

Responses of a wetland ecosystem to the controlled introduction of invasive fish

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SUMMARY

1. The ecological consequences of species invasions can vary in time and space, complicating efforts to generalise invader impacts across ecosystems. This challenge is particularly relevant when using small-scale experiments to derive predictions for freshwater ecosystems. In this study, our aims were to document the effects of a controlled fish introduction within an ecosystem-scale experiment and to test possible factors driving variation in invasion outcomes.

2. We measured community and ecosystem responses to the introduction of western mosquitofish (*Gambusia affinis*: Poeciliidae) using a 'Before-After-Control-Impact' design within a large, experimentally divided natural wetland in California, USA. We then used a replicated outdoor mesocosm experiment to address how two factors that vary widely in natural wetlands – habitat complexity and alternative prey availability – mediate the effects of mosquitofish on native wetland amphibians.

3. In the natural wetland, mosquitofish increased in population size by ~90-fold over the course of a single summer. Mosquitofish introduction was associated with a 50% decrease in macroinvertebrate density and a 90% decrease in zooplankton abundance relative to a fishless control treatment. We observed no effects of mosquitofish on the abundance or total biomass of two native pond-breeding amphibians – the Pacific chorus frog (*Pseudacris regilla*: Hylidae) and California newt (*Taricha torosa*: Salamandridae) – likely because more preferable alternative prey were abundant, vegetation provided refuges from predation, and the mosquitofish introduction occurred after amphibian larval stages were most susceptible to predation. Surprisingly, mosquitofish were also associated with a 50% decrease in both relative phytoplankton fluorescence and total phosphorus, and a sharp increase in N:P ratios in the water column, possibly due to the assimilation of fish biomass acting as a nutrient sink.

4. In contrast to our ecosystem experiment, mosquitofish consumed native amphibians and reduced their growth rates in outdoor mesocosms. The strength of predation within the smaller scale venue, however, varied with the availability of alternative prey (i.e. zooplankton), and the complexity of the habitat (i.e. presence of aquatic macrophytes). Our mesocosm results support the hypothesis that alternative prey and habitat complexity facilitated coexistence between invasive mosquitofish and native amphibian larvae in our ecosystem experiment.

5. Our findings highlight the potential for invasive fish to drive rapid shifts in freshwater ecosystems while also emphasising the roles of environmental characteristics in mediating whether native and non-native species will coexist.

Keywords: amphibian decline, freshwater pond, introduced species, mosquito biocontrol, non-native

Introduction

Predicting the outcome of species invasions is a key goal in ecology, yet it can be challenging to achieve due to variation in invader impacts through time and space (Ricciardi *et al.*, 2013). A range of variables, including individual traits, community structure, the densities of interacting species and abiotic factors, can directly or indirectly mediate the strength of species interactions (Abrams, 2001; Peacor & Werner, 2004). As a result, the effects of invasive species can vary across ecological scales, between discrete ecosystems, or over time (Strayer *et al.*, 2006; Ricciardi *et al.*, 2013; Latzka *et al.*, 2016). For instance within the Laurentian Great Lakes Basin, where >180 invasive species have established, the effects of non-native round gobies on benthic invertebrates can vary from strongly positive to strongly negative depending on the relative importance of top-down versus bottom-up controls on community dynamics (Pagnucco & Ricciardi, 2015). It remains relatively uncommon, however, to quantify such variation, examine its underlying drivers, or incorporate it into predictions or management efforts (Thomsen *et al.*, 2011; Hulme *et al.*, 2013; Dick *et al.*, 2014).

Variation in the consequences of species invasions can also manifest in experimental venues, in some cases influencing the degree to which observed effects can be extrapolated to natural systems. For instance experimental design, including the scale of experimental venue, can drive variation in the strength of observed species interactions (Skelly & Kiesecker, 2001; Lunde, Resh & Johnson, 2012). Variation in interaction strengths may be of interest if it is relevant to the ecological questions being asked, or at worst, it may lead to artifactual outcomes that have little relevance in nature (Carpenter, 1996; Schindler, 1998). One promising approach to understand variation in the strength of interactions between native and non-native species is to combine experiments at multiple scales (Petersen, Cornwell & Kemp, 1999; Sandel & Smith, 2009). Small and medium-scale experiments (e.g. laboratory trials, microcosms, mesocosms) are valuable in isolating and testing possible mechanisms, whereas large-scale experiments (e.g. ecosystem manipulations) are then useful in testing the relative magnitude of effects in a more natural setting (e.g. Vredenburg, 2004). In this way, combining multi-scale experiments can be a powerful approach to understand invasive species effects because it integrates mechanistic understanding with realism.

