The Relative Importance of Predation Risk and Water Temperature in Maintaining Bergmann's Rule in a Marine Ectotherm

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abstract: Bergmann's rule—an increase in body size with latitude—correlates with latitudinal declines in ambient temperature and predation risk, but relatively few studies simultaneously explore the relative importance of these factors. Along temperate Atlantic shorelines, the isopod *Idotea balthica* from high latitudes are 53% longer on average than isopods from low latitudes. When reared at 6°–24°C, juveniles increased growth and development rates with temperature. Because the increase in growth rate with temperature outstripped increases in development rate, female size at maturity increased with temperature. This thermal sensitivity of growth cannot account for the latitudinal pattern in body size. Within temperature treatments, females from low latitudes reached sexual maturity at younger ages and at a smaller size than did females from higher latitudes. This shift in life-history strategy is predicted by latitudinal declines in predation pressure, which we tested using field-tethering experiments. Overall, isopods at low latitudes had a 44% greater mortality risk from daytime predators relative to isopods at higher latitudes. We conclude that a latitudinal gradient in predation risk, not temperature, is principally responsible for Bergmann's rule in *I. balthica*. Increases in body size during future warming of oceans may be constrained by local patterns of predation risk.

Keywords: body size, predation risk, temperature-size rule, ectotherm, latitudinal cline.

Introduction

Body size is central to organismal fitness and displays profound variation among individuals, populations, and species (Schmidt-Nielsen 1984; Peters 1986; Bonner 2006). One commonly cited pattern in body size is Bergmann's rule, which originally held that endothermic species in colder environments generally possess larger body sizes than do congeners in warmer environments (Bergmann 1847, translated in part by James [1970]). Bergmann's rule

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has been observed across species and populations of endotherms (Ashton et al. 2000; Meiri and Dayan 2003) as well as ectotherms (Arnett and Gotelli 1999; Ashton and Feldman 2003; Blanckenhorn and Demont 2004). But the generality of the rule has been debated (Mousseau 1997; Blackburn 1999) because many counterexamples exist (Ashton et al. 2000; Ashton 2002; Ashton and Feldman 2003; Meiri and Dayan 2003; Blanckenhorn and Demont 2004; Adams and Church 2008). Moreover, whether Bergmann's rule simply describes a pattern or implies a particular mechanism—such as the one proposed for endotherms (which makes the rule inapplicable and untestable for ectotherms)—has also fueled debate (Pincheiro-Donoso 2010; Watt et al. 2010; Meiri 2011; Olalla-Táraga 2011). We take the view (similar to Angilletta et al. 2004a) that Bergmann's rule describes a pattern observed between latitudinally separated congeners (or conspecifics) in both endotherms and ectotherms and that the important questions center on describing the evolutionary mechanisms that underlie these patterns.

Despite the fact that Bergmann's rule has been discussed for well over a century, we still have relatively little information on two important aspects of its evolution. First, surprisingly few studies have tested the relative contributions of genetic and environmental variation that underlie these patterns (Stillwell 2010; e.g., Lonsdale and Levinton 1985 and Partridge and Coyne 1997). Second, most studies have focused on the role of temperature in shaping latitudinal increases in body size even though multiple biotic and abiotic forces also grade with latitude (Ho et al. 2010). Temperature has remained at the forefront of explanations for Bergmann's clines in both endotherms and ectotherms, partly because of the widely confirmed temperature-size rule, which holds that body size at a recognizable developmental stage (e.g., maturation) declines with rearing temperature (Ray 1960). This phenotypic response has been observed in approximately 80% of ec-

tothermic species in published studies (Ray 1960; Atkinson 1994) and is consistent with a latitudinal decline in body size.

However, latitudinal changes occur across many environmental factors—including predation risk, food quantity and quality, and seasonality—that independently (or in combination) could affect body size (Wallerstein and Brusca 1982; Arnett and Gotelli 1999; Blanckenhorn and Demont 2004; Ho et al. 2010; Husby et al. 2011). Most of these factors remain largely overlooked in the literature on Bergmann's rule. Predation has been proposed as a potentially important driver of Bergmann's rule (Wallerstein and Brusca 1982; Angilletta et al. 2004*a*, 2004*b*) because predation risk tends to decline with latitude (Peterson et al. 2001; Freestone et al. 2011) and can reduce body size by plastic or genetic means. Predation risk reduces average body size in a population when larger individuals are consumed (e.g., Cooper 1965) or individuals use predator cues to mature more quickly and at a smaller size (e.g., Stibor 1992). Optimality models predict that populations with high juvenile mortality should evolve genotypes that mature earlier at smaller sizes (Abrams and Rowe 1996; Angilletta et al. 2004*b*). These predictions are more complicated when predators indirectly reduce competition for food or habitat and may thus increase the sizes of survivors (e.g., Matthews et al. 1992). Importantly, relatively few studies have simultaneously assessed the relative roles of the latitudinal gradients in temperature and predation risk in driving Bergmann's rule, despite increased calls for such multivariate and mechanistic approaches (Angilletta et al. 2004*b*; Shelomi 2012).

In this study, we attempt to fill this gap by examining Bergmann's rule among populations of the marine isopod *Idotea balthica* (Pallas). *Idotea balthica* is a brooding species with direct-developing juveniles occurring from estuaries in Virginia northward through Maine, where it principally inhabits subtidal seagrass beds dominated by *Zostera marina* (Bell and Sotka 2012). Using laboratory-based common-garden experiments and field-based surveys and manipulative experiments, we sought to answer two principal questions relating to this pattern: (1) What is the relative importance of genetic, environmental, and/or genetic by environment (G × E) variation in dictating the observed body pattern in *I. balthica*? (2) What is the relative importance of latitudinal gradients in temperature and predation risk in the evolution of this pattern?

