

Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity

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Abstract

Understanding the extent to which phylogenetic constraints and adaptive evolutionary forces help define the physiological sensitivity of species is critical for anticipating climate-related impacts in aquatic environments. Yet, whether upper thermal tolerance and plasticity are shaped by common evolutionary and environmental mechanisms remains to be tested. Based on a systematic literature review, we investigated this question in 82 freshwater fish species (27 families) representing 829 experiments for which data existed on upper thermal limits and it was possible to estimate plasticity using upper thermal tolerance reaction norms. Our findings indicated that there are strong phylogenetic signals in both thermal tolerances and acclimation capacity, although it is weaker in the latter. We found that upper thermal tolerances are correlated with the temperatures experienced by species across their range, likely because of spatially autocorrelated processes in which closely related species share similar selection pressures and limited dispersal from ancestral environments. No association with species thermal habitat was found for acclimation capacity. Instead, species with the lowest physiological plasticity also displayed the highest thermal tolerances, reflecting to some extent an evolutionary trade-off between these two traits. Although our study demonstrates that macroecological climatic niche features measured from species distributions are likely to provide a good approximation of freshwater fish sensitivity to climate change, disentangling the mechanisms underlying both acute and chronic heat tolerances may help to refine predictions regarding climate change-related range shifts and extinctions.

Keywords: acclimation ability, climate change, critical thermal maxima, niche conservatism, species' distributions, thermal safety margins

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Introduction

Understanding the mechanisms underlying large-scale variation in thermal tolerances is considered fundamental to assessing species vulnerability to climate change (Huey *et al.*, 2012; Chown & Gaston, 2016). Mounting evidence for terrestrial species suggests a relative degree of invariance in upper physiological limits across latitude (Addo-Bediako *et al.*, 2000; Sunday *et al.*, 2011; Hoffmann *et al.*, 2013), thus challenging the long-standing view that thermal conditions shape physiological tolerances and species' range limits. Such findings support the hypothesis that conservatism exists in physiological tolerances to heat, perhaps representing a latent artifact of ancestral regimes, whereas the importance of adaptation to current experienced temperatures is minor (Kellermann *et al.*, 2012; Araújo *et al.*, 2013). An emerging consequence of this research is that species inhabiting lower latitudes may be more physiologically susceptible to the direct effects of climate change than species at higher latitudes, despite the lower climatic exposure anticipated in these areas (Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008).

There exists substantial variation in the extent of conservatism across clades and geographical areas (e.g., Grigg & Buckley, 2013; Khaliq *et al.*, 2015). In particular, striking differences in the extent of range filling between marine and terrestrial ectotherms have been consistently reported, where evidence suggests that marine species are more physiologically adapted to the current experienced temperature than their terrestrial counterparts (Sunday *et al.*, 2012). Although several hypotheses have been proposed to explain this pattern (e.g., oxygen dependence of thermal tolerances; Pörtner & Peck, 2010), the general extent to which phylogenetic constraint, adaptation, and their interactions help define the physiological sensitivity of aquatic – including freshwater – species remains an unanswered question. Deciphering the underlying causes of phylogenetic signal (i.e., the tendency for closely related species to display similar trait values) in physiological traits may provide a more mechanistic basis to anticipate species' responses to climate change (Kellermann *et al.*, 2012; Hoffmann *et al.*, 2013).

Based on the current thermal tolerances of species, widespread climate change-related extinctions have been predicted in the coming decades (Urban, 2015). However, the ability to tolerate and buffer the

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short-term consequences of increases in temperatures and variability will depend not only on species' upper tolerance limits, but also on their acclimation ability (Bozinovic *et al.*, 2011; Seebacher *et al.*, 2015). If species show scope for plastic responses, then extinction risks may be lower than currently estimated, especially in aquatic environments where physiological plasticity may be favored over behavioral plasticity (Gunderson & Stillman, 2015). Whether similar selective pressures have driven the evolution of upper thermal tolerance and its associated plasticity and whether plasticity itself displays phylogenetic conservatism is still a matter of debate (Stillman, 2003; Calosi *et al.*, 2008; Nyamukondiwa *et al.*, 2011; Overgaard *et al.*, 2011; Magozzi & Calosi, 2015).

