# Barnacle larvae in ice: Survival, reproduction, and time to post settlement metamorphosis

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# Abstract

Late stage larvae (cyprids) of the barnacle *Semibalanus balanoides* frequently encounter freezing conditions along the northwest Atlantic coast. *Semibalanus balanoides* cyprids survived for more than four weeks embedded in sea ice, and a significant fraction of larvae held in ice up to two weeks successfully settled and metamorphosed after thawing. Larvae that completed metamorphosis continued to develop and reproduce. In settlement experiments with cyprids of known age and where settled cyprids were removed every other day from the experimental containers, cyprids held in ice for two weeks settled and metamorphosed more than non-frozen larvae. Mean time to metamorphosis was longer for frozen cyprids than for non-frozen ones, and maximum time to metamorphosis was 38 d for cyprids held in sea ice for two weeks and 26 d for cyprids in non-frozen treatments. Larval tolerance to freezing conditions greatly expands the environmental tolerance repertoire of marine invertebrates and may help explain the ecological success of this widespread intertidal species.


## Introduction

Understanding of species' distributions, including response to global climatic change (Southward et al. 1995; Walther et al. 2002), species redistribution after major biogeographic events (Vermeij 1991), and survival in extreme environments (Peck 2004), requires understanding how environmental stresses (Helmuth et al. 2002; Wethey 2002) constrain population abundance and distribution. The case for marine animals living associated with the bottom is complex because environmental stresses can influence the

adults, which are sedentary and live on the bottom, and their larvae, which drift and inhabit the water column. Stress tolerant larvae capable of colonizing variable environments might confer advantages for the population in ecological time, and in the long run may prevent local extinction and influence geographic ranges. The apparently extraordinary ability of a larval barnacle we report below may help explain its success in the western north Atlantic intertidal, an extreme environment, and underscores the necessity of considering all phases of the life cycle to understand past, present and future species distributions.

*Semibalanus balanoides,* a very abundant and functionally important intertidal species throughout north Atlantic rocky shores (Lewis 1964; Bertness 1999), release early stage nauplii larvae that feed on water column plankton, live in bays and open coastal waters, and molt five times before metamorphosing into the non-feeding late stage cyprid, which subsequently returns to shore, settles, metamorphoses into juveniles, and develop into reproductive adults, which are obligate cross-fertilizers. Larval development lasts from 10 to 30 d (Harms 1984) and in Woods Hole waters late stage larvae are abundant from January to March (Fish 1925; Pineda et al. 2002). In the laboratory, cyprids can live up to 8 weeks (Holland and Walker 1975). *Semibalanus balanoides* larval settlement in Woods Hole, Massachusetts USA occurs from January to May, coinciding roughly with the seasonally coldest water and air temperatures (Pineda, et al. 2002). Freezing air temperatures in northeast US shores are common, leading to frequent sea-ice formation; from 1881 to 1980, 20% winters featured sea freezing conditions, when at least one month had a mean air temperature colder than -5°C (Wethey 1985). It is known that *Semibalanus balanoides* adults (Barnes 1958; Petersen

1962;1966; Crisp and Ritz 1967; Crisp et al. 1977) and larvae, for a short time, can tolerate freezing conditions. Late stage larvae (cyprids) held for 18 h at about -7.5°C had less than 15% survival (Crisp and Ritz 1967). It is not known, however, whether survivors retained the ability to metamorphose or ultimately to reproduce.

In 2003 we collected late stage larvae (cyprids) embedded in intertidal ice (Fig. 1). After about 1 h in sea water, the initially immobile individuals were swimming vigorously (CDB, per. obs.). Cyprids were often found embedded in or on the surface of ice samples surveyed in 2003 and 2004 on both Massachusetts and Rhode Island shores. Crisp and Ritz (1967) showed that larvae can survive freezing for less than a day. Yet, in 2003 and 2004 intertidal sites in New England were frozen for several weeks (see below). Here we address the viability of late stage larvae (cyprids) kept in sea ice, their ability to settle and metamorphose into juveniles, and their subsequent growth to reproduction. In another set of experiments, we test whether time to post settlement metamorphosis is prolonged when larvae are kept in sea ice.

# Methods and Results

**Winter intertidal temperature and settlement of a marine invertebrate**

Before the intertidal freezes, air temperature is often below freezing and colder than seawater. A week long time series of pressure, a proxy for sea level, and intertidal and subtidal temperature at Buzzards Bay, northeast USA, show that intertidal temperatures dropped and rose with the tide and rarely rose above 0 °C (Fig. 2). Subtidal temperature fluctuated around 0°C and was less well correlated with the tide. These conditions can lead to extended freezing in the intertidal.

The cold 2003 and 2004 winters in the northeast USA caused extensive sea ice formation in shore habitats. Ice sheets totally or partially covered semi enclosed bays in Maine, Massachusetts, and Rhode Island, Northeast USA. Ice formations on open coastlines were mostly restricted to the shore, but in Buzzards Bay, Massachusetts and Damariscotta River, Maine weekly visual surveys in 2003 and 2004 indicated that the ice sheets extended throughout most or the entire bays (Fig. 1). In Narragansett Bay, Rhode Island, ice formation was less extensive, with tidal channels free from semi-permanent ice. Sea-ice was restricted to the intertidal in open coastal sites, including Noyes Neck (Rhode Island, 41.327° N, 71.755° W), Oak Bluffs (Massachusetts, 41.460° N, 70.557° W) and Cape Newagen (Maine, 43.786° N, 69.655° W). Intertidal ice formations occurred intermittently from December 2002 to March 2003 and January to March 2004, and ice cover at a single time varied from patchy (cm to m variability) to extensive, where large portions of the intertidal shore (>100's m) were fully covered by sea-ice.

