

Cross-habitat effects shape the ecosystem consequences of co-invasion by a pelagic and a benthic consumer

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Abstract Invasive species can have major impacts on ecosystems, yet little work has addressed the combined effects of multiple invaders that exploit different habitats. Two common invaders in aquatic systems are pelagic fishes and crayfishes. Pelagic-oriented fish effects are typically strong on the pelagic food web, whereas crayfish effects are strong on the benthic food web. Thus, co-invasion may generate strong ecological responses in both habitats. We tested the effects of co-invasion on experimental pond ecosystems using two widespread invasive species, one pelagic (western mosquitofish) and one benthic (red swamp crayfish). As expected, mosquitofish had strong effects on the pelagic food web, reducing the abundance of *Daphnia* and causing a strong trophic cascade (increase in phytoplankton). Crayfish had strong effects on the benthic food web, reducing the abundance of benthic filamentous algae. Yet, we also found evidence for important cross-habitat effects. Mosquitofish treatments reduced the biomass of benthic filamentous algae, and crayfish treatments increased *Daphnia* and phytoplankton abundance. Combined effects of mosquitofish and crayfish were primarily positively or negatively additive, and completely offsetting for some responses, including gross primary production (GPP). Though co-invasion did not affect GPP, it strongly shifted

primary production from the benthos into the water column. Effects on snail abundance revealed an interaction; snail abundance decreased only in the presence of both invaders. These results suggest that cross-habitat effects of co-invaders may lead to a variety of ecological outcomes; some of which may be unpredictable based on an understanding of each invader alone.

Keywords Indirect effects · Benthic–pelagic coupling · *Gambusia* · *Procambarus* · Biological invasions

Introduction

Invasive species are a threat to native biodiversity and can change important aspects of ecosystem function (Vitousek 1990; Simon and Townsend 2003; Ehrenfeld 2010; Simberloff 2011). Many ecosystems have been invaded by two or more non-native species, making it critical to evaluate how interactions among invaders shape community and ecosystem processes (Kuebbing et al. 2013). A recent meta-analysis showed the effects of simultaneous invasion by two invaders (co-invasion) using the same or similar habitats and resources are often not predicted by their individual effects (Jackson 2015). However, co-invaders commonly use very different habitats and resources (e.g., Ricciardi 2001). Despite a lack of direct interactions, resource use in different habitats can cause indirect, cross-habitat effects. These combined indirect ecosystem effects might also be challenging to predict based on an understanding of each invader alone.

Two ubiquitous invaders of lentic freshwater systems are fishes and crayfishes (Strayer 2010). Many fishes are pelagic predators, while crayfishes are typically benthic omnivores, but both of these invasive consumers can have

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cross-habitat effects. Zooplanktivorous fishes drive strong pelagic trophic cascades (increases in phytoplankton abundance due to numerical declines or grazing rate declines of zooplankton) with marked impacts at the ecosystem-level (Hurlbert et al. 1972; Carpenter et al. 1985; Brett and Goldman 1996), and crayfish foraging has engineering effects through bioturbation and modification to habitat structure (reviewed in Gherardi 2007). We examined the ecological effects of co-invasion by western mosquitofish (*Gambusia affinis*) and red swamp crayfish (*Procambarus clarkii*), both widespread invasives, to understand their habitat-specific and cross-habitat impacts on pond ecosystems.

Mosquitofish (*G. affinis* and *G. holbrooki*) are a live-bearing, pelagic-oriented fish which were introduced worldwide from the southeast USA throughout the 20th century as a biocontrol agent, and are today one of the most abundant and widespread freshwater fishes in the world (reviewed in Pyke 2005, 2008). Their global distribution, aggressive nature, and marked ecosystem impacts have resulted in their listing as one of the world's 100 worst invaders (Lowe et al. 2000). Based on prior studies, we expect mosquitofish impacts in the pelagic zone to be strong, particularly through their consumption of large-bodied zooplankton (primarily *Daphnia*). Reductions of zooplankton abundance by mosquitofish can cause extreme increases in phytoplankton abundance (i.e. a pelagic trophic cascade), which can even have strong impacts for ecosystem properties, such as water temperature and pH (Hurlbert et al. 1972; Fryxell et al. 2015).