The principle of allocation predicts that upper thermal tolerances have evolved at the expense of maintaining acclimation capacity of these limits, thus reflecting an evolutionary trade-off between these two traits (Stillman, 2003; Bozinovic *et al.*, 2011). Consequently, the most heat-tolerant species may be at greater risk from warming, not only because they are already living close to their thermal tolerance limits, but also because they are likely to exhibit reduced resilience to change via either phenotypic or evolutionary rescues, including genetic assimilation (Sgrò *et al.*, 2016). Another implication is that if thermal tolerances are phylogenetically conserved, one might expect a strong phylogenetic signal where closely related species display similar levels of plasticity and thermal tolerance. Conversely, higher levels of plasticity are expected to have evolved for species that occupy heterogeneous thermal habitats (e.g., greater range size; Calosi *et al.*, 2008), but be limited or lost for species from more stable environments. Inherent to this prediction is the notion that the potential to respond to changing environments incurs a cost that is selected against (Somero, 2010; Overgaard *et al.*, 2011). As a consequence, the spatiotemporal variability of species' thermal habitats may have synergistic or decoupled effects on the evolution of these two physiological traits, complexity that should be accounted for when assessing species vulnerability to climate change.

Here, we provide the first attempt to assess the contribution of common selection pressures and phylogenetic constraints in thermal tolerances and acclimation capacity for freshwater fishes. Fish represent the most taxonomically diverse group of vertebrates – among which most species inhabit fresh waters and play a central role in the structure and function of these ecosystems (Closs *et al.*, 2016). However, analyses of the spatial patterns of freshwater fish vulnerability are relatively few compared with other taxonomic groups. To do so, we investigated the links between upper tolerance limits, acclimation capacity, and species' thermal

habitat, in terms of seasonal temperatures, variability, and extent of thermal specialization. We further evaluated the degree of conservatism in these two physiological traits and tested whether similarities between closely related species can be explained by evolution in response to shared environmental constraints (phylogenetically structured adaptation) or by retention of ancestral niche characters (phylogenetic inertia).

Materials and methods

Upper tolerance reaction norms

We conducted a systematic review of the primary and gray literature to collect critical thermal maxima (CT_{max}) of freshwater fish species obtained under laboratory conditions involving a dynamic change in temperature at a constant rate until a critical thermal end point was reached (e.g., loss of equilibrium; Lutterschmidt & Hutchison, 1997). We considered species that live all (stenohaline species), or a critical part of their life (euryhaline species) in either freshwater inland or brackish estuaries, excluding coral-associated fish that spawn in mangroves or marine species only occasionally found in freshwaters (Moyle & Cech, 2000). We selected species for which at least two different temperatures of acclimation were used across a thermal window spanning more than 5 °C but with consecutive acclimation temperatures separated from <10 °C. The final database included 82 species from 27 families representing a total of 829 CT_{max} experiments (based on combinations of species \times acclimation temperatures; range = 2–54, mean = 10 per species; Table S1).

Plasticity, defined here as the ability of species to increase their thermal tolerances when exposed to higher temperature, was determined using upper thermal limit temperature reaction norms (Murren *et al.*, 2014). While the relationship between upper thermal limit and acclimation temperature is typically nonlinear, species may display considerable variability in their responses to temperature increase (Claussen, 1977; Magozzi & Calosi, 2015). We thus used a model selection approach considering either linear, quadratic, or asymptotic regression models to best describe the relationship between species' CT_{max} and the temperatures of acclimation based on the Akaike information criterion (AIC). We chose the model with the fewest number of parameters based on the differences in AIC with the lowest AIC model ($\Delta AIC < 2$). If more than one experimental protocol was used (i.e., capture location, heating rate, endpoint), we included the ID of the experiments as a random factor in the model and determined the optimal random effects structure (random intercepts only or both random intercepts and random slopes for linear models, random asymptote only or both random asymptote and intercept for asymptotic models) using the same selection procedure. The acclimation response ratios ($ARR_{CT_{max}}$), describing the change in thermal tolerance across the range of acclimation temperature (Claussen, 1977), were then calculated as the slope for every small temperature intervals of 0.5 °C along the fitted reaction norms and then averaged to get an overall

estimate per species. This allowed quantifying the magnitude of plasticity while accounting for potential nonlinearity (shape) in plastic responses.

Similarly, CT_{\max} was calculated as the mean thermal tolerance obtained across the range of acclimation temperatures. This approach accounted for the fact that we were interested in between-species differences in thermal tolerances with respect to a range of thermal environments experienced within their range (as opposed to seasonal extremes only, e.g., Sunday *et al.*, 2014) and that the maximum acclimation temperature used for each species might be somewhat arbitrary with respect to the highest temperature for long-term survival (Richard *et al.*, 2012). By doing so, we assumed that the thermal windows used during the experiments were likely to be representative of the temperatures experienced by the species in their habitat; an assumption supported by the strong association found between the temperatures of acclimation and the temperatures experienced by species across their range (Fig. S1).

