





Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q_{10} effects: Why methodology matters

Justin C. Havird¹  | Jennifer L. Neuwald^{2,3}  | Alisha A. Shah^{2,4}  | Alexander Mauro^{2,3} |
Craig A. Marshall² | Cameron K. Ghalambor^{2,3} 

¹Department of Integrative Biology,
University of Texas at Austin, Austin, TX,
USA

²Department of Biology, Colorado State
University, Fort Collins, CO, USA

³Graduate Degree Program in Ecology,
Colorado State University, Fort Collins, CO,
USA

⁴Division of Biological Sciences, University
of Montana, Missoula, MT, USA

Correspondence

Justin C. Havird
Email: jhavird@utexas.edu

Funding information

The University of Texas at Austin; NSF,
Grant/Award Number: IOS-1457383

Handling Editor: Charles Fox

Abstract

1. Characterizing thermal acclimation is a common goal of eco-physiological studies and has important implications for models of climate change and environmental adaptation. However, quantifying thermal acclimation in biological rate processes is not straightforward because many rates increase with temperature due to the acute effect of thermodynamics on molecular interactions. Disentangling such passive plastic responses from active acclimation responses is critical for describing patterns of thermal acclimation.
2. Here, we reviewed published studies and distinguished between different study designs measuring the acute (i.e. passive) and acclimated (i.e. active) effects of temperature on metabolic rate. We then developed a method to quantify and classify acclimation responses by comparing acute and acclimated Q_{10} values. Finally, we applied this method using meta-analysis to characterize thermal acclimation in metabolic rates of ectothermic animals.
3. We reviewed 258 studies measuring thermal effects on metabolic rates, and found that a majority of these studies (74%) did not allow for quantifying the independent effects of acclimation. Such studies were more common when testing aquatic taxa and continue to be published even in recent years.
4. A meta-analysis of 96 studies where acclimation could be quantified (using 1,072 Q_{10} values) revealed that 'partial compensation' was the most common acclimation response (i.e. acclimation tended to offset the passive change in metabolic rate due to acute temperature changes). However, 'no acclimation' and 'inverse compensation', in which acclimation further augmented the acute change in metabolic rate, were also common.
5. Acclimation responses differed among taxa, habitats and with experimental design. Amphibians and other terrestrial taxa tended to show weak acclimation responses, whereas fishes and other aquatic taxa tended to show stronger compensatory responses. Increasing how long the animal was allowed to adjust to a new test temperature increased the acclimation response, but body size did not. Acclimation responses were also stronger with longer acclimation durations.

6. Collectively, these results highlight the importance of using the appropriate experimental design to investigate and estimate thermal acclimation of biological rates. To facilitate and guide future studies of thermal acclimation, we end with some suggestions for designing and interpreting experiments.

KEYWORDS

acclimation, arrhenius, climate change, metabolic theory of ecology, oxygen and capacity limited thermal tolerance, oxygen consumption, Q_{10} , thermal performance curve

1 | INTRODUCTION

Many organismal traits exhibit phenotypic plasticity in response to environmental variation. A fundamental, yet unresolved problem, is distinguishing whether such plasticity represents a passive or active response (Ghalambor, McKay, Carroll, & Reznick, 2007; Gotthard & Nylin, 1995; Schulte, Healy, & Fanguie, 2011; Whitman & Agrawal, 2009). For example, many biological rates increase exponentially with increasing temperature solely due to the thermodynamics of molecular interactions (Arrhenius, 1915). Such automatic responses that solely reflect the constraints of biochemical and biophysical laws can be termed 'passive' plasticity because they are not regulated by the organism (Ghalambor et al., 2007; Gotthard & Nylin, 1995; Kingsolver, 2009; Schulte et al., 2011; Whitman & Agrawal, 2009). On the other hand, traits displaying 'active' plasticity respond to environmental change through mechanisms which can be shaped by evolution at different levels of biological organization (Schulte, 2015; Whitman & Agrawal, 2009). Active plasticity in response to temperature may include changes in gene expression, membrane composition, enzyme concentrations or isoform profiles that increase performance at different temperatures (Angilletta, 2009; Cossins & Bowler, 1987; Hochachka & Somero, 2002). It should be noted that most plastic responses to the environment, even those involving thermodynamic processes, most likely reflect both 'passive' and 'active' processes (Whitman & Agrawal, 2009). Yet, disentangling passive and active plasticity is challenging because a trait may be shaped by both simultaneously.

The tension between active and passive thermal plasticity is exemplified when considering how biological rates respond to thermal acclimation. Acclimation (or acclimatization in natural environments) is a specific type of active phenotypic plasticity that is usually defined as a reversible phenotypic change due to exposure to an environment on the timescale of days to months (Angilletta, 2009; Chown, Haupt, & Sinclair, 2016; Holzman & McManus, 1973; Huey & Berrigan, 1996; Powell & Watts, 2006; Russell & Chappell, 2007; Schulte et al., 2011; Seebacher, Beaman, & Little, 2014; Tsuji, 1988; Vezina et al., 2006). In many cases, thermal acclimation will increase tolerance and performance at that temperature (Angilletta, 2009) and populations that show robust acclimation responses may be more resilient to variation in temperature and climate change

(Colinet, Sinclair, Vernon, & Renault, 2015; Rohr et al., 2018; Seebacher et al., 2014). Yet, many phenotypes that undergo thermal acclimation also show passive responses to temperature described by the Q_{10} coefficient—the factorial change in a biological rate associated with a 10°C increase in temperature. A fundamental challenge is that acute exposure causes a passive Q_{10} response, while continued exposure might result in an entirely different Q_{10} due to active acclimation effects. Although relatively rare, others have also differentiated between acute Q_{10} effects (passive plasticity) and thermal acclimation (active plasticity), which is paramount to predicting the effects of climate change (see Cossins & Bowler, 1987; Einum et al., 2019; Payne & Smith, 2017; Schulte et al., 2011; Seebacher, White, & Franklin, 2015).

Thermal acclimation in a biological rate can result in several types of responses (Cossins & Bowler, 1987; Huey & Berrigan, 1996; Kelsch & Neill, 1990; Precht, 1958; Prosser, 1958; Withers, 1992). Here we follow Huey and Berrigan (1996) in distinguishing five possible responses (Figure 1a): (a) *No acclimation*—rates increase with temperature simply due to passive plasticity, but no additional change is observed (i.e. only a strictly passive Q_{10} effect is observed); (b) *Partial compensation*—the passive rate change is counteracted by acclimation and the acclimated rate approaches the original rate; (c) *complete compensation*—the acclimated rate returns to the original rate, neutralizing the passive response (referred to as 'metabolic homeostasis' by Cossins & Bowler, 1987); (d) *over-compensation*—the acclimated rate 'overshoots' the original rate, more than compensating for the passive response; and (e) *inverse compensation*—the passive rate change is amplified with acclimation. Indeed, Einum et al. (2019) recently criticized studies of thermal acclimation for not distinguishing among these acclimation responses.

However, categorizing a thermal acclimation dataset into one of these responses is not straightforward. Many experimental designs are unable to distinguish between active versus passive responses to temperature (see Figure 2). For example, in an 'acclimation only' experimental design, animals are acclimated at one temperature, then tested at the same temperature. This is repeated across at least two temperatures, resulting in an 'acclimated Q_{10} ' (Table 1; Figure 2a). In an 'acute only' experimental design, a single acclimation temperature is used, but many test temperatures. Here, only passive thermal plasticity is quantified, resulting in a 'passive Q_{10} '

