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HORIZONS

Global patterns in copepod thermal tolerance

MATTHEW SASAKI * AND HANS G. DAM

DEPARTMENT OF MARINE SCIENCES, UNIVERSITY OF CONNECTICUT, 1080 SHENNECOSSETT RD, GROTON, CT 06340, USA

*CORRESPONDING AUTHOR: matthew.sasaki@uconn.edu

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Copepods are key components of aquatic habitats across the globe. Understanding how they respond to warming is important for predicting the effects of climate change on aquatic communities. Lethal thermal limits may play an important role in determining responses to warming. Thermal tolerance can vary over several different spatial and temporal scales, but we still lack a fundamental understanding of what drives the evolution of these patterns in copepods. In this Horizons piece, we provide a synthesis of global patterns in copepod thermal tolerance and potential acclimatory capacities. Copepod thermal tolerance increases with maximum annual temperature. We also find that the effects of phenotypic plasticity on thermal tolerance are negatively related to the magnitude of thermal tolerance, suggesting a potential trade-off between these traits. Our ability to fully describe these patterns is limited, however, by a lack of spatial, temporal and phylogenetic coverage in copepod thermal tolerance data. We indicate several priority areas for future work on copepod thermal tolerance, and accompanying suggestions regarding experimental design and methodology.

KEYWORDS: Copepod; thermal tolerance; plasticity; acclimation; macrophysiology

INTRODUCTION

Copepods are some of the most abundant animals on the planet (Humes, 1994; Turner, 2004; Huys *et al.*, 2016). By nature of their abundance, they play important roles in aquatic food webs (Turner, 2004; Menden-Deuer and Kiørboe, 2016; Dam and Baumann, 2017), transferring energy from phytoplankton and microzooplankton to higher trophic levels. Copepods are also key components of biogeochemical cycles (Ducklow *et al.*, 2001; Buitenhuis

et al., 2006; Steinberg and Landry, 2017). How copepods respond to climate change will affect the fate of aquatic communities.

Thermal limits, temperatures at which performance and fitness drop to zero, strongly affect patterns in vulnerability to warming (Deutsch *et al.*, 2008; Pinsky *et al.*, 2019). Several large projects have examined global patterns in lethal thermal limits, or thermal tolerance, for diverse taxa (Pinsky *et al.*, 2019; Sunday *et al.*, 2019; Vinagre *et al.*, 2019; Cereja, 2020). Data on copepod

lethal thermal limits, however, have been omitted from these projects for a variety of reasons; consequently, global patterns in thermal tolerance are unresolved for this important group. Elucidating these patterns and the underlying evolutionary mechanisms is important for our understanding of the processes that shape diversity, abundance and distribution of copepods, and therefore, our ability to predict the effects of climate change on aquatic communities.

Variation in thermal limits may stem from several sources, including the effects of phenotypic plasticity and genetic differentiation. Phenotypic plasticity refers to the ability of a single genotype to produce different phenotypes based on the environmental conditions experienced. This encompasses a wide range of physiological phenomena including acclimation, which refers to the general phenotypic effects of exposure to different non-stressful environments (unlike hardening, which refers specifically to the effects of exposure to stressful environments), irrespective of the timing of the exposure (compared with developmental phenotypic plasticity, for example which specifically refers to the effect of the environment experienced during development). The effects of plasticity on thermal tolerance are important to incorporate when considering how populations may respond to warming (Sasaki and Dam, 2019; Cavieres *et al.*, 2020; Leung *et al.*, 2021). In general, plasticity is expected to buffer organisms from the effects of warming by increasing thermal tolerance as temperatures increase. While often well below perfect acclimation (a 1:1 relationship between the increase in temperature and an increase in thermal tolerance), the effects of plasticity on thermal tolerance can be substantial (Gunderson and Stillman, 2015; Morley *et al.*, 2019).

Genetic differentiation also produces large amounts of variation in thermal limits. Often discussed in the context of local adaptation, several studies have documented genetic variation in the innate ability of populations to withstand temperature stress, as well as genetic variation in the effect of plasticity on thermal tolerance in copepods (Kelly *et al.*, 2012; Leong *et al.*, 2018; Pereira *et al.*, 2017; Healy *et al.*, 2019; Sasaki and Dam, 2019, 2020). These effects of genetic differentiation are apparent both between and within species, even in planktonic copepods (Sasaki *et al.*, 2019; Sasaki and Dam, 2019). The implication is that all of these mechanisms (plasticity in thermal tolerance, genetic differentiation and variation in plasticity) can play an important role in shaping patterns in copepod thermal tolerance across various scales in the natural environment.

Here, we present the first global examination of patterns in copepod thermal tolerance and potential acclimatory responses of thermal tolerance. We show that

thermal tolerance generally increases with annual maximum temperature, that intertidal and freshwater copepods generally have higher thermal tolerance values than marine copepods, and that the potential for acclimation of thermal tolerance may be negatively related to thermal tolerance itself. Our results also highlight the limited spatial, temporal and phylogenetic coverage of copepod thermal tolerance data. We therefore provide several recommendations for research priorities and methods related to expanding the coverage of copepod thermal tolerance data.