Thermal habitat

Fish species' distributions were collected from the International Union for Conservation of Nature (IUCN, 2016). Monthly mean air surface temperatures for the period 1951–2000 at a $0.5^\circ \times 0.5^\circ$ grid resolution were obtained from the Climatic Research Unit (CRU v3.23; CRU, 2015) – a spatially interpolated high-resolution dataset from climate stations. To account for the sigmoidal-shaped relationship between air and water temperature (Mohseni & Stefan, 1999), monthly air temperatures of each grid cell were converted to water temperatures using previously derived nonlinear regressions for the different Köppen–Geiger climate zones (Punzet *et al.*, 2012) and were then averaged over the period 1951–2000. Note that this calculation did not take into account fine-scale temporal climatic variability or the existence of local thermal refuges offered by riparian shading or phreatic groundwater inputs. The species' spatial distributions and the water temperatures were then both projected onto a grid of $1^\circ \times 1^\circ$ cells, and species presence within each grid cell was defined if any part of the distribution overlapped with the respective cell.

A number of summary metrics were calculated. First, to describe the seasonal extremes experienced by a species across its range, we calculated the mean temperature of the coldest ($Thab_{\min}$) and warmest ($Thab_{\max}$) months among occupied grid cells. Second, to describe the temporal variation in climatic conditions experienced by a species across its range, we calculated the mean annual temperature seasonality (i.e., $Thab_{\text{var}}$; standard deviation of the monthly temperatures) among occupied grid cells. We used a measure of temperature change over the course of the year, instead of say, the annual temperature range, as temperature seasonality is generally considered as a main driver of macrophysiological variation (Deutsch *et al.*, 2008). Although the physiologies of organisms are more likely to evolve in response to extreme temperature events or local experienced temperature regimes (Gutschick & BassiriRad, 2003), we considered only average measures to circumvent errors arising from the difficulty in identifying

species' ranges accurately as well as the relative coarseness of the available climatic data. Finally, to describe the extent of thermal specialization of species, we used the outlying mean index (OMI; Doledec *et al.*, 2000) to estimate species' niche breadth (Niche breadth) in a multivariate climatic space defined by the three aforementioned variables. This allowed to account for the multidimensional nature of species' realized niche across temporally and spatially varying thermal conditions, which may be more adequate to understand the variability and evolutionary patterns in thermal limits (Gouveia *et al.*, 2014).

Phylogeny

Phylogenetic associations among freshwater fish species were based on the dated phylogeny of Rabosky *et al.* (2013), which is available at <http://dx.doi.org/10.5061/dryad.j4802>.

Statistical analyses

To determine whether phylogeny and the thermal conditions experienced by species across their range influence their physiological thermal limits and associated plasticity, we initially tested for the presence of phylogenetic signal in CT_{\max} and $ARR_{CT_{\max}}$ using a suite of analytical approaches. The first two methods, Pagel's λ and Bloomberg's K , measure the deviation from a Brownian motion process where traits evolve randomly at a constant rate following a random walk (Freckleton *et al.*, 2002; Blomberg *et al.*, 2003). For both indices, a value close to zero indicates phylogenetic independence, whereas a value of one indicates that the trait under study is evolving according to Brownian motion (i.e., the differences in trait value between species pairs are proportional to the time since divergence).

We also considered the area under the phylogenetic signal-representation (PSR) curve built upon phylogenetic eigenvector regression (PVR) as an alternative estimate of the amount of phylogenetic signal (Diniz-Filho *et al.*, 2012). PVR extracts eigenvectors from the phylogenetic distance matrix among species, and these are subsequently used in a multiple regression to provide an overall R^2 that indicates the degree of variation of the trait under study that is explained by phylogenetic structure. In the PSR curve, the eigenvectors are sequentially used to fit PVR models of increasing complexity and to plot the R^2 against the cumulative eigenvalues. This provides a representation of the shape of the relationship between trait variation and phylogenetic structure at distinct hierarchical levels along the phylogeny. Deviation from the 45° line, expressed as the PSR area, then indicates how fast the trait evolved relative to Brownian motion expectations (PSR < 0 indicates phylogenetic signal smaller than expected). These indices have proven to provide reliable measures of the strength of phylogenetic dependence among species trait values due to their phylogenetic relatedness that are comparable across different traits and phylogenies (but see Revell *et al.*, 2008; Münkemüller *et al.*, 2012 for further discussions about the relationship between phylogenetic signal and other evolutionary processes).