Red swamp crayfish were also transplanted worldwide from their native range in the southern USA and northern Mexico (Hobbs et al. 1989; Loureiro et al. 2015), and are today found on five continents (IUCN Invasive Species Specialist Group 2015). Red swamp crayfish have strong negative impacts for native macrophytes (Smart et al. 2002), amphibians (Gamradt and Kats 1996; Cruz et al. 2006), egg-laying fishes (Ilhéu et al. 2007), and snails (Gherardi and Acquistapace 2007; Klose and Cooper 2013). They also modify ecosystems through the destruction of macrophyte beds, bioturbation (Angeler et al. 2001; Rodríguez et al. 2003; Matsuzaki et al. 2009; Carreira et al. 2014; Rodríguez-Pérez et al. 2016), and detrital processing (Alp et al. 2016). Red swamp crayfish are primarily herbivorous (Gutiérrez-Yurrita et al. 1998; Alcorlo et al. 2004), and we thus expect strong impacts on benthic primary producers.

Direct interactions between small fishes (<15 cm total length) and crayfishes are uncommon, except at the egg stage (Reynolds 2011). Gape-limitation of small fishes and crayfish defense traits (e.g., burrowing with only the anterior cephalothorax and claws exposed) often prevent consumption of crayfish by small fish, while agility often

prevents consumption of small fish by crayfish (Stein 1977). While indirect evidence suggests live mosquitofish can be consumed by crayfish if mutually trapped (Gutiérrez-Yurrita et al. 1998; Alcorlo et al. 2004), studies executed in realistic field environments show that red swamp crayfish do not typically prey upon live adult mosquitofish (Gherardi and Acquistapace 2007; Anastácio et al. 2011), and that mosquitofish show no behavioral modifications in the presence of crayfish (Leite et al. 2005). Interestingly, this lack of direct interactions likely plays a role in the success of these co-invaders; the lack of a crayfish-vulnerable egg stage in live-bearing mosquitofish may facilitate their co-invasion with red swamp crayfish. Because mosquitofish and red swamp crayfish show a little evidence of direct interactions (e.g., interference competition or predation), use different habitats, and primarily use different resources; the nature of the combined effects of these invaders is likely to be the result of indirect effects. Here, we use a factorial manipulation of crayfish and mosquitofish presence in experimental ponds to determine their separate and combined ecosystem effects. Because effects of sympatric invaders are frequently non-additive, factorial designs elucidate the nature of combined impacts of multiple invaders (Jackson 2015).

Mosquitofish and red swamp crayfish have co-invaded many freshwater systems, including those in arid regions of the American Southwest (e.g., Moody et al. 2012) and the Iberian Peninsula (e.g., Gutiérrez-Yurrita et al. 1998), where endemic fishes are highly imperiled and crayfishes were historically absent. Co-invaded watersheds also occur in Italy (e.g., Gherardi and Acquistapace 2007), France (e.g., Poulin et al. 2007), Japan (Maehata 2012), and Israel (Wizen et al. 2008), and possibly also in a number of other countries containing records of both species' independent occurrences (IUCN Invasive Species Specialist Group 2015). Thus, an understanding of the independent and combined effects of these invaders is of global relevance.

Methods

Consumer source

We collected western mosquitofish (*G. affinis*) and red swamp crayfish from the upper pond at Warm Springs, Inyo County, California (37°16'01"N 118°16'21"W), a site restored as a refuge for endangered Owens Pupfish (*Cyprinodon radiosus*) (Online Resource 1.1). Western mosquitofish and red swamp crayfish are the only macro-consumers currently inhabiting this site, as the Owens Pupfish population at this site became extirpated in 2008

(Finger et al. 2013). Co-invasion by mosquitofish and red swamp crayfish is common for aquatic ecosystems in this region (D. Fryxell, unpublished data).

Mesocosm setup

We established an outdoor mesocosm experiment at the Long Marine Laboratory in Santa Cruz, California, USA. We set up 36 mesocosms (1136 L Rubbermaid® stock tank) in a 6 × 6 array (Online Resource 1.2), and then filled each with 19 L of sand and 1000 L of dechlorinated municipal water. We then inoculated each mesocosm with 4 L of homogenized sediment and a homogenous zooplankton community sample (dominated by *Daphnia*, collected using 80 µm mesh) taken from West Lake (Santa Cruz, CA, USA). We placed two unglazed ceramic tiles (2.8 × 4.3 cm²) on top of a cinderblock (9.3 × 19.0 × 39.3 cm³) in the center of each mesocosm to measure periphyton. A HOBO Pendant® (Onset Corporation) data logger was affixed to the cinderblock and set to measure temperature and light intensity continuously at 15-min intervals. Finally, we submerged ~8.5-g dried sycamore (*Platanus racemosa*) leaves (binder-clipped) as substrate for benthic animals and to estimate litter decomposition. We waited one week after establishing mesocosms to apply treatments.

