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Reproductive biology of *Pterois volitans* in the Baconao Biosphere Reserve, southern Cuba

Biología reproductiva de *Pterois volitans* en la Reserva de la Biosfera Baconao, sur de Cuba

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ABSTRACT

Background: *Pterois volitans* Linnaeus 1758 is an invasive exotic species established in the Atlantic and Caribbean, threatening the native marine biodiversity. It has led to reduced recruitment and biomass of small reef fish of ecological interest, altering the fish community structure by reducing the number of herbivores. In Cuba, it was first reported in 2007. **Goals**: This research aims to characterize the reproductive biology of *P. volitans* in the western part of the Baconao Biosphere Reserve, in southern Cuba. **Methods**: Sampling was carried out monthly from April 2012 to May 2013 (n = 535 fish). Total length (L_T, cm), total mass (M_T, g), and weight of the liver, gonads, and abdominal fat were recorded for each individual. To analyze spawning, the gonadosomatic (I_G) and hepatosomatic indices (I_H) were determined. **Results**: Mean size and weight of adults were 26.8 cm and 290.9 g, respectively. The size structure revealed that females are dominant in lengths of 22-31 cm, whereas males dominate from 31 cm onwards. The sex ratio was 1:1. I_G showed seasonal spawning peaks between March and May 2013. No significant differences were observed amongst monthly means, but I_H monthly means and monthly rates of abdominal fat showed significant differences. The size at first maturity was 30.07 cm in males and 26.2 cm in females. **Conclusions**: The results of this study suggest that the lionfish *P. volitans* shows an asynchronous reproductive cycle characterized by high gonadal index values during the breeding season and an inverse trend of I_H and I_F, suggesting that the liver and body fat store nutrients for use in reproduction.

Key words: gonadosomatic index, invasive exotic species, size of first maturity, protected areas, sex ratio.

RESUMEN

Antecedentes: *Pterois volitans* Linnaeus 1758 es una especie exótica invasora establecida en el Atlántico y el Caribe que amenaza la biodiversidad marina nativa. En consecuencia, su presencia ha reducido el reclutamiento y la biomasa de los peces pequeños del arrecife de interés ecológico, alterando la estructura de la comunidad al reducir el número de herbívoros. En Cuba, se informó de su presencia por primera vez en 2007. **Objetivos**: Esta investigación tiene como objetivo caracterizar la biología reproductiva de *P. volitans* en la zona occidental de la Reserva de la Biosfera Baconao, costa sur oriental de Cuba. **Métodos**: El muestreo se realizó mensualmente de abril de 2012 a mayo de 2013 (n = 535 peces). A cada pez capturado se le registró la longitud total (L_T, cm), peso total (M_T, g), peso del hígado (g), gónadas (g) y grasa abdominal (g). Para analizar la época de desove, se determinaron los índices gonádico-somático (I_G) y hepático-somático (I_H). **Resultados**: La talla promedio fue 26.8 cm con un peso medio de 290.9 g. La estructura poblacional, revela que las hembras son dominantes en longitudes que van desde 22-31 cm y los machos a partir de los 31 cm. La proporción de sexos fue de 1: 1. EL I_G mostró picos estacionales de desove, entre (marzo y junio), aunque no se encontraron diferencias significativas entre las medias mensuales, pero sí entre las medias mensuales del I_H y el índice de grasa abdominal. La talla de primera madurez se determinó en los machos de 30 cm y en las hembras de 26.2 cm. **Conclusiones**: Los resultados de este estudio indican que el pez León *P. volitans* tiene un ciclo reproductivo asincrónico, con altos valores del índice gonadosomático durante el periodo reproductivo y con una tendencia inversa con el I_H y el I_F, lo que sugiere que el hígado y la grasa del cuerpo contienen nutrientes que se utilizan durante el periodo reproductivo.

Palabras claves: índice gonadosomatico, especies exóticas invasoras, talla de primera madurez área protegidas, proporción de sexos.