The significance of Pagel's λ was estimated using a likelihood ratio test against a model with $\lambda = 0$ (no phylogenetic dependence), whereas significance of Bloomberg's K and PSR area was estimated using permutation tests by comparing the observed values to 1000 values expected under random trait distribution among the tips of the phylogenetic tree (Diniz-Filho *et al.*, 2012; Münkemüller *et al.*, 2012). To test whether the evolution of the two traits significantly deviate from a pure Brownian motion process, we also contrasted the observed values against a model with $\lambda = 1$ using a likelihood ratio test as described above, and by simulating a null distribution of 1000 K and PSR area values using a Brownian motion model of trait evolution.

The associations between CT_{max} , $ARR_{CT_{max}}$ and species' thermal habitat features ($Thab_{max}$, $Thab_{min}$, $Thab_{var}$, and Niche breadth) were then determined using phylogenetic generalized least squares (PGLS) where Pagel's λ was used to adjust the variance-covariance matrix to account for phylogenetic nonindependence among species (Freckleton *et al.*, 2002). We performed an information theoretic model averaging approach using the Akaike information criterion corrected for small sample size (AICc). We developed the full set of models including all the combinations of predictors and averaged the parameter estimates for each predictor over the best set of models encompassing 95% of the summed Akaike weights (Grueber *et al.*, 2011). Predictors were standardized to z-scores to facilitate the interpretation of their strength (effect size) and direction (positive or negative) in relation to one another.

Finally, we performed a variance partitioning analysis to quantify the portion of CT_{max} and $ARR_{CT_{max}}$ variation that could be attributed to either phylogenetic inertia, adaptation to the thermal conditions experienced by species across their range or phylogenetically structured adaptation because closely related species tend to occur in spatial proximity and thus share similar selection pressures. To that end, we use PVR in which the eigenvectors were used in partial regressions to quantify the amount of variation in trait explained by the phylogenetic structure, the effect of species' thermal habitat after accounting for the effect of phylogenetic structure and the shared variation between them (Desdevises *et al.*, 2003). This analysis was performed using both the full set of climatic predictors and separately for each species' thermal habitat feature. Niche breadth was log-transformed to reduce skewness in all the analyses.

All analyses were performed in R version 3.1.2 (R Development Core Team, 2014) using the packages ADE4 (Dray & Dufour, 2007), APE (Paradis *et al.*, 2004), NLME (Pinheiro *et al.*, 2015), PHYTOOLS (Revell, 2012), PVR (Santos *et al.*, 2013), RASTER (Hijmans, 2015), and RGDAL (Bivand *et al.*, 2015).

Results

CT_{max} ranged from 24.67 to 42.59 °C (mean = 34.34 °C \pm 4.02 SD) and $ARR_{CT_{max}}$ from 0.07 to 0.91 (mean = 0.37 \pm 0.16 SD). In general, higher CT_{max} was observed at higher acclimation temperature, although the shape and magnitude of plastic responses varied across species (Fig. 1; Table S2). Both CT_{max} and

$ARR_{CT_{max}}$ were nonrandomly distributed across the phylogeny and demonstrated a significant phylogenetic signal irrespective of the method used (Fig. 2a; Table 1). CT_{max} was more closely tied to the phylogeny ($\lambda = 0.95$, $K = 0.44$, PSR area = -0.10), whereas $ARR_{CT_{max}}$ displayed a moderate phylogenetic signal ($\lambda = 0.58$, $K = 0.23$, PSR area = -0.19). In both cases, estimates of $\lambda < 1$ indicated that physiological limits to heat stress were not evolving strictly under a Brownian motion model of evolution ($P < 0.01$), a finding that was not entirely supported by K and PSR area.

$ARR_{CT_{max}}$ showed a significant association with CT_{max} and species displaying the highest upper thermal tolerances exhibited the lowest acclimation capacity (PGLS, $P < 0.001$; Fig. 2b). The model selection procedure revealed that CT_{max} was strongly related to the mean temperature of the warmest month ($Thab_{max}$) and to a lesser extent to species' climatic niche breadth (Fig. 3a–d; Table 2; Fig. S2a). Species exposed to warmer seasonal temperatures and found across a greater range of climatic conditions tended to display higher upper thermal limits. Thermal safety margins – the difference between CT_{max} and $Thab_{max}$ – varied from 2.26 to 19.94 °C with a mean value of 11.63 °C. No association was found with respect to temperature seasonality ($Thab_{var}$) or the temperature of the coldest month ($Thab_{min}$).

