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The ecosystem consequences of trait variation in a globally important freshwater predator

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THE ECOSYSTEM CONSEQUENCES OF TRAIT VARIATION IN A GLOBALLY IMPORTANT FRESHWATER PREDATOR

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by

David C. Fryxell

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ABSTRACT

The ecosystem consequences of trait variation in a globally important freshwater predator

David C. Fryxell

Much can be learned of complex ecological communities by identifying the ecologically-important species and studying the contingencies of their interactions. Recent evidence suggests that a major mediator of species interactions is trait variation within such species. Western mosquitofish (*Gambusia affinis*) is a globally invasive predator with potent effects on ecosystems and substantial trait variation among populations. In this dissertation, I sought to determine the traits and environmental conditions that mediate variation in the community and ecosystem effects of mosquitofish populations. In **Chapter 2** I tested for the effects of mosquitofish sex ratio variation on experimental pond ecosystems. Mosquitofish exhibit sexual dimorphism and their sex ratios vary widely across their global range from highly female-biased to highly male-biased. In experimental ponds, female-biased populations had higher rates of prey depletion and nutrient excretion which induced stronger pelagic trophic cascades, causing significant increases to important ecosystem variables such as water temperature and pH. In **Chapter 3** I tested how temperature mediates the importance of mosquitofish trait variation for experimental pond ecosystems. A growing literature demonstrates that top-down effects increase under warming. Thus, I predicted that the differences in ecological role among mosquitofish populations would become larger under warming. In support of my prediction, warming increased top-down effects and increased the ecological divergence between mosquitofish populations. In **Chapter 4** I tested how evolutionary adaptation to temperature affects traits and ecological effects of mosquitofish. Mosquitofish exhibited increased mortality, and evolved slower growth and earlier maturity at higher temperatures. Warm-adapted mosquitofish also consumed smaller pelagic zooplankton prey and did not show a strong ontogenetic niche shift to consuming large benthic macroinvertebrates. Because warm-adapted mosquitofish grew slower, reproduced earlier, and continued to consume zooplankton throughout life, they exacerbated warming-induced shifts towards smaller zooplankton communities. This dissertation reveals considerable effects of mosquitofish demographic variation, interpopulation variation, and contemporary evolution on community and ecosystem ecology. These results suggest that the structure and functioning of an ecosystem can depend strongly upon the traits of the ecologically important species present.

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CHAPTER 1 - Introduction

Ecological communities represent the pinnacle of natural complexity. A complete understanding of communities would require knowledge integration across the natural sciences, from physics to chemistry to genetics to evolution to species interactions. This complexity makes the study of communities appear intractable. However, ecological interaction networks reveal that communities may commonly have just a few widely, strongly interacting species (McCann et al. 1998, Thebault and Fontaine 2010). Thus, the study of these ecologically important species holds the potential to reveal much about communities while being relatively tractable.

Top predators are of universal ecological importance and strongly shape communities through predation. Predators tend to feed on abundant, competitively dominant prey taxa. This numerical reduction in competitively dominant prey bolsters diversity by allowing competitively inferior taxa to persist (Paine 1966, Lubchenco 1978). In this way, predation is thought to be a common mechanism for the maintenance of diversity. *Top* predators on the other hand can play a unique role by determining the biomass distribution across trophic levels, and particularly that of the primary producers (Hairston et al. 1960, Carpenter et al. 1985). Through their cascading effects on primary producers, top predators can determine critical ecosystem functions and services like carbon sequestration (Atwood et al. 2013). Because top predators strongly shape many community attributes, much about communities and ecosystems can potentially be understood by the study of top predators.

An understanding of the context-dependence of top predator effects is important for predicting the strength of a top predator's community level effects. For example, if two top predator species are present, reciprocal predation between them (i.e. intraguild predation) can dampen their effects down the trophic chain (Finke and Denno 2004, 2005). Thus, predators may play a more potent role when there are just one or a few predator species in a community. For another example, environmental warming increases the food demand of top predators more than it increases primary productivity (O'Connor et al. 2011), so the effects of predation down the food chain are often stronger at higher temperatures (O'Connor et al. 2009, Barton et al. 2009, Kratina et al. 2012, Shurin et al. 2012). Thus, warming may also generally increase the importance of predators.

Variation in the abundance and size distribution of top predator species can have cascading community effects (Pace et al. 1999, Borer et al. 2005, Frank et al. 2005, DeLong et al. 2015). What is less known is how other aspects of intraspecific variation in predator populations may cascade to affect communities and ecosystems. For example, rapid evolutionary responses to anthropogenic stressors such as warming have now been reported in predator populations (Merilä and Hendry 2014), but whether these changes are important for communities is largely unknown. These effects may be important because predator trophic levels are depauperate, meaning they are often occupied by one or a few species. Thus, trait variation within a top predator species can have stark ecological consequences because it is not overshadowed by the ecological effects of variation among species within the predator guild.

In this dissertation, I use a predator species to understand how ecological context interacts with trait variation within the predator species to influence communities and ecosystems. My focal predator is the western mosquitofish (*Gambusia affinis*), a small fish which is a predator in small pond ecosystems globally. In **Chapters 2, 3, and 4**, I used trait assays and experimental ponds to explain variation in the effects of mosquitofish populations due to sex ratio variation, pond temperature, and evolutionary thermal adaptation, respectively. This body of work reveals the far-reaching importance of understanding trait variation in top predators for communities and ecosystems, especially as predator traits are changing in the Anthropocene.

CHAPTER 2 - Sex ratio variation shapes the ecological effects of a globally introduced freshwater fish

Abstract

Sex ratio and sexual dimorphism have long been of interest in population and evolutionary ecology, but consequences for communities and ecosystems remain untested. Sex ratio could influence ecological conditions whenever sexual dimorphism is associated with ecological dimorphism in species with strong ecological interactions. We tested for ecological implications of sex ratio variation in the sexually dimorphic western mosquitofish, *Gambusia affinis*. This species causes strong pelagic trophic cascades and exhibits substantial variation in adult sex ratios. We found that female-biased populations induced stronger pelagic trophic cascades compared to male-biased populations, causing larger changes to key community and ecosystem responses including zooplankton abundance, phytoplankton abundance, productivity, pH, and temperature. The magnitude of such effects indicates that sex ratio is important for mediating the ecological role of mosquitofish. Because sex ratio variation and sexual dimorphism are common features of natural populations, our findings should encourage broader consideration of the ecological significance of sex ratio variation in nature, including the relative contributions of various sexually dimorphic traits to these effects.

Introduction

Sex ratio variation is a longstanding theme in evolutionary biology. Fisher (Fisher 1930) famously theorized that natural selection should maintain 1:1 sex ratios by continuously favoring the rare sex, thereby always returning skewed sex ratios to equality. In nature, skewed sex ratios are a common observation across the tree of life, and explanations include differential mortality rates for males and females (Arendt et al. 2014), inbreeding and local competition for mates (Hamilton 1967), endocrine-disrupting environmental pollutants (Mills and Chichester 2005, Rodríguez et al. 2007), and adaptive maternal effects that allow differential investment in male or female offspring (Trivers and Willard 1973, Clutton-Brock and Iason 1986, Kahn et al. 2013). Despite the attention paid to the causes of sex ratio variation in nature, and in some cases its consequences for population growth (Thresher et al. 2013), theory and tests of its effects on communities and ecosystems are lacking. This lack of attention may in part owe to a presumption that the sexes of most species are ecologically equivalent in their effects on communities and ecosystems.

However, many species show marked sexual dimorphism in body size and other traits related to ecological function. Sexual size dimorphism has the potential to influence resource use because prey capture is size-dependent (Brose et al. 2006) and body size influences overall feeding rates (Rall et al. 2012). Body size and physiology also influence rates of nutrient excretion (Hall et al. 2007), which have important effects for ecosystem processes (Vanni 2002). Males and females can also be dimorphic in behavioral or morphological traits, affecting resource use (Shine 1989). The widespread observations that sex ratios vary in nature, and that males and females often differ in key ecological traits, suggest that sex ratio variation may have impacts on communities and ecosystems. These effects may be particularly prevalent when sex ratio variation is present in ecologically important species, such as keystone, foundation, dominant, or invasive species.

We examined the ecological consequences of sex ratio variation in the western mosquitofish (*Gambusia affinis*). Mosquitofish (*G. affinis* and its congener *G. holbrooki*) are small (< 6 cm) livebearing fishes (Poeciliidae), which lack sexual dimorphism in age and size at maturity (Pyke 2005), but show pronounced sexual size dimorphism (Fig. 1) due to differences in post-maturation growth rates. Empirical tests show female mosquitofish display higher feeding rates per unit body size (Reddy 1975, Shakuntala 1977) and typically show greater niche breadths, with a notable preference for prey of larger body sizes (Bence and Murdoch 1986, Homski et al. 1994). Females also spend relatively more time foraging when in the presence of other females compared to when in the presence of males (Pilastro et al. 2003, Arrington et al. 2009). In addition to these dimorphic foraging characteristics, the relatively large size of females could increase overall nutrient excretion rates for the same density of fish in female-biased populations compared to male-biased populations, which could in-turn affect primary production (Vanni 2002). As a result of these sex-specific tendencies, we predict that female-biased populations induce stronger pelagic trophic cascades compared to male-biased populations. We expect these effects to be strong because mosquitofish play a major role in aquatic ecosystems by altering invertebrate communities and driving strong trophic cascades that can even change abiotic conditions, including light penetration and nutrient dynamics (Hurlbert et al. 1972, Lancaster and Drenner 1990).

We performed a pond mesocosm experiment to test this prediction. The experiment was conducted in California (USA), where the western mosquitofish was introduced in 1922 and has since been spread by mosquito vector control districts (MVCD's) as a means of disease control (Lenert 1923), most notably West Nile Virus (Mosquito and Vector Control Association of California 2015). We obtained fish for our experiments from the Sacramento-Yolo MVCD (Elk Grove, CA, USA). This district alone stocks about one million mosquitofish annually over an area of about 5,000 hectares of agricultural fields, wildlife refuges, and private lands in Sacramento and Yolo Counties, California (Sacramento Yolo Mosquito Vector Control District 2005). Mosquitofish were historically introduced for the same purposes elsewhere and are today one of the most widespread and abundant freshwater fishes in the world ((Pyke 2008), Fig. 1). Their strong negative consequences for native fauna across their global range has resulted in their being listed as one of the world's 100 worst invasive species by the IUCN (IUCN Invasive Species Specialist Group 2015).

Mosquitofish sex ratios vary substantially across their present-day range (Fig. 1). The mechanism of sex determinism is chromosomal, and primary and secondary sex ratios have rarely been found to differ from 1:1 (but see (Kahn et al. 2013)). Therefore, sex ratio variation has primarily been attributed to differential postpartum mortality induced by a variety of physiological and ecological mechanisms, including temperature, salinity, seasonality, and interactions with other species. Predation may be a particularly important driver. For example, avian predators prefer females over males, leading to sex ratio estimates as high as 97% males in some locations (Britton and Moser 1982). Female-biased populations are more common, and are often attributed to the greater longevity of females (often > 6 months) relative to males (often < 6 months) (Haynes and Cashner 1995). Therefore, mosquitofish are a relevant system to understand whether sex ratio variation shapes ecological effects because they are dimorphic, ecologically important, and show substantial sex ratio variation across their global range.

Materials and Methods

Design

We created a 6x6 array of experimental pond mesocosms (1136 L Rubbermaid® stock tanks) on a level field on the grounds of Long Marine Laboratory at the University of California, Santa Cruz. Each mesocosm was layered with 19 L of sand and filled with dechlorinated municipal water. We inoculated each mesocosm with 4 L of thoroughly homogenized sediment from Westlake Pond (Santa Cruz, CA) to introduce nutrients and benthic communities. After the sediment settled (~5 days) a cinderblock (9.3 x 19.0 x 39.3 cm^3) was placed in the center of each mesocosm for habitat cover, and the water was inoculated with a homogenized plankton community comprised of multiple horizontal plankton tows (80 µm mesh) at Westlake Pond.

Experimental fish were obtained from the husbandry facility operated by the Sacramento-Yolo MVCD. Fish at or above the approximate size at maturity were sexsorted before being assigned to treatments. Fish were identified as adult males if they had evidence of a gonopodium and as adult females if they were larger than the smallest adult male and lacked evidence of a gonopodium (Krumholz 1948). Our experiment thus incorporated the normal range of sexual size dimorphism, as well as other aspects of dimorphism, inherent to the species. We then counted out sex ratio treatments of 0, 25, 50, 75, and 100 percent males at a density of 12 individual fish per mesocosm. We chose this density because it is consistent with stocking densities used by MVCD's and is within the range of densities used in similar experiments (Hurlbert et al. 1972, Lancaster and Drenner 1990). Our design included a fishless reference treatment, yielding a grand total of six treatments, each with six replicates. Treatments were assigned to the mesocosm array using a latin-squares random number generator. Fish were introduced to the mesocosms on March 31, 2014, one week after adding plankton to the pond communities. On the same day, we placed two unglazed ceramic tiles (2.8 x 4.3 cm^2) on the cinderblock in each mesocosm to measure periphyton accrual. A 1.27 cm mesh bird-netting was used over all mesocosms throughout the experiment to prevent catastrophic avian and mammalian disturbance, meanwhile allowing for exposure to all other natural physical, chemical, and biological elements, including oviposition by insects and amphibians.

Sampling

Mesocosms were sampled two and four weeks after fish introduction for various pelagic responses including zooplankton abundance, phytoplankton abundance, primary productivity and respiration, pH, temperature at dusk, and water nutrient concentrations. Zooplankton samples were taken from a 1 L depth-integrated water sample filtered through 80 µm mesh and preserved in 80% ethanol. All zooplankters were thereafter counted and identified to the lowest taxonomical distinction possible at 100x magnification. Phytoplankton abundance was estimated as the pelagic chlorophyll *a* concentration in 1 L of water. Water was filtered through a 0.7 µm glass microfiber filter (Whatman® GF/F) and the filters frozen for less than four weeks when we extracted the chlorophyll *a* with 90% acetone for 24 hours at 2° C (American Public Health Association 2012). The concentration of chlorophyll *a* in the acetone was then measured fluorometrically on a Trilogy Laboratory Fluorometer (Turner Designs®) using the Turner Designs non-acid module. Ecosystem respiration (ER), net primary productivity (NPP), and gross primary productivity (GPP) were estimated using diel change in dissolved oxygen measured at dawn, dusk, and the following dawn, using a handheld sonde (YSI® Pro 2030) as in (Harmon et al. 2009). Responses are reported in units of dissolved oxygen concentration (mg/L) change over time. ER is, by convention, reported with negative values which represent the decrease in dissolved oxygen with increasing respiration. Pond temperature and pH were measured with a handheld sonde (Oakton® PTTestr 35). To determine water nutrient concentrations 50 mL water samples were taken from 5 cm below the surface of each mesocosm, filtered through 0.7 µm glass microfiber filters (Whatman® GF/F), and frozen. A week later the water samples were thawed and analyzed for soluble reactive phosphorous (PO4) and nitrate+nitrite (NOx) concentrations on a flow injection analyzer (Lachat® QuickChem) following standard methods (American Public Health Association 2012).

At the end of the experiment (April 28, week 4), we collected samples for benthic community composition, snail abundance, periphyton accrual, and larval amphibian abundance. While mosquitofish primarily feed on pelagic food resources (Pyke 2005), their use of benthic resources has also been observed to cause changes to benthic communities (Hurlbert et al. 1972). Benthic community composition was determined from invertebrate counts taken from an 18 cm diameter benthic core in a common central location of each mesocosm. The dry mass of the dominant benthic invertebrate group, Chironomidae (nonbiting midges), was then determined after drying at 60° C for 48 hours. All snails (Planorbidae and Physidae) were then picked from each mesocosm, counted, and then dried and weighed in the same manner as the chironomids above. Periphyton tiles were scrubbed and rinsed into a filter apparatus and then filtered through a 0.7 µm glass microfiber filter (Whatman® GF/F). The filters were frozen and then analyzed for chlorophyll *a* as in the phytoplankton abundance estimation above. Larval Pacific tree frogs (*Pseudacris regilla*) were counted in mesocosms where they were present, euthanized using an overdose of tricaine methanesulfonate (MS-222), and dry mass obtained using methods above.

On April 29, we captured all fish from each mesocosm and ran excretion trials to estimate mesocosm-level fish N and P excretion. Fish within a mesocosm were temporarily held in a 10 L floating tub until all fish were captured. Thereafter, all fish from a given mesocosm were introduced to a 2 L Nalgene® bottle filled with 1.5 L of dechlorinated city water, and floated in their respective mesocosm for 30-60 minutes. In fishless treatments, a Nalgene® bottle was floated in the same manner with the same water, however without any fish added. A water sample was then taken from each bottle as in the NOx and PO4 measurements above, and its ammonium (NH4) and PO4 concentrations were determined using the same instrumentation and standard practice (American Public Health Association 2012). Excretion rate was calculated for all fish treatments as the concentration measured in each tank minus the mean-fishless concentration, all divided by the excretion trial time length. After the excretion assay, we euthanized experimental fish using an overdose of MS-222. Fish were then dried and weighed as above.

Analyses

At the end of the experiment we discovered that four fish had been initially misidentified to sex and three individuals had died (0.8% overall mortality). No fish in the even sex ratio treatment died nor were misidentified to sex, so to avoid dropping replicates, we combined the 0% and 25% male treatments into a single ‘female-biased’ treatment (N=12 replicates) and the 75% and 100% male treatments into a single ‘male-biased’ treatment (N=12 replicates). We then ran two separate but identical analyses to determine the differences between 1) the male-biased treatment versus the female-biased treatment, representing the ecological effects of sex ratio variation and 2) the fishless treatment versus the ‘even’ (50:50 female:male) treatment, representing the ecological effects of addition of mosquitofish as occurs in the context of an introduction or invasion. Mosquitofish introduction is known to have very strong impacts on most of the ecological variables measured, and the main purpose of this latter analysis was to provide a frame of reference effect size to better interpret the relative importance of sex ratio effects.

