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4
5 Received Date : 27-Apr-2016

6 Revised Date : 13-Dec-2016

7 Accepted Date : 14-Dec-2016

8 Article type : Standard Paper

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11 Running head: Invasive fish and freshwater wetlands

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

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27 **Keywords:** introduced species, nonnative, freshwater pond, amphibian decline, mosquito
28 biocontrol

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/fwb.12900](https://doi.org/10.1111/fwb.12900)

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Summary

1. The ecological consequences of species invasions can vary in time and space, complicating efforts to generalize invader impacts across ecosystems. This challenge is particularly relevant when using small-scale experiments to derive predictions for freshwater ecosystems. In the present 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

61 complexity of the habitat (i.e., presence of aquatic macrophytes). Our mesocosm results support
62 the hypothesis that alternative prey and habitat complexity facilitated coexistence between
63 invasive mosquitofish and native amphibian larvae in our ecosystem experiment.

64 5. Our findings highlight the potential for invasive fish to drive rapid shifts in freshwater
65 ecosystems while also emphasizing the roles of environmental characteristics in mediating
66 whether native and nonnative species will coexist.

67 68 **Introduction**

69 Predicting the outcome of species invasions is a key goal in ecology, yet it can be
70 challenging to achieve due to variation in invader impacts through time and space (Ricciardi *et*
71 *al.* 2013). A range of variables, including individual traits, community structure, the densities of
72 interacting species, and abiotic factors, can directly or indirectly mediate the strength of species
73 interactions (Abrams 2001; Peacor & Werner 2004). As a result, the effects of invasive species
74 can vary across ecological scales, between discrete ecosystems, or over time (Strayer *et al.* 2006;
75 Ricciardi *et al.* 2013; Latzka *et al.* 2016). For instance, within the Laurentian Great Lakes Basin,
76 where >180 invasive species have established, the effects of nonnative round gobies on benthic
77 invertebrates can vary from strongly positive to strongly negative depending on the relative
78 importance of top-down versus bottom-up controls on community dynamics (Pagnucco &
79 Ricciardi 2015). It remains relatively uncommon, however, to quantify such variation, examine
80 its underlying drivers, or incorporate it into predictions or management efforts (Thomsen *et al.*
81 2011; Hulme *et al.* 2013; Dick *et al.* 2014).

82 Variation in the consequences of species invasions can also manifest in experimental
83 venues, in some cases influencing the degree to which observed effects can be extrapolated to
84 natural systems. For instance, experimental design, including the scale of experimental venue,
85 can drive variation in the strength of observed species interactions (Skelly & Kiesecker 2001;
86 Lunde, Resh & Johnson 2012). Variation in interaction strengths may be of interest if it is
87 relevant to the ecological questions being asked, or at worst, it may lead to artifactual outcomes
88 that have little relevance in nature (Carpenter 1996; Schindler 1998). One promising approach to
89 understand variation in the strength of interactions between native and nonnative species is to
90 combine experiments at multiple scales (Petersen, Cornwell & Kemp 1999; Sandel & Smith
91 2009). Small and medium-scale experiments (e.g., laboratory trials, microcosms, mesocosms) are

92 valuable in isolating and testing possible mechanisms, while large-scale experiments (e.g.,
93 ecosystem manipulations) are then useful in testing the relative magnitude of effects in a more
94 natural setting (e.g., Vredenburg 2004). In this way, combining multiscale experiments can be a
95 powerful approach to understand invasive species effects because it integrates mechanistic
96 understanding with realism.

97 Among freshwater invasive species, mosquitofish (*Gambusia affinis* and *G. holbrooki*:
98 Poeciliidae) are one of the most widespread and there is a need to understand variation in their
99 effects across temporal and spatial scales. Mosquitofish are native to the eastern United States
100 and have been introduced to freshwaters on all continents except Antarctica as a biocontrol agent
101 of mosquito larvae (Pyke 2008). Mosquitofish are generalist predators and can prey on a wide
102 range of invertebrates (e.g., Hurlbert & Mulla 1981; Leyse, Lawler & Strange 2004; Merkley,
103 Rader & Schaalje 2015), amphibians (e.g., Webb & Joss 1997; Zeiber, Sutton & Fisher 2008;
104 Shulse, Semlitsch & Trauth 2013) and fishes (e.g., Mills, Rader & Belk 2004;
105 Henkanathgedara & Stockwell 2014). Most native amphibian species from the western United
106 States are susceptible to mosquitofish predation (Gamradt & Kats 1996; Goodsell & Kats 1999;
107 Preston, Henderson & Johnson 2012), although at least one wetland experiment (Lawler *et al.*
108 1999) and our own observations in northern California suggest that mosquitofish coexist with
109 native amphibians in some settings. Testing how variability in environmental characteristics
110 between lentic water bodies mediates invasion consequences for native communities will
111 therefore be useful to predict and manage undesired mosquitofish impacts.