Treatment application

On 30 March, 2015, we applied treatments consisting of nine replicates each of (1) no consumers (“None”), (2) mosquitofish, (3) crayfish, or (4) mosquitofish plus crayfish (“Both”). We assigned treatments randomly to the array. We stocked consumers at densities of one crayfish (density~0.5m⁻²) and 10 adult mosquitofish (density~1 per 100 L at a sex ratio 7 females:3 males) per mesocosm. Crayfish stocking density (Angeler et al. 2001; Rodríguez et al. 2003; Correia and Anastácio 2008) and the mosquitofish density and sex ratio (Hurlbert et al. 1972; Lancaster and Drenner 1990; Fryxell et al. 2015) were consistent with natural densities and those used in similar experiments. The experimental duration was 4 weeks following treatment application.

Sampling

We measured a variety of responses in the benthic and pelagic zones weekly, biweekly, or at a single time-point. Weekly pelagic responses included specific conductivity, pH, phytoplankton density (estimated fluorometrically as chlorophyll-*a* using the standard methods (as in Fryxell et al. 2015) (Online Resource 1.3), and *Daphnia* density (taken from 2-L depth-integrated water samples). We

averaged continuous temperature data from each logger over each week. We cleared light loggers of debris in the early morning of each weekly sampling point to quantify maximum daytime light. Lastly, at the final sample point, week 4, we used standardized pelagic net sweeps to quantify mosquito larvae abundance.

We sampled benthic responses less frequently, so as not to create overwhelming disturbance. Tile periphyton was measured biweekly (estimated fluorometrically as chlorophyll-*a* in the same manner as for phytoplankton above) by removing one tile at week 2 and another at week 4. Chironomid (midge larvae) density was quantified at week 3 from five randomly placed 5.2-cm diameter cores. At week 4, we removed leaf packs, picked snails from them to measure snail abundance, and then rinsed and dried leaves at 50 °C for 48 h. We calculated decomposition as the initial leaf pack dry mass minus the final dry mass. At week 4, all visible filamentous algae were removed from each mesocosm, dried at 50 °C for 48 h, and weighed to get algae mass.

To assess the aggregate (benthic plus pelagic) effects for primary production, we estimated gross primary production at week 2 and week 4 using diel change in dissolved oxygen concentrations (as in Fryxell et al. 2015) (Online Resource 1.4). We frequently checked each mesocosm to ensure consumer survival throughout the experiment. At the end of the experiment (week 4), fish and crayfish were removed to assess survival rates during the experiment.

Analyses

We tested for independent and interaction effects of crayfish and mosquitofish using ANOVA on linear regression with two dummy coded predictors, crayfish presence and mosquitofish presence. Responses with multiple sampling points were analyzed using MANOVA of repeated measures (rANOVA), and single time-point responses were analyzed using ANOVA. Some count data were $\log(x + 1)$ transformed and other data $\log(x)$ transformed as needed to meet model assumptions (see Table 1). Therefore, additivity of responses was assessed on the scales at which the responses were analyzed (Wootton 1994). Transformations were insufficient to overcome the zero-inflated nature of algae mass data, and thus, we used generalized regression with a zero-inflated gamma (GR-ZIG) distribution. Parameters for the GR-ZIG were estimated using the “Adaptive Lasso” technique, where variable selection (AICc) is part of the fitting procedure, and thus, predictors can be dropped (see JMP Pro® 12 online documentation “Generalized Regression Models”). We used JMP Pro® 12 for all analyses.

We considered combined effects to be “compounding” in cases, where interaction terms were not significant, and