Methods

### Field Collections and Laboratory Cultures

Idotea balthica samples were collected in the field in June 2010 from sites in Massachusetts, New Jersey, and Virginia

(fig. 1*A*). Isopods were collected using a dip net, swept through seagrass beds or floating algae at each site. Half of the collected animals were haphazardly selected and immediately placed in ethanol (100%) for measuring body traits. The remaining animals were placed in aerated buckets and returned to the Grice Marine Laboratory (Charleston, SC) to seed cultures. Because of the limited numbers of animals collected from one site (Goodwin Island), all collected specimens were returned to the laboratory for culturing. To balance the design for body size analysis, *I. balthica* from Goodwin Island collected in 2005 (collected in the same month and manner as 2010) were used for body trait analysis. All populations are interfertile and share common mitochondrial haplotypes, as would be expected within a single species (Bell 2009).

Within 10 days of collection, two cultures per population were seeded within aerated containers (approximately 6 L) at 18°C on a 12L : 12D cycle and regularly fed a diet of fresh mixed algae (*Ulva* spp., *Cladophora* spp., and *Gracilaria* spp.). Collapse of cultures from Wellfleet and Stone Harbor resulted in only four populations (Magnolia, Nahant, Goodwin Island [GI], and Cape Charles [CC]) that could be used for culture analysis. Water was changed every 2 days with fresh seawater (salinity = 25‰–30‰). We generated data from these isopod cultures in two ways. First, laboratory-birthed offspring (hereafter, the F_1 generation) of field-collected mothers were used in the temperature-growth experiment. To partially account for the effect of the maternal environment, all mothers were maintained in culture under these common conditions for at least 6 weeks before the experiment was initiated. Second, offspring (the F_2 generation, also termed "laboratory cultured") of adults that had been reared to reproductive maturity at 18° and 24°C in the temperature-growth experiment were measured for body traits. Thus, we reduced (though may not have eliminated) the influence of the native maternal environment, and any population-level differences observed in F_1 and F_2 generations likely have a genetic basis.

Body Traits

We measured body length and width of the 50 largest males and females from each field-collected population (note that only 19 males and 31 females were collected and measured at Stone Harbor, NJ) using a ruler and rounding to the nearest 0.5 mm. Isopods from the temperature-growth experiment were measured using digital methods (see below). The digital and manual methods yielded comparable results ($R^2 = 0.96$, slope = 0.997, $P > .0001$; $n = 200$). All eggs were removed from the broods of 50 field-collected females from each of the four populations, and digital images were taken of all eggs. Ten eggs in each
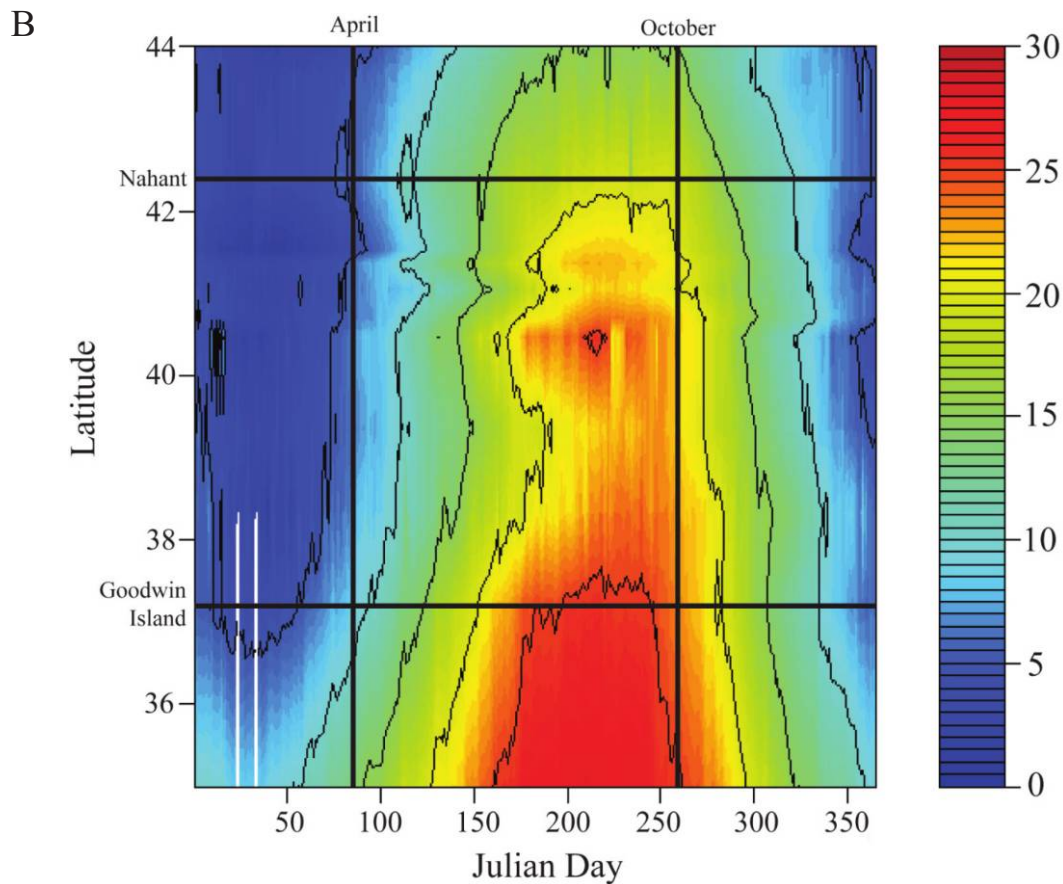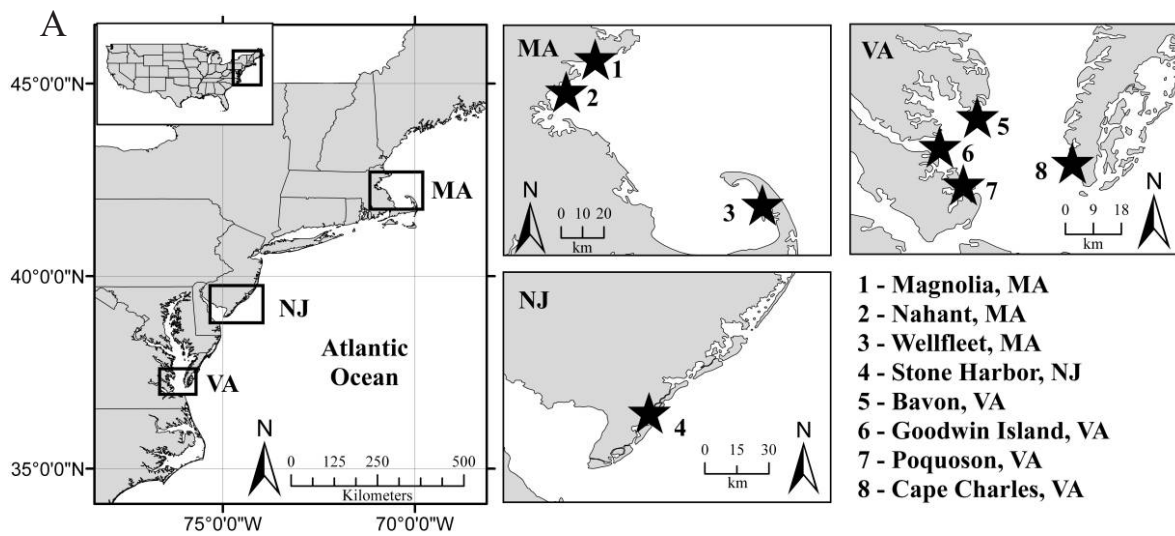
Figure 1: *A*, Map of field collection locations of *Idotea balthica*. See table A1 (available online) for details. *B*, Daily averages of water temperature from 13 tide stations along the northwestern Atlantic shoreline. Contour lines denote isoclines of 5°C. Horizontal lines denote Nahant and Goodwin Island latitudes. Daily temperatures were collected from 2006 through 2009 and are available from the National Oceanic and Atmospheric Administration's National Oceanic Service Center for Operational Oceanographic Products and Services at http://tidesandcurrents.noaa.gov.

