

Multiple predator effects and native prey responses to two non-native Everglades cichlids

Ashley E. Porter-Whitaker¹, Jennifer S. Rehage², Shawn E. Liston³, William F. Loftus⁴

¹Oceanographic Center, Nova Southeastern University, Dania Beach, FL, USA

²Earth and Environment Department, Southeast Environmental Research Center, Florida International University, Miami, FL, USA

³Audubon of Florida, Corkscrew Swamp Sanctuary, Naples, FL, USA

⁴Aquatic Research & Communication, LLC, Vero Beach, FL, USA

Accepted for publication January 31, 2012

Abstract – Non-native predators may have negative impacts on native communities, and these effects may be dependent on interactions among multiple non-native predators. Sequential invasions by predators can enhance risk for native prey. Prey have a limited ability to respond to multiple threats since appropriate responses may conflict, and interactions with recent invaders may be novel. We examined predator–prey interactions among two non-native predators, a recent invader, the African jewelfish, and the longer-established Mayan cichlid, and a native Florida Everglades prey assemblage. Using field enclosures and laboratory aquaria, we compared predatory effects and antipredator responses across five prey taxa. Total predation rates were higher for Mayan cichlids, which also targeted more prey types. The cichlid invaders had similar microhabitat use, but varied in foraging styles, with African jewelfish being more active. The three prey species that experienced predation were those that overlapped in habitat use with predators. Flagfish were consumed by both predators, while riverine grass shrimp and bluefin killifish were eaten only by Mayan cichlids. In mixed predator treatments, we saw no evidence of emergent effects, since interactions between the two cichlid predators were low. Prey responded to predator threats by altering activity but not vertical distribution. Results suggest that prey vulnerability is affected by activity and habitat domain overlap with predators and may be lower to newly invading predators, perhaps due to novelty in the interaction.

Key words: multiple predators; invasion; fishes; antipredator behaviour; functional traits; wetlands

Introduction

Natural systems are threatened by ‘cocktails’ of non-native species (*sensu* Johnson et al. 2009), whose impact on ecosystem structure and function is dependent on interactions among the non-native species themselves (Richardson et al. 2000; Ricciardi 2001; O’Dowd et al. 2003; Grosholz 2005). Non-native species may interact in positive ways, facilitating their invasion success (Adams et al. 2003), increasing impacts (Johnson et al. 2009) and contributing to invasion meltdown (Simberloff & Von Holle 1999; Simberloff 2006 and references therein). Alternatively, non-native species may interact in negative ways, resulting in the inhibition of one species’ invasion

success (Harvey et al. 2004; Britton et al. 2010), or impact (Griffen et al. 2008), and even replacement of one invader by another (Lohrer & Whitlatch 2002). Yet, in other circumstances, no effect of one invasive species on another has been detected (Cope & Winterbourn 2004). Thus, the outcome of multiple invasions is difficult to predict, requiring closer examination of invader interactions (Johnson et al. 2009), including of underlying behavioural mechanisms that help mediate evolutionarily novel interactions between non-native predators and native prey (Sih et al. 2011).

Effects of predators are also strongly influenced by predator–predator interactions (Sih et al. 1998; Schmitz 2007), overall predator diversity (Bruno &

Correspondence: A.E. Porter-Whitaker, Oceanographic Center, Nova Southeastern University, 8000 North Ocean Drive, Dania Beach, FL 33004, USA. E-mail: ashport@nova.edu

Cardinale 2008) and predator hunting modes (Schmitz 2007). Multiple predators may interact in a linear fashion to affect prey mortality rates (i.e., additive effects) or demonstrate a variety of nonlinear interactions because they consume one another (i.e., intra-guild predation, Crumrine & Crowley 2003), influence one another's foraging behaviour (i.e., interference competition, Griffen & Byers 2006) or influence prey behaviour (Lima 1998).

Multiple predators also present conflicting demands on prey (Lima 1998; Sih et al. 1998; Relyea 2003). The ability of prey to respond adaptively to multiple predators simultaneously is thus somewhat limited, especially when predators are non-native (e.g., Bryan et al. 2002). Prey may lack antipredator responses or exhibit ineffective or inappropriate responses when exposed to novel predators (Cox & Lima 2006; Sih et al. 2010). Even if novel predators are similar to native predators (e.g., both are fish), variation in morphological and behavioural foraging adaptations can result in some degree of predator novelty (Cox & Lima 2006; Dunlop-Hayden & Rehage 2011). Maladaptive antipredator responses will increase prey mortality (Nannini & Belk 2006), often resulting in stronger consumptive effects of non-native predators relative to native predators, as seen in freshwater ecosystems (Cox & Lima 2006; Salo et al. 2007; Sih et al. 2010). Thus, a key component of understanding the effects of non-native predators and the variation in native prey vulnerability is examining how multiple non-native predators interact, and how multiple prey species respond to these complex predation threats.

During the past 40 years, fish diversity in the southern Florida Everglades has increased by at least 40% due to species introductions (mostly cichlids). Seventeen non-native, freshwater fish species are currently established in Everglades National Park (ENP; J. Kline personal communication; Shafland et al. 2008). Despite this high invasion rate, our understanding of non-native fish impacts throughout the system remains poor (Trexler et al. 2000), and empirical work that mechanistically examines interactions between native and non-native aquatic taxa and the underlying behaviours remains limited (Brooks & Jordan 2010). In this study, we examined predator–predator and predator–prey interactions among the two most abundant non-native fishes in the Everglades ecosystem and an assemblage of native prey. Our main objective was to compare predation effects between a recent invader, the African jewelfish (*Hemichromis letourneuxi*, Sauvage 1880), and a well-established invader, the Mayan cichlid (*Cichlasoma urophthalmus*, Günther 1862), to better understand native species vulnerability, and how vulnerability may be affected by multiple predator interactions.

Both cichlids are generalist predators in the Everglades, where they consume fishes, macroinvertebrates and some plant material during ontogeny (Loftus 1987; Bergmann & Motta 2005; W.F. Loftus, unpublished data), but little is known about diets or prey preferences in their native range. Mayan cichlids were first detected in 1983 (Loftus 1987), and since then have become widespread and numerous, particularly in estuarine mangrove regions (Trexler et al. 2000; Lorenz & Serafy 2006). The African jewelfish is a more recent invader (since 2000), highly abundant in short-hydroperiod marshes and currently undergoing rapid spread, including into coastal regions where Mayan cichlids dominate (Langston et al. 2010; Rehage et al., unpublished data). Whether these two invaders may facilitate each other's invasion success, exacerbating their impact, or may interact in a negative way, leading to displacement or a predation release for affected prey, remains unknown.

