Thermal history drives trait divergence and alters the ecological role of a freshwater consumer (*Gambusia affinis*)

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Environmental Science, the University of Auckland, 2019.

Abstract

Increasing temperature, as a result of climate change, is predicted to have numerous effects on species, including altered geographic distributions, shifts in phenologies, and decreased body size at maturity. Theory predicts that reduced body size and rising temperature, particularly from a metabolic perspective, should lead to changes in trophic interactions and ultimately ecosystem function. However, contemporary adaptation may influence the outcomes of warming, an issue that is not commonly considered in climate change research. In this thesis, I used populations of the globally invasive mosquitofish (*Gambusia affinis*) from a wide geothermal temperature gradient as a model system to examine how thermal history influences individual traits and, ultimately, ecological function. I measured mosquitofish metabolic rates *in-situ* and after acclimation in a laboratory to understand how adaptation may lead to deviation in the rates predicted by metabolic theory. I found evidence of counter-gradient variation in metabolic traits of wild populations that offset predicted energetic demand of warming. My data show that, across populations, allometric slopes increased predictably with temperature and that size-corrected metabolic rates were unrelated to temperature. My laboratory data show that the temperature sensitivity of metabolism was reduced in warm-source populations, leading to a convergence in aerobic scope between acclimation treatments. Moreover, there was a relationship between metabolism and behaviour, this was only apparent when measured in certain contexts. I further analysed dietary variation and body elemental composition across a wide temperature range to determine if diet and body stoichiometry varied with temperature rise. Finally, I used a mesocosm experiment to examine the ecological role of body size of thermally divergent populations. Mosquitofish diet changed strongly with temperature, which was reflected by gut morphology and body elemental composition. Finally, my experimental data show that

ecological responses to different body size distributions were often dependant on source population. Overall, my research suggests considerable physiological adaptive flexibility to temperature and suggests that thermal history may mediate the ecological outcome of future body size declines.

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Publications

Chapter 2 of this thesis was prepared for publication as allowed under the 2016 PhD Statute and Guidelines.

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Eric P Palkovacs	Data collection, editing.
David C Fryxell	Data collection, laboratory sample analysis, editing.
Michael T Kinnison	Data collection, editing.

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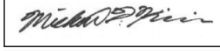
Michael T Kinnison		25 Nov 2018

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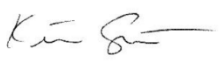
K S Simon		22 November 2018

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Eric P Palkovacs	Data collection in NZ, funding, editing.
Finnbar Lee	Laboratory sample analysis, statistical analysis, editing.

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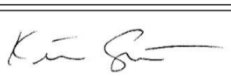
Kevin S Simon		22 November 2018
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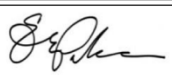
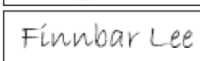
Eric P Palkovacs		26 Nov 2018
Finnbar Lee		22 November 2018

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David C Fryxell	Data collection, study design, laboratory sample analysis, editing.

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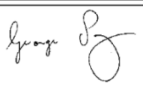
George L W Perry		23 November 2018
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Chapter 1

General Introduction

Understanding how animals interact with each another and their environment is a complex goal that requires a breadth of knowledge spanning multiple disciplines. Ecologists and evolutionary biologists have traditionally viewed ecosystems in different ways (Pelletier, Grant, & Hendry, 2009). Ecologists have typically considered evolutionary change as a process that occurs over long-time scales. Embedded in this notion is the idea that evolutionary change will have little impact on ecological dynamics, especially when looking within or across populations, and thus need not be considered. In contrast, evolutionary biology emphasises the role of trait change in determining organismal fitness, with less focus on the implications for populations, communities, or ecosystems. However, there is now much evidence to suggest that evolutionary change occurs on contemporary time-scales (Bennett et al., 2018; Ellner, Geber, & Hairston, 2011; Hairston, Ellner, Geber, Yoshida, & Fox, 2005; Norberg, Urban, Vellend, Klausmeier, & Loeuille, 2012). Such contemporary adaptation has led to interest in combining evolutionary and ecological theories of ecology, particularly where human activities impact natural systems (e.g. invasive species, global warming) (Hendry & Kinnison, 1999; Kinnison & Hairston, 2007; Palkovacs, Kinnison, Correa, Dalton, & Hendry, 2012).

Where evolutionary and ecological processes occur at the same rate reciprocal feedbacks between the processes may occur, whereby changes in the environment influence evolutionary processes and evolutionary changes influence ecological processes (eco-evolutionary dynamics) (Palkovacs & Post, 2008; Pelletier et al., 2009). For

example, in Trinidadian guppies (*Poecilia reticulata*) difference in predation pressure between populations plays a key role in regulating life histories and has consequences for ecosystem function (e.g. nutrient cycling) (Bassar et al., 2015; Dalton & Flecker, 2014). Therefore, understanding the processes driving local adaptation in contemporary time will play a key role in predicting future ecological responses.

Much of the focus on the ecological effects of trait change has been on dominant consumers or predators. Top-consumers play an important role in ecosystems because of the significant effects they have on prey communities through consumption, which ultimately drives cascading abundance and biomass changes through all trophic levels (e.g. trophic cascade) (Carpenter et al., 1987). These cascading effects are significant not only for the structure of ecosystems but also for ecosystem functioning. For example, top-down increases in consumption from fish leads to an increase in the biomass of primary producers which then influences larger scale processes such as carbon and nutrient cycling (Kitchell & Carpenter, 1993; Schindler, 1990). Thus, changes in the abundance or biomass of consumers driven by environmental change may have substantial ecological effects.

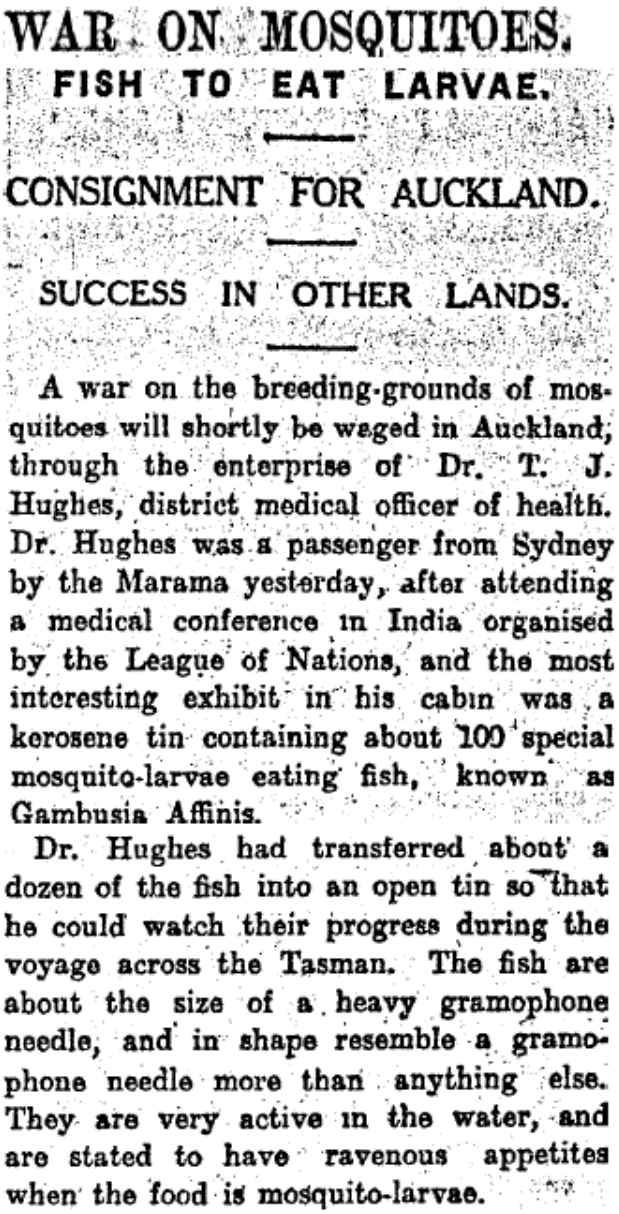
Environmental warming caused by human-induced climate change is a significant stressor for natural systems (Carpenter, Fisher, Grimm, & Kitchell, 1992). Increasing temperature will alter species distributions and phenologies and reduce body size at maturity (Comte, Buisson, Daufrense, & Grenouillet, 2013; Sheridan & Bickford, 2011). Such changes are likely to drive changes in eco-evolutionary dynamics (Crozier & Hutchings, 2014; Lavergne, Mouquet, Thuiller, & Ronce, 2010; Norberg et al., 2012). However, it is not known whether trait change (e.g. physiological, morphological, behavioural) will moderate or exacerbate environmental change (Palkovacs et al., 2012; Tuckett, Simon, & Kinnison, 2017). For example, if metabolic rates, and consumption

rates, increase with warming the top-down effect of consumers may strengthen (Brown, Gillooly, Allen, Savage, & West, 2004; Fryxell & Palkovacs, 2017; Kratina, Greig, Thompson, Carvalho-Pereira, & Shurin, 2012; O'Connor, Piehler, Leech, Anton, & Bruno, 2009; Shurin, Clasen, Greig, Kratina, & Thompson, 2012). Further, the effects of warming may not be equal across trophic levels, with some evidence to suggest that primary production may not increase commensurately to respiration, thus further increasing the strength of top-down effects of consumers on communities if metabolic demand cannot be moderated (Allen, Gillooly, & Brown, 2005; Yvon-Durocher, Jones, Trimmer, Woodward, & Montoya, 2010).

However, many studies that have investigated the effects of temperature rise on trait and ecosystem change have used individuals adapted to today's climate or have used smaller-bodied populations as a surrogate to measure biological and ecological response to warming (Bernhardt, Sunday, & O'Connor, 2018; Shurin et al., 2012; Yvon-Durocher et al., 2012). These approaches may be flawed as they do not allow for contemporary adaptation across all traits. Commemoratory adaptation is important to consider as this may mediate ecological responses. For example, differences in developmental temperature and body mass influence life history traits such as fecundity and may alter individual morphology or body nutrient stoichiometry (Bjorkman et al., 2018; Riesch et al., 2018; Savage, Gilloly, Brown, & Charnov, 2004). In addition, thermal selection and concomitant evolutionary responses may mediate the body-size scaling of metabolic rates (Bradford et al., 2019; Englund, Öhlund, Hein, & Diehl, 2011; Padfield et al., 2017; Schaum et al., 2018; West & Post, 2016). Therefore, there is a crucial need to understand the broader consequences of the suite of trait changes expected under warming.

In this thesis I aim to understand how thermal history (or potential local adaptation) influences trait change and how this trait change influences ecosystems.

Throughout my thesis I use a dominant consumer species, mosquitofish (*Gambusia affinis*), as a model organism. Mosquitofish are a global invader, spread for their ability to control mosquito-larvae in freshwater (Fig. 1.1 & 1.2). Here, I use populations of mosquitofish from geothermally influenced ponds spanning a wide temperature range to understand how thermal history influences trait and ecological change. In this thesis I use a combination of comparative and experimental methods to answer my key aim. This thesis is arranged as follows; **Chapter 2** describes the effects of thermal history on metabolic rates; **Chapter 3** investigates the effects of thermal history on metabolic plasticity and behaviour; **Chapter 4** describes the effects of thermal history on diet and associated morphological and body elemental composition changes (Fig. 1.3). Finally, **Chapter 5** describes the ecological effects of thermal history and reduced body size.



WAR ON MOSQUITOES.

FISH TO EAT LARVAE.

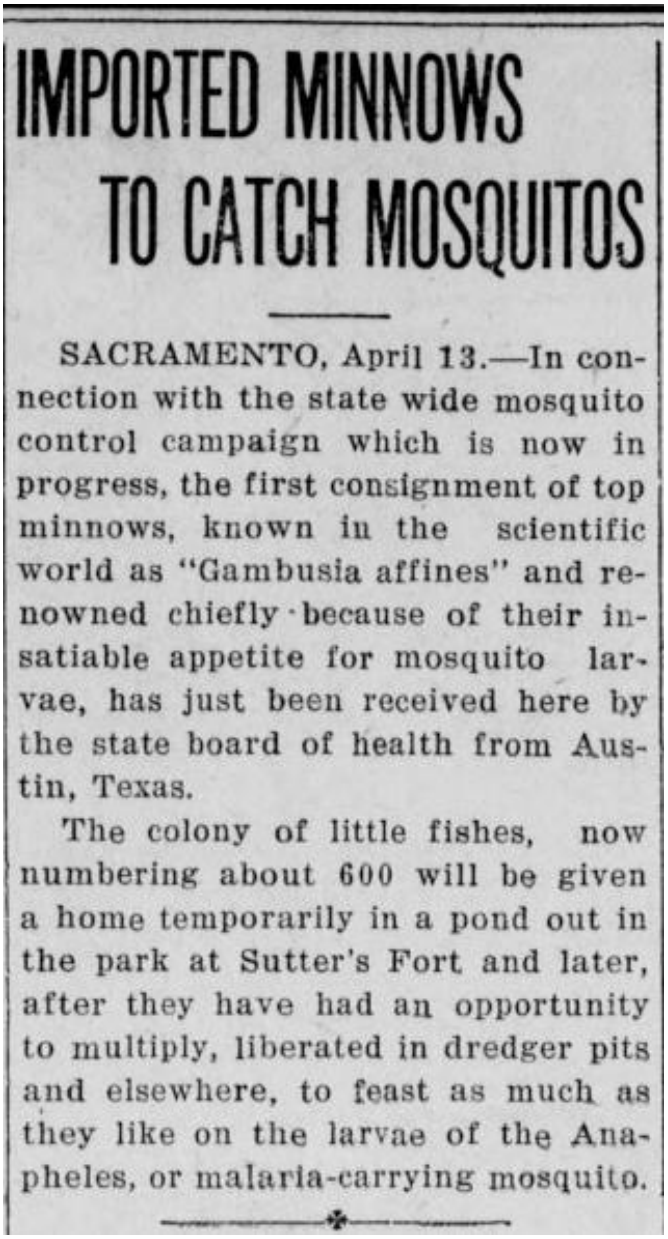
CONSIGNMENT FOR AUCKLAND.

SUCCESS IN OTHER LANDS.

A war on the breeding-grounds of mosquitoes will shortly be waged in Auckland, through the enterprise of Dr. T. J. Hughes, district medical officer of health. Dr. Hughes was a passenger from Sydney by the Marama yesterday, after attending a medical conference in India organised by the League of Nations, and the most interesting exhibit in his cabin was a kerosene tin containing about 100 special mosquito-larvae eating fish, known as Gambusia Affinis.

Dr. Hughes had transferred about a dozen of the fish into an open tin so that he could watch their progress during the voyage across the Tasman. The fish are about the size of a heavy gramophone needle, and in shape resemble a gramophone needle more than anything else. They are very active in the water, and are stated to have ravenous appetites when the food is mosquito-larvae.

Figure 1.1. Newspaper article from 28th of March 1928 describing the shipment of mosquitofish, *Gambusia affinis*, to New Zealand for mosquito larvae control. Newspaper article was published by New Zealand Herald Volume LXV, issue 19907 (NLNZ, 2018).



IMPORTED MINNOWS TO CATCH MOSQUITOS

SACRAMENTO, April 13.—In connection with the state wide mosquito control campaign which is now in progress, the first consignment of top minnows, known in the scientific world as "Gambusia affines" and renowned chiefly because of their insatiable appetite for mosquito larvae, has just been received here by the state board of health from Austin, Texas.

The colony of little fishes, now numbering about 600 will be given a home temporarily in a pond out in the park at Sutter's Fort and later, after they have had an opportunity to multiply, liberated in dredger pits and elsewhere, to feast as much as they like on the larvae of the Anapheles, or malaria-carrying mosquito.

Figure 1.2. Newspaper article from 13th April 1922 describing the introduction of *Gambusia affinis*, into California for mosquito larvae control. Newspaper article was published by Santa Cruz Evening News, page 6 (CDNC, 2019).

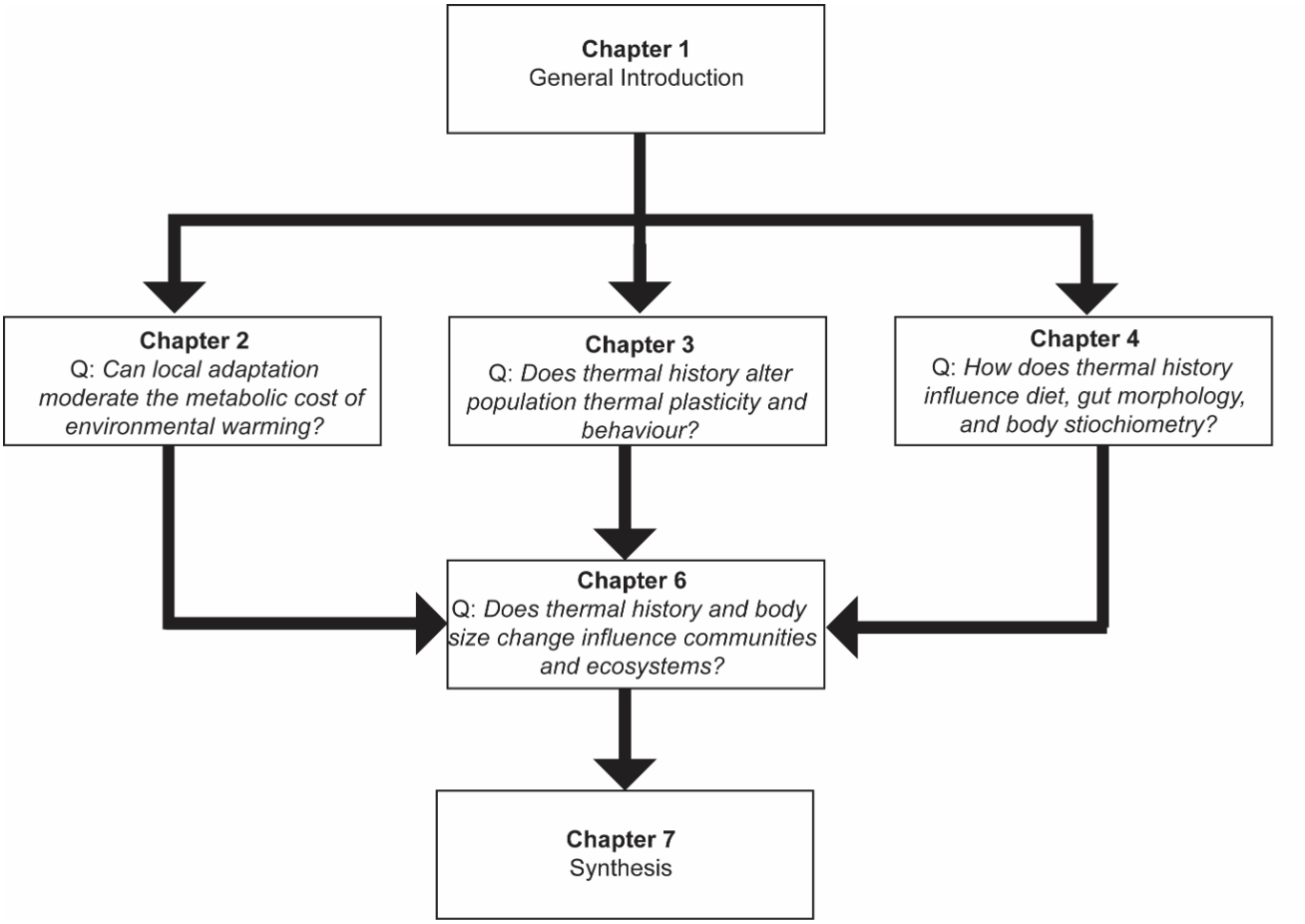


Figure 1.3. Thesis structure, including key questions from each chapter are shown with arrows indicating how chapters are inter-related. Chapters use a combination of comparative (Chapters 2, 3, and 4) and experimental (Chapter 5) methods to answer key aims.

Chapter 2

Local adaptation reduces the metabolic cost of environmental warming

2.1. Abstract

Metabolism shapes the ecosystem role of organisms by dictating their energy demand and nutrient recycling potential. Metabolic theory of Ecology (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 years. 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 counter-gradient 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.

2.2. Introduction

Concern over climate change has spurred interest in predicting how changing thermal regimes will influence ecological systems (Bellard, Bertelsmeier, Leadley, Thuiller, & Courchamp, 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 (e.g. 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 & Holzapfel, 2006). Such contemporary adaptation can substantially alter ecological outcomes derived from expectations of fixed phenotypes (Fryxell & Palkovacs, 2017; Woodward et al., 2005). 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 (Bruno, Carr, & O'Connor, 2015; Gilbert et al., 2014; Schramski, Dell, Grady, Sibly, & Brown, 2015). In particular, MTE predicts that metabolic rate (B) scales predictably in relation to body size and temperature using the equation (Equation 1):

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

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, Brown, West, Savage, & Charnov, 2001). Alpha (α) relates metabolic rate to body size, typically assuming ¾ 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 ~ -0.65eV. 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, Peters, Kearney, Joseph, & Heinsohn, 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. 2.1). If body size declines and MTE predictions hold 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. 2.1). This prediction suggests a potential energy constraint that could 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.

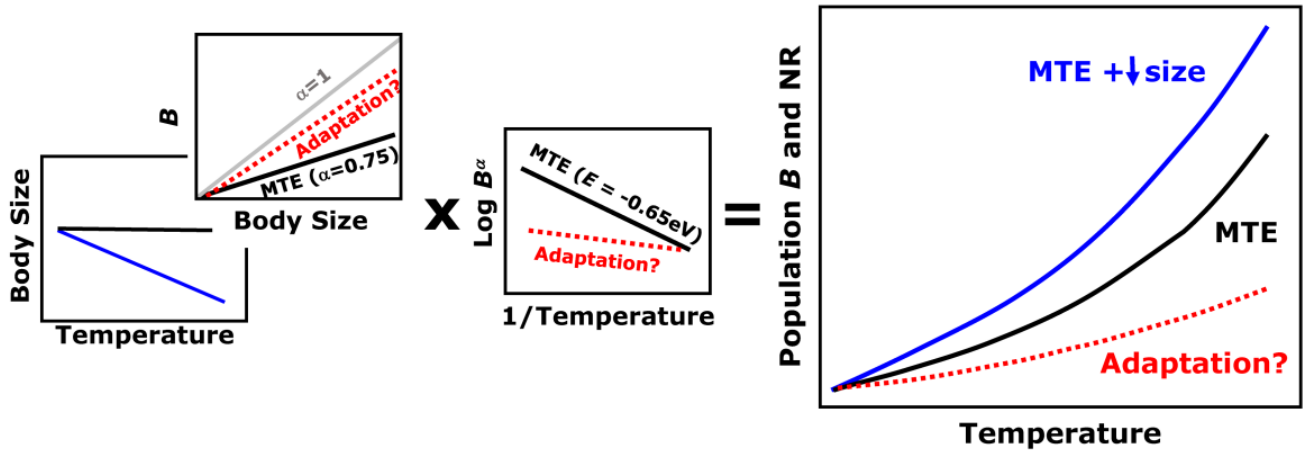


Figure 2.1. Conceptual diagram showing the change in population metabolic rate (B) and nutrient recycling rate (NR) with rising temperature under three scenarios. Metabolic theory (MTE, solid black lines) predicts exponentially rising metabolic and recycling rates with increasing temperature due to the temperature dependency of metabolism and invariance of metabolic scaling parameters (α and E, see eqn. 1 in text). Declining body size (MTE + ↓ 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. α =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 < -0.65eV), then population metabolic and excretion rates are far less sensitive to temperature change than predicted by MTE (Adaptation, dashed red lines).

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, Pawar, & Savage, 2011), particularly among divergent phylogenetic taxa (Uyeda, Pennell, Miller, Maia, & McClain, 2017). Variation in metabolic scaling parameters across and within some species has been linked to consumer body form and temperature (Killen, Atkinson, & Glazier, 2010; Ohlberger, Mehner, Staaks, & Hölker, 2012). It remains unclear though if variation in metabolic scaling parameters track 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. 2.1). 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. 2.1). Furthermore, adaptive changes in alpha towards 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, U.S.A.). 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.

2.3. Materials and methods

2.3.1. Study organism and populations

Gambusia affinis were introduced to New Zealand in the 1930s and to California in the 1920s (McDowall, 1978; Stockwell & Weeks, 1999). Those fish ultimately derived from populations in Texas, U.S.A.. *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 (Langerhans, Gifford, & Joseph, 2007; Stearns, 1983; Stockwell & Weeks, 1999). We studied five populations of *Gambusia* in geothermal springs spanning a wide gradient of temperatures in California, U.S.A. and another five populations on the North Island of New Zealand (Fig. S1.1). California populations were in springs that were pond-like. Some sites have barriers to dispersal allowing for precise local adaptation (Table S1.1); 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.

2.3.2. 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. Respirometers comprised clear, 40mL 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-minute runs using four respirometers in each run. During runs the respirometers were held in a 50L clear container of site water that was submerged to maintain ambient water temperature. *Gambusia* were captured with a

hand-net and held in a bucket of site water for 20 minutes 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, 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, Sandblom, & Jutfelt, 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 $\mu g\ O_2\ min^{-1}$.

Nutrient excretion rates were estimated by change in NH_4^+-N concentrations in the closed respirometers over the 20-minute assays (Whiles, Huryn, Taylor, & Reeve, 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 15mL 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, CA, U.S.A) in California, and by colorimetry

using a Lachat QuikChem® 8500 Series 2 Flow Injection Analysis System (Lachat Instruments, Loveland, CO, U.S.A.) 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 hours at 60ºC, and weighed to determine dry mass.

2.3.3. Population size structure

To determine *Gambusia* population size structure in each site a 5m seine (1.6mm 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 weight were later measured as described above.

2.3.4. 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 & Weisberg, 2011; R Development Core Team, 2017). 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 ¾ 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 500mg at 5mg 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 analysed similarly to metabolic rate. We also calculated an average (±SE) nitrogen quotient (NQ) for each population, as the ratio of excretion rate to metabolic rate. We used NQ to estimate the % of metabolism fuelled by protein, 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 analysed separately. Quantile regression was conducted at every 0.10 quantile from 0.10 to 0.90.

We modelled 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) across all populations, applied to a constant a body size distribution which was 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 was 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 (1kg) 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 Equation 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 , bootstrapped (replication = 1000) confidence intervals were produced for each data point using the R packages 'MASS' v.7.3.49 and 'Hmsic' v.4.1(Harrell, 2006; Venables & Ripley, 2002). Statistical significance was assumed at $\alpha < 0.05$.

2.4. Results

2.4.1. Population structure

All populations were female biased with male:female ratios ranging from 0.09-0.89 and this ratio was not significantly correlated with temperature (r_s = -0.243, P = 0.468) (Table S1.2). In separate analyses, body mass of females and males declined at every size quantile with increasing habitat temperature (quantile regression, $P < 0.0001$) (Fig. 2.2). The decline in body size was strongest at larger body sizes, as evident in the slope estimates. For example, in males slopes decreased 5 fold from -0.0004 to -0.0020 from quantile 0.1 to 0.9, respectively (Table S1.3).

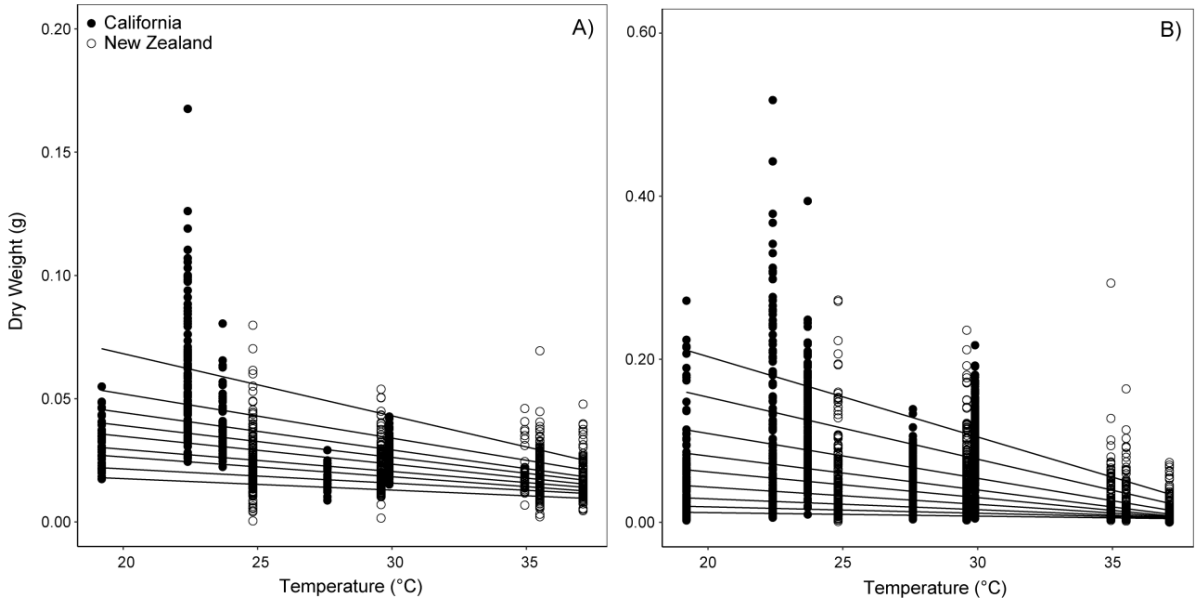


Figure 2.2. Mosquitofish population size distribution data for A) males (n = 1042) and B) females (n = 2939) across geothermal populations in California and New Zealand with quantile regression lines (0.1-0.9) shown.

2.4.2. 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) (Fig. S1.2). 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. 2.3A, r^2 = 0.534, P = 0.016). Arrhenius plots relating metabolism, corrected assuming ¾ power scaling in all populations, and temperature revealed a statistically significant relationship (P < 0.0001), but the slope (0.27eV) was far below expectations of MTE (~0.65eV) and the fit was poor (r^2 = 0.14)(Fig. S1.3A). Similarly, when estimated using population-specific scaling factors E was low (0.10eV) compared to MTE expectations (Fig. S1.3B). 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. 2.3B), a consequence of the rise in allometric slope with increasing temperature (Fig. 2.3A).

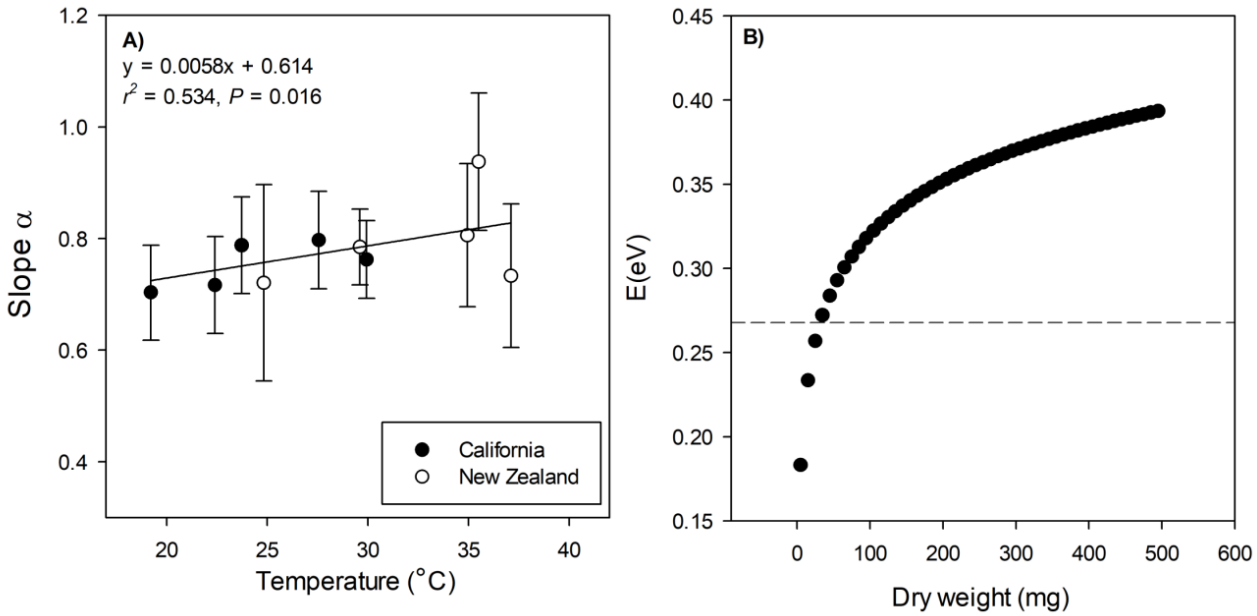


Figure 2.3. Relationship between site temperature and A) the allometric scaling coefficients for metabolic rate (α), error bars are mean absolute error (MAE) (n = 10) and B) the predicted E for different size classes across our thermal gradient.

2.4.3. 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 countries (Fig. S1.4). 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 (Fig. S1.5). 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 NZ (0.85:1) compared to CA (0.62:1) (Fig. 2.4). There was no relationship between metabolic and excretion slopes (Fig. S1.6). Nitrogen quotient values increased with temperature across populations, ranging from 0.065±0.004 at 19ºC to 0.150±0.012 at 37 ºC. This translates into an increase in the amount of metabolism supported by proteins from 23.9%±1.5 to 55.7%±4.3 across the temperature range (r^2 = 0.463, P = 0.0304).