Among freshwater invasive species, mosquitofish (*Gambusia affinis* and *G. holbrooki*: Poeciliidae) are one of

the most widespread and there is a need to understand variation in their effects across temporal and spatial scales. Mosquitofish are native to the eastern United States and have been introduced to freshwaters on all continents except Antarctica as a biocontrol agent of mosquito larvae (Pyke, 2008). Mosquitofish are generalist predators and can prey on a wide range of invertebrates (e.g. Hurlbert & Mulla, 1981; Leyse, Lawler & Strange, 2004; Merkley, Rader & Schaalje, 2015), amphibians (e.g. Webb & Joss, 1997; Zeiber, Sutton & Fisher, 2008; Shulse, Semlitsch & Trauth, 2013) and fishes (e.g. Mills, Rader & Belk, 2004; Henkanathgedara & Stockwell, 2014). Most native amphibian species from the western United States are susceptible to mosquitofish predation (Gamradt & Kats, 1996; Goodsell & Kats, 1999; Preston, Henderson & Johnson, 2012), although at least one wetland experiment (Lawler *et al.*, 1999) and our own observations in northern California suggest that mosquitofish coexist with native amphibians in some settings. Testing how variability in environmental characteristics between lentic water bodies mediates invasion consequences for native communities will therefore be useful to predict and manage undesired mosquitofish impacts.

In this study, we combined an ecosystem-level experiment with an outdoor mesocosm study to examine the effects of mosquitofish in California wetlands. To quantify community and ecosystem responses to mosquitofish invasion, we performed an experimental introduction of mosquitofish into a divided natural wetland. We used a 'Before-After-Control-Impact' design to document mosquitofish effects on water chemistry, phytoplankton, zooplankton, macroinvertebrates and native amphibians over the course of a summer. We then used an outdoor mesocosm experiment to test how alternative prey availability and habitat complexity affected the strength of mosquitofish predation on focal amphibians. Our mesocosm study was specifically designed to test possible mechanisms that could explain the coexistence of native amphibians and mosquitofish observed in our ecosystem experiment.

Methods

Ecosystem experiment

We used a 'Before-After-Control-Impact' (BACI) design to quantify the effects of mosquitofish introduction within a natural wetland (Hog Lake) located at the Hopland Research and Extension Center in Mendocino County, California (39.0316N, 123.0789W). A BACI

design is useful to examine the effects of invasive species because it allows comparisons before and after invasion at the same site, as well as across invaded and uninvaded replicates over space (Smith, 2002). Hog Lake, which is ~3200 sq. metres and ~1.5 m deep when full (Fig. 1), was divided into two equal sections using an impermeable rubber pond liner that prevented the passage of organisms and water (see Lunde *et al.*, 2012 for additional details). The barrier extended approximately 0.5 m into the wetland substrate and 0.5 m above the water line at the start of the study. Hog Lake was chosen because it supported a diverse native community, including two pond-breeding amphibian species, and it provided a unique opportunity for a reversible species introduction. Because the system dries by the end of the summer (August or September) and is relatively isolated from other wetlands, added fish could not persist between years or escape to other habitats. The wetland is naturally fish-free. Our prior surveys of >200 wetlands in northern California indicated that the invertebrate and amphibian communities within Hog Lake were very similar to permanent wetland systems in the region (Preston *et al.*, 2012, 2013; Johnson *et al.*, 2013). Rushes (*Juncus* spp.: Juncaceae) constitute the dominant emergent vegetation around Hog Lake.

Due to the logistical challenges associated with controlled introductions of non-native species to a natural

ecosystem, we performed our experiment within a single unreplicated system. The BACI design using a split wetland improved our ability to interpret whether treatment effects were driven by mosquitofish because we collected detailed information on pre-invasion wetland characteristics. In addition, both wetland treatments were highly similar at the start of the experiment. While this approach precludes extrapolation of our results to other ecosystems, it ensures that environmental variability between wetlands – such as differences in community structure or productivity – is not confounded with treatment effects.

We sampled both sides of Hog Lake every 2 weeks over the summer of 2011 for a total of six sampling dates. We quantified nutrient concentrations, relative phytoplankton fluorescence and the abundances of zooplankton, macroinvertebrates and amphibian larvae. The experiment was initiated on June 9th of 2011, when we introduced 60 adult male and 60 adult female mosquitofish (*Gambusia affinis*) to the west side of Hog Lake. We did not add the fish earlier in the season because we needed to ensure that the water level in the wetland was low enough to prevent introduced mosquitofish from moving over the top of the dividing barrier or escaping to nearby waterbodies that are more connected during the wet season. For all responses except nutrients and phytoplankton, the first two sampling dates



Fig. 1 Hog Lake in Mendocino County, California. Invasive western mosquitofish (*Gambusia affinis*) were added to the west side of Hog Lake and community and ecosystem responses were measured using a 'Before-After-Control-Impact' experimental design. The bottom images show the fence dividing the wetland (Left) and the school of introduced mosquitofish (Right). [Colour figure can be viewed at wileyonlinelibrary.com]

occurred prior to fish introduction (May 16 and June 1), and the last four occurred after fish introduction (June 17, July 4, July 15 and July 25). Nutrients were sampled on the last five dates and phytoplankton fluorescence was measured on the last four dates. For nutrient analyses, three water samples per wetland side per sampling date were collected within acid-washed Nalgene bottles (100 mL), frozen and analysed for total nitrogen and total phosphorus using standard protocols (see: <https://instaar.colorado.edu/research/labs-groups/kiowa-environmental-chemistry-laboratory/>). We quantified relative phytoplankton fluorescence on five water samples per wetland side per date using a Turner Designs Laboratory Fluorometer (Sunnyvale). Zooplankton were sampled with six 10 m horizontal tows of a 60 µm zooplankton net per wetland side per date. The first author conducted all of the tows by pulling the zooplankton net at a rate of 1 m per s across a distance of 10 m. After preservation, zooplankton collections were standardised in volume and subsampled (10 mL) to identify the abundance of five major taxa: *Daphnia* (Daphniidae), *Bosmina* (Bosminidae), Copepoda, Sididae or Rotifera.

