Community ecology of invasions: direct and indirect effects of multiple invasive species on aquatic communities

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Abstract. With many ecosystems now supporting multiple nonnative species from different trophic levels, it can be challenging to disentangle the net effects of invaders within a community context. Here, we combined wetland surveys with a mesocosm experiment to examine the individual and combined effects of nonnative fish predators and nonnative bullfrogs on aquatic communities. Among 139 wetlands, nonnative fish (bass, sunfish, and mosquitofish) negatively influenced the probability of occupancy of Pacific treefrogs (*Pseudacris regilla*), but neither invader correlated strongly with occupancy by California newts (*Taricha torosa*), western toads (*Anaxyrus boreas*), or California red-legged frogs (*Rana draytonii*). In mesocosms, mosquitofish dramatically reduced the abundance of zooplankton and palatable amphibian larvae (*P. regilla* and *T. torosa*), leading to increases in nutrient concentrations and phytoplankton (through loss of zooplankton), and rapid growth of unpalatable toad larvae (through competitive release). Bullfrog larvae reduced the growth of native anurans but had no effect on survival. Despite strong effects on natives, invaders did not negatively influence one another, and their combined effects were additive. Our results highlight how the net effects of multiple nonnative species depend on the trophic level of each invader, the form and magnitude of invader interactions, and the traits of native community members.

Key words: amphibian decline; community structure; food web; freshwater pond; Gambusia affinis; introduced species; invasion biology; Lithobates catesbeianus; Rana catesbeiana.

Introduction

While many invasion biology studies focus on interactions between a single invader and a single native species, there is a growing need to address the net consequences of multiple invasions within a community context (Olden and Poff 2003). Continued homogenization of the planet's biota has increased the number of nonnative species in many communities, a trend that is especially evident in aquatic ecosystems (Ricciardi and MacIsaac 2011). Yet the consequences of multiple invasions within native communities are challenging to disentangle because invaders can interact with one another, affect native species in highly variable ways, and cause subtle indirect effects (Simberloff and Von Holle 1999, Grosholz 2005). The net community-level effects of multiple invaders depend on both the trophic level of each invader and how they affect one another (Levin et al. 2002). If co-occurring invaders occupy different trophic levels (e.g., a primary consumer and secondary consumer), their combined effects could be reduced if one invader consumes the other, or amplified if the invasive predator indirectly releases the invasive

consumer from competition with natives (Grosholz 2005). Disentangling interactions among multiple invaders within native communities becomes even more challenging when nonnative species occur simultaneously with other types of environmental change, which can facilitate invaders or directly alter community structure (MacDougall and Turkington 2005).

In the western United States, American bullfrogs (*Lithobates catesbeianus* [=*Rana catesbeiana*]) and nonnative fish (i.e., sunfish, bass, and mosquitofish) are common wetland invaders that frequently co-occur in human-modified habitats, making it challenging to determine their individual roles in structuring native communities. Various studies have shown the potential for nonnative fish and bullfrogs to negatively influence native aquatic species through predation and competition (e.g., Kupferberg 1997, Goodsell and Kats 1999), although only two studies have simultaneously examined impacts of both nonnative fish and bullfrogs on a native species (Kiesecker and Blaustein 1998, Lawler et al. 1999). Understanding the combined effects of nonnative fish and bullfrogs is particularly important in light of evidence for multi-invader facilitation within this system. Sunfish (*Lepomis* spp.) can indirectly facilitate bullfrogs by preying on predators of amphibians, which occurs in the native ranges of both taxa (Werner and McPeek 1994) and in their introduced

ranges in the western United States (Adams et al. 2003). Bullfrog larvae are relatively unpalatable to most fish predators (Kruse and Francis 1977, Woodward 1983) and the indirect positive effects of fish on bullfrogs outweigh any direct negative effects. Less clear, however, is how fish and bullfrogs, alone and in combination, influence entire communities of native species, as single-taxon focused studies can often omit important indirect effects mediated through food web changes.