Our observations in Massachusetts in early 2003 and 2004 show that ice occupied the intertidal for up to 4 weeks. At one Massachusetts' intertidal site, sea-ice dampened temperature variability from about 18 January to 21 February 2004 and provided a less varying environment (Fig. 3; see also Fig. 1 for a picture of the site on 19 January). At another site 12 km south, temperature magnitudes and temporal patterns were similar. Patterns of reduced temperature variability at the two sites and weekly surveys in New England support the observation that large portions of the intertidal were covered by sea-ice for extended periods of time.

**Laboratory experiments, 2003: viability, metamorphosis and survival to reproduction**

**Viability.** We tested the viability of larvae held in frozen seawater from 0.5 to 56 d. Late stage *Semibalanus balanoides* larvae were collected in 2003 with a plankton net in Narragansett Bay, Rhode Island, transported to the laboratory where they were sorted in chilled seawater, placed in 50 ml containers with 30 ml filtered sea-water, fast frozen at -20 °C for 1-2 h until the water in the beakers was frozen solid, and then transferred to an incubator where they were kept at -5 to -7 °C. Replicate trials started on 15, 18, and 31 March, and the number of larvae per container was 28-31 in trials 1 and 3 and 37-40 in trial 2, with three beakers per treatment. Samples were thawed after 0.5, 1, 2, 5, 9, 10, 14, 28, 42 and 56 d and maintained at 4 °C for further inspection. Larvae were inspected daily for a period of two to three d after thawing and were scored as viable if they swam regularly and vigorously, dead if they remained immobile at the bottom of the beaker for 2-3 d, and unviable if larvae resting at the bottom of the beaker appeared hurt (e.g. extruded thorax), movement was restricted to some body parts (e.g., typically a single ramous swimming organ) or swimming was erratic (e.g. crawling sideways at the bottom).

A few hours after thawing, the majority of larvae that were frozen up to 14 d swam actively and were scored as viable (Fig. 4). A sharp decline in viability occurred after freezing between 2 and 4 weeks, yet even after being frozen for 4 weeks an average of 22% of the larvae were actively swimming. Few larvae (about 1%) were active after being frozen for 6 weeks, and none after 8 weeks. To test whether the mean proportion of viable larvae differed between the treatments, a 2 way ANOVA was performed for an

143 unbalanced design with arc-sine transformed data. Day was the fixed factor with 9 levels
144 for each frozen time treatment and trial was a random factor with 3 levels. Mean
145 proportion of viable larvae differed between days ($p$ = 0.000018). The interaction trial x
146 day was significant ($p$ = 0.000003) but variation between trials was not significant
147 ($p$ = 0.99).

148 **Settlement and metamorphosis.** The ability of cyprids to survive being
149 embedded in ice for extended periods of time was unexpected. Still, surviving larvae
150 must be able to settle and metamorphose if they are going to reproduce. This ability was
151 tested in 3 experiments by keeping individuals in frozen sea-water for periods ranging
152 from 0.5 to 14 d and then monitoring the settlement and metamorphosis of the thawed
153 samples of larvae. Larvae were collected as above and the sample was split with a
154 Folsom plankton splitter (McEwen et al. 1954) as many times as needed to have at least
155 three replicates per treatment. Each split fraction was randomly assigned to a treatment.
156 In the frozen treatment, late stage larvae in seawater were frozen for 0.5, 1, 2, or 14 d,
157 after which the ice was thawed, and the larvae deposited in plastic 1 - L beakers with
158 filtered sea-water and settlement plates, suspended ridged clay tiles. In the non-frozen
159 treatments, larvae were introduced into the beakers at time 0 and settlement plates were
160 added at time 0, 0.5, 1, 2 or 14 d. Each trial had three replicates per treatment except in
161 three treatments, which had 4 or 6 replicates. The number of larvae per replicate ranged
162 from 78 to 1013 individuals. Experiments 1, 2 and 3 were started 28 Feb, 18 Mar and 24
163 Mar 2003, and ended after 24-25 d when every other day observations of settlement on
164 the plates revealed little settlement.

Larvae settled and metamorphosed on the clay tiles, as expected, but they also settled on the bottom of the plastic beakers. At the end of the experiment, late stage larvae, settled larvae and metamorphs were quantified, where metamorphs are individuals that have lost their larval shells and have assumed a juvenile morphology (Anderson 1994). To test whether the mean proportion of metamorphs (metamorphs over total number of initial larvae) differed between the treatments, a two-way ANOVA was performed with two-factors: freezing (frozen and non-frozen) and time (Exp. 1: 0, 0.5, 1 d; Exp. 2 and 3: 0, 0.5, 1, 2, 14 d). The design was unbalanced because there was no frozen 0 d combination. The dependent variable was proportion of metamorphs relative to total larvae per treatment. This included all metamorphs observed on settlement plates, bottom of the beakers, and loose in the water. The analysis was performed on each experiment separately using GLM with SYSTAT. There was high variability within and between experiments with mean percent metamorphosis for the frozen treatments ranging from 17 to 74 %, and 12 to 67% in the non-frozen treatments (Fig. 5). Mean percent metamorphosis was not significantly different between the frozen treatments in any experiment ($p$ = 0.48, 0.92, and 0.61 for freezing treatment effects in experiments 1, 2 and 3, and $p$ = 0.97, 0.82, and 0.91 for time effects in experiments 1, 2 and 3). Thus, freezing does not appear to affect the final proportion of larvae that metamorphose. Results did not change by excluding the loose metamorphs from the analysis.

Metamorphosis in naturally frozen larvae collected in the field was also observed. On 19 Feb 2004, 255 larvae were collected in intertidal ice in Buzzards Bay, Massachusetts. In the laboratory, the cyprids were thawed, the larvae kept at ambient

187 seawater temperature and allowed to settle and metamorphose. Survivorship (79%) and
188 metamorphosis rate (55%) were high.