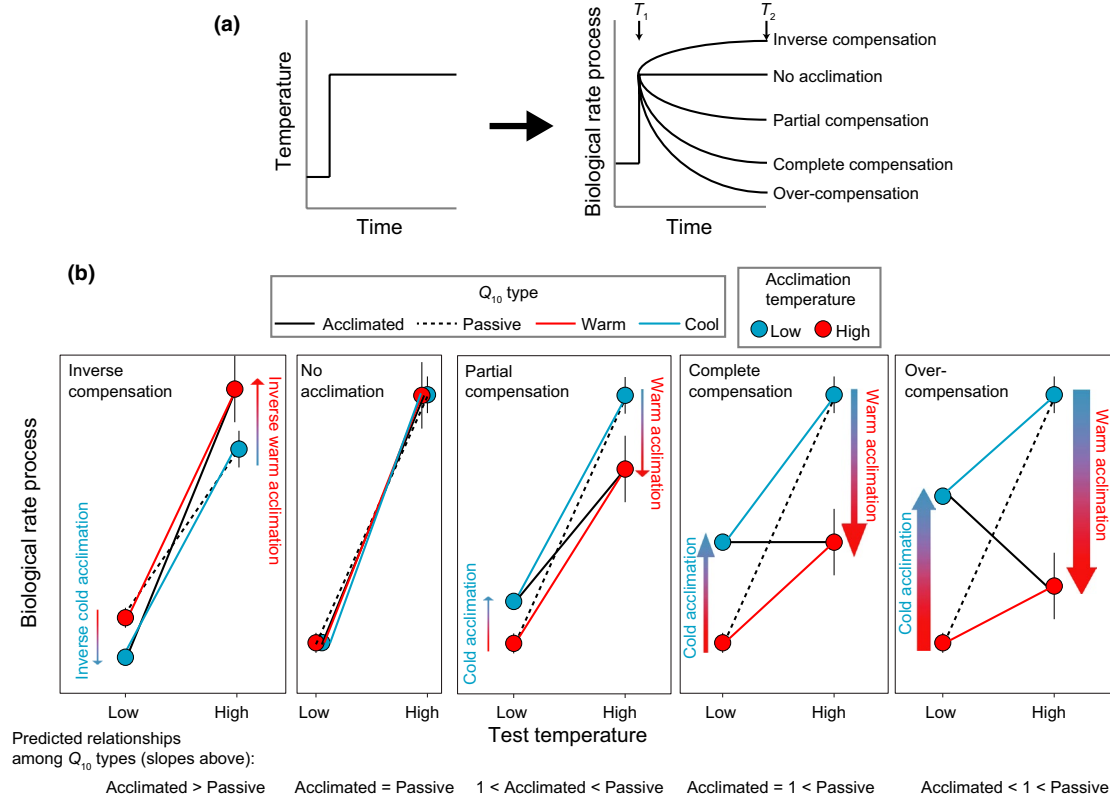


FIGURE 1 Thermal acclimation of biological rates. (a) After transfer to an elevated temperature, biological rate processes will increase passively due to thermodynamics (time T_1). With further exposure to the new temperature, one of five acclimation response types may become apparent (T_2). After Huey and Berrigan (1996). (b) Examples of different acclimation response types that can be distinguished between comparing acclimated and passive Q_{10} s

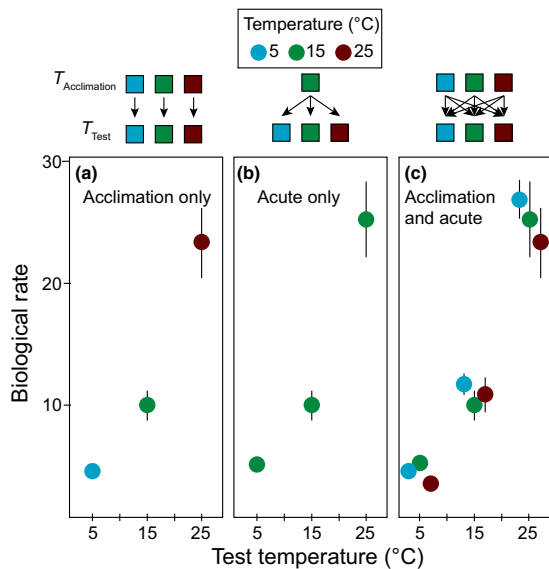


FIGURE 2 Schematic transfers (top) and representative data (bottom) illustrating three types of experimental designs for measuring biological rates at different temperatures. (a) In ‘acclimation only’ studies, animals acclimated to a particular temperature ($T_{Acclimation}$) are tested (T_{Test}) at that same temperature. (b) In ‘acute only’ studies, animals acclimated to a single temperature are tested across a range of temperatures. (c) In ‘acclimation and acute’ studies, animals are acclimated and tested at all temperatures

(Table 1; Figure 2b). Finally, in a fully factorial ‘acclimation and acute’ experimental design, organisms are acclimated and acutely tested at all temperatures, allowing for quantification of both passive and acclimated Q_{10} (Table 1; Figure 2c). Only in this last design can the acclimation response be binned into one of the five acclimation categories (Figure 1b).

As an example, consider animals acclimated to either a low or high temperature. When tested at their respective acclimation temperatures, they show the same rate (acclimated $Q_{10} = 1$). But, testing a cold acclimated animal at a warm temperature results in large increase in the rate, while testing a warm acclimated animal at the cool temperature produces a large decrease in the rate (passive $Q_{10} \gg 1$). With these data, it is clear that acclimation has completely compensated for the passive Q_{10} effect (see complete compensation in Figure 1b), but this is only apparent when both the passive and acclimated Q_{10} are known.

While the above arguments likely apply to quantifying acclimation for any biological rate process, here we focus on metabolic rate, which is a fundamental property of animals linking their physiology and ecology (McNab, 2002). Measures of metabolic rates are sensitive to temperature and have been used to formulate hypotheses such as the metabolic theory of ecology (MTE) and oxygen and capacity limited thermal tolerance (OCLTT), which provide a framework for predicting animal responses to warming

	Passive plasticity	Active plasticity	Notes
Study design	'Acute only' 'Acclimation and acute'	'Acclimation and acute'	'Acclimation only' measures both simultaneously
Q ₁₀ type	'Passive'	Difference between 'acclimated' and 'passive'	'Acclimated' by itself takes into account passive and active plasticity
Compensation responses	'No acclimation'	'Partial compensation' 'Complete compensation' 'Over-compensation' 'Inverse compensation'	'Unknown' can also be assigned if 'warm' and 'cool' Q ₁₀ are considered

TABLE 1 The consequences of different study designs when attempting to distinguish between the influence of passive and active plasticity on biological rate processes

and climate change (Brown, Gillooly, Allen, Savage, & West, 2004; Duarte, 2007; Portner, 2001, 2010; Portner & Farrell, 2008; Pörtner & Knust, 2007).

Here we used meta-analyses to explore the consequences of experimental design in the thermal acclimation of metabolic rates. We asked (a) In what proportion of studies can acclimation be quantified? (b) Which type(s) of acclimation are most common? (c) Do acclimation responses increase with acclimation duration? (d) Do acclimation responses increase with body size (as recently proposed; Rohr et al., 2018)? and (e) Do acclimation responses differ among taxa or habitats?

2 | MATERIALS AND METHODS

2.1 | Data collection

We searched the literature for publications addressing how metabolic rate changes with temperature using combinations of keywords such as "metabolic rate," "oxygen consumption," "temperature," "thermal acclimation," "plasticity" and certain taxonomic phrases (e.g. "insect"). We primarily used the Web of Science database but also employed other databases and reference-based searches. Only papers that investigated whole-animal metabolic rates of metazoans at multiple temperatures and were indexed in or before February 2017 were selected. In some papers, interpreting thermal acclimation of metabolic rates was not a primary goal of the study. Some studies were excluded due to difficulty in interpreting the experimental design. Although our searching was thorough, it was not meant to be exhaustive and we likely overlooked many papers, especially those not written in English or indexed in popular literature databases. A breakdown of the numbers of papers examined at each stage of our analysis is presented as a PRISMA flow chart in Figure S1.

2.2 | Classifying studies

Papers were classified into one of three experimental designs based on which Q₁₀ types they quantified: 'acclimation only',

'acute only' or 'acclimation and acute' as described above (see Table 1; Figure 2). We took 24 hr as a minimum time for acclimation because in many studies animals were allowed to incubate to test temperatures (and experimental apparatus) for several hours before measuring metabolic rate. Although the time course for determining acute versus acclimation responses is 'fuzzy' (Schulte et al., 2011), 24 hr very clearly delimited studies explicitly investigating acclimation as all such studies used at least 24 hr for an acclimation duration. In papers where multiple experiments with different designs were undertaken, we never classified a paper as an 'acclimation only' study if 'acute' or 'acclimation and acute' experiments were performed. Additionally, when animals acclimated to different temperatures were tested at a common (often intermediate) test temperature, the paper was classified as 'acclimation and acute' because this was often performed in an attempt to disentangle acclimation and test temperatures (e.g. Miller, Chen, & Stillman, 2014). However, it should be noted that acclimation cannot be quantified in this study design using our methods because only passive Q₁₀s can be extracted (see below).

Metadata collected for each paper included taxonomy of study organisms, acclimation duration, the type of metabolic rate investigated (e.g. basal, resting, routine), publication details and habitat. For a subset of 100 of the studies (spanning the organismal diversity we observed), a second reviewer confirmed the experimental design classification in 94 cases, indicating our classification scheme was fairly robust. Finally, for 'acclimation only' papers, we reviewed whether data were explicitly interpreted as assessing thermal acclimation in metabolic rates.