To better understand the direct and indirect effects of nonnative fish and bullfrogs on native aquatic communities, we combined field surveys of 139 wetlands with a mesocosm experiment designed to assess the mechanisms underlying aquatic interactions between native and nonnative species (see Plate 1). Our aims were to (1) evaluate how nonnative bullfrogs and fish (centrarchids and mosquitofish) influence the site occupancy of native amphibian taxa (*Pseudacris regilla*, *Anaxyrus* [=*Bufo*] *boreas*, *Taricha torosa*, and *Rana draytonii*) and (2) understand the direct and indirect mechanisms by which these invaders influence native aquatic communities in an experimental setting. While site occupancy of amphibians in ponds will depend on a myriad of factors influencing all amphibian life stages, our experiment focused on how invaders influenced the aquatic larvae of native amphibians, for which we predicted that fish and bullfrogs would have strong effects. We hypothesized that in the mesocosm experiment the invasive predator (mosquitofish) would decrease densities of palatable native species (amphibians and zooplankton), while the invasive herbivore (bullfrog larvae) would decrease growth of native grazers (anuran larvae and snails; see interaction web in Appendix A for detailed predictions). Because bullfrog larvae and mosquitofish occupy different trophic levels and are expected to have neutral direct effects on one another (Woodward 1983), we also suspected that the combined effects of both invaders would be additive.

Materials and Methods

Field surveys.—Between May and July of 2009, we surveyed 139 ponds in the San Francisco Bay Area of California (Contra Costa, Alameda, Santa Clara, and San Mateo counties). Because nonnative fish and bullfrogs cannot become established in temporary ponds, we restricted our analysis to permanent ponds (Adams 2000). To detect fish and amphibians, we used a combination of visual encounter surveys (VES) around the pond margins and standardized net sweeps with D nets (1.4 mm mesh, 2600-cm^2 opening) every 3–5 m around the perimeter. In 67 ponds, we also conducted three to four collections using a seine net (4 mm mesh, 1 × 2 m net) to sample the deeper waters. We identified all amphibians and fish captured before releasing them back into the pond. We defined sites as "occupied" only if breeding activity was detected (i.e., presence of eggs, larvae, or recent metamorphs). We also recorded pond elevation and pond area, the percentage of the shoreline

that was vegetated (as opposed to bare), the number of trees within 15 m of the pond, and measured water turbidity with a turbidometer (LaMotte, Chestertown, Maryland, USA).

Occupancy models.—We used single-season occupancy models (MacKenzie et al. 2002) to examine how the presence of nonnative species influenced the occupancy of four native amphibians (*P. regilla*, *A. boreas*, *T. torosa*, and *R. draytonii*). Occupancy models account for imperfect species detection and estimate both the probability of occurrence at a site (ψ) and the probability of detection conditional on presence (p). We used the three different survey methods (netsweeps, visual encounter surveys, and seines) as repeat observations within each model. Although a multi-method occupancy model exists, the one-species, single-season model provided similar results with more reliable model convergence. Our models for probability of occupancy (ψ) of each amphibian were variations of a global model that included separate covariates for the presence of bullfrogs, presence of fish (mosquitofish and centrarchids), pond elevation, pond surface area, shoreline vegetation, and tree density. Detection probability (p) was modeled with a general equation that included the same covariates used to model occupancy probability, with the addition of survey type, survey date (days since first survey), water temperature (°C), and water turbidity. Continuous covariates were standardized to have a mean of zero and standard deviation of one and the independent variables were tested for collinearity (all $r <$ 0.7; Quinn and Keough 2002). All occupancy analyses were conducted with the program PRESENCE (*available online*).[4]

We used a model-selection approach (Burnham and Anderson 2010) to determine the most important covariates in predicting amphibian occupancy. We tested all possible combinations of the six covariates in the occupancy equation (64 models per species), which included a model with constant occupancy probability between sites ($\psi(.)$). We assessed the fit of the global models for each species by generating 10 000 parametric bootstraps using the goodness-of-fit test in PRESENCE. Data for three of the four species demonstrated overdispersion ($\hat{c} > 1$), so we ranked models using QAIC and inflated standard errors by $\sqrt{\hat{c}}$ (Burnham and Anderson 2010). We then used the most supported models for each amphibian species (ΔQAIC < 2) to generate model-averaged estimates of occupancy (ψ) and coefficients (β) of the six parameters in the top occupancy models (B. Mitchell, *unpublished spreadsheets*).

