Spring 5-10-2019

Cascading Community Consequences of Fish Adaptation

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Recommended Citation
Wood, Zachary T., "Cascading Community Consequences of Fish Adaptation" (2019). *Electronic Theses and Dissertations*. 3046.
https://digitalcommons.library.umaine.edu/etd/3046

CASCADING COMMUNITY CONSEQUENCES OF FISH ADAPTATION

By

Zachary T. Wood

B.A. Dartmouth College, 2015

A DISSERTATION

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Ecology and Environmental Sciences)

The Graduate School

The University of Maine

May 2019

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CASCADING COMMUNITY CONSEQUENCES OF FISH ADAPTATION

By Zachary T. Wood

Dissertation Advisor: Dr. Michael T. Kinnison

An Abstract of the Dissertation Presented
in Partial Fulfillment of the Requirements for the
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Trophic interactions are an enduring framework for ecological thought. Broad and growing evidence for contemporary evolution has demonstrated that ecology and evolution dynamically interact on similar time scales. In this dissertation, I seek to understand how genetic and plastic trait change in human-influenced systems shape trophic dynamics, how such trait changes are constrained by inherent tradeoffs, and the broad implications of such trait change for ecological communities. I advance the premise that competition-defense tradeoffs are the essential mechanism behind many eco-evolutionary trophic dynamics that can reshape multi-trophic communities. In support of this view, I assess the presence of ecologically relevant genetic evolution along a competition-defense tradeoff in a model species. I also employ models and experiments to quantify how the particularly strong genetic and plastic trait changes in population phenotypes generated by humans can rearrange ecological communities by altering trophic interaction strengths.

ACKNOWLEDGEMENTS

I am extremely grateful to my entire committee for selflessly advising me on all aspects of research, teaching, and professional development. All five of them went well beyond the duties expected of committee members, including one-on-one teaching training, friendly reviews, and innumerable drinks. My advisor, Dr. Michael Kinnison, has been a tireless advocate for my research, taking time away from his family to travel with me to conferences and the field. Dr. Eric Palkovacs has been a gracious and generous host on my many trips to Santa Cruz. I look forward to continued collaboration with all of them.

Most chapters of this dissertation represent larger, collaborative projects with numerous co-authors. Mike Kinnison, Eric Palkovacs, Rebecca Robinson, Dave Fryxell, Emma Moffett, Kevin Simon, Laura Lopez, Celia Symons, Cowan Sikora, and Rachel Van Gorden have all contributed greatly to the science presented here and have all been excellent coauthors and friends.

Beyond my committee, several other scientists provided essential feedback, encouragement, support, and advocacy during this dissertation. Sarah Nelson, Kate Ruskin, and Michelle Smith all served as unofficial mentors, to whom I am extremely grateful.

Numerous undergraduate researchers have also contributed hundreds of hours of service to this dissertation, and this work would truly have been impossible without them. Cowan Sikora, Rachel Van Gorden, Ethan Cantin, Jeff Parsons, Emma Garner, and Bruno Zicarella are all to thank for this work.

Much of this work involved collaboration between myself and various mosquitofish production facilities in California. Specifically, Chris Miller and Tony Hedly provided fish and bountiful information on the mosquitofish rearing process that greatly informed by labwork and writing.

I am very grateful to my parents, Dawn and Jeff Wood, who embraced the odd habits of their aspiring scientist son at a young age and taught me the courage I needed to get this PhD. I love you both very much.

Finally, I would not have survived this degree without the love and support of some amazing friends: Isaac Shepard, Jared Homola, Vanessa Hensley, Mitch Paisker, Brad Erdman, Kevin Duffy, and Cool Joe Mohan.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

1.1.1 Evolution is contemporary

Overwhelming evidence has demonstrated that evolution can occur on ecologically relevant, “contemporary” time scales of just a few generations (Carroll et al., 2007; Hairston et al., 2005; Hendry and Kinnison, 1999; Kinnison and Hendry, 2001). This evidence supplants the classical Darwinian view that: “we see nothing of these slow changes in progress, until the hand of time has marked the long lapse of ages.” (Darwin, 1859; Pianka, 2011). Murmurings of contemporary evolution occurred in the 1950s (Fenner and Marshall, 1957; Kettlewell, 1955). Pimentel’s “genetic feed-back” (Pimentel, 1961) and Van Valen’s “Red Queen hypothesis” (Van Valen, 1973) both posited that dynamic, adaptive, evolution over ecological timescales was a stabilizing force for populations and communities. Meta-analyses by Kinnison and Hendry in the late 1990s (Hendry and Kinnison, 1999; Kinnison and Hendry, 2001) showed that evolution appeared to proceed more quickly when observed over shorter timeframes. This pattern suggested that longer studies of evolution with few observation points neglected more rapid—often dynamic or reversing—episodes of evolution by essentially smoothing them over.

There are now numerous synchronic examples allowing us to deduce divergent evolution over a few generations (reviewed in (Carroll et al., 2007; Hairston et al., 2005; Hendry and Kinnison, 1999; Kinnison and Hendry, 2001; Post and Palkovacs, 2009)). In addition, a few high-resolution allochronic studies have documented evolution occurring in real, contemporary time in a single population:

- Beak size evolution in Galapagos finches in response to environmental change and competition (Grant and Grant, 2002, 2006).
- Evolution of armor plating in threespine stickleback (Bell et al., 2004).
- Evolution of chemical defenses in algal-rotifer chemostats (Yoshida et al., 2003).
- Antler morphology in red deer (Hoffmann et al., 2016).
- Horn size in bighorn sheep (Pigeon et al., 2016).
- Body size in great tits (Garant et al., 2004).

Aside from being academically interesting, the consequences of evolution over ecological time scales are of broad applied concern as well.

1.1.2 Humans and evolution

Contemporary evolution may even be faster if humans are the root cause (Darimont et al., 2009a; Hendry et al., 2008). Humans generate strong selection through harvest (Fenberg and Roy, 2008; Heino et al., 2015; Hutchings and Fraser, 2008; Jørgensen et al., 2007; Sharpe and Hendry, 2009), habitat change (Fountain et al., 2016; Hoffmann and Sgrò, 2011; Kinnison and Hairston, 2007; Stockwell and Weeks, 1999; Stockwell et al., 2003), species introductions (Lambrinos, 2004; Prentis et al., 2008; Sax et al., 2007) and removals (Khater et al., 2014; Palkovacs et al., 2011), captive propagation (Bilio, 2007; Hutchings and Fraser, 2008; Lorenzen et al., 2012; Teletchea and Fontaine, 2012), and numerous other intentional and unintentional actions. It is unclear whether the relatively higher strength of human-induced evolution is due to stronger, more consistent, or simpler selection (i.e. humans select on a few, obvious traits, rather than complex, correlated networks of traits). The high strength and frequency of human-induced ecological and evolutionary change suggests that eco-evolutionary dynamics caused by humans may be among the most prevalent and the most likely to generate lasting ecological change.

1.1.3 Eco-evolutionary dynamics

Ecology and evolution both have the same currencies: births, deaths, immigration and emigration (Urban and Skelly, 2006; Urban et al., 2008; Vellend, 2010). When these demographic processes, which are driven by ecological interactions, change allele frequencies (i.e. through directional, stabilizing, or frequency-dependent selection), contemporary evolution has occurred. When changes in allele frequencies drive ecological change (e.g. through changes in population size or ecologically-relevant functional traits), then contemporary evolution has affected ecology. Thus, ecology and contemporary evolution can interact through what are sometimes referred to as “eco-to-evo” and “evo-to-eco” pathways (Hendry, 2016).

Eco-to-evo. While recognition of eco-to-evo interactions is as old as the premise of adaptation itself, our understanding of its prevalence and extent in contemporary time has changed dramatically in the past few decades. Adaptive contemporary evolution has been documented in response to biotic (e.g. predators (Khater et al., 2014), abiotic (e.g. temperature (Meffe et al., 1995) and anthropogenic (e.g. domestication (Duarte et al., 2007) perturbations. In some cases, it has been feasible to show that such trait change has a heritable basis, and in other cases it is assumed that the trait change represents some combination of genetic change and plastic responses to environments. Regardless, the general view is that such contemporary evolution often aids populations in persisting or thriving under changing ecological environments (‘adaptive tracking’ or ‘evolutionary rescue’: Bell and Gonzalez, 2009; Carlson et al., 2014; Gonzalez et al., 2013; Kinnison and Hairston, 2007; Tallmon et al., 2004; Whiteley et al., 2015). Interestingly, this commonly assumed benefit of adaptation itself constitutes a form of eco-evolutionary dynamic in that it implies that ongoing evolution has some ecological effect on population growth through the shared link of absolute fitness (gross reproductive success) (Gomulkiewicz and Holt, 1995; Kinnison and Hairston, 2007; Stockwell et al., 2003).

Evo-to-eco. Contemporary evolution can also have a much wider array of ecological consequences beyond a population’s growth rate. Contemporary evolution often shapes the functional traits that determine how organisms broadly interact with one another and their abiotic environments (Des Roches et al., 2018; Palkovacs et al., 2012; Schmitz et al., 2008). Example traits can include feeding morphology (Palkovacs and Post, 2009; Palkovacs et al., 2011), habitat use (Des Roches et al., 2013; Harmon et al., 2009; Tuckett et al., 2017), antipredator defenses (Friman et al., 2014; Yoshida et al., 2003), body size (Audzijonyte et al., 2013a), and growth and maturation rate (Audzijonyte et al., 2014; Kuparinen et al., 2016). Changes in these traits can generate novel predator-prey cycling (Hiltunen et al., 2014), modify interaction strengths (terHorst et al., 2010), alter nutrient fluxes (Carlson et al., 2011), and even mask trophic interactions (Yoshida et al., 2007), all of which can have broad ecological consequences.

Eco-evolutionary dynamics. While it is interesting to understand how ecology shapes evolution and how ongoing evolution shapes ecology, a particularly intriguing aspect of eco-evolutionary dynamics are the emergent feedbacks (Fussmann et al., 2007; Hendry, 2016; Post and Palkovacs, 2009; Schoener, 2011) that come with coupling the eco-to-evo and evo-to-eco pathways. Eco-evolutionary feedbacks are defined as bidirectional interactions between evolution and ecology (Hendry, 2016; Post and Palkovacs, 2009). Eco-evolutionary feedbacks are considered *strong* when the same trait that generates ecological change is selected upon by the ecological change (Hendry, 2016). Negative, or stabilizing eco-evolutionary feedbacks occur when ecological change and trait change are mutually offsetting. Positive, or destabilizing eco-evolutionary feedbacks occur when ecological change and trait change are mutually reinforcing; these feedbacks can result in “runaway” eco-evolutionary dynamics. Thus, when ecology and evolution are interconnected, even relatively small perturbations in ecology or evolution can have effects that are long lasting and difficult to reverse (Abrams and Matsuda, 1997a; Cortez et al., 2018; Marrow and Cannings, 1993).

Eco-evolutionary dynamics may be difficult to detect—and therefore frequently overlooked—due to their often-cryptic nature (Kinnison et al., 2015). For example, adaptation may prevent prey densities from responding to changes in predator densities, thereby masking predator-prey interactions (Yoshida et al., 2007). Such cryptic dynamics could have important roles in food webs (Griffiths et al., 2018), but would be missed by classical ecological measurements (e.g. abundance counts). Adaptation in the face of environmental change may also prevent declines in population size, thus masking the potential impact of environmental change (i.e. cryptic evolutionary rescue: Kinnison et al., 2015). Given this potentially cryptic nature, it is important to study systems by coupling theory with rigorous observation that includes traits and interaction strengths in addition to simple densities.

1.1.4 The primacy of competition-defense tradeoffs

I suggest that competition-defense tradeoffs are an essential—but painfully overlooked—component of contemporary evolution and eco-evolutionary dynamics. Constraints on the long-term evolution of defenses (i.e. the evolution of defenses seldom proceeds without limit) imply that defenses must eventually come at a cost (Agrawal, 2007; Bazzaz et al., 1987; Koricheva, 2002; Tollrian and Harvell, 1999). Repeated evolution of prey naivete when predators are removed (Palkovacs and Post, 2009; Reznick et al., 1990) also suggests that there are benefits to being undefended when predators are absent, indicating that defenses have inherent competitive costs. Resource limitations that constrain allocation of material to feeding morphology or armor, or force budgeting of time for either hiding or foraging create numerous scenarios where competitive ability and defendedness are negatively related. Competition-defense tradeoffs have been explored in a wide range of organisms, including fish, (Langerhans, 2009; Palkovacs et al., 2011) plants, (Mole, 1994) insects, (Fellowes et al., 1999; Kraaijeveld et al., 2002) algae, (Agrawal, 1998; Kasada et al., 2014; Yoshida et al., 2003) and bacteria (Lennon and Martiny, 2008). Such tradeoffs may be behavioral, (Conrad et al., 2011; Langerhans, 2009) morphological, (Langerhans, 2009; Palkovacs et al., 2011) physio-chemical, (Agrawal, 1998; Lind et al., 2013) or life-historical. (Reznick et al., 1990; Stearns, 1983b, 1983a, 1989). While the specific traits behind competition-defense tradeoffs are diverse, their functional outcomes for interaction strengths between focal species and their predators and prey may be generalizable.

This ability to generalize competition-defense tradeoffs could provide an important mechanistic link between evolution and community ecology. If a predator introduction results in defense evolution in prey, then the competitive cost of such evolution should result in prey having weaker growth or feeding efficacy (Yoshida et al., 2004), potentially leading to cascading food web consequences. Furthermore, the competitive cost of evolved defenses implies that prey will evolve increased competitive ability (and decreased defendedness) if the abundance of predators ever decreases (Palkovacs et al., 2011; Reznick and Endler, 1982; Reznick et al., 1990). Thus, when prey trait evolution is constrained by a competition-defense tradeoff, predator abundance and prey traits are directly coupled, creating the potential for strong eco-evolutionary dynamics.

Contemporary evolution along competition-defense tradeoffs can substantially influence the abundance and stability of both predator and prey populations (Abrams, 2009; Abrams and Matsuda, 1997a, 1997b; Ehrlich et al., 2018; Kasada et al., 2014; Yoshida et al., 2003). Specifically, the slope of the competition-defense tradeoff can itself determine whether eco-evolutionary dynamics will be present, and whether they will be stabilizing or destabilizing (Abrams, 2009; Kasada et al., 2014; Loeuille et al., 2002).

Despite the potential universality and significance of competition-defense tradeoffs, they can be surprisingly challenging to quantify, particularly in terms of such functional slopes. In part this challenge is because tradeoffs can shape and be shaped by a range of processes, including trait correlations, pleiotropy, or resource allocation (e.g. time can be allocated to feeding or hiding). Furthermore, these tradeoffs are likely context-dependent, as the efficacy of competitive or defended traits likely depends on the density of prey, conspecifics, and predators (Siemens et al., 2003; Tollrian and Harvell, 1999). Moreover, what we understand as tradeoffs may vary in strength and pattern at different scales, such as across or within populations (Hahn and Maron, 2016).

1.1.5 Evolution and trophic dynamics

One way that eco-evolutionary dynamics may extend beyond a focal species is via food web interactions. Contemporary evolution can be a cause and consequence of trophic cascades. Contemporary evolution in upper trophic levels can cause cascading top-down effects on food webs (Ousterhout et al., 2018; Palkovacs et al., 2012; Walsh et al., 2012). The rearranging of relative interaction strengths during a classic density-mediated trophic cascade can also alter selection pressures on lower trophic levels, driving contemporary evolution (Wood et al., 2018). Ecological compensation, which is essentially evolution across rather than within species (i.e. community evolution) can also dampen trophic cascade strength (Fahimipour et al., 2017). However, the specific role of contemporary trait change in contemporaneously modifying trophic cascade strength is largely unknown.

While trophic cascades remain among the strongest, most widely-tested paradigms in ecology (Borer et al., 2005; Pace et al., 1999; Ripple et al., 2016), only a few studies have incorporated evolutionary processes into trophic cascade theory (Griffiths et al., 2018; Mooney et al., 2010; Ousterhout et al., 2018; Start, 2018). One body of work that comes close is the trait-mediated / non-consumptive literature, which focuses on the role of prey traits (i.e. behavior) in generating food web impacts of introduced predators (Preisser and Bolnick, 2008b, 2008a; Schmitz et al., 1997, 2004). This work has examined the relative strength and commonness of density- versus trait-mediated processes in trophic dynamics (Křivan and Schmitz, 2004; Peacor et al., 2013; Preisser and Bolnick, 2008b; Schmitz et al., 2004; Trussell et al., 2006). However, density- and trait-mediated interactions are likely inter-dependent (Griffiths et al., 2018), as evolution of prey traits that generate trait-mediated trophic cascades may also increase survival (Sih et al., 2010).

Through intra-generational plasticity (Lönnstedt et al., 2012; McCormick and Holmes, 2006), inter-generational plasticity (e.g. epigenetic and maternal effects) (Storm and Lima, 2010), and genetic evolution (Wund et al., 2015), prey may display antipredator adaptations in the face of shifting predator densities. If these defenses are linked to typical competition-defense tradeoffs (see earlier), then evolution of defense during trophic cascades may have its own cascading effect through changes in competitive ability (feeding).

1.1.6 Genetic and plastic contributions

A major nuance that is missing in many eco-evolutionary studies is the assessment of the genetic basis for focal traits. Therefore, the relative contributions of genetic evolution and plastic change to evo-to-eco interactions are poorly understood. Furthermore, with the approaches often used, it is easy to overlook how trait change is not limited to 'evolution or plasticity' but also includes 'evolution of plasticity'. Thus, the bulk of evo-to-eco studies may be grossly overestimating the ecological impacts of genetic evolution if the real mechanism behind their focal traits is transient phenotypic plasticity or a reaction norm removed from its realistic context. On the other hand, evo-to-eco studies may be grossly underestimating the ecological impacts of contemporary trait change if common rearing masks reaction norms by which genetic and plastic change work synergistically (e.g. genetic evolution of a reaction norm by which plasticity amplifies existing genetic differences).

Evidence suggests that phenotypic plasticity may buffer against genetic evolution (Oostra et al., 2018; Price et al., 2003) or facilitate it (Ghalambor et al., 2007, 2015), depending on the environmental context. Thus, some trait differences across populations may be mostly due to plasticity, while others are mainly due to genetic evolution. Teasing apart the two is necessary for understanding the timescale and reversibility of contemporary trait change.

1.2 DISSERTATION STRUCTURE

In this dissertation, I argue that human perturbations generate ecologically impactful evolution along competition-defense tradeoffs that may in turn generate important eco-evolutionary feedbacks in such systems. Specifically, I seek to advance the following theoretical and empirical considerations:

1. The slope, trophic level, and organizational level of competition-defense tradeoffs determines the strength, stability, and trophic reach of eco-evolutionary dynamics.
2. Adaptive evolution produces two ecologically important effects: the direct ecological impact of the altered functional traits, and the indirect ecological impact of the increased abundance of the adapting population. Both need to be considered together to understand the full ecological role of contemporary evolution, particularly when trait and density effects are opposing in direction.
3. Adaptive evolution in response to humans produces combinations of traits not seen in wild environments, and thus leads to novel ecological impacts of human-adapted populations.
4. Cascading ecological effects of trait change, which are often attributed to plastic antipredator defenses, are actually generated by a combination of plastic and genetic adaptation to predators and local environments.

To support these arguments, I have assembled five chapters, with each tackling multiple sub-questions (Figure 1.1). These chapters span a mixture of theoretical and empirical work. Many of the chapters include western mosquitofish (*Gambusia affinis*) as a focus species, but with generalized implications for different systems strongly considered. Each chapter also centers around applied issues associated with human effects on populations and ecosystems, including harvest, captive propagation, and predator invasion, under the general premise that humans are now the world's greatest evolutionary driver.

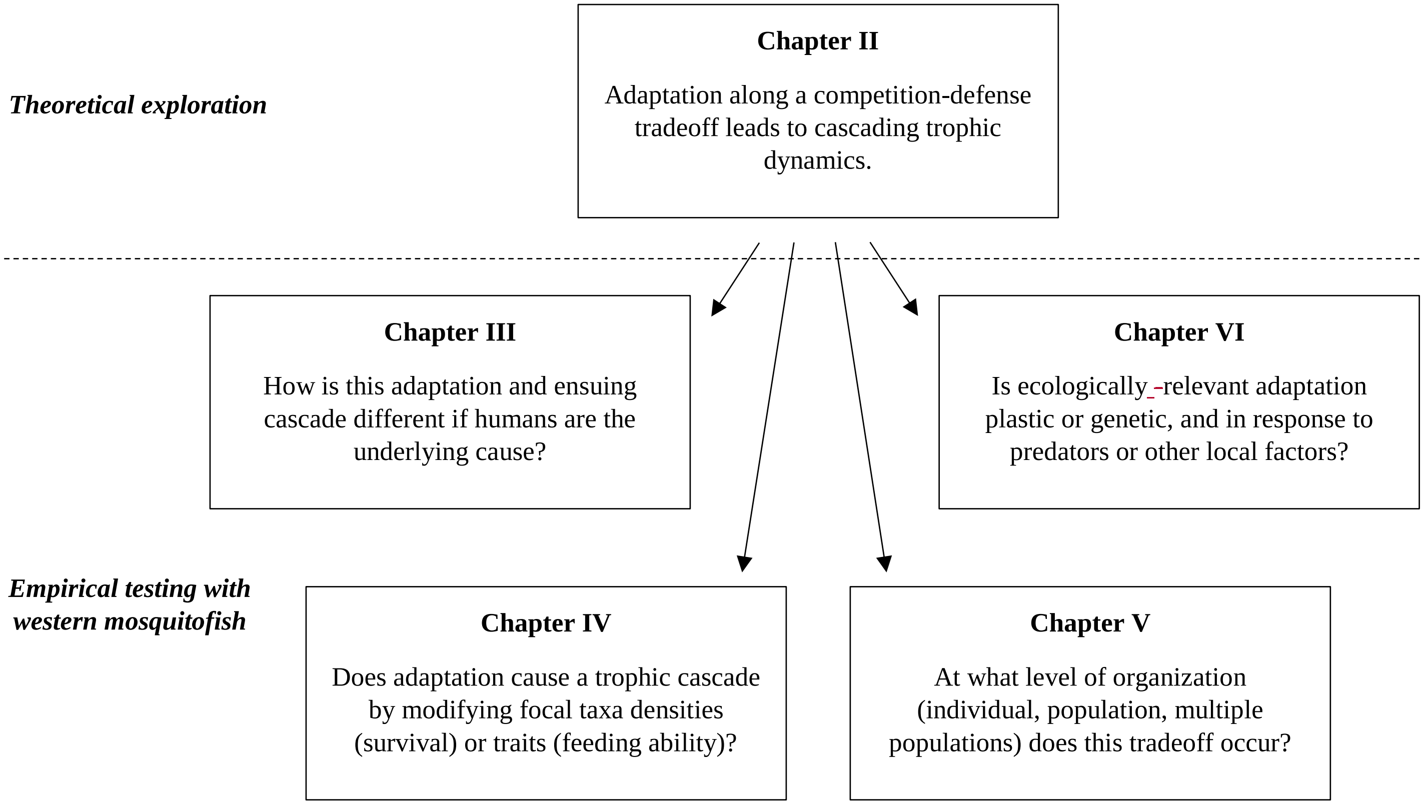


Figure 1.1. Dissertation structure schematic.

1.2.1 Chapter 2: Eco-evolutionary feedbacks from nontarget species influence harvest yield and sustainability

In this chapter, I use individual-based model simulations to investigate how the slope and trophic level of a competition-defense tradeoff lead to eco-evolutionary dynamics during the harvest of a top-predator. Through these simulations, I provide an example of how competition-defense tradeoffs can be an essential mechanism in multi-trophic eco-evolutionary dynamics, tying prey traits to predator abundance. Limited empirical and theoretical work has shown that the slope of competition-defense tradeoffs can determine system stability and the potential for eco-evolutionary dynamics (Abrams, 2009; Ehrlich et al., 2018; Kasada et al., 2014). However, much of this work is limited to very simple predator-prey systems in which only the prey evolve, which constrains the relevance of such theory to many real-world systems.

Here I use a four trophic level individual-based model to investigate evolution along competition-defense tradeoffs in the lowest three trophic levels of a food chain while the top trophic level is subjected to harvest. I demonstrate extensive theoretical evidence that the slope of competition-defense tradeoffs in all trophic levels predictably sets the potential for contemporary evolution before and during harvest. I also show that evolution along this tradeoff predictably exacerbates or dampens the effect of harvest, depending on which trophic level(s) evolve(s). I argue that these results demonstrate the primacy of competition-defense tradeoffs in driving eco-evolutionary dynamics in complex systems. I also argue that the role of evolution in trophic dynamics can reach many trophic levels beyond the evolving trophic level, depending on food web structure.

1.2.2 Chapter 3: Phenotypic and community consequences of captive propagation in mosquitofish

In this chapter, I investigate how captive propagation by humans potentially alters the phenotypes of mosquitofish, and how these altered phenotypes change pond mesocosm communities. Adaptation to human environments (i.e. domestication) can lead to marked trait divergence from wild populations (Bilio, 2007; Huntingford, 2004; Lorenzen et al., 2012; Price, 2002; Teletchea and Fontaine, 2012), but is such anthropogenic evolution unique relative to patterns of adaptation in nature (e.g., is domestication similar to evolution of prey naivete (Langerhans et al., 2004; Lönnstedt et al., 2012; Wund et al., 2015))? Moreover, does trait change associated with human environments lead to different ecological outcomes when human-impacted phenotypes are introduced back into wild habitats (Araki et al., 2007, 2008, 2009)?

Here I compare the phenotypes and ecological impacts of mosquitofish from captive propagation facilities with those from high- and low-predation wild ponds. I posit that captive propagation in mosquitofish produces unique mixtures of traits due to intense competition and harvest in propagation facilities. I also show that this unique mixture of traits leads to a completely novel ecological niche for captive propagated mosquitofish in the wild. As in my first chapter, I show that the ecological effects of trait change can extend well beyond the changing taxa, particularly when humans are the agents of change.

1.2.3 Chapter 4: Prey experience cryptically shifts trophic cascades from density- to trait-mediated

In this chapter, I examine how adaptation along a competition-defense tradeoff in mosquitofish facilitates density- or trait-mediated trophic cascades in the face of bass predation, using pond mesocosms. In my first chapter, I demonstrate how adaptation along a competition-defense tradeoff can have cascading impacts on lower trophic levels. However, there are two potential drivers in cascading impacts of adaptation:

1. Density: adaptation positively affects population size, leading to a cascade
2. Traits: adaptation alters feeding ability or mode, leading to a cascade

Here we introduced mosquitofish from two populations: predator-experienced or predator-naïve, into mesocosms with largemouth bass, measuring mosquitofish survival and mosquitofish ecological impact. I demonstrate that these populations fall along a competition-defense tradeoff axis and argue that density- and trait-mediated effects of mosquitofish are opposed, with higher survival (density effects) connected to lower feeding ability (trait effects). I thus show that prey adaptation along this tradeoff axis determines whether trophic cascades are dominated by density- or trait-mediated effects. I also evaluate the relative strength of density- versus trait-mediated trophic cascades and discuss implications for detecting adaptation during trophic cascades.

1.2.4 Chapter 5: Levels of competition-defense tradeoffs in mosquitofish

In this chapter, I investigate individual-, population-, and background-level genetic competition-defense tradeoffs in mosquitofish, using a serial growth and antipredator survival experiment.

While competition-defense tradeoffs are an essential component in eco-evolutionary dynamics and appear in numerous taxa (see earlier review), few studies have examined the genetic basis of competition-defense tradeoffs. Furthermore, given that tradeoffs may shape both population evolution and divergence, I ascertain whether competition-defense tradeoffs are expressed and comparable at within versus among population scales.

Here I use growth and survival trials in mosquitofish to quantify competitiveness and defendedness in individuals from numerous common-reared populations representing a few different predation regimes. I demonstrate that within a background (i.e. within multiple populations of fish that have similar predator regimes), there is compelling evidence for competition-defense tradeoffs linked to body size. I also show that across landscapes, competitiveness and defendedness are positively related, and I argue that this trend indicates ongoing local adaptation due to the fairly recent introduction of mosquitofish. I note that this genetic competition-defense tradeoff provides a compelling explanation for the abundant evidence of eco-evolutionary dynamics in mosquitofish, including in other chapters of this dissertation.

1.2.5 Chapter 6: Ecological and phenotypic causes and consequences of eco-evolutionary trophic cascades in mosquitofish

In this chapter, I investigate the plastic and genetic contributions to antipredator adaptation in mosquitofish, and the ecological impacts of such adaptation in pond mesocosms. Numerous experiments have shown contemporary local and antipredator adaptation in poecilid fishes (Bassar et al., 2010, 2012; Langerhans, 2009; Langerhans and Makowicz, 2009; Langerhans et al., 2004; Meffe, 1991; Palkovacs et al., 2009, 2011; Reznick and Endler, 1982; Reznick et al., 1990; Stearns, 1983b, 1983a; Stockwell and Weeks, 1999), and many have shown that this adaptation can cause contemporary ecological change (Bassar et al., 2010, 2012; Palkovacs et al., 2009, 2011). Much of this (and other (Des Roches et al., 2013; Palkovacs and Post, 2009)) evo-to-eco work has assumed that adaptation is a mixture of plastic and genetic change, without disentangling the two.

Here I assess morphological and behavioral traits in replicate populations of western mosquitofish from three backgrounds: low-predation, bass-predation, and bluegill predation, which have each undergone multi-generational common-rearing with or without bass cues. This crossed, population-replicated design allows me to quantify genetic and plastic local- and anti-predator adaptation. I then use pond mesocosms experiments to examine the ecological consequences of the above fish adaptation. I compare the relative effect sizes of adaptation versus fish density. I show that trait variation in mosquitofish is caused by a diverse mix of genetic and plastic processes. I also show broadly-reaching impacts of mosquitofish trait variation on food web form and function.

CHAPTER 2

ECO-EVOLUTIONARY FEEDBACKS FROM NON-TARGET SPECIES INFLUENCE HARVEST YIELD AND SUSTAINABILITY

2.1 ABSTRACT

Evolution in harvested species has become a major concern for its potential to affect yield, sustainability, and recovery. However, the current singular focus on harvest-mediated evolution in target species overlooks the potential for evolution in non-target members of communities. Here we use an individual-based model to explore the scope and pattern of harvest-mediated evolution at non-target trophic levels, and its potential feedbacks on abundance and yield of the harvested species. The model reveals an eco-evolutionary trophic cascade, in which harvest at top trophic levels drives evolution of greater defense or competitiveness at subsequently lower trophic levels, resulting in alternating feedbacks on the abundance and yield of the harvested species. The net abundance and yield effects of these feedbacks depends on the intensity of harvest and attributes of non-target species. Our results provide an impetus and framework to evaluate the role of non-target species evolution in determining fisheries yield and sustainability.

Keywords: Harvest-induced evolution, eco-evolutionary feedbacks, individual based modeling

2.2 INTRODUCTION

Harvest by humans is frequently associated with significant accumulating changes in the traits of targeted species (Darimont et al., 2009b; Sharpe and Hendry, 2009). In marine and freshwater systems, selective harvesting of the largest and oldest fish often favors smaller fish that mature earlier (Audzijonyte et al., 2013b; Carlson et al., 2007; Heino et al., 2015; Jørgensen et al., 2007), thereby driving trait changes that increase the fishing effort necessary to yield a consistent biomass haul (Conover and Munch, 2002; Law et al., 2015). Harvest-induced trait changes can also alter demographic parameters linked to sustainable stock growth or recovery of stocks following moratoria (Enberg et al., 2009). Concerns for these effects on sustainable yield and population abundance have prompted growing demand for evolutionary impact assessments (Laugen et al., 2014; Mollet et al., 2015) that link different harvest intensities and patterns to anticipated dynamics of trait change, abundance and yield over contemporary time scales (i.e., 50-100 years).

Recently, concerns for sustainability have further expanded to consider ways in which evolution in harvested species can cause cascading ecological changes in lower trophic levels (Audzijonyte et al., 2013a, 2014; Fenberg and Roy, 2008), sometimes reducing community stability and resilience (Kuparinen et al., 2016). To date, the common denominator to this work has been an almost singular attention to evolution of the harvested species themselves. In this study, we expand focus to ways in which harvested species abundance and yield could be impacted by evolution in lower trophic levels. We use an eco-evolutionary dynamic modelling framework (Fussmann et al., 2007; Kinnison and Hairston, 2007; Post and Palkovacs, 2009) to explore whether there is reason to cast our Darwinian net more broadly to consider trait changes and their ecological feedbacks in non-harvested members of communities.

Cascading community changes caused by harvest are likely to generate contemporary evolution in trophic levels below those occupied by harvested species. Many harvested species are at or near the apex trophic level in many communities (Pauly et al., 1998). Harvest-induced depression of these top trophic levels can therefore cause cascading density- and behaviorally-mediated changes in lower, non-harvested trophic levels (Frank et al., 2005; Schmitz et al., 2004). Such changes in community structuring mirror changes in predation regimes that have generated many examples of contemporary evolution in lower trophic levels (Langerhans, 2009; Magurran, 1990; Palkovacs et al., 2011). Indeed, contemporary evolution in response to changes in predation has been documented in all levels of pelagic food chains: piscivorous fishes (Carlson et al., 2007), planktivorous fishes (Bassar et al., 2010; Langerhans et al., 2004; Palkovacs et al., 2011), zooplankton (Hairston et al., 2005), and phytoplankton (Kasada et al., 2014; Yoshida et al., 2003). This body of work not only suggests that cascading ecological changes due to harvest might drive evolution in lower trophic levels, but also hints at a trophic pathway by which such evolution could reciprocally feed back on the abundance dynamics of harvested species.

Although specific adaptations to predator or prey regimes are diverse, the nature of these adaptions can be broadly classified along a competition-defense tradeoff spectrum, in which feeding ability and vulnerability to predators are positively related. Such tradeoffs have been explored in a wide range of organisms, including fish (Langerhans, 2009; Palkovacs et al., 2011), plants (Mole, 1994), insects (Fellowes et al., 1999; Kraaijeveld et al., 2002), algae (Agrawal, 1998; Kasada et al., 2014; Yoshida et al., 2003), and bacteria (Lennon and Martiny, 2008). Competition-defense tradeoffs may be behavioral (Conrad et al., 2011; Langerhans, 2009), morphological (Langerhans, 2009; Palkovacs et al., 2011), physio-chemical (Agrawal, 1998; Lind et al., 2013), or life-historical (Reznick et al., 1990; Stearns, 1983b, 1983a, 1989).

Importantly, a substantial body of theory and experimentation, much beginning with Pimentel's (Pimentel, 1961) pioneering work on the 'genetic feed-back', indicates that contemporary evolution along this tradeoff can substantially influence the abundance and stability of both predator and prey populations (Abrams, 2009; Abrams and Matsuda, 1997a, 1997b; Kasada et al., 2014; Yoshida et al., 2003). The population dynamical signatures of evolution along this trade-off have recently been uncovered in many "classic" predator-prey experiments, in which evolution was not originally considered (Hiltunen et al., 2014).

Here we investigated the eco-evolutionary consequences of contemporary evolution in non-harvested species during harvest. We sought to develop a generalized model to explore the scope (capacity) and pattern of evolution of non-target evolution and feedbacks on stability and yield of the harvested species, with the intent that our findings might serve to generate baseline predictions for future empirical and theoretical exploration. We took an eco-evolutionary dynamics approach, employing a multi-trophic-level, individual-based model (Fussmann et al., 2007; Post and Palkovacs, 2009). Because other models exist to predict trait and ecological consequences of evolution in harvested species, we focus here on the evolutionary outcomes and feedbacks originating in non-harvested members of the community, which were modeled as discreet trophic levels. Genotypes and phenotypes of specific non-harvested trophic levels were allowed to evolve (eco-evolutionary model), or not (ecology-only model), in bifurcated model runs that split after the onset of harvest. These bifurcated runs allowed us to isolate eco-evolutionary from purely ecological processes. Although greater complexity might be added to our approach, the consistent and generalizable patterns we observed provide insight into specific conditions under which non-target evolution and eco-evolutionary feedbacks might be most overt and critical to harvest sustainability.

2.3 METHODS

2.3.1 Model overview

We used a generalized individual-based model to extend our eco-evolutionary framework beyond classic two-level predator-prey models to simulate a four trophic-level community in which the top or penultimate trophic levels (referred to as the top predator and secondary consumer, respectively) were subjected to a range of harvest intensities. We simulated evolution and ecology dynamically along a competition-defense tradeoff axis separately in each trophic level below the harvested level by allowing genotypes and phenotypes at that focal level to undergo selection based on the reciprocally interacting abundance dynamics of their own predators and prey. Evolution was an emergent property of this system (Epstein, 1999), with selection a byproduct of predator and prey dynamics and inheritance determined by genetic and environmental components. We analyzed the effect of contemporary evolution in non-harvested species on abundance and yield of the harvested species by comparing bifurcated models: an *eco-evolutionary model*, in which evolution was allowed to continue after the initiation of harvest, and an *ecology-only model*, in which genotypes were frozen at the harvest onset mean, although environmental variation was retained. This approach allowed us to compare models to determine the extent to which evolution in non-harvested species impacts yield and sustainability of harvest for targeted species.

Competition-defense tradeoffs. Preliminarily, we tested a wide range of competition-defense tradeoff slopes (Fig. A.1) in trophic levels below the harvested species to determine the trait space within which evolution in response to harvest would occur. We calculated tradeoff slope as:

(2.1)

$$S = \frac{\frac{\partial a}{\partial G}}{\frac{\partial v}{\partial G}}$$

In which *S* = tradeoff slope, *a* = attack rate on resources, *v* = vulnerability to predators, and *G* = genotype (coded as a continuous quantitative trait). We ran simulations at moderate harvest levels (levels that would visibly reduce the abundance of the harvested species but would almost never lead the harvested species to extirpation). For these initial runs we simulated evolution separately for each trophic level (i.e. we allowed only one trophic level to evolve at a time). For all tested trophic levels in all model structures, we observed a central range of competition-defense tradeoff slopes that led to marked evolution after harvest onset (see Results and Discussion). Because our primary goal was to discern generalized patterns of potential feedbacks from non-target evolution (to inform future data collection and investigations), we selected competition-defense tradeoff ratios roughly in the middle of these slope ranges for the following analyses.

Single trophic level evolution. Using the above selected values for competition-defense tradeoff ratios, we simulated a range of consistent effort harvest from negligible harvest to overharvest resulting in harvested species extirpation. We ran a separate model for each non-harvested trophic level, allowing only that trophic level to evolve. We observed differences in eco-evolutionary and eco-only model results for harvested species abundance, yield, and stability across the harvest intensity gradient. We attributed differences between these two models to eco-evolutionary processes rooted in evolution in the lone evolving trophic level.

Multi-trophic evolution. We subsequently tested for effects of evolution in multiple non-harvested trophic levels on the harvested species. Using our four-trophic level model in which the top predator was harvested, we allowed pairs of trophic levels to evolve to examine the potential for evolutionary reinforcement or compensation at multiple trophic levels. We also ran models in which all trophic levels below the harvested species evolved and varied food web length (four or three trophic levels total) to examine how “fishing down the food web”(Pauly et al., 1998) would change the net effect of evolution on the harvested species.

2.3.2 Model design and details

We built an individual-based model framework using Matlab R2015b software. The model uses iterative Monte Carlo methods to simulate four discrete populations, with each population constituting an entire trophic level (Figure 2.1).

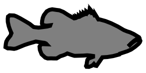
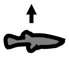
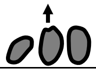
Trophic level		Harvest-mediated abundance change	Direction of evolution	Effect of evolution on harvested top predator abundance
	Harvested top predator	-		
	Secondary consumer	+	↑ Competitiveness ↓ Defense	+
	Primary consumer	-	↓ Competitiveness ↑ Defense	-
	Producer	+	↑ Competitiveness ↓ Defense	+

Figure 2.1. Harvest-induced eco-evolutionary trophic cascades. Cascading harvest-mediated abundance changes cause evolution in lower, non-target species, which then feeds-back to bolster or undermine the harvested top predator. Directions of abundance changes, evolution, and feedbacks alternate predictably down the food chain. Results are from an individual-based model with four trophic levels, each feeding exclusively on the level below it. Patterns are robust to models in which the penultimate trophic level (secondary consumer) was harvested instead of the top predator.

Representing populations, genotypes and phenotypes. Each population was represented by a data table, in which each row represented an individual and each column represented a trait. Births were appended to each table; deaths were deleted.

Each individual in each population had a quantitative trait genotype influencing its competition-defense phenotype. Our assumption of polygenic inheritance is grounded in the premise that such performance phenotypes likely reflect the additive influence of many interacting traits that may or may not be polygenic themselves. For efficiency and generalizability, we produced subsequent generations by sampling directly from standing genetic variation (i.e. asexually) and applying a mutational component. Sampling to produce offspring was weighted by the survival and reproductive probabilities of current organisms. Such inheritance is generalizable as an additive polygenic trait (Hill et al., 2008), and is robust to most dominance and recombination structures, while avoiding variance deflation (Fisher, 1930). We allowed a small chance of mutational input to variation during each reproductive event:

(2.2)

$$G_{oumCrineyffspring} = G_{parent} + Binomial(P_{mutation}) * Normal(0, \sigma_{mutation})$$

G = genotype; $P_{mutation}$ = mutation probability; and $\sigma_{mutation}$ = average mutation severity. Mutation probability was set to 0.12 for all trophic levels, consistent with mutational inputs for a highly polygenic phenotype (i.e. mutation is rare (Baer et al., 2007; Drake et al., 1998; Eyre-Walker and Keightley, 2007), but an effectively large number of loci makes mutations likely). The standard deviation of mutation severity was set to 0.05 for all trophic levels. The starting mean and standard deviation for *G* were 0 and 0.25, respectively. In our model, the individual's genotype simultaneously defines its competitiveness and defense, defined proximately as its attack rate, vulnerability, and death rate. Therefore, these three characteristics covaried, consistent with observed competition-defense tradeoffs (see Introduction). Finally, consistent with quantitative polygenic inheritance, attack rate, vulnerability, and death rate were all affect by a coefficient of environmental variation error term (noise), with the net effect that effective heritability was roughly 0.60.