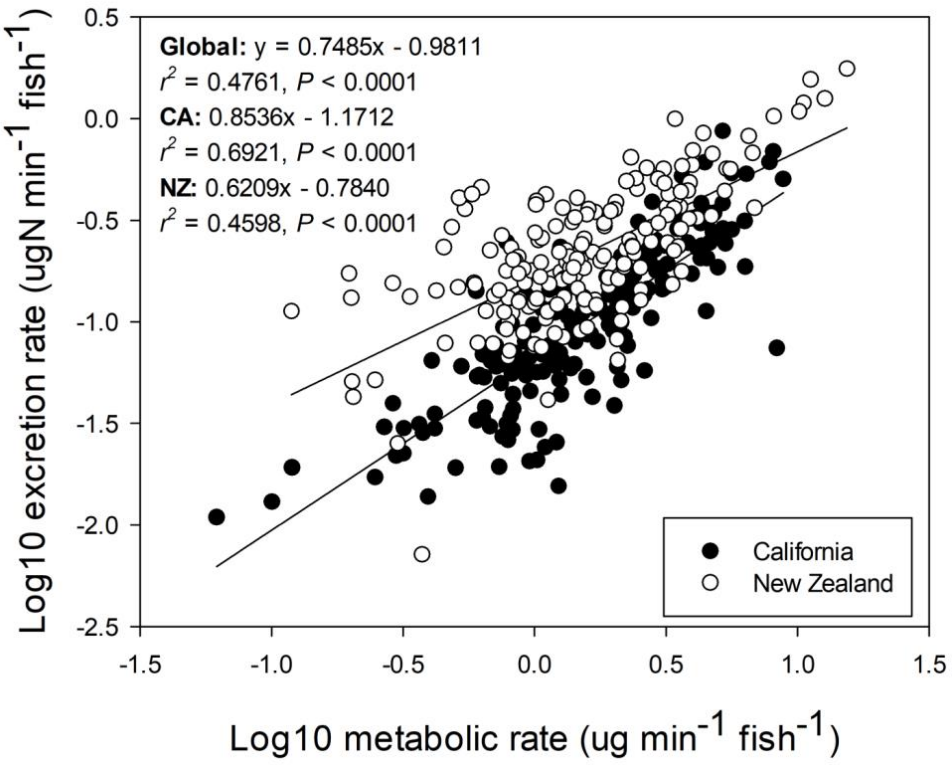


Figure 2.4. Relationship between excretion and metabolic rates of all fish measured in California and New Zealand populations (n = 373). Regressions are for linear fits to each country individually (solid lines). Global regression statistics are for all individuals pooled.

2.4.4. 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. 2.5). 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 electron volts, eV). 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 = 0.26eV) 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. 2.5). Differences were even more dramatic when compared to use of 'universal' MTE scaling.

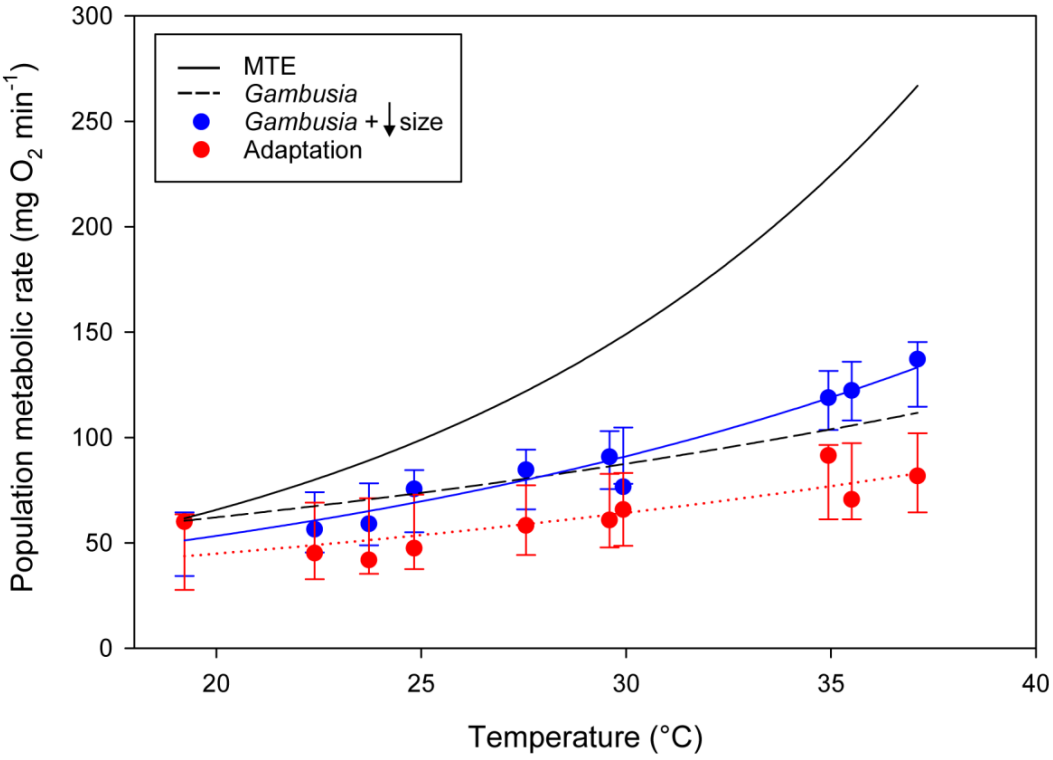


Figure 2.5. Predicted change in mosquitofish population-level metabolic rate with rising temperature derived from 3 scenarios: 1) MTE with no body size change with rising temperature (MTE, solid black line), 2) MTE with our observed E for *Gambusia* (E = 0.27eV) (*Gambusia*, dashed line), 3) MTE with the observed E for *Gambusia* and changes in body size distributions across out mosquitofish populations (*Gambusia* + ↓ size, blue solid line)**,** and 4) observed metabolic scaling relationships and change in body size distributions in mosquitofish populations (Adaptation, red dotted line) (see Statistical analysis section and Fig. 2.1 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.

Models of nutrient excretion that assumed MTE and *Gambusia*-specific scaling followed patterns similar to metabolic rate (Fig. S1.7; 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 = 0.40eV). 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.

2.5. 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 versus 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.

2.5.1. 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 & Bickford, 2011). This

prediction has strong empirical and theoretical support (Angilletta & Dunham, 2003; Bergmann, 1847; James, 1970), although there are exceptions (Adams et al., 2013; Walters & Hassall, 2006). 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, Lengfellner, Sommer, & Carpenter, 2009; Forster, Hirst, & Atkinson, 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. 2.2). 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 modelling 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.

2.5.2. Body size scaling and metabolic rate

A body mass scaling exponent of 0.757 across all fish in our study fit neatly with MTE prediction of ¾ scaling, but this masked important underlying variation in scaling across populations (Fig. S1.1). The range in scaling coefficients we found across populations of one fish species (0.70 to 0.87) equalled the range of variation among different fish species with highly divergent body morphologies (0.69 to 0.86) (Killen et

al., 2010). Variation in metabolic scaling coefficients is now well recognized (Dodds, Rothman, & Weitz, 2001; Glazier, 2005, 2010; Kozlowski & Konarzewski, 2004). 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. 2.3A) in both NZ 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 (e.g. Gilchrist, Huey, & Serra, 2001; Kinnison, Unwin, & Quinn, 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 (Fig. S1.8) 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.

2.5.3. Temperature sensitivity of metabolic rate

Under MTE, metabolic rate is predicted to increase with temperature under a mass-normalised activation energy (E) of 0.65eV (Gillooly et al., 2001). We found a lower *Gambusia*-specific activation energy of 0.27eV in our combined dataset, indicating low temperature sensitivity of *Gambusia* in general (Fig. 2.3B). This result again supports other studies that call into question the generality of E = 0.65eV. Indeed, while a meta-analysis (Dell et al., 2011) supported an average value of 0.66eV across 1072 individuals, the median value was lower (E = 0.55eV), and species-specific values are highly variable (Killen et al., 2010; Marshall & 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 counter-gradient trait

variation (Conover & Schultz, 1995; Kinnison et al., 2003), such that cold-adapted populations attain higher than expected metabolic rates, warm-adapted populations achieve lower than expected metabolic rates, or both.

2.5.4. Metabolic rate and nutrient excretion

Variation in consumer metabolic rates may strongly influence rates of resource uptake, use, and excretion (Allen & Gillooly, 2009; Brown et al., 2004), 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. 2.4), 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 (Fig. S1.4 & Fig. S1.5). 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 & 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 (Uliano et al., 2010; Wood, 2001). 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 account for such variation.

2.5.5. 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 (Ezard, Cote, & Pelletier, 2009; Hairston et al., 2005). MTE suggests population energetic demand and excretion rate will rise with increasing temperature and our modelling 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. 2.5). Indeed, under this counter-gradient 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 counter-gradient 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 counter-gradient changes in scaling relationships may substantially impair accurate predictions of future biodiversity responses to global change.

Chapter 3

Thermal history alters temperature sensitivity of metabolism and behaviour of an invasive consumer, *Gambusia affinis*

3.1. Abstract

Energy demand and behaviour are important in shaping the ecosystem-level effects of consumers, and both are sensitive to temperature change. However, it is unknown if thermal history modifies the temperature sensitivity of metabolism and behaviour. Here, we aimed to understand whether the thermal history of a dominant consumer, *Gambusia affinis*, influences temperature sensitivity of metabolic rate and behaviour. We measured routine, standard, and maximum metabolic rates, in addition to behaviour across eight populations of *Gambusia* with differing thermal histories at two acclimation temperatures. Our data reveal a divergence in thermal sensitivity with thermal history, in which populations from warm habitats were less sensitive to warming than were populations from cooler habitats. Both acclimation and source-population temperature strongly shaped behaviour across populations, with individuals from warm-source populations and individuals acclimated to warm conditions being bolder. Finally, as metabolic rates increased individuals were bolder, but these factors were only related when metabolic rate was measured as routine metabolic rate. Further, our data suggest that the relationship between metabolism and behaviour is best described alongside other factors. Overall, our data reveal an important role of consumer thermal history in moderating temperature sensitivity of metabolic rate and behaviour, which may facilitate population persistence under warming.

3.2. Introduction

Climate change is predicted to have numerous effects on species, including reduced body size, change in geographic distributions, and shifts in phenology (Hickling, Roy, Hill, Fox, & Thomas, 2006; Parmesan, 2006; Sheridan & Bickford, 2011). Physiologically, organismal metabolism is predicted to increase with both warming and smaller body size, imposing greater energetic constraints on individuals and leading to alterations in other traits (e.g. behaviour, life history) (Holt & Jorgensen, 2015; Norin, Malte, & Clark, 2016; Sibly, Brown, & Kodric-Brown, 2012). Therefore, measures of metabolic rate are often used to understand how organismal metabolism will change with warming (Clark et al., 2013). Metabolic rate can be measured as maximum metabolic rate (MMR), routine metabolic rate (RMR), or standard metabolic rate (SMR). MMR is the maximum metabolic rate possible by an individual and therefore sets the upper limit on organismal metabolic performance (Fry, 1971). In contrast, SMR is the minimal requirement of an animal to sustain life. The difference between SMR and MMR is an organism's aerobic scope (AS), this is the amount of energy available for activity, maintenance, growth, or reproduction beyond maintenance costs (Fry, 1971). RMR lies between SMR and MMR, and is metabolic rate measured under less stringent conditions. For example, measures of RMR may represent the typical metabolic state of an individual as this measure incorporates variation in activity, and therefore may better relate to other traits (e.g. behaviour) (Mathot & Dingemanse, 2015).

Decreased body size is predicted to increase metabolic rates (Brown et al., 2004). The relationship between body size and metabolism should scale at 0.75 (or ¾) when plotted as a log-log relationship, thus increases in metabolic rate are predicted to be greater per unit mass for smaller individuals. Furthermore, metabolic rate should increase exponentially with temperature, with a negative activation energy scaling slope of -0.65 electron volts (eV) (Brown et al., 2004; Gillooly et al., 2001). These predictions are

significant for models predicting the ecological effects of warming because they assume an increase in metabolic demand under warming, which may increase the top-down effects of dominant consumer species. However, despite initial support for the generality of the mass and temperature scaling relationships there is now much evidence against fixed scaling exponents. In particular, the validity of the ¾ power law has received much attention, with many studies finding exponents significantly different to 0.75 (Bokma, 2004; Clarke & Johnston, 1999; Glazier, 2005, 2010). For example, variation in scaling exponents has been linked to variation in thermal history, ecology, and lifestyle (Glazier, 2005, 2010; Killen et al., 2010). In our recent work with *Gambusia affinis* we showed that a history of higher temperature modified the body mass scaling of metabolism when measured *in-situ*, reducing the metabolic cost of small body size under warming (see Chapter 2, Moffett, Fryxell, Palkovacs, Kinnison, & Simon, 2018). Such findings are significant as they suggest an important role of thermal history in regulating metabolic rates.

Differences in metabolic rate may lead to consistent differences in behaviour, where activities that provide (e.g. consumption) or use (e.g. reproduction) energy are dictated by energetic requirements (Biro & Stamps, 2010). Therefore, the increasing metabolic costs of rising temperature should be reflected in an organism's behaviour. For example, if metabolic rates increase with warming then risk-taking behaviours may also increase in frequency to maximise energy intake (Mathot & Dingemanse, 2015). Intraspecific variation in behaviour in response to temperature may be explained by the pace-of-life syndrome (POLS), in which populations subject to different conditions may vary in metabolic and co-evolved life-history and personality traits (Réale et al., 2010; Sih, Bell, & Johnson, 2004). Broadly, individuals from a population with a fast POL tend to be bolder, have higher metabolic demands, and low parental investment. However, if

population thermal history moderates metabolic rates, behavioural variation among populations should be minor. Further, within populations there is often substantial inter-individual variation in behaviour, which may be driven by metabolism and is believed to contribute to population persistence (Biro & Stamps, 2010; Oers & Mueller, 2010; Réale et al., 2010). Recently, there has been increased interest in linking metabolism to personality as this may explain why individuals within a population or over their lifetime show variation in metabolic rates (Dingemanse, Kazem, Reale, & Wright, 2010; Mathot & Dingemanse, 2015; Royauté, Berdal, Garrison, & Dochtermann, 2018). Furthermore, a mechanistic understanding of the relationship between metabolism and behaviour may help to explain why co-existing life strategies persist in populations and how these may change under warming.

Therefore, the aims of our research were to understand how consumer thermal history influences metabolism and behaviour, and to determine if there is a relationship between energetic demand and behavioural variation. We used populations of a globally dominant freshwater consumer species (*Gambusia affinis* or mosquitofish). Mosquitofish show intraspecific and inter-individual variation in behavioural traits (Cote, Fogarty, Weinersmith, Brodin, & Sih, 2010; Polverino, Santostefano, Díaz-Gil, & Mehner, 2018) and make an ideal model organism as they have invaded habitats with differing thermal regimes. Across populations mosquitofish show differences in life history characteristics, such as smaller body size at maturity and variation in *in-situ* metabolic scaling linked to thermal history (Fryxell & Palkovacs, 2017; Moffett et al., 2018; Stockwell & Weeks, 1999). Consequently, we hypothesized that consumer thermal history would lead to intraspecific variation in allometric scaling, temperature sensitivity of metabolism, and behaviour.

3.3. Materials and methods

3.3.1. Fish populations and collection

Mosquitofish were introduced to New Zealand in the 1930s and have spread throughout the North Island of New Zealand, including into sites with geothermal influence (McDowall, 1978). We collected mosquitofish from the 19th to the 27th of January 2016 from eight sites in the North Island of New Zealand that differ in thermal regime (Table S2.1). Four sites had geothermal influence and four were subject to daily and seasonal temperature change. At each site we collected a minimum of 15 male and 15 female individuals using hand-nets. Fish were placed into 20 L insulated buckets with water collected on-site and a portable aerator. We collected 20 L of water from each site to use in our aquaria. At the time of fish collection, we measured dissolved oxygen, pH, conductivity, and temperature using hand-held meters (YSI Professional Plus; YSI ProODO).

3.3.2. Temperature Acclimation

In the laboratory, 24-30 fish from each population were randomly allocated to one of two 20 L tanks, with at least 6 males and 6 females in each. Where females were pregnant, males and females were separated for acclimation; however, self-fertilization was persistent throughout acclimation. Tank temperatures were initially set to the collection temperature of each fish population and subsequently increased or decreased by a maximum of 1°C every two days until the desired temperatures were reached. Fish were acclimated to one of two experimental temperatures (20±0.5 and 30±0.5°C) over a four-month period. In each aquarium we started with water from the appropriate field site, which was combined with treated (API Stress Coat) water and progressively replaced by treated water over two weeks. Fish were fed twice daily with freeze-dried *Daphnia* and Nutrafin MAX small tropical fish micro-granules and a light cycle of 12:12 was maintained over the course of the experiment. Each aquarium had artificial macrophytes

and stones to provide refuge. Water was continuously filtered using sponge air filters which were cleaned every second day. Fish mortality was low among most of our populations but did occur in one of our geothermal populations (Akatarewa Stream), reducing the number of individuals used from this population. The number of *Gambusia* used in the study was $n = 201$ (Table S2.1 for more details). Individuals were fasted for 24 hours prior to measuring behavioural and metabolic traits to encourage exploratory behaviour for food and to control for the metabolic costs of food digestion.

3.3.3. Metabolic Rate Measurement

We measured individual metabolic rate as routine metabolic rate (RMR), standard metabolic rate (SMR), and maximum metabolic rate (MMR). Routine and maximum metabolic rates were measured using static respirometry and SMR was measured using intermittent flow-through respirometry (Clark et al., 2013; Steffensen, 1989). Respirometers were 40 mL acrylic chambers with magnetic stir bars in the base of the chambers to ensure mixing of the water over the course of our oxygen measures. Metabolic rate was measured as oxygen consumption (M_{O2}) using a FireSting four-channel oxygen logger with optical oxygen sensors (PyroScience, Germany). Measurements were performed at the acclimation temperature of the fish in an 80 L aquaria fitted with a UV filtration system, an aerator, and a 100W aquarium heater.

Immediately following behavioural trials, we measured RMR by placing individuals into chambers and measuring oxygen consumption over a 15 minute period. Chambers were then connected to a recirculating pump and slowly flushed with oxygenated water for five minutes before beginning SMR measurements. Oxygen consumption measurements for SMR were taken overnight over an approximately 18 hour period. Chambers were intermittently flushed by a computer-controlled aquarium pump for five minutes to ensure a complete turnover of water inside the chambers, once

flushing stopped chambers were sealed and an oxygen measurement period of 15 minutes began after a 30 second wait period. The oxygen pump and data logging intervals were controlled through a PC connected to a USB-1208LS data acquisition device with a relay unit and data were logged using open-source software (Svendsen, 2017). Following SMR measurements MMR was measured using an exhaustive chase protocol to induce maximum oxygen consumption (Clark et al., 2013; Norin & Clark, 2016). Fish were removed from chambers one-by-one and placed into a circular tank; in this tank we used an aquarium net to chase the fish until they were exhausted (defined as the lack of ability for burst swimming) (Norin & Clark, 2016). Fish were then immediately placed into a static respirometer and oxygen consumption measured for 5 minutes. We chose to exercise the fish following SMR measurements to ensure recovery from any handling stress was achieved over our SMR measurement period.

Following the measurement of MMR, *Gambusia* were immediately euthanized using clove oil, measured for weight, length, sex, and volume, then dried at 60°C for 48 hours and re-weighted for dry weight.

We controlled for microbial oxygen consumption by subtracting the oxygen consumption in blanks (respirometers with water only) which were run prior to every run. We assumed a linear increase in microbial oxygen consumption between measurements.

We calculated metabolic rate (Equation 1) as;

$$M_{O2} = \left(V_r - V_f\right) \times \frac{\Delta C_{wO2}}{\Delta \mathrm{t}} \quad (1)$$

where: V_r is respirometer volume, V_f is fish volume, ΔC_{wO2} is the change in oxygen concentration, Δt is the change in time.

SMR was calculated using the mean of the lowest 10 % of all measurements, excluding any outliers (± 2 standard deviations [SD] from the mean) (Chabot, Steffensen, & Farrell,

2016; Clark et al., 2013). Aerobic scope was calculated as MMR-SMR. Allometric scaling relationships were calculated using least squares linear regression models of $\log_{10}$ metabolic rate (μg O_2 min^{-1}) data against $\log_{10}$ mass (mg) data. Scaling exponents (*b*) are presented ± mean absolute error (MAE). Simple linear regression models were used to show the allometric relationships between mass and metabolic rate for each acclimation treatment and Analysis of covariance (ANCOVA), using body size as a covariate, was used to determine difference in allometric scaling between acclimation treatments. Temperature sensitivity was determined using Arrhenius plots of mass corrected metabolic rates, using population-level scaling exponents (*b*) and temperature as an inverse function (1/kT) where T is temperature in degrees Kelvin and *k* is the Boltzmann constant (8.62×10^{-5} eV K^{-1}).

We calculated the temperature coefficient (Q_{10})(Equation 2) as;

$$Q_{10} = \left(\frac{MR_{T2}}{MR_{T1}}\right)^{\frac{10}{T2-T1}} \quad (2)$$

where: *MR* is average metabolic rate (μg O_2 min^{-1}) from each population at 20ºC (T1) and 30ºC (T2).

We used a one-way Analysis of variance (ANOVA) to determine difference in Q_{10} values between acclimation treatments.

3.3.4. Behaviour

Behavioural assays using two metrics of boldness were conducted on individuals in a 60 L aquarium with a water depth of 20 cm and temperature set to acclimation temperature. The aquarium tank was fitted with an air pump and a UV 3 w aquarium purifier to maintain high oxygen saturation and control microbial respiration. We measured individual 'boldness' when exploring a novel area as emergence latency and

exploration time (Cote et al., 2010; Wilson, Godin, & Ward, 2010). For these boldness measures, an individual was placed into a small enclosed and darkened area ('safe area') at one end of the 60 L aquaria. The aquarium was covered on all but one side to allow for observation. In the safe area, we provided refuge in the form or artificial macrophytes and river stones. Fish were left in the safe zone for 10 minutes before a 4 × 4 cm door was opened remotely via a pulley system allowing fish to exit and explore the remainder of the tank ('open area'). In the open area macrophytes were placed opposite the safe area opening as a visual cue for exploration. Emergence latency was measured as the time it took the fish to leave the safe area. Fish that did not leave were assigned a maximum latency time of 600 seconds (Brown, Burgess, & Braithwaite, 2007; Sih, Cote, Evans, Fogarty, & Pruitt, 2012; Yoshida, Nagamine, & Uematsu, 2005). Once the fish began exploring, we recorded the time spent exploring, as time spent moving over the five-minute period following their emergence from the safe area.

As our data were censored we used a binomial logistic regression model followed by ANOVA to identify any factors that influenced emergence latency using the following factors; acclimation temperature, source population temperature, gender, pregnancy, body mass, and metabolic rate. Taking only individuals that left the safe area we used a quasi-Poisson generalized linear model (GLM) with the same factors as listed above to understand which factors influenced exploration time.

We tested for changes in metabolic rate with behaviour using quantile regression using the R package 'quantreg' v.5.33 (Koenker, 2017). Quantile regression was conducted for quantiles 0.1 to 0.8. All analyses were performed using R version 3.5.0 (R Development Core Team, 2017) in base R packages unless specified. Statistical significance was determined at the $\alpha = 0.05$ level.

3.4. Results

3.4.1. Allometric scaling of metabolism

Across all individuals, scaling exponents for MMR, RMR, SMR, and AS were greater for individuals acclimated to 20°C compared to 30°C (Fig. 3.1). Thus, the metabolic rates of smaller individuals were most sensitive to increasing acclimation temperature. Differences in allomeric scaling relationships were not significantly different between acclimation treatments for SMR ($F_{1,193} = 0.515$, $p = 0.474$), MMR ($F_{1,195} = 1.828$, $p = 0.178$), or RMR ($F_{1,195} = 0.108$, $p = 0.742$). While individuals with higher AS did tend to come from the warm acclimation treatment, there was no significant difference in AS between acclimation treatments ($F_{1,189} = 0.912$, $p = 0.341$). Measures of metabolic rate were highly correlated and overall MMR was 1.6-times greater than SMR and RMR was 1.3-times greater than SMR (Fig. S2.1).

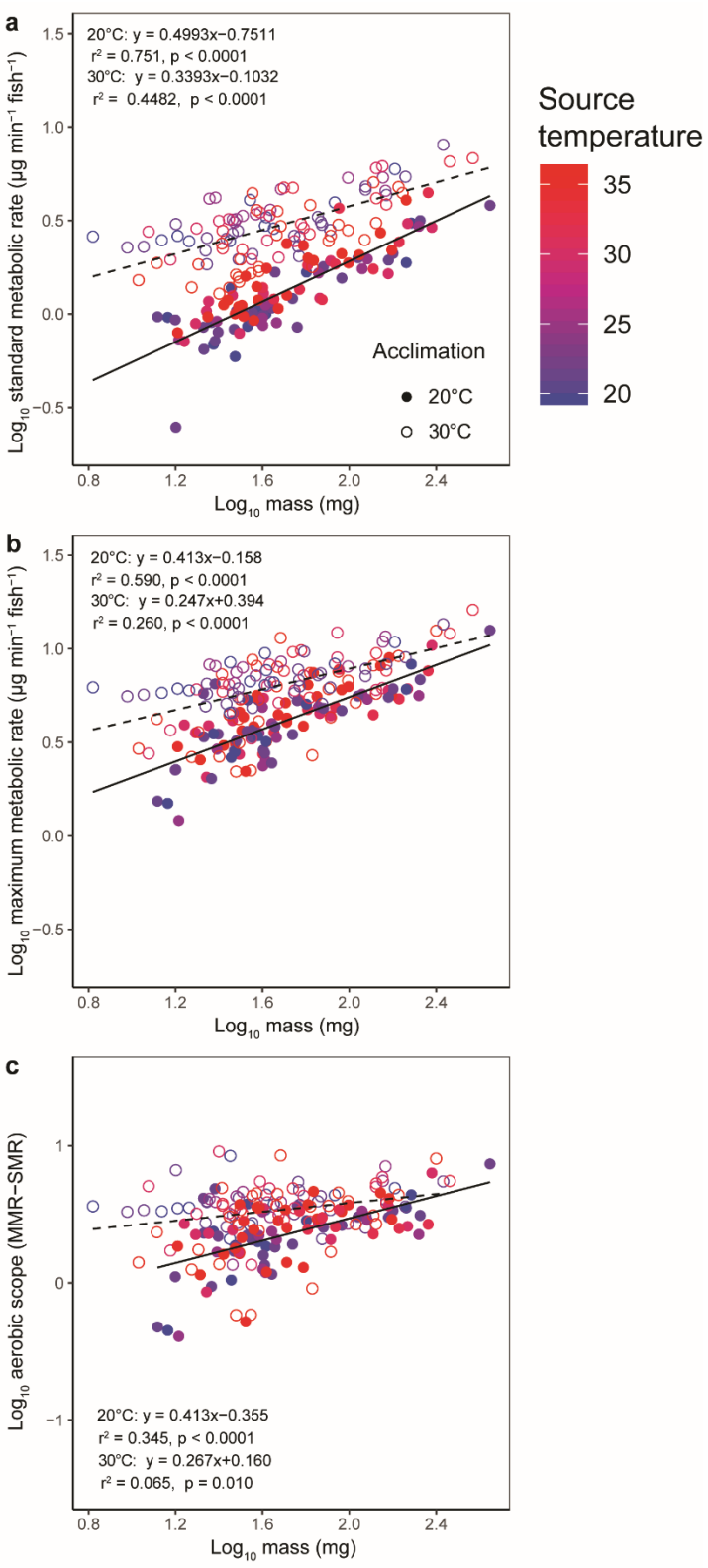


Figure 3.1. Relationship between fish mass and a) standard metabolic rate (SMR), b) maximum metabolic rate (MMR) and c) aerobic scope (AS). Data points are coloured by source population temperature; *n* = 201.

Allometric scaling exponents varied among populations, but this was not related to source population temperature (Fig. S2.3). At 20°C exponents varied among populations from 0.43 to 0.60 for SMR, from 0.49 to 0.65 for RMR, and from 0.35 to 0.52 for MMR. At 30°C exponents tended to be lower and varied from 0.22 to 0.55 for SMR, from 0.26 to 0.61 for RMR, and from 0.17 to 0.58 for MMR. Exponents were lower for MMR compared to SMR in 13 of our 16 treatments (Table S2.2).

Difference in MMR between the 20 and 30°C treatments decreased predictably with increasing temperature (r^2 =0.825, p = 0.021, Fig. 3.2); however this relationship did not exist for SMR (p = 0.414) and RMR (p = 0.620).

Pregnant fish had significantly lower mass normalised metabolic rates than non-pregnant females ($F_{1,94}$ = 6.70, p = 0.011), and metabolic rates of females were consistently higher in warm acclimated individuals ($F_{1,94}$ = 34, p < 0.0001) (Fig. S2.4a).

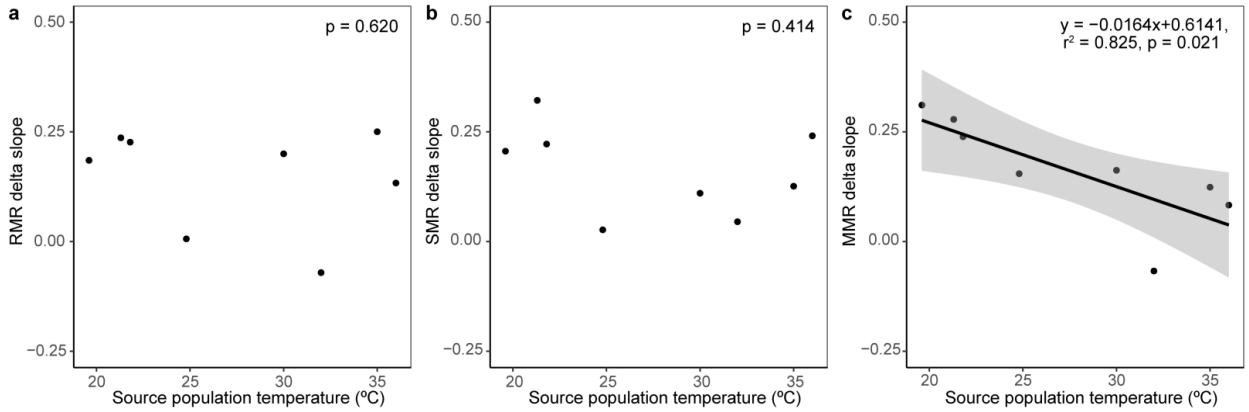


Figure 3.2. Difference in allometric slopes of metabolism between 20ºC and 30ºC acclimation treatments (delta slope) across populations, data are a) RMR, b) SMR, and c) MMR; *n* = 8.

3.4.2. Temperature scaling

Mass-normalised SMR was sensitive to temperature change with an activation energy of -1.1 eV across all individuals (y = -1.1x + 41.274, r^2 = 0.662, p < 0.0001), this relationship was also apparent for MMR (y = -0.719x + 28.158, r^2 = 0.411, p < 0.0001)

and RMR ($y = -0.953x + 36.381$, $r^2 = 0.590$, $p < 0.0001$). Across populations there was significant variability in activation energies, largely driven by the variation in mass corrected metabolic rates at 30°C. For MMR, variation in activation energies was negatively related to source population temperature ($r^2 = 0.576$, $p = 0.029$; Fig. 3.3). Variation in activation energies was not significantly related to source population temperature for SMR ($p = 0.236$) or RMR ($p = 0.303$).

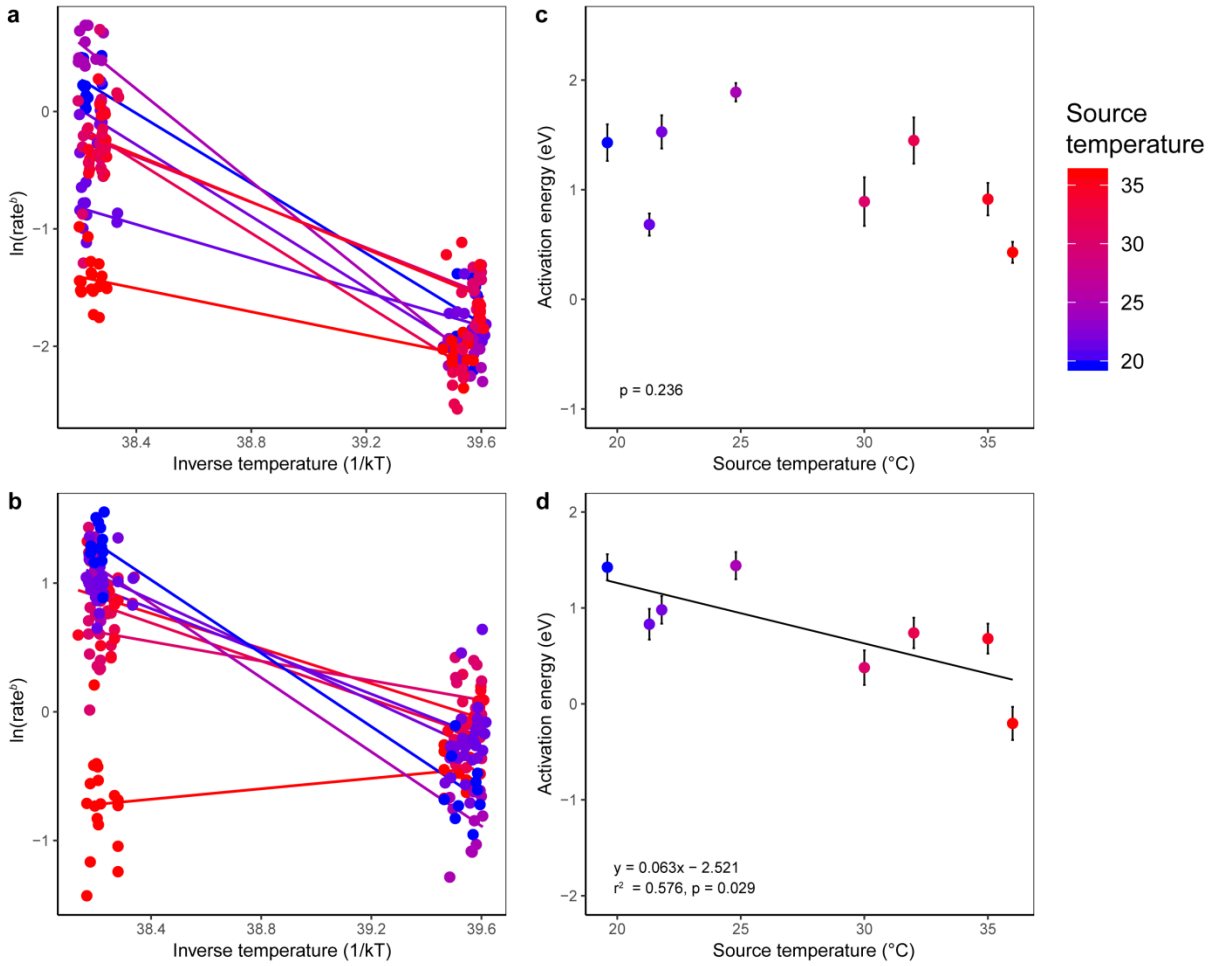


Figure 3.3. Thermal sensitivity of metabolism across laboratory acclimated populations of *Gambusia affinis*. Panels a) and b) are Arrhenius plots SMR and MMR, respectively ($n = 201$). Panels c) and d) are activation energies of metabolism across populations derived from Arrhenius plots ($n = 8$) for SMR and MMR, respectively. Data points in a) and b) are individual fish. T is Temperature in kelvin and k is the Boltzmann constant (8.62×10^{-5} eV K^{-1}); $n = 201$.