Experimental manipulation.—We conducted a 2 × 2 factorial experiment manipulating the presence of bullfrog larvae and mosquitofish in outdoor mesocosms (378 L) maintained at the Hopland Research and Extension Center, California, USA. Each treatment
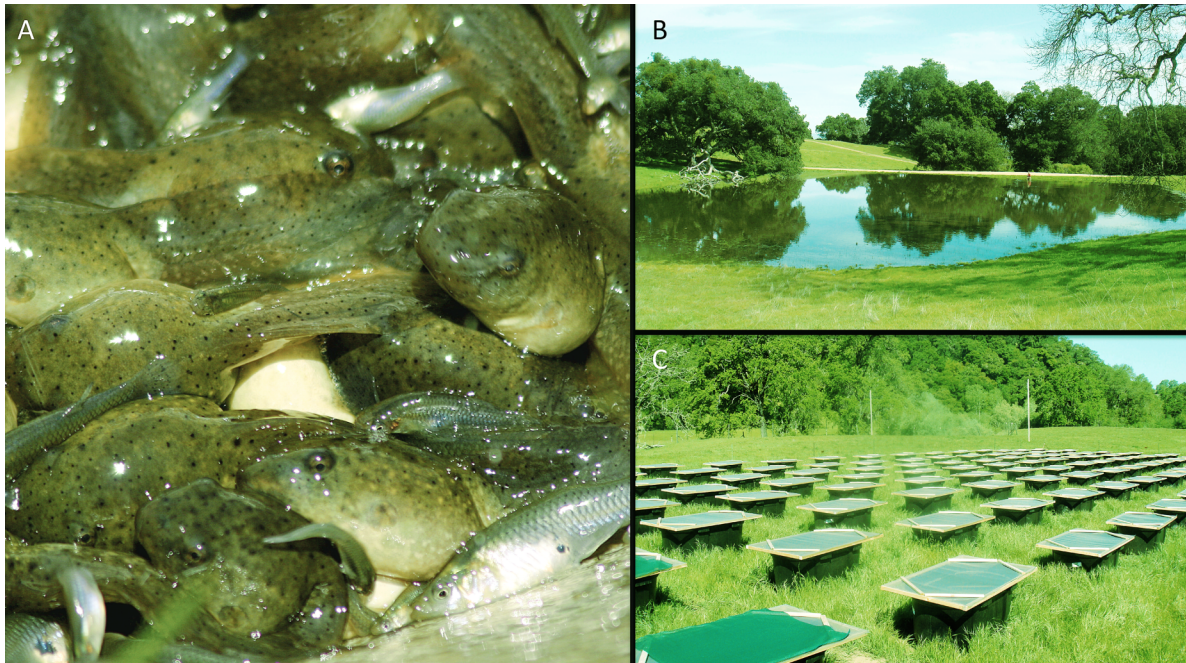
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PLATE 1. (A) Nonnative western mosquitofish (*Gambusia affinis*) and bullfrog tadpoles (*Lithobates catesbeianus*) dominate the contents of a seine net haul from a California pond. (B) The pond where photo A was taken, which is representative of the wetlands in our field surveys. Alongside the nonnative mosquitofish and bullfrogs, this pond also supports breeding populations of the three native amphibians that were focal species in our mesocosm experiment (Pacific treefrogs [*Pseudacris regilla*], western toads [*Anaxyrus boreas*], and California newts [*Taricha torosa*]). (C) Outdoor mesocosms used to experimentally evaluate the effects of mosquitofish and bullfrog tadpoles on a native aquatic community. The mesocosm experiment was conducted at the Hopland Research and Extension Center in Mendocino County, California (USA). Photo credits: A, Jeremy Monroe/Freshwaters Illustrated; B and C, D. L. Preston.

was replicated five times, for a total of 20 mesocosms. Mesocosms were established using standard techniques (see Appendix B). After allowing communities of algae, zooplankton, and snails (*Helisoma* sp.) to establish, we added 15 Pacific treefrog (*P. regilla*) tadpoles, 15 western toad (*A. boreas*) tadpoles, and 10 California newt (*T. torosa*) larvae. Native amphibians were collected locally as embryos and were hatched in the laboratory. Amphibians were matched for size (snout–vent length, SVL) and developmental stage before being randomly assigned to mesocosms (Appendix B). To the invader treatments we added three locally collected second-year bullfrog tadpoles (*Lithobates catesbeianus*) and/or five adult mosquitofish (*Gambusia affinis*; three females, two males). Densities were chosen to lie within the ranges observed in the field. We focused on aquatic invaders and amphibian larval stages, both because we expected strong interactions and because predatory and competitive interactions between metamorphosed bullfrogs and native amphibians have been previously documented (Kiesecker and Blaustein 1998, Pearl et al. 2004).