2.3 | Extracting Q₁₀s, metadata and classifying acclimation responses

The different Q₁₀ types described in Figure 1b and Table 1 were extracted from papers classified as 'acclimation and acute'. Metabolic rates and their variances were extracted from tables or from figures using ImageJ. We extracted all possible pairwise comparisons for each set of temperatures. Four different metabolic rates

were extracted (e.g. $MR_{\text{TestTemp1AcclimTemp1}}$, $MR_{\text{TestTemp2AcclimTemp1}}$, etc.) and each of the four different Q_{10} s (Figure 1b) were finally calculated using the equation $Q_{10} = (\text{rate at } T_2 / \text{rate at } T_1)^{10/(T_2 - T_1)}$, where T_2 and T_1 refer to upper and lower test temperatures, respectively. Specifically, we refer to these Q_{10} s as (a) acclimated Q_{10} ($MR_{\text{TestTemp1AcclimTemp1}}$, $MR_{\text{TestTemp2AcclimTemp2}}$), (b) passive Q_{10} ($MR_{\text{TestTemp1AcclimTemp2}}$, $MR_{\text{TestTemp2AcclimTemp1}}$), (c) warm Q_{10} ($MR_{\text{TestTemp1AcclimTemp2}}$, $MR_{\text{TestTemp2AcclimTemp2}}$) and (d) cool Q_{10} ($MR_{\text{TestTemp1AcclimTemp1}}$, $MR_{\text{TestTemp2AcclimTemp1}}$). Multiple species, temperatures or experimental manipulations were used in some papers, resulting in many sets of Q_{10} s. To control for this, we added a random effect of study in the meta-analyses described below, but results were not altered significantly in these models. For each set of Q_{10} s, metadata were extracted as above with the addition of the incubation duration and approximate weights of each study organism (as stated in the paper or based on additional literature searches).

We binned each set of Q_{10} s into one of the acclimation responses described in Figure 1. This was performed using two methods: either utilizing only passive and acclimated Q_{10} s or using all four Q_{10} s. Dichotomous keys and additional information for classifying acclimation responses using both methods are provided in File S1 (<https://doi.org/10.6084/m9.figshare.10125248.v2>).

2.4 | Meta-analyses

Random effects meta-analyses were conducted using the metafor package in R (Viechtbauer, 2010). Q_{10} was used as the effect size. Variance in Q_{10} was calculated using the delta method (Hoef, 2012) when sample sizes and variances in metabolic rates were provided by original authors (using a similar method as Heine et al., 2019; see File S2: <https://doi.org/10.6084/m9.figshare.10125248.v2>). Studies where variances in Q_{10} s could not be calculated were excluded from meta-analyses but included when graphing results. Four Q_{10} s that showed very high or very low variances were also excluded from meta-analyses to obtain stable results with metafor. Data from the Supplementary Information of Seebacher et al. (2015) were also included in meta-analyses if: whole-animal (not tissue-specific) metabolic rate was examined, acclimation (not acclimatization) was investigated, the study used an 'acclimation and acute' study design, and the study was not found in our searches.

We also quantified the strength of an acclimation response by comparing the different types of Q_{10} s. For example, assuming compensation is common, passive Q_{10} s should increase and/or acclimated Q_{10} s decrease with acclimation duration if longer durations result in stronger acclimation responses. Essentially, this amounts to testing for an interaction between Q_{10} type and acclimation duration. This interaction and ones between Q_{10} type and taxa, weight, acclimation duration, incubation duration, habitat, acclimation/compensation type, the lower test temperature, and whether the

data came from our identified studies or from Seebacher et al. (2015) were calculated.

3 | RESULTS

3.1 | Multiple experimental designs are common

The final dataset for classifying experimental designs included 258 papers (Figure S1). Acclimation times averaged 31 ± 2 days SEM. While 'acclimation and acute' study designs were the most common (49%; Figure 3), 'acclimation only' studies where passive and active plastic responses are confounded were also numerous (34%). 'Acute only' studies were the rarest (17%). Similar trends were found using the dataset from Seebacher et al. (2015; e.g. 35% 'acclimation only').

Studies of different taxa (Figure 3a,b) and habitats (Figure 3c,d) used significantly different proportions of study designs ($p < .001$ for both, Fisher's exact test). For example, with molluscs and crustaceans, 'acclimation only' study designs were common (59% and 74% respectively), but were rare with insects (18%). 'Acclimation only' studies were also common in studies of aquatic, but not terrestrial organisms (61% vs. 9%). We included 60 studies from endotherms, even though the complex relationships between acclimation, test and body temperatures make quantifying acclimation responses difficult in endotherms. Nonetheless, 'acclimation only' studies were rare in endotherms (1/60 studies), but common in ectotherms (86/198). We found no evidence that 'acclimation only' study designs were more frequent in older papers (Figure S2). We also tested an effect of journal on experimental design (Figure S3) for journals that had at least five studies and were not taxa- or habitat-specific ($n = 6$ journals, representing 60% of the studies). Although we are well aware of the limitations of journal impact factors (IF; Chang, McAleer, & Oxley, 2011; Neff & Olden, 2010; Postma, 2007; Tort, Targino, & Amaral, 2012; Wilson, 2007), we used IF as a quantitative metric to distinguish journals. There was a significant negative correlation between IF and the frequency of 'acclimation only' studies (Figure S3, $p = .007$, $r^2 = .87$).

3.2 | Ectotherms show a variety of acclimation responses

We estimated the different Q_{10} types in 52 of the 127 papers classified as 'acclimation and acute' (Figure S1). We excluded studies of endotherms (60 papers) because endotherms use energy to maintain relatively stable body temperatures, making our comparison of passive and acclimated Q_{10} largely specific to ectotherms. In all, 15 papers were excluded because animals acclimated to multiple temperatures were measured at a common test temperature, preventing estimation of all Q_{10} types. Therefore, only in 26% of papers examining ectotherms could acclimation be characterized. Another 46 papers were added

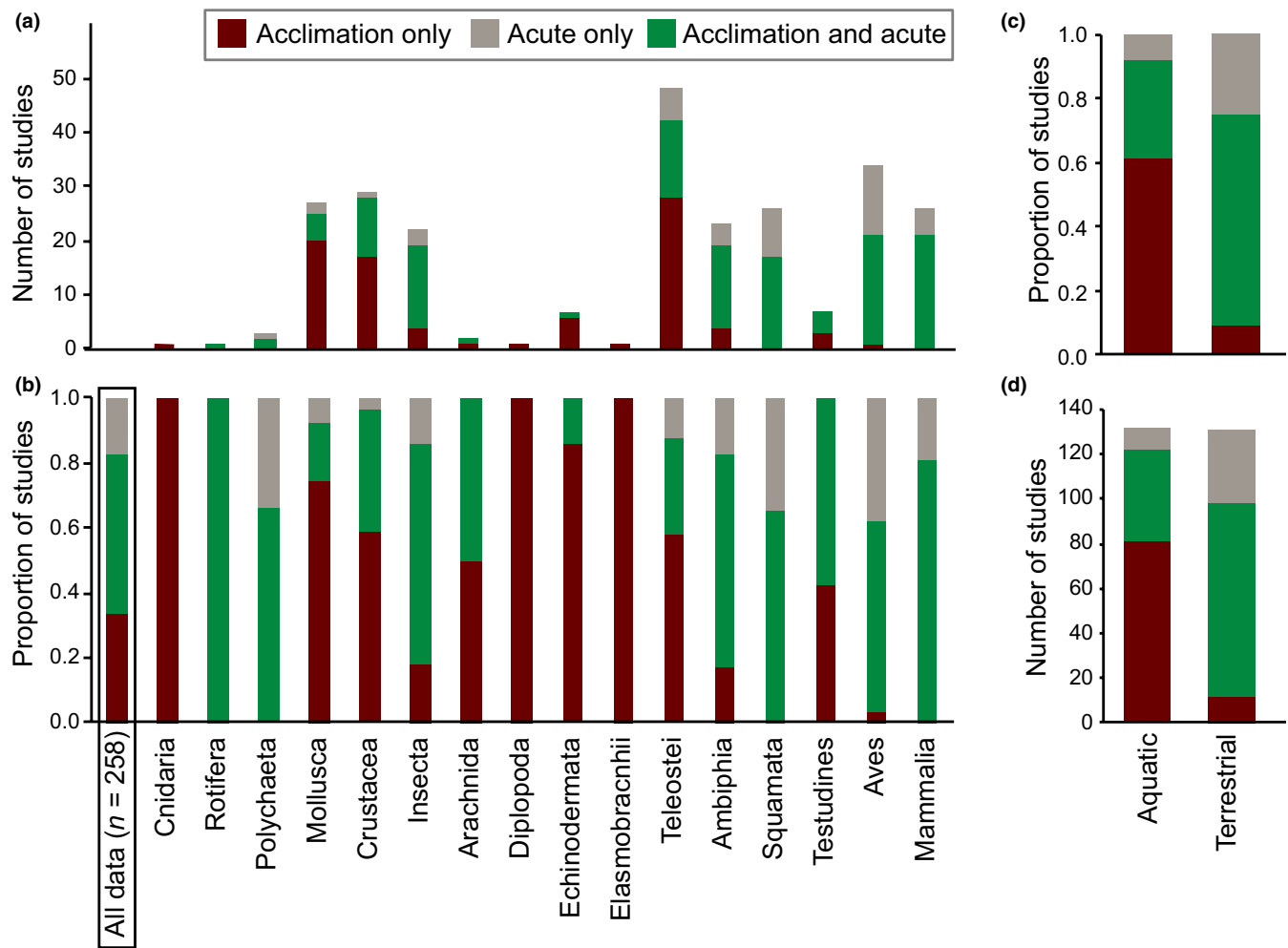


FIGURE 3 Experimental designs in our dataset as a function of taxa (a, b) and environment (c, d). The proportion of ‘acclimation only’ studies varied significantly based on both variables ($p < .001$, Fisher’s exact test). The leftmost column in (b) shows the proportion of study types for the entire dataset ($n = 258$ papers)

from Seebacher et al. (2015) so that 96 papers were used to classify acclimation responses in meta-analyses. From these papers, we extracted 268 different sets of Q_{10} s (each set consisted of the four Q_{10} types, for a total of 1,072 Q_{10} values; File S3, <https://doi.org/10.6084/m9.figsh.10125248.v2>). It should be noted that a small proportion (~5%) of extracted Q_{10} values were <1 . Based on the test temperatures used in these studies (-1 to 35°C), we assume these measurements occur during the exponential ascending phase of the TPC. Low acclimated Q_{10} values are also predicted with over-compensation. However, because the exact shape of the TPC for metabolic rate is unknown for most of the taxa examined here, other interpretations are possible (see Section 4) and caution should be exercised in interpreting these low Q_{10} values.

