1

2

3

4 Article type   : Article

5

6

7

8

9   **Nutrient availability and invasive fish jointly drive**

10   **community dynamics in an experimental aquatic system**

11

12   Daniel L. Preston[1,†], Hayden D. Hedman[2], Pieter T. J. Johnson[3]

13

14 [1]Department of Integrative Biology, Oregon State University, Corvallis, Oregon 97331

15 USA

16 [2]School of Natural Resources and the Environment, University of Michigan, Ann Arbor,

17 Michigan 48109 USA

18 [3]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder,

19 Colorado 80309 USA

23

24

25

26

27

28

29

30

31

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 2×3 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: nonnative, introduced, eutrophication, pollution, freshwater, pond, food web, pond-breeding amphibian, trophic cascade

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

75

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 enhance 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 co-exist 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

91   invasions are influenced by other ecosystem changes is thus a priority for effective

92   prediction and management of invasion impacts (Pyšek and Richardson 2010).

93   Alongside species invasions, freshwaters are often simultaneously affected by

94   nutrient pollution (Carpenter et al. 2011, Ricciardi and MacIsaac 2011). Indeed, these two

95   disturbances represent two of the most commonly implicated drivers of freshwater

96   ecosystem change (Carpenter et al. 1998, Smith and Schindler 2009, Strayer 2010). For

97   instance, aquatic systems within the Great Lakes region support ~180 invasive species

98   and have a long history of non-point nutrient pollution from the terrestrial environment

99   (Beeton 1965, Mills et al. 1994, Ricciardi 2001). Similarly, the Rift Valley Lakes in East

100   Africa have been invaded by numerous nonnative species—including water hyacinth and

101   Nile perch—while simultaneously experiencing nutrient pollution from agriculture and a

102   growing human population (Ogutu-Ohwayo et al. 1997, Odada et al. 2003). In both

103   examples, the co-occurrence of invasive species and nutrient pollution was associated

104   with wholesale shifts in community structure and ecosystem processes, leading to

105   declines in native species and economic losses totaling billions of dollars (Pitcher and

106   Hart 1995, Pimentel et al. 2005).

107   Despite the common co-occurrence of non-native species and elevated nutrient

108   loading, predicting how these two factors interact to drive ecosystem change remains a

109   key challenge (Flores-Moreno et al. 2016, Tabassum and Leishman 2016, Teixeira et al.

110   2017). The net effect of nutrient availability on species invasions will depend on

111   characteristics of the invasive species (e.g., resource needs, resource use efficiency,

112   trophic position) and the invaded ecosystem (e.g., relative roles of top-down versus

113   bottom-up factors in regulating community dynamics; Kolar and Lodge 2000, González

114   et al. 2010). Successful invaders have been predicted to have high resource needs and to

115   be efficient at utilizing excess resources relative to natives (Blumenthal 2006, González

116   et al. 2010). For example, prior studies indicate that invasive primary producers are often

117   able to more rapidly utilize excess nutrients than natives, thereby facilitating invader

118   spread and a shift towards dominance by nonnatives (e.g., water hyacinth, Eurasian

119   milfoil, nonnative phytoplankton; Chase and Knight 2006, Coetzee et al. 2007). Nutrients

120   can also influence invaders in higher trophic levels through bottom-up food web effects

121   and indirect changes in community structure. Many invasive primary and secondary

122   consumers have high nutrient demands and relatively fast life-histories, in which case

123   they may disproportionately benefit from increased nutrients relative to natives (e.g.,

124   Tibbets et al. 2010, Jackson et al. 2013). Lastly, rapid shifts in nutrient availability may

125   alter the conditions under which native species have adapted such that

126   invaders gain a competitive advantage in ecosystems where they would normally be

127   outcompeted by natives (Byers 2002).

128   Alternatively, it is also possible that elevated nutrient availability could

129   ameliorate the negative effects of invaders by lowering colonization success or

130   weakening invasion impacts on any specific native community member. For instance,

131   nutrients can facilitate the coexistence of invasive species with native community

132   members by enhancing the overall availability of resources to the community, thereby

133   weakening interspecific competition (Firn et al. 2010). Bottom-up fertilization effects can

134   also increase prey availability, thereby preventing predators from extirpating native

135   community members (Balciunas and Lawler 1995). In general, it has been posited that

136   increased resource supply is one mechanism that can facilitate species coexistence

137   (Fargione and Tilman 2002), and productivity is often associated positively with species

138   richness at large spatial scales (Field et al. 2009, Chase 2010). Although such patterns are

139   likely context-dependent (Dodson et al. 2000, Mittelbach et al. 2001), they support the

140   potential for elevated nutrients to allow coexistence between nonnative and native

141   species.

142   In the present study, our primary aim was to examine whether nutrients attenuate

143   or amplify the effects of invasive fish within pond communities using outdoor

144   mesocosms. We focused on the western mosquitofish (*Gambusia affinis*), which is a

145   widespread invasive species that has been implicated in declines of multiple native taxa

146   (Pyke 2008; see also *Study system* below). We utilized outdoor mesocosms, which are

147   useful for testing ecological mechanisms, as they allow replication of controlled

148   experimental treatments, and they are particularly relevant for studies of small ponds

149   (Semlitsch and Boone 2010, Spivak et al. 2011). We sought to experimentally assess the

150   relative influence of two competing hypotheses. First, increases in nutrients could

151   disproportionately increase the growth and reproduction of mosquitofish through bottom-

152   up effects (the invader amplification hypothesis). Under this hypothesis, increased

153 nutrient loading could amplify the negative predatory effects of mosquitofish on the
154 native aquatic community due to increased mosquitofish biomass. In contrast, increases
155 in nutrients could enhance primary and secondary production, thereby weakening the
156 population-level effects of mosquitofish on any focal prey taxon through increases in
157 overall prey availability (the invader attenuation hypothesis). In this scenario, increased
158 nutrients could facilitate the coexistence of predatory mosquitofish with native prey,
159 including zooplankton, invertebrates, and amphibians.
160
161 **Methods**
162 *Study system*
163 We examined interactions between nutrient concentrations and western mosquitofish
164 (*Gambusia affinis*) within the community context of wetlands in the San Francisco Bay
165 Area of northern California, USA. Wetlands are the most imperiled habitat type in North
166 America, and within California >90% of natural wetlands have been lost to agriculture
167 and development (Nichols et al. 1986, Dahl 2000, Brinson and Malvárez 2002). Many of
168 the existing wetlands around the San Francisco Bay Area were artificially constructed as
169 livestock watering sites and now serve as important habitat refuges for species of
170 conservation concern, including native pond-breeding amphibians (i.e., Pacific chorus
171 frogs, California newts, western toads, California red-legged frogs, and California tiger
172 salamanders; Joseph et al. 2016). Concurrently, wetlands in this region also support
173 multiple invasive species, including American bullfrogs (*Lithobates catesbeianus*), bass
174 (*Micropterus* spp.), sunfish (*Lepomis* spp.), and western mosquitofish (*Gambusia affinis*;
175 Preston et al. 2012). Western mosquitofish, which are native to the Mississippi River
176 drainage, have a long history of intentional introduction into California wetland, and
177 waterways worldwide, for use as a biological control agent of mosquito larvae (Downs
178 1991). However, mosquitofish are generalist predators that also prey on a wide diversity
179 of non-target organisms, including zooplankton, invertebrates, amphibians, and fishes
180 (e.g., Goodsell and Kats 1999, Mills et al. 2004, Shulse et al. 2013, Merkley et al. 2015,
181 Holbrook and Dorn 2016) and their use as mosquito biocontrol has been controversial
182 (Azevedo-Santos et al. 2017).
183 *Mesocosm experiment*

184   To examine how nutrient availability influenced interactions between mosquitofish and

185   native aquatic organisms, we conducted a 2×3 factorial outdoor mesocosm experiment

186   involving two levels of mosquitofish presence (yes or no) and three levels of nutrient

187   concentrations (low, medium, or high). Mesocosms were located at the University of

188   California Hopland Research and Extension Center in Mendocino County, California.

189   Each treatment was replicated five times for a total of 30 mesocosms. Mesocosms

190   consisted of 378 L livestock watering tanks filled with well water and fitted with mesh

191   screen lids. To each mesocosm we added 6 kg of silica sand and 25 grams of dry leaves

192   (*Quercus* and *Arbutus* spp.) as substrate and cover. To each tank we also added two

193   square clay tiles (15.2 cm^2 area) to provide a surface from which to quantify periphyton.