We used field and laboratory experiments to answer three questions: (i) Do Mayan cichlids and African jewelfish have similar predation rates and prey preferences (i.e., are they functionally redundant)? (ii) Do their interactions alter predatory effects on native prey? (iii) Do prey exhibit effective antipredator responses to both non-native predators? Using *in situ* field enclosures, we compared predation rates by the two cichlids on an assemblage of five native prey (four fish species and one macroinvertebrate). Field enclosures are less likely to constrain species interactions than laboratory experiments, providing an appropriate context for understanding the functional roles of predators in nature (Schmitz 2007). Next, we examined variation in predator tactics and antipredator behaviour in laboratory aquaria. We predicted higher predation rates for the African jewelfish because of greater prey naiveté. We hypothesised that interference competition among the cichlids would lead to lower predation rates when together, resulting in prey being released from predation in mixed predator treatments. We expected prey to exhibit stronger antipredator responses to the Mayan cichlid, due to greater experience with this predator.

Methods

Field collections

For both the field and laboratory experiments, we used a natural prey assemblage consisting of the five most abundant Everglades fish and macroinvertebrate taxa (Trexler et al. 2000): riverine grass shrimp (*Palaeomonetes paludosus*, Gibbes 1850), eastern mosquito-fish (*Gambusia holbrooki*, Girard 1859), bluefin killifish (*Lucania goodei*, Jordan 1880), least killifish (*Heterandria formosa*, Girard 1859) and flagfish

(*Jordanella floridae*, Goode & Bean 1879). We used unbaited minnow traps and dip nets to collect prey from several locations in the Everglades. Collections of African jewelfish and smaller Mayan cichlids were made with unbaited minnow traps along with the prey collections at ENP and the Big Cypress National Preserve, while larger Mayan cichlids were collected by angling in canals bordering ENP and northern Water Conservation Area 3A.

Prior to the field experiment, prey were held in 1-m³ *in situ* enclosures at the field site, while predators were held in 1200-l outdoor tanks at ENP. For the laboratory experiment, predators and prey were held in 795-l tanks at Nova Southeastern University's Oceanographic Center. Predators were collected over the course of 10 weeks, while prey were collected up to 2 weeks before starting both experiments, which falls within the range of holding periods for similar predator-prey studies (4–24 weeks; Ioannou et al. 2008; Cote et al. 2010; Harcourt et al. 2010; Schurch & Heg 2010). During the holding period, predators were fed an assortment of live prey *ad libitum* (including experimental prey taxa), but were starved the evening before behavioural trials. For the field experiment, prey fed on ambient resources (e.g., periphyton and infauna added to cages), whereas in the laboratory experiment, prey were fed commercial flake food *ad libitum*. In both experiments, prey sizes (carapace or standard length) and wet weights (± 1 SE), based on a random sample of 60 individuals of each species, were as follows: riverine grass shrimp, 10.33 ± 0.34 mm, 0.10 ± 0.01 g; eastern mosquitofish, 18.76 ± 0.45 mm, 0.11 ± 0.01 g; bluefin killifish, 23.59 ± 0.44 mm, 0.20 ± 0.01 g; least killifish, 14.25 ± 0.42 mm, 0.08 ± 0.01 g; and flagfish, 21.01 ± 0.51 mm, 0.35 ± 0.02 g. Prey sizes were within the natural range of prey found throughout marshes (Trexler et al. 2005), as well as found in gut content analyses of the two predator species (W.F. Loftus, unpublished data).

Because the two predators differ significantly in adult size, we used adult African jewelfish and both adult and subadult Mayan cichlids in each experiment, allowing us to evaluate the role of body size on predator-predator interactions and predatory effects (Taylor et al. 2001; Mills et al. 2004). African jewelfish mature at ~ 40 mm standard length (A.L. Jungman et al., unpublished data), while Mayan cichlids mature at ~ 120 mm SL in Florida (Faunce & Lorenz 2000). Across the two experiments, predator sizes (SL ± 1 SE) averaged 49.7 ± 1.7 mm for all African jewelfish ($N = 44$), 66.3 ± 3.6 mm for subadult Mayan cichlids ($N = 33$) and 123.7 ± 5.0 mm for adult Mayan cichlids ($N = 11$).

Field enclosure experiment

We conducted the *in situ* enclosure experiment in a wet prairie wetland near Paurotis Pond in the southern region of ENP ($25^{\circ}16'58''\text{N}$, $80^{\circ}47'53''\text{W}$). This intermediate-hydroperiod site was selected because both non-native predators inhabit the region, and the site has an abundance of floating periphyton and emergent spikerush (*Eleocharis* spp.), both characteristic of Everglades marshes and known to increase microhabitat complexity for aquatic fauna (Liston & Trexler 2005).

Field enclosures consisted of 1-m³ PVC frames that supported 5-sided 1-mm polypropylene mesh cages (no tops). Two weeks prior to the start of the experiment, we erected 25 enclosures at the field site and stocked each enclosure with ambient volumes of floating and benthic material: 3.8 l of floating periphyton and 12 l of flocculent organic detritus (typically lying atop the sediment). To mimic habitat structure provided by emergent macrophytes but excluded by the mesh floor (primarily *Eleocharis* spp.), we randomly placed 100 artificial stems (1.6-mm-diameter steel rods extending through water column) in each enclosure. Enclosures were arranged in a single row (approximately 1.5 m apart) oriented perpendicular to water flow (east-west) to minimise the potential effect of chemical cues among enclosures.

Using a randomised block design, we assigned five predator treatments to the 25 enclosures: two African jewelfish (JJ), two subadult Mayan cichlids (MM), one subadult Mayan cichlid + 1 African jewelfish (MJ, size-matched), one adult Mayan cichlid + 1 African jewelfish (MJ, Mayan cichlid is 2.5 times larger than African jewelfish) and a no-predator treatment (NP). We used a replacement series experimental design, where predator density remained constant, allowing us to examine inter- and intraspecific interactions between the cichlids (Sih et al. 1998; Schmitz 2007). Stocked prey assemblages in each cage consisted of six individuals per prey species (6×5 spp. = 30 prey m⁻²), a prey density that fell within the natural range of Everglades marshes ($1\text{--}55$ fish m⁻², Trexler et al. 2005; $21\text{--}31$ fish m⁻², $0\text{--}431$ grass shrimp m⁻², Rehage & Trexler 2006).

The experiment was conducted at the peak of the 2007 wet season (25–29 October) at 33.4 ± 0.6 cm ($N = 25$) water depth and 26.7 ± 0.2 °C water temperature ($N = 93$). Prey were introduced into enclosures and allowed to acclimate for 48 h before predator stocking. During this time, we checked for and replaced any prey mortalities ($<0.5\%$ of prey were replaced). The experiment ran for 4 days, after which we collected all remaining individuals using dip nets, and by turning enclosures on their side, and manually sorting through the benthos and periphyton. Specimens

were preserved in 10% formalin and returned to the laboratory for identification and enumeration.

