

Local adaptation reduces the metabolic cost of environmental warming

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Abstract. Metabolism shapes the ecosystem role of organisms by dictating their energy demand and nutrient recycling potential. Metabolic theory (MTE) predicts consumer metabolic and recycling rates will rise with warming, especially if body size declines, but it ignores potential for adaptation. We measured metabolic and nutrient excretion rates of individuals from populations of a globally invasive fish that colonized sites spanning a wide temperature range (19–37°C) on two continents within the last 100 yr. Fish body size declined across our temperature gradient and MTE predicted large rises in population energy demand and nutrient recycling. However, we found that the allometry and temperature dependency of metabolism varied in a countergradient pattern with local temperature in a way that offset predictions of MTE. Scaling of nutrient excretion was more variable and did not track temperature. Our results suggest that adaptation can reduce the metabolic cost of warming, increasing the prospects for population persistence under extreme warming scenarios.

Key words: allometric scaling; body size; climate change; countergradient variation; ecology; excretion; *Gambusia*; metabolic rate; mosquitofish; temperature.

INTRODUCTION

Concern over climate change has spurred interest in predicting how changing thermal regimes will influence ecological systems (Bellard et al. 2012). Much of our current approach to making these predictions is based on studies that take subsets of communities from a single ecosystem, expose them to elevated temperature over relatively short periods of time (i.e., within the lifespan of some constituent organisms), and gauge ecological responses (Shurin et al. 2012, Yvon-Durocher et al. 2012). Often, the results of these experiments are either compared to or used to parameterize models that incorporate expected temperature dependency of various ecological processes (Gilbert et al. 2014). In essence, this approach forces today's organisms into the context of tomorrow's climate. This may be a major shortcoming because it fails to account for potential adaptation. Here, we consider adaptation as trait change through either developmental plasticity or evolution of genetically fixed traits to a range of drivers (Palkovacs et al. 2012), including changing climate (Bradshaw and Holzapfel 2006). Such contemporary adaptation can substantially alter ecological outcomes derived from expectations of fixed phenotypes (Woodward et al. 2005, Fryxell and Palkovacs 2017). Anticipating future climate change outcomes may thus depend on our ability to develop a general mechanistic understanding of how contemporary thermal adaptation alters fundamental physiological and ecological functions of populations.

The Metabolic Theory of Ecology (MTE) addresses temperature, body size, and metabolic rate and proposes “universal” scaling rules among these parameters to explain a wide range of ecological processes (Brown et al. 2004). Consequently, MTE is now embedded in models aimed at

predicting how rising temperature can alter a diverse array of attributes such as population abundance, community composition, trophic interactions, and whole ecosystem processes (Gilbert et al. 2014, Bruno et al. 2015, Schramski et al. 2015). In particular, MTE predicts that metabolic rate (B) scales predictably in relation to body size and temperature using the equation:

$$B = b_0 M^\alpha e^{-E/kT} \quad (1)$$

where b_0 is a normalization constant, M is body mass, α (alpha) is a “universal” scaling coefficient, E is the activation energy of metabolism, k is Boltzmann's constant, and T is the absolute temperature (Gillooly et al. 2001). Alpha (α) relates metabolic rate to body size, typically assuming 3/4 power scaling, such that smaller individuals should have a predictably higher metabolic rate per unit mass than large individuals (i.e., small individuals are metabolically less efficient than large individuals). Metabolic rate is also predicted to rise exponentially with increasing temperature following a Boltzmann-Arrhenius factor wherein $E \sim -0.65$ eV. Processes that are coupled to metabolic rate and ecologically important, like nutrient recycling through excretion (Vanni 2002), should behave similarly (Brown et al. 2004).

It has been recently proposed that a decline in body size within communities and populations may be a universal outcome of rising temperature (Gardner et al. 2011). This has important implications when linked to MTE and scaled up to population-level processes in ecosystems. MTE predicts that warming alone should drive up population-level metabolic rates due to the temperature dependence of metabolism (Fig. 1). If body size declines and MTE holds true, populations in a warmer future should have even higher energy demand and nutrient recycling rates than expected under MTE alone because populations shift to groups of less metabolically efficient smaller individuals (Fig. 1). This prediction suggests a potential energy constraint that could