Aquatic macroinvertebrates (Table S1) and amphibian larvae, including Pacific chorus frogs (*Pseudacris regilla*: Hylidae) and California newts (*Taricha torosa*: Salamandridae), were sampled using ten stovepipe samples per wetland side per date and measured for body size (snout-vent length) and converted into dry biomass densities using length-to-mass regressions (Anderson, Daring & Benke, 1998; Benke *et al.*, 1999; Edwards *et al.*, 2009; Preston *et al.*, 2013). Our stovepipe sampler measured 53 cm in diameter and 74 cm tall (0.223 m² of wetland bottom per sample) and we used a D-frame dipnet (1.4 mm mesh; 2600 cm² opening) to remove organisms from within the sampler until five consecutive sweeps yielded zero additional organisms. Abundance and biomass data from stovepipe samples were converted into densities (or biomass densities) of organisms per square metre to facilitate comparison with other studies. Our protocols for sampling amphibians and invertebrates were developed from prior standardised methods and have been adapted for the area and depth of our study site (Olson, Leonard & Bury, 1997; Turner & Trexler, 1997; Meyer, Peterson & Whiles, 2011).

To collect additional data on amphibian abundances and body sizes, we performed ten dipnet sweeps from the shore and three seine net hauls in the deepest portion of the wetland on every sampling date. These additional methods allowed us to capture possible variation

in amphibian abundances in the shallowest and deepest portions of the wetland respectively. Each dipnet sweep was performed perpendicular to the shoreline by extending the net 1 m out and pulling it rapidly towards shore. Our seine net hauls were ~3 m in length and the net measured 1 × 3 m in area with 4 mm mesh. On July 28, we collected a haphazard sample of chorus frog metamorphs from each side of the wetland to quantify amphibian abnormalities that can be caused by mosquitofish attacks (Preston *et al.*, 2012; Shulse & Semlitsch, 2014). Lastly, on August 16, we estimated the total population size of mosquitofish on the west side of Hog Lake using standardised dipnet sweeps (as described for amphibians). By this time, the wetland had dried down to a small area that we measured (c. 110 sq. m), making it possible to sample a large fraction of the population efficiently and convert dipnet catches into a fish density.

Mesocosm experiment

Our aim in the outdoor mesocosm experiment was to examine how two features of natural wetlands – the complexity of the habitat and the availability of alternative prey – influenced the strength of mosquitofish predation on Pacific chorus frogs. To achieve this aim, we conducted a 2 × 2 factorial experiment manipulating prey availability (high or low) and habitat complexity (high or low). Prior work has demonstrated the negative effects of mosquitofish on Pacific chorus frogs within similar outdoor mesocosms (Preston *et al.*, 2012). Given this past work, we did not include fishless treatments in our experiment because our aim was to understand factors mediating negative mosquitofish impacts (rather than to demonstrate that mosquitofish can prey on chorus frogs). Each treatment was replicated five times within 378 L outdoor mesocosms that measured 134 cm in length, 63 cm in height and 79 cm in width. Each mesocosm was covered with a screen lid and contained 6 kg sand, and a mixture of 50 g of dry leaves of *Quercus* (Fagaceae) and *Arbutus* (Ericaceae), supplemented nitrogen and phosphorus sources (KH₂PO₄ and NaNO₃), and algae and zooplankton inocula from a local wetland. The algae inoculum was collected by shaking macrophytes into a bucket of pond water and then filtering the resulting material through a 1.4 mm mesh sieve to obtain water containing algal cells. The zooplankton was collected with a zooplankton net (60 µm), homogenised in a five gallon bucket, and then allocated into equal volumes before addition to the mesocosms. In the high prey availability treatments, we added an additional 200 mL

of concentrated *Daphnia* and copepods daily to each mesocosm. In the high habitat complexity treatments, we added ~1 kg wet mass of aquatic macrophytes to each mesocosm (native *Myriophyllum sibiricum*: Haloragaceae). We thoroughly rinsed macrophytes to remove invertebrates and other organisms prior to addition. To initiate the experiment, we added 15 chorus frog larvae and three adult mosquitofish (two female, one male) to each mesocosm. Approximately 3 weeks after the addition of mosquitofish, we ended the experiment and quantified amphibian survival and growth (wet mass and snout-vent length), and the density of zooplankton. We combined five zooplankton samples collected per mesocosm with a tube sampler (70 cm length × 5 cm diameter; 2.35 L volume per sample). Zooplankton samples were passed through a mesh screen (58 µm) and preserved in ethanol for subsequent quantification. The experiment was ended at this time to preclude metamorphosis of amphibian larvae inside the mesocosms.

