

NOTE

Mangrove use by the invasive lionfish *Pterois volitans*

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ABSTRACT: Lionfish *Pterois volitans* are successful invasive predators in the sub-tropical and tropical Western Atlantic. Their invasion of coral reef ecosystems is a major conservation concern, as previous studies in the Western Atlantic have found that this top predator (1) occurs at higher densities and forages more successfully than in its native range, (2) lacks significant predators as an adult, and (3) reduces reef fish recruitment. The effects of lionfish on coral reef communities have been examined, but it is not known if these predators use critical non-reef habitat such as mangroves, which serve as nurseries for many reef fish species. To investigate lionfish usage of non-reef critical habitat, we compared population size-structure and stomach contents of *P. volitans* in mangroves and coral reefs on the island of San Salvador, Bahamas. In each habitat, >80% of lionfish stomachs contained prey items with similar gut content wet weight (0.98 ± 0.35 g in reef habitat and 0.99 ± 0.43 g in mangrove habitat) despite differences in total lionfish length (231.1 ± 10.30 mm in reef habitat and 176.3 ± 16.83 mm in mangrove habitat). Prey items detected by genetic analysis included individuals of the families Palaemonidae, Penaeidae, Gobidae, Scaridae, Mullidae and Grammatidae. These results demonstrate that lionfish colonize and feed in mangrove habitat in the Bahamas. Further study of lionfish outside reef habitat is required to understand the effects of this marine invasion.

KEY WORDS: Marine invasion · Nursery habitat · Recruitment · San Salvador · Stomach contents · Scorpaenidae · Genetic analysis

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INTRODUCTION

Two species of invasive Indopacific lionfish, *Pterois volitans* and *P. miles*, invaded Western Atlantic waters in the 1990s and have established populations ranging from North Carolina to the Caribbean (Whitfield et al. 2002, Hamner et al. 2007). *P. volitans* is now common in the Bahamas and appears to have rapidly expanding populations (Albins & Hixon 2008). Bahamian populations of *P. volitans* are estimated to be denser than in their native range (Green & Côté 2009), while *P. miles* has not been found in Bahamian waters (Freshwater et al. 2009). The lionfish invasion could have serious effects on native ecology due to a lack of known predators as an adult (Maljkovic et al. 2008), higher prey

consumption rates in the Atlantic than in their native range (Fishelson 1997), an ability to consume a wide range of trophic levels and reduce reef fish recruitment (Albins & Hixon 2008), and extensive dispersal capabilities (Fishelson 1975).

Most Bahamian lionfish research has focused on coral reef habitat; no information exists on colonization effects in mangrove habitat—a critical habitat that influences reef community structure and overall reef fish biomass (Nagelkerken et al. 2002, Mumby et al. 2004, Adams et al. 2006). Lionfish utilizing these habitats may affect recruitment to reef habitat by reducing the survival of reef fish in juvenile habitat through predation and/or competition, exacerbating known lionfish effects on reef fish recruitment.

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The aim of this study was to examine lionfish presence in Bahamian mangroves, comparing mangrove and reef lionfish through size-structure and stomach content analyses.

MATERIALS AND METHODS

Study site. Collection of *Pterois volitans* occurred within Graham's Harbor (24° 07' N 74° 27' W) and Pigeon Creek (28° 58' N 74° 29' W), San Salvador, Bahamas. Graham's Harbor is a protected, low energy area on the northeast end of San Salvador characterized by multiple fringing, patch, and barrier reefs, as well as artificial structure and seagrass beds. Sampling within the harbor occurred at multiple sites: Dump Reef (24° 07' 47" N, 74° 27' 35" W), Government Pier (24° 07' 12" N, 74° 28' 14" W), Golans Reef (24° 07' 47" N, 74° 27' 35" W), and an unnamed hardened structure site (24° 07' 11" N, 74° 27' 57" W). Pigeon Creek is a tidal mangrove creek in the southeast corner of San Salvador, characterized by dense mangroves (*Rhizophora mangle*, *Avicennia germinans*), seagrass beds, a pock-marked CaCO₃ substrate, and a central channel ~1 to 3 m deep. Pigeon Creek is a presumed nursery habitat for reef fish (Diehl et al. 1988).