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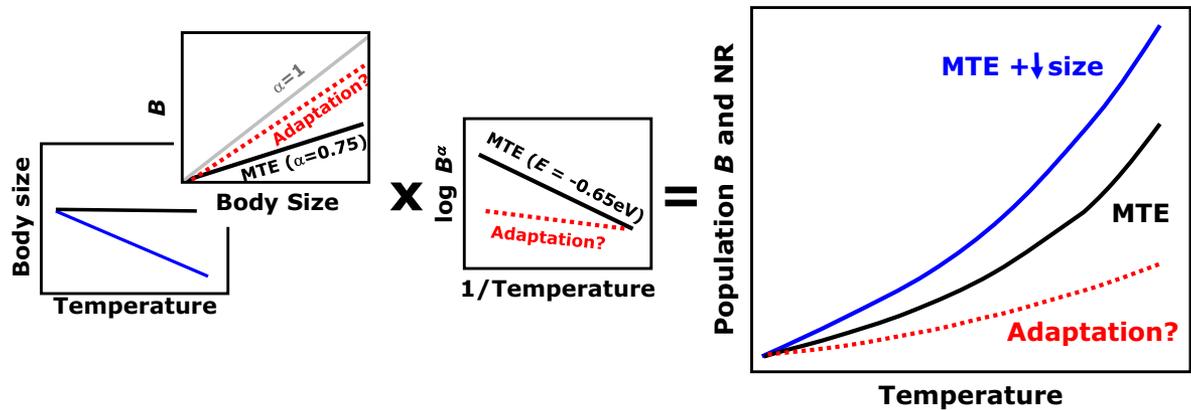


FIG. 1. Conceptual diagram showing the change in population metabolic rate (B) and nutrient recycling rate (NR) with rising temperature under three scenarios. Metabolic theory (MTE, solid black lines) predicts exponentially rising metabolic and recycling rates with increasing temperature due to the temperature dependency of metabolism and invariance of metabolic scaling parameters (α and E , see Eq. 1). Declining body size (MTE + \downarrow size, blue lines) with increasing temperature accelerates the rise in metabolic and excretion rates because of the assumed scaling coefficient for metabolism relative to body size (i.e., $\alpha = 0.75$) in MTE. If body size declines, but adaptation (1) equalizes metabolic rate per unit mass across body sizes (i.e., α approaches 1 with rising temperature) and (2) reduces the temperature sensitivity of metabolism (i.e., $E < -0.65$ eV), and then, population metabolic and excretion rates are far less sensitive to temperature change than predicted by MTE (Adaptation, dashed red lines).

limit population abundance, similar to self-thinning, where individual size increases while population density decreases (Jonsson 2017), if food supply does not rise to match consumer demand.

Here, we ask whether adaptation can mitigate the metabolic cost of increasing temperature by shifting metabolic scaling factors. While MTE proposes relatively universal scaling, there is substantial variation in scaling parameters for body size (Glazier 2005) and temperature (Dell et al. 2011), particularly among divergent phylogenetic taxa (Uyeda et al. 2017). Variation in metabolic scaling parameters across and within some species has been linked to consumer body form and temperature (Killen et al. 2010, Ohlberger et al. 2012). It remains unclear though if variation in metabolic scaling parameters tracks temperature within species as populations adapt on time frames similar to expectations of future climate warming.

We hypothesize that selection shifts scaling parameters in ways that mitigate the expected greater energetic demands of smaller body size and warmer temperatures (Fig. 1). For example, an adaptive reduction in temperature sensitivity of metabolism (i.e., $E < 0.65$ eV) would minimize the direct metabolic increase resulting from rising temperature (Fig. 1). Furthermore, adaptive changes in alpha toward 1 under warmer conditions could reduce the relative metabolic penalty of being small (i.e., more similar metabolic rate per unit mass across body sizes). Such metabolic adaptations should be evident in metabolically related processes, like nutrient recycling capacity, that have strong effects in ecosystems beyond energy demand by consumers.

To test this hypothesis, we examined populations of a globally invasive freshwater fish, *Gambusia affinis* (western mosquitofish), that has recently colonized geothermal systems spanning a large temperature gradient in geographically diverse areas (New Zealand and California, USA). Using these geographically diverse areas allowed us to determine whether the species response to temperature was convergent between regions. We used these populations to

(1) determine if rising temperature leads to reductions in consumer body size, (2) measure the amount of intraspecific variation in metabolic and nutrient recycling scaling parameters and examine their relationship to temperature, and (3) compare the population-level outcomes of warming derived from models using scaling parameters predicted by MTE alone and models using measured intraspecific parameters and body size change that have been subject to potential evolution.

MATERIALS AND METHODS

Study organism and populations

Gambusia affinis were introduced to New Zealand in the 1930s and to California in the 1920s (McDowall 1978, Stockwell and Weeks 1999). Those fish ultimately derived from populations in Texas, USA. *Gambusia* are livebearers, can reach high densities in the wild, and are found across a wide range of environmental conditions (salinity, temperature, pH, turbidity; Pyke 2008). Assuming two generations per year since introduction (Pyke 2008), there may have been approximately 180 generations in California and in New Zealand. Rapid evolutionary divergence has been noted in *Gambusia* in response to novel habitats, predation pressure, and thermal environments (Stearns 1983, Stockwell and Weeks 1999, Langerhans et al. 2007). We studied five populations of *Gambusia* in geothermal springs spanning a wide gradient of temperatures in California, USA and another five populations on the North Island of New Zealand. California populations were in springs that were pond like. Some sites have barriers to dispersal allowing for precise local adaptation (Appendix S1: Table S1); however, we cannot discount movement of fish among sites due to human activity. New Zealand populations inhabited slow flowing, spring-fed streams, and four of the five systems were potentially open to fish movement. All measurements were carried out in summer with California sites sampled between 1 and