When comparing passive and acclimated Q_{10} s, we found partial compensation was the most common acclimation response (32%), although no acclimation and inverse compensation were also common (30% and 25% respectively; Figure 4a). Complete and over-compensation were relatively rare (8% and 4% respectively; Figure 4a). We also repeated this analysis using all four Q_{10} s (passive, acclimated, warm and cool) and found these general patterns were supported (Figure S4).

3.3 | Overall characteristics of the meta-analyses

Here, we report results when using passive and acclimated Q_{10} s only, although results were similar when using all four Q_{10} s. In all meta-analyses performed, heterogeneity between study effect sizes was large ($I^2 > 99\%$). Examination of a funnel plot (Figure S5) suggested little publication bias and overall avoidance of the ‘file drawer problem’ (Rosenthal, 1979), as did a formal Egger’s test for publication bias (Egger, Davey Smith, Schneider, & Minder, 1997). Overall, passive Q_{10} s were significantly higher than acclimated Q_{10} s ($p < .001$) and warm and cool Q_{10} s were intermediate (Figure 4b). Data from Seebacher et al. (2015) had higher Q_{10} s overall and a stronger partial compensation response (interaction between Q_{10} type and dataset $p < .001$; Figure S6). Finally, in each individual meta-analysis, moderators explained only a small percentage of the variation in Q_{10} values (e.g. $\max r^2 = .314$). When all shared moderators (Q_{10} type, acclimation type, taxa, acclimation duration, habitat and dataset) and their interactions were used, a majority of the variation in Q_{10} could be explained ($r^2 = .747$).

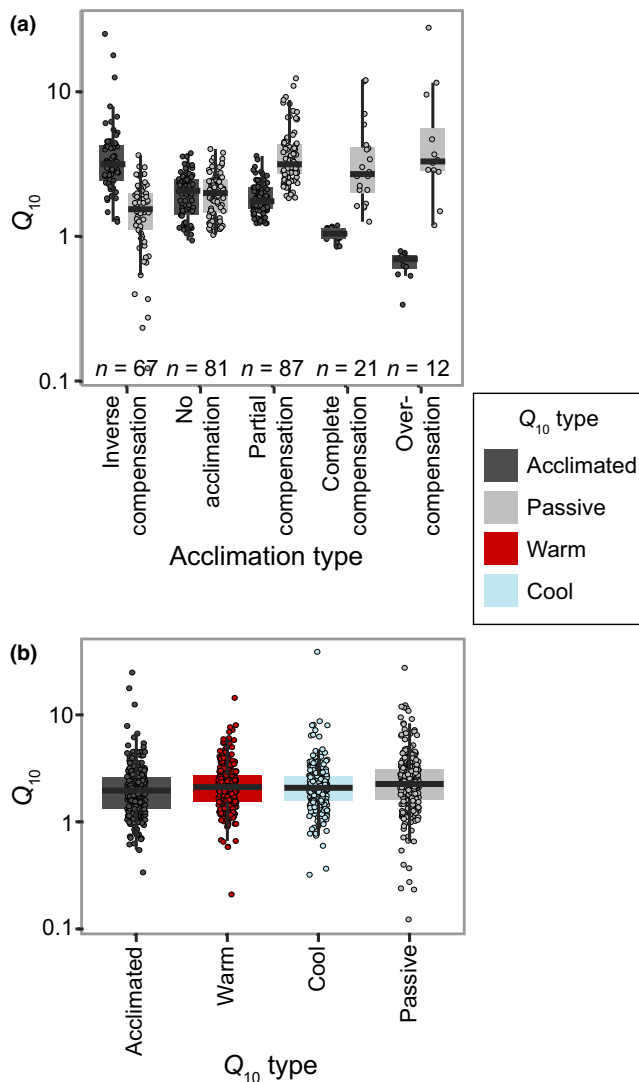


FIGURE 4 Metabolic rates show many thermal acclimation responses. (a) Complete and over-compensation responses were relatively rare, while other acclimation responses were common in our dataset (based on $n = 268$ sets of Q_{10} s). (b) When all data were analysed together, passive Q_{10} s were significantly larger than acclimated Q_{10} s, with warm and cool Q_{10} s falling in between, generally supporting that partial compensation is a common response to acclimation. On boxplots here and throughout: lower and upper hinges correspond to the first and third quartiles, and whiskers extend to values no further than 1.5 times the interquartile range

3.4 | Acclimation responses are different among taxa, habitats and experimental designs

As expected due to our classification scheme, there was a strong, significant interaction between Q_{10} type and the classified acclimation response ($p < .001$, $r^2 = .314$; Figure 4a). Although Q_{10} s did not vary significantly among taxa ($p = .369$), there was a significant interaction between Q_{10} type and taxonomic group ($p = .006$; Figure 5a). For example, in amphibians, squamates and turtles ($n = 87, 30$ and 9 respectively), passive and acclimated Q_{10} s were about the same, suggesting a

lack of acclimation. In teleosts, molluscs and insects ($n = 62, 30$ and 22 respectively), passive Q_{10} s were significantly larger than acclimated Q_{10} s (Figure 5a), indicating partial compensation. There was also a significant interaction between Q_{10} type and habitat ($p < .001$), with terrestrial animals showing a lack of acclimation, and aquatic animals showing partial compensation (Figure 5b). When aquatic animals were further divided, the difference between Q_{10} types was most pronounced in marine taxa compared with freshwater taxa (Figure S7).

There was a significant interaction between Q_{10} type and acclimation duration ($p < .001$), such that with each additional 30 days of acclimation, passive Q_{10} s increased by 0.29 while acclimated Q_{10} s decreased by 0.09 (Figure 5c). Incubation durations were reported for 88% of the data and averaged about 5 hr, although 1 hr was by far the most common incubation time (used in 35% of the studies, median = 1 hr). Q_{10} s did not significantly change with incubation duration ($p = .409$), but there was a significant interaction between Q_{10} type and incubation duration ($p = .017$), such that each additional hour of incubation resulted in passive Q_{10} s increasing by 0.03 , while acclimated Q_{10} s decreased by 0.01 (Figure 5d). Body size was provided or inferred for 94% of measurements, but there was no significant relationship between body size and Q_{10} ($p = .312$) or the interaction between Q_{10} type and body size ($p = .448$). Test temperatures also influenced Q_{10} s (both passive and acclimated Q_{10} similarly): Q_{10} decreased by 0.46 with each 10°C increase in lower test temperature ($p = .001$, Figure S8).

4 | DISCUSSION

4.1 | Characterizing thermal acclimation of biological rate processes

Thermal acclimation has been studied for decades, yet quantifying how much of the observed variation is due to the intrinsic, passive properties of biological rate processes versus active physiological adjustments has received relatively little attention. Because passive plasticity causes phenotypes such as metabolic rate to change more-or-less automatically with temperature, a persistent challenge when quantifying *active* plasticity in the form of acclimation is to design experiments that separate these two responses.

