

Consumptive effects of fish reduce wetland crayfish recruitment and drive species turnover

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Abstract Predators and dry-disturbances have pronounced effects on invertebrate communities and can deterministically affect compositional turnover between discrete aquatic habitats. We examined the effect of sunfish (*Lepomis* spp.) predators on two native crayfish, *Procambarus alleni* and *P. fallax*, that regionally coexist in an expansive connected wetland in order to test the hypotheses that sunfish predation limits crayfish recruitment (both species) and that it disproportionately affects *P. alleni*, the species inhabiting temporary wetlands. In replicate vegetated wetlands (18.6 m²), we quantified summertime crayfish recruitment and species composition across an experimental gradient of sunfish density. Separately, we quantified effects of sunfish on crayfish growth, conducted a complimentary predation assay in mesocosms, and compared behavior of the two crayfish. Sunfish reduced *P. alleni* summertime recruitment by >99% over the full sunfish gradient, and most of the effect was caused by low densities of sunfish (0.22–0.43 m⁻²). *P. alleni* dominated wetlands with few or no sunfish, but the composition shifted towards *P. fallax*

dominance in wetlands with abundant sunfish. *P. fallax* survived better than *P. alleni* over 40 h in smaller mesocosms stocked with warmouth. Sunfish reduced *P. fallax* recruitment 62% in a second wetland experiment, but growth rates of caged crayfish (both species) were unaffected by sunfish presence, suggesting predatory effects were primarily consumptive. Consistent with life histories of relatively fish-sensitive invertebrates, *P. alleni* engaged in more risky behaviors in the laboratory. Our results indicate that sunfish predators limit recruitment of both species, but disproportionately remove the more active and competitively dominant *P. alleni*. Spatially and temporally variable dry-disturbances negatively co-varying with sunfish populations allow for regional coexistence of these two crayfish and may release populations of either species from control by predatory fishes.

Keywords Community structure · Everglades · Growth rate–predation risk tradeoff · Habitat template · Pulsed production

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Introduction

Identifying the mechanisms that result in population success and species turnover in space and time is a perennial issue in ecology (Paine 1966; McPeck 1990; Chase and Leibold 2003). The life-history traits making a species flourish in one habitat often make the same species a loser when faced with opposing mortality risks in other habitats (McPeck 1990). Aquatic animal community structure in freshwater habitats often follows a pattern of species sorting across habitats according to variation in habitat permanence (i.e., hydroperiod) and top predator identity (McPeck 1990; Wellborn et al. 1996; McCauley 2008). Invertebrate assemblages

inhabiting semi-permanent water bodies without large vertebrates (fish or salamanders) include large invertebrate top predators (Wellborn et al. 1996; Wissinger et al. 1999a; Garcia and Mittelbach 2008) and other invertebrates with highly active lifestyles, relatively fast development, and greater competitive abilities (Wellborn et al. 1996). In contrast, invertebrate communities inhabiting longer-hydroperiod habitats, where fish or salamanders are the top predators, tend to lack the largest predatory invertebrates, and are dominated by species with relatively lower activity levels (with lower growth rates) and potentially smaller sizes that make them less vulnerable to fast-moving visual vertebrate predators (Wellborn et al. 1996; McCauley 2008). A byproduct of these correlations is the observation that predatory interactions between vertebrate predators and invertebrate species with which they do not coexist tend to be stronger than interactions between those predators and coexisting species (Wellborn et al. 1996; Wissinger et al. 1999b; Garcia and Mittelbach 2008). Recent studies suggest that the presence of large-bodied fishes may be more important than fish presence (i.e., small or large-bodied fish) for determining the life histories of co-existing invertebrates (Dorn 2008; McCauley 2008; McCauley et al. 2008).

While effects of hydroperiod gradients on crayfish species turnover have not been well examined (Dorn and Trexler 2007), the predator–prey interactions between fish and crayfish assemblages have been examined for a few crayfish species pairs inhabiting lakes and streams (e.g., Didonato and Lodge 1993; Garvey et al. 1994; Fortino and Creed 2007). The traditional ideas about crayfish species turnover and the favorable traits in fish-dominated systems are in contrast with the life-history concepts previously explained (Dorn and Trexler 2007). Fish, especially large-bodied fishes like sunfishes (Centrarchidae), are major predators of crayfish in North America, and cause crayfish to reduce their activity levels, feeding rates, and seek shelter more frequently (Stein and Magnuson 1976; Garvey et al. 1994; Keller and Moore 2000). Large body sizes are thought to be favored in environments with high fish predation because crayfish can reach an effective size-refuge from most freshwater fish (Stein 1977; Kershner and Lodge 1995; Englund and Krupa 2000) and because large size (and fast growth) coupled with aggression should give a species advantages when competing for shelter (Garvey et al. 1994; Vorburger and Ribic 1999).

Crayfish (*Procambarus* spp.) are prominent members of the shallow wetland communities in south Florida; they make up a large portion of macroinvertebrate standing stocks (Turner et al. 1999) and densities commonly range from 2 to 10 m⁻². Two species of crayfish *Procambarus fallax* (the Slough crayfish) and *Procambarus alleni* (the Everglades crayfish) (Hendrix and Loftus 2000; Dorn and Trexler 2007) inhabit the Everglades, and although there are

no strict habitat boundaries delineating temporary, semi-permanent and permanent wetlands in the Everglades, *P. alleni* dominates most short-hydroperiod areas that dry annually in the winter, while *P. fallax* tends to dominate sloughs (i.e., shallow flowing wetlands) with longer hydroperiods and infrequent dry-disturbances (Hendrix and Loftus 2000; Dorn and Trexler 2007). Within a single slough, the crayfish composition can switch between years depending on the recent history of drying events (Dorn and Trexler 2007). Both species have relatively short life spans (1–2 years), and can grow to mature sizes within 2–3 months.