Table 1 Summary of results and statistical analyses for all responses

Response	Habitat	Time-points	Transform	Method	Mfish	<i>p</i>	Crayfish	<i>p</i>	<i>p</i> (interaction)	Combined effect
<i>Daphnia</i> density	Pelagic	4	log(1 + <i>x</i>)	rANOVA	↓	<0.0001	↑	0.0062	0.2984	Opposing
Phytoplankton	Pelagic	4	log(<i>x</i>)	rANOVA	↑	<0.0001	↑	0.0051	0.7903	Compounding
Conductivity	Pelagic	4	None	rANOVA	↓	0.0178	↑	<0.0001	0.4318	Offsetting ^a
pH	Pelagic	4	None	rANOVA	↑	0.0048	↓	<0.0001	0.1304	Offsetting ^a
Temperature	Pelagic	4	None	rANOVA	NA	0.5394	NA	0.9126	0.8673	NA ^b
Light	Pelagic	4	None	rANOVA	NA	0.2029	↓	0.0402	0.9007	NA ^b
Mosquito counts	Pelagic	1 (W4)	log(1 + <i>x</i>)	ANOVA	↓	<0.0001	NA	0.4211	0.9650	NA ^b
Chironomid density	Benthic	1 (W3)	log(<i>x</i>)	ANOVA	NA	0.5561	NA	0.7922	0.9526	NA ^b
Periphyton	Benthic	2 (W2, W4)	log(<i>x</i>)	rANOVA	NA	0.8706	↑	0.0001	0.7456	NA ^b
Leaf decomposition	Benthic	1 (W4)	log(<i>x</i>)	ANOVA	NA	0.4121	NA	0.1435	0.1161	NA ^b
Algae mass	Benthic	1 (W4)	None	GR-ZIG	↓	0.0003	↓	0.0437	NA ^c	Compounding
Snail counts	Benthic	1 (W4)	log(1 + <i>x</i>)	ANOVA	↑	0.8771 ^d	NA	0.0142 ^d	0.0110	Synergistic
GPP	Aggregate	2 (W2, W4)	None	rANOVA	↑	<0.0001	↓	0.0401	0.8590	Offsetting ^a

Significant *p*-values ($\alpha = 0.05$) are bolded in the columns for the mosquitofish effect (Mfish), crayfish effect (Crayfish), and interaction effect (interaction). Effect directions are shown with *arrows*. For responses not sampled weekly, the number of time-points sampled and the week of sampling (e.g., W4) is noted

^a Combined effect was offsetting for opposing effects without differences between the *Both* and *None* treatments ($\alpha = 0.05$)

^b Combined effect was not applicable (NA) for responses lacking both a mosquitofish and a crayfish effect

^c Adaptive Lasso estimation with AICc validation dropped the interaction term as not significant

^d Significance of main effects cannot be interpreted when the interaction term is significant

independent effects of crayfish and mosquitofish were each significant and acted additively on the response in the same direction (i.e., positive additivity). We considered the opposite “opposing” combined effects (i.e., negative additivity). We employed post-hoc rANOVA to determine whether the *Both* treatment was different from the *None* treatment in the cases of opposing combined effects. In these cases, we considered the opposing combined effect to be “offsetting” if the *Both* and *None* treatments were statistically indistinguishable ($\alpha = 0.05$). Finally, responses with a significant interaction term, where combined impacts were greater than the sum of their individual parts, were considered “synergistic” effects.

Results

Consumer mortality was low throughout the experiment. Four individual mosquitofish died, yielding four mesocosms with 9 instead of 10 fish (98 % survival overall). Two crayfish died (89 % survival overall), but this occurred within the last three days of the experiment. Because of this low mortality, we did not replace any organisms and we did not drop any replicates from the analyses. Variation in reported degrees of freedom below results from data lost due to processing errors for conductivity (two lost), chironomid density (three lost), and decomposition (one lost); importantly, lost data were rare and were unrelated to assigned treatment.

Within-habitat effects

In the pelagic zone, mosquitofish treatments severely decreased *Daphnia* density ($F_{1,32} = 171.5$, $p < 0.0001$) and mosquito counts ($F_{1,32} = 52.1$, $p < 0.0001$), increased phytoplankton density ($F_{1,32} = 1020.9$, $p < 0.0001$), decreased conductivity ($F_{1,30} = 6.3$, $p = 0.0178$), and increased pH ($F_{1,32} = 9.2$, $p = 0.0048$) (Figs. 1, 2a). Mosquitofish treatments had no effects on water temperature ($F_{1,32} = 0.4$, $p = 0.5394$) or light penetration ($F_{1,32} = 1.7$, $p = 0.2029$). In the benthos, crayfish treatments caused a reduction in filamentous algae (Wald $\chi^2 = 4.1$, $p = 0.0437$) (Fig. 2b), an increase in tile periphyton ($F_{1,32} = 19.1$, $p = 0.0001$) (Fig. 3a), and had no effects on chironomid density ($F_{1,29} = 0.1$, $p = 0.7922$) or leaf decomposition ($F_{1,31} = 2.3$, $p = 0.1435$).