Warming increased average population aerobic scope in all but one population ($F_{1,13} = 32.2$, $p < 0.0001$) (Fig. 3.4a). Average (±1 SE) aerobic scope varied between

2.1±0.3 and 3.3±0.3 for fish acclimated at 20°C and between 3.2 ± 0.5 and 4.4± 0.6 for fish acclimated at 30°C.

Aerobic scope at 20 (p = 0.269) and 30°C (p = 0.124) was not significantly related to population source temperature, but the difference in aerobic scope between acclimation temperatures (i.e. the plasticity in aerobic scope) was related to population temperature (r^2 = 0.610, p = 0.022). In particular, the difference in aerobic scope declined with increasing source population temperature (Fig. 3.4b).

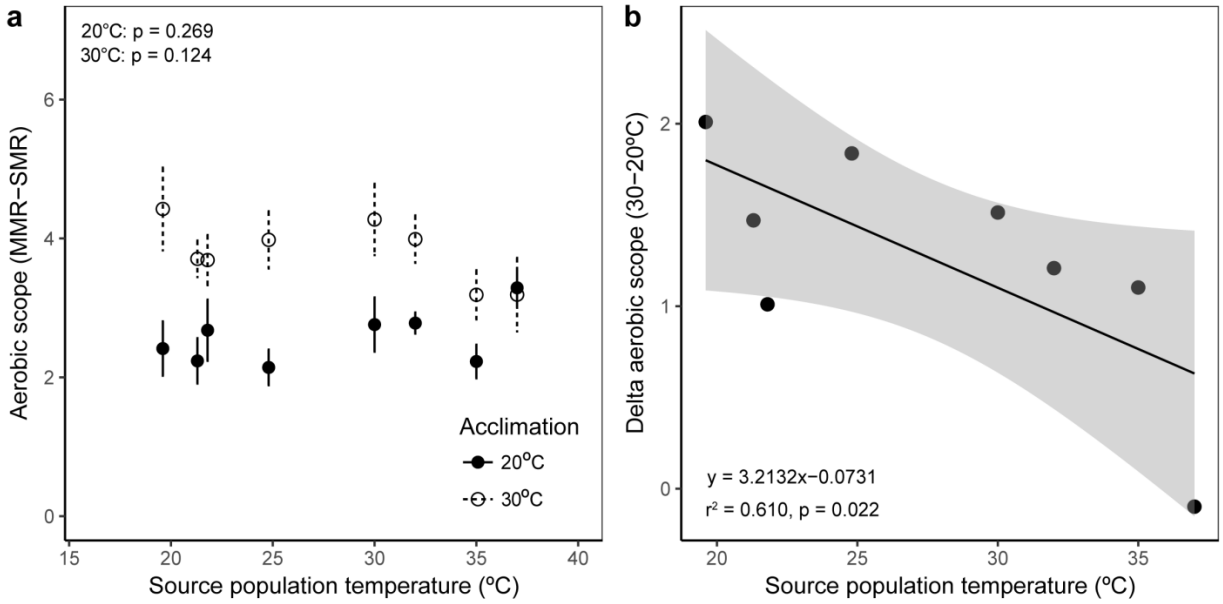


Figure 3.4. Relationship between temperature and aerobic scope for each study population (a) and difference aerobic scope at 30 and 20ºC across populations (b). Points in panel a are average +- SE and standard error is shown in grey in panel b; *n* = 8.

Q_{10} values varied between 0.75 and 4.69 across all populations, and metabolism measurements were not related to source population temperature for SMR (p = 0.158), RMR (p = 0.111), and MMR p = 0.158) (Fig. S2.5). Q_{10} values were highest for MMR across all populations and lowest for SMR for all but one population; however, these were not significantly different ($F_{2, 20}$ = 1.292, p = 0.297).

3.4.3. Behaviour

Individuals had shorter emergence latency times if they were acclimated at 30°C ($z = 2.711$, $p = 0.007$), from a warmer source population ($z = 2.908$, $p = 0.004$), and not pregnant ($z = -1.326$, $p = 0.015$). Our data also show that gender ($z = -1.514$, $p = 0.130$), body mass ($z = 0.910$, $p = 0.363$), and SMR ($z = -1.016$, $p = 0.310$) were not strong predictors of behaviour. MMR and RMR were similarly poor predictors of emergence latency ($p > 0.05$, Table S2.4 & 2.5). Fewer individuals from cooler source populations left the safe area compared to warmer populations and individuals from warmer temperatures tended to have shorter emergence latency (Fig. 3.5).

When considering the behaviour of individuals who left the safe area, individuals spent more time exploring where they were acclimated at 30°C ($t = 5.671$, $p < 0.0001$), from warmer source populations ($t = -2.026$, $p = 0.043$), male ($t = 2.412$, $p = 0.018$), not pregnant ($t = 2.294$, $p = 0.025$), and had higher metabolic rates (RMR, $t = -2.116$, $p = 0.038$; Fig. 3.5c, Table S2.7). Individuals with high RMRs spent more time exploring than did those with low RMRs who showed substantial variation in their exploration times. The relationship between RMR and exploration time was significant across the 10^{th} to the 80^{th} percentiles, but the slope of this relationship became lower with increasing percentiles (Table S2.9). Individuals with lower routine metabolic rates showed substantial variation in their exploration times, compared to those with higher routine metabolic rates who spent more time exploring. The relationship between exploratory behaviour and metabolic rate was non-significant for MMR ($t = -1.839$, $p = 0.070$) and non-significant for SMR ($t = -1.250$, $p = 0.215$; Tables S8 & 9).

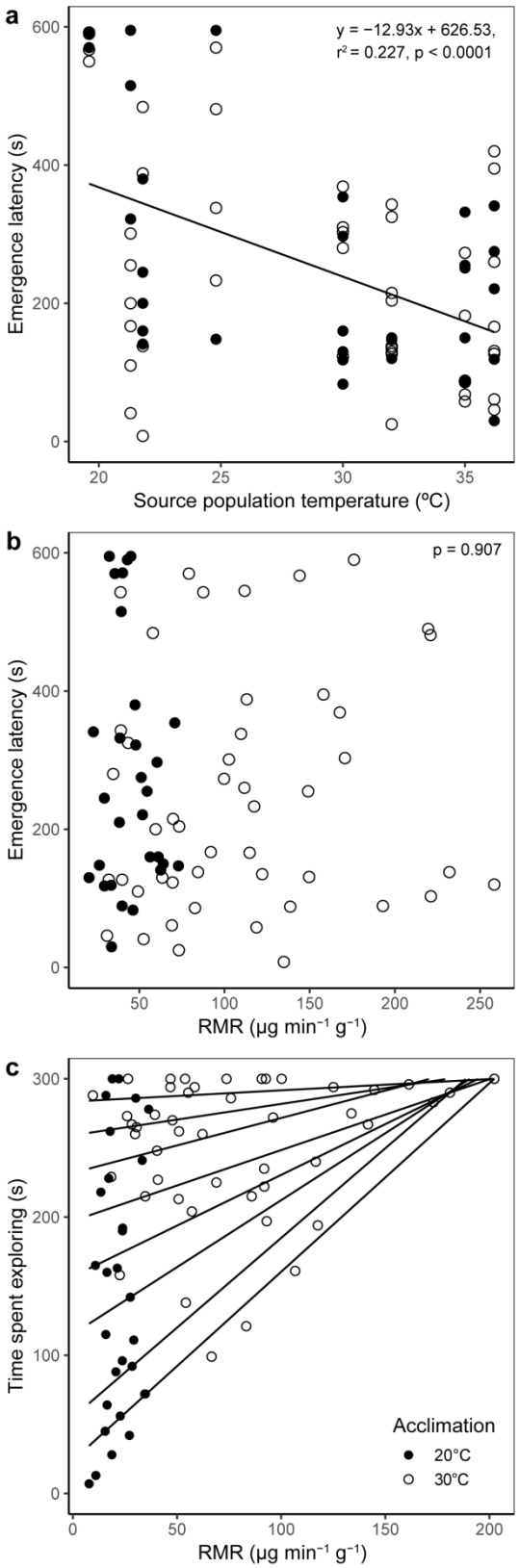


Figure 3.5. Relationship between a) source population temperature and behaviour; b) routine metabolic rate (RMR) and behaviour as emergence latency; and c) RMR and behaviour as time spent exploring. Note that individuals who did not leave the safe area are not shown; n = 86 per plot.

3.5. Discussion

Population thermal history may alter plasticity in physiological and behavioural responses to temperature rise; however, thermal history is often not considered in models projecting future population change (Cheung et al., 2012; Holt & Jorgensen, 2015; Persson, Leonardsson, de Roos, Gyllenberg, & Christensen, 1998; West, Brown, & Enquist, 1997). Our results demonstrate that populations of mosquitofish with varying thermal histories show differences in the plasticity of temperature sensitivity of metabolism for MMR, which reduced the temperature sensitivity of aerobic scope in populations derived from warmer habitats. However, population thermal history had little effect on allometric scaling. We also demonstrate that population thermal history and acclimation temperature are linked to behavioural variation. Finally, while individual behaviour (boldness) is related to organismal metabolism, this relationship was only apparent under certain conditions.

3.5.1 Body size scaling and metabolic rate

Metabolic rates increased with mass and with warming; however, scaling exponents were low compared with other reported values and decreased with temperature. Intraspecific scaling exponents are known to vary widely with reported variation in exponents from 0.40 to 1.29 (Clarke & Johnston, 1999). Our MMR, RMR, and SMR scaling exponents (0.25-0.50) are therefore lower than what is typically reported. In *Gambusia* species slope values have been reported between 0.61 and 0.91 (Mitz & Newman, 1989; Moffett et al., 2018; Srean, Almeida, Rubio-Gracia, Luo, & García-Berthou, 2017). However, a study on the repeatability of standard metabolic rate measurements in brown trout found slope values between 0.98 to 1.51, with variability thought to be due to feeding and growth (Norin & Malte, 2011). Thus, although it is unclear why our scaling exponents were relatively low, substantial variation in scaling exponents is common and differences have been linked to physiology, ecology, or

lifestyle, bringing into question the validity of fixed scaling exponents, especially when these may be used to predict larger-scale ecosystem effects (e.g. MTE) (Bokma, 2004; Glazier, 2005, 2010; Killen et al., 2010; Watson et al., 2014).

Relationships between temperature and allometric scaling exponents are mixed, with some studies noting positive (Carey & Sigwart, 2014; Moffett et al., 2018) but others noting negative relationships (Killen et al., 2010; Ohlberger et al., 2012), such as shown in this study. Our data show that allometric scaling exponents are lower at higher temperatures following a four-month acclimation period, suggesting that mosquitofish are more sensitive to increased temperatures at smaller body sizes. However, when measured *in-situ* there was a positive relationship between temperature and population-level scaling exponents, which reduced the metabolic penalty of small body-size (see Chapter 2, Moffett et al., 2018). Our field data covered a similar range in body sizes (3 – 320 mg, dry mass) compared to what was measured in this study (7 – 445 mg, dry mass), suggesting it is not our range in body size which is limiting interpretation of this pattern. The compensatory response to the imposed laboratory temperature regime (e.g. acclimation) may explain why relationships between temperature and scaling exponents varied between studies (Bennett & Dawson, 1976). For example, in the laboratory environment, individuals were acclimated to a novel and unchanging temperature regime and fed routinely, potentially influencing metabolic rates. Overall, the differences in these data suggest that acclimation alters species-specific metabolic responses to warming.

Across our populations the difference in allometric scaling exponents between acclimation treatments was predicted by temperature, but this trend was only significant for MMR. Populations adapted to warmer temperatures showed less variation in their population allometric scaling exponents than did populations from cooler sites, suggesting that metabolic sensitivity to warming is reduced with thermal history in warm-

source populations. Further, the change in temperature sensitivity with thermal history was reflected in population aerobic scope values, suggesting an increased thermal plasticity in populations derived from warmer habitats. Our aerobic scope data show a convergence of scope values between temperature acclimation treatments as population temperature rises. While it is predicted that AS should increase until a thermal optimum is reached (Lee et al., 2003), mosquitofish can downregulate developmental modifiers to assume a constant AS to maximise fitness (Seebacher, Beaman, & Little, 2014; Seebacher et al., 2010). Further, AS may decrease to a constant level overtime with acclimation (Norin, Malte, & Clark, 2014). Our data thus provide further evidence that AS may be modulated to meet daily energy requirements, which varies with thermal history.

3.5.2. Population metabolic rate and thermal sensitivity

The temperature sensitivity of metabolic rate when measured as MMR decreased predictably with source population temperature, varying from -0.2 to 1.4eV across our populations (Fig. 3.3). This range in values is similar to that reported in other species common (-0.2 to 1.2eV) (Brown et al., 2004; Dell et al., 2011; Gillooly et al., 2001). Unlike MMR, SMR did not show a predictable relationship with the temperature sensitivity of metabolic rate between our two-acclimation temperatures, which suggests that consumer thermal history may play an important role in moderating an organism's aerobic capacity at its upper limit. Similarly, several recent studies have noted that traits may be better expressed under stressful conditions, such as hypoxia, starvation, or high temperatures (Killen, Marras, & McKenzie, 2011; Killen, Marras, Ryan, Domenici, & McKenzie, 2012; Norin et al., 2016). Thus, our data suggest that difference in metabolic temperature sensitivity is best reflected through MMR and suggests an important role of consumer thermal history among our populations (e.g. Gilchrist et al., 2001; Kinnison et al., 2003).

Variability in temperature sensitivity of metabolism among our populations was further reflected by AS. Our data show that the difference between population AS average values narrowed with increasing source temperatures and that this decrease in AS between acclimation treatments was predictable by source temperature. Across all populations, there was little difference in AS at 20ºC acclimation, but at 30ºC MMR increased relative to SMR, which increased AS values in our cooler populations (Fig. S2.4). A decrease in variation in AS from cool to warm populations suggests the degree to which metabolic rate is lifted above resting becomes increasingly unaffected by warming in our warm adapted populations. This narrowing of AS with increased temperaure provides further evidence that AS is moderated to meet energy requirements and may not be a good predictor of peak performance (e.g. Ern, Huong, Phuong, Wang, & Bayley, 2014; Gräns et al., 2014; Norin et al., 2014). Furthermore, this relationship suggests that consumer thermal history plays a key role in controlling metabolic performance (Clarke & Johnston, 1999).

3.5.3. Behaviour and temperature

Increasing temperature may impose energetic constraints on individuals through increasing metabolic rates, leading to alterations in behaviour (Gillooly et al., 2001; Holt & Jorgensen, 2015). In-line with this expectation, our data show an increase in boldness (lower emergence latency times) with increased acclimation temperature. Even small increases in temperature can alter animal behaviour when measured in a laboratory setting, for example an increase of 3 °C altered individual personalities in coral reef fish such that they became more aggressive and active (Biro & Stamps, 2010). Significantly, our data also show that source population temperature influenced boldness, where individuals from warmer-source populations were bolder compared to those from populations in cooler habitats, few of which left the safe area (Fig. 3.5a). Such

intraspecific variation in behaviour was also noted in Trinidadian guppies (*Poecilia reticulata*) in response to different predation regimes, in which fish were bolder in the presence of predators (Fraser & Gilliam, 1987). Thus, variation among populations may play an important role in dictating behaviours. Overall, our data suggest that both consumer thermal history and *in-situ* temperature play important roles in moderating individual behaviour.

Among our populations there was considerable variation in behaviour that was not explained by temperature. Our data show a decrease in boldness of females compared to males, and particularly in pregnant females. Pregnancy in mosquitofish comes with high energetic costs of both egg development and behavioural changes (Biro & Stamps, 2010; Seebacher, Ward, & Wilson, 2013). Pregnancy in mosquitofish may increase aggressive behaviour and may limit aerobic scope for other activities (Seebacher et al., 2013); it is therefore not surprising that pregnant females had higher emergence latency times and lower exploratory times. Because pregnancy in female mosquitofish can be difficult to detect in its early stages and mosquitofish may self-fertilize (Pyke, 2008), our pattern of decreased boldness with pregnancy may also explain why males were bolder than females in our experiment. Increased boldness in males was also noted in field and F1 individuals from a poecilid species, thought to be explained to some degree by difference in hormones (Brown et al., 2007). Boldness in males may also confer a selective advantage for mating, with bold guppies having greater mating success (Evans, Pilastro, & Ramnarine, 2003). Together, these data suggest that boldness in mosquitofish is influenced by several factors.

3.5.4. Behaviour and metabolism

While our data show that metabolic rate could not be used to predict emergence latency, we did find a relationship between exploratory behaviour and metabolic rate (Fig. 3.5). Exploratory behaviour was best described by source population temperature, acclimation temperature, gender, pregnancy and RMR. Our data suggest that RMR may place a limit on exploratory behaviour, where, as RMR increases, minimum exploratory time also increases. Our data further suggest that physiological measures need to be taken in a way that allows for, rather than controls against, variation in behaviour. Here, behaviour was only related to metabolism when measured as RMR, but not MMR or SMR, which suggests that SMR does not capture behavioural variation among individuals. Careau *et al* (2008) argued that strict physiological measures of measures of metabolic rate (e.g. SMR) inherently attempt to minimise behavioural variation among individuals in favour of physiological accuracy. Our data support this assertion that less stringent measures of metabolic rate, such as RMR, may be better connected with behaviour.

Variation in behaviour, especially at lower metabolic rates in our data, is an important characteristic of natural populations that may facilitate population persistence under different contexts (Biro & Stamps, 2008; Careau et al., 2008; Sih et al., 2004; Sih et al., 2012). Though our data show that an increase of time spent exploring is linked to RMR there is substantial variation that is not explained by RMR alone. Inter-individual variation is common and an important characteristic of populations. Therefore, while our behavioural data do show a relationship between behaviour and metabolism, this was not consistent across behavioural measures and there was substantial inter-individual variation within populations.

Attempts to marry behavioural and metabolic variation have had varied outcomes. For example, Biro and Stamps (2010) reviewed 27 experiments, of which 20 found positive relationships between behaviour and boldness. More recent meta-analyses have found little support for a simple relationship between behaviour and metabolism; rather these studies suggest that this relationship is complex and cannot be predicted via linear relationships (Niemela & Dingemanse, 2018; Royauté et al., 2018). Similarly, our data show that individual behaviour is influenced by several factors, including metabolism. However, this relationship was only apparent when behaviour was measured as exploratory behaviour. When measured as emergence latency other factors (temperature, gender, and pregnancy) played a key role in dictating whether an individual would take the risk of leaving a safe area. In the same way, Hoogenboom *et al* (2013) found variation in behavioural responses to food resources, in which only certain behaviours were related to metabolism. Differences in success combining metabolic and behavioural measurements suggest that only certain aspects of behaviour may be related to individual physiology.

3.5.5. Conclusion

Overall, our data suggest that consumer thermal history plays an important role in regulating the temperature sensitivity of metabolism and determining boldness. Reductions in metabolic temperature sensitivity at warmer temperatures will reduce the energy required to fuel metabolism and may therefore facilitate population persistence under warming. While we did find a relationship between behaviour and metabolism, this relationship was complex and best described alongside other individual characteristics. Our data add to the existing literature demonstrating substantial intraspecific variation among populations which may have significant ecological effects (e.g. Des Roches et al., 2018), and therefore, should be considered in projections of future ecological change.

Chapter 4

Shift in diet with temperature alters gut morphology and body nutrient composition

4.1. Abstract

Temperature rise will have numerous effects on species including shifts in phenology, altered geographic distributions, and reduced body size. However, little is known about how temperature rise will influence dietary demands, and whether changes in diet will lead to changes in the way nutrients are used by organisms. Here, we used populations of *Gambusia affinis* in New Zealand and California, which span a wide temperature gradient to understand how temperature influences fish diet and morphological and stoichiometric phenotypes. Our data show that dietary patterns in New Zealand and California were divergent with rising temperature. However, in both regions a shift toward a plant-based diet was associated with an increasing gut length to body length ratio and fuller guts. Further, populations with predominantly plant-based diets had lowered elemental carbon or increased nitrogen with warming. Together, our data suggest that temperature is a pervasive stressor driving dietary changes, which leads to gut morphological and stoichiometric divergence among populations.

4.2. Introduction

Changing thermal regimes will have numerous effects on ecosystems, including alterations in species composition and abundances (Hickling et al., 2006; Parmesan, 2006). Such alterations to local communities may lead to a shift in diet for many organisms by increasing mass-specific feeding rates and decreasing diet breadth (O'Gorman et al., 2012; Petchey, Beckerman, Riede, & Warren, 2008). For example, warming is predicted to lead to increased energetic demand while decreasing primary production, leading to an increase consumption rates in consumers while limiting

resources at the base of food webs (Brown et al., 2004; Padfield et al., 2017). Further, communities may become restricted to species tolerant of warming, or diets may shift from being animal-based to plant-based with warming if prey species cannot adapt to temperature rise. Such shifts in consumption, driven by temperature rise, may have numerous ecological effects (e.g. community change, nutrient cycling, food-web stability) as nutrients are acquired, digested, assimilated, and egested to meet the increasing metabolic requirements of warming by exploiting a different resource base (Brown et al., 2004; Kondoh, 2003; Vanni, 2002).

Shifts in diet driven by rising temperature may select for traits which maximise nutrient uptake from the environment. For example, plant-rich diets are associated with longer gut lengths across and within species due to the lower nutrient content of plant-based diets and the greater processing times required due to the high amount of refractory materials in plant material (Karasov & Douglas, 2013; Sullam et al., 2015; Wagner, McIntyre, Buels, Gilbert, & Michel, 2009). Organisms may also demonstrate compensatory feeding, that is increasing their feeding rates to meet their energetic demands (van de Waal, Verschoor, Verspagen, van Donk, & Huisman, 2010). Understanding how temperature affects gut morphology is important as this directly reflects how organisms are using nutrients from their environment. Further, because increasing gut length comes with a high maintenance cost gut morphology should reflect a balance between maximising nutrient uptake and lowering tissue maintenance costs (Sullam et al., 2015).

Change in diet may lead to changes in an organism's elemental composition. Organismal elemental demands of nitrogen (N), phosphorous (P), and carbon (C) depend on the stoichiometry (C:N:P) of these elements in an organism's body tissue (Sterner & Elser, 2002). While stoichiometry varies widely among taxa, it was thought to be

consistent within species (Sterner & Elser, 2002). However, there is now much evidence to suggest that intraspecific differences in stoichiometry may be widespread and occur in response to diet, trophic status, ontogeny, predation pressure, seasonal variation, or evolutionary divergence (Boros et al., 2012; Dalton et al., 2017; Durston & El-Sabaawi, 2017; El-Sabaawi, Zandona, et al., 2012; Leal, Best, Durston, El-Sabaawi, & Matthews, 2017; Mozsár, Sály, Antal, Nagy, & Boros, 2019; Pilati & Vanni, 2007; Tuckett, Kinnison, Saros, & Simon, 2016). Though, little is known about the effect of temperature change on elemental stoichiometry (but see, O'Gorman et al., 2016). Thus, changes in diet with temperature rise may lead to changes in body stoichiometry and fitness traits (e.g. growth) via altered nutrient demand (Vrede, Dobberfuhl, Kooijman, & Elser, 2004). Such changes in stoichiometry may have ecosystem consequences where nutrient requirements shift alongside environmental change, leading to an alteration in the ratio in which nutrients are ingested an excreted, influencing larger-scale ecosystem processes (Vanni, 2002).

Here, we use a widespread freshwater consumer, *Gambusia affinis* (mosquitofish), to understand how temperature change influences gut morphology and body elemental composition. We use mosquitofish populations in two countries than span a parallel temperature gradient. We explored how shifts in diet driven by temperature change lead to divergence in phenotypic traits (gut length) and body elemental composition. We hypothesized that individuals with diets lacking in nutrient rich foods should have longer guts and a body elemental composition which reflects the nutrient limitation of their diet. Thus, our aims were to: 1) understand how consumer diets shift with rising temperature, and 2) describe the effects of dietary change on gut morphology and body nutrient stoichiometry.

4.3. Methods

4.3.1. Study organism and populations

Gambusia affinis were introduced to New Zealand (NZ) in the 1930s and to California (CA) in the 1920s from populations in Texas, U.S.A. (McDowall, 1978; Stockwell & Weeks, 1999). *Gambusia* are live-bearers, reach high densities in the wild, and are found across a wide range of environmental conditions (salinity, temperature, pH, turbidity) (Pyke, 2008). *Gambusia* are omnivorous, feeding on a variety of foods including algae, detritus, zooplankton, invertebrates, and fish (Lee, Simon, & Perry, 2018; Pyke, 2005). We studied 18 populations of *Gambusia* in geothermal springs and streams in California, U.S.A and in the North Island of New Zealand. In California sites were commonly closed pond systems where barriers isolated populations, whereas, in New Zealand populations often inhabited slow flowing spring-fed streams open to potential fish movement (Table 4.1).

Table 4.1. Site characteristics, sites in New Zealand (NZ) and California (CA) are ordered by temperature. Water temperature, dissolved oxygen (DO), and specific conductivity measurements were taken at the time of fish collection.

Region	Site	Open/ Closed	Temperature (°C)	DO (mg/L)	Conductivity (mS/cm)
NZ	PP	O	19.2	8.59	0.201
	AL	O	22.7	8.15	0.145
	AS	O	23.4	5.67	0.344
	PK	O	24.2	2.54	3.566
	MR	O	30.9	4.49	0.740
	WA	O	33.5	4.49	1.092
	SP	O	35	5.43	0.424
	AWK	O	37.7	8.5	0.391
	AA	C	38	5.54	0.473
CA	NE	C	20.9	11.32	0.339
	WW5	O	23.6	9.84	0.492
	AW	C	23.7	4.67	0.391
	FS	C	24	8.11	0.156
	WSU	C	27.8	6.42	0.461
	HC	O	29.9	5.14	0.391
	FC	C	33.4	12.82	0.962
	LHC	O	36.7	2.42	0.452
	K2	C	38.9	6.37	0.827

4.3.2. Sampling

We collected *Gambusia* from sites along parallel temperature gradients in both NZ (19.2 - 38°C) and CA (20.9 - 38.9°C) during summer (Table 4.1). In CA, we sampled between the 30th of May and the 1st of June 2016 and in New Zealand, from the 8th of February to the 21st of February 2017. Temperature, specific conductivity, and dissolved oxygen were measured at each site using YSI ProODO and an YSI Professional Plus meters. At each site *Gambusia* were captured using a 5 m seine (1.6 mm mesh) which was pulled through the water at several locations at each site. All fish were immediately

euthanized with MS-222 in CA or clove oil in NZ and transported back to the laboratory on ice and immediately frozen.

Macroinvertebrate communities were surveyed at each of the sites using a standard kick-net protocol using a D-net (0.5 mm mesh) (P2, Stark et al 2001). At each site we sampled 10 0.5 m^2 areas of stream, targeting as many habitat types as possible. At each site, the contents of the D-net were pooled and preserved in 80 % ethanol on site. In the laboratory, macroinvertebrates were identified to the lowest practical taxonomic unit (typically genus) under a 10-80× magnification microscope and counted to give relative abundances of each taxon and taxa richness in each sample.

Zooplankton were collected using a 63µm Wisconsin plankton net. At each site this net was dragged through the water column for approximately 20 m. All plankters were preserved in 80 % ethanol. In the laboratory zooplankter were enumerated and identified to the lowest possible taxonomic unit under a 10-80× magnification microscope.

4.3.3. Sample preparation

We randomly selected 40 (20 males and 20 females, where possible) individuals from each of the 18 sites (n = 720) for diet and morphometric analysis. *Gambusia* were weighed (±1 mg), and lateral photographs were taken for body length measurements (± 1 mm). We then removed the intestine of each photographed fish, photographed the intestine for length and preserved it in 70 % ethanol until gut content analysis. Fish (less intestines) were dried at 60°C for 48 hours, before being ground for stoichiometric analysis (see below).

4.3.4. Gut content analysis

We first visually estimated gut fullness which was scored from 1 (empty) to 5 (full). Individual gut contents were removed and placed onto a petri dish with a 1 mm

graticule. Contents were identified under an 80- 100× microscope to the finest taxonomic resolution possible. The proportion of the total volume of each prey item was visually estimated (Baker et al 2014). Finally, area of gut contents was quantified by gently pressing a microscope cover slip over the gut contents to a uniform depth and counting how many 1 mm^2 cells on the graticule were filled (Brooker et al. 2018). Digested material that could not be identified was summed as amorphous.

4.3.5. Nutrient stoichiometry

Body elemental composition was measured on 20 individuals (10 males and 10 females) from each of our 18 sites (n = 360). Elemental C and N were measured using a vario EL cube elemental analyser, for each individual measured we used ~5 mg of dried and ground tissue (Elementar, Germany). For total P analysis ~2-3 mg of dried and ground fish tissue was ashed in a furnace at 500°C for 4 hours. Combusted samples were digested by adding 10 mL of distilled water and 2mL of 2N HCl into each tube, tubes were then placed into an oven at 105°C for 2 hours. Following digestion, 0.5 mL of each sample was removed and diluted to 10 mL using distilled water. Samples were analysed manually on a spectrophotometer according to the ascorbic acid method (APHA, 2000; 4500- P.E.).

4.3.6. Statistical analysis

Variation in diet was summarised for each population using a relative importance index (RIi) (Equation 1). This index accounts for both frequency of occurrence of a diet item (%F) and percent volume in the gut (%V) (Chucholl, 2013).

$$RI_i = \frac{(Al_i\ 100)}{\sum_{i=1}^{n} Al_i} \quad (1)$$

where: Al_i was calculated as %F ×%V for food item i.

We summarised variation in diet among populations using ordination by non-metric multidimensional scaling (NMDS). NMDS was carried out on the RI_i data for each site using the weighted Jaccard distance metric. We fitted temperature as an environmental gradient onto the ordination, where the arrow points to the direction of change and the length of the arrow represents the strength of the correlation between diet and temperature.

We used Levin's standardized Index (BA) to quantify dietary niche breadth among our populations (Equation 2) (Wallace, 1981). Levin's index values range from 0 to 1, where low values indicate a specialist diet which is composed of fewer items and high values indicate a generalist diet composed of a wide variety of items.

$$B_a = \frac{(\left(\frac{1}{\sum d_i^2}\right)-1)}{n-1} \quad (2)$$

where: d_i is the proportion of diet that was made up of prey item *i* and *n* is the number of prey categories.

Finally, to determine if fish were preferentially selecting prey we calculated prey selectivity using the Ivlev's prey selectivity index (Equation 3) (Kohler & Ney, 1982). Values range from 1 to -1 with values closer to 1 indicating the food item is selected by the predator by a greater proportion than what is available in the environment. In contrast, values between 0 and -1 indicate that the food item is less frequently selected for consumption compared to its abundance in the environment. This index is calculated as;

$$E_i = \frac{r_i - p_i}{r_i + p_i} \quad (3)$$

where: E_i is the prey selectivity for item *i*, r_i is the proportional abundance of prey *i* in the diet, and p_i is the proportional abundance of prey *i* in the environment.

Trends in volume of prey items in guts across all individuals from each NZ and CA population were analysed using generalized linear models (GLM). We used site temperature, body length, and gender as independent factors. Gut fullness was analysed using multinomial logistic regression. The baseline condition was set as empty (1) for analysis and significance was determined using z-tests.

To compare differences in gut length across populations we calculated relative gut length (RGL) (Equation 4) as;

$$\mathrm{RGL} = \frac{\mathrm{GL}}{\mathrm{SL}} \quad (4)$$

Where: GL is gut length (mm) and SL is standard length (mm).

We used simple linear regression models to determine if trends in diet (RI_i, Levin's index) and relative gut length were related to population temperature. For plots of RI_i and temperature we pooled the major dietary categories (algae + detritus; invertebrates + amorphous material) for comparison of the dominant trends.

We used separate GLMs to understand the factors which influenced %C, %N, %P, C:N, C:P, and N:P. We used temperature, gender, and dry weight as our independent variables. Data were $\log_{10}$ transformed prior to analysis to meet the model's normality assumption. All statistical analyses were performed using R version 3.5.0 and using the R packages 'NNET' version 7.3-12 and 'Vegan' version 2.5-3 (Oksanen et al., 2018; R Development Core Team, 2017; Venables & Ripley, 2002). All plots were created in R using ggplot2 version 3.0.0 (Wickham, 2016).

4.4. Results

4.4.1. Site invertebrate and zooplankton communities

In New Zealand (NZ), the number of invertebrates captured, and species richness tended to decline as temperature increased, but this relationship was not significant ($p > 0.05$; Fig. S3.1). In California (CA), invertebrate count and richness data were not related to temperature and species richness remained relatively high across all sites. There were fewer zooplankton in NZ sites when compared to CA sites and their presence was not related to site temperature (Fig. S3.1c). In CA, there was a significant decrease in zooplankton numbers as site temperature increased ($r^2 = 0.645$, $p = 0.009$) in which both copepods and rotifers became less abundant and *Ceriodaphnia* became more abundant with increased site temperature. In both NZ ($p = 0.523$) and CA ($p = 0.893$) there were no distinct patterns of change invertebrate community composition with temperature (Fig. 4.1a,b).

