



## Nutrient availability and invasive fish jointly drive community dynamics in an experimental aquatic system

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**Citation:** Preston, D. L., H. D. Hedman, and P. T. J. Johnson. 2018. Nutrient availability and invasive fish jointly drive community dynamics in an experimental aquatic system. *Ecosphere* 9(3):e02153. 10.1002/ecs2.2153

**Abstract.** Species invasions increasingly occur alongside other forms of ecosystem change, highlighting the need to understand how invasion outcomes are influenced by environmental factors. Within freshwaters, two of the most widespread drivers of change are introduced fishes and nutrient loading, yet it remains difficult to predict how interactions between these drivers affect invasion success and consequences for native communities. To test competing theories about interactions between nutrients and invasions, we conducted a  $2 \times 3$  factorial mesocosm experiment, varying western mosquitofish (*Gambusia affinis*) presence and nutrient availability within aquatic communities. Based on theory, increased nutrients could either (1) facilitate coexistence between predatory mosquitofish and native species by increasing prey availability (the invader attenuation hypothesis) or (2) strengthen predation effects by enhancing fish productivity more than native community members (the invader amplification hypothesis). In outdoor mesocosms designed to mimic observed nutrient conditions and local community structure, mosquitofish directly reduced the abundances of zooplankton and three native amphibian species, leading to indirect increases in phytoplankton, periphyton, and freshwater snail biomass through trophic cascades. Nutrient additions increased native amphibian growth but had especially pronounced effects on the productivity of invasive mosquitofish. The elevated nutrient condition supported ~5 times more juvenile mosquitofish and 30% higher biomass than the low nutrient condition. Increased nutrients levels did not weaken the top-down effects of mosquitofish on invertebrates or amphibians. Collectively, our results support the invader amplification hypothesis, suggesting that increased nutrient loading may benefit invasive species without attenuating their undesirable effects on native community members.

**Key words:** eutrophication; food web; freshwater; introduced; nonnative; pollution; pond; pond-breeding amphibian; trophic cascade.

**Received** 9 December 2017; **revised** 28 December 2017; **accepted** 10 January 2018. Corresponding Editor: Stephanie M. Carlson.

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### INTRODUCTION

Ecosystems that support invasive species increasingly experience other concurrent drivers of ecosystem change, including habitat alteration, pollution, resource extraction, and climate change (Kolar and Lodge 2000, MacDougall and Turkington 2005, Rahel and Olden 2008). The

outcome of species invasions—including invader establishment, spread, and consequences for native species—can be moderated by such co-occurring drivers of ecosystem change (Vitousek et al. 1997, Dukes and Mooney 1999, Hall et al. 2003, Didham et al. 2007, Tylianakis et al. 2008, Silva et al. 2013). If concurrent ecosystem changes lower biotic resistance or enhances the

growth and reproduction of invaders relative to natives, they can increase invasion success and subsequent spread (Davis et al. 2000, Winsome et al. 2006, Crooks et al. 2011, Penk et al. 2016). Alternatively, if other forms of ecosystem change create barriers to invader establishment or enhance the ability of native species to coexist with nonnatives, they may prevent successful invasion or reduce the magnitude of invasion consequences (Zenni and Nuñez 2013). Understanding the potentially complex mechanisms through which species invasions are influenced by other ecosystem changes is thus a priority for effective prediction and management of invasion impacts (Pyšek and Richardson 2010).

Alongside species invasions, freshwaters are often simultaneously affected by nutrient pollution (Carpenter et al. 2011, Ricciardi and MacIsaac 2011). Indeed, these two disturbances represent two of the most commonly implicated drivers of freshwater ecosystem change (Carpenter et al. 1998, Smith and Schindler 2009, Strayer 2010). For instance, aquatic systems within the Great Lakes region support ~180 invasive species and have a long history of non-point nutrient pollution from the terrestrial environment (Beeton 1965, Mills et al. 1994, Ricciardi 2001). Similarly, the Rift Valley Lakes in East Africa have been invaded by numerous nonnative species—including water hyacinth and Nile perch—while simultaneously experiencing nutrient pollution from agriculture and a growing human population (Ogutu-Ohwayo et al. 1997, Odada et al. 2003). In both examples, the co-occurrence of invasive species and nutrient pollution was associated with wholesale shifts in community structure and ecosystem processes, leading to declines in native species and economic losses totaling billions of dollars (Pitcher and Hart 1995, Pimentel et al. 2005).

Despite the common co-occurrence of nonnative species and elevated nutrient loading, predicting how these two factors interact to drive ecosystem change remains a key challenge (Flores-Moreno et al. 2016, Tabassum and Leishman 2016, Teixeira et al. 2017). The net effect of nutrient availability on species invasions will depend on characteristics of the invasive species (e.g., resource needs, resource use efficiency, trophic position) and the invaded ecosystem (e.g., relative roles of top-down versus bottom-up factors in regulating community dynamics; Kolar

and Lodge 2000, González et al. 2010). Successful invaders have been predicted to have high resource needs and to be efficient at utilizing excess resources relative to natives (Blumenthal 2006, González et al. 2010). For example, prior studies indicate that invasive primary producers are often able to more rapidly utilize excess nutrients than natives, thereby facilitating invader spread and a shift toward dominance by nonnatives (e.g., water hyacinth, Eurasian milfoil, nonnative phytoplankton; Chase and Knight 2006, Coetze et al. 2007). Nutrients can also influence invaders in higher trophic levels through bottom-up food web effects and indirect changes in community structure. Many invasive primary and secondary consumers have high nutrient demands and relatively fast life histories, in which case they may disproportionately benefit from increased nutrients relative to natives (e.g., Tibbets et al. 2010, Jackson et al. 2013). Lastly, rapid shifts in nutrient availability may alter the conditions under which native species have adapted such that invaders gain a competitive advantage in ecosystems where they would normally be outcompeted by natives (Byers 2002).

