Barnacle larvae in ice: Survival, reproduction, and time to postsettlement metamorphosis

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Abstract

Late stage larvae (cyprids) of the barnacle *Semibalanus balanoides* frequently encounter freezing conditions along the northwest Atlantic coast. *S. balanoides* cyprids survived for more than 4 weeks embedded in sea ice, and a significant fraction of larvae held in ice up to 2 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 2 weeks settled and metamorphosed more than nonfrozen larvae. Mean time to metamorphosis was longer for frozen cyprids than for nonfrozen ones, and maximum time to metamorphosis was 38 d for cyprids held in sea ice for 2 weeks and 26 d for cyprids in nonfrozen 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.

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 nonfeeding late stage cyprid, which subsequently returns to shore, settles, metamorphoses into juveniles, and develops 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). *S. balanoides* larval settlement in Woods Hole, Massachusetts, occurs from January to May, coinciding roughly with the seasonally coldest water and air temperatures (Pineda et al. 2002). Freezing air temperatures in northeast U.S. 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 *S. 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 (C.D. unpubl. data). 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 postsettlement 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 in-

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

tertidal and subtidal temperature at Buzzards Bay, northeast United States, shows 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 United States caused extensive sea ice formation in shore habitats. Ice sheets totally or partially covered semienclosed bays in Maine, Massachusetts, and Rhode Island. Ice formations on open coastlines were mostly restricted to the shore, but in Buzzards Bay, Massachusetts, and Damariscotta River,
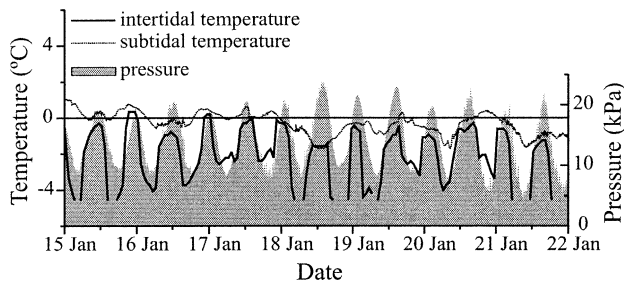

Fig. 2. Pressure and intertidal and subtidal temperature in 2003 in eastern Buzzards Bay, Massachusetts (41.533°N, 70.671°W). Subtidal temperature and pressure, uncorrected for atmospheric fluctuations, were measured every 5 min with a Seabird SBE39 sensor located about 50 cm below the lowest tidal sea level (10 kPa are ~1 m). An Onset Stowaway thermistor (−4°C to +37°C range) recorded every hour and was placed in a midintertidal 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.

Maine, weekly visual surveys in 2003 and 2004 indicated that the ice sheets extended throughout most or all of the bays (Fig. 1). In Narragansett Bay, Rhode Island, ice formation was less extensive, with tidal channels free from semipermanent 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 (centimeter to meter variability) to extensive, where large portions of the intertidal shore (more than hundreds of meters) 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 *S. 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
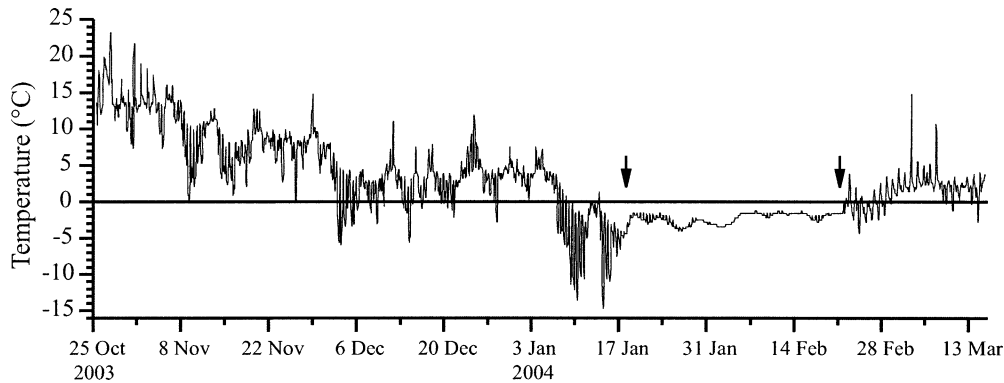
Fig. 3. Hourly temperature record at a midintertidal location in eastern Buzzards Bay, Massachusetts (41.643°N, 70.652°W). Time, in GMT, and temperature measured with Onset Stowaway thermistors (-20°C to 50°C range). Exposure by the tide caused semidiurnal temperature variability due to seawater/air 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.