Cross-habitat effects

Each consumer treatment had cross-habitat effects. Mosquitofish treatments caused a decrease in filamentous algae (Wald $\chi^2 = 12.9$, $p = 0.0003$) (Fig. 2b), but had no independent effects on other benthic responses (see Table 1). Crayfish treatments caused increases in pelagic phytoplankton ($F_{1,32} = 9.1$, $p = 0.0051$), *Daphnia* ($F_{1,32} = 8.6$, $p = 0.0062$), and conductivity ($F_{1,30} = 24.4$, $p < 0.0001$), and decreases in pH ($F_{1,32} = 29.1$, $p < 0.0001$) (Fig. 1) and light penetration ($F_{1,32} = 4.6$, $p = 0.0402$), but had

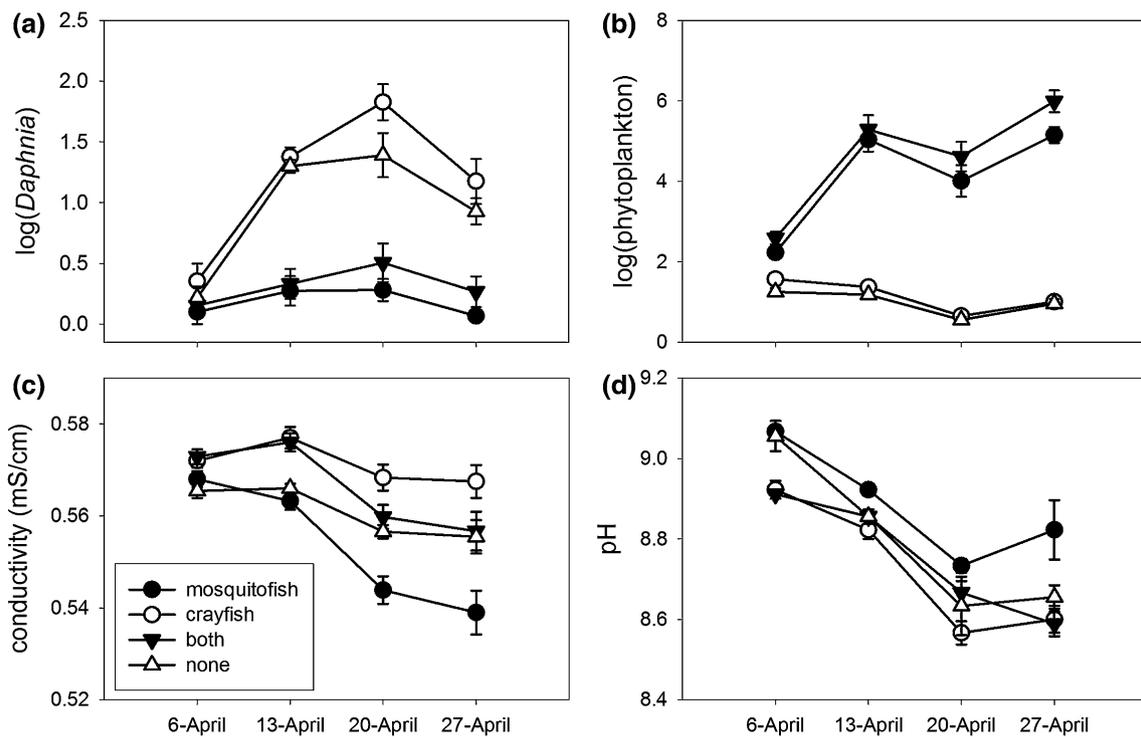


Fig. 1 Time-series of pelagic responses to our factorial manipulation of red swamp crayfish (*Procambarus clarkii*) and western mosquitofish (*Gambusia affinis*) presence. *Daphnia* density per liter (a) is $\log(1 + x)$ transformed, phytoplankton density (b) in μg chlorophyll-*a* per liter is $\log(x)$ transformed, and conductivity (c) and pH (d) are untransformed to show data on scales at which they were analyzed.

6 April 2015 was the first sampling point, which was one week after consumer introduction (treatment application). Each treatment had nine replicates for all responses except conductivity, for which the Crayfish treatment had seven replicates due to processing errors. Data points are treatment mean \pm SE

no effects on mosquito counts ($F_{1,32} = 0.7, p = 0.4211$) or water temperature ($F_{1,32} = 0.01, p = 0.9126$).

Combined effects

Some combined effects of crayfish and mosquitofish were compounding, some opposing, and one synergistic (interacting) (Table 1). The GR-ZIG Adaptive Lasso fitting procedure dropped the interaction term from the model predicting filamentous algae abundance, suggesting no significant interaction term. In fact, the *Both* treatment lacked filamentous algae altogether, as mosquitofish and crayfish treatments both independently reduced filamentous algae, such that there was none when both consumers were together (Fig. 2b). Snail (*Physa* sp.) counts from leaf packs were the only response showing a significant interaction effect ($F_{1,32} = 7.3, p = 0.0110$) (Table 1). The mosquitofish treatment alone increased the number of snails relative to the *None* treatment, the crayfish treatment had little or no effect, but together mosquitofish and crayfish (the *Both* treatment) had the lowest snail counts (Fig. 2c).