The variance partitioning analysis further indicated that the pattern of phylogenetic conservatism in CT_{max} was mainly driven by phylogenetically structured adaptation (38%; shared variation between phylogenetic structure and the thermal conditions experienced by species across their range: $Thab + Phylo$) rather than

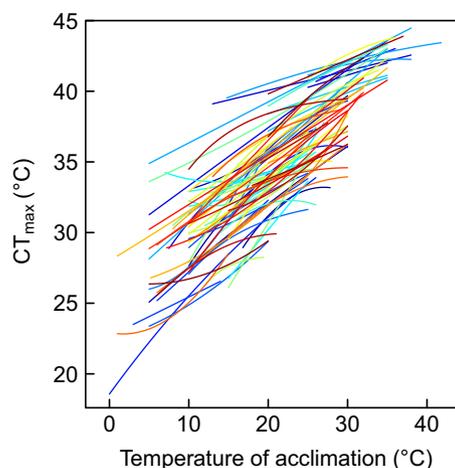


Fig. 1 Species' response curves fitted between critical thermal maxima and the temperature of acclimation for the 82 species considered in this study. Each species is represented by a different color.

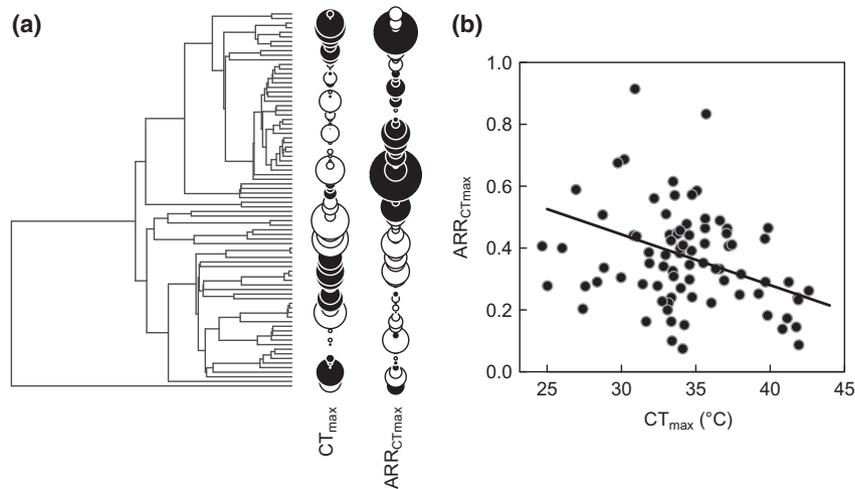


Fig. 2 (a) Distribution of upper thermal tolerance (CT_{max}) and acclimation capacity ($ARR_{CT_{max}}$) with respect to species' phylogeny. Circle size is defined according to the absolute scaled trait values (i.e., centered and scaled to have a mean of zero and standard deviation of one) and thus represent the deviation from the average value where black indicates smaller values than average (lower thermal tolerance and plasticity) and white higher values than average (higher thermal tolerance and plasticity). (b) Fitted relationship between acclimation capacity ($ARR_{CT_{max}}$) and upper thermal tolerance (CT_{max}) using a phylogenetic generalized least squares (PGLS; $P < 0.001$).

Table 1 Phylogenetic signal in (a) upper thermal tolerance (CT_{max}) and (b) acclimation capacity ($ARR_{CT_{max}}$) assessed using Pagel's λ , Bloomberg's K , and the area of a phylogenetic signal-representation (PSR) curve. P_{random} and P_{BM} indicate whether the values significantly differed from a random (no phylogenetic dependence) and a Brownian motion processes, respectively

| Trait | Phylogenetic signal | P_{random} | P_{BM} |
|----------------------|---------------------|--------------|----------|
| (a) CT_{max} | | | |
| λ | 0.95 | <0.001 | <0.001 |
| K | 0.44 | 0.001 | 0.135 |
| PSR area | -0.10 | <0.001 | 0.642 |
| (b) $ARR_{CT_{max}}$ | | | |
| λ | 0.58 | <0.001 | <0.001 |
| K | 0.23 | 0.016 | 0.002 |
| PSR area | -0.19 | <0.001 | 0.823 |

by phylogenetic inertia (9%; Phylo) (Fig. 4). Adaptation to species' thermal habitat after accounting for the effect of phylogenetic structure ($Thab$) also represents a non-negligible proportion of variation in CT_{max} (21%), mainly driven by variation in seasonal temperature extremes ($Thab_{max}$ and $Thab_{min}$; Fig. S3a). No association was found between $ARR_{CT_{max}}$ and any characteristics of species' thermal habitat using either PGLS (Fig. 3e–h; Table 2; Fig. S2b) or variance partitioning (4% of variance explained; Fig. 4; Fig. S3b). In contrast to CT_{max} , the pattern of phylogenetic conservatism in $ARR_{CT_{max}}$ appeared to result mostly from phylogenetic inertia rather than phylogenetically structured

adaptation, although the former explained only a fraction of the trait variation (24%).