189 **Survival and growth to reproduction.** To test whether metamorphs in the
190 metamorphosis experiments survive and grow to reproduce, metamorphs in the frozen
191 and non-frozen treatments were transplanted to the field. Transplanted individuals were
192 on bottoms (9 cm diameter) that were cut away from the beakers and kept in running
193 seawater at ambient temperature. On 1 and 15 April and 8 May (Experiments I, II and III)
194 the beaker bottoms were fixed to rectangular wooden boards, one board per experiment,
195 and transplanted to the intertidal of a coastal lagoon (Eel Pond, Woods Hole,
196 Massachusetts). Initial number of metamorphs ranged from 6 to 580 per beaker bottom in
197 frozen treatments and 6 to 700 in non-frozen treatments. Beaker bottoms were arranged
198 in a randomized blocked design where the blocking factor was intertidal height with three
199 levels. Approximately every 6 weeks beaker bottoms were photographed to follow
200 individuals at which time invertebrates that had settled on the plates were removed. After
201 7.4 to 8.6 months under ambient conditions, survivorship, final size and reproductive
202 stage were assessed for transplanted barnacles in the three experiments. Three beaker
203 bottoms that had $n < 5$ individuals at the start of the deployment were not analyzed. No
204 results are presented for experiment 3, frozen treatment 2 d because (1) one replicate
205 started with less than 5 individuals, (2) another replicate had one individual at the end of
206 the experiment and therefore could not reproduce, and (3) the remaining replicate had no
207 survivors (see Fig. 6). Survival was assessed from following individuals in time series
208 photographs. Size was estimated as maximum opercular diameter. Individuals were
209 classified into two reproductive stages, those that had embryos (nauplii) with well formed

limbs and eye spots, corresponding to the most advanced embryonic development (categories 9-11, in Anderson 1994, p.184) and are evidence of reproduction, and those that did not, including barnacles with no egg masses to translucent eggs with no eye spots.

Results were variable within and between experiments. Survival was 8 to 62% in frozen treatments, and 20 to 73% in non-frozen treatments. Individual mean size was 3.2 to 4.5 mm for barnacles kept in ice and 3.3 to 4.6 mm for non-frozen barnacles. Finally, 25 to 79% of individuals from larvae kept in ice had well developed embryos, while the range was 26 to 86% for non-frozen treatments (Fig. 6). Survival, size and reproductive stage of individuals transplanted to the field were analyzed separately in each experiment. An unbalanced randomized blocks design was performed with (1) freezing treatment (frozen and non-frozen) and (2) time treatment (Exp. 1: 0, 0.5, 1 d; Exp. 2 and 3: 0, 0.5, 1, 2, 14 d) as the two factors, and row (intertidal height) as the blocking factor. All times had a frozen and non-frozen treatment except at time 0 which had no frozen treatment; this accounts for the unbalanced design. Analyses were performed using GLM with SYSTAT. Experiment 3 had low densities, so the proportion of barnacles with developed embryos may have been a conservative estimate of reproductive potential because *Semibalanus balanoides* are obligate cross fertilizers and some barnacles on some plates were isolated. There was no significant time effect or freezing treatment effect on survival, size and reproductive stage, except for experiment 3, where non-frozen metamorphs had higher survival ($p < 0.05$) and larger opercular diameters ($p < 0.05$) than frozen treatments. Row effect (intertidal height) was significant for survival (experiments 1 and 3, $p < 0.001$ and $p < 0.01$) and reproductive stage (experiment 1, $p < 0.001$).

**Laboratory experiments, 2004: prolongation of time to post settlement metamorphosis in cyprids of known age kept in ice**

We tested the hypothesis that keeping cyprid larvae in ice prolongs time to post settlement metamorphosis. Experiments were conducted with cyprids of known age with larvae kept in ice for two weeks, treatment 1, and with non-frozen larvae, treatment 2. Settlement opportunities were minimized (1) by removing attached settlers from experimental containers every other day, thus minimizing gregarious settlement (Knight-Jones 1953) and (2) by offering larvae smooth settlement surfaces, and thus depriving them of their favorite substrate, cracks and pits (e.g. Wethey 1984; Hills et al. 1998).

Barnacle nauplii were collected from plankton tows taken at the Woods Hole Oceanographic Institution dock on 6 dates from 19 February to 12 March 2004. Nauplii retained in a 500 μm sieve were placed into 1 μm filtered seawater in 1 - L beakers and kept at 4$^{o}$C, where some metamorphosed to cyprids. Each day for 3 d cyprids were removed from the beakers. Because these larvae had metamorphosed into cyprids sometime during the previous 24 h, the date of metamorphosis and cyprid age was known. Cyprids were kept at 4° C for 1 to 3 d before starting the experiments. Cyprids were pipetted into 6 equal portions and the portions were assigned randomly to two different experimental treatments, non-frozen and frozen, with 3 replicates each. Number of larvae per replicate per experiment ranged from 56 to 390 individuals.