We employed MANOVA of repeated measures for pelagic (time series) responses. Treatment and Time x Treatment interaction effects were of primary interest. Treatment effects were also tested independently for each time point using student's t-tests, after checking for equality of variances using Levene's test ($\alpha = 0.05$) (Levene 1960). If Levene's test was significant for a given response, then Welch's t-test for unequal variances was used (Welch 1947). Bonferroni corrections were implemented for interpretation of significance for the two non-independent t-tests performed on time-series data (i.e. those performed at both times separately). Benthic responses, excretion rates, and fish biomass were measured once at the end of the experiment, and thus were analyzed using t-tests as above. Analyses were performed in JMP® Pro (11.2.0) and R Programming Environment (3.1.2) (R Core Team 2014). Zooplankton abundance data were log10(x+1) transformed in order to meet assumptions of normality of residuals. A summary of all the above statistical tests is provided in Table S2.

Calculations of effect size (Cohen's *d*, where $d=(M_1 - M_2)/s_{\text{pooled}}$, $s_{\text{pooled}} =\sqrt{[(s_1^2 + s_2^2) / 2]}$, *M*=mean, and s=standard deviation) (Cohen 1992) were used to compare sex ratio effects to the effect of mosquitofish intoduction. For pelagic responses, which were measured twice, we used data from the sampling time point showing the greatest sex ratio effect. *Post hoc* path analyses were used to explore the strength of potential causal linkages among response variables. These analyses were performed on standardized data in R with the package '*lavaan*' (Rosseel 2012). Lastly, we examined whether total fish biomass explained variation in important pelagic responses within grouped sex ratio treatments using simple linear regression.

Results

Female-biased treatments had lower abundances of crustacean zooplankton (dominated by *Daphnia*, *Bosmina*, *Sida*, and calanoid copepods) than male-biased treatments ($F_{1,22}$=11.620, P=0.003). Rotifer abundance (dominated by the family Brachionidae) did not differ between sex ratio treatments ($F_{1,22}$=1.069, P=0.312). Female-biased treatments had higher phytoplankton abundance ($F_{1,22}$=3.953, P=0.059) and higher rates of NPP ($F_{1,22}$=4.819, P=0.039), ER ($F_{1,22}$=12.315, P=0.002), and GPP ($F_{1,22}$=9.441, P=0.006). Although the Sex Ratio effect was nonsignificant for pH ($F_{1,22}$=2.084, P=0.163) and temperature ($F_{1,22}$=2.698, P=0.115), the Time x Sex Ratio interaction effects were significant (pH: $F_{1,22}$=4.400, P=0.048, temperature: $F_{1,22}$=9.333, P=0.006). Overall these results suggest that female-biased populations induced stronger pelagic trophic cascades when compared with male-biased populations by driving a relative decrease in crustacean zooplankton abundance, an increase in phytoplankton abundance, and an increase in ecosystem production and respiration (Fig. 2). Concentrations of NOx and PO4 were not influenced by Sex Ratio (NOx: $F_{1,22}$=0.021, P=0.887, PO4: $F_{1,22}$=1.742, P=0.201) or the Time x Sex Ratio interaction (NOx: $F_{1,22}$=0.078, P=0.783, PO4: $F_{1,22}$=2.046, P=0.167). The t-tests performed to analyze pelagic response trends at each time separately were in general support of results from the MANOVA of repeated measures. Pelagic responses, other than crustacean zooplankton abundance, were significant at week 2 but not at week 4 (Table S2).

Benthic invertebrate samples were dominated by chironomid larvae (98% of all organisms counted). Sex ratio had no effect on their abundance (t_{22}=0.164, P=0.871), total mass (t_{22}=0.499, P=0.622), or mean individual mass (t_{22}=0.154, P=0.879). Mean individual snail mass was larger in female-biased treatments (mean=0.061 g, S.D.=0.025) than male-biased treatments (mean=0.045 g, S.D.=0.013) (Welch's $t_{14.83}$=1.883, P=0.040), but there were no differences in overall snail abundance (t_{22}=1.189, P=0.247) or biomass (t_{22}=0.242, P=0.811). Periphyton accrual was not affected by sex ratio (t_{22}=1.243, P=0.227).

Female-biased treatments had a mean fish biomass of 1.877 g (S.D.=0.1707) and excretion rates of 4.470 µg N per minute (S.D.=1.0197) and 0.485 µg P per minute (S.D.=0.4430), while male-biased treatments had a mean fish biomass of 0.962 g (S.D.= 0.2454) and excretion rates of 1.807 µg N per minute (S.D.=0.4667) and 0.132 µg P per minute (S.D.=0.3445). These differences were significant for biomass of mosquitofish (t_{22}=10.602, P<0.001) and excretion rates for both N (t_{22}=8.225, P<0.001) and P (t_{22}=2.178, P=0.040).

Amphibian eggs were deposited in at least one replicate of each treatment within the first week of fish introduction. However, larval amphibians were only recovered in three replicates of the fishless treatment at week 4. The biomass of tadpoles in those replicates ranged from 0.051 g to 0.289 g.

Mosquitofish addition (relative to fishless conditions) affected all pelagic responses except for NOx concentration (Table S2, Fig. S1). Mosquitofish addition also had no effect on the number of snails ($t_{10}=0.282$, $P=0.784$) and chironomids ($t_{10}=0.772$, $P=0.458$), the mean individual snail mass ($t_{10}=0.444$, $P=0.667$) and the mean individual chironomid mass ($t_{10}=1.031$, $P=0.327$), or the overall snail mass ($t_{10}=0.005$, $P=0.996$) and overall chironomid mass ($t_{10}=1.367$, $P=0.202$). Periphyton accrual was higher in the mosquitofish addition treatment (mean=0.172 $\mu g/cm^2$, S.D.=0.1229) than the fishless (mean=0.049 $\mu g/cm^2$, S.D.=0.0158) treatment ($t_{10}=2.433$, $P=0.035$).

Upper effect sizes were calculated on week 2 data for all pelagic responses except for zooplankton abundance, which was performed on week 4 data. Sex ratio effect sizes for all significant responses were “large” (i.e. greater than 0.8, (Cohen 1992))(Fig. 3). Interestingly, although sex-ratio effects were less than half those of mosquitofish introduction (presence versus absence) female-biased sex-ratio effects nearly always reinforced introduction effects (Fig. 3). Path analyses were performed to investigate the drivers of phytoplankton abundance, NPP, and snail size across all treatments. We found that phytoplankton abundance at week 2 across all treatments with fish was affected by fish excretion rather than by crustacean zooplankton abundance (Fig. S2). NPP was found to be driven by direct effects of both temperature and phytoplankton abundance (Fig. S3). Snail size was related to the number of females present in a mesocosm, but not significantly related to temperature or the amount of primary production (Fig. S4). Linear regressions of significant pelagic responses on biomass were not significant ($\alpha=0.10$) in any case (Table S3).

Discussion

Mosquitofish are a globally introduced freshwater fish showing pronounced sexual and ecological dimorphism and widespread variation in adult sex ratios (Fig. 1, (Krumholz 1948, Pyke 2005)). Female mosquitofish are larger than males, prefer larger food items (Bence and Murdoch 1986, Homski et al. 1994), exhibit higher feeding rates (Reddy 1975, Shakuntala 1977), and spend more time foraging in the presence of other females (Pilastro et al. 2003, Arrington et al. 2009). We therefore predicted that female-biased populations would induce stronger trophic cascades than male-biased populations. Our results show that female-biased populations also exhibit higher nitrogen and phosphorous excretion rates, as expected given the sexual size dimorphism, which could also lead to an increase in trophic cascade strength.

Consistent with this prediction, we found that experimental ponds with female-biased populations had reduced crustacean zooplankton abundances and increased phytoplankton abundances relative to ponds with male-biased populations (Fig. 2). Female-biased ponds had higher water temperatures, likely caused by the increase in turbidity associated with increased phytoplankton abundance, which can increase the absorption of solar heat energy (Mazumder et al. 1990). Female-biased ponds also had higher NPP and pH compared to male-biased ponds. Increases in NPP can be attributed to both the increase in pond temperature and the increase in phytoplankton abundance (Fig. S3). Pond pH increases when primary production reduces the amount of dissolved carbon dioxide, shifting the equilibrium in the aqueous carbonate system (Dodson 2005). While the large effect of mosquitofish introduction on pond temperature and pH has been noted in the past (Hurlbert et al. 1972, Hurlbert and Mulla 1981), our results suggest that sex ratio has a surprisingly large role mediating the magnitude of these effects (Fig. 3).

Early on in our experiment (week 2), the effect of sex ratio for trophic cascades was clear, despite relatively modest effects of sex ratio on crustacean zooplankton abundance. Excretion is known to play a large role in contributing to trophic cascades [43], and thus, treatment differences in excretion could have driven this early cascade (Fig. S2). This trend may additionally highlight the importance of behavioral changes induced by perceived threats of predation (Schmitz et al. 1997). In this case, zooplankton could have reduced their foraging rates to a greater extent in female-biased treatments relative to male biased treatments. Later in our experiment (week 4) the cascading effects of sex ratio diminished, while zooplankton effects greatly strengthened. It is possible that proliferation of grazing-resistant forms of phytoplankton led to the observed reduction in trophic cascade strength through time (Fig. 2, Fig. S1, (Persson 1999)) or that these patterns represent natural cycling of zooplankton and phytoplankton abundances.

Mosquitofish are typically consumers of pelagic and epipelagic resources (Pyke 2005). However, mosquitofish may also consume benthic food resources, especially in the littoral zone. In our experiment, mosquitofish introduction did not decrease chironomid density, which may be due to greater resources available in the pelagic zone of our artificial pond mesocosms. Indeed, the only significant effect of mosquitofish on benthic resources (P=0.04) was for snails. Snails are not a major diet item, yet there is evidence that they are occasionally consumed, especially by large females (Hubbs 1990). Surprisingly, sex ratio influenced the average individual size of snails while fish introduction had no effect on snail size. Predation is known to plastically induce higher growth rates in snails (Crowl and Covich 1990). Female-biased mesocosms had larger snails than male-biased mesocosms, which could be the result of faster growth rates of snails in response to the presence of snail-consuming females. Alternatively, both the higher temperatures and the relatively high rate of primary production in ponds with female-biased sex ratios could have led to faster snail growth. Path analysis suggests that the number of females present had a larger effect on snail size than did temperature or NPP (Fig. S4); however, the mechanism driving this effect remains unclear.

Mosquitofish have been introduced worldwide throughout the past century for mosquito biocontrol purposes, resulting in unintended consequences for native biodiversity. Their negative impacts on amphibians and native fishes have been the subject of extensive study (reviewed in [15]). In our experiment, amphibians deposited eggs in all treatments, but larval amphibians were found only in mesocosms lacking mosquitofish. The introduction of mosquitofish into historically fishless habitats (e.g. isolated springs) has led to declines in native invertebrates as well

(Schaefer et al. 1994). Because of these global impacts, mosquitofish have been nicknamed the 'plague minnow' (Pyke and White 2000) and identified as one of the world's 100 worst invasive species (Lowe et al. 2000). Where mosquitofish have invaded, population control efforts are commonly employed to mitigate such negative consequences. Traditional methods of mosquitofish removal such as the use of minnow traps may selectively remove females due to their larger body size [20]. A recently described control method proposes to use 'Trojan sex chromosomes' to control mosquitofish abundance by generating females that can only produce male offspring, with the goal of creating male-biased populations in order to lower reproductive output and increase the probability of local extinction [46]. Our results suggest that control measures that reduce the relative abundance of females may have added benefits for aquatic communities and ecosystems since it is the females that cause the strongest ecological effects.

Our experimental design sought to control fish density while allowing for natural variation in biomass associated with sexual size dimorphism. Controlling density was necessary because density strongly influences behavior of these social fish (Smith 2007, Cureton et al. 2010). At the same time, including the effects of sexual size dimorphism was important because it is a principal expression of sexual dimorphism in mosquitofish, a widespread form of dimorphism in general, and theoretically important due to its effects on consumption and excretion. Biomass and sex ratio were strongly collinear in our experiment (Fig. S5), suggesting sexual size dimorphism is indeed an important component of our findings, but it is important to recognize that our study design does not preclude effects of other forms of ecological dimorphism. Indeed biomass variation from size dimorphism did not explain a significant amount of variation in pelagic responses within treatments (Table S3). Moreover, one might predict some amount of sex ratio effects tied to other ecological aspects of sexual dimorphism. Empirical evidence suggests females have higher feeding rates than males per unit body size (Reddy 1975, Shakuntala 1977) and spend more time feeding when in the presence of other females (Pilastro et al. 2003, Arrington et al. 2009). In addition, mosquitofish size dimorphism is largely related to differences in post-maturation growth rates. Since excretion rates are determined partly by instantaneous growth rate (Kraft 1992), it is likely that there exists some size-specific sexual dimorphism in excretion rates. Future investigation of sex-ratio effects might thus profitably employ study designs to isolate and estimate the ecological effects of size- and non-size components of sexual dimorphism.

Conclusions

Our work demonstrates that sex ratio variation in ecologically important species showing sexual dimorphism can lead to marked ecological effects. This study adds to a growing literature suggesting that intraspecific variation may be important for shaping ecology (Post and Palkovacs 2009, Schoener 2011, Bolnick et al. 2011). Indeed, sexual dimorphism is one of the most common and well-known forms of intraspecific trait variation in the wild and many populations in nature show marked demographic differences in sex ratios from the commonly assumed expectation of 1:1

(Hamilton 1967, Clutton-Brock and Iason 1986, Mills and Chichester 2005, Rodríguez et al. 2007, Arendt et al. 2014). As such, we suggest that sex ratios may be a common driver of community and ecosystem variation across a wide diversity of organisms and habitat types. We recommend future investigations into other study systems where there is known sexual dimorphism in functional traits, where the focal species is ecologically important (e.g. keystone species, invasive species, dominant species), and where there is substantial sex ratio variation in the wild. Subsequent work with size dimorphic species should aim to isolate biomass-dependent and biomass-independent sex ratio effects. Such work can further inform our understanding of the ecological importance of one of the most common forms of intraspecific trait variation in nature.

Ethics

All handling of vertebrates was approved under UCSC IACUC protocol Palke1306-2.

Authors' Contributions

DF, HA, MK, and EP designed the experiment based on a pilot concept by HA, EP and MK. DF, HA, and TA carried out the experiment and analyzed samples. DF performed statistical analyses. TA created the map. DF and EP led writing of the manuscript with contributions from all authors.

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Figures

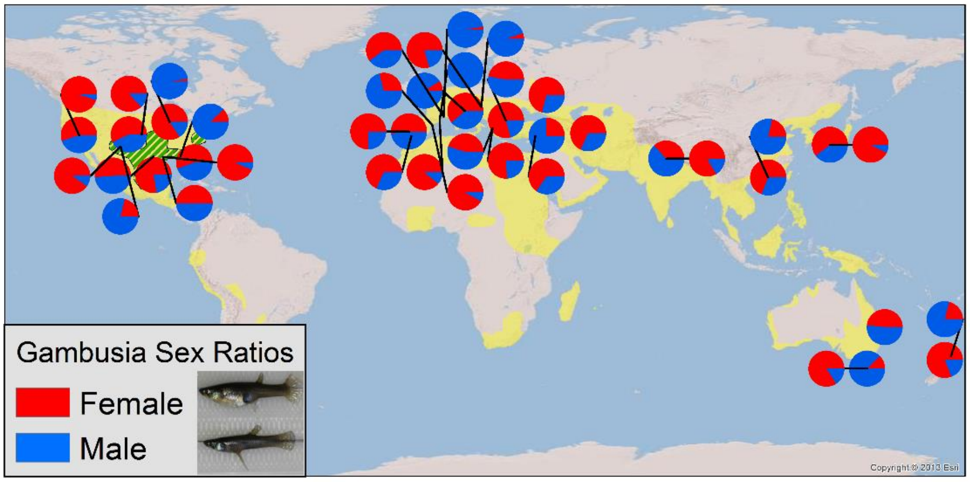

Figure 1 Sex ratio variation over part of the present day range of mosquitofish (*Gambusia affinis* and *G. holbrooki*). These species are both widely introduced for mosquito control and were grouped together into a single species until 1988 (Wooten et al. 1988). Therefore, data for both species are presented. Yellow represents the approximate present day range of mosquitofish, while green hatching represents their native range (modified from (Lloyd et al. 1986), with supplemental data from (Encyclopedia of Life 2015, IUCN Invasive Species Specialist Group 2015)). Their range is likely to be larger than presented due to the unreported nature of historical transplantations. For studies reporting more than two sex ratios (within a region or in a single location through time), two pie charts were plotted representing the most female-biased and most male-biased sample taken. Data and sources are reported in Table S1.