Dorn and Trexler (2007) argued that a competition–colonization tradeoff could not explain the distributional pattern of the two species; *P. alleni* grows faster, is better able to burrow and survive dry-disturbances (loss of surface water) in marl soils that are found in the southern Everglades and competitively dominates *P. fallax* for both food and shelter. While their work could mechanistically explain why the composition switches to *P. alleni* dominance in short-hydroperiod wetlands, the reverse switch to *P. fallax* dominance in long-hydroperiod wetlands was unexplained and they suggested selective predation by fish on juvenile *P. alleni* might explain the compositional shift. As catches of large-bodied fishes negatively co-vary with intensity and frequency of dry-disturbances (Trexler et al. 2002; Parkos et al. 2011), fish predation is expected to be more common in long-hydroperiod sloughs that function akin to fish-dominated, albeit still semi-permanent, habitats (see distinction in Batzer et al. 2006).

The objective of our research was to examine the net effects of native sunfishes (*Lepomis* spp.) on recruitment of the two species of crayfish found in the Everglades. We conducted two studies in experimental wetland environments (18.6 m² outdoor wetlands) with manipulations of juvenile crayfish and sunfish starting densities. We report the effects of sunfish on crayfish summertime (8–13 weeks) recruitment (both wetland experiments) and assemblage structure (first wetland experiment) and we expected total crayfish recruitment and biomass to be inversely related to sunfish biomass in both experiments. We did not know whether the impacts of fish on the assemblage would be manifested primarily through consumptive or non-consumptive effects, but our initial results compelled us to investigate the potential for important non-lethal effects of predators, via growth reduction by *P. alleni*. Increased non-lethal growth responses may be predicted to occur by imperfect predator risk assessment (Abrams 1994) and so we caged each species in the presence and absence of sunfish to explore non-consumptive effects of sunfish on crayfish growth. We also report results from a predation assay in mesocosms where both species were preyed on by predatory warmouth (*Lepomis gulosus*). Finally, we examined activity levels of both species in the laboratory.

Materials and methods

Experimental wetlands

We conducted our primary experiments in 12 aboveground experimental tanks measuring 3.1×6.1 m (18.6 m²) by 85 cm deep at the University of Florida's Institute for Food and Agricultural Sciences (I.F.A.S.) Station located in Davie, Florida. In 2009, we established wetland conditions similar to Everglades wet prairies in the tanks (Online Resource 1). Tanks were cleaned, dried, and were filled with Everglades peat soil from a local supplier to a depth of 15 cm. We planted each experimental wetland with common emergent and floating-leaf macrophytes (*Eleocharis cellulosa*, *Nymphaea odorata*, and *Crinum americanum*) found in Everglades wetlands and brought the water to a final experimental depth of 55 cm. *Chara* sp., which can also be found in some Everglades wetlands, sprouted and established in all wetlands. We added small groups of eastern mosquitofish (*Gambusia holbrooki*) and grass shrimp (*Palaemonetes paludosus*) to every experimental wetland and both species established self-sustaining populations. Eastern mosquitofish and grass shrimp are two of the most common small animals (1–2 cm) in sloughs (Turner et al. 1999; Trexler et al. 2002). Other aquatic invertebrates, including gastropods and *Libellulid* dragonflies, also established populations in the wetlands. Initial conditions were similar between replicate wetlands at the start of both experiments. Mean stem densities ranged from 24 to 85 stems m⁻² in the first experiment and 210–567 stems m⁻² in the second experiment. *Chara* sp. was present in both experiments, but was more abundant in the first experiment.

The early wet season (June–July) is the pre-dominant season for juvenile recruitment for *P. alleni* (Dorn and Trexler 2007) and is often also a season of high *P. fallax* juvenile densities, although juvenile *P. fallax* and berried females are often observed in other seasons too (N.J.D., personal observation). We bred adults of both species in captivity in May–June and stocked newly hatched and free-swimming juveniles in the experimental wetlands. Adult crayfish were bred under drying conditions in 68-L plastic tubs containing typical Everglades peat or marl substrates, similar to those used by Dorn and Volin (2009). The tubs were covered with fiberglass window screen and were placed inside a large empty cement tank. We lowered the water levels, forcing adult crayfish underground, and recovered females with eggs or young after 4–5 weeks. The adult females were moved to the laboratory until the young were ready for stocking (1–10 days after independence). All juveniles from a single female were evenly distributed between all experimental wetlands in both wetland experiments so that offspring from 4–8 females per species were represented in each wetland.

Two common sunfish species, the dollar sunfish (*Lepomis marginatus*) and warmouth (*L. gulosus*), were used as typical sunfish predators for these experiments. The sunfish were stocked in a 3:1 ratio of dollar sunfish to warmouth in order to favor the more numerous and smaller gape-sized species. Both species of sunfish are known to feed on crayfish (Loftus 2000), and we estimate that, with the fish sizes we used, small to medium-sized juvenile crayfish [3–14 mm carapace length (CL)] are capable of being consumed by one or both fish species. Sunfish were stocked at different densities to simulate increasing predatory fish densities associated with shorter versus longer hydroperiods (Trexler et al. 2002; Parkos et al. 2011). The experimental densities were approximated from a combination of various fish sampling methods and observations (Online Resource 2). The fish treatments were conservative tests of fish predation in the sense that the largest predatory fish size classes (>8 cm SL; Parkos et al. 2011) were not included.

Sunfish effects on the crayfish assemblage

The first experiment examined the joint responses of both crayfish species to sunfish density and was conducted in the summer of 2009. Sunfish were added to the experimental wetlands 3 days prior to additions of crayfish. Dollar sunfish [46.3 mm mean standard length (SL) \pm 0.04 SE] and warmouth (56.8 mm mean SL \pm 0.10 SE) were stocked at densities of 0, 4, 8, 16, and 32 sunfish wetland⁻¹ (0–1.72 sunfish m⁻²); the control (no sunfish) and highest density treatments were replicated three times and the intermediate densities were replicated twice. Using a gradient approach allowed us to determine the shape of the relationship between sunfish density and juvenile crayfish survival/recruitment (Cottingham et al. 2005). Wetlands with sunfish were regularly observed for a week after the initial sunfish additions to look for sunfish mortality, but no deaths were observed. Crayfish added to the tanks had mean starting sizes of 3.43 mm \pm 0.04 (mean CL \pm SE) for *P. alleni* and 3.27 mm \pm 0.09 (mean CL \pm SE) for *P. fallax*. Crayfish were added at night (2100 hours), were distributed throughout each wetland and were transported immediately to the substrate by washing them down a 10-cm-diameter PVC tube slide. All additions took place over 2 weeks. Different numbers were added each night, but the stocking numbers (per species) were equal across all wetlands on each night. A total of 258 *P. alleni* and 130 *P. fallax* were stocked in each experimental wetland resulting in a ratio of 2:1 *P. alleni* to *P. fallax*. We had fewer *P. fallax* juveniles available and that limited us to 130 per experimental wetland. These stocking densities approximated the reproduction of 2–4 typical female crayfish of each species in each wetland.

