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## Temperature Adaptations of Embryos from Intertidal and Subtidal Sand Dollars (*Dendraster excentricus*, Eschscholtz)

### Abstract

Animals living in the marine intertidal zone have a suite of adaptations that allow them to survive thermal stresses associated with low tides. We hypothesized that adjustments of adult organisms to their thermal environment might be reflected in the thermal tolerances of their developing offspring (i.e., adults that survive large temperature fluctuations might produce embryos that have similarly large tolerances). Comparison of the thermal biology of developing sand dollar embryos (*Dendraster excentricus*) which were offspring of adults living in intertidal or subtidal habitats revealed subtle differences in effects of temperature on survival and developmental rates. Intertidal and subtidal *D. excentricus* showed similar fertilization success at temperatures from 7° to 19°C and could develop from fertilized egg to pluteus larva at temperatures from 7° to 26°C. Within this common tolerance range, however, subtidal animals' offspring were more likely to develop normally at lower temperatures (8° - 16°C), while the intertidal animals' offspring were more likely to develop normally at higher temperatures (22° - 26°C). There were also significant differences between subtidal and intertidal *Dendraster* groups in the effects of temperature on developmental rates. In particular, cleavage rates of embryos from intertidal adults remained constant or increased as temperature was raised from 7 to 15°C. In contrast, cleavage rates of embryos from subtidal adults decreased with increasing temperature. Ecological observations suggest that these differences are due to physiological acclimatization effects rather than genetic differentiation of the groups. To contrast *D. excentricus* with a different species that is largely confined to subtidal depths, we also examined temperature tolerances of developing sea urchin embryos (*Strongylocentrotus droebachiensis*). We found that its temperature tolerance during early development was slightly narrower and shifted to lower temperatures than that of *D. excentricus*. This correlates well with differences in habitat and reproductive season of these species and may help explain their distributions and abundances.

### Introduction

Invertebrates living in the marine intertidal zone can experience enormous changes in their physical environment as they are repeatedly covered and uncovered by tides. Adult organisms living here generally show behaviors that help minimize their exposure to the fluctuations or physiological adaptations that enable them to tolerate the stress. However, factors affecting early developmental stages of intertidal species also critically test adaptation. Temperature is a particularly important environmental parameter because many animals, during early development, are less tolerant of temperature variation than during other parts of their lives (Cossins and Bowler 1987).

Responses of developing embryos to temperature variation have been extensively investigated in echinoid echinoderms. Many early investigations were basic descriptions of effects temperature has on development (for references see Tyler

1936, Hoadley and Brill 1937, Harvey 1956). Subsequent research revealed correlations between responses of developing embryos to temperature and the particular temperatures encountered during the normal breeding season (Andronikov 1975, Amy 1983, Strathmann 1987, Fujisawa 1989, Fujisawa and Shigei 1990). Others have investigated responses of developing embryos to thermal extremes (e.g., Clarke 1982, Bosch et al. 1987, Chen and Chen 1992).

For some species there may be only one characteristic set of temperature responses during development. Members of such species could breed in any given geographic region when environmental temperatures there reach the optimal range for development (Sastry 1975, Marcus 1983). There are, however, reports of intraspecific geographic variation in developmental temperature responses in some echinoids (Fox 1936, Stephens 1972, Strathmann 1987) and other echinoderms (Johnson and Babcock 1994). These geographic differences in effects of temperature on development could result from genetic differentiation of

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populations (Hilbish et al. 1994), physiological acclimatization (Cossins and Bowler 1987), or a combination of the two. In some species with extended breeding seasons or more than one breeding season per year, responses of developing embryos to temperature in a single location can change seasonally (Hørstadius 1975, O'Connor and Mulley 1977). If members of a species breed throughout an extended period in one location, any seasonal changes in temperature tolerances of the embryos are attributable to the parents' physiological response to temperatures (acclimatization). Thermosensitivity of developing embryos is largely or exclusively determined by the egg (maternal acclimatization) and not by the fertilizing sperm (Fujisawa 1993). Maternal effects, resulting in such seasonal changes, have been demonstrated in other invertebrates (Tester 1985, Pedersen and Tande 1992).