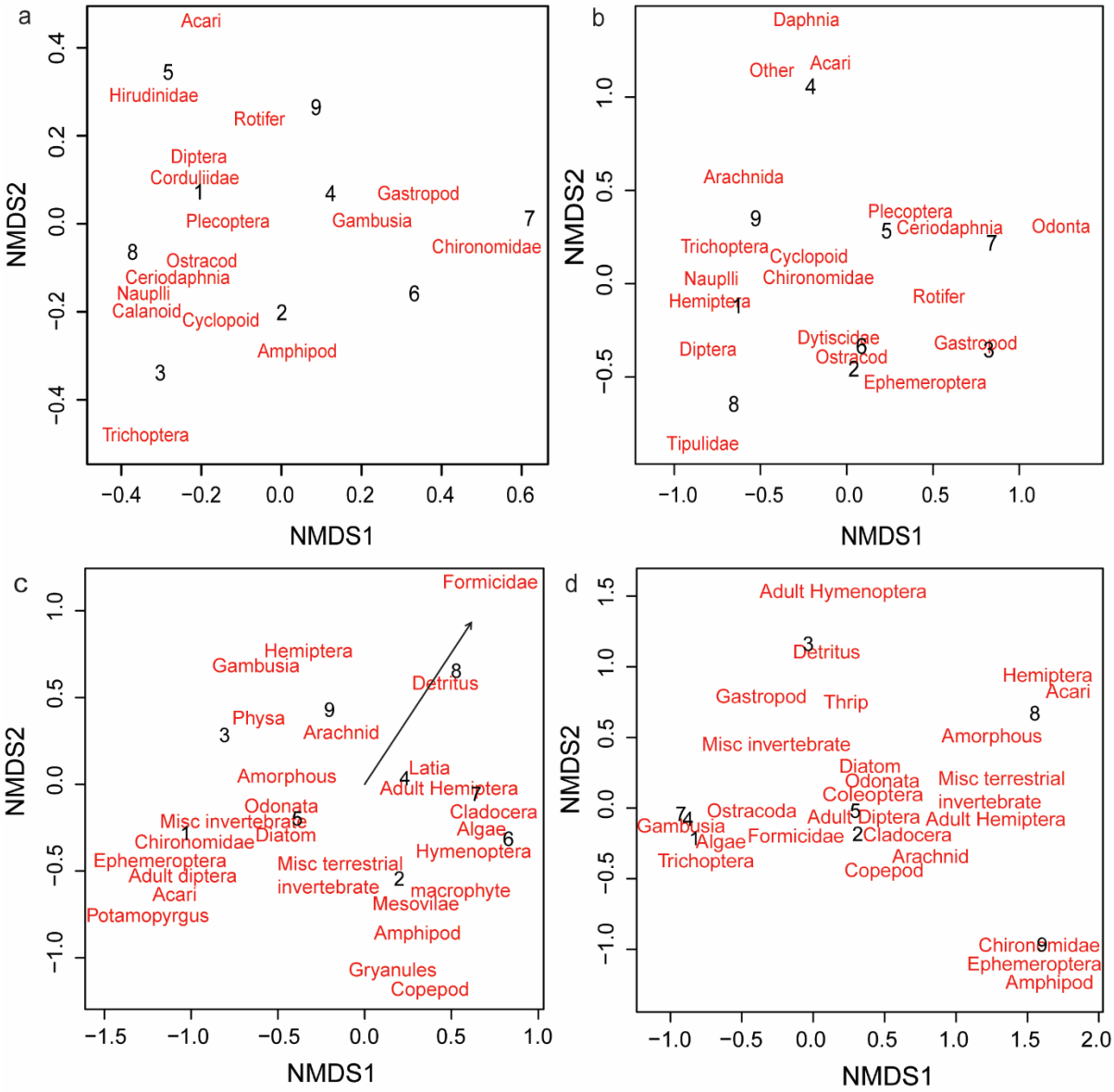


Figure 4.1. Nonmetric multidimensional scaling (NMDS) ordination representing how the invertebrate communities differed across sites in a) NZ (stress = 0.132) and c) CA (stress = 0.112) and how the diet of *Gambusia affinis* changes across populations in NZ (stress = 0.015) c) and CA (stress = 0.012) d). Sites are shown as numbers, which are sorted from coolest (1) to warmest (9) in both regions. The black arrow on plot c) indicates the direction of community change with warming ($r^2 = 0.694$, $p = 0.0311$), no other covariate showed a significant relationship to temperature ($p > 0.05$).

4.4.2. Diet and temperature

The volume of food in fish guts increased with increasing temperature in NZ ($t = 3.680$; $p < 0.0001$) but decreased with increasing temperature in CA ($t = -3.484$, $p < 0.0001$; Table S3.1). In both NZ and CA, longer body length was associated with an increase in the volume of food in guts (NZ, $t = 4.844$, $p < 0.0001$; CA, $t = 10.792$, $p < 0.0001$) and males had less food volume in their guts compared to females (NZ, $t = -5.701$, $p < 0.001$; CA, $t = -2.553$, $p = 0.011$). Gut fullness increased with temperature in NZ ($Z = 0.059$, $p = 0.030$), while decreasing with temperature in CA ($Z = 0.307$, $p = 0.006$; Table S3.2 & S3.3). Gender affected gut fullness with males having a lower gut fullness in NZ ($Z = 0.330$, $p = 0.008$) and in CA ($Z = 0.200$, $p < 0.0001$). In neither region did body length affect gut fullness ($p > 0.05$).

In NZ and CA there were clear shifts in diet with warming; however, these trends were orthogonal to one another. In NZ, RI_i values show a shift from invertebrate-dominated to algal and detritus-dominated diet with temperature, while the opposite was observed in CA (Fig. 4.2; Table S3.4). Similarly, the NZ NMDS ordination shows an increasing dominance of detritus, algae, and terrestrial invertebrates in fish diets with temperature ($r^2 = 0.694$; $p = 0.031$; Fig. 4.1). In CA, NMDS trends were less clear and differences were not related to temperature ($p = 0.355$). In particular, the ordination shows the warmest population in CA (Site number 9, K2, 38.9°C) was characterised by a distinct diet likely due to the prevalence of prey items at this site and a selective diet (Fig. S3.1; Table S3.4 and S3.5).

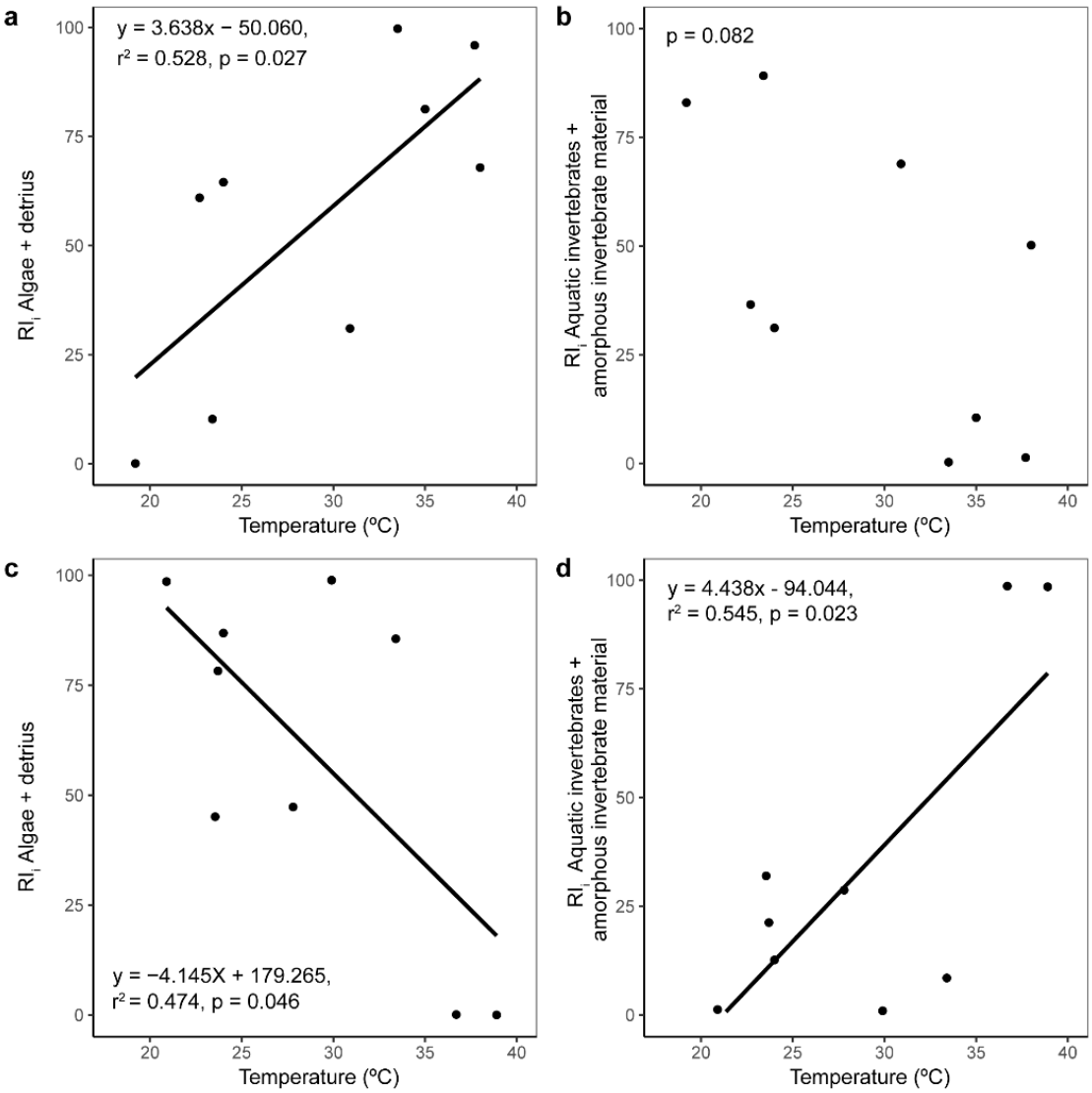


Figure 4.2. Relative importance of either algal and detrital matter or aquatic invertebrates and amorphous material in the diet of *Gambusia affinis* across populations of different temperatures. Data are relative importance index scores calculated for each site and are shown for New Zealand (a,b) and California (c,d). $N = 9$ per region.

Diet niche breadth (Levin's index) tended to decline with temperature, suggesting a more specialised diet with rising temperature; however, this trend non-significant in NZ ($p = 0.083$) and CA ($p = 0.182$; Fig. S3.2). Levin's index scores were not related to species richness in the environment ($p > 0.05$).

In NZ, the prey selectivity (Ei) values show that aquatic and terrestrial invertebrate species were selected for across all sites, with the exception of chironomids,

which had mixed selection across all populations (Table S3.5). The selection of chironomids, amphipods, and ostracods increased with site temperature. In CA, terrestrial invertebrates, acari, and Hemiptera were always positively selected for, whereas zooplankton was always negatively selected for. Across categories with mixed selection there were no obvious trends with site temperature.

4.4.3. Gut length

Fish gut length varied with source population temperature. In NZ, relative gut length increased with temperature ($r^2 = 0.561$, $p = 0.020$; Fig. 4.3a). In contrast, in CA, relative gut length decreased with temperature ($r^2 = 0.861$, $p = 0.0003$; Fig. 4.3b). In both NZ and CA relative gut length did not covary with body length (ANCOVA, $p > 0.05$).

In NZ, gut length increased predictably with the relative importance of algae and detritus in fish guts ($r^2 = 0.451$, $p = 0.047$; Fig. 4.3c). There was a similar tendency for relative gut length to increase with algae and detritus in fish diets in CA although this trend was not significant ($p = 0.214$; Fig. 4.3d).

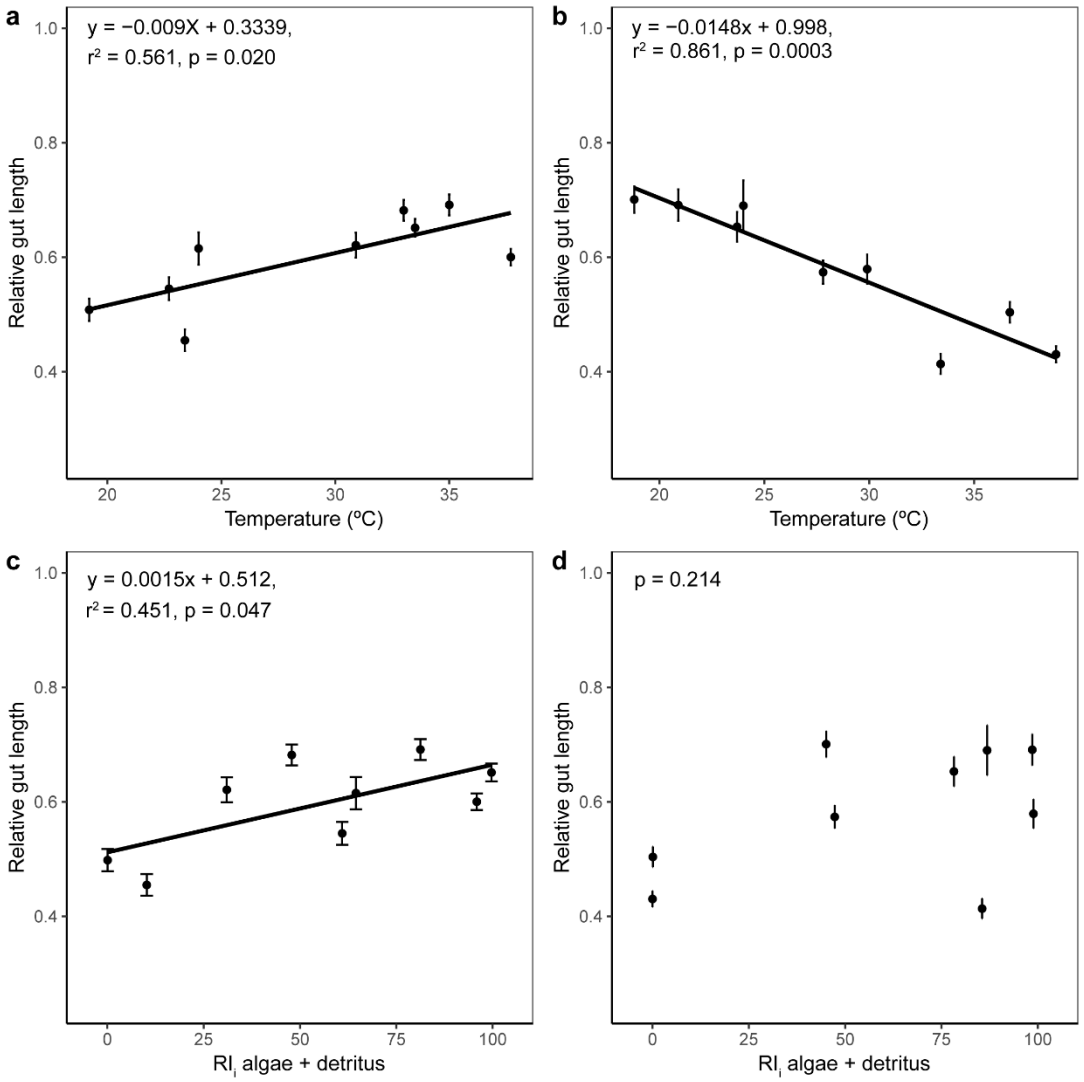


Figure 4.3. Relationship between relative gut length and temperature across populations of *Gambusia affinis* in a) NZ and B) CA. Relative gut length is also compared to relative importance index (RI_i) of algae and detritus in each population diet for c) NZ and d) CA. Data are averages ± SE. $N = 9$ for each region.

4.4.4. Elemental tissue composition

Mosquitofish populations exhibited a wide range of elemental variability with %C values ranging from 33 to 51 %, %N values ranging from 7.2 to 11.2 %, and %P varying from 1.1 to 8.2 %. Coefficients of variation (CV) were 27.8 % for P, followed by 8.2 % for C, then 7.4 % for N. The range in values of elemental nutrient was similar in NZ and CA (Fig. S3.3).

In both NZ and CA temperature affected fish elemental composition, whereby nutrients increased and carbon decreased with increasing population temperature; however, this played out in different ways. In NZ, %C decreased with increasing site temperature ($t = -2.039$, $p = 0.043$), while %P increased ($t = 1.993$, $p = 0.048$; Fig. 4.4). There was no change in %N across the temperature gradient ($p = 0.890$; Table 4.2). In NZ, there was no direct effect of temperature on elemental ratios; however, there was a significant interactive effect between C:N, temperature, dry weight, and sex ($t = 2.119$, $p = 0.035$; Fig. S3.3). Dry weight and sex had no direct effect on elemental nutrients or ratios ($p > 0.05$).

In comparison, in CA %N increased with increasing site temperature ($t = -2.042$, $p = 0.043$). In addition, in CA there were significant interactive effects between temperature and dry weight for %N ($t = -3.071$, $p = 0.002$) and C:N ($t = 2.569$, $p = 0.011$). Similarly, we found interactions between temperature and sex for % N ($t = -2.493$, $p = 0.0136$) and C:N ($t = 2.761$, $p = 0.006$; Fig. 4.4). There was a significant effect of dry weight × sex on %C (2.020, $p = 0.045$) and C:N ($t = 2.331$, $p = 0.021$). These also show that in CA larger individuals had increased elemental %N ($t = 2.524$, $p = 0.0125$), and males had increased elemental %N ($t = 2.319$, $p = 0.022$) and reduced elemental %C ($t = -2.344$, $p = 0.020$) compared to females. Finally, there was a significant interaction between temperature × dry weight × gender for C:N ($t = -2.032$, $p = 0.044$). The prevalence of interactive effects CA suggests that stoichiometric differences between populations commonly varied with sex and body size.

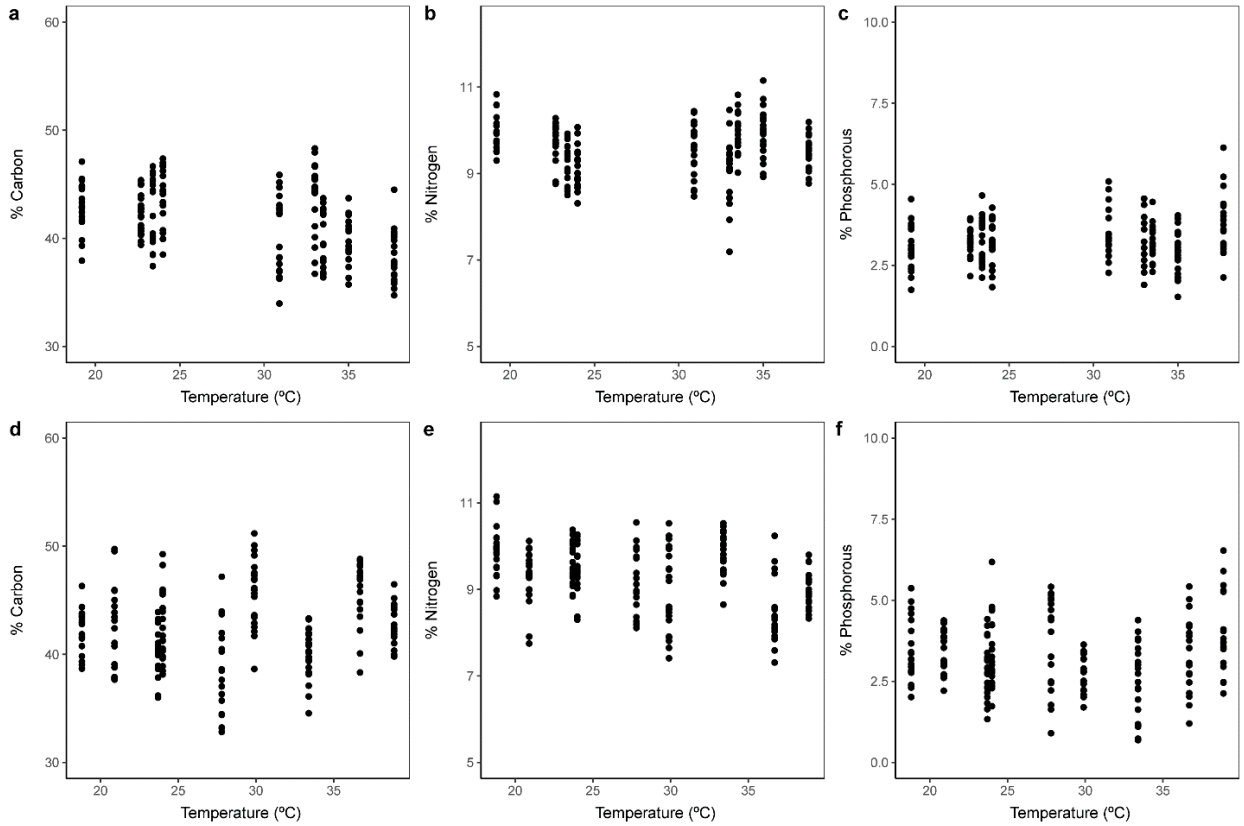


Figure 4.4. Relationship between site temperature and % body elemental carbon, nitrogen, and phosphorous. Data are shown for New Zealand (a,b,c), and California (d,e,f) populations of *Gambusia affinis*. Data are individual fish, n = 40 per population. Results of the statistical analysis are shown in Table 4.2.

Table 4.2. Generalized linear model (GLM) results describing which factors explain the nutrient percentages and ratios in fish body tissue of *Gambusia affinis* in New Zealand (NZ) and California (CA). Data are t-values and significance is noted as: < 0.0001 '***' < 0.001 '**' < 0.01 '*' < 0.05 '.' < 0.1, significant t-values are bolded.

Region	Source	DF	%C	%N	%P	C:N	N:P	C:P
NZ	Temperature	179	**-2.039***	0.138	**1.993***	-1.432	-0.336	-1.011
	Dry weight		-0.148	1.002	1.101	-0.703	0.314	-0.064
	Sex		0.606	-0.616	0.116	0.877	0.449	0.730
	Temperature × dry weight		-0.013	-1.065	-1.099	0.639	-0.216	0.130
	Temperature × sex		-1.186	1.409	-0.033	-1.784 .	-0.363	-1.004
	Dry weight × sex		-1.137	0.721	1.053	-1.353	-0.904	-1.421
	Temperature × dry weight × Sex		1.334	0.093 .	-0.848	**2.119***	0.570	1.427
CA	Temperature	177	-0.411	**-2.042***	0.750	-0.870	-0.592	-0.794
	Dry weight		-0.536	**2.524 ***	1.333	-1.856 .	-0.876	-1.375
	Sex		**-2.344***	**2.319***	1.515	**-2.897****	-1.803	-1.891 .
	Temperature × dry weight		1.135	**-3.071****	-1.119	**2.569***	0.568	1.284
	Temperature × sex		1.972 .	**-2.493***	-1.048	**2.761****	0.601	1.374
	Dry weight × sex		2.020*	-1.724 .	-1.223	**2.331***	0.879	1.551
	Temperature × dry weight × Sex		-1.702 .	1.564	1.043	**-2.032***	-0.737	-1.318

4.5. Discussion

Warming is predicted to lead to an increase in energetic demand and a shift in species composition and abundance in natural environments, thereby altering consumer diets (O'Gorman et al., 2012; Shurin et al., 2012; Somero, 2010; Walther, 2010). Our data show that dietary patterns in New Zealand and California were divergent with rising temperature. Changes in diet were reflected in gut length and fullness, where populations with a detrital and algal based diet had longer and fuller guts. Finally, trends in elemental composition varied with temperature, suggesting a divergence in stoichiometric phenotype across our populations to meet nutrient requirements.

4.5.1. Diet and temperature

Temperature rise is expected to impose an increased energetic demand on individuals (Brown et al 2004). This increased energetic demand can be met through two strategies: first, increasing the quantity and frequency of consumption of low-quality food items (e.g. algae and detritus), or, second, having a diet of high nutritional quality (e.g. insects) (Day, Tibbetts, & Secor, 2014; Hopcraft, Olff, & Sinclair, 2010; O'Gorman et al., 2016). Our data show the prevalence of both feeding strategies in mosquitofish. In NZ, invertebrate food resources became less common as population temperatures increased, leading to a diet dominated by algal and detrital matter. Further, fish had a greater volume of food in their guts, suggesting compensatory feeding with temperature rise to meet energy demand (van de Waal et al., 2010). Trends were reversed in CA, where invertebrate food resources were still available at the warmest sites, leading to diets dominated by invertebrates, lower gut fullness, and less food volume in fish guts with warming. Differences in invertebrate resources between NZ and CA may be related to the site geomorphologies and hydrologies, in CA, sites were often closed pond systems whereas in NZ all sites were flowing streams. These differences in site structure may affect prey availability, through differences in habitat and resources.

While we observed divergent dietary responses to warming, related to resource availability, other research suggests a shift toward high nutritional value prey with warming to maximise energy intake (O'Gorman et al., 2016; Vucic-Pestic, Ehnes, Rall, & Brose, 2011). However, this feeding strategy requires sufficient invertebrate resources, which may not occur if prey species cannot adapt to local conditions or are displaced by warm-adapted species (Walker et al 2006; Freiberg et al 2009). Our data suggest that mosquitofish are modifying their feeding strategies to maximise fitness based on the resources available at each site. Further, these data indicate that ecosystems which are composed of similar species will be affected differently under climate change where food resources differ.

4.5.2. Gut length

Our data support other studies that have found longer gut lengths both between and within species with a shift toward plant-based diets (German & Horn, 2006; Kohl et al., 2016; Leigh, Nguyen-Phuc, & German, 2018; O'Grady, Morando, Avila, & Dearing, 2005; Olssson, Quevedo, Colson, & Svanback, 2007; Sullam et al., 2015). Notably, increasing gut length and temperature rise are both likely to impose a metabolic cost through tissue-maintenance and elevated organismal metabolic rates. For example, in NZ, where nutrient rich resources were less common with warming and gut maintenance costs are higher (e.g. greater relative gut length), fish may have lowered energetic efficiencies or face starvation if metabolic demand cannot be met with local resources (Vucic-Pestic et al., 2011). Thus, understanding how species diets will change with temperature rise is critical in predicting future ecological responses.

The change in gut length with food resources suggests that mosquitofish can adjust their phenotype to meet their energetic demands. Plasticity in gut morphology is common and may occur through ontogeny, with diet changes, or with fasting (Day et al.,

2014; German & Horn, 2006; Leigh et al., 2018; Olssson et al., 2007; Wagner et al., 2009). However, adaptive change may occur concomitant with plastic changes, particularly in spatially separated populations where there is potential for local adaptation. For example, Herrel *et al* (2008) found evolutionary divergence in lizard gut morphologies 36 years after introduction to novel environments. Similarly, diet trials in Trinidadian guppies (*Poecilia reticulata*) and prickleback (Family Stichaeidae) fishes did not alter gut lengths and in both studies gut length remained longest in species with a low-quality diet, suggesting local adaptation (German & Horn, 2006; Sullam et al., 2015). Thus, while plasticity in gut morphology has been documented in some species, local evolutionary adaptation can lead to divergence in gut morphology between populations. The mechanism driving the trends in gut length in mosquitofish is unknown; however, the presence of parallel patterns of gut length with dietary shifts in NZ and CA highlights the functional significance of morphological changes in mosquitofish.

4.5.3. Elemental tissue composition

Mass balance models predict that consumers maintain fixed whole-body nutrients (%N, %P) (Sterner & Elser, 2002). However, there is now evidence of substantial inter- and intra-specific variation in the elemental composition of organisms (Dalton et al., 2017; Leal, Seehausen, & Matthews, 2017; Sullam et al., 2015). Such variation may occur in response to local environmental conditions (e.g. season, nutrients, predation) or organismal traits (body size, diet, morphology, gender) (El-Sabaawi, Kohler, et al., 2012; El-Sabaawi, Zandona, et al., 2012; Mozsár et al., 2019; Tuckett et al., 2016). Our data show a shift in mosquitofish elemental composition in response to temperature, likely driven by the associated dietary change. The range of variation across our populations in the elemental composition of mosquitofish is in-line with values found in other species in response to different biotic and abiotic factors (Boros et al., 2012; El-

Sabaawi, Zandona, et al., 2012; Sterner & George, 2000; Tuckett et al., 2016). This variation is significant as population phenotypic divergence in response to temperature change may lead to ecosystem changes (e.g. Post & Palkovacs, 2009). For example, stoichiometric variation at the population level may change the balance of nutrients available in the environment through changing feeding patterns as organismal nutrient requirements change (Leal, Seehausen, et al., 2017)

Low-quality diets were consistently associated with increased N and reduced C in mosquitofish elemental composition. While diet shifted in the opposite direction with temperature in NZ and CA, elemental composition tracked diet shift in the same manner. In NZ, %C decreased as temperature increased, and in CA %N decreased with temperature. Thus, overall an increase in algae and detritus in fish diet is associated with increased body nutrients or reduced body carbon. Similarly, Sullam *et al* (2015) found that fish that had high quality diets had elevated elemental C:N thought to be due to the C-rich lipids in fish diets. Moreover, in NZ the %P in fish body tissue increased as diet quality decreased, suggesting fish are retaining P where diets are generally low in P (El-Sabaawi, Zandona, et al., 2012; Karasov & Douglas, 2013). Overall, these data suggest that dietary shifts associated by temperature change lead to consistent responses across geographically isolated populations of mosquitofish, indicating that such stoichiometric phenotypes in response to dietary quality may be a widespread occurrence (Leal, Seehausen, et al., 2017; Pilati & Vanni, 2007).

While our overall trends were similar between regions, there were some differences in the relationships between elemental stoichiometry, temperature, body size, and gender. In NZ, population temperature was related to %C and %P, whereas in CA %N was related to temperature. Further, in CA there were frequent interactions between dry weight, sex, and temperature on elemental C:N. Change in lipid demand through

ontogeny via bone formation or between sexes can shape variation in organismal stoichiometry (Mozsár et al., 2019). For example, larval zebrafish and gizzard shads have lower C:P and N:P ratios compared to adults, suggesting an increased demand for nutrients in younger individuals (Pilati & Vanni, 2007). In contrast, in CA larger fish and males had increased body %N, leading to a significant interaction in body nutrients between gender and body size. Difference in body nutrients through ontogeny between studies may be explained by a difference in resources within local environments. Thus, our study and others show that nutrients may be allocated differently through ontogeny and between sexes, suggesting these factors should be accounted for in models of stoichiometric variation.

In conclusion, we observed increasing temperature to alter the diet of a widespread consumer, with predictable changes in both gut morphology and organismal stoichiometry. Here, a low-quality diet was associated with longer and fuller guts and a decrease in elemental carbon or increase in nitrogen in body tissue. These data are unique as there has been limited research done on the effect on temperature on diet and phenotypic changes. In this research we show a distinct relationship from environmental to dietary and phenotypic change. Interestingly, populations in NZ and CA showed divergent dietary trends with temperature rise, suggesting that models predicting these relationships need to be region-specific. Further, the appearance of stoichiometric phenotypes in our research and others indicates a divergence in the nutrient requirements of organisms which may have consequences for larger-scale ecological processes in streams (e.g. Palkovacs et al., 2009). Our data demonstrate that temperature is a pervasive stressor which alters consumer diet and leads to phenotypic change, understanding how such changes may interact with the environment to generate ecological change will be key to understanding the effects of future climate change.

Chapter 5

Thermal history alters the ecological role of consumer body size

5.1. Abstract

Body size is a key determinant of the ecological role of consumers and size is expected to decline with temperature rise predicted by climate change. The ecological effects of consumers may therefore change in the future as body size declines. However, the ecological outcome of body size declines may depend on the thermal history of consumers. We addressed this issue by examining populations of a globally invasive fish (*Gambusia affinis*) recently established in geothermal systems of differing temperatures. We conducted a mesocosm experiment in which we manipulated source population (cool-source versus warm-source) and fish body size, while holding fish biomass constant. We measured the metabolic and excretion rates of the experimental populations and measured community and ecosystem response variables, from zooplankton biomass to greenhouse gas emissions. Body size change had strong effects on most ecological responses, but these effects depended on population thermal history. Interestingly, the effects of body size were often opposing between populations, indicating that thermal adaptation and body size change generally buffered the direct effects of warming on community and ecosystem responses. Overall, these results suggest that thermal history may moderate the ecological changes caused by warming-induced body size declines.

5.2. Introduction

Increasing temperature as a result of climate change is having numerous effects on species, including altered geographic distributions, shifts in phenology, and decreased body size at maturity (Hickling et al., 2006; Parmesan, 2006; Sheridan & Bickford, 2011). Decreasing body size is likely to be ecologically important and particularly conspicuous in water-breathing organisms due to the lower oxygen solubility in water with rising

temperature (Daufrense et al., 2009; Forster et al., 2012; Gardner et al., 2011). Body size influences essential biological rates (e.g. metabolism), which may in turn influence community abundances, trophic positions, and ecosystem stability (Brose, 2010; Brose et al., 2006; Schoener, 1989; Segura, Franco-Trecu, Franco-Fraguas, Arim, & Tonn, 2015).

A common approach to testing the ecological outcome of warming involves exposing animals from current climatic conditions to warmer temperatures over a short period of time (Shurin et al., 2012; Yvon-Durocher et al., 2012). Recent work steps beyond this initial work to examine how these short-term effects interact with effects of reduced body size (models using temperature size rule (TSR), e.g. Bernhardt et al., 2018). While an important step forward, this work considers smaller-bodied populations surrogates for those of future conditions. Importantly, the relevance of this strategy may be limited because changing body size is just one possible facet of trait response to warming.

Increased temperature can mediate the body size dependence of important functional traits (Englund et al., 2011; Rall et al., 2012). For example, difference in developmental temperature and body mass influence life history traits such as fecundity and may alter individual morphology or body nutrient stoichiometry (Bjorkman et al., 2018; Riesch et al., 2018; Savage et al., 2004). In addition to developmental temperature, thermal selection and concomitant evolutionary responses may also mediate the body size scaling of metabolic rate (Schaum et al., 2018; West & Post, 2016). For example, in our recent work we showed that a history of higher temperature modified the body mass scaling of metabolism (see Chapter 2; Moffett et al., 2018). Importantly, because thermal history may mediate body size effects, there is a crucial need to understand the broader consequences of these suites of trait changes expected under warming.

With smaller body size an increase in energetic demand due to the allometry of metabolic scaling and shifts in prey selectivity are expected (Brown et al., 2004; Gillooly et al., 2001; Rall et al., 2012; Sheridan & Bickford, 2011). If consumer body size declines under warming, but overall biomass remains the same, stronger top-down controls may occur due to increased metabolic demand. Consequently, body size changes at high trophic levels may limit trophic efficiency as trophic interactions are often moderated by body size (Barnes, Maxwell, Reuman, & Jennings, 2010; Trebilco, Baum, Salomon, & Dulvy, 2013). For example, body size decline under warming may alter energy flow though food webs if metabolic demand and therefore consumption rates increase, thus strengthening the top-down controls of consumers on ecosystems (Segura et al., 2015; West & Post, 2016). However, differences in consumer thermal history my alter traits to offset required increases in energy demand and therefore moderate ecological effects.