Predator and prey behaviour laboratory experiment

To examine the behavioural interactions among African jewelfish, Mayan cichlids and the five native prey species, we conducted behavioural trials in indoor 56.8-l tanks ($50 \times 24.5 \times 40$ cm height). The same five treatments were assigned to 15 tanks in a randomised block design. Behavioural trials were conducted in two 2-day blocks, with 12 replicates per treatment (three replicates per day). Block 1 was conducted on 5–6 January 2008 and block 2 on 12–13 January 2008. Though the prey assemblage was comprised of the five species used in the field experiment, prey number was reduced to 10 prey/tank because of the smaller container size and the difficulty in observing a large number of prey accurately (2 prey \times 5 spp. \times 5 treatments \times 12 replicates = 600 total prey).

To minimise observer disturbance, lateral observations were conducted from behind a blind that surrounded the entire tank area. Tanks were covered on three sides and bottom with a white vinyl covering. We provided habitat structure to simulate submerged macrophytes by attaching black plastic strips (4×22 cm) to a weighted 16×16 cm black plastic grid, covering 1/3 of the tank bottom and extending through 2/3 of the water column. The water depth matched field conditions (33.4 cm), and photoperiod over the course of the experiment was maintained at 12 L/12 D.

The evening before trials began, prey were randomly placed in conspecific pairs and added to the observation tanks to acclimate, while predators were isolated in individual 5.7-l containers ($27.5 \times 14.5 \times 11$ cm). The following morning, tanks were checked for prey mortality prior to the start of the experiment, and mortalities were replaced prior to data collection (2% of prey were replaced). For the predator treatments, predators were released into a randomly selected tank, and data collection began 10 min after release. We quantified predator and prey behaviour through multiple spot check observations conducted at each of the 15 replicate tanks (Martin & Bateson 2007). Tanks were observed in a random order, and a new randomisation was done daily. Spot check observations were made in each tank every hour for 6 h, after which the six scores were averaged (Dunlop-Hayden & Rehage 2011).

At each spot check, we recorded the activity and microhabitat use of the predators and of each prey. For the prey, we averaged the score of the two individuals of each species to obtain a single value per replicate. Activity was scored as '0' if immobile in one location,

'1' if there was a slight movement of the body (slow and stop and go swimming for the fishes and movement of appendages for grass shrimp) and '2' if there was constant and/or faster swimming for the fish, or any walking or swimming that resulted in a change in location for grass shrimp. Microhabitat use by the predators and prey was assessed by noting their vertical distribution within the tank ('1' for bottom third, '2' for middle and '3' for the top third of the water column), and whether fish were in or out of the habitat structure ('1' or '0'). All observations were conducted between 0900 and 1600 h.

Predator–predator interactions were counted from spot observations and totalled for the entire trial. They included approaches by one predator to another, schooling of both predators in close proximity (within one body length) and chases with or without physical contact. Predators were free ranging, so a small number of predation events were recorded during our observations, accounting for 8% of the prey in the experiment (37 shrimp, five flagfish, five least killifish and two bluefin killifish). Consumed prey were not replaced, and data for consumed prey were averaged or counted for period of observation.

Data analyses

For the enclosure study, we compared total and prey-specific predation rates (number of prey stocked – number recovered after 4 days) using generalised linear mixed models (GLMM) that tested prey species, treatment and interaction effects (all fixed) and treated block as a random effect (Bolker et al. 2009). We calculated expected predation rates for the MJ multiple predator treatment, assuming that each predator had independent effects. We used Griffen's (2006) formula for calculating expected predation: $E_{MJ} = (M_{JJ} \times M_{MM})^{0.5}$, where M_{JJ} and M_{MM} were the prey mortalities in African jewelfish and Mayan cichlid predator pairs, respectively. We calculated expected mortality rates separately for each block (Crumrine & Crowley 2003) and then tested for an emergent multiple predator effect using an ANOVA that compared expected and observed mortalities for the MJ treatment. For the laboratory data, we used GLMMs to examine predator (two-way: treatment, block) and prey (three-way factorial: treatment, prey species, treatment \times prey species and block) activity, use of habitat structure and vertical distribution, as well as predator–predator interactions. These models treated species and treatment as fixed effects, and block as a random effect.

Based on the behaviour of residuals (Kery & Hatfield 2003), raw prey counts and activity values were used, vertical distributions were square root-transformed, while use of habitat structure and

predator interactions were arcsin(square root)-transformed to satisfy normality assumptions. In preliminary analyses, we examined the effect of trial time (the six hourly observations per trial) on response variables using repeated measures ANOVAs. No significant treatment or species effects were found over the course of the trials (all $P > 0.05$), so averaged observations over the entire trial period were used for all analyses. Least significant difference (LSD) pairwise comparisons and planned orthogonal contrasts (i.e., all predator treatments versus no predator control) were used to compare means. For the pairwise comparisons, we denote significant differences at $P = 0.05$ using letters in bar graphs (Figs 1–5). All analyses were performed using Proc Mixed and Proc GLM in SAS[®] 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Predation rates in field enclosures

Predation rates over the 4-day field experiment varied as a function of treatment and prey taxa (Table 1). As expected, prey mortality was higher in the presence versus absence of predators (Fig. 1). Prey recovery at

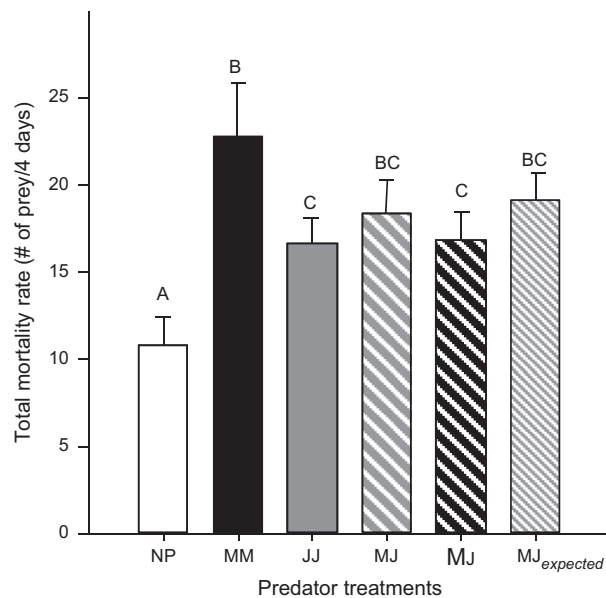


Fig. 1. Total predation rate (all prey \pm 1 SE) across treatments in field enclosures (NP = no predators, MM = 2 Mayan cichlids, JJ = 2 African jewelfish, MJ = 1 juvenile Mayan cichlid + 1 African jewelfish and MJ = 1 adult Mayan cichlid + 1 African jewelfish). Predator pairs were size-matched, except for MJ where the Mayan cichlid was larger. Solid bars show single predator treatments, and striped bars show multiple predator treatments. Expected predation of an African jewelfish and Mayan cichlid pair is shown as MJ_{expected}. Significant pairwise differences among treatments are indicated by letters above bars ($P \leq 0.05$).

the end of the experiment in the no-predator enclosures was rather low, 64%, but was significantly higher than recovery across predator treatments, 28% (NP versus all predator treatments: $F_{1, 16} = 15.3$, $P = 0.0012$). Predation rates on all prey combined were highest by the Mayan cichlid pair (MM). Over the 4 days of the experiment, Mayan cichlids consumed on average 23 of the 30 prey stocked, compared to 17 prey consumed by the African jewelfish pair, and 18 prey consumed by the size-matched MJ treatment (Fig. 1).

