Adaptation to climate change: contrasting patterns of thermal-reaction-norm evolution in Pacific versus Atlantic silversides

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How organisms may adapt to rising global temperatures is uncertain, but concepts can emerge from studying adaptive physiological trait variations across existing spatial climate gradients. Many ectotherms, particularly fish, have evolved increasing genetic growth capacities with latitude (i.e. countergradient variation (CnGV) in growth), which are thought to be an adaptation primarily to strong gradients in seasonality. In contrast, evolutionary responses to gradients in mean temperature are often assumed to involve an alternative mode, ‘thermal adaptation’. We measured thermal growth reaction norms in Pacific silverside populations (Atherinops affinis) occurring across a weak latitudinal temperature gradient with invariant seasonality along the North American Pacific coast. Instead of thermal adaptation, we found novel evidence for CnGV in growth, suggesting that CnGV is a ubiquitous mode of reaction-norm evolution in ectotherms even in response to weak spatial and, by inference, temporal climate gradients. A novel, large-scale comparison between ecologically equivalent Pacific versus Atlantic silversides (Menidia menidia) revealed how closely growth CnGV patterns reflect their respective climate gradients. While steep growth reaction norms and increasing growth plasticity with latitude in M. menidia mimicked the strong, highly seasonal Atlantic coastal gradient, shallow reaction norms and much smaller, latitude-independent growth plasticity in A. affinis resembled the weak Pacific latitudinal temperature gradient.

Keywords: countergradient variation; growth capacity; conversion efficiency; latitudinal gradients; temperature; seasonality

1. INTRODUCTION

The need to understand how organisms adapt to climatic variability has increased with the evidence for unprecedented global climate change [1]. Because temperature greatly influences the expression and fitness of many phenotypic traits, adaptive landscapes will be altered substantially by long-term changes in mean temperature and seasonality [2]. Anticipating evolutionary responses to climate change remains a complex challenge. Retrospective analyses or other temporal approaches are often inconclusive owing to a lack of replication and difficulties in distinguishing genetic from plastic responses [3,4]. An alternative is to study adaptations to climate change across spatial scales among extant populations. Many species occur across temperature and seasonality clines along latitudinal, altitudinal, depth or continentality gradients, and exhibit apparent adaptive variations in morphological and physiological traits [5]. Spatial climate gradients provide opportunities to rigorously identify mechanisms of adaptation that, by analogy, may elucitate evolutionary responses to temporal climate change.

One form of spatial adaptation that has gained strong empirical support across taxa is countergradient variation (CnGV; reviewed by [6]). CnGV occurs when genotypes with a higher (or lower) value for a given trait are predominantly found in environments that tend to decrease (or increase) the trait’s phenotypic value (figure 1). The most common form of CnGV involves metabolic compensation and is displayed mostly in physiological traits, for example, in the genetically higher growth capacities of many poikilotherms at high versus low latitudes (e.g. [7] (Reptilia); [8] (Pisces); [9] (Insecta); [10] (Gastropoda); [11] (Amphibia)). In cases of CnGV in growth, genetic growth capacities are adjusted upward or downward over a species’ entire viable temperature range, with thermal reaction norms (=temperature-specific phenotypic trait expressions, figure 1) shifted in parallel to higher or lower levels. This mode of adaptation is currently interpreted as an evolutionary response to gradients in seasonality, i.e. the degree of seasonal temperature fluctuations influencing the length of the growing season [6,12,13]. However, latitudinal adaptations could also evolve without changing the overall growth capacity but via shifts in thermal reaction norms toward a higher or lower range of temperatures in accordance to those most often experienced in nature [14]. In this case, thermal reaction norms would cross (figure 1) and represent a form of genotype × environment interaction rather than CnGV [15]. Such thermal adaptation is thought to be the primary adaptive response to gradients in mean temperature [15,16]. However, this distinction is still uncertain, as it remains largely based on studies that examined trait variations in single species and across single latitudinal gradients where mean temperature and seasonality varied concomitantly (e.g. along the North-American Atlantic coast [8,15,17]). Hence, the confounding effects of mean temperature versus seasonality as agents of selection have yet to be disentangled. This uncertainty could be effectively addressed by a