5 September 2015 and New Zealand sites sampled between 23 January and 1 February 2016.

Field metabolism and nutrient excretion

Field metabolic rate (FMR) was measured as oxygen consumption (MO_2) by individual *Gambusia* held in situ in closed-system respirometers (Sinclair et al. 2006). We measured FMR because it is more ecologically relevant than basal metabolic rate (Hudson et al. 2013) and because we coupled metabolism with nutrient excretion, which is influenced by diet necessitating the need for measurement of fish in the field. Respirometers comprised clear, 40 mL rectangular acrylic chambers with valves on each end. At each site, we captured 50 fish spanning a wide size range for the measurement of RMR in a series of 20-min runs using four respirometers in each run. During runs, the respirometers were held in a 50-L clear container of site water that was submerged to maintain ambient water temperature. *Gambusia* were captured and held in a bucket of site water for 20 min in advance of each run to establish uniform holding times and conditions prior to measurements. At the start of a run, each respirometer was filled with water from the container, a single fish was added, the respirometer lid was sealed, and container water was flushed through the valves, which were then closed. After a few minutes, dissolved oxygen concentration and temperature in each respirometer were monitored using a FireSting four-channel oxygen logger with optical oxygen sensors (PyroScience, Aachen, Germany). An exception to this protocol was made for the smallest juveniles (<16 mm), which were assayed as pairs.

Fish settled rapidly in the respirometers and held position with minimal body movement. Fish-mass:respirometer-volume was sufficient to provide mixing with fin movement only and provide linear declines in oxygen over time (Clark et al. 2013). Changes in oxygen concentration over time were estimated from linear fits to the data, and only fits with $r^2 > 0.9$ were used. In some instances where aberrations occurred (e.g., fish contacting the sensors), the difference in oxygen concentrations at the start and end of the run was used (9% of all measurements). Microbial MO_2 was controlled for by subtracting the MO_2 in blanks (respirometers with water only) that were completed every other run. MO_2 was calculated per fish as $\mu\text{g O}_2/\text{min}$.

Nutrient excretion rates were estimated by change in NH_4^+ -N concentrations in the closed respirometers over the 20-min assays (Whiles et al. 2009). Change in nitrogen concentration was determined by difference in concentrations between respirometers with fish and blanks. At the end of each run, a water sample was withdrawn from each respirometer, filtered (Whatman GF/F, Buckinghamshire, UK) into 15-mL HDPE tubes, and frozen until analysis. Ammonium (NH_4^+) concentration was measured by fluorometric technique (Holmes et al. 1999) using a Trilogy Laboratory Fluorometer (Turner Designs, San Jose, California, USA) in California and by colorimetry using a Lachat QuikChem 8500 Series 2 Flow Injection Analysis System (Lachat Instruments, Loveland, Colorado, USA) in New Zealand. After withdrawing water samples, *Gambusia* were euthanized with MS-222 or clove oil, measured for length and sex on site, and frozen. Later in the laboratory, *Gambusia*

volume was by measured by water displacement following the Archimedes principle, dried for 48 h at 60°C, and weighed to determine dry mass.

Population size structure

To determine *Gambusia* population size structure in each site, a 5-m seine (1.6-mm mesh) was hauled repeatedly in several locations at each site to capture diversity among habitat types. All seined fish were immediately euthanized, transported to the laboratory on ice, and frozen. Fish length, sex, and dry mass were later measured as described in *Field metabolism and nutrient excretion*.

Statistical analysis

The allometric relationship between body size and FMR was determined for each population, and for the combined data set, using simple linear regression on log-transformed data. For all individuals, the allometric slope was compared to the MTE slope of 0.75 with a Wald Test, allowing us to test for difference between models. This analysis was done using the package car v2.1-6 in R v3.3.3 (Fox and Weisberg 2011, R Development Core Team 2015). Population-specific slopes and intercepts were related to site temperatures by linear regression. We estimated E using an Arrhenius relationship between mass-normalized metabolic rate and site temperature in three ways. First, E was estimated using metabolic rate normalized to mass assuming 3/4 power for all populations following Gillooly et al. (2001). Second, we calculated E with metabolic rate normalized to observed allometric slopes (α) for each population. Third, we estimated E at multiple body sizes in each population along the temperature gradient by using the observed allometric relationships between metabolic rate and body size specific to each population. For each population, we calculated metabolic rate of individuals between 5 and 500 mg at 5 mg increments using the intercept and slope of the allometric relationship from our observed data. Activation energy (E) for each body size increment was then determined from an Arrhenius relationship between metabolic rate and temperature across populations at each body size. The allometry of nitrogen excretion rate with body size was analyzed similarly to metabolic rate. We also calculated an average (\pm SE) nitrogen quotient (NQ) for each population, as the ratio of excretion rate to metabolic rate. We used NQ to estimate the percentage of metabolism fueled by protein and this equation assumes that diet of 100% protein has a NQ of 0.27 (Wood 2001).