The major conclusions of this study are as follows: (a) only 'acclimation and acute' study designs allow for partitioning of variation due to passive and active plasticity and (b) these studies reveal 'partial compensation' and 'no acclimation' (Figures 1 and 4) to be the most common form of acclimation, but with considerable variation across taxa, habitats and experimental designs. Partial compensation implies that acclimation responses are often masked because metabolic rates increase with increasing temperature due to passive plasticity, while active plasticity due to acclimation acts to counteract this response. Schulte et al. (2011, p. 697) make the insightful observation that 'This leads to the apparently absurd conclusion that the only way to achieve a reaction norm demonstrating a lack of plasticity is for the organism to exhibit substantial plasticity'. Similarly, others have noted that if thermal compensation were perfect, rates

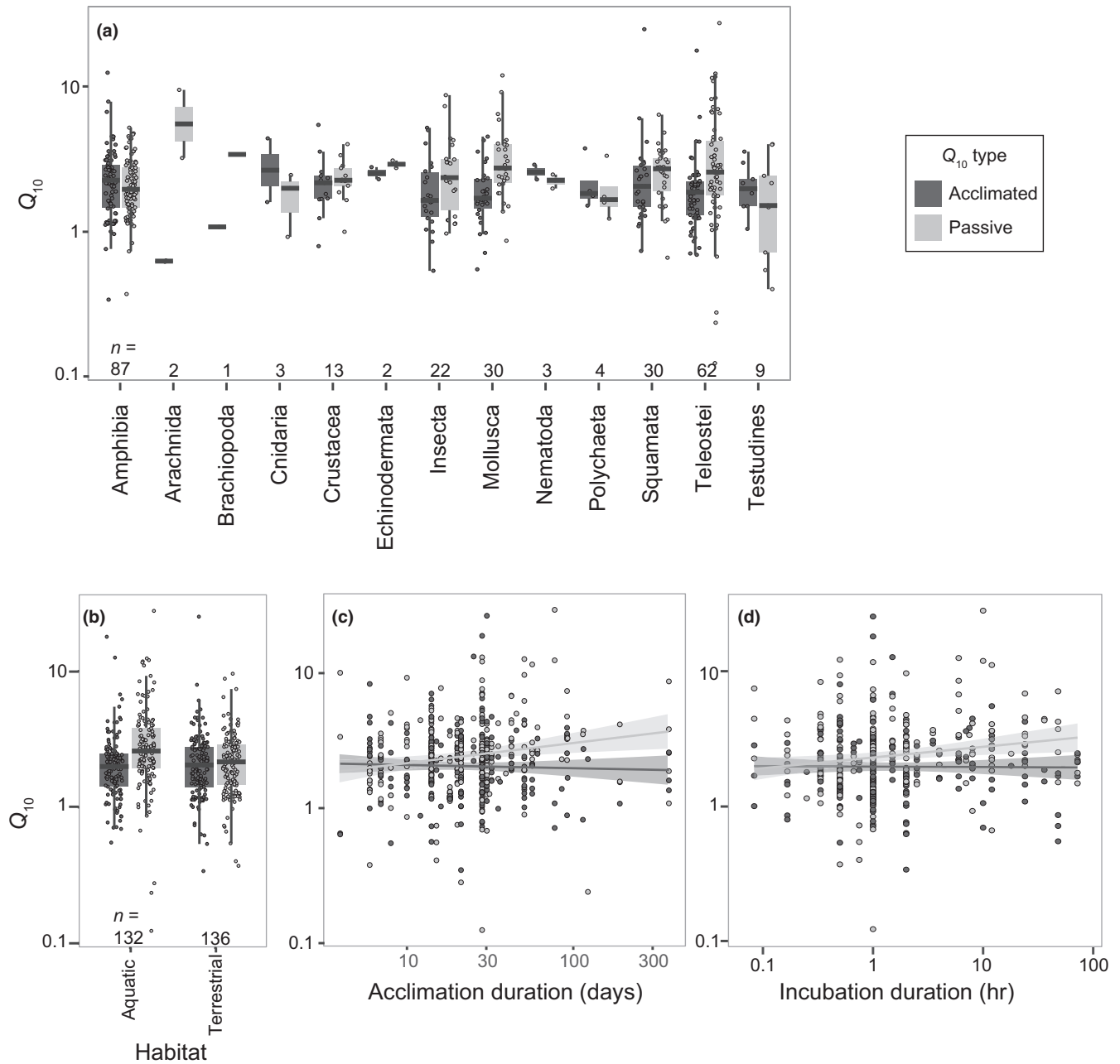


FIGURE 5 Many moderators significantly influence thermal acclimation in metabolic rates. (a) Acclimation responses varied among taxa, with amphibians, crustaceans and squamates showing no acclimation response, while partial compensation was common in insects, molluscs and teleosts. (b) Terrestrial animals generally lacked an acclimation response, while aquatic animals showed partial compensation. (c) Acclimated Q_{10} s decreased while passive Q_{10} s increased with acclimation duration, suggesting that acclimation responses increase with acclimation duration. (d) Acclimation responses also increase with incubation duration (the time an animal was allowed to adjust to a new test temperature before measurements began). There was a significant interaction ($p \leq .017$) between Q_{10} type and each moderator shown here in meta-analyses. In (c) and (d), 95% confidence intervals are shown around lines of best fit

would exhibit no change across temperatures (Seebacher et al., 2015; Wilson & Franklin, 2002). Having said this, in nearly one-third of the studies no acclimation response was observed (Figures 1 and 4), suggesting that the passive change in metabolic rate with temperature is sufficient to meet the biological demands of many organisms.

Importantly, acclimation is not a passive effect resulting from biophysics, but rather an active physiological and biochemical

response shaped by the mechanisms of evolution (Cossins & Bowler, 1987; Schulte et al., 2011). While others have noticed this problem, we feel this distinction warrants considerably more attention in the context of experimental design. For example, Seebacher et al. (2015) recognized and quantified differences between acclimated versus passive Q_{10} s. However, because acclimated Q_{10} s by themselves do not allow for estimating acclimation responses (because the passive component remains unknown), we argue that studies only examining

acclimated Q_{10} cannot be included along with 'acclimated and acute' studies. Specifically, acclimated Q_{10} s that are near 1 may indicate a strong compensatory response, but such an interpretation can only be made if passive Q_{10} s are >1 . In other words, acclimation can only be quantified when the passive effect without any acclimation is known (as in 'acclimation and acute' studies).

Although the diversity of acclimation responses can be complex (Figure 1), there is renewed interest in quantifying thermal acclimation and understanding the selection pressures that generate variation in acclimation capacity. Such studies are increasingly being used to predict responses to global climate change and investigate environmental adaptation (Deutsch et al., 2008; Duarte, 2007; Einum et al., 2019; Gunderson & Stillman, 2015; Kingsolver, 2009; Pörtner & Knust, 2007; Rohr et al., 2018; Seebacher et al., 2015; Wythers, Reich, & Bradford, 2013). For example, the climate variability hypothesis suggests that animals living in thermally variable high latitudes should have broader thermal niches and exhibit stronger thermal acclimation responses than those from the less variable tropics (Angilletta, Condon, & Youngblood, 2019; Compton, Rijkenberg, Drent, & Piersma, 2007; Ghalambor, Huey, Martin, Tewksbury, & Wang, 2006; Janzen, 1967; Shah et al., 2017). Most studies investigating this hypothesis have avoided examining biological rate processes, but instead focus on phenotypes that represent components of TPCs, such as CT_{max} and thermal breadth. Payne and Smith (2017) challenged the prevailing view of climate variability by arguing that narrower thermal breadths in tropical organisms could be explained simply by the passive plasticity of biological rates to temperature. Yet, Payne and Smith (2017) had to estimate thermal breadth because too few studies have empirically estimated TPCs, and even fewer have accounted for how much acclimation alters these curves. Using the framework and methods here, clearer predictions can be made when testing passive and active responses to temperature under the climate variability hypothesis (see Section 4.6).

4.2 | Experimental designs for investigating thermal acclimation vary widely

When estimating metabolic rates over temperatures, multiple experimental designs are common (Figure 3). Differences among taxa and habitats in study designs may be due to historical legacies, where certain study designs become engrained in the scientific culture. The high proportion of terrestrial endotherms in this dataset may also account for why 'acclimation only' designs were more common in aquatic animals, which were all ectotherms. While interesting, it is difficult to speculate why journals with higher impact factors may have a lower proportion of 'acclimation only' study designs (Figure S3). For example, reviewers at specific journals may be more prone to reject certain study designs. 'Acclimation and acute' experiments also necessitate larger studies, which may be more appealing to journals that are more highly cited.

Why do 'acclimation only' study designs remain common (Figure S2), despite this design confounding passive and active plasticity?

Although these confounded designs warrant concern under some conditions, there are multiple reasons why such studies may be appropriate. First, 'acclimation only' may be the most biologically relevant type of study design in relation to climate change. Many researchers may only be interested in how metabolic rates will change with temperature and not what proportion of the effect is due to passive plasticity versus acclimation. For example, in one study examining the OCLTT hypothesis and variation in salmon TPCs, metabolic rates were only quantified at acclimation temperatures (i.e. an 'acclimation only' study; Eliason et al., 2011), presumably because these are the most ecologically relevant temperatures. Second, as mentioned above, 'acclimation and acute' study designs require more resources—animals, equipment and time. Scaling metabolic rate experiments to accommodate this study design may be more feasible in particular systems, offering another explanation for variation in study designs. Third, quantifying acclimation may not be a goal of many studies. We found many instances where 'acclimation' was not mentioned throughout entire 'acclimation only' papers. Metabolic rates were also often a peripheral part of such studies.

A fourth consideration for adopting an 'acclimation only' design that deserves more attention is minimizing stress. Both passive plasticity and stress can cause acute changes with temperature in biological rates (Barton, Peter, & Paulencu, 1980). Drawing a line between passive versus stress responses may be difficult (see Sections 4.4 and 4.6). 'Acclimation only' studies sidestep this problem by ensuring measurements are taken long after any stress response has concluded. Therefore, in systems where stress may play a large role or when the strength of thermal acclimation is not of interest, 'acclimation only' study designs may be preferred.