Alternatively, it is also possible that elevated nutrient availability could ameliorate the negative effects of invaders by lowering colonization success or weakening invasion impacts on any specific native community member. For instance, nutrients can facilitate the coexistence of invasive species with native community members by enhancing the overall availability of resources to the community, thereby weakening interspecific competition (Firn et al. 2010). Bottom-up fertilization effects can also increase prey availability, thereby preventing predators from extirpating native community members (Balciunas and Lawler 1995). In general, it has been posited that increased resource supply is one mechanism that can facilitate species coexistence (Fargione and Tilman 2002), and productivity is often associated positively with species richness at large spatial scales (Field et al. 2009, Chase 2010). Although such patterns are likely context-dependent (Dodon et al. 2000, Mittelbach et al. 2001), they support the potential for elevated nutrients to allow coexistence between nonnative and native species.

In the present study, our primary aim was to examine whether nutrients attenuate or amplify the effects of invasive fish within pond

communities using outdoor mesocosms. We focused on the western mosquitofish (*Gambusia affinis*), which is a widespread invasive species that has been implicated in declines of multiple native taxa (Pyke 2008, see also *Study system* below). We utilized outdoor mesocosms, which are useful for testing ecological mechanisms, as they allow replication of controlled experimental treatments, and they are particularly relevant for studies of small ponds (Semlitsch and Boone 2010, Spivak et al. 2011). We sought to experimentally assess the relative influence of two competing hypotheses. First, increases in nutrients could disproportionately increase the growth and reproduction of mosquitofish through bottom-up effects (the invader amplification hypothesis). Under this hypothesis, increased nutrient loading could amplify the negative predatory effects of mosquitofish on the native aquatic community due to increased mosquitofish biomass. In contrast, increases in nutrients could enhance primary and secondary production, thereby weakening the population-level effects of mosquitofish on any focal prey taxon through increases in overall prey availability (the invader attenuation hypothesis). In this scenario, increased nutrients could facilitate the coexistence of predatory mosquitofish with native prey, including zooplankton, invertebrates, and amphibians.

## METHODS

### *Study system*

We examined interactions between nutrient concentrations and western mosquitofish (*Gambusia affinis*) within the community context of wetlands in the San Francisco Bay Area of northern California, USA. Wetlands are the most imperiled habitat type in North America, and within California, >90% of natural wetlands have been lost to agriculture and development (Nichols et al. 1986, Dahl 2000, Brinson and Malvárez 2002). Many of the existing wetlands around the San Francisco Bay Area were artificially constructed as livestock watering sites and now serve as important habitat refuges for species of conservation concern, including native pond-breeding amphibians (i.e., Pacific chorus frogs, California newts, western toads, California red-legged frogs, and California tiger salamanders; Joseph et al. 2016). Concurrently, wetlands in this region also support

multiple invasive species, including American bullfrogs (*Lithobates catesbeianus*), bass (*Micropterus spp.*), sunfish (*Lepomis spp.*), and western mosquitofish (*Gambusia affinis*; Preston et al. 2012). Western mosquitofish, which are native to the Mississippi River drainage, have a long history of intentional introduction into California wetland, and waterways worldwide, for use as a biological control agent of mosquito larvae (Downs 1991). However, mosquitofish are generalist predators that also prey on a wide diversity of non-target organisms, including zooplankton, invertebrates, amphibians, and fishes (e.g., Goodsell and Kats 1999, Mills et al. 2004, Shulse et al. 2013, Merkley et al. 2015, Holbrook and Dorn 2016), and their use as mosquito biocontrol has been controversial (Azevedo-Santos et al. 2017).

### *Mesocosm experiment*

To examine how nutrient availability influenced interactions between mosquitofish and native aquatic organisms, we conducted a  $2 \times 3$  factorial outdoor mesocosm experiment involving two levels of mosquitofish presence (yes or no) and three levels of nutrient concentrations (low, medium, or high). Mesocosms were located at the University of California Hopland Research and Extension Center in Mendocino County, California. Each treatment was replicated five times for a total of 30 mesocosms. Mesocosms consisted of 378-L livestock watering tanks filled with well water and fitted with mesh screen lids. To each mesocosm, we added 6 kg of silica sand and 25 grams of dry leaves (*Quercus* and *Arbutus spp.*) as substrate and cover. To each tank, we also added two square clay tiles (15.2-cm<sup>2</sup> area) to provide a surface from which to quantify periphyton. The nutrient levels used in the experiment were informed by field nutrient measurements from 231 wetlands in the San Francisco Bay Area of California (see Appendix S1 for details). One month prior to the start of the experiment (12 May), and again three weeks later (4 June), we added chemical nutrients ( $\text{KH}_2\text{PO}_4$  and  $\text{NaNO}_3$ ) to all mesocosms at a molar nitrogen-to-phosphorus ratio of 38:1, which was the median value from field data (see Appendix S1: Fig. S1). On each occasion, we added  $\text{KH}_2\text{PO}_4$  in amounts of 0.032 g to the low nutrient condition, 0.13 g to the medium nutrient condition (a fourfold increase), or 0.51 g to the high nutrient condition (a 16-fold

