

## Research Article

## Population ecology and genetics of the invasive lionfish in Puerto Rico

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### Abstract

The lionfish (*Pterois volitans*), has become an abundant alien species along the southeast coast of the United States of America and in Caribbean coastal waters. Although, they represent a major concern in conservation, no studies have yet assessed their population ecology and genetic structure simultaneously. We collected 227 lionfish from 24 sites around Puerto Rico to investigate, the length distribution, length-weight relationships, relative condition factor, and mitochondrial DNA (mtDNA) genetic diversity. The total length distribution ranged between 37 and 349 mm with a single peak between 130 and 149 mm. Our analysis indicated isometric growth for mature individuals and a positive allometric growth for immature lionfishes. These results suggest a different allocation of resources, most likely reproduction for mature individuals and somatic growth for the immature lionfish. The relative condition factor (KN) was similar for immature and mature individuals. Genetic analyses indicated that *P. volitans* is the only lionfish species present in Puerto Rico, and that its origin was likely dispersal from the epicenter of introduction off the coast of Florida. Interestingly, only four of the nine mtDNA haplotypes identified in the Atlantic were observed in the Puerto Rican population. A fine-scale population genetic analysis suggested a significantly different population structure between the west and south coasts. Finally, our data suggests a second founder effect, but with a reduction of haplotype diversity.

**Key words:** *Pterois* spp., relative condition factor (KN), COI, mtDNA control region, Caribbean, invasive reef-fish

### Introduction

Biological invasions are the second major cause of biodiversity loss after habitat degradation (Lowe et al. 2000; Stachowicz et al. 1999). They also cause alterations in ecosystem functions that may result in significant economic losses (Pimentel et al. 2005). In comparison to terrestrial and freshwater habitats (Carlton 1999; Drake et al. 1989), biological invasions have been poorly documented in tropical and subtropical marine ecosystems (e.g., Baltz 1991; Randall 1987). However, a recent increase in the number of alien marine species in these systems has been noted, apparently introduced via ballast waters or the aquarium trade (Hickey et al. 2004; Huxel 1999; Mooney and Cleland 2001; Rahel and Olden

2008; Semmens et al. 2004). Just along the coastal waters of Florida, at least 31 alien marine reef-fish species have been reported over the last 6 years (Semmens 2004; Schofield 2009). Of these, the red-lionfish *Pterois volitans* (Linnaeus, 1758) and the devil firefish (*P. miles* (Bennett, 1928) have established breeding populations along the southeast coast of North America and in the Caribbean (Albins and Hixon 2008; Betancur-R et al. 2011). Even though the species are readily differentiated at mitochondrial levels (Freshwater et al. 2009), morphologically they are nearly indistinguishable (Schultz 1986; Schofield et al. 2009). Thus, the inability to visually differentiate between the two lionfish species has precluded a precise report of their distributions.

Lionfish were first sighted along the southern coast of Florida during the 1980's (Morris and

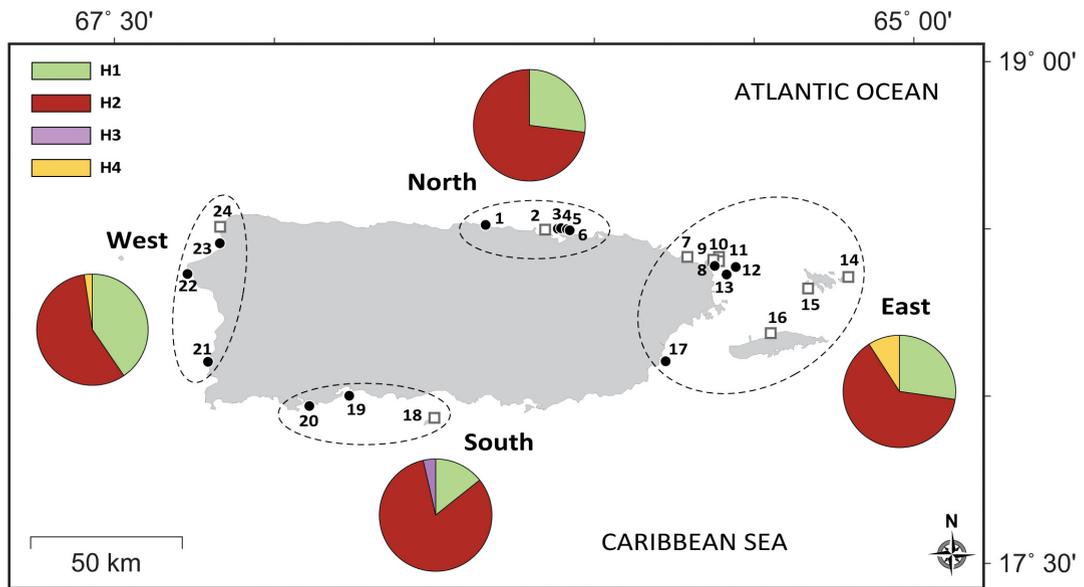
Akins 2009). Since 1999, they have spread along the eastern US coast as far north as Rhode Island (Whitfield et al. 2002; Meister et al. 2005). The red lionfish has also expanded its range to the Bahamas, Bermuda, Turks and Caicos, the Greater Antilles, and the Caribbean coasts of Central and South America (Whitfield et al. 2002; Meister et al. 2005; Whitfield et al. 2007; Guerrero and Franco 2008; Gonzalez et al. 2009; Green and Côté 2009; Morris and Akins 2009; Schofield 2009; Lasso-Alcalá and Posada 2010; Santander-Monsalvo et al. 2012). The invasion of lionfish to the sub-tropical Atlantic and the Caribbean occurs during a period of ecological crisis. Overfishing has reduced the populations of many large marine predatory vertebrates to levels near functional extinction (Hughes 1994; Jackson et al. 2001). As well, bleaching and infectious diseases have all but exterminated some of the most important reef-building corals (Carpenter et al. 2008; Rogers 2009; Weil and Rogers 2011), and global warming and ocean acidification are altering the physico-chemical parameters of seawater, heightening the already stressful conditions for coral reef ecosystems (Anthony et al. 2008; Munday et al. 2009). Consequently, coral reefs may have exceeded their resilience threshold and thus their capacity of response to invasive species has diminished (Stachowicz et al. 1999).