As amphibians began to metamorphose (4 weeks), we destructively sampled the mesocosms and recorded the SVL, wet mass, developmental stage (Gosner 1960), presence/absence of injuries on each individual and the combined dry mass for each species. For mosquitofish, we recorded the number of fish removed from each mesocosm and their lengths. We also enumerated all snails and measured their combined dry mass. At the beginning and end of the experiment, we analyzed water samples for total nitrogen, total phosphorus, and total carbon (procedures *available online*).[5] We measured phytoplankton abundance indirectly as chlorophyll fluorescence using a fluorometer (Turner Designs, Sunnyvale, California, USA) and quantified zooplankton density based on samples collected with a tube sampler (70 cm in length × 5 cm in diameter, five combined samples per mesocosm).

Mesocosm data analysis.—Survival (0 vs. 1) of native amphibians nested within mesocosms was analyzed with a generalized linear mixed-effects model with binomial errors using the lme4 package in R (Zuur et al. 2009). We specified bullfrogs, mosquitofish, and their interaction as fixed effects, and mesocosm as a random effect. For all other response variables, analyses were conducted on mesocosm means. Two-way MANOVA with mosquitofish and bullfrogs as independent variables were used to analyze mesocosm data on response variables of native amphibian species (Gosner stage, SVL, and individual wet mass), snails (log-transformed abundance and wet mass), zooplankton, and phyto-

[5] http://snobear.colorado.edu/Kiowa/Kiowaref/procedure.html

TABLE 1. Model-averaged coefficients (β), $\sqrt{\hat{c}}$-inflated standard errors (SE), and 95% confidence intervals for the covariates from the most well-supported models (ΔQAIC < 2) of native amphibian occupancy.

Covariate	β	SE	Lower CI	Upper CI
Pacific treefrog				
Elevation	0.148	0.145	−0.600	0.895
Surface area	0.011	0.024	−0.293	0.315
Vegetation	−0.007	0.034	−0.367	0.353
Trees	−0.003	0.020	−0.280	0.274
Fish	−2.241	0.958	−4.159	−0.323
Bullfrogs	0.062	0.226	−0.870	0.994
Western toad				
Elevation	0.501	0.195	−0.366	1.367
Surface area	1.050	0.275	0.023	2.077
Vegetation	−1.116	0.180	−1.949	−0.284
Trees	−0.005	0.012	−0.215	0.206
Fish	−0.113	0.184	−0.955	0.728
Bullfrogs	2.429	1.565	−0.023	4.880
California newt				
Elevation	0.006	0.010	−0.191	0.202
Surface area	0.376	0.615	−1.160	1.913
Vegetation	0.054	0.045	−0.361	0.469
Trees	−0.092	0.228	−1.027	0.844
Fish	−0.092	0.228	−1.027	0.844
Bullfrogs	0.774	1.548	−1.665	3.212
California red-legged frog				
Elevation	0.005	0.010	−0.190	0.200
Surface area	1.413	0.415	0.151	2.675
Vegetation	0.018	0.018	−0.244	0.281
Trees	0.004	0.007	−0.165	0.173
Fish	−0.148	0.275	−1.176	0.881
Bullfrogs	−0.058	0.111	−0.711	0.595

Notes: Models used to calculate parameter estimates are shown in Appendix E: Table E1. For an explanation of $\sqrt{\hat{c}}$-inflated standard errors, see Burnham and Anderson (2010).