The experiment was run for a total of 8 weeks (first crayfish addition to throw trap sampling), long enough for considerable crayfish growth for both species (Dorn and Trexler 2007) and a large amount of predation, but not long enough for the young-of-year crayfish to reproduce themselves. Thus, we examined the summertime recruitment (survival and growth) of single cohorts. All experimental wetlands were sampled with 1-m² throw traps after 8 weeks to estimate crayfish densities. Three throw traps were set simultaneously at randomly chosen positions and cleared using the methods outlined in Dorn et al. (2005). In the process of capturing the crayfish, we also captured grass shrimp and mosquitofish, and we note their density responses in the results. To assess crayfish assemblage composition and average individual sizes of *P. alleni* for each experimental wetland, we had to conduct additional sampling because catches were so low in some of the replicates with higher sunfish densities. All efforts were employed equally in every experimental wetland and included minnow trapping, additional throw traps and 1-m bar seine sweeps in areas with previously undisturbed vegetation. Recovered crayfish were preserved and moved to the laboratory where they were identified to species using reproductive organs and coloration (Hendrix and Loftus 2000) and measured to the nearest 0.1 mm CL. We converted CL to dry mass (g) using regressions (Online Resource 3).

In 2005, we conducted a predation assay with warmouth feeding on medium-sized juvenile crayfish in 1.4-m² outdoor mesocosms at the Daniel Beard Center in Everglades National Park. In each mesocosm, we created typical slough conditions; water depth was 50 cm deep in each mesocosm and the habitat consisted of a thin sand substrate covered by 5 cm of soft dark organic sediments equal amounts of *Utricularia foliosa* from long-hydroperiod sloughs. We added 15 juveniles of each crayfish species to each mesocosm [starting sizes of *P. alleni*: 8.3 mm ± 0.3 (mean CL ± SE) and *P. fallax*: 8.3 mm ± 0.3]. Two hours after adding the crayfish, we introduced a single warmouth (74–86 mm SL) to each of eight randomly chosen mesocosms. Four mesocosms served as controls to document crayfish survival and recoverability in the absence of warmouth. Previous observations indicated that these sizes would be highly vulnerable to the warmouth used in this study. After 40 h of predation (2 nights), we drained the mesocosms and sifted the mud to recover and identify all surviving crayfish.

Sunfish effects on *P. fallax* recruitment

Because *P. alleni* is the dominant competitor (Dorn and Trexler 2007), we reasoned that the recruitment of *P. fallax* may have been muted in the controls during the first experi-

ment. In 2010, we examined the effects of sunfish on recruitment of *P. fallax* alone, without the presence of *P. alleni*. Between experiments, nine of the wetlands were dried for 8 weeks to eliminate fish and crayfish from the previous experiment. The experimental wetlands were re-flooded with water and populations of mosquitofish and grass shrimp were re-established. Sunfish were stocked at densities of 0, 4, and 24 sunfish wetland⁻¹ (0, 0.24, 1.45 m⁻²) with the same 3:1 ratio of dollar sunfish (mean SL ± SE; 48.0 mm ± 0.6) to warmouth (59.0 mm ± 1.0). Each stocking density was replicated three times. We added 505 small juvenile *P. fallax* (mean CL: 3.1 mm ± 0.04 SE) to each wetland over 2 weeks using the same methods as in the first experiment. The experiment ran for 13 weeks, at which time we quantified crayfish in each wetland with three randomly placed throw traps.

Effects of sunfish on crayfish growth

The results from the first experiment (2009) suggested that *P. alleni* might have reduced its growth in the presence of abundant sunfish, so during the second experiment we also examined the non-lethal effects of sunfish on the growth of each species. In each experimental wetland, we placed two 1 m² × 97 cm tall mesh (mesh size = 1.5 mm) cages (with mesh bottoms) and stocked them with either juvenile *P. alleni* or *P. fallax*. The cages were positioned in the corners, 20 cm from the wetland walls, and exposed caged crayfish to the presence of sunfish with no lethal contact (picture in Online Resource 1). Cages were supported by a PVC frame and tops were covered with fiberglass window screen to inhibit colonization by large predatory insects. A total of 200 ml of *Chara* sp. and 10 L of peat soil were added to the cages as food and substrate for the juvenile crayfish. Four juvenile crayfish (mean CL ± SE; *P. alleni*: 7.48 mm ± 0.06; *P. fallax*: 7.12 mm ± 0.05) were added to each cage. Individuals were not marked so growth was measured using group average sizes. After 6 weeks of growth, the crayfish were collected and re-measured.

Activity levels of *Procambarus* crayfish

We compared the activity levels of the crayfish species with behavioral observations in the laboratory. All observations were on mono-specific groups of *P. alleni* or *P. fallax* and were directed towards comparing movement rates and risky behaviors of the two species. The observations were conducted in four glass aquaria measuring 45.7 × 121.9 cm. Sand was spread thinly over the bottom of each tank, water was added and maintained at 17 cm, and 12 PVC shelters of two diameters (21 and 26 mm) were added to each tank and spaced evenly throughout. Individual lights were placed above each aquaria with a 14:10 h light:dark cycle. Six

crayfish ranging from 12.3 to 22.0 mm CL (40:60 M:F sex ratio in each tank) were added to each tank. Each species was replicated twice at one time and then the entire procedure was repeated (runs 1 and 2) with new crayfish, providing four replicates of each species. Each crayfish in a tank was distinctly marked using nail polish so that individual behavior, as well as group-averaged movements could be analyzed. Crayfish activity in each tank was recorded with a video camera (Canon S95) twice per day (0700 and 1600 hours) for three consecutive days providing a total of 120 observation min tank⁻¹.