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

122

123 **Materials and Methods**

124 *Ecosystem experiment* – We used a ‘Before-After-Control-Impact’ (BACI) design to
125 quantify the effects of mosquitofish introduction within a natural wetland (Hog Lake) located at
126 the Hopland Research and Extension Center in Mendocino County, California (39.0316N,
127 123.0789W). A BACI design is useful to examine the effects of invasive species because it
128 allows comparisons before and after invasion at the same site, as well as across invaded and
129 uninvaded replicates over space (Smith 2002). Hog Lake, which is ~3,200 sq. meters and ~1.5 m
130 deep when full (Fig. 1), was divided into two equal sections using an impermeable rubber pond
131 liner that prevented the passage of organisms and water (see Lunde, Resh & Johnson 2012 for
132 additional details). The barrier extended approximately 0.5 m into the wetland substrate and 0.5
133 m above the water line at the start of the study. Hog Lake was chosen because it supported a
134 diverse native community, including two pond-breeding amphibian species, and it provided a
135 unique opportunity for a reversible species introduction. Because the system dries by the end of
136 the summer (August or September) and is relatively isolated from other wetlands, added fish
137 could not persist between years or escape to other habitats. The wetland is naturally fish-free.
138 Our prior surveys of >200 wetlands in northern California indicated that the invertebrate and
139 amphibian communities within Hog Lake were very similar to permanent wetland systems in the
140 region (Preston, Henderson & Johnson 2012, Preston *et al.* 2013; Johnson *et al.* 2013). Rushes
141 (*Juncus* spp.: Juncaceae) constitute the dominant emergent vegetation around Hog Lake.

142 Due to the logistical challenges associated with controlled introductions of nonnative
143 species to a natural ecosystem, we performed our experiment within a single unreplicated
144 system. The BACI design using a split wetland improved our ability to interpret whether
145 treatment effects were driven by mosquitofish because we collected detailed information on pre-
146 invasion wetland characteristics. Additionally, both wetland treatments were highly similar at the
147 start of the experiment. While this approach precludes extrapolation of our results to other
148 ecosystems, it ensures that environmental variability between wetlands – such as differences in
149 community structure or productivity – is not confounded with treatment effects.

150 We sampled both sides of Hog Lake every two weeks over the summer of 2011 for a total
151 of six sampling dates. We quantified nutrient concentrations, relative phytoplankton
152 fluorescence, and the abundances of zooplankton, macroinvertebrates, and amphibian larvae. The
153 experiment was initiated on June 9th of 2011, when we introduced 60 adult male and 60 adult

154 female mosquitofish (*Gambusia affinis*) to the west side of Hog Lake. We did not add the fish
155 earlier in the season because we needed to ensure that the water level in the wetland was low
156 enough to prevent introduced mosquitofish from moving over the top of the dividing barrier or
157 escaping to nearby water bodies that are more connected during the wet season. For all responses
158 except nutrients and phytoplankton, the first two sampling dates occurred prior to fish
159 introduction (May 16 and June 1), and the last four occurred after fish introduction (June 17, July
160 4, July 15, and July 25). Nutrients were sampled on the last five dates and phytoplankton
161 fluorescence was measured on the last four dates. For nutrient analyses, three water samples per
162 wetland side per sampling date were collected within acid-washed Nalgene bottles (100 mL),
163 frozen, and analyzed for total nitrogen and total phosphorus using standard protocols
164 (see: [https://instaar.colorado.edu/research/labs-groups/kiowa-environmental-chemistry-](https://instaar.colorado.edu/research/labs-groups/kiowa-environmental-chemistry-laboratory/)
165 [laboratory/](https://instaar.colorado.edu/research/labs-groups/kiowa-environmental-chemistry-laboratory/)). We quantified relative phytoplankton fluorescence on five water samples per
166 wetland side per date using a Turner Designs Laboratory Fluorometer (Sunnyvale, California).
167 Zooplankton were sampled with six 10 m horizontal tows of a 60 µm zooplankton net per
168 wetland side per date. The first author conducted all of the tows by pulling the zooplankton net at
169 a rate of 1 m per s across a distance of 10 m. After preservation, zooplankton collections were
170 standardized in volume and subsampled (10 ml) to identify the abundance of five major taxa:
171 *Daphnia* (Daphniidae), *Bosmina* (Bosminidae), Copepoda, Sididae, or Rotifera.