4.3 | Why are there different acclimation responses?

Acclimation responses vary widely (Figures 4 and 5). When all data were summed together, acclimation tended to partially compensate for the passive increase in metabolic rate caused by increased temperatures (Figure 4b). However, inverse compensation and lack of an acclimation response were also common (Figure 4a). Inverse compensation is particularly interesting because it suggests that physiological adjustments are needed to increase or decrease biological rates beyond those occurring passively. In those cases where no acclimation is observed, it is possible that passive responses to temperature are sufficient to maintain energy balance and organismal performance. Complete and over-compensation may be rare because most animals may not be able to completely compensate for changes due to passive plasticity. It is unclear at this time what evolutionary mechanisms drive these different responses, but our results highlight recent criticisms of assuming one particular response is common across diverse organisms and experiments (Einum et al., 2019).

While these results suggest which types of acclimation are most common, a major unresolved problem is that they are unable

to distinguish if particular acclimation responses are beneficial. An adaptive reason can be used to explain any of the acclimation response types (Huey & Berrigan, 1996). For example, compensation for passive thermal changes may act to return rates to an 'optimum' level. However, lack of a response might suggest maintenance of a new optimum rate. Making specific a priori hypotheses on a case-by-case basis for why one would predict certain types of acclimation responses versus others is critical, but is rare, especially in studies critical of the benefits of acclimation (e.g. Wilson & Franklin, 2002). For example, in systems where thermal stress may play a large role, an inverse compensation response might be predicted if organisms are 'losing control'. Similarly, when elevated metabolic rates are not costly, the lack of an acclimation response may be optimal. At the very least, the variety of acclimation responses shown here suggest that different strategies may be adaptive under different scenarios.

Acclimation responses varied significantly among taxonomic groups and habitats (Figure 5a,b). For those taxa where at least 10 sets of Q_{10} s were compared, only 'no acclimation' and 'partial compensation' responses were found when data were summed. These responses are largely correlated with differences between terrestrial and aquatic animals (Figure 5b), which have been noted previously (Seebacher et al., 2015). Aquatic animals may tend to show stronger acclimation responses because water has a high specific heat capacity, resulting in body temperatures more closely tracking environmental temperatures and making behavioural thermoregulation more difficult. Aquatic acclimation responses may therefore be under stronger selection, whereas terrestrial animals may rely more strongly on behavioural thermoregulation in more thermally heterogeneous environments. Alternatively, thermal transfers in aquatic animals may cause greater stress responses, causing elevated passive Q_{10} s and therefore necessitating stronger acclimation responses. However, it is difficult to generalize across taxa or habitats, as aquatic crustaceans and terrestrial insects did not follow habitat-specific patterns, showing no acclimation and partial compensation responses, respectively. Whatever the reasons, extending general conclusions to all members of a taxonomic group is likely an oversimplification. Importantly, taxa that lack an acclimation response and live closer to their thermal limits may be more sensitive to climate change (Rohr et al., 2018; Seebacher et al., 2015).

4.4 | Time courses of thermal acclimation are important

As might be predicted, acclimation responses increased with acclimation duration: when an animal is acclimated to a temperature longer, passive Q_{10} s are elevated and acclimated Q_{10} s are reduced (Figure 5c). Indeed, passive Q_{10} s were 3.2 times more strongly influenced by acclimation duration than acclimated Q_{10} s, suggesting passive responses become more pronounced with longer acclimation durations. This may be due to a more extreme initial stress response that diminishes over time. In contrast, acclimated Q_{10} s may decrease with acclimation duration because with increased time, acclimation

can become more pronounced. For example, it is possible that partial compensation might start to approach complete compensation at later time points. Differences in acclimation duration may also explain the significantly stronger acclimation response in Seebacher et al.'s (2015) dataset (Figure S6), which included a minimum acclimation duration of 10 days, while ours was only 24 hr (the minimum acclimation duration in our final dataset was only 4 days).

Interestingly, our analyses suggest that on average, acclimation responses might not be measurable until more than 30 days of acclimation (Figure 5c). Previous studies generally describe acclimation in metabolic rate on a much shorter time scale. For example, Barrioneuvo and Fernandes (1998) found that after large acute changes in metabolic rates following thermal transfers, rates tended to stabilize 2–3 days following transfer and did not change further when measured 15 days after transfer in the freshwater fish *Prochilodus scrofa*. Differences in taxa, habitat and other factors among experiments may influence how long it takes to mount an acclimation response, but based on the results here, quantifying rates at extended time points may be warranted.

Incorporating an incubation time into metabolic rate experiments allows animals to adjust to a new temperature and mitigates stress responses. Therefore, we predicted that passive Q_{10} s should decrease with longer incubation times. However, our data indicate the opposite (Figure 5d). This may be because stress responses were still being initiated even after incubation, or because acclimation responses were becoming apparent after longer incubations (i.e. acclimation may be happening during longer incubation times). Responses such as upregulation of heat shock proteins or changes in protein phosphorylation states can occur on very short time scales (Healy et al., 2010; Park & Jang, 2011), suggesting that acclimation response on the order of hours is possible.

Ultimately, a researcher must decide what time courses correspond to stress, passive and active responses in their systems, and acknowledge that a measured rate is likely always influenced by all these responses. This time course consideration has been discussed before (Chown & Terblanche, 2007; Kingsolver, Izem, & Ragland, 2004; Pigliucci, 2001; Schulte et al., 2011) and essentially amounts to deciding what type of Q_{10} is being measured (Table 1; Figure 1). Unfortunately, time courses likely vary widely, and few studies have attempted to disentangle these responses (Feder, Gibbs, Griffith, & Tsuji, 1984). The methods outlined here could be useful in addressing these concerns during experimental design (see below).

Many other questions relating to experimental design in thermal acclimation experiments also warrant further discussion. For example, Chown, Jumbam, Sorensen, and Terblanche (2009) found that effects from the rate of temperature change in measuring critical thermal limits were different depending on the traits and species examined. Similar effects should be investigated in thermal responses of metabolic rates. For example, rapid transfer to a new, drastically different temperature (as was common in our dataset) may result in a large stress response that could obscure an otherwise modest passive Q_{10} response. Examining how acclimation responses vary due to constant versus fluctuating acclimation

conditions is also worthwhile (Bozinovic, Catalan, Estay, & Sabat, 2013).

4.5 | Do metabolic rates follow a thermal 'performance' curve?

The finding that Q_{10} s tended to decrease with increased test temperatures (Figure S8) supports other findings that metabolic rates do not follow strict Arrhenius-like patterns. Rather, when summed across taxa they may show a TPC similar to other biological rates that increase to a peak and then decline as temperature increases (DeLong et al., 2018; Schulte, 2015; Schulte et al., 2011). Recent studies have argued that by converting temperature to biological rates, differences in habitat temperature can explain the width of these performance curves (Payne & Smith, 2017). However, our results clearly demonstrate that TPCs are not static, but are plastic and can be influenced by thermal acclimation. Genetic variation can also likely influence TPCs (Kingsolver & Gomulkiewicz, 2003; Kingsolver et al., 2015; Knies et al., 2006; Latimer, Foley, & Chenoweth, 2015).

While metabolic rates follow a TPC, it may be unwise to assume that maximal metabolic rates represent higher 'performance' or an animal's optimal 'operating' temperature. Higher rates might represent a greater ability for terrestrial ectotherms to forage in cold weather (Huey et al., 2012), whereas lower rates might indicate needing fewer resources to maintain basal functions and allow for a greater aerobic scope (Portner, 2010). Indeed, the OCLTT hypothesis suggests that maximal aerobic scopes should be beneficial because animals will have more energy to pursue food and reproductive opportunities when this value is maximized (Portner, 2010). Additionally, metabolic rates or aerobic scopes may peak at one temperature while other traits (e.g. fecundity) may peak at other temperatures. Supporting this, a recent study showed that TPCs can differ substantially among different traits in the same organism (Kellermann et al., 2019).

4.6 | Providing future direction

The complications of active plasticity, passive plasticity and stress are likely debated internally during the course of designing any study measuring the effects of temperature on metabolic rate. Yet, few studies openly discuss how to resolve these problems. Below, we offer some suggestions for future studies when considering experimental designs.

1. 'Acclimation only' design (Figure 2a).

If the question is: 'what is the metabolic rate of a fully acclimated animal to a given temperature?', then this is the best design. It minimizes any confounding effects of handling and thermal stress and provides the best estimate of what is likely the 'functional' metabolic rate at that temperature. Ideally, acclimation periods of

different durations would be examined with the expectation that after some time period, metabolic rates will stabilize as the animal achieves a fully acclimated state. Our results here suggest it may take up to 1 month before these fully acclimated states are achieved (Figure 5c). However, because these metabolic rates reflect the combined effects of passive and active plastic responses to temperature, a drawback of this study design is that the effects of either response cannot be disentangled.

2. 'Acute only' design (Figure 2b).