seawater, placed in 50-ml containers with 30 ml of filtered seawater, 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°C 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 2 to 3 d after thawing and were scored as viable if they swam regularly and vigorously, dead if they remained immobile on the bottom of the beaker for 2–3 d, and unviable if larvae resting on 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 on 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 two-way analysis of variance (ANOVA) was performed for an unbalanced design with arcsine transformed data. Day was the fixed factor with nine levels for each frozen time treatment, and trial was a random factor with three levels. Mean proportion of viable larvae differed between days ($p = 0.000018$). The interaction trial × day was significant ($p = 0.000003$), but variation between trials was not significant ($p = 0.99$).

Settlement and metamorphosis: The ability of cyprids to survive being embedded in ice for extended periods of time was unexpected. Still, surviving larvae must be able to settle and metamorphose if they are going to reproduce. This ability was tested in three experiments by keeping individuals in frozen seawater for periods ranging from 0.5 to 14 d and then monitoring the settlement and metamorphosis of the thawed samples of larvae. Larvae were collected as above, and the sample was split with a Folsom plankton splitter (McEwen et al. 1954) as many times as needed to have at least three replicates per treatment. Each split fraction was randomly assigned to a treatment. In the frozen treatment, late stage larvae in seawater were frozen for 0.5, 1, 2, or 14 d, after which the ice was thawed and the larvae deposited in plastic 1-liter beakers with filtered seawater and settlement plates, suspended ridged clay tiles. In the nonfrozen treatments, larvae were introduced into the beakers at time 0 and settlement plates were added at 0, 0.5, 1, 2, or 14 d. Each trial had three replicates per treatment except in three treatments, which had four or six replicates. The number of larvae per replicate ranged from 78 to 1,013 individuals. Experiments 1, 2, and 3 were started 28 February, 18 March, and 24 March 2003, and ended after 24–25 d when every other day observations of settlement on 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
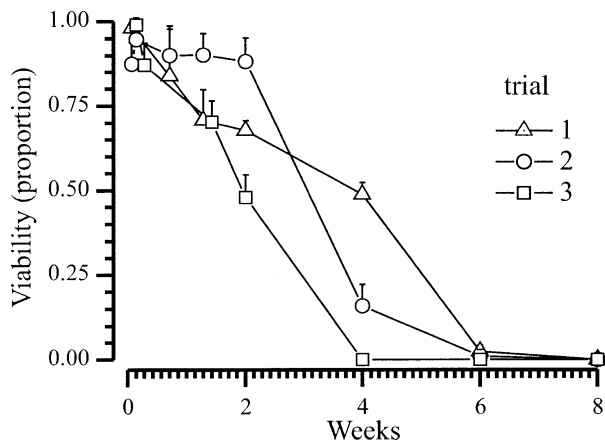