Analyses

For the ecosystem experiment, we used linear models with wetland treatment (fish or fishless), sampling time point (one to six), and their interaction as predictors. We expected that the effects of mosquitofish would manifest as an interaction between wetland side (i.e. treatment) and time, with the magnitude of effects increasing with time in the fish addition treatment. For invertebrate and amphibian body sizes responses, we pooled all individuals on the same date per wetland side from multiple samples and included a random intercept term for the stovepipe sample identity (ten per sampling date) using linear mixed effects models (Zuur *et al.*, 2009). We pooled individuals across samples because many stovepipe samples contained zero or only a few individuals of certain taxa. In these cases, sample level mean body sizes would have been highly sensitive to variation in the number of individuals per sample. For all other responses from Hog Lake (i.e. density and biomass of organisms, water chemistry and phytoplankton), we used sample means (rather than individuals nested within samples) and general linear models with transformations if they improved model assumptions (Ives, 2015). We emphasise that because our analyses treat samples from the same side of the wetland on the same date as independent samples, we consider the two sides of the wetland as our populations of interest, rather than multiple wetlands across the landscape. As a result, extrapolation of our results is restricted to the specific wetland under study (Fig. 1).

For the mesocosm experiment, we used linear mixed effects (LME) models with fixed effects of prey availability (high or low), habitat complexity (high or low), and their interaction. We used a binomial error distribution for survival data and included a random intercept term for mesocosm identity (Zuur *et al.*, 2009). For amphibian growth responses (wet mass and snout-vent-length) we used the same model structure with a Gaussian distribution, and for zooplankton abundance we used means per mesocosm (log-transformed), as the response in a general linear model. All analyses were conducted using the R computing environment (R Core Team 2014).

Results

Ecosystem experiment

The introduced mosquitofish in Hog Lake reproduced rapidly, increasing from the initial 120 individuals to an estimated population size of ~11 074 fish by mid-August (95% confidence interval = 7096–15 051). We did not detect mosquitofish on the east side of the wetland at any point during our sampling, nor did mosquitofish reappear the following year.

Mosquitofish caused significant reductions in zooplankton and macroinvertebrate abundances. Total zooplankton abundance decreased by 90% in the fish treatment by the final sampling date (LM, treatment*time, $t = -2.69$, $P = 0.009$). This effect was driven primarily by reductions in *Bosmina* cladocerans (LM, treatment*time, $t = -3.43$, $P = 0.001$; Fig. 2a) and brachiopods in the family Sididae (LM, treatment*time, $t = -2.51$, $P = 0.015$; Fig. 2b), which together represented 76% of the total zooplankton individuals across all samples. *Daphnia* (Fig. 2c) and rotifers (Figure S1) also decreased with mosquitofish presence, although their abundances were much lower overall (*Daphnia*: LM, treatment*time $t = -2.11$, $P = 0.038$; rotifers: LM, treatment*time, $t = -2.04$, $P = 0.044$), and we did not observe an effect on copepods (LM, treatment*time, $t = -0.97$, $P = 0.34$; Fig. 2d).

In addition to zooplankton, mosquitofish decreased the total abundance (LM, treatment*time, $t = -3.37$, $P = 0.001$) and total biomass (LM, treatment*time, $t = -2.70$, $P = 0.008$) of aquatic macroinvertebrates. Damselflies (*Lestes* and Coenagrionidae; LM, treatment*time, $t = -3.91$, $P = 0.0001$) and mayflies (*Calibaetis*; LM, treatment*time, $t = -3.02$, $P = 0.003$) were the most abundant aquatic insect taxa and exhibited the strongest reductions, showing densities that were two to five times lower on the side with fish (Fig. 2e, f).