(2.3)

$$a_i = (a_0 + \epsilon)(100 + AG_i)$$

(2.4)

$$v_i = (v_0 + \epsilon)(100 + VG_i)$$

(2.5)

$$d_i = (d_0 + \epsilon)(100 + MG_i^2)$$

a_i = attack rate; a_0 = inherent attack rate; ϵ = normally distributed error term; A = contribution of focal gene to attack rate; G_i = genotype; v_i = vulnerability; v_0 = inherent vulnerability; V = contribution of focal gene to vulnerability; d_i= death rate; d_0 = inherent death rate; M = contribution of focal gene to death rate.

A heritability of 0.6 is relatively high (Serbezov et al., 2010), but we suggest this is justifiable on the grounds that contemporary evolution of performance tradeoffs leverages heritability of multiple traits, including component traits with higher than average heritability (but see Discussion).

We manipulated the tradeoff ratio (*A*/*V*) to determine the slope of the competition (*A*) – defense (*V*) tradeoff for each population. A high tradeoff ratio indicated “cheap” competition, while a low tradeoff ratio indicated “cheap” defense (Fig. A.1). Death rates increased quadratically with extreme phenotypes in order to prevent runaway evolution in cases of complete predator absence and to ensure some measure of canalization (Abrams and Matsuda, 1997b).

Iterating events. Births were dependent on the total number of prey consumed by each individual, which were in turn dependent on its phenotypic attack rate and the pool of available prey (determined by the death rate of the next lower trophic level):

(2.6)

$$L_i = b_i \frac{\frac{a_i}{1 + h_i a_i N_{prey}} N_{prey\ eaten}}{\overline{\left(\frac{a}{1 + haN_{prey}}\right)} N}$$

L_i = birth probability; b_i = conversion efficiency; a_i = attack rate; h_i = handling time; N_{prey} = prey abundance; $N_{prey\ eaten}$ = number of prey whose deaths were from predation (see death formula).; N = number of consumers. Note that the denominator is a mean quantity for the entire population.

We determined deaths through an intrinsic mortality rate plus deaths from predation, as determined by an individuals' phenotypic vulnerability to predators:

(2.7)

$$d_{P_i} = \overline{\left(\frac{a_{pred}}{1 + h_{pred} a_{pred} N_{pred}}\right)} v_i N_{pred}$$

d_{Pi} = death probability from predation; a_{pred} = predator attack rate; h_{pred} = predator handling time; N_{pred} = number of predators; v_i = vulnerability to predators.

(2.8)

$$D_i = 1 - (1 - d_i)\left(1 - d_{P_i}\right)$$

D_i = total death probability; d_i = intrinsic death probability; d_{Pi} = death probability from predation.

We accounted actual births by sampling a Poisson distribution with a mean of L_i. We accounted actual deaths by sampling a binomial distribution with a mean of D_i. To quantify which deaths were actually from predation, we summed the proportion of the death probability that was due to predation for all dead individuals:

(2.9)

$$N_{eaten} = \sum_i \left(\frac{d_{P_i}}{D_i} \middle| dead \right)$$

We used this term to inform the number of births at the next higher trophic level (see above). Producers consumed a finite but replenishing resource.

Adding harvest. We assumed a consistent effort harvest with a small normally-distributed (5%) error term. We added harvest by modifying the death rate of the top or penultimate predator:

(2.10)

$$D_i = 1 - (1 - d_i)\left(1 - d_{P_i}\right)\left(1 - f - \epsilon_f\right)$$

D_i = death rate of the harvested species; d_{Pi} = death rate from predation sources; d_i = inherent death rate; f = harvest intensity; ϵ_f = harvest error from variability, sampled from a normal distribution.

We calculated yield as the proportion of deaths each iteration that were due to harvest:

(2.11)

$$Y = \sum_i \left(\frac{f + \epsilon_f}{D_i} \middle| dead \right)$$

Y = harvest yield; f = harvest intensity; ϵ_f = harvest error; D_i = total death rate of the harvested species.

We tested a range of harvest intensities from harvests that had no appreciable impact on the harvested species to overharvests that led to harvested species collapse.

Parameterizing the model. We used parameter combinations that generated a primary producer population size that was computationally manageable. We then used geometric changes in attack rate, handling time, and death rate to create a community in which population size decreased and generation time increased with increasing trophic level (Table 2.1). Attack rates, vulnerability, and death rates were modified by genotype in focal, evolving trophic levels (see *Representing populations, genotypes, and phenotypes*); the values in Table 1 represent the starting values that were then modified by genotypes.

Symbol	Parameter	Producer value	Geometric change with increasing trophic level
a_0	Attack rate	$6*10^{-6}$	/ 2
v_0	Vulnerability	1	* 1
b	Conversion efficiency	$1*10^{-3}$	* 1
h	Handling time	$2*10^{-10}$	* 100
d_0	Death rate	$1*10^{-3}$	/ 4

Table 2.1. Starting population parameter values for individual-based model simulations.

Running the model. We ran about 200 batches of simulations for each trophic level below the harvested species. In each batch, we allowed only one, non-harvested trophic level to evolve (Figure 2.2). We initially ran the model for 12,500 iterations (when the lowest trophic level evolved; 20,000 when the next higher trophic level evolved, and 50,000 when the penultimate trophic level evolved) to attain quasi-equilibrium. We then initiated harvest and ran for an additional 12,500 iterations under harvest conditions (when the lowest trophic level evolved; 20,000 when the next higher trophic level evolved, and 50,000 when the penultimate trophic level evolved). In this model each iteration represents a finite time step. While the total number of time steps is very large, the effective generation time (T) of each trophic level spans 1.5 to 640 iterations. Using the definition of a generation as the mean time between the birth of an individual and the birth of its offspring, a 10,000 iteration time window in our model equated with 16 generations of the top predator ($T = 639.50$), 185 generations of the secondary consumer ($T = 54.15$), 580 generations of the primary consumer ($T = 17.19$), and 6450 generations of the producer ($T = 1.55$).

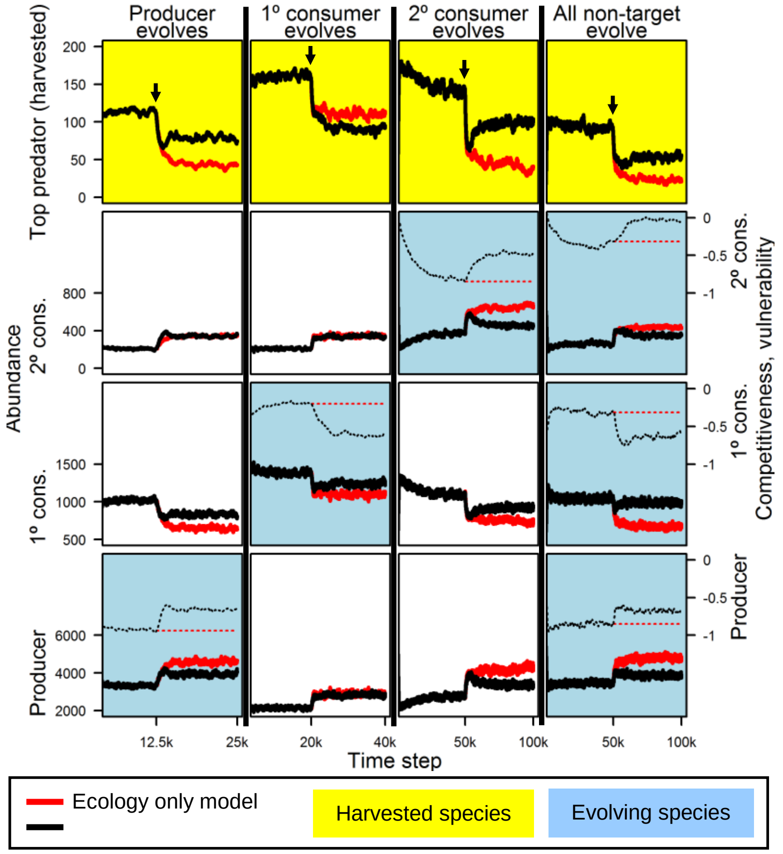


Figure 2.2. Eco-evolutionary consequences of non-target species evolution during harvest. Harvest-mediated evolution in non-target trophic levels feeds-back to undermine or bolster the harvested top predator. Each column represents a unique set of simulations. Odd-numbered trophic levels below the harvested species evolve increased competitive ability and decreased defense (increased vulnerability) during harvest, which bolsters the harvested species; even numbered trophic levels evolve decreased competitive ability and increased defense (decreased vulnerability), undermining the harvested species. **Black** lines show average results from 12 simulations with evolution on (eco-evolutionary models); **red** lines show average results from 12 simulations in which trait values were fixed at pre-harvest means (ecology only models). Thick lines show population abundances; thin dashed lines show genotype means, which code for competitiveness and vulnerability to predators. Harvest of top predator begins at arrows.

Competition-defense tradeoff ratios were set to maximize evolutionary potential (Figure 2.3). See Figure 2.1 for model structure schematic.

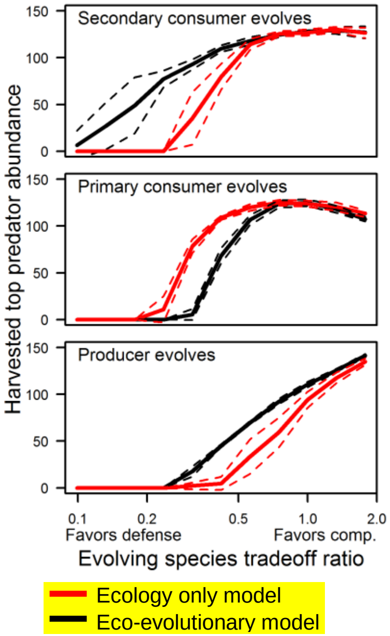


Figure 2.3. Competition-defense tradeoffs and eco-evolutionary potential. Competition-defense tradeoff ratio in non-target species influences the difference between ecological (**red**) and eco-evolutionary (**black**) model predictions of harvest-species abundance. “Cheap defense” tradeoffs (left side) lead to system destabilization prior to harvest. “Cheap competition” tradeoffs (right side) lead to similar predictions from both models. Intermediate competition-defense tradeoffs lead to harvest-induced evolution in non-target species (Figure 2.2), which feeds-back to bolster or undermine the harvested top predator. Solid and dashed lines indicate mean ± one standard deviation for 12 model runs. See Figure 2.1 for model structure schematic.

At initiation of harvest, we split the model into two parallel models: one in which evolution continued and one in which genotypes of the evolving population were frozen at pre-harvest means. The former model therefore includes eco-evolutionary effects of harvest, while the latter includes only ecological effects. We ran these simulations with harvest of either the top or penultimate trophic level (referred to as the top predator and secondary consumer, respectively). To determine the range of harvest intensities to explore with our model, we ran the models with no evolution occurring at a wide range of harvest intensities and selected an initial harvest intensity that produced a modest depression in the abundance of the harvested species (0.0009 for the top predator; 0.0077 for the secondary consumer). Using this harvest intensity as a setpoint, we ran simulations across a wide range of competition-defense tradeoff ratios (Figures 2.3, A.7) to examine the sensitivity of harvest stability and yield to trade-off ratio. We then subsequently selected a setpoint tradeoff ratio at each trophic level that generated strong differences between eco-only and eco-evolutionary models and assessed the sensitivity of evolution and feedbacks to varying harvest strengths (Figures 2.4, A.8). For the simulations involving *top predator harvest* (and fishing down the food web example) our setpoint ratios (*A*, *V*) were (19.1, 52.5),(22.0, 45.4), (29.5, 39.3) for the secondary consumer, primary consumer, and producer, respectively. For simulations involving *secondary consumer harvest*, the setpoint ratios (*A*, *V*) were (17.0, 58.8), (20.5, 48.8) for the primary consumer and producer, respectively. For all models, canalization parameters (*M*, see *Representing populations, genotypes and phenotypes*) were set to 25.0 for evolving trophic levels.

2.3 RESULTS AND DISCUSSION

Comparing bifurcated simulations consistently revealed a harvest-driven eco-evolutionary trophic cascade, in which upper trophic level harvest led to dynamically coupled evolutionary and ecological changes at proximate and distant trophic levels (Figures 2.1, 2.2).

2.3.1 Harvest induces an eco-evolutionary trophic cascade

For any given trophic level, we found that evolution followed a general pattern. Odd-numbered trophic levels (starting with the level below harvested) evolved increased competition and lower defense and even-numbered trophic levels evolved greater defense and lower competitive ability (Figures 2.1, 2.2). This flip-flopping pattern is analogous to the common depiction of alternating dominance of predation and competition as regulators of abundance in classical density-mediated trophic cascades (Pace et al., 1999). Notably, selection at each intermediate trophic level was mutually reinforced by the relative abundances of its predators and prey, providing particularly strong and consistent selection along the competition-defense tradeoff. An abundant trophic level with relatively few predators and prey faces weaker selection for defense and stronger selection for competition (e.g. secondary consumers, Figure 2.1), whereas a less abundant trophic level with numerous predators and prey faces stronger selection for defense and weaker selection for competitiveness (e.g. primary consumers, Figure 2.1). In this respect, abundance and competitiveness tended to track together (Figure 2.2). Shortening of the food chain to three trophic levels and harvesting the secondary consumer (“fishing down the food web”) (Pauly et al., 1998) led to evolutionary reversal in subsequent non-target trophic levels (Figure A.4), suggesting that the most important factor in determining the direction of evolution in any trophic level was that level’s food web distance from the harvested species.

Importantly, our model shows the direction of causation was not exclusively one of ecology driving evolution; evolution also fed back to affect ecology. For example, adaptive evolution of more competitive but vulnerable prey of a harvested predator tended to feed back positively on that predator's abundance, causing an increase in the population size of the harvested predator. These results are consistent with studies of antipredator evolution and predator abundance in simpler predator-prey systems (Duffy and Sivars-Becker, 2007; Kasada et al., 2014; Lennon and Martiny, 2008; Pimentel, 1988; Pimentel and Al-Hafidh, 1965).

2.3.2 Evolution in lower trophic levels feeds-back to bolster or undermine harvested species

Eco-evolutionary feedbacks again followed a consistent pattern that transmitted in a bottom-up fashion to the harvested species. Evolution in odd-numbered trophic levels (again starting with the level below harvested) fed back positively on abundances of the harvested species, though the net extent of the population increase depended on reinforcing or opposing evolution at other trophic levels (Figures 2.2, 2.4, A.3). Evolution in even-numbered trophic levels fed back negatively on the abundance of the harvested species. Simultaneous evolution at multiple trophic levels resulted in stronger or weaker net demographic effects on the harvested species, depending on which levels evolved (Figures 2.2, A.3). Neighboring trophic levels' eco-evolutionary feedbacks tended to cancel (a form of cryptic eco-evolutionary dynamics) (Kinnison et al., 2015) while once-removed trophic levels' eco-evolutionary feedbacks were reinforcing (Figure A.3).

These eco-evolutionary feedbacks were able to significantly affect yield and sustainability in our simulated harvest fishery (Figure 2.4). Evolution of increased competition in odd-numbered trophic levels below the harvested species increased yield and sustainability. Evolution of increased defense in even-numbered trophic levels decreased yield, and lowered the harvest rates associated with fisheries collapse (Figure 2.4). Evolutionary effects on yield and abundance (i.e. differences between eco-evolutionary and ecology only models) were most pronounced at high harvest levels, whereas evolution of non-target species was at times the difference between obtaining the highest possible yields and complete harvest collapse (Figure 2.4). Results were similar when the penultimate trophic level (secondary consumer) was harvested (Figures A.5-A.7), although abundance changes in the harvested species were lesser in magnitude.

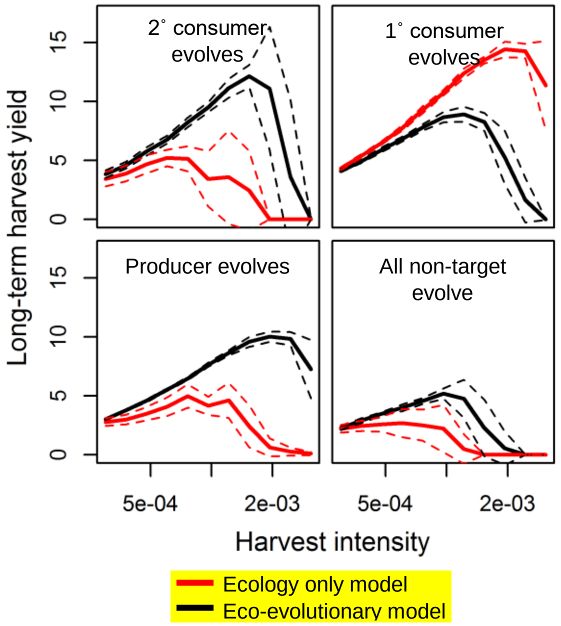


Figure 2.4. Harvest yield predictions from ecology only versus eco-evolutionary models. Evolution in non-target trophic levels can lead to significantly higher or lower long-term harvest yield, maximum sustainable yield, and appropriate harvest intensity. Considering ecological (**red**) processes only and neglecting eco-evolutionary (**black**) model predictions can lead to top predator overharvest, extirpation, or foregone yield, depending on which trophic levels evolve.

Dashed lines show ± one standard deviation for 12 model runs. See Figure 2.1 for model structure schematic.

2.3.3 Competition-defense tradeoff slope dictates eco-evolutionary potential

At all trophic levels, evolution and its ecological feedbacks were most pronounced at intermediate to somewhat defense-biased tradeoffs, where fitness gains or losses from defense were accompanied by similar or slightly smaller changes in competitiveness (Figures 2.3, A.7). Tradeoffs that favored very cheap defense (large changes in defense come with small changes in competitiveness) resulted in community destabilization and extinctions before harvest had started, whereas tradeoffs that favored cheap competition (large change in competitiveness associated with small changes in defense) led to little evolutionary change in response to altered predation regimes. The competition-defense tradeoff bias that led to the greatest difference between eco-evolutionary and ecology only models became more defense-biased with increasing trophic level (Figure 2.3, A.7). These findings are consistent with experiments showing stronger eco-evolutionary feedbacks from balanced than unbalanced tradeoffs in a simpler predator-prey system (Kasada et al., 2014).

2.3.4 Model expansions and future work

Our models reveal consistent patterns that should inform future study in real-world ecosystems. Future work for specific systems should consider nuances and complexities that fall within three broad categories:

1) Factors affecting the pace of evolution. Factors affecting the relative pace of evolution at different trophic levels (e.g. genetic variation, generation time, heritability), should not necessarily change the scope of the patterns we observed here, but will change the timescales on which patterns could be observed. Lower trophic levels in particular, by virtue of their larger population sizes and often shorter generation times, could evolve more quickly in the initial face of harvest on higher trophic levels and thus dominate in early harvest-driven feedbacks. We ran our models with simple assumptions about the relative generation times (see Methods), and long enough to accommodate quasi-equilibrium evolution in any trophic level, but a more nuanced understanding of relative evolutionary rates and transitory dynamics is likely important for detecting patterns and understanding specific outcomes on the management timeframes of real world harvest systems.

2) Factors affecting the scope of evolution. Eco-evolutionary dynamics in our models are strongly nuanced by the form of the competition-defense tradeoff ratios (Figure 2.3). While there is strong theoretical and empirical basis for this tradeoff across diverse species and ecosystems (see Introduction), it remains poorly characterized for many species in harvested ecosystems. Because the goal of this study was to assess the scope for evolution and its feedbacks, our models centered on tradeoff-ratios for which evolution was likely to happen, but it should be noted there exist areas of parameter space for which the eco-evolutionary and ecological expectations were relatively similar (Figure 2.3). This result suggests that future research should place a priority on quantifying the form and slope of these tradeoff ratios for species in harvested ecosystems.

The underlying mechanisms for competition-defense tradeoffs may be both shared and unique across trophic levels. Certain changes in size or life history may be common tradeoff responses at multiple trophic levels (Lind et al., 2013; Mole, 1994; Reznick et al., 1990; Stearns, 1989). Other responses may be more unique, such as greater reliance of primary producers on investments in defense compounds that reduce growth rate (Agrawal, 1998; Lennon and Martiny, 2008; Yoshida et al., 2003, 2007), and greater reliance in upper trophic levels on complex behavioral competition-defense tradeoffs (Conrad et al., 2011; Langerhans, 2009). Although we would contend that competition-defense tradeoffs are broadly universal, it remains to be empirically determined whether and how specific tradeoff mechanisms might modify the scope for eco-evolutionary feedbacks, as might occur if some of these mechanisms are associated with very different tradeoff slopes.

Plastic, rather than genetic, trait change along a competition-defense tradeoff axis could lead to similar patterns to those observed in this model, and should not be ignored when considering real-world systems. Furthermore, although phenotypic plasticity may modify the strength of the genetic evolutionary response to harvest (Ghalambor et al., 2007; Price et al., 2003), plastic responses to harvest-induced trophic cascades may be at least as ecologically impactful as genetic responses (Schmitz et al., 1997, 2004), and likely occur on more immediate time scales.

We modelled a necessarily simplified food chain with discrete evolving trophic levels. The broad phenotypic and feedback patterns we describe are likely robust to the number of evolving species at a given trophic level so long as there is genetic variation (within or among species) along a competition-defense tradeoff. Indeed, the typically greater phenotypic and genetic variation among species could provide faster rates of net phenotypic change. In complex food webs, cascading responses are likely to involve change in both species and intraspecific diversity (Estes et al., 2011; Pace et al., 1999).

3) Factors affecting the scope of eco-evolutionary feedbacks. Ecosystems vary in their connectivity and interspecific interaction strengths, and this variation can dictate the potential for cascading ecological impacts and system stability (Gross et al., 2009; Kondoh, 2003; LeCraw et al., 2014). For example, omnivory, in which one organisms consumes individuals from multiple adjacent trophic levels, might be predicted to alter the strength of the eco-evolutionary dynamics we described here, either by dampening the net feedback—if omnivores integrate opposing feedbacks at neighboring trophic levels—or strengthening the net feedback—if large changes in omnivore abundance generate selection in the same direction at multiple adjacent trophic levels below the omnivore. Along these lines, we would suggest that ecological metrics of cascade strength (Borer et al., 2005; Shurin et al., 2002) or interaction strength (Berlow et al., 1999; Paine, 1980) might be used as an initial means to identify harvested communities where strong non-target evolution and eco-evolutionary feedbacks may be most evident and influential.

2.3.5 Conclusions

The capacity for large differences between purely ecological versus eco-evolutionary models (Figures 2.2-2.4) in our study supports many prior calls for greater consideration of evolutionary processes when managing biological resources (Kinnison and Hairston, 2007; Schoener, 2011; Smith et al., 2014; Stockwell et al., 2003). Under some scenarios, the predictions from ecology-only simulations differed substantially from eco-evolutionary simulations, suggesting that failure to account for evolution in non-target species could appreciably influence risks associated with overharvest or sacrificed yield (Figure 2.4). Likewise, our results hint that harvesting at some trophic levels might be more prone to positive or negative feedbacks than others due to compensatory effects at other trophic levels. Taken broadly our findings suggest that evolutionary impact assessments that focus exclusively on evolution in harvested species may provide an incomplete picture of evolution's role in harvested ecosystems. However, this result does not imply that evolutionary management is complex beyond reach, or that non-target evolution is intrinsically bad for fisheries outcomes. While our model highlights some considerations that may ostensibly complicate real-world resource management, it also highlights general patterns that are testable in natural systems and processes that could convey a degree of resiliency in harvested ecosystems. Finally, our results a logical next step of investigating the interaction between evolutionary processes in harvested trophic levels (i.e. harvest-induced evolution) and evolutionary processes in lower trophic levels.

2.4 ACKNOWLEDGMENTS

We thank Tim Waring [University of Maine] and Ariel Wertheim [Dartmouth College] for advice on model management, as well as Steve Munch [NOAA] and Anna Kuparinen [University of Helsinki] for manuscript comments and advice. The University of Maine Ecology and Evolution of Everything group also provided invaluable comments during the early stages of this project.

Funding was provided by:
University of Maine Graduate Student Government
University of Maine Agricultural and Forest Experiment Station

US National Science Foundation: DEB 1457112; DEB 1457333

NOAA Cooperative Institute for Marine Ecosystems and Climate

CHAPTER 3

PHENOTYPIC AND COMMUNITY CONSEQUENCES OF CAPTIVE PROPAGATION IN MOSQUITOFISH

3.1 ABSTRACT

Captive propagation can lead to phenotypic change in fish populations, but the broader community-level consequences of captive phenotypes remain largely unknown. We investigate the degree to which captive propagation alters the phenotypes and ecological roles of fish stocked into wild communities. We focus on captive propagation of western mosquitofish (*Gambusia affinis*) for biocontrol, which represents one of the largest-scale production efforts for any fish released into the wild. Captive propagation in mosquitofish consistently generated novel mixtures of morphological and behavioural traits that deviate from those of wild populations. A mesocosm experiment showed that mosquitofish from captive propagation facilities differ from wild fish in their effects on aquatic community structure by shifting their consumption to less-mobile, benthic prey. Captive-propagated and translocated wild fish stocks not only differ in phenotype, but can have substantially different ecological effects on the communities into which they are introduced. Therefore, captive propagation programs involving continual release should expand their concerns beyond altered phenotypes and fitness to include whether propagated fish actually provide the intended ecological roles and services associated with their wild counterparts. Infusions of wild alleles and captive environments that mimic wild conditions are recommended strategies to retain the desired ecological role of captive-propagated fish.

Keywords: biological control, domestication, captive propagation, trophic cascades, fish introductions, antipredator evolution, mosquitofish

3.2 INTRODUCTION

Captive propagation and stocking of fishes constitutes a massive human intervention in many wild ecosystems. Hundreds of species are currently reared at least partially in captive environments (e.g. hatcheries), with limited genetic input from wild populations (Bilio, 2007; Teletchea and Fontaine, 2012). Captive propagation and release programs are commonly operated with the intent to bolster declining wild populations (Araki and Schmid, 2010; Brown and Day, 2002), support commercial or sport-fishing activities (Halverson, 2008), or produce fish for biological control of pest species (Chandra et al., 2008; Swanson et al., 1996). Growing focus has been given to whether captive-propagated fish provide the socio-economic or ecological services associated with wild fish (Ham and Pearsons, 2001; Pearsons and Hopley, 1999; Pister, 2001). Whereas captive-propagated fish can fill some socio-economic roles of wild fish—providing food or recreation—we know much less about the extent to which captive-propagated fish serve as true ecological surrogates for wild fish. Recent evidence suggests that divergent phenotypes of fishes and other organisms can lead to markedly different ecological conditions (Des Roches et al., 2018). Here we test the extent to which phenotypic (plastic and genetic) divergence of captive-propagated and wild fish may change the functional traits and ecological roles of captive lines in ways that alter aquatic community structure and ecosystem function following stocking.

Multi-generational captive rearing is widely associated with marked divergence from wild sources in morphology, life-history, and behaviour. Captive-propagated fish are often larger and have different fin placement and body shapes than wild fish (Pulcini et al., 2013). Propagated fish also tend to grow and mature more rapidly (Lorenzen et al., 2012; Vøllestad et al., 2004).

Behaviourally, propagated fish are generally less aware of predators, worse at identifying profitable food sources, and have inappropriate levels of aggression towards conspecifics (Fenderson and Carpenter, 1971; Huntingford, 2004; Swain and Riddell, 1990). Such phenotypic change can markedly decrease survival and reproduction in the wild (Araki et al., 2007, 2008; Bowlby and Gibson, 2011; Christie et al., 2014; Milot et al., 2013). However, such a focus on reduced fitness does not fully address the extent to which captive-propagated fish fill the same ecological roles as wild fish.

While little work has directly examined the ecological role of captive-propagated fish in wild environments, suspected changes in the functional traits of propagated fish hint at important consequences for communities and ecosystems. Divergence in morphological traits that affect feeding mode and efficiency—i.e. head shape and body streamlining (Albertson et al., 2003; Domenici et al., 2008; Langerhans, 2009)—are especially likely to have strong community impacts by determining the type and quantity of prey consumed and ability to avoid predators. Divergence in behavioural traits may also be important, including traits associated with habitat use, risk avoidance, and foraging patterns (Schluter, 1993; Werner et al., 1983).

Here we assess phenotypic divergence and resulting ecological consequences around introductions of captive-propagated versus wild western mosquitofish (*Gambusia affinis*). Mosquitofish are the most commonly used form of mosquito biocontrol in the world (Swanson et al. 1996). We evaluate two hypothetical mechanisms for how captive propagation might shape the traits and community effects of propagated mosquitofish relative to wild-sourced fish:

1. A main goal of captive propagation is to increase survival by removing predation. Therefore, captive propagation may produce functional traits and ecological consequences similar to those of wild, predator-naïve populations.
2. Additionally, captive environments may differ from wild environments—not only through predator absence but also in a host of other important ways (e.g., higher densities, artificial feeding, simplified environments). Therefore, captive propagation may produce novel combinations of functional traits, which lead captive-propagated fish to have unique ecological consequences when released into the wild.

We examined behavioural and morphological traits across multiple captive-propagated and wild mosquitofish populations. We also used replicated pond mesocosms to assess cascading community effects of stocking captive-propagated versus wild-translocated fish. By comparing the community-level effects of mosquitofish from mosquito-control propagation facilities with those caused by mosquitofish from wild populations, we assess the ecological implications of stocking captive-propagated fish versus the alternative of translocating wild fish. As both methods are widely employed by mosquito control districts (Swanson et al., 1996), this comparison not only provides novel insights into ecological consequences of captive propagation, but also illuminates yet unknown environmental impacts of these alternative biocontrol approaches.

3.3 METHODS

We studied 11 populations of western mosquitofish (*Gambusia affinis*) from central California, USA (Figure B.1, Table B.1 in Supporting Information) representing three different source-types: (1) mosquitofish produced for biocontrol in captive propagation facilities, (2) wild mosquitofish from low-predation ponds lacking largemouth bass (*Micropterus salmoides*), and (3) wild fish from high-predation ponds with largemouth bass present. Largemouth bass are a dominant predator of mosquitofish and have been shown to play a strong role in shaping the functional traits of mosquitofish populations (Langerhans, 2009; Langerhans et al., 2004).

Western mosquitofish were introduced to California in the 1920s for mosquito control (Lenert, 1923). Although detailed stocking records and population genetic surveys are lacking these original fish were widely stocked and translocated throughout the region over a period of decades. As such, any differences among wild populations are presumed to reflect a combination of recent local divergence (through genetic adaptation and genetic drift) and phenotypic plasticity.

In central California, millions of mosquitofish are stocked annually with the intent to control mosquito borne diseases (Contra Costa Mosquito Vector Control, 2016; Sacramento-Yolo Mosquito & Vector Control, 2016). The potential for ecologically-relevant phenotypic change in captive-propagated mosquitofish is high due to their capacity for contemporary adaptation (Langerhans, 2009; Langerhans et al., 2004; Stearns, 1983b; Stockwell and Weeks, 1999). The widespread introduction of mosquitofish, despite their strong, often harmful ecological effects on aquatic communities (Hurlbert and Mulla, 1981; Hurlbert et al., 1972; Pyke, 2008) makes it particularly important to assess how captive propagation might shape these effects. In mosquitofish propagation facilities, fish breed volitionally in indoor tanks or in covered outdoor ponds where they are surface fed a powdered diet of fish meal. A subset of fish are periodically netted for stocking.

We compared the following traits and ecological effects among captive-propagated, wild high-predation, and wild low-predation mosquitofish populations: 1) morphology: 10 populations (two captive-propagated, five wild low-predation, and three wild high-predation); 2) exploratory behaviour: 10 populations (three captive-propagated, four wild low-predation, and three wild high-predation); 3) boldness and activity behaviour: 3 populations (one captive-propagated, one wild low-predation, one wild high-predation; 4) ecological effects: 3 populations (the same as (3)). Differences in the number of population sources for each response were due to differences in availability of fish sources at the time of each study and infrastructure requirements (small tanks versus large mesocosms). For wild sources, we collected mosquitofish using a mixture of seine- and hand-netting; for captive sources, we used hand-nets, only. The predation regime of each pond was determined by performing repeated fish surveys over multiple dates using beach seines.

3.3.1 Morphology

For morphological analyses, we used adult mosquitofish collected in July and August 2015. We euthanized all fish on-site and froze them for later morphometric analyses. We also supplemented our morphology dataset with individuals from the populations that were used in the subsequent mesocosm experiment (see below).

3.3.2 Exploratory behaviour

To assay exploratory behaviour, we collected adults in June, July, and August 2016. We held these fish at the University of California-Santa Cruz, Coastal Science Campus for 18-24 hours to acclimate without food prior to experimentation.

We assayed mosquitofish exploratory behaviour within a social context by measuring the latency time it took male-female pairs of the same population source to exit a refuge. We assessed exploratory behaviour in pairs because mosquitofish are extensively social, and males are almost always found in close company of females. Here we define exploratory behaviour as "willingness to investigate novel environments" (Conrad et al., 2011). We used an experimental arena consisting of an opaque plastic tank (49 cm x 34 cm x 21.5 cm) with a cylindrical PVC refuge (20.5 cm tall, 11 cm diameter) secured to the inside wall of the tank. The PVC cylinder (refuge) contained a remotely operated door that could be opened or closed by rotating the cylinder. We placed cardboard around each trial enclosure to minimize exposure of the fish to movement outside the tank and observed fish through a small hole.

For each trial, we placed one female and one male fish inside the PVC refuge and allowed them to acclimate for five minutes. After allowing the fish to acclimate, we opened the refuge door and recorded the time each fish remained in the refuge before exiting, with a maximum trial time of 10 minutes. After each trial, we measured total length (including caudal fin) of each fish.

We calculated individual latency to exit the refuge as the proportion of the 600-second trial spent inside the starting refuge by each fish. We arcsine-square-root transformed these proportions for normality, then calculated the mean transformed latency of each pair of fish. We analyzed mean transformed latency of each pair, rather than individuals, as latency times of individuals within a pair were correlated (Figure B.2) (Arrington et al., 2009).

3.3.3 In-mesocosm boldness and activity behaviour

We examined predation/propagation source-type differences in mosquitofish boldness and activity level using in-mesocosm behavioural assays (see mesocosm setup below) on day 18 after mosquitofish introduction. Here we define boldness as “reaction to a situation perceived as dangerous” and activity as degree of movement in the same situation (Conrad et al., 2011; Réale et al., 2007). Mosquitofish tended to associate in tight shoals within our mesocosms. The same (treatment-blind) observer stood motionless next to each mesocosm and observed the shoal of mosquitofish for 150 seconds. At 30 second intervals, after a 30 second waiting period, the observer noted depth, distance from the observer, and activity level of the shoal, all on a scale of 1-4. We calculated depth and distance by visually dividing the mesocosms into four equal thickness zones (perpendicular to the observer), then recording which zone the majority of the shoal occupied at the time of recording. Activity level ranks were based on type of shoal movement: 1-no movement; 2-slow, steady movement; 3-exploring, foraging; 4-burst-swimming, aggressive interactions.

3.3.4 Ecological consequences

To assess ecological consequences of mosquitofish introduction and trait divergence, we used fish collected in May 2016. Our captive line was provided by Contra Costa Vector Control in Concord, California. This lineage has been reared in captivity with very limited genetic input for over 10 years, or roughly 30 mosquitofish generations (Chris Miller, personal communication). Our wild low-predation line for this experiment (Table B.1) was taken from the primary wild source location used to establish the Contra Costa captive-propagated population (Chris Miller, personal communication). These three populations exhibited morphological and behavioural traits distributions that mirrored those of other populations from their respective source-type (captive-propagated, wild high, wild low; Figures B.3, B.4, B.5). We held all three populations in outdoor 330 L Rubbermaid cattle tanks for a week before experimentation.

We established 48 1,100 L mesocosms in Santa Cruz, California, which we seeded with benthic and pelagic components of local pond ecosystems. We added unfiltered water (4 L total) equally from West Lake (N 36.976083°, W 122.045683°) and Antonelli Pond (N 36.955566°, W 122.060489°), both in Santa Cruz, to foster colonization by a diverse pelagic community. We added 20 L air-dried sediment and 1 L active sediment from West Lake, Santa Cruz. We added equal aliquots of zooplankton (> 80 μm) from tows taken at both West Lake and Antonelli Pond to all mesocosms. We allowed mesocosms to settle and develop for 5 days before fish addition. We added dechlorinated city water to the mesocosms halfway through the experiment to compensate for evaporation.

We established four mesocosm treatments, one for each mosquitofish background (captive-propagated, wild low-predation, wild high-predation), plus a fish-free control. Each mesocosm, except those in the control treatment, received six fish, which were a haphazard mixture of mature males and females. Fish remained in each mesocosm for three weeks. The three-week duration of this experiment is similar to that of other mosquitofish mesocosm experiments, which documented consistent ecological consequences of mosquitofish phenotype (Fryxell and Palkovacs, 2017), and was long-enough for us to observe consistent ecological effects of mosquitofish presence and phenotype (see Results).

We sampled common mosquitofish prey (zooplankton and benthic chironomid larvae) and primary producers to examine cascading ecological effects of mosquitofish presence and phenotypic divergence. Starting on the day of fish addition, we collected zooplankton weekly from 4 L water samples on 80 µm mesh and phytoplankton from 1 L water samples on a 0.7 µm filter. We later identified zooplankton to common taxonomic clades (cladocerans and copepods [adult and nauplii]) under a dissecting microscope. We estimated phytoplankton abundance as chlorophyll-a concentration measured fluorometrically (Turner Designs, Trilogy Module CHL-NA). We measured 24-hour gross primary production (GPP) using three dissolved O_2 measurements over a 24-hour period (dawn, dusk, and dawn) 1 and 2.5 weeks after fish addition. We calculated GPP as the amount of oxygen produced during one day + the amount of oxygen consumed during the following night (Harmon et al., 2009). We added plastic tiles (28 cm^2 surface area) on the day of fish addition. These were removed two and three weeks after fish addition to measure benthic chlorophyll a and benthic chironomid larva abundance. At the end of the experiment, we measured total adult fish dry biomass, adult sex ratio, and counted the number of fry present in each mesocosm.

3.3.5 Analyses

We used R software (R Core Team, 2016) for all statistical analyses. All general and generalized linear mixed models were fit with the functions *lmer* and *glmer,* respectively, in the *lme4* package (Bates et al., 2015). We conducted likelihood ratio tests on the components of all models with the default *anova*(*glmer* or *lmer*) functions. We also categorized source-type level differences within all models (except exploratory behaviour) using *post-hoc* multiple comparison tests with the function *glht* in the *multcomp* package (Hothorn et al., 2008).

We used allometric models to examine differences in mosquitofish morphology across captive propagation and wild source-types. We used Image J (Schneider et al., 2012) to measure several morphometric distances on each adult mosquitofish: snout – posterior edge of eye, snout – dorsal fin, snout – anal fin, minimum caudal peduncle depth, and total body length (excluding caudal fin). We fit the following allometric model for each morphometric variable:

(3.1)

$$\ln(Y) = a \cdot S + b \cdot \ln(L) + P$$

Y is a morphometric variable, S is source-type, L is body length, P is a random effect term for population within source-type, and a and b are model coefficients. We fit models using ln-ln-transformed data, separately for each sex.

To analyze the effects of source-type, body length, and the interaction thereof on pair exploration latency, we fit the following general linear mixed model:

(3.2)

$$E_{pair} = a \cdot S + b_1 \cdot S \cdot L_f + b_2 \cdot S \cdot L_m + P$$

E_{pair} is pair mean transformed exploration latency, S is source type, L_f and L_m are female and male length, respectively, P is a random effect term for population within source-type, and a, b_1, and b_2 are model coefficients.

We tested for source-type differences in shoal distance from observer and depth using generalized linear mixed models:

(3.3)

$$D = a \cdot S + M + T$$

D is distance or depth, S is source-type, M is a random effect term for mesocosm identity within source-type, T is a random effect term for time since the start of observation, and a is a model coefficient.

We tested for predation/hatchery source differences in activity level using two binary activity metrics: activity > 1 (any movement) and activity > 2 (any foraging, fast-swimming, or aggressive behaviour). We used a generalized linear mixed model with a binomial distribution and a logit link function to predict activity:

(3.4)

$$A = \frac{e^X}{1 + e^X}$$

(3.5)

$$X = a \cdot S + M + T$$

A is activity, *S* is source-type, *M* is a random effect term for mesocosm identity within source-type, *T* is a random effect term for time since the start of observation, and a is a model coefficient.

To test for fish source effects on community composition, we built models that predicted each producer or consumer metric:

(3.6)

$$N_{producer} = a \cdot S + b_1 \cdot B + b_2 \cdot R + b_3 \cdot F$$

(3.7)

$$N_{consumer} = \mathrm{e}^{a \cdot S + b_1 \cdot B + b_2 \cdot R + b_3 \cdot F}$$

N is abundance, *S* is source-type, *B* is adult fish biomass, *R* is adult sex ratio (males/total), *F* is number of fry, and a, b_1, b_2, b_3 are model coefficients. We used only mesocosms that contained fish for these models. We used a general linear model for each producer metric, and a generalized linear model with a log link function and a Poisson distribution for each consumer metric.

3.4 RESULTS

We found significant differences among population sources (captive-propagated, wild high predation, wild low predation) in morphology, behaviour, and community effects. Captive-propagated fish did not simply resemble low predation fish; they showed traits and community effects that differed from both wild source types. All model coefficient estimates and likelihood ratio test outputs can be found in Appendix B.1.

3.4.1 Morphology

There was significant variation in mosquitofish morphology across source-types, with the largest differences between captive-propagated and wild sources (Figure 3.1, Table B.2). Captive-propagated mosquitofish had more posterior eyes (larger heads) than fish from wild populations (likelihood ratio test for source-type effect: $\chi^2 > 10$, df = 2, $p < 0.01$ for both males and females; Figure 3.1; Table B.2). Captive-propagated and wild high-predation male mosquitofish both had deeper caudle peduncles than male wild low-predation fish (likelihood ratio test for source-type effect: $\chi^2 = 11.0$, df = 2, $p < 0.01$; Figure 3.1; Table B.2).