194   The nutrient levels used in the experiment were informed by field nutrient measurements

195   from 231 wetlands in the San Francisco Bay Area of California (see Appendix S1 for

196   details). One month prior to the start of the experiment (12 May), and again three weeks

197   later (4 June), we added chemical nutrients (KH_2PO_4 and $NaNO_3$) to all mesocosms at a

198   molar nitrogen-to-phosphorus ratio of 38:1, which was the median value from field data

199   (see Appendix S1: Fig. S1). On each occasion we added KH_2PO_4 in amounts of 0.032 g

200   to the low nutrient condition, 0.13 g to the medium nutrient condition (a fourfold

201   increase), or 0.51 g to the high nutrient condition (a 16-fold increase). Similarly, we

202   added $NaNO_3$ in amounts of 0.75 g (low nutrients), 3.0 g (medium), or 12.0 g (high). We

203   seeded each mesocosm with algae, zooplankton collected with a 243 μm net (mostly

204   cladocerans and copepods), and nine taxa of local wetland invertebrates, including snails,

205   hemipterans, odonates, and amphipods (Appendix S1: Table S1). At the start of the

206   experiment (13 June), we added larvae of three native amphibians to each mesocosm: 15

207   Pacific chorus frogs (*Pseudacris regilla*), 20 western toads (*Anaxyrus boreas*), and 10

208   California newts (*Taricha torosa*; see Appendix S1: Table S2 for initial body sizes). In

209   the replicates assigned to the mosquitofish addition treatments, we added four adult male

210   and three adult female mosquitofish (Appendix S1: Table S2). All organisms added to

211   mesocosms were locally collected from natural wetlands in Mendocino County,

212   California, and the densities of organisms were within the range of densities observed in

213   the field (Preston et al. 2012, Joseph et al. 2016, Preston et al. 2017). The experiment

214   lasted for approximately four weeks (from 13 June to 16 July; see Appendix S1: Table S3

215   for a detailed timeline).

216   In the mesocosm experiment we quantified nutrient concentrations (total

217   dissolved nitrogen and total dissolved phosphorus), phytoplankton (relative

218   fluorescence), periphyton (biomass), zooplankton (abundance), invertebrates (abundance

219   and biomass), amphibians (growth, survival, and biomass), and mosquitofish (abundance

220   and biomass). We measured nutrient concentrations at three time points from the

221   beginning, mid-point, and end of the study. To quantify nutrients, we collected water

222   samples from each mesocosm in acid-washed Nalgene bottles, which were frozen until

223   analysis at the University of Colorado (see http://niwot.colorado.edu/ research/kiowa-

224   lab/the-arikaree-environmental-laboratory for methodological details). Relative in vivo

225   phytoplankton fluorescence was quantified weekly (6 sampling dates from 9 June to 13

226   July) using a fluorometer (Turner Designs, Sunnyvale, California, USA). Water samples

227   for phytoplankton measurements were collected from the center of the mesocosm using

228   Nalgene bottles, briefly stored in a cooler, and then immediately processed on each

229   sampling date. Periphyton dry biomass on clay tiles was measured on two dates early in

230   the study (3.3 cm^2 sampled per replicate on 9 June and 17 June) and at the end of the

231   experiment from the mesocosm walls (12 × 40 cm sampled per replicate on 12 July). We

232   switched from measuring periphyton on clay tiles to measuring it on the mesocosm walls

233   because periphyton on tiles was removed by grazers at the mid-point of the study.

234   Periphyton samples were oven-dried at 70°C prior to weighing. We measured

235   zooplankton abundance at five time points spanning the length of the study (9 June–13

236   July) by pooling five samples per mesocosm collected with a vertical tube sampler (70

237   cm in length × 5 cm in diameter). Zooplankton samples were filtered onto 50 μm mesh

238   and preserved in 80% ethanol prior to enumeration and identification as either copepods

239   or cladocerans (primarily *Daphnia* spp.) in the laboratory. Invertebrates were sampled

240   using two pooled samples per mesocosm collected with a benthic stovepipe sampler (28

241   cm in diameter). At the conclusion of the experiment (15 July), we counted and removed

242   all amphibians, snails, and mosquitofish and measured their length and wet-mass. For

243   frogs and toads, we also recorded their developmental stage (Gosner 1960) or days-to-

244   metamorphosis for those individuals that emerged before the experiment ended.

246 To analyze responses that were quantified on multiple dates of the experiment (nutrients

247 concentrations, phytoplankton, zooplankton, periphyton from tiles), we used linear mixed

248 effects models with main effects of fish, nutrient levels, time and their interactions. We

249 also included a random intercept term for mesocosm identity. The data for each of these

250 responses involved one time point that was collected prior to the start of the experiment

251 (i.e., before mosquitofish introduction), and multiple time points during the study

252 (Appendix S1: Table S3). For responses that were measured only at the conclusion of the

253 study, we omitted the effect of time and the random intercept term. Periphyton collected

254 on clay tiles was analyzed separately from periphyton collected on the mesocosm walls.

255 Fish presence was coded as a categorical variable (present or absent) while nutrient levels

256 were coded as a continuous variable representing the relative differences in nutrient

257 inputs (1, 4 or 16). For amphibian survival data, we used a generalized linear mixed

258 effects model with a binomial error distribution, a random intercept term for mesocosm

259 identity, and survival of each individual within a mesocosm as the response (Zuur et al.

260 2009). For all other responses we used a single mean value per mesocosm one each

261 sampling date.

262 Because our initial analyses suggested that indirect effects were important in

263 explaining our mesocosm results, we used path analysis to further evaluate potential

264 mechanisms linking fish and nutrients with the response variables (Kline 2015). The path

265 analysis included nutrient levels as a continuous variable (1, 4 or 16) and mosquitofish

266 biomass at the end of the study as continuous predictors (see Fig. 5 for the path diagram).

267 Within our path analysis, nutrients were linked to phytoplankton fluorescence and

268 periphyton biomass through bottom-up effects, while mosquitofish were linked to

269 zooplankton density, amphibian biomass, and snail biomass through top-down predatory

270 effects. Phytoplankton and periphyton were also linked to zooplankton and amphibians,

271 respectively (through grazing), and snails were linked to periphyton (via bottom-up

272 resource availability; our initial analysis did not suggest the presence of a strong top-

273 down link from snails to periphyton via grazing). Model fit was evaluated using the

274 Comparative Fit Index (CFI) and the Standardized Root Mean Square Residual (SRMR),

275 which are both robust to violations of normality and effective with small sample sizes

276 (Hu and Bentler 1999). Log transformations were used when they improved variable

277 distributions and all analyses were conducted in the R computing environment (R Core

278 Team 2014), using the lavaan package for path analysis (Rosseel et al. 2011).

279

280 **Results**

281 *Nutrient effects in mesocosms*

282 Observed nutrient concentrations in the mesocosms (Fig. 1) indicated that the

283 manipulation was effective, with low, medium, and high nutrient treatments showing

284 consistent differences throughout the study in total dissolved nitrogen (LMM, nutrients, t

285 = 13.48, P < 0.001) and phosphorus (LMM, nutrients, t = 5.37, P < 0.001). We did not

286 detect any significant effects of fish, time or a fish-by-time interaction on total dissolved

287 nitrogen (LMM, fish, t = −1.16, P = 0.25; time, t = 0.71, P = 0.48) or phosphorus (LMM,

288 fish, t = 0.46, P = 0.65; time, t = 1.29, P = 0.20).