We detected no evidence that the prey assemblage experienced a release or enhanced predation when both predators were present. Total predation by the MJ pair was not significantly different from rates when predators were alone (MM and JJ, Fig. 1). Based on the substitutive model, expected predation for the MJ pair was 19.1 prey, which did not differ from the observed predation rate of 18.4 prey ($F_{1, 8} = 0.07$, $P = 0.80$). Further, predation rates did not vary between MJ and MJ, suggesting that size may not play a critical role in mediating multiple predator effects (at least in the absence of intraguild predation). No African jewelfish mortality was recorded in the MJ treatment, despite the size differential.

The MM pair consumed not only more prey, but also more prey types than the JJ treatment (3 versus 1 prey species, Fig. 2). Flagfish were readily consumed by all predator combinations and had the lowest mortality in the absence of predators (81.7% across predation treatments versus 13.3% in NP). Bluefin killifish experienced high mortality only when the predators were MM and MJ, while eastern mosquitofish experienced no predation. Riverine grass shrimp suffered significant mortality relative to the control only in MM treatments (72.5% versus 53%; Fig. 2), while least killifish experienced high mortality both in the presence and absence of predators (78.3% versus 70%). Comparing the effects of single versus multiple predators on individual prey species, we found little evidence of changes in risk. Flagfish was the only prey consumed in both JJ and MM treatments, and their mortality in single predator treatments was similar to that in the two mixed treatments (Fig. 2).

Predator behaviour in laboratory experiment

Activity levels for the JJ pair were more than double those of the other predator treatments (Table 1, Fig. 3a). Activity levels for the mixed-predator treatments were similar to those of the MM pair and were unaffected by size. Variation in predator–predator interactions mirrored the predator activity results (Table 1, Fig. 3b). Interactions were highest among JJ and relatively low in the other two treatments. Despite the large disparity in activity levels between

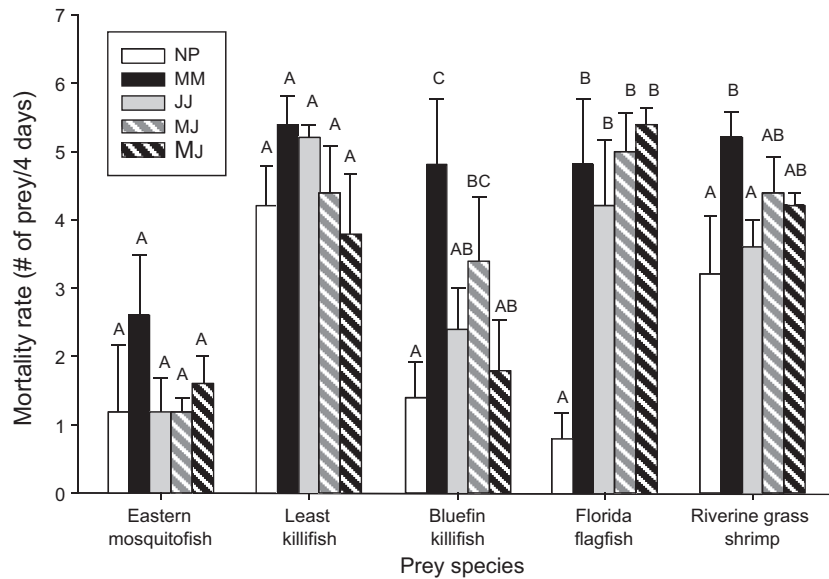


Fig. 2. Field predation rates (± 1 SE) shown separately for the five prey species across treatments (NP = no predators, MM = 2 Mayan cichlids, JJ = 2 African jewelfish, MJ = 1 Mayan cichlid + 1 African jewelfish-size-matched and MJ = 1 adult Mayan cichlid + 1 African jewelfish). Solid bars show single predator treatments, while striped bars show multiple predator treatments. Significant pairwise differences among treatments (compared separately by spp.) are indicated by letters above bars ($P \leq 0.05$).

African jewelfish and Mayan cichlids, their use of tank microhabitats was similar (Table 1). Predators spent 50–60% of the time in cover (Fig. 4a) and mainly occupied the mid- to low water column, with the use of the lower areas increasing for the MJ pair (Fig. 4b).

Prey behaviour in laboratory experiment

Strong antipredator responses, particularly prey- and predator-specific responses, were seen only in the prey's activity and not in their use of tank microhabitats. Prey did not alter vertical distributions in response to predators (Table 1). In the use of habitat structure, there was lower usage with the MJ predator pair (MJ versus all other treatments: $P < 0.0149$). Instead, we saw consistent prey-specific microhabitat use patterns (Fig. 4c and d). Eastern mosquitofish and least killifish occurred highest in the water column and used habitat structure the least, while the opposite pattern was seen for flagfish and riverine grass shrimp. Bluefin killifish were found midwater, and their use of structure was intermediate. Thus, the more demersal species also appeared to use cover more frequently, suggesting variation in the habitat domain of these species.

Prey species differed in overall activity levels, but also exhibited distinct responses to the predator treatments (Table 1). In general, eastern mosquitofish and least killifish were most active, flagfish and bluefin killifish showed intermediate levels of activity, and riverine grass shrimp were least active (Fig. 5). Yet, prey showed the full gamut of activity responses to

predation threats: generalised (i.e., same response to all predator combinations), specific and no response. Generalised responses were seen in flagfish and riverine grass shrimp; both species lowered their activity indiscriminately to all predator combinations relative to the control (Fig. 5). Specific antipredator responses were measured for the poeciliids. Eastern mosquitofish lowered their activity markedly in the presence of the African jewelfish pair, while least killifish responded in the opposite manner by becoming more active in the African jewelfish + adult Mayan cichlid treatment (Fig. 5). Lastly, bluefin killifish did not alter activity across predator treatments.