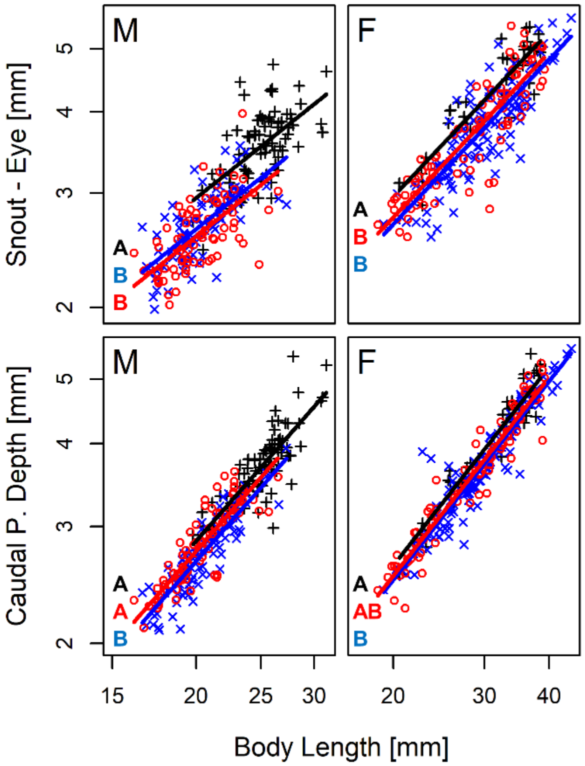


Figure 3.1. Mosquitofish morphology. Morphology measurements for *Gambusia affinis* from three source types: captive-propagated (**black** +), high-predation wild (**red** o), and low-predation wild (**blue** x), with population-level variation within source-types removed. Captive-propagated *G. affinis* had more posterior eyes and deeper caudal peduncles than wild fish. Lines show

predictions from general linear mixed models. Note the log-scaled axes. Letters show groupings based on Tukey *post-hoc* tests.

3.4.2 Exploration latency

The slope and direction of the size-latency relationship varied across source-types and sexes (likelihood ratio test for source-type × male length: $\chi^2 = 9.36$, df = 2, $p < 0.01$; likelihood ratio test for source-type × female length: $\chi^2 = 9.03$, df = 2, $p = 0.011$; Figure 3.2, Table B.3). Captive-propagated females had similar exploration patterns to wild high-predation females, while captive-propagated males had similar exploration patterns to wild low-predation males. Specifically, pair latency increased (exploration decreased) with female size in both captive-propagated and wild high-predation populations; whereas, pair latency decreased (exploration increased) with larger female size in low-predation populations (Figure 3.2). Both the captive-propagated and wild low-predation pairs showed increased pair latency (decreased exploration) with increased male size, whereas wild high-predation pairs showed a weak negative relationship between latency (positive with exploration) and male length (Figure 3.2).

3.4.3 In-mesocosm boldness and activity behaviour

Captive-propagated mosquitofish had similar in-mesocosm boldness and activity behaviour to low-predation wild fish. Captive-propagated and low-predation wild mosquitofish stayed farther from the observer and deeper in the water column than high-predation wild fish (Likelihood ratio test on source-type effect: $\chi^2 > 10$, df = 2, $p < 0.01$ for distance; $\chi^2 = 6.32$, df = 2, $p = 0.042$ for depth; Figure 3.3; Table B.6). Captive-propagated and low-predation wild fish were also less active than high-predation wild fish during observation (Likelihood ratio test on source-type effect: $\chi^2 > 10$, df = 2, $p < 0.01$; Figure 3.4; Table B.6).

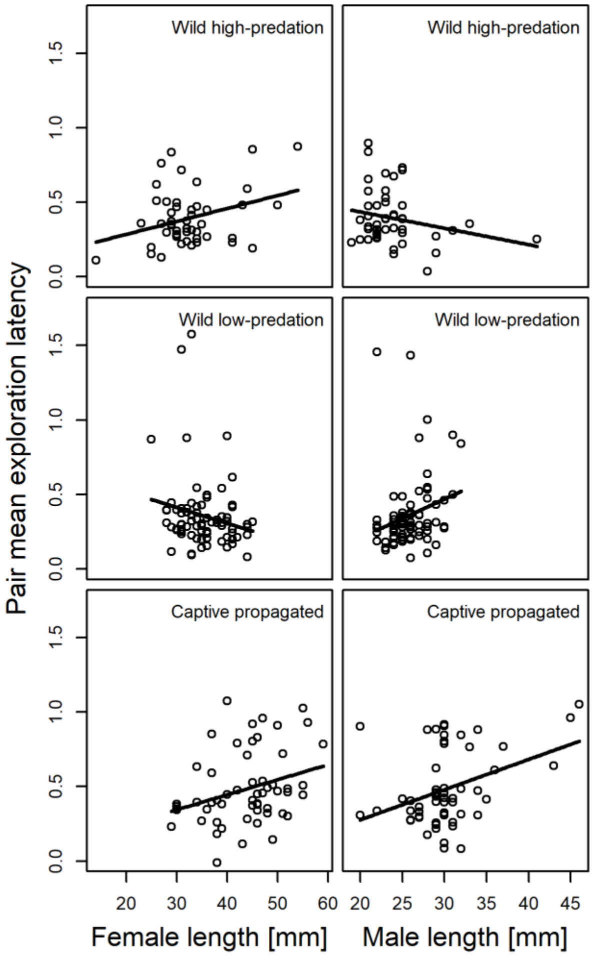


Figure 3.2. Mosquitofish exploration. Exploration latency for pairs (male-female) of western mosquitofish (*Gambusia affinis*) based on source-type and individual body length, with male and female contributions to pair latency shown separately. Vertical axis data are arcsine-square root transformed proportions of a 600-second trial spent in an initial refuge before exploring, with variation from non-focal fish length (i.e. male length in the female length panels, etc.) and population within source-type removed. Lines show predictions from master model (Equation.

3.2). Pair latency was analyzed, rather than individual latency, as individuals within a pair had correlated leave times (Figure B.2).

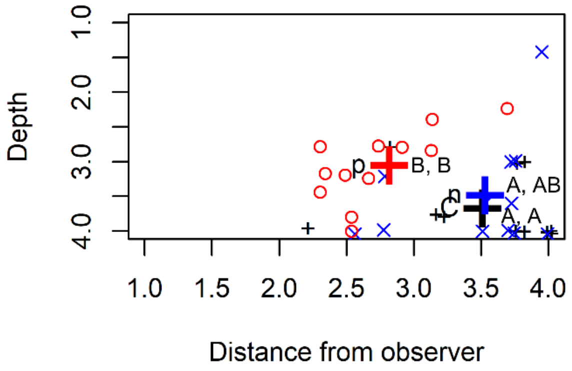


Figure 3.3. In-mesocosm mosquitofish behaviour. *Gambusia affinis* from a high-predation wild source (**red** o, p) lingered closer to a human observer and higher in the water column than captive-propagated (**black** +, C) or wild low-predation (**blue** x, n) fish during 150 seconds of observation. Source-type means indicated with bold +. Average distances and depths based on repeated measurements within each mesocosm are shown as single jittered points. Letters show categorization of distance and depth, respectively, based on Tukey *post-hoc* tests.

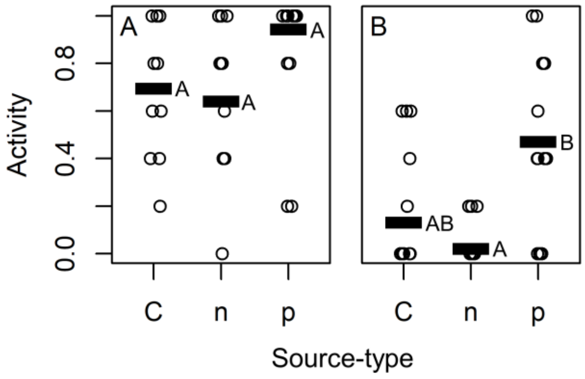


Figure 3.4. Mosquitofish activity. Wild *Gambusia affinis* shoals from a high-predation source (p) were more active than captive-propagated (C) or wild low-predation (n) fish during 150

seconds of observation. **A** shows average frequency of behaviour > rank 1 (see text); **B** shows average frequency of behaviour > rank 2. Thick bars show logit means for each source-type. Average activity values based on repeated measurements within each mesocosm are shown as single points. Letters show categorizations based on Tukey *post-hoc* tests.

3.4.4 Ecological consequences

Fish additions resulted in a trophic cascade, as evident in the generally lower zooplankton and higher producer abundances in mesocosm with fish, compared to fishless control mesocosms (Figure 3.5). We observed source-type differences in the impact of mosquitofish on all measured consumers, but none of our producer metrics (likelihood ratio test for source-type effect on each measured consumer: $\chi^2 > 10$, df = 2, $p < 0.01$; Figure 3.5; Tables B.4, B.5). Compared to our two wild populations, captive-propagated fish led to reductions in the number of benthic chironomid larvae and increases in the number of copepod nauplii (Figure 3.5). Compared to low-predation wild fish, high-predation wild fish generated decreases in every pelagic consumer and increases in benthic chironomid larvae, which were most abundant in the high-predation source mesocosms (Figure 3.5).

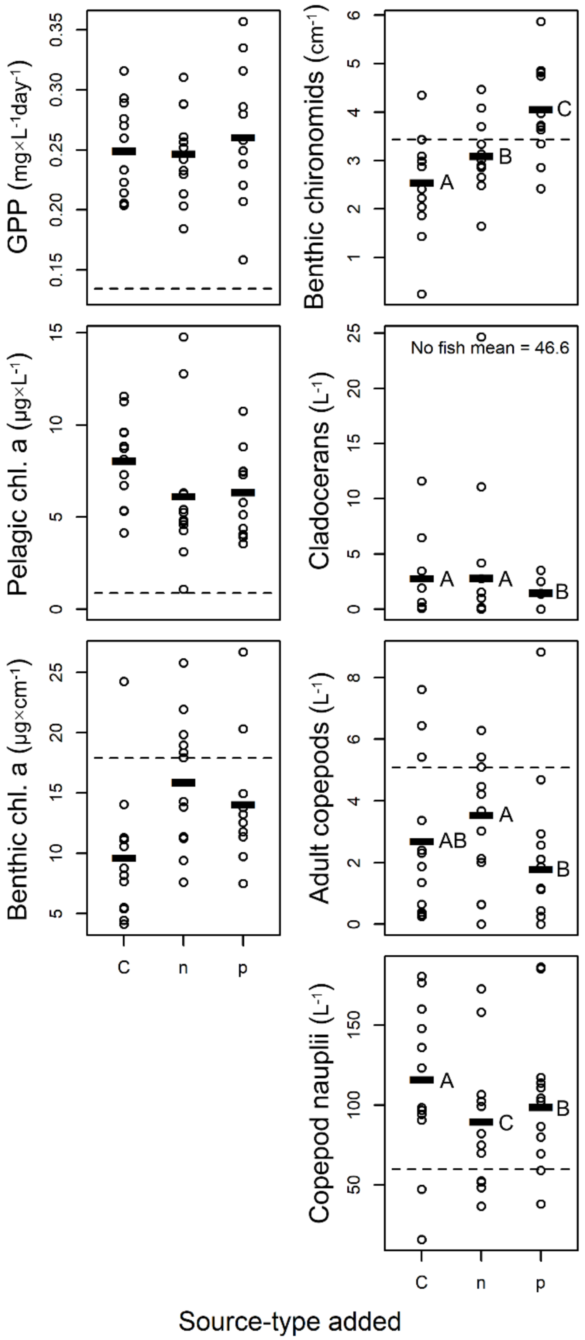


Figure 3.5. Ecological effects of mosquitofish introduction and source-type. Mesocosm community responses to different *Gambusia affinis* source-types: captive-propagated fish (C), low-predation wild fish (n), and high-predation wild fish, with variation from fish biomass, sex ratio, and fry count removed. Dashed lines show means from fishless control mesocosms. Fish

presence caused a trophic cascade in almost all community variables. Fish source-type had little effect on producers (Table B.4), but captive-propagated fish caused a consumer shift that strongly favored copepod nauplii and cladocerans and lowered benthic chironomid abundance (Table B.7). All responses above were measured 3 weeks after fish introduction, but are largely consistent with trends observed earlier in experimentation (Figure B.5). Letters represent significant differences among fish population sources based on Tukey *post-hoc* tests.

3.5 DISCUSSION

3.5.1 Captive propagation breaks wild trait patterns

Multiple populations of captive-propagated mosquitofish consistently demonstrated combinations of morphological and behavioural traits that deviated from typical suites of high- or low-predation wild traits. This result suggests that captive rearing conditions are not merely equivalent to other low-predation environments. Captive environments have high densities and few predators—much like low-predation wild environments—but also have abundant food and periodic removal of individuals—much like high-predation wild environments. Fitting with this unique combination of traits, we found evidence that captive-propagated phenotypes had cascading effects on pond communities unlike those from either wild type.

Multiple lines of captive-propagated mosquitofish had larger heads and caudle peduncles than wild fish (Figure 3.1), which is the opposite trend reported in the bulk of hatchery fish studies (which mostly cover salmonids) (Fleming and Einum, 1997; Fleming et al., 1994; Swain et al., 1991), but see (Von Cramon-Taubadel et al., 2005). Larger tails have been associated with high predation in wild mosquitofish (Langerhans, 2009; Langerhans et al., 2004) and other poeciliids

(Magurran et al., 1992; Palkovacs et al., 2011; Reznick and Endler, 1982), but head size changes are reportedly variable in direction (Langerhans, 2009; Langerhans et al., 2004; Magurran et al., 1992; Palkovacs et al., 2011; Reznick and Endler, 1982). While fish in most captive propagation systems are free from interspecific predation, the enhanced burst-swimming ability conferred by a larger caudle peduncle (Langerhans, 2009) may also aid fish in frenzied food capture, net evasion (van Wijk et al., 2013), or escape from cannibalism—which can be intense in dense populations of captive-propagated mosquitofish (Dionne, 1985). Furthermore, the larger head sizes we observed in captive-propagated mosquitofish may increase perceptive ability, aiding in all of the above as well.

Female captive-propagated and wild high-predation mosquitofish both exhibited negative size-exploration relationships, while wild low-predation females drove positive size-exploration relationships (Figure 3.2). Decreased exploration with size may be a signature of selection against exploratory individuals more easily detected by largemouth bass (which are not gape limited when feeding on mosquitofish) in high predation wild females (Blake and Gabor, 2014) and more easily captured by nets for stocking-out in captive-propagated females (van Wijk et al., 2013). In males, captive-propagated mosquitofish and wild low-predation mosquitofish both showed decreased exploration tendency with larger size. Our observed trend may indicate an energetic tradeoff driven by determinate growth in males, in which smaller, more sexually aggressive (Hughes, 1985), exploratory males allocate fewer energetic and material resources to growth. As we observed, this tradeoff may be stronger in captive propagation facilities and wild low-predation ponds compared to wild high-predation ponds due to more intense competition.

During exposure to an observer, captive-propagated and wild low-predation mosquitofish were less active than high-predation wild mosquitofish and remained farther in lateral distance and depth from the observer (Figures 3.3 and 3.4). Studies of other poeciliids (Archard and Braithwaite, 2011; Brown et al., 2005; Magurran et al., 1992) and of European minnows (*Phoxinus phoxinus*) (Magurran, 1990) show increased inspection activity and decreased inspection distance in high-predation fish. Such behaviour suggests that efficient risk-perception can be a component of antipredator adaptation, and as such high-predation individuals may take better advantage of times when predators are not nearby.

3.5.2 Captive propagation alters communities

Captive-propagated mosquitofish significantly rearranged consumer communities. Relative to both wild source-types, captive-propagated mosquitofish reduced the abundance of benthic invertebrates (chironomids) and increased the abundance of pelagic invertebrates (zooplankton) (Figure 3.5). This community change suggests a substantial shift in captive fish foraging niche and/or reduced ability to capture smaller, more mobile prey. The more posterior eyes of captive-propagated fish (Figure 3.1) may decrease recognition and capture of small, mobile prey. Captive lines that become adapted or habituated to food that settles to the bottom of tanks might maintain these benthic feeding habits in the wild. Furthermore, the behavioural changes in captive propagated fish—which may be due to avoidance of human "predators" (see above)—may shift the preferred habitat of captive propagated mosquitofish to benthic areas away from possible net capture. Indeed, changes in feeding style or efficiency have been noted for captive populations of other species (Huntingford, 2004), including masu salmon (*Oncorhynchus masou*) (Reinhardt, 2001), rainbow trout (*Oncorhynchus mykiss*) (Lucas et al., 2004), Atlantic salmon (Einum and

Fleming, 1997), and zebra danio (Robison and Rowland, 2005). These prior studies did not examine the effects of feeding differences on foodwebs, but widespread observations of feeding changes caused by captive propagation suggest that broader ecological effects, as shown here, could be common. Although rarely considered, such a niche shift—as opposed to some general reduction in vigor—may be at least partly responsible for observations of low fitness of captive fish lines stocked into wild environments. Likewise, niche shifts might partly explain why captive-propagated fish sometimes fail to achieve biocontrol goals (Blaustein, 1992).

While mosquitofish presence significantly altered pelagic production through a trophic cascade (Figure 3.5), differences among mosquitofish source-types in invertebrate community structure did not overtly extend to primary producers. Cascading effects of captive propagation on producers could have occurred on longer or shorter timescales than our sampling schedule, or could have been missed by our particular producer metrics. Community compensation in the producer or consumer trophic levels could also dampen the effect of mosquitofish phenotype on producers (Pace et al., 1999) and could be missed by measures like total chlorophyll-a and GPP (i.e. cryptic eco-evolutionary trophic cascades, Kinnison et al., 2015). In another model system for evo-to-eco effects, evolutionary divergence in stickleback (*Gasterosteus aculeatus*) feeding modes led to consumer community divergence, but this consumer restructuring did not always cascade down to the producer level (Des Roches et al., 2013).

In the ecological frame, a shifting niche in captive fish lines has potentially broad ecosystem implications. Phenotypic divergence leading to shifts from pelagic to benthic feeding, as we observed in captive-propagated sources, has the potential to alter food web interactions and nutrient pathways (Tuckett et al., 2017) potentially leading to a range of unintended ecological consequences, from simple community composition shifts to full ecosystem regime shifts (Evans and Loftus, 1987; Jackson et al., 2014; Tuckett et al., 2017). Given that much fish stocking occurs into already stressed ecosystems, the scope and consequences of such divergence deserves much broader consideration.

The consistent morphological and behavioural differences between replicate populations of captive and wild mosquitofish suggest that captive propagation has an identifiable phenotypic and ecological signature in mosquitofish. While we focused on mosquitofish taken directly from their respective sources to reflect the reality of stocking and translocation programs (i.e. instead of using common garden rearing), there is widespread evidence for genetic local adaptation in *Gambusia* spp. (Brown, 1985; Meffe et al., 1995; Stearns, 1983a), suggesting that at least some of the trait variation we observed has a genetic basis. Thus, the community changes wrought by captive-propagated mosquitofish suggest that the syndrome of captive propagation extends to the ecological role of fish after release. This syndrome may eventually be reversed through plastic and genetic re-adaptation to the wild (i.e. feralization (Daniels and Bekoff, 1989; Stringwell et al., 2014)), dampening the environmental impact of one-time fish introductions. However, continued introduction of captive propagated fish to the same area (e.g. supplementation) is likely to sustain the persistence and ecological impacts of captive phenotypes. Based on the high frequency of continued intentional (Pister, 2001) or unintentional (Naylor et al., 2005) releases of captive-propagated organisms into the wild, the degree to which captive propagation alters the ecological role and ecosystem services of fish demands greater attention.

3.5.3 Conclusions

A unique combination of environmental conditions and selective processes—likely including competition, harvest, and cannibalism—creates unique combinations of morphological and behavioural traits in captive-propagated mosquitofish. Such trait change also appears to shift the foraging niche of captive bred mosquitofish to favor consumption of relatively immobile, benthic food resources. This change in feeding niche altered aquatic prey community outcomes of fish introduction. Despite the potential negative environmental consequences of mosquitofish introductions (Pyke, 2008), mosquitofish remain a widespread tool for biocontrol, and large-scale introduction is likely to continue (Swanson et al., 1996). We suggest that the choice of stocking from wild translocations or captive sources should be a conscious decision that weighs the potential broader ecological effects and services afforded by different phenotypes. Infusions of wild fish into captive programs, and captive environments that more closely mimic wild conditions are common recommendations to slow inadvertent domestication (Hutchings and Fraser, 2008). Our results suggest that these tools may also have broader roles in achieving some ecosystem service goals of stocking.

3.6 ACKNOWLEDGEMENTS

We would like to thank Bruno Zicarelli (Bates College), Chris Miller (Contra Costa CA Vector Control), Tony Hedley (Sac Yolo CA Vector Control), Emma Garner (UMaine), Brian Olsen (UMaine), Amanda Klemmer (UMaine), Ben Wasserman (UCSC), Emma Moffett (University of Auckland), Kevin Simon (University of Auckland), Wes Wright (UMaine), Travis Apgar (UCSC), Devin Chance (UCSC), Jeff Parsons (UMaine), Cassidy Bigos (UMaine), and the Santa Cruz CA Homeless Garden Project for generous help with this project.

Funding was provided by:

US National Science Foundation DEB 1457112; DEB 1457333

CHAPTER 4

PREY EXPERIENCE CRIPTICALLY SHIFTS TROPHIC CASCADES FROM DENSITY- TO TRAIT-MEDIATED

4.1 ABSTRACT

Trophic cascades have become a dominant paradigm in ecology, yet considerable debate remains about the relative strength of density- (consumptive) and trait-mediated (non-consumptive) effects in trophic cascades. This debate has largely ignored the role of prior prey experience with predators, which shapes prey traits (through genetic and plastic adaptation) and influences prey survival (and therefore density). Here, we investigate the cascading role of prey experience through the addition of mosquitofish (*Gambusia affinis*) from predator-experienced or predator-naïve sources to mesocosms containing piscivorous largemouth bass (*Micropterus salmoides*), zooplankton, and phytoplankton. Predator-naïve mosquitofish suffered higher predation rates, which drove a density-mediated cascade, whereas predator-experienced mosquitofish exhibited higher survival but fed less, which drove a trait-mediated cascade. Both cascades were similar in strength, leading to indistinguishable ecological signatures. Therefore, the accumulation of prey experience with predators can cryptically shift cascade mechanisms from density- to trait-mediated.

Keywords: predator experience, trophic cascades, consumptive effects, non-consumptive effects, trait-mediated indirect interactions, cryptic dynamics, Gambusia affinis

4.2 INTRODUCTION

Trophic cascades—effects of predators that propagate downward through food webs (Ripple et al., 2016)—are a dominant but frequently-revisited (Borer et al., 2005; Polis et al., 2000; Strong, 1992) paradigm in ecology. Understanding the mechanisms underlying trophic cascades is essential to understanding how manipulation of one trophic level will effect entire food webs (Fahimipour et al., 2017; Finke and Denno, 2004). Trophic cascades are of broad environmental concern since the addition of novel predators (e.g. invasive species) (Walsh et al., 2016) or removal of top-predators (e.g. harvest) (Daskalov et al., 2007; Frank et al., 2005) can reshape entire food webs, often in undesirable ways.

Trophic cascades can be driven by *consumptive*—or *density-mediated—effects*, in which changes in prey abundance propagate through food webs, and *non-consumptive*—or *trait-mediated—effects*, in which changes in prey functional traits (often behavior) propagate (Grabowski and Kimbro, 2005; Preisser and Bolnick, 2008a; Schmitz et al., 1997). There has been persistent debate in the literature over which mechanism is stronger or more common, with little practical consensus (Křivan and Schmitz, 2004; Peacor et al., 2013; Preisser and Bolnick, 2008b; Schmitz et al., 2004; Trussell et al., 2006). However, density- and trait-mediated interactions are likely inter-dependent, as prey traits that generate trait-mediated trophic cascades (e.g. predator avoidance) also increase survival (Sih et al., 2010). Thus, interacting density- and trait-mediated processes should be considered together to assess the nature and strength of trophic cascades and maximize predictive power (Griffiths et al., 2018). Our work here aims to do just this, elucidating the role of prey experience with predators in shaping whether density- or trait-mediated processes will dominate.

Prior experience with predators can mediate the extent to which predator introductions cause density and trait changes in prey populations. Through phenotypic plasticity (Lönnstedt et al., 2012; McCormick and Holmes, 2006), trans-generational plasticity (e.g. epigenetic and maternal effects) (Storm and Lima, 2010), and genetic evolution (Wund et al., 2015), accrued experience with predators can lead prey populations to display antipredator behaviors that enhance survival. Experience with native predators may also increase prey survival rates in the face of a novel predator (Dunlop-Hayden and Rehage, 2011). Antipredator behaviors that increase prey survival include fleeing (Langerhans, 2009), aggregating (Magurran et al., 1992; Seghers, 1974), freezing (Eilam, 2005; Vilhunen and Hirvonen, 2003), hiding (Romare and Hansson, 2003; Templeton and Shriner, 2004), and predator inspection (Magurran, 1986; Magurran et al., 1992). Such behaviors may lead to a decrease in feeding in a given habitat if they restrict the space or time available for foraging (Schmitz et al., 2004). Thus, natural selection imposed by predators may shift populations along a feeding-survival tradeoff where individuals from predator-experienced populations survive longer but feed less in the face of predators. This evolutionary tradeoff could shape the strength of trait- and density-mediated processes (Peacor et al., 2013). Furthermore, evolution in the face of predators—presumably along this tradeoff—has already been theoretically and empirically shown to have cascading ecological effects by modifying feeding behavior, morphology, and effectiveness (Bassar et al., 2010; Ousterhout et al., 2018; Palkovacs et al., 2011; Start, 2018; Wood et al., 2018).

We hypothesize that prior prey experience with predators dictates the extent to which trophic cascades induced by predators are density- or trait-mediated (Figure 4.1). Naïve prey are less likely to exhibit antipredator behaviors, and thus may suffer high mortality upon predator introduction, driving a density-mediated trophic cascade. Experienced prey are more likely to employ antipredator behaviors, which decrease mortality rates (Sih et al., 2010). However, reduced feeding in risky habitats should alternatively generate a trait-mediated trophic cascade (Trussell et al., 2006). In theory, the nature of this feeding-survival tradeoff should dictate the relative strength of interacting density- versus trait-mediated trophic cascades.

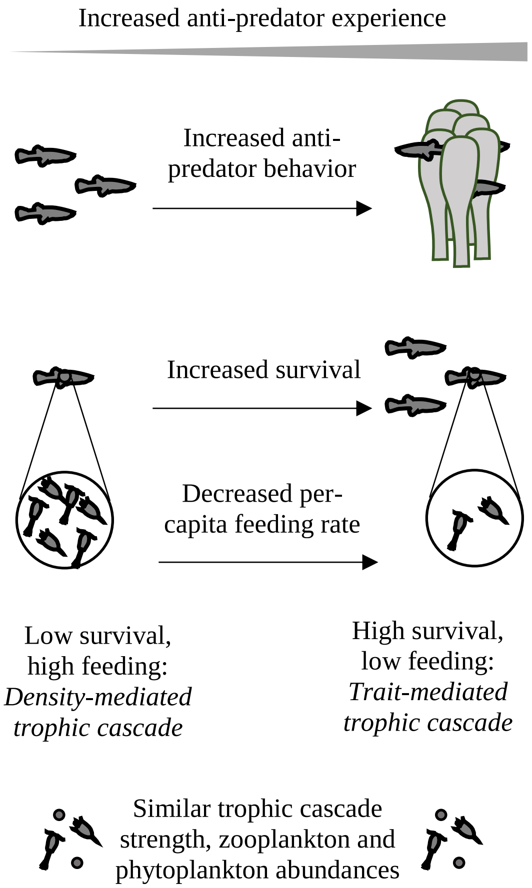


Figure 4.1. **Mosquitofish experience and trophic cascade mode.** Anti-predator experience, which is driven by genetic and plastic adaptation, may shape the strength of density- and trait-mediated trophic cascades. Predator-naïve prey are predicted to have high feeding and high mortality rates, leading to a density-mediated trophic cascade. Predator-experienced prey are predicted to have low feeding and low mortality rates, driving a trait-mediated trophic cascade.

Here we investigate the cascading ecological consequences of prior prey experience with predators for western mosquitofish (*Gambusia affinis*) exposed to piscivorous largemouth bass (*Micropterus salmoides*). In our experiment, prey experience represents a combination of genetic evolution, maternal effects, and phenotypic plasticity. Mosquitofish are an ideal study system to address the cascading effects of prey experience because they have strong effects on pelagic communities (Hurlbert and Mulla, 1981; Hurlbert et al., 1972; Pyke, 2008) and show potential for adaptation and plasticity in response to predators (Langerhans and Makowicz, 2009; Langerhans et al., 2004). We established pond mesocosms seeded with phytoplankton and zooplankton, then added bass and predator-experienced or predator-naïve source mosquitofish. We quantified cascading effects of predators as contingent on mosquitofish experience, separating density-mediated (survival) and trait-mediated (per-capita consumption) effects. We detected trophic cascades mechanistically by relating changes in mosquitofish density throughout the experiment – caused by bass predation – to changes in zooplankton density. Mosquitofish introduction in this system has a clear trophic cascade signature, with most zooplankton abundances decreasing and phytoplankton increasing as a result (Fryxell and Palkovacs, 2017; Fryxell et al., 2015, 2016; Hurlbert et al., 1972). Therefore, we expected bass introduction to generally cause the opposite response, decreasing mosquitofish abundances, increasing zooplankton, and decreasing phytoplankton.

4.3 METHODS

4.3.1 Fish sources

We collected mosquitofish using hand nets and beach seines from two ponds in eastern California in March 2016: Fish Slough Northeast Spring (N 37.518003°, W 118.400157°), which is protected from downstream predators (United States Bureau of Land Management. Bishop Resource Area and U.S. Fish and Wildlife Service, 1985) by a dam and diligent predator monitoring and removal (US Fish and Wildlife Service, 2005), and Furnace Creek Pond (N 36.460453°, W 116.872978°), which contains a high density of largemouth bass (*Micropterus salmoides*) and no other piscine mosquitofish predators. Both of these ponds are small, clearwater, spring-fed desert ponds. Largemouth bass are a common mosquitofish predator and play a strong role in mosquitofish functional ecology(Langerhans, 2009; Langerhans et al., 2004). We classified mosquitofish from these ponds as predator-naïve and predator-experienced, respectively. Mosquitofish density and body size in these populations are representative of other experienced and naïve populations (Table C.1). As we were concerned with the maximum functional scope of mosquitofish antipredator experience (plastic and genetic), we used fish collected from the wild in this experiment. We held fish in 100 L tanks (3 tanks per population) for 8 days on a diet of TetraMin tropical flakes before they were stocked into experimental mesocosms.

4.3.2 Mesocosm experiment

Our experiment used 16 mesocosms in Santa Cruz, California in March 2016. We filled each 1,100 L mesocosm with municipal water and 18.5 L of sand, then added 4 L of homogenized sediment from two nearby ponds: West Lake (N 36.976083°, W 122.045683°) and Antonelli Pond (N 36.955566°, W 122.060489°). We also added homogenized zooplankton from the above ponds. We installed plastic mesh cylinders (29 cm diameter, 22 mm mesh) filled with plastic artificial macrophytes as mosquitofish refuges from predation (mosquitofish could enter the refuge and used it freely; bass were excluded by the mesh). We allowed mesocosms to equilibrate for 15 days prior to fish introductions. We introduced 10 predator-experienced or predator-naïve mosquitofish into each mesocosm (8 mesocosms per fish source). This density of mosquitofish (3.64 m^{-2}) falls within the range of mosquitofish densities observed in the wild from ponds with largemouth bass (Table C.1). We introduced a single one-year-old bass from a hatchery source (Freshwater Fish Co., Elk Grove CA) into each mesocosm.

To measure the effects of largemouth bass on mosquitofish density, we conducted weekly visual counts of the mosquitofish in each mesocosm. An observer standing adjacent to each mesocosm counted all mosquitofish seen during a 5-minute interval. A second observer repeated this process for an additional 5-minute interval. If the mosquitofish counts differed between observation periods, a third observer did an additional 5-minute observation. At the end of the experiment, we used paired visual counts (using the methods above) and exhaustive netting of all mosquitofish in each mesocosms to build an observed ~ actual mosquitofish count relationship (see Appendix C.1, Figure C.1, Table C.2). We used this relationship to correct our mosquitofish counts from earlier in the experiment; these corrected counts were used for all successive analyses.

We sampled zooplankton and phytoplankton weekly for 6 weeks after fish introduction. Zooplankton were collected from 1 L depth-integrated water samples, preserved in 80% ethanol, and identified under a dissecting microscope. Phytoplankton concentrations were measured using pelagic chlorophyll-*a* (chl-*a*), collected using 1L depth integrated water samples, filtered onto 0.7 μm filters (Whatman GF/F), and measured using fluorometry (Turner Designs, Trilogy

Module CHL-NA). We estimated 24-hour gross primary production (GPP) using diel changes in dissolved oxygen concentrations (Harmon et al., 2009).

4.3.3 Analyses

Cascading effects of mosquitofish experience. We conducted all analyses in this study in R (R Core Team, 2016), using default packages, except where specified. We analyzed per-capita effects of mosquitofish on consumers (zooplankton) and producers (phytoplankton) using a two-step general linear mixed model. The first step predicted responses based solely on a random time effect. The second step predicted the residuals from the first step with a fixed, source-specific fish abundance effect and a random mesocosm identity effect. Removing time effects before considering fish abundance effects avoided conflating successional changes in mesocosm communities with top-down effects of mosquitofish abundance. Our model formulations were:

(4.1.a)

$$N = \beta_0 + T + \varepsilon_0$$

(4.1.b)

$$\varepsilon_0 = \beta_1 + F^{\frac{1}{3}} + C_S + M + \varepsilon_1$$

where N is the concentration or density of each response variable, β_0 and β_1 are intercepts, T is a random time effect, ε_0 and ε_1 are residuals, F is number of mosquitofish (calculated as the average of fish observed at the beginning and end of each week), C_S is a source-specific coefficient, and M is a random mesocosm identity effect. We $\ln(X + 1)$ transformed zooplankton abundances for normality. We included the effect of mosquitofish as a nonlinear term due to obvious non-linear trends in the data (see Results). We included all time points for this analysis (i.e. mosquitofish survival data from weeks 1-6 and ecological data from weeks 2-6—due to the way F was calculated). We used maximum-likelihood (non-restricted) model fitting within the *lme4* package in R for all mixed models (Bates et al., 2015).

For each response variable, we used likelihood ratio tests to sequentially test for effects of mosquitofish density, then source-specific effects of mosquitofish density. Significant source-specific effects of mosquitofish density indicated that mosquitofish from the different sources had different per-capita consumptive or cascading effects on zooplankton or producers, respectively. We analyzed the following broad taxonomic groups for zooplankton: all adult crustaceans, copepods (together or separated into adults, copepodites, and nauplii), cladocerans (together, or separated into *Bosmina*, *Daphnia*, and *Ceriodaphnia*), and rotifers. Mosquitofish consume all of the above taxa (Mansfield and Mcardle, 1998) and commonly reduce their abundances upon introduction (Hurlbert and Mulla, 1981).

Mosquitofish survival. We analyzed weekly mosquitofish survival rate using a general linear model of the form:

(4.2)

$$F_{t+1} = F_t \cdot L_S$$

where F is number of mosquitofish, t is time (in weeks), and L_S is a source-specific coefficient. We used a likelihood ratio test to determine whether L_S varied significantly across predator-experienced and predator-naïve sources.

4.4 RESULTS

Our mesocosms showed a clear temporal pattern of a bass-mosquitofish-zooplankton-phytoplankton trophic cascade, such that after bass were added, 1) mosquitofish were frequently consumed or confined to cover, 2) most pelagic zooplankton increased (Figure 4.2, C.2) and 3) pelagic chlorophyll-a decreased during the course of the experiment (GPP, which includes pelagic and benthic producers and turnover rates, increased) (Figure C.2). These patterns were consistent with our expectations of a trophic cascade driven by bass consumption of mosquitofish, as they were roughly the opposite of documented trophic cascades generated by mosquitofish introduction (Fryxell and Palkovacs, 2017; Fryxell et al., 2015, 2016; Hurlbert et al., 1972). Interestingly, there were no major differences in any observed zooplankton or producer abundances across mosquitofish treatments (Figure 4.2, C.2).

The strength of the cascade, measured in terms of zooplankton dynamics, was approximately equal between the predator-naïve and predator-experienced mosquitofish populations, but the mechanism differed. The density-mediated effect was stronger when mosquitofish were predator-naïve—bass increased zooplankton density by reducing mosquitofish density. In contrast, the trait-mediated effect was greater when mosquitofish were predator-experienced—bass increased zooplankton density by suppressing mosquitofish feeding. Thus, the tradeoff between feeding and survival across mosquitofish populations shaped the relative strength of the density- and trait-mediated cascade mechanisms in this experiment.

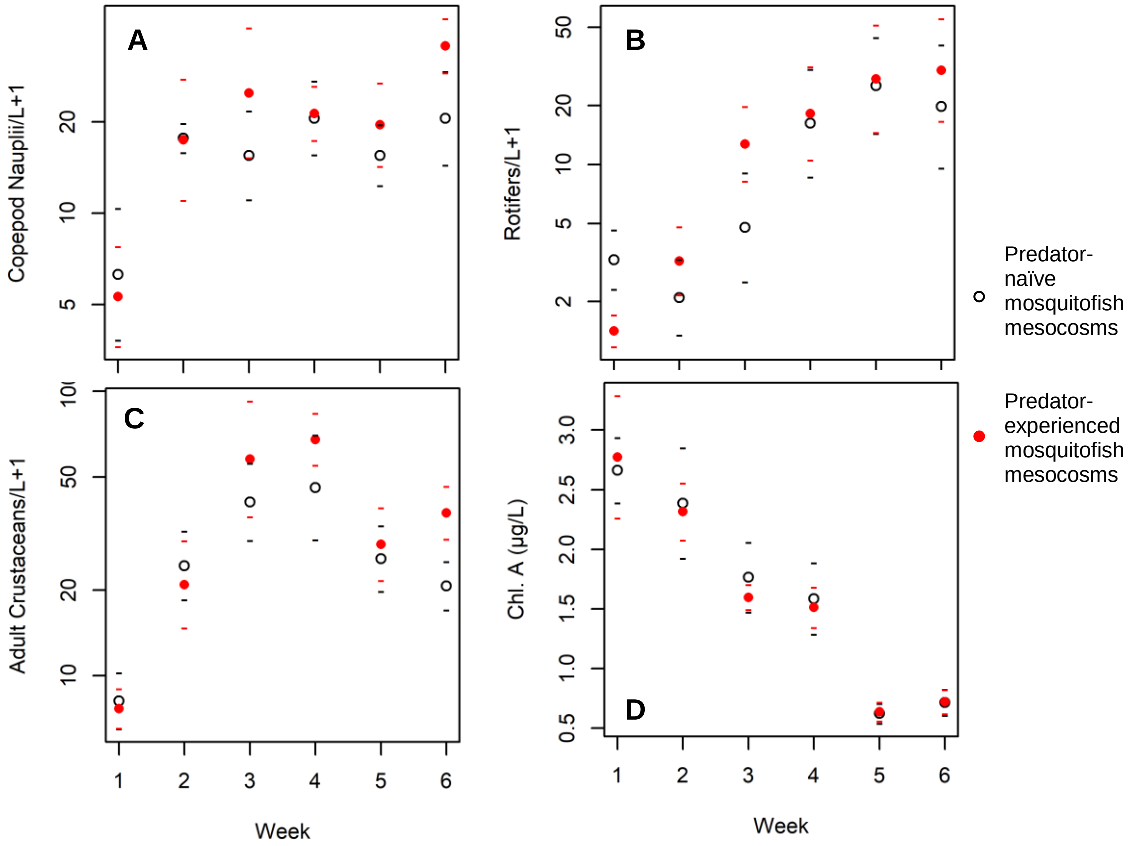


Figure 4.2. Temporal emergence of trophic cascades following bass additions. Abundances of copepod nauplii **(A),** rotifers **(B)**, and adult crustacean**s (C)** all increased sharply after bass introduction. Pelagic Chl-a **(D)** decreased over time. The strength of this cascade was similar in mesocosms with predator-naïve and predator-experienced mosquitofish despite significantly lower survival (and therefore lower abundance) of predator-naïve mosquitofish with bass. Points and bars are mean ± one standard error; *N* = 8 per point.

4.4.1 Cascading effects of mosquitofish source

Mosquitofish had negative, source-specific, per-capita effects on copepods and rotifers (Table 4.1, Figure 4.3), with the trend in copepods mostly driven by nauplii (Table 4.1). Mosquitofish also had a negative marginally-significant effect on total adult crustaceans (Table 4.1, Figure

4.3). In all cases, predator-naïve mosquitofish had stronger estimated per-capita consumptive effects on zooplankton than predator-experienced mosquitofish. Nauplii and rotifers were by far the most numerous zooplankton, and both increased during the experiment as a result of the trophic cascade (Figure 4.2). Mosquitofish had no effect on any other zooplankton, GPP, or pelagic chl-*a* (Table 4.1).

4.4.2 Mosquitofish survival

Predator-experienced mosquitofish had significantly higher weekly survival rates compared to predator-naïve mosquitofish (0.86 ± 0.04 vs. 0.52 ± 0.09, respectively; $\chi^2 = 11.4$, df = 1, $p < 0.001$; Figure 4.4). This survival difference led predator-experienced mosquitofish to persist much longer in mesocosms than predator-naïve mosquitofish (Figure 4.4). Combining the estimated source-specific effects of mosquitofish on zooplankton (above) and survival rates revealed a feeding-survival tradeoff, in which the predator-naïve population had higher apparent zooplankton consumption rates but lower survival rate (Figure 4.5).