172 Aquatic macroinvertebrates (Table S1) and amphibian larvae, including Pacific chorus
173 frogs (*Pseudacris regilla*; Hylidae) and California newts (*Taricha torosa*; Salamandridae), were
174 sampled using ten stovepipe samples per wetland side per date and measured for body size
175 (snout-vent length) and converted into dry biomass densities using length-to-mass regressions
176 (Anderson, Darring & Benke 1998; Benke *et al.* 1999; Edwards *et al.* 2009; Preston *et al.* 2013).
177 Our stovepipe sampler measured 53 cm in diameter and 74 cm tall (0.223 m² of wetland bottom
178 per sample) and we used a D-frame dipnet (1.4 mm mesh; 2,600 cm² opening) to remove
179 organisms from within the sampler until five consecutive sweeps yielded zero additional
180 organisms. Abundance and biomass data from stovepipe samples were converted into densities
181 (or biomass densities) of organisms per square meter to facilitate comparison with other studies.
182 Our protocols for sampling amphibians and invertebrates were developed from prior
183 standardized methods and have been adapted for the area and depth of our study site (Turner &
184 Trexler 1997; Olson *et al.* 2007; Meyer *et al.* 2011).

185 To collect additional data on amphibian abundances and body sizes, we performed ten
186 dipnet sweeps from the shore and three seine net hauls in the deepest portion of the wetland on
187 every sampling date. These additional methods allowed us to capture possible variation in
188 amphibian abundances in the shallowest and deepest portions of the wetland, respectively. Each
189 dipnet sweep was performed perpendicular to the shoreline by extending the net 1 m out and
190 pulling it rapidly toward shore. Our seine net hauls were ~3 m in length and the net measured 1
191 m x 3 m in area with 4 mm mesh. On July 28, we collected a haphazard sample of chorus frog
192 metamorphs from each side of the wetland to quantify amphibian abnormalities that can be
193 caused by mosquitofish attacks (Preston, Henderson & Johnson 2012; Shulse & Semlitsch 2014).
194 Lastly, on August 16, we estimated the total population size of mosquitofish on the west side of
195 Hog Lake using standardized dipnet sweeps (as described for amphibians). By this time, the
196 wetland had dried down to a small area that we measured (~110 sq. m), making it possible to
197 sample a large fraction of the population efficiently and convert dipnet catches into a fish
198 density.

199 *Mesocosm experiment* – Our aim in the outdoor mesocosm experiment was to examine
200 how two features of natural wetlands – the complexity of the habitat and the availability of
201 alternative prey – influenced the strength of mosquitofish predation on Pacific chorus frogs. To
202 achieve this aim, we conducted a 2 x 2 factorial experiment manipulating prey availability (high
203 or low) and habitat complexity (high or low). Prior work has demonstrated the negative effects of
204 mosquitofish on Pacific chorus frogs within similar outdoor mesocosms (Preston, Henderson &
205 Johnson 2012). Given this past work, we did not include fishless treatments in our experiment
206 because our aim was to understand factors mediating negative mosquitofish impacts (rather than
207 to demonstrate that mosquitofish can prey on chorus frogs). Each treatment was replicated five
208 times within 378 L outdoor mesocosms that measured 134 cm in length, 63 cm in height, and 79
209 cm in width. Each mesocosm was covered with a screen lid and contained 6 kg sand, 50 g of dry
210 leaves (*Quercus*: Fagaceae and *Arbutus*: Ericaceae), supplemented nitrogen and phosphorus
211 sources (KH_2PO_4 and NaNO_3), and algae and zooplankton inocula from a local wetland. The
212 algae inoculum was collected by shaking macrophytes into a bucket of pond water and then
213 filtering the resulting material through a 1.4 mm mesh sieve to obtain water containing algal
214 cells. The zooplankton was collected with a zooplankton net (60 μm), homogenized in a five
215 gallon bucket, and then allocated into equal volumes before addition to the mesocosms. In the

216 high prey availability treatments, we added an additional 200 ml of concentrated *Daphnia* and
217 copepods daily to each mesocosm. In the high habitat complexity treatments, we added ~1 kg
218 wet mass of aquatic macrophytes to each mesocosm (native *Myriophyllum sibiricum*:
219 Haloragaceae). We thoroughly rinsed macrophytes to remove invertebrates and other organisms
220 prior to addition. To initiate the experiment, we added 15 chorus frog larvae and three adult
221 mosquitofish (two female, one male) to each mesocosm. Approximately three weeks after the
222 addition of mosquitofish, we ended the experiment and quantified amphibian survival and
223 growth (wet mass and snout-vent length), and the density of zooplankton. We combined five
224 zooplankton samples collected per mesocosm with a tube sampler (70 cm length × 5 cm
225 diameter; 2.35 L volume per sample). Zooplankton samples were passed through a mesh screen
226 (58 µm) and preserved in ethanol for subsequent quantification. The experiment was ended at
227 this time to preclude metamorphosis of amphibian larvae inside the mesocosms.