Discussion

The study of physiological trait variation across large spatial and temporal scales has gained substantial attraction over recent years, notably due to its implications for understanding the susceptibility of species to climate change (Chown & Gaston, 2016). Here, we expand on earlier investigations based on terrestrial and marine ectotherms by providing a comprehensive assessment of how physiological tolerances to heat and acclimation capacity are linked to species distributions and evolutionary history of freshwater fishes.

We demonstrated that upper thermal tolerances were correlated with the temperatures experienced by species across their range, thus supporting the hypothesis that natural selection modulates physiological adaptation of species to warm temperatures in aquatic environments (Eliason *et al.*, 2011; Magozzi & Calosi, 2015). The analysis further revealed that the strong phylogenetic signal in thermal tolerance was largely related to spatially autocorrelated adaptive processes, in which closely related species are likely to be similar because they display limited dispersal from ancestral environments, and thus share common selection pressures, rather than phylogenetic inertia (Desclaves *et al.*, 2003). This pattern is likely the result of constraints imposed by the structure of hydrographic network in

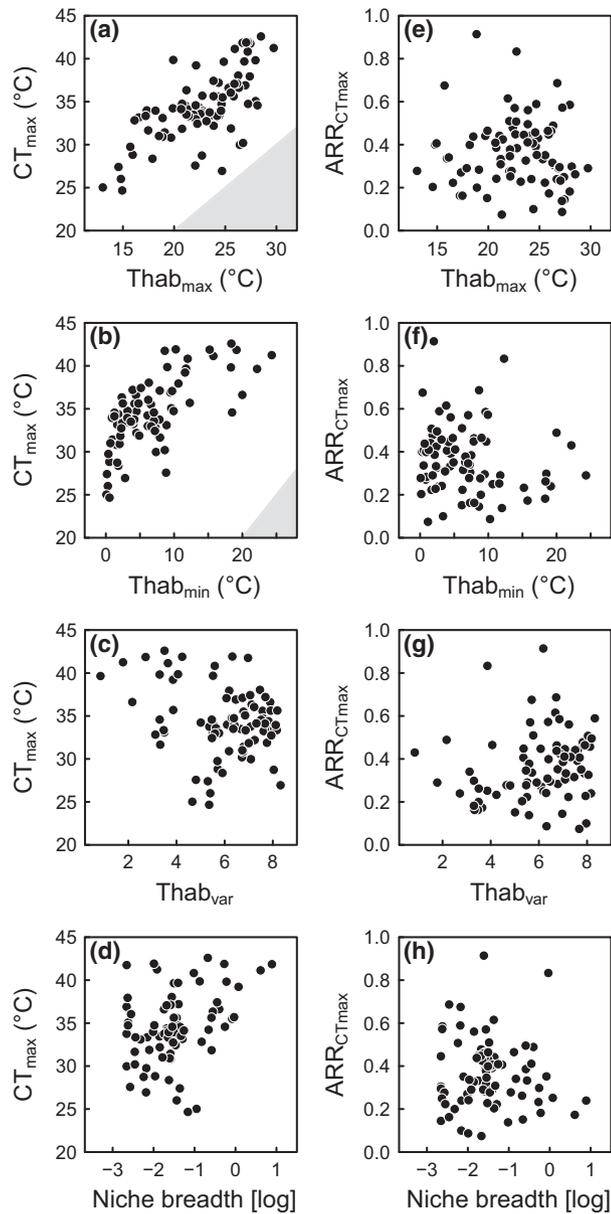


Fig. 3 Relationship of (a–d) upper thermal tolerance (CT_{\max}) and (e–h) acclimation capacity ($ARR_{CT_{\max}}$) with species' thermal habitat. Climate variables include: $Thab_{\max}$ (mean temperature of the warmest month); $Thab_{\min}$ (mean temperature of the coldest month); $Thab_{\text{var}}$ (mean temperature seasonality); Niche breadth (multivariate climatic niche breadth). The gray shaded zone in (a, b) corresponds to the climatic space where $CT_{\max} \leq Thab_{\max}$ or $Thab_{\min}$ (null or negative thermal safety margins).

which the dispersal of freshwater species is contingent on the existence of basin boundaries and hydrological connections (Olden *et al.*, 2010). The ultimate mechanism underpinning this relationship may lie as much with the availability of dissolved oxygen as it does with the reduced possibility for behavioral thermoregulation