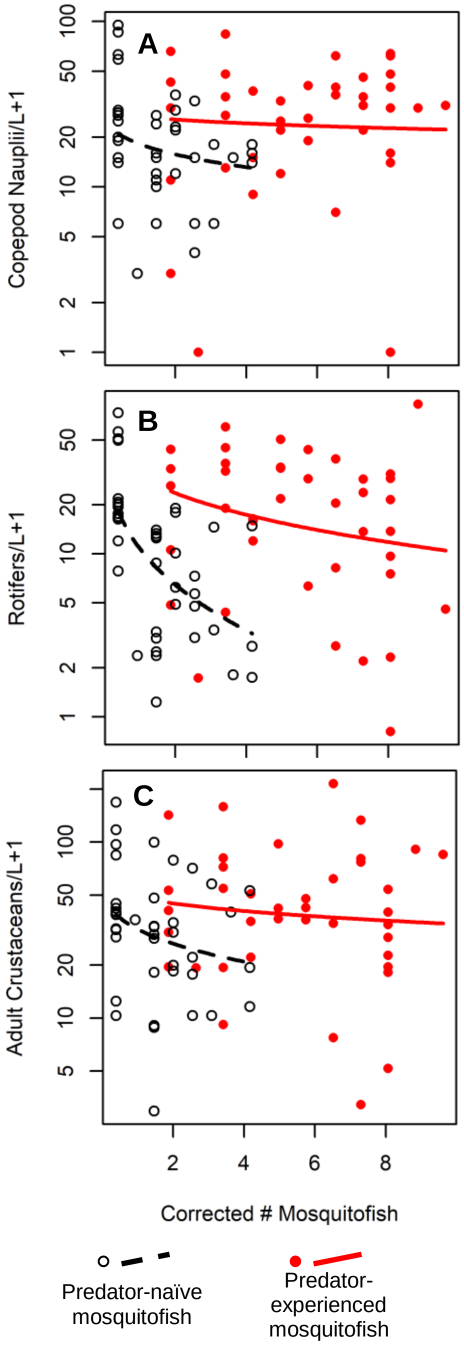


Figure 4.3. Effects of mosquitofish density on zooplankton density. Prey densities were negatively related to mosquitofish densities, with the slope of the relationship dependent on mosquitofish source. Mosquitofish from a predator-naïve source had a strong per-capita effect (i.e. consumption rate) on copepods nauplii **(A),** rotifers **(B)**, and adult crustaceans **(C)**, while mosquitofish from a predator-experienced source had a weak per-capita effect. Lines show

general linear mixed model predictions for each source-type. Data from weeks 2-6 are pooled, with variation from time and mesocosm identity effects removed; $N = 80$.

	Explanatory variable					
Dependent variable	Fish$^{1/3}$			Fish$^{1/3}$ · Experience		
	χ^2	df	p	χ^2	df	p
All adult crustaceans	0.0122	1	0.91	3.67	1	**0.055**
All copepods	0.11	1	0.74	10.5	1	**0.0012**
Adult copepods	1.05	1	0.30	0.90	1	0.34
Copepodites	0.49	1	0.48	0.40	1	0.53
Copepod nauplii	0.21	1	0.65	3.93	1	**0.047**
All cladocerans	0.025	1	0.87	2.01	1	0.16
Bosmina	0.085	1	0.77	1.74	1	0.19
Daphnia	0.17	1	0.68	0.13	1	0.72
Ceriodaphnia	0.31	1	0.58	2.84	1	0.09
Rotifers	2.67	1	0.10	6.01	1	**0.014**
Chlorophyll *a*	0.41	1	0.52	0.94	1	0.33
GPP	1.54	1	0.22	0.73	1	0.39

Table 4.1. Mosquitofish effects on zooplankton and producers. Mosquitofish had source-specific per-capita effects on some groups of zooplankton but not primary producers. Tests shown are likelihood ratio tests, conducted sequentially (fish, then fish · experience). Null model includes fixed time factor effect and random mesocosm identity effect. $N = 80$ for each model.

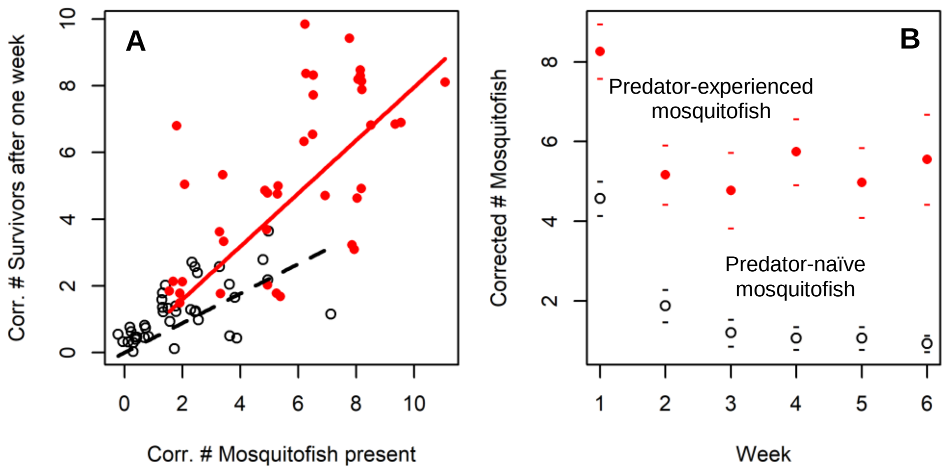


Figure 4.4. Mosquitofish survival in the face of bass predation. Mosquitofish from a from a predator-experienced source had higher week-to-week survival rates than mosquitofish from a predator-naïve source **(A)** and thus persisted longer in mesocosms with bass **(B)**. Data from weeks 1-6 are pooled for (A), and points are jittered to aid visual comprehension. Lines on (A) show general linear model predictions for each source-type. Bars on (B) show ± one standard error. *N* = 80 for (A) and 8 per point for (B).

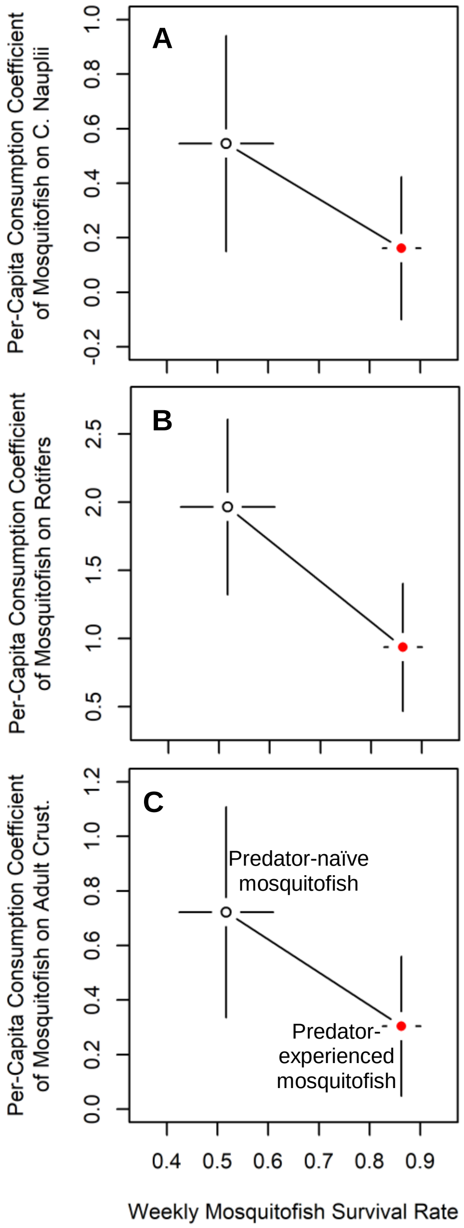


Figure 4.5. Feeding-survival tradeoffs. Mosquitofish exhibited a feeding-survival tradeoff: predator-experienced mosquitofish had higher survival but lower consumption rates on copepods **(A)** and rotifers **(B)**. Points are source-level estimates (i.e. L_S and C_S from Equations 4.2 and 4.1.b, respectively). Bars indicate ± 1 standard error.

4.5 DISCUSSION

Our results show that prey experience with predators—likely resulting from a mixture of genetic and plastic adaptation—determined the extent to which mosquitofish density versus traits drove the bass-mosquitofish-zooplankton trophic cascade. Predator-naïve mosquitofish had lower survival in the presence of bass but higher per-capita feeding rates, whereas predator-experienced mosquitofish had higher survival and lower per-capita feeding rates. Thus, the high mortality of predator-naïve mosquitofish facilitated a stronger density-mediated trophic cascade, whereas the low feeding rate of predator-experienced mosquitofish facilitated a stronger trait-mediated trophic cascade (Figure 4.1). Importantly, the feeding-survival tradeoff exhibited across mosquitofish populations (Figure 4.5) generated net trophic cascades of roughly equal strength. In other words, there was no significant difference in zooplankton abundances between the two treatments despite the predator-naïve treatment having significantly lower mosquitofish density following bass addition. Thus, increasing prior prey experience with predators cryptically shifted the dominant trophic cascade mechanism from density-mediated to trait-mediated.

Accumulating prey experience with predators through plasticity and adaptation may mediate the cascading effects of predator introductions, shifting the cascade mechanism from density-mediated to trait-mediated as prey become more experienced. The degree to which this shift remains ecologically cryptic depends on the relative strength of opposing density (survival) and trait (feeding) mechanisms. In our experiment, these opposing mechanisms were roughly equal in effect size. The decrease in density-mediated effects was compensated by an increase in trait-mediated effects, leading the net strength of the cascade to remain about constant. This scenario represents a cryptic shift in the mechanism driving the trophic cascade. However, such complete compensation may not be present in all instances. Indeed, the relative strength of density and trait effects may determine whether antipredator traits reduce trophic cascades (density > trait), remain cryptic (density ≈ trait), or amplify trophic cascades (density < trait). For predator introductions into communities with predator-experienced prey, trait-mediated processes may dominate trophic cascades from onset (Carpenter et al., 1987).

This work adds to the growing literature suggesting that population adaptation, in all its genetic and plastic forms, can generate or alter trophic cascades. Contemporary adaptation in upper trophic levels can cause cascading top-down effects on food webs (Ousterhout et al., 2018; Palkovacs et al., 2012; Start, 2018; Walsh et al., 2012). The rearranging of relative interaction strengths during a classic density-mediated trophic cascade can also alter selection pressures on lower trophic levels, driving contemporary adaptation (Wood et al., 2018). Prey adaptation at the population (Mooney et al., 2010) or community level (Fahimipour et al., 2017) can mediate the strength and reach of trophic cascades (Wood et al., 2018). Our work here demonstrates that adaptation can not only affect the strength and extent of trophic cascades, but also the specific mechanisms underlying trophic cascades.

A cryptic shift from density- to trait-mediated trophic cascades may be a stabilizing force in food webs. The increased survival of predator-experienced mosquitofish could take the form of a rescue dynamic (Gonzalez et al., 2013), which keeps mosquitofish at higher densities, buffering them against direct extirpation or indirect risks from processes like Allee effects (Gascoigne and Lipcius, 2004). A more stable, defended mosquitofish resource may also prevent bass from self-extirpating through resource depletion (Yamamichi and Miner, 2015). Weaker interaction strengths between bass, mosquitofish, and zooplankton generated by antipredator traits may also contribute to increased overall food-web network stability (McCann et al., 1998; Neutel et al., 2002).

While we found strong evidence for a cascading effect of bass on producers, we did not detect a measurable effect of mosquitofish experience on pelagic producers (Table 4.1, Figure C.2). This result could be because cladocerans, especially *Daphnia*, were uncommon in all mesocosms throughout the experiment (Figure C.2). *Daphnia* are highly efficient grazers, and their reduction by mosquitofish is known to have large effects on the abundance and composition of pelagic phytoplankton (Hurlbert et al., 1972). Thus, with *Daphnia* comparatively rare throughout this experiment, effects of mosquitofish experience on zooplankton may not have been transmitted strongly to phytoplankton. Alternatively, changes in phytoplankton community composition (i.e. compensation) can sometimes mask overall changes in phytoplankton density (Bell, 2002; Sommer et al., 2003; Tessier and Woodruff, 2002). Thus, we may have failed to detect compositional changes that might have occurred in the phytoplankton community.

4.5.1 Conclusions

Our results show that density- and trait-mediated effects are functionally interconnected through tradeoffs underlying the antipredator traits of prey, and their relative importance in trophic cascades can be driven by prior prey population experience with predators. Here we found that increasing prey experience cryptically shifted the trophic cascade mechanism from density-mediated to trait-mediated. Our results suggest that debates about the dominant cascade mechanism in nature may be a false dichotomy, since traits that reduce feeding in the presence of predators also increase survival. It may therefore be profitable to broadly consider cascade mechanisms along a continuum of prey experience with predators.

4.6 ACKNOWLEDGEMENTS

We thank B. Zicarelli and J. Centoni for help with data collection. The students in the Spring 2016 Freshwater Ecology Lab course at UC Santa Cruz helped with mesocosm setup and weekly mosquitofish counts. Hamish Grieg, Amanda Klemmer, Tim Waring, and Isaac Shepard provided feedback on data analysis and interpretation.

Funding was provided by:

US National Science Foundation (DEB 1457333 and DEB 1457112)

UC Santa Cruz

Maine Agricultural and Forest Experiment Station

University of Maine Janet Waldron Fellowship Program

NOAA Cooperative Institute for Marine Ecosystems and Climate

CHAPTER 5

ECO-EVOLUTIONARY COMPETITION-DEFENSE TRADEOFFS IN WESTERN MOSQUITOFISH

5.1 ABSTRACT

Evolution can contemporaneously interact with ecology, leading to eco-evolutionary dynamics. Competition-defense tradeoffs are an essential mechanism connecting contemporary evolution and ecology by linking traits of taxa to densities of their predators and prey, thereby setting the stage for eco-evolutionary dynamics. Here we investigate the nature of genetic competition-defense tradeoffs in western mosquitofish (*Gambusia affinis*), a model species for eco-evolutionary dynamics. We use competition (growth) and defense (survival around bass) mesocosm trials on a mixture of common-reared mosquitofish from replicate populations within four backgrounds: predator-free, bass, bluegill, and captive propagation. We document a competition-defense tradeoff dependent on body size within backgrounds, but not populations. This finding suggests that selection along tradeoffs is very strong within populations leading local optimization that nonetheless varies among populations within backgrounds. Therefore, such variation is likely a key determinant of the strength and scope of eco-evolutionary dynamics.

Key words: eco-evolutionary dynamics, tradeoffs, competition, antipredator defenses, Gambusia affinis, evolution of body size

5.2 INTRODUCTION

Eco-evolutionary dynamics are a growing paradigm linking evolution and ecology (Hendry, 2016; Schoener, 2011). Mounting evidence demonstrates that evolution can occur contemporaneously with ecology (Carroll et al., 2007; DeLong et al., 2016; Hendry and Kinnison, 1999; Kinnison and Hendry, 2001), suggesting that evolutionary and ecological processes can interact dynamically (Fussmann et al., 2007; Hairston et al., 2005). Such dynamic interactions allow for environmental change to cause contemporary trait evolution (Gonzalez et al., 2013; Grant and Grant, 2002), and for contemporary trait evolution to cause environmental change (Des Roches et al., 2018; Palkovacs et al., 2012). When these pathways mutually interact through the same traits, eco-evolutionary feedbacks can occur (Post and Palkovacs, 2009). While certain (negative) eco-evolutionary feedbacks can be a source of food web stability, others can be destabilizing (Cortez et al., 2018; Marrow and Cannings, 1993), or allow small perturbations to cause large food web changes (Audzijonyte et al., 2013a; Kuparinen et al., 2016).

One major focus for eco-evolutionary dynamics has been evolution during predator-prey interactions. In some cases, evolution of prey defenses can dampen the impact of introduced predators (Pimentel, 1988; terHorst et al., 2010; Yamamichi and Miner, 2015). In other cases, evolution of prey in ecological time can lead to novel predator-prey density cycling (Abrams and Matsuda, 1997b; Jones and Ellner, 2007; Yoshida et al., 2003). This eco-evolutionary cycling tends to be longer in nature than classic predator-prey cycling, and has a stronger offset, with predator and prey densities ½ phase offset (antiphase) rather than ¼ phase (Abrams and Matsuda, 1997b; Yoshida et al., 2003). In some cases, prey evolution may occur quickly enough to prevent prey densities from changing at all (i.e. a cryptic evolutionary rescue (Kinnison et al., 2015)), causing only predator densities to cycle (Yoshida et al., 2007). These cycles may be a source of system instability, either by being inherently destabilizing (Abrams and Matsuda, 1997a), or by frequently allowing predator or prey densities to drop dangerously low (Zhou et al., 2005). Eco-evolutionary predator-prey cycling has been documented in numerous “classical” predator-prey dynamics (Hiltunen et al., 2014).

Eco-evolutionary predator-prey dynamics can have broader food web impacts. Prey evolution at the population (Mooney et al., 2010; Ousterhout et al., 2018) or community level (Fahimipour et al., 2017) can alter the cascading impact of predator dynamics. Specifically, the trophic level at which contemporary evolution occurs can determine whether evolution dampens or exaggerates trophic cascades (Wood et al., 2018). In addition to affecting the strength of trophic cascades, prey adaptation can also affect the mode of trophic cascades, with predator-naïve prey facilitating density-mediated cascades, and predator-adapted prey facilitating trait-mediated cascades (Wood et al., in prep).

The theoretically shared mechanism behind eco-evolutionary predator-prey dynamics is a competition-defense tradeoff, in which feeding ability and vulnerability to predators are positively related (Yoshida et al., 2004). This tradeoff facilitates eco-evolutionary dynamics by tying prey traits to predator densities: when predators are dense, prey evolve costly defenses, driving down predator abundances. When predator abundances are low and prey competition increases, prey evolve increased competitive ability and decreased defendedness, allowing predator abundances to climb. Competition-defense tradeoffs have been observed in numerous taxa—including fish, (Langerhans, 2009; Palkovacs et al., 2011) plants, (Fernandez et al., 2016;

Mole, 1994; Züst and Agrawal, 2017) insects, (Fellowes et al., 1999; Kraaijeveld et al., 2002) algae, (Agrawal, 1998; Kasada et al., 2014; Yoshida et al., 2003) and bacteria (Lennon and Martiny, 2008)—and can be behavioral, (Conrad et al., 2011; Langerhans, 2009) morphological, (Langerhans, 2009; Palkovacs et al., 2011) physio-chemical, (Agrawal, 1998; Lind et al., 2013) or life-historical (Reznick et al., 1990; Stearns, 1983b, 1983a, 1989) (Table 5.1).

Tradeoff type	Example	Reference
Physio-chemical	Algae that produce compounds reducing edibility grow more slowly	(Yoshida et al., 2004)
Morphological	Mosquitofish can have body forms hydrodynamically optimized for efficient feeding or fast escape	(Langerhans, 2009)
Life-historical	Earlier maturation in guppies to avoid predation results in decreased reproductive output	(Reznick et al., 1990)
Behavioral	Hiding increases survival in spiders, but decreases feeding ability	(Schmitz et al., 1997)

Table 5.1. Example tradeoffs of various trait types.

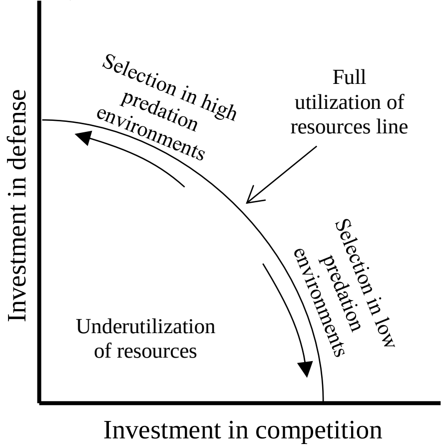
Theoretical and empirical work has tied competition-defense tradeoff slope to system stability. When prey have “expensive defense” tradeoffs—increasing defense comes at a large competitive cost—prey are unlikely to evolve, and eco-evolutionary dynamics are unlikely to occur (Kasada et al., 2014). When prey have “cheap defense” tradeoffs—defense comes at little competitive cost—then rapid and extensive evolution of prey defenses is likely to lead to destabilizing eco-evolutionary dynamics or outright predator extirpation (Wood et al., 2018). Intermediate “tit for tat” tradeoffs allow for potentially stable or oscillating eco-evolutionary dynamics (Abrams, 2009; Ehrlich et al., 2018; Kasada et al., 2014), which can cascade throughout ecosystems (Wood et al., 2018).

The underlying genetic mechanisms behind competition-defense tradeoffs are very poorly understood. Theoretically, at least three mechanisms are possible depending on how many genes or gene networks underly competitive and defended phenotypes (Figure 5.1, 5.2):

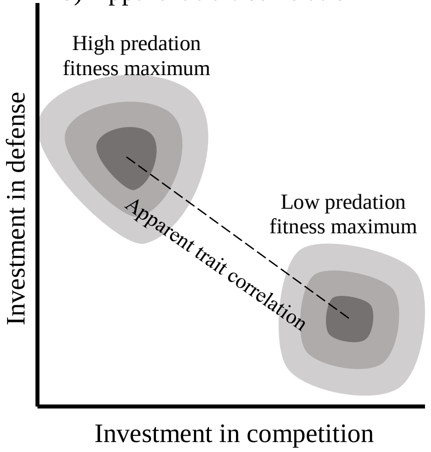
1. *Pleiotropy or epistasis*, in which competitive traits and defended traits are inversely regulated by the same single gene or gene network (Conner et al., 2011; Pavlicev Mihaela et al., 2011; Roff, 1994);
2. *Trait correlations*, in which competitive and defended traits are regulated by two separate genes, but the alleles behind each are negatively correlated (Roff and Fairbairn, 2012), possibly through linkage; and
3. *Resource constraint*, in which largely independent genes control competitive and defended traits, but the nature of finite structural, energy or time budgets necessitate that increases in competition or defense necessitate reductions of the other (Arnold, 1992).

The mode of a given tradeoff should have consequences for its persistence in time and ability to constrain evolutionary divergence. Trait correlations may be readily broken under changed selection, especially if the traits are weakly linked (Calafell et al., 2001; McVean, 2007), whereas traits that are pleiotropic may be slower to remodel (Conner et al., 2011), and tradeoffs based on functional constraints may be very difficult to alter (Arnold, 1992). The same tradeoff mechanisms apply to plastic traits as well, as they apply to the genes underlying mechanisms for plasticity.

a) Resource constraint



b) Apparent trait correlation



c) Pleiotropy

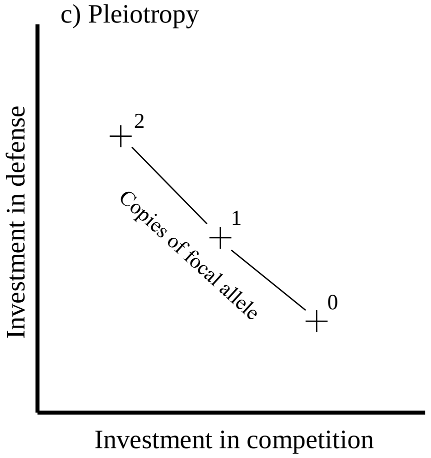


Figure 5.1. Theoretical mechanisms for competition-defense tradeoffs. Competition and defense will trade-off when structures, chemicals, and/or behaviors that favor competition and

defense: **a)** pull from the same resource pool, **b)** produce low fitness when occurring together, or **c)** are controlled inversely by the same gene.

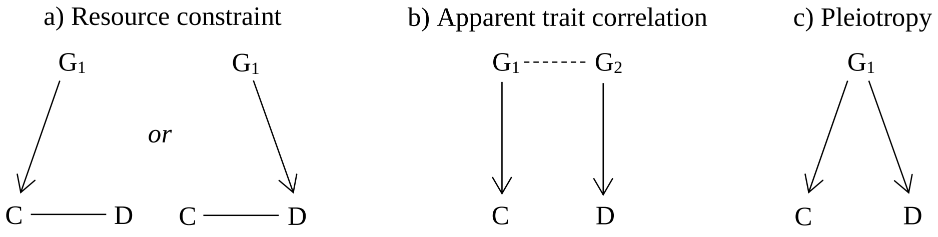


Figure 5.2. Genetic frameworks for three types of competition-defense tradeoffs. G indicates a gene; C indicates competitiveness; D indicates defensiveness. In limited resource allocation tradeoffs (a), a genetic change increases a competitive trait, which leaves limited resources for defense, or vice-versa. In apparent trait correlation tradeoffs (b), correlated genes underlay competitive and defensive traits. In pleiotropic tradeoffs (c), one gene directly controls separate competitive and defensive traits.

One trait that can generate a relationship between competition and defense is body size. Body size can affect competitive ability by determining prey capture success, metabolic demands, and resource use efficiency (Bence and Murdoch, 1986; Clarke and Johnston, 1999; Mittelbach, 1981; Nakayama and Fuiman, 2010; Schmitt and Holbrook, 1984). Smaller organisms have higher success feeding on smaller prey and lower net metabolic demands, but larger organisms have higher success feeding on larger prey, use resources more efficiently, and may prevail during interference competition. Body size can affect survival by determining escape ability, detection chances, and gape limitation (Christensen, 1996; Hansen et al., 2013; Lundvall et al., 1999; Nowlin et al., 2006; Wardle, 1975). Smaller organisms are less likely to be detected by predators, while larger organisms are more likely to be able to escape predators or may be too large for predators to eat. Thus, the relative balance of these size-dependent advantages and disadvantages will determine whether larger organisms are more or less competitive or defended, and thus whether size drives a competition-defense tradeoff. Body size can be regulated genetically through genes that code for growth rate, maturation rate, and maximum size (Gutierrez et al., 2015; Paibomesai et al., 2010; Tao and Boulding, 2003).

Here we investigate genetic competition-defense tradeoffs linked to body size and source population in western mosquitofish (*Gambusia affinis*). Poeciliid fishes—like mosquitofish—have been model taxa for evolutionary ecology, showing strong phenotypic responses to predator introductions and removals (Langerhans and Makowicz, 2009; Langerhans et al., 2004; Magurran et al., 1992; O'Steen et al., 2002; Reznick and Endler, 1982), as well as strong ecological impacts of phenotypic change (Bassar et al., 2010, 2012; Palkovacs et al., 2011; Fryxell et al., in review; Wood et al., in prep). We examined 10 populations of common-garden-reared mosquitofish from a variety of predator-rich or predator-free backgrounds in central California, USA. We used mesocosms with and without largemouth bass (*Micropterus salmoides*) to measure competitiveness and defendedness, which we linked to individual (sex and mass) and population-level traits. We focused on two main questions:

1. Are competition and defense genetically linked in mosquitofish, creating a competition-defense tradeoff?
2. If so, on what demographic level do tradeoffs occur?

We tested three hypothesized tradeoff levels: individual-, population-, and background-level. At the individual level, we examined tradeoffs related specifically to body size (see above). At the population- and background-levels, we examined generalized tradeoffs linked to population identity—i.e. whether more defended populations have less-competitive individuals. The level at which competition-defense tradeoffs occur should dictate the scope of eco-evolutionary dynamics within or across populations. The level at which competition-defense tradeoffs occur should also determine whether tradeoffs are universal or differ based on population history or predation background.

5.3 METHODS

5.3.1 Fish sources

We collected western mosquitofish from multiple sources in central California in May 2017. Western mosquitofish were introduced to California in the 1920s for mosquito control (Lenert, 1923). Although detailed stocking records and population genetic surveys are lacking these original fish were widely stocked and translocated throughout the region over a period of decades. Thus, we assumed at least some common ancestry among populations within the region. We collected individuals from at least two populations (ponds) from each of four background types: captive propagated, wild predator-free, wild with bluegill (*Lepomis macrochirus*) predators, and wild with bass predators (Figure 5.3, Table D.1). Depending on fish availability at the time of collection, some populations were directly wild captured, while others were obtained from new breeding stocks at the University of California Santa Cruz. Fish from these breeding stocks were direct offspring of wild fish and had been in captivity less than three months. We transported the fish to the University of Maine mosquitofish breeding facility after roughly one week of holding in Santa Cruz, CA. We bred mosquitofish for one additional generation in separate 300L cattle tanks for each population. We used floating mesh refugia to passively separate fry from adults, after which we moved fry to separate 36 L tanks for growth and holding. There was a long delay (ca. six months) between fish importation and volitional fish breeding. We fed all fish a mixture of tropical flake food and dried bloodworms. We used the offspring of the imported fish (F1s) for experimentation.

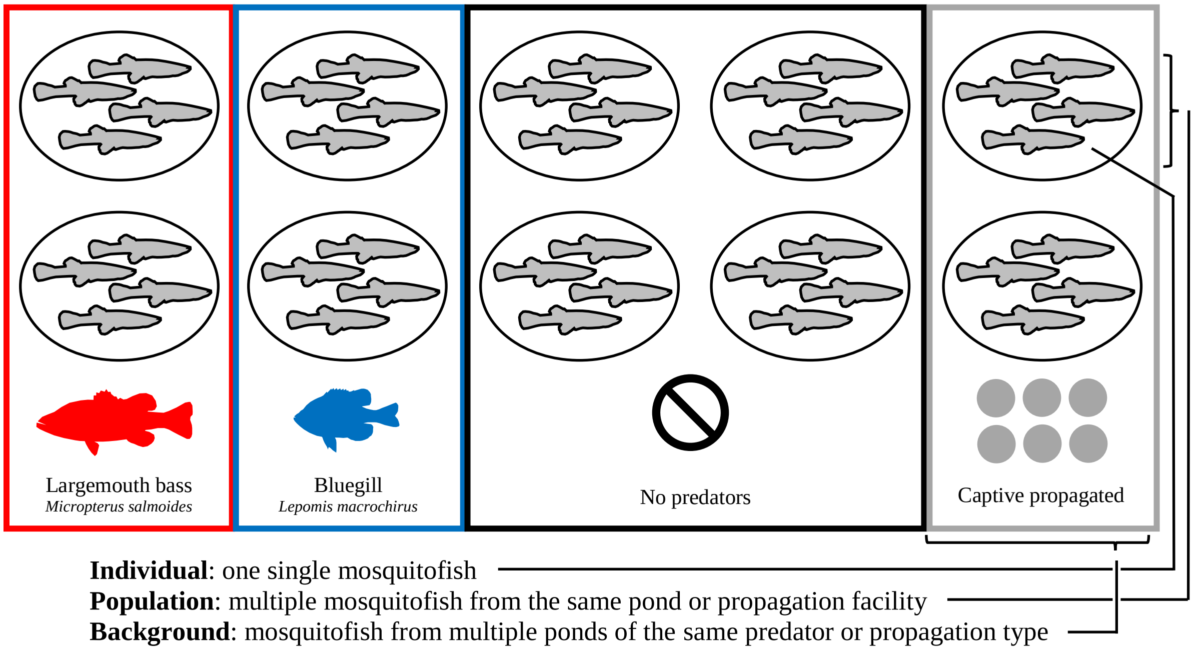


Figure 5.3. Mosquitofish population classifications. Our experiment sourced mosquitofish from four different predator or propagation backgrounds. Within those four backgrounds, we sourced fish from at least two separate populations (i.e. ponds or captive propagation facilities). We used multiple F1 individuals from each population in subsequent competitiveness and defendedness trials.

5.3.2 Competition and defense trials

We placed elastomer-tagged (VIE Northwest Marine Technology) mixtures of mosquitofish from the multiple populations in mesocosms with or without bass to study defendedness and competitiveness, respectively. We established ten (five bass-present and five bass-absent) 1,100

L mesocosms in the University of Maine Roger Clapp Greenhouses in October 2018. On October 11, we added to each mesocosm 3.6 L of benthic sediment from an unnamed pond in Orono, ME (N 44.900467°, W 68.724374°), and a mixture of zooplankton and whole water from Perch Pond (Mud Pond; N 44.946917°, W 68.777578°) and Pushaw Lake (N 44.946527°, W 68.801038°), both in Old Town, ME. We allowed mesocosms to equilibrate for 40 days prior to fish addition. We included a 15 cm diameter cylindrical mesh (1 cm square opening to allow mosquitofish entry but exclude bass) refuge filled with artificial macrophytes in the center of each mesocosm.

We introduced nine or ten tagged adult mosquitofish into each mesocosm on November 20, 2017. Other work has shown that mosquitofish consumptive effects on zooplankton saturate above roughly 6 individuals per 1,100 L mesocosm (Wood et al., in prep); thus, we expected our stocking density of 9-10 individuals per mesocosm to generate intense intraspecific mosquitofish competition. This other work also shows that bass predation causes mosquitofish abundances to quickly drop below this saturation threshold, thus limiting the extent of competition in the bass-present mesocosms. Therefore, competition and defense were likely the dominant forces driving mosquitofish success in the bass-absent and bass-present mesocosms, respectively. Most mosquitofish populations were represented in every mesocosm, with some variability due to high or variable numbers of offspring production during breeding. We tagged mosquitofish on November 15—5 days before introduction to mesocosms—using 1-2 mm of elastomer. We subcutaneously placed a single tag of either red, orange, or yellow elastomer in one of four possible locations on each fish, creating unique identifiers within each mesocosm. We measured length and towel-blotted wet mass of each fish before and after experimentation.

We placed largemouth bass (length range: 11-15 cm) collected from either Pushaw Lake or Hermon Pond in Hermon, ME (N 44.779098°, W 68.950479°) in five of the mesocosms roughly one hour after mosquitofish introduction. Largemouth bass are a natural predator of mosquitofish, and while wild mosquitofish are not present in Maine (Nico et al., 2019), we habituated these bass to consuming mosquitofish for several weeks in the lab prior to experimentation. We also confirmed that each bass could readily consume mosquitofish from the entire size range present in our lab.

We censused each mesocosm every three or four days for a period of 29 days. We removed bass from their respective mesocosms prior to censusing to ensure that census activities did not lead to mosquitofish depredation. We recorded which fish were present based on elastomer tags, using netting when necessary. We concluded the experiment when only one mosquitofish was remaining in the bass mesocosms. There was no mortality in the bass-absent mesocosms.

5.3.3 Analyses

We used absolute growth rate over the study period as an assay for competitiveness. We used days survived around bass and number of conspecifics present at death as assays for individual defendedness in the bass-present mesocosms; these two defendedness metrics offer absolute and relative measures of survival, respectively. Although we analyzed competitiveness and defendedness in different fish, we related competitiveness to defendedness by linking both to individual-, population-, and background-level traits by applying competitiveness functions from our bass-absent treatments to our depredation treatment fish.

We calculated absolute growth rate (competitiveness) over the 29-day study period for individuals in the bass-absent mesocosms:

(5.1)

$$G = \ln\left(M_{final}\right) - \ln(M_{initial})$$

G = absolute growth rate; M_{final} = final mass; $M_{initial}$ = initial mass.

We used the bass-present mesocosms to calculate two defendedness metrics: days survived, and number of mosquitofish present at death (which is inversely related to death order):

(5.2)

$$U = \frac{T_{present}+T_{absent}}{2}$$

(5.3)

$$L = \frac{N_{T_{present}} + N_{T_{absent}}}{2}$$

U = days survived, $T_{present}$ = last day a fish was observed present; T_{absent} = first day a fish was observed absent; L = number of mosquitofish present at death of the focal fish; $N_{T_{present}}$ = number of fish alive in the focal mesocosm at the last census in which the focal mosquitofish was present; $N_{T_{absent}}$ = number of fish alive in the focal mesocosms at the first census in which the focal mosquitofish was absent.

These equations assumed that deaths happened at the midpoint between the latest fish present observation and the first fish absent observation. A few fish in the bass-present mesocosms (five total across all five bass-present mesocosms) died from causes besides bass consumption, i.e.

were found dead in their mesocosms. We excluded these individuals from both defendedness metrics.

Population- and background-level differences in competitiveness and defendedness. We tested background- and population-specific identity effects on competitiveness and defendedness using maximum-likelihood (non-restricted) general linear mixed models in R (Bates et al., 2015; R Core Team, 2016):

(5.4)

$$G, U, L = B + B \times P + S + \ln(M_{initial}) + R$$

G = absolute growth rate; U = days survived; L = number of fish remaining at death; B = background; $B \times P$ = population, nested within background; S = sex; $M_{initial}$ = initial mass; R = random effect for mesocosm (and therefore bass).

We used type II Wald analysis of deviance tests to examine the significance of all model terms (Fox and Weisberg, 2011).

Competition-defense tradeoffs. We tested statistically for several scales of tradeoffs (see Figure 5.3 for definitions of individual, population, and background):

1. *Individual universal*: across all populations and backgrounds, individuals with higher competitiveness are less defended
2. *Individual within population*: within each population, individuals with higher competitiveness are less defended

3. *Individual within background*: within each background type, individuals with higher competitiveness are less defended
4. *Population*: populations that on average are more competitive are less defended
5. *Background*: backgrounds that on average are more competitive are less defended

To test for each tradeoff, we fit a model predicting relative competitiveness (growth rate) based on focal traits: for individual-level tradeoffs our focal traits were sex and mass (i.e. body size); for population- or background-level tradeoffs, our focal traits were population or background identity, respectively. We also included traits for which we wanted to control: for individual-level tradeoffs we included no controls (individual universal tradeoff), population identity (individual within population tradeoff) or background identity (individual within background tradeoff); for population- or background-level tradeoffs, we included mass and sex as controls. The basic model form was:

(5.5)

$$G = X + A + R$$

G = absolute growth rate; *X* = focal trait(s); *A* = controlled-for traits; *R* = random mesocosm effect.

We then created a predicted competitiveness metric (*C*) based only on the focal traits from the above model:

(5.6)

$$C = X$$

We did not fit Equation 5.6, we calculated *C* directly from parameters determined from fitting the model in Equation 5.5 (see Table D.2). We then used this competitiveness metric to create estimated competitiveness values for the individuals in the bass mesocosms. We then fit a model predicting days survived (U) or number of conspecifics present at death (L) (defendedness) with estimated competitiveness (*C*), also including the same earlier controlled-for traits (*A*) and a random mesocosm effect (*R*):

(5.7)

$$U, L = C + A + R$$

For expanded model equations for each tradeoff, see Table D.2. We used type II Wald analysis of deviance tests to examine the significance of each competition-defense tradeoff (i.e. the *C* term in Equation 5.7) (Fox and Weisberg, 2011).

5.4 RESULTS

5.4.1 Background- and population-level differences in competitiveness and defendedness

Absolute growth rate (competitiveness) was significantly related to background (Figure 5.4, Table 5.2), with the captive propagated background conferring the highest growth rate, bass background conferring the lowest growth rate, and bluegill and predator-free backgrounds having intermediate growth rates (Figure 5.4). We found no significant effect of population within background on absolute growth rate. Initial mass was also a significant predictor of growth rate (Table 5.2), with larger fish having lower growth rates (Figure 5.5). Sex did not significantly predict growth rate.

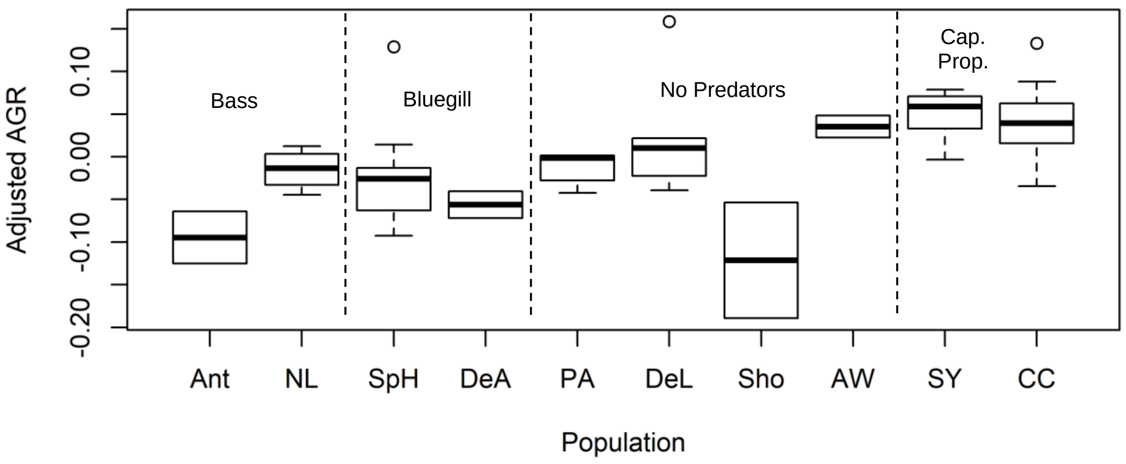


Figure 5.4. **Mosquitofish competitive ability.** Competitiveness (absolute growth rate, adjusted for body size and sex) was dependent on mosquitofish backgrounds, with mosquitofish from captive propagation (Cap. Prop.) facilities or ponds with no predators having higher absolute growth rates than fish from ponds with bass or bluegill predators.

Independent variable	**Dependent variable**								
	Absolute growth rate			**Days survived**			**Conspecifics at death**		
	$\chi 2$	df	*p*	$\chi 2$	df	*p*	$\chi 2$	df	*p*
Sex	0.53	1	0.47	1.77	1	0.18	1.15	1	0.28
ln(Mass)	11.63	1	**0.0006**	0.22	1	0.64	0.92	1	0.34
Background	15.49	3	**0.0014**	5.54	3	0.14	4.86	3	0.18
Population within Background	17.17	6	**0.0087**	14.73	6	**0.023**	17.44	6	**0.0078**

Table 5.2. Testing competitiveness and defendedness. Wald type II analysis of deviance test results for models predicting absolute growth rate, days survived, and number of conspecifics present at death for western mosquitofish. *N* = 47, 49, and 49 for absolute growth rate, days survived, and conspecifics at death, respectively.

Number of days survived and conspecifics present at death (defendedness) were both related to population identity, but not background, mass (size), or sex (Figure 5.6, Table 5.2). However, refitting model 4 for days survived (*U*) without the population term (*B*×*P*) results in larger fish having significantly higher survival ($\chi^2 = 3.92$, df = 1, $p = 0.048$), due to a significant interaction between population and body size ($\chi^2 = 27.55$, df = 9, $p = 0.0011$).