We tested for changes in body size distributions across the temperature gradient using quantile regression using the R package quantreg v.5.33 (Koenker 2017). Quantile regression was used for its ability to discern whether changes in size distributions occurred uniformly across size classes within population or disproportionately with respect to larger or smaller size contingents of populations. Because *Gambusia* display sexual size dimorphism, males and females were analyzed separately. Quantile regression was conducted at every 0.10 quantile from 0.10 to 0.90.

We modeled population metabolic and nutrient excretion rates across the observed temperature range of our sites in four scenarios. In scenario 1, we used "universal" MTE

scaling (MTE; $\alpha = 0.75$ and $E = 0.65$ eV) applied to a constant body size distribution equivalent to our overall coldest population. In scenario 2, we used *Gambusia*-specific scaling parameters (*Gambusia*; $\alpha = 0.75$ and $E = 0.27$ eV, which were derived from our data) applied to the same static body size distribution as in Scenario 1. In scenario 3, we used the *Gambusia*-specific scaling parameters combined with the observed body size distributions in each population (*Gambusia* + size). In scenario 4, we used observed metabolic and nutrient excretion scaling parameters and body size distributions for each population (Adaptation). We held total population biomass static (1 kg) across all populations and scenarios. Total population biomass was distributed into 50 equal body size bins according to the observed distribution of biomass for the populations determining body size distributions in each model. The number of individuals in each bin was determined by dividing biomass in the bin by mean body size of the bin. For models 1 and 2, metabolic rates of individual fish were calculated according to Eq. 1 and parameterized using the scenarios described in the current paragraph and summed across all fish to generate total population metabolic and excretion rate. For model 3, the observed relationship between metabolism or excretion and body size at each site was used to calculate individual rates, which were summed for population rate. Non-linear models were run using the nls function in base R, and bootstrapped (replication = 1,000) confidence intervals were produced for each data point using the R packages MASS v.7.3.49 and Hmisc v.4.1 (Venables and Ripley 2002, Harrell 2006). Statistical significance was assumed at $\alpha < 0.05$.

RESULTS

Population structure

All populations were female biased with male:female ratios ranging from 0.09 to 0.89, and this ratio was not significantly

correlated with temperature ($r_s = -0.243$, $P = 0.468$; Appendix S1: Table S2). In separate analyses, body mass of females and males declined at every size quantile with increasing habitat temperature (quantile regression, $P < 0.0001$; Fig. 2). The decline in body size was strongest at larger body sizes, as evident in the slope estimates. For example, in males slopes decreased fivefold from -0.0004 to -0.0020 from quantile 0.1 to 0.9, respectively (Appendix S1: Table S3).

Body size scaling and temperature sensitivity and of metabolic rate

Across all individuals, metabolic rate increased with mass ($r^2 = 0.763$, $P < 0.0001$) with a slope (0.757) very similar to the 0.75 expectation of MTE (Wald Test, $P = 0.754$; Appendix S1: Fig. S1). However, slopes, or the rate at which metabolic rate increases with mass, for individual populations varied considerably across our populations (0.70–0.87), and the slopes increased with temperature (Fig. 3A, $r^2 = 0.534$, $P = 0.016$). Arrhenius plots relating metabolism, corrected assuming 3/4 power scaling in all populations, and temperature revealed a statistically significant relationship ($P < 0.0001$), but the slope (0.27 eV) was far below expectations of MTE (~ 0.65 eV), and the fit was poor ($r^2 = 0.14$; Appendix S1: Fig. S2A). Similarly, when estimated using population-specific scaling factors, E was low (0.10 eV) compared to MTE expectations (Appendix S1: Fig. S2B). When E was estimated at individual body size increments using observed allometric relationships in each population, we found that E increased with body size (Fig. 3B), a consequence of the rise in allometric slope with increasing temperature (Fig. 3A).