289 Nutrient concentrations strongly affected phytoplankton but had relatively weak

290 effects on periphyton in mesocosms. Across all mesocosms, an increase from low to high

291 nutrient concentrations increased mean phytoplankton fluorescence by 300%. Effects of

292 nutrients were strongest towards the end of the study, leading to a nutrients-by-time

293 interaction on phytoplankton (Fig 2a; LMM, nutrients*time, t = 2.40, P = 0.017). On the

294 second sampling time point, periphyton biomass was ~50% lower on the clay tiles in the

295 low nutrient conditions than the medium or high, however this effect was not statistically

296 significant (Appendix S1: Fig. S2; LMM, nutrients*time, t = 0.24, P = 0.81). Periphyton

297 biomass at the end of the study on the mesocosm walls was not different between nutrient

298 conditions (Fig. 3a; t = 0.15, P = 0.88).

299 Nutrients generally had smaller effects on invertebrate and amphibians in the

300 mesocosms compared to mosquitofish. At the intermediate sampling time point, the high

301 and medium nutrient treatments supported approximately three-fold more zooplankton

302 than the low nutrient treatment; however, this effect was not significant for either

303 cladocerans (Fig. 2b; LMM, nutrients*time, t = −0.43, P = 0.66) or copepods (Fig. 2c;

304 LMM, nutrients*time, t = 0.13, P = 0.90). Of the benthic invertebrates added to the

305 mesocosms, only aquatic snails (*Helisoma* and *Physa*) reproduced over the course of the

306 experiment. Snail biomass was not different across nutrient treatments at the conclusion

307 of the study (Fig. 3b). Nutrients also did not affect amphibian survival (Fig. 4; GLMM, t

308 = −0.26, P = 0.79), but increased the average developmental stage of chorus frog larvae

309 (LM, t = 2.51, P = 0.025) and the average wet mass of newt larvae (Fig. 4b; LM, t = 2.80,

310 P = 0.015). Nutrients also slowed time-to-metamorphosis of toads (LM, t = 2.85, P =

311 0.008).

312   Among the aquatic consumers present, nutrient additions most strongly affected

313 mosquitofish in mesocosms (Fig. 4d). Total mosquitofish biomass was ~30% higher in

314 the high nutrient treatments relative to the low nutrient treatments (LM, t = 2.28, P =

315 0.039). Based on the size distribution of mosquitofish at the end of the study, this effect

316 was driven by increases in fish reproduction and/or greater survival of offspring at higher

317 nutrient levels. Juvenile mosquitofish produced within the mesocosms were differentiated

318 from the adults that were added at the start of the experiment based on their body sizes

319 (14.9 mm mean length for juveniles vs. 34.5 mm mean length for adults at the end of the

320 study). On average, mesocosms in the high nutrient conditions supported ~5 times more

321 juvenile mosquitofish (mean = 23.4 individuals) than in the low nutrient treatment (mean

322 = 4.6 individuals).

323 *Mosquitofish effects in mesocosms*

324 Mosquitofish influenced invertebrates and periphyton in mesocosms. Fish presence was

325 associated with a >10-fold reduction in total mean zooplankton density (Figs. 2b and 2c).

326 Fish decreased the density of both copepods (Fig. 2c; LMM, fish, t = −3.47, P = 0.002)

327 and cladocerans (Fig. 2b; LMM, fish, t = −3.14, P = 0.004). Densities of both types of

328 zooplankton peaked at intermediate time points, leading to significant effects of time as

329 well (copepods LMM, time, t = −4.45, P < 0.001; cladocerans GLMM, time, t = −2.41, P

330 0.018). The total biomass of aquatic snails was 26% higher in mesocosms with fish than

331 in mesocosms without fish at the end of the experiment (Fig. 3b; LM, fish, t = 2.61, P =

332 0.015). Fish presence also increased benthic periphyton biomass along the mesocosm

333 walls by approximately ten-fold at the end of the study (Fig. 3a; LM, t = 4.43, P =

334 0.0001). This effect of fish was not observed at the two early time points on clay tiles

335 (Appendix S1: Fig. S2). Mean phytoplankton fluorescence over the entire study was 70%

336 higher in the presence of fish, however this effect was not statistically significant (Fig.

337 2a; LMM, fish, t = −0.27, P = 0.81).

338   The presence of mosquitofish strongly reduced amphibian survival (Fig. 4).

339   Among all mesocosms with fish, only two chorus frogs (<1%) and ten California newts

340   (7%) survived to the end of the experiment (Fig. 4; total amphibian survival, GLMM, t

341   = −8.43, P < 0.0001). Western toad survival was higher in the presence of fish than the

342   other two amphibian species (mean = 43%), but was still reduced by half relative to

343   mesocosms without fish (Fig. 4c). Fish presence also accelerated time-to-metamorphosis

344   of toads (LM, t = 3.00, P = 0.006), but decreased their average individual wet mass at the

345   end of the study (LM, t = −3.11, P = 0.004).

346   *Path analysis*

347   The path analysis provided support for several indirect effects (trophic cascades) of

348   mosquitofish in mesocosms (Fig. 5). Mosquitofish were negatively associated with

349   zooplankton density, which in turn was negatively associated with phytoplankton

350   fluorescence, resulting in a net positive pathway from mosquitofish to phytoplankton

351   fluorescence (standardized indirect path coefficient = 0.27, P = 0.007; Fig. 5). A similar

352   association with mosquitofish was observed involving the benthic community;

353   mosquitofish were negatively associated with amphibian biomass, which was negatively

354   associated with periphyton biomass, leading to a net positive pathway from fish to

355   periphyton (standardized indirect path coefficient = 0.48, P = 0.001; Fig. 5). Periphyton

356   also associated positively with snail biomass, leading to a positive indirect path from

357   mosquitofish to snails (standardized indirect path coefficient = 0.22, P = 0.034; Fig. 5).

358   Lastly, the fit indices indicated that the model provided an adequate fit to the data (CFI =

359   0.94; SRMR = 0.062).

360

361   **Discussion**

362   Invasive fish and nutrient pollution represent two ubiquitous drivers of ecosystem

363   change in freshwaters. We hypothesized that increased nutrients could either 'dilute' the

364   negative predatory effects of mosquitofish on lower trophic levels by increasing prey

365   availability (the invader attenuation hypothesis), or alternatively, amplify their effects on

366   native taxa by disproportionately benefiting mosquitofish (the invader amplification

367   hypothesis). Overall, our data supported the invader amplification hypothesis:

368   mosquitofish biomass increased by 30% while population density increased by five-fold

369 under the high nutrient treatments, with no reduction in their predatory effects on lower

370 trophic levels. Effects of nutrients on zooplankton and native amphibians were weak

371 compared to the effects of nutrients on mosquitofish. These findings indicate that

372 mosquitofish may benefit from increases in nutrients more than the native organisms that

373 they prey upon.

374   The expected outcome of interactions between species invasions and nutrient

375 inputs remains a relatively open question, with some studies reporting positive effects of

376 elevated nutrients on invaders and increased invasion impacts (e.g., Chase and Knight

377 2006, Coetzee et al. 2007, Zhao et al. 2015), while in other cases nutrients facilitate co-

378 existence between native and introduced species or disproportionately promote natives

379 (e.g., Firn et al. 2010). Many invaders tend to have fast life histories, with rapid

380 maturation times and a large numbers of offspring, and thus high resource demands

381 (Blumenthal 2006, González et al. 2010, Tibbets et al. 2010). Our experimental results

382 support this idea, suggesting that mosquitofish can effectively utilize excess resources,

383 perhaps more so than native community members. Within our experimental

384 manipulation, increases in nutrient availability led to enhanced reproduction and survival

385 of young mosquitofish. Mosquitofish are known to cannibalize their young, which may

386 have occurred more readily under low resource availability conditions (Pyke 2008).

387 Because mosquitofish are generalist predators that feed on zooplankton, small

388 invertebrates, and amphibians (García-Berthou 1999), the increase in nutrient input likely

389 promoted fish biomass through increased food availability (Lancaster and Drenner 1990).