Non-frozen and frozen treatments started on the same day in each of six dates, and lasted 91 d in all cases except in experiment 6 were all larvae died before 89 d. (1) In the non-frozen treatments larvae were deposited in three 800 ml beakers filled with 500 ml of filtered seawater on day 1. (2) In the frozen treatments cyprids were deposited in

three 50 ml plastic containers with 25 ml of filtered seawater, and on day 1 containers were frozen for 1.5 h at -20° C and then transferred to a -8 °C freezer. On day 14 the frozen containers were placed into a seawater table with ambient temperature seawater. After 2 to 4 h the ice had thawed, and the water and cyprids were poured into an 800 ml beaker filled with 500 ml of filtered seawater. All beakers were placed in a water bath at ambient seawater temperature and covered loosely with Parafilm. Water table temperature in the experimental period increased from 1 °C on 19 February to 17 °C on 11 June. Every other day the content in each beaker was decanted off into a new beaker. Old beakers were examined for attached metamorphs and attached cyprids. Cyprids that were attached to the beaker were left in the beaker with filtered seawater, placed into a 4°C incubator, and checked every other day to determine day of metamorphosis. Metamorphosis was usually observed within 4 d, but in two cases cyprids failed to metamorphose. For attached metamorphs and attached cyprids the day of metamorphosis was noted. The beakers also contained loose metamorphs, partial metamorphs (see below) and dead larvae, which were removed from the experiment. All analyses and statistics are based on attached metamorphs. Loose metamorphs are not included in this analysis because the lack of preferred substrate for settlement and the very long experimental period (91 d) might have resulted in spontaneous metamorphosis (e.g. Crisp 1974; Pechenik 1980; Zimmerman and Pechenik 1991; Gebauer et al. 1998). Because the container had smooth surfaces, it is likely that some loose metamorphs become detached after settling.

More cyprids metamorphosed in the frozen treatments than in the non-frozen treatments (Fig. 7). The differences were significant (paired samples *t* - test on

279 proportions, $p = 0.014$) and analysis including the loose metamorphs did not change the
280 results. The proportions of attached metamorphs ranged from 0 to 4.9% in the non-frozen
281 treatments and from 3.2 to 21.6% in the frozen treatments. Including the loose
282 metamorphs the proportions ranged from 0 to 6.7% in the non-frozen treatments and 6.4
283 to 26.3% in the frozen treatments. Time to metamorphosis, the interval between the start
284 of the experiment and observed metamorphosis, was higher in the frozen than in the non-
285 frozen treatments (Fig. 8). Variability in the non-frozen treatments, with mean values
286 ranging 6 to 22 d, was higher than in the frozen treatments, with values ranging 20 to
287 26.4 d (or 6 to 11 d after thawing). The longest time to metamorphosis in attached larvae
288 was 26 d in non-frozen treatments and 38 d in the frozen treatments. By the end of the
289 experimental 91 d period, some cyprids on the bottom of the container showed restricted
290 movement and displayed erratic behavior. The longest time to metamorphosis in loose
291 metamorphs was 77 d and 89 d in the non freezing and the freezing treatments. Partial
292 metamorphs, individuals with morphological characteristics of metamorphs but still
293 enclosed in a cyprid chitinous shell, were observed in the two treatments predominantly
294 during the last 30 d of the experiments. All partial metamorphs occurred after 55 days,
295 with the exception for one individual on day 30 in Experiment 3. The maximum number
296 of partial metamorphs per treatment in each experiment was 6.

297 To test whether time to metamorphosis differed in the non-frozen and the frozen
298 treatments, the mean time to metamorphosis for each replicate in each treatment and
299 experiment combination was obtained. Replicates were excluded from the computation of
300 the average if no cyprids metamorphosed. This occurred for two replicates in non-frozen
301 experiment 1 and one replicate in the non-frozen treatment experiment 6. Mean time to

metamorphosis was longer in the frozen treatments than in the non-frozen (paired samples *t* - test, $p = 0.00014$). Differences were consistent and significant when loose metamorphs were included in the analysis.

Cyprids kept in ice could not settle during the 14 d freezing period. Thus, to test whether mean time to metamorphosis differed for cyprids in the 2 treatments when cyprids were able to settle for the same amount of time, 14 d was subtracted from the mean date to metamorphosis of each frozen treatment replicate, and a new mean time to metamorphosis computed for each experiment and treatment. (The averages for the non-frozen treatments did not change.) Removing 14 d to the mean time to metamorphosis resulted in shorter times for the frozen treatments, with a grand mean of 10.1 d, than for the non-frozen treatments, with a grand mean of 12.5 d, and the differences were significant (paired samples *t* - test, $p = 0.035$)

## Discussion

We have shown that *Semibalanus balanoides* late stage larvae held in sea ice can survive for extended periods of time and subsequently settle, metamorphose, and grow to reproduce. We are not aware of any other marine larvae that can tolerate frozen environmental conditions and retain the ability to metamorphose, grow, and develop reproductively, but perhaps larvae of other near-shore species that experience freezing might also be tolerant. Late stage larvae of another local barnacle species (*Balanus* sp.) held in ice yielded no survivors. Our results expand considerably the environmental repertoire of marine invertebrate larvae (Pechenick 1987). We do not know whether

larvae kept in ice had their tissue fluids frozen, but in adults 80% of fluids freeze at about -16°C (Crisp, et al. 1977).

Cyprids of known age kept in ice for 2 weeks metamorphosed later than those in the non-frozen treatments. The delayed time to metamorphosis must be, in part, the result of the freezing process which prevented settlement for two weeks. Removing this period of time from the statistical analysis not only removed the differences, but changed the sign of the differences, with frozen larvae metamorphosing earlier than non-frozen larvae. Rates of metamorphosis were lower in the 2004 than in the 2003 experiments. Settlement in 2004 was prevented by minimizing gregarious settlement and by depriving the larvae of their preferred settlement substrate textures, cracks and pits. Another factor influencing metamorphosis rates might be cyprid age heterogeneity. In the 2003 experiments, cyprids of unknown and presumably heterogeneous age were used, while in 2004 cyprids were about the same age. Age heterogeneity might offer some cyprids a larger window in which they are attracted to other settlers and thus increase settlement.

A striking result in the 2004 experiments, where cyprids were discouraged from settling, was that there were more metamorphs in treatments where larvae were kept in ice than in non-frozen treatments. This result contrasts with the 2003 experiments, where metamorphosis in frozen and non-frozen larvae did not differ. Freezing and preventing settlement for two weeks might suppress cyprid choosiness but only in the experiments where settlement opportunities were minimized. The two experiments differed in the three factors discussed above, gregariousness, substrate texture and cyprid age, and it is unclear what combination of factors explains our results.