Metabolic rate and nutrient excretion

Nitrogen excretion rates rose with body size at similar slopes (0.66–0.67), but with different intercepts, in the two

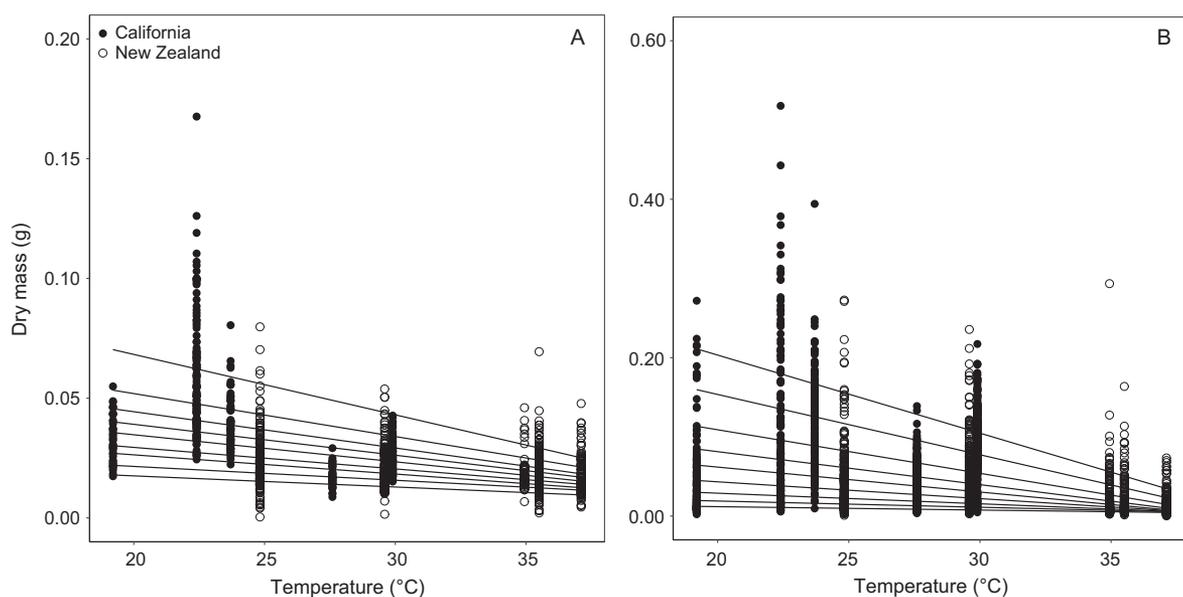


FIG. 2. Mosquitofish population size distribution data for (A) males ($n = 1,042$) and (B) females ($n = 2,939$) across geothermal populations in California and New Zealand with quantile regression lines (0.1–0.9) shown.

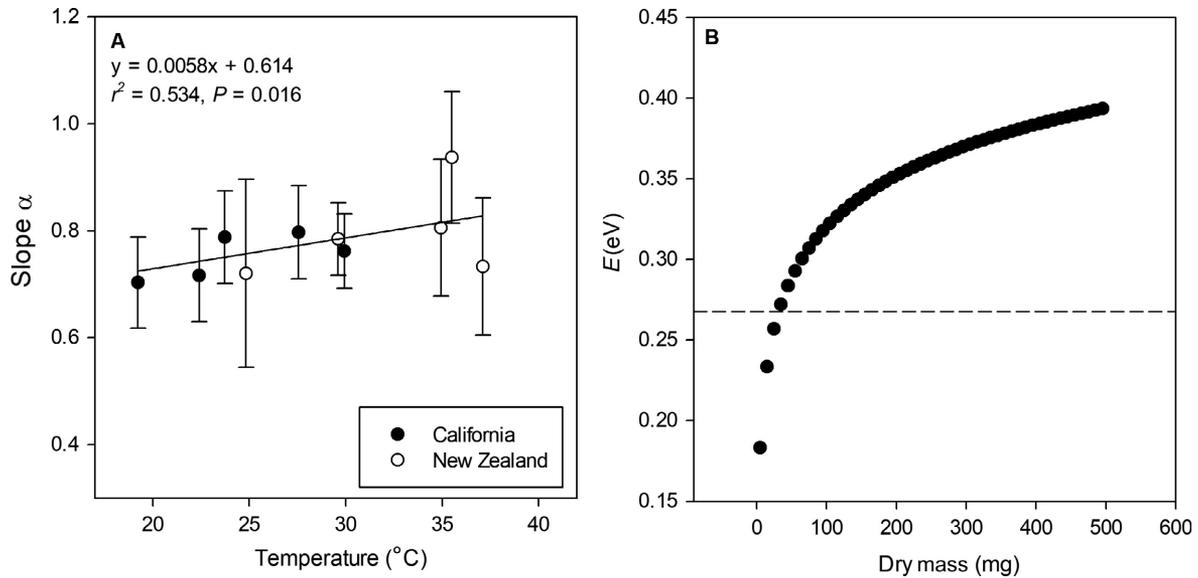


FIG. 3. Relationship between site temperature and (A) the allometric scaling coefficients for metabolic rate (α ; error bars are mean absolute error [MAE]; $n = 10$) and (B) the predicted E for different size classes across our thermal gradient.