Since the establishment of lionfish in the subtropical Atlantic waters of the southeastern USA and the subsequent expansion into the Caribbean in 2007, the majority of the studies addressing their ecological impact are mainly located in Florida and the Bahamas (Albins and Hixon 2008; Freshwater et al. 2009; Arias-González et al. 2011; Jud and Layman 2012; Albins 2012). These studies have revealed that lionfish in the Atlantic exhibit relatively rapid growth and high reproductive rates compared to those in their native range. Consequently, their population growth and density may far exceeds those in their native regions (Green and Cote 2009). Lionfish can occupy a variety of habitats, ranging from wrecks and artificial submerged structures to mangroves (Albin and Hixon 2008, 2011; Barbour et al. 2010) and compete directly for food and habitat with local fishes. Despite these results, clear information on population growth and habitat partitioning, size structure, life stage (i.e. immature and mature), and physiological wellbeing of lionfish in the most recently invaded habitats of the Caribbean are scarce (e.g., Biggs and Olden 2011).

Similarly, limited information is available from genetic studies of the latest colonized regions of the lionfish invasion. Hamner et al. (2007) characterized the populations of *Pterois* spp. using cytochrome *b* mtDNA sequences and detected a strong founder effect in the invasive populations of both lionfish species. Subsequent studies investigated the variability of the control region of the mtDNA, a less conserved marker, to address the connectivity between the invasive populations and to investigate possible routes of dispersal. Freshwater et al. (2009) assessed and compared lionfish populations from their native range with two invasive populations in the Western Atlantic and Betancur-R et al. (2011) investigated the genetics of populations in the Caribbean and determined the geographic extent of the two invasive species and their haplotype composition. While these studies have provided a general understanding of the origin, extent, and haplotype composition of the lionfish invasion, none of them attempted to characterize the fine scale genetic composition, which is at the forefront of the invasion.

To derive a more complete picture of the impact and mode of expansion of the lionfish invasion, it is necessary to obtain information from many sites across their invaded range. This is especially important to monitor and understand the similarities and differences between established populations of the mid-Atlantic and the more recent invasion in the Caribbean. Lionfish were first reported in Puerto Rico around 2008 (Schofield 2009) and have been sighted regularly since then. Puerto Rico provides a unique opportunity to investigate the progression of the lionfish invasion in the Caribbean because it is strategically located between the Caribbean Sea and the North Atlantic Ocean and connects the Greater to the Lesser Antilles. Thus studies in the waters of Puerto Rico provide the opportunity to obtain information in real time regarding the progression of the lionfish invasion, its ecological effects, and changes in the genetic structure of the population.

In this study, 24 sites around the coastal waters of Puerto Rico were sampled to 1) investigate the population size structure, length-weight relationships and relative condition factor ( $K_N$ ) of lionfish; 2) identify the local genetic diversity and structure by sequencing the same mtDNA regions used in the two most recent lionfish genetic studies (Freshwater et al. 2009; Betancur-R et al. 2011); and 3) compare the genetic data from this study