Statistical analyses

Data analyses were performed with SAS v.9.2 and all crayfish population density data were log₁₀-transformed to better fit linear model assumptions. MANOVA was performed on log-transformed *P. alleni* biomass density (g m⁻²), *P. fallax* biomass density, mosquitofish density (no. m⁻²), and grass shrimp density (no. m⁻²) to look for significant responses to stocked sunfish biomass. Each response variable was further analyzed individually with linear regression using the total biomass of sunfish stocked in the experimental wetlands as the predictor. Multiple regression analyses including vegetation abundance indicated that vegetation parameters added relatively little explanatory value to the models of crayfish population responses; they explained almost nothing as individual independent variables, they did not alter the affect of fish biomass, and for purposes of clarity were not included in the results. Because the crayfish biomass density responses to fish were non-linear, even after log-transformation, we further evaluated the effect of sunfish biomass on the untransformed crayfish biomass data using negative exponential models (Proc NLIN, SAS v.9.2). The *P. alleni* biomass density response was decomposed into mean density and mean individual mass for additional analyses. We looked for evidence of a compositional shift by calculating the proportional contribution of *P. alleni* (by nos.) to the final crayfish assemblage. The proportion data were logit transformed with a small value added to the numerator and denominator to make 0 and 1 defined, following suggestions by Warton and Hui (2011), and analyzed using linear regression. The analysis was repeated with weightings, by total number of crayfish captured, but the results remained the same.

For the mesocosm predation assay, we tested for deviation from a null hypothesis of equal survival of the two species in the mesocosms using a paired *t* test (paired differences were normally distributed). The same results could be obtained with a non-parametric signed-rank test.

In the second wetland experiment, we looked for effects of sunfish on mean *P. fallax* biomass density (g m⁻²), mos-

quitofish density (no. m⁻²), and grass shrimp density (no. m⁻²) with MANOVA and one-way ANOVAs with pairwise comparisons (Tukey's HSD). The *P. fallax* biomass density was decomposed into density and mean individual mass for additional analyses.

The growth of caged crayfish was analyzed for each species using one-way ANOVA treating each group in a cage as a replicate. The instantaneous growth for each species [ln(final mass)–ln(initial mass)] was analyzed in two ways. We first analyzed the instantaneous growth using the starting and ending mean individual masses within each cage. We also analyzed the change in mass of the largest crayfish in each group. The second quantification of growth assumed that the largest individual stocked in a particular cage was also the largest individual coming out of that cage, and it was conducted because social interactions could potentially skew the growth of the group towards a single individual in some cases. Starting sizes of juvenile *P. fallax* and the largest individual *P. alleni* stocked in cages did not vary between sunfish treatments (*P* values >0.23), but the average *P. alleni* were modestly larger in the cages placed in low sunfish wetlands ($F_{2,6} = 4.59$; *P* = 0.062; 0.092 vs. 0.076 and 0.078 g). Because instantaneous growth changes with initial size, we conducted analyses with and without initial size as a covariate, but the covariate did not change the results and we present only the model results without the covariate.

Preliminary analyses of the crayfish activity levels indicated no important day effects or interactions between day and species. We calculated a single value for each tank (crayfish group) by totaling the crayfish movement time (min) out of shelter and dividing by six. We calculated the mean number of risky behaviors (tail flips, chasing or fighting with conspecifics) per individual over the total 120 min, again treating groups in tanks as replicates. Both responses were analyzed using a blocked ANOVA with run as the block parameter and included a block × species interaction. We also categorized individual crayfish (*n* = 24 per species) by the number of risky behaviors exhibited over the 120 min of observations. Crayfish were placed in one of four categories (0, 1–5, 6–10, and >10 risky behaviors) and the distributions were compared with a Fisher's Exact Test. The results did not appear to depend on the categorization scheme.

Results

Sunfish effects on the crayfish assemblage

Every aquatic animal response variable except *P. fallax* biomass density was affected by sunfish biomass in this experiment (MANOVA Wilk's Lambda *P* < 0.01). *P. alleni*

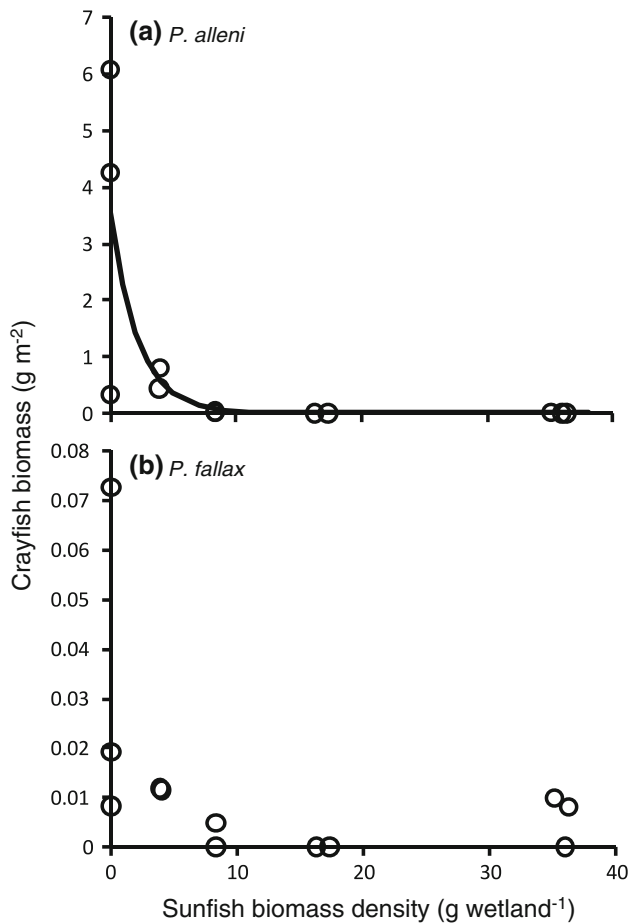


Fig. 1 Crayfish biomass density (g dry mass m⁻²) of **a** *Procambarus alleni* and **b** *P. fallax* after 8 weeks of summertime recruitment in wetlands (18.6 m²) stocked with five different densities of sunfish, *Lepomis* spp. Each point is a mean crayfish biomass density from three 1-m² throw traps taken in each experimental wetland. The prediction line in (a) was calculated from a negative exponential, non-linear regression. No regression line was included for (b) because of the non-significant effect of sunfish biomass on *P. fallax* biomass density in this experiment (notice scale change between panels). The sunfish stocking densities (no. m⁻²) in the wetlands were 0, 0.22, 0.43, 0.86 and 1.72