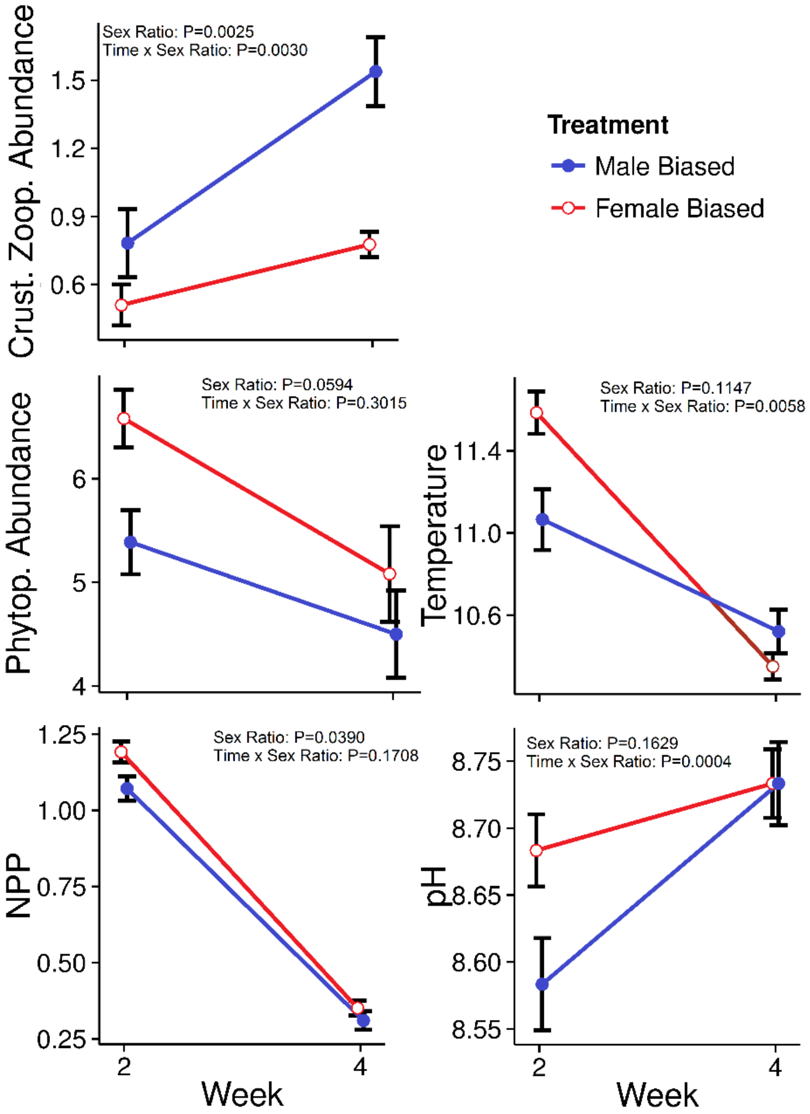

Figure 2 Impacts of mosquitofish sex ratio on the strength of various components of the pelagic trophic cascade (mean ± SE). Ponds with female-biased sex ratios had relatively lower densities of crustacean zooplankton, higher densities of phytoplankton, higher net primary productivity (NPP), higher pH, and higher temperature. P-values are reported for the Sex Ratio and Time*Sex Ratio effects from the MANOVA of repeated measures.

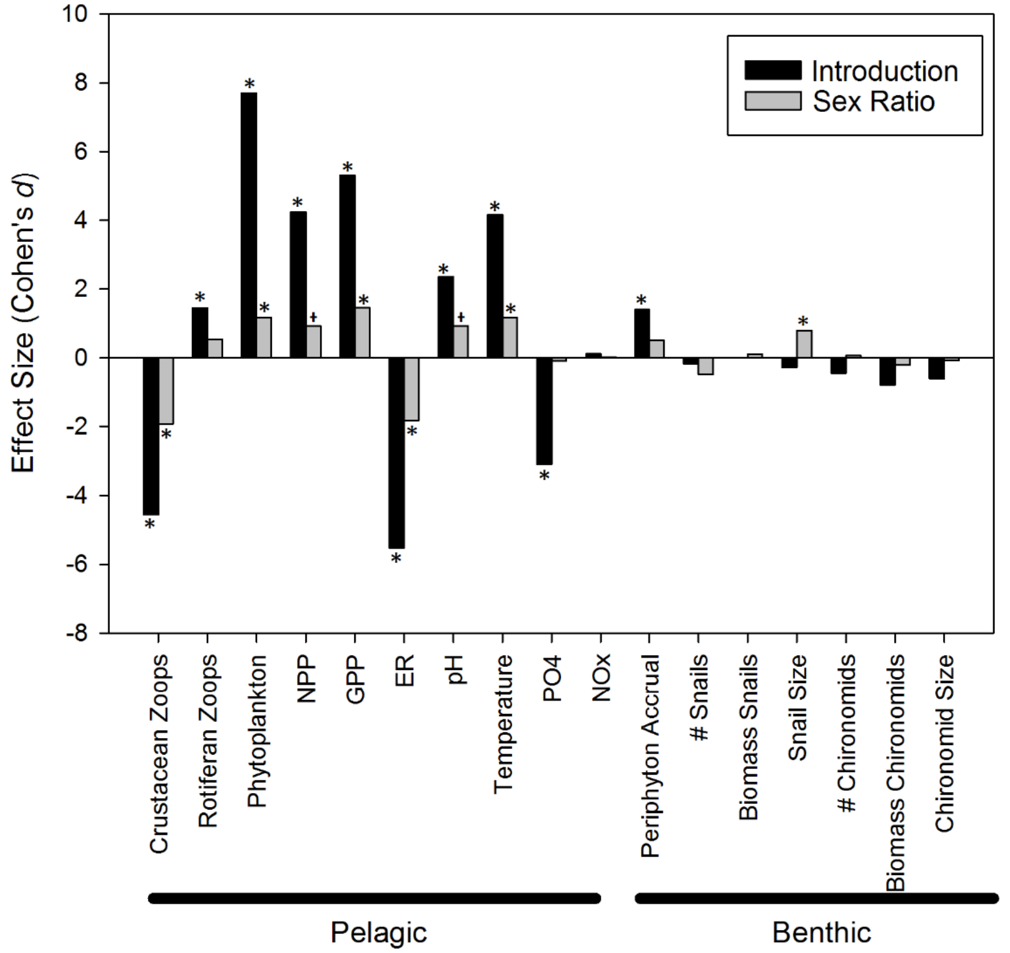

Figure 3 Comparisons of upper sex ratio effect sizes to effect sizes of mosquitofish introduction at an even sex ratio. Significance symbols from t-tests ($p < 0.10^{+}$, $p < 0.05$*) (Table S2) are based on unadjusted p-values for benthic responses and Bonferroni-adjusted p-values (i.e. 2 x p) for Pelagic responses since they were measured twice. Effects going in the same direction (positive or negative) indicate that female-biased treatments exacerbated the effects of mosquitofish introduction relative to male-biased treatments.

CHAPTER 3 - Warming strengthens the ecological role of intraspecific variation in a predator

Abstract

Recent work shows communities and ecosystems can be shaped by predator intraspecific variation, but it is unclear whether the magnitude and direction of these influences are context-dependent. Temperature is an environmental context of strong ecological influence and widespread relevance given global warming trends. Warming should increase per-capita predator effects on prey through increases in predator metabolic rate, potentially exacerbating intraspecific differences in ecological effects. Here, we used two populations of the potent pelagic freshwater predator, Western Mosquitofish (*Gambusia affinis*), to test how experimental pond temperature mediates the differences between their ecological impacts. Mosquitofish introduction induced a strong pelagic trophic cascade, causing a large reduction of crustacean zooplankton biomass, an increase in phytoplankton biomass, and changes to ecosystem-level response variables. Warming (+2° C above unwarmed treatments) exacerbated fish-induced reduction of zooplankton biomass, but moderated the cascade to phytoplankton, primary productivity, and nutrient concentrations. Effects of intraspecific variation were apparent only on zooplankton, and only at warmed environmental temperatures. The traits underlying this divergence may be related to the population source thermal environments. Overall, results show that warming may increase the ecological importance of predator intraspecific variation. In general,

extrinsic environmental drivers, such as those associated with climate change, may reshape the effects of intraspecific trait variation on ecosystems.

Introduction

Intraspecific trait variation in ecologically-important species is becoming a widely recognized potential driver of community- and ecosystem- level characteristics and processes (reviewed in Whitham et al. 2003; Hairston et al. 2005; Fussmann et al. 2007; Bailey et al. 2009; Post and Palkovacs 2009; Bolnick et al. 2011; Matthews et al. 2011; Schoener 2011). Intraspecific effects can be strong even contrasted with traditional ecological factors like presence of a dominant species (Bailey et al. 2009a, Palkovacs et al. 2015, Gómez et al. 2016) and habitat size (Farkas et al. 2013). A typical study design is a “common gardening” experiment (*sensu* Matthews et al. 2011), in which one tests the ecological impacts of intraspecific trait variants in a common environmental context (e.g. Schweitzer et al. 2004; Palkovacs and Post 2009; Ingram et al. 2011; Lundsgaard-Hansen et al. 2014; Fryxell et al. 2015; Rudman and Schluter 2016). However, the role of environmental context in determining the strength and direction of intraspecific effects is not well known. Some studies find intraspecific effects depend on biotic context (i.e. presence of another species or another species’ particular phenotype) (Palkovacs et al. 2009; Ingram et al. 2012; Rudman et al. 2015), but few studies evaluate how intraspecific effects may depend on the abiotic environment, especially in animals (but see El-Sabaawi et al. 2015; Lajoie and Vellend 2015; Tuckett et al. THIS VOLUME).

Because abiotic context strongly shapes ecological interactions (Chamberlain et al. 2014), impacts of intraspecific variation likely also depend on abiotic context.

Temperature is an abiotic variable with profound impacts across levels of biological organization. The ecological influence of temperature is fundamental in that it shapes organismal metabolism (Gillooly et al. 2001, Brown et al. 2004), which itself may help explain higher-level ecological patterns such as biodiversity and carbon-flow through ecosystems (Allen et al. 2002, Schramski et al. 2015). Temperature is highly variable across space and through time, and has been increasing rapidly on average across the globe in recent history (IPCC 2014). Because of its pervasive ecological role and immediate relevance, it is important to understand how temperature may mediate the ecological role of intraspecific variation.

Metabolism increases exponentially with temperature within the range of temperatures typically encountered by an organism (Gillooly et al. 2001), so small increases in body temperature can greatly increase metabolic demand. This increased demand must be met by increased ingestion rates at the individual level (Rall et al. 2012), which, in consumers and predators, could underlie warming-induced strengthening of top-down effects on ecosystems (Sanford 1999, O’Connor et al. 2009, Barton et al. 2009, Barton and Schmitz 2009, Hoekman 2010, Harley 2011, Kratina et al. 2012, Shurin et al. 2012). If feeding-related trait variation occurs among populations, warming-induced increases in per-capita feeding rates could increase the ecological effects differences between populations of ectotherm predators.

Here, we test the prediction that predator intraspecific differences have stronger effects for freshwater communities in a warmed versus an unwarmed environment. We test this prediction using Western Mosquitofish, *Gambusia affinis*, which prey heavily upon crustacean zooplankton in the pelagic zone of ponds (Hurlbert and Mulla 1981, Pyke 2005), causing trophic cascades whereby producer biomass increases, primary productivity increases, and nutrient concentrations decline (Hurlbert et al. 1972, Fryxell et al. 2016). Mosquitofish have been spread globally (Pyke 2008) and today inhabit a wide diversity of environments to which they have acclimated and adapted (Pyke 2005). There is considerable trait variation within and among Mosquitofish populations. Body size variation and sex ratio variation are common, and can mediate a population's ecological effects. An increasing proportion of females, which are generally larger, can induce stronger trophic cascades (Fryxell et al. 2015). Morphological differences among Mosquitofish populations can emerge via evolutionary responses to predation pressure (Langerhans et al. 2004). Mosquitofish are also known to exhibit rapid evolution of life history traits in response to habitat size variation (Stearns 1983) and temperature (Stockwell and Weeks 1999). Such contemporary trait change might also have community and ecosystem effects.

In this experiment, we used wild-caught Mosquitofish from two recently divergent populations of different thermal environment. We crossed three fish treatments (fishless, cool-source, warm-source) with two ecosystem temperature treatments (unwarmed, warmed) to test our predictions that 1) warming exacerbates top-down effects of fish introduction and 2) warming exacerbates the ecological differences between warm- and cool-source fish. Specifically, we expect 1) fish will more strongly suppress zooplankton at warmed versus unwarmed temperatures, which should cascade to affect phytoplankton, productivity, and nutrients, and 2) the ecological differences between populations for these same response variables will be larger at warmed versus unwarmed temperatures. Our use of recently divergent wild-caught fish from populations of different source temperatures may additionally allow us to address how predator trait variation generated along the temperature axis interacts with thermal context to shape ecological conditions.

Materials and methods

Source population

We used Mosquitofish from two geothermal sites near Bishop, CA, USA. The “warm-source” site was Keough’s Hot Ditch (37°15'33.6" N, 118°22'18.5" W) which has a mean temperature of 31.6° C, and the “cool-source” site was an unnamed dammed artesian well (37°21'02.1" N, 118°19'35.7" W) which has a mean temperature 23.7° C, as measured over the same period (Fig. 1). Mosquitofish were introduced to California from a common source population in Texas, USA in 1922 (Lenert 1923). Fish have occupied these particular sites since at least 1980, though gene flow could have occurred until as recently as 2001, when the translocation of Mosquitofish among sites was discontinued by the Owens Valley Mosquito Abatement Program (Bob Kennedy, personal communication). There is little potential for natural gene flow between these sites because they are aquatic islands in a desert landscape, and are both dammed pools upstream from separate tributaries of the Owens River watershed.

Mesocosm setup

We established 36 experimental ponds (1136 L Rubbermaid ® stock tanks) in a 6x6 array at Long Marine Laboratory in Santa Cruz, CA, USA. On 5 August 2013 we filled tanks with city water, evenly spread 19 L of sand each across their bottoms, and placed two cinderblocks ($9.3 \times 19.0 \times 39.3$ cm^3) adjacently in the center of each. We placed a smaller cinderblock ($6.0 \times 8.5 \times 16.0$ cm^3) between the two larger cinderblocks in the center of all ponds as cover and habitat structure for Mosquitofish. On 20 August 2013 we established a biological community in each pond by introducing a 4 L aliquot of a homogenized sediment sample and even aliquots of a large zooplankton sample taken from Antonelli Pond (36°57'18.6" N 122°03'37.8" W), Santa Cruz, CA, USA. The zooplankton community was dominated by crustacean zooplankton *Daphnia*, *Ceriodaphnia*, *Bosmina*, calanoid copepods, and cyclopoid copepods.

Design and treatment application

We assigned treatments in our 3 x 2 factorial design (fishless, cool-source, warm-source x unwarmed, warmed) to the 6 x 6 mesocosm array using latin-squares random assignment. We initiated warming on 12 August 2013 using 300-watt heaters

(Fluval Aquatics ®), placed on the small central cinderblock in ponds assigned the warmed treatment. Similar warming methods have been used in other experiments, and had maintained a ~3° C temperature above unwarmed treatments, with similar patterns of temperature variation between treatments (Kratina et al. 2012, Shurin et al. 2012). This temperature increase is within the range of those expected globally over the next century (IPCC 2014).

We collected experimental fish from both sites using seine and hand nets on 22 August 2013. We transported fish to Santa Cruz, CA and immediately introduced a random subsample of ten individuals to each experimental pond following treatment assignments. This density of Mosquitofish was equivalent to densities used in prior mesocosm experiments, which reflect approximate natural summertime densities (Fryxell et al. 2015, 2016).

Mesocosm sampling

We took repeated samples for water temperature, crustacean zooplankton (hereafter zooplankton) biomass, and phytoplankton biomass. Water temperature was monitored continuously at 15 minute intervals using HOBO Pendant (Onset Corporation ®) data loggers. We sampled mesocosm water for zooplankton and phytoplankton two and four weeks following fish introduction. We collected zooplankton from a 1 L water sample using a 40 μm sieve and preserved them in ethanol. Later, each zooplankter was identified to the lowest taxonomic group possible at 100x magnification, and measured to get the body length using the "Live measurements" module of Leica Microsystems ® Application Suite. Lengths were converted to mass by using published length-weight relationships for each taxonomic group (Bottrell et al. 1976), and masses of all zooplankters within a mesocosm sample were summed to get biomass. We collected phytoplankton from 1 L water samples filtered through Whatman GF/F © filters (pore size 0.7 µm). Filters were placed into a -20° C freezer for later analysis. Chlorophyll *a* was extracted with acetone for 24 hours at 4° C and its concentration analyzed using the non-acid module on a Trilogy Flourometer (Turner Designs ®). Chlorophyll *a* concentration was used as a proxy for phytoplankton abundance.

We took final time point (week 4) samples for net primary productivity (NPP) and phosphate concentrations. NPP was estimated as the change in dissolved oxygen concentration between dusk and the previous dawn (as in Harmon et al. 2009). Phosphate (soluble reactive phosphorus) was measured from 60 mL water samples filtered (0.7 µm pore size), frozen, and then thawed and analyzed on a LaChat ® flow injection analyzer following standard methods (American Public Health Association 2012).

After ecological sampling was complete, we removed fish and euthanized them with an overdose of tricaine methanesulfonate. Thereafter fish were measured for length, counted to get population growth (i.e. number of offspring), weighed to get total fish biomass per mesocosm, and sexed to get the adult sex ratio (proportion males).

Analyses

We used ANOVA to test the significance of warming, fish introduction, and intraspecific variation for each response variable. The “Warming” effect is the difference between the ‘warmed’ and ‘unwarmed’ treatments. Separate ANOVA analyses were used for the independent contrasts of interest – the “fish introduction effect” (FI) and the “intraspecific variation effect” (IV). The FI effect used all treatments but coded both populations as a single “fish present” factor level. The IV effect used only the treatments with fish to test for differences between the effects of the populations. Specifically, we used MANOVA of repeated measures for zooplankton and phytoplankton responses to account for correlations between sampling points, and used ANOVA for NPP and phosphate. All ecological responses were $\log_{10}$ transformed before analyses to improve adherence to ANOVA assumptions, though zooplankton biomass was $\log_{10}(1+x)$ transformed to avoid losing a replicate due to a 0 value. We used Bartlett’s tests to ensure approximate equality of variances for the FI effect, since it was an unbalanced comparison (6 replicates without fish, 12 with fish). For zooplankton, we additionally performed post hoc MANOVA tests for warmed and unwarmed treatments separately. We used principal components analysis (PCA) on correlations of the four transformed ecological responses at the final sampling point to understand how treatments arranged themselves in multivariate space. PCAs were run on data from all treatments, and then for warmed and unwarmed treatments separately.