Here, we aim to understand if consumer thermal history alters the ecological outcome of reduced body size. We used a globally dominant freshwater consumer species (*Gambusia affinis*) as a model system. *Gambusia* causes strong trophic cascades in which zooplankton biomass decreases, primary production increases, and nutrient concentrations decline (Carpenter et al., 1987; Hurlbert & Mulla, 1981). Populations of mosquitofish that have invaded habitats with differing thermal regimes show differences in life history characteristics, such as smaller body size at maturity, and differences in metabolic scaling relative to temperature and body size (Fryxell & Palkovacs, 2017; Moffett et al., 2018; Stockwell & Weeks, 1999). Consequently, we hypothesized that consumer thermal history would lead to differences in the ecological effects of body size among populations.

5.3. Materials and methods

Study organism and populations

Mosquitofish are a widely distributed and successful invader which were introduced to New Zealand in the 1930s from Texas, U.S.A., via Hawaii (McDowall, 1978). Since their introduction mosquitofish have invaded a range of systems, including geothermal springs spanning a wide temperature range (Moffett et al., 2018). We used mosquitofish collected from two geothermal populations in the Taupo volcanic region of New Zealand; hereafter we refer to these as 'ambient' and 'warm' (Table S4.1). Fish were collected at 22°C from the ambient population and 35°C from the warm population. Annual average temperatures vary with air temperature and rainfall at both sites, with an annual average (±SD) temperature of 21.3±4.2°C at our ambient site and 30.7±5.4°C at our warm site. These sites were chosen for comparison based on prior work, in which metabolic scaling exponents vary widely in the warmest and coolest sites (Moffett et al., 2018; See Chapter 2). Therefore, we expect the results of this study to be generalizable beyond these specific populations.

5.3.1. Experimental design

We used a 5 × 8 array of 600 L pond mesocosms at The University of Auckland's Ardmore Field Station, New Zealand from 24 January to 4 March 2017. On 24 January each mesocosm was filled with rainwater and stocked with 20 L of sand and 10 L of homogenised sediment from the Waikato River to introduce nutrients and benthic invertebrate communities. A cement block with artificial macrophytes attached was added to each mesocosm to provide a fish refuge. Two days later we introduced zooplankton and phytoplankton from a nearby pond. Zooplankton were collected by pulling a Wisconsin-style plankton net (80 µm mesh) through an equivalent volume of water for each mesocosm to replicate natural plankton densities. Zooplankton were held in pond

water and dispersed in equal aliquots to each mesocosm. Fish were collected on the 30th of January 2017 and placed in aquaria with oxygen pumps, filters, and heaters set to 25°C. Fish were held for two days prior to starting the experiment.

Our experimental design consisted of eight replicates of five treatments. Treatments were the two populations (warm and ambient) at two body sizes (small and large) and a fishless reference. Treatments were assigned using a randomised block design, with the five treatments randomly assigned within each of the eight rows (blocks). Fish were introduced to the mesocosms one week after zooplankton were added. All fish treatments contained the same total biomass of fish (target biomass of 1.7 g wet weight) and the same sex ratio (3 female: 1 male). Large fish treatments had four individuals (3 female, 1 male) whereas small fish treatments had eight individuals (6 females, 2 males). The sex ratio was based on population distribution data from these populations (Moffett et al., 2018). Length-weight relationships for each population were used to calculate the length of the males and females needed in each size treatment to normalise mass. Once sorted into size classes and organised into treatments, top-down photographs of fish were taken prior to their addition to the mesocosms to determine initial size.

5.3.2. Sampling

Fish metabolic and excretion rates were measured at the end of the experiment, immediately following sampling. Mesocosms were sampled for inorganic nutrient concentrations, zooplankton, and phytoplankton at the following time points: 0 (prior to adding fish) and 4, 8, 16, 24, and 32 days after adding fish.

5.3.3. Consumers

We measured field metabolic rate (FMR) as oxygen consumption (MO_2), and nitrogen excretion rates of fish in each mesocosm following Sinclair et al. (2006) and Moffett et al. (2018). All fish in each mesocosm were held together in closed-system

respirometers incubated in a control mesocosm to maintain ambient water temperature. We used four 120 mL cylindrical acyclic respirometers with valves on each end. Fish were placed into the respirometers, the valves were sealed, and left for one minute before beginning our measurements. Dissolved oxygen concentration and temperature in each respirometer was measured continuously using a FireSting four-channel oxygen logger with optical oxygen sensors (PyroScience, Germany). Declines in oxygen concentration over time were estimated from linear fits to the data and only fits with $R^2 > 0.9$ were used. 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 as $\mu g\ O_2\ g^{-1}$ fish min^{-1} using total fish dry mass in each chamber.

Nitrogen excretion rates were estimated by change in nitrogen as ammonium (NH_4^+-N) concentrations in the closed respirometers over the 20-minute 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 taken from each respirometer, filtered (Whatman GF/F) into 15 mL HDPE tubes, and frozen until analysis. NH_4^+ concentration was measured colorimetry using a Lachat QuikChem® 8500 Series 2 Flow Injection Analysis System (Lachat Instruments, Loveland, CO, U.S.A.). After withdrawing water samples, *Gambusia* were euthanized using clove oil, measured for length and sex on-site, and frozen. Population growth was calculated as the difference in fish mass from T0 and T5, this was calculated from length-weight regressions and divided by the length of the experiment (32 days).

We sampled pelagic macroinvertebrates by moving an aquarium net through all levels of the mesocosm water column in a half circle motion using a using a 20 × 20 cm aquarium net. We then sampled benthic macroinvertebrates by disturbing the benthos in half of each mesocosm ($0.8 m^2$) and sweeping just above the sediment to capture any

disturbed invertebrates. Pelagic and benthic invertebrates samples were preserved separately in 80 % ethanol and subsequently identified under a 20-80× microscope to at least family level.

Zooplankton were collected by filtering 1 L of water through a 50 µm sieve; zooplankton were placed into a narcotizing agent of carbonated water then preserved in 80 % ethanol. Zooplankton were counted, measured, and identified to the lowest practical taxonomic resolution at 80× magnification. In each sample, 50 individuals per taxon were photographed and their body length was measured using ImageJ v 1.8.0 (Schneider, Rasband, & Eliceiri, 2012). If there were more than 50 individuals these were counted and assigned the average length per taxa for the sample. Zooplankton biomass was then calculated for each taxon, using published weight-length regressions (Bottrell, 1976).

5.3.4. Primary producers

Phytoplankton biomass, as chlorophyll-*a*, was measured by vacuum filtering 1 L of water through a 0.7 mm glass fibre filter (Whatman GF/F). Filters were immediately frozen and analysed for chlorophyll *a* no longer than one month after collection by extraction in 10 mL of 90 % acetone for 24 hours at 4°C. Chlorophyll-*a* concentration was measured fluorometrically for time points T0 to T2 on an AquaFlor Trilogy Laboratory Fluorometer (TurnerDesigns, San Jose, CA, U.S.A.). We used a spectrophotometer for time points T3 to T5 on a Cintra 2020 UV-Vis spectrophotometer (GBC Science, Hampshire, IL, U.S.A.) (APHA, 2000) due to a logistical issue. The fluorometer was calibrated against the spectrophotometer to give equivalent chlorophyll *a* abundance readings. Periphyton was sampled in mesocosms on the final day of the experiment (32) by scraping a 4.5 × 10 cm area on the north-west facing side of each tank using a razor blade. Samples were placed in vials and immediately frozen until analysis for chlorophyll-*a* using spectrophotometry as above.

5.3.5. Ecosystem metabolism

Ecosystem metabolism, including net primary production (NPP), ecosystem respiration (ER), gross primary production (GPP), was measured after 7, 15, 21, and 31 days immediately prior to each sampling event using diurnal oxygen changes (Harmon et al., 2009). Dissolved oxygen was measured using a handheld oxygen probe (YSI ProODO) at dawn (M1), dusk (M2), and the following dawn (M3). Ecosystem respiration was estimated as M2-M3, NPP was estimated as M2-M1, and GPP was estimated as NPP+ER.

5.3.6. Greenhouse gas flux

Greenhouse gas flux was measured after 23 days at 11 AM and at 4 AM the subsequent day, between the diurnal dissolved oxygen measures. Gas flux was measured using floating 4 L open bottom polyethylene chambers (Cole, Bade, Bastviken, Pace, & Van de Bogert, 2010; Matthews, St.Louis, & Hesslein, 2003). We sampled blocks sequentially with 5 chambers over a two-hour period. The chambers were placed onto each tank for 15 minutes, a time period which yielded linear changes in gas concentrations in preliminary experiments. Atmospheric samples were collected with each block to determine starting gas concentrations. Headspace samples were collected through a valve on top on each chamber using 200 mL syringes and gas samples were stored in Tedlar bags. Gas samples were analysed the same day using a cavity ring down spectrometer which measured CO_2, N_2O, CH_4, and NH_3 gasses in ppm which were corrected for air moisture content (G2508, Picarro Inc., Santa Clara, California, USA). Emission rates (mg $min^{-1}m^{-2}$) were calculated as the difference in concentration between the background (ambient air) and treatment air concentrations.

5.3.7. Nutrients and physiochemical measurements

Inorganic nutrients were measured from a 50 mL sample of water filtered through a 0.7 mm glass fibre filter (Whatman GF/F). Samples were frozen until analysis using a Lachat QuikChem 8500 Flow Injection Analyser (Lachat Instruments, Loveland, CO, U.S.A.) for nitrate and nitrite (NO_X), soluble reactive phosphorus (SRP), and ammonium (NH_4^+).

Temperature was logged (HOBO Pendant, Onset, Bourne, MA) continuously in 12 of the mesocosms, with at least one logger in every row and one on every edge. Conductivity and pH were measured after 16 and 32 days using a handheld meter (YSI Professional Plus).

5.3.8. Statistical analyses
Statistical analyses

We were particularly interested in detecting body size × population interactions that signalled shifts in the ecological role of body size. For our individual trait and community and ecosystem level data we used a linear mixed effect model (LMM) with body size, source-population, and their interaction as terms and mesocosm rows as blocks. Models were constructed as *response ~ body size*population* + (1|block)* in R (v.3.3.3; R Development Core Team, 2017) using the Package 'lme4' (v. 1. 1.17; Bates, Mächler, Bolker, & Walker, 2015). P values were calculated using the 'ImerTest' package in R via Satterthwaite's degrees of freedom method (v.3.0.0; Kuznetsova, Brockhoff, & Christensen, 2017). Data were $\log_{10}$ transformed prior to analyses if they did not meet the model assumptions (see Table S4.2). We show data for controls (no fish) to illustrate effect direction and size of each of the fish treatments but do not address them statistically. We chose to focus on community and ecosystem effects at T4 (24 days), which allowed time for effects in our experimental systems to emerge for all variables. Data for other time-points are plotted as time-series (Fig. S4.1 & S4.2). Notably, there

was a rain storm to between T2 and T3 sampling which diluted our mesocosms and reduced zooplankton biomass.

One-way ANOVA was used to test for a temperature difference from the temperature logger data. Linear regression models were used to determine the relationship between ER or GPP and CO_2 flux. All analyses were conducted using R version 3.5.0 and all plots were created using 'ggplot2' (v. 3.0.0) (R Development Core Team, 2017). We interpret effects to be significant where $p < 0.05$, and marginal where $p < 0.10$.

5.4. Results

5.4.1. Consumers

Fish metabolic, excretion, and growth rates were higher for small individuals, but differences between populations were not consistent across these parameters (Fig. 5.1; Table 5.1). We found no interaction between population and body size for metabolic rate ($p = 0.714$), nutrient excretion rate ($p = 0.705$) or growth rate ($p = 0.5633$) of fish. There was an effect of body size ($t_{23} = 2.61$, $p = 0.016$) and a marginal effect of population ($t_{23} = -1.85$, $p = 0.077$) on metabolic rate. Metabolic rates were 36-44 % higher for small individuals compared to large individuals and 34-41 % higher in warm source fish compared to ambient source fish (Fig. 5.3a). Nitrogen excretion rate was influenced by body size ($t_{24} = 2.53$, $p = 0.019$) and differed between populations ($t_{23} = 2.53$, $p = 0.018$). Excretion rates were 15-26 % higher for small individuals and 16-27 % higher for ambient population fish than the warm population fish (Fig. 5.3b). Growth rate was 15-40 % lower at larger body size ($t_{31} = 2.33$, $p = 0.027$) and 46-70 % higher in warm population fish ($t_{31} = 35.845$, $p < 0.001$).

Chironomids were least abundant in the small fish treatments and predatory invertebrates were reduced across all fish treatments (Table 5.1). There was no interactive ($p = 0.778$) or population ($p = 0.995$) effect on chironomid abundance, but there was an

effect of body size (t_{20} = -2.363, p = 0.0284, Fig. S4.3a). In particular, chironomid numbers were 40 % greater in the large body size treatments than the small size treatments. Other invertebrate taxonomic groups showed no significant individual or interactive treatment effects (p > 0.05). Predatory invertebrates (Dytiscid beetles/ larvae and Notonectidae) were 14-times more common in the no fish control mesocosms than in mesocosms containing fish (Fig. S4.3b).

The effect of body size on zooplankton biomass depended on source population (Fig. 5.2a). We found a marginal interactive effect, a body size main effect, but no effect of population (Table 5.1). In ambient population treatments zooplankton biomass was similar to the control tanks for large individuals but was enhanced for small individuals. In warm population treatments, zooplankton remained similar to the controls for small and large individuals. By T4 *Daphnia* were absent from most of our treatments and rotifer abundance increased, particularly in our small treatments (Fig. S4.4).

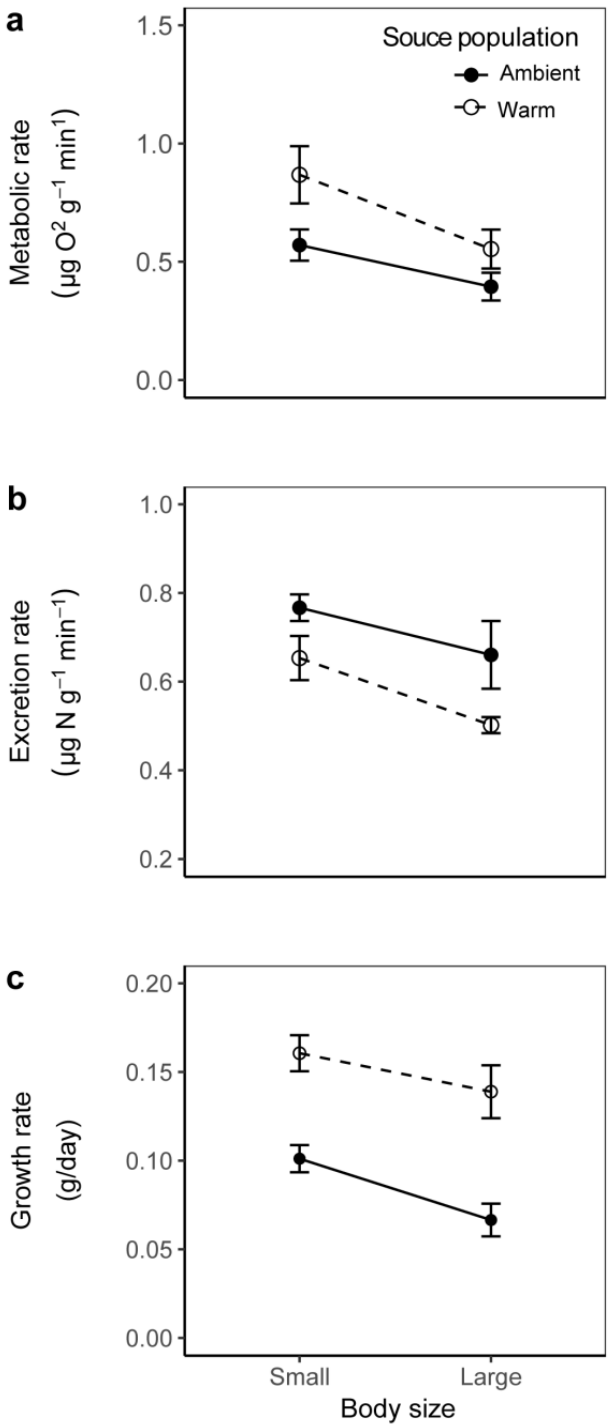


Fig 5.1. Metabolic (a), excretion (b), and growth (c) rate interaction plots. Data are split by body size and population and are averages ± 1 SE. Growth was measured as the difference in body mass over the duration of our experiment.

Table 5.1 Summary of experimental response variables, negative (-) and positive (+) responses are denoted for both size and population effects. Negative responses indicate a lower response for large body size or warm population, positive responses indicate the opposite. An asterisk (*) indicates a significant response (at $\alpha = 0.05$), where directionality depends on body size and source population. Where interaction effects are present these are denoted with a tick. Non-significant effects are denoted by ns.

Response	Body size *Small~Large*	Source population *Ambient~Warm*	Interaction *Population*Size*
Fish growth rate	-	+	ns
Fish metabolic rate	-	*+marginal*	ns
Fish excretion rate	-	-	ns
Chironomid abundance	-	ns	ns
Zooplankton biomass	+	ns	*marginal*
Phytoplankton	*	*	✓
Periphyton	-	ns	ns
GPP	ns	ns	ns
CO_2- day	*	**marginal*	✓
CO_2- night	*-marginal*	ns	ns
N_2O- day	*	*	✓
N_2O- night	ns	ns	ns
Conductivity	ns	*	✓

5.4.2. Primary producers

Periphyton was highest in small body size treatments for both populations, whereas, the effect of body size on phytoplankton biomass was dependant on source population (Fig. 5.2b). We found no interactive ($p = 0.341$) or population effect ($p = 0.120$) on periphyton biomass, but there was a body size effect ($t_{26} = 4.18$, $p < 0.005$). At large body size fish had no effect on periphyton (values similar to controls) whereas at

small body size periphyton concentrations were 32 % higher (Fig. S4.5). We found an interactive (t_{23} = 2.59, p = 0.018), body size (t_{23} = -2.13, p = 0.046), and population (t_{23} = -3.10, p = 0.006) effect of body size and population on phytoplankton biomass. Fish enhanced phytoplankton biomass in all cases, but in ambient population treatments the effect was stronger for large fish than small fish. The pattern was reversed in warm population treatments where small fish caused the largest increase in phytoplankton.

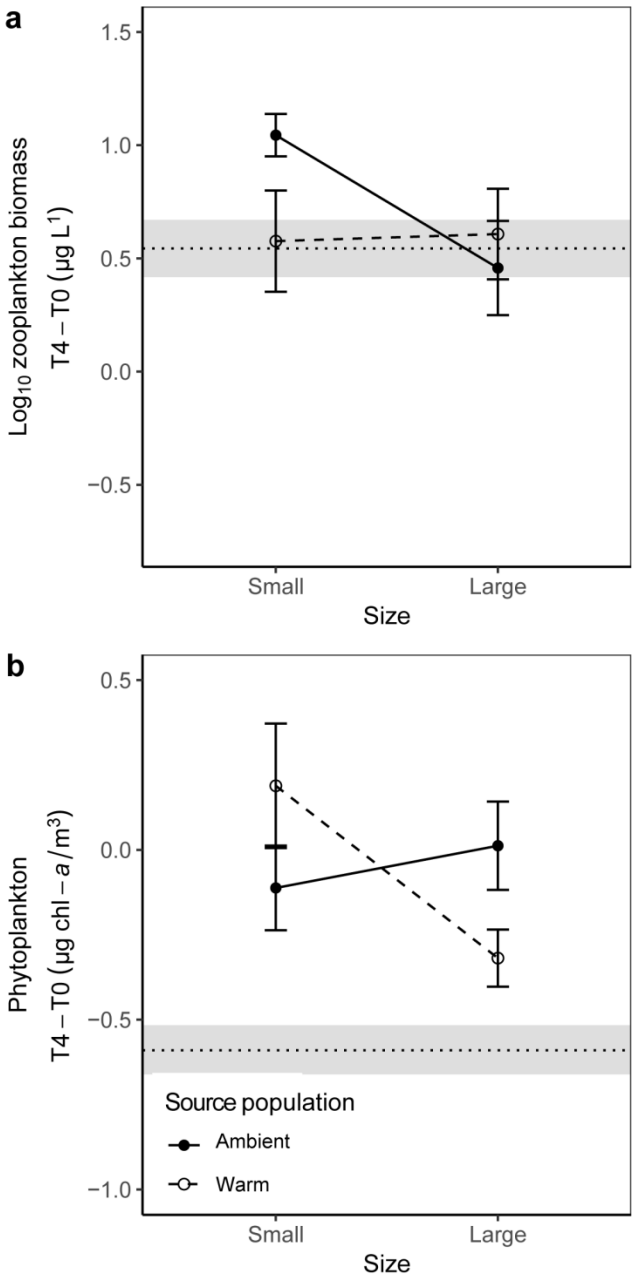


Figure 5.2. Relationships between body size and population for (a) zooplankton biomass as T4-T0, and (b) phytoplankton as T4-T0 at T4. Data are averages ± 1 SE. Dotted lines and shaded zones represent control tank average ± SE values respectively. *N* = 8 for each treatment.

5.4.3. Ecosystem metabolism and greenhouse gas flux

We found no treatment effects on EPP, NPP, or ER ($p > 0.05$, Table S4.2). Overall, NPP and GPP increased from T1 to T5; however, there was a decrease at T3 following rainfall (Fig. S4.6). ER decreased until T3 before increasing, following the same overall trend as NPP and GPP.

During the day the greenhouse gasses, CO_2 and NH_3 was absorbed by and N_2O and CH_4 was emitted by mesocosms (Table S4.3). The effect of body size on day-time CO_2 and N_2O flux rates depended on source population, but the direction of these relationships was reversed between variables. For both CO_2 and N_2O the difference between populations was most pronounced at large body size. We found significant interactive (t_{22}=3.82, p = 0.001), population (t_{22} = -3.12, p = 0.005), and body size (t_{22} = -2.57, p = 0.18) effects on CO_2 flux rates. In ambient population treatments, CO_2 uptake was enhanced relative to controls under small body size while the pattern was reversed in the warm population treatment (Fig .5.3a). The pattern for N_2O was the reverse of the CO_2 pattern, with significant interactive (t_{29} = -2.45, p = 0.021), population (t_{29} = 2.44, p = 0.021), and body size (t_{29} = 2.32, p = 0.027) effects on N_2O flux rates (Fig. 5.3b). Flux of CO_2 was weakly related to a decrease in ER ($F_{1,38}$ = 9.495, r^2 = 0.179, p = 0.004) and NPP ($F_{1,38}$ = 4.714, r^2 = 0.140, p = 0.010; Fig. S4.7). We found no interaction (p = 0.643) or population (p = 0.771) effect on day-time CH_4, but there was a marginal effect on body size (t_{24} =1.912, p = 0.068). In both population treatments, CH_4 was suppressed relative to controls in the presence of large fish. We found no interaction (p = 0.169), population (p = 0.751), or body size (p = 0.529) effect on day-time NH_3.

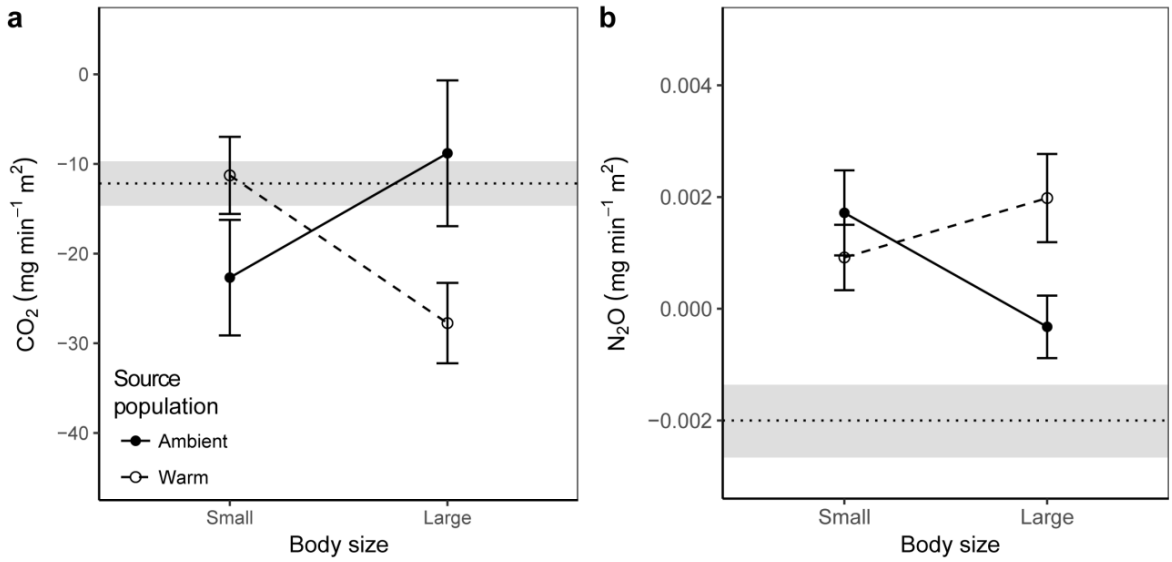


Figure 5.3. Daytime CO_2 (a) and N_2O (b) interaction plots from T4. Data are averages ± 1 SE. Dotted lines and shaded zones represent control tank average ± SE values respectively. *N* = 8 for each treatment.

During the night, CO_2 was released from mesocosms, but there were no interactive ($p = 0.683$) or main effects ($p = 0.311$ and $p = 0.152$ for body size and population, respectively). There was no interaction ($p = 0.921$) or population ($p = 0.942$) effect on night-time N_2O flux, but there was a body size ($t_{24} = 2.225$, $p = 0.036$) effect. In both populations, N_2O flux was enhanced relative to controls in the presence of small fish and suppressed relative to controls in the presence of large fish. We found no interaction ($p = 0.112$) or body size ($p = 0.618$) effect on NH_3 overnight, but there was a marginal effect of population ($t_{32} = -1.713$, $p = 0.096$). In our warm population NH_3 was supressed compared to controls, particularly for large individuals.

5.4.4. Water temperature and nutrients

Mean water temperatures across our mesocosms were similar with means ranging from 23.1 to 23.5°C. There were no treatment differences in temperature ($p > 0.05$, Table S4.5). There were no treatment effects on water column NH_4^+ ($p > 0.05$; Table S4.2) and

SRP and NO_X concentrations remained under detection limit (< 5µg/L) throughout our experiment.

5.5. Discussion

Body size and consumer thermal history are important moderators of ecological processes, so it is important to understand how each of these factors, and importantly, their interaction will alter ecosystems under warming (Brose et al., 2006; Gardner et al., 2011). In our experiment, consumer body size influenced a suite of ecological response variables, but the magnitude and directionality of the effect body size often depended on the population from which fish were derived. In other words, consumer thermal history influenced the ecological role of body size in our mesocosms. This divergence in ecological effects with body size highlights that consumer body size is an important moderator of ecological outcomes and suggests that consumer thermal history may moderate the outcome of environmental change. Our data add to the growing literature showing that rapid adaptation may lead to intraspecific divergence, which affects ecosystem function (Bassar et al., 2015; Palkovacs & Post, 2008; Post, Palkovacs, Schielke, & Dodson, 2008).

5.5.1 Individual response to body size change and consumer thermal history

Reduced body size led to an increase in metabolic, excretion, and growth rates, which followed theoretical expectations (Atkinson, 1994; Brown et al., 2004). However, there was no interaction between body size and population, indicating that body size change effects acted similarly between our populations on our individual-level traits. Between populations, the magnitude of individual level responses varied, warm-source fish had a higher metabolic demand, grew faster, but had lower excretion rates than our ambient population (Table 5.2). Together, the differences in individual traits between our populations suggests an increase in N allocated for growth, rather than being excreted as

waste, in our warm-source population (Liess, 2014; Vanni, 2002). Overall, these trait data suggest that a shift toward reduced body size without a change in total population biomass should lead to higher energy demand by consumers and, potentially, stronger top-down influence on prey.

5.5.2. Body size and consumer thermal history mediate fish effects

Difference in fish traits (growth, metabolism, and excretion) suggests a difference in the ecological effects of our two populations, but a similar role of body size. However, in our community response data the effect of body size was often dependant on source population. In our recent work with mosquitofish we demonstrated that warmer populations showed less change in metabolic rate with body size than did cool populations when measured *in-situ* (Moffett et al., 2018). The differences in these data may be due to change in metabolism over time, for example, thermal acclimation can alter resting metabolic rates (Norin & Gamperl, 2018; Norin et al., 2014). Here, fish were acclimated over a month to a novel temperature regime in which warm-source fish were cooled down and cool-source fish were close to their *in-situ* temperature. Overall, the divergence in trends between individual and community patterns in our data suggest that simply scaling up predictions from organismal traits to community changes, a common ecological goal, may be problematic (Lefevre, McKenzie, & Nilsson, 2017).

Zooplankton densities in our control treatments were equivalent to that in some of our fish treatments. While we expected zooplankton densities to be greater in the absence of fish and therefore predation there were large numbers of predatory invertebrates in our experimental tanks. Predatory invertebrates significantly reduce zooplankton abundance in ponds where fish are absent (Herwig & Schindler, 1996; Hurlbert & Mulla, 1981). In our experiment, Dytiscidae and Notonectidae, were 14-times more abundant in the control tanks compared to fish treatments. Invertebrates account for a large part of the

diet of mosquitofish, particularly in larger individuals (Lee et al., 2018); it is not therefore surprising that predatory invertebrates were suppressed in the fish treatments in our experiment.

High zooplankton biomass led to a decrease in primary production in our experiment, with the effect of body size again varying with population source. This top-down driven decrease in primary production follows the cascading trophic interactions hypothesis, in which phytoplankton are released from herbivorous plankton predation when fish exert strong top-down effects on plankton communities (Carpenter, Kitchell, & Hodgson, 1985; Carpenter et al., 1987; Jeppesen, Lauridsen, Mitchell, & Burns, 1997; Persson, 1999). Our data show that the presence of fish leads to an increase in primary production, even where total zooplankton biomass is not different between the fish and control treatments. This difference in primary productivity may be due to continued presence of efficient grazers, such as *Daphnia*, in the absence of fish. Alternatively, fish excretion may be contributing to the higher phytoplankton biomasses (Vanni & Layne, 1997); however, the patterns in our excretion and phytoplankton data are not consistent, suggesting that excretion was not strongly controlling primary production in our experiment. Overall, our data demonstrate that a decrease in fish body size plays an important role in moderating prey community abundance and primary productivity with consumer thermal history.

Like community effects, ecosystem processes were commonly responsive to interactions between body size and populations. CO_2 flux did not reflect patterns seen in our phytoplankton data, and, in fact, patterns were reversed (Fig. 5.3b & 5.4a). We expected that CO_2 and phytoplankton responses would show the same trends due the uptake of CO_2 by algae for the conversion to organic carbon, as part of the photosynthetic process (Demars et al., 2016). However, periphyton also contributed to whole tank

primary productivity, and when extrapolated to whole-mesocosm concentrations periphyton chlorophyll-*a* concentrations were, on average, 10× greater those of phytoplankton. Periphyton was measured at T5 when there was no interaction, which is consistent with our phytoplankton data (Fig. S4.5). Thus, although we cannot show that periphyton influenced CO_2 at earlier time points in our experiment, it may explain why primary production and CO_2 trends are not aligned.

Fish changed our experimental mesocosms from N_2O sinks to N_2O sources and the effect of body size differed between populations. N_2O flux is controlled by denitrification which is regulated by temperature, N and C availability, oxygen, and pH (Seitzinger, Kroeze, & Styles, 2000). The most likely mechanism by which fish may promote denitrification is by increasing the amount of organic carbon available for microbial transformation (Burgin & Hamilton, 2007). An increase in organic carbon may have occurred in our experiment due to the increased primary productivity in our fish treatments. Patterns in N_2O contrasted those in CO_2 such that N_2O emissions were high when mesocosms were stronger CO_2 sinks. It is unclear why trends in CO_2 and N_2O differed; however, N excretion was unlikely to have driven the N_2O pattern, even though N concentrations were low, because the pattern in N excretion did not match that of N_2O. Overall, these data suggest that consumers play an important role in promoting denitrification, through increasing organic matter deposition, where the magnitude of this effect is dependent on body size.

Climate change predictions are commonly made by acclimating animals from today's climate to warmer conditions. Here, fish from our warm population were cooled close to the temperature of our ambient population. Warm-source fish demonstrated not only differences in ecological effects, but also a complete reversal of the role of body size. Thus, our data show that consumer thermal history of two recently diverged

populations may moderate community and ecosystem effects of body size change. However, under warming all organisms will be subject to the physiological effects of temperature rise and ecosystem properties may be directly altered. For example, warmer temperatures may increase the activation energy of ecosystem respiration faster than the rate of primary production, leading to a reduction in carbon sequestration (Allen et al., 2005; Yvon-Durocher et al., 2010). If primary production is reduced with warming, fish predation may strengthen top-down controls, thus amplifying any effects of climate change on communities (Barton, Beckerman, & Schmitz, 2009; Kratina et al., 2012; O'Connor et al., 2009; O'Gorman et al., 2012; Schaum et al., 2018). However, our data and others (e.g. Padfield et al., 2017) suggest that consumer thermal history may compensate for some of the effects of warming.

Understanding how the ecological effects of temperature change are altered by intraspecific variation is important for models predicting future ecological outcomes (Lefevre et al., 2017). With temperatures rise, eco-evolutionary effects may exacerbate or buffer ecological interactions with climate change (Angilletta, 2009; Palkovacs et al., 2012; West & Post, 2016). Our data suggest that difference in consumer thermal history alongside body size change buffers the community and ecosystem effects. Given predictions of body size reduction with temperature rise such moderation of community and ecosystem effects may act as an important tool to buffer ecosystems from rapid change where organisms can adapt in contemporary time.

Chapter 6

Synthesis

Environmental warming is causing many ecological changes, from alterations to key life history traits to ecosystem functioning. Temperature rise alters body size, growth rates, life history events, and may limit biomass at the base of food webs (Gardner et al., 2011; Hickling et al., 2006; Padfield et al., 2017). In addition, temperature rise is theorised to impose a metabolic cost, which may, in turn, strengthen the top-down effects of consumer species (Brown et al., 2004). However, the role of thermal history in moderating or exacerbating the effects of temperature rise remains poorly understood, particularly where evolutionary change occurs over time-scales concordant with temperature rise. In this thesis, I sought to understand how thermal history in a widespread and dominant consumer species, mosquitofish (*Gambusia affinis*), influences a series of traits and how these trait changes influence ecosystems. Mosquitofish made an ideal model system for this work due to their global spread and occurrence in geothermal systems spanning a gradient of temperatures. As such, mosquitofish are now being used a model system to understand the future effects of warming (e.g. Fryxell & Palkovacs, 2017).