390 At the intermediate time point of the study (week three), we observed a three-fold

391 increase in zooplankton in the medium and high nutrient conditions relative to the low

392 nutrient condition. Densities of both cladocerans and copepods were highest at this point

393 in the study, indicating that there were likely large differences in total food availability to

394 mosquitofish across the nutrient treatments that could have driven the differences in fish

395 productivity. It is also possible that very small zooplankton taxa (e.g., rotifers and

396 ciliates) may have been a food source in the mesocosms that responded to nutrient

397 treatments. Additionally, the increase in mosquitofish biomass at high nutrients could

398 have been attributed in part to increases in detritus from turnover of phytoplankton and

399 periphyton. Detritus has been previously shown to be a component of the diet of

400   mosquitofish in some settings (Blanco et al. 2004). Analyses of the stomach content or

401   isotopic ratios of the mosquitofish would be useful for future studies to help clarify the

402   mechanisms driving the bottom-up effects that we observed here.

403   In contrast to mosquitofish, most native consumers in the mescosms did not show

404   strong responses to nutrient enrichment that could have facilitated coexistence with

405   mosquitofish or weakened their predatory effects. The only amphibian to show a positive

406   growth response with nutrient enrichment were California newts, which are strict

407   carnivores and may have occupied a similar trophic level as mosquitofish within the

408   mesocosms (Petranka 2010). Although the native amphibians were unable to reproduce in

409   the mesocosms (in contrast to mosquitofish), overall they showed weak growth responses

410   to nutrient enrichment, with two of three species being relatively unaffected. Aquatic

411   benthic invertebrates also did not show strong responses to nutrient enrichment,

412   collectively suggesting that mosquitofish benefitted more from bottom-up effects than

413   native community members.

414   Mosquitofish in our mesocosm experiment directly preyed on native amphibians,

415   completely eliminating them from over 50% of mesocosm replicates containing fish.

416   Prior work has also found that amphibian larvae are highly susceptible to predation by

417   mosquitofish, particularly in mescosms and laboratory experiments (Webb and Joss 1997,

418   Goodsell and Kats 1999, Zeiber et al. 2008, Preston et al. 2012, Shulse et al. 2013).

419   Mosquitofish directly consume amphibians and cause sublethal injuries when they

420   remove the legs and tails of developing larvae (Preston et al. 2012, Shulse and Semlitsch

421   2014). Of the three amphibian species in this experiment, western toads showed the

422   highest survival. This species is toxic in its larval stages and often less palatable to

423   vertebrate predators than other species (Gunzburger and Travis 2005). We also note,

424   however, that the predatory effects of mosquitofish on amphibians and invertebrates may

425   be context-dependent. Increases in habitat complexity and the availability of alternative

426   prey can weaken the predatory effects of mosquitofish on some amphibian species

427   (Lawler et al. 1999, Preston et al. 2017) and invertebrates (Knorp and Dorn 2016). As a

428   result, it may be difficult to generalize mosquitofish impacts across discrete wetlands that

429   vary in environmental conditions and community structure. In some systems, they will

430   strongly reduce amphibian populations (e.g., Shulse et al. 2013, Holbrook and Dorn

431 2016), whereas in others they may coexist (e.g., Reynolds 2009). Furthermore, although

432 we observed positive effects of nutrients on mosquitofish, this did not result in stronger

433 mosquitofish effects on amphibians at high nutrient levels. One explanation for this result

434 is that the additional mosquitofish at high nutrients were juveniles that may have been too

435 small to effectively prey on late-stage amphibian larvae. A longer-running study,

436 allowing the young mosquitofish produced in the mesocosms to mature into adults, could

437 have resulted in increased mosquitofish impacts on amphibians at high nutrients.

438   Mosquitofish also caused indirect positive effects on periphyton and freshwater

439 snails. Based on the path analysis, the increase in periphyton was likely driven by

440 reductions in grazing amphibian larvae due to mosquitofish predation. Snail biomass, in

441 turn, increased due to greater resource availability and the reduction in competition from

442 grazing amphibians. Such indirect positive effects of invasive species on native

443 community members have been documented before in a handful of cases (reviewed in

444 Rodriguez 2006). For instance, invasive European green grabs reduce abundances of

445 native clams and crabs, but increase the abundances of tube-building polychaete worms,

446 presumably through competitive release (Grosholz et al. 2000). Trophic cascades

447 involving increases in benthic algae due to decreases in herbivores after the introduction

448 of a nonnative predator have also been observed. Invasive brown trout in New Zealand,

449 for example, reduce periphyton from grazing pressure by native invertebrates, leading to

450 increases in benthic primary production (Townsend 1996, Huryn 1998). Further work is

451 needed to understand how commonly invasive species facilitate native species through

452 indirect interactions.

453   Our path analysis, in conjunction with past work, suggest that the overall effect of

454 mosquitofish on phytoplankton is an indirect effect driven by decreases in zooplankton

455 abundance (Hurlbert and Mulla 1981). Strong predatory effects of mosquitofish on

456 zooplankton, including the total loss of large-bodied taxa, have been observed in

457 mesocosms, artificial wetlands, as well as natural ponds (Hurlbert et al. 1972, Lancaster

458 and Drenner 1990, Margaritora et al. 2001, Nagdali and Gupta 2002). While mosquitofish

459 additions led to increased phytoplankton abundance in all treatments, this effect was

460 largest at high nutrients. While this effect was not statistically significant (likely due to

461   the high variability between mesocosms), it presents the potential for synergistic effects

462   of fish and nutrients on phytoplankton production.

463   In general, we found relatively few interactions between mosquitofish and

464   nutrients in our analyses, and most observed effects were additive. This is consistent with

465   a series of in-lake mesocosm experiments replicated in five countries in Europe (Stephen

466   et al. 2004). In these experiments, there were relatively few interactions between nutrient

467   availability and fish abundance (Moss et al. 2004, Vakkilainen et al. 2004, Van de Bund

468   et al. 2004), due likely in part to the presence of aquatic macrophytes, which have the

469   potential to regulate fish-zooplankton-phytoplankton interactions (Schriver et al. 1995).

470   In our experiment, it is possible that the effects of mosquitofish alone were so strong on

471   many responses (e.g., amphibians) that synergistic effects of fish and nutrients became

472   difficult to detect. Environmental conditions that result in weaker effects of fish may

473   enhance potentially subtle interactions between these two factors.

474   One consideration in interpreting the effects of mosquitofish at high nutrients is

475   that we focused on population-level effects, rather than per-capita effects. The increase in

476   fish numbers, and most of the increase in biomass, at high nutrients was due to young

477   individuals, making it likely that the per capita effects of mosquiofish were weaker at

478   high nutrients then at low nutrients (in contrast to the population-level effects).

479   Examining per capita effects (Wootton and Emmerson 2005) would provide

480   complementary insights into interactions between species invasions and nutrient inputs,

481   and should be a priority for future work.

482   A further consideration in our study is the time-scale and choice of experimental

483   venue. In a prior experiment (Preston et al. 2017), we found strong evidence for

484   reductions in zooplankton and invertebrates caused by mosquitofish introductions to a

485   natural wetland, consistent with the current mesocosm study. In contrast, however, we

486   did not find strong effects of mosquitofish on native amphibians, potentially because the

487   mosquitofish did not co-occur with the earliest and most susceptible developmental

488   stages of amphibian larvae, and the availability of alternative prey in the wetland was

489   higher than in the mesocosms. A multi-year field experiment would overcome these

490   limitations by testing how changes in nutrients influence mosquitofish interactions with

491   their prey, incorporating effects of age- and size-structured predator-prey populations

492   throughout the season. Under this scenario, we suspect that invader amplification would

493   be even stronger than in mesocosms because mosquitofish would co-occur with all life

494   stages of their prey and juvenile fish would mature and contribute to top-down effects. In

495   general, the possible differences in abiotic variables and community structure between

496   mesocosm studies and natural systems must always be considered when extrapolating

497   results to nature.