Discussion

Interactions between sequential invaders have the potential to drastically alter an earlier invader's abundance, distribution and per capita effects (e.g., O'Dowd et al. 2003; Grosholz 2005; Britton et al. 2010). The presence of one invader may facilitate subsequent invasions or may exacerbate impact by a second invader. Alternatively, interactions between invaders may result in displacement of one invader by a later invader. Our examination of sequential invasions by two cichlid predators in the Everglades revealed no evidence of interactions between the two predators that could alter each species' predation effects when sympatric. Predation rates of the two cichlids together were intermediate to predation rates by each cichlid alone, suggesting no emergent

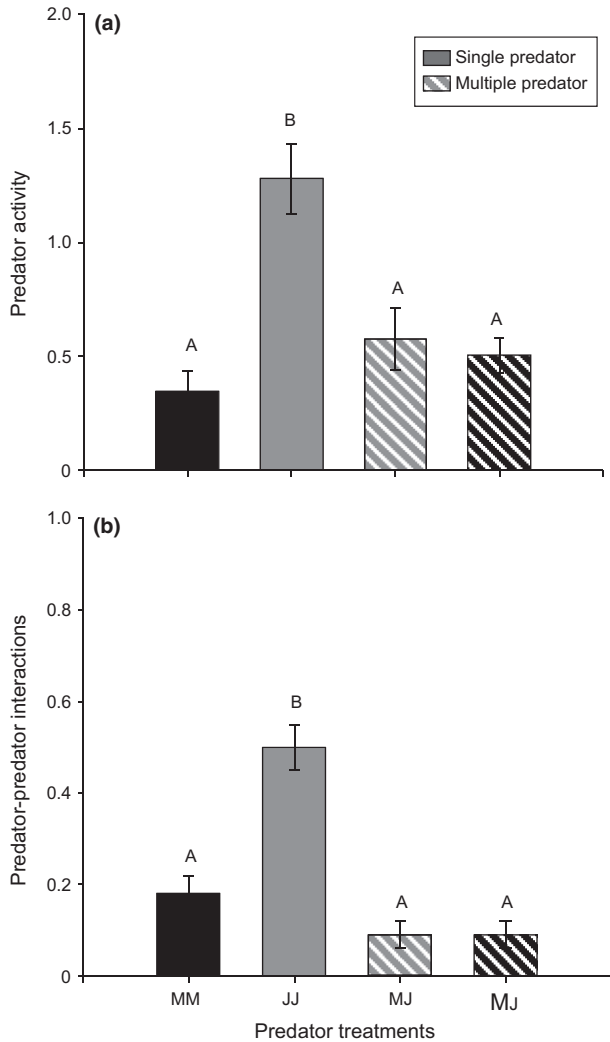


Fig. 3. (a) Laboratory predator activity and (b) number of predator–predator interactions ($\bar{X} \pm 1$ SE) across treatments (MM = 2 Mayan cichlids, JJ = 2 African jewelfish, MJ = 1 Mayan cichlid + 1 African jewelfish-size-matched and MJ = 1 adult Mayan cichlid + 1 African jewelfish). Activity was scored as 0–2 (0 = not active). Solid bars show single predator treatments, and striped bars show multiple predator treatments. Significant pairwise differences are indicated by letters above bars ($P \leq 0.05$).

multiple predator effects on Everglades prey assemblages. Although size is often an important modifier of predator–prey interactions (Taylor et al. 2001; Mills et al. 2004), we found predation rates to be similar between mixed predator treatments with larger, adult and size-matched juvenile Mayan cichlids.

Interactions among multiple predators often result in a reduction in risk for their common prey, as predation rates by one or both predators decrease in sympatry (Sih et al. 1998; but see Schmitz 2007). We found no evidence for reduced prey risk from either behavioural (i.e., interference; Griffen et al. 2008) or density-mediated (e.g., intraguild predators, Crumrine &

Crowley 2003; Relyea 2003; Vance-Chalcraft & Soluk 2005; Griffen & Byers 2006) interactions in our two experiments. Even though Mayan cichlids may readily consume African jewelfish that are of similar size to those used in our experiments (Whitaker et al., unpublished data), African jewelfish mortality did not occur when paired with adult Mayan cichlids in enclosures. Thus, in the absence of predator–predator interactions between African jewelfish and Mayan cichlids, predation rates did not differ between mixed and single predator treatments.

Instead, high levels of agonistic interactions were observed among pairs of African jewelfish. We suspect that these elevated intraspecific interaction rates among African jewelfish contributed to the difference in predation rates between the single predator treatments. Overall, African jewelfish pairs consumed less prey and fewer prey types than the similarly sized Mayan cichlid pairs. Time-consuming social interactions among predators can affect a predator’s rate of consumption (Abrams & Ginzburg 2000). Previous work shows that African jewelfish aggression toward conspecifics was higher than levels observed among native Everglades centrarchids (Schofield et al. 2007). Else, a difference in size-specific energy requirements could explain the higher predation rate of the larger-bodied Mayan cichlid (Baber & Babbitt 2003).

For individual prey, predation risk varied between the two single predator treatments, suggesting higher vulnerability for Everglades prey to the longer-established Mayan cichlid. In enclosures, African jewelfish pairs consumed only flagfish, while Mayan cichlid pairs consumed not only more prey items but also more prey types: flagfish, bluefin killifish and riverine grass shrimp. Surface-dwelling, highly active eastern mosquitofish appeared immune to predation in enclosures, likely due to low encounter rates with both cichlids. But, we suspect that eastern mosquitofish were responsible for the high mortality of least killifish (also surface-dwelling) observed across all treatments. Intraguild predation is common between these live-bearers (Belk & Lydeard 1994; Schaefer et al. 1994), and predation by mosquitofish is an important structuring force in assemblages of small-sized Everglades marsh fishes (Taylor et al. 2001).

Behavioural data collected in laboratory aquaria showed that the species that were significantly preyed upon in the field, riverine grass shrimp, bluefin killifish and flagfish, were the least active and more demersal taxa. Prey activity affects predator attack probabilities, foraging preferences and perceived pursuit cost (Sih & Christensen 2001). Similarly, vertical distribution in the water column can be a key determinant of predator–prey encounter rates (Baber & Babbitt 2003). Prey also responded to the presence of