228 *Analyses* – For the ecosystem experiment, we used linear models with wetland treatment
229 (fish or fishless), sampling time point (one to six), and their interaction as predictors. We
230 expected that the effects of mosquitofish would manifest as an interaction between wetland side
231 (i.e., treatment) and time, with the magnitude of effects increasing with time in the fish addition
232 treatment. For invertebrate and amphibian body sizes responses, we pooled all individuals on the
233 same date per wetland side from multiple samples and included a random intercept term for the
234 stovepipe sample identity (ten per sampling date) using linear mixed effects models (Zuur *et al.*
235 2009). We pooled individuals across samples because many stovepipe samples contained zero or
236 only a few individuals of certain taxa. In these cases, sample level mean body sizes would have
237 been highly sensitive to variation in the number of individuals per sample. For all other
238 responses from Hog Lake (i.e., density and biomass of organisms, water chemistry, and
239 phytoplankton), we used sample means (rather than individuals nested within samples) and
240 general linear models with transformations if they improved model assumptions (Ives 2015). We
241 emphasize that because our analyses treat samples from the same side of the wetland on the same
242 date as independent samples, we consider the two sides of the wetland as our populations of
243 interest, rather than multiple wetlands across the landscape. As a result, extrapolation of our
244 results is restricted to the specific wetland under study (Fig. 1).

245 For the mesocosm experiment, we used linear mixed effects (LME) models with fixed
246 effects of prey availability (high or low), habitat complexity (high or low), and their interaction.

247 We used a binomial error distribution for survival data and included a random intercept term for
248 mesocosm identity (Zuur *et al.* 2009). For amphibian growth responses (wet mass and snout-
249 vent-length) we used the same model structure with a Gaussian distribution, and for zooplankton
250 abundance we used means per mesocosm (log-transformed), as the response in a general linear
251 model. All analyses were conducted using the R computing environment (R Core Team 2014).

252

253 **Results**

254 *Ecosystem experiment* – The introduced mosquitofish in Hog Lake reproduced rapidly,
255 increasing from the initial 120 individuals to an estimated population size of ~11,074 fish by
256 mid-August (95% confidence interval = 7096 to 15051). We did not detect mosquitofish on the
257 east side of the wetland at any point during our sampling, nor did mosquitofish re-appear the
258 following year.

259 Mosquitofish caused significant reductions in zooplankton and macroinvertebrate
260 abundances. Total zooplankton abundance decreased by 90% in the fish treatment by the final
261 sampling date (LM, treatment*time, $t = -2.69$, $P = 0.009$). This effect was driven primarily by
262 reductions in *Bosmina* cladocerans (LM, treatment*time, $t = -3.43$, $P = 0.001$; Fig. 2a) and
263 brachiopods in the family Sididae (LM, treatment*time, $t = -2.51$, $P = 0.015$; Fig. 2b), which
264 together represented 76% of the total zooplankton individuals across all samples. *Daphnia* (Fig.
265 2c) and rotifers (Fig. S1) also decreased with mosquitofish presence, although their abundances
266 were much lower overall (*Daphnia*: LM, treatment*time $t = -2.11$, $P = 0.038$; rotifers: LM,
267 treatment*time, $t = -2.04$, $P = 0.044$), and we did not observe an effect on copepods (LM,
268 treatment*time, $t = -0.97$, $P = 0.34$; Fig. 2d).

269 In addition to zooplankton, mosquitofish decreased the total abundance (LM,
270 treatment*time, $t = -3.37$, $P = 0.001$) and total biomass (LM, treatment*time, $t = -2.70$, $P =$
271 0.008) of aquatic macroinvertebrates. Damselflies (*Lestes* and Coenagrionidae; LM,
272 treatment*time, $t = -3.91$, $P = 0.0001$) and mayflies (*Calibaetis*; LM, treatment*time, $t = -3.02$, P
273 $= 0.003$) were the most abundant aquatic insect taxa and exhibited the strongest reductions,
274 showing densities that were two to five times lower on the side with fish (Figs. 2e, 2f).
275 Dragonflies (*Sympetrum*: LM, treatment*time, $t = 0.63$, $P = 0.50$) and backswimmers
276 (*Notonecta*: LM, treatment*period, $t = -1.23$, $P = 0.22$) were not significantly reduced in the
277 presence of mosquitofish, although the latter group was two times less abundant on the side of

278 the wetland with fish on the final sampling date (Figs. 2g, 2h). We did not detect significant
279 differences in the abundances of water boatmen (Corixidae), aquatic beetles (Coleoptera),
280 California clam shrimp (*Cyzicus californicus*), Holarctic clam shrimp (*Lynceus brachyurus*),
281 midges (Chironomidae), leeches (*Erpobdella*), or rams horn snails (*Helisoma trivolvis*) (see
282 Table S1, Fig. S2 and Appendix for statistics).