Sampling procedure. We collected *Pterois volitans* using hand nets while snorkeling (Fishelson 1997). Sampling occurred before noon in Pigeon Creek, and throughout the day in Graham's Harbor. We measured total wet weight and total length, and removed prey items and remnants from the stomach and esophagus. We weighed contents wet and identified by microscope to the lowest possible taxonomic level.

Genetic analysis. DNA extraction of *Pterois volitans* and gut contents was conducted using the CTAB (cetyl trimethylammonium bromide) isolation protocol. PCR amplification of 2 mitochondrial genes 16S rRNA and cytochrome c oxidase subunit I (COI) was performed using the universal primers AR and BR for 16S rRNA, and HCOI and LCOI for COI, with the following PCR conditions: 1 mM deoxynucleoside triphosphates (dNTPs), 3 mM MgCl₂ and 10 μM primers. PCR products were cleaned using the QIA-GEN QIAquick PCR purification kit and sequenced using an ABI-PRISM electronic sequencer. Sequence products were edited using Geneius 4.6. and blasted using the GenBank database. For both *P. volitans* and gut contents, only 16S rRNA was positively amplified and considered for taxonomic identification.

Genetic data analysis. Summary statistics including nucleotide diversity (π), and the standard neutrality test, Tajima's D , were calculated using DNAsp v.5.0 both overall and by location. Population differen-

tiation among locations was estimated using the S_{nn} 'nearest neighbor' statistic of Hudson (2000), using DNAsp v.5.0 and by F_{ST} pairwise tests implemented in ARLEQUIN 3.11.

Statistical analysis. We calculated catch per unit effort (CPUE) as a measure of number of fish caught, per habitat type, divided by hours spent searching the habitat. To test if data conformed to assumptions of homogeneity of variances and normality, we used visual analysis of standardized residuals versus fitted values, F -tests, and Shapiro-Wilks tests. We compared mean total length, mean total weight, and mean stomach content weight using Welch's 2-sample t -tests. Statistical significance for all analyses was determined at $\alpha = 0.05$ and results are reported ± 1 SE.

RESULTS

Mean CPUE (fish caught h⁻¹) was 2.6 times higher in Pigeon Creek (mangrove habitat) (0.67 ± 0.33 vs. 0.26 ± 0.088). We found mean total length to be greater in Graham's Harbor (reef habitat) (231.1 ± 10.30 vs. 176.3 ± 16.83 mm) ($t = 2.78$, $p = 0.0126$).

Diet analysis was performed on 22 specimens of *Pterois volitans*: 10 from Graham's Harbor and 12 from Pigeon Creek. We found contents in 80% of Graham's Harbor and 83% of Pigeon Creek lionfish stomachs. Mean wet weight of diet contents from Graham's Harbor was 0.98 ± 0.35 g compared to 0.99 ± 0.43 g from Pigeon Creek. Of the 22 stomachs examined, 13 contained identifiable food items, including whole fish, fish parts and whole shrimp. Six families and three genera were positively identified as prey items using 16S rRNA mitochondrial DNA: Palaemonidae and Scaridae (*Scarus*)* in Graham's Harbor, and Penaeidae (3 ind.), Gobidae, Mullidae (*Pseudopeneus*), and Grammatidae (*Gramma*)* in Pigeon Creek. Samples confirmed with 99% similarity at the genus level denoted by (*).

A 445 bp section of the 16S rRNA mitochondrial gene of lionfish tissue was analyzed in 23 samples: 11 from Graham's Harbor and 12 from Pigeon Creek. Genetic analysis revealed that all lionfish 16S rRNA sequences belong to the species *Pterois volitans*. Nucleotide diversity registered a value of $\pi = 0.022$ at Graham's Harbor and $\pi = 0.024$ at Pigeon Creek, while Tajima's neutrality test showed values of $D_T = -0.61$ and -1.48 for Graham's Harbor and Pigeon Creek, respectively. The genetic differentiation parameters S_{nn} (Hudson 2000) and F_{ST} pairwise revealed a weak structure among the studied locations for *P. volitans* and suggest the existence of a single population on the island of San Salvador, Bahamas ($S_{nn} = 0.41$, not significant; $F_{ST} = -0.051$).