increase). Similarly, we added  $\text{NaNO}_3$  in amounts of 0.75 g (low nutrients), 3.0 g (medium), or 12.0 g (high). We seeded each mesocosm with algae, zooplankton collected with a 243  $\mu\text{m}$  net (mostly cladocerans and copepods), and nine taxa of local wetland invertebrates, including snails, hemipterans, odonates, and amphipods (Appendix S1: Table S1). At the start of the experiment (13 June), we added larvae of three native amphibians to each mesocosm: 15 Pacific chorus frogs (*Pseudacris regilla*), 20 western toads (*Anaxyrus boreas*), and 10 California newts (*Taricha torosa*; see Appendix S1: Table S2 for initial body sizes). In the replicates assigned to the mosquitofish addition treatments, we added four adult male and three adult female mosquitofish (Appendix S1: Table S2). All organisms added to mesocosms were locally collected from natural wetlands in Mendocino County, California, and the densities of organisms were within the range of densities observed in the field (Preston et al. 2012, 2017, Joseph et al. 2016). The experiment lasted for approximately four weeks (from 13 June to 16 July; see Appendix S1: Table S3 for a detailed timeline).

In the mesocosm experiment, we quantified nutrient concentrations (total dissolved nitrogen and total dissolved phosphorus), phytoplankton (relative fluorescence), periphyton (biomass), zooplankton (abundance), invertebrates (abundance and biomass), amphibians (growth, survival, and biomass), and mosquitofish (abundance and biomass). We measured nutrient concentrations at three time points from the beginning, mid-point, and end of the study. To quantify nutrients, we collected water samples from each mesocosm in acid-washed Nalgene bottles, which were frozen until analysis at the University of Colorado (see <http://niwot.colorado.edu/research/kiowa-lab/the-arika-ree-environmental-laboratory> for methodological details). Relative in vivo phytoplankton fluorescence was quantified weekly (six sampling dates from 9 June to 13 July) using a fluorometer (Turner Designs, Sunnyvale, California, USA). Water samples for phytoplankton measurements were collected from the center of the mesocosm using Nalgene bottles, briefly stored in a cooler, and then immediately processed on each sampling date. Periphyton dry biomass on clay tiles was measured on two dates early in the study (3.3  $\text{cm}^2$  sampled per replicate on 9 June and 17

June) and at the end of the experiment from the mesocosm walls (12  $\times$  40 cm sampled per replicate on 12 July). We switched from measuring periphyton on clay tiles to measuring it on the mesocosm walls because periphyton on tiles was removed by grazers at the mid-point of the study. Periphyton samples were oven-dried at 70°C prior to weighing. We measured zooplankton abundance at five time points spanning the length of the study (9 June–13 July) by pooling five samples per mesocosm collected with a vertical tube sampler (70 cm in length  $\times$  5 cm in diameter). Zooplankton samples were filtered onto 50- $\mu\text{m}$  mesh and preserved in 80% ethanol prior to enumeration and identification as either copepods or cladocerans (primarily *Daphnia* spp.) in the laboratory. Invertebrates were sampled using two pooled samples per mesocosm collected with a benthic stovepipe sampler (28 cm in diameter). At the conclusion of the experiment (15 July), we counted and removed all amphibians, snails, and mosquitofish and measured their length and wet mass. For frogs and toads, we also recorded their developmental stage (Gosner 1960) or days-to-metamorphosis for those individuals that emerged before the experiment ended.

### Analyses

To analyze responses that were quantified on multiple dates of the experiment (nutrients concentrations, phytoplankton, zooplankton, periphyton from tiles), we used linear mixed effects models with main effects of fish, nutrient levels, time, and their interactions. We also included a random intercept term for mesocosm identity. The data for each of these responses involved one time point that was collected prior to the start of the experiment (i.e., before mosquitofish introduction), and multiple time points during the study (Appendix S1: Table S3). For responses that were measured only at the conclusion of the study, we omitted the effect of time and the random intercept term. Periphyton collected on clay tiles was analyzed separately from periphyton collected on the mesocosm walls. Fish presence was coded as a categorical variable (present or absent), while nutrient levels were coded as a continuous variable representing the relative differences in nutrient inputs (1, 4, or 16). For amphibian survival data, we used a generalized linear mixed effects

model with a binomial error distribution, a random intercept term for mesocosm identity, and survival of each individual within a mesocosm as the response (Zuur et al. 2009). For all other responses, we used a single mean value per mesocosm one each sampling date.

Because our initial analyses suggested that indirect effects were important in explaining our mesocosm results, we used path analysis to further evaluate potential mechanisms linking fish and nutrients with the response variables (Kline 2015). The path analysis included nutrient levels as a continuous variable (1, 4, or 16) and mosquitofish biomass at the end of the study as continuous predictors (see Fig. 5 for the path diagram). Within our path analysis, nutrients were linked to phytoplankton fluorescence and periphyton biomass through bottom-up effects, while mosquitofish were linked to zooplankton density, amphibian biomass, and snail biomass through top-down predatory effects. Phytoplankton and periphyton were also linked to zooplankton and amphibians, respectively (through grazing), and snails were linked to periphyton (via bottom-up resource availability; our initial analysis did not suggest the presence of a strong top-down link from snails to periphyton via grazing). Model fit was evaluated using the Comparative Fit Index (CFI) and the Standardized Root Mean Square Residual (SRMR), which are both robust to violations of normality and effective with small sample sizes (Hu and Bentler 1999). Log transformations were used when they improved variable distributions and all analyses were conducted in the R computing environment (R Core Team 2014), using the lavaan package for path analysis (Rosseel et al. 2011).