CURRENT UNDERSTANDING OF COPEPOD THERMAL TOLERANCE PATTERNS

A detailed description of this study's methods is included in the Supplementary Data. Briefly, we used Web of Science to search the literature for papers reporting thermal tolerance values for copepods with the term “copepod AND (‘thermal tolerance’ OR ‘heat tolerance’ OR ‘thermal limit’ OR CTmax OR ‘lethal temperature’ OR LD50 OR ‘critical temperature’).” Our search yielded 265 thermal tolerance measurements for 41 copepod species from 29 studies that satisfied key criteria for inclusion (primarily: measuring thermal tolerance in degree Celsius, and including the geographic origin of copepods). Studies are summarized in Table I, with additional details about the studies and species included provided in Supplementary Tables I and II. Thermal tolerance was reported as either CTmax or LD50 values. There was no systematic difference between the two methodologies (Supplementary Fig. 1). No new thermal tolerance data are presented in this study. The earliest studies in our survey date from the 1970s (Fig. 1A). Some early work is not included in our analysis because it measured thermal tolerance in units of time (time to death or comatose state), rather than temperature (Bradley, 1978). Four major orders of copepods are represented, but the data set is dominated by Harpacticoida and Calanoida (Fig. 1B). This strong phylogenetic bias also corresponds to habitat type—the vast majority of harpacticoid thermal tolerance measurements come from intertidal taxa and most calanoid measurements are from the marine habitat. The limited number of cyclopoid measurements in the data set come from freshwater systems.

This taxonomic unevenness is even more prominent in the distribution of thermal tolerance measurements across genera (Fig. 2). A total of 23 genera have at least one thermal tolerance value represented in this data set, but the majority of measurements come from just two, the marine calanoid genus *Acartia* and the intertidal harpacticoid genus *Tigriopus*.

Box 1: Glossary

Thermal limit	Temperatures at which organismal performance and/or fitness is equal to zero. This multidimensional point is often approximated using individual traits, such as thermal tolerance.
Thermal tolerance	The ability to withstand exposure to high temperatures. Also commonly referred to as lethal thermal limits. Often quantified using measurements like Critical Thermal Maxima (CT_{max}) and Lethal Dosages (LD_{50}).
CT_{max}	A temperature at which organismal functions fail to maintain normal operations, generally resulting in the Loss of Righting Response (LRR), the Onset of Spasms (OS), and eventually mortality (Cowles and Bogert, 1944).
LD_{50}	A temperature resulting in 50% mortality over a given period of time, estimated from survivorship curves.
Genetic differentiation	Variation in the phenotype produced by the accumulation of differences at the genetic level.
Phenotypic plasticity	The ability of a single genotype to produce multiple phenotypes depending on the environmental conditions experienced.
ARR	Acclimation Response Ratio - the change in thermal tolerance per degree change in acclimation temperature. Often measured as the slope of a thermal tolerance reaction norm.

Table I: A list of studies included in this analysis from each of the major habitats

Habitat	Studies
Freshwater	Johnson, 1978; Verbitsky <i>et al.</i> , 2016; Buxton <i>et al.</i> , 2020
Intertidal	Damgaard and Davenport, 1994; Davenport <i>et al.</i> , 1997; Kelly <i>et al.</i> 2012; Kelly <i>et al.</i> , 2013; Li <i>et al.</i> , 2014; Pereira <i>et al.</i> , 2014, 2017; Tangwancharoen and Burton, 2014; Wallace <i>et al.</i> , 2014; Han <i>et al.</i> , 2018; Leong <i>et al.</i> , 2018; Healy <i>et al.</i> , 2019
Marine	Lahdes, 1995; González, 1974; Bradley, 1975; Bodoy <i>et al.</i> , 1977; Hirche, 1987; Wang <i>et al.</i> , 1990; Jiang <i>et al.</i> , 2008; Jiang <i>et al.</i> , 2009; Borchel <i>et al.</i> , 2018; Sasaki <i>et al.</i> , 2019; Sasaki and Dam, 2019, 2020
Subterranean	Castañó-Sánchez <i>et al.</i> , 2020

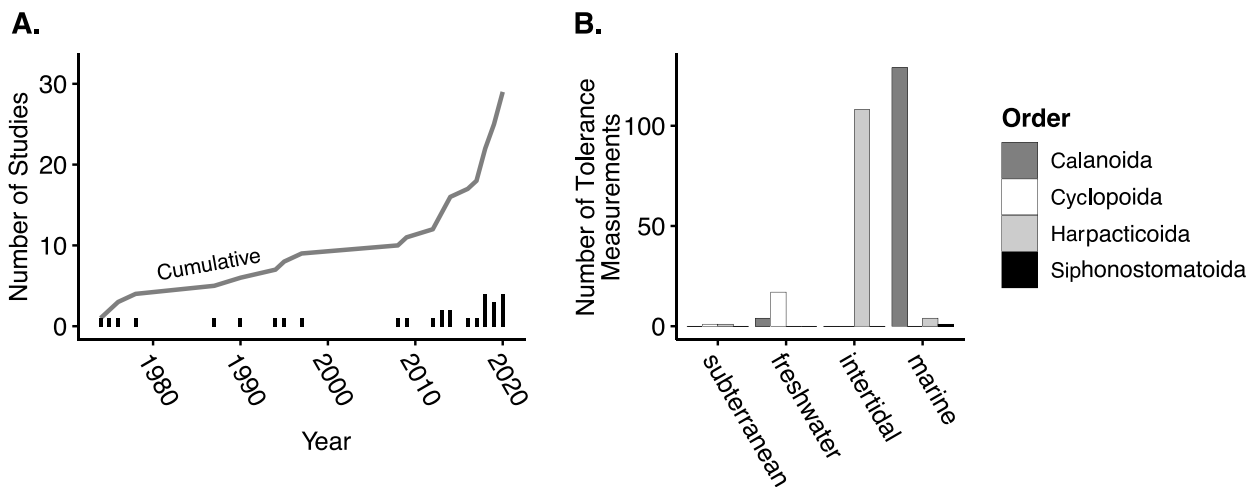


Fig. 1. A summary of published literature on copepod thermal tolerance. (A) The number of studies published per year, indicated by the bars, with a cumulative total over time (gray line). (B) The broad phylogenetic breakdown of copepod thermal tolerance measurements across the different habitats. Bars for the different Orders are differentiated by fill color.