**Figure 1.** Map of Puerto Rico showing the collection sites (in numbers) and detailed mtDNA control region haplotype structure of *Pterois volitans* distributed across the four cardinal points. Haplotype proportions within each cardinal point are represented by color-coded pies. Black dots represent sampling sites that were used for both ecological and genetic analyses. White squares represent sampling sites that were used only in the ecological analyses. Cerro Gordo (1); Palo Seco (2); Capitolio (3); Escambrón (4) Condado (5); Isla Verde (6); Playa Azul (7); Seven Seas (8); Playa Colorá (9); Las Croabas (10); Cabezas de San Juan (11); Cayo Diablo (12); Palomino (13); Culebrita (14); Luis Peña (15); Isabel II (16); Palmas del Mar (17); Caja de Muertos (18); Eco-eléctrica-Guayanilla (19); Guánica (20); Cabo Rojo (21); Rincón (22); El Natural (23); Crash Boat (24).

with those from previous studies to identify the origin and the number of species currently present in Puerto Rican waters. This work is contributing to the back casting of dispersal routes and the population structure of the lionfish in the Caribbean and provides baseline data to understand and monitor the progression of the invasion in the Antilles.

**Methods**

*Lionfish collection and surveyed sites*

Lionfish distribution was assessed through presence/absence surveys conducted at 24 locations around Puerto Rico, from July 2010 to October 2011 (Figure 1; Table 1S). The habitats surveyed included fringing coral reefs, hard ground habitats, seagrass beds, and submerged artificial structures (i.e. dock pillars and artificial reefs). Most sampling was conducted between 07:00 and 12:00. Depth of collection ranged from 1 m to ca. 30 m. Specimens were collected by divers (snorkeling or SCUBA) using hand nets and pole-spears when depths of collection were < 18 m. Fish traps were used to sample depths > 18 m.

*Life history traits*

The total length ( $L_T$  in mm) of each specimen was measured with calipers or a measuring tape and wet mass ( $M_T$  in g) was measured with a tabletop balance. All individuals were cut-open for sex identification through macroscopic examination of gonads. Each individual was then, classified either as immature or mature following Morris (2009). That is, males > 100 mm  $L_T$  and females > 174 mm  $L_T$  were classified as mature; otherwise individuals were classified as immature. Individuals in which gender could not be identified -mostly individuals < 80 mm  $T_L$ - were also classified as immature.

A linear regression pooling all individuals was performed between  $\log_{10}$ -transformed length and weight data. To assess whether increase in weight was isometric with length, we compared the slope (b) from the linear regression against 3.0 using a t-test (Sokal and Rohlf 1995; Table 1). As the slope was different from 3.0 ( $t=2.643$ ;  $n=227$ ;  $p<0.05$ ), the  $\log_{10}$ -transformed data were grouped into immature and mature fishes, and two additional linear regression analyses were performed to further assess whether increase in weight/length was isometric.

The relative body condition index (KN, LeCren 1951) was calculated for each fish using the slope and the intercept obtained from the regression analysis from the combined data, using the following equation:

$$K_N = M_T * (aL_T^b)^{-1} \quad (1)$$

The data were then re-grouped into immature and mature individuals, and a one-way ANOVA was performed to detect possible differences in  $K_N$  between these groups.

#### *Molecular characterization, identification, and population genetics*

Genomic DNA was extracted from pectoral fin tissue of lionfish using the QIAGEN® DNeasy kit according to manufacturer's instructions, eluted in a final volume of 50  $\mu$ l per reaction and stored at -20°C. The primers LionA-H (5'-CCA TCT TAA CAT CTT CAG TG-3') and LionB-H (5'-CAT ATC AAT ATG ATC TCA GTAC-3') were used to target a gene region of ca. 750 base pairs (bp) from the mitochondrial DNA (mtDNA) control region (Freshwater et al. 2009). This molecular marker was used to determine the proportion of the two invasive lionfish species (*i.e.* *Pterois volitans* and *P. miles*) represented in a subsample of 118 individuals.

Primers COIFishF.2 (5'- CGACTAATCATAAA GATATCGGCAC-3') and COIFishR.1 (5'-TTCA GGGTGACCGAAGAATCAGAA-3'; Tomas Hrbek (Federal University of Amazonas, Manaus, Brazil) were used to amplify ca. 600bp of the gene cytochrome oxidase I (COI) from the mtDNA and to further detect the presence of *P. miles*, in a subset of the individuals analyzed. This gene is used in most vertebrates as the universal region for species level identifications (Hebert and Gregory 2005). All PCR had a final volume of 8  $\mu$ l containing 1 $\mu$ l of genomic DNA, at ca. 20 ng. $\mu$ l<sup>-1</sup>; 0.25  $\mu$ M of each primer; 1X Taq Buffer (Invitrogen); 0.2 mM dNTPs; 2.5 mM of MgCl<sub>2</sub> and 1  $\mu$ l of 1X Taq ACME. The amplification protocol in all reactions had an initial denaturation step of 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 50 °C, 1min at 72 °C and a final extension step of 30 min at 72 °C. Final concentrations of PCR products were estimated by running 2  $\mu$ l of Low DNA Mass Ladder (Invitrogen) in a 1% agarose gel. Amplified fragments were sequenced and ran in both directions using respective PCR primers with ABI Big-dye® terminator chemistry on the automated

sequencer station ABI 3130xl (Applied Biosystems). Chromatograms of the forward and reverse sequences were assembled into contigs using Sequencher v.4.8 (Gene Codes). Unique sequences were identified by comparing all assembled sequences with a parameter of 100% similarity. For the control region mtDNA haplotypes, one sequence from each resulting contig was compared with haplotypes from Freshwater et al. (2009) and Betancur-R et al. (2011) and haplotype numeration follows these studies. For the barcoding region we identified haplotypes by comparing the sequences with public COI sequences stored at National Center for Biotechnology information (NCBI) and public repository of the Consortium for the Barcode of Life (BOLD, <http://www.bold.org>).