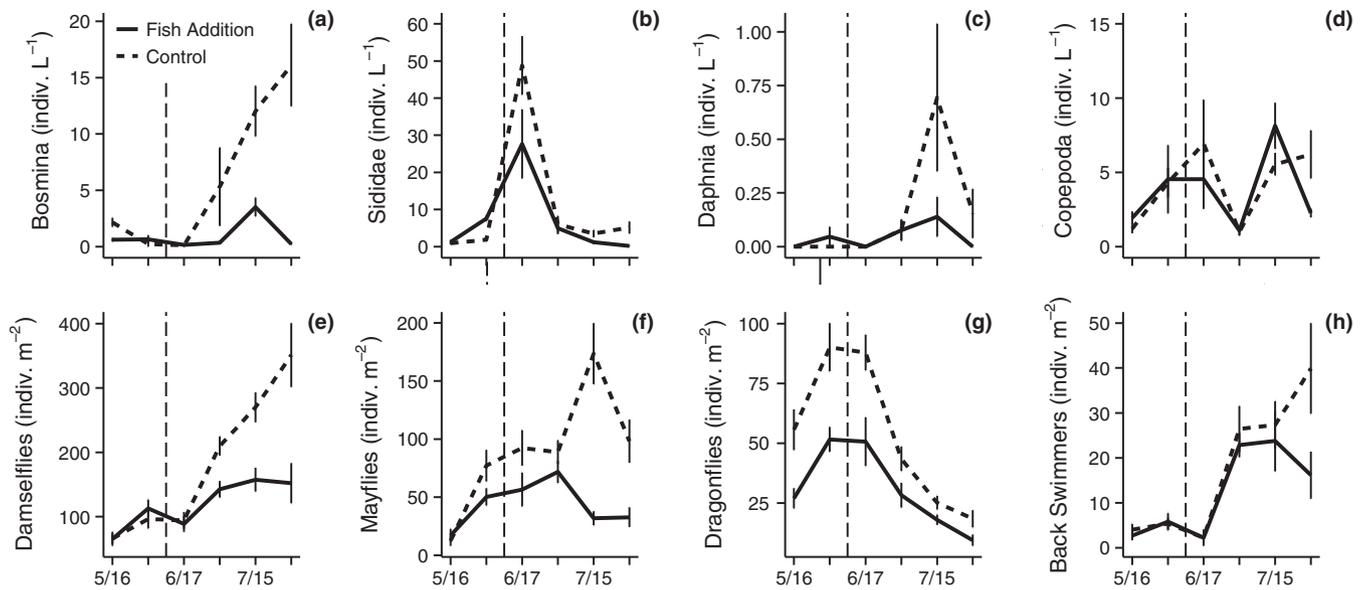


Fig. 2 Effects of mosquitofish on zooplankton and invertebrate taxa within a wetland ecosystem experiment. The dashed vertical line indicates the date of mosquitofish introduction and the legend for all plots is shown at the top left. The top row shows zooplankton responses including *Bosmina* (a), *Sididae* (b), *Daphnia* (c) and Copepoda (d). The bottom row shows macroinvertebrate responses including damselflies (e), mayflies (f), dragonflies (g) and back swimmers (h). Zooplankton data are means from net tows and macroinvertebrate data are means from stovepipe samples.

Dragonflies (*Sympetrum*: LM, treatment*time, $t = 0.63$, $P = 0.50$) and backswimmers (*Notonecta*: LM, treatment*period, $t = -1.23$, $P = 0.22$) were not significantly reduced in the presence of mosquitofish, although the latter group was two times less abundant on the side of the wetland with fish on the final sampling date (Fig. 2g, h). We did not detect significant differences in the abundances of water boatmen (*Corixidae*), aquatic beetles (*Coleoptera*), California clam shrimp (*Cyzicus californicus*), Holarctic clam shrimp (*Lyneceus brachyurus*), midges (*Chironomidae*), leeches (*Erpobdella*) or ramshorn snails (*Helisoma trivolvis*) (see Table S1, Figure S2 and Appendix S1 for statistics).

In contrast to our predictions and results of the smaller scale experiments, mosquitofish did not have strong effects on Pacific chorus frogs or California newts in Hog Lake. Chorus frog larvae numbers in stovepipe samples declined over the summer as frogs metamorphosed but there were no effects of mosquitofish on larval abundance (LM, treatment*time, $t = -0.23$, $P = 0.82$; Fig. 3a), total biomass (LM, treatment*time, $t = 0.55$, $P = 0.58$) or individual body mass (LME, treatment*time, $t = -1.41$, $P = 0.16$; Fig. 3b). The density of California newt larvae in stovepipe samples were higher in the fish treatment throughout the study, likely due to initial differences in egg oviposition, and mosquitofish did not alter newt abundance (LM, treatment*time, $t = -1.19$, $P = 0.24$; Fig. 3c) or total biomass (LM,

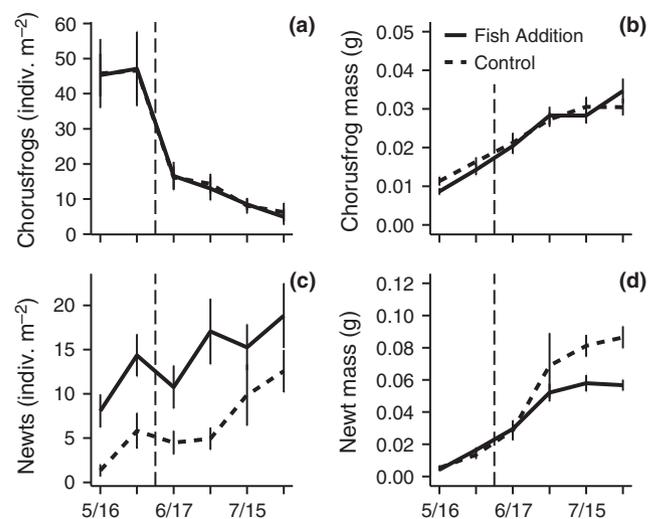


Fig. 3 Effects of mosquitofish on native amphibian taxa within a wetland ecosystem experiment. The dashed vertical line indicates the date of mosquitofish introduction and the legend for all plots is shown at the top right. Responses comprising Pacific chorus frog density (a), chorus frog individual body mass (b), California newt density (c) and California newt individual body mass (d). All data are means per sampling date from stovepipe samples.

treatment*time, $t = -0.24$, $P = 0.81$). Larval newts, however, did have a smaller individual body mass at the end of the experiment in the mosquitofish treatment (LME, treatment*time, $t = 3.13$, $P = 0.002$; Fig. 3d). Amphibian abundances in dipnet sweeps and seine net

hauls demonstrated the exact same patterns that were observed from stovepipe samples. At the end of the summer, we detected more chorus frog leg abnormalities in the mosquitofish treatment (4 out of 209) than on the control side (0 out of 145); however, the overall frequency of abnormalities was low and the difference was not significant (Pearson chi-square = 1.31, $df = 1$, $P = 0.25$).