The mosquitofish treatment decreased GPP ($F_{1,32} = 23.9, p < 0.0001$), while the crayfish treatment

increased GPP ($F_{1,32} = 4.6, p = 0.0401$) (Fig. 3b). Post-hoc rANOVA on such opposing responses revealed that the effects of mosquitofish and crayfish were completely offsetting (*None = Both*) for conductivity ($F_{1,16} = 4.1, p = 0.0588$), pH ($F_{1,16} = 3.5, p = 0.0809$), and GPP ($F_{1,16} = 3.6, p = 0.0752$), but not for *Daphnia* density ($F_{1,16} = 82.3, p < 0.0001$).

Discussion

Mosquitofish and crayfish each had a diversity of effects both within- and across-habitats, which led to combined effects that were compounding (additive in the same direction), opposing (additive in different directions), or synergistic (interacting). As expected, mosquitofish treatments had strong pelagic effects and crayfish treatments had strong benthic effects. Mosquitofish treatments reduced the number of *Daphnia* and mosquitoes, likely driving the marked increase in phytoplankton via a pelagic trophic cascade (Hurlbert et al. 1972; Fryxell et al. 2015). Mosquitofish treatments caused a decrease in conductivity, perhaps resulting from uptake of salts by the highly abundant

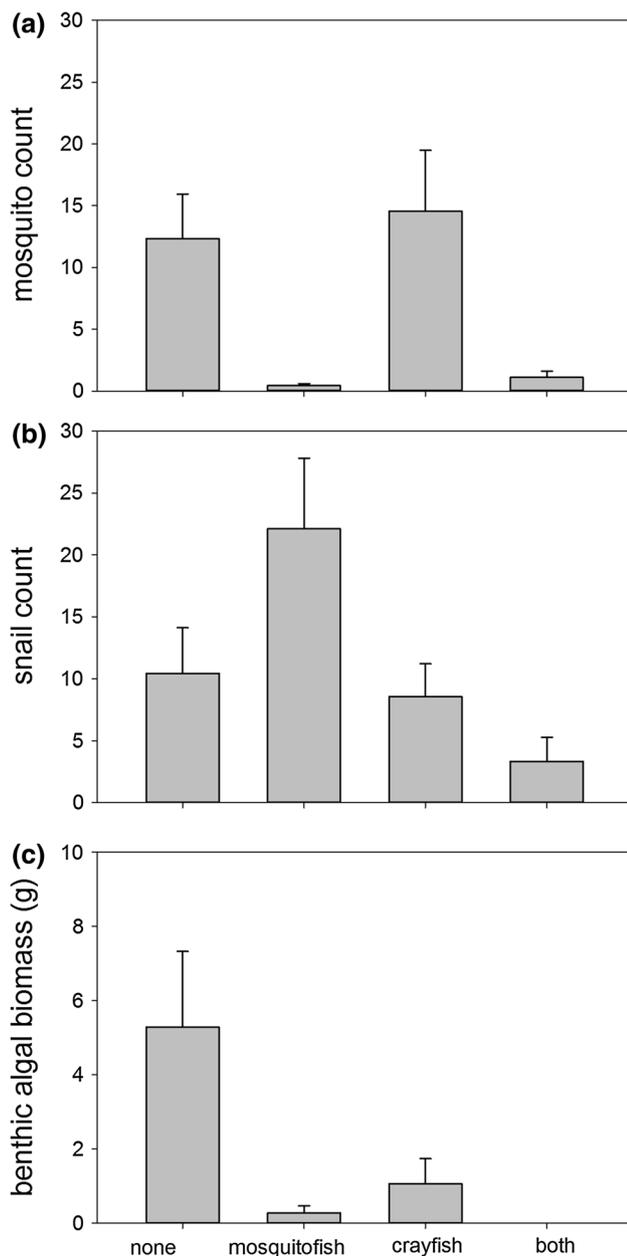


Fig. 2 Mosquito counts per standardized sweep (a), snail counts per leaf pack (b), and total benthic algae biomass in grams (c), as measured at the end of the experiment (end of week 4). Each treatment had nine replicates. Data are treatments mean \pm SE

phytoplankton in those treatments. Phytoplankton is hyperosmotic in freshwaters, and thus, a large bloom could theoretically cause a detectable reduction in free ions as they sequester those ions into tissue throughout the growth (Raven 1982). A decrease in conductivity at the ecosystem-level due to a strong pelagic trophic cascade has, to our knowledge, not been observed, and the mechanisms underlying this result, therefore, remain unclear. Crayfish treatments reduced benthic filamentous algae, which could