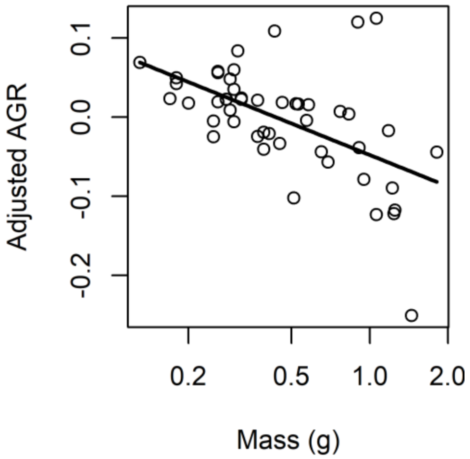


Figure 5.5. **Competitiveness and fish size**. Competitiveness (absolute growth rate) decreased with increasing fish mass. Points shown are adjusted for background and population.

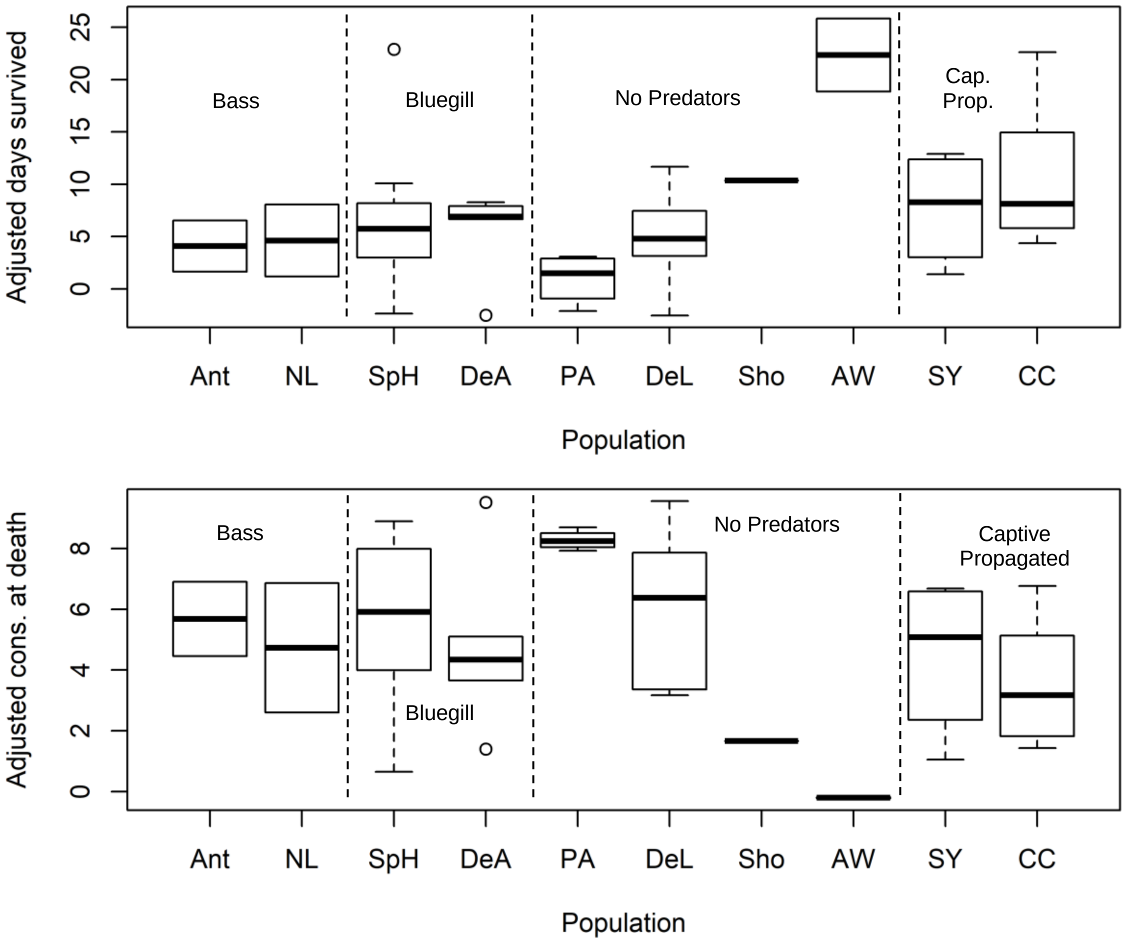


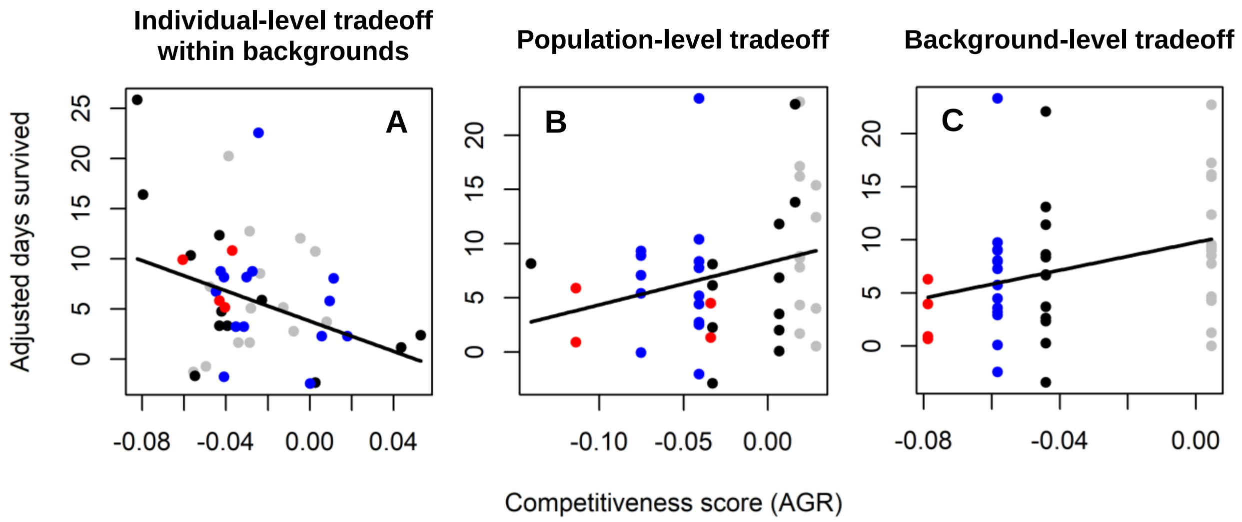
Figure 5.6. **Fish defendedness.** Defendedness (number of days survived around bass or number of conspecifics present at death, both adjusted for body size and sex) was dependent on mosquitofish population, but not mosquitofish predator or hatchery background.

5.4.2 Competition-defense tradeoffs

We found no significant universal relationship between individual-level predicted competitiveness and individual defendedness, using either defendedness metric (Table 5.3). We did, however, find a significant, negative relationship (i.e. a tradeoff) between individual-level predicted competitiveness and individual days survived around bass within backgrounds, but not within populations (Figure 5.7, Table 5.3). We also found a significant, positive relationship between both population- and background- level predicted competitiveness and days survived (Figure 5.7, Table 5.3), though the population-level relationship between competitiveness and defendedness was marginally significant (Table 5.3).

	Survival metric					
Tradeoff type	**Days survived**			**Conspecifics at death**		
	$\chi 2$	df	*p*	$\chi 2$	df	*p*
Individual within populations	0.05	1	0.83	0.34	1	0.56
Individual within background	3.99	1	**0.046**	2.29	1	0.13
Individual universal	2.55	1	0.11	1.74	1	0.19
Population-level	3.02	1	0.082	1.11	1	0.29
Background-level	3.98	1	**0.046**	2.96	1	0.09

Table 5.3. Testing for competition-defense tradeoffs. Wald type II analysis of deviance test results for the competitiveness term in tradeoff models predicting two metrics of defendedness: days survived around bass, or number of conspecifics present at death. *N* = 49.

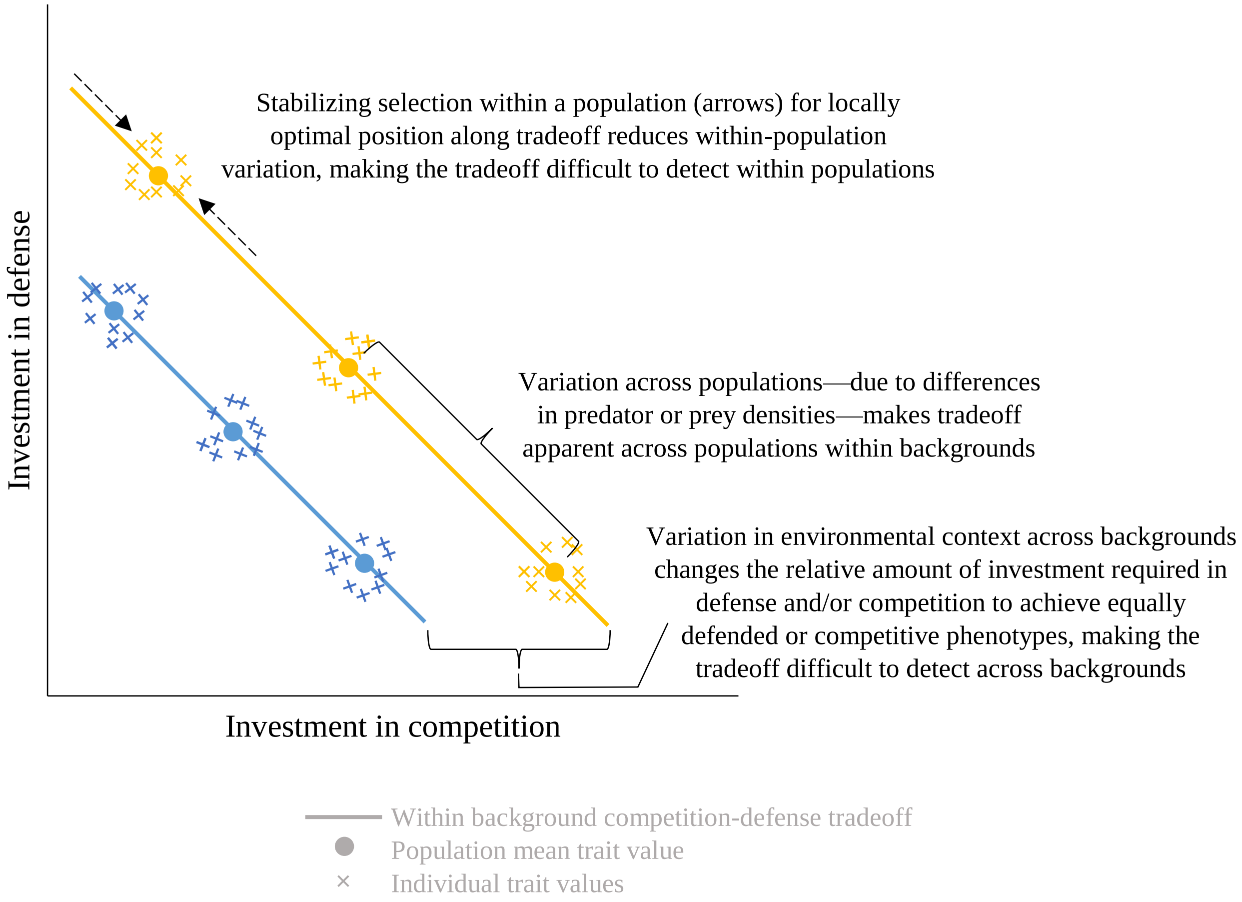


Black = no predator populations; **Blue** = bluegill populations; **Red** = bass populations; **Gray** = captive propagation populations.

Figure 5.7. Competition-defense tradeoffs. Days survived and predicted competitiveness were negatively related for individuals within backgrounds (**A**), suggesting a competition-defense tradeoff. On the population (**B**) and background (**C**) levels, days survived and predicted competitiveness were positively related, suggesting a gradient of local adaptation. Competitiveness score is based on mass and sex (controlled for background) for (A), and based on population and background (both controlled for sex and mass) for (B) and (C), respectively. Days survived were controlled for background for (A) and for mass and sex for both (B) and (C). *N* = 49.

5.5 DISCUSSION

Our results reveal a competition-defense tradeoff due to body size across individual mosquitofish within a background, as well as a positive relationship between competitiveness and defendedness on the population- and background-levels. These results indicate a mixture of competition-defense tradeoffs and local adaptation (Figure 5.8) that likely explains the abundant examples of eco-evolutionary dynamics in mosquitofish and closely-related taxa.


Stabilizing selection within a population (arrows) for locally optimal position along tradeoff reduces within-population variation, making the tradeoff difficult to detect within populations

Variation across populations—due to differences in predator or prey densities—makes tradeoff apparent across populations within backgrounds

Variation in environmental context across backgrounds changes the relative amount of investment required in defense and/or competition to achieve equally defended or competitive phenotypes, making the tradeoff difficult to detect across backgrounds

Figure 5.8. Conceptual model for detecting competition-defense tradeoffs across individuals, populations, and backgrounds.

5.5.1 Background- and population-level adaptation

Competitive ability was largely driven by size and background, with smaller individuals and those from predator-scarce backgrounds (wild no-predator and captive propagated) having higher absolute growth rates (Figure 5.4, 5.5). Selection for competition in these backgrounds is intuitive, as the paucity of predators and high density of competitors should select for highly competitive individuals (Palkovacs et al., 2011). Our finding that smaller individuals are more competitive contradicts some literature suggesting that larger individuals should have higher competitive success through interference competition (Van Buskirk et al., 2017) and ability to capture larger prey (Bence and Murdoch, 1986). Furthermore, as mosquitofish commonly grow to sizes well beyond those of individuals in our experiment (Pyke, 2005), our finding is unlikely to be due to the capping of growth in larger fish. Our findings may instead be a result of planktivore grazing patterns. Planktivores, like mosquitofish, tend to initially consume larger, slower zooplankton, like *Daphnia* (Hurlbert and Mulla, 1981; Hurlbert et al., 1972). In a highly competitive environment, these zooplankton should be quickly depleted, leaving smaller zooplankton like copepods and rotifers, which may be more readily consumed by smaller mosquitofish (Bence and Murdoch, 1986). Smaller fish should also be more competitive in heavily food-limited environments due to their lower net metabolic costs and higher attack rates on smaller prey (Hjelm and Persson, 2001).

Defendedness in mosquitofish was linked only to population—though in our tradeoff models, competitiveness calculated from size and sex within background was significantly related to one metric of defendedness (Figure 5.7, Table 5.5). Evolution of defenses at the population-, rather than the background-level suggests that predator-presence alone may not be a strong determinant of genetic (only) antipredator defense evolution in this system. Instead, defenses may be a result of plasticity or evolution of reaction norms that would be missed by our common-rearing approach. The efficacy of common modes of mosquitofish defense—avoidance and hiding (Smith and Belk, 2001; Winkelman and Aho, 1993)—are strongly dependent on the local environment, including water clarity and vegetation (Casterlin and Reynolds, 1977). Thus, adaptation to the local environment, rather than adaptation to specific predator regimes, may be the main determinant of antipredator success in mosquitofish.

Life-history defenses may also have driven our lack of observed background-level differences in survival. In the case of non-gape-limited predation, earlier maturation and increased investment in early reproduction ensures that prey are more likely to reproduce before being eaten, thus increasing their fitness. Such life-history evolution in response to increased mortality has been documented in mosquitofish (Stearns, 1983b, 1983a) and guppies (Reznick and Endler, 1982). Early maturation and reproduction often comes at a cost to growth investment (Stearns, 1989). Thus, our observed lower growth rates in bass- and bluegill-adapted mosquitofish (Figure 5.4) may be due to a defended life-history strategy in addition to decreased competitive ability.

5.5.2 Competition-defense tradeoffs within backgrounds

Within backgrounds, we found a negative relationship between individual predicted competitiveness and number of days survived around bass (Figure 5.7), indicating a genetic competition-defense tradeoff across individuals within backgrounds. We found no relationship between individual competitiveness and number of days survived without controlling for background (i.e. no universal individual-level tradeoff), suggesting that the tradeoff occurred in different trait space for each background. Food composition (Hambright et al., 1986; Lorenzen et al., 2012), predator feeding mode (or harvest by human "predators") (Olson et al., 1995; Turner and Mittelbach, 1990; Werner et al., 1983; van Wijk et al., 2013; Wood et al., in review), and conspecific density (Huntingford, 2004; Fryxell et al., in review) certainly vary greatly across the four backgrounds considered. Thus, while a competition-defense tradeoff may exist within each background, the trait values that are highly competitive or highly defended are likely contingent on the above ecological context. Therefore, the position of the competition-defense tradeoff in trait space should not be universal, and instead should vary across backgrounds.

While we found a significant competition-defense tradeoff across individuals within backgrounds, we interestingly found no tradeoff across individuals within populations. This dichotomy suggests that the competition-defense tradeoff is driven by variation across populations within backgrounds, which is supported by our finding that fish body size was significantly related to population (see Results). Furthermore, the tradeoff is the most visually compelling within the wild, predator-free background (Figure 5.7), for which we included four populations, rather than two. Within a population (i.e. a single pond), evolution may converge on a single adaptive peak along a background-specific competition-defense tradeoff, thus making the tradeoff difficult to detect within populations due to insufficient variation. The location of the adaptive peak along the tradeoff should differ across populations within backgrounds due to differences in predator density and prey availability. Thus, when multiple populations are considered within a background, there exists sufficient variation along a competition-defense tradeoff for the tradeoff to be detected.

5.5.3 Landscape-level adaptation

At the across-background level, competitiveness and number of days survived around bass were positively related (Figure 5.7). This relationship suggests that on the landscape level, some populations and backgrounds have a higher degree of general adaptedness, or vigor. As mosquitofish have been in California for less than a century (Lenert, 1923), local adaptation may be ongoing, and may be constrained by repeated stocking (Contra Costa Mosquito Vector Control, 2016; Sacramento-Yolo Mosquito & Vector Control, 2016; Swanson et al., 1996) or genetic bottlenecks (Bell and Gonzalez, 2009; Frankham, 1996; Gonzalez et al., 2013). Interestingly, captive propagated mosquitofish had the highest vigor on both the population- and background-levels (Figure 5.7), which contradicts evidence that hatchery fish often have lowered fitness in the wild (Araki et al., 2007, 2008). The trend in vigor appears to be driven mostly by predation history, with wild no-predator and captive-propagated populations having higher vigor than the bass and bluegill populations. Thus, while there may be a within-background competition-defense tradeoff related to body size, the general competitive advantage of fish from the predator-scarce backgrounds may also broadly confer higher survival, possibly through increased energetic stores for escape or hiding and decreased need for risk-taking (Godin and Crossman, 1994).

5.5.4 Tradeoffs, evolution, and eco-evolutionary dynamics

The presence of a competition-defense tradeoff related to body size in mosquitofish indicates significant potential for eco-evolutionary dynamics in this model species. This tradeoff also provides some explanation for the broad evidence of contemporary evolution to predators (or release therefrom) in mosquitofish and closely-related taxa (Langerhans, 2009; Langerhans et al., 2004; Reznick and Endler, 1982; Reznick et al., 1990). This tradeoff also supports the findings that release from predation in these taxa sparks evolution increasing their top-down per-capita effects on food webs (i.e. competitive ability) (Bassar et al., 2010, 2012; Palkovacs et al., 2011).

Interestingly, our findings of an individual-level tradeoff within background—but not populations—suggests that single populations may not initially contain sufficient variation along a tradeoff to generate eco-evolutionary dynamics. Indeed, during numerous predator-prey experiments examining eco-evolutionary dynamics, there is significant delay between the onset of ecological dynamics (i.e. predator-prey cycling) and the onset of tradeoff-driven eco-evolutionary dynamics (Hiltunen et al., 2014; Yoshida et al., 2003, 2004, 2007); this delay has been attributed to lack of genetic variation (Yoshida et al., 2003). Our findings thus suggest that—in mosquitofish and other taxa—variation along a competition-defense tradeoff may be a key factor in determining the timing and scope of eco-evolutionary dynamics. Thus, systems that are likely to retain variation over time—dynamic, interconnected metapopulations with predator and prey regimes varying in space and time—are the most likely to generate strong examples of eco-evolutionary dynamics.

5.5.5 Conclusions

A genetic competition-defense tradeoff dependent on body size exists within various predator and hatchery backgrounds of western mosquitofish—a model species for eco-evolutionary dynamics. However, lack of evidence for the tradeoff within populations suggests that single populations may not initially possess sufficient variation along the tradeoff to generate eco-evolutionary dynamics in response to ecological perturbations. Therefore, we suggest that standing variation along a competition-defense tradeoff, as well as the metapopulation characteristics that contribute thereto, are essential characteristics underlying potential eco-evolutionary dynamics.

5.6 ACKNOWLEDGEMENTS

We thank Ethan Cantin, Isaac Shepard, Mitch Paisker, and Brad Libby for help with data collection.

Funding was provided by:

US National Science Foundation (DEB 1457333 and DEB 1457112)

Maine Agricultural and Forest Experiment Station

University of Maine Janet Waldron Fellowship Program

CHAPTER 6

ECOLOGICAL AND PHENOTYPIC CAUSES AND CONSEQUENCES OF ECO-EVOLUTIONARY TROPHIC CASCADES IN MOSQUITOFISH

6.1 ABSTRACT

Evolution can occur contemporaneously with ecology, allowing ecological and evolutionary processes to mutually interact. One important type of contemporary trait change is antipredator adaptation in prey, which can influence the persistence of both predator and prey populations and generate or mediate trophic cascades. However, few studies have mechanistically linked genetic and plastic evolution in specific traits to ecological change. Here we assess morphological and behavioral traits in nine populations of common-garden reared western mosquitofish (*Gambusia affinis*) from three different predator backgrounds to quantify heritable and plastic local- and anti-predator evolution. We then use pond mesocosm experiments to examine the ecological consequences of variation in these traits. We also compare the relative ecological effect sizes of population divergence and fish density variation. Evolution in response to two different predators generates a similar mixture of heritable and plastic trait changes, likely focused on predator avoidance and energetic efficiency, but trait variation from antipredator evolution is significantly smaller than that from idiosyncratic local population divergence within predator backgrounds. Among-population variation in several mosquitofish traits associated with heritable and plastic predator naivete causes cascading zooplankton and primary producer change, likely through both top-down and bottom-up pathways. Ecological effects of mosquitofish trait divergence are far more extensive than those of fish density variation, reaching almost all studied taxa and compartments. As such, this study provides an impactful example of how contemporary trait change—through a variety of genetic and plastic mechanisms—can cascade through food webs and alter ecosystem function.

Keywords: Contemporary evolution, antipredator evolution, phenotypic plasticity, eco-evolutionary dynamics, trophic cascades, Gambusia affinis

6.2 INTRODUCTION

Contemporary evolution can drive ecological change. Overwhelming evidence for contemporary evolution—evolution on ecologically-relevant timescales—(Carroll et al., 2007; Hendry and Kinnison, 1999; Kinnison and Hendry, 2001), demonstrates that ecological and evolutionary processes can contemporaneously interact (Fussmann et al., 2007; Hendry, 2016; Post and Palkovacs, 2009; Schoener, 2011). Thus, ecology can shape evolution, and ongoing evolution can shape ecology. Ongoing evolution can shape ecology by influencing species persistence (Gonzalez et al., 2013; Kinnison and Hairston, 2007; Yamamichi and Miner, 2015), changing population sizes of adapting taxa (Lambrinos, 2004; Stockwell et al., 2003), or changing ecologically relevant functional traits (Ellner et al., 2011; Hairston et al., 2005). However, the specific mechanisms driving evo-to-eco processes—as well as their relevant effect sizes—are yet poorly understood.

When plastic or genetic evolution alters ecologically relevant functional traits, significant environmental change may occur. Several case-studies highlight examples of local adaptation in fish that have generated ecological change through functional trait change (Table 6.1). Example functional traits include feeding morphology, feeding mode (e.g. benthic versus limnetic), growth rate, and antipredator defenses (Palkovacs et al., 2008, 2011; Tuckett et al., 2013; Willacker et al., 2010). The common thread to each of these examples is that local adaptation affects what, where, and how much an organism eats, thereby leading to top-down ecological change. These top-down effects of species adaptation are often greater in magnitude than those of species introduction or removal (Des Roches et al., 2018). Understanding the functional links between traits and ecological dynamics is a challenge at the heart of any mechanistic understanding of eco-evolutionary dynamics.

Taxa	Trait Change	Ecological Impact	References
Alewives *Alosa pseudoharengus*	Loss of anadromy	Prey size-selectivity alters zooplankton communities	(Palkovacs and Post, 2008, 2009; Palkovacs et al., 2008; Post et al., 2008)
White perch *Morone americana*	Adaptation to eutrophication	Context-dependent shifts in pond productivity	(Tuckett et al., 2013, 2017)
Threespine stickleback *Gasterosteus aculeatus*	Benthic vs. limnetic habits	Feeding mode divergence alters water clarity, invertebrate composition	(Des Roches et al., 2013; Harmon et al., 2009; Rundle et al., 2000; Willacker et al., 2010)
Trinidadian guppies *Poecilia reticulata*	Antipredator adaptation	Cascading changes in invertebrate and algal biomass and composition	(Bassar et al., 2010, 2012; Palkovacs et al., 2009, 2011; Reznick and Endler, 1982; Reznick et al., 1990)

Table 6.1. Select examples of contemporary local adaptation in fish that have been linked to ecological changes.

One broadly relevant type of functional trait adaptation is the evolution of antipredator defenses. Early work suggested that antipredator adaptation in prey stabilizes both predator and prey abundances after predator introduction (Pimentel, 1961). Ongoing antipredator evolution in prey can determine the impact of introduced predators and the persistence of both predators and prey (Sax et al., 2007; Strauss, 2014; Yamamichi and Miner, 2015). In some cases, evolution in prey can reduce the effect size of predators on prey (terHorst et al., 2010). In other cases, adaptation of prey can lead to novel predator-prey cycling (Abrams and Matsuda, 1997b; Kasada et al., 2014; Yoshida et al., 2003), which may destabilize food webs (Abrams and Matsuda, 1997a; Cortez et al., 2018). The distinction between these outcomes—as well as their broader ecological impacts—depend on the specific form, lability, and tradeoffs of functional traits (Kasada et al., 2014; Wood et al., 2018).

Fish employ numerous behavioral or morphological defenses to avoid or escape predators. Antipredator behaviors include fleeing (Langerhans, 2009), schooling and shoaling (Magurran et al., 1992; Seghers, 1974), freezing (Eilam, 2005; Vilhunen and Hirvonen, 2003), hiding (Romare and Hansson, 2003; Templeton and Shriner, 2004), and predator inspection (Magurran, 1986; Magurran et al., 1992). Morphological defenses include crypsis (Cox et al., 2009; Donnelly and Dill, 1984; Donnelly and Whoriskey, 1993), armor (Bell et al., 2004), apparatus for predator detection (Brown, 2003; Cowan and Brown, 2000; McCormick and Manassa, 2008), and apparatus for predator escape (Domenici et al., 2008; Langerhans, 2009). A variety of life-history strategies to reduce the fitness impact of predation also exist; these typically involve early maturation to increase the chances of reproduction before predation or rapid growth to reach inedible sizes (Jennions and Telford, 2002; Reznick and Endler, 1982; Reznick et al., 1990). While attention to the ecological relevance of antipredator defenses is growing, fairly little work has examined whether contemporary evolution of defenses are consistent across multiple predator species (i.e. are generalist) or predator-specific (i.e. specialist) (Strauss et al., 2006).

Antipredator defenses can have cascading impacts on food webs through trait- and density-mediated processes. Many defenses come at a feeding cost, often because behaviors or morphologies suited to defense (e.g. hiding) reduce feeding ability (Tymchuk et al., 2007). Thus, defended phenotypes can cause defended populations to exert weaker feeding pressures on their prey, generating trait-mediated cascading food web dynamics (Griffiths et al., 2018; Ousterhout et al., 2018; Preisser and Bolnick, 2008a; Schmitz et al., 2004). These trait-mediated effects may be at least partially offset by the increased density of the defended population (Wood et al, in preparation). Evolution along the aforementioned feeding-defense tradeoff in intermediate trophic levels during a trophic cascade can dampen or exaggerate the cascade, depending on which trophic level evolves (Cadier et al., 2019; Wood et al., 2018). Certain defense behaviors, like habitat switching, can even shift trophic cascades to other taxa (Grabowski and Kimbro, 2005; Trussell et al., 2006). Thus, trait changes during a trophic cascade can alter the strength, mode, and reach of the cascade. However, the relative strengths of the evolutionary processes behind ecologically relevant trait variation are generally poorly understood.

Much of the work on contemporary antipredator evolution has focused on total phenotypic change, i.e. the combination of genetic and plastic effects (Hendry, 2016). While such an approach is sufficient to determine the immediate net ecological impact of a defended or naïve phenotype, it does not yield a complete mechanistic understanding of how evolution affects ecology. To understand the ecological role of antipredator evolution during predator invasion, requires a disentangling of genetic and plastic evolution—which should occur at different speeds (Ghalambor et al., 2007; Lande, 2015). Plastic change may buffer against genetic change in the short term (Oostra et al., 2018; Price et al., 2003), or facilitate it, depending on whether the plastic change is adaptive (Ghalambor et al., 2007, 2015). The evolution of reaction norms (gene × environment interactions) may cause some populations to have higher plastic capacity than others (Dodson, 1989; Tollrian and Harvell, 1999), and make the ecological impacts of genetic evolution contingent on environmental context. Therefore, thorough investigation of the plastic and genetic mechanisms of adaptation is necessary to assess the long-term fate and ecological-impact of evolving species.

Here, we investigate the cascading ecological consequences of genetic and plastic antipredator divergence among western mosquitofish (*Gambusia affinis*) populations in order to mechanistically link trait variation to ecological change. Specifically, we address the following questions:

1. How does generic or predator-specific antipredator adaptation change mosquitofish traits through plastic and genetic mechanisms?
2. How much trait variation does antipredator adaptation explain, relative to other sources of inter-and intra-population trait variation?
3. What are the cascading ecological impacts of functional trait divergence in mosquitofish?
4. How do the ecological impacts of trait divergence compare to those of fish introduction and density change?

We examined the morphology, behavior, and impact on pond mesocosms of replicate populations of mosquitofish from various depredation backgrounds. We used common-rearing, varied predator cues, and varied densities to separate genetic, plastic, and density-dependent effects of mosquitofish adaptation.

6.3 METHODS

6.3.1 Mosquitofish sources and rearing

Western mosquitofish were introduced to California in the 1920s for mosquito control (Lenert, 1923). Although detailed stocking records and population genetic surveys are lacking, these original fish were widely stocked and translocated throughout the region over a period of decades. Thus, we assume at least some common ancestry among all study populations.

We collected mosquitofish from 9 populations (i.e. ponds) in central California in early spring 2017 using beach seine hauls (Table E.1). These populations belonged to one of three predation backgrounds: no piscine predators, bluegill (*Lepomis macrochirus*) predators, or largemouth bass (*Micropterus salmoides*) predators. We bred each of these populations for two generations in captivity, keeping fish in 1,100 L outdoor mesocosms, where they were fed abundant flake food. We passively separated fry from adults in these mesocosms using floating mesh refugia, from which we moved fry to new mesocosms for subsequent breeding or holding. For each population, we reared two separate lines: one with bass cues present for the entire two generations of breeding, and one with bass cues absent. For the bass-present lines, we introduced a single largemouth bass from Freshwater Fish Co, Elk Grove CA into a plastic- and mesh-walled holding container within the breeding mesocosm. We periodically fed mosquitofish to this bass. Thus, mosquitofish in the bass-present mesocosms had visual and chemical cues of bass presence and mosquitofish depredation for two generations. This two-generation rearing ensured that we were able to observe the full scope of mosquitofish plastic responses to bass presence or absence, including maternal effects (Marsh-Matthews et al., 2005; Zhang et al., 2006).

We examined heritable and plastic population differences in mosquitofish morphology and behavior. Specifically, we partitioned trait variation into seven possible mechanistic sources:

1. *Generic antipredator evolution*—heritable trait divergence in response to piscine predator presence (i.e. not distinguishing between bass and bluegill predators)
2. *Specific antipredator evolution*—heritable trait divergence in response to a specific local predator (bass vs. bluegill)
3. *Local population evolution*—heritable trait divergence among populations (ponds) within predator-type backgrounds.
4. *General plasticity*—environmentally induced trait divergence due to a universal plastic response to bass cues
5. *Generic evolution of antipredator plasticity*—heritable divergence in environmentally-induced trait responses to bass cues, regardless of predator background (i.e. not distinguishing between bass and bluegill predators)
6. *Specific evolution of antipredator plasticity*—heritable divergence in environmentally-induced trait response to bass cues that is associated with a specific predator background (bass vs. bluegill)
7. *Local population evolution of plasticity*—heritable divergence in environmentally-induced trait responses to bass cues that differs across populations (ponds) within predator backgrounds

Types 1-3 represent presumed adaptive evolution, type 4 represents presumed plastic trait change, and types 5-7 represent potentially adaptive evolution of reaction norms (i.e. gene × environment interactions).

6.3.2 Fish morphology

We analyzed morphometric characteristics of all fish used in the mesocosm experiments after experimentation. We took standardized photographs of the left side of each fish. We marked 10 morphometric landmarks (Langerhans et al., 2004) on each fish picture using tpsdig2 (Rohlf, 2006), and measured fish length (excluding caudal fin) (Figure E.1). We then used these landmarks to calculate several morphological traits (Table E.2). Rather than holistically analyzing morphology using geometric morphometric methods (Bookstein, 1997; Zelditch et al., 2012), we analyzed these specific traits directly due to their adaptive divergence and environmental impact in other studies (Des Roches et al., 2013; Harmon et al., 2009; Langerhans et al., 2004; Magurran et al., 1992; Palkovacs et al., 2011).

6.3.3 Fish behavior

We conducted fish behavioral assays before and during mesocosm experimentation to determine feeding rate, activity, shoaling depth, and distance from a tank-side observer. As with our morphological analyses, we chose behavioral traits that have been linked to adaptive divergence and ecological impacts in the literature (Magurran, 1986, 1990; Magurran et al., 1992; Rehage and Sih, 2004; Rehage et al., 2005a, 2005b; Sih et al., 2010).

We measured activity level, shoaling depth, and shoaling distance from an observer during mesocosm experimentation (see below) using the protocol from Wood et al. (in press). For these measurements, a treatment-blind observer stood next to the mesocosm and observed the shoal. The observer waited for 5 minutes, then collected measurements every 1 minute for 10 minutes.

We measured activity on a 1-4 scale (1: no movement; 2: maintenance movement; 3: modest exploratory movement or foraging; 4: burst-swimming or aggressive interactions) (Wood et al., in press). We measured shoaling depth and distance by dividing the mesocosm into four zones vertically and four zones horizontally, then recording which zone was occupied by the majority of the shoal. We conducted these observations on days 1 and 2 after mosquitofish introduction, and again on days 7 and 8.

We conducted feeding rate assays before introducing fish into mesocosms. Feeding rate assays were conducted between 9:00 am and 5:00 pm. We caught groups of five mosquitofish, 3 females and 2 males, from each group of fish waiting to be introduced into mesocosms (48 groups total) using handheld nets, and placed them into plastic aquaria (15W x 20H x 25L cm). Each aquarium contained one PVC u-bend pipe positioned in the back-left corner, which provided shelter and reduced stress for fish during the trials. We allowed mosquitofish to acclimate to the aquaria for 30 minutes, after which trials commenced. Each trial ran for a total of 15 minutes. Following a 5-minute acclimation period to the presence of the observer (LKL), we added 10 bloodworms (Omega One brand) into the aquarium using a pipette. Over 10 minutes the number of bloodworms consumed by the group of mosquitofish was recorded. At the end of each assay, any remaining worms were removed. The feeding assay was run twice for each replicate mosquitofish group, once in the morning and once in the afternoon.

6.3.4 Ecological consequences of fish evolution

We used replicated pond mesocosms to assess the ecological consequences of mosquitofish introduction, density variation, and trait variation. We established 50 experimental 1,100 L mesocosms at the University of California Santa Cruz Long Marine Lab Campus in September, 2017. We added 2 L of active sediment from Schwann Lake (N 36.962655°, W 121.996843°), as well as 10 L of sand to the bottom of each mesocosm. We added whole water and filtered (> 80 μm) zooplankton from Antonelli Pond (N 36.956292°, W 122.060251°) and West Lake (N 36.976083°, W 122.045683°). We allowed mesocosms to equilibrate for 6 days prior to fish addition. We added 5 mosquitofish (3 females, 2 males) to each of 36 "low-density" mesocosms, representing 2 replicates each of every factorial population (9) and bass cue (2) combination. We added 10 mosquitofish (6 females, 4 males) to each of 12 "high-density" mesocosms, representing 2 replicates each of a factorial combination of a subset of populations (one from each predator background for a total of 3) and bass cues (2). We also retained two control mesocosms, which received no fish. We assigned the above treatments randomly across the 7 by 8 grid of mesocosms. Mesocosms containing mosquitofish that had been reared with bass cues present received bass cues (50 mL of water from a 100 L tank with ~12 juvenile bass that had been consuming mosquitofish) twice during the experiment to sustain the bass cue effect.

We sampled ecological characteristics 0, 3, 6, 9, and 27 days after fish addition. We measured gross primary production (GPP) by taking dissolved oxygen measurements at dawn, the following dusk, and the following dawn, then calculating GPP as the amount of oxygen consumed during the night + the amount of oxygen produced during the day (Harmon et al., 2009). We measured pelagic chlorophyll-a (chl-a), collected using 0.2-1.0 L depth integrated water samples, filtered onto 0.7 μm filters (Whatman GF/F), and measured using fluorometry (Turner Designs, Trilogy Module CHL-NA). We filtered 20 L depth-integrated zooplankton samples through 80 μm mesh, and preserved them with 70% ethanol. We sorted zooplankton to the following broad taxa: calanoids, cyclopoids, nauplii, *Daphnia, Bosmina*, *Ceriodaphnia*, chydroids, and ostracods. In this manuscript, we present data from the final sampling date, 27 days after fish addition.

6.3.5 Analyses

Fish morphology. We used the following general linear mixed model to analyze mosquitofish morphology:

(6.1)

M		morphometric (Table E.2) (natural log for lengths and areas; untransformed for angles and fin placement ratios)
=	P	generic antipredator evolution (any predators historically present)
+	B	specific antipredator evolution (bass or bluegill), nested within general antipredator evolution (within P)
+	S	local population identity, nested within backgrounds (within B within P)
+	C	general plasticity to bass cues
+	$C \times P$	generic evolution of antipredator plasticity
+	$C \times B$	specific evolution of antipredator plasticity, nested within backgrounds (within P)
+	$C \times S$	local population evolution of plasticity, nested within backgrounds (within B within P)
+	X	sex
+	$\ln(L)$	natural log body length
+	$X \times \ln(L)$	sex-length interaction
+	T	morphology technician who placed the landmarks (random)

We fit this model using a maximum-likelihood (non-restricted) algorithm via *lmer* in R (Bates et al., 2015; R Core Team, 2016). We analyzed the significance of each model term for each model metric using Wald type II analysis of deviance tests (Fox and Weisberg, 2011).

Fish behavior. We used the following general linear mixed model to analyze mosquitofish behavior (or generalized linear model when modeling proportion of food consumed):

(6.2)

A		average value for each behavioral assay across 10 mins of observation or proportion of food items consumed during a 10 min feeding trial
=	P	generic antipredator evolution (any predators historically present)
+	B	specific antipredator evolution (bass or bluegill), nested within general antipredator evolution (within P)
+	S	local population identity, nested within backgrounds (within B within P)
+	C	general plasticity to bass cues
+	$C\times P$	generic evolution of antipredator plasticity
+	$C\times B$	specific evolution of antipredator plasticity, nested within backgrounds (within P)
+	$C\times S$	local population evolution of plasticity, nested within backgrounds (within B within P)
+	N	fish stocking density
+	T	observer (random, not included for feeding trials)
+	U	observation date (random, not included for feeding trials)

We fit this model using a maximum-likelihood (non-restricted) algorithm via *glm* or *lmer* in R (Bates et al., 2015; R Core Team, 2016). We analyzed the significance of each model term for each model metric using Wald type II analysis of deviance tests (Fox and Weisberg, 2011).

Evolutionary effect sizes on traits. We also examined the relative effect size contributions of our seven focal types of population divergence to morphology and behavior. We re-fit the above models for morphology and behavior, but this time treated all the evolutionary variables as random. We again used maximum-likelihood (non-restricted) model fitting.

We extracted the standard deviations from each evolutionary variable, which indicate the average contribution of each variable to each morphological or behavioral trait. We calculated relative standard deviation for each evolutionary variable for each trait:

(6.3)

$$r_i = \frac{s_i}{\sum s_x + s_\varepsilon}$$

r_i = relative standard deviation; s_i = standard deviation for a single evolutionary variable within a trait model; Σs_x = sum of the standard deviations of all evolutionary variables within a trait model; s_ε = residual standard deviation for the trait model.

We then fit the following general linear mixed model predicting the values of r_i calculated in Equation 6.3:

(6.4)

$$r_i = V + T$$

r_i = relative standard deviation; V = type of evolution; T = random effect for trait considered.

We used Tukey tests using the *ghlt* function in R to rank the relative contributions of each type of evolution to mosquitofish trait variation (Hothorn et al., 2008). We used a likelihood ratio test to examine the significance of the V term.

Ecological consequences. We directly examined the effects of specific mosquitofish traits on our ecological metrics. We began by fitting basic allometric models for mosquitofish morphology:

(6.5)

$$M = X + \ln(L) + X \cdot \ln(L) + T$$

M = morphometric (Table E.2); X = sex; L = mass; and T is a random term for morphology technician. We used the same model forms and variable transformations as the earlier morphometric analyses (natural log for lengths and areas; untransformed for angles and fin placement ratios).

We also fit a basic observer and date model for our mosquitofish behavioral assays:

(6.6)

$$A = T + U$$

A = behavioral metric; *T* is a random term for observer; and *U* is a random term for observation date, which was not included for the feeding trials. We used the same data distributions as the earlier behavioral analyses.

We calculated standardized residuals from the above models for each of the 13 morphometric and behavioral variables. We averaged morphometric residuals for all individuals within a particular mesocosm and averaged shoal behavior residuals for all time points within a particular mesocosm. This created a per-mesocosm average residual or "group deviance" value for each morphological or behavioral trait, which we could use to predict our ecological metrics.