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large-scale comparative approach using latitudinal gradients that differ substantially in their seasonality and temperature change. Consider, for example, the highly contrasting latitudinal temperature and seasonality gradients that exist along the North American Atlantic versus Pacific coast [18]. We quantified this contrast by extracting mean coastal sea surface temperatures (SST) per week and degree latitude from a publicly available dataset of in situ and satellite observations (http://dss.ucar.edu/datasets/ds277_0, 1982–2008, figure 2). Between 27°N and 49°N, the absolute magnitude of temperature fluctuations along the Atlantic coast is twice as large (−0.9°C to 29.3°C, ΔTAtl = 30.2°C) as on the Pacific side (7.6–22.0°C, ΔTPac = 14.4°C). On average, mean temperatures decrease almost three times faster with latitude along the Atlantic (−1.11°C per latitude) than on the Pacific coast (−0.40°C per latitude). Seasonality, i.e. the latitude-specific maximum summer–winter difference, is small and independent of latitude along the Pacific coast (2.6–6.7°C), but strong and increasing with latitude along the Atlantic coast, particularly north of Cape Hatteras (ΔTAtl 28.5°N = 6.3°C, ΔTAtl 45.3°N = 18.6°C, figure 2).

We used these two gradients to contrast latitudinal growth adaptations between two broadly distributed, ecologically equivalent atherinopid species: Pacific topsmelt (*Atherinopsis affinis*) and the Atlantic silverside (*Menidia menidia*). To reveal extant genetic variation in growth capacity and efficiency, *A. affinis* offspring from four different populations were reared in common garden environments. Alternatively, local adaptation may involve CnGV, which leads to genetic increases in trait expression over the entire range of experienced temperature without changing the thermal optimum (2, red curve; population reaction norms do not cross).

expected *A. affinis* latitudinal growth adaptations to be either undetectable or occur via shifts in thermal optima of growth capacity (crossing thermal reaction norms, figure 1). Instead, we found novel evidence for CnGV, i.e. higher growth capacities with increasing latitude over the entire thermal range of *A. affinis*. While CnGV thus appears to be the prevalent mode of thermal-reaction-norm evolution even across simple temperature gradients (Pacific coast), a strong seasonality gradient (Atlantic coast) probably necessitates additional adaptive increases in growth plasticity in high-latitude populations.

2. MATERIAL AND METHODS

(a) Study species

*Menidia menidia* and *A. affinis* are two silverside species (Atherinopsidae) that occur over a broad and similar latitudinal range along the Atlantic and Pacific coast, respectively (*M. menidia*: 30–46°N [8]; *A. affinis*: 24–45°N [20]). Both are estuarine, schooling, omnivorous fish of equivalent trophic levels. Both are multiple batch spawners laying benthic, intertidal eggs on a semilunar cycle mainly between spring and summer [21,22]. In *M. menidia*, onset and length of the spawning season shift with latitude, while the same is not known for *A. affinis*. Both species mature and spawn at age 1 but differ in their maximum size and age: *M. menidia* is essentially an annual silverside, reaching as much as 15 cm in total length with less than 1 per cent of fish reaching age 2 [21], while *A. affinis* reaches up to 37 cm and typically lives to ages 4–5 [23].

(b) *Atherinops affinis* offspring collection

Fertilized *A. affinis* eggs were collected by strip-spawning ripe adults caught with beach seines in four Pacific estuaries. For the first year of experiments (2008), populations P233 N, P337 N and P443 N were sampled, whereas in the second year (2009), populations P128 N, P233 N and P337 N was sampled (table 1). Re-sampling of P233 N and P337 N was done to facilitate inter-annual comparisons. Sufficient genetic diversity representative of each population was assumed after strip-spawning at least 20 individuals of each sex. To transfer embryos to our laboratory facility at Flax Pond (Stony Brook University, Long Island, NY, USA), screens with attached egg masses were wrapped in moist paper towels and stored in common thermos cans. Upon arrival, eggs were placed in aerated 201 containers sitting in large (7001) temperature-controlled baths at three (i.e. 15, 21, 27°C in 2008) or four (15, 21, 24, 27°C in 2009) temperature treatments. Containers were equipped with screened holes to ensure water exchange with the baths. The photoperiod was 15 L:9 D. A salinity of 30 ± 2 psu was maintained during both years using water drawn from saline ground wells. Addition of commercial sea salt (Instant Ocean) allowed controlling for variation in salinity among years. Depending on the temperature, *A. affinis* larvae hatched 6–16 days post-fertilization at approximately 6 mm (population independent) and were start-fed with a mix of larval powder food (*Otohime Marine Weaning Diet, size A, Reed Mariculture*) and newly hatched brine shrimp nauplii (*Artemia salina*, San Francisco strain, Brine Shrimp Direct, Inc.).