In Chapters 2, 3, and 4 I used a comparative approach to understand how traits are altered with consumer thermal history. My work aimed to understand the effect of temperature on body size and metabolic rate (Chapter 2), on metabolic plasticity and behavioural variation (Chapter 3), and on diet, gut morphology, and elemental composition (Chapter 4). In Chapters 2 and 4 my sampling spanned populations in both New Zealand (NZ) and California (CA), allowing me to determine if responses to temperature were consistent across regions. In Chapter 2 my data show a similarity in

metabolic and body size responses to temperature in NZ and CA, whereby local adaptation (or thermal history) reduced the metabolic cost of warming. In Chapter 4, I show that a shift in consumer diet leads to consistent gut morphological and body elemental composition trends in NZ and CA. The consistency of these metabolic, morphological, and stoichiometric trends between geographically diverse regions provides evidence that such responses may be widespread. Finally, In Chapter 3, I used NZ mosquitofish populations with varying thermal histories to understand if population metabolic responses and behaviours differed. These data reveal a difference in the temperature sensitivity of metabolism across populations, suggesting greater plasticity in individuals from warm-source populations. Together, these data reveal a divergence in consumer traits in with thermal history.

In my thesis metabolic rate was measured on mosquitofish in three chapters. In Chapter 2, metabolic was measured *in-situ* as field metabolic rate (FMR), in Chapter 4 it was measured using standard physiological protocols in a laboratory setting at two temperatures. In Chapter 5 metabolic rate was measured as a FMR; however, fish from both populations were bought to a common ambient temperature, rather than being run at their population temperature (e.g. Chapter 2). In each of these Chapters different aspects of mosquitofish metabolism were determined. For example, FMR in Chapter 2 provided an ecologically relevant measurement of metabolism, where metabolic rates incorporated a population's thermal history, food resources, and behavioural variation. Whereas, the laboratory-based data in Chapter 3 provided a fundamental physiological measure of mosquitofish metabolic rates.

Using a combination of field and laboratory-based approaches to measuring metabolic rates provided me with a unique understanding how thermal adaptation alters the physiology of mosquitofish and how this plays out in different contexts. My data

show that not only are mosquitofish altering their metabolic rates to minimise energetic costs, they also show an increase in the plasticity of temperature sensitivity in warm-adapted populations which may also lower metabolic costs under warming. Both findings are novel and challenge central assumptions from metabolic theory that metabolic rates will increase predictably with both body size (allometric scaling) and temperature rise (temperature sensitivity of metabolism).

Finally, to understand the ecological effects of trait change in populations with different thermal histories I used a mesocosm experiment (Chapter 5). In this experiment I used individuals with different body sizes and from different populations to understand how thermal history and body size change influences ecological effects. My mesocosm measurements uniquely span individual traits (metabolism) to functional metrics (greenhouse gasses), where mesocosm experiments typically focus on change in prey communities and primary production measures (e.g. Des Roches et al., 2013). In this experiment the effects of intraspecific variation were considerable between populations. For example, small body size reduced phytoplankton abundance in our ambient population but increased it in our warm population. Interestingly, these data reveal that patterns in community data and greenhouse gas emissions between populations were often in conflict with one another, suggesting that future predictions need to take local adaption into consideration as this may significantly alter community and ecological patterns.

Data from my mesocosm experiment showed a lack of connection between traits (metabolic, excretion, growth rate) and ecological effects. This difference between traits and ecological effects is significant as it may indicate that metabolic traits cannot simply be up-scaled to provide broader ecological predictions. However, the interpretation of these data may be limited because metabolic rate was measured at the end of the

experiment. Metabolic rate measurements may, therefore, not reflect ecological changes that occurred during the experiment. Alternatively, the lack of connection between traits and ecological effects may suggest that all relevant traits were not measured. To address these issues, future studies may consider measuring metabolic rates immediately before experiments begin or acclimating fish to ambient temperatures prior to the beginning of an experiment, in addition to measuring a broader suite of traits. Using such methods may help to link metabolic demand to ecological effects.

My data show that consumer thermal history plays in an important role in trait divergence among populations of mosquitofish. Our data suggest under warming populations have the potential to substantially increase the scope for metabolic efficiency, increase their metabolic plasticity, lead to an alteration in diet which may moderate their gut morphology and body nutrients to maximise energetic efficiencies, and ultimately may moderate the top-down effects of consumers. While my research provides important and novel insights into the role of consumer thermal history on phenotypic divergence and ecological effects, future research should seek to understand how local adaptation influences prey communities, and how such adaptation may alter interactions between consumers and prey with warming. Further, understanding the ecological effects of consumer thermal history under warmed conditions will allow us to understand if consumer thermal history moderates or exacerbates the top-down effect of consumers.

In summary, my thesis demonstrates that temperature change can have significant effects on both trait variation and ecosystems. This outcome adds to a growing volume of literature emphasising the importance of intraspecific variation (Bailey et al., 2009; Des Roches et al., 2018; Harmon et al., 2009; Palkovacs, Mandeville, & Post, 2014). My research suggests that consumer thermal history, which is frequently not considered, should be in an important consideration in predictions of biological response to future

climate change. Failure to account for such changes with consumer thermal history may substantially impair accurate predictions of future biodiversity responses to global change.

Appendices

Appendix S1
Chapter 2: Local adaptation reduces the metabolic cost of environmental warming

Methods:

Temperature dependency in laboratory acclimated fish

Routine metabolic rate (RMR) was measured on fish from two geothermal populations in New Zealand. These populations were Awakeri Spring (35°C) and Akatarewa Spring (37.4°C). Fish were captured in January 2016 and bought back to the laboratory where they were held at their collection temperature for one week, before we adjusted the temperature to either 20 or 30°C over 1-2 weeks. Fish were then acclimated at their holding temperature for two months (Clark et al., 2013; Seebacher et al., 2014). Fish were fasted for 24 hours before individual RMR was recorded. RMR was measured on at least six males and six females from each population. Individuals were placed into one of four 40 mL acrylic respirometers with oxygen and temperature loggers and magnetic stir bars in the base of the repiometers to gently circulate the water. 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, Germany). Fish were left for 20 minutes, or until a sufficient decline in oxygen consumption was measured. Microbial oxygen consumption was controlled for by subtracting the oxygen consumption in blanks (respirometers with water only) which were run daily.

Table S1.1. Physiochemical characteristics of populations in California (CA) and New Zealand (NZ), data are averages with standard deviations. Populations are split by region then temperature. Temperature data are the temperature that field metabolism and excretion rate were run at *in-situ* at each site. Average temperature data are from temperature loggers in all populations with the exception of Waikato River Spring and Wairua Spring, at these sites seasonal spot measurements were taken.

Region	Population	Location	Dispersal barrier present	Temperature (°C)	Average annual temperature ±SD	DO (mg/L)	Specific Conductance (mS/cm)	pH
CA	Northeast Spring	37°31'04.8"N 118°24'00.5"W	Y	19.2	18.8(0.8)	8.26	0.36	8.3
CA	BLM Spring	37°28'49.39"N 118°24'10.30"W	Y	22.4	21.0(1.1)	6.98	0.47	8.2
CA	Artesian Well	37°21'02.18"N 118°19'35.43"W	Y	23.7	23.6(0.6)	4.49	0.45	7.4
CA	Warm Springs	37°16'00.38"N 118°16'20.19"W	Y	27.6	26.9(1.2)	7.91	0.51	7.8
CA	Keough Hot Ditch	37°15'33.71"N 118°22'18.53"W	Y	29.9	31.6(2.1)	5.31	0.86	8.4
NZ	Waikato River Spring	38°37'45.5"S 176°06'11.0"E	N	24.8	21.3(4.2)	7.96	0.22	7.7
NZ	Miranda Springs	37°12'26.0"S 175°19'54.0"E	N	29.6	32.7(2.5)	4.39	0.73	8.8
NZ	Akatarewa Spring	38°27'50.1"S 176°08'58.0"E	Y	34.9	30.7(5.4)	6.08	0.51	7.8
NZ	Awakeri Spring	38°00'19.0"S 176°51'38.5"E	N	35.5	36.6(2.0)	4.86	0.46	7.8
NZ	Wairua Spring	38°14'22.3"S 176°25'28.3"E	N	37.1	36.0(1.6)	7.76	1.18	8.0

Table S1.2. Summary of *Gambusia affinis* population length, weight, and gender data across our populations in California (CA) and New Zealand (NZ), data are averages with standard deviations in parentheses. Populations are split by region.

Region	Population	M:F	Male length (mm)	Female length (mm)	Male mass (mg)	Female mass (mg)
CA	Northeast Spring	0.31:1	24.7(2.2)	23.7(6.5)	33.1(9.6)	38.5(44.6)
CA	BLM Spring	0.79:1	27.6(2.3)	29.0(8.2)	57.8(24.8)	100.0(101.6)
CA	Artesian Well	0.20:1	28.0(1.6)	35.1(5.4)	111.7(56.9)	40.6(11.5)
CA	Warm Springs	0.18:1	24.0(1.6)	25.7(5.1)	17.3(4.4)	39.8(22.6)
CA	Keough Hot Ditch	0.09:1	24.5(1.6)	33.0(4.4)	25.2(6.3)	95.1(35.1)
NZ	Waikato River Spring	0.89:1	21.8(2.9)	24.2(6.1)	23.6(11.4)	44.3(45.6
NZ	Miranda Springs	0.36:1	22.2(2.8)	23.4(5.8)	21.5(9.1)	36.7(36.1)
NZ	Akatarewa Spring	0.15:1	20.8(2.9)	18.0(5.5)	19.7(7.8)	22.6(65.7)
NZ	Awakeri Spring	0.60:1	21.2(3.2)	20.3(5.8)	19.8(8.5)	24.3(26.5)
NZ	Wairua Spring	0.31:1	21.3(3.1)	16.7(4.1)	18.6(8.3)	11.0(28.6)

Table S1.3. Quantile regression statistics for dry weight versus site temperature across all populations in California and New Zealand, data were split by gender for analysis (see Figure 2.2).

Sex	Quantile	Intercept	Slope	*P*-value
Male	0.9	0.11869	-0.00252	<0.0001
	0.8	0.08777	-0.00179	<0.0001
	0.7	0.07464	-0.00152	<0.0001
	0.6	0.06487	-0.00129	<0.0001
	0.5	0.0573	-0.00113	<0.0001
	0.4	0.04742	-0.0009	<0.0001
	0.3	0.04235	-0.0008	<0.0001
	0.2	0.03305	-0.00058	<0.0001
	0.1	0.0268	-0.00046	<0.0001
Female	0.9	0.40036	-0.00985	<0.0001
	0.8	0.30583	-0.00761	<0.0001
	0.7	0.21898	-0.0055	<0.0001
	0.6	0.16462	-0.00416	<0.0001
	0.5	0.12486	-0.00315	<0.0001
	0.4	0.08574	-0.00212	<0.0001
	0.3	0.05567	-0.00134	<0.0001
	0.2	0.03476	-0.0079	<0.0001
	0.1	0.02049	-0.00043	<0.0001

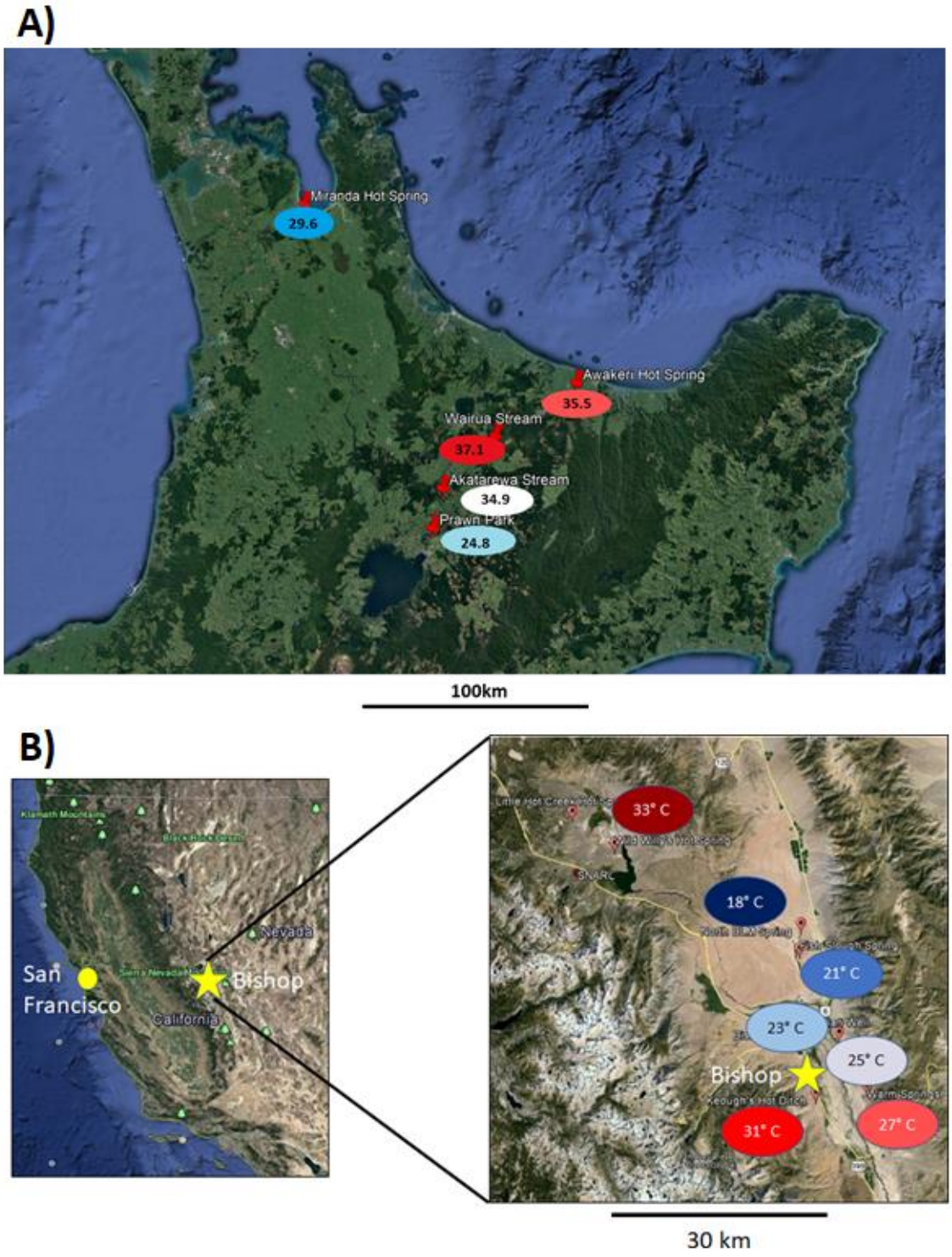


Figure S1.1. Map of field sites in A) New Zealand and B) California. Sites in each country are coloured from coolest (blue) to warmest (red) (Map credit: Google Earth, 2019).

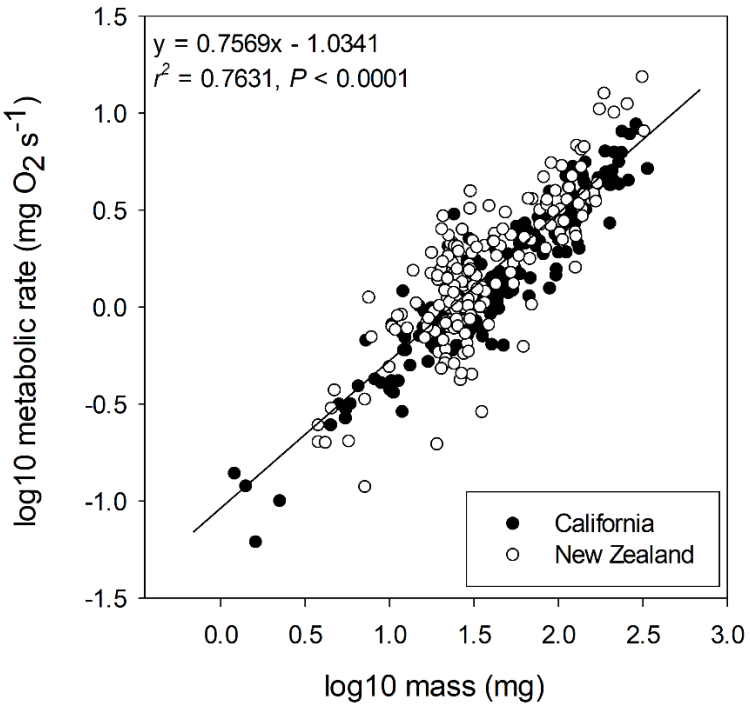


Figure S1.2. Relationship between metabolic rate and mass for all individuals in California and New Zealand, all data were log10 transformed (n = 386).

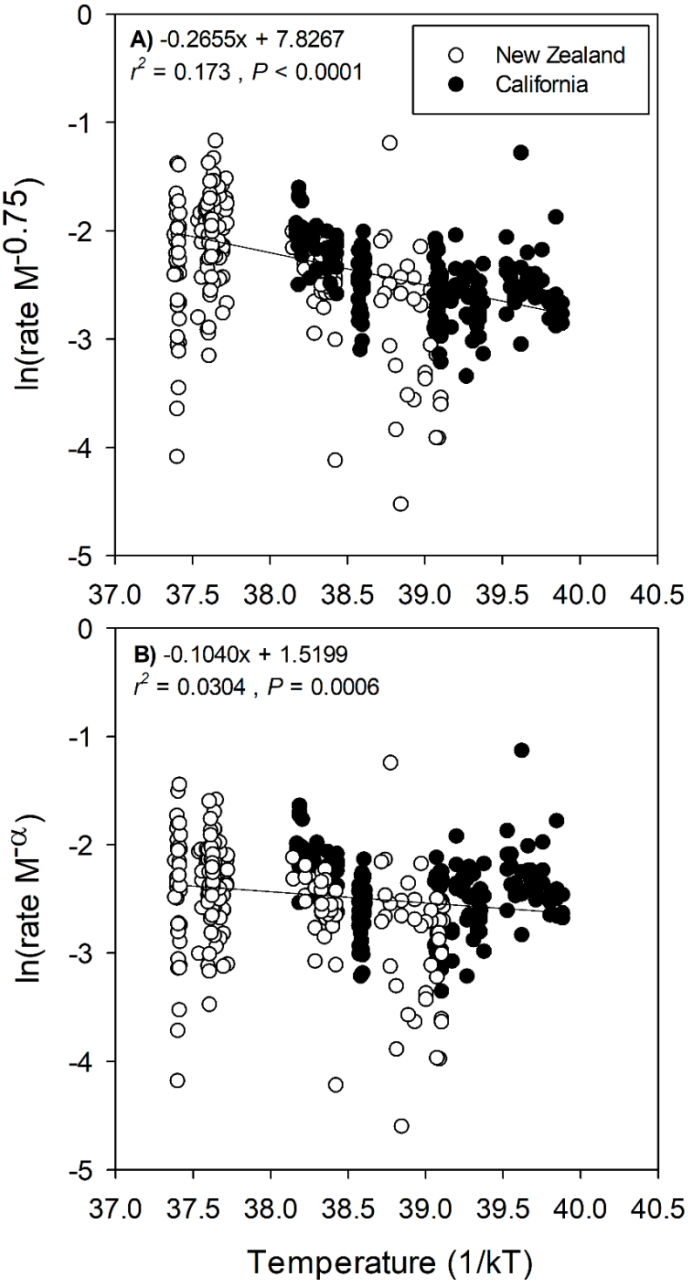


Figure S1.3. Arrhenius plots showing A) the scaling relationships between mass corrected metabolic rate and the inverse of temperature (n = 386), and B) the scaling relationships between mass corrected metabolic rate, using population level slope values and the inverse of temperature (n = 386). Data are plotted for all individuals from California and New Zealand. T is Temperature in kelvin and k is the Boltzmann constant (8.62×10^{-5} eV K^{-1}).

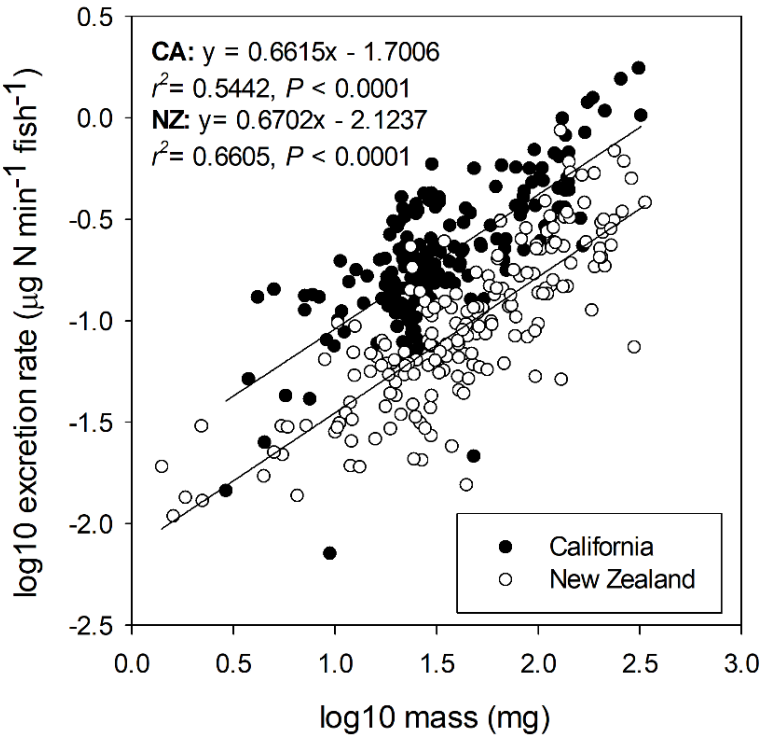


Figure S1.4. Relationship between body mass and excretion rate for all individuals in California and New Zealand. Linear regression slopes are shown for individuals in California and New Zealand separately (n = 381).

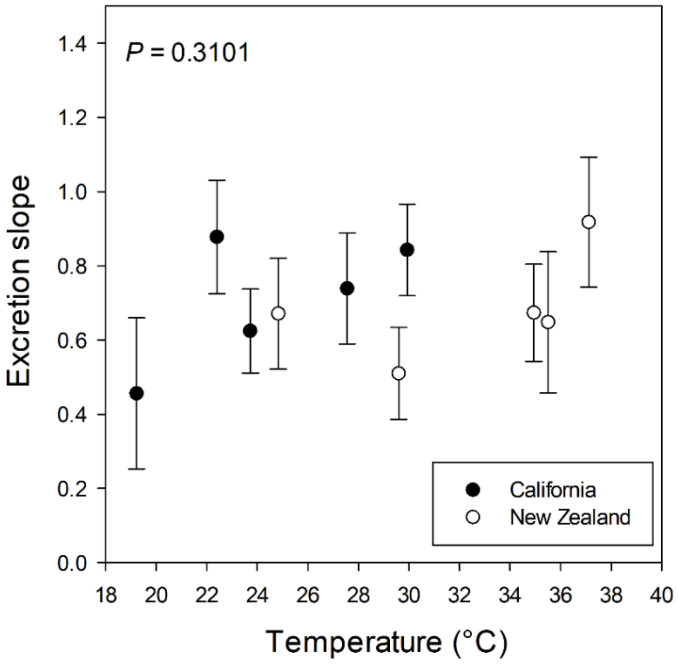


Figure S1.5. Relationship between excretion slope and site temperature for all populations individuals in California and New Zealand, error bars are mean absolute error (MAE) (*n* = 10).

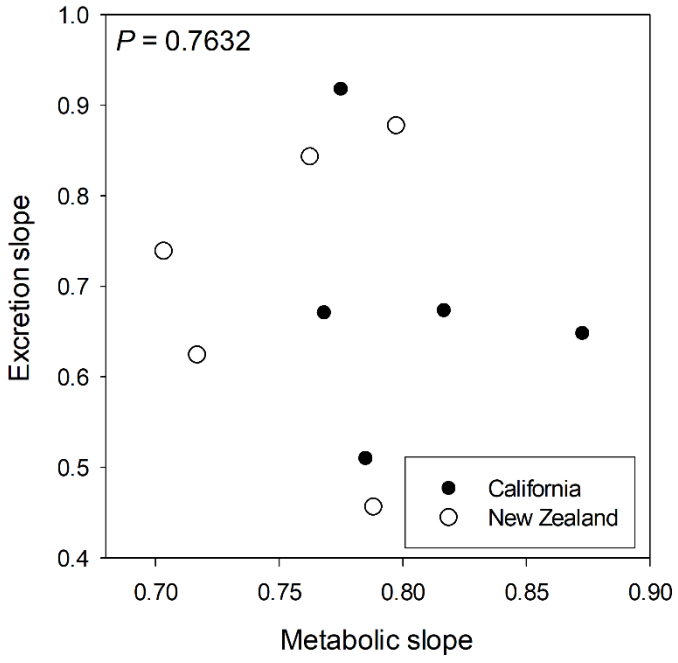


Figure S1.6. Relationship between excretion slope and metabolic slope for all populations in California and New Zealand (*n* = 10).

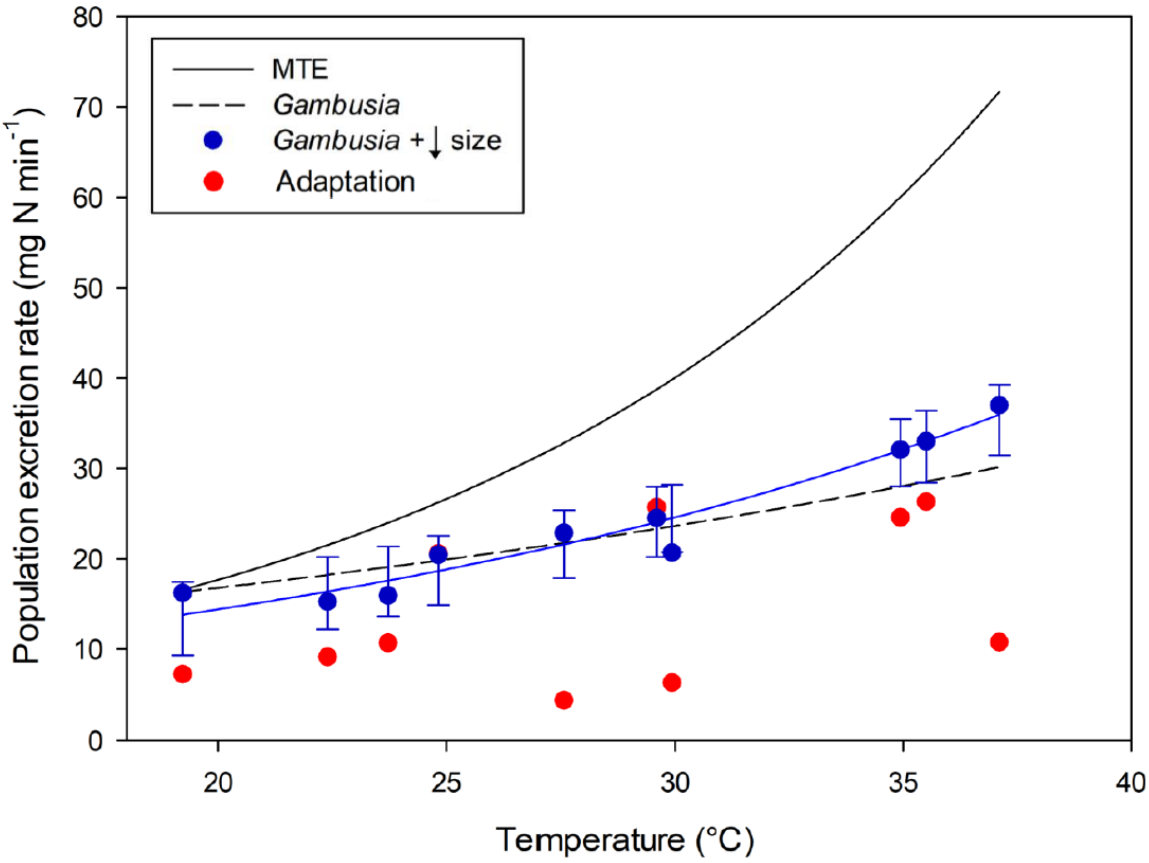


Figure S1.7. Predicted change in mosquitofish population-level excretion rate with rising temperature derived from 3 scenarios: 1) MTE with no body size change with rising temperature (MTE, solid black line), 2) MTE with our observed E for *Gambusia* (E = 0.27eV) (*Gambusia*, dashed line), 3) MTE with the observed E for *Gambusia* and changes in body size distributions across out mosquitofish populations (*Gambusia* + ↓ size, blue solid line)**,** and 4) observed metabolic scaling relationships and change in body size distributions in mosquitofish populations (Adaptation, red data points) (see text and Fig. 2.1 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 (see text for statistical details).

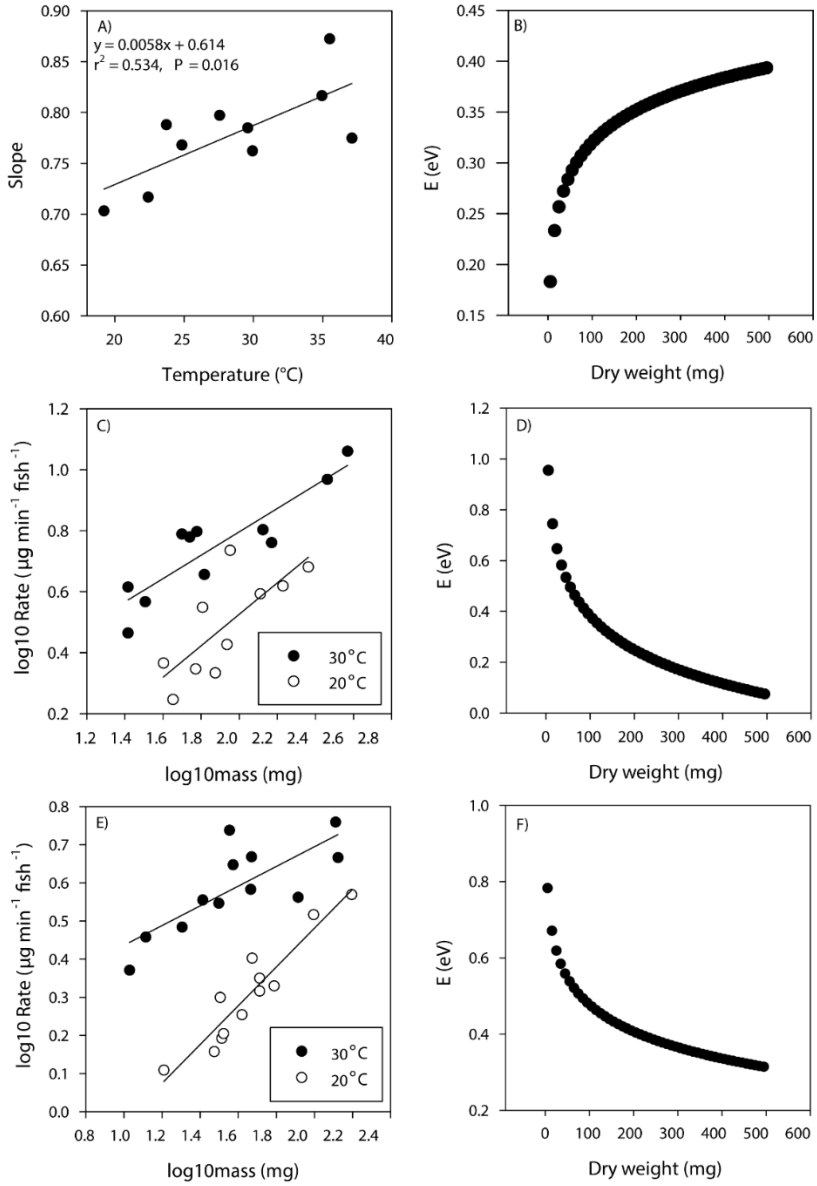


Figure S1.7. Relationship between temperature and the allometric scaling coefficients for metabolic rate (α) for our field metabolic data across all sites (A); and routine metabolic rate data from laboratory acclimated fish showing the relationship between mass and metabolic rate at two temperatures (20°C or 30°C), data are fitted with simple linear regression models. These sites were Awakeri Spring (C; 30°C: r^2 = 0.6093, P = 0.0027, y = 0.2583x +0.1782; 20°C: r^2 = 0.8840, P < 0.0001, y = 0.5085x – 0.5358) and Akatarewa Spring (E; 30°C: r^2 = 0.7962, P = 0.0002, y = 0.3832x + 0.030; 20°C: r^2 = 0.5704, P = 0.0115, y = 0.5164x + 0.5071). The relationship between activation energy (E) and dry weight are shown for our field data (B), laboratory data from Awakeri spring (D), and laboratory data from Akatarewa Spring (F). These data are based off population metabolic rates of individuals between 5 and 500mg at 5mg increments. Activation energy (E) for each body size was determined from an Arrhenius relationship between metabolic rate and temperature across populations.

Appendix S2
Chapter 3: Thermal history alters temperature sensitivity of metabolism and behaviour of an invasive consumer, *Gambusia affinis*

Table S2.1. Descriptions of site characteristics from with fish were collected. Temperature, conductivity, pH, and dissolved oxygen (DO) measurements were taken at the time of fish collection. Annual average variation in temperature measurements were taken as bi-monthly spot measurements over a year.

Site	Geothermal influence	Fish collection Temperature (°C)	Annual temperature bounds (°C)	Conductivity (µS/cm)	pH	DO (mg/L)
Waikato River Tributary	No	19.6	13 -20	140	7.03	6.05
Tahnua Torea Wetland	No	21.3	12 -23	275	7.55	6.91
Tourist Stream	No	21.8	13 -23	134	6.99	4.44
Auckland Domain	No	24.8	12- 25	307	7.64	4.61
Lake Ohakuri	Yes	30.0	26-30	337	7.51	7.23
Akatarewa Stream	Yes	32.1	29-35	483	7.88	4.97
Awakeri Spring	Yes	35.1	35-37	889	7.52	4.40
Miranda Hot Springs	Yes	36.0	32-37	632	7.43	9.88

Table S2.2. Summary of the number of individuals we used to measure SMR, RMR, MMR, behaviour, and excretion from each population and acclimation temperature. *N* = 201.