We calculated standardized effect sizes (Hedge’s G) for the effects of FI and IV separately for unwarmed and warmed treatments to compare the magnitude of effects between community-level responses variables. Effect sizes were calculated across all data (i.e. including data from both time-points). Effect sizes and confidence intervals were calculated using the “cohen.d” function with arguments “hedges.correction=T” and “pooled=T” in the package “effsize” (Torchiano 2016) on the R platform (R Core Team 2015).

To associate population traits with effects, we performed a number of tests. First, we used a t-test to test for population differences in sex ratio at the start of the experiment. Next, we tested how temperature and source population affected final fish biomass and population growth using ANOVA. Lastly, we tested how initial sex ratio, final fish biomass, and population growth may have affected zooplankton biomass using standard least-squares linear regressions, separately for unwarmed and warmed ponds. All ANOVA- and regression-related analyses, and PCAs were performed in JMP Pro 12 (SAS Institute ®).

Results

Warmed treatments (overall mean 25.0° C) were an average 2.21° C warmer than unwarmed treatments (overall mean 22.8° C), and tracked unwarmed diel temperature patterns closely (Fig. 2A). The treatment difference did vary with time, from as little as 1.30° C to as much as 3.60° C, over the course of the experiment (Fig 2B). Diel patterns in the magnitude of warming also emerged, where treatment warming was strongest in morning as temperatures naturally rose, and treatment warming was weakest in the evenings as temperatures naturally cooled (Fig. 2).

Fish introduction (FI) effects were significant at community and ecosystem levels, though there was little evidence for a FI x Warming interaction in most cases. Bartlett's test identified no violations ($p>0.01$) of the heteroscedasticity assumption of ANOVA (Appendix 2), allowing us to proceed with FI tests. FI and Warming reduced zooplankton biomass (FI: $F_{1,32}=2.70$, $p<0.0001$; Warming: $F_{1,32}= 7.03$, $p= 0.0124$; Fig. 3A,B), without a FI x Warming interaction ($F_{1,32}=1.06$, $p=0.3104$; Fig. 3A,B). Univariate tests revealed a reduction in the FI effect through time (Time x FI: $F_{1,32}=8.42$, $p=0.0067$), which was dominated by the unwarmed treatment (post hoc unwarmed only Time x FI: $F_{1,16}=9.18$, $p=0.008$; post hoc warmed only Time x FI: $F_{1,16}=1.08$, $p=0.3143$; Fig. 3 A,B), though the three-way interaction was nonsignificant (Time x FI x Warming: $F_{1,32}=2.13$, $p=0.1540$). We found evidence of a trophic cascade associated with FI, as phytoplankton biomass greatly increased with fish introduction ($F_{1,32}=38.04$, $p<0.0001$; Fig. 3C,D). Warming increased phytoplankton ($F_{1,32}=6.06$, $p=0.0194$; Fig. 3C,D), without an interaction with the FI effect ($F_{1,32}=0.9645$, $p=0.3334$; Fig. 3C,D), despite the result that the FI effect was weaker on average at warmer temperatures (Fig. 3C,D). NPP increased as a result of both FI and Warming, though Warming weakened FI effects (FI x Warming: $F_{1,32}=7.56$, $p=0.0097$; Fig. 4A). Lastly, FI reduced phosphate concentrations ($F_{1,32}=10.2826$, $p=0.0030$; Fig. 4B), and Warming may have moderated this impact (Warming: $F_{1,32}= 3.4948$, $p=0.0707$; FI x Warming: $F_{1,32}=2.7594$, $p=0.1065$; Fig. 4B).

Zooplankton biomass was affected by intraspecific variation (IV), though this effect only emerged at warmed temperatures (IV: $F_{1,20}=2.92$, $p=0.1031$; post hoc unwarmed only: $F_{1,10}=0.2634$, $p=0.6189$; post hoc warmed only: $F_{1,10}=6.04$, $p=.0338$, Fig. 3A,B). We did not detect cascading impacts of this effect, as IV effects were nonsignificant for phytoplankton biomass (IV x Warming: $F_{1,20}=0.03$, $p=0.8571$; IV: $F_{1,20}=0.001$, $p=0.9717$; Fig. 3C,D), NPP (IV x Warming: $F_{1,20}=0.01$, $p=0.9067$; IV: $F_{1,20}=0.04$, $p=0.8531$; Fig. 4A,B), and phosphate concentration (IV x Warming: $F_{1,20}=0.22$, $p=0.6406$; IV: $F_{1,20}=0.42$, $p=0.5244$; Fig. 4C,D).

The first principal axes of the three PCAs (across all mesocosms, or for warmed and unwarmed mesocosms separately) each explained ~60% of the variation in the data, while the second principal axes explained ~20% of the variation. Phytoplankton and NPP had positive loadings on the first axis, while phosphate and zooplankton had negative loadings on the first axis. The second principal axes showed variable loadings across PCAs, suggesting different relationships among responses due to warming. PCAs showed spatial separation of fish from fishless treatments, but significant overlap of the two treatments with fish (Appendix 3).

Effect size calculations showed very strong (>>1) effects of FI and little mediation of FI and IV effects by warming (Fig 5A,B) . For the IV effect, 90 percent confidence intervals overlap with zero except for effects on zooplankton in warmed environments (Fig 5B). Effect sizes generally declined down trophic levels from zooplankton to phytoplankton.

Fish survival was high. Only 2 of the 240 individuals that were stocked at the start of the experiment died over the course of the experiment. Because of this high survival, and because few fish offspring reached maturity by the end of the experiment, we estimated initial sex ratio as the final adult sex ratio in experimental ponds. The cool-source population (M=0.37, SD=0.16) had a significantly higher proportion males than the warm-source population (M=0.21, SD=0.16) (t_{22}=2.34, p=0.0285), though a range of 0.0 to 0.6 proportion males was present across mesocosms in both populations. Despite this, the cool-source population tended to have higher population growth ($F_{1,18}$=9.90, p=0.0056, outliers removed, Fig. 6A) though this was apparent only after removal of two large, influential outlier values, or a log transformation (Appendix 1). Both populations had similar final fish biomasses ($F_{1,20}$=0.78, p=0.388, Fig. 6B). Warming increased fish biomass ($F_{1,20}$=4.53, p=0.0458, Fig. 6B) and population growth ($F_{1,18}$=8.06, p=0.0109, Fig. 6A), with no interaction with source population ($F_{1,20}$=0.20, p=0.6599; $F_{1,18}$=0.06, p=0.9638) (see Appendix 1). Lastly, our linear regressions of zooplankton biomass on sex ratio, fish biomass, and population growth found no significant effects on zooplankton biomass (p>0.10) (Appendix 2). However, the variance in final zooplankton biomass explained by sex ratio (unwarmed: R^2=0.01; warmed: R^2=0.03), fish biomass (unwarmed: R^2=0.04; warmed: R^2=0.16) and population growth (unwarmed: R^2=0.02; warmed: R^2=0.14) was greater in warmed than unwarmed treatments. A summary of all regression and ANOVA-related statistical tests is available in Appendix 2.

Discussion

Warming mediates fish effects

Mosquitofish have strong top-down impacts in pond ecosystems and show considerable trait variation within and among populations across their global range, making them good candidates for tests of the ecological effects of intraspecific trait variation (Pyke 2005). As predicted, Mosquitofish introduction reduced crustacean zooplankton biomass, which induced a trophic cascade whereby phytoplankton bloomed, productivity spiked, and nutrient concentrations declined (Fig. 3,4). These cascading impacts are consistent with prior studies of Mosquitofish-driven trophic cascades (Hurlbert et al. 1972, Hurlbert and Mulla 1981, Fryxell et al. 2015, 2016) and set the stage for effects of intraspecific variation and their interaction with warming.

We predicted warming would increase the strength of the top-down effects of fish introduction. For zooplankton, this prediction was supported by the final time-point, despite the overall effect of fish introduction having no significant interaction with warming (Fig. 3A,B). This time-dependence was driven by changes in unwarmed treatments only, where fishless and fish present treatments converged through time. In warmed treatments, fish effects were stable. This difference may reflect accelerated biological and ecological processes at warmed temperatures (Brown et al. 2004). Beyond four weeks, it is unclear whether zooplankton biomasses would have further converged in unwarmed treatments.

Despite warming-induced increases in fish introduction effects on zooplankton biomass, we did not find that warming increased pelagic trophic cascades from fish to phytoplankton, because warming directly increased phytoplankton biomass (Fig. 3C,D). This result is contrary to another warming study in pelagic freshwater systems, which showed strengthened trophic cascades from fish to phytoplankton under warming, and hence, enhanced top-down control (Kratina et al. 2012). However, in that experiment, warming did not enhance zooplankton biomass reduction by fish (Shurin et al. 2012). Perhaps fish excretion or zooplankton body size were primary drivers of the observed warming-induced trophic cascade in that study. Warming generally increases excretion rates (Vanni and McIntyre 2016) which can increase nutrient supply for phytoplankton growth (Vanni and Layne 1997). Zooplankton community size structure mediates trophic cascades through relationships between body size and grazing rates (Carpenter et al. 1985). In our study, increased fish excretion would not explain warming-induced phytoplankton blooms, as these effects were primarily seen in fishless treatments (Fig. 3C,D). Simultaneous warming-induced increases in phytoplankton and decreases in zooplankton in fishless ecosystems could occur if warming favored defended or toxic phytoplankton phenotypes (e.g. small phytoplankton, gelatinous-sheathed phytoplankton, cyanobacteria). These phenotypes may be favored via increased per-capita predation by zooplankton (Peter and Sommer 2012), and via some combination of nutrients (Vanni 1987) and temperature (Rigosi et al. 2014, Yvon-Durocher et al.

2015). Without phytoplankton trait and/or community composition data, we are unable to directly address this hypothesis.

Increased productivity is a long-hypothesized (Carpenter et al. 1992) and common response to warming (Yvon-Durocher et al. 2015) and fish introduction (Carpenter et al. 1985). Ecosystem-level primary productivity is a process driven by metabolic rates of producers and producer standing stock, and is ultimately fueled by light and nutrient availability. As in similar studies (Shurin et al. 2012), we found warming and fish introduction increased NPP and decreased phosphate (Fig. 4). However, warming slightly reduced the effect of fish on these responses, perhaps because trophic cascades from fish to phytoplankton biomass were weaker with warming. Overall, we found support for our prediction that warming increases top-down effects of fish on zooplankton, but without cascading impacts for phytoplankton biomass and ecosystem characteristics. Bottom-up factors appear to have moderated this impact across trophic levels and to higher levels of organization.

Warming mediates intraspecific effects

We predicted that ecological effects of intraspecific variation would be stronger with warming if top-down effects were stronger with warming. We found intraspecific variation had stronger effects at warmed versus unwarmed temperatures for crustacean zooplankton, but not other responses. Bottom-up factors at warmed temperatures likely prevented cascading effects of intraspecific variation, as they did for fish introduction. However, the effect size of intraspecific variation was considerably smaller than fish introduction (Fig. 5B), so our power to detect its cascading influences was also smaller (Cohen 1992). Even at warmed temperatures, the effect of intraspecific variation was less than half that of fish introduction (Fig. 5B), which is a notably weaker effect than found in many other studies (Palkovacs et al. 2015, Hendry 2016).

The intraspecific effect from our study could be relatively weak for many reasons. Our focal populations are recently divergent (~90 years maximum, likely less), with human-assisted gene flow possible until very recently. Many other studies of intraspecific effects in fishes compare traits variants with a much longer or larger divergence – e.g. alewives at >300 years (Palkovacs et al. 2008), stickleback, which are post-Pleistocene incipient species (Rundle et al. 2000), and guppies, which are probably longer-derived, showing considerable within-drainage genetic divergence and incomplete phenotypic transformation after a 50-years-long transplant experiment (Willing et al. 2010). Second, populations of many fish species exhibit countergradient variation with respect to temperature - where natural selection favors phenotypic similarity across populations in different environments (Conover and Schultz 1995, Angilletta-Jr. 2009). Countergradient variation may have minimized trait differences between our focal populations and minimized ecological effects differences despite potential genetic differences (Kinnison et al. 2015). Third, other experiments are designed with a more thorough understanding of the trait differences between populations (e.g. alewives, guppies, stickleback), allowing implementation of methods aimed specifically at detecting or maximizing the ecological effects of the functional trait differences. Investigators might choose maximally divergent focal populations for ecological effects tests, might focus on contexts (i.e. experimental arenas) hypothesized to maximize effects, and/or might control for many aspects of ecologically-relevant natural variation like sex ratio, body size, and density, so as not to “swamp out” intraspecific effects. Lastly, studies with incomplete knowledge of ecological effects of focal population differences tend to measure a large number of ecological responses, and may emphasize responses showing relatively large intraspecific effects.

The effect sizes we measure in this study may more accurately reflect general intraspecific effect sizes among populations in nature. With little understanding of specific trait differences between our focal populations we were unable to cater our experimental arena for maximizing intraspecific effects. We chose to control only for initial fish density, so other sources of natural intraspecific variation were present. Lastly, we focused on measuring a few responses with well-known linkages and broad ecological relevance (i.e. trophic cascades). Using such a less-biased strategy in picking focal populations and focusing on one or a few ecological mechanisms of general relevance may better inform as to how strong intraspecific effects are more generally in nature.

Traits underlying intraspecific effects

After testing whether the populations diverged in ecological effects, we asked whether we could predict those effects from traits and attributes of known ecological relevance across experimental populations (i.e. mesocosms) – sex ratio, population growth (a reflection of life history and phenological differences), and final fish biomass. Temperature is known to have strong phenotypic effects on Mosquitofish traits like body size and life history (e.g. Vondracek et al. 1988; Meffe 1991; Meffe et al. 1995; Stockwell and Weeks 1999; Stockwell and Vinyard 2000), and may affect sex ratio via sex-specific responses to temperature (e.g. see Seebacher et al. 2015). In our experiment, cool-source fish ended with the same biomass of fish as warm-source populations, but had more male-biased initial sex ratios and had greater population growth overall (Fig. 6). Therefore, at a given temperature treatment, cool-source populations had the same biomass of fish but had more and smaller individuals. Nevertheless, initial sex ratio, population growth, and biomass were each independently not associated with final zooplankton biomass at either warmed or unwarmed temperatures. Despite these factors being nonsignificant predictors of zooplankton biomass, we did find stronger associations (i.e. explained variance) between them at warmed versus unwarmed temperatures, in line with our overall prediction.

We hypothesized that intraspecific effects would be greater at warmed versus unwarmed temperatures because of increased metabolic demand. This general prediction is an over-simplification. Metabolic demand does not increase exponentially over the range of all possible temperatures, and does not do so with the same rate across evolutionary lineages (Gillooly et al. 2001). Further, feedings rates and other ecological rates do not perfectly track this metabolic demand (Rall et al.

2012), usually increasing approximately linearly up until a performance maximum, beyond which performance declines (Angilletta-Jr. 2009). In this study, experimental temperatures were approximately at or below population source temperatures. Theory therefore predicts focal populations were tested within the range that performance should increase with increasing temperature. Cool-source fish were nearer the source temperature of their natural pond (23.7° C) in experimental treatments (unwarmed: 22.8° C; warmed: 25.0° C) than warm-source fish (31.6° C), and greater reduced zooplankton (Fig. 3A,B). Cool-source fish also greater suppressed zooplankton at warmed versus unwarmed temperatures, perhaps because organisms are commonly found to perform optimally at a temperature slightly above that to which they are acclimated or adapted (Angilletta-Jr. 2009). Intraspecific variation in thermal performance curves likely contributes to ecological effects differences at different temperatures.

Eco-evo dynamics and global change

"Eco-evolutionary dynamics" is an emerging worldview that posits evolutionary trait change and ecological change can interact on contemporary timescales (Hendry 2016). Trait responses to anthropogenic drivers can be particularly fast (Hendry et al. 2008a), so eco-evolutionary dynamics may be important in the face of anthropogenic change (Lavergne et al. 2010, Norberg et al. 2012, Urban et al. 2016). A question recently posed by researchers is whether global change drives eco-evolutionary dynamics such that population trait responses buffer or exacerbate further ecological or environmental change (Palkovacs et al. 2012). Tuckett et al. (2017) found that lake eutrophication causes trait changes in white perch that exacerbate nutrient loading. Urban et al. (2017) found that adaptive responses of anuran prey to climate-induced predator range expansion mitigated the predator's impact on prey survival. Trait responses may thus exacerbate or mitigate further change in the face of global change drivers. Our study focused on another potential global change driver of eco-evolutionary dynamics – temperature. We found the population from temperatures more similar to experimental conditions more strongly affected prey community biomass. If trait differences reflected recent adaptation, these results may suggest that predator thermal adaptation can mitigate ecological change by maintaining top-down effects on prey across different temperatures. However, our study only shows how a presumably relatively adapted versus a relatively non-adapted population might impact prey in one temperature range (i.e. both experimental temperatures were near the "home" temperature of one of the populations). Future studies might profitably test whether predator populations recently adapted to different temperatures have ecological effects more similar in their specific respective "home" temperatures than in their "away" temperatures.