Some of the adaptations to temperature seen in echinoid development may be related to variability of the habitats the animals occupy. For example, the especially broad developmental temperature tolerances reported for echinoid species that occur in reef flats (Rupp 1973) or shallow tropical waters that become very warm (Chen and Chen 1992) may be crucial for those species' reproduction, subjected as they are to very large temperature fluctuations.

The sand dollar, *Dendraster excentricus* Eschscholtz, occurs in both intertidal and subtidal habitats. Individuals living in intertidal habitats are exposed to considerably broader temperature ranges than are individuals living subtidally. This provided an opportunity to test the possibility that there is a relationship between parental habitat and temperature tolerance of developing embryos. We did this by comparing the effects of various exposure temperatures on development in *D. excentricus* individuals collected from one subtidal site and several nearby intertidal sites. We also compared effects of temperature on development of *Dendraster* with those on development of *Strongylocentrotus droebachiensis*, an echinoid that breeds earlier in the spring and is largely confined to low intertidal and subtidal habitats.

## Methods

### Adult temperature environments

Subtidal *Dendraster excentricus* (Eschscholtz 1831) were collected from Deepwater Bay on

Cypress Island. Intertidal *D. excentricus* came from East Sound (Orcas Island), from Bellingham Bay, and from Illahee State Park (Fig. 1). Sea urchins, *Strongylocentrotus droebachiensis* (Müller 1776), were collected from Burrows Bay (Fidalgo Island). All collections were made in the months of May, June, and July when the animals were reproductive. Subtidal animals were collected by divers at depths between 15 and 18 m. Animals were kept in flow-through seatables at the Shannon Point Marine Center and, except for those used in acclimation experiments, were used for experiments within five days of collection.

Water temperatures at -1.5 m were measured weekly for one year at Deepwater Bay, the collection site for subtidal *D. excentricus*. This area experiences strong tidal flushing and the waters are well-mixed; strong thermoclines did not seem to develop even in the warmest summer months (pers. obs.). We assume, therefore, that surface water temperatures at this site reflect those experienced by *D. excentricus* at 15-18 m. To document temperature variations experienced by animals at an intertidal site, we measured surface body temperatures of *D. excentricus* at the East Sound collecting site during 2 low-tide series: 14 April (a windy, overcast day, 11°C air temperature) and 25 April (a clear, sunny day, 13°C air temperature). Every 15 minutes throughout the low tide, a thermister probe was placed on the surfaces of 10 arbitrarily chosen animals and their temperatures were recorded.

### Fertilization

For all fertilization and development experiments, gametes were obtained by injecting 0.55 M KCl into the perivisceral coelom. Eggs were collected in 13°C filtered seawater but sperm were collected in a dry petri dish and diluted just prior to insemination. To mitigate effects of individual variability, sperm from 2-4 males and eggs from 2-4 females were mixed in all experiments.

To determine whether fertilization is temperature-dependent and differs between intertidal and subtidal *D. excentricus*, we examined fertilization success at a range of experimental temperatures. Five replicate 20 ml scintillation vials were filled with 16 ml of 5 µm-filtered seawater at 7, 11, 15 and 19°C. A monolayer of newly spawned eggs (with no germinal vesicle evident) and 5 drops of diluted sperm suspension (approximately 1.25