INTRODUCTION

The invasion of lionfish *Pterois volitans* Linnaeus, 1758 and *P. miles* (Bennett, 1828) in the northwest Atlantic and the Caribbean is one of the fastest invasions of marine fish recorded in history (Morris *et al.*, 2008). These species are considered as established in these seas (Morris *et al.*, 2009; Schofield, 2009; Schofield, 2010).

Lionfish are a major concern for managers of marine protected areas, governments, and local communities, and their impacts as predators affect the reef fauna, particularly the fish community (Albins & Hixon, 2008). *P. volitans* is far more abundant than *P. miles*; therefore,-current research is focused on the former species. It has been determined that the lionfish is able to cause a 79% reduction in the recruitment of forage fish (Whitfield *et al.*, 2007), which play a key role contributing to maintain the equilibrium in the algae-coral relationship. Meanwhile, Green *et al.* (2012a) reported biomass reductions in 65% of 42 species of small reef fish. Other authors have also documented the predatory impact of this species on the reef (Green & Côté, 2009; Brown *et al.*, 2009; Santander-Monsalvo *et al.*, 2012). From the economic viewpoint, impacts of lionfish are expected on fisheries and in the reduction of reef fish that are major attractions for tourism, thus deserving evaluation.

The first confirmed record of lionfish in Atlantic waters occurred in Florida, where a specimen was collected in October 1985 (Morris & Akins, 2009). Since 1992, lionfish were observed in Palm Beach, Boca Raton, and Miami, Florida; since 2000, they were recorded in North Carolina, South Carolina, Georgia, and Bermuda (Whitfield *et al.*, 2002; Hare & Whitfield, 2003; USGS, 2004; Reef, 2008). The species continued to expand rapidly in the Atlantic and the Caribbean (Schofield, 2009) as well as in the Gulf of Mexico (Brown & Ruiz-Carus, 2006; Aguilar-Perera *et al.*, 2012; Santander-Monsalvo *et al.*, 2012).

In Cuba, lionfish were first reported in 2007 off the coast of Santiago de Cuba, near the Aquarium of Baconao (Chevalier *et al.*, 2008), and rapidly colonized the rest of the coast, to the extent that since 2010, it was considered as a well-established species in the country.

The seasonality of lionfish reproduction throughout its natural range is unknown (Morris *et al.*, 2008). Ruiz-Carus *et al.* (2006) stated that this species may have been breeding in Florida during the first months of the year; their rapid colonization of other areas led to suspect that reproduction may occur throughout the year under suitable conditions (Morris *et al.*, 2009).

Therefore, this study was designed to fulfill the scarce information on the breeding process of lionfish in Cuba and the need to address recommendations to control their populations aiming to minimize their impacts on local reefs. Specific goals were to determine (1) seasonality of spawning, and (2) minimum recruitment size at first reproduction. With these results, we expect to provide data that will support the development of proposals to address lionfish management in the Baconao Biosphere Reserve.

MATERIALS AND METHODS

Study area. The selection of the study area followed criteria based on the monitoring protocol for the study of lionfish in Cuba (Acuario Nacional de Cuba, 2011). A site encompassing 10 km of coastline in the eastern sector of the Baconao Biosphere Reserve was selected (Fig. 1), including the Siboney-Juticí Ecological Reserve, one of the five core conservation areas of this biosphere reserve. As sampling biotope (1 km scale), spur and groove sites of coral reef were selected at a 0.1 km scale, considering access to the coast.

This area is characterized by a coast of tectonic origin, with no platform, but with abundant submarine terraces, where coral reef grows at short distance from the coastline (100-200 m in some areas). The surface sea current runs from east-to-west with a maximum speed of 50 $cm \cdot s^{-1}$. Salinity is stable, with values slightly above 36 ppt (Ionin *et al.*, 1977). Sea surface temperature fluctuates around 27.6°C across the entire area (García, 1989).