If the question is: 'what are the acute effects of temperature on metabolic rate in the absence of any acclimation?', then this is the best design. It isolates the passive plastic responses to temperature. However, this design may induce a stress response when moving animals from a single acclimation temperature to many test temperatures. Therefore, an ideal design would investigate if incubation duration to the new test temperature (on the order of hours) alters metabolic rates. The drawback of this design is that no strong inference can be made regarding the influence of thermal acclimation.

3. 'Acclimation and acute' design (Figure 2c).

If the question is: 'what are the independent and combined effects of passive and active thermal plasticity on metabolic rate?', then this is only appropriate design. It isolates both the acute (passive) and acclimated (active) responses and can be used to answer the same questions posed in 'acute only' and 'acclimation only' designs. However, it also requires simultaneous consideration of the same challenges of choosing an acclimation and incubation duration. These considerations can quickly become logistically challenging as the number of acclimation and test temperatures increase. Nevertheless, this is the only design that allows quantifying the type of acclimation response (Figure 1) by directly comparing the passive and acclimated Q_{10} s (Figure 1b).

Finally, in all cases we suggest caution when interpreting results. Explicitly recognizing how much of the observed response is due to passive or active plasticity, the degree to which a stress response is confounded, and other sources of variation is a critical first step. We argue that care must also be taken when applying an adaptive explanation to temperature-induced variation in metabolic rates. Depending on the taxon, environment or physiological state, elevated or reduced metabolic rates could be beneficial. There is a need for developing more sophisticated hypotheses that predict the outcome of interactions between temperature, organismal responses and metabolic rates across different taxa and environments.

ACKNOWLEDGEMENTS

We thank members of the Ghalambor, Havird and Hillis lab groups for providing comments on this work, which was supported by the University of Texas at Austin and NSF IOS-1457383. We also thank

several anonymous reviewers whose comments helped improve this manuscript.

AUTHORS' CONTRIBUTIONS

All authors conceived the ideas and collected data. J.C.H. analysed the data and led the writing of the manuscript.

DATA AVAILABILITY STATEMENT

All data extracted and analysed here, as well as R code used to perform meta-analyses, are provided in File S3, which is available via FigShare along with the other supplementary files: <https://doi.org/10.6084/m9.figshare.10125248.v2> (Havird, Shah & Ghalambor, 2019)

ORCID

Justin C. Havird  <https://orcid.org/0000-0002-8692-6503>

Jennifer L. Neuwald  <https://orcid.org/0000-0002-8850-5534>

Alisha A. Shah  <https://orcid.org/0000-0002-8454-7905>

Cameron K. Ghalambor  <https://orcid.org/0000-0003-2515-4981>

REFERENCES

- Angilletta, M. J. (2009). *Thermal adaptation: A theoretical and empirical synthesis*. New York, NY/Oxford: Oxford University Press.
- Angilletta, M. J. Jr., Condon, C., & Youngblood, J. P. (2019). Thermal acclimation of flies from three populations of *Drosophila melanogaster* fails to support the seasonality hypothesis. *Journal of Thermal Biology*, 81, 25–32. <https://doi.org/10.1016/j.jtherbio.2019.02.009>
- Arrhenius, S. (1915). *Quantitative laws in biochemical chemistry*. London, UK: Bell.
- Barrionuevo, W. R., & Fernandes, M. N. (1998). Time-course of respiratory metabolic adjustments of a South American fish, *Prochilodus scrofa*, exposed to low and high temperatures. *Journal of Applied Ichthyology*, 14, 37–41. <https://doi.org/10.1111/j.1439-0426.1998.tb00611.x>
- Barton, B. A., Peter, R. E., & Paulencu, C. R. (1980). Plasm-cortisol levels of fingerling rainbow-trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. *Canadian Journal of Fisheries and Aquatic Sciences*, 37, 805–811.
- Bozinovic, F., Catalan, T. P., Estay, S. A., & Sabat, P. (2013). Acclimation to daily thermal variability drives the metabolic performance curve. *Evolutionary Ecology Research*, 15, 579–587.
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85, 1771–1789. <https://doi.org/10.1890/03-9000>
- Chang, C. L., McAleer, M., & Oxley, L. (2011). What makes a great journal great in the sciences? Which came first, the chicken or the egg? *Scientometrics*, 87, 17–40. <https://doi.org/10.1007/s11192-010-0335-0>
- Chown, S. L., Haupt, T. M., & Sinclair, B. J. (2016). Similar metabolic rate-temperature relationships after acclimation at constant and fluctuating temperatures in caterpillars of a sub-Antarctic moth. *Journal of Insect Physiology*, 85, 10–16. <https://doi.org/10.1016/j.jinsphys.2015.11.010>
- Chown, S. L., Jumbam, K. R., Sorensen, J. G., & Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Functional Ecology*, 23, 133–140. <https://doi.org/10.1111/j.1365-2435.2008.01481.x>
- Chown, S. L., & Terblanche, J. S. (2007). Physiological diversity in insects: Ecological and evolutionary contexts. In S. J. Simpson (Ed.), *Advances in insect physiology* (pp. 50–152). London, UK: Academic Press.
- Colinet, H., Sinclair, B. J., Vernon, P., & Renault, D. (2015). Insects in fluctuating thermal environments. *Annual Review of Entomology*, 60(60), 123–140. <https://doi.org/10.1146/annurev-ento-010814-021017>
- Compton, T. J., Rijkenberg, M. J. A., Drent, J., & Piersma, T. (2007). Thermal tolerance ranges and climate variability: A comparison between bivalves from differing climates. *Journal of Experimental Marine Biology and Ecology*, 352, 200–211. <https://doi.org/10.1016/j.jembe.2007.07.010>
- Cossins, A. R., & Bowler, K. (1987). *Temperature biology of animals*. New York, NY/London, UK: Chapman and Hall.
- DeLong, J. P., Bachman, G., Gibert, J. P., Luhring, T. M., Montooth, K. L., Neyer, A., & Reed, B. (2018). Habitat, latitude and body mass influence the temperature dependence of metabolic rate. *Biology Letters*, 14(8), 20180442. <https://doi.org/10.1098/rsbl.2018.0442>
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, 105(18), 6668–6672. <https://doi.org/10.1073/pnas.0709472105>
- Duarte, C. M. (2007). Marine ecology warms up to theory. *Trends in Ecology & Evolution*, 22, 331–333. <https://doi.org/10.1016/j.tree.2007.04.001>
- Egger, M., Davey Smith, G., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, 315, 629–634. <https://doi.org/10.1136/bmj.315.7109.629>
- Einum, S., Ratikainen, I., Wright, J., Pelabon, C., Bech, C., Jutfelt, F., ... Burton, T. (2019). How to quantify thermal acclimation capacity? *Global Change Biology*, 25, 1893–1894. <https://doi.org/10.1111/gcb.14598>
- Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., ... Farrell, A. P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science*, 332, 109–112. <https://doi.org/10.1126/science.1199158>
- Feder, M. E., Gibbs, A. G., Griffith, G. A., & Tsuji, J. (1984). Thermal acclimation of metabolism in salamanders – Fact or artifact. *Journal of Thermal Biology*, 9, 255–260.
- Ghalambor, C. K., Huey, R. B., Martin, P. R., Tewksbury, J. J., & Wang, G. (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integrative and Comparative Biology*, 46, 5–17. <https://doi.org/10.1093/icb/icj003>
- Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21, 394–407. <https://doi.org/10.1111/j.1365-2435.2007.01283.x>
- Gotthard, K., Nylin, S., & Nylin, S. (1995). Adaptive plasticity and plasticity as an adaptation – A selective review of plasticity in animal morphology and life-history. *Oikos*, 74, 3–17. <https://doi.org/10.2307/3545669>
- Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20150401. <https://doi.org/10.1098/rspb.2015.0401>
- Havird, J. C., Shah, A. A., & Ghalambor, C. K. (2019). Data from: ThermalAcclimation. *figshare*, <https://doi.org/10.6084/m9.figshare.10125248.v2>
- Healy, T. M., Tymchuk, W. E., Osborne, E. J., & Schulte, P. M. (2010). Heat shock response of killifish (*Fundulus heteroclitus*): Candidate gene and heterologous microarray approaches. *Physiological Genomics*, 41, 171–184.
- Heine, K. B., Abebe, A., Wilson, A. E., & Hood, W. R. (2019). Copepod respiration increases by 7% per degrees C increase in temperature: A meta-analysis. *Limnology and Oceanography Letters*, 4, 53–61.
- Hochachka, P. W., & Somero, G. N. (2002). *Biochemical adaptation: Mechanism and process in physiological evolution*. New York, NY: Oxford University Press.
- Hoef, J. M. V. (2012). Who invented the delta method? *American Statistician*, 66, 124–127. <https://doi.org/10.1080/00031305.2012.687494>
- Holzman, N., & McManus, J. J. (1973). Effects of acclimation on metabolic rate and thermal tolerance in the carpenter frog. *Comparative Biochemistry and Physiology Part A: Physiology*, 45, 833–842.