image were randomly selected and measured using ImageJ image analysis software (ver. 1.43u; http://imagej.nih.gov). Any nonspherical eggs were excluded from the analysis. Dry mass was measured for 50 females (after egg removal) and 50 males individually by drying them to constant weight at 60°C (approximately 5 days).

Lengths of field-collected isopods were analyzed using a nested ANOVA model, with region and sex as fixed factors and population as a random factor nested within region. For analyses of body length of laboratory-cultured animals, we had too few populations for a nested ANOVA and instead constructed a traditional ANOVA model to compare body length across population and sex as fixed effects. The residuals of this model met assumptions. Tukey-Kramer post hoc analyses were performed when treatments were significantly distinct. Treating population as a random effect, which provides a more stringent test of the effect, led to conclusions that were qualitatively the same. Both egg number and dry mass were analyzed using ANCOVA, with length as a covariate and population as a fixed effect. Egg diameter was analyzed using an ANOVA with population as a fixed effect. A Tukey-Kramer test was run on significant results to detect differences between populations. Egg number and diameter data satisfied the assumptions of a parametric ANOVA, while dry masses were square-root transformed before analysis. All analyses were performed in R (ver. 2.10.1; R Development Core Team 2011) except where noted.

Temperature-Growth Experiment

To examine the role of temperature on life-history traits, isopods from Massachusetts (Magnolia, Nahant) and Virginia (Goodwin Island, Cape Charles) were raised to sexual maturity at five water temperatures (6°, 12°, 18°, 24°, and 30°C). On average, all field populations experience 6°, 12°, and 18° at some point during the year (fig. 1*B*). The highest temperatures assayed (24° and 30°C) are rarely experienced in Massachusetts but regularly occur in the summertime in Virginia. Reproductive adults are present from approximately April to October (A. Manyak-Davis, T. M. Bell, and E. E. Sotka, personal observation), during which time the Massachusetts animals typically experience temperatures from 5° to 18°C and Virginia animals experience temperatures from 9° to 25°C.

For unknown reasons, juvenile *I. balthica* exhibit higher survivorship when raised in groups of three than when isolated (A. Manyak-Davis, T. M. Bell, and E. E. Sotka, unpublished data). Thus, to initiate the experiment we placed three similarly sized juveniles from the cultures into 50-mL plastic centrifuge tubes with the bottom removed, a hole drilled in the cap, and each of the opened ends covered in mesh (300 μm) to allow for water exchange.

In total, there were 20 growth containers (=60 isopods) per population per temperature. We placed containers within one of five darkened water tables (salinity = 30‰) set to one of five experimental temperatures (±0.5°C). Water temperature was maintained with a re-circulating chiller, heater, or both. Water quality was checked weekly, and deionized water was added as needed to maintain a salinity of 30‰. Ammonia and pH remained at benign levels throughout the experiment. At 5 weeks, isopods were moved into individual growth chambers to avoid cannibalism. Isopods were offered ad lib. freeze-dried *Ulva linza* and *Ectocarpus* spp., which are readily consumed by both Massachusetts and Virginia isopods (Bell and Sotka 2012). The prepared food also contained 0.0045% tetracycline to reduce the potential for excessive death of isopods at high temperatures, which can be immunocompromised (Roth et al. 2010).

Each week we measured survivorship. All isopods were photographed under a dissecting microscope every 2 weeks for the first 4 weeks and weekly thereafter for the 18° and 24°C treatments. Because of low growth rates, isopods raised at 6° and 12°C were photographed monthly and bimonthly, respectively. Total body length and width was assessed using ImageJ. Length was measured from anterior to posterior. Given that the temperature-size rule pattern is based on size at stage (Atkinson 1994), we primarily focused on the size of females at sexual maturity. Relative to other growth stages in males or females, female sexual maturity is easily identifiable by the emergence of a brood pouch (marsupium). Once sexual maturity had been reached for all females in a temperature-population combination, we recorded length for all males and females. These animals were then used to seed new cultures. Some temperature-population combinations were terminated when significant mortality precluded future data collection or when it was apparent that isopods were growing too slowly to ever reach sexual maturity.