Capture method. Sea lionfish catches were performed on a monthly basis, from April 2012 until May 2013; the only exception was November 2012 because of the impact of Hurricane Sandy in Santiago de Cuba. Catches were carried out with scuba-diving equipment at 15 to 30 m depth by dive computer Mares Puck Pro®. Fish were collected by speargun fishing, and all the animals sighted during diving were caught, regardless of fish size. On the surface, fish were placed in a cooler chest and transported to the laboratory at the Siboney-Juticí Ecological Reserve, where they were kept refrigerated and processed within 24 hours.

Data collection. Total length (L_T, cm) of each fish was measured with a board to the nearest 1 cm. Total weight (M_T, g) was recorded with a dynamometer to the nearest 1 g (± 1 g weight error). Then, fish were dissected by following the procedures detailed in Green *et al.* (2012b). The Kruskal-Wallis test was run to test for differences between monthly medians of both total length and total weight. The significance level applied was α=0.05. The weight of gonads, liver, and fat were recorded with an analytical balance (to the nearest 0.001 g). The U Mann-Whitney test was used to explore the statistical significance of differences in size between males and females. A Chi-square test (α = 0.05) was used to determine significant differences between months.

Data processing to determine the breeding season. To determine the breeding season, the gonadosomatic index was calculated using the following equation:

$I_G = (M_G / M_T)\ 100$ (Maddock & Burton, 1998).

where:

I_G = Gonadosomatic index.

M_G = Weight of both gonads (g).

M_T = Total weight without stomach content (g).

Mean monthly IG values were plotted to determine the peak of spawning, when this index decrease in an annual cycle.

The hepatosomatic index (I_H) was used as a quantification of cyclical changes in accumulation of reserves.

$I_H = (LM / M_T)\ 100$ (Maddock & Burton, 1998)

where:

I_H = Hepatosomatic index

LM = Liver weight (g)

M_T = Total fish weight (g)