Mosquitofish also influenced nutrients and phytoplankton. The addition of mosquitofish was associated with a ~50% decrease in total phosphorus (LM, treatment*time, $t = 9.81$, $P < 0.0001$; Fig. 4a) and a 13% decrease in total nitrogen (LM, treatment*time, $t = 2.79$, $P = 0.009$; Fig. 4b). Correspondingly, N:P molar ratios in water samples were ~2× higher with mosquitofish (LM, treatment*time, $t = -6.74$, $P < 0.0001$; Fig. 4c). Relative phytoplankton fluorescence followed a similar pattern as phosphorus, with ~50% lower fluorescence values in the mosquitofish treatment by the final sampling date (LM, treatment*time, $t = 4.61$, $P < 0.0001$; Fig. 4d).

Mesocosm experiment

Results from the mesocosm experiment showed that alternative prey and habitat complexity reduced the predatory effects of mosquitofish on chorus frog larvae (Fig. 5). In the control treatments, chorus frog survival averaged 37%, whereas the survival increased to a mean

of 70% and 77%, respectively, in mesocosms receiving increased prey or increased habitat complexity (GLMM, prey availability, $z = 1.94$, $P = 0.052$; habitat complexity, $z = 2.05$, $P = 0.040$; Fig. 5a). Amphibian survival was highest (79%) with both increased prey availability and increased habitat complexity, although we did not detect any significant interactions (GLMM, prey*habitat, $z = -1.24$, $P = 0.216$). Chorus frog snout-vent length increased by 15% in the high habitat complexity treatments (LME, $t = 2.48$, $P = 0.025$), whereas added prey availability alone did not affect chorus frog body size (LME, $t = 0.264$, $P = 0.79$; Fig. 5b). We did not detect a significant prey availability-by-habitat complexity interaction (LME, $t = 0.67$, $P = 0.51$), although the addition of both macrophytes and zooplankton increased mean snout-vent length by 23% relative to controls. At the conclusion of the experiment, zooplankton densities were highest in the two treatments containing elevated habitat complexity (Figure S3). All mosquitofish survived to the end of the experiment.

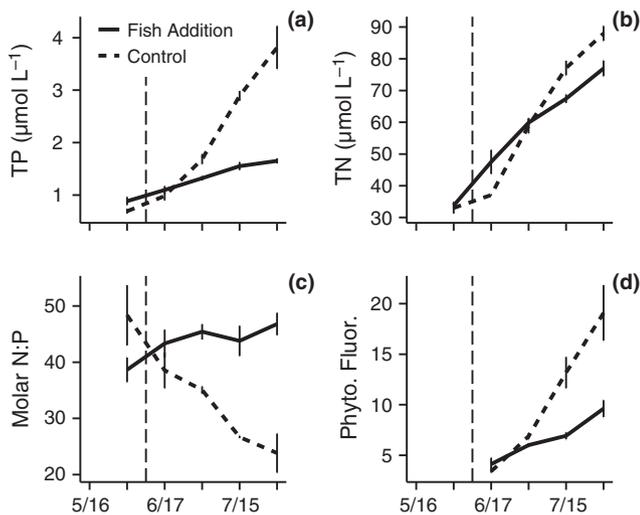


Fig. 4 Effects of mosquitofish on nutrients and phytoplankton within a wetland ecosystem experiment. The dashed vertical line indicates the date of mosquitofish introduction and the legend for all plots is shown at the top left. Responses include total phosphorus (a), total nitrogen (b), molar nitrogen to phosphorus ratio (c) and relative phytoplankton fluorescence (d).

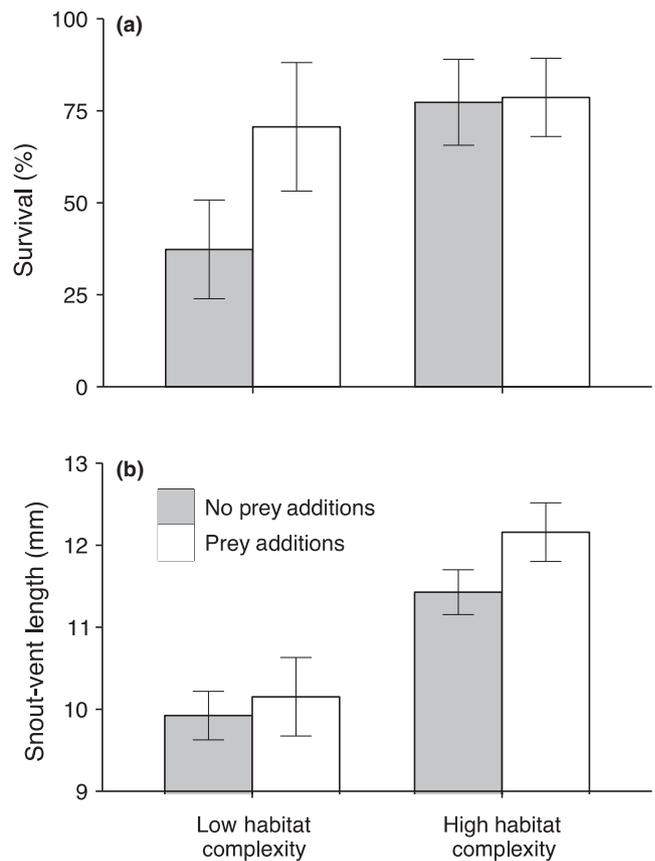


Fig. 5 Effects of mosquitofish on Pacific chorus frog larvae in an outdoor mesocosm experiment. An increase in alternative prey (zooplankton) and/or habitat complexity (aquatic plants) mediated the negative effects of mosquitofish on chorus frog survival (a) and growth (b).