Because we haphazardly collected juveniles of unknown age to initiate the experiment, differences in age at initiation could have generated a bias in time to maturity or growth rates. To test this, we generated a standard curve of juvenile growth by age for a single Massachusetts (Nahant) and a single Virginia (Goodwin Island, or GI) population using juveniles of known age reared for 3 weeks (linear regression results: Nahant $R^2 = 0.96$, GI $R^2 = 0.89$; $P < .001$ for each). While there were significant differences in the inferred age of juveniles among populations ($F_{3, 1,186} = 10.042$, $P < .0001$), the magnitude of the differences in age among treatments at experiment initiation was much smaller relative to time to sexual maturity (95% confidence interval of age: Nahant = 5.2–6.0 days, CC = 5.6–6.4 days, Magnolia = 6.4–7.4 days, GI = 6.8–7.8 days; time to maturity at end of experiment = 50–100 days).

Survivorship data were analyzed at weeks 4, 10, and 15 using JMP (ver. 9; SAS Institute). These time points were chosen due to incomplete survival curves resulting from death of isopods in the 30°C treatment group (week 4), removal of isopods in the 24°C treatment group once reproductive (week 10), and completion of the experiment (week 15; see fig. A2; figs. A1–A3 are available online). At each time point, survivorship data were subset by temperature, compared across populations, and analyzed using both the log rank and the Wilcoxon tests, two nonparametric survival curve analyses. When significant differences between populations were detected with both tests, pairwise population comparisons within temperature were similarly analyzed with a Bonferroni correction.

Given the death of all isopods in the 30°C treatment group and the removal of isopods at maturity (see "Results"), growth curves of immature isopods could not be compared across populations at all time points and temperatures. Instead, we analyzed growth rates (i.e., difference in length [mm] divided by time) between weeks 0 and 2 and between weeks 6 and 10. We grouped week 9 with week 10 isopods because of logistical constraints in recording images. Analyses of size at week 0 and growth rates between weeks 0 and 2 were relatively balanced, and we performed parametric ANOVAs to assess the effects of population, temperature, and their interaction. Sample sizes were unbalanced at the end of the experiment; as a result, permuted ANOVAs and post hoc analyses (Anderson 2001; custom-scripted in R) were performed on all analyses at weeks 4, 6, and 10.

Tethering Experiment

To assess latitudinal differences in daytime predation rates, tethering experiments were carried out during the summer of 2011. Four seagrass beds separated by between 1 and 90 km within each region were used in total (fig. 1; table A1; tables A1–A6 are available online). The isopods used as bait in all trials were male and were collected from Massachusetts because at the time of collection Virginia isopods were too scarce to perform reciprocal transplant experiments. Our experiment thus tests the susceptibility of Massachusetts isopods to daytime predators within both Massachusetts and Virginia estuaries.

Tethering sticks (termed "isopopsicles") were constructed using acrylic rods (3.2 mm in diameter and 30 cm in length), with a blade of seagrass (10–30 cm in length) secured to it using twist ties wound around the top and bottom of the blade. Isopods were measured for length and attached to the seagrass using superglue (Loctite; Henkel Corporation, Rocky Hill, CT) with their ventral side lying parallel to the seagrass blade. To ensure that removal of isopods from the isopopsicles was due

principally to predation rather than water motion or glue failure, we placed five control tethers at each transect in August (i.e., 15 tethers per site). These were constructed in the same manner as the experimental isopopsicles; however, 2–3-cm green pipe cleaners were glued directly to the seagrass to act as an isopod mimic. We assumed that the pipe cleaners would be uninteresting to predators but would generate drag comparable to that generated by isopods.

We chose sites with abundant seagrass and a water depth less than 1.5 m mean lower low water. Three transects of 20 isopopsicles each were laid parallel to the shore spaced by 10 m (note that for the August trial Bavon had only 15 tethers per transect because of low isopod numbers). Isopopsicles were inserted three-fourths of their length into the sediment and 1 m apart along each transect, with control tethers randomly interspersed. At each site, tethers were deployed during a morning low tide and collected the same day during the evening low (approximately 12-h deployment), except for the August deployment at Wellfleet, where inclement weather prevented us from collecting tethers until 24 h. Tethers were scored after collection for the presence or absence of the isopod or control object. Replicates were excluded from analysis if the seagrass blade was gone (June: 5 of 360 total tethers; August: 25 of 465 total tethers) or if the rod and blade were missing (June: 99 of 360 total tethers; August: 106 of 465 total tethers). We considered an isopod to be "absent" if more than half of the isopod was missing (six isopods in total; four other isopods had less than half remaining).

Survival (presence/absence) in the predation trials was a binomial variable and analyzed using an analysis of deviance (Zuur et al. 2009). For each month (June and August), we examined the effect of site, body length (as a covariate), and their interaction on survival. Post hoc analyses were performed by removing length from the logistic regression model and performing multiple pairwise comparisons across sites with a Bonferroni correction. All data were deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.7hg56 (Manyak-Davis et al. 2013).