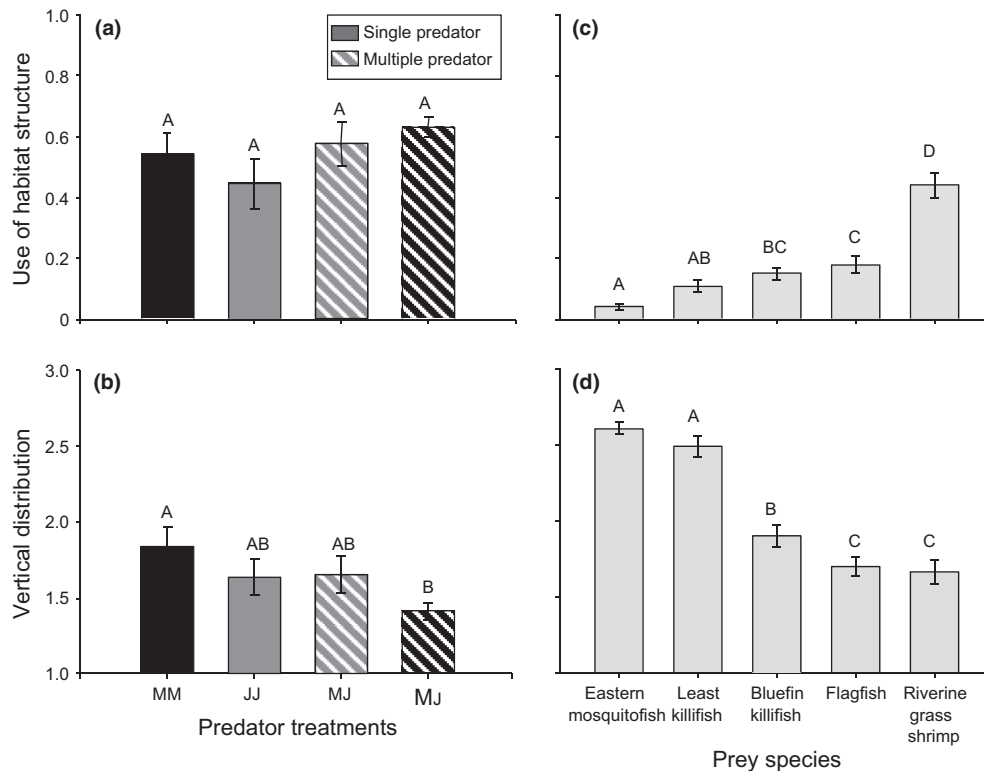


Fig. 4. Use of habitat structure and water column in laboratory aquaria (± 1 SE) by the predators (a and b) and the prey (c and d). Treatments are MM = 2 Mayan cichlids, JJ = 2 African jewelfish, MJ = 1 juvenile Mayan cichlid + 1 African jewelfish-size-matched and MJ = 1 adult Mayan cichlid + 1 African jewelfish. Use of habitat structure was scored as 0 = no use, 1 = use, and vertical distribution was scored as 1 = bottom, 2 = middle, 3 = top. In (a) and (b), solid bars show single predator treatments, and striped bars show multiple predator treatments. Significant pairwise differences are indicated by letters above bars ($P \leq 0.05$).

predators by altering activity levels but not microhabitat use, which appeared to be consistently varied across prey taxa. In our study, both predators were demersal, and the three species that experienced predation were also demersal, supporting the notion that habitat domain overlap between predators and prey is a key determinant of predation effects (Schmitz 2007). But, habitat domain overlap is not the only determinant since we saw differential predation between the two cichlids, with African jewelfish consuming only one of the three prey with which they overlapped in domain, flagfish.

We hypothesise two mechanisms for the exclusive predation of African jewelfish pairs on flagfish, despite high encounter rates with three bottom-dwelling prey. First, with the highest biomass of the prey used in the study, flagfish may provide the highest energetic content, and thus may have appeared more profitable to African jewelfish (Lima 1998). Second, prey novelty may have played a role. Non-native predator tactics may be less efficient at catching prey if the interaction is rather novel (Salo et al. 2007; Sih et al. 2010), despite the prey's ability to detect and respond to new invaders (Dunlop-Hayden & Rehage 2011). African jewelfish foraging tactics may have been

relatively ineffective at capturing Everglades prey, being only successful with the slowest, large-sized and most visible prey.

Overall, our results show that the vulnerability of native Everglades prey to non-native cichlid predators was higher for the longer-established Mayan cichlid, was unaffected by multiple predators interactions and varied among prey taxa in relation to prey activity levels and microhabitat use. Marsh species that were more demersal, overlapping in habitat domain with both predators and were less active appeared to be at higher risk. Vulnerability to African jewelfish was lower for two of the five prey tested, suggesting species-specific variation in the ability of prey to cope with novel predation, likely related to the prey's individual evolutionary history and resulting traits (Sih et al. 2011). Vulnerability to non-native predation was unaffected by sympatry, suggesting that the potential for synergistic and antagonistic effects where these predators co-occur is low. Regardless, these non-native cichlids shared prey resources, perhaps with African jewelfish being less effective or more 'choosy' and targeting larger prey, while Mayan cichlids had greater consumptive effects and on more prey taxa.

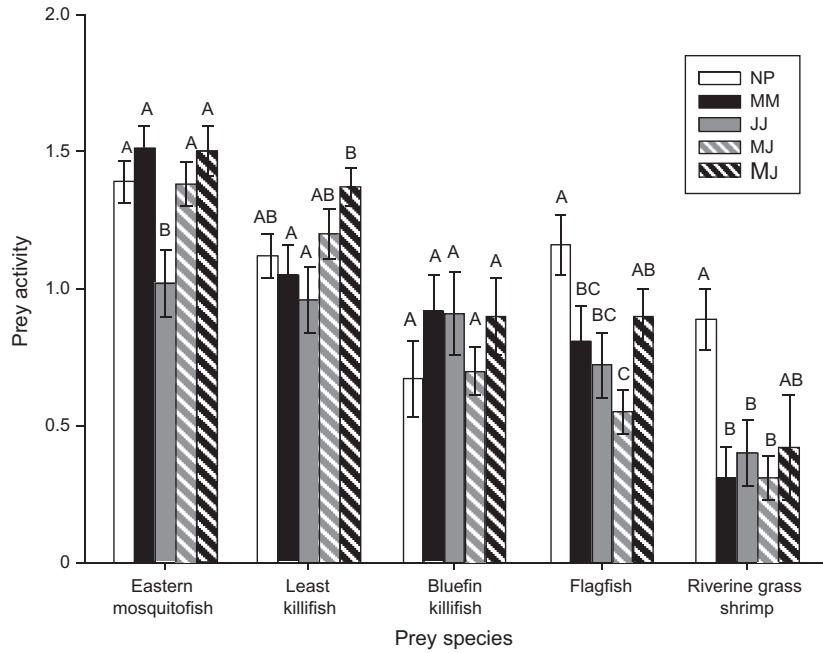


Fig. 5. Activity level of the five prey taxa in laboratory aquaria (\pm SE) across predator treatments (NP = no predators, MM = 2 Mayan cichlids, JJ = 2 African jewelfish, MJ = 1 = Mayan cichlid + 1 African jewelfish-size-matched and MJ = 1 adult Mayan cichlid + 1 African jewelfish). Activity was scored 0–2 (0 = not active). Solid bars show single predator treatments, and striped bars show multiple predator treatments. For each prey species, significant treatment differences are indicated by letters above bars ($P \leq 0.05$).