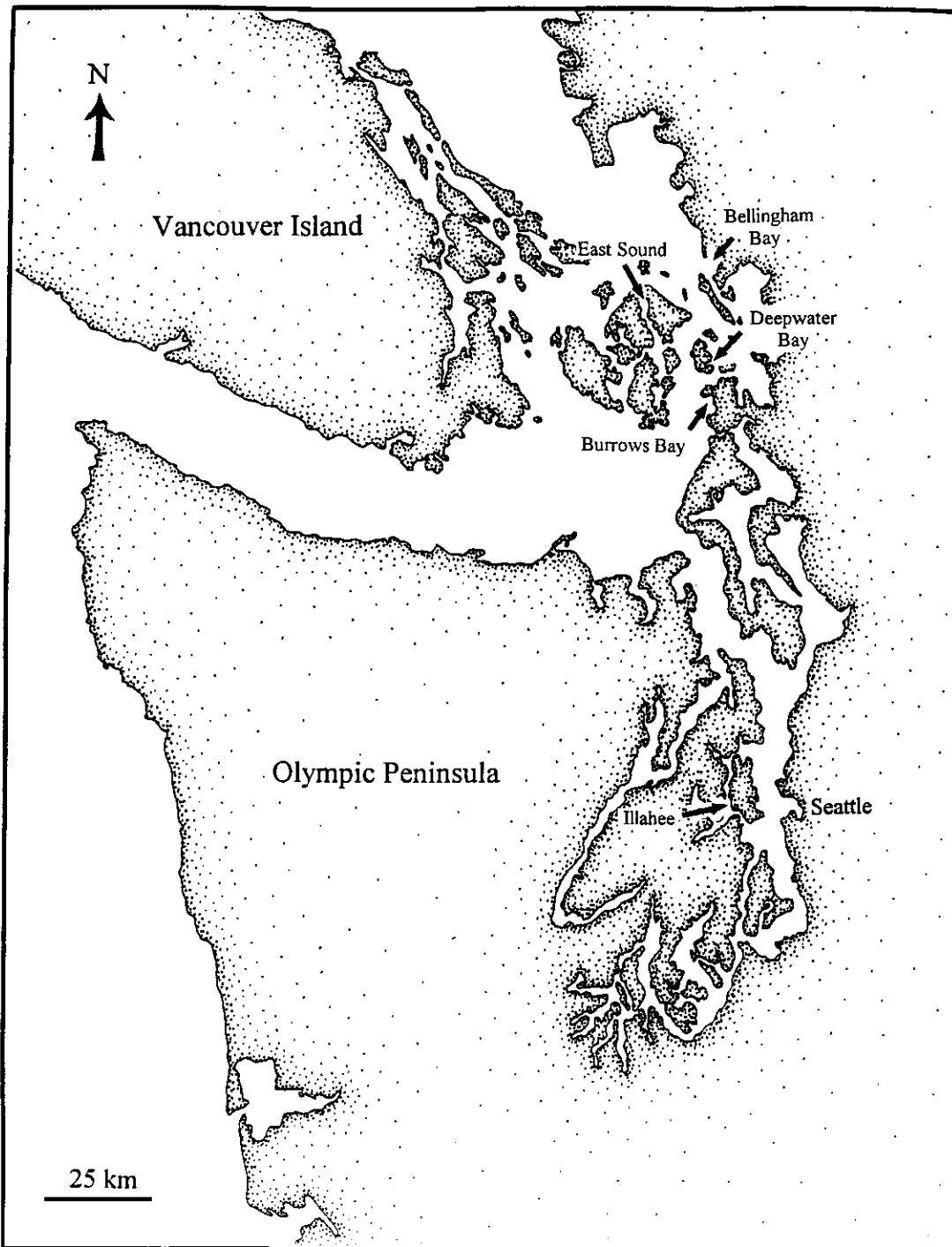


Figure 1. Collection sites: Bellingham Bay, East Sound (Orcas Island), and Illahee State Park where intertidal *D. excentricus* were collected at low tide; Deepwater Bay (Cypress Island) where subtidal *D. excentricus* were collected; Burrows Bay where subtidal *S. droebachiensis* were collected. All subtidal animals were collected from depths between 15 and 18 m.

$\times 10^5$  sperm per ml) were added to each vial. Contents of the vials were mixed and incubated at the experimental temperatures for 1 hour. Fertilization success was then measured by examining 100-egg samples from each vial for presence of elevated fertilization membranes.

