

## ORIGINAL ARTICLE

# Habitat use patterns of the invasive red lionfish *Pterois volitans*: a comparison between mangrove and reef systems in San Salvador, Bahamas

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## Keywords

Apex predator; feeding habits; isotopic niche; nursery habitat; predator–prey interactions.

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## Abstract

The Indo-Pacific red lionfish *Pterois volitans* is widespread both in its native and its non-native habitats. The rapid invasion of this top predator has had a marked negative effect on fish populations in the Western Atlantic and the Caribbean. It is now well documented that lionfish are invading many tropical and sub-tropical habitats. However, there are fewer data available on the change in lionfish abundance over time and the variation of body size and diet across habitats. A recent study in San Salvador, Bahamas, found body size differences between individuals from mangrove and reef systems. That study further suggested that ontogenetic investigation of habitat use patterns could help clarify whether lionfish are using the mangrove areas of San Salvador as nurseries. The aim of the present study is to determine temporal trends in lionfish relative abundance in mangrove and reef systems in San Salvador, and to further assess whether there is evidence suggesting an ontogenetic shift from mangroves to reef areas. Accordingly, we collected lionfish from mangrove and reef habitats and calculated catch per unit effort (a proxy for relative abundance), compared body size distributions across these two systems, and employed a combination of stable isotope, stomach content, and genetic analyses of prey, to evaluate differences in lionfish trophic interactions and habitat use patterns. Our results show that populations may have increased in San Salvador during the last 4 years, and that there is a strong similarity in body size between habitats, stark differences in prey items, and no apparent overlap in the use of habitat and/or food resources. These results suggest that there is not evidence for an ontogenetic shift from mangroves to reefs, and support other studies that propose lionfish are opportunistic foragers with little movement across habitats.

## Introduction

For hundreds of years, invasive plants, animals and pathogens have been changing both marine and terrestrial ecosystems, severely decreasing biodiversity (Meyerson & Mooney 2007; Ruiz *et al.* 2011). Over the last two decades, the Indo-Pacific lionfish *Pterois volitans* and *Pterois miles* have rapidly and successfully invaded the Western Atlantic from the northeast coast of the

United States to the Caribbean coasts of Colombia and Venezuela (Schofield 2010). The fast spread of these species has negatively affected Atlantic coral-reef fish populations via consumption and a resulting reduction in fish recruitment (Albins & Hixon 2008). This problem is exacerbated not only by lack of strong predatory pressures on lionfish, but also by the overfishing of top predators in the Caribbean (*e.g.* Jackson *et al.* 2001; Mumby *et al.* 2012).

The Indo-Pacific lionfish are successful apex-predators. The success of these species may be due to their physiological, morphological and behavioral characteristics (Albins & Lyons 2012). For instance, they possess defensive venomous dorsal, pelvic and anal fin spines, which can be fatal to other fish (Allen & Eschmeyer 1973). In addition, their slow movement, camouflaged coloration, and elongated fin ray projections result in low detectability by predators (Albins & Hixon 2008). As a consequence of these features, lionfish may be free from significant top-down control, with only a few occasional predators (Bernadsky & Goulet 1991; Pimiento *et al.* in press).

It has been proposed that lionfish preferentially (but not exclusively) settle in shallow habitats before moving to deep reefs when they reach larger sizes (Claydon *et al.* 2012). This pattern is oftentimes a consequence of fish ontogeny (*e.g.* Eggleston 1995; Green 1996; Mumby *et al.* 2004, 2007; Frias-Torres 2006; Koenig *et al.* 2007; Wilson *et al.* 2010). Hence, the shallow area where juvenile fish species occur at higher densities, seek refuge from predation, and potentially grow faster, is referred as nursery habitat (Beck *et al.* 2001).

Lionfish are common throughout the Western Atlantic and the Caribbean. Genetic studies have demonstrated that the red lionfish *P. volitans* is the only introduced species currently found in the Bahamas (Freshwater *et al.* 2009; Barbour *et al.* 2010). Little is known about how lionfish abundance changes over time and how they use various habitats. However, some studies have suggested that they appear to have rapidly increasing populations (Albins & Hixon 2008), particularly surrounding the island of New Providence (Green & Côté 2008; Côté & Maljković 2010; Green *et al.* 2012).