Table 2 Results from the model averaging procedure using phylogenetic generalized least squares (PGLS) between (a) upper thermal tolerance (CT_{\max}) and (b) acclimation capacity ($ARR_{CT_{\max}}$) and species' thermal habitat

| Trait | β | 95% CI | w_{imp} |
|-----------------------|-------------|---------------------|------------------|
| (a) CT_{\max} | | | |
| $Thab_{\max}$ | 1.73 | 0.92 to 2.55 | 0.78 |
| $Thab_{\min}$ | 1.81 | –0.001 to 3.63 | 0.49 |
| $Thab_{\text{var}}$ | –0.16 | –2.22 to 2.01 | 0.68 |
| Niche breadth | 0.74 | 0.10 to 1.38 | 0.94 |
| (b) $ARR_{CT_{\max}}$ | | | |
| $Thab_{\max}$ | –0.39 | –1.16 to 0.37 | 0.73 |
| $Thab_{\min}$ | 0.77 | –0.27 to 1.81 | 0.51 |
| $Thab_{\text{var}}$ | 0.54 | –0.29 to 1.37 | 0.56 |
| Niche breadth | – | – | – |

Climate variables include: $Thab_{\max}$ (mean temperature of the warmest month); $Thab_{\min}$ (mean temperature of the coldest month); $Thab_{\text{var}}$ (mean temperature seasonality); Niche breadth (multivariate climatic niche breadth). w_{imp} is the relative variable importance based on the sum of Akaike weights of the models that include the variable. β whose 95% confidence intervals (95% CI) do not encompass zero are given in bold.

in aquatic environments (but see Davis *et al.*, 2013). Although fish are mobile organisms, the higher thermal sensitivity of larval stages coupled with their limited swimming ability may promote physiological adaptation as a primary strategy for coping with environmental variability (Pörtner & Peck, 2010).

From a conservation standpoint, most of the species examined here were found to occupy waters far below their upper thermal tolerances, and as such may appear unlikely to experience lethal temperatures in the near future. Caution must be taken, however, not to oversimplify the relationship between species' thermal limits and realized climatic habitats. In addition to the resolution of the climate data, experimental protocols may not adequately account for other environmental factors (e.g., prey availability; Gilbert & Miles, 2016) or the time dependence of physiological thresholds (Rezende *et al.*, 2011). Regardless, the close coupling between thermal tolerance and current experienced temperatures suggests that macroecological climatic niche features measured from species distributions are likely to provide a more direct physiological basis to assess species' sensitivity to climate change. This contrasts with other terrestrial ectotherms for which a strong climatic disequilibrium is typically reported (Araújo *et al.*, 2013), and suggests that species' responses to climate change might be more predictable in aquatic – including marine and freshwater – environments (Sunday *et al.*, 2012).

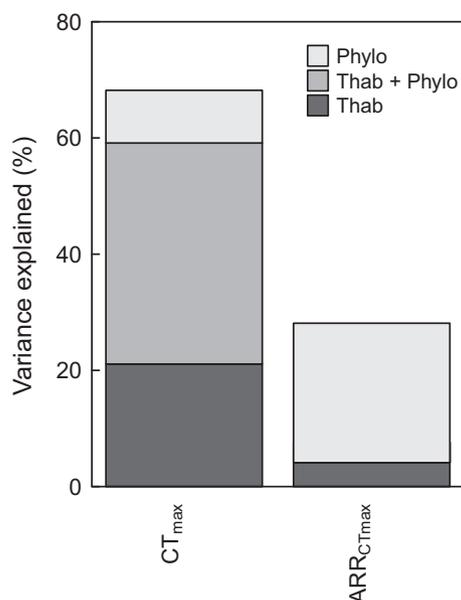


Fig. 4 Variance partitioning using phylogenetic eigenvector regressions (PVR) in upper thermal tolerance (CT_{max}) and acclimation capacity ($ARR_{CT_{max}}$) with respect to phylogenetic inertia (Phylo), adaptation to species' thermal habitat (Thab), and the shared variation between thermal habitat and phylogeny (Thab + Phylo). The overall percentage of variance explained by the phylogeny is the sum of phylogenetic inertia (Phylo) and the shared variation between thermal habitat and phylogeny (Thab + Phylo). The overall percentage of variance explained by species' thermal habitat is the sum of phylogenetically structured adaptation (Thab + Phylo) and adaptation to species' thermal habitat after accounting for the effect of phylogenetic structure (Thab).