biomass density declined dramatically with increasing sunfish ($P < 0.01$, linear regr. $R^2 = 0.59$; Fig. 1a), but *P. fallax* biomass density did not ($P = 0.16$; Fig. 1b). The non-linear regression results shown in Fig. 1 illustrate a >99.9% reduction in *P. alleni* biomass from controls to the high sunfish wetlands, but 97.7% of the biomass reduction came with the first eight fish (0.43 m⁻²; see biomass at 8 g sunfish in Fig. 1a). Although one control had a relatively lower biomass than the other two controls, the *P. alleni* biomass (0.34 g m⁻²) was still much higher than in the high sunfish density treatments (<0.01 g m⁻² in the 5 highest sunfish wetlands), and the discrepancy was probably related to excessively high *Chara* abundance in that replicate. The effect of sunfish biomass on *P. alleni* biomass density was a function of both average size and numbers; recruiting

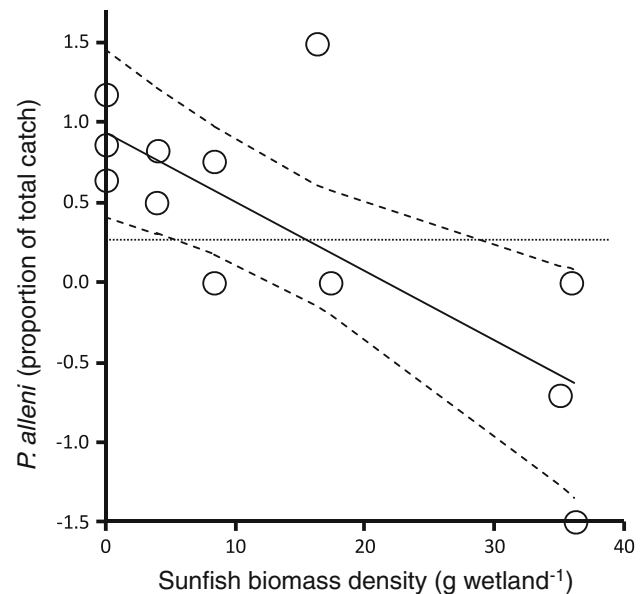


Fig. 2 Proportional contribution of the number of *P. alleni* to the total catch after 8 weeks of growth in wetlands stocked with five different densities of sunfish predators (g dry mass per wetland). Data were logit transformed; logit = 0 indicates 50% *P. alleni*. The solid black line indicates the best fit line from linear regression and dashed black lines indicate 95% confidence intervals on the mean. The horizontal dotted line indicates the transformed initial *P. alleni* stocking proportion of 67%

P. alleni were smaller (regression coefficient \pm SE: -0.03 ± 0.009 , $P < 0.01$, $R^2 = 0.56$) and less abundant (-0.01 ± 0.004 , $P = 0.01$, $R^2 = 0.45$) with increasing sunfish biomass. Compared to the average of the control replicates, *P. alleni* density was 97% lower and individual crayfish were 87% smaller in high sunfish wetlands. Grass shrimp (regression coefficient \pm SE: -0.02 ± 0.006 , $P = 0.01$, $R^2 = 0.45$) and mosquitofish (regression coefficient \pm SE: -0.013 ± 0.001 , $P < 0.01$, $R^2 = 0.86$) densities also declined significantly across the sunfish biomass gradient with 28 and 34% reductions, respectively; however, they were still abundant (mean densities ranged from 3.3 to 11.9 grass shrimp m⁻² and 48 to 142.3 mosquitofish m⁻²) in every wetland.

The proportion of the recruiting crayfish that were *P. alleni* declined with increasing sunfish biomass ($P < 0.01$, $R^2 = 0.64$; Fig. 2). In the control and lowest sunfish density wetlands, the final proportions were above the stocking proportions of 67% *P. alleni* in all five replicates while wetlands with the highest sunfish density (1.7 m⁻²) were below the stocking proportion of *P. alleni* in all the replicates. The 95% confidence interval on the mean did not overlap the 67% stocking proportion of *P. alleni* in the controls or at the highest sunfish densities (Fig. 2; note logit transformation).

In the mesocosm experiment, juvenile *P. fallax* (≈ 8 mm CL) survived better than *P. alleni* when preyed upon by warmouth ($t_7 = 3.8$, $P < 0.01$). In the seven mesocosms

with surviving crayfish, *P. fallax* survivors outnumbered *P. alleni* in every mesocosm, and by an average of almost 4–1 (mean no. survivors \pm 1 SE: *P. fallax*: 4.0 ± 1 , *P. alleni*: 1.1 ± 0.4). We recovered similar numbers of both species from the four control mesocosms (mean no. survivors \pm SE: *P. fallax*: 14 ± 0.6 , *P. alleni*: 13.8 ± 0.8), indicating that both were recoverable, and survived well in the mesocosms without warmouth.

Sunfish effects on *P. fallax* recruitment

In the second wetland experiment, in the absence of *P. alleni*, the multivariate test indicated significant effects of sunfish treatment on animal densities (Wilk's Lambda $P = 0.01$). *P. fallax* biomass density was reduced by sunfish ($F_{2,6} = 45.8$, $P < 0.01$; Fig. 3a) and mosquitofish densities were reduced by sunfish again in this study ($F_{2,6} = 6.6$, $P = 0.03$, data not shown), but grass shrimp densities were not affected by sunfish treatment ($F_{2,6} = 3.3$, $P = 0.11$). Both mosquitofish and shrimp were still abundant in all experimental wetlands at the end of the study (mean densities ranging from 6.7 to 44.7 grass shrimp m^{-2} and 39.7 to 109.7 mosquitofish m^{-2}). *P. fallax* density was affected by sunfish treatment ($F_{2,6} = 30.9$, $P < 0.01$; Fig. 3b), but mean individual mass was not ($F_{2,6} = 0.4$, $P = 0.66$; Fig. 3c). *P. fallax* density and biomass density were reduced $\geq 62\%$ in treatments with sunfish compared to the control (Tukey HSD $P < 0.01$), but *P. fallax* densities in the two sunfish treatments were not different from one another (P values > 0.1).