## RESULTS

### Nutrient effects in mesocosms

Observed nutrient concentrations in the mesocosms (Fig. 1) indicated that the manipulation was effective, with low, medium, and high nutrient treatments showing consistent differences throughout the study in total dissolved nitrogen (LMM, nutrients,  $t = 13.48, P < 0.001$ ) and phosphorus (LMM, nutrients,  $t = 5.37, P < 0.001$ ). We did not detect any significant effects of fish, time, or a fish-by-time interaction on total dissolved nitrogen (LMM, fish,  $t = -1.16, P = 0.25$ ; time,

$t = 0.71, P = 0.48$ ) or phosphorus (LMM, fish,  $t = 0.46, P = 0.65$ ; time,  $t = 1.29, P = 0.20$ ).

Nutrient concentrations strongly affected phytoplankton but had relatively weak effects on periphyton in mesocosms. Across all mesocosms, an increase from low to high nutrient concentrations increased mean phytoplankton fluorescence by 300%. Effects of nutrients were strongest toward the end of the study, leading to a nutrients-by-time interaction on phytoplankton (Fig. 2A; LMM, nutrients  $\times$  time,  $t = 2.40, P = 0.017$ ). On the second sampling time point, periphyton biomass was ~50% lower on the clay tiles in the low nutrient conditions than the medium or

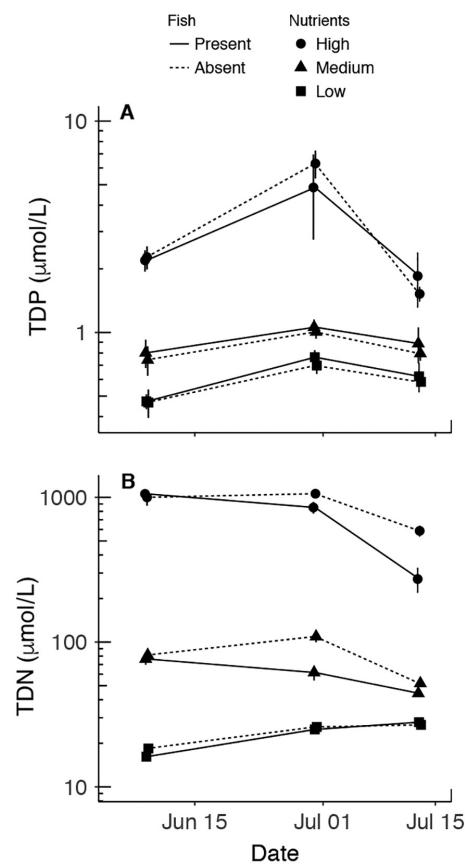


Fig. 1. (A) Total dissolved phosphorus and (B) total dissolved nitrogen from mesocosm water samples collected at three time points over the duration of the study. Fish presence (solid lines) or absence (dotted lines) is indicated by line type, and nutrient conditions are indicated by the point shapes. Error bars represent one standard error.

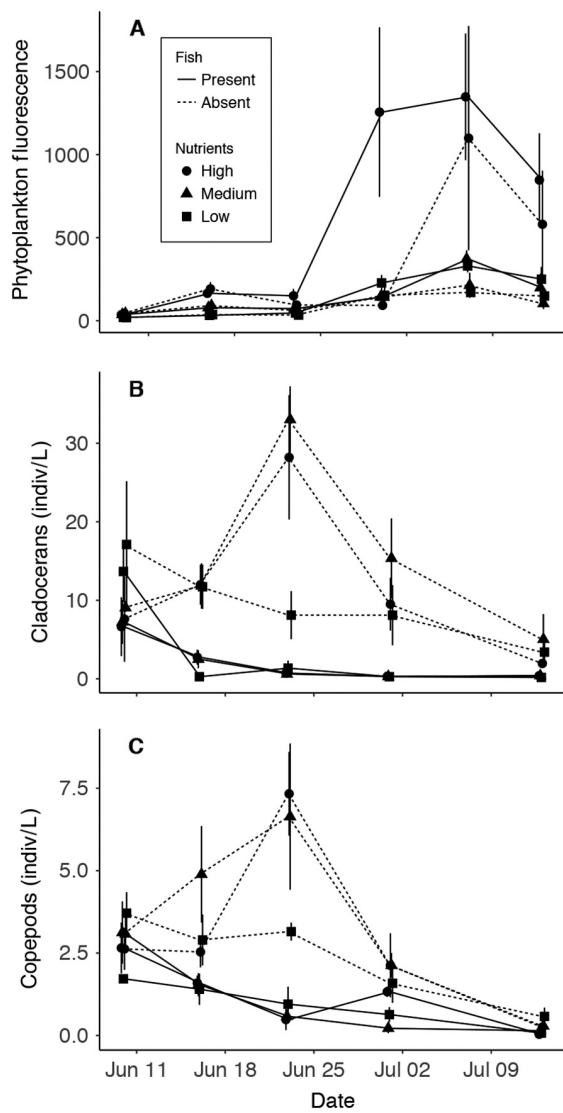


Fig. 2. (A) Relative phytoplankton fluorescence, (B) density of cladoceran zooplankton, and (C) density of copepod zooplankton from experimental mesocosms. Phytoplankton fluorescence is a unitless, relative measure obtained from a laboratory fluorometer. Zooplankton were measured from five combined tube samples (~4 L water volume per mesocosm) on each sampling date. Fish presence (solid lines) or absence (dotted lines) is indicated by line type, and nutrient conditions are indicated by the point shapes. Error bars represent one standard error.

high; however, this effect was not statistically significant (Appendix S1: Fig. S2; LMM, nutrients  $\times$  time,  $t = 0.24$ ,  $P = 0.81$ ). Periphyton biomass at the end of the study on the mesocosm

walls was not different between nutrient conditions (Fig. 3a;  $t = 0.15$ ,  $P = 0.88$ ).