Discussion

Introduced mosquitofish in Hog Lake reproduced rapidly and caused large changes in community- and ecosystem structure, including nutrient dynamics, phytoplankton production and the abundance of zooplankton and macroinvertebrates. In outdoor mesocosms, habitat complexity in the form of macrophytes and alternative prey in the form of zooplankton – which vary widely in abundance across lentic ecosystems – weakened the negative effects of mosquitofish on amphibian larvae. These factors likely contributed to the coexistence of mosquitofish and native amphibian larvae in our ecosystem experiment. Taken together, our results reinforce the strong effects of invasive fish on freshwater communities, while also emphasising the dynamic nature of species interactions and the potential for invasion impacts to vary across time and/or space.

The experiment in Hog Lake allowed us to examine the effects of mosquitofish within a natural wetland setting containing a complex food web. Predatory effects of mosquitofish were taxon-specific and varied with the abundances and traits of prey. Consistent with previous research from other venues, we found that mosquitofish strongly altered invertebrate abundances and community composition (Hurlbert, Zedler & Fairbanks, 1972; Miura, Takahashi & Wilder, 1984; Leyse *et al.*, 2004). The most abundant zooplankton and macroinvertebrate taxa showed suppressed population growth with mosquitofish, leading to significant reductions in total invertebrate numbers and biomass relative to the fishless treatment. The most affected insect taxa – damselflies and mayflies – are soft-bodied, which likely makes them more susceptible than taxa that did not show significant changes in numbers (e.g. dragonflies, hemipterans, clam shrimp, snails). Similar decreases in soft-bodied prey, with little change in abundance of hard-bodied prey, have been observed after mosquitofish introduction in rice fields (Miura *et al.*, 1984). Given that the most strongly affected insect groups also metamorphose into terrestrial adults, mosquitofish could reduce subsidies from aquatic environments to the surrounding terrestrial landscape (Merkley *et al.*, 2015). In general, the strength of mosquitofish effects on native species is likely dependent on the characteristics of native community members including palatability, niche overlap and life-history traits (Macdonald *et al.*, 2012).

The Hog Lake experiment also allowed us to measure ecosystem-level responses, including nutrient concentrations and phytoplankton abundance. Interestingly, the

effects of mosquitofish on nutrients and phytoplankton were opposite to our predictions and most past work. Fish addition led to a 50% reduction in total phosphorus in the water column, an increase in nitrogen-to-phosphorus ratios, and a 50% decrease in phytoplankton production. The reduction in phytoplankton became larger over the course of the summer despite the fact that mosquitofish reduced zooplankton abundance by 13-fold. These results contrast with past work showing that mosquitofish induce trophic cascades by removing top-down zooplankton controls on phytoplankton (Hurlbert *et al.*, 1972; Nagdali & Gupta, 2002). For instance prior work from artificial pools has found that mosquitofish decrease zooplankton abundance, leading to large algal blooms associated with a 100–200 fold increase in some phytoplankton groups (Hurlbert *et al.*, 1972). One possible explanation for our different results is that Hog Lake phytoplankton are more limited by nutrients (particularly phosphorus) than by zooplankton grazing, and that mosquitofish are compounding this nutrient limitation through the assimilation of biomass. Fish biomass can contain up to 75% of the limnetic phosphorus in lakes (Kitchell, Koonce & Tennis, 1975) and a rapidly growing fish population, as was present in our study, is most likely to act as a nutrient sink rather than a source (Kraft, 1992). The relatively high nitrogen to phosphorus ratio (c. 50) in the fish treatment at the end of the summer supports the idea that the wetland was phosphorus limited, particularly towards the end of the dry summer period when nutrient inputs are minimal.

The mosquitofish addition into Hog Lake had few detectable effects on native Pacific chorus frogs or California newts, despite considerable evidence that introduced fishes in general, and mosquitofish in particular, are a contributor to declines in amphibian populations in the western United States (Fisher & Shaffer, 1996; Kats & Ferrer, 2003; Vredenburg, 2004; Joseph, Preston & Johnson, 2016). Several, non-mutually exclusive mechanisms could underlie this result. Prior work suggests that mosquitofish can coexist with certain native amphibians, including California red-legged frogs in experimental wetlands (Lawler *et al.*, 1999) and several species of frogs in southwestern Australia wetlands (Reynolds, 2009). In the study with red-legged frogs, the authors posit that co-existence may have occurred due to a combination of high alternative prey availability, spatial refuges in cobbles and vegetation, and a decrease in predatory invertebrates that feed on amphibian larvae (Lawler *et al.*, 1999). In our study, it is possible that the dense shoreline vegetation and an abundance of zooplankton and aquatic macroinvertebrates alleviated

predation pressure on the native amphibians, both species of which are palatable to mosquitofish (Gamradt & Kats, 1996; Goodsell & Kats, 1999; Preston *et al.*, 2012). More broadly, a growing body of literature has highlighted how the outcome of species invasions can be context dependent, often depending on levels biotic or abiotic resistance from the invaded community (Strayer *et al.*, 2006; Ricciardi *et al.*, 2013; Zenni and Nuñez 2013).