Around the island of San Salvador, *P. volitans* is commonly observed on rocky reefs and artificial structures. A recent study by Barbour *et al.* (2010) found that this species use the mangrove creeks of San Salvador in addition to reefs. This research further suggests that lionfish forage within mangrove ecosystems potentially threatening fish families (*e.g.* Scaridae, Serranidae) that utilize mangrove systems as nursery areas (Mumby *et al.* 2004, 2007; Koenig *et al.* 2007). Barbour *et al.* (2010) also found that lionfish occurring in mangrove habitats are smaller relative to the individuals captured from reef habitats.

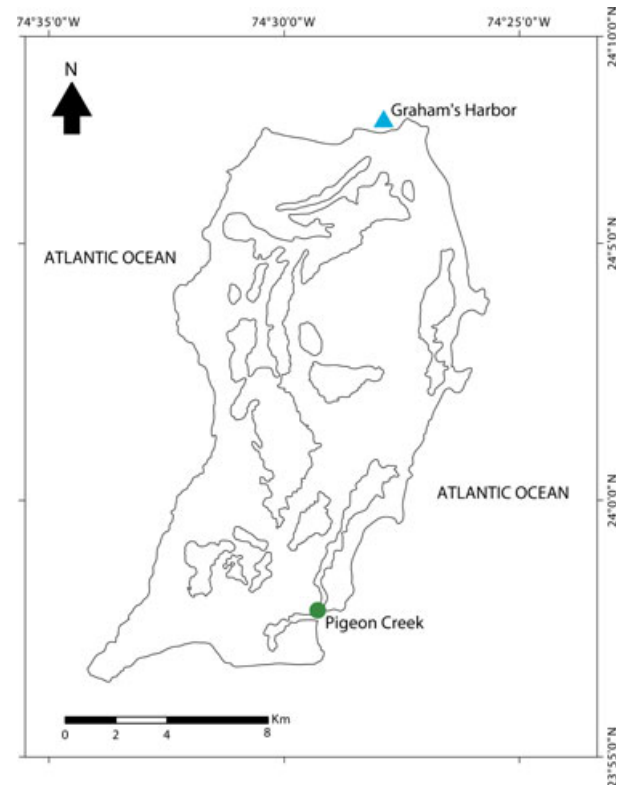
This finding, which highlights the lack of quantitative research on body size and diet of lionfish across habitats, drove us to ask whether lionfish use mangroves as nursery habitats. To begin to test this idea, we aim to describe trends in lionfish relative abundance, trophic interactions, and habitat use patterns in mangrove and reef ecosystems in San Salvador, Bahamas. Accordingly, the body sizes of lionfish captured in the different habitats were compared. In addition, we employed stable isotope analysis (SIA)

and stomach content analysis (SCA), using morphological and genetic identification of prey items. Knowing the population size trends and critical habitats for lionfish maturation could help focus efforts for management of the Western Atlantic invasion process.

## Material and Methods

### Study area

We collected *Pterois volitans* from a mangrove and a reef habitat in San Salvador, Bahamas. Pigeon Creek (23°58' N, 74°29' W) was selected as the mangrove study area since this system is known to serve as a nursery habitat for reef fish (Diehl *et al.* 1988). The tidal mangrove creek was located in Southeast San Salvador (Fig. 1), and has dense red and black mangroves, seagrass beds, and a central channel no deeper than 3 m. Graham's Harbor (24°07' N, 74°27' W) on Northeast San Salvador (Fig. 1) was selected as the reef habitat for lionfish collection. This reef ecosystem is a shallow-water (<5 m depth), protected, low-energy environment with a variety of artificial structures, seagrass beds and small patch reefs. Within



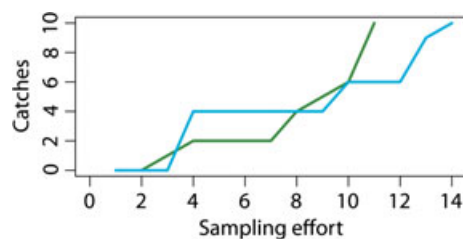
**Fig. 1.** Map of San Salvador, Bahamas, and the location of sampling sites. Green circle represents the mangrove habitat. Blue triangle represents the reef habitat.

the harbor, we took samples in Dump Reef and an unnamed hardened structure site. The distance between the mangrove and the reef habitat is ~18 km.