Site	Acclimation Temperature (°C)	Number of females	Number of males	Total number
Waikato River Tributary	20	7	5	12
	30	6	6	12
Tahnua Wetland	20	6	6	12
	30	6	6	12
Tourist Stream	20	6	6	12
	30	8	8	14
Auckland Domain	20	6	6	12
	30	6	6	12
Lake Ohakuri	20	6	7	13
	30	8	6	14
Akatarewa Stream	20	6	4	10
	30	6	5	11
Awakeri Spring	20	6	6	12
	30	6	6	12
Miranda Hot Springs	20	7	11	18
	30	6	7	13

Table S2.3. Scaling exponents (*b*) for standard metabolic rates (SMR), routine metabolic rates (RMR), and maximum metabolic rates (MMR) for *Gambusia affinis* from eight populations acclimated to either 20 or 30º C.

Site	Acclimation Temperature (ºC)	SMR *B*	RMR *b*	MMR *b*
Miranda Hot Springs	20	0.599	0.543	0.507
	30	0.554	0.614	0.575
Akatarewa Stream	20	0.597	0.516	0.387
	30	0.357	0.383	0.304
Awakeri Hot Spring	20	0.464	0.509	0.360
	30	0.338	0.258	0.236
Lake Ohakuri	20	0.433	0.492	0.347
	30	0.323	0.292	0.185
Auckland Domain	20	0.541	0.503	0.519
	30	0.219	0.267	0.240
Tourist Stream	20	0.548	0.652	0.420
	30	0.326	0.344	0.167
Waikato River Tributary	20	0.466	0.485	0.499
	30	0.261	0.300	0.188
Tahnua Wetland	20	0.449	0.542	0.381
	30	0.475	0.536	0.266
All sites	20	0.499	0.537	0.413
	30	0.339	0.371	0.179

Table S2.4. Summary of binomial logistic model coefficients for individual behaviour. The model describes which factors influenced the decision to leave a safe space. Separate models were run for SMR, RMR, and MMR. *N* = 198.

	Estimates	Std. Error	z value	Pr(>|z|)
Acclimation	-0.240	3.105	2.711	0.007 **
Source Temperature	0.133	0.049	2.908	0.004 **
Pregnant	-1.326	0.026	-2.428	0.015 *
Sex	-1.240	0.546	-1.514	0.130
Mass	0.004	0.819	0.910	0.363
SMR (fish)	-0.208	0.205	-1.016	0.310
Acclimation	0.107	0.051	2.077	0.038 *
Source Temperature	0.078	0.026	2.946	0.003 **
Pregnant	-1.262	0.541	-2.334	0.020 *
Sex	-1.172	0.814	-1.440	0.150
Mass	0.002	0.005	0.438	0.661
RMR (fish)	-0.052	0.183	-0.282	0.778
Acclimation	0.125	0.045	2.774	0.006 **
Source Temperature	0.077	0.026	2.914	0.004 **
Pregnant	-1.266	0.542	-2.336	0.020 *
Sex	-1.141	0.815	-1.400	0.162
Mass	0.003	0.004	0.824	0.410
MMR (fish)	-0.093	0.098	-0.956	0.339

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table S2.5. Analysis of deviance table for the behavioural binomial logistic model (Table S4). The model describes which factors influenced the decision to leave a safe space. *N* = 198.

	Df	Deviance Resid.	Df	Resid. Dev	Pr(>Chi)
NULL			197	269.92	
Acclimation	1	7.900	166	262.02	0.005 **
Source Temperature	1	6.923	195	255.10	0.008 **
Pregnant	1	10.102	194	245.00	0.001 **
Sex	1	1.942	193	243.06	0.163
Mass	1	0.114	192	242.94	0.735
SMR (fish)	1	1.036	191	241.91	0.309

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table S2.6. Generalized linear model (GLM) coefficients for exploratory behaviour using routine metabolic rate (RMR), *N* = 86.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2.0142	0.8633	2.333	0.022 *
Acclimation	0.0682	0.0120	5.671	<0.0001 ***
Source Temperature	-0.0129	0.0062	-2.0262	0.043 *
Sex	0.5422	0.2248	2.412	0.018 *
Pregnant	0.3350	0.1460	2.294	0.025 *
Mass	0.0005	0.0012	0.367	0.714
RMR (fish)	-0.0813	0.0138	-2.116	0.038 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table S2.7. Summary of quantile linear regression model data for the relationship between RMR and exploratory behaviour, *n* = 86.

Quantile	Intercept	Lower and upper bound	Slope	p-value
0.10	-6.657	-19.002 – 33.093	1.149	< 0.0001
0.20	33.658	-3.599 – 79.739	1.063	< 0.0001
0.30	93.741	28.165 – 155.336	0.789	< 0.0001
0.40	154.234	132.056 – 189.119	0.583	< 0.0001
0.50	201.495	150.327 – 231.848	0.369	<0.001
0.60	230.952	206.588 – 259.223	0.294	<0.001
0.70	256.933	225.493 – 276.133	0.186	<0.001
0.80	282.512	256.010 – 292.179	0.075	<0.01

Table S2.8. Generalized linear model (GLM) coefficients for exploratory behaviour using standard metabolic rate (SMR), *n* = 86.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2.050	0.884	2.318	0.023 *
Acclimation	0.060	0.011	5.238	<0.0001 ***
Source Temperature	-0.101	0.006	-1.734	0.087 .
Sex	0.555	0.231	2.409	0.018 *
Pregnant	0.318	0.152	2.084	0.040 .
Mass	-0.001	0.001	-0.556	0.580
SMR (fish)	-0.054	0.043	-1.250	0.215

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table S2.9. Generalized linear model (GLM) coefficients for exploratory behaviour using maximum metabo rate (MMR), *n* = 86.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2.002	0.865	2.313	0.023 *
Acclimation	0.061	0.011	5.826	<0.0001 ***
Source Temperature	-0.012	0.006	-1.981	0.0511 .
Sex	0.586	0.223	2.624	0.011 *
Pregnant	0.325	0.147	2.212	0.030 *
Mass	-0.000	0.001	-0.209	0.835
MMR	-0.040	0.022	-1.839	0.070 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

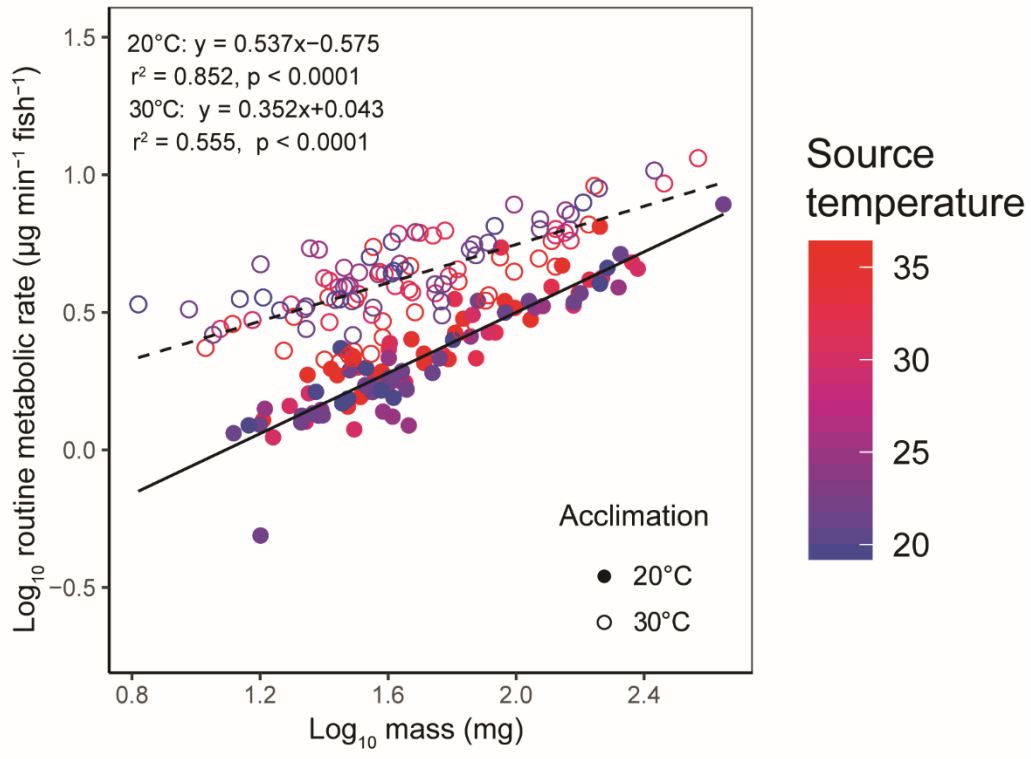


Figure S2.1. Relationship between fish mass and routine metabolic rate (RMR). Data points are individual fish and data are split by acclimation temperature. *N* = 201.

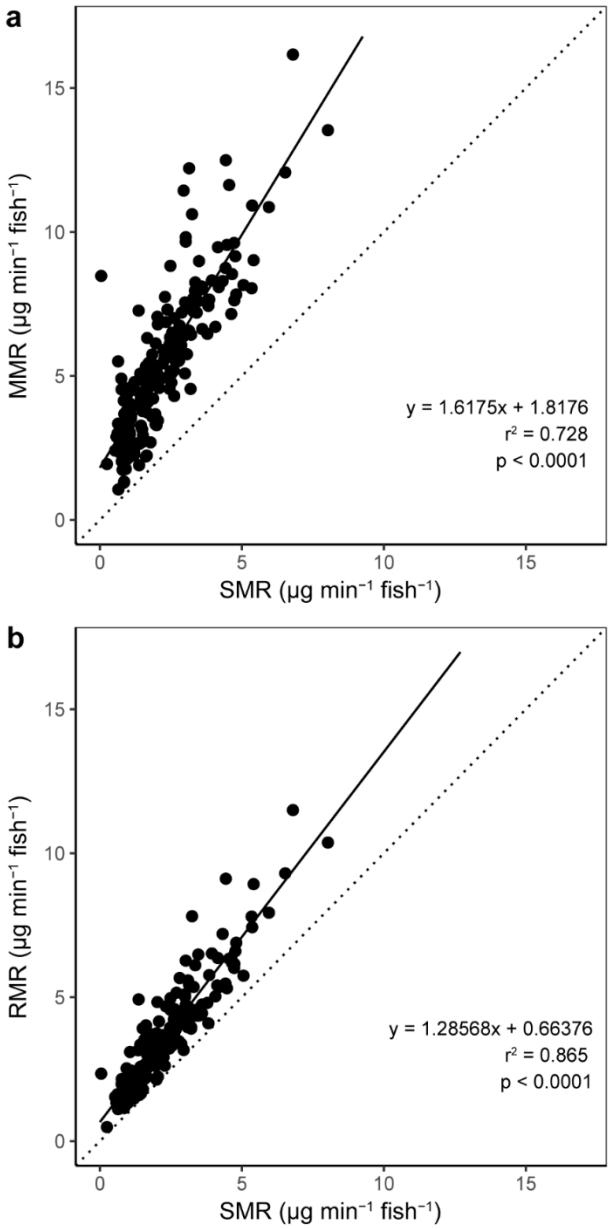


Figure S2.2. Comparison of metabolic rate data measured as MMR, RMR, and SMR. Data points represent individual fish. *N* = 201.

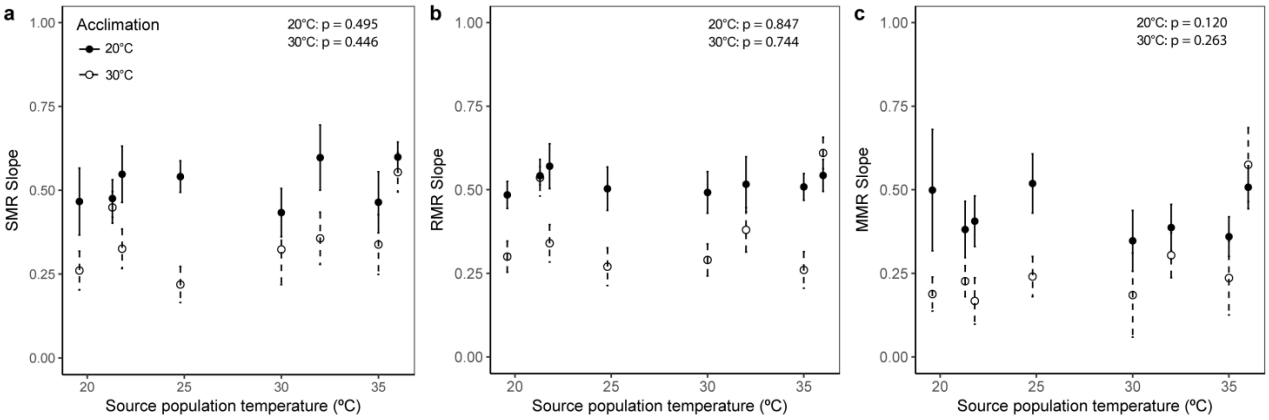


Figure S2.3. Relationship between source population temperature and scaling exponents (*b*) for a) standard metabolic rates (SMR), b) routine metabolic rates (RMR), and c) maximum metabolic rates (MMR). Data points are averages ± mean absolute error (MAE) for each population. Data are split by acclimation temperature.

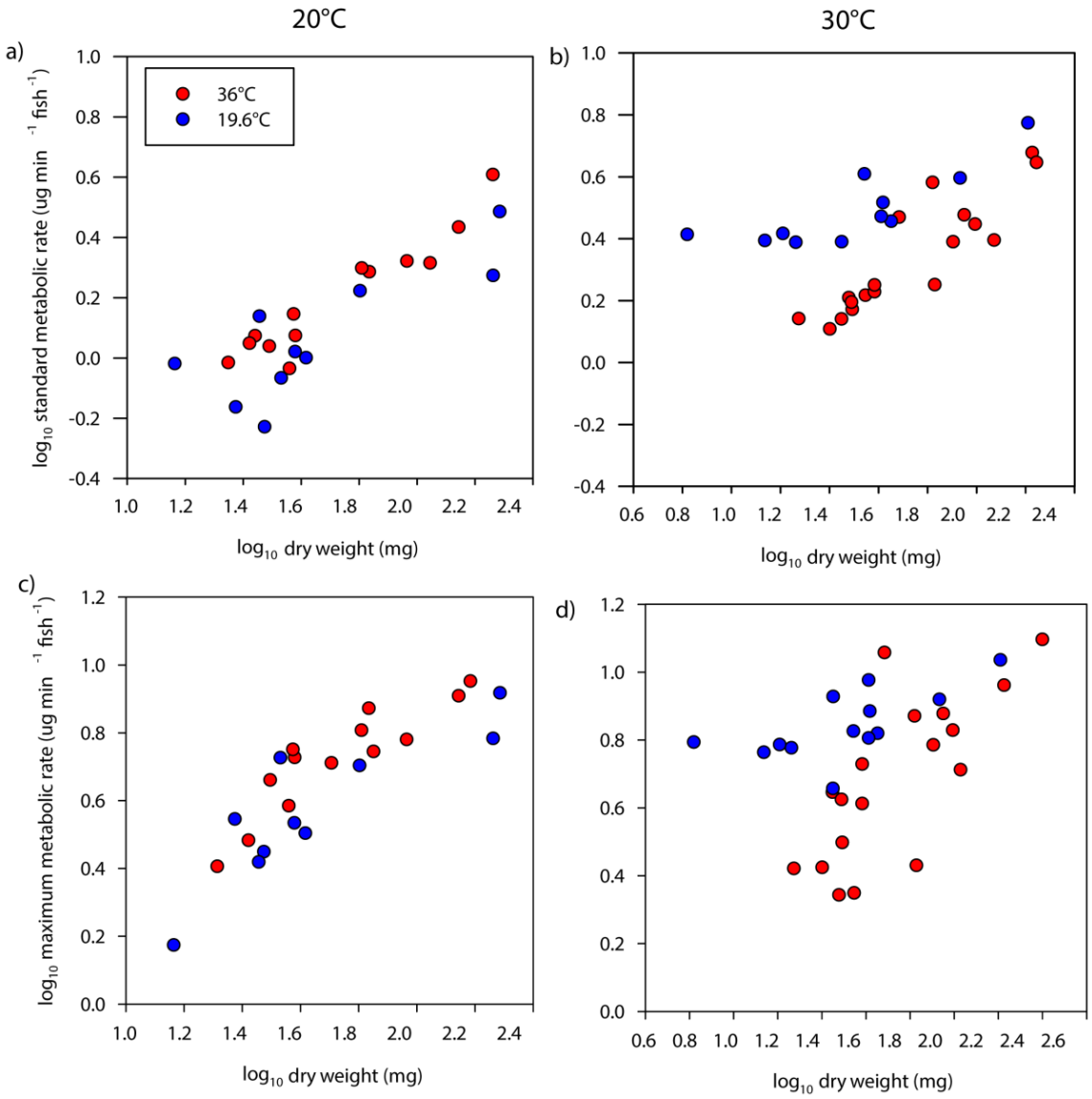


Figure S2.4. Relationship between mass and metabolic rate for the warmest (36; shown in red) and coolest (19.6; shown in blue) population in our study for standard metabolic rate (a,b) and maximum metabolic rate (c,d). Plots a) and c) are data from individuals acclimated at 20°C whereas plots b) and d) are data from individuals acclimated at 30°C.

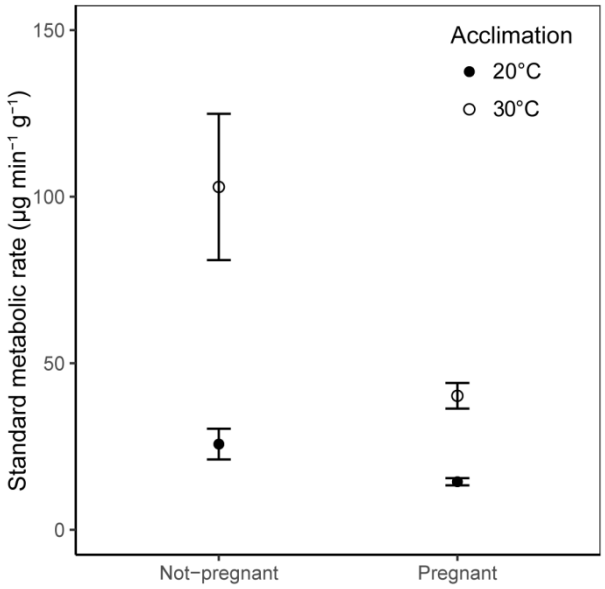


Figure S2.4. The effect of pregnancy on SMR. Data are average ±1SE and are spilt my acclimation treatment.

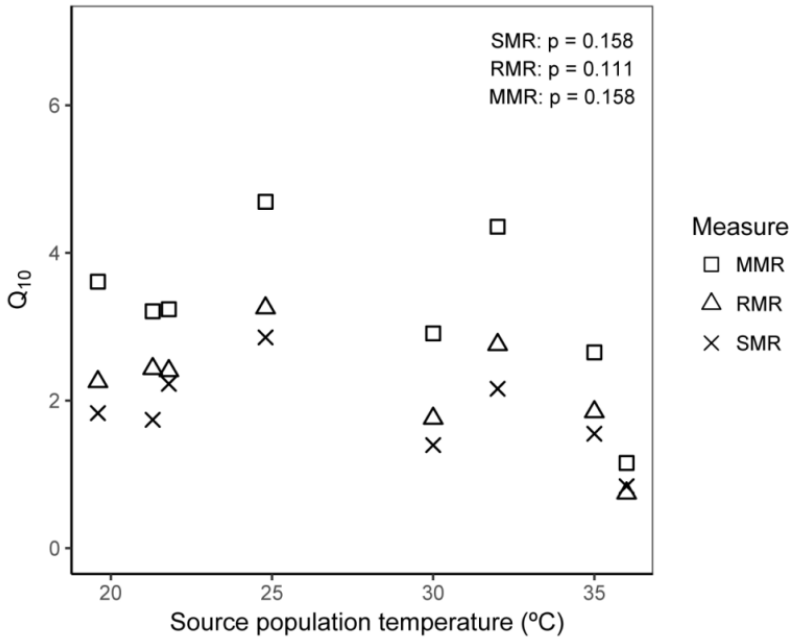


Figure S2.5. Relationship between population source temperature and Q_{10}, data are shown for MMR, RMR, and SMR.

Appendix S3
Chapter 4: Shift in diet with temperature alters gut morphology and body nutrient composition

Table S3.1. Generalized linear model (GLM) results describing the factors which explain the volume (%V) of food within the guts of *Gambusia affinis* in New Zealand (NZ) and California (CA). Significance is noted as: < 0.0001 '***' < 0.001 '**' < 0.01 '*' < 0.05 '.' < 0.1.

Region	Measure	Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
NZ	Volume (mm^2)	Intercept	2.424	3.247	0.747	0.456
		Temperature	0.339	0.092	3.680	<0.0001 ***
		Body length	87.266	18.017	4.844	<0.0001 ***
		Gender	-8.112	1.424	-5.701	<0.0001 ***
CA	Volume (mm^2)	Intercept	24.778	6.602	3.753	<0.0001 ***
		Temperature	-0.677	0.194	-3.484	<0.0001 ***
		Body length	219.524	20.341	10.792	<0.0001 ***
		Gender	-7.883	3.088	-2.553	0.011 *

Table S3.2. Multinomial logistic regression summary for gut fullness 1 (empty) to 5 (full) of *Gambusia affinis* in New Zealand (NZ) and California (CA). Empty (1) was used as the baseline.

	(Intercept)	Temperature	Gender	Body length
NZ				
Coefficients:				
2 (Quarter)	-1.393	0.046	0.115	0.757
3 (Half)	-1.988	0.075	0.313	1.367
4 (Three quarters)	-1.928	0.063	0.091	8.953
5 (Full)	-1.007	0.057	-1.261	5.707
Std Errors:				
2 (Quarter)	1.113	0.031	0.497	7.562
3 (Half)	1.040	0.289	0.463	7.062
4 (Three quarters)	1.022	0.290	0.455	6.220
5 (Full)	1.033	0.030	0.472	6.132
CA				
Coefficients:				
2 (Quarter)	2.094	-0.028	-0.878	-0.049
3 (Half)	1.306	-0.037	-1.154	-0.002
4 (Three quarters)	1.466	-0.070	-0.853	-0.025
5 (Full)	1.690	-0.060	-1.591	0.028
Std Errors:				
2 (Quarter)	1.606	0.028	0.455	0.039
3 (Half)	1.488	0.026	0.441	0.035
4 (Three quarters)	1.436	0.025	0.426	0.033
5 (Full)	1.320	0.024	0.403	0.030

Table S3.3. Multinomial logistic regression Z-test results on gut fullness data, where guts were ranked from 1 (empty)- 5(full). Empty (1) was used as the baseline from which significance was tested. Significance is noted as: < 0.0001 '***' < 0.001 '**' < 0.01 '*' < 0.05 '.' < 0.1.

	(Intercept)	Temperature	Gender	Body length
NZ				
2 (Quarter)	0.211	0.132	0.816	0.920
3 (Half)	0.056 .	0.009 **	0.499	0.847
4 (Three quarters)	0.059 .	0.030 *	0.842	0.150
5 (Full)	0.330	0.084 .	0.008 **	0.352
CA				
2 (Quarter)	0.192	0.313	< 0.0001 ***	0.457
3 (Half)	0.380	0.155	< 0.0001 ***	0.961
4 (Three quarters)	0.307	0.006 **	< 0.0001 ***	0.961
5 (Full)	0.200	0.011 *	< 0.0001 ***	0.354

Table S3.4. Diet summary of *Gambusia affinis* gut contents across geothermal populations in New Zealand (NZ) and California (CA). Data are relative importance index values (RI_i). Blank spaces indicate that this item was not present in the population's diet. Sites are arranged by temperature.

Region					NZ				
Site	PP	AL	AD	PK	MR	WA	SP	AWK	AA
Temp (°C)	19.2	22.7	23.4	24	30.9	33.5	35	37.7	38
Amorphous	47.987	29.606	80.636	29.361	46.868	0.185	10.390	1.327	42.593
Gambusia	0.060	0.000	0.328	0.004					0.342
Detritus	0.051	1.514	9.779	28.803	14.060	21.127	25.645	69.682	36.462
Algae	0.019	59.437	0.455	35.711	16.941	78.567	55.604	26.194	11.401
Amphipod		3.114							0.553
Acari	1.856	0.299	0.017	0.023			0.001		0.037
Ostracoda			0.000						0.024
Chironomidae	16.773	1.089	5.190	1.278	1.041	0.012			0.370
Hemiptera	0.294		0.177	0.070	0.034	0.009			2.132
Odonata	0.065	0.158	0.177						0.049
Mollusc	1.474	0.122		0.046			0.004		0.931
Mesovilae	0.013	0.107		0.065			0.002		
Ephemeroptera	0.326		0.036						
Zooplankton		0.598			0.002		7.538		
Terrestrial	16.872	1.900	0.263	4.314	0.103	0.015	0.691	2.779	1.592
Other invertbrate	14.209	2.057	2.941	0.325	20.951	0.085	0.125	0.019	3.514
Region					CA				
Site	NE	WW5	AW	FS	WSU	HC	FC	LHC	K2
Temp (°C)	20.9	23.6	23.7	24	27.8	29.9	33.4	36.7	38.9
Amorphous	0.102	22.174	13.236	7.335	25.740	0.024	3.015	89.588	6.944
Gambusia	0.014			0.023		0.001			
Detritus	0.150	1.456	65.216	3.532	15.826	0.387	0.051		
Algae	98.414	43.625	13.024	83.319	31.471	98.487	85.499	0.090	0.006
Amphipod		0.004							0.103
Acari		0.109	0.042	0.012	0.136			3.360	
Ostracoda		0.281	0.055	1.925	0.726	0.014	0.015	0.082	
Chironomidae	0.081	9.364	0.158	0.006	0.026	0.806		5.125	91.253
Hemiptera								0.115	
Odonata					0.056				
Mollusc	0.007	0.055	6.260	1.087	0.012	0.065	3.806		
Mesovilae									
Ephemeroptera						0.011			0.060
Zooplankton		11.931		0.005	1.058		0.001	0.003	
Terrestrial	0.175	11.000	0.512	0.477	22.942	0.177	5.946	1.269	1.503
Other invertebrate	1.057	0.000	1.497	2.280	2.006	0.028	1.667	0.368	0.132

Table S3.5. Prey selectivity (Ei) of *Gambusia affinis* for invertebrate prey categories at each site. Positive numbers indicate consumption was greater than the relative abundance of the given taxa in the environment; negative numbers indicate consumption was less than the relative abundance of the given taxa in the environment. Blank spaces indicate that the prey taxa were neither consumed nor present in the environment at the given site.

Region					NZ				
Site	PP	AL	AD	PK	MR	WA	SP	AWK	AA
Temp (˚C)	19.2	22.7	23.4	24.2	30.9	33.5	35	37.7	38
Positively selected									
Terrestrial invertebrate	0.69	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Acari	1.00	1.00	1.00					1.00	1.00
Odonata		1.00	1.00						1.00
Hemiptera			1.00						1.00
Ephemeroptera			1.00			1.00	1.00		
Negatively selected									
Other invertebrate	-0.81	-0.37	-1.00	-0.99		-1.00	-1.00		-1.00
Mixed selection									
Chironomid	1.00	-0.41	0.78	-1.00	-1.00	0.02	1.00	0.38	1.00
Amphipod		-0.07	-1.00			1.00			1.00
Ostracoda	1.00	-1.00	-1.00	1.00			1.00	1.00	1.00
Zooplankton	-0.94	-0.94	-1.00	0.40	-1.00		-0.96	-0.82	-0.95
Region					CA				
Site	NE	WW5	AW	FS	WSU	HC	FC	LHC	K2
Temp (˚C)	20.9	23.6	23.7	24	27.8	29.9	33.4	36.7	38.9
Positively selected									
Terrestrial_invert	1.00	0.99	1.00	1.00	1.00	1.00	1.00	0.96	0.96
Acari	1.00	0.28	1.00				1.00	1.00	0.82
Hemiptera				1.00	1.00		1.00	1.00	1.00
Negatively selected									
Zooplankton	-0.99	-0.99	-0.98	-1.00	-1.00	-0.94	-1.00	-0.84	-0.91
Mixed selection									
Chironomid	-1.00	-0.87	1.00	0.69	-0.82	-0.27	1.00	-0.35	-0.89
Amphipod		-1.00	1.00						
Ostracoda		0.75	1.00				1.00		-1.00
Odonata		1.00					-1.00	1.00	
Ephemeroptera	-1.00	-1.00						1.00	
Other invertebrate	0.88	0.43	-0.15	1.00	-1.00	-1.00	-0.96	-1.00	-1.00

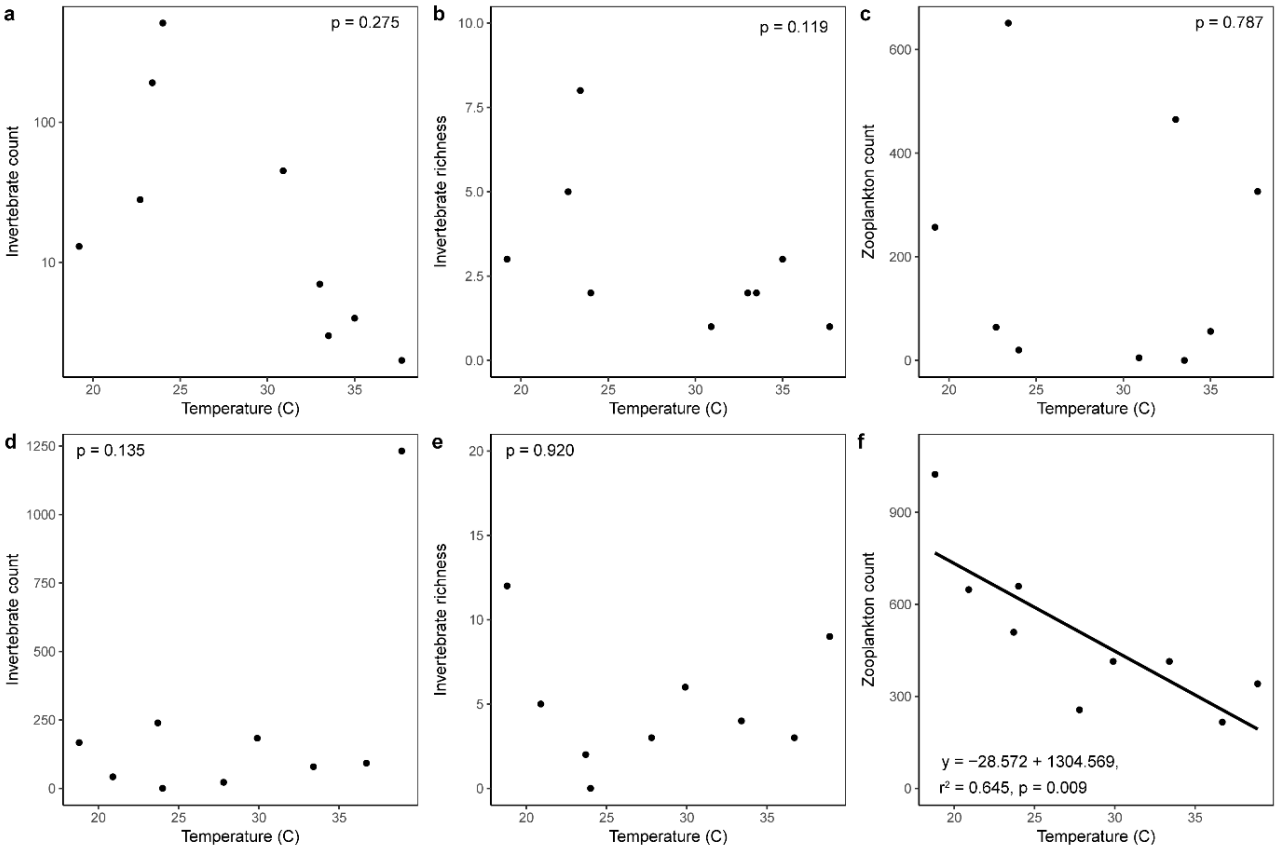


Figure S3.1. Invertebrate count, invertebrate richness and zooplankton count data for NZ (a,b,c) and CA (d, e, f) sites. Data are plotted against site temperature. *N* = 9/region.