283 In contrast to our predictions and results of the smaller scale experiments, mosquitofish
284 did not have strong effects on Pacific chorus frogs or California newts in Hog Lake. Chorus frog
285 larvae numbers in stovepipe samples declined over the summer as frogs metamorphosed but
286 there were no effects of mosquitofish on larval abundance (LM, treatment*time, $t = -0.23$, $P =$
287 0.82 ; Fig. 3a), total biomass (LM, treatment*time, $t = 0.55$, $P = 0.58$), or individual body mass
288 (LME, treatment*time, $t = -1.41$, $P = 0.16$; Fig. 3b). The density of California newt larvae in
289 stovepipe samples were higher in the fish treatment throughout the study, likely due to initial
290 differences in egg oviposition, and mosquitofish did not alter newt abundance (LM,
291 treatment*time, $t = -1.19$, $P = 0.24$; Fig. 3c) or total biomass (LM, treatment*time, $t = -0.24$, $P =$
292 0.81). Larval newts, however, did have a smaller individual body mass at the end of the
293 experiment in the mosquitofish treatment (LME, treatment*time, $t = 3.13$, $P = 0.002$; Fig. 3d).
294 Amphibian abundances in dipnet sweeps and seine net hauls demonstrated the exact same
295 patterns that were observed from stovepipe samples. At the end of the summer, we detected more
296 chorus frog leg abnormalities in the mosquitofish treatment (4 out of 209) than on the control
297 side (0 out of 145); however, the overall frequency of abnormalities was low and the difference
298 was not significant (Pearson chi-square = 1.31, $df = 1$, $P = 0.25$).

299 Mosquitofish also influenced nutrients and phytoplankton. The addition of mosquitofish
300 was associated with a ~50% decrease in total phosphorus (LM, treatment*time, $t = 9.81$, $P <$
301 0.0001 ; Fig. 4a) and a 13% decrease in total nitrogen (LM, treatment*time, $t = 2.79$, $P = 0.009$;
302 Fig. 4b). Correspondingly, N:P molar ratios in water samples were ~2x higher with mosquitofish
303 (LM, treatment*time, $t = -6.74$, $P < 0.0001$; Fig. 4c). Relative phytoplankton fluorescence
304 followed a similar pattern as phosphorus, with ~50% lower fluorescence values in the
305 mosquitofish treatment by the final sampling date (LM, treatment*time, $t = 4.61$, $P < 0.0001$;
306 Fig. 4d).

307 *Mesocosm experiment* – Results from the mesocosm experiment showed that alternative
308 prey and habitat complexity reduced the predatory effects of mosquitofish on chorus frog larvae

309 (Fig. 5). In the control treatments, chorus frog survival averaged 37%, whereas the survival
310 increased to a mean of 70% and 77%, respectively, in mesocosms receiving increased prey or
311 increased habitat complexity (GLMM, prey availability, $z = 1.94$, $P = 0.052$; habitat complexity,
312 $z = 2.05$, $P = 0.040$; Fig. 5a). Amphibian survival was highest (79%) with both increased prey
313 availability and increased habitat complexity, although we did not detect any significant
314 interactions (GLMM, prey*habitat, $z = -1.24$, $P = 0.216$). Chorus frog snout-vent length
315 increased by 15% in the high habitat complexity treatments (LME, $t = 2.48$, $P = 0.025$), whereas
316 added prey availability alone did not affect chorus frog body size (LME, $t = 0.264$, $P = 0.79$; Fig.
317 5b). We did not detect a significant prey availability-by-habitat complexity interaction (LME, $t =$
318 0.67 , $P = 0.51$), although the addition of both macrophytes and zooplankton increased mean
319 snout-vent length by 23% relative to controls. At the conclusion of the experiment, zooplankton
320 densities were highest in the two treatments containing elevated habitat complexity (Fig S3). All
321 mosquitofish survived to the end of the experiment.

323 Discussion

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

334 The experiment in Hog Lake allowed us to examine the effects of mosquitofish within a
335 natural wetland setting containing a complex food web. Predatory effects of mosquitofish were
336 taxon-specific and varied with the abundances and traits of prey. Consistent with previous
337 research from other venues, we found that mosquitofish strongly altered invertebrate abundances
338 and community composition (Hurlbert, Zedler & Fairbanks 1972; Miura, Takahashi & Wilder
339 1984; Leyse, Lawler & Strange 2004). The most abundant zooplankton and macroinvertebrate

340 taxa showed suppressed population growth with mosquitofish, leading to significant reductions
341 in total invertebrate numbers and biomass relative to the fishless treatment. The most affected
342 insect taxa – damselflies and mayflies – are soft-bodied, which likely makes them more
343 susceptible than taxa that did not show significant changes in numbers (e.g., dragonflies,
344 hemipterans, clam shrimp, snails). Similar decreases in soft-bodied prey, with little change in
345 abundance of hard-bodied prey, have been observed after mosquitofish introduction in rice fields
346 (Miura, Takahashi & Wilder 1984). Given that the most strongly affected insect groups also
347 metamorphose into terrestrial adults, mosquitofish could reduce subsidies from aquatic
348 environments to the surrounding terrestrial landscape (Merkley, Rader & Schaalje 2015). In
349 general, the strength of mosquitofish effects on native species is likely dependent on the
350 characteristics of native community members including palatability, niche overlap, and life-
351 history traits (Macdonald *et al.* 2012).