We created a general linear model predicting each ecological metric (GPP, chlorophyll a, or zooplankton density). We started with a model including all standardized residual morphological and behavioral traits, as well as density:

(6.7)

$$E = R_1 + R_2 \ldots + N$$

E = ecological metric ($\ln(Y)$ transformed for producers, $\ln(Y + 0.1)$ transformed for zooplankton);

R = per-mesocosm average standardized residual trait (morphological or behavioral) value; and

N = mosquitofish density.

We performed type II likelihood ratio tests on each model parameter. We sequentially removed the parameter with the lowest relative likelihood and re-fit the model until each parameter had $p < 0.05$; we left the mosquitofish density term (N) in the model regardless of its significance. We compared the AIC of the resulting model with the AIC from a null model including only density (N) to assess relative fit quality of the trait parameters (i.e. relative information gain (Burnham and Anderson, 2003)).

Ecological effect sizes. We compared the ecological effect sizes of mosquitofish trait variation and density variation. We re-fit the final models from the *Ecological consequences* section as standardized general linear models (i.e. all independent and dependent numeric variables had a mean of 0 and a standard deviation of 1). We then extracted the model slope coefficients for the various parameters, which provide a measure of effect sizes (Schielzeth, 2010): the standard deviation of ecological change expected from a standard deviation in trait change or a doubling of mosquitofish density.

6.4 RESULTS

6.4.1 Morphology

Mosquitofish morphology responded genetically and plastically to predators, but these responses were generally not predator-specific (Figures 6.1.a, 6.1.b, Table 6.2). Fish from either predator background had shallower heads and more posterior dorsal and anal fins than fish from predator-naïve sources. Plastically, fish had smaller, shallower tails when exposed to bass cues for two generations; this reaction was more pronounced in fish from the predator backgrounds. When exposed to bass cues, predator-naïve mosquitofish also had more posterior dorsal and anal fins. Plasticity causes opposite reactions in head depth for bass- and bluegill-impacted sources: when exposed to bass cues, fish from bass-impacted sources developed shallower heads, while fish from bluegill-impacted sources developed deeper heads. There was also population-specific genetic evolution within backgrounds for nearly all measured morphological traits (Figure 6.1.a, 2b, Table 6.2). Fish lateral eye position and eye angle ratio did not vary significantly with any of our evolutionary variables (Table 6.2).

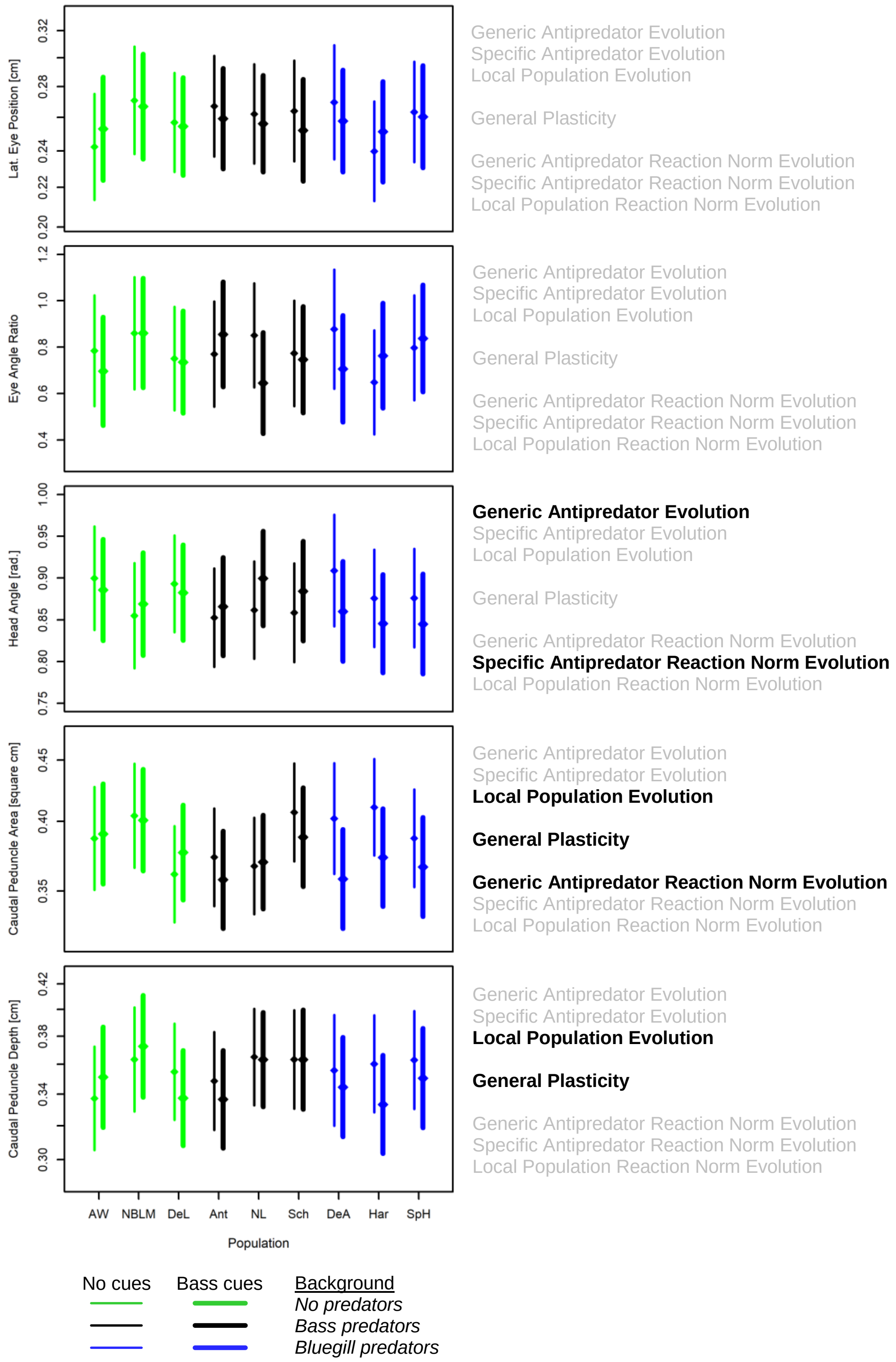
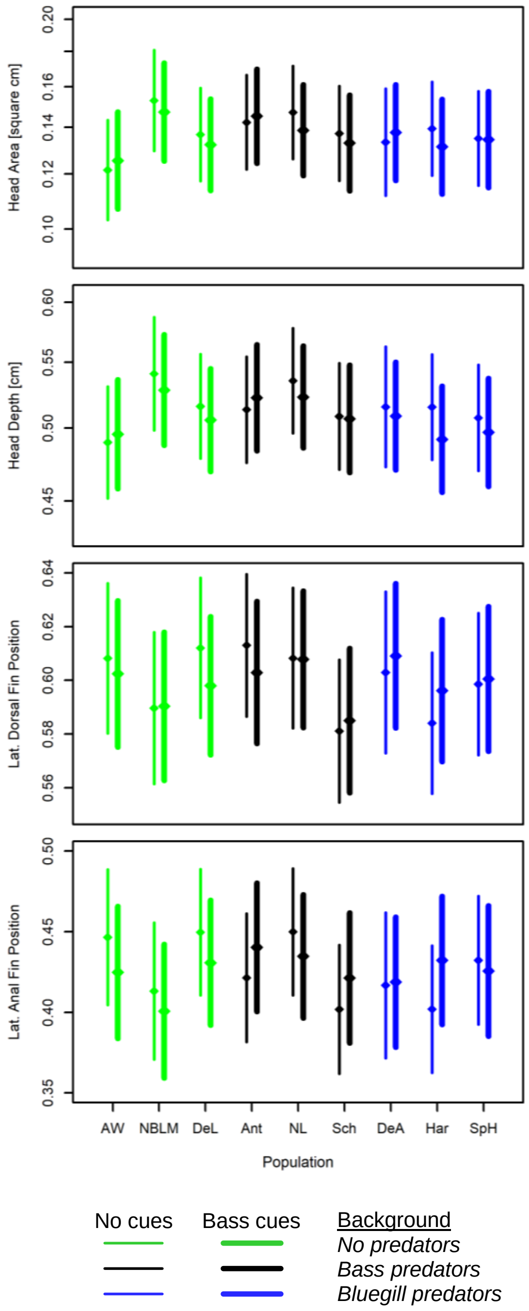


Figure 6.1.a. Evolution of mosquitofish morphology. Morphological traits for *Gambusia affinis* from various predator backgrounds, with some exposed to bass cues for two generations.

Bars show general linear mixed model predictions ± one standard error. Text next to each panel indicates significant effects of various types of genetic and plastic evolution on each trait.



Generic Antipredator Evolution
Specific Antipredator Evolution
Local Population Evolution

General Plasticity

Generic Antipredator Reaction Norm Evolution
Specific Antipredator Reaction Norm Evolution
Local Population Reaction Norm Evolution

Generic Antipredator Evolution
Specific Antipredator Evolution
Local Population Evolution

General Plasticity

Generic Antipredator Reaction Norm Evolution
Specific Antipredator Reaction Norm Evolution
Local Population Reaction Norm Evolution

Generic Antipredator Evolution
Specific Antipredator Evolution
Local Population Evolution

General Plasticity

Generic Antipredator Reaction Norm Evolution
Specific Antipredator Reaction Norm Evolution
Local Population Reaction Norm Evolution

Generic Antipredator Evolution
Specific Antipredator Evolution
Local Population Evolution

General Plasticity

Generic Antipredator Reaction Norm Evolution
Specific Antipredator Reaction Norm Evolution
Local Population Reaction Norm Evolution

Figure 6.1.b. Evolution of mosquitofish morphology, continued.

Trait	**Genetic Divergence (G)**			**General Plasticity (E)**	**Reaction Norm Divergence (G×E)**		
	Predator (Generic)	**Predator (Specific)**	**Pop.**		**Predator (Generic)**	**Predator (Specific)**	**Pop.**
	P	**B**	**S**	**C**	**C×P**	**C×B**	**C×S**
Eye Position	$\chi^2 = 0.42$ $p = 0.516$	$\chi^2 = 0.09$ $p = 0.758$	$\chi^2 = 11.43$ $p = 0.076$	$\chi^2 = 1.21$ $p = 0.272$	$\chi^2 = 0.65$ $p = 0.42$	$\chi^2 = 1.21$ $p = 0.272$	$\chi^2 = 2.89$ $p = 0.822$
Eye Angle Ratio	$\chi^2 = 0.18$ $p = 0.67$	$\chi^2 = 0.21$ $p = 0.646$	$\chi^2 = 9.36$ $p = 0.154$	$\chi^2 = 0.53$ $p = 0.466$	$\chi^2 = 0.03$ $p = 0.855$	$\chi^2 = 1.22$ $p = 0.268$	$\chi^2 = 10.05$ $p = 0.123$
Head Angle	**$\chi^2 = 6.2$** **$p = 0.013$**	$\chi^2 = 0.58$ $p = 0.447$	$\chi^2 = 8.01$ $p = 0.238$	$\chi^2 = 0.36$ $p = 0.548$	$\chi^2 = 0.11$ $p = 0.745$	**$\chi^2 = 14.69$** **$p < 0.001$**	$\chi^2 = 1.79$ $p = 0.938$
Tail Area	$\chi^2 = 0.49$ $p = 0.485$	$\chi^2 = 1.18$ $p = 0.276$	**$\chi^2 = 37.63$** **$p < 0.001$**	**$\chi^2 = 6.73$** **$p = 0.009$**	**$\chi^2 = 14.38$** **$p < 0.001$**	$\chi^2 = 2.75$ $p = 0.097$	$\chi^2 = 5.19$ $p = 0.52$
Tail Depth	$\chi^2 = 3.07$ $p = 0.080$	$\chi^2 = 2.45$ $p = 0.117$	**$\chi^2 = 17.25$** **$p = 0.008$**	**$\chi^2 = 5.23$** **$p = 0.022$**	$\chi^2 = 0.07$ $p = 0.792$	$\chi^2 = 2.16$ $p = 0.141$	$\chi^2 = 7.59$ $p = 0.270$
Head Area	$\chi^2 = 0.59$ $p = 0.441$	$\chi^2 = 1.2$ $p = 0.274$	**$\chi^2 = 22.56$** **$p = 0.001$**	$\chi^2 = 1.58$ $p = 0.209$	$\chi^2 = 0.01$ $p = 0.926$	$\chi^2 = 0.20$ $p = 0.654$	$\chi^2 = 2.6$ $p = 0.857$
Head Depth	$\chi^2 = 0.26$ $p = 0.609$	$\chi^2 = 3.36$ $p = 0.067$	**$\chi^2 = 20.31$** **$p = 0.002$**	$\chi^2 = 3.12$ $p = 0.077$	$\chi^2 = 0.00$ $p = 0.969$	$\chi^2 = 1.05$ $p = 0.306$	$\chi^2 = 2.33$ $p = 0.887$
Dorsal Fin Position	**$\chi^2 = 4.31$** **$p = 0.038$**	$\chi^2 = 2.45$ $p = 0.118$	**$\chi^2 = 34.72$** **$p < 0.001$**	$\chi^2 = 0.33$ $p = 0.565$	**$\chi^2 = 4.26$** **$p = 0.039$**	$\chi^2 = 0.43$ $p = 0.511$	$\chi^2 = 3.38$ $p = 0.760$
Anal Fin Position	**$\chi^2 = 4.75$** **$p = 0.029$**	$\chi^2 = 0.34$ $p = 0.561$	**$\chi^2 = 25.68$** **$p < 0.001$**	$\chi^2 = 0.09$ $p = 0.770$	**$\chi^2 = 8.50$** **$p = 0.004$**	$\chi^2 = 0.59$ $p = 0.442$	$\chi^2 = 7.96$ $p = 0.241$
Shoaling Depth	**$\chi^2 = 19.16$** **$p < 0.001$**	$\chi^2 = 0.89$ $p = 0.346$	**$\chi^2 = 21.94$** **$p = 0.001$**	**$\chi^2 = 5.65$** **$p = 0.017$**	$\chi^2 = 0.17$ $p = 0.678$	$\chi^2 = 2.09$ $p = 0.149$	$\chi^2 = 5.77$ $p = 0.449$
Distance from Observer	$\chi^2 = 0.71$ $p = 0.401$	$\chi^2 = 2.32$ $p = 0.128$	**$\chi^2 = 19.01$** **$p = 0.004$**	$\chi^2 = 0.48$ $p = 0.489$	**$\chi^2 = 13.83$** **$p < 0.001$**	$\chi^2 = 0.01$ $p = 0.906$	**$\chi^2 = 14.21$** **$p = 0.027$**
Activity	$\chi^2 = 0.74$ $p = 0.389$	$\chi^2 = 0.01$ $p = 0.921$	$\chi^2 = 7.03$ $p = 0.318$	$\chi^2 = 0.76$ $p = 0.384$	$\chi^2 = 0.57$ $p = 0.45$	$\chi^2 = 2.37$ $p = 0.123$	$\chi^2 = 3.04$ $p = 0.804$
Feeding	$\chi^2 = 2.35$ $p = 0.125$	$\chi^2 = 0.45$ $p = 0.502$	$\chi^2 = 6.8$ $p = 0.34$	$\chi^2 = 0.00$ $p = 0.954$	$\chi^2 = 1.95$ $p = 0.163$	$\chi^2 = 0.05$ $p = 0.824$	$\chi^2 = 7.09$ $p = 0.313$
	df = 1	df = 1	df = 6	df = 1	df = 1	df = 1	df = 6

Table 6.2. Evolution of mosquitofish traits. Type II Wald test results for genetic and plastic evolution of morphological and behavioral traits in *Gambusia affinis*. $N = 266$ for all morphological and 96 for all behavioral tests.

6.4.2 Behavior

Mosquitofish behavior also responded genetically and plastically to predators and local pond environments (Figure 6.2, Table 6.2). Fish from either predator-impacted background type shoaled at shallower depths. Shoaling depth and distance from observer also varied genetically across populations within backgrounds. Plastically, fish shoaled at even shallower depths after being exposed to bass cues for two generations. Fish from predator-naïve backgrounds shoaled farther from the observer if they had been exposed to bass cues. The shoaling distance – bass cue relationship also varied significantly across populations within backgrounds. Fish activity level and feeding rate did not vary significantly with any of our evolutionary variables (Table 6.2).

6.4.3 Evolutionary effect sizes on traits

We observed significant trait variation due to six of our seven evolution types (Table 6.3). There was significant variation in the contribution of these types of evolution to morphological and behavioral traits ($\chi^2 = 36.714$; df = 6; $p < 0.001$), with population-specific genetic evolution within backgrounds being greater than all other types (Figure 6.3). After population-specific genetic evolution, generic antipredator genetic evolution, general antipredator plasticity, and the various types of reaction norm evolution all contributed similar but variable amounts to trait variation. While the data for this model appear to be significantly skewed, inclusion of the random trait term in Equation 4 results in a roughly normal residual distribution (Figure E.2).

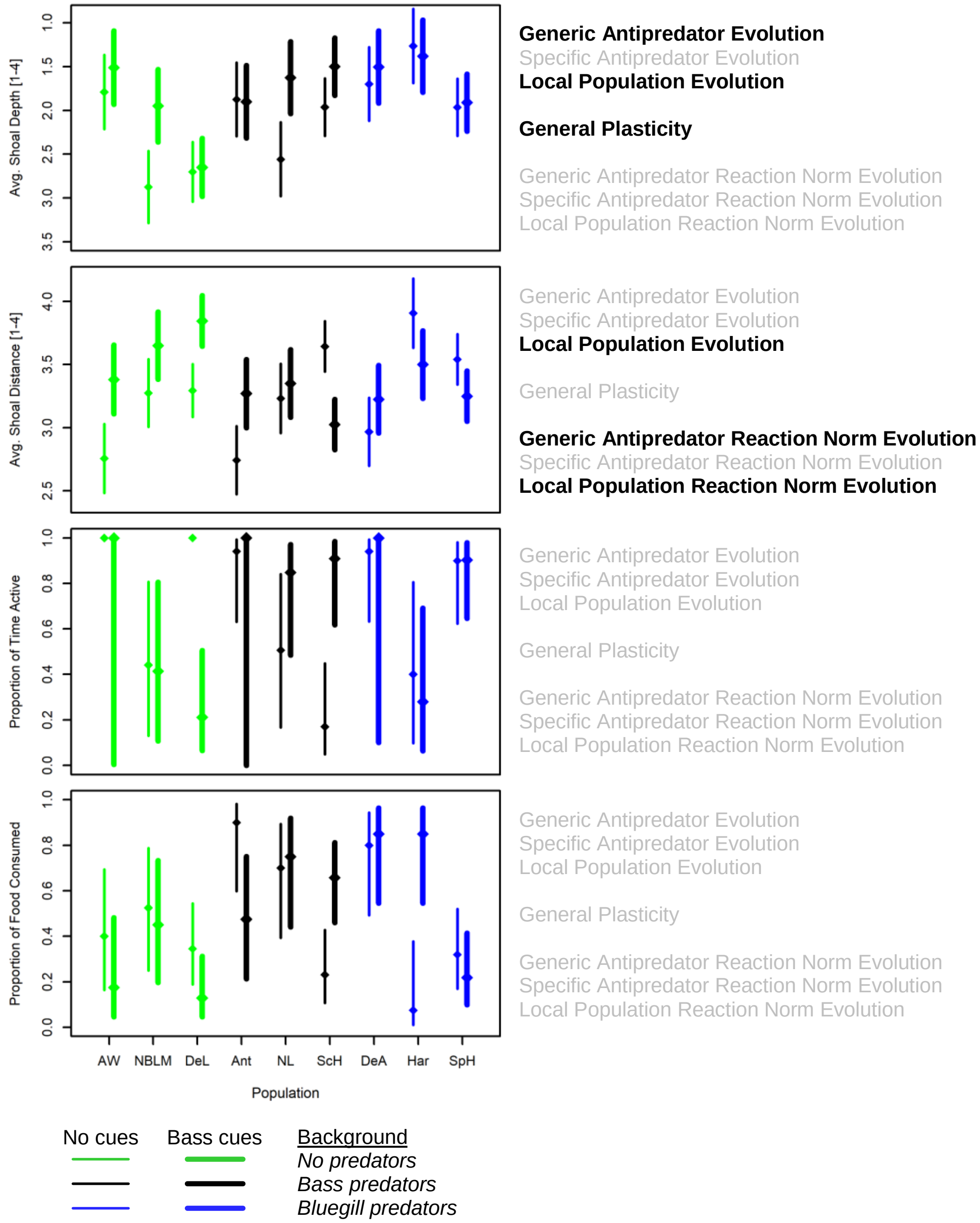


Figure 6.2. Evolution of mosquitofish behavior. Behavioral traits for *Gambusia affinis* from various predator backgrounds, with some exposed to bass cues for two generations. Bars show general[ized] linear [mixed] model predictions ± one standard error. Text next to each panel indicates significant effects of various types of genetic and plastic evolution on each trait.

Type of Evolution	**Results**
Generic Antipredator Evolution	Shallower heads in predator-experienced populations
	More posterior fins in predator-experienced populations
	Shoaling at shallower depths in predator-experienced populations
Specific Antipredator Evolution	None
Local Population Evolution	Changes in nearly all measured traits across populations within backgrounds
General Plasticity	Smaller, shallower tails in populations exposed to bass cues
	Shoaling at shallower depths in populations exposed to bass cues
Generic Antipredator Reaction Norm Evolution	Steeper tail size reaction norm. in predator-experienced populations
	Steeper dorsal and anal fish placement reaction norms in predator-naïve populations
	Steeper shoaling distance reaction norm in predator-naïve populations
Specific Antipredator Reaction Norm Evolution	Narrower heads in bass-experienced, but wider heads in bluegill-experienced populations exposed to bass cues
Local Population Reaction Norm Evolution	Shoaling closer to or farther from the observer when exposed to bass cues in various populations

Table 6.3. Results summary for mosquitofish morphological and behavioral evolution.

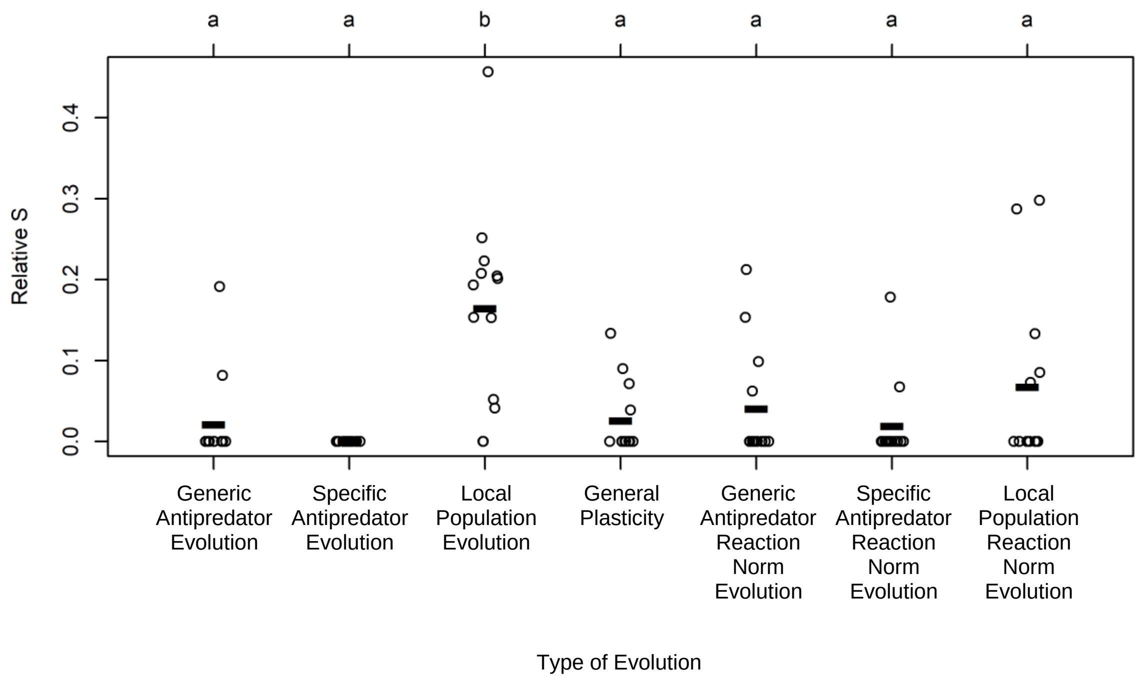


Figure 6.3. Relative contributions (see Equation 6.3) of various types of evolution to 13 morphological and behavioral mosquitofish traits. Bars show means. Letters show categorizations based on Tukey tests.

6.4.4 Ecological consequences

Mosquitofish introduction had a clear cascading effect, leading to declines in most cladocerans and increases in pelagic chlorophyll-a and mesocosm general primary production (GPP) (Figure 6.4). Doubling mosquitofish density generally strengthened these effects in cladocerans—leading to further declines—but did not cascade significantly to the producer level (Figure 6.4, Table 6.4).

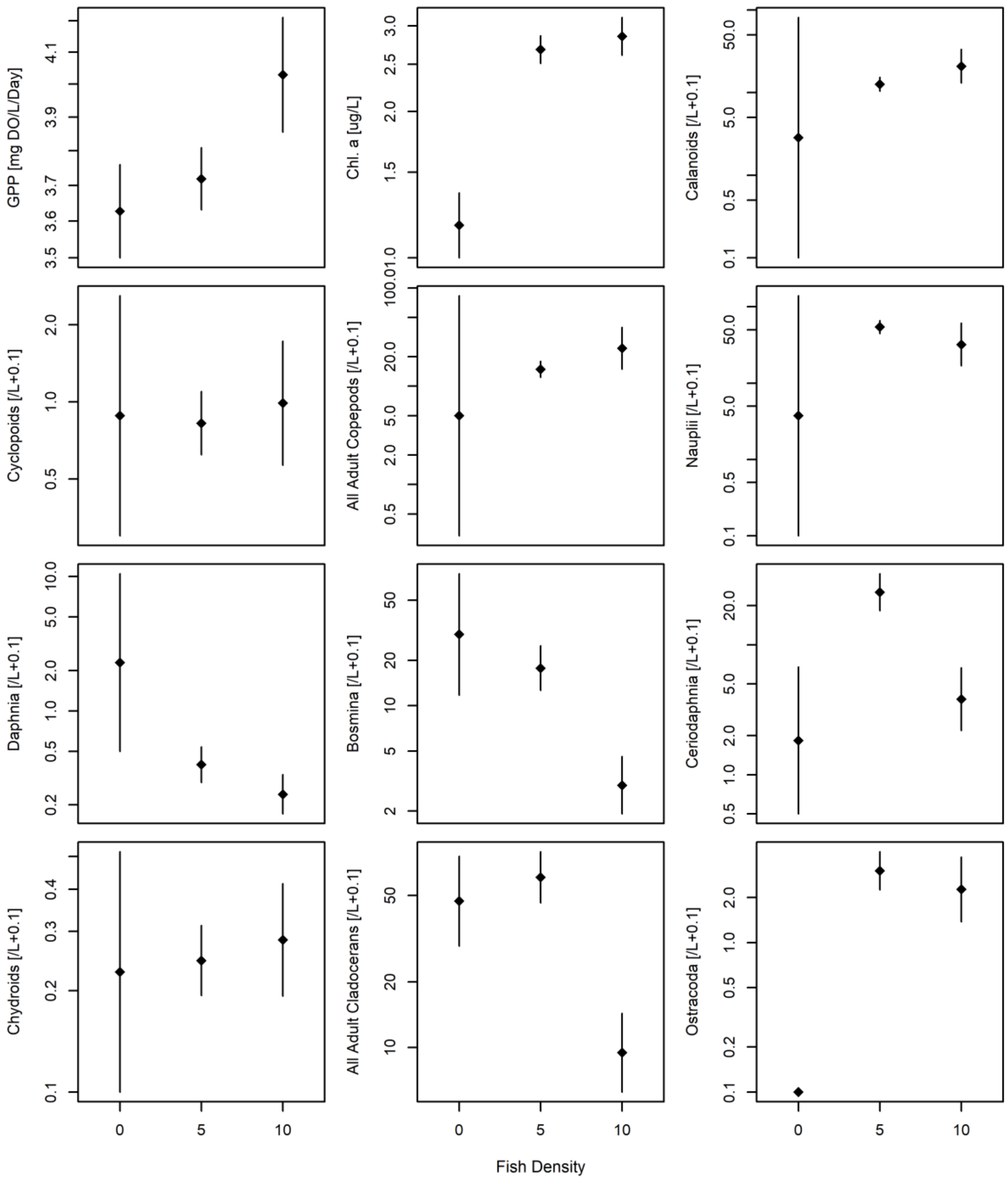


Figure 6.4. Ecological impacts of mosquitofish density. Bars show mean ± one standard error. Mosquitofish introduction led to a trophic cascade, reducing most cladocerans and increasing pelagic chlorophyll a and mesocosm primary production. N = 2, 36, and 12, from left to right.

	Trait													
Ecological Variable	**Eye Pos.**	**Eye Angle Ratio**	**Head Angle**	**Tail Area**	**Tail Depth**	**Head Area**	**Head Depth**	**Dorsal Fin Pos.**	**Anal Fin Pos.**	**Shoal Depth**	**Dist. from Obs.**	**Activ.**	**Feed. Rate**	**Dens.**	**ΔAIC from Null Model**
Calanoid Copepods	-	-	-	$\chi^2 = 11.8$ $p < 0.01$	-	-	-	$\chi^2 = 9.93$ $p < 0.01$	-	-	-	-	-	$\chi^2 = 0.24$ $p = 0.62$	-9.64
Cyclopoid Copepods	-	-	-	-	-	-	-	-	-	-	-	-	-	$\chi^2 = 0.09$ $p = 0.76$	0
All Adult Copepods	-	-	-	$\chi^2 = 15.3$ $p < 0.01$	-	-	-	$\chi^2 = 6.86$ $p < 0.01$	-	-	-	-	$\chi^2 = 4.52$ $p = 0.03$	$\chi^2 = 0.01$ $p = 0.93$	-11.5
Nauplii	-	-	-	$\chi^2 = 6.59$ $p = 0.01$	-	-	-	-	-	-	-	-	$\chi^2 = 5.56$ $p = 0.02$	$\chi^2 = 2.79$ $p = 0.10$	-5.25
Daphnia	-	-	-	-	-	-	-	-	-	-	-	-	-	$\chi^2 = 0.80$ $p = 0.37$	0
Bosmina	-	-	-	$\chi^2 = 7.24$ $p < 0.01$	-	-	-	$\chi^2 = 6.14$ $p = 0.01$	-	-	-	-	-	$\chi^2 = 12.3$ $p < 0.01$	-4.83
Cerio-daphnia	-	-	-	-	-	-	-	-	-	-	-	-	-	$\chi^2 = 8.46$ $p < 0.01$	0
All Adult Cladocera	-	-	-	$\chi^2 = 6.44$ $p = 0.01$	-	-	-	$\chi^2 = 6.31$ $p = 0.01$	-	-	-	-	-	$\chi^2 = 18.2$ $p < 0.01$	-4.42
Ostracoda	-	-	-	-	-	-	-	-	-	-	-	-	-	$\chi^2 = 0.24$ $p = 0.62$	0
Chl. A	-	-	-	-	-	-	-	-	-	-	-	-	-	$\chi^2 = 0.25$ $p = 0.62$	0
GPP	-	-	-	$\chi^2 = 4.71$ $p = 0.03$	-	-	-	-	-	-	$\chi^2 = 4.80$ $p = 0.03$	$\chi^2 = 6.22$ $p = 0.01$	-	$\chi^2 = 1.48$ $p = 0.22$	-5.86

Table 6.4. Mosquitofish trait effects on zooplankton and producers. Type II likelihood ratio tests for effects of mosquitofish traits and density on mesocosm ecology. Dashes indicate removed trait terms, which had $p > 0.05$. Density terms were included in all

models regardless of significance. ΔAIC indicates AIC improvement compared to a null model, which included only a mosquitofish density term. df = 1; *N* = 48 for all tests.

Mosquitofish traits had numerous significant effects on zooplankton. Increasing mosquitofish tail area, dorsal fin posteriority, and mosquitofish feeding rate led to declines in most copepods and cladocerans (Figure 6.5, Table 6.4). This effect was most noticeable in the most abundant taxa, i.e. calanoid copepods and *Bosmina*.

The effect of mosquitofish tail area cascaded to the producer level; increases in tail area led to increased mesocosm gross primary production (Figure 6.6, Table 6.4). Increasing mosquitofish shoaling distance from a human observer and activity level also led to increased gross primary production, though these traits had no effect on zooplankton (Figure 6.6, Table 6.4).

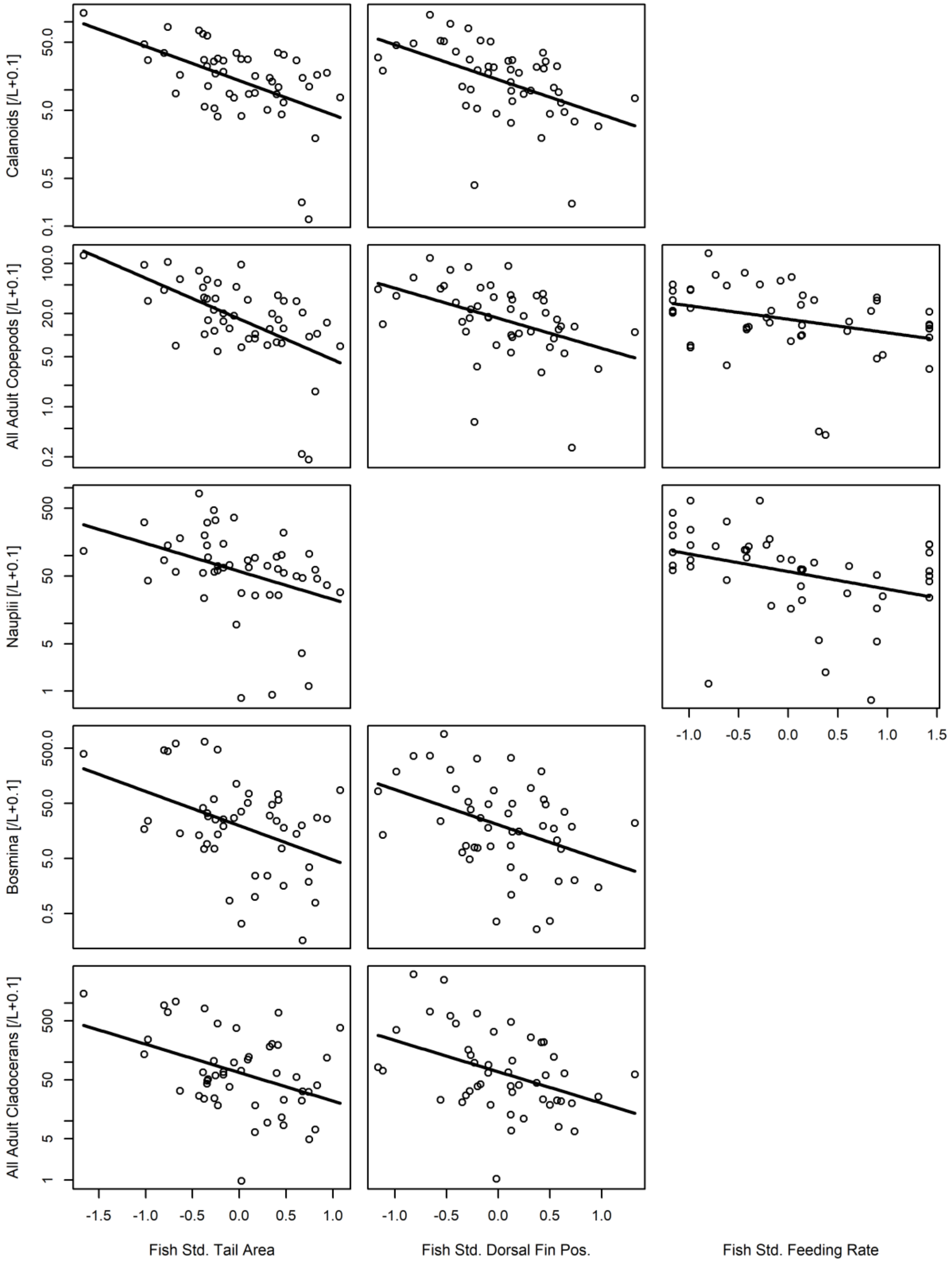


Figure 6.5. Effects of mosquitofish trait variation on zooplankton. Zooplankton densities are controlled for mosquitofish densities and other fish traits. Lines show predictions from general

liner model. Each X-value represents the average trait value for all fish within a single mesocosm, and each Y value represents zooplankton densities 27 days after fish introduction.

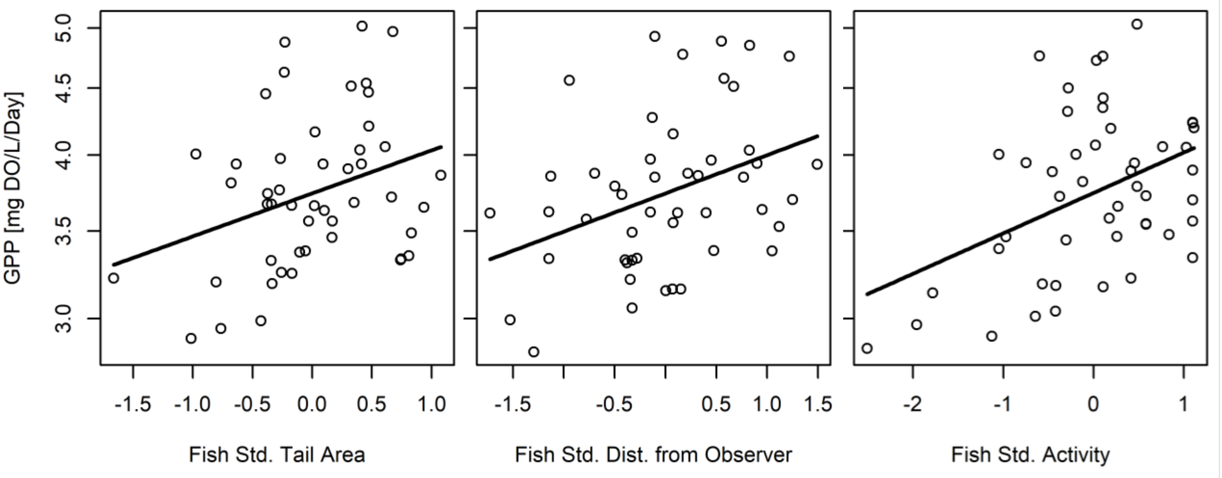


Figure 6.6. Effects of mosquitofish trait variation on GPP. Gross Primary Production increased with mosquitofish tail area, shoaling distance from an observer, and activity level. Each X-value represents the average trait value for all fish within a single mesocosm, and each Y value represents mesocosm GPP 27 days after fish introduction. GPP values are controlled for fish density and other fish traits. Lines show predictions from general liner model.

6.4.5 Ecological effect sizes

Significant ecological effects of mosquitofish trait variation were more extensive than those of mosquitofish density doubling (Table 6.4). Mosquitofish trait variation significantly affected copepods, cladocerans, and GPP, while mosquitofish density doubling significantly affected only cladocerans. A one standard deviation increase in tail area, dorsal fin posteriority, or feeding rate led to anywhere from a 0.29 to 0.58 standard deviation decrease in copepod density (Table 6.5). Tail area had the strongest trait-mediated effects on zooplankton; an increase of one standard deviation in tail area led to a 0.58 standard deviation decrease in adult copepod density (Table

6.5). In cladocerans, which were affected by both mosquitofish traits and density, doubling mosquitofish density led to a roughly one standard deviation decrease in cladoceran density, which was roughly 3-4 times stronger than the effects of a standard deviation of mosquitofish trait change (Table 6.5). Mosquitofish trait change and density change had roughly equal effects on GPP, though the effect of fish density change was not statistically significant (Tables 6.4, 6.5).

Ecological Variable	**SD Ecological Change Generated by 1 SD Fish Trait Change**					**SD Ecological Change Generated by Doubling Fish Density**
	Tail Area	Dorsal Fin Position	Distance from Observer	Activity	Feeding Rate	
Calanoid Copepods	-0.51	-0.50				0.15 ^{ns}
Cyclopoid Copepods						0.10 ^{ns}
All Adult Copepods	-0.58	-0.40			-0.29	0.02 ^{ns}
Nauplii	-0.36				-0.35	-0.53 ^{ns}
Daphnia						-0.30 ^{ns}
Bosmina	-0.40	-0.38				-1.05
Ceriodaphnia						-0.90
All Adult Cladocera	-0.36	-0.37				-1.24
Ostracoda						-0.16 ^{ns}
Chl. A						0.17 ^{ns}
GPP	0.29		0.35	0.40		0.38 ^{ns}

Table 6.5. Ecological effect sizes of mosquitofish trait and density change. Effect sizes were calculated from slope parameters of standardized general linear models. ^{ns} = not significant (Table 6.4).

6.5 DISCUSSION

We found numerous examples of contemporary divergence in western mosquitofish, including changes in head shape, tail shape, fin placement, and shoaling habits. These population differences were from a mixture of genetic and plastic antipredator adaptation and other local evolutionary mechanisms, though population-level genetic differences within backgrounds (i.e. local population divergence) were by far the greatest source of mosquitofish trait variation. Several traits—caudal peduncle size in particular—drove widespread changes in zooplankton abundance and gross primary production, demonstrating the cascading community and ecosystem effects of functional trait variation. The cascading effects of mosquitofish trait variation were weaker but reached more ecological compartments than those of density doubling, highlighting the role trait change can play in driving widespread ecological change.