plankton (density of *Daphnia* and relative phytoplankton fluorescence). We used repeated-measures MANOVA to analyze effects of invaders on nutrients in the water column. One-way MANOVAs were used to test for effects of bullfrogs on mosquitofish (total number of offspring and juvenile length) and of mosquitofish on bullfrogs (SVL and wet mass). Significant MANOVAs were analyzed with Bonferroni-adjusted univariate ANOVAs to determine which variables were responsible for significant main effects. To aid in interpretation of differences in multiple response variables between experimental treatments, we used the lavaan package in R to generate a structural equation model (SEM) that included bullfrogs and mosquitofish as exogenous variables that were either directly or indirectly linked to nutrients in the water, native amphibians, snails, zooplankton and phytoplankton (for detailed SEM methods see Appendix C; lavaan package *available online*).[6]

RESULTS

Field surveys.—We detected nonnative bullfrogs at 23%, and nonnative fish at 18% (of which 12% contained

[6] http://cran.r-project.org/web/packages/lavaan/index.html

centrarchids and 8% contained mosquitofish) of sampled ponds. Model-averaged estimates from the top occupancy models (ΔQAIC < 2) indicated occupancy estimates of 90% for Pacific treefrogs, 67% for California newts, 57% for California red-legged frogs, and 35% for western toads. Nonnative fish presence was the most important predictor of treefrog occupancy, with all five top models including this covariate (see Appendix E: Table E1 for a full list of top models and associated statistics for each species). Fish presence was the only covariate of Pacific treefrog occupancy to have a coefficient with a 95% confidence interval that excluded zero (Table 1; model-averaged $\beta = -2.24$; 95% CI = −4.16 to −0.32). Four models were well supported for western toad occupancy, and the most important covariates (Table 1) included a positive effect of surface area (model-averaged $\beta = 1.05$; 95% CI = 0.02 to 2.08) and a negative effect of vegetation (model-averaged $\beta = -1.12$; 95% CI = −1.95 to −0.28). Twelve models were well supported for newt occupancy, although the second-best model did not include any covariates of occupancy probability ($\psi(.)$) and none of the model-averaged coefficients had a 95% confidence interval that excluded zero (Table 1). The only strong predictor of California red-legged frog occupancy was a positive effect of surface area, which was included in all six top
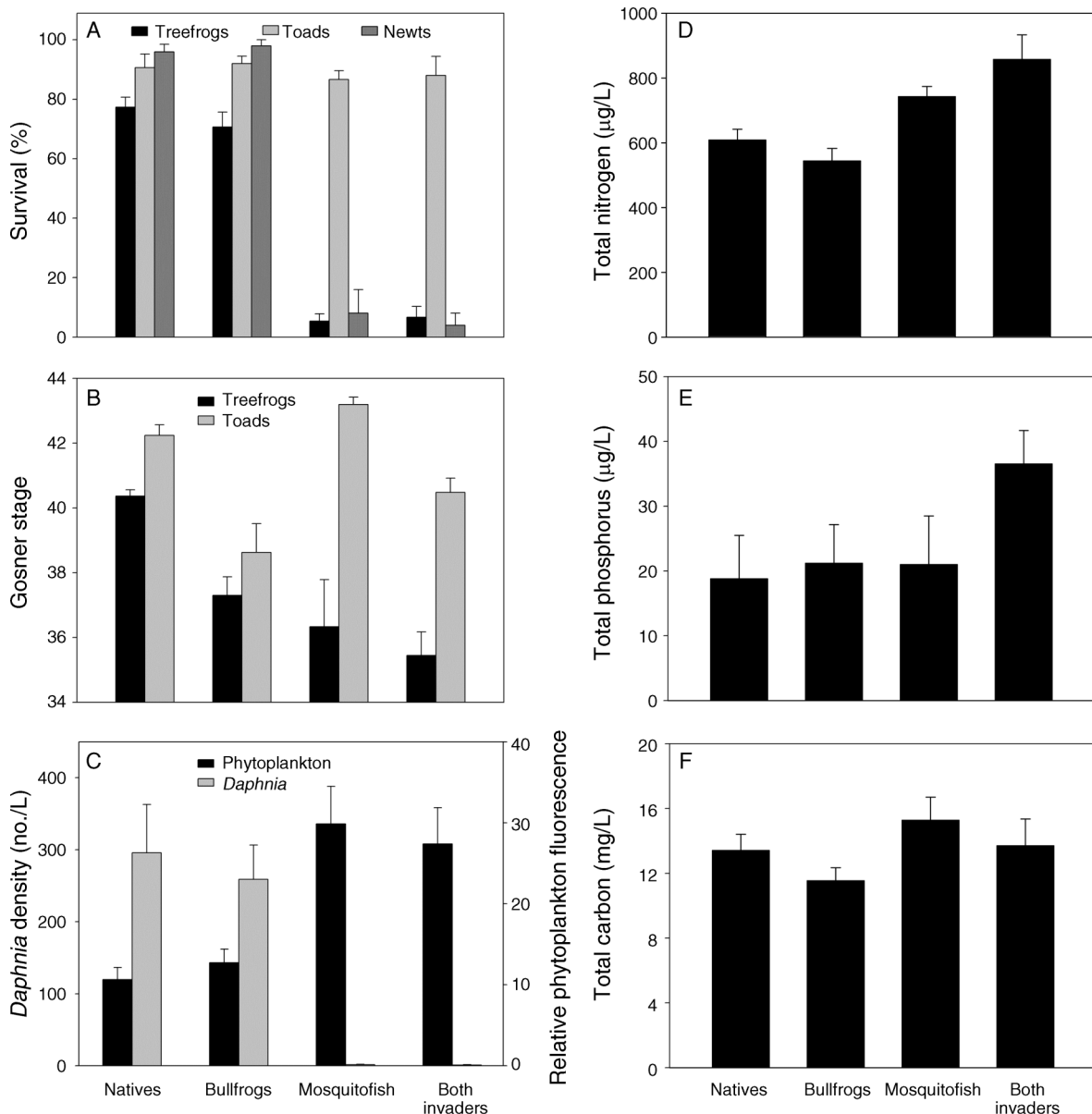
FIG. 1. Experimental mesocosm results examining effects of nonnative mosquitofish and bullfrogs on a native amphibian community, zooplankton, phytoplankton, and nutrients in the water column. All bars represent means plus one standard error. (A) Percentage survival of Pacific treefrogs (*Pseudacris regilla*), western toads (*Anaxyrus boreas*), and California newts (*Taricha torosa*). (B) Developmental stage of Pacific treefrogs and western toads at the end of the experiment. (C) Effects of wetland invaders on *Daphnia* density (left axis) and phytoplankton fluorescence (right axis). Effects of invaders on the increase in (D) total nitrogen, (E) total phosphorus, and (F) total carbon in the water column between the beginning and end of the experiment.