Fig. 4. Effects of freezing time on larval viability. Larvae were kept in ice for 0.5, 1, 2, 5, 9, 10, 14, 28, 42, and 56 d. Mean + 1 SE for trials started on 15, 18, and 31 March.
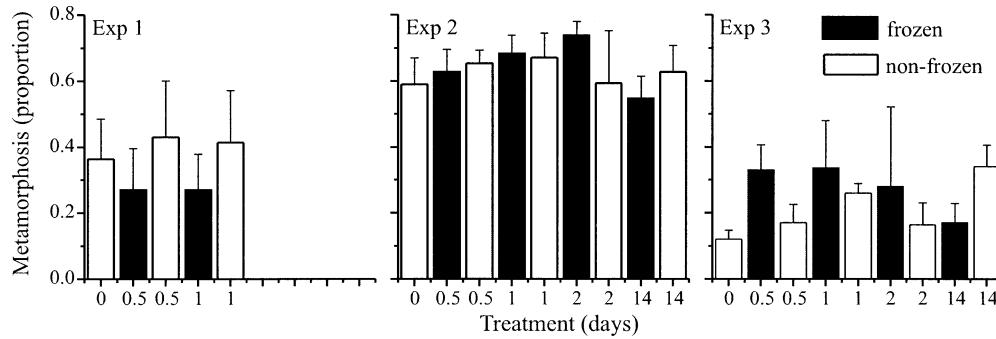
Fig. 5. Effects of freezing on postsettlement metamorphosis. For the frozen treatments, times are the days larvae were kept in sea ice before being offered a settlement substrate. For the nonfrozen treatments, times are days after which larvae were offered a settlement plate. Mean + 1 SE. Experiments started on 28 February, 18 March, and 24 March 2003 and ended 24–25 d later.

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 nonfrozen) and time (Experiment 1: 0, 0.5, 1 d; Experiments 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 the proportion of metamorphs relative to total larvae per treatment. This included all metamorphs observed on settlement plates, at the bottom of the beakers, and loose in the water. The analysis was performed on each experiment separately using GLM (General Linear Model) with SYSTAT. There was high variability within and between experiments, with mean percentage metamorphosis for the frozen treatments ranging from 17% to 74%, and 12% to 67% in the nonfrozen treatments (Fig. 5). Mean percentage 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 February 2004 in Buzzards Bay, Massachusetts, 255 larvae were collected in intertidal ice. In the laboratory, the cyprids were thawed and the larvae kept at ambient seawater temperature and allowed to settle and metamorphose. Survivorship (79%) and metamorphosis rate (55%) were high.

Survival and growth to reproduction: To test whether metamorphs in the metamorphosis experiments survive and grow to reproduce, metamorphs in the frozen and nonfrozen treatments were transplanted to the field. Transplanted individuals were on bottoms (9 cm diameter) that were cut away from the beakers and kept in running seawater at ambient temperature. On 1 and 15 April and 8 May (experiments 1, 2, and 3), the beaker bottoms were fixed to rectangular