Effects of sunfish on crayfish growth

A total of five *P. alleni* died during the course of the growth experiment with three deaths in control wetlands and one each in a low and a high sunfish replicate. The only *P. fallax* fatality occurred in a high sunfish replicate. Growth rates did not differ across sunfish density treatments for *P. alleni* ($F_{2,6} = 1.7$, $P = 0.25$; Fig. 4a) or *P. fallax* ($F_{2,6} = 1.3$, $P = 0.34$; Fig. 4b). Growth of the largest *P. fallax* did not differ across the treatments ($F_{2,6} = 1.0$, $P = 0.42$). Growth of the largest caged *P. alleni* varied some ($F_{2,6} = 4.0$, $P = 0.08$; data not shown), but mean growth was highest in the cages placed in high sunfish wetlands followed by control wetlands.

Activity levels of *Procambarus* crayfish

P. alleni was more active than *P. fallax* when measured by total walking time crayfish $^{-1}$ (species: $F_{1,4} = 13.1$, $P = 0.02$; Fig. 5a); however, there was also variability between blocks (i.e., experimental runs) ($F_{1,4} = 27.4$, $P < 0.01$) and a significant interaction ($F_{1,4} = 8.0$, $P = 0.05$)

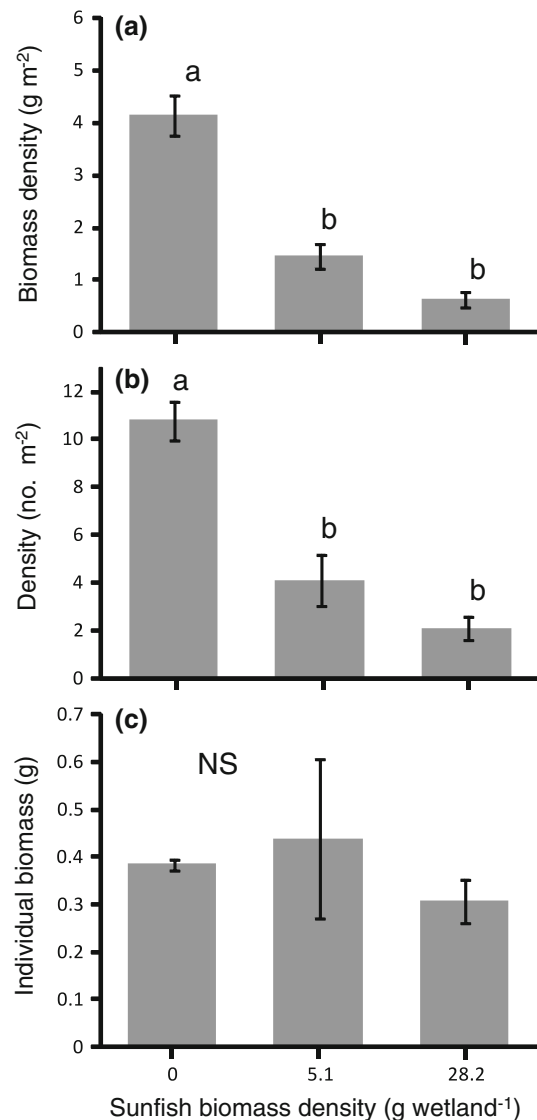


Fig. 3 Effect of initial sunfish biomass density (0, 5.1 and 28.2 g dry mass per wetland) on *P. fallax* **a** biomass density (g dry mass m^{-2}), **b** density (number m^{-2}), and **c** individual size (g), mean \pm 1 SE, $n = 3$ wetlands treatment $^{-1}$. Different letters indicate significant difference at $\alpha = 0.05$ with a Tukey test, NS not significant

because total activity of both species increased in the second run and the differences between species were less pronounced (Fig. 5a). Nevertheless, the activity for each *P. alleni* replicate within a run was always higher than activity for the *P. fallax* replicates in the same run (Fig. 5a). *P. alleni* engaged in more risky behaviors (i.e., swimming, fighting) than *P. fallax* (species: $F_{1,4} = 10.3$, $P = 0.03$; Fig. 5b; block: $F_{1,4} = 2.2$, $P = 0.21$; species \times block: $F_{1,4} = 6.9$, $P = 0.06$). One group in the second run appeared to be an exception to the rule (Fig. 5b); however, one individual *P. fallax* in that group accounted for 78% of risky behaviors in that group. When all individuals were categorized by the amount of risky behavior, the variation

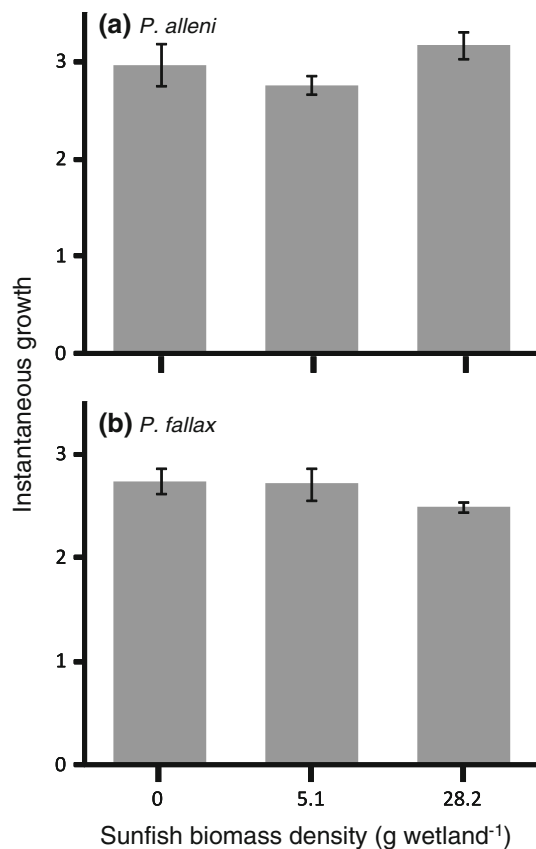


Fig. 4 Juvenile **a** *P. alleni* and **b** *P. fallax* instantaneous growth [$\ln(\text{final mass}) - \ln(\text{initial mass})$, mean \pm 1 SE] in the presence of sunfish predators at 0, 5.1 and 28.2 g wetland⁻¹

between species was striking (Fig. 6; Fisher's Exact $P < 0.01$); 15 *P. alleni* (63%) engaged in ≥ 6 risky behaviors and only 2 *P. fallax* individuals (8%) had similar levels of risky behavior.