Table 1. One- and two-way anovas comparing predation rates, and predator and prey behaviour.

| | Treatment | | Prey species | | Treatment \times prey species | | Block | | Model R^2 |
|-------------------------------|----------------------|---------------|-----------------------|---------------|---------------------------------|---------------|--------|--------|-------------|
| | $F_{d.f.}$ | P | $F_{d.f.}$ | P | $F_{d.f.}$ | P | Wald Z | P | |
| Field enclosures | | | | | | | | | |
| Total predation rate | 5.8 _{4,16} | 0.0046 | | | | | 0.82 | 0.2070 | 0.64 |
| Individual predation rate | 9.9 _{4,96} | 0.0001 | 20.6 _{4,96} | 0.0001 | 2.0 _{16,96} | 0.0181 | 1.08 | 0.1410 | 0.64 |
| Laboratory prey behaviour | | | | | | | | | |
| Activity | 5.0 _{4,272} | 0.0007 | 41.6 _{4,272} | 0.0001 | 2.4 _{16,272} | 0.0018 | 0.33 | 0.3716 | 0.47 |
| Use of habitat structure | 5.5 _{4,272} | 0.0003 | 32.9 _{4,272} | 0.0001 | 1.3 _{16,272} | 0.2083 | 0.61 | 0.2697 | 0.40 |
| Vertical distribution | 2.0 _{4,272} | 0.0935 | 50.6 _{4,272} | 0.0001 | 1.3 _{16,272} | 0.2067 | 0.62 | 0.2691 | 0.56 |
| Laboratory predator behaviour | | | | | | | | | |
| Activity | 9.2 _{3,44} | 0.0001 | | | | | 0.0 | 1.0000 | 0.39 |
| Use of habitat structure | 1.5 _{3,43} | 0.2244 | | | | | 0.13 | 0.4480 | 0.10 |
| Vertical distribution | 3.0 _{3,43} | 0.0412 | | | | | 0.64 | 0.2600 | 0.32 |
| Predator interactions | 9.5 _{3,43} | 0.0001 | | | | | 0.04 | 0.4860 | 0.39 |

Significant values are in bold.

Most importantly, we do not know whether invasion by these predators translates into increased overall predation (versus replacement of native predators), and thus greater consumptive effects in areas where invaders are present (Sih et al. 2010), which could be detrimental to higher consumers in the Everglades (i.e., wading birds). At minimum, our experiments suggest that African jewelfish may represent lesser of a predation threat than their confamilial Mayan cichlid due to their lower

predation rates and selective predation. However, seasonal drying limits the abundance of large native piscivores in shorter hydroperiod wetlands of the Everglades, resulting in lower predation regimes throughout much of the landscape (Trexler et al. 2005). But African jewelfish, with their small body size, seem to cope well with the higher frequency of drydown and are abundant in these shallow habitats (Kobza et al. 2004; Schofield et al. 2010), while larger fish predators, both native and non-native, are

excluded. Thus, despite their lower predatory effect, African jewelfish may have an important predation effect in parts of the ecosystem that are shallower and experience frequent drydown. As African jewelfish continue to expand into coastal areas, the potential for altered predation regimes in other shallow habitats across the Everglades landscape (e.g., forested wetlands, freshwater and tidal creeks) deserves further examination. Similarly, as the number of invaders continues to increase in the Everglades and across aquatic ecosystems globally, the need to better understand the relative impact of non-native predators, the importance of predator–predator interactions to invader success and impact and overall multiple non-native predator effects, will likely increase.

Acknowledgements

This project was funded by the Park Oriented Biological Support Initiative of the U.S. Geological Survey (POBS 06-086) and was developed in collaboration with the Florida Coastal Everglades Long-Term Ecological Research program under National Science Grant no. DEB-9910514. We wish to thank Everglades National Park personnel, especially P. J. Walker and J. Kline, for research permits and project support. We thank K. Dunker for conceptual input; K. Dunlop-Hayden, L. McCarthy and N. Katin for field assistance; A.J. Whitaker, A. Hayden and P.F. Rehage for assistance with field collections and laboratory work; and J.C. Trexler for loaning us field enclosures.

References

Abrams, P.A. & Ginzburg, L.R. 2000. The nature of predation: prey dependent, ratio dependent or neither? *Trends in Ecology & Evolution* 15: 337–341.

Adams, M.J., Pearl, C.A. & Bury, R.B. 2003. Indirect facilitation of anuran invasion by non-native fishes. *Ecology Letters* 6: 343–351.

Baber, M.J. & Babbitt, K.J. 2003. The relative impacts of native and introduced predatory fish on a temporary wetland tadpole assemblage. *Oecologia* 136: 289–295.

Belk, M.C. & Lydeard, C. 1994. Effect of *Gambusia holbrooki* on a similar-sized, syntopic poeciliid, *Heterandria formosa*: competitor or predator? *Copeia* 1994: 296–302.

Bergmann, G.T. & Motta, P.J. 2005. Diet and morphology through ontogeny of the nonindigenous Mayan cichlid *Cichlasoma (Nandopsis) urophthalmus* (Günther 1862) in southern Florida. *Environmental Biology of Fishes* 72: 205–211.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* 24: 127–135.

Britton, J.R., Harper, D.M., Oyugi, D.O. & Grey, J. 2010. The introduced *Micropterus salmoides* in an equatorial lake: a paradoxical loser in an invasion meltdown scenario? *Biological Invasions* 12: 3439–3448.

Brooks, W.R. & Jordan, R.C. 2010. Enhanced interspecific territoriality and the invasion success of the spotted tilapia (*Tilapia mariae*) in South Florida. *Biological Invasions* 12: 865–874.

Bruno, J.F. & Cardinale, B.J. 2008. Cascading effects of predator richness. *Frontiers in Ecology and the Environment* 6: 539–546.

Bryan, S.D., Robinson, A.T. & Sweetser, M.G. 2002. Behavioral responses of a small native fish to multiple introduced predators. *Environmental Biology of Fishes* 63: 49–56.

Cope, N.J. & Winterbourn, M.J. 2004. Competitive interactions between two successful molluscan invaders of freshwaters: an experimental study. *Aquatic Ecology* 38: 83–91.

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T. & Sih, A. 2010. Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society B-Biological Sciences* 277: 1571–1579.

Cox, J.G. & Lima, S.L. 2006. Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. *Trends in Ecology and Evolution* 21: 674–680.

Crumrine, P.W. & Crowley, P.H. 2003. Partitioning components of risk reduction in a dragonfly-fish intraguild predation system. *Ecology* 84: 1588–1597.

Dunlop-Hayden, K.L. & Rehage, J.S. 2011. Antipredator behavior and cue recognition by multiple Everglades prey to a novel cichlid predator. *Behaviour* 148: 795–823.

Fauce, C.H. & Lorenz, J.J. 2000. Reproductive biology of the introduced Mayan cichlid, *Cichlasoma urophthalmus*, within an estuarine mangrove habitat of southern Florida. *Environmental Biology of Fishes* 58: 215–225.

Griffen, B.D. 2006. Detecting emergent effects of multiple predator species. *Oecologia* 148: 702–709.

Griffen, B.D. & Byers, J.E. 2006. Intraguild predation reduces redundancy of predator species in multiple predator assemblage. *Journal of Animal Ecology* 75: 959–966.

Griffen, B.D., Guy, T. & Buck, J.C. 2008. Inhibition between invasiveness: a newly introduced predator moderates the impacts of a previously established invasive predator. *Journal of Animal Ecology* 77: 32–40.