498   The fish and nutrient manipulations influenced the variance of several responses,

499   which has been linked to regime shifts in freshwater systems (Carpenter and Bock 2006).

500   For instance, the standard deviation of mean phytoplankton fluorescence across all

501   mesocosms was ~10 fold higher at high nutrients relative to low nutrients. A similar

502   magnitude increase in standard deviation of periphyton biomass was observed from fish-

503   absent to fish-present treatments. Both nutrient additions and predatory fish introductions

504   have potential to drive shifts in stable states in freshwater ecosystems, and our results

505   support the idea that such shifts can be associated with a prior change in the variance of a

506   system (Carpenter and Bock 2006, Carpenter et al. 2011). Future work that allows a

507   system to reach equilibrium, would facilitate testing how nutrients and mosquitofish

508   jointly influence shifts in stable states and potential warning signals.

509   Collectively, our results demonstrate that nutrients have the potential to mediate

510   the success of mosquitofish, such that elevated nutrient loading may enhance

511   mosquitofish production more so than native community members. Increased nutrient

512   loading also has potential to enhance the establishment and possibly spread of

513   mosquitofish through connected waterways. Our findings parallel results from other

514   systems in which invasive species disproportionately benefit from increased nutrients

515   relative to natives (González et al. 2010). Our simplified mesocosm experiment provides

516   a mechanistic foundation for future studies to test the relationship between nonnative fish

517   impacts and nutrient availability in more complex natural ecosystems, for which we

518   emphasize the need to consider how additional factors, such as ontogenetic diet shifts and

519   size-structured predation, affect the potential for invader amplification. Results of such

520   studies will be useful for informing management strategies of freshwaters, particularly

521   considering the increasing co-occurrence of abiotic environmental change and aquatic

522   species invasions (MacDougal and Turkington 2005). In particular, it would be useful to

523 identify the nutrient conditions under which invasive species are most likely to have

524 undesirable impacts, and to prioritize management resources for these locations or time

525 periods.

526

527 **Acknowledgments**

537

538 **Literature Cited**

539 Azevedo-Santos, V. M., J. R. S. Vitule, F. M. Pelicice, E. García-Berthou, and D.

540   Simberloff. 2017. Nonnative fish to control *Aedes* mosquitoes: a

541   controversial, harmful tool. BioScience 67:84-90.

542 Balciunas, D., and S. P. Lawler. 1995. Effects of basal resources, predation, and

543   alternative prey in microcosm food chains. Ecology 76:1327–1336.

544 Beeton, A. M. 1965. Eutrophication of the St. Lawrence great lakes. Limnology and

545   Oceanography 10:240–254.

546 Blanco, S., S. Romo, and M.-J. Villena. 2004. Experimental study on the diet of

547   mosquitofish (*Gambusia holbrooki*) under different ecological conditions in a

548   shallow lake. International Review of Hydrobiology 89:250–262.

549 Blumenthal, D. M. 2006. Interactions between resource availability and enemy release in

550   plant invasion. Ecology Letters 9:887–895.

551 Brinson, M. M., and A. I. Malvárez. 2002. Temperate freshwater wetlands: types, status,

552   and threats. Environmental Conservation 29:115–133.

553   Byers, J. E. 2002. Impact of non-indigenous species on natives enhanced by

554   anthropogenic alteration of selection regimes. Oikos 97:449–458.

555   Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H.

556   Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen.

557   Ecological Applications 8:559–568.

558   Carpenter, S. R., E. H. Stanley, and M. J. Vander Zanden. 2011. State of the world's

559   freshwater ecosystems: physical, chemical, and biological changes. Annual

560   review of Environment and Resources 36:75–99.

561   Chase, J. M., and T. M. Knight. 2006. Effects of eutrophication and snails on Eurasian

562   watermilfoil (*Myriophyllum spicatum*) invasion. Biological Invasions 8:1643–

563   1649.

564   Coetzee, J. A., M. J. Byrne, and M. P. Hill. 2007. Impact of nutrients and herbivory by

565   *Eccritotarsus catarinensis* on the biological control of water hyacinth, *Eichhornia*

566   *crassipes*. Aquatic Botany 86:179–186.

567   Crooks, J. A., A. L. Chang, and G. M. Ruiz. 2011. Aquatic pollution increases the

568   relative success of invasive species. Biological Invasions 13:165–176.

569   Dahl, T. E. 2000. Status and trends of wetlands in the conterminous United States 1986 to

570   1997. US Fish and Wildlife Service. Onalaska, Wisconsin, USA.

571   Davis, M. A., J. P. Grime, and K. Thompson. 2000. Fluctuating resources in plant

572   communities: a general theory of invasibility. Journal of Ecology 88:528–534.

573   Didham, R. K., J. M. Tylianakis, N. J. Gemmell, T. A. Rand, and R. M. Ewers. 2007.

574   Interactive effects of habitat modification and species invasion on native species

575   decline. Trends in Ecology & Evolution 22:489–496.

576   Dodson, S. I., S. E. Arnott, and K. L. Cottingham. 2000. The relationship in lake

577   communities between primary productivity and species richness. Ecology

578   81:2662–2679.

579   Downs, C. W. 1991. Fishes in California mosquito control. California Mosquito and

580   Vector Control Association, Sacramento, California, USA.

581   Dukes, J. S., and H. A. Mooney. 1999. Does global change increase the success of

582   biological invaders? Trends in Ecology & Evolution 14:135–139.

583   Fargione, J., and D. Tilman. 2002. Competition and coexistence in terrestrial plants. Pgs

584   165–206. In: *Competition and Coexistence*. Springer. New York, New York,

585   USA.

586   Field, R., et al. 2009. Spatial species-richness gradients across scales: a meta-analysis.

587   Journal of Biogeography 36:132–147.

588   Firn, J., A. S. MacDougall, S. Schmidt, and Y. M. Buckley. 2010. Early emergence and

589   resource availability can competitively favour natives over a functionally similar

590   invader. Oecologia 163:775–784.

591   Flores-Moreno, H., et al. 2016. Climate modifies response of non-native and native

592   species richness to nutrient enrichment. Philosophical Transactions of the Royal

593   Society B 371:20150273.

594   García-Berthou, E. 1999. Food of introduced mosquitofish: ontogenetic diet shift and

595   prey selection. Journal of Fish Biology 55:135–147.

596   González, A. L., J. S. Kominoski, M. Danger, S. Ishida, N. Iwai, and A. Rubach. 2010.

597   Can ecological stoichiometry help explain patterns of biological invasions? Oikos

598   119:779–790.

599   Goodsell, J. A., and L. B. Kats. 1999. Effect of introduced mosquitofish on Pacific

600   treefrogs and the role of alternative prey. Conservation Biology 13:921–924.

601   Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes

602   on identification. Herpetologica 16:183–190.

603   Grosholz, E. D., G. M. Ruiz, C. A. Dean, K. A. Shirley, J. L. Maron, and P. G. Connors.

604   2000. The impacts of a nonindigenous marine predator in a California bay.

605   Ecology 81:1206–1224.

606   Gunzburger, M. S., and J. Travis. 2005. Critical literature review of the evidence for

607   unpalatability of amphibian eggs and larvae. Journal of Herpetology 2005:547–

608   571.

609   Hall Jr, R. O., J. L. Tank, and M. F. Dybdahl. 2003. Exotic snails dominate nitrogen and

610   carbon cycling in a highly productive stream. Frontiers in Ecology and the

611   Environment 1:407–411.

612   Holbrook, J. D., and N. J. Dorn. 2016. Fish reduce anuran abundance and decrease

613   herpetofaunal species richness in wetlands. Freshwater Biology 61:100–109.

614   Hu, L., and P. M. Bentler. 1999. Cutoff criteria for fit indexes in covariance structure

615   analysis: Conventional criteria versus new alternatives. Structural Equation

616   Modeling: a Multidisciplinary Journal 6:1–55.

617   Hurlbert, S. H., and M. S. Mulla. 1981. Impacts of mosquitofish (*Gambusia affinis*)

618   predation on plankton communities. Hydrobiologia 83:125–151.