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Figures

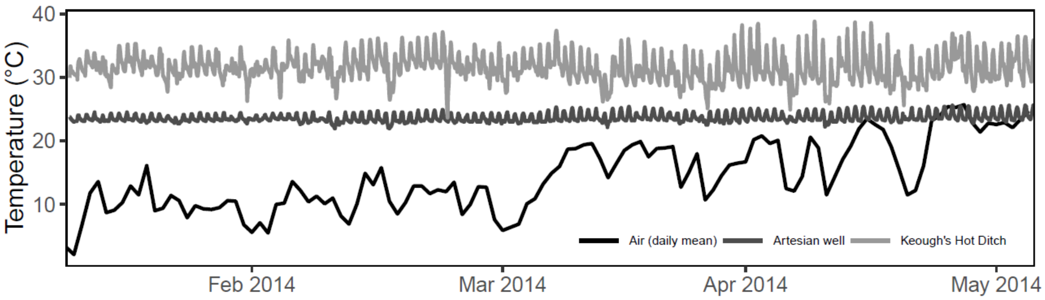

Fig. 1: The temperature profiles of both fish sources used in the study. “Cool-source” fish were taken from the artesian well and “warm-source” fish were taken from Keough’s Hot Ditch, both in Bishop, CA, USA. Daily average air temperatures are plotted from the Bishop Airport weather station, Bishop, CA (Station ID GHCND:USW00023157), measured over the same period. In these geothermal systems, air temperature has little influence on water temperature.

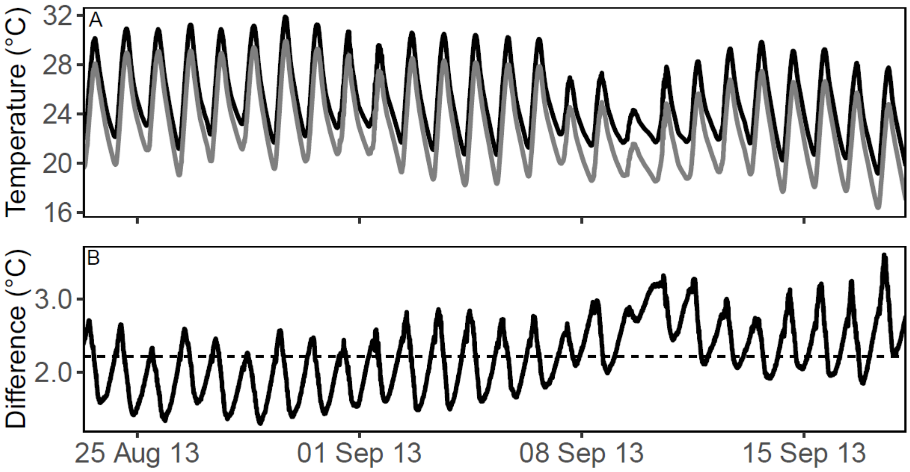

Fig. 2: A) The mean of each logged temperature for both the warmed (black) and unwarmed (grey) treatments. B) The difference between treatment means at each time-point, with the hatched line showing the overall mean temperature increase in the warming treatment: 2.21° C.

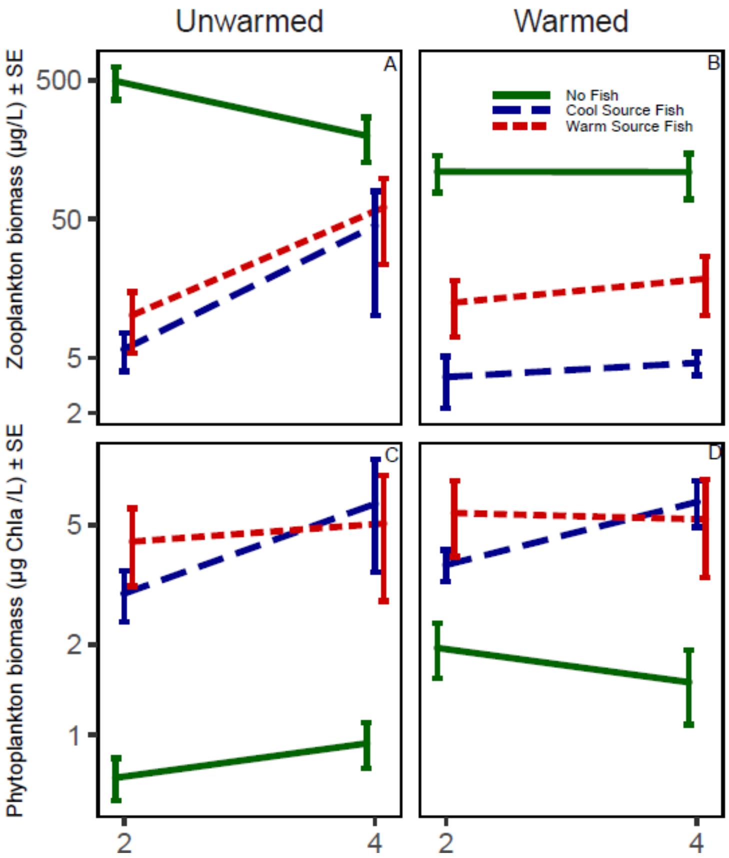

Fig. 3: Responses of zooplankton (A, B) and phytoplankton (C, D) to warming and fish treatments for both sampling points. Y-axes are plotted on the $\log_{10}$ scale.

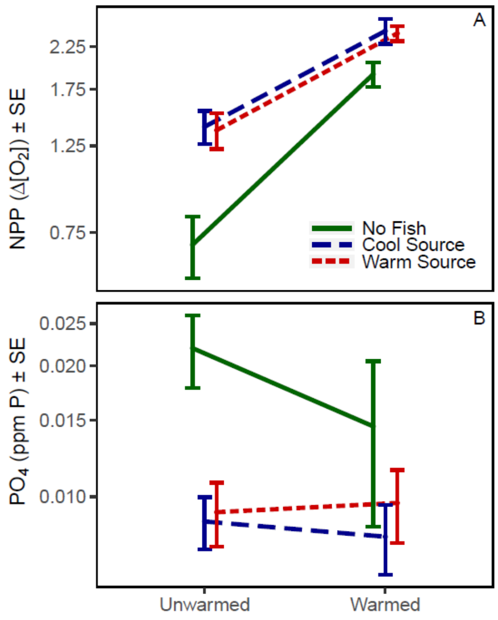

Fig. 4: Response of net primary production (A) and phosphate concentration (B) to warming and fish treatments at the final sampling point of the experiment – four weeks after fish introduction. Y-axes are plotted on the $\log_{10}$ scale.

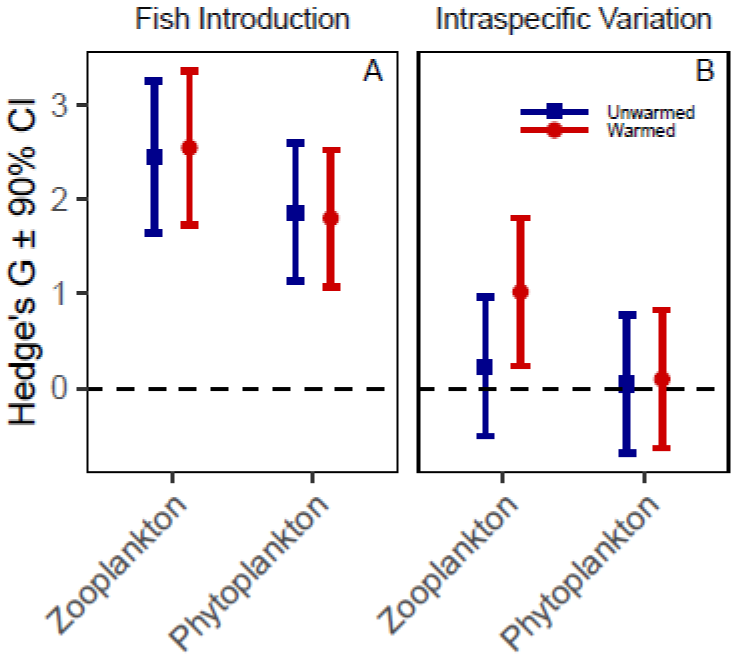

Fig. 5: The absolute value of effect sizes for all responses calculated over all sampling points, separately for A) the fish introduction effect and B) the intraspecific variation effect.

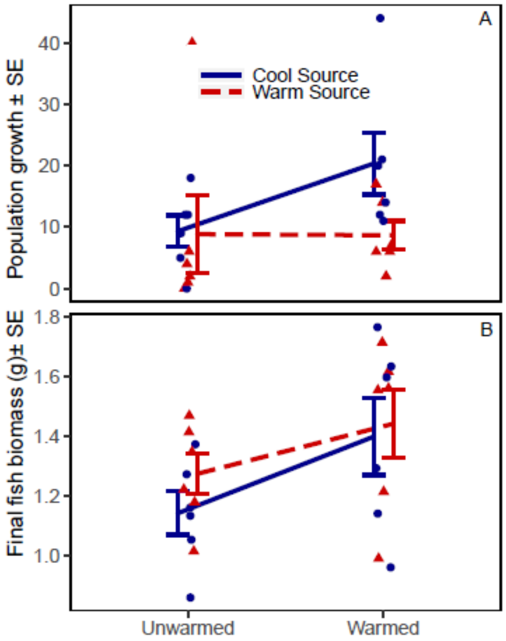

Fig. 6: Trait differences between populations at the end of the experiment. Fish population growth (A) was the number of offspring present at the end of the experiment and total fish biomass (B) was the dry mass of all fish within a mesocosm at the end of the experiment. Means and standard errors were calculated without removing outliers (Appendix 1). Raw data points each reflect a mesocosm (triangles are warm-source, and circles are cool-source).

CHAPTER 4 - Predator adaptation exacerbates community responses to warming

Abstract

Climate change is a strong agent of ecological and evolutionary change. However, little is known about how adaptive evolution occurring on timescales relevant to current climate change may mediate ecological outcomes. We investigated how an influential freshwater predator (*Gambusia affinis*) has adapted to increased temperature over a short time scale. We then tested how this thermal adaptation modifies community ecological responses to warming. Life history theory predicts the evolution of earlier maturity if warming increases mortality via the risks posed by elevated resource demand. Our data supported this theory. *G. affinis* exhibited increased mortality, and evolved slower growth and earlier maturity at higher temperatures. Warming has been shown to cause strong reductions in body size of aquatic invertebrate communities. Thus, we predicted that thermally-adapted predators would evolve increased propensity to consume small invertebrate prey. In support of this prediction, warm-adapted *G. affinis* consumed smaller pelagic zooplankton prey and did not show a strong ontogenetic niche shift to consuming large benthic macroinvertebrates. Finally, we tested the community effects of thermal adaptation in pond mesocosms. Because warm-adapted *G. affinis* grew slower, reproduced earlier, and continued to consume zooplankton throughout life, they exacerbated warming-induced shifts towards smaller zooplankton taxa. This study

reveals that eco-evolutionary processes can lead to dominance of small consumer taxa under warming. Importantly, our results show that ecological models and experiments ignoring the effects of evolution may underestimate climate change effects on some community and ecosystem responses.

Introduction

It is widely predicted that contemporary evolution will shape community responses to climate change (Lavergne et al. 2010, Norberg et al. 2012, Urban et al. 2016), yet little empirical evidence exists to support this claim. The paucity of evidence stems from the challenge of parsing ecological from evolutionary responses in nature (Gienapp et al. 2008, Merilä and Hendry 2014, Kinnison et al. 2015), especially given their potential for bi-directional interactions in the form of eco-evolutionary feedbacks (Fussmann et al. 2007, Post and Palkovacs 2009, Becks et al. 2012, Hendry 2016, Matthews et al. 2016). Controlled experiments have an advantage in this regard, but most current work applies sudden or unnaturally accelerated climate change stressors and measures responses over short time scales (e.g. less than one generation). Such experimental designs rarely permit adaptation *in situ*. This is particularly true for relatively large and long-lived organisms, such as predators, whose adaptation may strongly mediate community outcomes (Post and Palkovacs 2009, Hendry 2016). Evaluating how ecologically important species adapt to climate change stressors over relevant timescales, and whether this adaptation modifies community structure, is an important step toward understanding the potential for evolution to shape the ecological responses to climate change.

Declining body size appears to be a general trait response to climate change (Sheridan and Bickford 2011). A growing literature addresses interactions of changing body size with climate change stressors for predicting community and ecosystem outcomes (e.g. Shackell et al. 2010, Brose et al. 2012, Legagneux et al. 2014, Binzer et al. 2016, Osmond et al. 2017, Lindmark et al. 2018). Some of this work considers smaller-bodied populations surrogates for those adapted to future conditions. While important, the relevance of this strategy may be limited because changing body size is just one possible facet of adaptation to climate change. Selection acts on many traits, not just body size, and changing body size itself can arise from selection on other traits related to life history, including resource demand, growth rate, and age at maturity (Stearns 1992). Changes in these traits may have their own ecological effects. In addition, populations can adapt to both the direct effects of climate stressors as well as the associated suite of community and ecosystem changes (Bradshaw and Holzapfel 2006, Karell et al. 2011, Urban et al. 2017). Thus, predicting how adaptation mediates responses to climate change relies upon understanding how trait responses arise and how the full suite of adapted traits influences ecological interactions.

Warming is one aspect of climate change causing widespread body size declines (Daufresne et al. 2009, Gardner et al. 2011). In part, this response may reflect recent life history adaptation. For ectotherms, warming generally increases growth rates and thus resource demand (Angilletta 2009), increasing the risk of starvation and forcing consumers to make riskier foraging decisions. If warming increases mortality through this risk, life history theory predicts evolution of earlier maturity at a smaller size (Atkinson 1994, Sibly and Atkinson 1994, Berrigan and Koella 1994, Kozłowski et al. 2004). From this perspective adaptation to warming should manifest in two related traits. First, thermal adaptation should counteract warming-induced increased growth rates to offset mortality risks. This prediction is supported by countergradient latitudinal patterns in growth rate (Conover and Schultz 1995), although it is unclear whether these patterns evolve over timescales relevant under current climate change. Second, thermal adaptation should decrease age-at-maturity, as populations experiencing increased mortality should invest in earlier reproduction (Stearns 1992). Evolution of earlier maturity under increased mortality has strong empirical support, and is known to occur over short timescales (Reznick et al. 1996, Olsen et al. 2004). The predicted outcome of warming-induced increased mortality, evolution of slowed growth, and evolution of earlier reproduction is a decline in population body size distribution.

Thermal adaptation in a broader sense might also occur indirectly through warming-induced changes to communities. For example, warming may drive some populations extinct while favoring others. Those populations that persist may evolve in response to not only the direct effects of temperature change, but also the indirect effects of changes in community composition. In many cases, these community shifts will be towards smaller bodied species (Daufresne et al. 2009, Gardner et al. 2011). In the presence of smaller prey, predators might evolve increased propensity to consume small prey items. Evolution of predator feeding traits in response to changing prey communities can occur rapidly (Palkovacs & Post, 2008; Phillips & Shine, 2004), so such evolution might be pertinent over the timescales of climate change. Because thermal adaptation may modify predator life history, body size, and the size of prey consumed by predators, we hypothesize that predator thermal adaptation is likely to mediate the effects of warming on communities.

Here, we test this hypothesis in aquatic ecosystems using the focal predator *Gambusia affinis* (western mosquitofish). *G. affinis* are known to rapidly evolve (i.e. over ecological timescales) in life history traits (Stearns 1983), including in response to temperature (Stockwell and Weeks 1999). *G. affinis* also exhibit strong top-down effects on aquatic ecosystems (Hurlbert et al. 1972). They have particularly strong impacts in the pelagic zone through consumption of crustacean zooplankton, which can cause marked pelagic trophic cascades (Fryxell et al. 2015, 2016). However, as with many fish species, *G. affinis* shift consumption from small to large crustacean zooplankton, and eventually towards larger benthic macroinvertebrate prey through ontogeny (Bence and Murdoch 1986, García-Berthou 1999). Their diet shift towards macroinvertebrates at larger sizes may moderate their strong impact in the pelagic zone. Because thermal adaptation in *G. affinis* may decrease the size of prey consumed, it might also mediate their shift from pelagic (small-bodied) to benthic (large-bodied) prey.

We predict that *G. affinis* exhibits rapid thermal adaptation, thereby modifying its effects on community ecology under warming conditions. We used a multi-step approach to test this prediction. First, we surveyed the life history traits of seven recently diverged *G. affinis* populations along a natural thermal gradient to establish the connection between temperature and life history traits. Second, to determine the contribution of evolution versus plasticity to observed life history trait differences, we common-garden reared a subset of these populations for two generations at different temperatures. Third, we performed a diet experiment with common-reared fish to understand whether thermal adaptation has resulted in consumption of smaller prey via changes to body size and/or size-specific changes to feeding. Finally, we introduced common-reared fish to pond mesocosms for a longer-term experiment, to explicitly test how predator thermal adaptation mediates the ecological effects of warming for pond communities. This final experiment revealed how predator adaptation mediates the direct effects of a climate stressor on communities, parsing ecological and evolutionary aspects of how ecosystems may respond to warming conditions.

Materials and methods

Study sites

Gambusia affinis have been introduced worldwide since the early 20th century for mosquito control, creating a global experiment in rapid adaptation to novel environments (Pyke 2005, 2008). They were introduced into a single pond in

California in 1922 from one or two locations in Texas, and were subsequently spread across the state from that pond through a series of transplantations (Dill and Cordone 1997). Today in central eastern California, *G. affinis* populations exist in geothermally-influenced spring systems. These populations have had 14-93 years (<< 1000 generations) to adapt to local thermal conditions leading up to our study (Appendix S1). We focused on a subset of these systems (n=7), which have natural or manmade barriers (spillover dams) to *G. affinis* migration, hindering gene flow from populations experiencing other temperature regimes. All sites are small, clearwater, spring-fed ponds. Sites are within at least ~70 km linear distance of one another but differ markedly in their temperature profiles (Appendix S1: Fig S1a). Other influential biotic (competitors, predators) and abiotic factors (depth, area, pH, conductivity, dissolved oxygen, nutrients) do not significantly covary with temperature (Appendix S1: Table S1a). For these reasons, we expect population divergence among sites to be principally related to temperature differences.