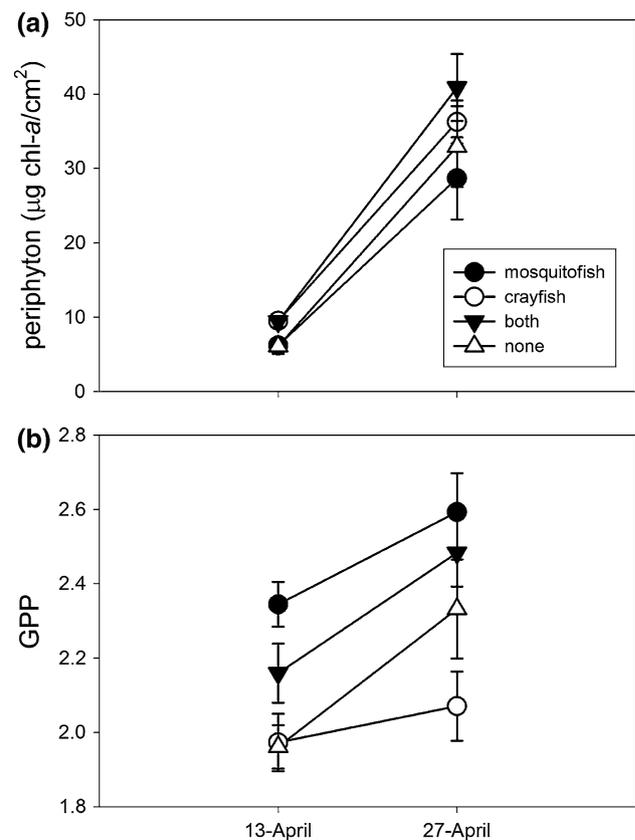


Fig. 3 Periphyton abundance on tiles present since the start of the experiment until week 2 (13 April 2015) and week 4 (27 April 2015) on an untransformed scale (a), and gross primary production (GPP) as estimated using diel changes in dissolved oxygen concentration (b). GPP is measured in units of $\Delta[\text{O}_2]$ adjusted for estimated respiration rates (Online Resource 1.4). Each treatment had nine replicates. Plotted are treatments mean \pm SE

have been a direct effect of foraging (Alcorlo et al. 2004) or an indirect effect of increased turbidity and the reduction in light available to the benthos (Sand-Jensen and Borum 1991). Crayfish treatments increased periphyton, likely by increasing resources through bioturbation, excretion, or egestion (Flint and Goldman 1975), or by modifying grazer activity (Turner et al. 1999, 2000).

Cross-habitat effects of each consumer were observed for a large number of responses. Reduction of benthic filamentous algae in mosquitofish treatments was, perhaps, due to increased grazing by the abundant *Physa* snails in those treatments (Kesler et al. 1986; Brönmark 1989). Alternatively, the mosquitofish-induced pelagic cascade could have resulted in decreased benthic algae if phytoplankton outcompeted benthic algae for resources, and especially light (Sand-Jensen and Borum 1991; Jäger and Diehl 2014). Crayfish treatments increased conductivity, decreased light, and increased phytoplankton density, likely through bioturbation and release of nutrients into the water

column (Angeler et al. 2001). Such drastic impacts of invasive red swamp crayfish bioturbation are documented in natural ecosystems (e.g., Rodríguez et al. 2003). The slight, albeit surprising, increase in *Daphnia* density induced by crayfish could be an indirect effect of the increase in phytoplankton, or a result of *Daphnia* avoidance of the benthos in the presence of crayfish (i.e., a habitat shift induced by the presence of crayfish). *Daphnia* are known to migrate to greater depths during the daytime in response to fish predators (Zaret and Suffern 1976), and other species of zooplankton have been found to migrate upwards in response to benthic invertebrate predators (Ohman et al. 1983). However, because crayfish are relatively inactive during the day (Correia 1998; Huber and Davis 2007), we do not think *Daphnia* avoidance of the benthos would occur during our daytime sampling. To avoid disturbing sediment, our depth-integrated *Daphnia* sample included water to within ~2 cm of the benthos; thus, we cannot directly assess the likelihood of such a habitat shift.