352 The Hog Lake experiment also allowed us to measure ecosystem-level responses,
353 including nutrient concentrations and phytoplankton abundance. Interestingly, the effects of
354 mosquitofish on nutrients and phytoplankton were opposite to our predictions and most past
355 work. Fish addition led to a 50% reduction in total phosphorus in the water column, an increase
356 in nitrogen-to-phosphorus ratios, and a 50% decrease in phytoplankton production. The
357 reduction in phytoplankton became larger over the course of the summer despite the fact that
358 mosquitofish reduced zooplankton abundance by 13-fold. These results contrast with past work
359 showing that mosquitofish induce trophic cascades by removing top-down zooplankton controls
360 on phytoplankton (Hurlbert, Zedler & Fairbanks 1972; Nagdali & Gupta 2002). For instance,
361 prior work from artificial pools has found that mosquitofish decrease zooplankton abundance,
362 leading to large algal blooms associated with a 100 to 200 fold increase in some phytoplankton
363 groups (Hurlbert, Zedler & Fairbanks 1972). One possible explanation for our different results is
364 that Hog Lake phytoplankton are more limited by nutrients (particularly phosphorus) than by
365 zooplankton grazing, and that mosquitofish are compounding this nutrient limitation through the
366 assimilation of biomass. Fish biomass can contain up to 75% of the limnetic phosphorus in lakes
367 (Kitchell, Koonce & Tennis 1975) and a rapidly growing fish population, as was present in our
368 study, is most likely to act as a nutrient sink rather than a source (Kraft 1992). The relatively
369 high nitrogen to phosphorus ratio (~50) in the fish treatment at the end of the summer supports

370 the idea that the wetland was phosphorus limited, particularly towards the end of the dry summer
371 period when nutrient inputs are minimal.

372 The mosquitofish addition into Hog Lake had few detectable effects on native Pacific
373 chorus frogs or California newts, despite considerable evidence that introduced fishes in general,
374 and mosquitofish in particular, are a contributor to declines in amphibian populations in the
375 western United States (Fisher & Shaffer 1996; Kats & Ferrer 2003; Vredenburg 2004; Joseph,
376 Preston & Johnson 2016). Several, non-mutually exclusive mechanisms could underlie this
377 result. Prior work suggests that mosquitofish can coexist with certain native amphibians,
378 including California red-legged frogs in experimental wetlands (Lawler *et al.* 1999) and several
379 species of frogs in southwestern Australia wetlands (Reynolds 2009). In the study with red-
380 legged frogs, the authors posit that co-existence may have occurred due to a combination of high
381 alternative prey availability, spatial refuges in cobbles and vegetation, and a decrease in
382 predatory invertebrates that feed on amphibian larvae (Lawler *et al.* 1999). In our study, it is
383 possible that the dense shoreline vegetation and an abundance of zooplankton and aquatic
384 macroinvertebrates alleviated predation pressure on the native amphibians, both species of which
385 are palatable to mosquitofish (Gamradt & Kats 1996; Goodsell & Kats 1999; Preston, Henderson
386 & Johnson 2012). More broadly, a growing body of literature has highlighted how the outcome
387 of species invasions can be context dependent, often depending on levels biotic or abiotic
388 resistance from the invaded community (Strayer *et al.* 2006; Ricciardi *et al.* 2013; Zenni and
389 Nuñez 2013).

390 It is possible that an earlier introduction of mosquitofish, such that fish overlapped more
391 with the earliest and most vulnerable amphibian larval stages, could have led to stronger
392 population-level effects on amphibians. Prior studies show that early larval stages are more
393 readily consumed than eggs or late-stage tadpoles for most amphibian species (Zeiber *et al.*
394 2008; Reynolds 2009; Smith & Smith 2015). If predation pressure is highest on early larval
395 stages, mosquitofish effects may vary temporally such that the strongest predatory effects occur
396 shortly after embryos emerge from egg masses. California newts at Hog Lake lay eggs in
397 February and March, while chorus frogs reproduce from February to June/July, such that larvae
398 are smallest in the early spring months. While small amphibian larvae are probably most
399 susceptible to predation, we have observed mosquitofish consume metamorphic chorus frogs in
400 the field, and fish attacks can cause sublethal injuries to the tails and limbs of amphibian larvae

401 that are approaching metamorphosis, indicating that effects may extend to later age/size classes
402 under some conditions (Preston, Henderson & Johnson 2012; Shulse & Semlitsch 2014).
403 Collectively, our findings suggest that negative effects of mosquitofish on native amphibians can
404 be strong under certain settings, but they may also be influenced by factors such as community
405 structure, habitat characteristics, and productivity. Prior research has also shown that
406 hydrological characteristics can mediate the effects of mosquitofish on some native species (Ho
407 et al. 2013). Repeating our mosquitofish introduction within a less complex or productive
408 habitat, and/or introducing mosquitofish earlier in the season, may have resulted in stronger
409 effects on amphibians.