It is possible that an earlier introduction of mosquitofish, such that fish overlapped more with the earliest and most vulnerable amphibian larval stages, could have led to stronger population-level effects on amphibians. Prior studies show that early larval stages are more readily consumed than eggs or late-stage tadpoles for most amphibian species (Zeiber *et al.*, 2008; Reynolds, 2009; Smith & Smith, 2015). If predation pressure is highest on early larval stages, mosquitofish effects may vary temporally such that the strongest predatory effects occur shortly after embryos emerge from egg masses. California newts at Hog Lake lay eggs in February and March, whereas chorus frogs reproduce from February to June/July, such that larvae are smallest in the early spring months. While small amphibian larvae are probably most susceptible to predation, we have observed mosquitofish consume metamorphic chorus frogs in the field, and fish attacks can cause sublethal injuries to the tails and limbs of amphibian larvae that are approaching metamorphosis, indicating that effects may extend to later age/size classes under some conditions (Preston *et al.*, 2012; Shulse & Semlitsch, 2014). Collectively, our findings suggest that negative effects of mosquitofish on native amphibians can be strong under certain settings, but they may also be influenced by factors such as community structure, habitat characteristics and productivity. Prior research has also shown that hydrological characteristics can mediate the effects of mosquitofish on some native species (Ho, Bond & Lake, 2011). Repeating our mosquitofish introduction within a less complex or productive habitat, and/or introducing mosquitofish earlier in the season, may have resulted in stronger effects on amphibians.

Results from our outdoor mesocosm experiment support the hypothesis that habitat complexity and/or alternative prey availability can weaken the predatory effects of mosquitofish on native amphibians. Aquatic macrophytes attenuated the negative effects of mosquitofish on amphibian survival by 50%, suggesting that more complex habitats may facilitate the coexistence of amphibians and predatory fish in natural wetlands (Babbitt & Tanner, 1997; Hartel *et al.*, 2007). Macrophytes provided cover from predation and also increased the abundance of zooplankton in mesocosms. At the conclusion of the

mesocosm experiment, the treatments with high habitat complexity supported the highest zooplankton densities, suggesting that the aquatic plants increased zooplankton populations and thereby weakened predation on native amphibians by increasing alternative prey. Zooplankton are probably the more preferred prey item of mosquitofish when they are sufficiently available (Hurlbert & Mulla, 1981; Miura *et al.*, 1984; García-Berthou, 1999). While our mesocosm experiment informed possible mechanisms underlying results in Hog Lake, we note that a longer duration mesocosm study and a higher density of fish due to reproduction could have led to greater depletion of alternative prey and stronger effects of mosquitofish on amphibian larvae, particularly in the treatments lacking zooplankton additions. It is also possible that long-term effects of mosquitofish introductions (i.e. effects manifesting over several seasons) would be stronger than the effects observed in our experiments. Nonetheless, our results suggest that variation in environmental factors between wetlands can mediate the effects of fish invasions on native communities. The presence of aquatic macrophytes and emergent vegetation such as *Typha* (Typhaceae) and *Juncus* (Juncaceae) in California wetlands varies dramatically from completely absent to 100% shoreline cover, often in association with livestock grazing intensity (Joseph *et al.*, 2016). Such variability emphasises the potential for disparate mosquitofish impacts across discrete wetlands, and for possible interactions between grazing intensity and local invasive fish effects.

Taken together, results of our ecosystem manipulation and mesocosm experiment reinforce the need to consider variation in invasive species impacts across natural systems. A more nuanced understanding of when and where invaders are likely to have the strongest effects will benefit from knowledge of the specific biotic and abiotic mechanisms that drive variation in invasion outcomes (e.g. environmental change or community composition) (Ricciardi *et al.*, 2013). Our results also complement and extend the considerable body of literature demonstrating that mosquitofish introductions can negatively affect native species, underscoring the need for land managers to limit new introductions and pursue mosquitofish removal if their goals are to conserve native wetland communities.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Responses of rotifers (a) and cladocerans (other than *Daphnia* spp.) (b) in the Hog Lake experiment. The dashed vertical line indicates the date of mosquitofish introduction and the legend for all plots is shown at the top left.

Figure S2. Additional macroinvertebrate responses in the Hog Lake experiment, including water boatmen (*Corixa*) (a), California clam shrimp (*Cyzicus californicus*) (b), beetles (Coleoptera) (c), Holarctic clam shrimp

(*Lynceus brachyurus*) (d), midges (Chironomidae) (e), leeches (*Erpobdella*) (f) and ramshorn snails (*Helisoma trivolvis*) (g). The dashed vertical line indicates the date of mosquitofish introduction and the legend for all plots is shown at the top left.

Figure S3. Density of zooplankton at the end of the mesocosm experiment. Zooplankton consist of *Daphnia* and copepods.

Table S1. Aquatic macroinvertebrate list from Hog Lake.

Appendix S1. Additional Hog Lake Results.

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