Nutrients generally had smaller effects on invertebrate and amphibians in the mesocosms compared to mosquitofish. At the intermediate sampling time point, the high and medium nutrient treatments supported approximately threefold more zooplankton than the low nutrient treatment; however, this effect was not significant for either cladocerans (Fig. 2B; LMM, nutrients  $\times$  time,  $t = -0.43$ ,  $P = 0.66$ ) or copepods (Fig. 2C; LMM, nutrients  $\times$  time,  $t = 0.13$ ,  $P = 0.90$ ). Of the benthic invertebrates added to the mesocosms, only aquatic snails (*Helisoma* and *Physa*) reproduced over the course of the experiment. Snail

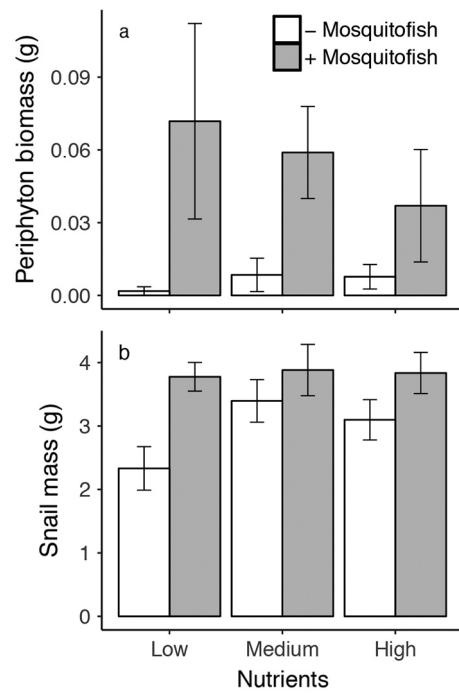


Fig. 3. (a) Results from experimental mesocosms showing periphyton biomass and (b) snail biomass (*Helisoma* sp. and *Physa* sp.) quantified at the conclusion of the experiment. Periphyton biomass was measured from a standardized area on the mesocosm walls ( $480 \text{ cm}^2$ ), and snail biomass was the total from all individuals with a mesocosm. Periphyton was also measured at the beginning of the study from clay tiles (see Appendix S1: Fig. S2). Nutrient concentrations are shown on the x-axis, and bar colors correspond with mosquitofish presence or absence. Error bars represent one standard error.

biomass was not different across nutrient treatments at the conclusion of the study (Fig. 3b). Nutrients also did not affect amphibian survival (Fig. 4; GLMM,  $t = -0.26$ ,  $P = 0.79$ ), but increased the average developmental stage of chorus frog larvae (LM,  $t = 2.51$ ,  $P = 0.025$ ) and the average wet mass of newt larvae (Fig. 4b; LM,  $t = 2.80$ ,  $P = 0.015$ ). Nutrients also slowed time-to-metamorphosis of toads (LM,  $t = 2.85$ ,  $P = 0.008$ ).

Among the aquatic consumers present, nutrient additions most strongly affected mosquitofish in mesocosms (Fig. 4d). Total mosquitofish biomass was ~30% higher in the high nutrient treatments relative to the low nutrient treatments (LM,  $t = 2.28$ ,  $P = 0.039$ ). Based on the size distribution of mosquitofish at the end of the study, this effect was driven by increases in fish reproduction and/or greater survival of offspring at higher nutrient levels. Juvenile mosquitofish produced within the mesocosms were differentiated from the adults that were added at the start of the experiment based on their body sizes (14.9 mm mean length for juveniles vs. 34.5 mm mean length for adults

at the end of the study). On average, mesocosms in the high nutrient conditions supported ~5 times more juvenile mosquitofish (mean = 23.4 individuals) than in the low nutrient treatment (mean = 4.6 individuals).

#### Mosquitofish effects in mesocosms

Mosquitofish influenced invertebrates and periphyton in mesocosms. Fish presence was associated with a >10-fold reduction in total mean zooplankton density (Fig. 2B, C). Fish decreased the density of both copepods (Fig. 2C; LMM, fish,  $t = -3.47$ ,  $P = 0.002$ ) and cladocerans (Fig. 2B; LMM, fish,  $t = -3.14$ ,  $P = 0.004$ ). Densities of both types of zooplankton peaked at intermediate time points, leading to significant effects of time as well (copepods LMM, time,  $t = -4.45$ ,  $P < 0.001$ ; cladocerans GLMM, time,  $t = -2.41$ ,  $P = 0.018$ ). The total biomass of aquatic snails was 26% higher in mesocosms with fish than in mesocosms without fish at the end of the experiment (Fig. 3b; LM, fish,  $t = 2.61$ ,  $P = 0.015$ ). Fish presence also increased benthic periphyton biomass along the mesocosm