LATITUDINAL PATTERNS

While the latitudinal coverage is broad, the geographic distribution is strongly biased toward the Northern Hemisphere (Fig. 3). Additionally, studies in the marine habitats are mostly restricted to coastal waters; freshwater and subterranean sampling sites are extremely limited and almost no thermal tolerance values have been reported for copepods, in any habitat, from tropical regions.

Studies have found that thermal tolerance is correlated with mean or maximum temperature climatologies in a wide range of taxa (Sunday *et al.*, 2019). The same pattern is evident here, with thermal tolerance increasing with maximum annual temperatures (Supplementary Table III; Fig. 4A). Climatological data were collected for a 1-km region around each sampling site from global high-resolution surface temperature data sets—the BIO-ORACLE or CHELSA data sets for

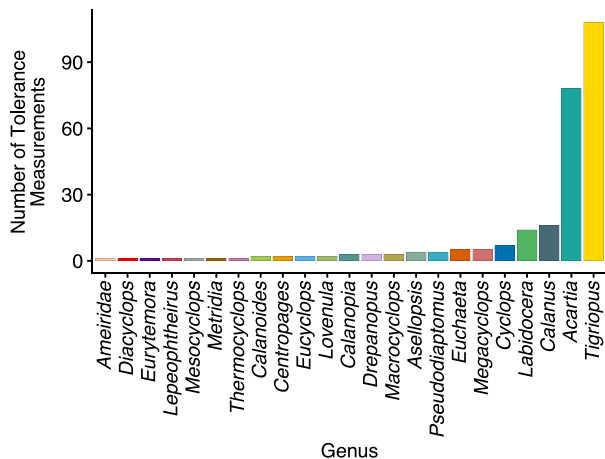


Fig. 2. The number of thermal tolerance values reported in the data set for each copepod genus.

marine and intertidal or freshwater sites, respectively (Karger *et al.*, 2017; Assis *et al.*, 2018). Maximum temperatures are the highest temperatures of the warmest month. It is possible that the temporal resolution of the temperature data does not capture extreme diel temperature fluctuations, as can be experienced in the intertidal environment by copepods such as *Tigriopus* (Leong *et al.*, 2018) and may therefore underestimate maximum temperatures in this case. It is also important to note that many marine species of copepods may experience large depth gradients in temperature. However, a large majority of thermal tolerance measurements for marine copepods come from shallow coastal waters, where diel vertical migrations are limited. The studies that did collect copepods from deeper waters were focused on the polar regions, where thermoclines are generally reduced. We therefore only consider sea surface temperature in this analysis. Climatological data were not available for the subterranean copepods ($n=2$; Castaño-Sánchez *et al.*, 2020), which are therefore excluded from the climatological analyses. As latitude and maximum temperature are strongly correlated, we focus on the environmental variable for these analyses. Intertidal and freshwater taxa (harpacticoids and cyclopoids) generally have higher thermal tolerance values than the (mostly calanoid) marine species (Supplementary Table III; Fig. 4A).

For studies that reported data for un- or minimally acclimated copepods, there was an asymptotic relationship between thermal tolerance and collection temperature, suggesting other factors may limit thermal tolerance in the field at high temperatures (quantity and quality of food resources, for example; Fig. 4B). The dashed 1:1 line in Fig. 4B indicates where thermal tolerance values would match collection temperatures. Unsurprisingly, no points fall below this line as this would indicate that copepods

were collected at temperatures above their lethal thermal limit. However, copepods collected at higher temperatures are closer to this threshold than copepods collected at lower temperatures.

This pattern of increased vulnerability in warm environments may apply across latitudinal temperature gradients as well. We estimated warming tolerance for all collection sites as the difference between thermal tolerance and maximum annual temperature at that site. Larger warming tolerances indicate that thermal tolerance well exceeds maximum temperatures. Copepods from warm, low latitude sites have smaller warming tolerances (Fig. 5). Negative warming tolerance should be interpreted with caution in this instance. As described below, acclimation to different conditions can strongly affect thermal tolerance, and the conditions experienced by copepods immediately prior to thermal tolerance measurements may differ from conditions experienced during the warmest times of the year.

POTENTIAL ACCLIMATION EFFECTS

Thermal tolerance values generally increased with acclimation temperature (Fig. 6). The colored lines represent regressions for individual genera that had thermal tolerance values measured at multiple acclimation temperatures. Note that by grouping data (across populations, species, studies, etc.), this representation combines the separate potential effects of acclimation and genetic differentiation on thermal tolerance. We, therefore, interpret this as an upper-bound to the relationship between acclimation temperature and thermal tolerance for various copepod genera.