countries (Appendix S1: Fig. S3). As was the case for metabolic rate, the scaling slopes for excretion rate across body size varied considerably among our populations (0.46–0.92). In contrast to metabolic rate, there was no relationship between scaling slopes for excretion and temperature across our populations (Appendix S1: Fig. S4). Nitrogen excretion rate scaled sub-equally with metabolic rate, where slopes differed between countries such that excretion rate increased faster relative to metabolic rate in New Zealand (0.85:1) compared to California (0.62:1; Fig. 4). There was no relationship between metabolic and excretion slopes (Appendix S1: Fig. S5). Nitrogen quotient values increased with temperature across populations, ranging from 0.065 ± 0.004 at 19°C to 0.150 ± 0.012 at 37°C. This translates into an increase in the amount of metabolism supported by proteins from $23.9\% \pm 1.5\%$ to $55.7\% \pm 4.3\%$ across the temperature range ($r^2 = 0.463, P = 0.0304$).

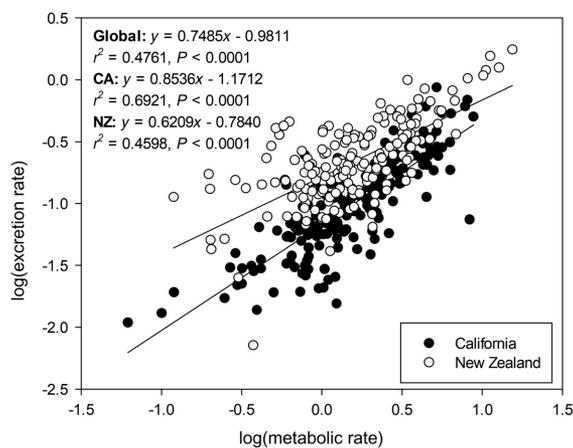


FIG. 4. Relationship between excretion ($\mu\text{g N}\cdot\text{min}^{-1}\cdot\text{fish}^{-1}$) and metabolic rates ($\mu\text{g}\cdot\text{min}^{-1}\cdot\text{fish}^{-1}$) of all fish measured in California and New Zealand populations ($n = 373$). Regressions are for linear fits to each country individually (solid lines). Global regression statistics are for all individuals pooled.

Scaling up and predicting the future

Application of “universal” MTE parameters to a “cool” population (scenario 1) predicted, as expected, an exponential increase in population metabolic rates with rising temperature (Fig. 5). Use of *Gambusia*-specific parameters (scenario 2) predicted rising population metabolic and excretion rate with temperature, but to a far lesser extent than assuming the specific activation energy associated with aerobic respiration (i.e., $E = 0.65$). When we applied the observed changes in population body size distributions to the *Gambusia* model (scenario 3), population metabolism was higher than expected without body size declines ($r^2 = 0.944, P < 0.0001, E = 0.40$ eV). At lower temperatures, the 95% confidence intervals overlapped with scenario 2, but at warmer temperatures, these began to diverge. Application of population-specific metabolic scaling parameters and body size distributions (scenario 4) yielded rates of population metabolism that rose with temperature ($r^2 = 0.693, P = 0.0028, E = 0.26$ eV) but at rates consistently lower (up to 3.3-fold at the highest temperature) than the model assuming the generalized species scaling and populations size shifts in scenario 3 (Fig. 5). Differences were even more dramatic when compared to use of universal MTE scaling.

Models of nutrient excretion that assumed MTE and *Gambusia*-specific scaling followed patterns similar to metabolic rate (Appendix S1: Fig. S6; scenarios 1 and 2). When we applied the observed changes in population body size distributions (scenario 3), population excretion diverged most at higher temperatures, with higher population excretion rates than expected without body size declines ($r^2 = 0.944, P < 0.0001, E = 0.40$ eV). Excretion based on observed scaling in populations tended to rise with temperature (scenario 4), but the pattern was not statistically significant ($P = 0.1861$). Population excretion rates based on population-specific scaling were consistently lower than all other models.