Growth capacity, i.e. the temperature-specific growth rate at unlimited feeding conditions, was measured during the first experimental period. Trials started 4–13 days

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Figure 1. Schematic diagram of two alternative modes of thermal reaction norm evolution across thermal gradients such as those across latitudes. Consider the thermal reaction norm (i.e. the trait expression at a range of temperatures) of a hypothetical organism adapted to some average temperature regime (blue curve). A shift to lower average temperature conditions (e.g. at higher latitudes) may lead to ‘thermal adaptation’, i.e. a horizontal shift in the reaction norm and a new lower thermal optimum (1, black curve). This results in crossing reaction norms of different populations reared in common garden environments. Alternatively, local adaptation may involve CnGV, which leads to genetic increases in trait expression over the entire range of experienced temperature without changing the thermal optimum (2, red curve; population reaction norms do not cross).
weeks per year, when average temperature conditions are above the growth permitting thermal threshold in Pacific (10°C). For orientation, geographical reference points are given next to each panel. Grey lines and values denote the number of weeks per year, when average temperature conditions are above the growth permitting thermal threshold in Pacific (10°C) and Atlantic silversides (12°C).

Table 1. *Atherinops affinis* sampling sites and dates during the two experimental years.

<table>
<thead>
<tr>
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<th>location</th>
<th>sampling date(s)</th>
<th>acronym</th>
</tr>
</thead>
<tbody>
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<td>P128N</td>
</tr>
<tr>
<td>Tijuana estuary, Oneonta Slough, California, USA</td>
<td>32.57° N 117.13° W</td>
<td>May 2008 (19)</td>
<td>P233N</td>
</tr>
<tr>
<td>Elkhorn Slough, South Marsh, California, USA</td>
<td>36.82° N 121.74° W</td>
<td>May 2008 (16 and 17)</td>
<td>P337N</td>
</tr>
<tr>
<td>Coos Bay, North Bend, Oregon, US</td>
<td>43.38° N 124.20° W</td>
<td>June 2008 (19)</td>
<td>P443N</td>
</tr>
</tbody>
</table>

Figure 2. Contour representation of latitude-specific, weekly mean SSTs along the (a) Pacific and (b) Atlantic coast, derived from long-term SST data (1982–2008). For every 1° of latitude, data from the grid cell next to land in the land-sea mask were used. For orientation, geographical reference points are given next to each panel. Grey lines and values denote the number of weeks per year, when average temperature conditions are above the growth permitting thermal threshold in Pacific (10°C) and Atlantic silversides (12°C).

post-hatch after larvae had reached a mean ± s.d. total length (TL) of 8.0 ± 0.9 mm (measured to the nearest 0.1 mm via calibrated digital pictures and ImagePro software). A random sample of 10–20 larvae per temperature/population was measured for initial TL and wet weights (W) nearest 0.1 mg, Mettler AE163), followed by randomly placing 35 ± 2 larvae in each of three replicate containers (20 l) per temperature/population. Fish were subsequently reared on ad libitum rations of newly hatched brine shrimp nauplii until reaching a mean ± s.d. TL of 23.2 ± 2.1 mm (consistent with [8,15]). At this point, a sub-sample of at least 10 fish per container was measured for TL (0.1 mm, using callipers) and W (same scale). During the first 3 days of each trial, dead specimens were replaced to correct for initial mortality owing to handling. Total mortality was low, averaging 4.2 (2008) and 2.3 fish per replicate (2009). Growth capacity in length (mm d⁻¹) and weight (dimensionless) was calculated for each replicate by dividing the difference in mean TL and W (cube-root transformed) by the corresponding duration of the first experimental period (i.e. 18–80 days).