Some cyprids were alive after 91 d in the experiments conducted in 2004, although many had died and live cyprids were lying on the bottom, not swimming. Holland and Walker (1975) found that cyprids of unknown age could survive up to 8 weeks in the laboratory, but they did not observe metamorphosis. The longest time to metamorphosis in the non-freezing and freezing treatments was 26 and 38 d for the attached cyprids, and 77 and 89 d in the loose metamorphs. Lucas et al (1979) calculated an energy budget for *Semibalanus balanoides* cyprids, and concluded that cyprids would loose their competence to metamorphose successfully at 21-28 d. Lucas et al (1979) used captured cyprids, where age was unknown and therefore 21-28 d might underestimate the maximum competency period. Lucas et al. (1979) experiments were conducted at 10°C, which is generally warmer than in our experiments, where 10°C was reached in late April. Higher temperature increases metabolic rates which might yield shorter time to metamorphosis. Our results from the non-frozen treatment with attached metamorphs fall within Lucas' et al. range, but keeping the larvae in ice extends this period. Freezing presumably reduces larval metabolism, and this would account for the higher maximum time to metamorphosis in frozen larvae than in non-frozen larvae.

We found loose metamorphs well beyond the 21-28 d range. It is not clear whether loose metamorphs become detached after settlement or whether they metamorphosed spontaneously. Most loose metamorphs were found at the same date as the attached ones, suggesting that at least some detached from the smooth experimental surfaces. Conversely, the appearance of partial metamorphs enclosed in a cyprid shell at the end of the experiment suggests spontaneous metamorphosis.

Freezing conditions were adverse to larvae, as survival and size were reduced for barnacles in frozen treatments relative to non-frozen ones, though the differences were not consistent for all experiments. Reduced survival and size in experiment 3 suggest that late freezes might be more deleterious to larvae than early freezes, but more experiments are needed to test this idea. Zooplankton in high latitude environments might be able to avoid freezing conditions because as sea-ice forms at the surface, individuals might escape to ice-free depths. *Semibalanus balanoides*, a boreal circumpolar sessile species, must settle on intertidal habitats where ice formation occurs. Hence, freezing conditions are inevitable for intertidal adults, and sometimes for larvae found near their settlement sites. This is true in the Massachusetts and Rhode Island shores, where peak settlement in late February roughly coincides with annually coldest water and air temperatures. Freezing conditions for intertidal larvae might also be expected in coastlines north of Massachusetts, along the Canadian Maritime Provinces coastline, where low temperatures might occur in late spring, when settlement occurs (Bousfield 1955). In western Greenland north of 70 ° latitude *Semibalanus balanoides* might find freezing settling conditions in early fall (Petersen 1966).

Freezing tolerance might allow *Semibalanus balanoides* to establish populations where few other species succeed. Larvae settling during the winter can grow and attain a refuge in size against predators inactive at low temperatures (Menge 1976). Survival in low water temperatures also prolongs the settlement competency period. Intertidal environments are very variable, and some intertidal species have wide geographic range (Jackson 1974). Freezing tolerance in *S. balanoides* adults and larvae might help explain this species' widespread range and results suggesting its survival through the last

390 glaciation in the Western Atlantic (Wares and Cunningham 2001). Finally, larvae caught
391 in drifting intertidal ice provides a variation on traditional mechanisms of larval dispersal,
392 as larvae in ice would follow different dispersal pathways than free swimming larvae, as
393 floating ice blown by the wind follows different trajectories than seawater.

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468

**List of Figures**

Fig. 1. Frozen intertidal in Buzzards Bay, Massachusetts (41.643°N, 70.652°W) photographed on 19 January 2004, and cyprid larvae embedded in ice sampled in the Buzzard's Bay intertidal.

Fig. 2. Pressure and intertidal and subtidal temperature in 2003 in eastern Buzzards Bay, Massachusetts (41.533 °N, 70.671° W). Subtidal temperature and pressure, uncorrected for atmospheric fluctuations, were measured every 5 minutes with a Seabird SBE39 sensor located about 50 cm below the lowest tidal sea level (10 kPa are ~ 1 m). An Onset Stowaway thermistor (-4 to + 37 °C range) recorded every hour and was placed in a mid intertidal location. No intertidal temperature is plotted when temperature was less than - 4.6°C, the lower limit of the sensor. High frequency variability in pressure indicates surface wave activity.

Fig. 3. Hourly temperature record at a mid-intertidal location in eastern Buzzards Bay, Massachusetts (41.643°N, 70.652°W). Time, in GMT, and temperature measured with Onset Stowaway thermistors (-20 to 50 °C range). Exposure by the tide caused semidiurnal temperature variability due to sea-water/air temperature differences. Arrows point to approximate start and end of ice cover at the site determined from visual observations and dampened patterns in temperature variability.

Fig. 4. Effects of freezing time on larval viability. Larvae were kept in ice for 0.5, 1, 2, 5, 9, 10, 14, 28, 42 and 56 d. Mean + 1 SE for trials started on 15, 18 and 31 March.

Fig. 5. Effects of freezing on post settlement metamorphosis. For the frozen treatments, times are the days larvae were kept in sea ice before being offered a settlement substrate. For the non-frozen treatments, times are days after which larvae were offered a settlement plate. Mean + 1 SE. Experiments started on 28 Feb, 18 Mar and 24 Mar 2003 and ended 24-25 days later.