Discussion

The results in our study suggest that dominance of the crayfish assemblage in long-hydroperiod sloughs could be driven by selective fish predation on juvenile crayfish. In wetlands, including the Florida Everglades, the fish predation gradient is expected to be correlated with the permanence gradient (Batzer et al. 2006; Dorn 2008; Parkos et al. 2011), but our experiment tested the influence of predatory sunfish controlling for hydroperiod and reproductive effort for each species of crayfish. The assemblages in low sunfish wetlands were dominated by *P. alleni* and this was consistent with expectations based on the competitive asymmetry between the species; *P. alleni* is known to be the stronger competitor for food and habitat (Dorn and Trexler 2007). *P. alleni* abundance declined exponentially across the sunfish gradient and the composition shifted towards more

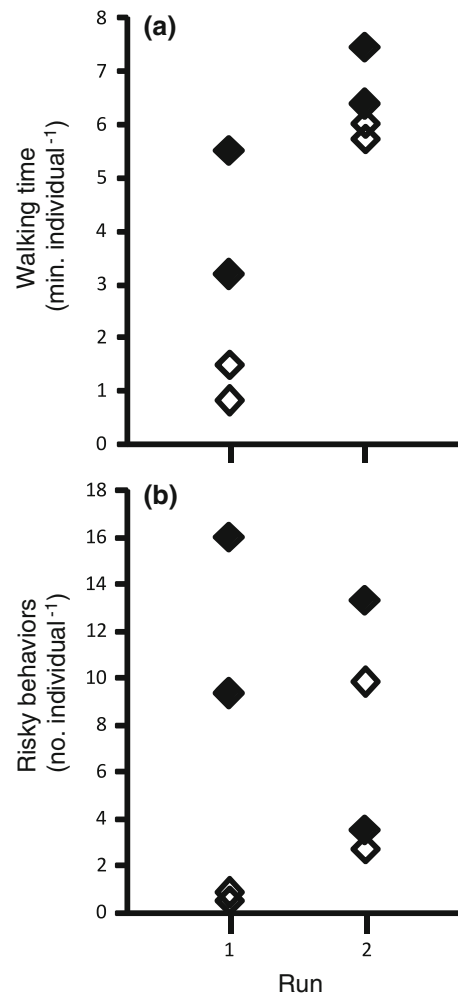


Fig. 5 *P. alleni* (solid diamonds) and *P. fallax* (open diamonds) activity measured as **a** walking time (min) and **b** number of risky behaviors per capita over 120 min of observations. Each datapoint shows the mean activity of a group of six animals; two runs are shown

P. fallax (the weaker competitor), such that, at the highest sunfish density, all replicate wetlands had low crayfish densities, and assemblages that favored *P. fallax* when compared to the stocked composition of juveniles. The results of the predation assay were consistent with the compositional shift observed in the experimental wetlands, suggesting *P. alleni* is relatively more vulnerable to sunfish predation and that selective predation can result in assemblages dominated by the weaker competitor. *P. alleni* grows faster than *P. fallax* (Dorn and Trexler 2007) and *P. alleni* activity levels and propensity for highly active, presumably risky, behaviors were greater than those of *P. fallax* in our laboratory study. The higher activity of *P. alleni* should make them more conspicuous to visual sunfish predators (Lima and Dill 1990; Werner and Anholt 1993; Elkin and Baker 2000).

The lack of significant sunfish effects on *P. fallax* in the first experiment was probably caused by population growth

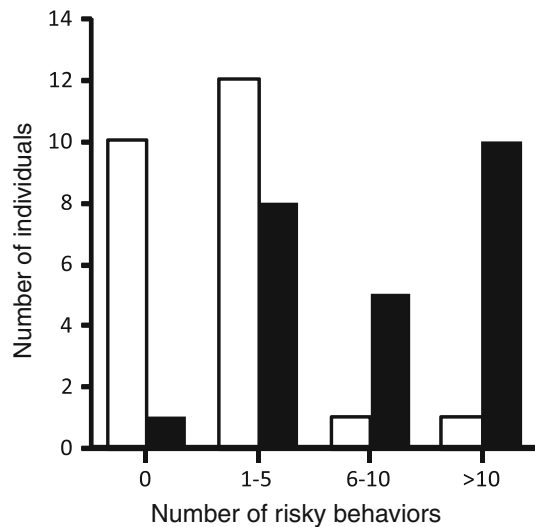


Fig. 6 Frequency distribution of *P. fallax* (white bars) and *P. alleni* (black bars) individuals displaying risky behaviors (tail flips, fights, chasing conspecifics) over 120 min of observations spread over 3 days. Twenty-four crayfish of each species were observed and each individual was embedded in a group of six conspecifics

inhibition by the dominant competitor on one end of the gradient and high fish predation on the other side of the gradient dampening an otherwise negative relationship between sunfish and *P. fallax* recruitment. This interpretation was supported by the significant effect of sunfish on *P. fallax* density and biomass in the second experiment (2010) when only *P. fallax* juveniles were stocked in the experimental wetlands. Wissinger et al. (1999b) demonstrated that the caddisfly *Asynarchus nigriculus* inhabiting temporary subalpine wetlands excludes slower growing caddisflies by intraguild predation; and we note it is also possible that *P. alleni* may prey upon *P. fallax*, but that interaction remains to be studied.