- Huey, R. B., & Berrigan, D. (1996). Testing evolutionary theories of acclimation. In I. A. Johnston & A. F. Bennett (Eds.), *Animals and temperature: Phenotypic and evolutionary adaptation* (Vol. 59, pp. 205–237). Cambridge, UK: Society for Experimental Biology Seminar Series.
- Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M., & Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 367, 1665–1679. <https://doi.org/10.1098/rstb.2012.0005>
- Janzen, D. H. (1967). Why mountain passes are higher in tropics. *The American Naturalist*, 101, 233.
- Kellermann, V., Chown, S. L., Schou, M. F., Aitkenhead, I., Janion-Schepers, C., Clemson, A., ... Sgro, C. M. (2019). Comparing thermal performance curves across traits: How consistent are they? *Journal of Experimental Biology*, 222(11), jeb193433. <https://doi.org/10.1242/jeb.193433>
- Kelsch, S. W., & Neill, W. H. (1990). Temperature preference versus acclimation in fishes – Selection for changing metabolic optima. *Transactions of the American Fisheries Society*, 119, 601–610. [https://doi.org/10.1577/1548-8659\(1990\)119<0601:TPVAIF>2.3.CO;2](https://doi.org/10.1577/1548-8659(1990)119<0601:TPVAIF>2.3.CO;2)
- Kingsolver, J. G. (2009). The well-temperated biologist (American Society of Naturalists Presidential Address). *The American Naturalist*, 174, 755–768. <https://doi.org/10.1086/648310>
- Kingsolver, J. G., & Gomulkiewicz, R. (2003). Environmental variation and selection on performance curves. *Integrative and Comparative Biology*, 43, 470–477. <https://doi.org/10.1093/icb/43.3.470>
- Kingsolver, J. G., Heckman, N., Zhang, J., Carter, P. A., Knies, J. L., Stinchcombe, J. R., & Meyer, K. (2015). Genetic variation, simplicity, and evolutionary constraints for function-valued traits. *The American Naturalist*, 185, E166–E181. <https://doi.org/10.1086/681083>
- Kingsolver, J. G., Izem, R., & Ragland, G. J. (2004). Plasticity of size and growth in fluctuating thermal environments: Comparing reaction norms and performance curves. *Integrative and Comparative Biology*, 44, 450–460. <https://doi.org/10.1093/icb/44.6.450>
- Knies, J. L., Izem, R., Supler, K. L., Kingsolver, J. G., & Burch, C. L. (2006). The genetic basis of thermal reaction norm evolution in lab and natural phage populations. *PLoS Biology*, 4, e201. <https://doi.org/10.1371/journal.pbio.0040201>
- Latimer, C. A., Foley, B. R., & Chenoweth, S. F. (2015). Connecting thermal performance curve variation to the genotype: A multivariate QTL approach. *Journal of Evolutionary Biology*, 28, 155–168. <https://doi.org/10.1111/jeb.12552>
- McNab, B. K. (2002). *The physiological ecology of vertebrates: A view from energetics*. Ithaca, NY: Cornell University Press.
- Miller, N. A., Chen, X., & Stillman, J. H. (2014). Metabolic physiology of the invasive clam, *Potamocorbula amurensis*: The interactive role of temperature, salinity, and food availability. *PLoS ONE*, 9(3), e91064. <https://doi.org/10.1371/journal.pone.0091064>
- Neff, B. D., & Olden, J. D. (2010). Not so fast: Inflation in impact factors contributes to apparent improvements in journal quality. *BioScience*, 60, 455–459. <https://doi.org/10.1525/bio.2010.60.6.9>
- Park, S., & Jang, M. (2011). Phosphoproteome profiling for cold temperature perception. *Journal of Cellular Biochemistry*, 112, 633–642. <https://doi.org/10.1002/jcb.22960>
- Payne, N. L., & Smith, J. A. (2017). An alternative explanation for global trends in thermal tolerance. *Ecology Letters*, 20, 70–77. <https://doi.org/10.1111/ele.12707>
- Pigliucci, M. (2001). *Phenotypic plasticity: Beyond nature and nurture*. Baltimore, MD: Johns Hopkins University Press.
- Portner, H. O. (2001). Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften*, 88, 137–146. <https://doi.org/10.1007/s001140100216>
- Portner, H. O. (2010). Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, 213, 881–893. <https://doi.org/10.1242/jeb.037523>
- Portner, H. O., & Farrell, A. P. (2008). Ecology physiology and climate change. *Science*, 322, 690–692. <https://doi.org/10.1126/science.1163156>
- Pörtner, H. O., & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, 315, 95–97. <https://doi.org/10.1126/science.1135471>
- Postma, E. (2007). Inflated impact factors? The true impact of evolutionary papers in non-evolutionary journals. *PLoS ONE*, 2(10), e999. <https://doi.org/10.1371/journal.pone.0000999>
- Powell, M. L., & Watts, S. A. (2006). Effect of temperature acclimation on metabolism and hemocyanin binding affinities in two crayfish, *Procambarus clarkii* and *Procambarus zonangulus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 144, 211–217. <https://doi.org/10.1016/j.cbpa.2006.02.032>
- Precht, H. (1958). Theory of temperature adaptation in cold-blooded animals. In C. L. Proser (Ed.), *Physiological adaptation* (pp. 50–78). Washington, DC: American Physiological Society.
- Prosser, C. L. (1958). General summary: The nature of physiological adaptation. In C. L. Proser (Ed.), *Physiological adaptation* (pp. 167–180). Washington, DC: American Physiological Society.
- Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*, 21, 1425–1439. <https://doi.org/10.1111/ele.13107>
- Rosenthal, R. (1979). The file drawer problem and tolerance for null results. *Psychological Bulletin*, 86, 638–641. <https://doi.org/10.1037/0033-2909.86.3.638>
- Russell, G. A., & Chappell, M. A. (2007). Is BMR repeatable in deer mice? Organ mass correlates and the effects of cold acclimation and natal altitude. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, 177, 75–87. <https://doi.org/10.1007/s00360-006-0110-y>
- Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, 218, 1856–1866. <https://doi.org/10.1242/jeb.118851>
- Schulte, P. M., Healy, T. M., & Fanguie, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*, 51, 691–702. <https://doi.org/10.1093/icb/51/6/691>
- Seebacher, F., Beaman, J., & Little, A. G. (2014). Regulation of thermal acclimation varies between generations of the short-lived mosquitofish that developed in different environmental conditions. *Functional Ecology*, 28, 137–148. <https://doi.org/10.1111/1365-2435.12156>
- Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, 5, 61–66. <https://doi.org/10.1038/nclimate2457>
- Shah, A. A., Gill, B. A., Encalada, A. C., Flecker, A. S., Funk, W. C., Guayasamin, J. M., ... Ghalambor, C. K. (2017). Climate variability predicts thermal limits of aquatic insects across elevation and latitude. *Functional Ecology*, 31, 2118–2127. <https://doi.org/10.1111/1365-2435.12906>
- Tort, A. B. L., Targino, Z. H., & Amaral, O. B. (2012). Rising publication delays inflate journal impact factors. *PLoS ONE*, 7(12), e53374. <https://doi.org/10.1371/journal.pone.0053374>
- Tsuji, J. S. (1988). Thermal-acclimation of metabolism in sceloporus lizards from different latitudes. *Physiological Zoology*, 61, 241–253. <https://doi.org/10.1086/physzool.61.3.30161237>
- Vezina, F., Jalvingh, K. M., Dekinga, A., & Piersma, T. (2006). Acclimation to different thermal conditions in a northerly wintering shorebird is driven by body mass-related changes in organ size (vol 209, pg 3141, 2006). *Journal of Experimental Biology*, 209, 3952–3952. <https://doi.org/10.1242/jeb.02512>
- Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, 36, 1–48.

- Whitman, D. W., & Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important? In D. W. Whitman & T. N. Ananthakrishna (Eds.), *Phenotypic plasticity of insects: Mechanisms and consequences* (pp. 1–63). Enfield, NH: Science Publishers Inc.
- Wilson, A. E. (2007). Journal impact factors are inflated. *BioScience*, 57, 550–551. <https://doi.org/10.1641/B570702>
- Wilson, R. S., & Franklin, C. E. (2002). Testing the beneficial acclimation hypothesis. *Trends in Ecology & Evolution*, 17, 66–70. [https://doi.org/10.1016/S0169-5347\(01\)02384-9](https://doi.org/10.1016/S0169-5347(01)02384-9)
- Withers, P. C. (1992). *Comparative animal physiology*. Fort Worth, TX: Harcourt Brace Jovanovich College Publishers.
- Wythers, K. R., Reich, P. B., & Bradford, J. B. (2013). Incorporating temperature-sensitive Q_{10} and foliar respiration acclimation algorithms modifies modeled ecosystem responses to global change. *Journal of Geophysical Research-Biogeosciences*, 118, 77–90.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Havird JC, Neuwald JL, Shah AA, Mauro A, Marshall CA, Ghalambor CK. Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q_{10} effects: Why methodology matters. *Funct Ecol*. 2020;34:1015–1028. <https://doi.org/10.1111/1365-2435.13534>