wooden boards, one board per experiment, and transplanted to the intertidal of a coastal lagoon (Eel Pond, Woods Hole, Massachusetts). The initial number of metamorphs ranged from 6 to 580 per beaker bottom in frozen treatments and 6 to 700 in nonfrozen treatments. Beaker bottoms were arranged in a randomized block design where the blocking factor was intertidal height with three levels. Approximately every 6 weeks beaker bottoms were photographed to follow individuals, at which time invertebrates that had settled on the plates were removed. After 7.4 to 8.6 months under ambient conditions, survivorship, final size, and reproductive stage were assessed for transplanted barnacles in the three experiments. Three beaker bottoms that had $n < 5$ individuals at the start of the deployment were not analyzed. No results are presented for experiment 3, frozen treatment 2 d because (1) one replicate started with fewer than five individuals, (2) another replicate had one individual at the end of the experiment and therefore could not reproduce, and (3) the remaining replicate had no survivors (*see Fig. 6*). Survival was assessed from following individuals in time series photographs. Size was estimated as maximum opercular diameter. Individuals were 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 providing 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 nonfrozen treatments. Individual mean size was 3.2 to 4.5 mm for barnacles kept in ice and 3.3 to 4.6 mm for nonfrozen barnacles. Finally, 25% to 79% of individuals from larvae kept in ice had well-developed embryos, while the range was 26% to 86% for nonfrozen 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 nonfrozen) and (2) time treatment (Experiment 1, 0, 0.5, 1 d; Experiments 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 nonfrozen treatment except at time 0, which
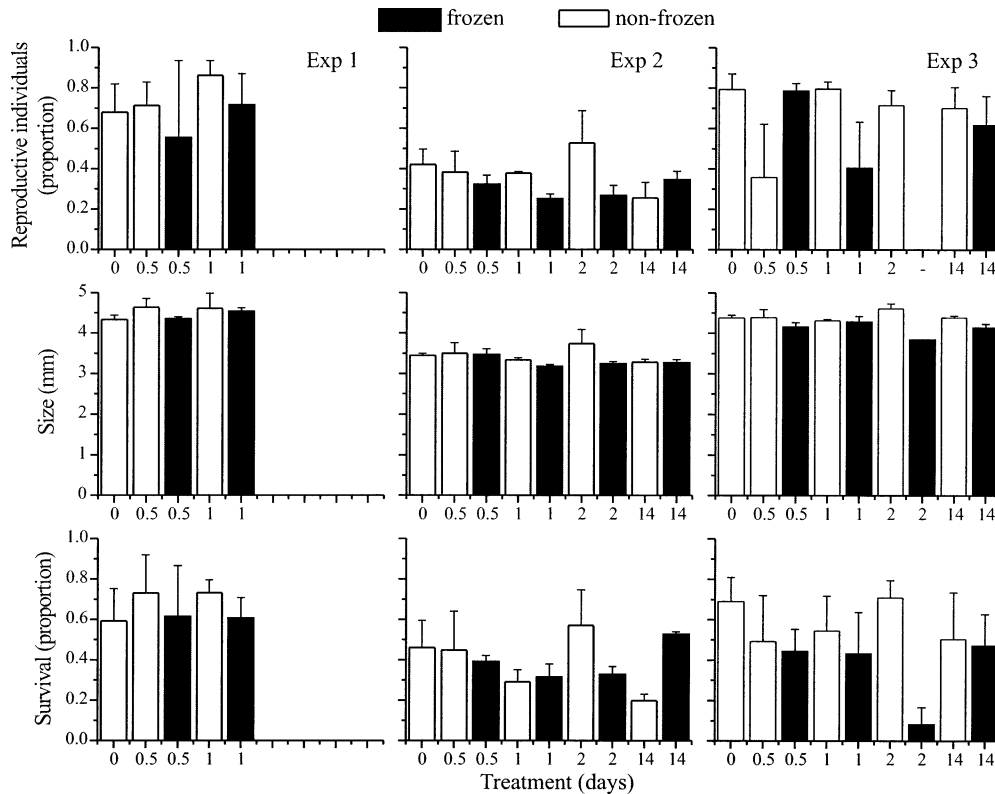
Fig. 6.   Reproductive stage (top panels), size (middle panels), and survival (bottom panels) for larvae kept in ice and for nonfrozen larvae in each one of three experiments (columns). Mean + 1 SE. Larvae that metamorphosed on the beaker bottoms were transplanted to the field in April and May 2003 and inspected about 8 months later. Size for experiment 3, frozen treatment 2 d is based on one individual. See the text for explanation of missing reproductive stage data in this treatment.

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 *S. 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 nonfrozen metamorphs had higher survival ($p = 0.046$) and larger opercular diameters ($p = 0.02$) than frozen treatments. Row effect (intertidal height) was significant for survival (experiments 1 and 3, $p = 0.0007$ and $p = 0.0001$) and reproductive stage (experiment 1, $p = 0.009$).

Laboratory experiments, 2004: prolongation of time to postsettlement metamorphosis in cyprids of known age kept in ice—We tested the hypothesis that keeping cyprid larvae in ice prolongs time to postsettlement metamorphosis. Experiments were conducted with cyprids of known age with larvae kept in ice for 2 weeks, treatment 1, and with nonfrozen 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 six dates from 19 February to 12 March 2004. Nauplii retained in a 500-μm sieve were placed into 1-μm filtered seawater in 1-liter beakers and kept at 4°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 six equal portions, and the portions were assigned randomly to two different experimental treatments, nonfrozen and frozen, with three replicates each. Number of larvae per replicate per experiment ranged from 56 to 390 individuals.