410 Results from our outdoor mesocosm experiment supports the hypothesis that habitat
411 complexity and/or alternative prey availability can weaken the predatory effects of mosquitofish
412 on native amphibians. Aquatic macrophytes attenuated the negative effects of mosquitofish on
413 amphibian survival by 50%, suggesting that more complex habitats may facilitate the
414 coexistence of amphibians and predatory fish in natural wetlands (Babbitt & Tanner 1997; Hartel
415 et al. 2007). Macrophytes provided cover from predation and also increased the abundance of
416 zooplankton in mesocosms. At the conclusion of the mesocosm experiment, the treatments with
417 high habitat complexity supported the highest zooplankton densities, suggesting that the aquatic
418 plants increased zooplankton populations and thereby weakened predation on native amphibians
419 by increasing alternative prey. Zooplankton are probably the more preferred prey item of
420 mosquitofish when they are sufficiently available (Hurlbert & Mulla 1981; Miura, Takahashi &
421 Wilder 1984; García-Berthou 1999). While our mesocosm experiment informed possible
422 mechanisms underlying results in Hog Lake, we note that a longer duration mesocosm study and
423 a higher density of fish due to reproduction could have led to greater depletion of alternative prey
424 and stronger effects of mosquitofish on amphibian larvae, particularly in the treatments lacking
425 zooplankton additions. It is also possible that long-term effects of mosquitofish introductions
426 (i.e., effects manifesting over several seasons) would be stronger than the effects observed in our
427 experiments. Nonetheless, our results suggest that variation in environmental factors between
428 wetlands can mediate the effects of fish invasions on native communities. The presence of
429 aquatic macrophytes and emergent vegetation (e.g., *Typha*: Typhaceae and *Juncus*: Juncaceae) in
430 California wetlands varies dramatically from completely absent to 100% shoreline cover, often
431 in association with livestock grazing intensity (Joseph, Preston & Johnson 2016). Such

432 variability emphasizes the potential for disparate mosquitofish impacts across discrete wetlands,
433 and for possible interactions between grazing intensity and local invasive fish effects.

434 Taken together, results of our ecosystem manipulation and mesocosm experiment
435 reinforce the need to consider variation in invasive species impacts across natural systems. A
436 more nuanced understanding of when and where invaders are likely to have the strongest effects
437 will benefit from knowledge of the specific biotic and abiotic mechanisms that drive variation in
438 invasion outcomes (e.g., environmental change or community composition) (Ricciardi et al.
439 2013). Our results also complement and extend the considerable body of literature demonstrating
440 that mosquitofish introductions can negatively affect native species, underscoring the need for
441 land managers to limit new introductions and pursue mosquitofish removal if their goals are to
442 conserve native wetland communities.

443 **Acknowledgements**

444 We thank N. Brown, O. Goodwin, T. Hayes, G. Hidalgo-Cuellar, S. Palmer, M.
445 Summerside and D. Warrendorf for assistance in data collection, and the staff of the
446 University of California Hopland Research and Extension Center for support, especially R.
447 Keiffer, A. Shrum, W. Marston, S. Poor, and S. Fierer. Funding came from the National Science
448 Foundation (DEB-0841758, DEB- 1149308, DEB-1311467, and a Graduate Fellowship to DLP),
449 the David and Lucile Packard Foundation, and the University of Colorado Undergraduate
450 Research Opportunities Program.

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601 **Figure Legends**

602 **Figure 1.** Hog Lake in Mendocino County, California. Invasive western mosquitofish
603 (*Gambusia affinis*) were added to the west side of Hog Lake and community and ecosystem
604 responses were measured using a 'Before-After-Control-Impact' experimental design. The
605 bottom images show the fence dividing the wetland (Left) and the school of introduced
606 mosquitofish (Right).

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

614 **Figure 3.** Effects of mosquitofish on native amphibian taxa within a wetland ecosystem
615 experiment. The dashed vertical line indicates the date of mosquitofish introduction and the
616 legend for all plots is shown at the top right. Responses comprising Pacific chorus frog density

617 (a), chorus frog individual body mass (b), California newt density (c), and California newt
618 individual body mass (d). All data are means per sampling date from stovepipe samples.

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

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

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Figure 1.