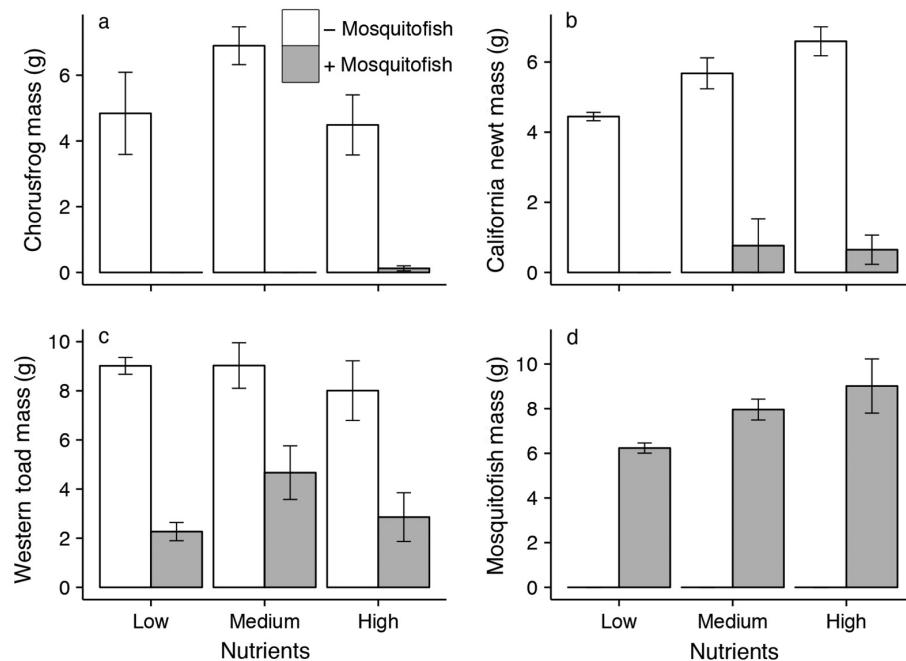


Fig. 4. Results from experimental mesocosms showing (a) chorus frog biomass (*Pseudacris regilla*), (b) California newt biomass (*Taricha torosa*), (c) western toad biomass (*Anaxyrus boreas*), and (d) mosquitofish biomass (*Gambusia affinis*). All values are means per mesocosm of total biomass at the end of the experiment. Nutrient concentrations are shown on the  $x$ -axis, and bar colors correspond with mosquitofish presence or absence. Error bars represent one standard error.

walls by approximately 10-fold at the end of the study (Fig. 3a; LM,  $t = 4.43$ ,  $P = 0.0001$ ). This effect of fish was not observed at the two early time points on clay tiles (Appendix S1: Fig. S2). Mean phytoplankton fluorescence over the entire study was 70% higher in the presence of fish; however, this effect was not statistically significant (Fig. 2A; LMM, fish,  $t = -0.27$ ,  $P = 0.81$ ).

The presence of mosquitofish strongly reduced amphibian survival (Fig. 4). Among all mesocosms with fish, only two chorus frogs (<1%) and ten California newts (7%) survived to the end of the experiment (Fig. 4; total amphibian survival, GLMM,  $t = -8.43$ ,  $P < 0.0001$ ). Western toad survival was higher in the presence of fish than the other two amphibian species (mean = 43%), but was still reduced by half relative to mesocosms without fish (Fig. 4c). Fish presence also accelerated time-to-metamorphosis of toads (LM,  $t = 3.00$ ,  $P = 0.006$ ), but decreased their average individual wet mass at the end of the study (LM,  $t = -3.11$ ,  $P = 0.004$ ).

#### Path analysis

The path analysis provided support for several indirect effects (trophic cascades) of mosquitofish in mesocosms (Fig. 5). Mosquitofish were negatively associated with zooplankton density, which in turn was negatively associated with phytoplankton fluorescence, resulting in a net positive pathway from mosquitofish to phytoplankton fluorescence (standardized indirect path coefficient = 0.27,  $P = 0.007$ ; Fig. 5). A similar association with mosquitofish was observed involving the benthic community; mosquitofish were negatively associated with amphibian biomass, which was negatively associated with periphyton biomass, leading to a net positive pathway from fish to periphyton (standardized indirect path coefficient = 0.48,  $P = 0.001$ ; Fig. 5). Periphyton also associated positively with snail biomass, leading to a positive indirect path from mosquitofish to snails (standardized indirect path coefficient = 0.22,  $P = 0.034$ ; Fig. 5). Lastly, the fit indices indicated that the model provided an adequate fit to the data (CFI = 0.94; SRMR = 0.062).

#### DISCUSSION

Invasive fish and nutrient pollution represent two ubiquitous drivers of ecosystem change in

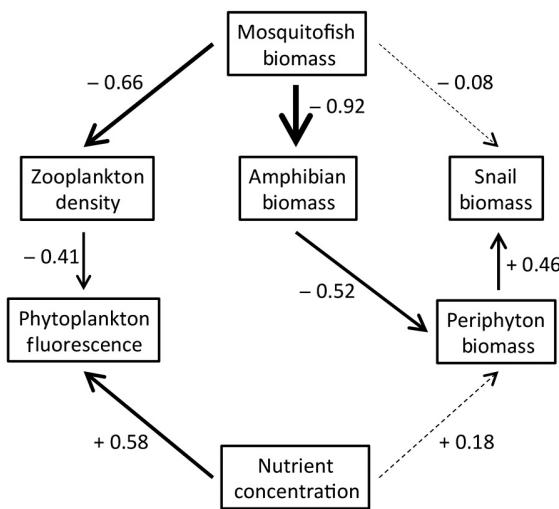


Fig. 5. Path diagram testing hypothesized links within the mesocosm experiment. Values for zooplankton and phytoplankton are from the conclusion of the study. All other variables correspond to the values shown in Figs. 3, 4. Solid arrows show statistically significant ( $P < 0.05$ ) paths, and dashed arrows show nonsignificant paths. The width of each arrow corresponds to the magnitude of the standardized path coefficient, which is also provided next to the arrow.

freshwaters. We hypothesized that increased nutrients could either “dilute” the negative predatory effects of mosquitofish on lower trophic levels by increasing prey availability (the invader attenuation hypothesis), or alternatively, amplify their effects on native taxa by disproportionately benefiting mosquitofish (the invader amplification hypothesis). Overall, our data supported the invader amplification hypothesis: Mosquitofish biomass increased by 30%, while population density increased by fivefold under the high nutrient treatments, with no reduction in their predatory effects on lower trophic levels. Effects of nutrients on zooplankton and native amphibians were weak compared to the effects of nutrients on mosquitofish. These findings indicate that mosquitofish may benefit from increases in nutrients more than the native organisms that they prey upon.