Nonfrozen and frozen treatments started on the same day in each of six dates and lasted 91 d in all cases except in experiment 6, where all larvae died before 89 d. (1) In the nonfrozen 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 contain-
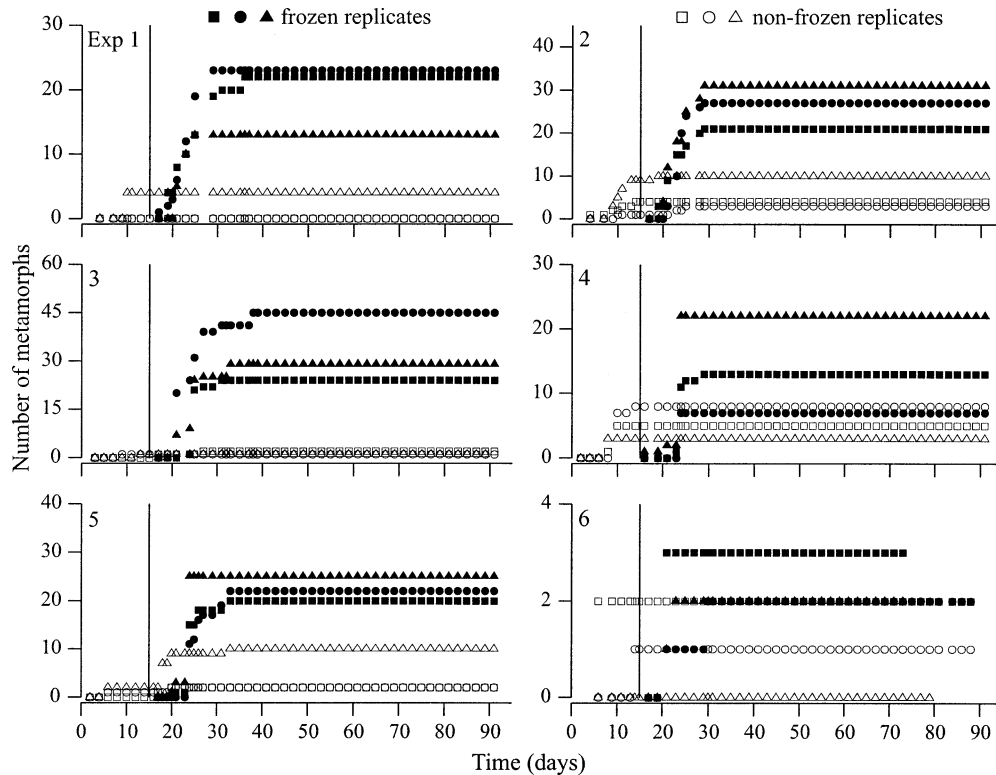
Fig. 7. Effects of freezing on postsettlement number of metamorphs in experiments with cyprids of known age and where settlement opportunities were minimized. Cumulative number of attached metamorphs for larvae kept in ice and in nonfrozen treatments. Vertical lines indicate thaw dates in the frozen experiments. Panels are different experiments. Experiments 1–6 started on 23 February, 23 February, 27 February, 5 March, 5 March, and 15 March 2004. Number of larvae per replicate in frozen treatments in experiments 1 to 6 was 107, 267, 258, 144, 404, and 63. In the nonfrozen treatments the number of larvae was 127, 267, 227, 165, 390, and 56.

ers 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 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 became detached after settling.