6.5.1 Trait change in mosquitofish

Our results show a consistent suite of generalist antipredator traits, including shallower and smaller caudal peduncles, more posterior fins, and shoaling shallower and farther from a disturbance (Figures 6.1.a, 6.1.b, 6.2). These changes were due to a mixture of genetic evolution, plastic trait change, and genetic evolution of plastic reaction norms (Table 6.3). With the exception of one trait—head angle—genetic antipredator evolution in mosquitofish was the same in bass and bluegill backgrounds, suggesting that the mechanisms or benefits of mosquitofish antipredator adaptation are not unique to a particular piscine predator (Dunlop-Hayden and Rehage, 2011). The generalist nature of these predator-induced trait changes, as well as their consistent parallel evolution, may explain the broad ecological success of mosquitofish under numerous piscivorous predation regimes (McPeek, 1996; Pyke, 2008).

Our results suggest that increased avoidance of predators and resource conservation, rather than enhanced predator detection or escape, may be the more important pattern of adaptive antipredator evolution in California mosquitofish. We saw no predator-driven divergence in eye position, suggesting that predator detection is not strongly selected for in mosquitofish (Hassell et al., 2012). Fish exposed to predator cues—particularly those from predator-rich backgrounds—developed smaller caudal peduncles, which have been empirically shown to reduce burst-swimming ability but enhance general swimming efficiency (Langerhans, 2009). Furthermore, mosquitofish from predator-impacted backgrounds or those exposed to bass cues tended to shoal farther from human observers and closer to the surface, suggesting that predator avoidance through selection of shallow-water refugia, rather than inspection (Magurran, 1986) is the common pattern of mosquitofish antipredator behavior. Indeed, smaller caudal peduncles in mosquitofish are associated with feeding in protected fringe habitats away from open areas (Ruehl and DeWitt, 2005). Thus, predator exposure over the short- and long-term appears to favor mosquitofish that use shallow refuge habitats, and efficiently subsist by locally browsing for forage. Such a predator avoidance strategy is typically most successful when food resources for prey are high, limiting the adaptive cost of reduced prey foraging time (Anholt and Werner, 1995). Such high-resource conditions are more likely to occur in mosquitofish populations with bass and bluegill, where predation, rather than resource competition, limits the population size and resource consumption of mosquitofish (Pyke, 2008).

Our findings contrast with other common-rearing studies suggesting that mosquitofish from high-predation environments have larger caudal peduncles and enhanced burst-swimming abilities (Langerhans, 2009; Langerhans et al., 2004). This may be due to differences in experimental environments across common-rearing studies—fish in other studies were reared in aquaria, while ours were reared in mesocosms with limited live food, more closely approximating natural foraging and competitive conditions. The unrealistic context of unlimited food in more controlled laboratory conditions might permit predator-adapted mosquitofish some heightened investment in caudal morphology (Lönnstedt et al., 2012; Magnhagen and Borcherding, 2008). Alternatively, it may be that the ecological context of fish predation in California mosquitofish habitats are not directly comparable to the predation contexts in other studies that often examined different mosquitofish species in different geographic regions and used fewer source populations.

Overall, heritable evolution across populations (ponds) within predation backgrounds was by far the strongest mode of mosquitofish divergence, influencing the greatest number of traits and explaining the greatest proportion of mosquitofish trait variation (Figure 6.3, Table 6.3). Thus, despite a common focus on antipredator adaptation in poecilids as a driver of ecologically relevant trait change (Bassar et al., 2010, 2012; Palkovacs et al., 2009, 2011), factors other than predators may play stronger roles in divergence, including competitors (Schmitt and Coyer, 1983; Seehausen and Schluter, 2004; Werner and Hall, 1977), parasites (Barber and Dingemanse, 2010; Huntingford, 2004), prey (Higham et al., 2007; Palkovacs and Post, 2008), and a variety of biotic and abiotic lake characteristics (Moffett et al., 2018; Seehausen et al., 2008; Tuckett et al., 2013). Genetic drift may also account for some of the divergence in mosquitofish traits (Vera et al., 2016). Thus, studies focusing on a single driver of trait change may underestimate the size of evo-to-eco interactions. Furthermore, studies that only include a small number of study populations are likely to include confounding effects of local population divergence—particularly when local population divergence is greater than divergence from the focal driver.

6.5.2 Ecological impacts of mosquitofish density and trait change

The effects of mosquitofish introduction and density change on zooplankton were mostly limited to large cladocerans (Figure 6.4). Mosquitofish introduction decreased cladoceran density greatly, suggesting that mosquitofish predation efforts had the greatest impact on larger, slow-moving taxa (Rehage et al., 2005b). However, mosquitofish have been shown to consume most zooplankton (Bence and Murdoch, 1986; García-Berthou, 1999; Mansfield and Mcardle, 1998; Rehage et al., 2005b), including copepods. Thus, the lack of copepod response to mosquitofish introduction and density change may be due to an offsetting mixture of mosquitofish consuming copepods and simultaneously releasing them from competition, rather than a lack of mosquitofish predation (Chase et al., 2002). Furthermore, the direct effects of mosquitofish traits—including feeding rate—on copepods (see below) suggest that mosquitofish consumed at least some copepods.

The observed reduction in cladocerans upon mosquitofish introduction clearly drove a trophic cascade (Ripple et al., 2016), leading to a higher pelagic chlorophyll-a concentration in mesocosms with mosquitofish compared to those without (Figure 6). Doubling mosquitofish density decreased cladoceran density further, but did not, however, result in further increases in chlorophyll-a standing stocks, though there was a nonsignificant increase in GPP related to mosquitofish doubling (Figure 6.4). The lack of chlorophyll-a increase in the doubled mosquitofish density treatments could be due to phytoplankton nutrient limitation (Tilman et al., 1982) or a compensatory response in other zooplankton or phytoplankton taxa (Fahimipour et al., 2017).

Mosquitofish trait divergence also influenced a broad array of zooplankton densities. Increasing mosquitofish caudal peduncle area, dorsal fin posteriority, and feeding rate all reduced copepod and cladoceran densities (Figure 6.5). The strongest and most consistent of these effects was due to tail size (Table 6.5); larger caudal regions were associated plastically and genetically with bass and bluegill absence, but also varied greatly across populations within predator regimes (Figure 6.1.b). Larger caudal regions—while less efficient—facilitate burst-swimming ability in mosquitofish (Langerhans, 2009). Such burst-swimming may be advantageous for both exploitative and interference competition for limited zooplankton resources. Larger caudal peduncles are associated with profitable (but vulnerable) pelagic feeding in mosquitofish (Bence and Murdoch, 1986; Ruehl and DeWitt, 2005). Furthermore, larger tails reduce the impact of aggression-induced damage on swimming ability (Sinclair et al., 2011). Therefore, it seems likely that increased investment in caudal regions increased resource acquisition of mosquitofish under competitive conditions. Thus, larger caudal peduncles led to reductions in most zooplankton, particularly more-mobile copepods (Link, 1996). We observed a similar pattern with dorsal fin posteriority, which may provide an additional swimming boost. Increases in fish feeding rate also decreased some zooplankton densities, which is, of course, intuitive.

Changes in caudal peduncle area in mosquitofish were associated with cascading effects down to the producer level, influencing mesocosm GPP (Figure 6.6), but not chlorophyll-a (Table 6.4).

Effects of mosquitofish caudal peduncle size on GPP could be top down through changing foraging ability on zooplankton (see above), or bottom-up through increased nutrient recycling (Horppila et al., 1998; Vanni and Layne, 1997), though the opposite directions of the trait-zooplankton and trait-GPP relationships (Figures 6.5, 6.6) suggest that the prominent mechanism is a top-down, trait-driven trophic cascade (Benndorf et al., 2002; McQueen et al., 1989; Power, 1992). The effects of mosquitofish density on chlorophyll-a appeared to saturate below our minimum density of five fish (Figure 6.4), so cascading effects of mosquitofish traits on chlorophyll-a may have been overwhelmed at our stocking densities. Two other fish traits—fish activity and fish distance from observer—both did not affect zooplankton, but did positively effect GPP (Figure 6.6). Fish activity level should relate positively to nutrient excretion, suggesting that fish activity level may also affect primary production through bottom-up mechanisms (Horppila et al., 1998; Vanni and Layne, 1997). Thus, we suggest trait variation in mosquitofish likely affects ecosystem function (primary production) through both top-down and bottom-up mechanisms.

Our work shows that the ecological effects of trait changes in mosquitofish are at least as wide-reaching as those of density changes. Where we found significant ecological effects of both mosquitofish density and trait change (cladocerans), the effects of one standard deviation of trait change were roughly one-third to one-quarter as strong as the effects of mosquitofish density doubling (Table 6.5). However, whereas doubling mosquitofish density impacted only cladocerans, variation in mosquitofish traits affected copepods, cladocerans, and mesocosm primary production (Table 6.4). Thus, while increasing the density of mosquitofish exacerbates some of the ecological effects of mosquitofish introduction, altering mosquitofish traits expands the cascading effects of mosquitofish introduction to new taxa and food web pathways. Thus, trait change in organisms can reshape ecosystems in ways difficult to predict from introduction effect sizes or initial species interaction strengths.

6.5.3 Conclusions

Morphological and behavioral traits in western mosquitofish are influenced by a diverse mixture of heritable, plastic, and reaction norm changes in response to predators and other local factors. Through a mixture of genetic evolution and plasticity, mosquitofish adapted or exposed to predators took on similar conservative body forms and behaviors that likely maximized efficiency and minimized predator exposure. Idiosyncratic evolution at the population level (to factors other than predators) was by far the strongest form of evolution, influencing the greatest number of traits and the highest proportion of trait variation. Trait variation in mosquitofish—associated in part with genetic and plastic predator naivete—cascaded through ecosystems, likely via both top-down and bottom-up pathways. Ecological effects of mosquitofish trait change—though weaker than some ecological effects of mosquitofish density change—were far more extensive, reaching almost all studied taxa and compartments. Thus, the drivers and ecological impacts of evolution are pervasive but complex, and singular focus on any particular selective agent, trait, mode of trait expression (evolution or plastic), or ecological impact, is to underestimate the strength and reach of eco-evolutionary dynamics.

6.6 ACKNOWLEDGEMENTS

We thank Rachel Van Gorden, Cowan Sikora, and Ethan Cantin for help with data collection.

Funding was provided by:

US National Science Foundation (DEB 1457333 and DEB 1457112)

University of Maine Janet Waldron Fellowship Program

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APPENDIX A: SUPPLEMENT TO CHAPTER 2

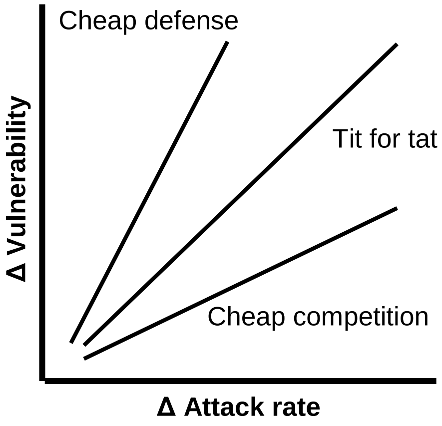


Figure A.1. Competition-defense tradeoffs. Competition – defense tradeoffs in our model were realized by modifying the degree to which attack rate and vulnerability increased with increasing genotype values. “Cheap competition” occurs when changes in genotype can increase attack rate with little increase in vulnerability, and vice-versa.

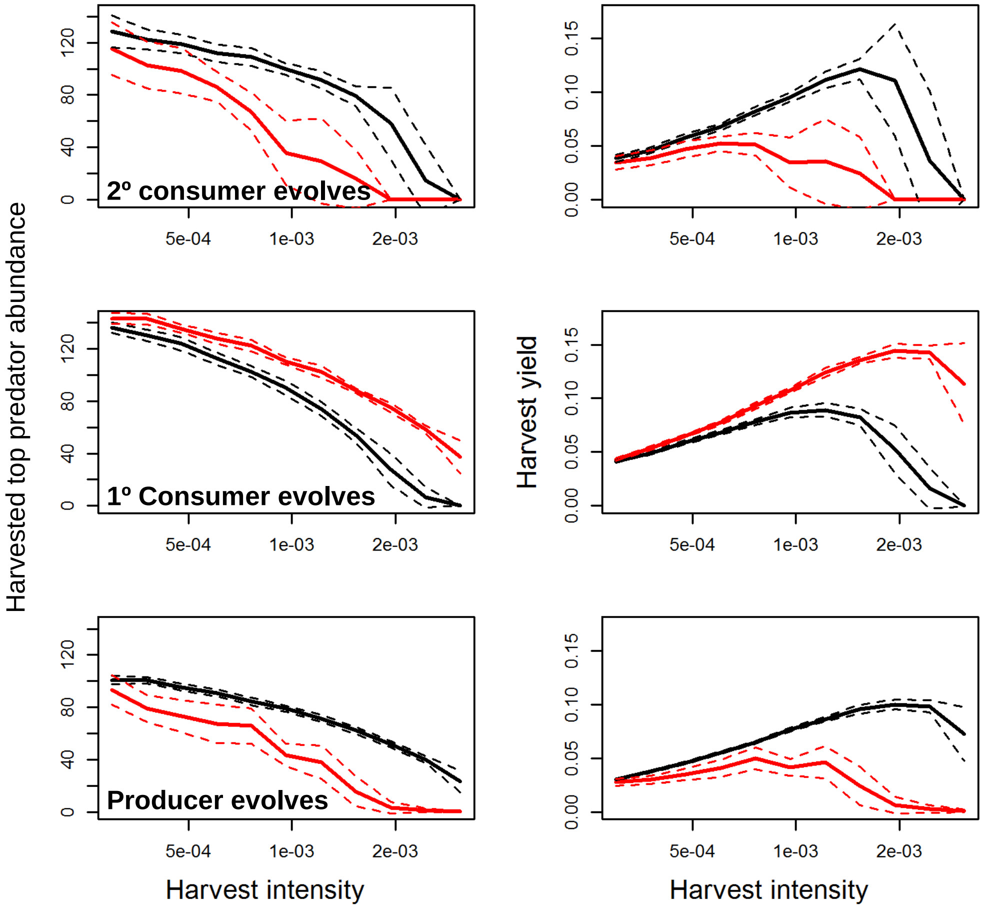


Figure A.2. Harvest yield and sustainability when lower, non-target trophic levels evolve. Evolution in trophic levels below the harvested top predator alternately dampened and exacerbated harvest effects with decreasing trophic level. **Black** lines represent outcomes with evolutionary and ecological processes included; **red** lines represent outcomes with evolution frozen and only ecological processes following the initiation of harvest. Evolution in odd-numbered trophic levels increased harvested species yield and stability, while evolution in even-numbered trophic levels decreased yield and stability. Lines represent mean ± one standard deviation for twelve runs per each point

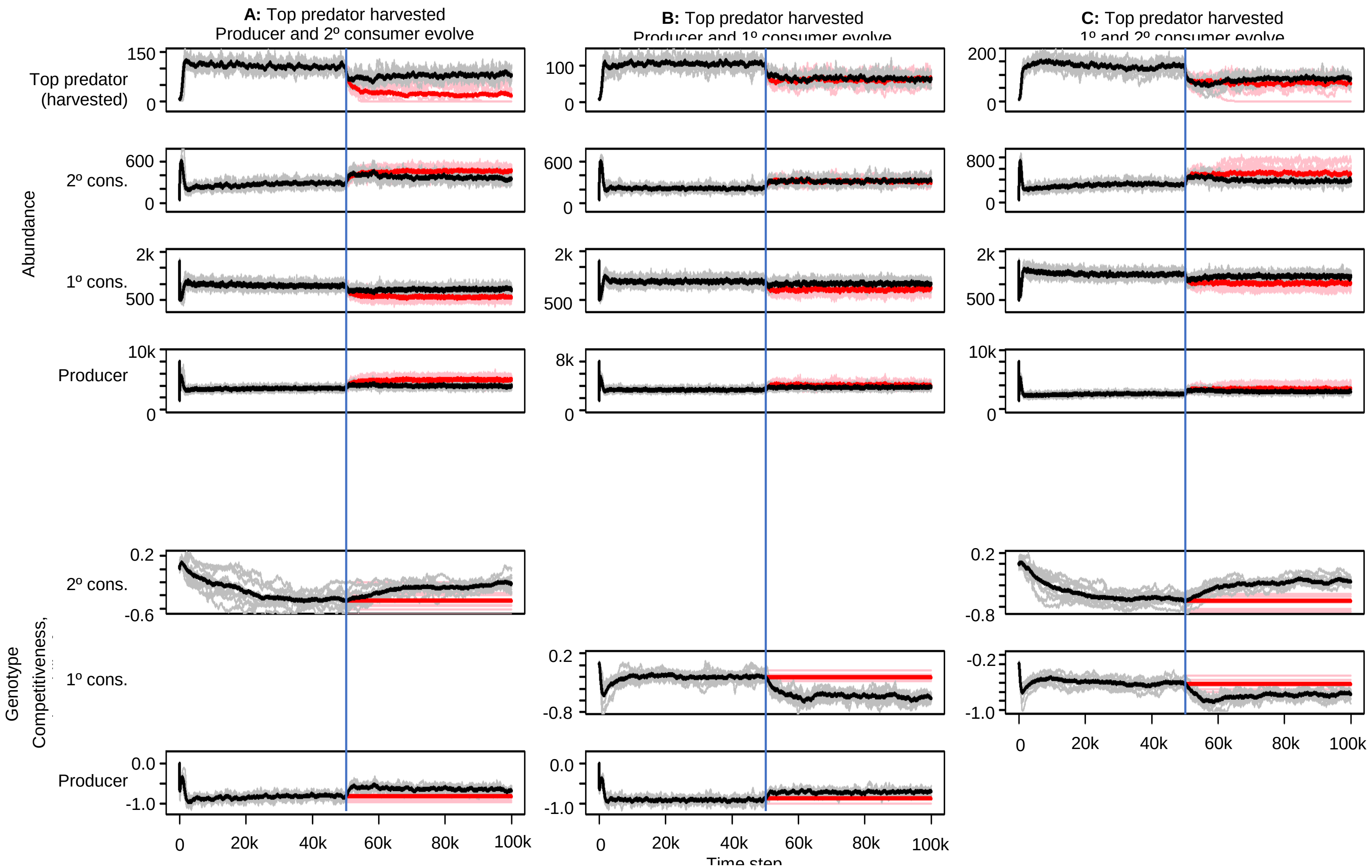


Figure A.3. Multi-trophic non-target evolution during harvest of a top predator. Once-removed trophic levels (panel A) evolved in similar directions to create a large combined bolstering effect on the harvested species. Neighboring trophic levels (panels B and C) evolved in opposite directions, with effects on the harvested species roughly cancelling. **Black** lines show the average (dark line) and 12 example runs (gray lines) when evolution was allowed to proceed. **Red** lines show the average (dark line) and 12 example runs (pink lines) when evolution was frozen at harvest onset (vertical **blue** lines).

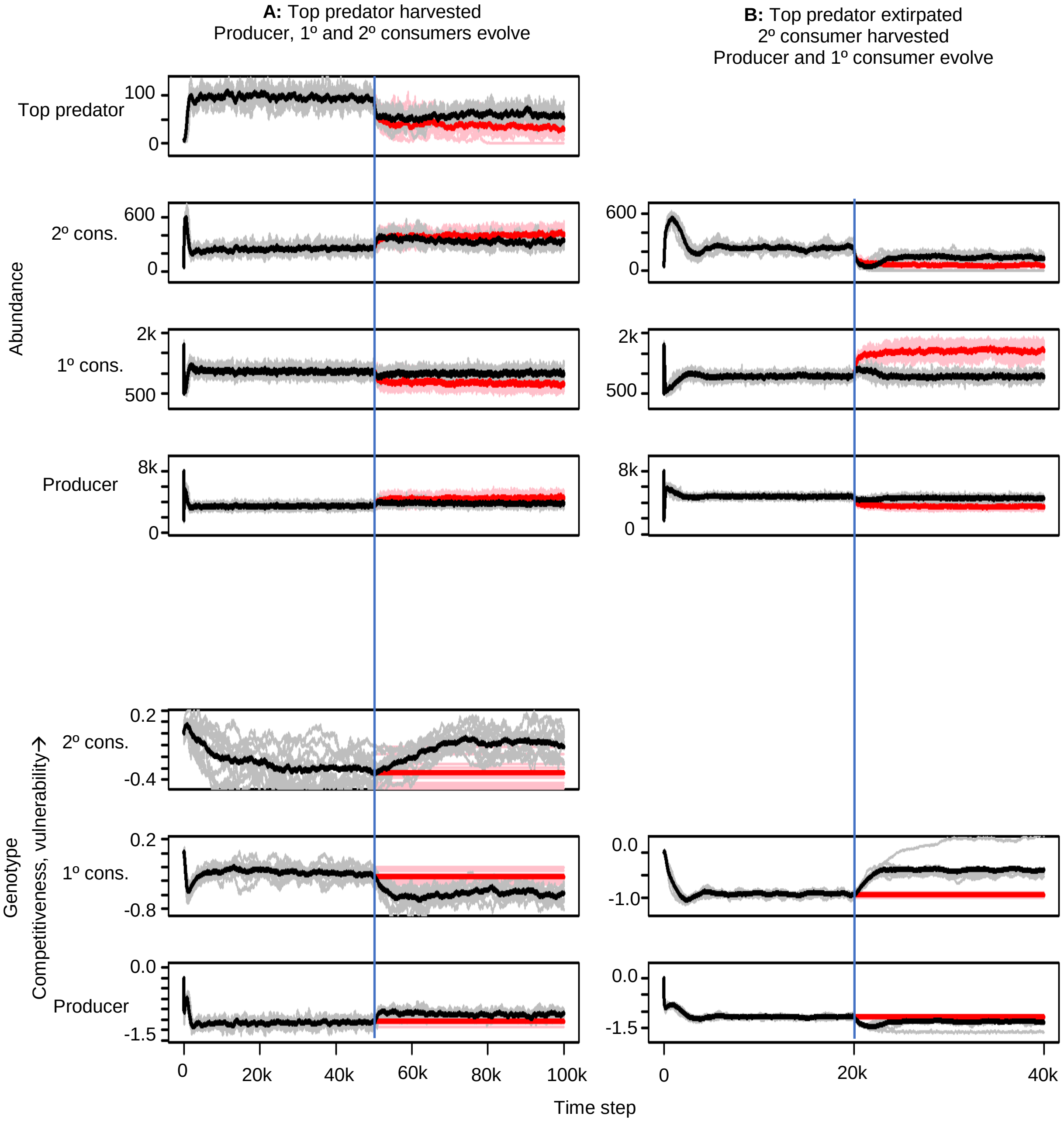


Figure A.4. Effects of fishing down the food web. Shortening the food chain from 4 (panel A) to 3 trophic levels (panel B) and harvesting secondary consumers (now the top tropic level) led to evolutionary reversal in lower trophic levels, though the net effect of evolution was to bolster the harvested species in both scenarios. **Black** lines show the average (dark line) and 12 example runs (gray lines) when evolution was allowed to proceed. **Red** lines show the average (dark line) and 12 example runs (pink lines) when evolution was frozen at harvest onset (vertical **blue** lines).

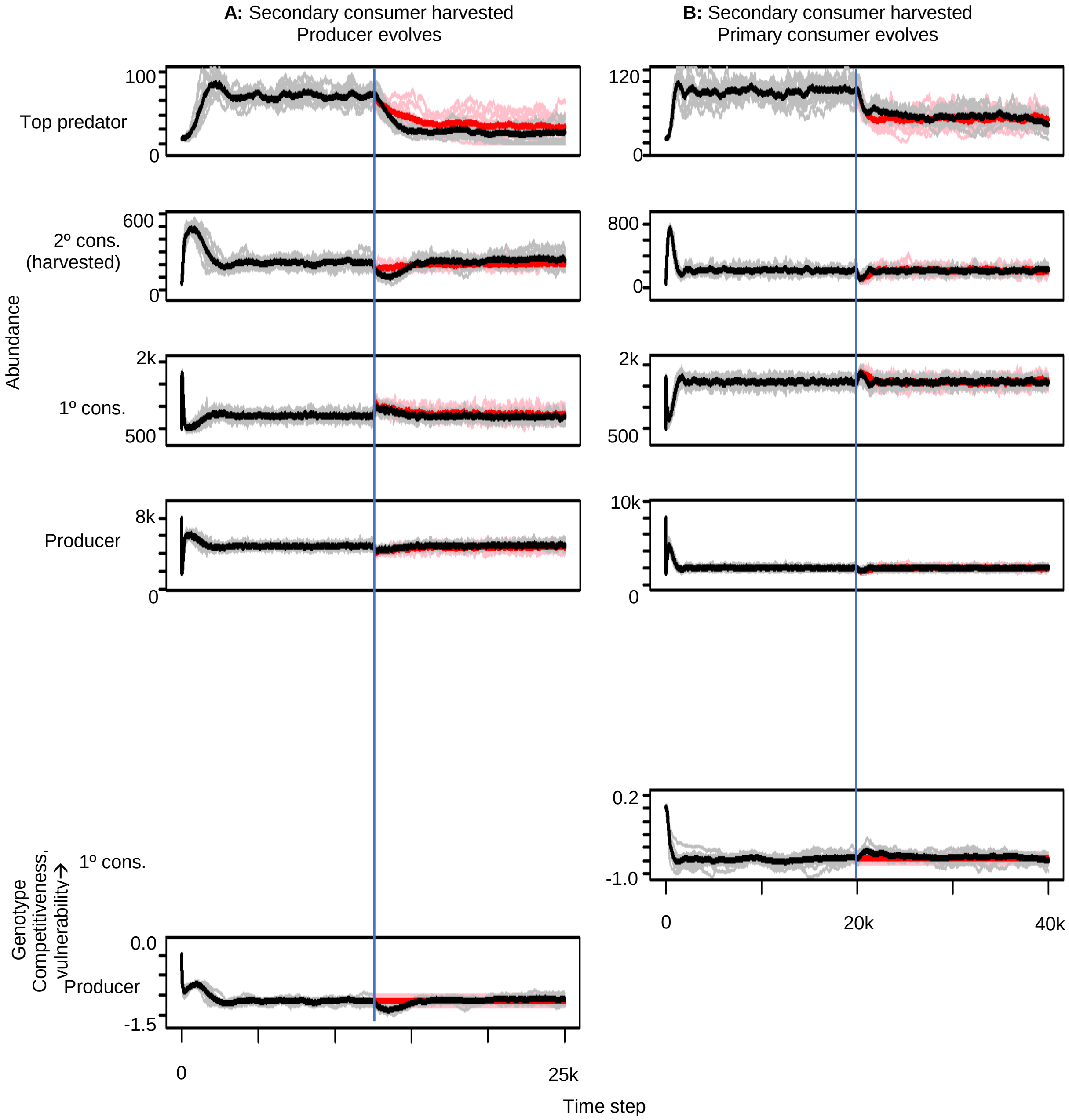


Figure A.5. Eco-evolutionary consequences of non-target species evolution during harvest of the secondary consumer (penultimate trophic level). Even-numbered trophic levels below the harvested species (panel A) evolved increased defense and weakly decreased the abundance of the top predator; odd-numbered trophic levels (panel B) evolved increased competitive ability and lead to a weak bolstering of the top predator. **Black** lines show the average (dark line) and 12 example runs (gray lines) when evolution was allowed to proceed. **Red** lines show the average (dark line) and 12 example runs (pink lines) when evolution was frozen at harvest onset (vertical **blue** lines).

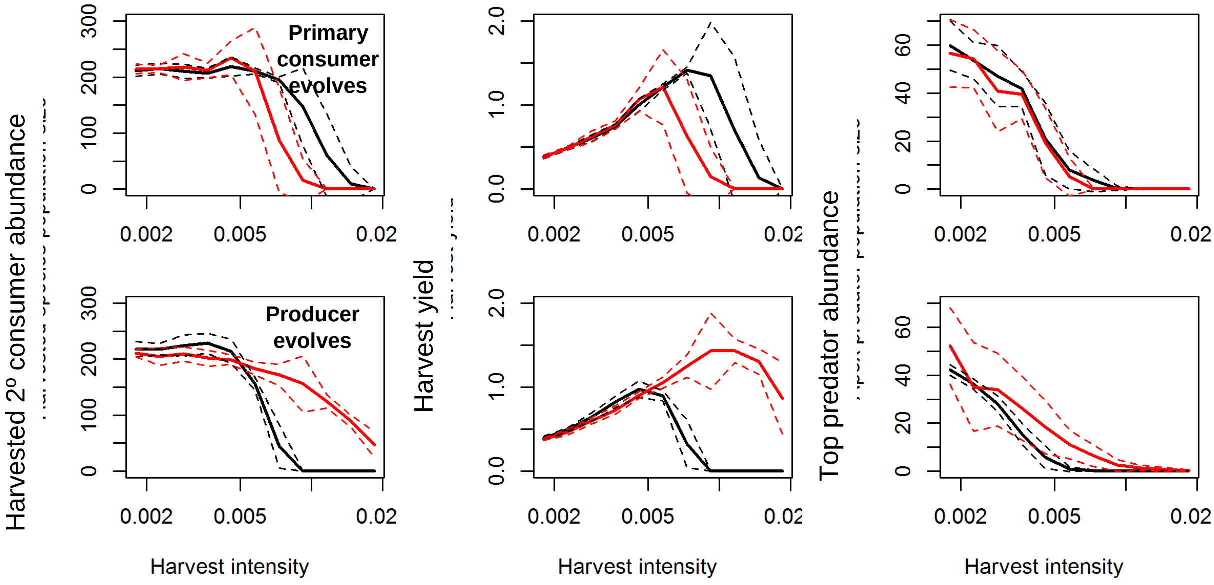


Figure A.6. Harvest yield and sustainability when lower, non-target trophic levels evolve. Secondary consumer (penultimate trophic level) harvested. Evolution in trophic levels below the harvested species alternately dampened and exacerbated harvest effects with decreasing trophic level, though these effects were largely attenuated by population size changes in the top predator. **Black** lines represent outcomes with evolutionary and ecological processes included; **red** lines represent outcomes with evolution frozen and only ecological processes following the initiation of harvest. Evolution in odd-numbered trophic levels increased harvested species yield and stability, while evolution in even-numbered trophic levels decreased yield and stability. Lines represent mean ± one standard deviation for twelve runs per each point.

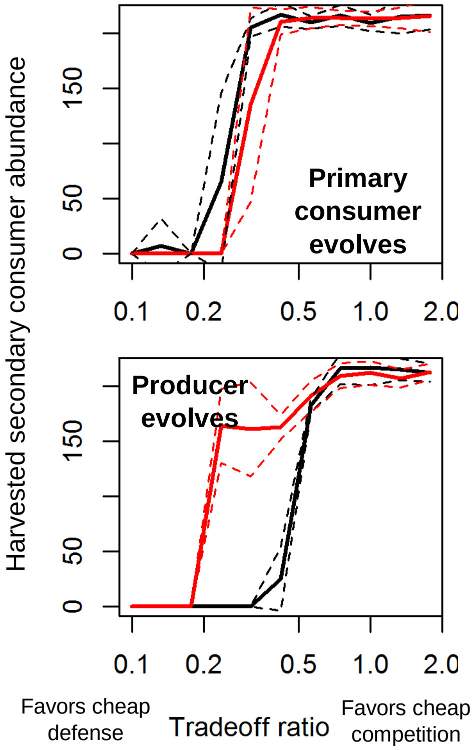


Figure A.7. Competition-defense tradeoffs and eco-evolutionary potential. Secondary consumer (penultimate trophic level) harvested. Intermediate competition-defense tradeoff ratios in non-harvested species led to divergence between eco-evolutionary (**Black**) and ecology-only (**Red**) model outcomes for harvested species abundance. Tradeoff ratios necessary to cause significant evolution in response to harvest became more biased towards inexpensive defense as trophic level increased. Tradeoffs biased strongly towards inexpensive defense led to food web collapse and extirpation of the harvested species; tradeoffs biased towards inexpensive competition caused no difference between eco-evolutionary and ecology-only models. Lines represent mean ± one standard deviation for twelve runs per each point.

APPENDIX B: SUPPLEMENT TO CHAPTER 3

Appendix B.1. Model parameters for all reported models.
(estimates ± standard errors shown)

Morphology: $\ln(Y) = a \cdot S + b \cdot \ln(L) + P$

Y is a morphometric variable, S is source-type, L is body length, P is a random effect term for population within source-type, and a and b are model coefficients.

	Males	Females
Snout – Eye	$a_C = -1.38 \pm 0.21$; $a_n = -1.49 \pm 0.20$; $a_p = -1.52 \pm 0.19$; $b = 0.82 \pm 0.06$	$a_C = -1.44 \pm 0.11$; $a_n = -1.54 \pm 0.10$; $a_p = -1.51 \pm 0.10$; $b = 0.84 \pm 0.03$
Snout – Dorsal Fin	$a_C = -0.19 \pm 0.09$; $a_n = -0.21 \pm 0.08$; $a_p = -0.20 \pm 0.08$; $b = 0.89 \pm 0.03$	$a_C = -0.57 \pm 0.05$; $a_n = -0.57 \pm 0.05$; $a_p = -0.58 \pm 0.04$; $b = 1.04 \pm 0.01$
Snout – Anal Fin	$a_C = -0.60 \pm 0.19$; $a_n = -0.57 \pm 0.18$; $a_p = -0.60 \pm 0.18$; $b = 0.92 \pm 0.06$	$a_C = -1.01 \pm 0.06$; $a_n = -1.03 \pm 0.06$; $a_p = -1.02 \pm 0.06$; $b = 1.14 \pm 0.02$
Caudle P. Depth	$a_C = -2.40 \pm 0.15$; $a_n = -2.47 \pm 0.14$; $a_p = -2.44 \pm 0.14$; $b = 1.15 \pm 0.05$	$a_C = -2.01 \pm 0.08$; $a_n = -2.06 \pm 0.08$; $a_p = -2.04 \pm 0.08$; $b = 0.99 \pm 0.02$

C = captive-propagated; n = wild low-predation; p = wild high-predation

Exploration latency: $E_{pair} = a \times S + b_1 \times S \times L_f + b_2 \times S \times L_m + P$

E_{pair} is pair mean transformed exploration latency, S is source type, L_f and L_m are female and male length, respectively, P is a random effect term for population within source-type, and a, b_1, and b_2 are model coefficients.

	Captive-propagated	Wild low-predation	Wild high-predation
a	-0.57 ± 0.28	0.06 ± 0.34	0.37 ± 0.25
b_1	0.010 ± 0.004	-0.011 ± 0.006	0.0087 ± 0.0047
b_2	0.020 ± 0.006	0.026 ± 0.011	-0.011 ± 0.009

In-mesocosm behavior: $D = a \times S + M + T$

$$A = e^{X}/(1+e^{X})$$

$$X = a \times S + M + T$$

D is distance or depth, S is source-type, M is a random effect term for mesocosm identity, T is a random effect term for time since the start of observation, A is activity, and a is a model coefficient.

	Captive-propagated	Wild low-predation	Wild high-predation
Depth	$a = 3.67 \pm 0.18$	$a = 3.48 \pm 0.17$	$a = 3.05 \pm 0.17$
Distance from observer	$a = 3.51 \pm 0.15$	$a = 3.53 \pm 0.14$	$a = 2.82 \pm 0.14$
Activity >1 (binary)	$a = 0.81 \pm 0.75$	$a = 0.55 \pm 0.71$	$a = 2.89 \pm 0.93$
Activity >2 (binary)	$a = -1.95 \pm 0.74$	$a = -3.88 \pm 0.95$	$a = -0.11 \pm 0.59$

Producers: $N = a \times S + b_1 \times B + b_2 \times R + b_3 \times F$

N is abundance, S is source-type, B is adult fish biomass, R is adult sex ratio (males/total), F is number of fry, and a, b_1, b_2, b_3 are model coefficients.

	GPP	Pelagic chl. a	Benthic chl. a[1]
a_C	0.26 ± 0.10	10.7 ± 6.3	-110 ± 332
a_n	0.26 ± 0.08	8.8 ± 4.8	65 ± 254
a_p	0.28 ± 0.08	9.1 ± 5.3	14 ± 279
b_1	-0.0067 ± 0.0685	-0.15 ± 4.38	197 ± 230
b_2	-0.0092 ± 0.0714	-4.9 ± 4.6	441 ± 241
b_3	-0.0003 ± 0.0007	0.017 ± 0.043	-1.4 ± 2.3

C = captive-propagated, n = wild low-predation, p = wild high-predation
1: µg per 28 cm^2 tile

Consumers: $N = \exp(a \times S + b_1 \times B + b_2 \times R + b_3 \times F)$

N is abundance, S is source-type, B is adult fish biomass, R is adult sex ratio (males/total), F is number of fry, and a, b_1, b_2, b_3 are model coefficients.

	Chironomids[1]	Cladocerans[2]	Adult copepods[2]	Copepod nauplii[2]
a_C	4.78 ± 0.21	-2.61 ± 0.78	2.08 ± 0.55	6.48 ± 0.10
a_n	4.98 ± 0.16	-2.61 ± 0.62	2.35 ± 0.42	6.23 ± 0.08
a_p	5.25 ± 0.18	-3.25 ± 0.71	1.67 ± 0.49	6.32 ± 0.09
b_1	-0.31 ± 0.015	3.89 ± 0.43	0.43 ± 0.36	-0.33 ± 0.07
b_2	-0.30 ± 0.15	4.80 ± 0.61	1.37 ± 0.41	0.37 ± 0.08
b_3	-0.0071 ± 0.0015	-0.041 ± 0.0059	-0.053 ± 0.006	-0.0204 ± 0.0009

C = captive-propagated, n = wild low-predation, p = wild high-predation
1: individuals per 28 cm^2 tile; 2: individuals per 4 L sample

Population source	Coordinates	Background	Exploration latency trials	Morphometrics	Mesocosm introductions, boldness & activity
1. De Laveaga Pond	N 36.998071° W 121.999344°	No bass	Summer 2016 *N* = 20 pairs	Summer 2015 *N* = 17m, 21f	
2. Dodero Pond	N 36.982388° W 122.048387°	No bass	Summer 2016 *N* = 20 pairs	Summer 2015 *N* = 22m, 25f	
3. Shorebirds Marsh	N 36.873470° W 121.821673°	No bass	Summer 2016 *N* = 20 pairs	Summer 2015 *N* = 15m, 25f	
4. Watsonville Lagoon	N 36.871364° W 121.818474°	No bass	Summer 2016 *N* = 20 pairs	Summer 2015 *N* = 15m, 16f	
5. Dow Wetland*	N 38.018818° W 121.836500°	No bass		Spring 2016 (post-mesos) *N* = 27m, 42f	Spring 2016 *N* = 12 groups
6. Antonelli Pond	N 36.956292° W 122.060251°	Bass	Summer 2016 *N* = 15 pairs	Summer 2015; Spring 2016 (post-mesos) *N* =18m, 25f; 35m, 33f	Spring 2016 *N* = 12 groups
7. Neary Lagoon	N 36.962687° W 122.029602°	Bass	Summer 2016 *N* = 13 pairs	Summer 2015 *N* = 8m, 5f	
8. Schwann Lake	N 36.965141° W 121.994765°	Bass	Summer 2016 *N* = 20 pairs	Summer 2015 *N* = 20m, 22f	
9. Sac Yolo MVC	N 38.424359° W 121.383089°	Captive Propagation	Summer 2016 *N* =20 pairs	Summer 2015 *N* = 25m, 25f	
10. Contra Costa MVC*	N 38.009202° W 122.037591°	Captive Propagation	Summer 2016 *N* = 20 pairs	Spring 2016 (post-mesos) *N* = 55m, 14f	Spring 2016 *N* = 12 groups
11. Contra Costa MVC albino	N 38.009202° W 122.037591°	Captive Propagation	Summer 2016 *N* = 17 pairs		

Table B.1. Mosquitofish collection and sample size information. Western mosquitofish (*Gambusia affinis*) sources, collection dates, and sample sizes for exploration, morphology, and mesocosm experiments. Exploration latency trials, morphometric analyses, and mesocosm introduction experiments were conducted on separate groups of individuals, except where noted. Numbers correspond to map (Figure B.1)

*The Contra Costa Mosquito Vector Control population was at least partially established from Dow Wetland individuals at least a decade prior to fish collection.

Morphometric	Sex	Source-type	
		χ^2_{df}	*p*
Snout – Eye	Male	16.0_2	**0.0003**
	Female	11.3_2	**0.004**
Snout – Dorsal Fin	Male	2.98_2	0.23
	Female	0.96_2	0.62
Snout – Anal Fin	Male	3.72_2	0.16
	Female	2.09_2	0.35
Caudle P. Depth	Male	11.0_2	**0.004**
	Female	4.54_2	0.10

Table B.2. Morphology likelihood ratio test results. Likelihood ratio tests for source-level differences in mosquitofish morphometrics. N = 257 males; 253 females

Component	χ^2	df	*p*
Source-type	6.24	2	**0.044**
Male length*	6.67	1	**0.010**
Female length*	2.72	1	0.10
Source-type × Male length	9.36	2	**0.009**
Source-type × Female length	9.03	2	**0.011**

Table B.3. Exploration behavior likelihood ratio test results. Likelihood ratio tests for male-female pair exploration latency in *Gambusia affinis*. *N* = 185.

*Male length and female length likelihood ratio tests were performed on models without their respective higher-order interactions.

Term (x)	Producer response (y)					
	GPP		Pelagic chl. a		Benthic chl. a	
	χ^2_{df}	p	χ^2_{df}	p	χ^2_{df}	p
Source-type	0.401_{2}	0.82	0.915_{2}	0.63	2.46_{2}	0.29
Fish mass	0.010_{1}	0.92	0.001_{1}	0.97	0.735_{1}	0.39
Sex ratio	0.016_{1}	0.90	1.17_{1}	0.27	3.36_{1}	0.067
Fry produced	0.196_{1}	0.66	0.148_{1}	0.70	0.344_{1}	0.56
Total ΔAIC	-9.45		-6.20		-4.94	

Table B.4. Primary producer likelihood ratio test results. General linear model likelihood ratio test results for producer metrics in mesocosms containing *Gambusia affinis* from domesticated captive-propagated and wild sources (no fish reference mesocosms excluded). Fish source-type had no detected effect on measured producer attributes (Figure 3.5). All responses above were measured 3 weeks after fish introduction, but are largely consistent with trends observed earlier in experimentation (Figure B.4). $N = 36$.