The relationship between acclimation temperature and thermal tolerance can be represented as Acclimation Response Ratio (ARR), which standardizes the difference in thermal tolerance by the difference in acclimation temperature; i.e. the change in thermal tolerance per degree difference in acclimation temperature (Claussen, 1977). We estimated potential ARR values in three ways, grouping data at the population (as defined by sampling location), species or genus level (as illustrated in Fig. 6). In all cases, after grouping, ARR is estimated as the slope of a linear regression of thermal tolerance against acclimation temperature. These standardized estimates allow us to examine patterns in the evolution of tolerance plasticity.

As with thermal tolerance, patterns in the evolution of tolerance plasticity may also be driven directly by climatological factors. The Climate Variability Hypothesis posits that populations from more variable environments should have larger capacity for acclimation of thermal tolerance (Janzen, 1967; Stevens, 1989). The ARR

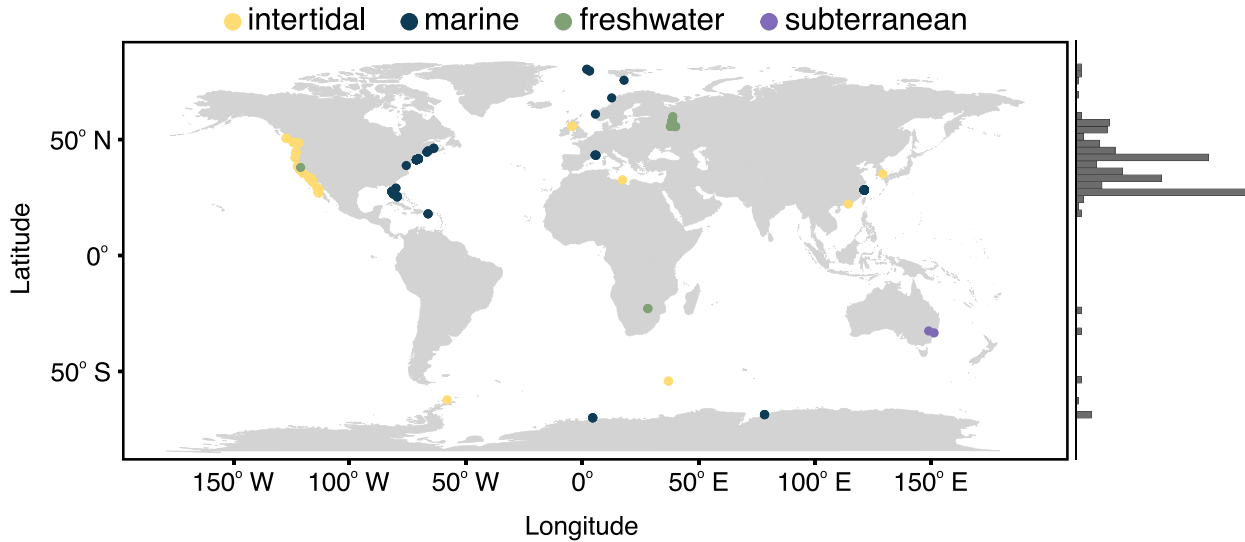


Fig. 3. Locations copepods were collected from. Sites in the different habitats are shown with different colored points. The marginal histogram along the right edge shows the latitudinal distribution of thermal tolerance measurements.

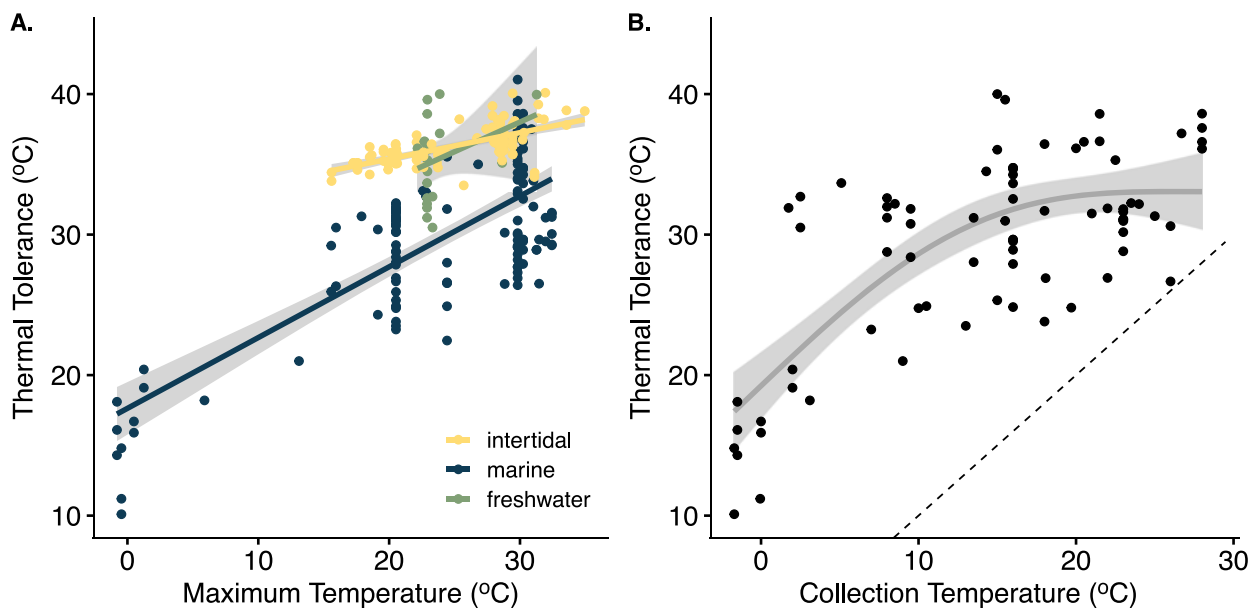


Fig. 4. (A) Thermal tolerance values plotted against maximum annual temperature at the site of collection. Thermal tolerance values for copepods from different habitats are shown with different colors. (B) The relationship between collection temperature and thermal tolerance values of un- or minimally acclimated copepods. A smoother (GAM; $k = 3$) is included. The dashed 1:1 line shows where collection temperature is the same as thermal tolerance values. Shading around regression lines represents the 95% confidence interval.