Effects of predators on prey populations and communities are manifested via both consumptive and non-consumptive mechanisms (Preisser et al. 2005). The small final sizes of *P. alleni* and the lower densities in the presence of abundant sunfish seemed a rather dramatic response, and we wondered whether *P. alleni* suffered increased mortality partly mediated by plasticity in growth/development; reduced growth could prolong individual susceptibility to gape-limited predators (Stein 1977) and possibly lead to developmental failure (Lewis 2001). Alternatively, the sunfish may have selectively removed the fastest-growing, and presumably more active/conspicuous, individuals of the *P. alleni* cohort, leaving only the individuals that were less active and growing slowly (i.e., the less active, slow-growing *P. alleni* persisted). When *P. alleni* were caged in the presence of sunfish (non-lethal risk cues) in 2010, we found no evidence for individual modulation of growth. In fact, both species of crayfish grew at similar rates across all

sunfish treatments. We reason, therefore, that sunfish selectively removed the fastest-growing *P. alleni* in the first wetland experiment and that effects of sunfish on *Procambarus* recruitment (both experiments), crayfish assemblage structure, and size structure of *P. alleni* were primarily direct consumptive effects. Mere sunfish presence (non-consumptive effects) had no obvious effect on small juvenile crayfish in these wetlands. While some studies indicate that the presence of large fish can reduce crayfish activity levels (Stein and Magnuson 1976; Garvey et al. 1994; Keller and Moore 2000) and even cause growth reductions (Nyström 2005), the direction of responses to fish cues is not entirely consistent; some species even enhance foraging activity with fish odor (Willman et al. 1994; Pintor et al. 2008). Our study differs from most previous studies of non-lethal effects in species composition (predator and prey), and also in experimental setup. In particular, predators were stocked at field densities in relatively large experimental wetlands with natural vegetation and abundant alternative prey and therefore the level of perceived risk may have been variable in time and space. The consistent size of surviving *P. fallax* outside the cages, regardless of fish treatment (Fig. 3), further suggests that effects were primarily consumptive and the cage growth results were meaningful (growth was not modulated in the presence of predators).

We have no reason to believe that the strong consumptive effects of the sunfish in these experiments were caused by artificiality of the experimental system; either low productivity of the wetlands, low habitat cover, or a lack of alternative prey for the fish. As mentioned in “Materials and methods”, other large (1–3 cm long) macroinvertebrates common to the Everglades were present (e.g., odonates and grass shrimp) in moderate or high densities throughout the experiments, mosquitofish were present at relatively high densities and stem densities (and *Chara* in 2009) were at moderate to high abundances for emergent prairies or sloughs in the Everglades. Further, the predatory sunfish were not starving, as both sunfish species grew during these experiments (mean length increase of 14.0 mm for warmouth and 5.2 mm for dollar sunfish) and dollar sunfish spawned in several wetlands.

To summarize these results and earlier work, the smaller, slower growing, and competitively subordinate *P. fallax* is favored in long-hydroperiod habitats by selective predators (this study), while *P. alleni* is favored in frequently drying short-hydroperiod marshes by relatively better burrowing abilities (Dorn and Trexler 2007; Dorn and Volin 2009) and competitive superiority (Dorn and Trexler 2007). The effect of drying on the distribution appears to be modified somewhat by substrate type (Dorn and Volin 2009), but overall, the life-history traits of crayfish that promote coexistence with sunfish in long-hydroperiod areas of the Everglades appear to follow the well-known patterns for other freshwater

invertebrates of smaller adult size (Wellborn et al. 1996; Garcia and Mittelbach 2008), slower growth and relatively lower activity levels (McPeck 1990; Wellborn et al. 1996; Wissinger et al. 1999b; McCauley 2008).

The crayfish traits favoring coexistence with sunfish in these wetlands seem to be the opposite of the traits promoting coexistence with fish in lake and riverine ecosystems. Large size, fast growth, aggressive behavior (Garvey et al. 1994; Fortino and Creed 2007) or highly active escape responses (Fortino and Creed 2007) favor coexistence with sunfishes in those systems. One difference between those communities and these wetlands may be related to habitat structure; the soft organic sediments found in many Florida sloughs and marshes contrast sharply with the rocky bottoms of lakes and streams with sturdy permanent shelters. Access to permanent shelters may make both juvenile survivorship higher and competition for shelters a key determinant for thriving in the presence of large-bodied fishes. Ability to obtain permanent shelter should favor larger sizes, faster growth, and aggressiveness (Vorburger and Ribí 1999). In soft-bottom habitats, the lack of cobble shelters may favor cryptic life histories, especially at small vulnerable juvenile stages, similar to traits of odonates coexisting with large-bodied fishes (McPeck 1990; McCauley 2008). Most fish–crayfish predator–prey studies have examined behaviors and risks for relatively larger (>10 mm CL) juvenile crayfish (e.g., Stein 1977; Garvey et al. 1994; Englund and Krupa 2000; Fortino and Creed 2007), and this is one of the few studies (also Nyström 2005) to look at recruitment through the earliest ontogenetic stages, starting with the smallest free-living juveniles. Roth et al. (2007) pointed out the potential importance of consumption by smaller sunfish on relatively small juvenile crayfish. We echo that sentiment and further suggest that considering activity-based vulnerability of juvenile stages could shed light on the observation that the invasive rusty crayfish (*Orconectes rusticus*) is most successful in hard-bottom lakes of the upper Midwest (USA), but fails to completely dominate native crayfishes or even reach high densities in macrophyte-dominated lakes (Garvey et al. 2003; Roth et al. 2007). Because predation rates on small juvenile rusty crayfish and native congeners in soft-bottom habitats have not been examined, this remains a hypothesis to explore.

While this discussion has highlighted the relative differences between crayfish species in ability to withstand fish predation and dry-disturbances, these experiments demonstrated strong direct impacts of sunfish predators on recruitment of both species of crayfish in experimental wetlands with natural vegetation and alternative prey for the sunfishes. We also know that, compared to their fish predators, both crayfish species are relatively more resistant to dry-disturbances; individuals of both species are able to burrow and survive under-

ground for several weeks or months (Dorn and Volin 2009). By extension, these observations suggest that crayfish populations of either species could be enhanced by local dry-disturbances that reduce fish populations (Dorn and Trexler 2007; Dorn 2008). Understanding these relationships has additional value as a mechanistic hypothesis to explain the pulsed-production hypothesis for wading birds in the Everglades (Frederick and Ogden 2001). Historical data suggest that years of high nesting activity of the white ibis (*Eudocimus albus*) came 1–2 years after relatively strong regional droughts (Frederick and Ogden 2001). These droughts presumably reduced fish populations (Trexler et al. 2005; Parkos et al. 2011) and, as the diets of white ibis chicks are frequently dominated by crayfish (Dorn et al. 2011), these relationships may indicate that the secondary production of the Everglades temporarily shifts to high crayfish productivity following droughts. Similar suggestions of drought-induced prey release in wetlands have been made by others (Chase and Knight 2003) and should be considered further here and in other shallow aquatic ecosystems.

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