Term (x)	Consumer response (y)							
	Benthic chironomids		Cladocerans		Adult copepods		Copepod nauplii	
	χ^2_{df}	p	χ^2_{df}	p	χ^2_{df}	p	χ^2_{df}	p
Source-type	64.6_{2}	**< 0.0001**	14.6_{2}	**0.0007**	14.7_{2}	**0.0006**	59.6_{2}	**< 0.0001**
Fish biomass	4.46_{1}	**0.034**	88.7_{1}	**< 0.0001**	1.45_{1}	0.22	21.2_{1}	**< 0.0001**
Sex ratio	4.02_{1}	**0.045**	70.3_{1}	**< 0.0001**	11.6_{1}	**0.0007**	23.9_{1}	**< 0.0001**
Fry produced	22.0_{1}	**< 0.0001**	58.8_{1}	**< 0.0001**	106_{1}	**< 0.0001**	624_{1}	**< 0.0001**
Total ΔAIC	97.4		752		337		3090	

Table B.5. Consumer likelihood ratio test results. Likelihood ratio test results for consumer abundances in mesocosms containing *Gambusia affinis* from captive-propagated (domesticated) and wild sources (no fish reference mesocosms excluded). Tests were conducted by removing each term individually (nonsequentially) from a poisson-distributed generalized linear model.

Fish source-type had a significant impact on all measured consumer abundances (Figure 3.5). All responses above were measured 3 weeks after fish introduction, but are largely consistent with trends observed earlier in experimentation (Figure B.3). Total ΔAIC is the improvement in AIC when each full model is compared to a null model. *N* = 36.

Metric (*y*)	Model	Δ AIC	χ^2	df	*p*
Depth	Linear	2.32	6.32	2	**0.042**
Distance from observer	Linear	9.63	13.63	2	**0.0011**
Activity >1 (binary)	Logit	1.37	5.38	2	0.068
Activity >2 (binary)	Logit	9.87	13.87	2	**0.00097**

Table B.6. In-mesocosm behavior likelihood ratio test results. Generalized linear model results for positioning and activity differences across *Gambusia affinis* source-types during a mesocosm experiment. Depth and distance from observer (Figure 3.3) models were linear; activity (Figure 3.4) models were binomial with a logit link function. Likelihood ratio test statistics for the source-type term in each model (see Eqns. 3.4-3.6) are presented here. *N* = 175.

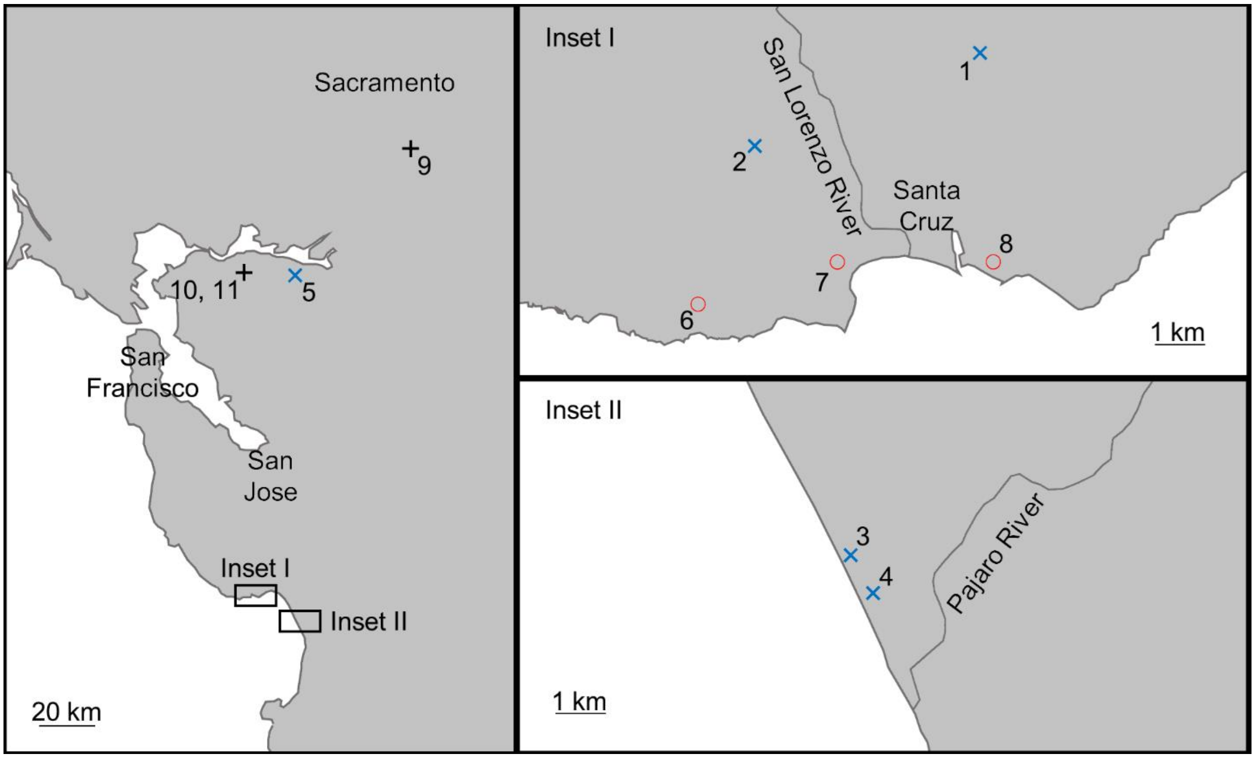


Figure B.1. Mosquitofish collection site map. Map of mosquitofish collection sites in east-central California. Numbers correspond to population information in Table B.1. Points denote populations from captive propagation (**black** +), high-predation wild (**red** o), and low-predation wild (**blue** x) environments

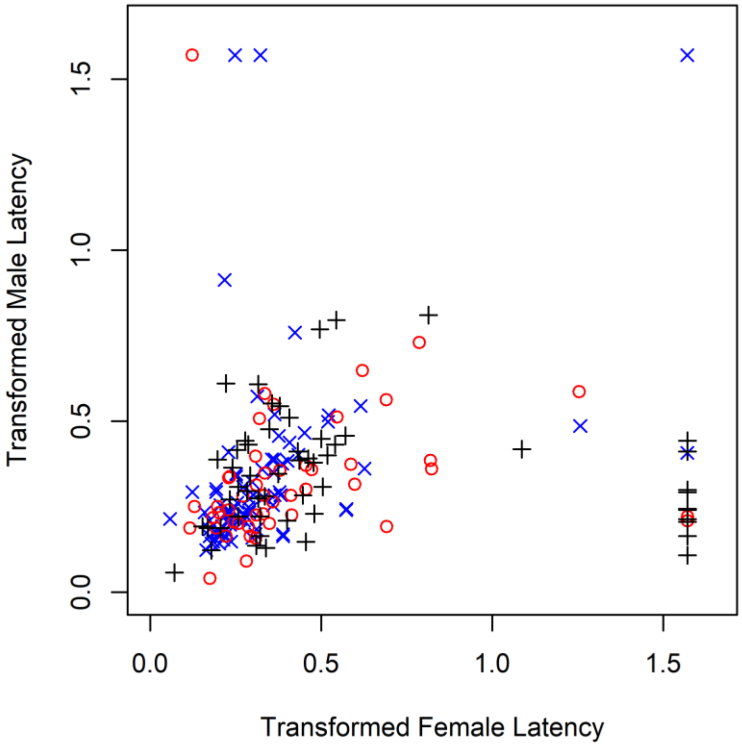


Figure B.2. Mosquitofish exploration latency correlation. Individual exploration latency times during paired trials of a single male and female were non-independent. *r* = 0.19. Points denote pairs from captive propagation (**black +**), high-predation wild (**red o**), and low-predation wild (**blue x**) environments.

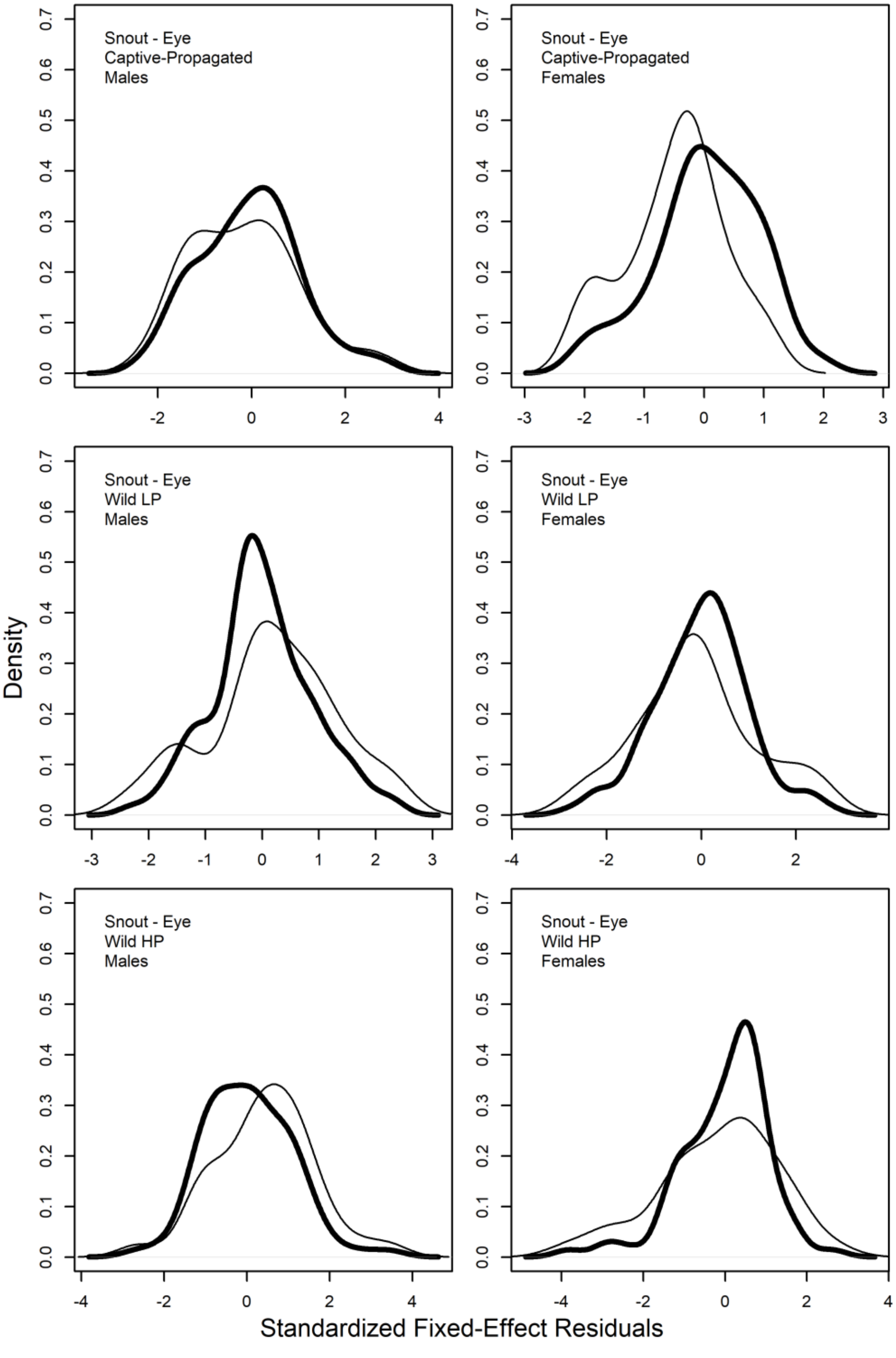


Figure B.3. Mosquitofish snout-eye morphology density plots. Density plots of snout-eye morphometric residuals for individuals used in mesocosm experiments (thin line) and all individuals studied (**thick line**).

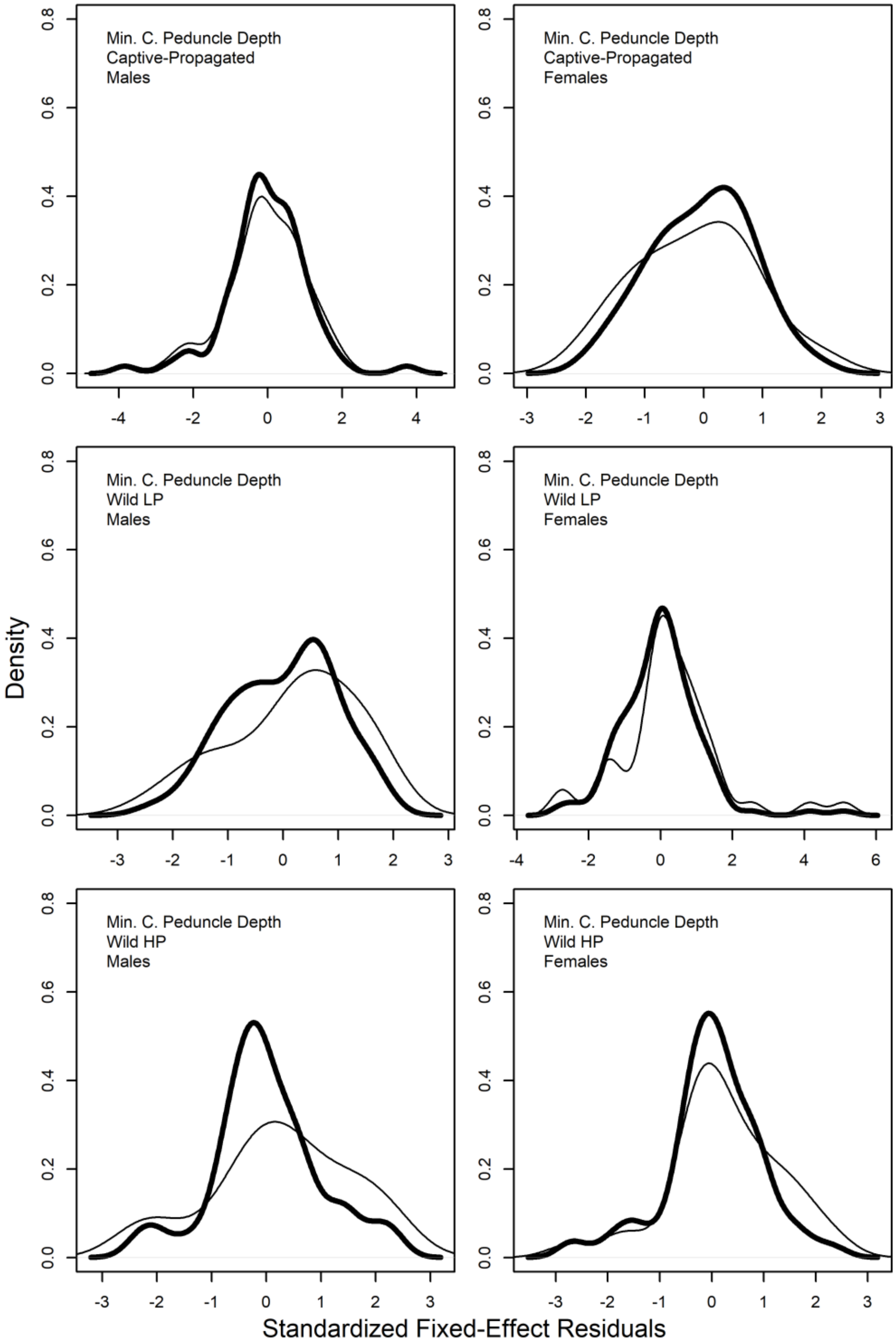


Figure B.4. Mosquitofish caudal peduncle morphology density plots. Density plots of caudle peduncle depth residuals for individuals used in mesocosm experiments (thin line) and all individuals studied (**thick line**).

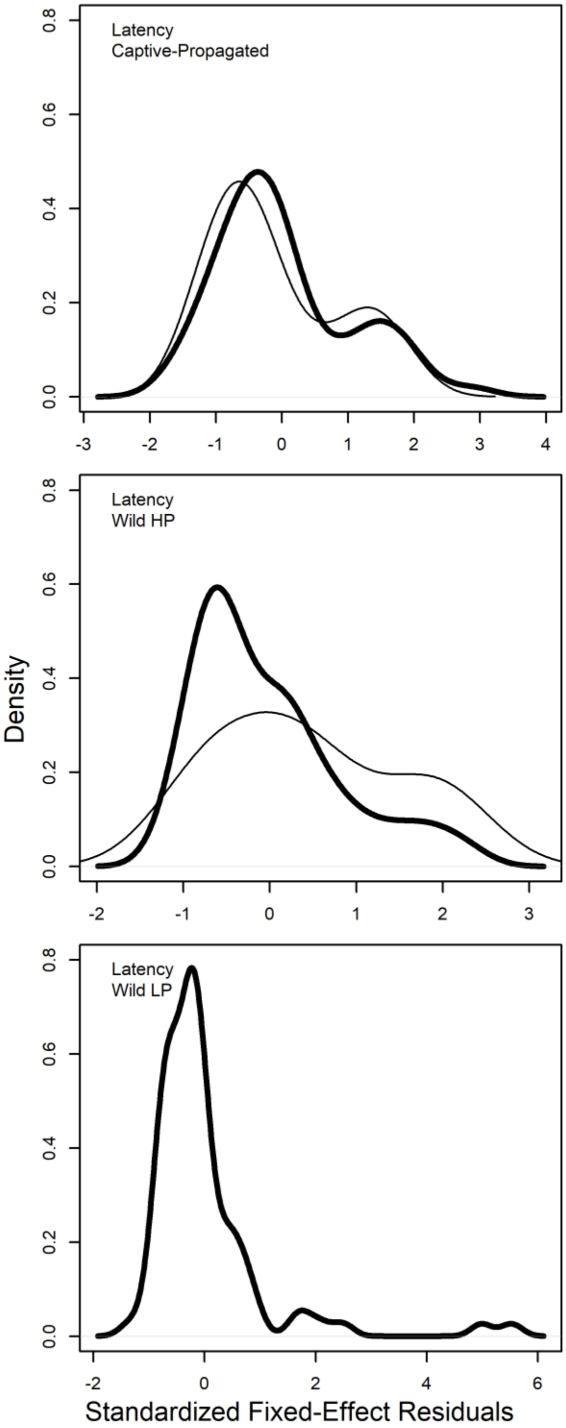


Figure B.5. Mosquitofish exploration latency density plots. Density plots of latency residuals for pairs from the same source populations as individuals in mesocosm experiments (thin line) and all individuals studied (**thick line**). For wild LP fish, no pairs were tested from the source population used for our mesocosm experiment.

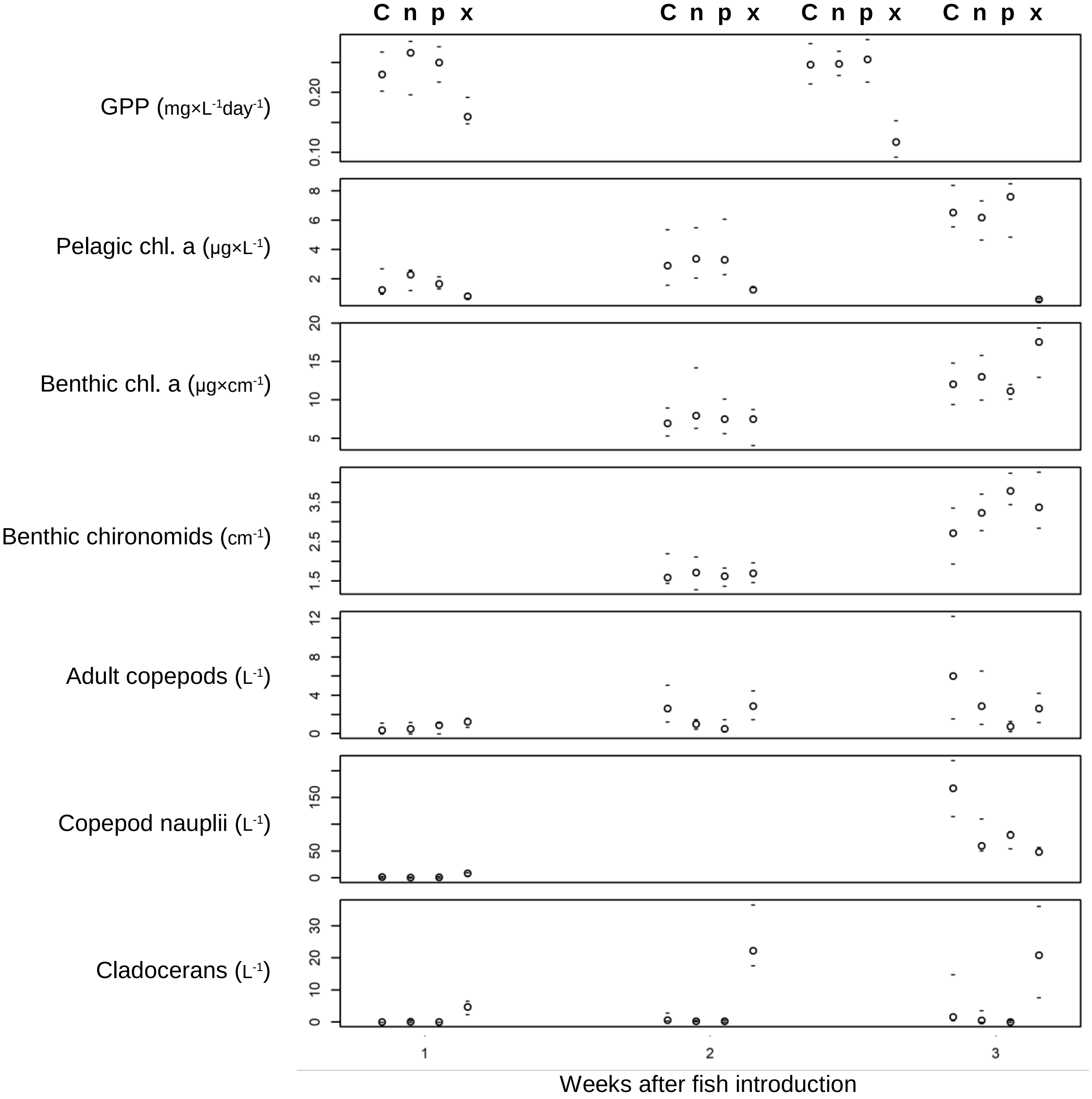


Figure B.6. Mesocosm time-series data plots. Mesocosm benthic and pelagic responses to mosquitofish introduction. Dots and dashes indicate median and first and third quartiles, respectively. C = captive propagated fish, n = wild low predation fish, p = wild high predation fish, x = no fish added. Points within the same week are spread horizontally to ease comparison.

APPENDIX C: SUPPLEMENT TO CHAPTER 4

Appendix C.1. Correcting mosquitofish densities.

We examined the accuracy of our visual counting methods at the end of experimentation (week 6). Using the same method as throughout our experiment, we visually counted all mosquitofish in all mesocosms: An observer standing adjacent to each mesocosm counted all mosquitofish seen during a 5-minute interval. A second observer repeated this process for an additional 5-minute interval. If the mosquitofish counts differed between observation periods, a third observer did an additional 5-minute observation, which generally confirmed the higher count.

To determine the actual number of mosquitofish in each mesocosm, we exhaustively captured all fish using hand nets. In addition to the mesocosms we studied for this experiment, we included mesocosms from a concurrent experiment using the exact same design, except that mosquitofish were periodically replenished—for a total sample size of 32.

Analyses

We used a general linear model in R (R Core Team, 2016) with a poisson independent variable distribution (identity link function) to predict the number of mosquitofish missed by the visual counts:

(C.1)

$$A - C = \beta_{0_S} + \beta_{1_S} \times C$$

Where A is the actual number of mosquitofish present, C is the number of visually counted mosquitofish, and β_{0_S} and β_{1_S} are mosquitofish source-specific parameters determined by the model fitting process. A - C conforms roughly to a poisson distribution, with a minimum of zero (C was never greater than A) and a mild right-skew.

We transformed the model after fitting to generate the predicted number of fish in each mesocosm (F) based on visual counts:

(C.2)

$$F = \beta_{0_S} + (1 + \beta_{1_S}) \times C$$

We used this equation to adjust visual fish counts from earlier time points in the experiment.

Results

We found source-specific differences in β_0 and β_1 (Table S-2; Type II likelihood ratio tests (Fox and Weisberg, 2011): $\chi^2 = 21.13$, df = 1, $p < 0.001$; and $\chi^2 = 2.60$, df = 1, $p = 0.11$; respectively), resulting in higher rates of visually missed mosquitofish from high-predation sources (Figure C-1).

Population	**Location (CA County)**	**Predation Regime**	**Density (fish/m^2)**	**Female Length (mm)**	**Male Length (mm)**
Antonelli Pond	Santa Cruz	Bass	5.75	23.79	19.06
Furnace Creek Pond*	Inyo	Bass	18.89	25.08	19.33
Little Artesian Well	Inyo	Bass	7.60	25.81	18.13
Neary Lagoon	Santa Cruz	Bass	<0.01	23.52	17.91
Schwann Lake	Santa Cruz	Bass	4.31	27.36	19.35
		With Bass Mean (SE)	**7.31 (3.15)**	**25.11 (0.70)**	**18.76 (0.31)**
Artesian Well	Inyo	No Bass	6.37	29.77	22.61
Camp Kennolyn Pond	Santa Cruz	No Bass	11.91	30.02	21.14
Corcoran Lagoon	Santa Cruz	No Bass	39.77	25.41	19.36
De Laveaga Pond	Santa Cruz	No Bass	6.02	32.56	18.30
Dodero Spring	Santa Cruz	No Bass	31.90	30.51	21.86
BLM Spring	Inyo	No Bass	10.40	26.73	22.81
Keough Hot Ditch 2	Inyo	No Bass	34.36	27.68	20.84
Keough Hot Ditch 5	Inyo	No Bass	29.64	28.89	20.78
Larsen Pond	Santa Cruz	No Bass	77.89	32.13	22.95
Little Hot Creek	Mono	No Bass	9.51	27.82	19.35
Northeast Spring*	Inyo	No Bass	27.38	28.94	21.65
Shorebirds Pond	Santa Cruz	No Bass	1.64	26.74	19.69
Warm Springs Upper	Inyo	No Bass	61.12	24.67	20.72
Watsonville Lagoon	Santa Cruz	No Bass	8.90	25.19	19.88
		No Bass Mean (SE)	**25.49 (6.03)**	**28.36 (0.66)**	**20.85 (0.38)**

* Source populations used in the mesocosm experiment.

Table C.1. **Mosquitofish densities.** Mosquitofish density in ponds with predatory largemouth bass and no fish predators, determined with repeated beach seine hauls in 2016.

Source	β_0	β_1
Predator-experienced (p)	1.87 ± 0.65	0.55 ± 0.22
Predator-naïve (n)	0.39 ± 0.23	0.08 ± 0.14

Table C.2. Correcting mosquitofish counts. Poisson GLM parameter estimates ± standard error for actual vs. counted mosquitofish model. See Equation C.1 for model formulation.

	Time model (first)		Fish model (second)				
N	β_0	$s(T)$	β_1	$C_{\text{naïve}}$	$C_{\text{experienced}}$	$s(M)$	$s(\varepsilon_1)$
All adult crustaceans*	3.53 ± 0.16	0.27	0.66 ± 0.43	-0.72 ± 0.39	-0.30 ± 0.26	0.00	0.83
All copepods*	3.39 ± 0.08	0.00	0.85 ± 0.34	-0.92 ± 0.31	-0.40 ± 0.20	0.00	0.66
Adult copepods*	1.68 ± 0.15	0.23	0.73 ± 0.53	-0.67 ± 0.47	-0.42 ± 0.32	0.48	0.80
Copepodites*	0.71 ± 0.14	0.25	0.35 ± 0.38	-0.32 ± 0.34	-0.21 ± 0.23	0.00	0.74
Copepod nauplii*	3.01 ± 0.10	0.00	0.44 ± 0.44	-0.55 ± 0.40	-0.16 ± 0.26	0.00	0.85
All cladocerans*	3.25 ± 0.21	0.40	0.48 ± 0.49	-0.54 ± 0.43	-0.21 ± 0.29	0.00	0.95
*Bosmina**	2.16 ± 0.31	0.64	0.43 ± 0.51	-0.50 ± 0.46	-0.18 ± 0.30	0.00	0.99
*Daphnia**	1.40 ± 0.34	0.72	0.26 ± 0.48	-0.23 ± 0.43	-0.15 ± 0.28	0.00	0.92
*Ceriodaphnia**	1.91 ± 0.33	0.68	0.38 ± 0.52	-0.50 ± 0.46	-0.12 ± 0.31	0.00	1.00
Rotifers*	2.46 ± 0.39	0.77	1.88 ± 0.76	-1.97 ± 0.64	-0.94 ± 0.47	0.92	1.03
Chl. a	1.39 ± 0.29	0.62	-0.36 ± 0.34	0.35 ± 0.30	0.20 ± 0.20	0.24	0.55
GPP	1.82 ± 0.27	0.60	-0.17 ± 0.20	0.06 ± 0.17	0.16 ± 0.13	0.28	0.26

Table C.3. Model parameters for mesocosm response variables. Based on equations 2a and 2b:

$$N = \beta_0 + T + \varepsilon_0$$

$$\varepsilon_0 = \beta_1 + F^{\frac{1}{3}} \times C_S + M + \varepsilon_1$$

where N is the concentration or density of each producer assay or consumer, β_0 and β_1 are intercepts, T is a random time effect, ε_0 and ε_1 are residuals, F is number of mosquitofish (calculated as the average of fish observed at the beginning and end of each week), C_S is a source-specific coefficient, M is a random mesocosm identity effect, and $s()$ is standard deviation.

*N is $\ln(X + 1)$ transformed for all consumers

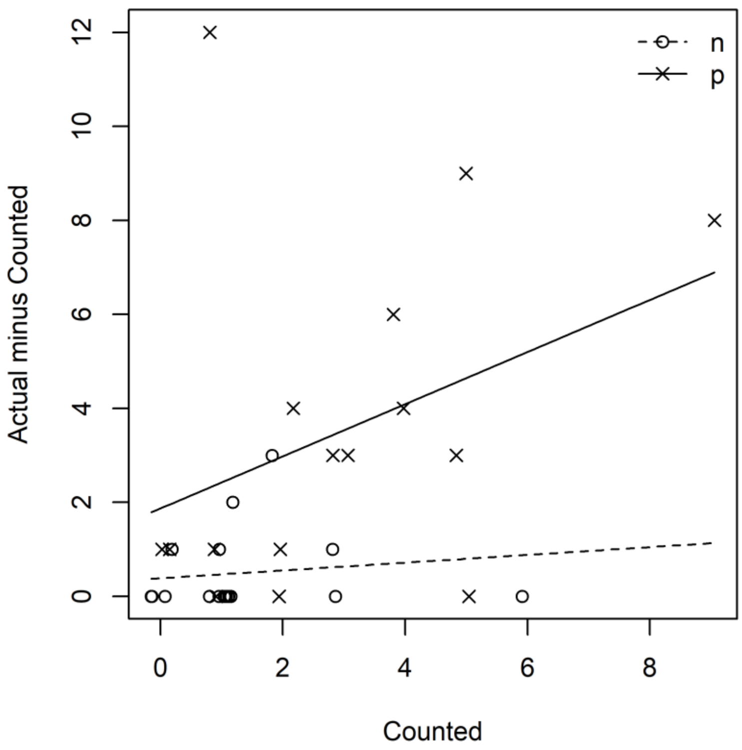


Figure C.1. Correcting mosquitofish densities. Actual minus counted (i.e. missed) mosquitofish abundances from a low- (n) and high-predation (p) source. Lines show poisson GLM mean predictions. Points are jittered slightly to aid viewing.

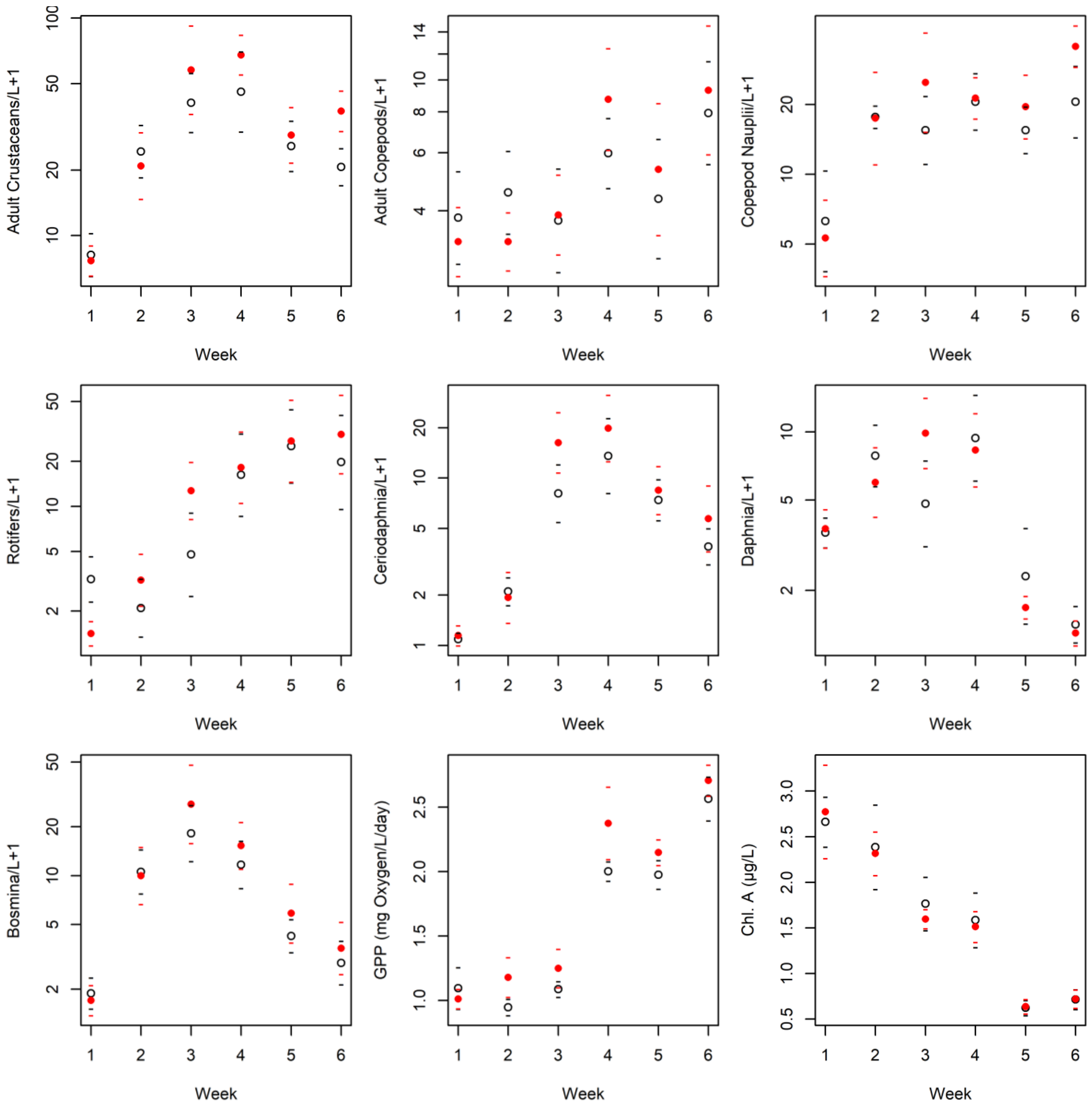


Figure C.2. **Ecological variable time-series**. Average abundances of all measured producers and consumers in predator-naïve (○) and a predator-experienced (●) mosquitofish treatments. We found no major differences in any measured producer or consumer across the two treatments. Bars indicate ± one standard error.

APPENDIX D: SUPPLEMENT TO CHAPTER 5

Population source	Code	Coordinates	Background
De Laveaga Pond	DeL	N 36.998071° W 121.999344°	No bass
Shorebirds Marsh	Sho	N 36.873470° W 121.821673°	No bass
Dow Wetland*	PA	N 38.018818° W 121.836500°	No bass
Artesian Well	AW	N 37.350584° W 118.326576°	No bass
Antonelli Pond	Ant	N 36.956292° W 122.060251°	Bass
Neary Lagoon	NL	N 36.962687° W 122.029602°	Bass
DeAnza Pond	DeA	N 36.951278° W 122.061323°	Bluegill
Spring Hills Pond	SpH	N 36.980472° W 121.756520°	Bluegill
Sac Yolo MVC	SY	N 38.424359° W 121.383089°	Captive Propagation
Contra Costa MVC*	CC	N 38.009202° W 122.037591°	Captive Propagation

Table D.1. Western mosquitofish sources. MVC = mosquito vector control.

*The Contra Costa MVC population was at least partially established from Dow Wetland individuals at least a decade prior to fish collection.

Tradeoff	**Model predicting AGR** ***Fit model***	**Competitiveness metric** ***Calculations based on highlighted parameters left, not fit model***	**Model predicting survival** ***Fit model***
Individual—universal	$G = \ln(M) + S + R$	$C = \ln(M) + S$	$U, L = C + R$
Individual within population	$G = \ln(M) + S + P + R$	$C = \ln(M) + S$	$U, L = C + P + R$
Individual within background	$G = \ln(M) + S + B + R$	$C = \ln(M) + S$	$U, L = C + B + R$
Population	$G = \ln(M) + S + P + R$	$C = P$	$U, L = C + \ln(M) + S + R$
Background	$G = \ln(M) + S + B + R$	$C = B$	$U, L = C + \ln(M) + S + R$

G = absolute growth rate (AGR); *M* = initial mass; *S* = sex; *P* = population; *B* = background; *R* = mesocosm identity random effect; *C* = estimated competitiveness; *U* = days survived; *L* = conspecifics present at death

Table D.2. Models and formulas used to calculate competitiveness and predict survival. First, we fit a model that predicted absolute growth rate (left column). Second, we used the calculated parameters from that model (highlighted terms) to generate an estimate of competitiveness (*C*) (i.e. through arithmetic, not model fitting). Third, we fit a model predicting defendedness using estimated competitiveness and controlled-for parameters.

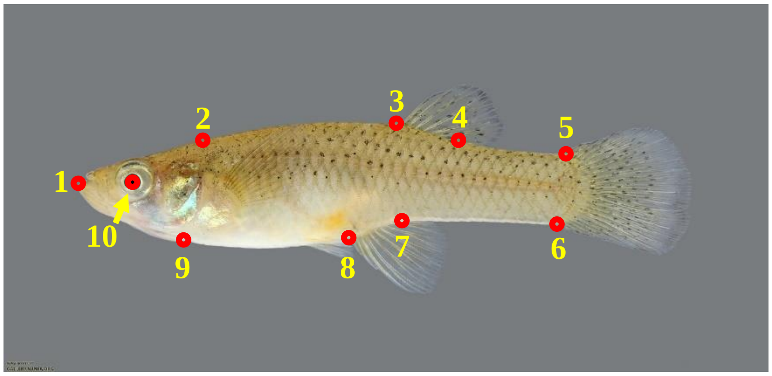
APPENDIX E: SUPPLEMENT TO CHAPTER 6

Population source	Code	Coordinates	Background
De Laveaga Pond	DeL	N 36.998071° W 121.999344°	No bass
Artesian Well	AW	N 37.350584° W 118.326576°	No bass
Northeast Spring, Fish Slough	NBLM	N 37.518003° W 118.400157°	No bass
Antonelli Pond	Ant	N 36.956292° W 122.060251°	Bass
Schwann Lagoon	Sch	N 36.962655° W 121.996843°	Bass
Neary Lagoon	NL	N 36.962687° W 122.029602°	Bass
DeAnza Pond	DeA	N 36.951278° W 122.061323°	Bluegill
Harkin Slough	Har	N 36.911600° W 121.803942°	Bluegill
Spring Hills Pond	SpH	N 36.980472° W 121.756520°	Bluegill

Table E.1. Mosquitofish source ponds in California.

Trait	Measurement	Description
Eye Angle Ratio	Angle 2—1—10 / angle 2—1—9	Ratio of the eye angle (from the top of the head to the snout to the eye) to the head angle (from the top of the head to the snout to the bottom of the head)
Head Angle	Angle 2—1—9	Angle from the top of the head to the snout to the bottom of the head
Tail Area	Quad. 3—5—6—7	Area of the caudal peduncle posterior to the dorsal and anal fins, excluding the caudal fin
Tail Depth	Segment 5—6	Depth of the tail where the caudal fin meets the caudal peduncle
Head Area	Triangle 1—2—9	Area of the head
Head Depth	Segment 2—9	Maximum depth of the head
Dorsal Fin Position	Segment 1—midpoint(3,4) / (segment 1—midpoint(3,4)+segment midpoint(3,4)—5)	Relative lateral placement of the dorsal fin along the dorsal side
Anal Fin Position	Segment 1—midpoint(7,8) / (segment 1—midpoint(7,8)+segment midpoint(7,8)—6)	Relative lateral placement of the anal fin along the ventral side

Table E.2. Morphological trait definitions for mosquitofish.



Point	Description
1	Snout
2	Dorsal head line
3	Anterior dorsal fin insertion
4	Posterior dorsal fin insertion
5	Dorsal caudal fin insertion
6	Ventral caudal fin insertion
7	Posterior anal fin insertion
8	Anterior anal fin insertion
9	Ventral head line
10	Eye center

Figure E.1. Landmarks used in mosquitofish morphometric analyses. Modified from (Langerhans et al., 2004).

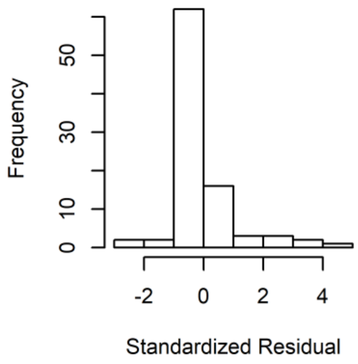


Figure E.2. Standardized residuals for type of evolution general linear mixed model

(Equation 6.4).

BIOGRAPHY OF THE AUTHOR

Zachary T. Wood was born in Schenectady New York in 1993. He took an early interest in research, studying trees in New York before graduating as his high school's Valedictorian. He studied ecology at Dartmouth College, earning a Bachelor of Arts with High Honors in 2015. He is a candidate for the Doctor of Philosophy degree in Ecology and Environmental Sciences from the University of Maine in May 2019.