In contrast to upper thermal tolerances, acclimation capacity was largely uncorrelated to species' thermal habitat, including both the magnitude and variability of water temperatures. Thermal generalists or species occupying more temporally variable environments did not display higher acclimation response ratios when compared to species occupying narrower or more stable thermal niches. Our findings corroborated two recent meta-analyses (Gunderson & Stillman, 2015; Seebacher *et al.*, 2015) that challenged the relative common assumption that the physiological flexibility of species should increase with latitude (i.e., climatic variability hypothesis; Bozinovic *et al.*, 2011). There was also little evidence for common adaptations to shared environments in closely related species. Instead, phylogenetic signal – albeit moderate – was related to phylogenetic inertia. Our findings thus suggest that the evolution of acclimation capacity may be associated with costs (e.g., antagonist selection on tolerance traits; Willett, 2010), reflecting an evolutionary trade-off with the evolution of thermal tolerances, rather than adaptation to species' thermal habitat. Despite this pattern, deciphering

whether the association between trait mean and plasticity resulted from a shared or independent mechanism may prove challenging (Sgrò *et al.*, 2016). For instance, the fact that thermal tolerances and acclimation capacity showed differential responses to the seasonal temperatures experienced by species suggests that adaptation to particular temperature regimes can evolve at least partly independently from the physiological flexibility of this limit (Seebacher *et al.*, 2005).

Our conclusions should be tempered, however, by the coarseness of the climatic variables used to describe species' thermal habitat, which importantly preclude the ability to account for fine-scale temporal climatic variability or the existence of local thermal refuges allowing thermoregulatory behaviors (Davis *et al.*, 2013). A growing body of evidence suggests that micro-geographic divergence in physiological trait, including acclimation capacity, is more widespread than is commonly appreciated, with strong local adaptations of populations to prevailing abiotic conditions (Eliaison *et al.*, 2011; Sorte *et al.*, 2011; Kelly *et al.*, 2012). Because plasticity may differ between populations as well as life stages, another implication is that measurements from single population and/or environment may not be applicable to species as a whole. Efforts should thus be made to account for variation in climatic conditions at a much finer spatial scale when investigating physiological adaptation in order to verify the patterns identified here.

We found that freshwater fish species with the highest upper thermal tolerances displayed the lowest plasticity of these limits, consistent with the hypothesis that specialization to thermal extremes is accompanied by a decrease in the ability to respond to changing environments (Somero, 2010; Overgaard *et al.*, 2011). Such trade-offs have been previously reported in marine crabs (Stillman, 2003) and prawns (Magozzi & Calosi, 2015) but not in aquatic beetles (Calosi *et al.*, 2008) and in some terrestrial flies (Nyamukondiwa *et al.*, 2011), thus suggesting limited generalization across taxonomies (Gunderson & Stillman, 2015). These discrepancies have been proposed to arise from contrasted evolutionary trajectories of thermal tolerances due to differences between extent and ancestral environments (Bozinovic *et al.*, 2011). By focusing on a group that occurs in both freshwater and marine environments but share a common freshwater ancestor such as fishes (Carrete Vega & Wiens, 2012), one might evaluate whether the transition from relatively variable to more stable thermal habitat has led to diverging selective pressures for marine species.

As ecologists seek to reduce uncertainty and improve accuracy in model projections, it is becoming abundantly clear that incorporating key parameters linked to

physiological limits, plasticity, and adaptive evolution has an important role to play (Catullo *et al.*, 2015). Although the analyses presented here provide an important step in understanding how thermal tolerances and acclimation capacity are distributed across species, the fact that for freshwater fishes the portion of trait variation that can be attributed to purely phylogenetic inertia is small does not imply that thermal tolerances may evolve rapidly and free from phylogenetic restrictions. Indeed, evolutionary responses to climate change may still be limited by many genetic and demographic constraints in which adaptation to past selection regimes may become tomorrow's constraint (Blomberg & Garland, 2002). Characterizing the tempo and mode of evolution of these two traits and their evolutionary trade-off will thus be of prime importance to predict and quantify climate-related impacts on biodiversity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of species examined in this study and characteristics of the thermal experiments.

Fig. S1. Comparison between the mean temperature of acclimation used during the experiments and the mean annual temperature across species' ranges.

Fig. S2. Model-averaged standardized regression coefficients (β) for phylogenetic generalized least squares (PGLS) between (a) upper thermal tolerance (CT_{max}) and (b) acclimation capacity ($ARR_{CT_{max}}$) and species' thermal habitat.

Fig. S3. Variance partitioning using Phylogenetic eigenVector Regressions (PVR) in (a) upper thermal tolerance (CT_{max}) and (b) acclimation capacity ($ARR_{CT_{max}}$) performed separately for each species' thermal habitat feature.