Haplotypic ( $h$ ) and nucleotide ( $\pi$ ) diversity indices of the mtDNA control region haplotypes were estimated with Arlequin v.3.5 (Excoffier and Lischer 2010). Arlequin v.3.5 was also used to estimate Tajima's  $D$  and test the neutrality of the molecular marker (Tajima 1989). A pairwise analysis using  $F_{ST}$  statistics (Wright 1969) was used to test for genetic structure among lionfish aggregations across Puerto Rico. The molecular distances for  $F_{ST}$  analyses were estimated under the best-fit model for nucleotide substitution as identified using jModelTest2 (Guindon and Gascuel 2003; Durriba et al. 2012). To investigate the effect of geography over population structure, a test of isolation by distance was conducted via a Mantel test implemented in Arlequin. For the Mantel test, the matrix of genetic distances, based on  $F_{ST}$ , between main areas in Puerto Rico, and the matrix geographical distances calculated as the shortest overwater distance between geographical midpoints of all sites within an area. The statistical significance of Tajima's  $D$  test,  $F_{ST}$ , and Mantel test was assessed establishing 10,000 permutations.

To investigate how genetic diversity is partitioned among populations, a spatial analysis of molecular variance (SAMOVA, Dupanloup et al. 2002) was conducted to define groups of populations of lionfish that are geographically homogeneous and maximally differentiated (rather than defining *a priori* groupings). SAMOVA incorporates geographic positions, which were obtained using Google Earth. The analysis was run using 1000 simulated annealing processes. To assess the correct number of groups (K) for SAMOVA, the FCT statistic from AMOVA (calculated *a posteriori*) was compared for values of K ranging from 2 to 5 (Dupanloup et al. 2002).

## Results

### *Life history traits*

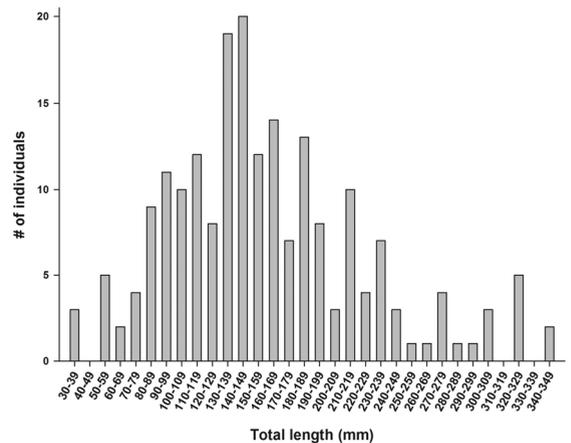
Lionfish were observed and caught at all sites except at four out of the ten sites surveyed on the east coast (Figure 1; Table 1S and Table 2S). All but 17 of the 227 individuals were collected by divers. The  $L_T$  ranged from 57.09 mm to 349 mm with an overall mean  $\pm$  SD of  $163.0 \pm 61.131$  mm and a single mode between 120 to 140 mm (Figure 2). The length distribution is not normal (Shapiro-Wilk test  $W=0.959$ ,  $P < 0.001$ ), and resembled a lognormal distribution given that the mode was to the left of the mean. The  $M_T$  ranged from 0.5 g to 459.9 g, with an overall mean  $\pm$  SD of  $69.857 \pm 86.141$  g.

The slope of the weight-length regression for the immature group was significantly  $> 3.0$  ( $t = 3.931$ ;  $n = 87$ ;  $P < 0.05$ ; Table 1), whereas the slope for the mature group did not differ significantly from 3.0 ( $t = 0.55$ ;  $n = 140$ ;  $P > 0.05$ ; Table 1). Thus, immature fish showed a positive allometric growth, while mature individuals showed isometric growth (Table 1).

Individual values of  $K_N$  ranged from 0.0003 to 0.003 with mean of  $0.0008 \pm 0.0002$ .  $K_N$  for immature fish ranged from 0.0005 to 0.003 with mean  $\pm$  SD of  $0.0008 \pm 0.0003$ . Mature fish exhibited a  $K_N$  ranging from 0.0003 to 0.003, with mean  $\pm$  SD of  $0.0008 \pm 0.0002$ . Mean  $K_N$  between immature and mature individuals was not significantly different ( $F_s = 0.031$ ;  $n = 227$ ;  $P > 0.05$ ).

