than anaerobically metabolizing animals held for

the same period in an incubator, additional time under aerobic conditions results in a marked loss of tolerance. In contrast, up to 20 h of continued anaerobic exposure increases freezing tolerance. Our results suggest, therefore, that prolonged exposure to severe metabolic stress may be adaptive to Mytilus living in extreme conditions. On the other hand, loss of a stressor is quickly associated with dramatic reduction in adaptation, a phenomenon of potential significance for mussels living in sub-tidal zones, where they are only occasionally required to metabolize anaerobically. The results also emphasize the importance of providing for stimulus flux during investigations of physiological function for animals normally exposed to changing environmental variables.

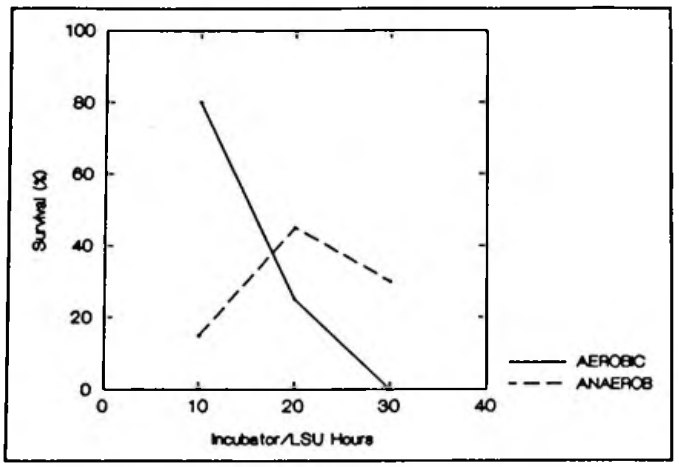

Figure 2. Tolerance of midtidal Mytilus samples to varying hours of aerobic or anaerobic conditions at 3 °C and 12 h exposure to -10 °C.

This work was partially supported by NIH EHS-P30-ES03828 to DAM and a grant from the Pew Charitable Trusts to JR.