models (Table 1; model-averaged β = 1.41; 95% CI = 0.15 to 2.68).

Mesocosm experiment.—Mosquitofish reduced treefrog survival from 74% to 7% (GLMM, $Z = -7.04$, $P < 0.001$) and newt survival from 97% to 6% (GLMM, $Z = -6.08$, $P < 0.001$), but had no effect on toad survival (Fig. 1A). Surviving treefrogs from mosquitofish treatments also developed more slowly and were smaller than frogs from fishless treatments, despite the lower overall density of conspecifics (Fig. 1B; ANOVA, stage $F_{3,12} =$

16.80, $P = 0.001$; SVL $F_{3,12} = 16.61$, $P = 0.002$; wet mass $F_{3,12} = 13.92$, $P = 0.003$). Among surviving native amphibians in the treatments with mosquitofish, 44% of the treefrogs, 34% of the toads, and 17% of the newts displayed injuries (missing tail or limb elements; Appendix D: Fig D1). No amphibians raised in fishless treatments displayed abnormalities. Nevertheless, toads developed more rapidly in the presence of mosquitofish (Fig. 1B; ANOVA, $F_{3,16} = 7.04$, $P = 0.017$) and their combined dry mass was higher in mosquitofish treat-

ments (ANOVA, $F_{3,16} = 5.22$, $P = 0.036$). The SEM analysis (Appendix C: Fig. C1) suggested that the enhanced growth of toads in mosquitofish treatments was an indirect effect; mosquitofish strongly reduced treefrog biomass (standardized path coefficient = −0.95) while treefrogs had a moderate negative effect on toad biomass (standardized path coefficient = −0.35). Bullfrogs reduced treefrog developmental stage (Fig. 1B; ANOVA, $F_{3,12} = 7.59$, $P = 0.017$), toad developmental stage, and size (Fig. 1B; ANOVA, stage $F_{3,16} = 35.63$, $P < 0.001$; SVL $F_{3,16} = 18.52$, $P = 0.001$; wet mass $F_{3,16} = 13.45$, $P = 0.002$), but not newt size or the survival of any natives. The SEM (Fig. C1) indicated that the relative strength of the negative effect of bullfrogs on toad biomass (standardized path coefficient = −0.90) was considerably higher than that of bullfrogs on treefrog biomass (standardized path coefficient = −0.33). We did not detect a mosquitofish-by-bullfrog interaction on any response variables.