data for copepods do not support this hypothesis—ARR is not significantly related to temperature range at the site of collection when estimated at the population, species or genus level (Supplementary Table IV; Supplementary Fig. 2). However, other forms of temperature variation (temperature range during the season of occurrence, predictability of variation, etc.) may have a stronger influence on evolutionary patterns in ARR, which are not examined here (Bitter *et al.*, 2020).

Alternatively, the evolution of tolerance plasticity may be more strongly affected by a trade-off, wherein the evolution of increased tolerance reduces the capacity for acclimation (often referred to as the Trade-off Hypothesis; Stillman, 2003). This is supported by the data for copepod genera, with a strong negative relationship observed between ARR and thermal tolerance (Fig. 7A). There are, however, processes other than a mechanistic trade-off that can produce a similar negative relationship between tolerance and acclimation (van Heerwaarden

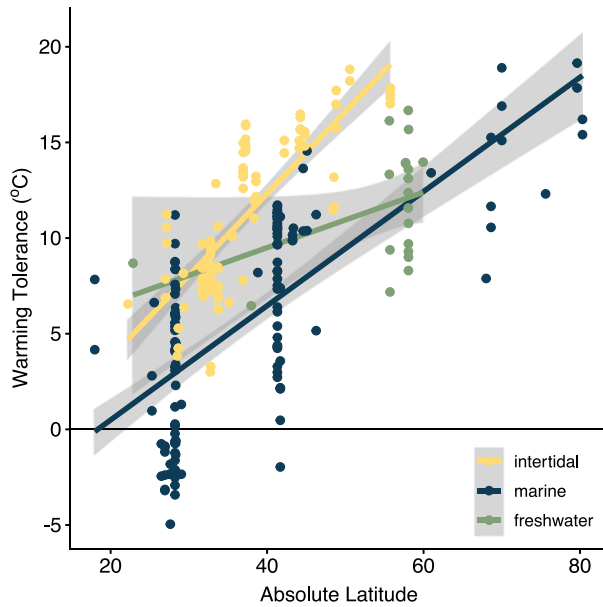


Fig. 5. Warming tolerances (thermal tolerance—annual maximum temperature) plotted against absolute latitude of collection locations. Small warming tolerances indicate that maximum temperatures are close to the thermal tolerance values of the copepods. Values from different habitats are shown as points with different colors. Shading around regression lines represents the 95% confidence interval.

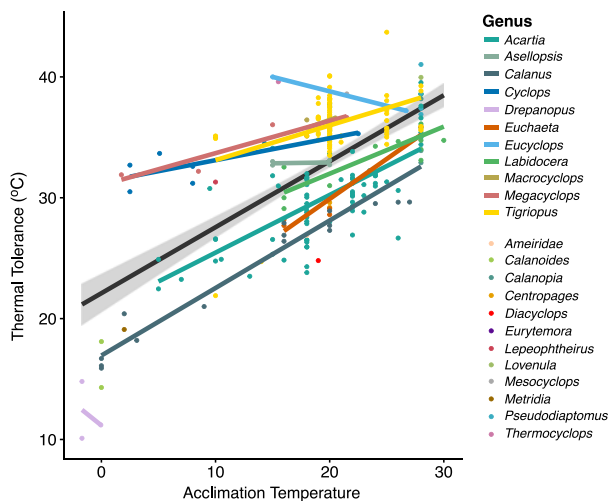


Fig. 6. Thermal tolerance values plotted against acclimation temperature. Each point represents a thermal tolerance measurement. The black line in the background represents the global regression of all thermal tolerance values against acclimation temperature. Separate regressions are included for genera represented by multiple thermal tolerance values. Shading around the regression line represents the 95% confidence interval.

and Kellermann, 2020). Regardless of the specific mechanism, this negative relationship with thermal tolerance also appears to shape patterns in the evolution of tolerance plasticity within copepod species, over

both spatial and seasonal scales (Sasaki *et al.*, 2019; Sasaki and Dam, 2019, 2020). This may be a general pattern in the evolution of tolerance plasticity in copepods.

This otherwise clear result is complicated, however, by the genus *Drepanopus*, represented by a single polar species, *Drepanopus bispinosus*. This species had both the lowest reported thermal tolerance values and a strongly negative ARR (indicating thermal tolerance decreased as acclimation temperature increased). Inclusion of this value results in a unimodal relationship between ARR and thermal tolerance (Fig. 7B). The same result is observed at the population and species levels as well (Supplementary Fig. 3). This fits with the common perception of polar species as cold-specialists with limited capacity to accommodate warming. This data point, however, is from a field study reporting thermal tolerance values for unacclimated individuals from just two collections (Wang *et al.*, 1990). Therefore, this strong, negative ARR value may be affected by other non-thermal environmental conditions. Combined with the considerable gap in the data between *Drepanopus* and the other genera, this precludes any large degree of confidence in this unimodal shape of the relationship between ARR and thermal tolerance values. This highlights the dire need to expand the spatial, temporal and phylogenetic coverage of thermal tolerance and ARR data in order to resolve the global relationships between temperature, thermal limits and acclimation capacity in copepods.