The expected outcome of interactions between species invasions and nutrient inputs remains a relatively open question, with some studies reporting positive effects of elevated nutrients on invaders and increased invasion impacts (e.g., Chase and Knight 2006, Coetzee et al. 2007, Zhao

et al. 2015), while in other cases nutrients facilitate coexistence between native and introduced species or disproportionately promote natives (e.g., Firn et al. 2010). Many invaders tend to have fast life histories, with rapid maturation times and a large numbers of offspring, and thus high resource demands (Blumenthal 2006, González et al. 2010, Tibbets et al. 2010). Our experimental results support this idea, suggesting that mosquitofish can effectively utilize excess resources, perhaps more so than native community members. Within our experimental manipulation, increases in nutrient availability led to enhanced reproduction and survival of young mosquitofish. Mosquitofish are known to cannibalize their young, which may have occurred more readily under low resource availability conditions (Pyke 2008). Because mosquitofish are generalist predators that feed on zooplankton, small invertebrates, and amphibians (García-Berthou 1999), the increase in nutrient input likely promoted fish biomass through increased food availability (Lancaster and Drenner 1990). At the intermediate time point of the study (week three), we observed a threefold increase in zooplankton in the medium and high nutrient conditions relative to the low nutrient condition. Densities of both cladocerans and copepods were highest at this point in the study, indicating that there were likely large differences in total food availability to mosquitofish across the nutrient treatments that could have driven the differences in fish productivity. It is also possible that very small zooplankton taxa (e.g., rotifers and ciliates) may have been a food source in the mesocosms that responded to nutrient treatments. Additionally, the increase in mosquitofish biomass at high nutrients could have been attributed in part to increases in detritus from turnover of phytoplankton and periphyton. Detritus has been previously shown to be a component of the diet of mosquitofish in some settings (Blanco et al. 2004). Analyses of the stomach content or isotopic ratios of the mosquitofish would be useful for future studies to help clarify the mechanisms driving the bottom-up effects that we observed here.

In contrast to mosquitofish, most native consumers in the mesocosms did not show strong responses to nutrient enrichment that could have facilitated coexistence with mosquitofish or weakened their predatory effects. The only amphibian to show a positive growth response

with nutrient enrichment was California newts, which are strict carnivores and may have occupied a similar trophic level as mosquitofish within the mesocosms (Petránka 2010). Although the native amphibians were unable to reproduce in the mesocosms (in contrast to mosquitofish), overall they showed weak growth responses to nutrient enrichment, with two of three species being relatively unaffected. Aquatic benthic invertebrates also did not show strong responses to nutrient enrichment, collectively suggesting that mosquitofish benefitted more from bottom-up effects than native community members.

Mosquitofish in our mesocosm experiment directly preyed on native amphibians, completely eliminating them from over 50% of mesocosm replicates containing fish. Prior work has also found that amphibian larvae are highly susceptible to predation by mosquitofish, particularly in mesocosms and laboratory experiments (Webb and Joss 1997, Goodsell and Kats 1999, Zeiber et al. 2008, Preston et al. 2012, Shulse et al. 2013). Mosquitofish directly consume amphibians and cause sublethal injuries when they remove the legs and tails of developing larvae (Preston et al. 2012, Shulse and Semlitsch 2014). Of the three amphibian species in this experiment, western toads showed the highest survival. This species is toxic in its larval stages and often less palatable to vertebrate predators than other species (Gunzburger and Travis 2005). We also note, however, that the predatory effects of mosquitofish on amphibians and invertebrates may be context-dependent. Increases in habitat complexity and the availability of alternative prey can weaken the predatory effects of mosquitofish on some amphibian species (Lawler et al. 1999, Preston et al. 2017) and invertebrates (Knorp and Dorn 2016). As a result, it may be difficult to generalize mosquitofish impacts across discrete wetlands that vary in environmental conditions and community structure. In some systems, they will strongly reduce amphibian populations (e.g., Shulse et al. 2013, Holbrook and Dorn 2016), whereas in others they may coexist (e.g., Reynolds 2009). Furthermore, although we observed positive effects of nutrients on mosquitofish, this did not result in stronger mosquitofish effects on amphibians at high nutrient levels. One explanation for this result is that the additional mosquitofish at high nutrients

were juveniles that may have been too small to effectively prey on late-stage amphibian larvae. A longer-running study, allowing the young mosquitofish produced in the mesocosms to mature into adults, could have resulted in increased mosquitofish impacts on amphibians at high nutrients.

Mosquitofish also caused indirect positive effects on periphyton and freshwater snails. Based on the path analysis, the increase in periphyton was likely driven by reductions in grazing amphibian larvae due to mosquitofish predation. Snail biomass, in turn, increased due to greater resource availability and the reduction in competition from grazing amphibians. Such indirect positive effects of invasive species on native community members have been documented before in a handful of cases (reviewed in Rodriguez 2006). For instance, invasive European green grabs reduce abundances of native clams and crabs, but increase the abundances of tube-building polychaete worms, presumably through competitive release (Grosholz et al. 2000). Trophic cascades involving increases in benthic algae due to decreases in herbivores after the introduction of a nonnative predator have also been observed. Invasive brown trout in New Zealand, for example, reduce periphyton from grazing pressure by native invertebrates, leading to increases in benthic primary production (Townsend 1996, Huryn 1998). Further work is needed to understand how commonly invasive species facilitate native species through indirect interactions.