Field collections and common rearing

We collected *G. affinis* from the seven focal populations in March 2015 using hand and seine nets. Fish were preserved in ethanol, and later sexed and measured for total length. Individuals with a gonopodium were identified as mature males. Size-at-maturity was determined as adult male total length, since males and females mature at about the same size, and males stop growing at maturity (Pyke 2005). We used linear regression to relate mean site temperature (Appendix S1: Fig S1a) to size-at-maturity across populations. This regression used raw size data, in effect weighting the regression by the number of observations per population.

We chose two focal populations for common garden rearing and experimentation - “Artesian Well” (mean 23.7° C, “cool-source”) and “Keough Hot Ditch” (mean 31.6° C, “warm-source”). We chose these populations because they spanned the temperature range we could maintain in the lab environment, and they were within the range of expected experimental pond temperatures (see below). We collected F0 (field generation) fish in February 2014 from both sites with seine and hand nets. We immediately transported fish to the lab for common rearing. Fish were held in the lab at 27° C (intermediate between fish source temperatures) through the F1 (first lab) generation. We reared F1 fish to standardize the parental environment to control for maternal effects on F2 (second lab generation) phenotype. We collected one group of F2 fish from these F1 parents each for three experiments. Fish were fed flake food *ad libitum,* supplemented with brine shrimp nauplii (zooplankton, *Artemia* sp.) and frozen chironomids (benthic midge larvae, *Chironomus* sp.). See Appendix S2 for details on rearing.

Experiment 1: Trait assay

For trait assays, we raised F2 fish at three temperatures (23°, 27°, 31° C) under otherwise identical conditions. Rearing at multiple temperatures may allow inferences of local adaptation through evaluation of thermal performance curves (i.e. thermal reaction norm) (Angilletta-Jr. 2009). Groups of four fish born on the same day were transferred to their rearing temperature and measured for mean length at ages 0, 15, and 30 days. We used twelve replicate groups per population x rearing temperature. At the end of rearing, mature males were identified, and all fish were later euthanized and measured for total length. Growth rates were estimated as slope regression coefficients from size-at-age data for each group of fish, after determining that growth was approximately linear (Appendix S2e). To test for population source and temperature effects on growth, we fit a multiple linear regression model with a population source predictor crossed with a quadratic rearing temperature predictor using the poly() function in R version 3.4.1 (R Core Team 2017) to create orthogonal polynomials. This approach represents one type of thermal performance curve (Angilletta Jr 2006).

Experiment 2: Diet experiment

To test for effects of thermal adaptation on diet, we performed a diet study in pond mesocosms. We used a pond diet experiment instead of controlled feeding trials to evaluate diet in an ecologically realistic setting. We established 36 pond mesocosms (1135 L mesocosms) in a 6 x 6 array in late June in Santa Cruz, CA, with city water, local pond sediment, and a rich inoculum of local pond zooplankton (Appendix S4: Fig S4a). Mesocosms were open to oviposition by macroinvertebrates. We used 12 of the 36 pond mesocosms for the diet study. The twelve mesocosms we used were those which had been previously been assigned the fishless treatment for the experimental pond study (see below). Half the mesocosms in each treatment were warmed (~2 °C above ambient) with 300 watt heaters (Appendix S5: Fig S5A). However, we did not expect temperature to affect fish diet independently of effects on prey availability, so we did not use temperature as a predictor in statistical models. Six weeks after we established the mesocosms, we introduced populations of ten F2 fish to each pond (for rearing conditions see Appendix S2d). Fish in each group of ten were chosen to span a broad range in body sizes. Each mesocosm had fish from one population source, resulting in six replicate mesocosm s per population source. At the start of the experiment, we sampled the benthic communities with a centrally-placed 18 cm diameter sediment core to estimate benthic prey availability. Benthic samples were preserved in ethanol. Fish were left in mesocosm s for ten days to ensure acclimation to the mesocosm environment. On day ten, we captured, euthanized, and froze fish for later diet analysis. We found no evidence that fish had reproduced during the diet experiment. We then took a 2 L depth-integrated zooplankton sample (>80 µm) in each pond to get an estimate of zooplankton availability. Zooplankton were preserved in ethanol.

We analyzed diet contents and prey availability in the lab. First, we measured the length of each fish and removed their guts to examine diets. We ranked gut fullness on a scale of 1-5 based on gut distension. We spread diet contents of each fish on a petri dish labelled with 1 mm^2 grids, and we then lightly pressed a cover slip over contents to a uniform depth (Brooker et al. 2010). Under a dissecting microscope, we counted the number of grids occupied by the diet contents in total and by each cladoceran zooplankton (pelagic prey) and chironomids (benthic prey). We then measured the length of up to 20 cladoceran zooplankton in the diet using an ocular micrometer. Finally, to assess availability, we quantified mesocosm sample abundances of pelagic cladocerans and benthic chironomids. We also quantified the body length of each available cladoceran using an ocular micrometer, as in the diet sample analysis above. We focused on pelagic cladocerans and benthic chironomids because of their ecological importance in pond ecosystems and prevalence in *G. affinis* diets. We focused on the size of cladocerans as opposed to the size of all crustacean zooplankton (i.e. including copepods) because cladocerans remained intact and unbent after consumption.

We tested for effects of population source on diet responses. Because individuals within a pond were not independent, we averaged responses across all individuals in each mesocosm (i.e. took “mesocosm means”) before analysis (Murtaugh 2007). First, we used a t-test to determine if gut fullness differed by population source. Second, we tested how population source interacted with prey availability to predict diet contents. We focused on three diet responses: mean cladoceran length, the proportion of the total gut content grids with cladocerans, and the proportion of the total gut content grids with chironomids. Availability metrics were mean cladoceran length, total cladoceran count per 2 L sample, and total chironomid count per 18 cm diameter core, respectively. We used multiple linear regression to test the effect of availability x population source on each diet response.

We also constructed linear mixed models to understand the effect of population source x fish body size on diet responses. We included a random intercept for mesocosm ID to account for nonindependence across individual fish within a mesocosm. Focal diet responses were cladoceran size selectivity (the mean body length of an individual's diet cladocerans minus the mean length of available cladocerans), the proportion of the total gut content grids with cladocerans, and the proportion of the total gut content grids with chironomids. We excluded individual fish with less than three diet cladocerans from the analysis for cladoceran size selectivity. Model assumptions were evaluated with diagnostic plots. We arcsine square-root transformed proportional responses to improve heteroscedastity and to achieve normality of residuals (Appendix S4: Fig S4c). Mixed models were created using the lmer() function in the lme4 package (Bates et al. 2015). ANOVA tables were constructed with Satterthwaite approximations for degrees of freedom using the anova() function in the lmerTest package (Kuznetsova et al. 2016).

Experiment 3: Pond mesocosm experiment

We raised a large group of F2 fish under common conditions at 27° C until maturity to study how predator thermal adaptation may cause community ecological divergence under warming. We established 36 pond mesocosms (1135 L mesocosms) in a 6 x 6 array in late June in Santa Cruz, CA, with city water, local pond sediment, and a rich inoculum of local pond zooplankton (Appendix S4: Fig S4a). We used a 2 x 3 factorial design of temperature (unwarmed, warmed) x fish treatment (cool-source fish, warm-source fish, or a fishless reference). The fishless reference was used as a reference for effect size calculations (see below). Treatments were assigned to the array using latin-squares assignment. Warmed treatments were heated 2.12° C above ambient (Appendix S5:Fig S5a) with a benthic, centrally-placed 300 watt heater (Greig et al. 2012, Kratina et al. 2012, Shurin et al. 2012). Warming was initiated just prior to seeding the pond mesocosms. We introduced populations of ten adult *G. affinis* per fish treatment two weeks after seeding. These fish were 2-3 months old (Appendix S2c). Population sex ratio was controlled at 7 females to 3 males, which is a commonly observed sex ratio in the wild (Fryxell et al. 2015). Other than sex, individual fish were haphazardly chosen from rearing tanks to create experimental populations. We terminated the experiment four weeks after fish introduction.

We sampled pond mesocosms for pelagic consumer and producer community responses weekly. We sampled benthic communities once, at the end of the experiment. We focused on pelagic responses because effects of *G. affinis* in the pelagic zone of pond ecosystems tend to be much stronger than in the benthic zone and because benthic sampling represented a larger disturbance which had the potential to alter ecological conditions (Fryxell et al., 2015, 2016; Hurlbert et al., 1972).

Zooplankton (>80 µm) were filtered from a 2 L depth-integrated water sample and preserved in ethanol. Each zooplankter was later identified to the lowest taxonomic level possible at 100x magnification (Appendix S5: Fig S5b), and measured for body length using an ocular micrometer. Thirty individuals per taxon were measured for length per sample. Thereafter, each remaining zooplankter was counted and assigned the average length for that taxon from the sample. We calculated two zooplankton community responses. First, to determine the extent to which fish depleted pelagic prey, we estimated crustacean zooplankton biomass using published length-mass regressions for each taxonomic group (Bottrell et al. 1976). Second, we calculated the proportion of all zooplankters that were rotifers. Rotifers are a small-bodied taxon of zooplankton (Appendix S5: Fig S5b) that are not commonly consumed by adult *G. affinis,* and tend to bloom after *G. affinis* deplete cladocerans (Hurlbert et al., 1972; Hurlbert & Mulla, 1981; Lancaster & Drenner, 1990). We did not test for treatment effects within narrower zooplankton taxonomic groupings because we were interested in community-level responses.

To test for potential cascading effects of predator adaptation, we measured producer responses. Phytoplankton were filtered from a 1 L depth-integrated water sample on 0.7 µm mesh and frozen. We estimated phytoplankton concentration as the concentration of pelagic chlorophyll *a*. We extracted chlorophyll *a* from phytoplankton filters with acetone for 24 hours at 4° C, and determined concentration fluorometrically using the non-acid module on a Trilogy® fluorometer (Turner Designs, USA). To estimate filamentous algal abundance, which grew into the pelagic zone and became a dominant feature in many ponds (Fig S5e in Appendix S5), we took level top-down photos of each experimental pond weekly. We used ImageJ (Schneider et al. 2012) to estimate the proportion of the area of each pond occupied by filamentous algae.

We analyzed time-series responses (zooplankton, primary producers) with mesocosm ID as a random intercept in linear mixed effects models to account for non-independence of observations within a pond over time. Models took the form *response ~ temperature * population source * time + 1|mesocosm ID*. Time was treated as a factor. Model assumptions were evaluated with diagnostic plots. Zooplankton biomass, zooplankton body size, and phytoplankton concentration were $\log_{10}$ transformed to improve heteroscedastity and to achieve normality of residuals (Appendix S5: Fig S5c,d). Models were created using the lmer() function in the lme4 package (Bates et al. 2015). ANOVA tables were constructed with Satterthwaite approximations for degrees of freedom using the anova() function in the lmerTest package (Kuznetsova et al. 2016).

During the final sampling, we additionally sampled benthic communities and fish populations. We counted and identified to the lowest taxonomic level possible all macroscopic organisms from a randomly placed 18 cm diameter benthic core in each experimental pond. Communities from benthic cores were dominated by chironomid larvae (89.5% of all individuals across treatments). We thus focused on abundance of this taxon and tested for effects of temperature x fish population source on chironomid abundance using ANOVA. At the final timepoint we euthanized fish, counted fish population sizes, and measured fish population biomass. We used ANOVA to test for the effect of temperature x population source on fish population wet biomass, total fish abundance, and total adult fish abundance.

Lastly, to evaluate the relative strength of effects within and across experiments, we calculated standardized effect sizes. We calculated this for three effects – predator addition (fish relative to fishless), warming (warmed relative to unwarmed, for treatments with fish), and predator thermal adaptation (warm-source relative to cool-source). We calculated the overall effect size across all individuals or sampling time points with the cohen.d() function using the arguments hedges.correction=T and pooled=T in the package effsize (Torchiano 2016). Data were scaled as in statistical models (e.g. $\log_{10}$ transformed).

We performed all analyses in R version 3.4.1 (R Core Team 2017). We considered significance tests with $p<0.10$ to be of interest.

Results

Field survey and experiment 1: Life history divergence

The field survey revealed a decline in fish size-at-maturity with increasing pond temperature. Size-at-maturity declined at a rate of 0.04 cm/°C (Fig 1a, slope=-0.040, $R^2=0.502$, $F_{1,188}=189.6$, $p<0.001$, see also Appendix S1). The focal cool-source population (Fig 1a, blue points) matured at a mean size of 2.78 cm. The focal warm-source population (Fig 1a, red points) matured at a mean size 15.9% smaller, at 2.33 cm.

In the trait assay common rearing experiment, there were no population differences in survival. However, fish mortality was highest at the highest rearing temperature. Of 48 fish per population x temperature, cool-source fish had 2, 0, and 6 mortalities, and warm-source fish had 1, 0 and 5 mortalities at 23°, 27°, and 31° C rearing temperatures, respectively.

Warm-source fish evolved smaller size-at-age and slower growth than cool-source fish. Populations did not differ in size at birth, but exhibited evolutionary divergence in size-at-ages 15 and 30 days (Appendix S3: Fig S3a), and thus showed evolution of growth rates (Fig 1c). By age 30 days, warm-source fish were an average 9.7% smaller (shorter) than cool-source fish. The ANOVA predicting growth rate showed a strong effect of temperature ($F_{2,66}=44.68$, $p<0.001$) and population source ($F_{1,66}=37.45$, $p<0.001$), but no interaction between the two ($F_{2,66}=0.9717$, $p=0.384$). Thus, the response to temperature did not differ by population source. Instead, the warm-source population grew consistently slower at all temperatures, consistent with a countergradient pattern of selection (Fig 1c). These growth differences remained similar when we excluded data from groups with at least one mature male (which matured and stopped accumulating size sometime between ages 15 and 30 days) (Appendix S3: Fig S3b, Table S3a). Interestingly, cool-source fish had only slightly slower growth reared at their source temperature (mean 0.027 cm/day at 23° C) than warm-source fish reared at their source temperature (mean 0.032 cm/day at 31° C) ($t_{22}=-2.038$, $p=0.054$).

Warm-source fish evolved younger age-at-maturity and smaller size-at-maturity than cool-source fish. At age 30 days, 21%, 96%, and 60% (assuming birth sex ratios were 50:50 (Krumholz 1948)) warm-source fish had reached maturity in the 23°, 27°, and 31° C temperature treatments, respectively. At the same age, despite that they were, on average, larger, only 4%, 8%, and 0% cool-source fish had reached maturity at 23°, 27°, and 31° C temperatures, respectively. We euthanized all fish to assess size-at-maturity several days after the last-born F2 fish were 41 days old. Fish were thus between 41 and 65 days old, were of identical age distributions among temperatures within population, and were of non-significantly different age distributions between populations (Kolmogorov-Smirnov test, D = 0.17, p=1.0, Appendix S2e). Every mature warm-source fish was smaller than the smallest mature cool-source fish at each rearing temperature (Fig 1b).

Experiment 2: Diet divergence

We did not detect differences in the gut fullness between population sources, but we did find differences in the size and type of prey consumed (Fig 2,3). Gut fullness did not differ by population source (t_{10}=0.278, p=0.787, Appendix S4: Fig S4b). Both population sources relied heavily on pelagic cladoceran and benthic chironomid prey. 110/117 fish had cladocerans and 94/117 had chironomids in their diet (3 fish were not recovered), and these taxa were consumed in high proportions (Fig 2, 3). One availability sample yielded no cladocerans (Fig 2b), so we excluded it from the analysis predicting the size of cladocerans in diets (Fig 2a). Population source interacted with availability to determine the size of consumed cladocerans ($F_{1,7}$=9.234, p=0.019, Fig 2a). Cool-source fish consumed larger cladocerans than warm-source fish, except when large cladocerans were not available. Warm-source populations consumed the same mean size cladocerans (340 µm mean length across all fish) regardless of availability. Availability, population source, and their interaction each did not predict the mean proportion of diet contents with cladocerans or chironomids ($p>0.10$, Fig 2b,c). Linear mixed effects models did not detect effects of fish body size, population source, or their interaction on selectivity for cladoceran body size ($p>0.10$, Fig 3a). However, fish body size interacted with population source to predict the proportion of diets with cladocerans ($F_{1,107.4}=2.812$, $p=0.096$) and chironomids ($F_{1,107.9}=9.993$, $p=0.002$). Warm-source fish shifted relatively weakly away from cladocerans and towards chironomids with increasing body size (Fig 3b,c). Full ANOVA tables for diet analyses are available in Appendix S4: Table S4a,b.

Experiment 3: Ecological divergence

Warming (mean +2.12° C, Fig S5b) and fish thermal adaptation caused additive shifts in zooplankton communities (Fig 4). Except for seven ostracods we encountered, all zooplankton in mesocosms were either rotifers or crustaceans. The mean length of rotifers was 141 µm while the mean length of crustacean zooplankters was 513 µm (Appendix S5: Fig S5b). Warming caused a decline in crustacean zooplankton biomass ($F_{1,20}=20.84$, $p<0.001$) and a community shift towards rotifers ($F_{1,20}=6.472$, $p=0.019$). Fish thermal adaptation (warm-source relative to cool-source) additively exacerbated the warming-induced decline in crustacean zooplankton ($F_{1,20}=3.399$, $p=0.080$) and the community shift towards rotifers ($F_{1,20}=3.416$, $p=0.079$). Interactions between warming and adaptation for these responses were nonsignificant, though three-way interactions between time, adaptation, and warming, and two-way interactions between warming and time neared significance (see full ANOVA tables for zooplankton responses in Appendix S5: Table S5a).

We detected effects of warming but not fish thermal adaptation ($p>0.10$) on producers (Appendix S5: Fig S5e). Warming interacted with time to predict phytoplankton concentration ($F_{3,60}=3.802$, $p=0.015$); unwarmed treatments had more phytoplankton during the first two weeks of the experiment whereas the warmed treatments had more phytoplankton during the last two weeks of the experiment. However, phytoplankton concentrations were generally low, and filamentous algae was instead a dominant feature of many mesocosms. Warming increased filamentous algae ($F_{1,20}=5.455$, $p=0.030$). Full ANOVA tables for producer responses are available in Appendix S5: Table S5b.