#### Early development

We also compared effects of temperature on survival of embryos from intertidal and subtidal *D. excentricus*. Batches of eggs were fertilized at an intermediate temperature (13°C) and then transferred to 20-ml scintillation vials. These cultures were maintained in incubators set at 5, 8, 10, 13, 16, 19, 22, 26, 29 and 31°C. All treatments were replicated (4 vials/treatment). Because cultures were unstirred, egg densities were kept low (2-5 eggs/ml). When four-armed pluteus larvae had developed, the cultures were terminated by addition of 4% buffered formalin. All individuals were then examined and the percentages of normal plutei were determined for each vial. The percentage data were arcsine transformed to stabilize variances and treatments were compared with two-way ANOVA with temperature and habitat (i.e., subtidal vs. intertidal) as the main effects.

To contrast *D. excentricus* with another echinoid that seldom occurs intertidally, we also measured temperature tolerance of developing green sea urchin embryos (*S. droebachiensis*). Methods were identical to those described above except that initial fertilization was done in 10°C water.

Early embryonic cleavage rates are generally proportional to rates for longer periods of development (Fujisawa 1989). Therefore, we compared effects of temperature on developmental rates of a subtidal (Deepwater Bay) and 2 intertidal (Illahee State Park and Bellingham Bay) *D. excentricus* groups by measuring early cleavage rates in cultures held at 7 and 15°C. When some embryos in the cultures had reached the 16-cell stage (after 3 hr at 15° and 11 hr at 7°), the cultures were terminated by addition of 4% buffered formalin and cleavage stages of 100 embryos per vial (5 vials per treatment) were recorded. Mean numbers of cleavages completed were calculated and the relationship between number of cleavages and temperature for each group was evaluated with linear regression. Effects of exposure temperature on developmental rate for each group were examined by comparing slopes of the regression lines.

#### Acclimation

To test effects of recent temperature exposure of parents on development of offspring and to determine whether tolerance limits could be shifted experimentally, we conducted acclimation experiments in which we placed newly collected *D. excentricus* and *S. droebachiensis* in 80-l aquaria adjusted to 7, 10, and 13°C. We defined temperature acclimation as physiological adjustment made by organisms subjected experimentally to environments differing only in temperature. Acclimatization, in contrast, is physiological adjustment made by organisms subjected in the field to a full range of environmental variation. The aquaria were aerated and held in incubators that maintained the acclimation temperatures. Animals were fed every 2 days (TetraMin™ fish food or algae, primarily *Laminaria* spp.) and the water was changed semi-weekly. There were no apparent negative effects of this treatment and animals were successfully made to spawn by injection of 0.55M KCl after a 21-day acclimation. Cultures of embryos were produced as described above and survival to the four-armed pluteus was determined at 5, 7, 10, 13, 16, 20, 24, and 31°C.

#### Results

Temperature measurements in the field showed that subtidal and intertidal animals experience substantially different temperature regimes. Temperatures in Deepwater Bay ranged from 7°C in January to 13°C in July/August. This seasonal temperature change occurred gradually, and extreme values were rare. In contrast, temperature changes at the intertidal site were more frequent, more sudden, and much greater. Animals in this habitat experienced particularly large temperature fluctuations during low tides; we measured an eight-degree increase (from 13°C to 21°C) in *D. excentricus* body surface temperature during a single 90-minute period (Fig. 2). Mid-summer low tides may expose the animals to even greater fluctuations as air temperatures reach 20-30°C.

We found no biologically relevant among-group differences in *D. excentricus* fertilization success at temperatures from 7-19°C. Fertilization percentages ranged from 90-100 in all treatments and the only temperature effect seemed to be a slight decrease in fertilization of all groups at 7°C.