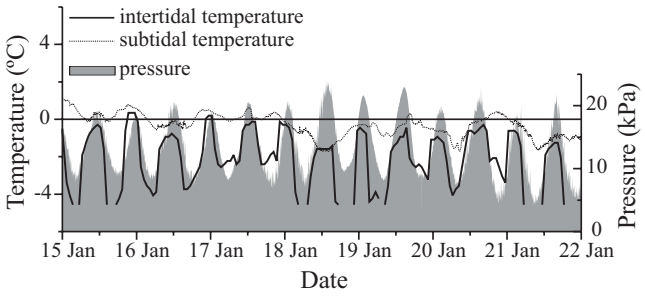
Fig. 6. Reproductive stage (top panels), size (mid panels) and survival (bottom panels) for larvae kept in ice and for non-frozen larvae in each one of three experiments (columns). Mean + 1 SE. Larvae that metamorphosed on the beaker bottoms were transplanted to the field in April and May 2003 and inspected about 8 months later. Size for experiment 3, frozen treatment 2 days is based on one individual. See the text for explanation on missing reproductive stage data in this treatment.

Fig. 7. Effects of freezing on post settlement number of metamorphs in experiments with cyprids of known age and where settlement opportunities were minimized. Cumulative number of attached metamorphs for larvae kept in ice and in non-frozen treatments. Vertical lines indicate thaw dates in the frozen experiments. Panels are different experiments. Experiments 1-6 started on 23 Feb, 23 Feb, 27 Feb, 5 Mar, 5 Mar and 15 Mar 2004. Number of larvae per replicate in frozen treatments in experiments 1 to

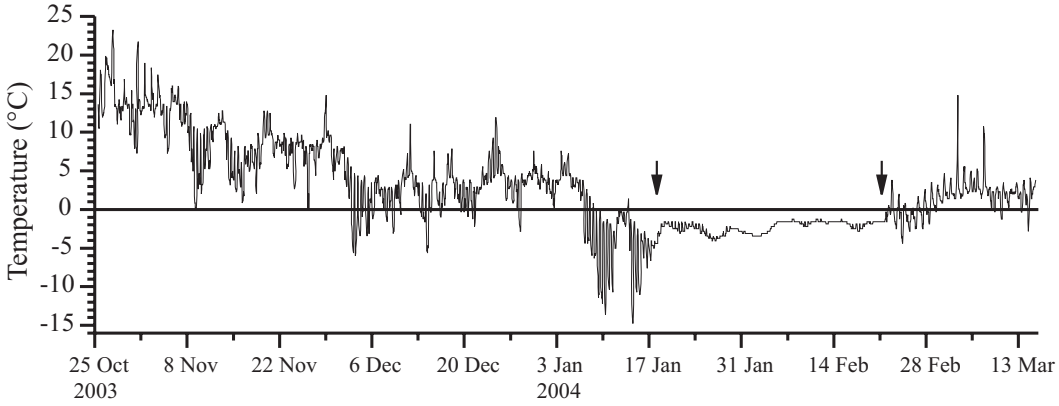
6 was 107, 267, 258, 144, 404 and 63. In the non-frozen treatments the number of larvae was 127, 267, 227, 165, 390 and 56.

Fig. 8. Effects of freezing on post settlement time to metamorphosis in experiments with cyprids of known age and where settlement opportunities were minimized. Mean time to metamorphosis of attached metamorphs with minimum and maximum date of metamorphosis per replicate per treatment. Vertical axes in frozen treatments displaced to thawing dates. Non-frozen treatments with no metamorphs are not plotted. Panels show experiments 1 to 6.

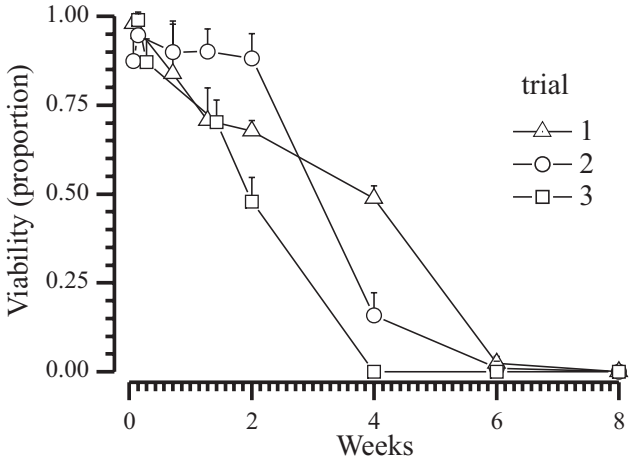
Pineda Fig 1


intertidal temperature
subtidal temperature
pressure
Temperature (ºC)
4
0
-4
20
10
0
Pressure (kPa)
15 Jan
16 Jan
17 Jan
18 Jan
19 Jan
20 Jan
21 Jan
22 Jan
Date

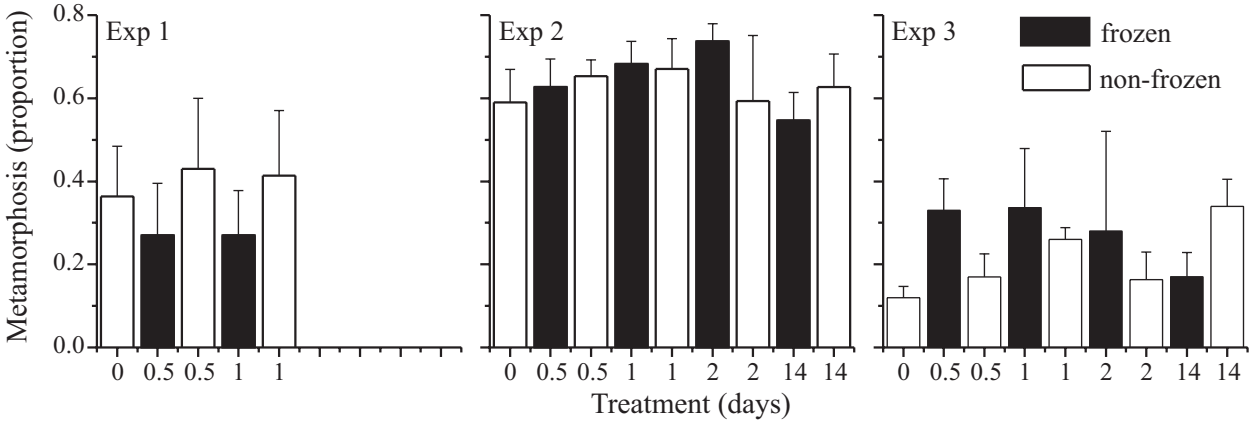
Pineda Fig 2


Temperature (°C)
25
20
15
10
5
0
-5
-10
-15
25 Oct
2003
8 Nov
22 Nov
6 Dec
20 Dec
3 Jan
2004
17 Jan
31 Jan
14 Feb
28 Feb
13 Mar