### Sampling methods

We collected *Pterois volitans* using a spear gun while snorkeling during daytime. Over the course of 4 days, 20 lionfish were captured across two habitat types. The same collection method was used in the two systems. Our sampling unit was person-hours (*i.e.* hours spent searching, multiplied by the number of people searching). We searched for a total of 25 person-hours (Table 1). In the mangrove habitat, we collected for 2 days. We actively explored transects along mangrove roots (edges) and the deep channel. We searched for 11 person-hours and collected 10 individuals. The first day we searched for six person-hours, whereas the second day we searched for five person-hours. In the reef habitat, we collected for 4 days. We randomly explored rocky piles, patch reefs, and any available structure in the nearby vicinity. We searched for 14 person-hours and collected 10 individuals. The 1st day, we searched for six person-hours, on the 2nd day for four person-hours, and on the 3rd and 4th days for two person-hours each (Table 1).

Two accumulation curves (Fig. 2) were produced to analyse the sampling effort (person-hours) in relation to the number of individuals collected in each habitat. These curves were created using the *vegan* package (Oksanen *et al.* 2010) of the R program (R Development Core Team 2012). We used the method 'collector', which adds sites in the order they occur in the data (Oksanen *et al.* 2010). The accumulation (collector) curves for both sites



**Fig. 2.** Accumulation curves showing the sampling effort (person hours) in relation with the number of individuals collected in each habitat. Green line represents the mangrove habitat. Blue line represents the reef habitat.

are very similar, indicating that sampling effort was comparable; however, we reached the total number of individuals slightly faster in the mangrove than in the reef system.

### Catch per unit effort and body size comparisons

We calculated the catch per unit effort (CPUE), a proxy for relative abundance, by dividing the number of fish caught by person-hours. We compared the CPUE results from mangrove and reef systems. Although there are more effective methods to assess population growth, we used CPUE because individuals needed to be removed in order to study their habitat use patterns. Accordingly, we measured and compared total length (TL) and mass (M) of the captured individuals from the two systems, and plotted the correlation between these two variables. Finally, we compared our results with Barbour *et al.* (2010). Data comparisons were made using Welch's two-sample *t*-tests ( $\alpha = 0.05$ ). Our analyses were made using

**Table 1.** Lionfish collection in mangrove and reef habitats in San Salvador, Bahamas.

Habitat	Date	Site/Transect	Person-hours	Catches	Sample
Reef	5 March 2012	Graham's Harbor	4	4	R01-R04
		Dump Reef	2	0	
	6 March 2012	Dump Reef	2	0	R05-R06
		Unnamed structure	2	2	
Mangrove	7 March 2012	Graham's Harbor	2	3	R07-R08
	9 March 2012	Graham's Harbor	2	1	R09-R10
	6 March 2012	Edge 1	1	0	M01
		Edge 2	1	0	
		Edge 3	1	1	
		Channel 1	1	1	
7 March 2012	Channel 2	1	0	M02	
	Edge 4	1	0	M03-M04	
	Edge 1	1	0		
	Edge 2	1	2		
Edge 3	1	1			
Total		Channel 1	1	1	M06
		Channel 3	1	4	M07-M10
Total			25	20	20

the statistics software R (R Development Core Team 2012).

### Stable isotope analysis

Stable isotope analysis of carbon- $\delta^{13}\text{C}$  and nitrogen- $\delta^{15}\text{N}$  is commonly used in ecological studies for a wide range of purposes ranging from the determination of resource use patterns and estimating trophic position, to calculating community-wide metrics of trophic structure (for review see Layman *et al.* 2012).  $\delta^{13}\text{C}$  is often used to determine the origin of resources, as it most often closely reflects the isotopic composition of resources used.  $\delta^{15}\text{N}$  is often used to indicate relative trophic position (Post 2002). When combined with additional information such as stomach content data,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can be a powerful tool to compare trophic interactions and food web structure. In this study, we use SIA to assess differences in the trophic interactions of lionfish captured in two habitats by assessing the location and width of their isotopic niches.

### Sample treatment

Approximately 1 cm<sup>3</sup> of dorsal muscle tissue from each lionfish ( $n = 20$ ) was preserved in ethanol, washed with deionized water, dried at 60 °C for 48 h, and ground to a fine powder using mortar and pestle. Ground samples were packed into 95-mm tin capsules for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis. Analyses were performed using a Finnigan-MAT DeltaPlus XL mass spectrometer at the Geology Stable Isotope Laboratory, University of Florida, Gainesville, Florida, USA. Stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) are expressed in standard per mil notation (‰), where  $\delta\text{‰} = [R_{\text{sample}}/R_{\text{standard}} - 1] \times 1000$ , relative to Vienna Pee Dee Belemnite for  $\delta^{13}\text{C}$  and Atmospheric Nitrogen-AIR for  $\delta^{15}\text{N}$ . Mean standard deviations of laboratory standard USGS-40 (L-glutamic acid) from five in-run samples were 0.12‰ for  $\delta^{15}\text{N}$  and 0.05‰ for  $\delta^{13}\text{C}$ . Following recommendations by Post *et al.* (2007) we did not correct  $\delta^{13}\text{C}$  for lipid content since the C:N ration of tissues was found to average <3.5 across all samples (mean  $\pm$  SD,  $3.16 \pm 0.06$ ,  $n = 20$ ). Although ethanol, among other preservation agents, has been shown to influence stable isotope composition over time (Kaehler & Pakhomov 2001), in this study all samples were preserved in the same manner and any influence of preservation on isotopic composition would have affected all samples equally.