619   Hurlbert, S. H., J. Zedler, and D. Fairbanks. 1972. Ecosystem alteration by mosquitofish

620   (*Gambusia affinis*) predation. Science 175:639–641.

621   Huryn, A. D. 1998. Ecosystem-level evidence for top-down and bottom-up control of

622   production in a grassland stream system. Oecologia 115:173–183.

623   Jackson, M. C., R. Allen, J. Pegg, and J. R. Britton. 2013. Do trophic subsidies affect the

624   outcome of introductions of a non-native freshwater fish? Freshwater Biology

625   58:2144–2153.

626   Jeppesen, E., T. L. Lauridsen, T. Kairesalo, and M. R. Perrow. 1998. Impact of

627   submerged macrophytes on fish-zooplankton interactions in lakes. Pages 91–114

628   *in* The structuring role of submerged macrophytes in lakes. Springer, New York,

629   New York, USA.

630   Joseph, M. B., D. L. Preston, and P. T. Johnson. 2016. Integrating occupancy models and

631   structural equation models to understand species occurrence. Ecology 97: 765-

632   775.

633   Kline, R. B. 2015. Principles and practice of structural equation modeling. Guilford

634   Publications, New York, New York, USA.

635   Knorp, N. E., and N. J. Dorn. 2016. Mosquitofish predation and aquatic vegetation

636   determine emergence patterns of dragonfly assemblages. Freshwater Science

637   35:114–125.

638   Kolar, C. S., and D. M. Lodge. 2000. Freshwater nonindigenous species: interactions

639   with other global changes. Pages 330 *in* Invasive species in a changing world.

640   Island Press, Washington, D.C., USA.

641   Lancaster, H. F., and R. W. Drenner. 1990. Experimental mesocosm study of the separate

642   and interaction effects of phosphorus and mosquitofish (*Gambusia affinis*) on

643   plankton community structure. Canadian Journal of Fisheries and Aquatic

644   Sciences 47:471–479.

645 Lawler, S. P., D. Dritz, T. Strange, and M. Holyoak. 1999. Effects of introduced

646   mosquitofish and bullfrogs on the threatened California red-legged frog.

647   Conservation Biology 13:613–622.

648 MacDougall, A. S., and R. Turkington. 2005. Are invasive species the drivers or

649   passengers of change in degraded ecosystems? Ecology 86:42–55.

650 Margaritora, F. G., O. Ferrara, and D. Vagaggini. 2001. Predatory impact of the

651   mosquitofish (*Gambusia holbrooki* Girard) on zooplanktonic populations in a

652   pond at Tenuta di Castelporziano (Rome, Central Italy). Journal of Limnology

653   60:189–193.

654 Merkley, S. S., R. B. Rader, and G. B. Schaalje. 2015. Introduced western mosquitofish

655   (*Gambusia affinis*) reduce the emergence of aquatic insects in a desert spring.

656   Freshwater Science 34:564–573.

657 Mills, E. L., J. H. Leach, J. T. Carlton, and C. L. Secor. 1994. Exotic species and the

658   integrity of the Great Lakes. BioScience 44:666–676.

659 Mills, M. D., R. B. Rader, and M. C. Belk. 2004. Complex interactions between native

660   and invasive fish: the simultaneous effects of multiple negative interactions.

661   Oecologia 141:713–721.

662 Mittelbach, G. G., C. F. Steiner, S. M. Scheiner, K. L. Gross, H. L. Reynolds, R. B.

663   Waide, M. R. Willig, S. I. Dodson, and L. Gough. 2001. What is the observed

664   relationship between species richness and productivity? Ecology 82:2381–2396.

665 Moss, B., et al. 2004. Continental-scale patterns of nutrient and fish effects on shallow

666   lakes: synthesis of a pan-European mesocosm experiment. Freshwater Biology

667   49:1633–1649.

668 Nagdali, S. S., and P. K. Gupta. 2002. Impact of mass mortality of a mosquito fish,

669   *Gambusia affinis* on the ecology of a fresh water eutrophic lake (Lake Naini Tal,

670   India). Hydrobiologia 468:45–51.

671 Nichols, F. H., J. E. Cloern, S. N. Luoma, and D. H. Peterson. 1986. The modification of

672   an estuary. Science 231:567–573.

673 Odada, E. O., D. O. Olago, F. Bugenyi, K. Kulindwa, J. Karimumuryango, K. West, M.

674   Ntiba, S. Wandiga, P. Aloo-Obudho, and P. Achola. 2003. Environmental

675   assessment of the East African Rift Valley lakes. Aquatic Sciences 65:254–271.

676   Ogutu-Ohwayo, R., R. E. Hecky, A. S. Cohen, and L. Kaufman. 1997. Human impacts on
677   the African great lakes. Environmental Biology of fishes 50:117–131.
678   Penk, M. R., J. M. Jeschke, D. Minchin, and I. Donohue. 2016. Warming can enhance
679   invasion success through asymmetries in energetic performance. Journal of
680   Animal Ecology 85:419–426.

681   Petranka, J. W. 2010. Salamanders of the United States and Canada. Smithsonian Books,
682   Washington, D.C., USA.
683   Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and
684   economic costs associated with alien-invasive species in the United States.
685   Ecological Economics 52:273–288.
686   Preston, D. L., H. D. Hedman, E. R. Esfahani, E. M. Pena, C. E. Boland, K. B. Lunde,
687   and P. T. Johnson. 2017. Responses of a wetland ecosystem to the controlled
688   introduction of invasive fish. Freshwater Biology 62:767–778.
689   Preston, D. L., J. S. Henderson, and P. T. Johnson. 2012. Community ecology of
690   invasions: direct and indirect effects of multiple invasive species on aquatic
691   communities. Ecology 93:1254–1261.
692   Pyke, G. H. 2008. Plague minnow or mosquito fish? A review of the biology and impacts
693   of introduced *Gambusia* species. Annual Eeview of Ecology, Evolution, and
694   Systematics 39:171–191.
695   Pyšek, P., and D. M. Richardson. 2010. Invasive species, environmental change and
696   management, and health. Annual Review of Environment and Resources 35:25–
697   55.
698   R Core Team (2014). R: a language and environment for statistical computing. R
699   Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-
700   project.org/.
701   Rahel, F. J., and J. D. Olden. 2008. Assessing the effects of climate change on aquatic
702   invasive species. Conservation Biology 22:521–533.
703   Reynolds, S. J. 2009. Impact of the introduced poeciliid *Gambusia holbrooki* on
704   amphibians in southwestern Australia. Copeia 2009:296–302.

705   Ricciardi, A. 2001. Facilitative interactions among aquatic invaders: is an invasional

706   meltdown occurring in the Great Lakes? Canadian Journal of Fisheries and

707   Aquatic Sciences 58:2513–2525.

708   Ricciardi, A., and H. J. MacIsaac. 2011. Impacts of biological invasions on freshwater

709   ecosystems. Pages 211–224 *in* Fifty years of Invasion Ecology: the legacy of

710   Charles Elton. Wiley-Blackwell, West Sussex, UK.

711   Rodriguez, L. F. 2006. Can invasive species facilitate native species? Evidence of how,

712   when, and why these impacts occur. Biological Invasions 8:927–939.

713   Rosseel, Y. et al. 2011. lavaan: latent variable analysis. R Package Version 04-11. URL:

714   https://cran.r-project.org/web/packages/lavaan/index.html.

715   Schriver, P. E. R., J. Bogestrand, E. Jeppesen, and M. Sondergaard.1995. Impact of

716   submerged macrophytes on fish-zooplanktotn-phytoplankton interactions: large-

717   scale enclosure experiments in a shallow eutrophic lake. Freshwater Biology

718   33:255–270.