DISCUSSION

Based on the available data, large-scale spatial patterns in copepod thermal tolerance are generally similar to patterns observed in a range of diverse taxa—upper thermal limits increase with decreasing latitude or increasing maximum annual temperature. Patterns in ARR are better explained by patterns in the evolution of thermal tolerance than annual temperature range. There is significant uncertainty in these patterns, though, that hampers predictions about how copepods will respond to warming. This uncertainty is underlain by a lack of spatial, temporal and phylogenetic coverage of copepod thermal tolerance data.

Caveats and future directions

As is often the case for this type of literature survey, the latitudinal patterns in thermal tolerance and ARR values described here are based on a combination of sampling and experimental designs and, therefore, likely incorporate variation in thermal tolerance that would ideally be

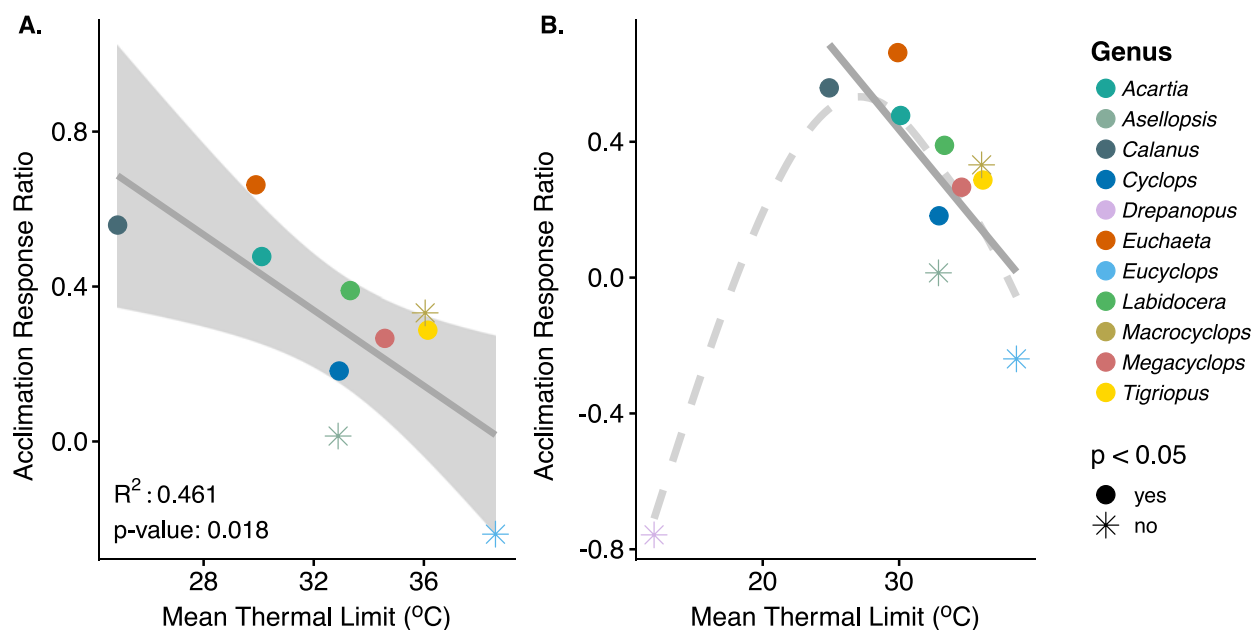


Fig. 7. ARRs plotted against mean thermal tolerance for several copepod genera. ARR values estimated from significant regressions are indicated with solid points, while values estimated from non-significant regressions are shown as asterisks. (A) ARR values for all copepod genera except *Drepanopus*, with the fitted linear regression. (B) All ARR values estimated, including that of *Drepanopus*. The same linear regression from panel A is included, as well as a GAM smoother across all data. Shading around regression lines represents the 95% confidence interval.

attributed to either genetic differentiation, the effects of acclimation or variation in ARR, and the influence of other environmental or biotic factors (food abundance, etc.). Hence, what is described here should serve primarily as a set of baseline predictions for global patterns in copepod thermal adaptation. We also note that the lethal temperature limits described here represent the most extreme biological effects of temperature, and there are many sub-lethal effects (on development, egg production, respiration, ingestion, gene expression, etc.) that will also play an important role in determining the response of copepods to ongoing climate change. These traits are affected by similar mechanisms and warrant their own treatment (Marshall *et al.*, 2020).

One other crucial consideration is the timescale of warming. Copepods are generally characterized by large populations and short generation times. As such, we generally expect adaptation to play a large role in determining how populations respond to a changing climate. A robust understanding of contemporary patterns in the evolution of thermal tolerance establishes a predictive framework for how populations may adapt to warming. For example, the significant relationship between thermal tolerance and maximum temperature suggests that, unsurprisingly, populations may evolve increased thermal tolerance as temperature increases, buffering copepod populations against the most extreme effects. However, if it comes at the expense of acclimation capacity,

as suggested by the negative relationship observed between ARR and thermal tolerance, this evolution of increased thermal tolerance may not actually reduce vulnerability to warming, as acclimation capacity likely carries its own fitness benefits in variable natural environments (Burggren, 2018). By characterizing how taxa have adapted to contemporary patterns in the thermal environment, we gain insight into how a rapidly changing climate may shape future patterns in adaptation.