DISCUSSION

This study presents the first evidence that lionfish use mangrove habitat. Our CPUE results qualitatively suggest (Pine et al. 2003) a higher lionfish density in Pigeon Creek (mangrove habitat) than in Graham's Harbor (reef habitat). Despite a smaller average *Pterois volitans* size in Pigeon Creek, fish from both habitats had similar gut content weight. Prey item diversity (number of families) was also higher in Pigeon Creek. Moreover, genetic analysis supports previous observations that *P. volitans* is the only species of lionfish present in the Bahamas (Freshwater et al. 2009). Genetic data suggest the existence of a single population of *P. volitans* on San Salvador, which is consistent with the strong connectivity and reduced structure observed in previous studies (Freshwater et al. 2009).

Lionfish colonization of Pigeon Creek occurred rapidly, as our group has studied Pigeon Creek for the past 6 yr, but had not seen lionfish in this creek until the present study (B. R. Silliman pers. obs.). We caution extrapolation of these results due to pseudoreplication of habitat types and the small sample size collected from study sites. Also, our exploration of Pigeon Creek was spatially restricted to the lower reach, approximately 1.0 km from the mouth, an area that may be used by non-resident species to utilize food resources (Layman & Silliman 2002). Although we explored only one mangrove system in the Bahamas, conversations with scientists in the Bahamas suggest lionfish colonization of mangrove habitat is occurring and imminent (P. Mumby, J. Bruno, L. Akins pers. comm.).

Additionally, we frequently observed juvenile Nassau grouper *Epinephelus striatus* residing near similarly sized lionfish in Pigeon Creek, suggesting direct competition for resting sites and potential competition for food resources near resting sites. Predation upon and competition with juvenile fish is likely to decrease recruitment to the adult population through an increase in juvenile mortality (Houde 1987, Connell & Jones 1991). As lionfish have been shown to drastically reduce reef fish recruitment (Albins & Hixon 2008), it is highly likely that their presence in mangrove habitat poses an immediate threat to economically and ecologically important fish families (e.g. Scaridae, Serranidae) utilizing mangrove systems as nursery habitat (Mumby et al. 2004, 2007, Koenig et al. 2007).

Molecular methods confirmed that lionfish consume over a broad trophic range, including (1) invertivores, (2) herbivores, (3) invertebrates, and (4) planktivores. This is of concern as invasions are especially destructive when the invasive species lacks predators and is polyphagous (Pimm 1987). Another concern is the potential for lionfish prey consumption to generate

powerful trophic cascades (Silliman & Bertness 2002). For example, lionfish could decrease herbivorous reef fish populations vital for controlling macroalgal growth. Without these controls, macroalgal proliferation can inhibit coral reef recruitment, growth, and recovery after physical disturbance (Mumby et al. 2006, Mumby et al. 2007). Albins & Hixon (2008) demonstrated that lionfish presence on experimental patch reefs reduced overall reef fish recruitment by 79%, including herbivorous parrotfishes. This novel consumption of native herbivorous reef species by an invasive may enhance macroalgal production on coral reefs, contributing to widespread phase shifts on Caribbean coral reefs (Hughes 1994, McCook 1999, Bellwood et al. 2004).

It may be prudent to expand current management actions to include the targeting of lionfish in mangrove habitat. Attention to lionfish is primarily devoted to areas of high human traffic (e.g. coral reefs); therefore, less trafficked areas should be explored for their presence, especially since there is potential for rapid colonization. Additional work needs to be conducted to determine if lionfish presence in mangroves decreases survival of native juveniles, subsequently affecting adult communities. Since lionfish occurred in Pigeon Creek (mangrove habitat) at a smaller average size, and mangrove habitat acts as a nursery for various reef fishes, we think it prudent to suggest an ontological investigation of lionfish habitat usage.

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