The afore-mentioned cross-habitat effects of crayfish and mosquitofish underscore the coupling of benthic and pelagic habitats in freshwater ecosystems through a variety of direct and indirect pathways (Schindler and Scheuerell 2002). Mosquitofish treatments increased overall GPP, likely as a result of the substantial increase in pelagic producers. Crayfish treatments decreased overall GPP, likely because they reduced filamentous algae without considerably increasing pelagic primary producer density. Mosquitofish-driven increases in GPP completely offset the crayfish-driven decrease in GPP, but co-invasion strongly shifted primary production from the benthos into the water column. Co-invasion by these two species may thus have extreme consequences for lentic ecosystems, initiating a switch between alternate stable states—from clear-water/benthic production to turbid-water/pelagic production (Scheffer et al. 1993; Dent et al. 2002).

In addition to GPP, crayfish and mosquitofish offset one another's effect on pH. GPP and pH are often related in these small aquatic environments (Fryxell et al. 2015), as primary production decreases CO₂, leading to an increase in pH (Dodson 2005).

Synergistic effects of multiple invasives are important, because they are difficult to predict based on our understanding of each invader alone. Our experiment revealed a synergistic combined effect for snail abundance; snails were only negatively impacted in the presence of both invaders. Both mosquitofish and red swamp crayfish are known to prey upon snails (Dorn 2013). However, only the largest female mosquitofish are capable of snail consumption (Hubbs 1990). It is therefore unlikely that mosquitofish had direct consumptive effects on snails, as evidenced by the increase in snail abundance in ponds with only mosquitofish relative to

the control. This suggests snail survival was higher when with only mosquitofish than without macro-consumers, as reproduction of *Physa* snails with growth to the adult stage is unlikely over the course of our one-month-long experiment (DeWitt 1954). In ponds with both consumers, perhaps transient positive effects of mosquitofish on snail abundance caused red swamp crayfish to prey-switch from detritus and algae to snails. Because we did not sample snail abundance through time, we are unable to directly address this hypothesis. The mechanism by which mosquitofish increased snail abundance is also unclear. Positive effects of mosquitofish on snails could be the result of snails being released from competition with more palatable invertebrates (Gresens 1995). Future investigation is necessary to understand the underlying causes of these effects. Such work is warranted, because snails have high rates of endemism (Hershler and Sada 2002), particularly within the springs systems of the American Southwest, where mosquitofish and red swamp crayfish co-invasion is common (Sada and Vinyard 2002), and where red swamp crayfish have been implicated with the decimation of endemic snail populations (Kilburn 2012).

Conservation of ecosystem properties and services requires evaluating tradeoffs before determining management action (Chan et al. 2006). Because invasive species impact a variety of ecological parameters, and these parameters are often inextricably linked, managers must prioritize parameters for conservation and carefully plan actions. If, in systems co-invaded by mosquitofish and red swamp crayfish, managers targeted the conservation of the ecosystem conductivity, GPP, or pH; our data suggest that neither invader need be managed, because the species offset one another's impact for those responses. Alternatively, if managers want to conserve native snail abundance, they could target either invader alone for removal, since our data suggest there are only negative impacts on snails when both invaders are present. Or, in systems with only one invader, resources should be directed towards preventing invasion by the other consumer. Of course, any strategy should be informed by evidence from a variety of studies and sources, as the effects of invasion and co-invasion are context-dependent. A thorough knowledge of the independent and combined effects of multiple invasives assists in prioritizing responses and actions for conservation.

Conclusion

Understanding the impacts of multiple invaders is a critical next step in invasion biology (Kuebbing et al. 2013). Studies of effects of freshwater co-invasion for communities

and ecosystems have primarily focused on species inhabiting the same habitats or using the same resources (Kuhns and Berg 1999; Nyström et al. 2001; Johnson et al. 2009; Porter-Whitaker et al. 2012; Preston et al. 2012; Jackson et al. 2014). Our study shows that within- and across-habitat effects of non-overlapping, but impactful invaders can, in combination, yield a variety of outcomes for different responses—compounding, opposing, and synergistic. Our results suggest that co-invasion by mosquitofish and red swamp crayfish may contribute to ecosystem state shifts and may cause severe declines in native snail abundances. The lack of our ability to predict the latter based on an understanding of the independent effects of each invader underscores the importance of factorial experiments for understanding the impacts of co-invasion.

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Author contribution statement D.C.F., M.A.F., and E.P.P. designed the experiment. All authors carried out the experiment and analyzed samples. D.C.F. performed statistical analyses. D.C.F. led writing.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethics Vertebrate use was approved under the IACUC protocol Palke 1306-2. All applicable institutional and/or national guidelines for the care and use of animals were followed.

Data accessibility Data are available at dryad doi:10.5061/dryad.n34s9/1.

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