### Niche location and width

We compared the lionfish isotopic niche location and measures of central tendency using methods from Turner *et al.* (2010). We used nested linear models and residual permutation procedures (RPP) to generate null models

which were used to make comparisons between the mean centroid location and three measures of central tendency (mean distance to centroid, MDC; mean nearest neighbor, MNN; and eccentricity, E) for lionfish captured in mangrove and reef habitats. MDC and MNN are commonly used metrics to describe isotopic data (Layman *et al.* 2007). E, a metric described by Turner *et al.* (2010), is a measure which describes the shape of scatter surrounding the centroid location in isotopic space. A value of  $E = 0$  describes a perfectly circular scatter and a value of  $E = 1$  describes a perfectly linear relationship where the direction of the line is defined by loadings from principal component analysis (see Turner *et al.* 2010 for detailed description and formulation). The mean centroids of groups being compared occupy different locations if the Euclidean distance between them is significantly greater than zero (Turner *et al.* 2010). Significant differences in centroid location indicate fundamental differences in the trophic interactions experienced by groups of interest. Additionally, we performed a parametric test (Hotelling's  $T^2$ ) to compare results from the RPP test for difference in mean centroid location. Script for running these statistical tests in R and additional information can be found in Turner *et al.* (2010).

To compare isotopic niche widths of lionfish groups we used a Bayesian framework to establish multivariate ellipse-based metrics following methods of Jackson *et al.* (2011). These analyses generate standard ellipse areas ( $\text{SEA}_B$ ), which are the bivariate equivalents of the standard deviation in univariate space.  $\text{SEA}_B$  is a robust metric of isotopic niche space, since it represents the core area occupied by a group, can be estimated for small sample sizes, and is not heavily influenced by outliers (Jackson *et al.* 2011). We calculated and compared  $\text{SEA}_B$  and convex hull areas (total area, TA, for lionfish groups; Layman *et al.* 2007). TA is measured as the space occupied by a convex hull polygon encompassing all individuals of a group within a  $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$  bi-plot. TA provides an estimate of the isotopic niche space occupied by a group, although it is strongly influenced by outliers, since all samples are taken into account when calculating TA (Layman *et al.* 2007).  $\text{SEA}_B$  and TA were calculated following methods of Jackson *et al.* (2011) and the R package SIAR (Parnell *et al.* 2010).

### Stomach content analysis

We removed prey items from the stomach, weighed wet contents and identified the contents to the lowest possible taxonomic level. All relatively intact (minimally digested) prey items were thoroughly washed and carefully separated into clean containers for the DNA analysis. To limit DNA contamination from the lionfish and other prey items, an internal section of tissue was cored from the

prey item when possible. Tissue samples were stored in 95% EtOH. Genomic DNA was isolated using DNeasy Blood and Tissue kits (Qiagen, Valencia, CA, USA). Cytochrome oxidase 1 primers LCO1490 (5'-GGTCAA-CAAATCATAAAGATATTGG-3') and HCO2198 (5'-TA-AACTTCAGGGTGACCAAAAAATCA-3'; Folmer *et al.* 1994) were used to generate the DNA barcode for species identification. The PCR reaction conditions were as follows: 10 ng DNA, 1 × PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.001% gelatin; Sigma-Aldrich, Inc., St. Louis, MO, USA), 0.8 mM dNTP, 3 mM MgCl<sub>2</sub>, 0.24 μM of each primer, 0.04 units of Sigma Jump Start *Taq* DNA polymerase. PCR cycling profile: 5 min at 94 °C; then 35 cycles of 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C, and 10 min at 72 °C. PCRs included both positive and negative controls to check for reaction success and contamination, respectively. PCR products were examined under ultraviolet light following electrophoresis on an ethidium bromide-stained 1.0% agarose gel. Amplified products were purified with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). DNA sequencing was accomplished in the DNA Sequencing Core at the University of Florida, Gainesville, Florida, USA, with the BigDye terminator protocol developed by Applied Biosystems (Foster City, CA, USA) using fluorescently labeled dideoxynucleotides. Sequences were trimmed, checked for quality scores, and aligned in GENEIOUS 5.3.5 (Drummond *et al.* 2011). CO1 fragments were sequenced in the 5'-3' heavy-strand orientation. Basic local alignment (BLAST) analysis was completed using all barcode records in the Barcode of Life Database (BOLD). If no records were found in BOLD, GenBank (<http://www.ncbi.nlm.nih.gov>) was searched. Prey items were identified at the species level if query results displayed sequence similarity values >97% (Lowenstein *et al.* 2009; Braley *et al.* 2010), otherwise, identification was made at the family or order level. Samples with low quality scores or low match likelihood were removed from the study.