Dry weight-based food consumption and conversion efficiency (= the increase in body mass per unit food ingested, %) was assessed during the second experimental period, when the remaining fish grew at excess feeding conditions from 23.2 mm TL (see above) to an average (±s.d.) of 32.5 ± 2.7 mm TL. The number of remnant nauplii was estimated daily by taking three 5 ml water samples from each container and counting all live nauplii in each using Bokorov chambers and a dissecting microscope. Nauplii added to containers were similarly quantified by counting sub-samples from nauplii hatching cones. The method had an estimated average precision of 20.9 per cent (CV). Daily nauplii consumption per container, i.e. remaining + added nauplii on any given day minus nauplii remaining on the following day, was then converted to dry weights (dW) using a value of 2.1 μg nauplius⁻¹ consistent with Present & Conover [19]. Fish W was converted to dW using the relationship dW = 0.0706 W¹.2046 (r² = 0.997, p < 0.001, n = 56) derived from a representative sub-sample that was oven-dried at 65°C for 98 h. Mean daily consumption estimates (% body dW) were scaled by the daily mean dW of all fish in each replicate, derived via calculating a mean dW growth rate of each replicate during the second period. Food conversion efficiency (%) was calculated for each replicate as the mean total increase of all fish dW during the second period relative to the total dW of consumed nauplii.
(c) Minimum temperature permitting juvenile growth
To determine the lowest growth-permitting temperature in *A. affinis*, some excess juveniles from reservoir containers in 2009 were reared at ad libitum rations of brine shrimp nauplii at 12°C, 10°C, and 8°C for four to five weeks. Prior to each trial, 30 juveniles of similar size were randomly assigned to each of three (12°C) or two replicates (10°C, 8°C) per population and acclimatized for 5–6 days to target temperature and excess food. Trials started by sacrificing a random of 10–12 fish for initial W measurements and ended 25–38 days later by determining W of all remaining specimens. Mean W of initial and final samples were tested for significant differences by t-tests. Average mortality was less than one fish per replicate.

(d) Menidia menidia growth capacity and efficiency
We used original data from two analogous common garden experiments conducted at the same laboratory facility with identical culturing equipment [8]. Laboratory-spawned offspring from three populations originating from South Carolina (SC82 N), New York (NY41 N) and Nova Scotia (NS44 N) were reared over a similar larval size range (7–18 mm TL) on excess brine shrimp nauplii and at four replicated temperatures (17, 21, 28, 32°C). Estimates of *M. menidia* food consumption and conversion efficiencies were derived from slightly smaller fish than *A. affinis*, monitored either individually over 24 h (consumption) or in small groups over 5 days (efficiency) as described in Present & Conover [19].

Statistical analyses were conducted in SPSS Statistics 17.0 (SPSS, Inc.) using replicate means (containers) as individual statistical entities. Growth capacities (length, weight), food consumptions and conversion efficiencies were first tested for significant (p < 0.05) effects of temperature using separate analyses of variance (ANOVA) per year and population. Likewise, population effects were assessed through ANOVAs per year and temperature. Least-significant difference (LSD) or Dunnet-T3 post hoc tests were used in case of homogeneous or heterogeneous variances between groups, respectively. For growth capacities (GC, length, weight), data from both years were used to construct general linear models (LM) of the form GC = T + P + T × P + Y + ε to test for significant overall effects of temperature (T), population (P), temperature × population interaction and year (Y, ε = error).

3. RESULTS
(a) Atherinops affinis thermal reaction norms
In both years, growth capacity significantly increased with temperature and from southern to northern populations (13 separate ANOVAs, p < 0.05, figure 3a,b). In 2008, the P44 N population grew on average 0.30, 0.55 and 0.76 mm d⁻¹ at 15, 21 and 27°C, respectively, which was significantly faster than the P3 N (LSD, p < 0.001) and P23 N populations (LSD, p < 0.001). Population growth differences of about 0.05 mm d⁻¹ remained similar across experimental temperatures in the first year (i.e. temperature × population interaction term not significant, LM, F₃,₁₀₈ = 1.09, p = 0.39, figure 3a). During the second year, P3 N fish again grew significantly faster at all temperatures than P2 N (Dunnet-T3, p < 0.05), which in turn grew faster than those from P1 N (Dunnet-T3, p < 0.05). Second year growth rates were 7–26% higher in the repeated P23 N and P3 N populations. There was also a weak but significant temperature × population interaction (LM, F₃,₃₅ = 4.07, p = 0.006), mainly due to steeper growth capacity increases with temperature in the P3 N population (figure 3b). The 24°C treatment proved meaningful in revealing the general nonlinearity of the *A. affinis* growth reaction norm, suggesting 27°C to be close to the thermal growth capacity maximum of *A. affinis*. Initial trials at 12°C and 30°C failed owing to poor hatching success and near total mortality of larvae within the first few days, thus indicating the thermal tolerance limits of the youngest *A. affinis* life stages. Patterns in weight growth were the same as those described for length. Overall, an LM with data from both years returned statistically significant effects (p < 0.001, electronic supplementary material, table S1) of temperature, population and year with a weak temperature × population interaction (p = 0.017).