Invaders also had direct and indirect effects on plankton and nutrients. In the presence of mosquitofish, zooplankton (*Daphnia* spp.) were virtually undetectable (Fig. 1C; ANOVA, $F_{3,16} = 44.58$, $P < 0.001$) and phytoplankton fluorescence was about three times higher than in treatments lacking mosquitofish (Fig. 1C; ANOVA, $F_{3,16} = 10.64$, $P = 0.005$). The SEM provided evidence for a strong positive indirect effect of mosquitofish on phytoplankton (mosquitofish to *Daphnia*, standardized path coefficient = −0.99; *Daphnia* to phytoplankton, standardized path coefficient = −0.85). Mosquitofish also enhanced total nitrogen and total phosphorus (Fig. 1D; repeated-measures ANOVA, nitrogen $F_{1,16} = 19.46$, $P < 0.001$; Fig. 1E; phosphorus $F_{1,16} = 5.92$, $P = 0.027$), but there were no significant differences in carbon (Fig. 1F). Finally, the SEM suggested a moderate negative effect of bullfrogs on snail biomass (Fig. C1; standardized path coefficient = −0.41).

In contrast to their strong effects on native species, invaders did not significantly affect one another. Mosquitofish reproduced within mesocosms during the course of the experiment but neither the number of offspring nor the length of juvenile fish were affected by bullfrogs (MANOVA, Wilks' λ = 0.759, $F_{2,6} = 0.951$, $P = 0.438$). The mean number of fish removed from mosquitofish and mosquitofish plus bullfrog treatments was 30 ± 4.84 (mean ± SE) and 21 ± 2.97, respectively. Similarly, mosquitofish did not affect bullfrog SVL or wet mass (MANOVA, Wilks' λ = 0.907, $F_{2,7} = 0.36$, $P = 0.711$), and all bullfrogs survived the experiment.

DISCUSSION

Increases in the diversity, abundance, and rate of spread of introduced species in freshwater ecosystems underscore the need to address the impacts of aquatic invaders within complex communities composed of multiple native and nonnative species (Ricciardi and MacIsaac 2011). By combining field surveys and mechanistic experiments, we evaluated impacts of

multiple invaders within a community context and found both direct changes in community structure as well as subtler, indirect effects mediated by native community members. The effects of nonnative mosquitofish and bullfrogs depended on traits of the native species, including palatability to the invasive predator and dietary overlap with the invasive herbivore. Despite their strong effects on native species, the invaders had no significant effects on one another, and their combined effects on native communities were additive.

Field data from California wetlands indicated that nonnative fish (centrarchids and mosquitofish) negatively influenced the probability of occupancy of Pacific treefrogs, but that neither invader had strong effects on occupancy of the other native amphibians. The effects of fish on Pacific treefrog occupancy are consistent with the predatory effects of mosquitofish in our experiment, and with previous field surveys in central California (Fisher and Shaffer 1996). While mosquitofish sharply reduced newt survival in mesocosms, the occupancy models did not show a strong negative association between these taxa. This suggests that amphibian survival across multiple life stages (i.e., egg, larvae, juvenile, adult) and at varying spatial scales (i.e., pond, landscape, and region) is important in nature. While nonnative fish probably reduce survival of newt larvae in ponds, survival of their terrestrial life stages may be more important in maintaining stable populations (Biek et al. 2002). We also acknowledge that additional factors outside the scope of our field data may influence amphibian distribution in the study region (e.g., land use, habitat fragmentation, road density, patterns of amphibian disease) and that factors protecting amphibian larvae from predation may have been omitted from our mesocosm experiment (e.g., additional alternative prey, increased habitat complexity).