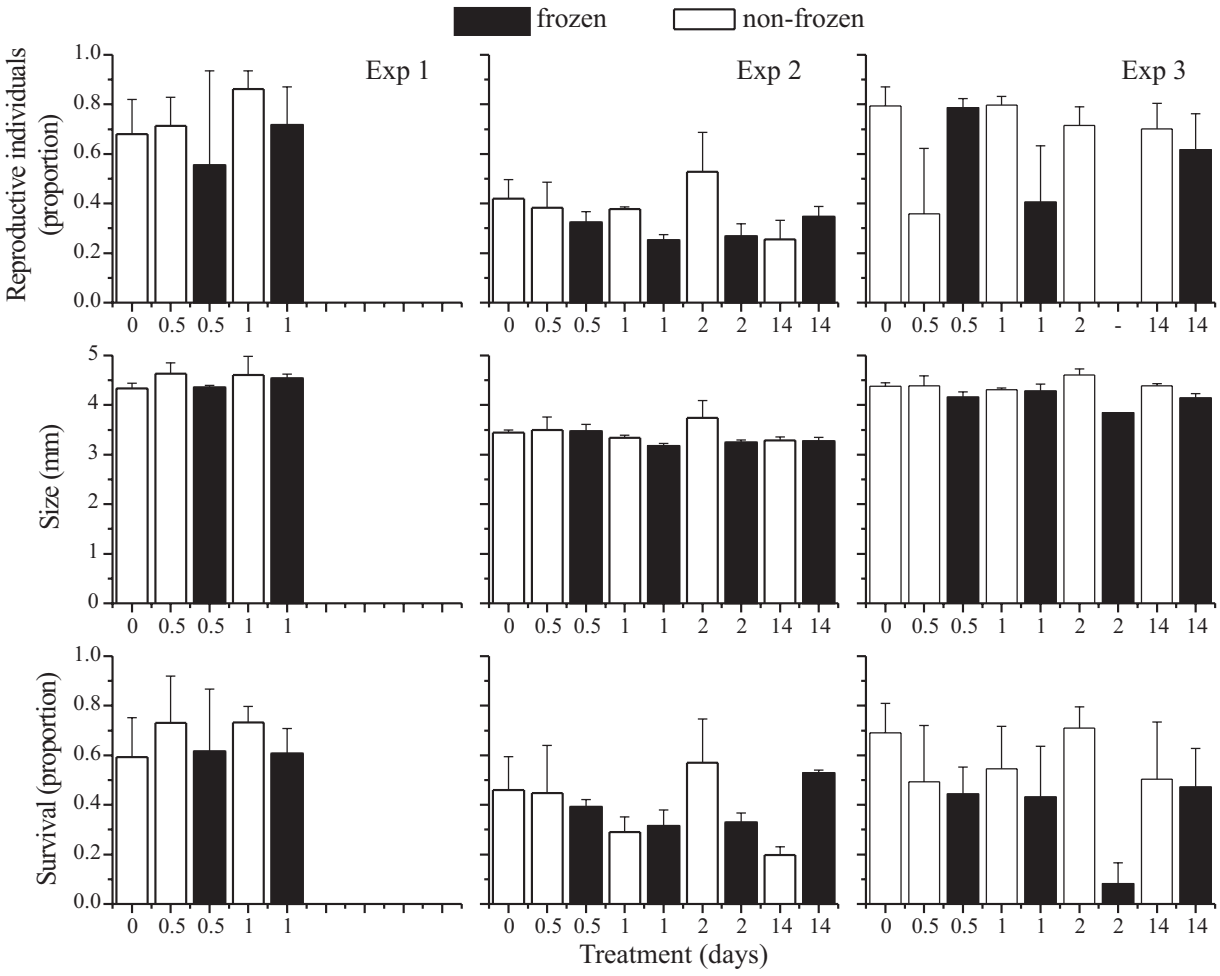
Pineda Fig 3


1.00
0.75
0.50
0.25
0.00
Viability (proportion)
trial
1
2
3
0
2
4
6
8
Weeks

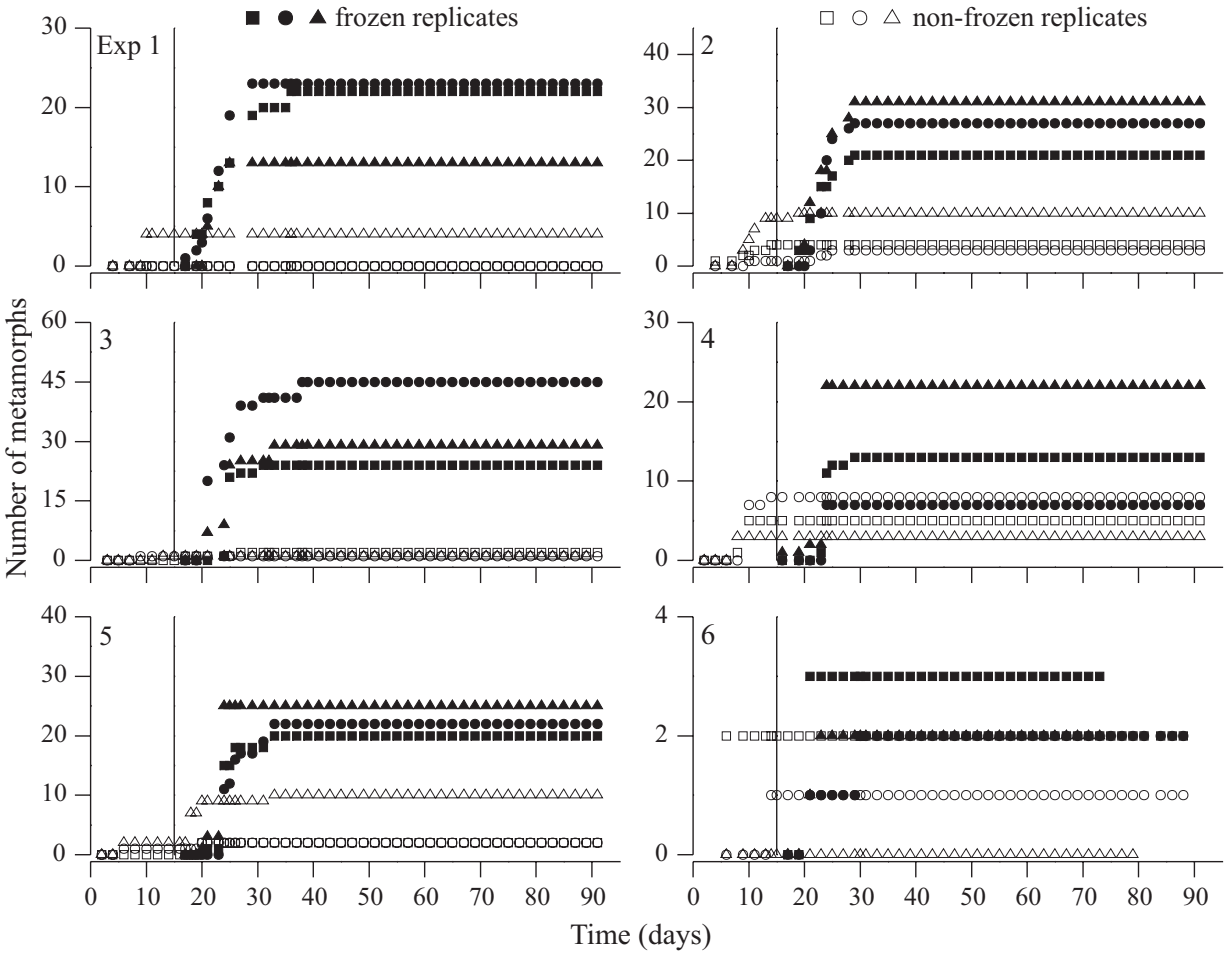
Pineda Fig 4


Metamorphosis (proportion)
0.0
0.2
0.4
0.6
0.8
Exp 1
Exp 2
Exp 3
frozen
non-frozen
0 0.5 0.5 1 1
0 0.5 0.5 1 1 2 2 14 14
0 0.5 0.5 1 1 2 2 14 14
Treatment (days)

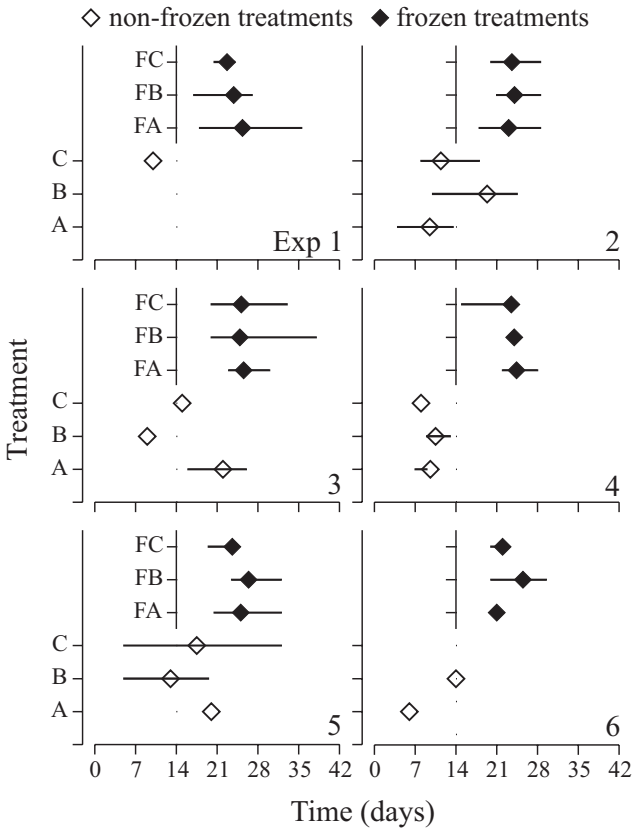
Pineda Fig 5


frozen
non-frozen
Exp 1
Exp 2
Exp 3
Reproductive individuals (proportion)
Size (mm)
Survival (proportion)
Treatment (days)
0 0.5 0.5 1 1
0 0.5 0.5 1 1 2 2 14 14
0 0.5 0.5 1 1 2 - 14 14
0 0.5 0.5 1 1 2 2 14 14

Pineda Fig 6


frozen replicates
non-frozen replicates
Exp 1
2
3
4
5
6
Number of metamorphs
Time (days)

Pineda Fig 7


◇ non-frozen treatments
◆ frozen treatments
Treatment
FC
FB
FA
C
B
A
Exp 1
2
3
4
5
6
0
7
14
21
28
35
42
Time (days)

Pineda Fig 8