In both years, weight-specific food consumption increased significantly with temperature (ANOVA, F(2008)₂,₂₄ = 8.7, p < 0.001; F(2009)₂,₃₂ = 42.0, p < 0.001) from daily mean values of 21–39% body dW at 15°C to 45–53% body dW at 27°C (electronic supplementary material, figure S1a,b). However, there were no significant differences between populations, except for lower values of P4 N at 15°C compared with P3 N and P2 N (ANOVA, F(15°C)₂,₇₆ = 27.6, p = 0.01). In both years, food conversion efficiencies showed a tendency to increase with latitude, however, data were very heterogeneous and most differences non-significant (electronic supplementary material, figure S1c,d). In 2008, P4 N converted on average 16, 20 and 17 per cent of consumed food into weight at 15, 21 and 27°C respectively; values that were 5 per cent higher (ANOVA, d.f. = 2, p < 0.05) than P3 N and P2 N efficiencies (electronic supplementary material, figure S2c). The two repeated populations showed significantly (ANOVA, d.f. = 1, p < 0.05) higher conversion efficiencies in the second year at 15°C and 21°C (electronic supplementary material, figure S2d) consistent with the observed overall increase in growth rates. For P23 N and P3 N, highest mean efficiencies of 21.2 and 24.6 per cent occurred at 24°C, while P1 N values peaked at 21°C (electronic supplementary material, figure S2d).

(b) Juvenile growth at low temperatures and unlimited food
At 12°C, juveniles from the three populations tested (P128 N, P23 N, P33 N) grew in mean weight, although increases were only significant (t-test, p < 0.01, table 2) for the two northernmost populations. At 10°C, weight changes were still positive, but not significantly different from zero. At 8°C, both P128 N replicates and one of two P23 N replicates showed slight weight losses, while weight in P33 N did not change significantly (table 2).

(c) Atlantic compared with Pacific patterns of CnGV in growth capacity
Both in *M. menidia* and *A. affinis*, latitudinal growth adaptations were achieved via increases in growth capacity across all temperatures from low- to high-latitude populations, not by intra-specific horizontal shifts in thermal reaction norms. Still, reaction norms differed greatly between species but the divergence was owing to the
much greater increase in thermal growth plasticity with increasing latitude in *M. menidia*. When averaged across populations, *M. menidia* growth capacity increased from 0.32 mm d
−1 (17°C) to 1.12 mm d
−1 (28°C), which corresponds to an average slope of 0.074 mm d
−1°C
−1 or a Q₁₀ of 3.12 (electronic supplementary material, figure S2a). In contrast, average *A. affinis* growth capacities increased only from 0.26 mm d
−1 at 15°C to 0.78 mm d
−1 at 27°C, corresponding to a much smaller slope of 0.043 mm d
−1°C
−1 (Q₁₀ = 2.49, P₄₁₃, N values adjusted for year effect). More importantly, the slopes of growth reaction norms were relatively similar between *A. affinis* populations, but differed greatly between *M. menidia* populations, thus causing the temperature × population interaction term to be very strong in Atlantic but relatively weak in Pacific silversides. *Menidia menidia* from SC₃₂, N grew 0.29–0.88 mm d
−1 (17–28°C), while those from NS₄, N grew 0.33–1.36 mm d
−1 (17–28°C), which means a doubling in slopes from 0.049 to 0.098 mm d
−1°C
−1. In contrast, southernmost *A. affinis* (P₁₂₈, N) grew 0.20–0.66 mm d
−1 (15–27°C), while northernmost P₄₄₃, N grew 0.33–0.90 mm d
−1 (15–27°C, adjusted for year effect), corresponding to similar slopes of 0.038 and 0.048 mm d
−1°C
−1, respectively.

Food consumption and conversion efficiency of *M. menidia* increased more strongly with temperature and latitude than in *A. affinis* (electronic supplementary material, figure S2b, [19]). Southernmost *M. menidia* had average efficiencies of 12.5 and 21.3 per cent (at 17 and 28°C, respectively) that were comparable to values observed in *A. affinis*. Efficiencies beyond 30 per cent, on the other hand, as measured in northernmost *M. menidia* at 28°C, were considerably above all *A. affinis* estimates. However, the general heterogeneity of both datasets and slightly differing experimental protocols place limitations on consumption/efficiency comparisons between the two species.