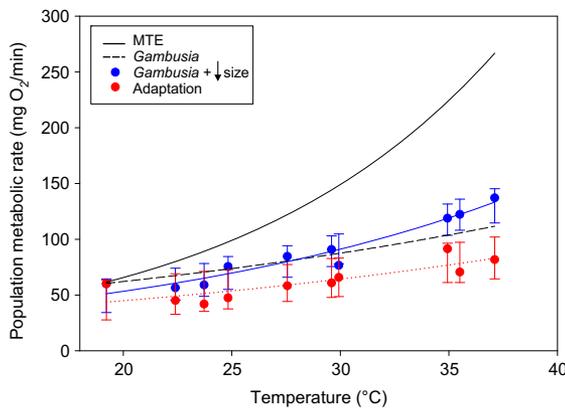


FIG. 5. Predicted change in mosquitofish population-level metabolic rate with rising temperature derived from three scenarios: (1) MTE with no body size change with rising temperature (MTE, solid black line), (2) MTE with our observed E for *Gambusia* ($E = 0.27$ eV; *Gambusia*, dashed line), (3) MTE with the observed E for *Gambusia* and changes in body size distributions across our mosquitofish populations (*Gambusia* + \downarrow size, blue solid line), and (4) observed metabolic scaling relationships and change in body size distributions in mosquitofish populations (Adaptation, red dotted line; see Materials and Methods, Statistical analysis and Fig. 1 for details). Error bars on models are bootstrapped 95% confidence intervals. Symbols are individual populations ($n = 10$), and lines are exponential fits for each model.

DISCUSSION

Projections of ecological response to increasing temperature are often based on bioenergetics models or studies in which populations or communities from the present day are exposed to elevated temperature over relatively short periods meant to simulate future thermal conditions. Our findings suggest such approaches may be generally flawed for species that demonstrate substantial capacity for contemporary adaptation in metabolic scaling traits. We do not know to what extent the intraspecific variation in our study is being shaped by plasticity vs. evolution. However, any trait change offsetting the costs of changing environmental conditions may reasonably be assumed adaptive. Regardless of the mechanisms underlying the trait change, the ecological outcome suggests that ignoring predictable variation in metabolic scaling traits can vastly overestimate consumer population energy demand and nutrient cycling and may ultimately underestimate the scope for persistence under future warming.

Body size and temperature

Body size reduction in response to temperature rise is thought to be the “third universal response” to warming (Gardner et al. 2011, Sheridan and Bickford 2011). This prediction has strong empirical and theoretical support (Bergmann 1847, James 1970, Angilletta and Dunham 2003), although there are exceptions (Walters and Hassall 2006, Adams et al. 2013). This phenomenon may be exacerbated in freshwater environments where body size changes are thought to be greater than in terrestrial environments, due to a lower oxygen availability (Daufrense et al. 2009, Forster et al. 2012). Despite their recent establishment in the

last century, our mosquitofish populations conform to this theorized general response to warming. Interestingly, we found that reductions in size were not equally distributed across size distributions, but rather disproportionately impacted upper size quantiles (Fig. 2). Thus, warming appears to have its greatest effects in constraining upper sizes attainable in populations. This would seem to present an added metabolic challenge for populations facing warming because smaller size individuals have higher mass-specific metabolic rates than large individuals under MTE (Brown et al. 2004). Our modeling showed that the observed size shifts in populations across the temperature gradient of our sites could drive up total population metabolism, but mostly at higher temperatures. This pattern is likely muted by the fact that all populations contained a range of smaller individuals, likely because of multiple cohorts. The size-driven boost in population energy demand only occurs though if metabolic scaling parameters are static, which was not true in our study.

Body size scaling and metabolic rate

A body mass scaling exponent of 0.757 across all fish in our study fits neatly with MTE prediction of $3/4$ scaling, but this masked important underlying variation in scaling across populations (Appendix S1: Fig. S1). The range in scaling coefficients we found across populations of one fish species (0.70–0.87) equaled the range of variation among different fish species with highly divergent body morphologies (0.69–0.86; Killen et al. 2010). Variation in metabolic scaling coefficients is now well-recognized (Dodds et al. 2001, Kozłowski and Konarzewski 2004, Glazier 2005, 2010). Ultimately, what is important is whether that variation is related to environmental drivers, subject to adaptive trait change, and of a magnitude and pattern that is ecologically meaningful.

The wide range in scaling exponents for our populations was predictable, with slopes increasing with temperature (Fig. 3A) in both New Zealand and California populations. Such repeated patterns of phenotype–environment associations in the introduced ranges of species are commonly interpreted as support for the importance of environmental gradients and the likely adaptive basis of contemporary trait change (Gilchrist et al. 2001, Kinnison et al. 2003). The pattern of rising slope with increasing temperature indicated reduced temperature sensitivity at low body size across populations. It appears the metabolic advantage of being large (i.e., low metabolic rate per unit mass) is lost under adaptation to locally higher temperatures. Therefore, our findings not only support the hypothesis that thermal adaptation shapes metabolic scaling, but also suggest that such adaptation might partly facilitate decreasing mean body sizes as the “third universal response” to warming. The pattern across our populations could reflect an inherently lower temperature sensitivity of small individuals in *Gambusia*, but we cannot disentangle this mechanism as we have not examined the temperature sensitivity across size classes within each of our mosquitofish populations. However, we have measured routine metabolic rate on fish acclimated at multiple temperatures in the laboratory for a few of our populations. Interestingly, those data (Appendix S1: Fig. S7) show higher temperature sensitivity of smaller fish, a pattern

reverse of what we found in our cross-population comparison. Those data are quite limited, and resolving the issue fully will require common garden rearing and further within population analysis.