### *Species identification, genetic diversity and population structure*

Twelve randomly chosen individuals were sequenced for COI and a single polymorphic site defined two haplotypes (HPa = 9, HPb = 3), identical to those recently reported for *P. volitans* in the Caribbean coast of Mexico (Valdez-Moreno et al. 2012). Seven polymorphic sites in the mtDNA d-loop determined four haplotype sequences from 118 lionfish individuals collected at four major locations along the Puerto Rican coast (north = 37, south = 28, east = 11, and west = 42). These four haplotypes are a subset of the nine d-loop haplotypes reported for the Western Atlantic by Freshwater et al. (2009). Haplotype H2 was the most common (68.64%), followed by H1 (28.81%), H4 (1.7%) and H3 (0.85%, Table 2). While H2 and H1 were present throughout all



**Figure 2.** Total length distribution of *Petrois volitans* captured across Puerto Rico.

locations, H3 was detected only at one site in the southern coast; H4 was detected twice, once at the eastern side and the other at the western side (Table 2 and Figure 1). The eastern side of Puerto Rico had the highest gene and nucleotide diversity, while populations from the southern side exhibited the lowest estimates (Table 2).

Except for the northern side of Puerto Rico (Tajima's  $D_{north}=2.324$ ,  $P=0.98$ ), all estimates of Tajima's  $D$  were negative (Tajima's  $D_{south}=-0.876$ ,  $P=0.21$ ; Tajima's  $D_{west}=-0.288$ ,  $P=0.43$ ; Tajima's  $D_{east}=-0.353$ ,  $P=0.38$ ), but in all cases, the null hypothesis of neutrality of the molecular marker was accepted. The Tamura-Nei (TrN) model of nucleotide substitution was the second best-fit model, after HKY+I, as suggested by the Akaike Information Criteria (AIC) in jModeltest. The differences between these two models, however, were negligible ( $dAIC= 1.27$ ). Therefore, the TrN model (implemented in Arlequin) was used to conduct tests of population differentiation. A weak, but significant difference in haplotype composition between the western and southern sides of the island ( $F_{ST}=0.097$ ,  $P < 0.05$ ) was observed, but no significant structure was apparent among other sites. The analysis of molecular variance indicated that 97.23 % of the genetic variation was distributed within each site rather than between sites ( $P > 0.05$ ). Isolation by distance was not detected by the Mantel test ( $P = 0.87$ ).

Given the relative weak genetic structure between sample sites around Puerto Rico, the 118 sampled individual sequences were pooled together to

**Table 1.** Regression parameters (log10-transformed data) for relationships between total length (in mm) and total weight (in g) of *Pterois volitans* from Puerto Rico.

Categories	N	a	95% CL of a	b	95% CL of b	r <sup>2</sup>	P
All sizes combined	227	8.0E-2	4.30E-6 - 1.03E-5	3.11	3.03 - 3.20	0.958	<< 0.001
Immature fishes	87	2.48E-6	1.15E-6 - 5.33E-6	3.32	3.16 - 3.48	0.951	<< 0.001
Mature fishes	140	1.23E-5	6.54E-6 - 2.33E-5	2.99	2.88 - 3.12	0.945	<< 0.001

N: sample size. a: intercept. b: slope. CL: confidence interval. r<sup>2</sup>: regression coefficient

**Table 2.** Number of individuals of *Pterois volitans* detected for each haplotype of the mtDNA control region for various locations at the Northern Sub-tropical Atlantic and the Wider Caribbean Regions. Sampling sites in Puerto Rico were grouped into cardinal regions following divisions in Figure1 (gray).

Haplotype	North Carolina <sup>1</sup>	Bermuda <sup>2</sup>	Bahamas <sup>1</sup>	Cayman <sup>2</sup>	San Andres <sup>2</sup>	Santa Marta <sup>2</sup>	Puerto Rico <sup>3</sup>			
							East	North	West	South
H01	102	18	61	21	18	43	3	10	17	4
H02	98	21	44	56	29	107	7	27	24	23
H03	15	1	7	1						1
H04	15		4	1	3	19	1		1	
H05	9		5							
H06	14	4	1							
H07	10	1	4							
H08			1							
H09	1									
Total	264	45	127	79	50	169	11	37	42	28