4. DISCUSSION

This study determined thermal growth reaction norms of Pacific silverside populations to test the paradigm that species distributed across simple temperature gradients evolve local adaptations via shifting thermal growth optima towards each population’s average temperature experience (i.e. ‘thermal adaptation’, figure 1). Instead, we found that Pacific silversides evolved CnGV in growth (figure 1), an alternative mode of reaction norm evolution previously assumed to be an adaptation to strong seasonality gradients (e.g. in Atlantic silversides [15]). If CnGV is the prevalent adaptive mechanism across simple temperature gradients in space, it probably plays an important role, too, for adaptations in time across thermal gradients, such as those elicited by global warming. In addition, our novel coast-to-coast comparison has broadened the current understanding about CnGV by suggesting a strong link between the characteristics of latitudinal climate gradients and the different, corresponding CnGV patterns in reaction norm evolution.

(a) Latitudinal growth adaptation in Pacific silversides

Our results clearly indicated CnGV in growth capacity among populations of *A. affinis*, thereby documenting the first case of growth CnGV in a coastal Pacific fish. Thermal reaction norms in growth capacity were generally parallel and differed primarily in elevation, with more northern populations growing faster than those from the south. Because mean growth capacity changed in rank order with latitude, this pattern of variation is probably the outcome of natural selection, not random genetic drift [5]. This suggests that sub-maximal growth capacities are adaptive, probably because evolutionary incentives for maximizing body size are countered by physiological trade-offs of fast growth [6], with the balance being temperature- and therefore latitude-dependent. Selection for increased body size via faster growth follows from survival advantages during the early life stages of fish known as ‘bigger-is-better’ or ‘stage-duration’ paradigms [24–26]. In many fishes, larger body size also increases juvenile survival during the first winter [27–29] and confers higher fertility during

Figure 3. *Atherinops affinis*. Thermal reaction norms of growth capacity in offspring from four populations along the US and Mexican Pacific coast, as revealed by common garden experiments in (a) 2008 and (b) 2009. Lines intersect means ± 1 s.e. For clarity, means are slightly jittered along the x-axis. Dash-dotted line: P₁₂₈, N (Laguna Manuela), solid line: P₂₃₅, N (Tijuana estuary), dashed line: P₃₇, N (Elkhorn Slough), dotted line: P₄₄₃, N (Coos Bay).
seasonal temperature fluctuations, changes greatly with gradient is three times steeper than the Pacific temperature decreases with latitude along both coasts, the Atlantic adaptation, CnGV, has evolved despite such contrasting when comparing Pacific with Atlantic silversides, it is relatively weak or because high efficiencies are associated or efficiencies in northern compared with southern populations. This mimics the presence versus absence of a latitudinal seasonality gradient along the Pacific coast (figure 4c,d). Evidence for growth CnGV in Pacific silversides suggests that this form of adaptation can evolve in response to changes in mean temperature alone, independent of changes in seasonality [8,17]. In M. menidia, size-selective overwinter mortality acts as a stronger agent of selection in driving the evolution of CnGV in growth [12,15]. In many species with distributions across large seasonality gradients, growth is limited to a fraction of the year when temperatures exceed a species-specific threshold (e.g. 12°C [8]). In Atlantic silversides, this results in a threefold decrease in growing season length from southern- to northermost populations (figure 2b), yet a reduction in body size is penalized by the increasingly severe and size-selective winter mortality in latitudes above approximately 36°N (28,41) figure 4d). In contrast, winter mortality is unlikely to be responsible for growth CnGV in Pacific silversides, because ambient temperatures would permit year-round growth if food is not a constraining factor (figure 2). Ad libitum-fed juveniles in our experiments were able to sustain growth above 10°C. Temperatures exceeding this threshold occur year-round in coastal Pacific waters south of 45°N (figures 2 and 4c). Other studies have documented latitudinal growth CnGV in fish species, where size-selective winter mortality is equally unlikely (e.g. M. peninsulæ [15]). Hence, size-selective winter mortality is not necessary to trigger the evolution of CnGV in growth rate. Even though CnGV is the common mode of adaptation in both species/coasts, the norms of reaction differed greatly in a manner that reflected the gradients in which they evolved. Average growth plasticity is much greater in Atlantic than Pacific silversides, resulting in a two-fold difference in average slopes of reaction norms between the two species. This coincides with the steeper latitudinal decrease in mean temperature along the Atlantic than the Pacific coast (figure 4). More importantly, growth plasticity increases greatly from southern to northern Atlantic silverside populations but remains similar between Pacific silverside populations. This mimics the presence versus absence of a latitudinal seasonality gradient along the Atlantic versus Pacific coast (figures 2 and 4). Northern populations of M. menidia must accelerate their growth rate rapidly with temperature in order to compensate for Table 2. *Atherinops affinis*. Growth of juveniles (45–50 mm TL) at low temperatures and unlimited food. Average fish wet weights (g) at the beginning (*W*$_{ini}$) and end (*W*$_{end}$) of trials at 12, 10 and 8°C, and trial lengths are given per replicate. Italicized pairs denote weight loss, bold pairs with asterisks denote significant weight changes (*t*-test).