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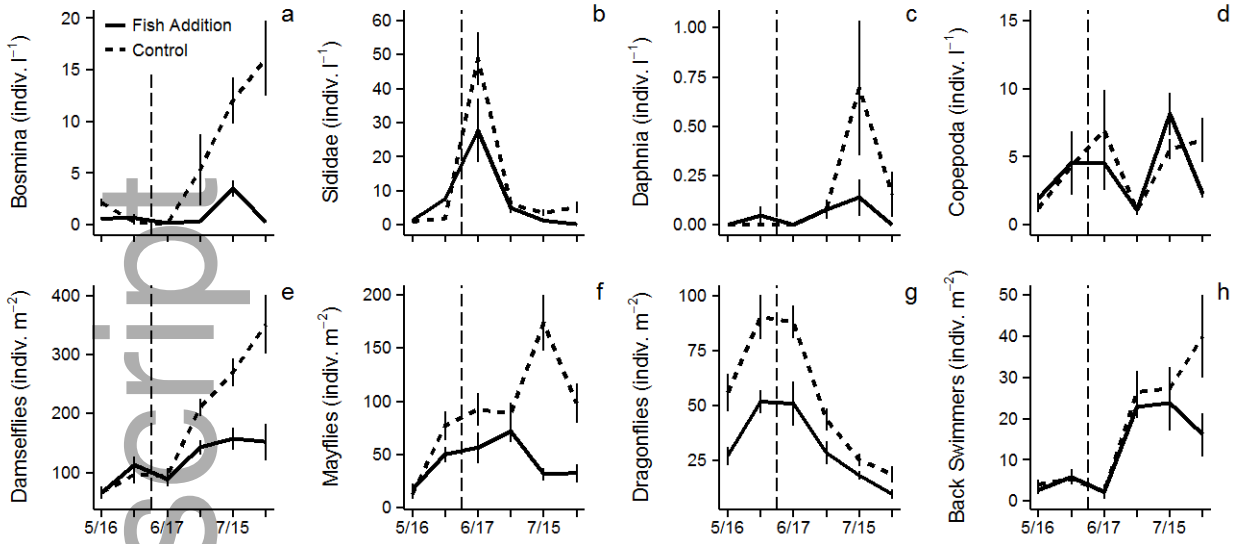


Figure 2.

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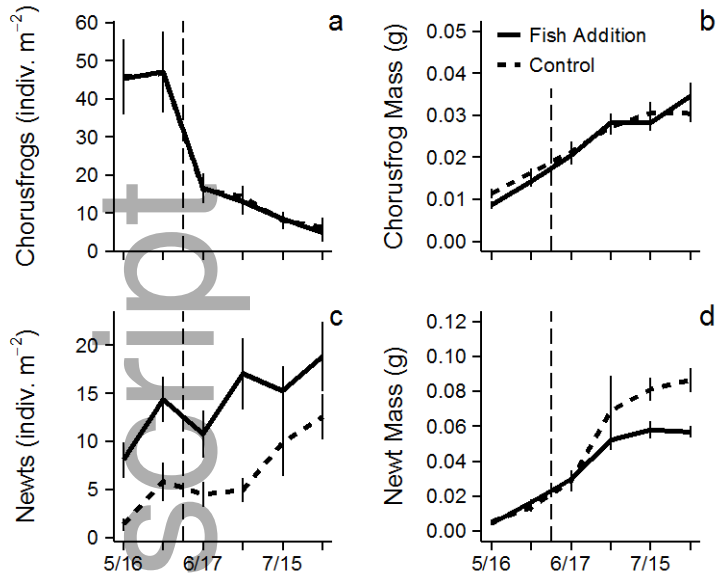


Figure 3

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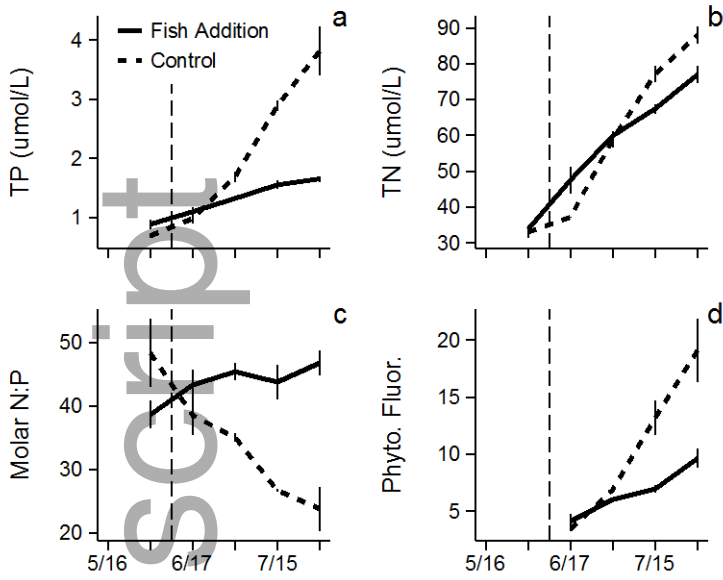


Figure 4.

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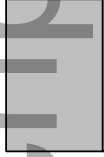
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Figure 5.