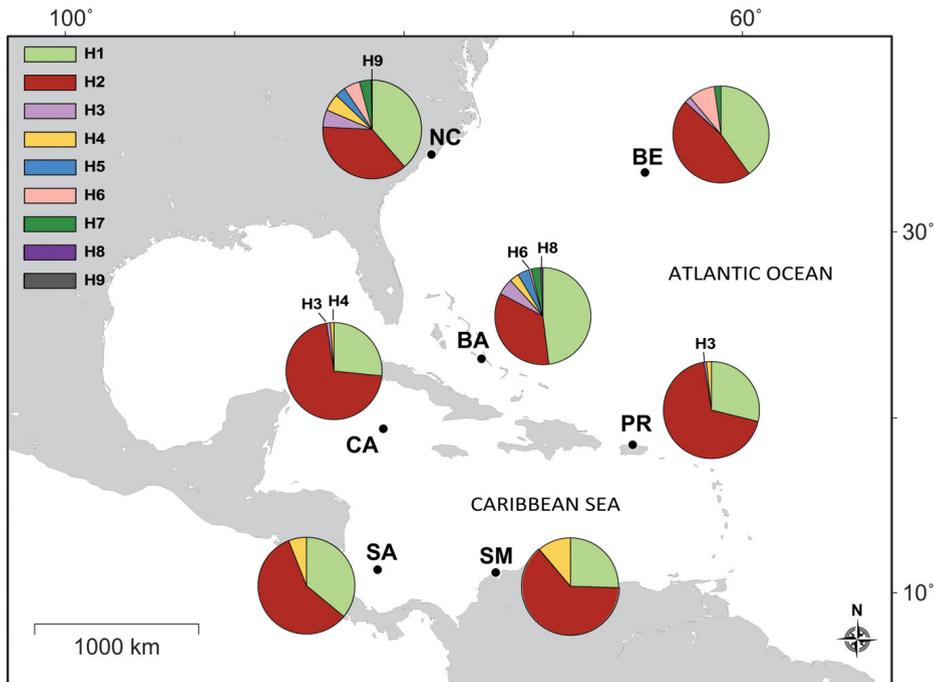
<sup>1</sup>data from Freshwater et al. (2009); <sup>2</sup>data from Betancur-R et al. (2011); <sup>3</sup>data from this study

**Table 3.** Sample size, number of mtDNA control region haplotypes, and associated statistics for various locations of the Northern Sub-tropical Atlantic and the Wider Caribbean Regions. Origins of samples are the same as described in Table 2. N: sample size. PS: polymorphic sites. HP: haplotypes. h: haplotypic diversity.  $\pi$ : nucleotide diversity. SD: standard deviation.

Population	N	PS	HP	h (+/-SD)	$\pi$ (+/-SD)
North Carolina	264	12	8	0.7037 (0.0176)	0.0037 (0.0022)
Bermuda	45	9	5	0.6273 (0.0413)	0.0029 (0.0018)
Bahamas	127	12	8	0.6477 (0.0284)	0.0032 (0.0020)
Cayman	79	9	4	0.4320 (0.0488)	0.0020 (0.0014)
San Andres	50	7	3	0.5412 (0.0434)	0.0028 (0.0018)
Santa Marta	169	7	3	0.5249 (0.0321)	0.0031 (0.0019)
Puerto Rico	East	11	7	0.5636 (0.1340)	0.0032 (0.0021)
	North	37	3	0.4054 (0.0688)	0.0018 (0.0013)
	West	42	7	0.5215 (0.0374)	0.0025 (0.0016)
	South	28	6	0.3148 (0.1024)	0.0015 (0.0012)
ALL	118	9	4	0.4492 (0.0371)	0.0021 (0.0014)

estimate the overall genetic and nucleotide diversity and to compare its genetic variability with previously published results. The genetic and nucleotide diversity for the whole Puerto Rican lionfish population turned out to be low ( $h = 0.449 \pm 0.037$ ,  $\pi = 0.002 \pm 0.001$ ) relative to other invasive lionfish populations examined across the Greater Caribbean, including the recently invaded sites of San Andres and Santa Marta, and only slightly higher compared to the estimates reported for Cayman Islands ( $h = 0.432 \pm 0.049$ ,  $\pi = 0.002$

$\pm 0.001$ , Table 3 and Figure 3). Moreover, when the molecular variance was computed using data obtained in this study, together with the rest of the d-loop sequences available from previous lionfish studies (Freshwater et al. 2009; Betancur-R et al. 2011) it was observed that 94.68% of the variation was distributed among individuals, whereas only 5.32% distributed among populations. The results from the simulations conducted with SAMOVA suggested the best  $K=2$ , based on the values of  $F_{CT}$ . The largest  $F_{CT}$  calculated was for



**Figure 3.** Geographic distribution of mtDNA control region haplotypes of the *Pterois volitans* across the Sub-Tropical Atlantic (NC=North Carolina, BE=Bermuda) and the Greater Caribbean (BA=Bahamas, CA=Cayman Islands, PR=Puerto Rico, SA=San Andres, SM=Santa Marta, Betancur-R et al. 2011; Freshwater et al. 2009). Haplotype proportions within each location are represented by color-coded pie.