<table>
<thead>
<tr>
<th>population</th>
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<th>8°C</th>
<th>10°C</th>
<th>12°C</th>
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<td><em>W</em>$<em>{ini}$–<em>W</em>$</em>{end}$ (g)</td>
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</tbody>
</table>
| P3$_{37}$°N | 1         | 0.57–0.64 | 0.53–0.61 | **0.49–0.73**
|            | 2         | 0.58–0.60 | 0.56–0.61 | —
| P2$_{33}$°N | 1         | 0.66–0.77 | 0.70–0.70 | **0.43–0.61**
|            | 2         | 0.70–0.64 | 0.67–0.70 | **0.46–0.61**
|            | 3         | —      | —      | **0.48–0.68**
| P1$_{28}$°N | 1         | **1.04–0.78** | 0.78–0.81 | 0.39–0.46 | 32
|            | 2         | 0.91–0.83 | 0.75–0.84 | 0.44–0.51 | 32
|            | 3         | —      | —      | 0.45–0.57 | 32

*p < 0.05.

*p < 0.01.

adulthood [30]. Trade-offs of fast growth, on the other hand, include smaller activity scopes and thus poorer burst and routine swimming of faster compared with slower growing fish of the same size [31–33], which implies higher predation vulnerabilities for fast growers [34–36]. The present findings for *A. affinis* suggest that the countervailing selection pressures on growth capacity operate even across latitudinal scales where the change in mean temperature is very small. For example, mean annual temperature differed by as little as 1.2°C between our Pacific study sites (figure 4c), but still evoked detectable shifts in growth capacity. The existence of CnGV in Pacific silversides therefore indicates that growth is finely tuned to local selection pressures even across modest changes in climate.

Growth capacity variations necessitate changes in either food consumption, conversion efficiency or both, yet physiological efficiencies within a species are often assumed to become maximized by natural selection [37,38]. Common garden experiments on vertebrate and invertebrate species have challenged that notion by finding higher food consumptions and higher conversion efficiencies in faster growing, higher latitude populations (e.g. [19,39,40]). Our data for *A. affinis* also showed higher conversion efficiencies in northern compared with southern populations. Changes in consumption were not evident, either because the small magnitude of *A. affinis* growth CnGV did not require them or because the differences were masked by the large uncertainty of our nauplii counting method. Overall, this study supports the notion that sub-maximal growth rates in fish involve sub-maximal growth efficiencies, either because directional selection for maximizing efficiency is weak or because high efficiencies are associated with so far unknown trade-offs, related or conceptually similar to the trade-offs of fast growth.

(b) **Countergradient growth variation across different gradients**

When comparing Pacific with Atlantic silversides, it is relevant that the same general mode of latitudinal growth adaptation, CnGV, has evolved despite such contrasting gradients in climate. Although mean temperature decreases with latitude along both coasts, the Atlantic gradient is three times steeper than the Pacific temperature gradient. Moreover, seasonality, i.e. the degree of seasonal temperature fluctuations, changes greatly with latitude along the Atlantic coast, while being latitude-independent along the Pacific coast (figure 4c,d).
the thermally constrained growing season at higher latitudes ([8], figure 2). The greater acceleration of growth capacity with temperature in northern Atlantic silversides might therefore be the result of additive selection forces, i.e. those generally compensating for latitudinal decreases in mean temperature plus those compensating for the decrease in growing season.