## Results

### Relative abundance

Mean CPUE was 3.4 times higher in the mangrove (0.97 ± 0.63) than in the reef system (0.29 ± 0.25). This result is consistent both with the species accumulation curves, which show that lionfish are slightly easier to catch in the mangrove systems (Fig. 2), and with the previous study in the area. Barbour *et al.* (2010) collected 12 lionfish individuals during 63 person-hours from the mangrove system, and 11 individuals during 18 person-hours from the reef system in March 2009. They reported that mean CPUE was 2.6 higher in the mangrove than in the

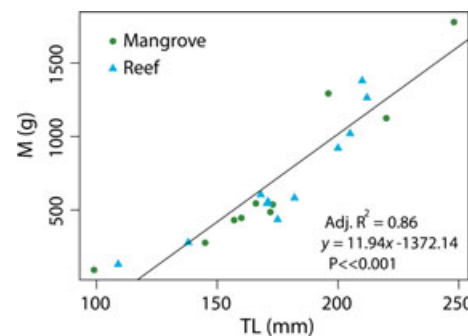
reef habitat (0.67 ± 0.33 *versus* 0.26 ± 0.08). Mean CPUE values in both systems were higher in this study than in the previous study (1.44 and 1.10 times higher in mangrove and reef systems, respectively). Barbour *et al.* (2010) spent 49 additional person-hours and found only three more individuals (23 lionfish and 74 person-hours in the previous study *versus* 20 lionfish and 25 person-hours in this study). This suggests that the relative abundance of the lionfish population may have increased in San Salvador in the last 3 years. However, the mean CPUE differences between studies are not significantly different (CPUE; mangrove:  $t = 1.00$ ,  $P = 0.50$  reef:  $t = 1.59$ ,  $P = 0.17$ ) and should be interpreted with caution.

### Body size

In terms of fish size, we found that lionfish from both mangrove and reef habitats captured in this study were smaller than those reported by Barbour *et al.* (2010). On average, body size differences were significant in the reef but not the mangrove habitat (TL; mangrove -2.73 mm;  $t = 0.13$ ,  $P = 0.89$ ; reef -54.1 mm;  $t = 2.47$ ,  $P = 0.02$ ). Furthermore, both the mean total length (TL; mangrove 173.60 mm; reef 177.00 mm;  $t = 0.20$ ,  $P = 0.84$ ) and the mean mass (M; mangrove 701.09 g; reef 716.30 g;  $t = 0.07$ ,  $P = 0.94$ ) were very similar in the two systems. This contrasts with Barbour *et al.* (2010), who found smaller individuals in the mangrove than reef habitats (mean TL: 176.30 *versus* 231.1 mm;  $t = 2.78$ ,  $P = 0.01$ ). In addition, TL and M from both systems in this study present exactly the same linear correlation ( $R^2 = 0.86$ ; Fig. 3), suggesting that there is no difference in size between lionfish inhabiting mangrove and those inhabiting reef systems.

### Stable isotope analysis

Both mean  $\delta^{13}\text{C}$  values ± SD (mangrove -12.72 ± 0.42‰; reef -14.93 ± 0.81‰;  $t = 7.67$ ,  $P < 0.001$ ) and  $\delta^{15}\text{N}$

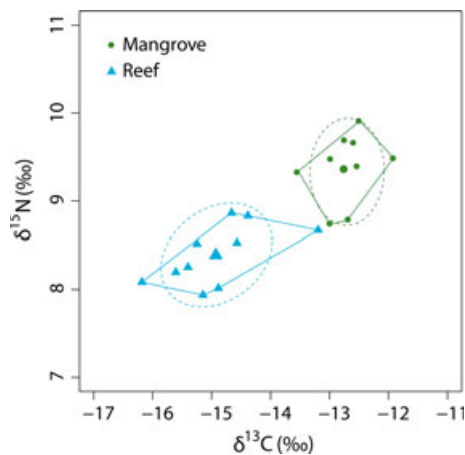


**Fig. 3.** Body size (TL: total length, M: mass) linear correlation for lionfish captured in mangrove and reef systems in San Salvador, Bahamas.