719   Semlitsch, R. D. and Boone, M. D. 2010. Aquatic mesocosms. Pages 97–102 *in*

720   Amphibian Ecology and Conservation. Oxford University Press, New York, New

721   York, USA.

722   Shulse, C. D., and R. D. Semlitsch. 2014. Western mosquitofish (*Gambusia affinis*)

723   bolster the prevalence and severity of tadpole tail injuries in experimental

724   wetlands. Hydrobiologia 723:131–144.

725   Shulse, C. D., R. D. Semlitsch, and K. M. Trauth. 2013. Mosquitofish dominate

726   amphibian and invertebrate community development in experimental wetlands.

727   Journal of Applied Ecology 50:1244–1256.

728   Silva, L. C., R. S. Corrêa, T. A. Doane, E. I. Pereira, and W. R. Horwath. 2013.

729   Unprecedented carbon accumulation in mined soils: the synergistic effect of

730   resource input and plant species invasion. Ecological Applications 23:1345–1356.

731   Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: Where do we go from

732   here? Trends in Ecology & Evolution 24:201–207.

733   Spivak, A. C., M. J. Vanni, and E. M. Mette. 2011. Moving on up: Can results from

734   simple aquatic mesocosm experiments be applied across broad spatial scales?

735   Freshwater Biology 56:279–291.

Stephen, D., et al. 2004. Continental-scale patterns of nutrient and fish effects on shallow
     lakes: introduction to a pan-European mesocosm experiment. Freshwater Biology
     49:1517–1524.

Strayer, D. L. 2010. Alien species in freshwaters: ecological effects, interactions with
     other stressors, and prospects for the future. Freshwater Biology 55:152–174.

Tabassum, S., and M. R. Leishman. 2016. Trait values and not invasive status determine
     competitive outcomes between native and invasive species under varying soil
     nutrient availability. Austral Ecology 41:875–885.

Teixeira, M. C., L. M. Bini, and S. M. Thomaz. 2017. Biotic resistance buffers the effects
     of nutrient enrichment on the success of a highly invasive aquatic plant.
     Freshwater Biology 62:65–71.

Tibbets, T. M., A. C. Krist, R. O. Hall Jr, and L. A. Riley. 2010. Phosphorus-mediated
     changes in life history traits of the invasive New Zealand mudsnail
     (*Potamopyrgus antipodarum*). Oecologia 163:549–559.

Townsend, C. R. 1996. Invasion biology and ecological impacts of brown trout *Salmo
     trutta in* New Zealand. Biological Conservation 78:13–22.

Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change
     and species interactions in terrestrial ecosystems. Ecology Letters 11:1351–1363.

Vakkilainen, K., et al. 2004. Response of zooplankton to nutrient enrichment and fish in
     shallow lakes: a pan-European mesocosm experiment. Freshwater Biology
     49:1619–1632.

Van de Bund, W. J., et al. 2004. Responses of phytoplankton to fish predation and
     nutrient loading in shallow lakes: a pan-European mesocosm experiment.
     Freshwater Biology 49:1608–1618.

Vitousek, P. M., C. M. D'antonio, L. L. Loope, M. Rejmanek, and R. Westbrooks. 1997.
     Introduced species: a significant component of human-caused global change. New
     Zealand Journal of Ecology 1997:1–16.

Webb, C., and J. Joss. 1997. Does predation by the fish *Gambusia holbrooki*
     (Atheriniformes: Poeciliidae) contribute to declining frog populations? Australian
     Zoologist 30:316–324.

766 Winsome, T., L. Epstein, P. F. Hendrix, and W. R. Horwath. 2006. Competitive
767   interactions between native and exotic earthworm species as influenced by habitat
768   quality in a California grassland. Applied Soil Ecology 32:38–53.
769 Wootton, J. T., and M. Emmerson. 2005. Measurement of interaction strength in nature.
770   Annual Review of Ecology, Evolution, and Systematics 36:419–444.
771 Zeiber, R. A., T. M. Sutton, and B. E. Fisher. 2008. Western mosquitofish predation on
772   native amphibian eggs and larvae. Journal of Freshwater Ecology 23:663–671.
773 Zenni, R. D., and M. A. Nuñez. 2013. The elephant in the room: the role of failed
774   invasions in understanding invasion biology. Oikos 122:801–815.
775 Zhao, H., W. Yang, L. Xia, Y. Qiao, Y. Xiao, X. Cheng, and S. An. 2015. Nitrogen-
776   enriched eutrophication promotes the invasion of *Spartina alterniflora* in coastal
777   China. CLEAN–Soil, Air, Water 43:244–250.
778 Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, G. M. Smith. 2009. Mixed effects
779   models and extensions in ecology with R. Springer. New York, New York, USA.
780
781 **Figure Legends**
782 **Figure 1.** (a) Total dissolved phosphorus and (b) total dissolved nitrogen from mesocosm
783 water samples collected at three time points over the duration of the study. Fish presence
784 (solid lines) or absence (dotted lines) is indicated by line type and nutrient conditions are
785 indicated by the point shapes. Error bars represent one standard error.
786 **Figure 2.** (a) Relative phytoplankton fluorescence, (b) density of cladoceran
787 zooplankton, and (c) density of copepod zooplankton from experimental mesocosms.
788 Phytoplankton fluorescence is a unitless, relative measure obtained from a laboratory
789 fluorometer. Zooplankton were measured from five combined tube samples (~4 L water
790 volume per mesocosm) on each sampling date. Fish presence (solid lines) or absence
791 (dotted lines) is indicated by line type and nutrient conditions are indicated by the point
792 shapes. Error bars represent one standard error.
793 **Figure 3.** (a) Results from experimental mesocosms showing periphyton biomass and (b)
794 snail biomass (*Helisoma* sp. and *Physa* sp.) quantified at the conclusion of the
795 experiment. Periphyton biomass was measured from a standardized area on the
796 mesocosm walls (480 cm^2) and snail biomass was the total from all individuals with a

797   mesocosm. Periphyton was also measured at the beginning of the study from clay tiles

798   (see Appendix S1: Fig. S2). Nutrient concentrations are shown on the x-axis and bar

799   colors correspond with mosquitofish presence or absence. Error bars represent one

800   standard error.

801   **Figure 4**. Results from experimental mesocosms showing (a) chorusfrog biomass

802   (*Pseudacris regilla*), (b) California newt biomass (*Taricha torosa*), (c) western toad

803   biomass (*Anaxyrus boreas*), and (d) mosquitofish biomass (*Gambusia affinis*). All values

804   are means per mesocosm of total biomass at the end of the experiment. Nutrient

805   concentrations are shown on the x-axis and bar colors correspond with mosquitofish

806   presence or absence. Error bars represent one standard error.

807   **Figure 5**. Path diagram testing hypothesized links within the mesocosm experiment.

808   Values for zooplankton and phytoplankton are from the conclusion of the study. All other

809   variables correspond to the values show in Fig. 3 and Fig. 4. Solid arrows show

810   statistically significant (P < 0.05) paths and dashed arrows show nonsignificant paths.