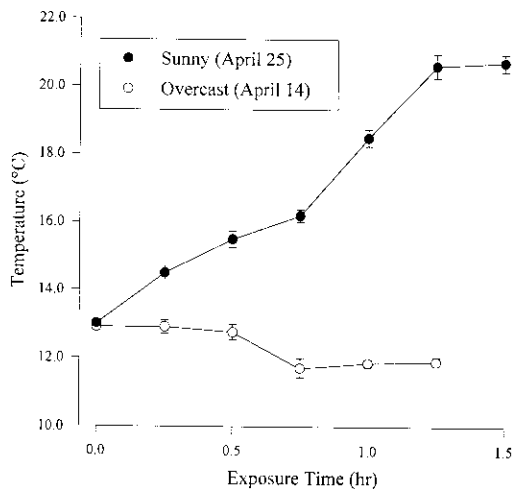


Figure 2. Body surface temperatures of *Dendraster excentricus* measured with a thermister on two low tides (East Sound, Orcas Island). Means  $\pm$  one standard error are shown ( $n = 10$  for each data point).

Study of developing embryos from both intertidal and subtidal *D. excentricus* at temperatures between 5° and 31° C, revealed that development to four-armed pluteus larvae was normal at temperatures from 7° C to 26° C; there were no significant differences between the two groups in the limits of their temperature tolerance during development. In contrast, the urchin *S. droebachiensis*, which occurs in the same area but is normally found subtidally, developed four-armed plutei across a lower range of temperatures, from 5° to 19° C.

Two-way analysis of variance revealed statistically significant differences, associated with temperature and habitat, between the two *D. excentricus* groups within their common tolerance range (Table 1). Because the significant temperature  $\times$  habitat interaction made interpretation of the main effects difficult, results were examined through simple main effects (i.e., *Dendraster* groups were compared within each level of temperature, Fig. 3; Winer et al. 1991). With a Bonferroni correction to control Type I error, the analysis showed that a significantly greater proportion of subtidal animals successfully completed development to the pluteus stage at lower temperatures (8, 10, 13, and 16°C), whereas a significantly greater proportion of offspring of intertidal animals developed to plutei at the higher temperatures (22 and 26°C).

TABLE 1. Two-way analysis of variance for the effects of temperature and habitat on *D. excentricus* development. Percent data were arcsine transformed prior to analysis to homogenize variances.

Source	df	MS	F	p
Temperature	8	2.775	344.21	<0.01
Location	1	0.095	11.90	<0.01
Temp $\times$ Location	8	0.144	17.51	<0.01
Error	54	0.008		
Total	71			

Percentages of normal development, however, were very low in both groups at 26°C (Fig. 3). Temperature tolerance of developing *S. droebachiensis* embryos was shifted to lower temperatures; normal plutei were found in temperature treatments between 5° and 19°C.

The effect of temperature on developmental rates of *D. excentricus* from the subtidal group (Deepwater Bay) differed significantly from that of the 2 intertidal groups (Fig. 4). A significantly negative slope ( $b = -0.35$ ,  $p = 0.02$ ) indicated that the Deepwater Bay embryos developed more slowly at 15°C than at 7°C. In contrast, there was no significant temperature effect for the Bellingham Bay group ( $p = 0.52$ ), and there was a significantly positive effect (i.e., embryos developed more rapidly at 15°C) for the Illahee State Park group ( $b = 1.01$ ,  $p = 0.0003$ ).

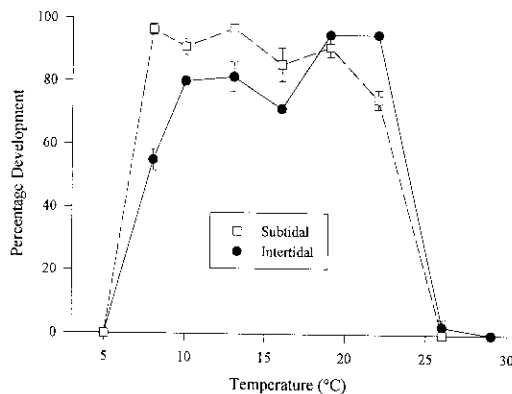


Figure 3. Percentages of intertidal and subtidal *D. excentricus* individuals developing normally to pluteus larvae at various exposure temperatures. Each point represents the mean  $\pm$  one standard error ( $n = 4$  vials for each point). In several cases, the length of the error bar is smaller than the plotted diameter of the point.