values  $\pm$  SD (mangrove  $9.38 \pm 0.37\text{‰}$ ; reef:  $8.39 \pm 0.34\text{‰}$ ;  $t = 6.24$ ,  $P \ll 0.001$ ) were found to be significantly different between lionfish from the two habitats. With regard to niche location, through a random permutation procedure (RPP) with 1000 iterations and Hotteling's  $T^2$  test, we found the Euclidian distance between mean centroid location of lionfish groups to differ significantly from zero (distance = 2.42,  $P = 0.001$ ; Hotteling's  $T^2 = 67.67$ ,  $P \ll 0.001$ ). Of the three measures of central tendency assessed (MDC, MNN and E), only absolute differences in E were found to differ significantly from zero when comparing lionfish groups (difference = 0.41,  $P = 0.027$ ). Lionfish captured within mangrove habitats ( $E = 0.91$ ) were less independently scattered around the mean centroid location in isotopic space, compared with lionfish captured in reef habitats ( $E = 0.48$ ). In terms of niche width, mean estimates of standard ellipse area ( $SEA_B$ ; mangrove mean 1.10, median 1.03, 95% Bayesian credible intervals, CI, 0.51–1.8; reef mean 1.52, median 1.41, CI 0.75–2.52;) as well as total area (TA; mangrove 0.99; reef 1.27) show no overlap in isotopic space of lionfish groups (Fig. 4). Furthermore, these estimates and measurements show that reef lionfish have a greater niche width than that of mangrove lionfish (TA ~28% greater for reef lionfish). Results were interpreted cautiously due to the small sample sizes used.

#### Stomach content analysis

Of the 20 fish sampled for SCA, six had empty stomachs. Three of these six empty-stomach lionfish were collected on the same day (7 March 2012). Four (40%) empty-



**Fig. 4.**  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot of lionfish captured in mangrove and reef systems in San Salvador, Bahamas. Small circles and triangles are individual isotopic values. Larger circle and triangle are the calculated mean centroid. Dashed lines are the standard ellipse area ( $SEA_B$ ) representation of the isotopic niches, estimated using Bayesian methods. Solid lines are the convex hull polygon [total area (TA)] representation of the isotopic niche. Note different scales on x- and y-axis.

stomach lionfish (samples R01, R07–R10; Table 1) were found in the reef and only two (20%) in the mangrove habitat (samples M07, M10; Table 1). This differs from Barbour *et al.* (2010), who found only 20% and 17% of empty stomachs in the reef and mangrove habitats, respectively. All empty-stomach lionfish from the reef habitat of this study were collected from the same site, Graham's Harbor (Table 1). Similarly, all empty-stomach lionfish from the mangrove habitat were found in the same transect, channel 3 (Table 1).

The stomach contents of 14 individuals contained a total of 36 prey items (Table 2). Of these, 24 individual prey items could be sampled and genetically identified. The number of individual prey items ranged per fish from zero to nine, with a mean of 1.8. Eight fish had more than two prey items. Genetic analysis was able to resolve high quality sequences for seven prey items to species level (Table 3). Prey items were from two main categories, Perciformes fish ( $n = 19$ ) and crustaceans ( $n = 5$ ). The Mullidae family (goatfishes) was the most common in the guts of lionfish from the reef habitat, whereas fish from the mangrove had a mixture of prey taxa, with a majority of Gobiidae (gobies) and Labrisomidae (labrisomids; Table 2). No prey taxa co-occurred in stomach contents of fish collected from both reef and mangrove habitats.

The spotted goatfish (*Pseudupeneus maculatus*) was the most frequently encountered prey ( $n = 7$ ), and was only found in reef lionfish. Four previously unreported dietary items for lionfish were identified (see a in Table 3), three of them in the reef habitat. These include *Eurythenes gryllus* (amphipod), *Pachygrapsus transverses* (grapsid crab), *Sparisoma chrysopterum* (parrotfish) and a specimen from the Majidae (crab) family. Aside from these, the prey taxa were consistent with what has been previously identified in lionfish stomach contents (*e.g.* Morris & Akins 2009; Morris & Whitfield 2009; Muñoz *et al.* 2011; Valdez-Moreno *et al.* 2012). Previously, *Coryphopterus hyalinus* and *Grama loreto* have been reported as the most frequent prey in the Bahamas (Morris & Whitfield 2009). Although *Coryphopterus tortugae* was found five times in this study, we did not find *C. hyalinus*. Also interesting to note is that *E. gryllus*, with 99% sequence similarity, which is described as a scavenger amphipod typically confined to deep waters (Blankenship & Yayanos 2005; Escobar-Briones *et al.* 2010), was collected in the shallow depths of the reef habitat.