Temperature dependency of allometric scaling of metabolism has been shown across fish species and within some fish species. Killen et al. (2010) found negative temperature dependence of allometric exponents across species. Within species, negative temperature dependency is more common than positive dependency, but few species have been examined (Ohlberger et al. 2012). Notably, prior studies have examined basal metabolic rates of fish collected from single populations and experimentally acclimated to different temperatures over short time frames. Our results show temperature dependency plays out in a predictable way as populations adapt in contemporary time to rising temperature and in a likely more ecologically relevant parameter, field metabolic rate. Whether the positive effect of temperature on allometric exponents we found is more representative of likely change in the wild or simply a feature of mosquitofish will require studies of more species. Regardless, our results support the notion that temperature dependency of metabolic exponents may have profound ecological consequences and places this in the context of contemporary adaptation.

Temperature sensitivity of metabolic rate

Under MTE, metabolic rate is predicted to increase with temperature under a mass-normalized activation energy (E) of 0.65 eV (Gillooly et al. 2001). We found a lower *Gambusia*-specific activation energy of 0.27 eV in our combined dataset, indicating low temperature sensitivity of *Gambusia* in general (Fig. 3B). This result again supports other studies that call into question the generality of $E = 0.65$ eV. Indeed, while a meta-analysis (Dell et al. 2011) supported an average value of 0.66 eV across 1,072 individuals, the median value was lower ($E = 0.55$ eV), and species-specific values are highly variable (Killen et al. 2010, Marshall and McQuaid 2011, Watson et al. 2014). Our results further suggest that E can vary by size in a manner linked to changing temperature. Low E values and variation in E with size could occur if local adaptation leads to countergradient trait variation (Conover and Schultz 1995, Kinnison et al. 2003), such that cold-adapted populations attain higher than expected metabolic rates, and warm-adapted populations achieve lower than expected metabolic rates, or both.

Metabolic rate and nutrient excretion

Variation in consumer metabolic rates may strongly influence rates of resource uptake, use, and excretion (Brown et al. 2004, Allen et al. 2005), which are important to nutrient cycling dynamics in freshwater systems (Vanni 2002). For this reason, it is expected that nutrient excretion rates should scale proportionally with metabolic rate. Measuring field metabolic rate and excretion in situ provided us with a unique opportunity to assess this relationship.

Our data show that excretion rates rise slower than metabolic rates (Fig. 4), and while there was substantial intraspecific variation in body size excretion scaling coefficients (0.46–0.88), they were not related to metabolic scaling

coefficients or temperature (Appendix S1: Figs. S4, S5). In a recent synthesis of in situ nutrient excretion rates in aquatic animals, scaling coefficients were commonly lower than 0.75, with global average values of 0.684 for N and 0.566 for P (Vanni and McIntyre 2016). The tendency for excretion scaling to fall short of MTE predictions suggests that factors beyond metabolism, such as diet, play a role in regulating nutrient excretion (Wood 2001, Uliano et al. 2010). We found that protein accounts for an average of 24% energy requirements at our overall coolest population to 56% at our overall warmest population, so some positive relationship between temperature and nitrogen excretion might still be expected. Hence, other factors like ingestion rates could be more locally variable and important in predicting nutrient recycling. Nonetheless, this result supports the broader premise that local and regional processes are important and that neither MTE predictions nor simple extrapolation from metabolic rate accounts for such variation.

Scaling up and predicting the future

Comparing predictions from eco-evolutionary models against predictions from base ecological models that ignore such variation can provide important insights into the potential fitness and ecological consequences of contemporary trait change (Hairston et al. 2005, Ezard et al. 2009). MTE suggests population energetic demand and excretion rate will rise with increasing temperature, and our modeling shows temperature-induced size reductions observed in real populations would exacerbate this phenomenon. Without any adaptive accommodation, these compounded costs are expected to impose a substantive energetic limitation on population biomass and persistence under warming. However, incorporating the real-world pattern of variation in metabolic scaling parameters from our study predicted a far lower increase in population energy demand than a static scenario assuming universal MTE scaling or even species-specific scaling (Fig. 5). Indeed, under this countergradient pattern, a population adapted to 30°C conditions would achieve a 35% reduction in metabolic rate when compared to our model using *Gambusia*-specific parameters and observed body size changes. This suggests that countergradient shifts in population metabolic scaling relationships have the potential to substantially increase the scope for metabolic efficiency, reproduction, and population persistence in the types of populations expected to otherwise be at greatest risk under warming. Hence, failure to account for such countergradient changes in scaling relationships may substantially impair accurate predictions of future biodiversity responses to global change.

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DATA AVAILABILITY

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.vg89038>