Results

Field Collections

Consistent with Bergmann's rule, field-collected females and males of *Idotea balthica* from northern latitudes (Massachusetts) were on average 53% longer than those from lower latitudes (New Jersey and Virginia; fig. 2*A*; table A2). The latitudinal increase in body length reflects an increase in overall body mass (r^2 ranged from 0.70 to 0.86 across populations; fig. A1*A*; table A3) and likely has a genetic basis, as the pattern was maintained among isopods
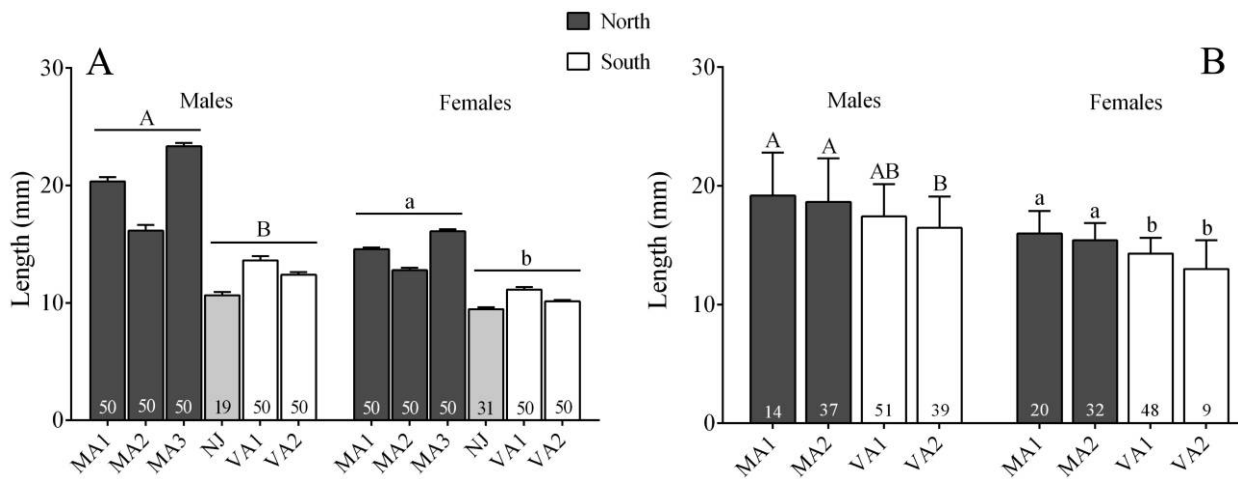
Figure 2: Body length (mean ± SE) of *Idotea balthica* from field-collected populations (*A*) and populations cultured in the laboratory for two generations (*B*). Dark gray bars indicate higher-latitude populations (MA), and light gray and white bars indicate lower-latitude populations (NJ and VA, respectively). Numbers within bars indicate sample sizes, and letters connect statistically indistinguishable means. MA1 = Magnolia, MA2 = Nahant, MA3 = Wellfleet, NJ = Stone Harbor, VA1 = Goodwin Island, VA2 = Cape Charles.

cultured for two generations under common-garden conditions (fig. 2*B*; table A2). In both field-collected and laboratory-cultured collections, males were longer and increased in size with latitude more sharply than did females. Female fecundity also increases with latitude because egg production strongly and positively correlates with body length (fig. 3; r^2 ranged from 0.34 to 0.44 across populations; table A3). Egg diameter was independent of female body length and did not differ among populations (data not shown; $F_{3,101} = 0.39$, $P = .7613$).

Temperature-Growth Experiment

When reared in the laboratory, the survivorship of isopods from two lower-latitude populations and two higher-latitude populations did not differ with temperatures between 6° and 24°C. At 30°C, however, virtually all individuals from two higher-latitude populations died within 4 weeks, while lower-latitude isopods survived these elevated temperatures at a rate comparable to that at lower temperatures (fig. 4*A*). Overall survivorship differed among populations for reasons that were unclear (fig. A2).

Juvenile growth rate was strongly affected by an interaction between population and temperature. During the first 2 weeks of the experiment (weeks 0–2), populations grew uniformly faster with the temperature between 6° and 24°C. At 30°C, however, the growth rate of lower-latitude populations tended to be greater than of higher-latitude populations (fig. 4*B*; table A4). Between weeks 6 and 10, growth rate increased with temperature between 6° and 18°C, and there was a significant interaction be-

tween temperature and population. Specifically, populations did not differ in growth rate at 6°C, while one lower-latitude population (Cape Charles) had far greater growth relative to other populations at 12° and 18°C (fig. A3; table A4).

Temperature substantially increased development rates (measured as time to female sexual maturity) across all populations, but the magnitude of the temperature effect differed across populations (fig. 5*A*; table A5). At 18°C, both lower-latitude populations and a single higher-latitude population (Magnolia) reached sexual maturity in less than 60 days on average, while a second higher-latitude population (Nahant) reproduced significantly more slowly
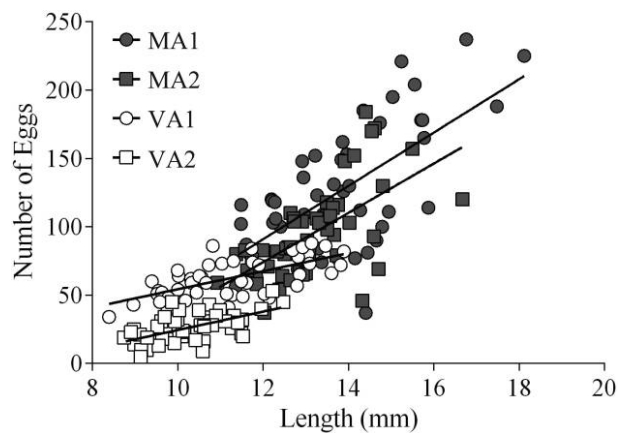
Figure 3: Fecundity of *Idotea balthica* collected from Massachusetts and Virginia. Population key is as in figure 2.

(80 days). At 24°C, females from both higher-latitude populations reached sexual maturity approximately 6 days later than did lower-latitude females.

At sexual maturity, females were significantly larger at 24°C relative to females at 18°C, and this temperature-dependent increase in body length occurred at a similar rate across populations. In addition, females from lower-latitude populations were consistently shorter (17% by length) than those from higher-latitude populations (fig. 5B; table A5). The additive effect of population and temperature on body size is reflected by a shift in the intercepts but not the slopes of the thermal reaction norms.

Tethering Experiment

An alternative explanation for the presence of smaller-bodied isopods in warmer, lower-latitude habitats is a latitudinal decline in predation pressure on isopods. To test this possibility, we tethered isopods onto seagrasses in high- and low-latitude seagrass beds and measured daytime consumption rates. In June, isopods tethered in two Massachusetts seagrass beds had greater survivorship than did isopods in three Virginia seagrass beds (51% vs. 14% on average; fig. 6A). The relatively low survivorship (25%) at Wellfleet, Massachusetts, likely reflects isopod consumption by a large flock of seabirds observed near the transects during the assay. In August, there was uniformly greater survivorship in Massachusetts relative to Virginia seagrass beds (58% vs. 13% on average; fig. 6B; table A6). It is unlikely that geographic differences in survivorship are attributed to differences in water motion or glue failure of the isopopsicles, as recovery rates of control tethers still possessing an isopod mimic were high (average = 90%) and did not differ between regions (fig. 5B). In both June and August, we detected no significant effect of isopod body length or an interaction between length and site on survivorship.