In addition to providing an initial appraisal of large-scale patterns in copepod thermal tolerance, this dataset highlights several interesting and important unanswered questions. How does maximum temperature and temperature variability interact to shape latitudinal patterns in the evolution of thermal tolerance and ARR? Are patterns similar between temperate and tropical species? Between coastal and open ocean habitats? Are inter- and intra-specific patterns of thermal adaptation similar? Are differences in thermal tolerance between freshwater, intertidal and marine copepods driven by phylogeny or adaptation to different habitats? How does relatively fine scale temporal variation in temperature affect thermal tolerance? Does seasonal variation in temperature produce seasonal variation in thermal tolerance? Do large diel vertical migrations (and the resulting large daily temperature ranges) affect the evolution of thermal tolerance and ARR? Where will future warming challenge copepod thermal limits? Can acclimation of thermal

tolerance provide respite from these challenges? Several of these questions are further discussed below, but all of them highlight how copepods can serve as an ideal model system for investigating fundamental questions about how variation in the thermal environment affects patterns in thermal adaptation.

Priority areas

Given the ubiquity of copepods in both marine and freshwater habitats, taking advantage of local assemblages can be an effective means to increase the spatial, temporal and phylogenetic coverage of copepod thermal tolerance measurements, and to answer some of the important questions outlined above. We suggest prioritizing projects that examine copepods from low latitudes, polar regions and the open ocean; that break the taxonomy-habitat relationships that characterizes the current data set or that examine seasonal variation in thermal tolerance.

Coverage in low latitudes, polar regions and the open oceans

Almost no thermal tolerance or ARR measurements have been made for copepods from the Tropics. By extrapolating the limited coverage presented in this data set, we might expect these copepods to be among the most vulnerable to climate change (Nguyen *et al.*, 2011). Even though these species may have high thermal tolerance values, their thermal safety margins are small, and they may generally have low ARR values. This, however, is largely based on data from low latitude populations of temperate species of copepods. Data for tropical species is needed to test this prediction. A similar argument can be made for polar species, which have larger thermal safety margins, but potentially lack capacity for acclimation of thermal tolerance, making them vulnerable to increases in temperature. The open ocean is also drastically under-sampled relative to its spatial extent and its important role in global systems. Predictions for marine copepods are based mostly on data from copepods sampled from fringing coastal regions, which vary drastically from off-shore waters in many aspects of the thermal environment (Steele *et al.*, 2019). Taking advantage of the small space requirements and general portability of the equipment required to measure thermal tolerance, collaborating with regularly scheduled open ocean time series cruises (BATS, HOTS, etc.) may be an effective strategy for generating *in situ* thermal tolerance measurements in the open ocean. Generating ARR measurements for both coastal and open ocean species across a large latitudinal range may be especially useful for disentangling the effects of temperature variability and patterns in thermal tolerance on the evolution of ARR, as the open ocean exhibits

similar latitudinal temperature gradients but is generally much less variable than nearshore environments at similar latitudes.

Phylogenetic diversity of thermal tolerance measurements

Increasing the phylogenetic coverage of thermal limit data is crucial for breaking the order-habitat correlation that currently dominates the data set. Determining whether the large differences observed between the copepod orders are driven by phylogeny or adaptation to differences between aquatic habitats is crucial for understanding the underlying mechanisms of evolutionary patterns in thermal tolerance and ARR. Luckily, this can be readily addressed: harpacticoids, cyclopoids and calanoids co-occur in many freshwater and marine habitats. For example, the cyclopoid genus *Oithona* is numerically dominant across the global ocean but lacks a single lethal thermal limit measurement. Examining intra-specific patterns in *Oithona* thermal tolerance may yield interesting and important insights into adaptation in the plankton when compared with patterns observed in marine calanoids (Sasaki and Dam, 2019). It is also important to note, however, that generating thermal tolerance values for a diverse range of cyclopoids and harpacticoids in both marine and freshwater habitats is perhaps more important for resolving whether thermal tolerance differences between copepod orders are driven by phylogeny or environmental adaptation.

Seasonal variation in thermal tolerance

Seasonal variation in temperature likely generates seasonal variation in thermal tolerance in copepods (Sasaki and Dam, 2020). These fine scale temporal patterns are largely unexplored, but the fluctuating selection imparted by seasonality may generate or maintain adaptive genetic variation for both thermal tolerance and acclimation capacity, priming populations to respond to a rapidly changing climate. The precise effects of seasonality on the evolution of copepod thermal tolerance and ARR likely depend on generation time. Short-lived copepods experience the majority of environmental variation across generations, whereas relatively long-lived taxa such as *Calanus* can experience the entirety of an annual temperature cycle within a generation. The scaling of this temporal temperature variability relative to generation time will have a strong influence on how copepod taxa adapt to seasonal variation and how they will respond to climate change. Taking advantage of the relative

ease with which copepods can be collected from local habitats, fine temporal scale sampling (at sub-generational timescales) can provide important information regarding this source of adaptive variation.