811   The width of each arrow corresponds to the magnitude of the standardized path

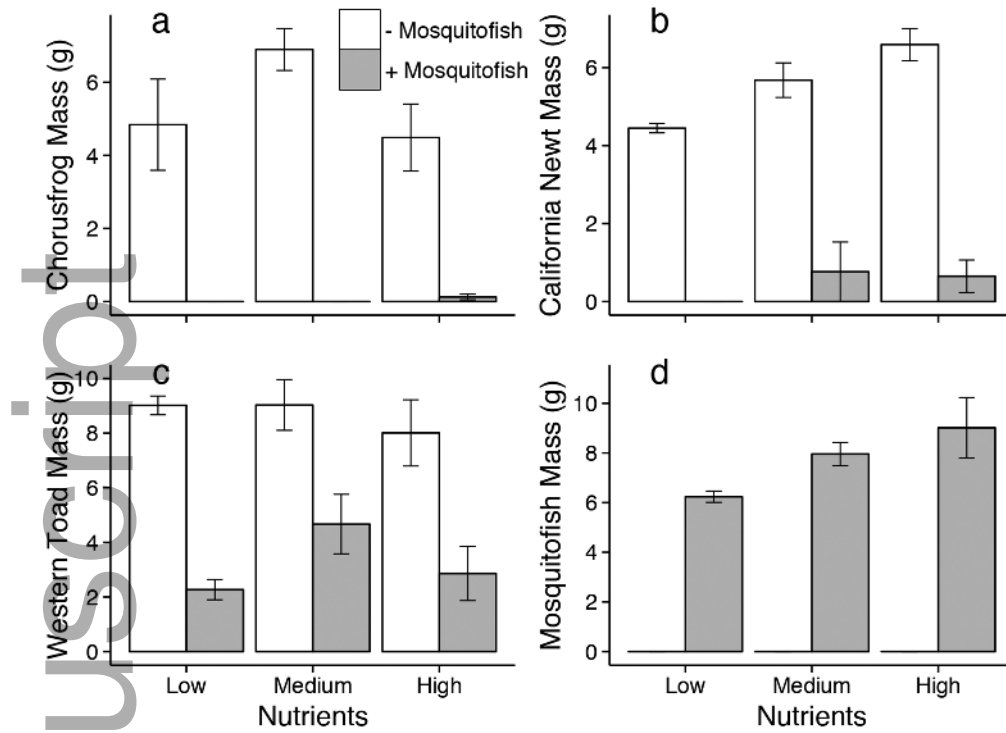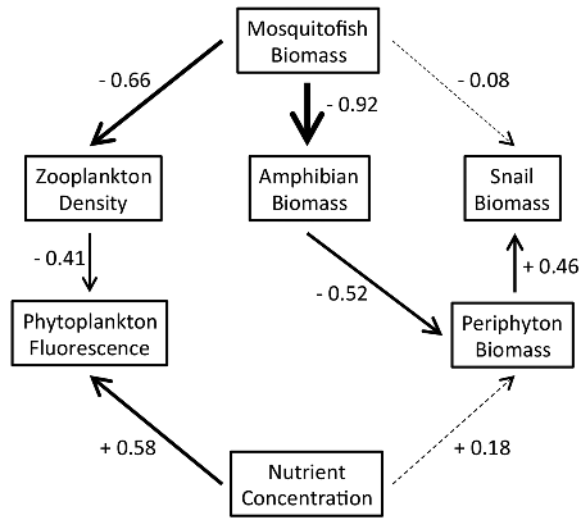812   coefficient, which is also provided next to the arrow.
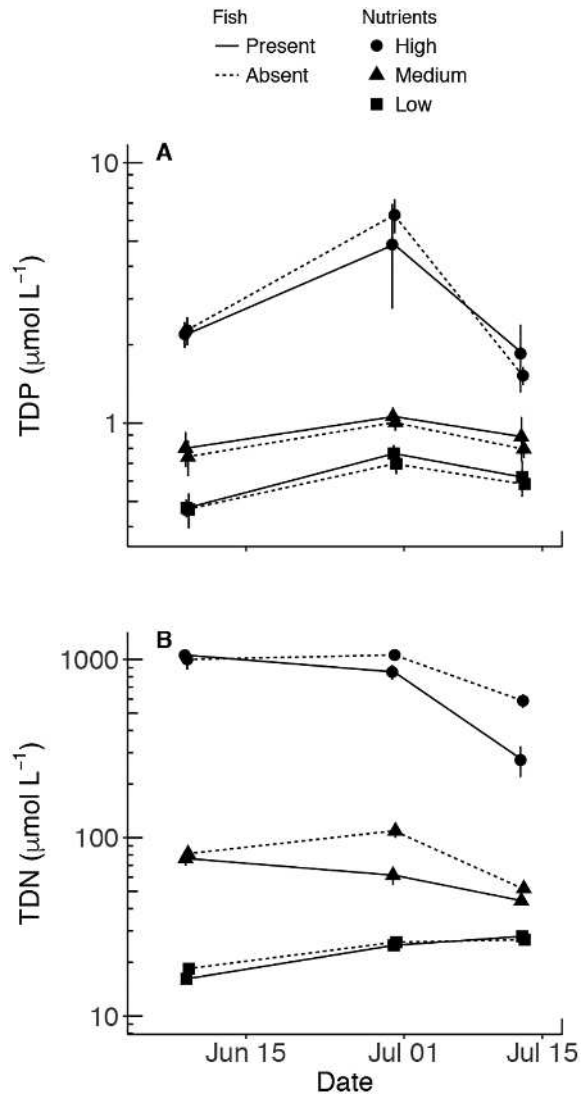
ecs2_2153_f1.tif
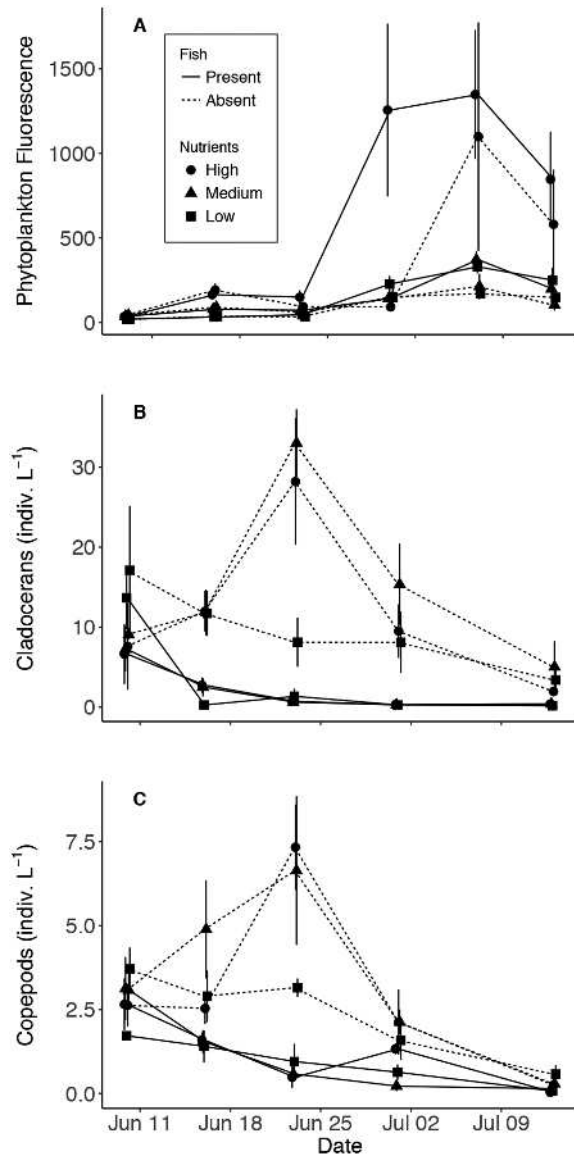
ecs2_2153_f2.tif
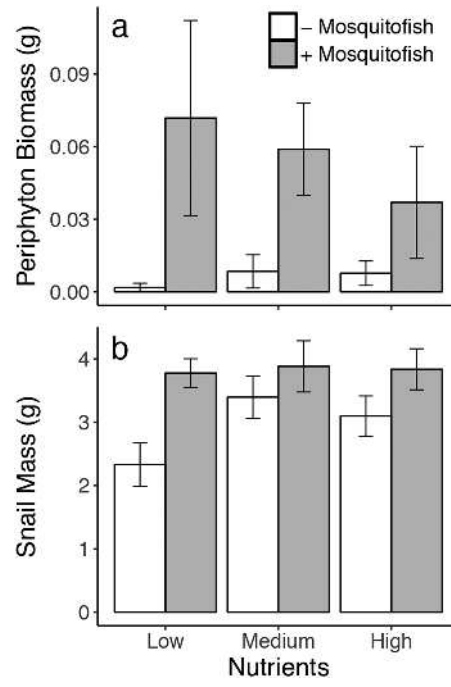
ecs2_2153_f3.tif

ecs2_2153_f4.tif

ecs2_2153_f5.tif