More cyprids metamorphosed in the frozen treatments than in the nonfrozen treatments (Fig. 7). The differences were significant (paired samples *t*-test on proportions, $p = 0.014$), and analysis including the loose metamorphs did not change the results. The proportions of attached metamorphs ranged from 0 to 4.9% in the nonfrozen treatments and from 3.2% to 21.6% in the frozen treatments. Including the loose metamorphs the proportions ranged from 0 to 6.7% in the nonfrozen treatments and 6.4% to 26.3% in the frozen treatments. Time to metamorphosis, the interval between the start of the experiment and observed metamorphosis, was higher in the frozen than in the nonfrozen treatments (Fig. 8). Variability in the nonfrozen treatments, with mean values ranging from 6 to 22 d, was higher than in the frozen treatments, with values ranging from 20 to 26.4 d (or 6 to 11 d after thawing). The longest time to metamorphosis in attached larvae was 26 d in nonfrozen treatments and 38 d in the frozen treatments. By the end of the experimental 91-d period, some cyprids on the bottom of the container showed restricted movement and displayed erratic behavior. The longest time to metamorphosis in loose metamorphs was 77 and 89 d in the nonfreezing and the freezing treatments. Partial metamorphs, individuals with morphological characteristics of
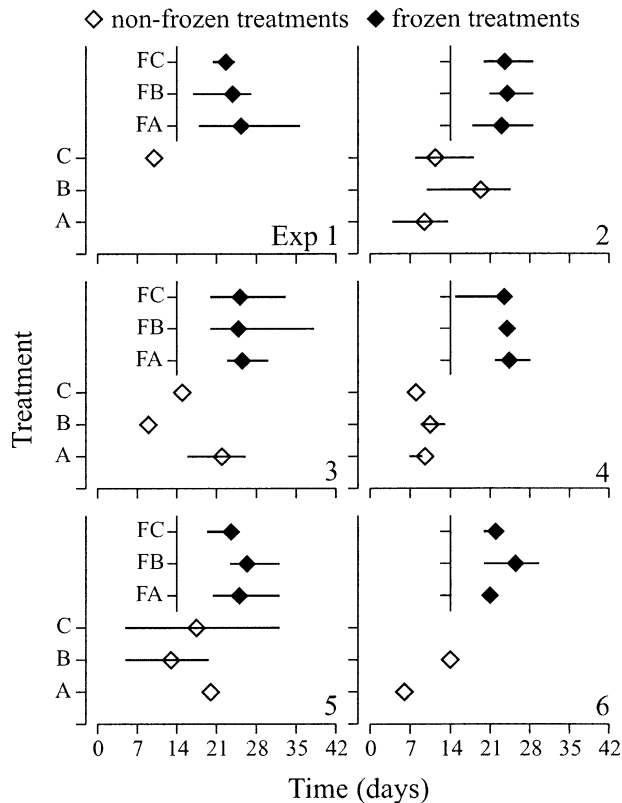
Fig. 8. Effects of freezing on postsettlement time to metamorphosis in experiments with cyprids of known age and where settlement opportunities were minimized. Mean time to metamorphosis of attached metamorphs with minimum and maximum date of metamorphosis per replicate per treatment. Vertical axes in frozen treatments displaced to thawing dates. Nonfrozen treatments with no metamorphs are not plotted. Panels show experiments 1 to 6.

metamorphs but still enclosed in a cyprid chitinous shell, were observed in the two treatments predominantly during the last 30 d of the experiments. All partial metamorphs occurred after 55 d, with the exception of one individual on day 30 in Experiment 3. The maximum number of partial metamorphs per treatment in each experiment was 6.