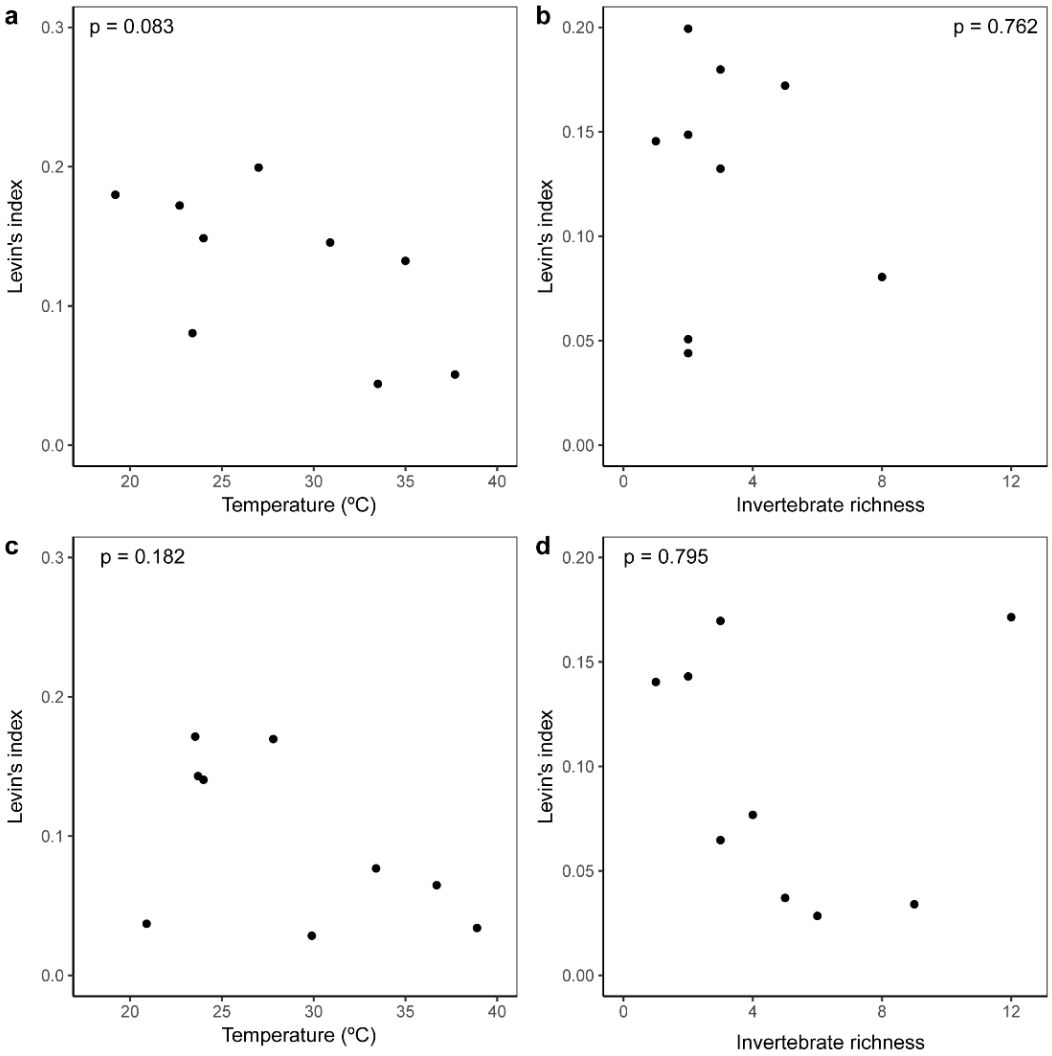


Figure S3.2. Relationship between Levin's index values calculated for each site and temperature for NZ (a) and CA (c), where low values indicate a specialist diet whereas larger values indicate a generalist diet. Levin's index is also shown against site invertebrate species richness data for NZ (b) and CA (d). *N* = 9 per region.

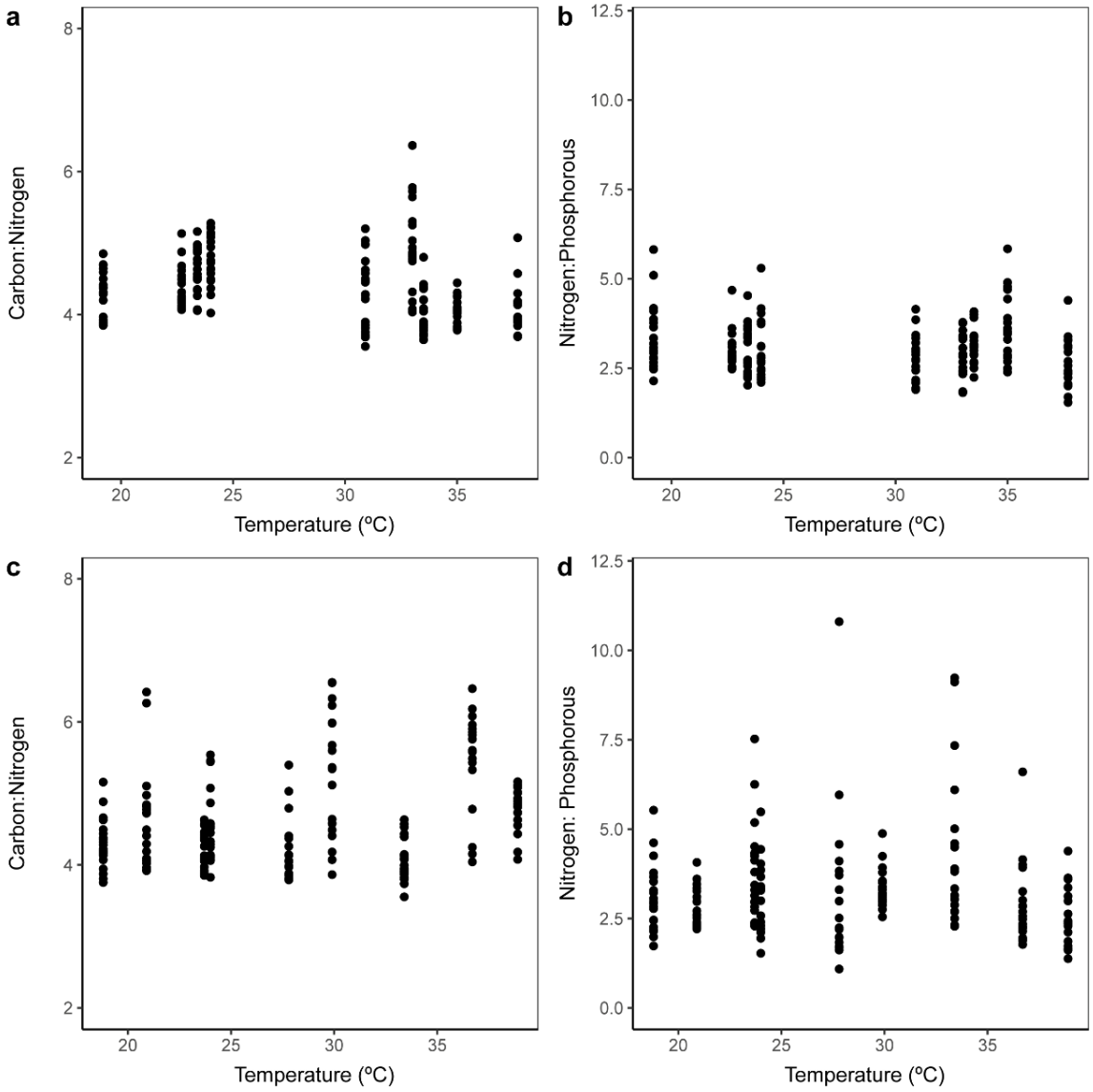


Figure S3.3. Relationship between site temperature and body carbon to nitrogen and nitrogen to phosphorous ratios for New Zealand (a,b) and California (c,d) populations for *Gambusia affinis.* Data are individual fish, n = 40 per population. Results of the statistical analysis are shown in Table 4.2.

Appendix S4
Chapter 5: Thermal history alters the ecological role of consumer body size

Results

pH and conductivity

There was an interactive effect ($t_{24} = -2.888$, $p = 0.008$) of body size and population on conductivity after 16 days. Fish reduced conductivity; in ambient population treatments conductivity declined with increasing body size, but that pattern was reversed in the presence of warm population fish (Fig. S4.9). After 32 days, changes in conductivity were small and we found no interactive ($p = 0.706$), population ($p = 0.678$), or body size ($p = 0.529$) effect. Similarly, we found no interactive, population, or body size effect on pH after 16 or 32 days ($p > 0.05$).

Table S4.1. Physiochemical characteristics of our study populations. Measurements were taken at fish collection with the exception of the average annual temperature values which were taken as spot measurements over a year.

Population	Barrier to dispersal	Collection Temperature (°C)	Average annual temperature (±SD)	DO (mg/L)	Specific conductivity (mS/cm)	pH
Akatarewa Hot Spring	Yes	35	30.7(5.4)	6.08	0.51	7.8
Waikato River Spring	No	22	21.3(4.2)	7.96	0.22	7.7

Table S4.2. Linear mixed effects model results for NPP, EPP, ER and inorganic nutrient concentrations. Significance is noted as . <0.10, *<0.05, **<0.005, ***<0.0005.

Response	Effect	*t*-statistic	Coefficient	SE	p-value	Log_{10} transformed
GPP_T4	Size	1.606	0.186	0.116	0.121	No
	Population	0.571	0.066	0.116	0.573	
	Size × Pop	-0.755	-0.124	0.164	0.458	
NPP_T4	Size	1.344	0.099	0.074	0.192	No
	Population	0.493	0.036	0.074	0.626	
	Size × Pop	-0.746	0.077	0.104	0.463	
ER_T4	Size	1.749	0.088	0.050	0.093.	No
	Population	0.600	0.030	0.050	0.554	
	Size × Pop	-0.654	-0.046	0.071	0.5195	
NH_4^+_T4	Size	-0.298	-0.384	0.777	0.705	No
	Population	-0.225	-0.289	0.777	0.775	
	Size × Pop	0.565	0.523	1.080	0.606	
pH @ 16 days	Size	0.314	0.01	0.04	0.756	No
	Population	-0.251	-0.01	0.04	0.804	
	Size × Pop	-0.222	-0.01	0.06	0.826	
pH @ 32 days	Size	0.383	0.05	0.12	0.705	Yes
	Population	-0.706	-0.09	0.12	0.487	
	Size × Pop	-0.307	-0.05	0.18	0.762	
Conductivity @ 16 days	Size	1.702	1.38	0.81	0.102	No
	Population	2.352	1.90	0.81	0.027*	
	Size × Pop	-2.888	-3.30	1.14	0.008*	
Conductivity @ 32 days	Size	0.638	1.06	1.67	0.529	No
	Population	-0.42	-0.70	1.67	0.678	
	Size × Pop	-0.38	-0.90	2.36	0.706	
Growth Rate	Size	2.33	0.03	0.01	0.027*	No
	Population	5.047	0.07	0.01	0.000***	
	Size × Pop	-0.624	-0.01	0.02	0.537	
Metabolic rate	Size	2.61	0.22	0.08	0.016*	Yes
	Population	-1.85	-0.15	0.08	0.077.	
	Size × Pop	-0.37	-0.04	0.12	0.714	
Excretion Rate	Size	2.53	0.11	0.04	0.019*	Yes
	Population	2.53	0.10	0.04	0.018*	
	Size × Pop	-0.38	-0.02	0.06	0.705	
Periphyton Abundance	Size	4.18	6.16	1.47	0.000***	Yes
	Population	1.61	2.28	1.42	0.120	
	Size × Pop	-0.97	-2.02	2.08	0.341	

Phytoplankton Abundance T4-T0	Size	-2.13	-0.29	0.14	0.046*	
	Population	-3.10	-0.42	0.14	0.006**	Yes
	Size × Pop	2.59	-0.53	0.21	0.018*	
Zooplankton Biomass T4-T0	Size	2.27	0.58	0.26	0.033*	
	Population	0.60	0.15	0.25	0.555	Yes
	Size × Pop	-1.71	-0.61	0.36	0.100.	
Copepod Biomass T4-T0	Size	-2.111	-0.41	0.19	0.045*	
	Population	-1.446	-0.28	0.19	0.161	Yes
	Size × Pop	2.258	0.60	0.27	0.034*	
Rotifer Biomass T4-T0	Size	2.186	0.44	0.20	0.041*	
	Population	-0.935	-0.20	0.22	0.360	Yes
	Size × Pop	-0.892	-0.26	0.29	0.383	
Chironomids	Size	-2.363	-0.31	0.13	0.028*	
	Population	-0.006	0.00	0.13	0.995	Yes
	Size × Pop	-0.286	-0.05	0.18	0.778	
Predatory invertebrates	Size	-1.089	-0.12	0.11	0.284	
	Population	0.076	0.01	0.11	0.940	Yes
	Size × Pop	1.445	0.22	0.15	0.158	
CO_2 day-time	Size	-2.57	-14.49	5.63	0.018*	
	Population	-3.12	-18.33	5.87	0.005*	No
	Size × Pop	3.82	29.74	7.79	0.001**	
N_2O day-time	Size	2.32	0.0020	0.0009	0.027*	
	Population	2.44	0.0023	0.0009	0.021*	No
	Size × Pop	-2.45	0.0031	0.0013	0.021*	
CH_4 day-time	Size	1.912	0.014	0.007	0.068.	
	Population	0.294	0.002	0.007	0.771	No
	Size × Pop	-0.469	-0.005	0.013	0.643	
NH_3 day-time	Size	-0.639	-0.02	0.03	0.529	
	Population	0.321	0.01	0.03	0.751	No
	Size × Pop	-1.418	0.07	0.05	0.169	
CO_2 night-time	Size	1.479	26.05	17.62	0.152	
	Population	1.034	18.22	17.62	0.311	No
	Size × Pop	-0.414	-10.31	24.92	0.683	
N_2O night-time	Size	2.225	0.006	0.003	0.036*	
	Population	0.075	0.000	0.003	0.941	No
	Size × Pop	0.1	0.000	0.004	0.921	
CH_4 night-time	Size	0.448	0.004	0.009	0.658	
	Population	1.407	0.013	0.009	0.172	No

	Size × Pop	-1.378	-0.018	0.013	0.181	
NH_3 night-time	Size	-0.504	-0.04	0.08	0.618	
	Population	-1.713	-0.14	0.08	0.096.	No
	Size × Pop	1.635	0.18	0.11	0.112	

Table S4.3. Average ± 1 SE greenhouse gas (GHG) flux values.

GHG (time)	Large, Ambient	Small, Ambient	Large, Warm	Small, Warm
N_2O (night)	-0.0015±0.0040	0.0045±0.0033	-0.0013±0.0030	0.0052±0.0040
N_2O (day)	-0.0003±0.0006	0.0017±0.0008	0.0020±0.0008	0.0009±0.0006
CO_2 (night)	23.14±21.52	49.19±22.48	41.36±10.81	57.10±23.27
CO_2 (day)	-8.81±8.13	-22.68±6.44	-27.75±4.48	-11.27±4.30
NH_3 (night)	0.0916±0.0812	0.0515±0.04120	-0.0447±0.0628	0.0993±0.0468
NH_3 (day)	0.0012±0.0434	-0.0204±0.0430	0.0121±0.0328	-0.0774±0.0401
CH_4 (night)	0.0291±0.0352	0.0331±0.0314	0.0418±0.0338	0.0282±0.0326
CH_4 (day)	-0.0164±0.0055	-0.0025±0.0010	-0.0142±0.0026	-0.0052±0.0078

Table S4.4. Summary of ANOVA model data for the mesocosm temperature data.

Response	Effect	F	*df*	p-value
Temperature	Size	2.481	1,8	0.176
	Population	3.009	1,8	0.143
	Size × Pop	1.993	1,8	0.219

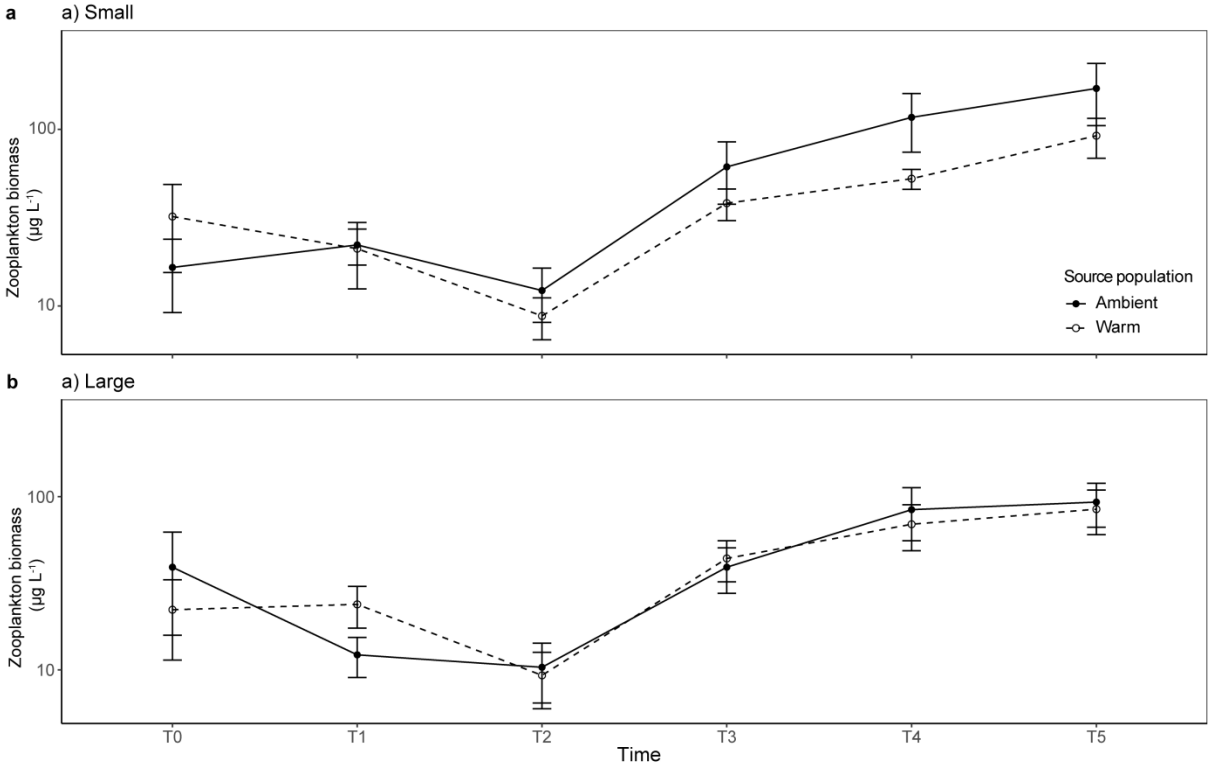


Figure S4.1. Zooplankton time series plots, data are average (± 1SE). The y-axes are shown on a logarithmic scale. Zooplankton biomass in control tanks is not shown.

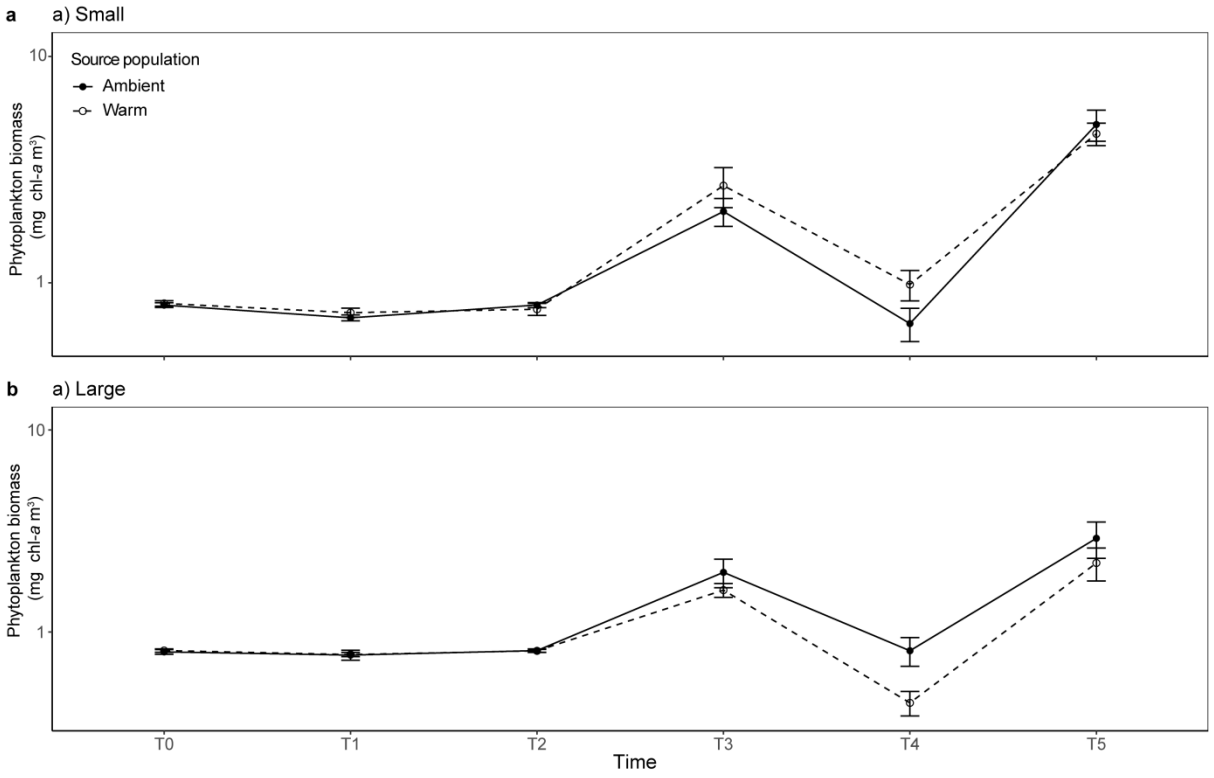


Figure S4.2. Phytoplankton time series plots, data are average (± 1SE). The y-axes are shown on a logarithmic scale. Phytoplankton biomass in control tanks is not shown.

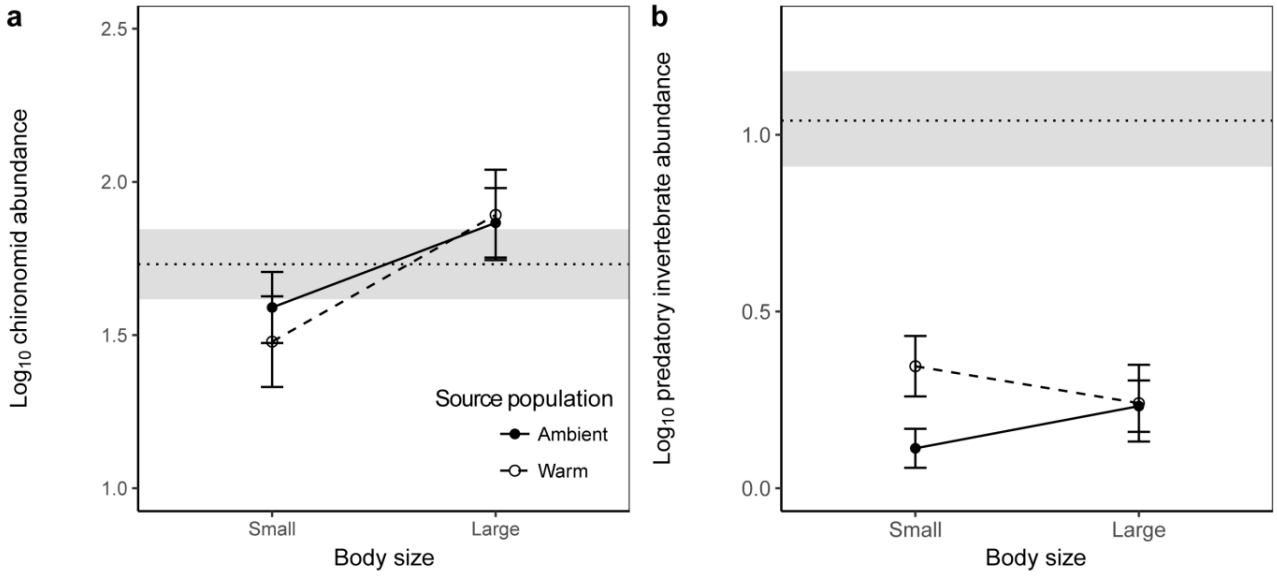


Figure S4.3. Invertebrate data showing a) chironomid abundance and b) predatory invertebrate abundance across our treatments, data are averages ± 1SE. Dotted lines and shaded zones represent control tank average and standard error values respectively.

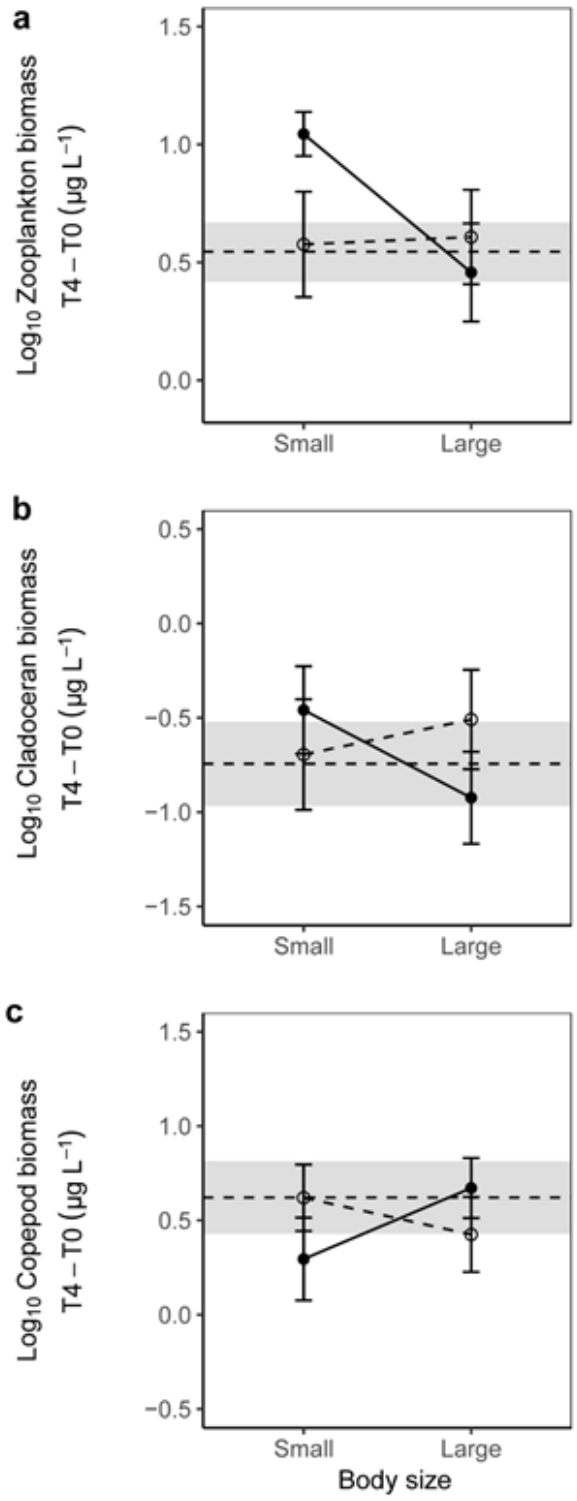


Figure S4.4. Interaction plots showing a,b) zooplankton biomass, c,d) *Daphnia* biomass, e,f) copepod biomass. Biomass data as T1-T0 are on the left and data as T4-T0 are on the right. Data are averages ± 1SE. Dotted lines and shaded zones represent control tank average and standard error values respectively.

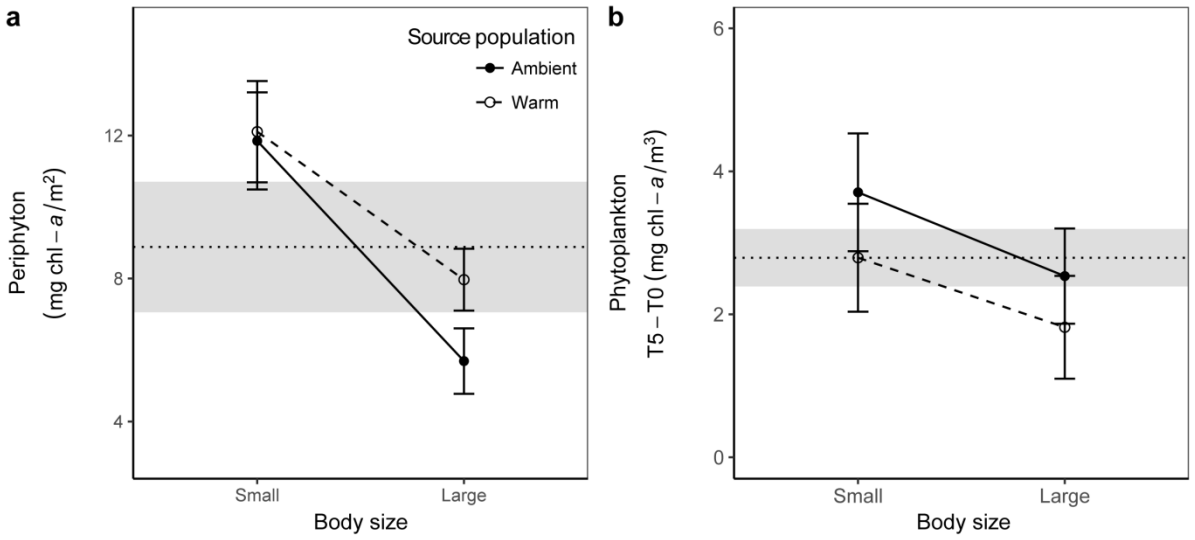


Figure S4.5. Periphyton (a) and phytoplankton (b) interaction plots. Data are averages ± 1SE. Dotted lines and shaded zones represent control tank average and standard error values respectively.

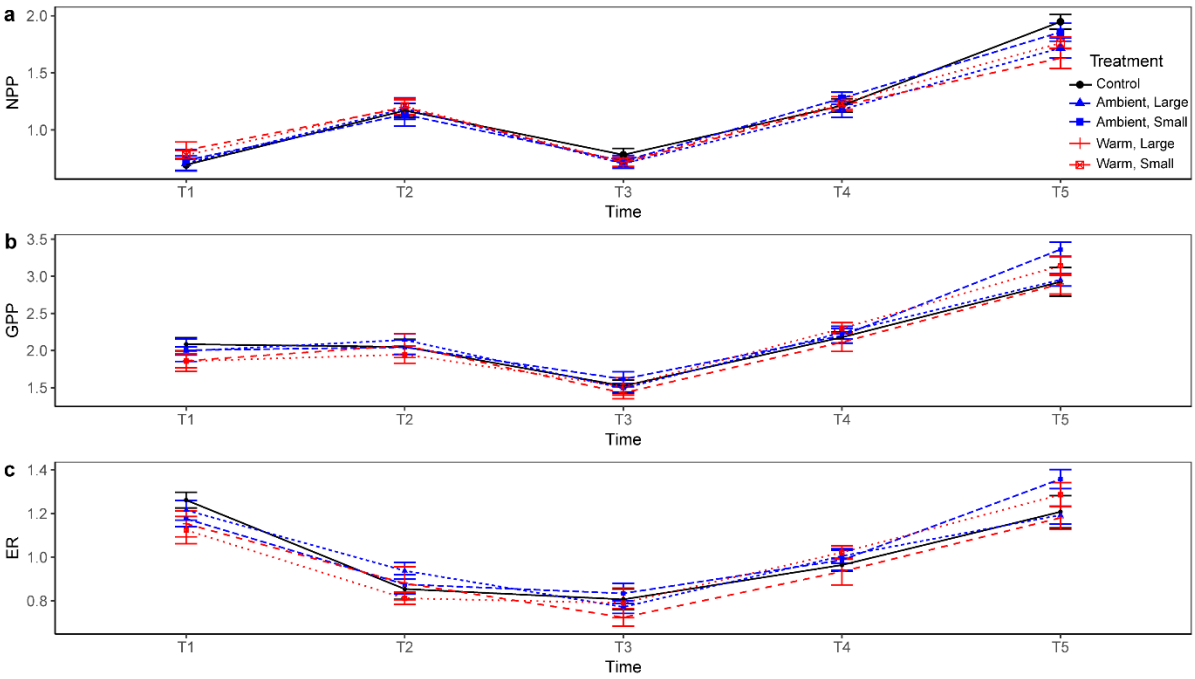


Figure S4.6. Net primary production (NPP; a), Gross primary production (GPP; b), and Ecosystem respiration (ER; c) measurements over our experimental period. Data are averages ± 1SE.

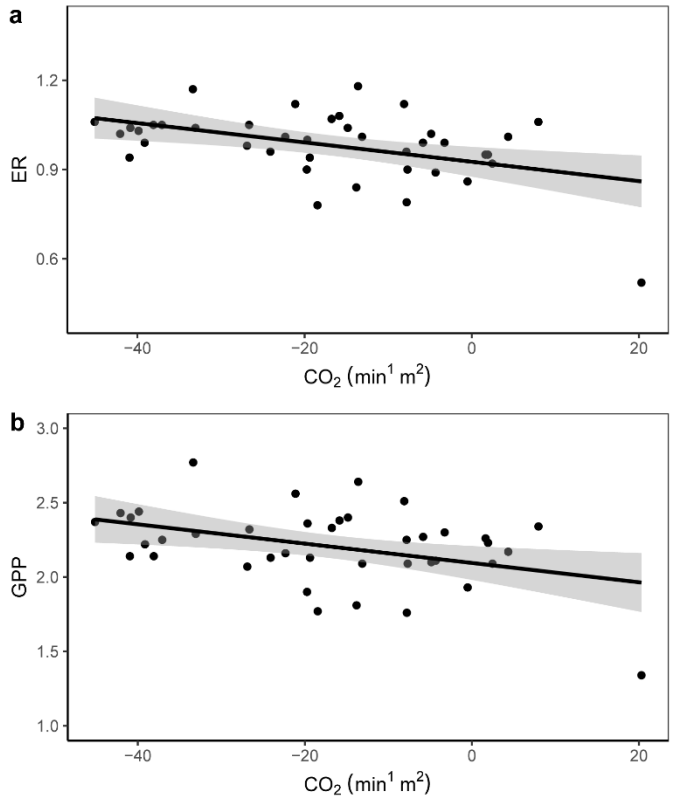


Figure S4.7. Relationship between CO_2 and a) ecosystem respiration (ER) and b) gross primary production (GPP), data are fit with a linear regression model with a 95 % confidence interval shown in grey.

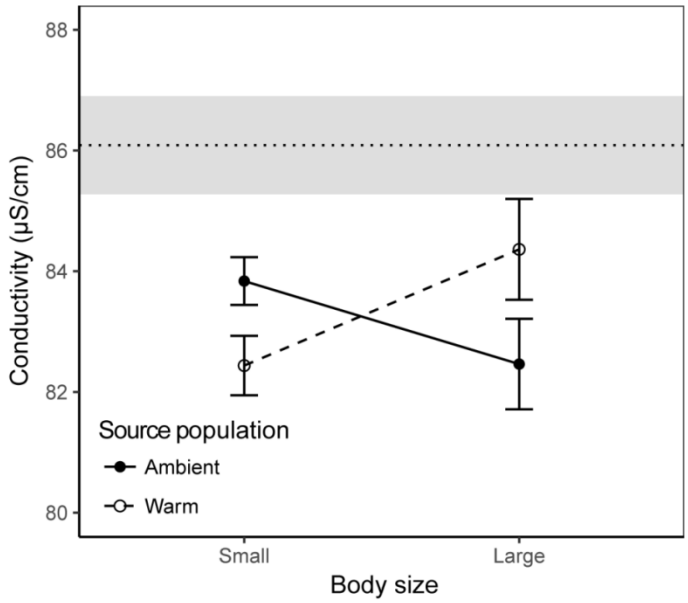


Figure S4.8. Conductivity interaction plot, data are from T3 (day 16 of our experiment). Data are averages ± 1SE. Dotted lines and shaded zones represent control tank average and standard error values respectively.

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