K=2 ( $F_{CT}=0.058$ ) and it decreased as K increased (K = 3,  $F_{CT} = 0.054$ ; K = 4,  $F_{CT} = 0.052$ ; K = 5,  $F_{CT} = 0.049$ ). The two groups defined under K=2 by SAMOVA were composed of North Carolina, Bermuda, Bahamas and Puerto Rico in one group and Cayman, San Andres and Santa Marta in the other group. Using this a-priori grouping, highly significant structure was observed between the two groups ( $F_{ST}=0.062$ ,  $P < 0.001$ ).

**Discussion**

This study revealed that lionfish are ubiquitous in Puerto Rico, though they appear to be scarcer at the eastern coast. Fishing pressure could explain their scarcity at the eastern coast, as we witnessed fishing for lionfish at some of the eastern sites, *i.e.* Playa Colorá, Playa Azul and Seven Seas. However, fishing alone cannot explain the observed differences in their distribution. Another possible explanation is that the eastern shore has the most recently colonized locations with suitable habitat. Nevertheless more research needs to be conducted to confirm these observations and also to monitor the progression and

dynamics of lionfish invasion in the eastern coast of Puerto Rico.

Our data indicates that individuals between 100 to 140 mm in  $T_L$  are the most common. This structure is likely reflecting a bias toward sampling sites, as the great majority of our visited sites were relatively shallow reef habitats, dominated by medium sized lionfish. Sites such as mangroves and seagrass, where small lionfish are reported to be more abundant, and deep reef habitats where larger individuals are apparently more abundant (Barbour et al. 2010; Biggs and Olden 2011; Pimiento et al 2013; Claydon et al. 2012), were seldom visited or not visited at all in this study. Accordingly, more effort toward these sites has to be allocated in order to have an unbiased sample of the population size structure in Puerto Rico. Moreover, means  $T_L$  of lionfish from seagrass (173.6 mm), reef habitats (176.3 mm) and mangroves (176.3 mm) from Bahamas (Barbour et al. 2010; Biggs and Olden 2011; Pimiento et al 2012) exceeded the 95% confidence interval of our mean  $T_L$  (155.25–171.72 mm), but not by much. In contrast, mean  $T_L$  of lionfish (113.1 mm) from Honduras were smaller than ours (Biggs

and Olden 2011), and those from El Salvador were larger (231 mm; Barbour et al. 2010). These discrepancies in size structure are perhaps reflecting differences in habitat quality among the expanding range of lionfish. However, we can not discard the possibility toward sampling bias in some of these studies.

#### *Distribution and life history traits of lionfish in Puerto Rico*

Similar to the majority of teleost fishes, lionfish undergo ontogenic changes in body proportion or body shape throughout their life cycle (Sogard 1997). In our study, immature lionfish showed positive allometric growth, which suggests a faster increase in their length than in their height while mature individuals exhibited isometric growth, which suggests that height and length increase at a proportional rate (Froese 2006). As mortality is intimately correlated to body sizes and shapes, a faster elongation of the body, as opposed to height, may improve the survivorship of juveniles by conferring them a higher capacity to swim and thus to escape from predators and to also obtain food (Sogard 1997). Hence, allometric growth should continue until reaching a “rescue size” where vulnerability to predation is drastically reduced while the capacity to obtain food continues to improve (Sogard 1997). Although such results are not extremely surprising given the fact that allometric growth is a common strategy among pre-reproductive teleost fish (Gisbert 1999), this is the first report for the lionfish. These shifts in growth form (length vs. height) may also help to explain the ontogenic shift in habitat, (shallow-water vs. deep-water habitat) during the lionfish life histories.

Another aspect addressed in this study is the assessment of the condition of lionfish in Puerto Rico by means of  $K_N$ . In this particular study, the  $K_N$  provided information about the welfare of lionfish of different life-stages. And it reveals that the wellbeing is in general, similar between immature and mature lionfish. Interestingly, our mean  $K_N$  (0.0008) is slightly higher than the mean  $K_N$  (0.0005) estimated using data from recent studies at Bahamas (Pimiento et al. 2012), suggesting that the individuals caught at this study are in a better physiological state than that from San Salvador, Bahamas. However, caution must be taken when interpreting results from the  $K_N$  index as this could be affected by the, stage of development, reproductive season and stomach content among other.

#### *Population genetics of lionfish invasion in Puerto Rico*

The two mitochondrial markers examined (COI and d-loop) suggest that *P. volitans* is the only lionfish present in Puerto Rican waters. The COI gene showed less than 2% genetic divergence from sequences of *Pterois volitans* published in BOLD, confirming that the species attribution has 100% accuracy. Since no mtDNA haplotypes of *P. miles* were detected in any of the samples examined from Puerto Rico, the results validate that *P. miles* is still restricted to the U.S East Coast and Bermuda (and thus is absent from the Caribbean; Betancur-R et al. 2011; Schultz et al., 2013) and that *P. volitans* is likely the only lionfish species that has invaded Puerto Rican waters. The absence of *P. miles* may indicate that the southern distribution limit of this species along the US east coast is too far north, that their population numbers are too low for them to overcome the barriers to disperse across the Florida Strait, or that this species does not exhibit the adaptive potential to overcome the environmental challenges encountered in the Caribbean ecosystems. It has been noted that *P. volitans* is far more abundant than *P. miles*, even at co-occurring locations, and is genetically more diverse (Betancur-R et al. 2011).