#### Discussion

Populations of the invasive lionfish have been dispersing rapidly in the Western Atlantic over the last 20 years (*e.g.* Schofield 2010; Claydon *et al.* 2012; Green *et al.* 2012).

**Table 2.** Prey families per fish sampled in mangrove and reef systems in San Salvador, Bahamas.

Habitat	Lionfish		Stomach		Reef				Mangrove				Total			
					Fish		Crustaceans		Fish		Crustaceans					
					Mul	Sca	Gra	Maj	Gob	Lab	Labr	Ste		Lys	Dec	
	Sample	TL	M	No. of prey items	M											
Reef	R01	182	582	0												0
	R02	210	1380	4	10.3	4										4
	R03	212	1264	2	3.5	1										1
	R04	109	130	2	0.8			1	1							2
	R05	138	278	1	0.1											0
	R06	168	604	2	2.1	2										2
	R07	205	1020	1	2.3		1									1
	R08	175	435	0												0
	R09	200	921	0												0
	R10	171	549	0												0
Mangrove	M01	145	277	1	1.0							1				1
	M02	172	486	5	2.8					5						5
	M03	220	1125	4	2.3								1	1		2
	M04	248	1779	1	1.8								1			1
	M05	166	545	1	2.0											0
	M06	196	1293	9	7.1					1	2					3
	M07	157	431	0												0
	M08	160	446	1	1.8						1					1
	M09	173	537	2	1.1							1				1
	M10	99	92	0												0
Total				36	39	7	1	1	1	6	3	2	1	1	1	24

TL = total length (mm); M = mass (g). Family abbreviations are as follows: Mul = Mullidae; Sca = Scaridae; Gra = Grapsidae; Maj = Majidae; Gob = Gobiidae; Lab = Labrisomidae; Labr = Labridae; Ste = Stenopodidae; Lys = Lysianassoidea; Dec = Decapoda (order level).

**Table 3.** List of prey taxa found in lionfish captured in mangrove and reef systems in San Salvador, Bahamas.

Prey	Mangrove	Reef
Amphipod ( <i>Eurythenes gryllus</i> ) <sup>a</sup>	1	
Banded cleaner shrimp ( <i>Stenopus hispidus</i> )	1	
Blenny ( <i>Malacoctenus</i> )	3	
Crab (Majidae indet.) <sup>a</sup>		1
Decapod (Decapoda indet.)	1	
Goby ( <i>Coryphopterus</i> )	1	
Mottled Shore Crab ( <i>Pachygrapsus transversus</i> ) <sup>a</sup>		1
Redtailed parrotfish ( <i>Sparisoma chrysopterus</i> ) <sup>a</sup>		1
Sand goby ( <i>Coryphopterus tortugae</i> )	5	
Slippery dick ( <i>Halichoeres bivittatus</i> )	2	
Spotted goatfish ( <i>Pseudupeneus maculatus</i> )		7
Total	14	10

<sup>a</sup>New taxa.

This has been referred to as the fastest invasion documented for marine fish (Morris & Whitfield 2009). It is therefore not surprising that our data suggest that the relative abundance of lionfish in San Salvador has also

increased from 2009 until the present. However, given the lack of statistical significance, this observed pattern could also be the result of a population fluctuation rather than a persistent directional trend. Similarly, it could be due to sampling variability. For instance, catchability could be lower in the reef habitat because it provides more structure for lionfish to hide relative to mangroves. Nevertheless, in comparing the relative abundance of lionfish over time in the same area, this study provides an indication of a possible increase in abundance in multiple habitats.