The mesocosm results added mechanistic insight into species interactions that were likely to occur within the surveyed ponds. Invasive predators exhibited strongly lethal effects on palatable amphibian larvae (treefrogs and newts), which far outweighed growth-related effects of bullfrogs. These findings add further experimental evidence to document the predatory effects of mosquitofish on amphibians (Gamradt and Kats 1996, Goodsell and Kats 1999). In contrast, unpalatable toad larvae exhibited no decrease in survival in the presence of mosquitofish, but did show strong reductions in growth in the presence of bullfrog larvae. The lack of predation on toads is likely due to chemical protection, which often protects bufonid larvae from fish (Kruse and Stone 1984). The sublethal injuries caused by mosquitofish also support the role of predators in contributing to missing-limb abnormalities in amphibians (Bowerman et al. 2010). Finally, newts were unaffected by bullfrog larvae, likely because direct competition for food resources and indirect effects were minimal (i.e., bullfrogs did not deplete resources that altered newt prey availability). We must also note that mesocosm

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experiments simplify natural communities, which are inherently complex. By isolating species of interest, we gained greater mechanistic understanding of their interactions, but many native species (and effects of their interactions) were omitted as a result.

Our study revealed an indirect positive effect of one invader on a native species. Western toads were larger and metamorphosed more quickly in the presence of mosquitofish. This positive association was evident even though many toad larvae from mosquitofish treatments showed injuries consistent with fish attacks (Appendix D: Fig. D1). Our SEM suggests this result is an indirect positive effect of mosquitofish that occurred due to reductions in abundance of treefrog tadpoles, which are competitors of toad tadpoles. Mosquitofish sharply decreased treefrog survival and treefrogs had a negative effect on toad biomass in the SEM. The SEM did not support an alternative mechanism, whereby the increased toad growth was due to enhanced phytoplankton abundance in the presence of mosquitofish and we are unaware of a direct interaction where mosquitofish would promote toad tadpoles. There is evidence to suggest that nonnative fish also promote toads in nature; Welsh et al. (2006) found western toads to be six times more abundant in the presence of nonnative salmonids in lake and pond habitats in northern California. To date, there are relatively few studies documenting this type of facilitation of native species by invaders through competitive release (e.g., Grosholz 2005). One interesting example involves nonnative golden eagles, which prey upon native foxes in the Channel Islands, leading to the competitive release of native skunks (Roemer et al. 2002).

Results of the mesocosm study also suggested that invasive predators can influence primary production and nutrient cycling. Consistent with other reports, mosquitofish strongly reduced zooplankton within mesocosms (Ning et al. 2009), leading to sharp increases in phytoplankton (see SEM). Relative phytoplankton fluorescence in mosquitofish treatments was about three times greater in treatments lacking mosquitofish. Including links from nutrients (TDN and TDP) to phytoplankton decreased model fit in the SEM, suggesting the top-down effect of zooplankton loss explained changes in phytoplankton (Appendix C), which has also been reported in nature (Nagdali and Gupta 2002). We also observed increases in total nitrogen and phosphorus within mosquitofish treatments, possibly due to direct excretion of nutrients by mosquitofish (Schaus et al. 1997). We note, however, that the observed effects on nutrients, phytoplankton, and zooplankton occurred over a short time span in mesocosms with a relatively small water volume and simplified community. Of particular note is that our mesocosms did not contain submerged macrophytes, which are known to provide refuge for zooplankton from predatory fish (Schriver et al. 1995). Further research into how multiple aquatic invaders influence native communities and ecosystem processes, especially whole-ecosystem manipulations, will yield valuable insights into how the effects reported here "scale-up" to real wetlands.

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SUPPLEMENTAL MATERIAL

Appendix A

Diagram of hypothesized species interactions in the presence of nonnative bullfrogs and mosquitofish (*Ecological Archives* E093-108-A1).

Appendix B

Mesocosm establishment methods (*Ecological Archives* E093-108-A2).

Appendix C

Structural equation model (SEM) methods and results (*Ecological Archives* E093-108-A3).

Appendix D

Images of sublethal injuries caused by mosquitofish in the mesocosm experiment (*Ecological Archives* E093-108-A4).

Appendix E

Table with top occupancy models and associated statistics for each native amphibian species (*Ecological Archives* E093-108-A5).

Reports