The molecular analysis of Puerto Rican *P. volitans* indicated the presence of only four out of the nine haplotypes reported throughout the invasive range (Freshwater et al. 2009; Betancur-R et al. 2011). The four haplotypes (H1, H2, H3 and H4) present in Puerto Rico (eastern Caribbean) are the same as those found in the Grand Cayman (north-western Caribbean). Genetic diversity from the mid-western and southern Caribbean populations (San Andres and Santa Marta, Colombia) is further reduced, evidencing only three (H1, H2, and H4) of the four northern Caribbean haplotypes (Betancur-R et al. 2011). When data from this study are analyzed together with those generated by previous studies, an interesting pattern emerges: lionfish populations in the Caribbean Sea lack more than half of the northwestern mitochondrial haplotypes reported by Freshwater et al. 2009 (H5, H6, H7, H8 and H9). Moreover, it was found that the haplotype dominance observed in the northwestern population (H1>H2) has shifted in the Caribbean towards a dominance of H2 (H2>H1). However, our data suggest that neither of these two haplotypes conferred better fitness over the other. This preliminary finding, however, does not preclude the effect of genetics

over lionfish fitness, but recognizes the lack of power of a single locus to make this kind of inference. Still, the dominance of H1 and H2 observed in the northwestern populations remains in the Caribbean. The dominance of these haplotype can be explained as the result of the initial abundance at the original invasion, while the lack of many haplotypes that were observed at the onset of the invasion can be simply the result of a combination of rarity and limited sampling. A continuous genetic monitoring of invasive population will help clarify that. The differences in haplotypic composition and relative frequency resulted in significant population genetic structure when the groups suggested after the spatial analysis are treated as two discrete units. At the level of local population genetics, lionfish populations in Puerto Rico reveal a subtle but significant differentiation between the western and southern coasts of the island (Figure 1). Despite strict data interpretation, this study represents the only attempt to explore the genetic structure of the lionfish invasion at a fine geographical scale, providing an important resource for future comparative studies.

In summary, our results are consistent with a previous study (Betancur-R et al. 2011) reporting a decrease in genetic diversity of lionfish in the Caribbean, associated with dispersal outside the epicenter of introduction in Florida. It is most probable that the structure observed between the Caribbean and the northwestern Atlantic populations is likely the result of the Bahamas and the Turks and Caicos/Caribbean connectivity break reported for the Western Atlantic (Cowen et al. 2006). This pattern is also consistent with the temporal lag in the arrival of lionfish in the Caribbean in 2007, which took place seven years after the major population expansion in the northwestern Atlantic. Lack of population structure across other recognized barriers in the Greater Caribbean (e.g., U.S East Coast-the Bahamas break, northwestern Caribbean barrier, and others; Betancur-R et al. 2011: Figure 3) may not necessarily reflect continuous gene flow but rather failure of mtDNA control region sequences to detect structure given the short time since the invasion of the lionfish. It is important to note that despite the inherent limitations of using a single loci for fine-scale population structure, it was possible to detect a subtle genetic differentiation at both regional (Northwestern Atlantic and the Caribbean) and local (Puerto Rico) scales. The next logical step will be to examine more powerful and sensitive

genome-wide markers (e.g. RAD Tags, Hohenlohe et al. 2010a or GBS, Elshire et al. 2011) to test for key factors linked to dispersal and colonization of invasive species. A recent study of stickleback fishes has found fine-scale geographic/genetic differentiation in short periods of time, but the only way to detect these fine-scales differences was by using other techniques such as genomics (Hohenlohe et al. 2010b). A genomic approach could provide a critical understanding on lionfish population dynamics, connectivity among lionfish populations, the genetic basis of rapid adaptation, gene flow and the spatial extent of population dynamics at both regional and local scales. Ultimately, the lionfish invasion provides a unique natural experiment to assess phylogeographic breaks for reef organisms in real time.

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## Supplementary material

The following supplementary material is available for this article:

**Table 1S.** Study sites names and their co-ordinates around Puerto Rico.

**Table 2S.** Records of *Pterois volitans* in coastal waters of Puerto Rico, from October 2010 to July 2011.

This material is available as part of online article from:

[http://www.aquaticinvasions.net/2014/Supplements/AI\\_2014\\_ToledoHernandez\\_etal\\_Supplement.xls](http://www.aquaticinvasions.net/2014/Supplements/AI_2014_ToledoHernandez_etal_Supplement.xls)