Discussion

Bergmann's rule has been attributed to latitudinal declines in ambient temperature and predation risk, but relatively few studies simultaneously explore their effects along a latitudinal gradient. Here, we use a combination of field surveys and laboratory and field experiments to demonstrate that a latitudinal increase in body size in a marine ectotherm does not reflect latitudinal declines in ambient temperature because size at maturity in females increases with temperature (i.e., does not follow the temperature-size rule). Rather, Bergmann's rule in this species reflects evolutionary responses of the life history to a latitudinal decline in predation risk.
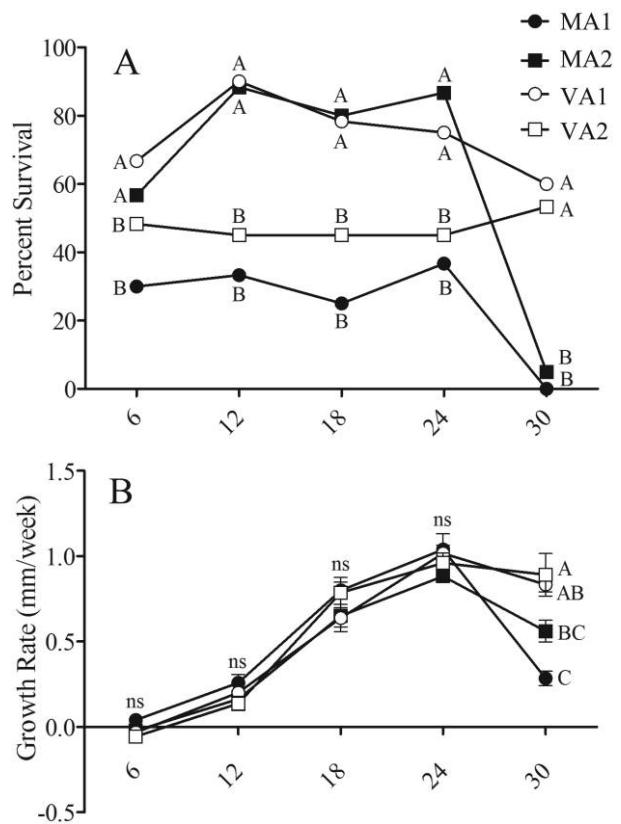

Figure 4: Juvenile survivorship at week 4 (*A*) and growth rates (mean ± SE) between weeks 0 and 2 (*B*) of juvenile *Idotea balthica* in the laboratory experiment. Letters connect statistically indistinguishable means. Population key is as in figure 2.

Latitudinal Decline in Upper Thermal Tolerance

As seen among other marine crustaceans with wide geographic distributions (Lonsdale and Levinton 1985; Gaston and Spicer 1998; Willett 2010), isopods from two high-latitude populations grew more slowly and died more readily at 30°C than did those from two lower-latitude populations (fig. 3). At lower temperatures, there was virtually no difference in growth rates or survivorship between populations. Thus, the populations from lower latitudes tolerated a wider range of temperatures, and this wider breadth did not appear to incur a measurable cost at lower temperatures (at least in terms of survivorship).

The geographic difference in thermal tolerance breadth reflects water temperatures in local environments. This subtidal isopod is subjected to water at 4°–28°C in the Chesapeake Bay (average daily mean; fig. 1B), while Massachusetts isopods experience 3°–19°C on average. To the extent that National Oceanic and Atmospheric Administration tide-station data reflect isopod habitats, the winter, spring, and fall temperatures do not dramatically differ between
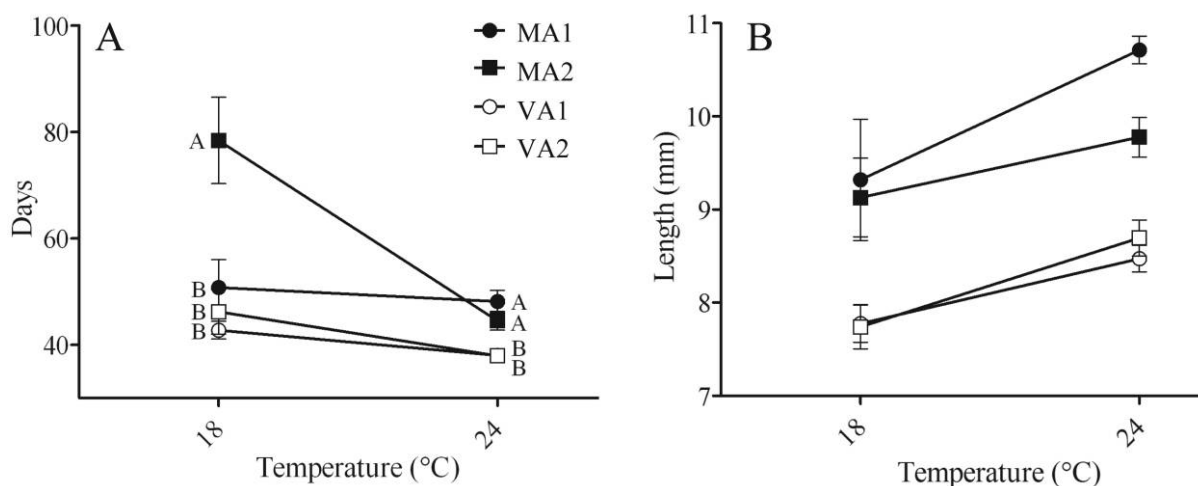
Figure 5: Days to maturity (*A*) and size at maturity (*B*) for all females reared at 18° and 24°C in a laboratory experiment. Letters connect significantly indistinguishable means. Population means ± SE are indicated. Population key is as in figure 2.

locations in Virginia and Massachusetts. Rather, the Virginia populations experience summer temperatures (including 30°C) that are rarely, if ever, experienced by Massachusetts isopods (see Sotka and Giddens 2009 for similar conclusions). It is likely that population-level differences in upper thermal tolerance reflect local adaptation because of the low dispersal potential created by the direct development of *Idotea balthica*. A genetic analysis of mitochondrial sequences, microsatellites, and morphological traits indicate highly restricted gene flow between Virginia and populations north of Cape Cod (Wares 2001; Wares et al. 2007). However, animals used in the temperature-growth experiment were the offspring (i.e., F_1) of field-collected mothers that had been collected at different temperatures between the two regions (15°C in Massachusetts and approximately 30°C in Virginia); thus, these results could reflect transgenerational plasticity in thermal tolerance (Crill et al. 1996).