Our path analysis, in conjunction with past work, suggests that the overall effect of mosquitofish on phytoplankton is an indirect effect driven by decreases in zooplankton abundance (Hurlbert and Mulla 1981). Strong predatory effects of mosquitofish on zooplankton, including the total loss of large-bodied taxa, have been observed in mesocosms, artificial wetlands, and natural ponds (Hurlbert et al. 1972, Lancaster and Drenner 1990, Margaritora et al. 2001, Nagdali and Gupta 2002). While mosquitofish additions led to increased phytoplankton abundance in all treatments, this effect was largest at high nutrients. While this effect was not statistically significant (likely due to the high variability between mesocosms), it presents the potential for synergistic effects of fish and nutrients on phytoplankton production.

In general, we found relatively few interactions between mosquitofish and nutrients in our analyses, and most observed effects were additive. This is consistent with a series of in-lake mesocosm experiments replicated in five countries in Europe (Stephen et al. 2004). In these experiments, there were relatively few interactions between nutrient availability and fish abundance (Moss et al. 2004, Vakkilainen et al. 2004, Van de Bund et al. 2004), due likely in part to the presence of aquatic macrophytes, which have the potential to regulate fish–zooplankton–phytoplankton interactions (Schriver et al. 1995). In our experiment, it is possible that the effects of mosquitofish alone were so strong on many responses (e.g., amphibians) that synergistic effects of fish and nutrients became difficult to detect. Environmental conditions that result in weaker effects of fish may enhance potentially subtle interactions between these two factors.

One consideration in interpreting the effects of mosquitofish at high nutrients is that we focused on population-level effects, rather than per capita effects. The increase in fish numbers, and most of the increase in biomass, at high nutrients was due to young individuals, making it likely that the per capita effects of mosquitofish were weaker at high nutrients than at low nutrients (in contrast to the population-level effects). Examining per capita effects (Wootton and Emmerson 2005) would provide complementary insights into interactions between species invasions and nutrient inputs, and should be a priority for future work.

A further consideration in our study is the time-scale and choice of experimental venue. In a prior experiment (Preston et al. 2017), we found strong evidence for reductions in zooplankton and invertebrates caused by mosquitofish introductions to a natural wetland, consistent with the current mesocosm study. In contrast, however, we did not find strong effects of mosquitofish on native amphibians, potentially because the mosquitofish did not co-occur with the earliest and most susceptible developmental stages of amphibian larvae, and the availability of alternative prey in the wetland was higher than in the mesocosms. A multi-year field experiment would overcome these limitations by testing how changes in nutrients influence mosquitofish interactions with their prey, incorporating effects of age- and size-structured predator-prey

populations throughout the season. Under this scenario, we suspect that invader amplification would be even stronger than in mesocosms because mosquitofish would co-occur with all life stages of their prey and juvenile fish would mature and contribute to top-down effects. In general, the possible differences in abiotic variables and community structure between mesocosm studies and natural systems must always be considered when extrapolating results to nature.

The fish and nutrient manipulations influenced the variance of several responses, which has been linked to regime shifts in freshwater systems (Carpenter and Brock 2006). For instance, the standard deviation of mean phytoplankton fluorescence across all mesocosms was ~10-fold higher at high nutrients relative to low nutrients. A similar magnitude increase in standard deviation of periphyton biomass was observed from fish-absent to fish-present treatments. Both nutrient additions and predatory fish introductions have potential to drive shifts in stable states in freshwater ecosystems, and our results support the idea that such shifts can be associated with a prior change in the variance of a system (Carpenter and Bock 2006, Carpenter et al. 2011). Future work that allows a system to reach equilibrium would facilitate testing how nutrients and mosquitofish jointly influence shifts in stable states and potential warning signals.

Collectively, our results demonstrate that nutrients have the potential to mediate the success of mosquitofish, such that elevated nutrient loading may enhance mosquitofish production more so than native community members. Increased nutrient loading also has potential to enhance the establishment and possibly spread of mosquitofish through connected waterways. Our findings parallel results from other systems in which invasive species disproportionately benefit from increased nutrients relative to natives (González et al. 2010). Our simplified mesocosm experiment provides a mechanistic foundation for future studies to test the relationship between nonnative fish impacts and nutrient availability in more complex natural ecosystems, for which we emphasize the need to consider how additional factors, such as ontogenetic diet shifts and size-structured predation, affect the potential for invader amplification.

Results of such studies will be useful for informing management strategies of freshwaters, particularly considering the increasing co-occurrence of abiotic environmental change and aquatic species invasions (MacDougall and Turkington 2005). In particular, it would be useful to identify the nutrient conditions under which invasive species are most likely to have undesirable impacts, and to prioritize management resources for these locations or time periods.

## ACKNOWLEDGMENTS

We thank Clara Boland for assistance with the experiment and Katie Richgels, Brett Goodman, Michelle Baragona, and Holly Hughes for assistance with field work. The staff of the University of California Hopland Research and Extension Center provided valuable help in completing our experiment, especially R. Keiffer, A. Shrum, W. Marston, S. Poor, and S. Fierer. Four anonymous reviewers helped improve the manuscript. Funding came from the National Science Foundation (DEB-0841758, DEB-1149308, DEB-1311467, DEB-1027341, and a Graduate Fellowship to DLP), the David and Lucile Packard Foundation, and the University of Colorado. Publication of this paper was funded by the University of Colorado Boulder Libraries Open Access Fund. Daniel L. Preston and Hayden D. Hedman made equal contributions to this article.

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