To test whether time to metamorphosis differed in the nonfrozen and the frozen treatments, the mean time to metamorphosis for each replicate in each treatment and experiment combination was obtained. Replicates were excluded from the computation of the average if no cyprids metamorphosed. This occurred for two replicates in nonfrozen experiment 1 and one replicate in the nonfrozen treatment experiment 6. Mean time to metamorphosis was longer in the frozen treatments than in the nonfrozen (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 two 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 nonfrozen treatments did not change.) Removing 14 d from the mean time to metamorphosis resulted in shorter times for the frozen treatments, with a grand mean of 10.1 d, than for the nonfrozen 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 *S. 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 nearshore 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 (Pechenik 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 nonfrozen treatments. The delayed time to metamorphosis must be, in part, the result of the freezing process that prevented settlement for 2 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 nonfrozen 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 nonfrozen treatments. This result contrasts with the 2003 experiments, where metamorphosis in frozen and nonfrozen larvae did not differ. Freezing and preventing settlement for 2 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 nonfreezing 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 *S. balanoides* cyprids and concluded that cyprids would lose 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 nonfrozen treatment with attached metamorphs fall within the range of Lucas et al., 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 nonfrozen 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, since survival and size were reduced for barnacles in frozen treatments relative to nonfrozen 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. *S. 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 *S. balanoides* might find freezing settling conditions in early fall (Petersen 1966).

Freezing tolerance might allow *S. 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 glaciation in the Western Atlantic (Wares and Cunningham 2001). Finally, larvae caught in drifting intertidal ice provide a vari-

ation on traditional mechanisms of larval dispersal. Larvae in ice would follow different dispersal pathways than free swimming larvae, since floating ice blown by the wind follows different trajectories than seawater.

References

ANDERSON, D. T. 1994. Barnacles. Structure, function, development and evolution. Chapman Hall.

BARNES, H. 1958. The northern limits of *Balanus balanoides* (L.). Oikos **8:** 1–15.

BERTNESS, M. D. 1999. The ecology of Atlantic shorelines. Sinauer.

BOUSFIELD, E. L. 1955. Ecological control of the occurrence of barnacles in the Miramichi estuary. Natl. Mus. Can. Bull. **137:** 1–69.

CRISP, D. J. 1974. Factors influencing the settlement of marine invertebrate larvae, p. 177–265. *In* P. T. Grant and A. M. Mackie [eds.], Chemoreception in marine organisms. Academic Press.

———, J. DAVENPORT, AND P. A. GABBOT. 1977. Freezing tolerance in *Balanus balanoides.* Comp. Biochem. Physiol. **57A:** 359–361.

———, AND D. A. RITZ. 1967. Changes in temperature tolerance of *Balanus balanoides* during its life-cycle. Helgol. Wissenschaftliche Meeresunters. **15:** 98–115.

FISH, C. J. 1925. Seasonal distribution of the plankton of the Woods Hole region. Bull. U.S. Bur. Fish. **41:** 91–179.

GEBAUER, P., I. WALTER, AND K. ANGER. 1998. Effects of substratum and conspecific adults on the metamorphosis of *Chasmagnathus granulata* (Dana) (Decapoda: Grapsidae) megalopae. J. Exp. Mar. Biol. Ecol. **223:** 185–198.

HARMS, J. 1984. Influence of water temperature on larval development of *Elminius modestus* and *Semibalanus balanoides* (Crustacea, Cirripedia). Helgol. Meeresunters. **38:** 123–134.

HELMUTH, B., C. D. G. HARLEY, P. M. HALPIN, M. O'DONNELL, G. E. HOFMANN, AND C. A. BLANCHETTE. 2002. Climate change and latitudinal patterns of intertidal thermal stress. Science **298:** 1015–1017.

HILLS, J. M., J. C. THOMASON, J. L. MILLIGAN, AND T. RICHARDSON. 1998. Do barnacle larvae respond to multiple settlement cues over a range of spatial scales? Hydrobiologia **375/376:** 101–111.

HOLLAND, D. L., AND G. WALKER. 1975. The biochemical composition of the barnacle *Balanus balanoides* L. J. Cons. Int. Explor. Mer. **36:** 162–165.

JACKSON, J. B. C. 1974. Biogeographic consequences of eurytopy and stenotopy among marine bivalves and their evolutionary significance. Am. Nat. **108:** 541–560.