Lionfish population growth in the Caribbean is alarming given that this species has negative effects on Atlantic coral-reef fish populations via consumption (Albins & Hixon 2008). In addition, lionfish lack strong predatory pressures, as potential predators are overfished (e.g. Jackson *et al.* 2001; Mumby *et al.* 2012; for discussion see Hackerott *et al.* 2013). Urgent action is therefore needed (e.g. Barbour *et al.* 2011) to slow the continued growth of this invasive species, which likely affects both mangrove and reef systems. To understand the community- and ecosystem-level impacts of lionfish in the Western

Atlantic, it is also important to know what they are consuming. In recent years, the diet of *Pterois volitans* has been the subject of several studies (e.g. Morris & Akins 2009; Morris & Whitfield 2009; Muñoz *et al.* 2011; Valdez-Moreno *et al.* 2012). Although our sampling size for the SCA was small (*i.e.* from the 20 fish collected, 14 had stomach contents), this study found four new prey taxa (*Eurythenes gryllus*, *Pachygrapsus transverses*, *Sparisoma chrysopteron* and an unidentified Majidae). Hence, it provides important information on the feeding ecology of this species that should help to predict some of the community-wide effects of this species in invaded habitats.

We found that lionfish from this study were smaller than those captured in a previous study (Barbour *et al.* 2010). However, the sampling effort was different between the two studies. Additional and systematic work is therefore needed to determine whether this trend continues in the future years and whether this is due to increased recruitment or to less fishing pressure. Our results also suggest that lionfish are not smaller in mangrove relative to reef systems, as would be expected if mangroves served as a nursery for this fish. Based on differences in lionfish TL, the previous study by Barbour *et al.* (2010) recommended further studies on the ontogenetic trends in lionfish to assess whether they are using the mangrove areas of San Salvador as nurseries. Claydon *et al.* (2012) proposed that lionfish in Turks and Caicos (~300 km southeast of San Salvador) move from shallow habitats to deep reefs; however, they found no difference in body size among shallow-water habitats. Based on the fact that fish did not differ in average body size (TL or M) and showed indistinguishable length–mass relationships between the mangrove and reef habitats of this study (Fig. 3), we found no evidence to support an ontogenetic shift in lionfish from mangrove to rock reef areas in San Salvador. An alternative explanation for the smaller body size found in the mangrove habitat in the previous study of Barbour *et al.* (2010) could be that at that time, mangroves had been recently colonized by lionfish, hence their small body size relative to reef habitats. Additional studies with larger sample sizes, replicated sampling, and an extended time period could shed more light on these patterns.

Due to low freshwater input (Gerace *et al.* 1998), the salinity of mangrove habitats in San Salvador is similar to that of reef habitats. Similarity in abiotic conditions between habitats suggests that lionfish have the potential to move freely between mangrove and reef systems. However, our SIA data showed that lionfish captured from mangrove and reef habitats have significant differences in mean centroid location and exhibit no overlap in isotopic niche space (Fig. 4). Lionfish isotopic composition is determined by the habitat in which they reside and their

trophic interactions; therefore, the differences in the isotopic niche of lionfish groups are most likely explained by dissimilarities in habitat use and associated differences in the isotopic values of prey residing in those habitats. Furthermore, SCA data indicated stark differences between the prey items identified from reef and mangrove habitats, confirming the lack of niche overlap. This difference in diet and trophic interactions can be explained potentially by individual specialization of a generalist species, high site-fidelity or differences in prey resource availability among habitat types (Layman & Allgeier 2012).

A toxic species like the lionfish with few predators may not need to seek refuge in nursery areas. In this case, habitat preference is not determined by predation avoidance (Claydon *et al.* 2012). Moreover, having a nursery in the mangrove would imply movement to the nearby reef. Jud & Layman (2012) found the mean maximum range occupied by lionfish to be 28 m in a mangrove-lined Florida estuary. Our isotopic data may also suggest that lionfish from San Salvador occupy relatively small home range areas. Replication of the study with additional sites is needed.

In addition, our genetic identification of the stomach contents further confirms many past studies indicating that lionfish are generalist and opportunistic foragers that maintain relatively small foraging ranges (Morris & Akins 2009; Barbour *et al.* 2010; Côté & Maljković 2010; Jud *et al.* 2011; Muñoz *et al.* 2011). Based on the distance between sites (~18 km), our quantitative data and field observations, it is unlikely lionfish regularly forage across these habitat boundaries. Our results are in agreement with the widely accepted idea that lionfish are versatile generalist feeders with high site-fidelity (e.g. Layman & Allgeier 2012), but we further propose that it is the habitat and the unique prey found there that drives habitat differences in lionfish diet. Additionally, we propose that mangroves are one of many structured habitats in which lionfish are capable of foraging and do not necessarily act as nursery grounds. Consequently, we suggest that management efforts to reduce or eliminate this invasive species should be focused on multiple habitats (e.g. mangroves, shallow reefs and deep reefs) rather than one particular habitat.

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