Methodological considerations

Experiments to measure thermal tolerance are straight forward—expose copepods to a range of temperatures and record survivorship. While these conditions do not directly mirror conditions experienced by organisms in the field, there is a large body of literature linking these thermal tolerance measurements to range extent and habitat utilization, seasonal occurrence, vulnerability to heat waves and other stochastic events, and other important ecological dynamics (Stillman, 2002; Madeira *et al.*, 2012; Hoffmann *et al.*, 2013; Shultz *et al.*, 2016; Campbell-Staton *et al.*, 2017; Stuart-Smith *et al.*, 2017; Sasaki and Dam, 2019; Sorte *et al.*, 2019). Many of these linkages are unlikely to be direct consequences of variation in lethal thermal limits but instead indicate that thermal tolerance is a robust proxy for several different levels of thermal adaptation—increased thermal tolerance likely also indicates adaptation of metabolic processes, cell and membrane structure, etc., to increased temperatures. As in other groups, two metrics, CT_{max} and LD_{50} , are most commonly used for measuring thermal tolerance in copepods. It is important to consider the differences between them carefully, as it will affect how measurements can (or should) be interpreted.

Both CT_{max} and LD_{50} are commonly used metrics to quantify thermal tolerance. However, these metrics provide different information about how temperature affects organisms. CT_{max} indicates a temperature where organismal functions fail to maintain normal operations, generally resulting in the loss of righting response (LRR), the onset of spasms (OS) and eventually mortality (Cowles and Bogert, 1944). These values are typically estimated by exposing a single individual to a temperature ramp and observing them until the endpoint of choice (LRR, OR, mortality, etc.) is reached. The temperature at that point is recorded as the CT_{max} value for that individual.

In contrast, LD_{50} is the temperature inducing 50% mortality in a random sample of the population. This metric is typically measured by exposing replicate samples of a population to some static temperature for a fixed period of time. The survivorship curve is estimated by repeating these exposures across a range of temperatures and fitting a logistic regression to the survival data. LD_{50} is then estimated from the resulting curve. Thus, this metric represents a single point on a curve of the time-dependent likelihood of mortality across a range of temperatures.

Both metrics provide valuable information. CT_{max} is particularly useful when data for individual copepods

are required (for example, genome-wide association studies, heritability measurements or correlations with other traits such as body size). Where possible, however, we recommend using LD_{50} measurements to represent thermal tolerance. The more nuanced population-level, time-dependent likelihoods of mortality that this approach provides may be more useful for modeling vulnerability to both long-term trends and acute heat shock events such as heat waves than a static threshold value such as CT_{max} . While requiring more individuals, this approach is not infeasible for smaller, abundant copepod taxa that dominate many aquatic habitats (Willett, 2010; Pereira *et al.*, 2017; Sasaki and Dam, 2019, 2020). When dealing with larger, or less abundant taxa, LD_{50} measurements may be more difficult to make, in which case we recommend measuring CT_{max} on as many individuals as possible, using temperature ramping rates between 0.1 and 0.3°C per minute (Jiang *et al.*, 2009; Harada and Burton, 2019).

Field sampling vs. common garden experiments

Both *in situ* patterns of thermal tolerance observed via field sampling and the experimental disentangling of the effects of genetic differentiation and acclimation on thermal tolerance are important for clarifying some of the global patterns in thermal adaptation described here. There is value in understanding the spatial and temporal patterns of thermal tolerance of copepods acclimated to field conditions for predicting vulnerability to climate change, as it can help identify where other factors (salinity, food availability, etc.) affect thermal limits. Unraveling the evolutionary dynamics and adaptive mechanisms behind these patterns may provide more robust predictions about responses to long-term climatic changes. Examining the relative effects of genetic differentiation and acclimation on thermal tolerance is most commonly accomplished via common garden laboratory experiments (Kelly *et al.*, 2012; Pereira *et al.*, 2017; Sasaki and Dam, 2019, 2020). These experiments can be used to quantify ARR (by acclimating or rearing copepods at multiple temperatures under controlled conditions), allowing for more robust inference how acclimation to predicted future conditions may affect thermal tolerance and vulnerability to warming (Sasaki and Dam, 2019, 2020). Ideally, copepods should be cultured under common garden conditions for several generations to minimize the confounding effects of previous environmental acclimation before experiments are undertaken. Long-term laboratory culturing should be avoided, however, as selection by these environments can affect both thermal tolerance and tolerance plasticity (Sasaki and Dam, 2021).

CONCLUDING REMARKS

In many ways, copepods represent one of the best model systems for studying global patterns in thermal adaptation: They are abundant, diverse and exceptionally ecologically important in aquatic habitats across the globe. Several species are cultivable, allowing for the laboratory experiments needed to disentangle the effects of genetic differentiation and acclimation on patterns in thermal tolerance (Dam, 2013). The experimental set-up commonly used to measure thermal tolerance is generally portable, reusable and both cost- and space-efficient. Altogether, we have an opportunity to examine global patterns in the evolution of thermal tolerance and tolerance plasticity in a meaningful and cohesive way. By increasing the phylogenetic, spatial and temporal coverage of copepod thermal tolerance data, we can test well-established hypotheses and address fundamental questions about the evolution of thermal adaptations, while providing important baseline information about the vulnerability of aquatic communities to climate change.

DATA ARCHIVING

All data and code involved in these analyses are available in a publicly accessible repository at <https://doi.org/10.6084/m9.figshare.14776371.v2>.

SUPPLEMENTARY DATA

Supplementary is available at *Journal of Plankton Research* online.

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