Warming and fish thermal adaptation affected the dominant benthic macroinvertebrate – chironomids (Fig 5a). Warming caused a decline in chironomids ($F_{1,20}=3.114$, $p=0.093$). Cool-source fish treatments had fewer chironomids than warm-source fish treatments ($F_{1,20}=4.255$, $p=0.052$). Fish source did not interact with warming ($F_{1,20}=1.628$, $p=0.217$).

Fish populations grew in pond mesocosms from the ten adult fish initially stocked. Final fish biomass did not significantly differ by treatment ($p>0.10$, Fig 5b). Final fish counts were higher at warmed temperatures ($F_{1,20}=13.02$, $p=0.002$), with no differences in or interaction with source population ($p>0.10$, Fig 5c). Thus, on average, warmed mesocosms had more and smaller fish (Fig S5f). Consistent with their decreased age-at-maturity, warm-source populations had higher final adult counts than cool-source populations, particularly at warmed temperatures (population x temperature interaction: $F_{1,20}$=4.038, p=0.058, Fig 5d), as more of their offspring reached maturity by the end of the experiment. Full ANOVA tables for final time point responses are available in Appendix S5: Table S5c.

Effect size calculations allow a comparison of the strength of effects across experiments. Fish thermal adaptation caused strong declines in growth and size at maturity and a moderate decline in prey size, but had weaker effects on zooplankton community responses (Fig 6). Fish addition, warming, and fish thermal adaptation had the same effect direction on zooplankton communities, but of different magnitudes. The effect size generally weakened from fish addition to warming to fish thermal adaptation (Fig 6).

Discussion

Humans are causing rapid trait changes in animal populations worldwide (Hendry et al. 2008b, Alberti et al. 2017), yet trait changes have been incompletely accounted for in short-term experimental tests of community ecological responses to climate change. Namely, populations have the potential to adapt, changing ecologically-meaningful traits and possibly mediating ecological responses (Lavergne et al. 2010, Palkovacs et al. 2012, Norberg et al. 2012, Urban et al. 2016). We tested this idea using a widespread freshwater predator (*Gambusia affinis*), and found that evolutionary thermal adaptation occurring on ecological timescales affects the community outcomes of warming. In natural ponds, recently introduced *G. affinis* populations declined in size-at-maturity with increasing pond temperature. Common rearing showed an evolutionary basis to this divergence, supporting the prediction that thermal adaptation can lead to body size declines under warming. Indeed, warm-source populations evolved relatively slower growth and younger age-at-maturity, resulting in smaller body size. In a diet experiment, we found that thermal adaptation caused shifts toward smaller zooplankton prey consumption and a weaker niche shift towards large benthic macroinvertebrate prey at large fish sizes. Finally, in pond mesocosms, predator thermal adaptation exacerbated warming-induced shifts towards small zooplankton taxa. Importantly, the effect size of predator thermal adaptation approached that of warming for consumer responses (Fig 6), suggesting predator thermal adaptation may play an important role in shaping community responses to climate warming. Our results reveal an eco-evolutionary pathway to observed "universal" body size declines under climate change (Daufresne et al. 2009, Gardner et al. 2011, Sheridan and Bickford 2011). We demonstrate warming-induced body size declines are shaped by interacting ecological and evolutionary mechanisms: 1) warming causes the evolution of smaller predator size, 2) warming changes community composition and shifts consumer communities to smaller size, 3) availability of smaller-bodied prey selects for predators that consume smaller prey items, and 4) these factors together favor an even smaller consumer community. Thus, our results suggest that ignoring effects of predator adaptation may yield underestimations of the true extent of body size declines and ecological shifts under climate change.

Adaptation to warming

Predicting trait responses to warming requires understanding the mechanisms underlying trait changes. There has been a strong emphasis on plasticity because many traits show plastic responses to temperature (Dell et al. 2011, Merilä and Hendry 2014). The "temperature-size rule" describes the propensity of warm-reared individuals to mature at a smaller size and a younger age despite faster growth than cool-reared individuals (Atkinson 1994). In our trait assay, fish did not neatly follow this rule. Most fish matured by age 30 days at the intermediate temperature (27° C), revealing a nonlinear temperature-size response. On the other hand, rapid evolution played a clear role in reducing age- and size- at-maturity. Warm-source fish had considerably slower growth, shorter times to maturity, and smaller body sizes at each rearing temperature. Clearly, evolutionary responses to temperature can operate on timescales relevant to current warming and may contribute to observed body size declines. Generally, the temperature-trait response curve (i.e. thermal reaction norm) generated from individuals of a single population may be of little utility for predicting future trait responses, because populations adaptively evolve.

Evolution can shape thermal reaction norms to offset environmental effects of temperature on phenotype, an idea clearly illustrated by examples of countergradient variation in nature (Conover and Schultz 1995). In our study adaptation to warming opposed the environmental effect of warming on growth rates. Recent work shows evolution of countergradient variation can occur rapidly for metabolic traits related to growth as well (Padfield et al. 2016). Other recent work suggests that the pace of life history rapidly evolves in tandem with metabolic rate (Auer et al. 2018). In alignment with these findings, our other work in five of the populations used here suggests local adaptation results in countergradient variation in routine metabolic rate (Moffett et al. in review). Thus, the evolved reduction in growth rate under warming found here may correspond to an evolved reduction in metabolic rate.

The evolved reduction in growth rate under warming almost completely compensated for the environmental effects on growth rate; warm-source fish reared in their home temperature grew at similar rates to cool-source fish at their source temperature, despite the 8° C difference. To put this into perspective, within-genotype or within-population responses to 10° C increases (i.e. Q10) commonly yield more than a doubling of physiological rates like growth across a diversity of taxa (Dell et al. 2011). Models predicting responses to climate change generally assume these invariant species-level Q10 values. If rapid evolution of countergradient variation in growth and metabolic traits is common, models projecting climate change responses based on invariant species-level Q10's for these traits will produce substantial over-estimates of future physiological rates. While some other work shows seemingly opposite (i.e. cogradient) patterns (e.g. Schaum et al. 2018), we emphasize that the otherwise cryptic pattern of countergradient variation can only be elucidated by isolating the genetic component to phenotypic variation (i.e. through common garden rearing).

Understanding *why* traits evolve (i.e. the agents, targets, and correlated responses to selection) will help us to predict when traits will evolve in response to climate change. The widespread tendency of genotypes, populations, species, and communities to exhibit smaller body sizes at higher temperatures suggests that small body size is adaptive at higher temperatures (Angilletta 2009). Life history theory predicts this downsizing response if fast growth under warming increases mortality (Atkinson 1994, Sibly and Atkinson 1994, Berrigan and Koella 1994, Kozłowski et al. 2004). Our data support this idea. First, mortality in the trait assay was highest at the warmest rearing temperature. Second, warm-source fish evolved a reduction in growth rate, presumably moderating the growth-induced increased mortality experienced at higher temperatures. Fast growth could increase mortality for a variety of reasons. Most obviously, rapid growth increases metabolic demand. Heightened resource demand decreases the time to starvation. In the presence of predators, this mortality risk is heightened; animals are forced to spend more time foraging, increasing their vulnerability to predation (Lima and Dill 1990). In this way, rapid evolutionary responses to increased mortality induced by predation (Reznick et al. 1996) and harvest (Olsen et al. 2004) may inform the study of thermal adaptation (Waples and Audzijonyte 2016).

We predicted that predator populations should adapt not only to warming itself, but also to the compositional changes in prey communities caused by warming. Declining zooplankton body size has been widely observed under warming due to ecological and evolutionary processes (Moore and Folt 1993, Daufresne et al. 2009, Forster et al. 2012). Our experiment corroborated this response, as short-term warming caused marked declines in crustacean zooplankton biomass and a shift toward zooplankton community dominance by a small-bodied taxon - rotifers. In support of our prediction, we found that warm-source fish evolved to consume significantly smaller zooplankton on average. Moreover, warm-source fish evolved to exhibit a weaker ontogenetic niche shift towards consuming larger macroinvertebrate prey. Importantly, changing body size is just one trait that is systematically shifting under warming. We suggest that thermal adaptation in a broader sense may commonly involve evolutionary responses to warming-induced shifts to community structure that are themselves direct responses to warming.

Eco-evolutionary responses to warming

Life history evolution has conspicuous consequences for population ecology. In our *G. affinis* populations, increasing temperature led to the evolution of earlier maturity. In the mesocosms, early reproduction, in turn, led to a decline in fish population body size distribution (see Appendix S5: Fig S5f). Thus, faster life histories associated with thermal adaptation may lead to reduced individual body sizes because of reduced individual growth, but may also shift population size distributions towards smaller size through consistently earlier reproduction at a smaller size. Population-level body size declines observed under warming may commonly be an eco-evolutionary response caused by the evolution of faster life history.

In pond mesocosms, predator thermal adaptation exacerbated warming-induced community shifts in the pelagic towards smaller zooplankton. This pattern aligns with the trait differences we detected in trait and diet assays. First, *G. affinis* thermal adaptation caused declines in fish population body sizes. Smaller *G. affinis* are known to consume more zooplankton relative to other, larger diet items like macroinvertebrates (Bence and Murdoch 1986, García-Berthou 1999). Our diet study corroborated this response, as fish shifted away from pelagic zooplankton and towards benthic macroinvertebrates at large sizes. Moreover, we found that thermal adaptation caused size-independent shifts toward consumption of smaller zooplankton prey, and a weaker shift to benthic macroinvertebrate prey at large sizes. Thus, fish may have exacerbated warming-induced zooplankton body size declines because of their direct adaptation to warming which reduced their body sizes and their adaptation to the small prey available in warmer environments.

A common pattern in the eco-evolutionary dynamics literature is a decline in the effect size of evolution from traits to community-level effects (Bailey et al. 2009a). Here, strong effects of thermal adaptation on growth, size at maturity, and prey size corresponded with weaker effects of thermal adaptation on zooplankton communities (Fig 6). One possible reason for this pattern is that offspring were produced earlier in warm-source treatments compared with cool-source treatments, and *Gambusia* offspring are known to consume rotifers early in life. Thus, although warm-source fish had a higher propensity to consume crustacean zooplankton versus benthic macroinvertebrates as adults, their early reproduction could have led to an opposing effect on zooplankton responses because juveniles consume rotifers.

While thermal adaptation exacerbated the consequences of fish on pelagic zooplankton communities, thermal adaptation reduced the effect of fish on benthic macroinvertebrate communities. Warm-source fish less suppressed benthic macroinvertebrates. This relative reduction in the effect of warm-adapted fish on benthic macroinvertebrates likely arose due to a combination of their smaller body sizes and their reduced propensity to switch consumption towards large benthic macroinvertebrates at large sizes. As many animals exhibit ontogenetic diet shifts to ecologically-different prey (Werner and Gilliam 1984), warming-induced changes to predator body size may fundamentally alter their niche, and thus the ecology of warming systems. The evolution of altered niche shifts has the potential to change communities in non-linear ways, potentially leading to threshold community changes (Groffman et al. 2006).

Evolutionary responses to warming can occur in species at all trophic levels, but these responses might be most ecologically-meaningful in top trophic levels. First, because predators often exhibit keystone predation, any changes to their populations have the potential to multiply climate change effects down the trophic chain (Zarnetske et al. 2012). Furthermore, top-down effects appear to be increasing more strongly than bottom-up factors with warming, causing increased top down control in diverse ecosystem types (O’Connor et al. 2009, Barton and Schmitz 2009, Hoekman 2010, Kratina et al. 2012, Shurin et al. 2012, Symons and Shurin 2016).

Increased top-down control under warming could increase the importance of predator trait change for communities and ecosystems (Fryxell and Palkovacs 2017). Moreover, in lower, more speciose trophic levels, species sorting may dominate the ecological response to warming. For example, Padfield et al. (2017) found biomass-specific primary productivity was independent of temperature across a considerable temperature gradient. They detected a mild effect of adaptation, but species turnover was the primary driver of this pattern. Similarly, (Nelson et al. 2017) found secondary production in invertebrate communities was temperature-insensitive, but this was due to changing species composition. For these interrelated reasons, we suggest that evolution at the predator trophic level may be a relatively strong driver of ecological outcomes under warming.

Several authors have claimed that adaptation may considerably alter community and ecosystem responses to climate change (Lavergne et al. 2010, Norberg et al. 2012, Urban et al. 2016); however, sparse data exist to support this claim. Adaptive responses to climate change have indeed been detected (e.g. Karell et al., 2011), and local adaptation occurring over long timescales can mediate ecological responses (e.g. Barton 2011, De Block et al. 2013, Peterson et al. 2017). Our work here is among the first to show that rapid evolutionary adaptation can mediate the community outcomes of climate change. Our work demonstrates the potential diverse ecological roles of ongoing evolutionary adaptation. First, adaptation can counteract environmental effects on metabolic-related traits like growth. Second, adaptation can contribute to the body size declines widely observed under warming. Third, adaptation of predators can exacerbate the community and ecosystem consequences of warming. Altogether, this work suggests ongoing evolutionary adaptation could strongly mediate responses to climate change.

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Figures

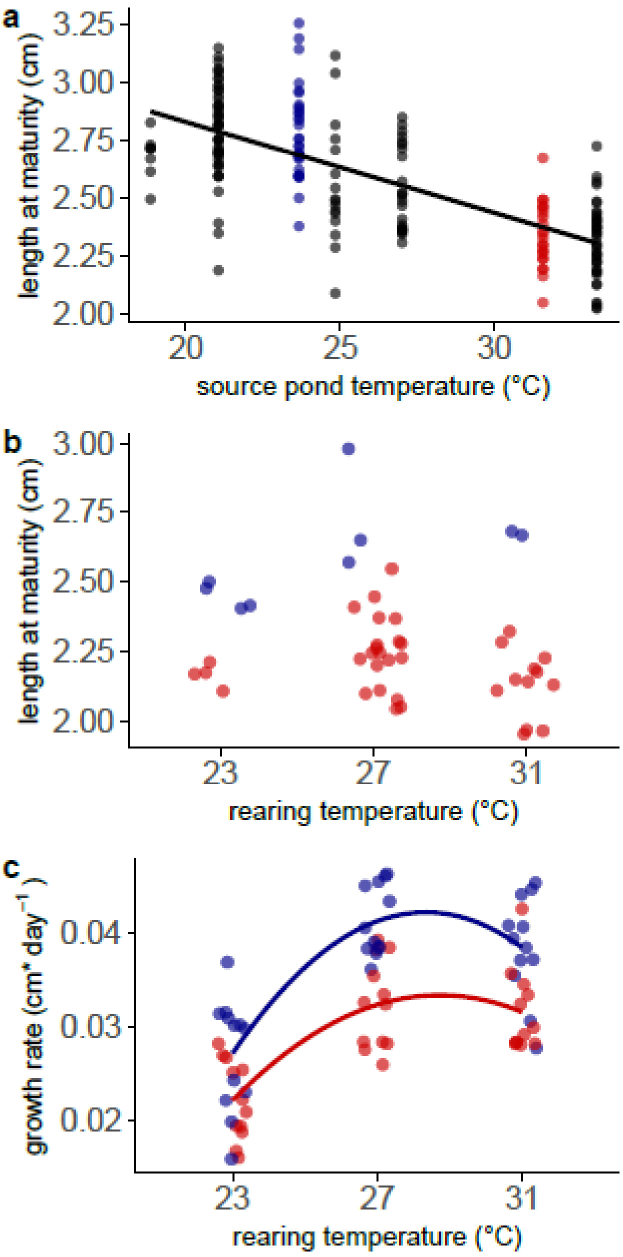

Figure 1 (left) Life history traits of wild and common reared *Gambusia affinis* (western mosquitofish) at different temperatures. **(a)** Size-at-maturity of wild-caught *G. affinis* regressed on mean ecosystem temperature. *G. affinis* were introduced to these sites sometime after 1922 and are derived from the same common ancestors. The trendline is the least-squares linear regression (slope -0.040, R^2=0.502, $F_{1,188}$=189.6, p<0.001). The Artesian Well site (blue) was used as the focal cool-source population and the Keough Hot Ditch site (red) was used as the focal warm-source population for subsequent experimental work. **(b)** Size-at-maturity of fish which had matured by the end of the common rearing experiment. Each jittered point represents a single F2 mature male fish. **(c)** Growth rates of fish from the rearing experiment. Curves represent least squares linear regressions with a

quadratic age coefficient to create hump-shaped population thermal growth performance curves. Jittered points each represent growth rates from mean size-at-age data over four fish reared per group. Twelve groups of four fish were reared in each population x temperature treatment.

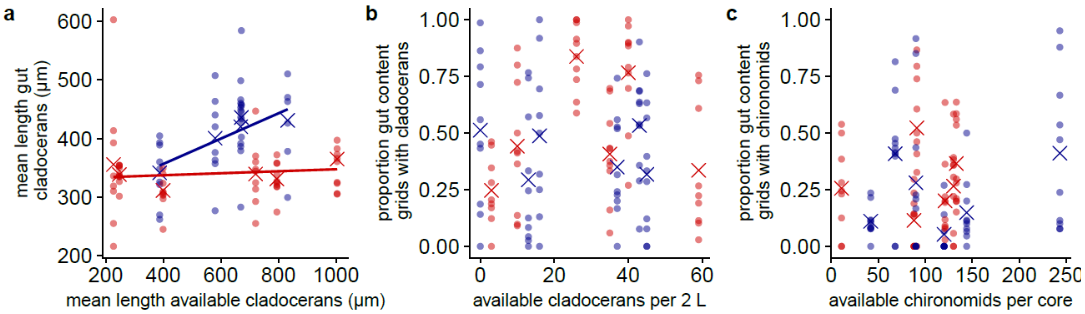

Figure 2 Effects of availability and *Gambusia affinis* population source on mean diet metrics from the diet experiment. Ten F2 fish (points) were introduced each to twelve experimental ponds. Half of the ponds had cool-source fish (blue) and half had warm-source fish (red). We used multiple linear regression to test the effect of pond prey availability x population source on pond mean diet contents (x's).