Grosholz, E.D. 2005. Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. *Proceedings of the National Academy of Sciences* 102: 1088–1091.

Harcourt, J.L., Biau, S., Johnstone, R. & Manica, A. 2010. Boldness and information use in three-spined sticklebacks. *Ethology* 116: 440–447.

Harvey, B.C., White, J.L. & Nakamoto, R.J. 2004. An emergent multiple predator effect may enhance biotic resistance in a stream fish assemblage. *Ecology* 85: 127–133.

Ioannou, C.C., Payne, M. & Krause, J. 2008. Ecological consequences of the bold-shy continuum: the effect of predator boldness on prey risk. *Oecologia* 157: 177–182.

Johnson, P.T.J., Olden, J.D., Solomon, C.T. & Vander Zanden, M.J. 2009. Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia* 159: 161–170.

Kery, M. & Hatfield, J.S. 2003. Normality of raw data in general linear models: the most widespread myth in statistics. *Bulletin of the Ecological Society of America* 84: 92–94.

Kobza, R.M., Trexler, J.C., Loftus, W.F. & Perry, S.A. 2004. Community structure of fishes inhabiting aquatic refuges in a

- threatened karst wetland and its implications for ecosystem management. *Biological Conservation* 116: 153–165.
- Langston, J.N., Schofield, P.J., Hill, J.E. & Loftus, W.F. 2010. Salinity tolerance of the African jewelfish *Hemichromis letourneuxi*, a non-native cichlid in South Florida USA. *Copeia* 3: 475–480.
- Lima, S.L. 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Advances in the Study of Behavior* 27: 215–290.
- Liston, S.L. & Trexler, J.C. 2005. Spatiotemporal patterns in community structure of macroinvertebrates inhabiting calcareous periphyton mats. *Journal of the North American Benthological Society* 24: 832–844.
- Loftus, W.F. 1987. Possible establishment of the Mayan cichlid, *Cichlasoma urophthalmus* (Günther) (Pisces: Cichlidae), in Everglades National Park, Florida. *Florida Scientist* 50: 1–6.
- Lohrer, A.M. & Whitlatch, R.B. 2002. Interactions among aliens: apparent replacement of one exotic species by another. *Ecology* 83: 719–732.
- Lorenz, J.J. & Serafy, J.E. 2006. Subtropical wetland fish assemblages and changing salinity regimes: implications for Everglades restoration. *Hydrobiologia* 569: 401–422.
- Martin, P. & Bateson, P. 2007. *Measuring behavior: an introductory guide*, 3rd edn. Cambridge: Cambridge University Press.
- Mills, M.D., Rader, R.B. & Belk, M.C. 2004. Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. *Oecologia* 141: 713–721.
- Nannini, M.A. & Belk, M.C. 2006. Anti-predator responses of two native stream fishes to an introduced predator: does similarity in morphology predict similarity in behavioural response? *Ecology of Freshwater Fish* 15: 453–463.
- O’Dowd, D.J., Green, P.T. & Lake, P.S. 2003. Invasional meltdown on an oceanic island. *Ecology Letters* 6: 812–817.
- Rehage, J.S. & Trexler, J.C. 2006. Assessing the net effect of anthropogenic disturbance on aquatic communities in wetlands: community structure relative to distance from canals. *Hydrobiologia* 569: 359–373.
- Relyea, R.A. 2003. How prey respond to combined predators: a review and an empirical test. *Ecology* 84: 1827–1839.
- Ricciardi, A. 2001. Facilitative interactions among aquatic invaders: is an invasional meltdown occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Sciences* 58: 2513–2525.
- Richardson, D.M., Allsopp, N., D’Antonio, C.M., Milton, S.J. & Rejma’nek, M. 2000. Plant invasions – the role of mutualisms. *Biological Reviews* 75: 65–93.
- Salo, P., Korpimäki, E., Banks, P.B., Nordstrom, M. & Dickman, C.R. 2007. Alien predators are more dangerous than native predators to prey populations. *Proceedings of the Royal Society Series B* 274: 1237–1243.
- Schaefer, J.F., Heulett, S.T. & Farrell, T.M. 1994. Interactions between two poeciliid fishes (*Gambusia holbrooki* and *Heterandria formosa*) and their prey in a Florida marsh. *Copeia* 1994: 516–520.
- Schmitz, O.J. 2007. Predator diversity and trophic interactions. *Ecology* 88: 2415–2426.
- Schofield, P.J., Loftus, W.F. & Brown, M. 2007. Hypoxia tolerance of two centrarchid sunfishes and an introduced cichlid from karstic Everglades wetlands of southern Florida, USA. *Journal of Fish Biology* 41: 87–99.
- Schofield, P.J., Loftus, W.F., Kobza, R.M., Cook, M.I. & Slone, D.H. 2010. Tolerance of nonindigenous cichlid fishes (*Cichlasoma urophthalmus*, *Hemichromis letourneuxi*) to low temperature: laboratory and field experiments. *Biological Invasions* 12: 2441–2457.
- Schurch, R. & Heg, D. 2010. Life history and behavioral type in the highly social cichlid *Neolamprologus pulcher*. *Behavioral Ecology* 21: 588–598.
- Shafland, P.L., Gestring, K.B. & Stanford, M.S. 2008. Florida’s exotic freshwater fishes – 2007. *Florida Scientist* 71: 219–245.
- Sih, A. & Christensen, B. 2001. Optimal diet theory, when does it work, and when and why does it fail. *Animal Behaviour* 61: 379–390.
- Sih, A., Englund, G. & Wooster, D. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution* 13: 350–355.
- Sih, A., Bolnick, D.I., Luttbeg, B., Orrock, J.L., Peacor, S.D., Pintor, L.M., Preisser, E., Rehage, J.S. & Vonesh, J.R. 2010. Predator-prey naiveté, anti-predator behavior, and the ecology of predator invasion. *Oikos* 119: 610–621.
- Sih, A., Ferrari, M.C.O. & Harris, D.J. 2011. Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications* 4: 367–387.
- Simberloff, D. 2006. Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecology Letters* 9: 912–919.
- Simberloff, D. & Von Holle, B. 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1: 21–32.
- Taylor, R.C., Trexler, J.C. & Loftus, W.F. 2001. Separating the effects of intra- and interspecific age-structured interactions in an experimental fish assemblage. *Oecologia* 127: 143–152.
- Trexler, J.C., Loftus, W.F., Jordan, F., Lorenz, J.J., Chick, J.H. & Kobza, R.M. 2000. Empirical assessment of fish introductions in a subtropical wetland: an evaluation of contrasting views. *Biological Invasions* 1: 21–32.
- Trexler, J.C., Loftus, W.F. & Perry, S. 2005. Disturbance frequency and community structure in a twenty-five year intervention study. *Oecologia* 145: 140–152.
- Vance-Chalcraft, H.D. & Soluk, D.A. 2005. Estimating the prevalence and strength of non-independent predator effects. *Oecologia* 146: 452–460.