KNIGHT-JONES, E. W. 1953. Laboratory experiments on gregariousness during settling in *Balanus balanoides* and other barnacles. J. Exp. Biol. **30:** 584–598.

LEWIS, J. R. 1964. The ecology of rocky shores. English Univ. Press.

LUCAS, M. I., D. L. HOLLAND, AND D. J. CRISP. 1979. An energy budget for the free-swimming and metamorphosing larvae of *Balanus balanoides* (Crustacea: Cirripedia). Mar. Biol. (Berlin) **55:** 221–229.

MCEWEN, G. F., M. W. JOHNSON, AND T. R. FOLSOM. 1954. A statistical analysis of the performance of the Folsom plankton sample splitter, based upon test observations. Arch. Met. Geophys. Bioklim. **7:** 502–527.

MENGE, B. A. 1976. Organization of the New England rocky intertidal community: Role of predation, competition, and environmental heterogeneity. Ecol. Monogr. **46:** 355–393.

PECHENIK, J. A. 1980. Growth and energy balance during the larval

lives of three prosobranch gastropods. J. Exp. Mar. Biol. Ecol. **44:** 1–28.

————. 1987. Environmental influences on larval survival and development, p. 551–608. *In* A. C. Giese, J. S. Pearse, and V. B. Pearse [eds.], Reproduction of marine invertebrates, Volume IX. General aspects: Seeking unity in diversity. Blackwell Scientific.

PECK, L. S. 2004. Physiological flexibility: The key to success and survival for Antarctic fairy shrimps in highly fluctuating extreme environments. Freshw. Biol. **49:** 1195–1205.

PETERSEN, G. H. 1962. The distribution of *Balanus balanoides* (L.) and *Littorina saxatilis,* Olivi, var. *groenlandica,* Mencke in northern west Greenland. Meddelelser om Grønland **159:** 1–42.

————. 1966. *Balanus balanoides* (L.) (Cirripedia) life and growth in Greenland. Meddelelser om Grønland **159:** 1–114.

PINEDA, J., D. RIEBENSAHM, AND D. MEDEIROS-BERGEN. 2002. *Semibalanus balanoides* in winter and spring: Larval concentration, settlement, and substrate occupancy. Mar. Biol. (Berlin) **140:** 789–800.

SOUTHWARD, A. J., S. J. HAWKINS, AND M. T. BURROWS. 1995. Seventy years' observations of changes in distribution and abundance of zooplankton and intertidal organisms in the western English channel in relation to rising temperature. J. Therm. Biol. **20:** 127–155.

VERMEIJ, G. J. 1991. Anatomy of an invasion: The trans-Arctic interchange. Paleobiology **17:** 281–307.

WALTHER, G.-R., E. POST, P. CONVEY, A. MENZEL, C. PARMESAN, T. J. C. BEEBEE, J.-M. FROMENTIN, O. HOEGH-GULDBERG, AND F. BAIRLEIN. 2002. Ecological responses to recent climate change. Nature **416:** 389–395.

WARES, J. P., AND C. W. CUNNINGHAM. 2001. Philogeography and historical ecology of the north Atlantic intertidal. Evolution **55:** 2455–2469.

WETHEY, D. S. 1984. Spatial pattern in barnacle settlement: Day to day changes during the settlement season. J. Mar. Biol. Assoc. U.K. **64:** 687–698.

————. 1985. Catastrophe, extinction, and species diversity: A rocky intertidal example. Ecology **66:** 445–456.

————. 2002. Biogeography, competition, and microclimate: The barnacle *Chthamalus fragilis* in New England. Integr. Comp. Biol. **42:** 872–880.

ZIMMERMAN, K. M., AND J. A. PECHENIK. 1991. How do temperature and salinity affect relative rates of growth, morphological differentiation, and time to metamorphic competence in larvae of the marine gastropod *Crepidula plana*? Biol. Bull. (Woods Hole) **180:** 372–386.