Reversal of the Temperature-Size Rule

The temperature-size rule, or T-S rule, has been observed in the vast majority of ectotherms examined (Ray 1960: 80% of ectotherms; Atkinson 1994: 83% of ectotherms). In stark contrast, we found that *I. balthica* females from two Massachusetts populations and two Virginia populations do not follow the "rule" and are larger at sexual maturity with increased temperature (fig. 4*B*). The reversal of the T-S rule arises because the thermal sensitivity of growth outstrips that of development (Walters and Hassall 2006; Forster et al. 2011). Thus, *I. balthica* joins a minority of ectotherms that reverse the rule within nonlethal temperatures, including grasshoppers (Walters and Hassall

2006), beetles (Lamb and Gerber 1985), mayflies (Vannote and Sweeney 1980), copepods (Johnston and Dykeman 1987), and diatoms (Fawley 1984).

A previous study with a different population of *I. balthica* (from Nova Scotia; 45°N) found that final body size was negatively correlated with the water temperature of optimal growth efficiency, which supports the growth efficiency model (Strong and Daborn 1980) and, by extension, the T-S rule (Angilletta and Dunham 2003). At first glance, the reversal in the T-S rule among Massachusetts and Virginia isopods versus the confirmation of the growth efficiency model (and, by extension, the T-S rule) among Nova Scotia isopods seems contradictory. However, there are several possible resolutions. Even though *I. balthica* confirms the growth efficiency model, Strong and Daborn (1980) never reared animals at multiple temperatures and measured body size; thus, they never explicitly tested the T-S rule. It is also possible that the growth efficiency model applies only to final body size in *I. balthica* (examined by Strong and Daborn [1980] but not us) and not to isopods that matured only recently (examined by us but not Strong and Daborn [1980]). Finally, microevolutionary differences in the thermal reaction norms for body size may exist between populations, as has been shown in other ectotherms (Kingsolver et al. 2007). Resolving these alternatives will require future studies that rear isopods from Massachusetts and Virginia to final body size, estimate thermal reaction norms of Nova Scotia isopods, or both.

The Pattern and Mechanism of Bergmann's Rule

A latitudinal increase in body size among populations or species is common among ectotherms (Watt et al. 2010).
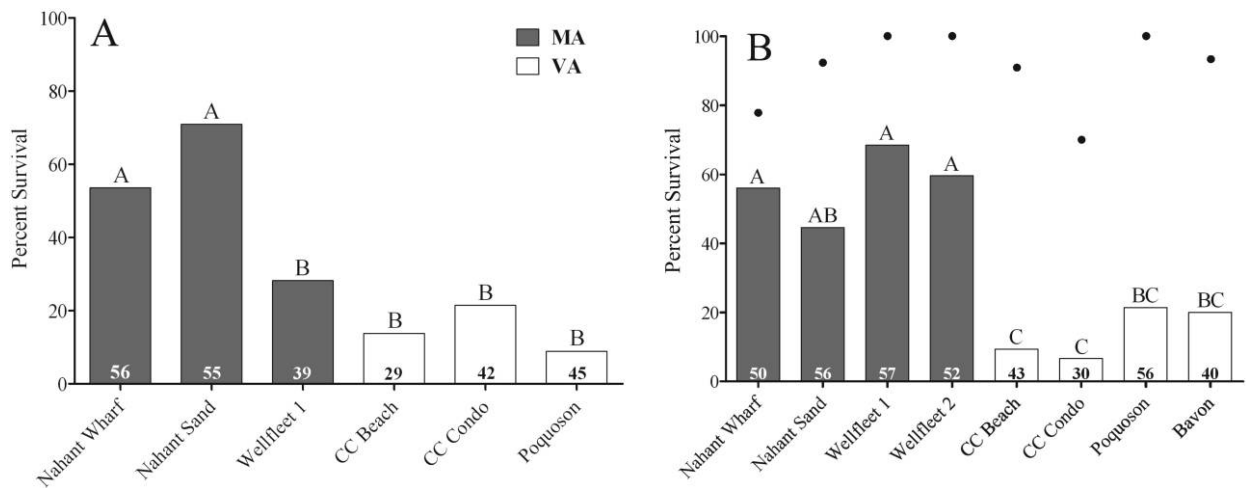
Figure 6: Survival of *Idotea balthica* during 12-hour field predation trials in June (*A*) and August (*B*) of 2011. Numbers within bars indicate sample size. Dots on *B* indicate retrieval rate of control tethers. Letters connect sites that were statistically indistinguishable. CC = Cape Charles.

Although the literature on such latitudinal patterns is large, *I. balthica* helps to explain three of the current gaps. First, among marine organisms, most studies of latitudinal body size patterns involve interspecific rather than intraspecific comparisons (e.g., Wallerstein and Brusca 1982; Daufresne et al. 2009; Shackell et al. 2010; but see Lonsdale and Levinton 1985; Conover and Present 1990; Ho et al. 2010). Second, most studies of size clines measure field-collected individuals and rarely test whether size clines have a genetic basis (Stillwell 2010) and thus might be attributable to local adaptation. In *I. balthica*, the intraspecific comparison of field-collected and laboratory-reared animals clearly established a Bergmann's cline that has a strong genetic basis (see also Bell and Sotka 2012). Third, while several environmental factors simultaneously grade across latitude (e.g., predation pressure, food quality, and seasonal length), most studies of latitudinal body size patterns focus exclusively on temperature (James 1970; Van Voorhies 1996; Walters and Hassall 2006), largely because the predictions of the temperature-size rule and Bergmann's rule coincide (Angilletta and Dunham 2003). However, it is highly unlikely that temperature explains the body size pattern in *I. balthica*. Isopods from both regions were larger when reared at warmer temperatures (i.e., they reversed the temperature-size rule), but isopods collected in the lower-latitude and warmer Virginia habitats were smaller than isopods from Massachusetts populations. If plastic responses to temperature are adaptive, the genetic difference in body size is not likely an adaptive response to local temperatures.