We conclude that small latitudinal gradients in mean temperature are sufficient to elicit adaptive CnGV in Pacific silversides, which display parallel growth reaction norms across latitudes. In contrast, Atlantic silversides display much higher plasticity in growth and also increased plasticity at higher latitudes. It is the plasticity of growth that represents adaptation to highly seasonal environments driven by the size-selectivity of winter mortality. Thus, seasonality gradients have a strong magnifying effect on thermal growth plasticity of high-latitude populations. These conclusions are not restricted to fish, but probably shape local adaptation patterns in other vertebrate and invertebrate taxa in a similar way. Metabolic compensation via CnGV is equally prominent in amphibians [11], molluscs [10] and insects [9,40,42], hence gradient effects should also be evaluated across taxa, e.g. by a meta-analysis of all published cases of growth CnGV.

Noteworthy constraints of our approach include, first, its focus on a brief early period in both species life cycle. While growth differences in Atlantic silversides are known to persist until adult life [43], this is not known yet for Pacific silversides, although growth differences in our experiment persisted well beyond the reported growth interval (until approx. 4–5 cm TL; H. Baumann 2010, unpublished data). Second, it is not known yet for *A. affinis*, how traits like growth rate or body size vary phenotypically in the wild, hence, whether the genetic differences partially compensate, equalize or overcompensate for latitudinal temperature differences. Third, comparing the Pacific (weak-temperature/no-seasonality) with the Atlantic gradient (strong-temperature/strong-seasonality),

**Figure 4.** Correspondence of latitudinal growth adaptations in (a) Pacific and (b) Atlantic silversides to large-scale climate gradients along the (c) Pacific and (d) Atlantic coast. (a,b) Population-specific colour shading depicts areas between 10th and 90th percentiles of temperature-specific growth capacity, calculated from combined data by dividing the percentile differences in TL before and after each growth trial by its duration (days). P443 N data were adjusted for year effect. (c,d) Colour shading depicts areas between average long-term annual minima (winter) and maxima (summer) of temperature per 1° latitude along the Atlantic and Pacific coast. The characteristic mid-latitude depression in Pacific summer temperatures coincides with the extent of the California Current upwelling system [51]. Dotted lines denote the latitudinal origin of investigated silverside populations, solid lines depict the thermal threshold of species-specific growth potentials (*A. affinis*: 10°C; *M. menida*: 12°C). (a) Pacific—*Atherinops affinis*, red lines/area, P128 N; green lines/area, P233 N; blue lines/area, P337 N; black lines/area, P443 N. (b) Atlantic—*Menidia menidia*, red lines/area, SC32 N; green lines/area, NY41 N; blue lines/area, NS44 N.
and contrasting two species (even if ecologically and taxonomically equivalent) is inevitably imperfect from a strict ‘experimental design’ point of view. *Atherinops affinis* lives longer, attains larger sizes and spawns repeatedly (iteroparity), more so than *M. menidia*. Species-specific life-history differences may have contributed to the observed differences in CnGV patterns.

(c) Implications for a changing climate

We posit that CnGV will be the principal mechanism by which ectotherms adapt to temporal gradients such as those elicited by global climate change. If so, our findings indicate that even small increases in mean temperature will alter local genotype frequencies in many species. Within species ranges, phenotypic similarity rather than divergence is expected owing to the opposing effects of genetic and plastic responses. In silversides, for example, some sub-maximal growth capacity presently confers the highest fitness at a given latitude. A rise in temperature would result in ‘too fast’ growth of the previously fittest genotypes, meaning that they will incur higher physiological growth costs than previously less-successful slower growing genotypes, which then will become the fittest at this location. The overall effect is a poleward migration of genotypes, either literally (if possible) or via natural selection, which remains masked by plastic responses to temperature. Empirical evidence for such temporal CnGV is still sparse but increasingly emerging [44–47].

Genetic shifts may produce little phenotypic change within a species range [6], but at its extremes climate change will have visible consequences. Species ranges should gradually shift towards higher latitudes, because at low latitudes, the genetic potential to evolve lower growth capacities in response to higher temperatures is exhausted, and habitat will be lost. At high latitudes, new habitat will become available to the most extreme genotypes, i.e. those with the highest species-specific growth capacities. This prediction is consistent with the already large and expanding evidence for shifting distributions in many marine and terrestrial taxa worldwide (e.g. [48–50]). In addition, the predicted increases in poleward heat transport [1] entail that warming will likely be disproportional at higher than lower latitudes, thus altering seasonality gradients and implying poleward expansions rather than uniform shifts of species ranges.

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