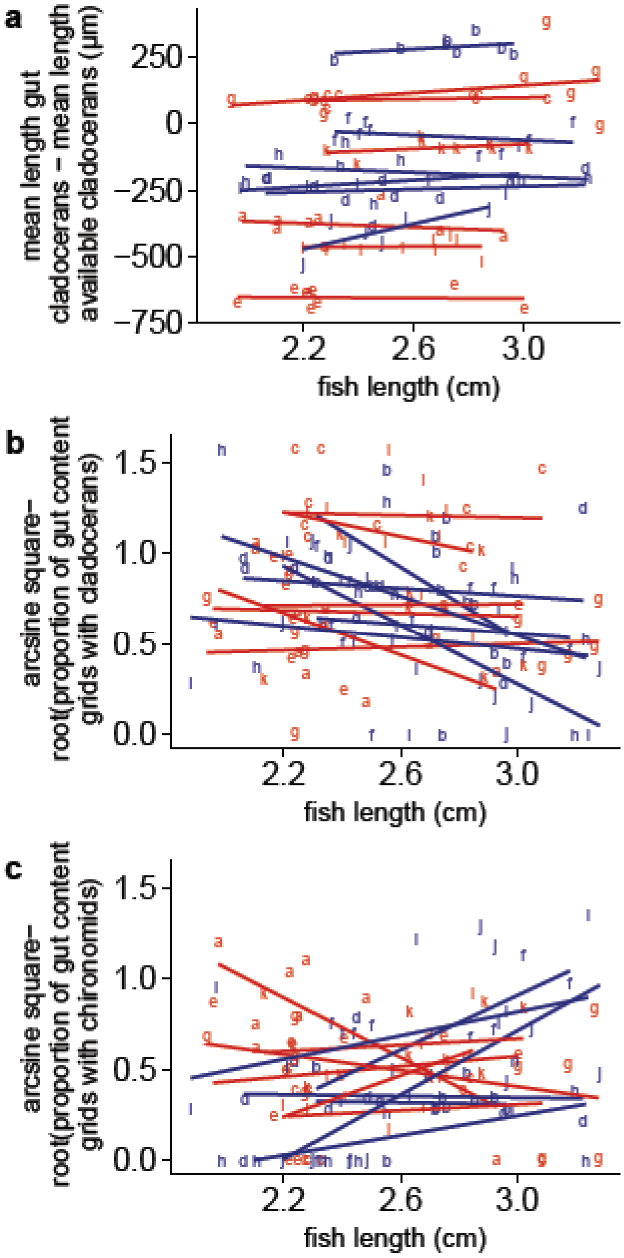

Figure 3 (left) Effects of *Gambusia affinis* body size and population source on diet metrics from the diet experiment. Ten F2 fish (individual letters) were introduced each to twelve experimental ponds. Half of the ponds had cool-source fish (blue) and half had warm-source fish (red). Fish guts were dissected and contents examined under a gridded slide. Plotted are least squares linear regressions for each pond separately to aid in visualizing the linear mixed effects models (with pond ID as a random effect) that we used to test the effects of fish body size x population source on each diet response. Responses were **(a)** cladoceran size selectivity **(b)** cladoceran abundance, and **(c)** chironomid abundance. Y axes for (b) and (c) span the range 0-100% in untransformed values (i.e. 0 to pi/2 in transformed values).

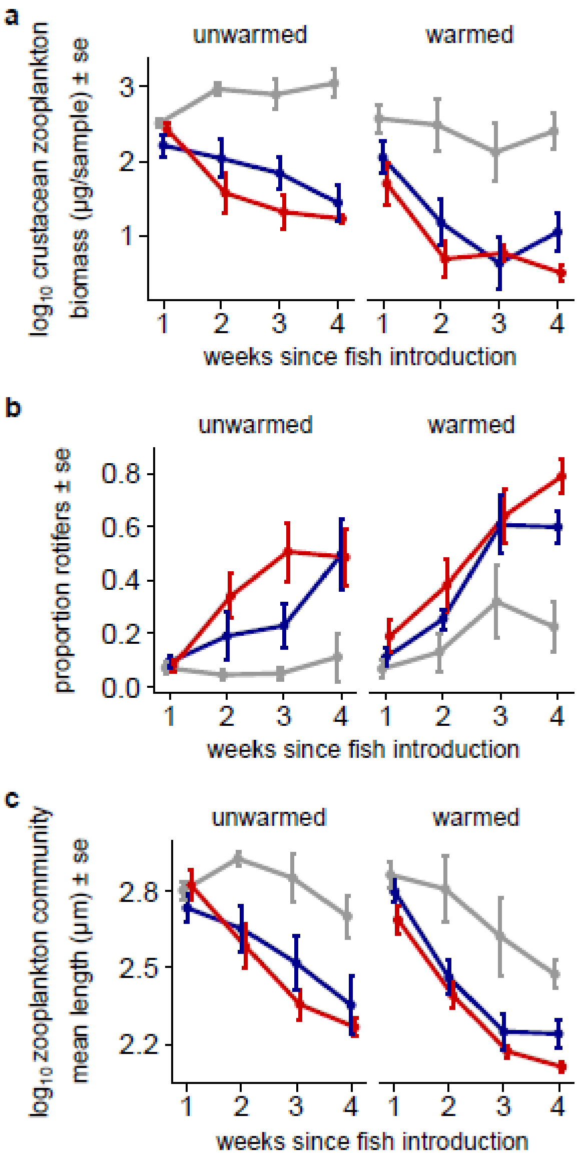

Figure 4 (left) Pelagic consumer responses in the pond experiment testing for effects of F2 cool-source *Gambusia affinis* populations (blue) versus F2 warm-source *G. affinis* populations (red), crossed with ecosystem temperature (warmed versus unwarmed). Grey lines are fishless reference treatments. Consumer responses were **(a)** crustacean zooplankton biomass, **(b)** proportion of the count of total zooplankters that were rotifers (a small-sized taxon), and **(c)** zooplankton community mean body length.

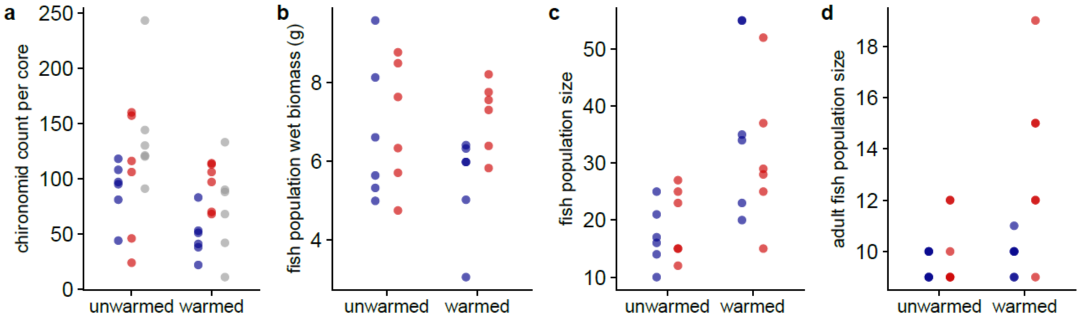

Figure 5 Final time-point responses in the pond experiment testing for effects of F2 cool-source *Gambusia affinis* populations (blue) versus F2 warm-source *G. affinis* populations (red), crossed with ecosystem temperature (warmed versus unwarmed). Points represent replicate experimental ponds. Responses were **(a) c**hironomid (midge larvae) counts from 18 cm diameter benthic cores (grey points are fishless reference treatments), **(b)** fish population biomass, **(c)** fish population size, and **(d)** adult fish population size.

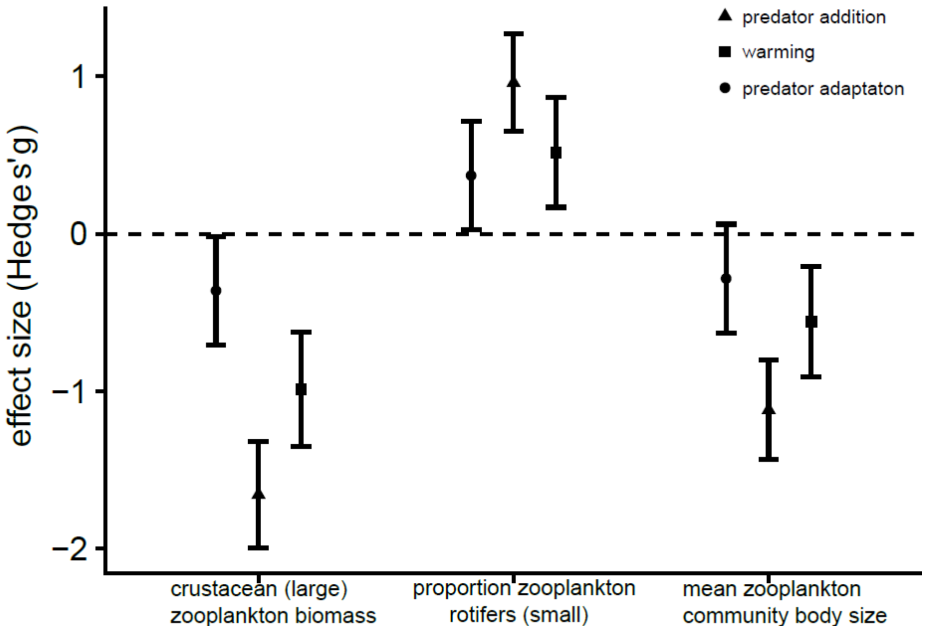

Figure 6 Standardized effects size calculations for three effects – predator addition, warming, and predator evolutionary thermal adaptation – on focal consumer responses in our experimental pond study. Effect sizes were calculated across the entire time series (Fig 4). Bars represent 90% confidence intervals.

CHAPTER 5 - Conclusions

Top predators alter communities by consuming competitively dominant taxa (Paine 1966, Lubchenco 1978) and controlling primary productivity through trophic cascades (Hairston et al. 1960, Carpenter et al. 1985). The top predator trophic guild also generally lacks species diversity. Together, these facts suggest that variation within top predator species may play an important role influencing community and ecosystem properties. In this dissertation, I tested this idea using a globally important predator species - western mosquitofish (*Gambusia affinis*). Mosquitofish were intentionally transplanted across the globe throughout the 20th century and today are becoming a model system in evolutionary ecology. In my experiments, I found that mosquitofish intraspecific variation had effects on a diverse set of community and ecosystem responses.

Across all three of the mesocosm experiments, the effects of intraspecific variation in mosquitofish were considerable, even when compared to the effects of mosquitofish presence itself. Standardized effect size calculations can be compared across these and other experiments. The effects of sex ratio variation (Chapter 2), interpopulation variation (Chapter 3), and thermal adaptation (Chapter 4) were each approximately ¼ to ½ the strength of that for predator addition for zooplankton responses. These results are consistent with studies on the ecological effects of intraspecific variation in other predators, which also show tendencies towards stronger predator addition effects (Palkovacs et al. 2015), especially for “direct” effects of predators on their prey (Des Roches et al. 2018). Thus, although mosquitofish are unique among predators in their global distribution, they do not seem to exhibit uniquely strong effects of intraspecific variation. These results indicate that mosquitofish are a promising model system for predator intraspecific effects in general.

Intraspecific variation had stronger direct effects on prey than indirect effects on other aspects of pond communities and ecosystems. This is perhaps unsurprising as intraspecific effects may generally be expected to weaken as they propagate across the trophic chain and to higher levels of ecological organization (Bailey et al. 2009b). Given this expectation, workers trying to detect the cascading influence of predator intraspecific variation beyond prey should keep in mind that more statistical power and thus larger sample sizes may be required. Indeed, in Chapters 3 and 4, there were six replicates per treatment and I was unable to detect many cascading effects of intraspecific variation, even though those effects are hypothesized based on the direct effects of intraspecific variation that I found on mosquitofish prey (e.g. see Chapter 2).

The only response for which intraspecific effects were larger than those of predator addition was that of snail size in Chapter 2. Predator addition had no effect on snail size while female-biased populations induced larger snail sizes. This response may have arisen as an induced response in shell size by snails which perceived a greater threat from females than males. This interpretation is consistent with the observation that only large female *Gambusia* consume snails (Hubbs 1990).

This result highlights the surprising and unpredictable effects of intraspecific variation on some ecologically relevant community attributes, and even those that are not affected by species presence itself.

I found that the effects of predator trait variation were context-dependent. Temperature affected the strength of intraspecific effects. According to the metabolic theory of ecology (MTE), metabolic demand and (concomitantly) feeding rates increase universally with temperature (Gillooly et al. 2001, Rall et al. 2012). Thus, in my experiments, temperature-induced changes to feeding rates may have partly driven ecological divergence among mosquitofish populations. In Chapter 2, female-biased mosquitofish sex ratios caused a greater pelagic trophic cascade, leading to an increase in water temperature. Interestingly, this increase in temperature may have reinforced the cascade by increasing the feeding rates of female mosquitofish. In Chapter 3, my direct manipulation of water temperature showed greater prey depletion by mosquitofish at warmed temperatures. However, the extent of prey depletion differed by population. As the focal populations in this chapter came from different thermal environments, this may suggest that adaptation or acclimation modified the feeding rate response to temperature itself (calling into question simple MTE predictions). On the other hand, populations could have consumed prey at the same rate but of different prey categories. Under this scenario, predator-induced community divergence should be consistently stronger under warming, as increased predator feeding rates should exacerbate ecological differences that emerge based on different prey preferences. Future studies should aim to parse the effects of population differences in feeding rates versus feeding preferences in studies of the community and ecosystem effects of intraspecific variation.

Intraspecific variation in body size may also contribute to variation in the ecological effects among mosquitofish populations. The MTE predicts that metabolic demand and feeding rates should also increase universally with body size (Gillooly et al. 2001, Rall et al. 2012). In Chapter 2, female-biased populations greater depleted prey, inducing a stronger trophic cascade than male-biased populations. As females are larger and continue growing throughout life (unlike males), they likely have a higher metabolic demand and thus higher feeding rates. Interpopulation variation in body size is common, and its effects are probably potent. Perhaps unfortunately, most studies of intraspecific ecological effects control for this important component of interpopulation variation.

My experiments likely also reflect some temperature- and size-independent aspects of predator intraspecific effects. In Chapter 2, the ecological effects of sex ratio could have emerged in ways that are independent of body size dimorphism. In addition to being larger, female mosquitofish spend less time avoiding males when in female-biased populations (Pilastro et al. 2003, Arrington et al. 2009), and thus more time feeding. In this way, individual feeding rates can depend on the social context within populations. In Chapter 3, as noted above, population divergence could have emerged via divergent prey preferences among populations. Most interestingly, in Chapter 4, I found that recent thermal adaptation in predators modifies intraspecific variation in ways which produce community ecological consequences. Warm-adapted populations evolved slower growth and earlier maturity, and evolved to consume smaller prey relative to cool-adapted populations when populations were reared for two generations at the same temperature. Because warm-adapted mosquitofish grew slower, reproduced earlier, and continued to consume zooplankton throughout life, they caused a greater change in zooplankton communities compared with cool-adapted populations at the same ecosystem temperature.

Anthropogenic disturbances are “pressing” environmental conditions, thereby changing the ecological context and probably modifying the effects of intraspecific variation. However, at the same time anthropogenic disturbances act as agents of selection, modifying intraspecific variation itself. As a growing body of evidence suggests that intraspecific variation is important for communities and ecosystems (Bailey et al. 2009b, Palkovacs et al. 2015, Des Roches et al. 2018), that environmental context matters for its cascading consequences (El-Sabaawi et al. 2015, Lajoie and Vellend 2015, Tuckett et al. 2017), and that populations are rapidly evolving in response to changing contexts (Merilä and Hendry 2014), a timely question worth addressing is how adaptation of the organisms in a community modifies the ecological outcomes of environmental change. I addressed this question in Chapter 4; I tested for an interaction between thermal context and recent thermal adaptation in predators on community outcomes. As each warming and predator thermal adaptation favored small zooplankton taxa, I concluded that adaptation exacerbated the ecological effects of warming itself. Because adaptation is an inevitable occurrence under environmental change, much more work should be done using similar techniques as those used here to determine whether adaptation may generally moderate or exacerbate ecological change.

In summary, this dissertation adds to a growing literature demonstrating the ecological effects of intraspecific variation (Bailey et al. 2009b, Palkovacs et al. 2015, Des Roches et al. 2018). While much has been learned from these studies, we still know little about the contexts under which these effects could be significant in nature. Future work should aim to determine which focal populations, which types of trait variation, and which ecological and environmental settings lead to meaningful intraspecific ecological effects. My work here suggests that previously overlooked aspects of intraspecific variation like sex ratio variation can be important. My work also suggests that variation in predators may be especially potent, and may be increasingly so under warming. Altogether, these results call for a broader consideration of the ecological effects of predator intraspecific variation into the future.

SUPPLEMENTAL FILES

Supplemental files are available online.

Chapter 2:

2_PRSBfryxellSD1	Supplement 1 - data
2_PRSBfryxellST2	Supplement 2 - table

Chapter 3:

3_Copeia_reprint_permission_request_signed	publisher reprint permission
3_Appendix 1_Outlier_ Diagnosis	Appendix 1
3_Appendix 2_ANOVA_Summary	Appendix 2
3_Appendix 3_PCA	Appendix 3
3_Appendix 4_Data	Appendix 4

Chapter 4:

4_Appendix_Meso2014	Appendix

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