Predation is a largely overlooked mechanism by which latitudinal patterns of body size might occur (Wallerstein

and Brusca 1982; Angilletta et al. 2004*a*, 2004*b*). At least three lines of argument support the hypothesis that geographic variation in predation risk plays an important role in the evolution of the latitudinal pattern of body sizes of *I. balthica*. First, a tethering experiment revealed that daytime predation risk is greater in Virginia than in Massachusetts seagrass beds (fig. 5). A latitudinal decline in predation risk within seagrass beds has been witnessed in other studies of predation pressure along this same coastline (Virnstein et al. 1984; Heck and Wilson 1987; Peterson et al. 2001). Virnstein et al. (1984) explored how predation might play a role in the latitudinal cline in amphipod body sizes, but they did not assay rates of predation. Therefore, our tethering experiment uniquely showed a latitudinal difference in predation pressure on an organism that conforms to Bergmann's rule.

Second, as would be predicted from life-history theory in predator-rich versus predator-poor habitats (Abrams and Rowe 1996), isopods from Virginia populations tended to reach sexual maturity at a smaller size and in less time than did isopods from Massachusetts populations. Similarly, Reznick and Endler (1982) showed that when Trinidadian guppies were raised in an environment where predators selectively consumed large prey, the guppies became reproductive in less time at a smaller size. In instances of nonselective predation, organisms in high-risk environments may show variable responses in time to maturity; however, smaller adult body size is a consistent result when food is not a limiting resource (Abrams and Rowe 1996).

Third, other phenotypic traits indicate that predation risk affects the evolution of Virginia populations more

than the evolution of Massachusetts populations. When held at room temperature (20°C), Massachusetts isopods are noticeably active and swimming, while Virginia isopods rarely swim or move at all (A. Manyak-Davis, T. M. Bell, and E. E. Sotka, personal observation). This difference is consistent with the notion that mobility increases predation rates by visual fish predators (Magnhagen and Wiederholm 1982) and that the sedentary nature of Virginia isopods acts to reduce predation in Virginia relative to Massachusetts seagrass beds. One caveat to our principal conclusion that the latitudinal decline in predation risk drives the cline in isopod body size is that we have not calculated all mortality sources for these populations. Thus, it remains possible that another ecological force (e.g., extreme temperatures, infections by parasites, and food shortage) may vary with latitude and play a role in isopod body size evolution.

Isopods from Virginia reared under laboratory conditions were considerably larger than those collected in the field (compare fig. 2A with 2B). Our laboratory environment lacked chemical or visual cues of predators, which might have caused Virginia isopods to mature at a smaller final size (cf. Stibor 1992). It is also possible that predators remove large-bodied (and presumably older) isopods in the field, leading to a different mean body size than that observed in the laboratory. However, the minimal size of reproductive females in the laboratory was also larger (11 mm) than that found in the field (8 mm). Finally, while we did not explicitly examine the role of food quality in the evolution of a body size cline, shifting to a higher-quality diet in the laboratory could have driven an increase in body size, as food quality can increase body sizes in cooler latitudes in both terrestrial (Langvatn and Aldon 1986) and marine (Bolser and Hay 1996; Ho et al. 2010) environments. Isopods from Massachusetts (especially males) were larger in the field than those reared in the laboratory, likely because field-collected populations have adults one or more years in age (Strong and Daborn 1979).

There are clear consequences of the latitudinal decline in body size for fecundity, as smaller-sized females from the lower latitudes produce fewer offspring per generation than higher-latitude females (fig. 3). This suggests that a life history that reduces predation risk (i.e., shorter generation time) may trade off against a different component of individual fitness of females (lower fecundity). A caveat is that we did not measure other demographic and fitness traits that differ between habitats and that could help to explain the fitness benefits or costs of being small in warmer habitats. For instance, warmer temperatures tend to decrease life span (Loeb and Northrop 1917), and shorter life spans would seem to reinforce the cost of being small at lower-latitude habitats. Warmer temperatures also increase development rate, and in an iteroparous species

like *I. balthica* this shortening of time to maturity could yield more generations per year. This beneficial effect of higher temperature could minimize the cost of small size and earlier reproduction and thus help to explain the persistence of small body sizes in Virginia populations.

Conclusions

A large number of environmental features vary with latitude. Although ambient temperature is among the most obvious abiotic factor influencing organismal biology and ecology, a dynamic suite of biotic and abiotic factors shapes the evolution of species with wide geographic distributions. In *I. balthica*, temperature increases growth rates, lowers development time, and can yield population-level differences in survivorship. Importantly, however, *I. balthica* does not follow the temperature-size rule and, thus, thermal plasticity cannot explain the presence of a Bergmann's cline in the field. Rather, increased predation risk in lower-latitude habitats appears to favor faster development in *I. balthica* and, as a consequence, smaller size of lower-latitude populations relative to higher-latitude populations. Because the estuaries of the eastern United States are warming by approximately 0.5°C on average per decade (Nixon et al. 2004; Allen et al. 2008), our results suggest (on the basis of first principles) that increased water temperatures have yielded larger bodies, greater fecundity, and lower generation times relative to historical *I. balthica* populations. However, our data also suggest that evolutionary or plastic responses of isopod populations to increased water temperature will be constrained by predation risk. Our study emphasizes the importance of documenting phenotypic and genetic bases that underlie geographic variation in body size (Conover et al. 2009; Stillwell 2010). We implore that others simultaneously examine multiple biotic and abiotic environmental forces to discern their evolution.

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