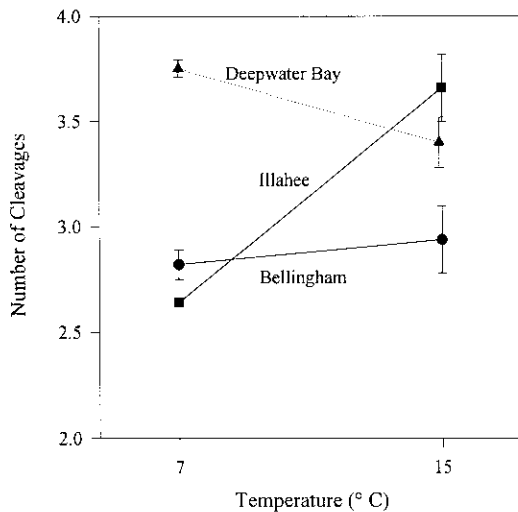


Figure 4. Early developmental rate of intertidal (Illehee and Bellingham) and subtidal (Deepwater Bay) *D. excentricus* as indicated by number of cleavages completed. Each point represents the mean  $\pm$  one standard error ( $n = 5$  vials for each point).

We found no evidence that a 21-day laboratory acclimation of adult *D. excentricus* or *S. droebachiensis* affects the temperature tolerance of their developing offspring. Survival to pluteus did not differ significantly among the treatment groups for either species; temperature tolerances of the embryos reflected what we had seen in the offspring of unacclimated animals (i.e., Fig. 3).

## Discussion

The effects of exposure temperature on *Dendraster excentricus* development suggest a relationship between parental habitat and temperature tolerance of developing offspring. Of particular interest was the drop in cleavage rate we observed for the subtidal *D. excentricus* embryos when they were placed at a warmer temperature (Fig. 4). This was surprising (cleavage rates generally increase with increasing temperature as was more evident in the intertidal groups) and indicates an adjustment to the temperatures embryos are most likely to encounter during early development. Differences in the responses of intertidal and subtidal embryos to temperature could arise from genetic differentiation of populations, from physiological acclimatization, or from a combination of the two. The length of the larval period in *D.*

*excentricus* (10–23 weeks at 7–13°C; Strathmann 1978) and the high potential for dispersal in the current- and wind-swept waters of the Puget Sound (Emlet 1986) make it unlikely that these differences result from genetic differentiation and suggest that such differentiation might not be adaptive, since larvae would seldom settle in their parents' immediate environment. We think that the observed differences result from a physiological acclimatization effect and could enhance survival of offspring of the respective groups under the conditions likely to be encountered early in development. Laboratory acclimation can alter temperature responses of adult echinoids (Farmanfarmaian and Giese 1963, Ulbricht 1973), and there is evidence that parental acclimatization in nature can shift temperature tolerance of developing embryos in some echinoids (Hørstadius 1975, O'Connor and Mulley 1977, Johnson et al. 1990) and asteroids (Johnson and Babcock 1994). We, however, did not observe any effect of laboratory acclimation on the higher or lower limits of temperature tolerance in embryos of either *D. excentricus* or *S. droebachiensis* nor did we see any differences in the range of temperatures intertidal and subtidal *D. excentricus* embryos could tolerate. Thus, we do not think that changes in temperature tolerance of embryos occurs simply through a shift in the absolute range of tolerance. Rather, the differences we observed in intertidal versus subtidal *D. excentricus* appear to result from a more complex form of acclimatization involving the relative rotation of developmental temperature response curves (Cossins and Bowler 1987) of the two groups. We suggest that such subtle fine-tuning of responses to environmental parameters during early development is adaptive; it may favor survival of embryos in their earliest, most vulnerable stages. Such a possibility should be further investigated in these, and other, marine organisms.

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