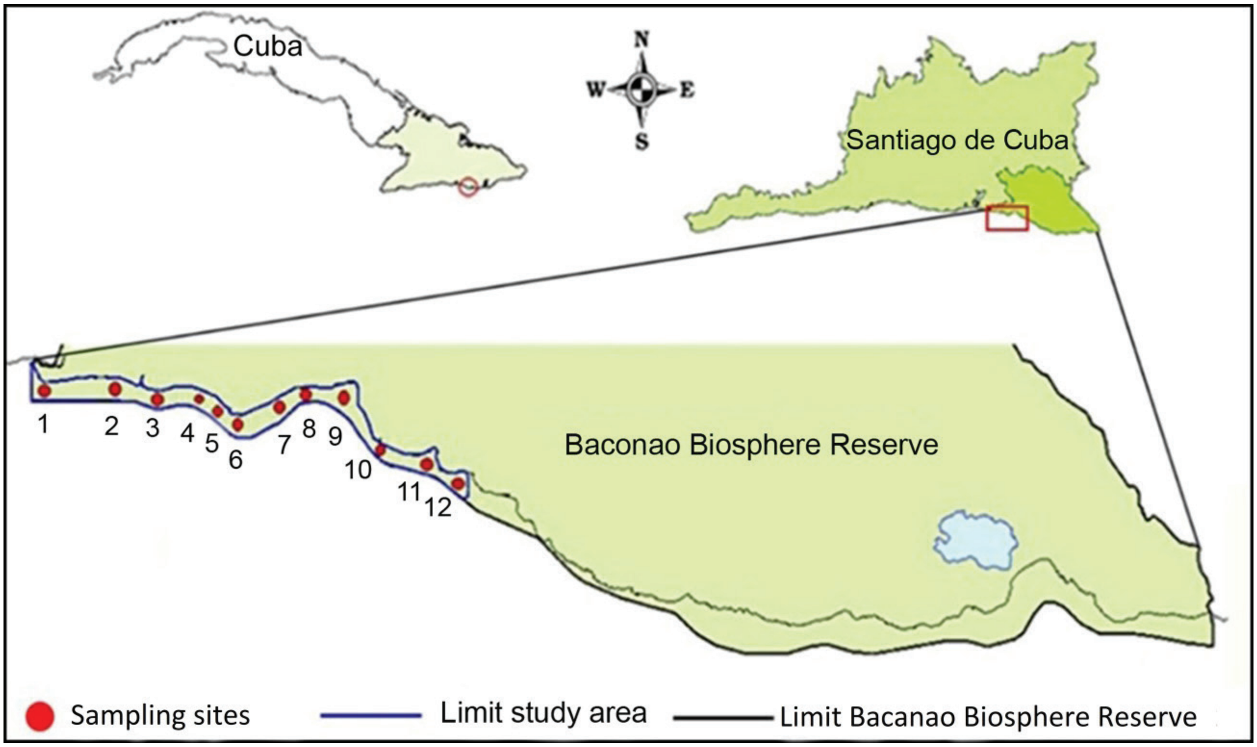

Figure 1. Study area in the Baconao Biosphere Reserve, south-eastern coast of Cuba. 1. Aguadores Este; 2. Sardinero Oeste; 3. Sardinero Este; 4. El Mangle; 5. Juticí Oeste; 6. Juticí Este; 7. Caballo Blanco; 8. La Cantera; 9. Playa Siboney; 10. Bucanero; 11. Playa Juraguá; 12. Playa Damajayabo.

Additionally, a body fat index was determined, as this is a reserve substance that accumulates before the onset of breeding; the following formula was applied:

$I_F = (MF / M_T)\ 100$

where:

I_F = Fat somatic index

MF = Weight of fat (g)

M_T = Total weight of fish (g)

Data processing of average size at maturity. Organisms in the maturity, spawning and post-spawning stages (It was determined based in GDS III, IV, V, and VI according to Morris *et al*. (2011) and Priyadharsini et al. (2013), regardless of sex) were used to estimate the size at first maturity ($L_{0.5}$) defined as the length at which 50% of organisms are sexually mature. Results were plotted and fitted to a logistic function not linear (Gaertner & Laloe, 1986; Sparre & Venema, 1997).

$$H_P = \frac{1}{1 + e^{a+b*Lt}} \quad (1$$

Where:

H_p: Percentage of sexually mature individuals corresponds to:

$$L_{0.5} = a / b \quad (2$$

RESULTS

We caught a total of 535 individuals with an average size of 26.8 (standard deviation SD = 2.1 cm). The mean total weight was 290.6 g (SD = 197.6). The largest and smallest fish caught were 42.2 cm and 13.8 cm, respectively. In general, individuals over 35 cm were found at depths greater than 30 m, while fish under 10 cm were observed at depths of less than 10 m. To note, the latter were not collected.

The Kruskal-Wallis test showed significant differences between the monthly medians of total length (K-W = 58913, p< 0.001) and total weight (K-W = 53936, p< 0.001). Females had a mean total length of 24.7 cm (SD = 3.9) and a mean weight of 207.8 g (SD = 96.1); males reached a mean length of 29.5 cm (SD = 6.1) and a mean weight of 382.1 g (SD = 231.3). The largest fish caught was a 42.3 cm long male weighing 1600 g, while the smallest (undifferentiated) measured 13.8 cm and weighted 10 g.

The microscopic examination of all fish caught showed that 218 fish were females and 233 were males; however, sex could not be determined in 84 fish (Fig. 2). In the remaining specimens, gonads were either damaged or immature, thus precluding the identification of the sex. The Chi-square test (X^2 = 0.499, p = 0.480) showed a 1:1 (F:M) sex ratio; only in May 2012, when 27 males and 13 females were sampled, there was a significant difference (X^2 = 4.225, p = 0.03983).

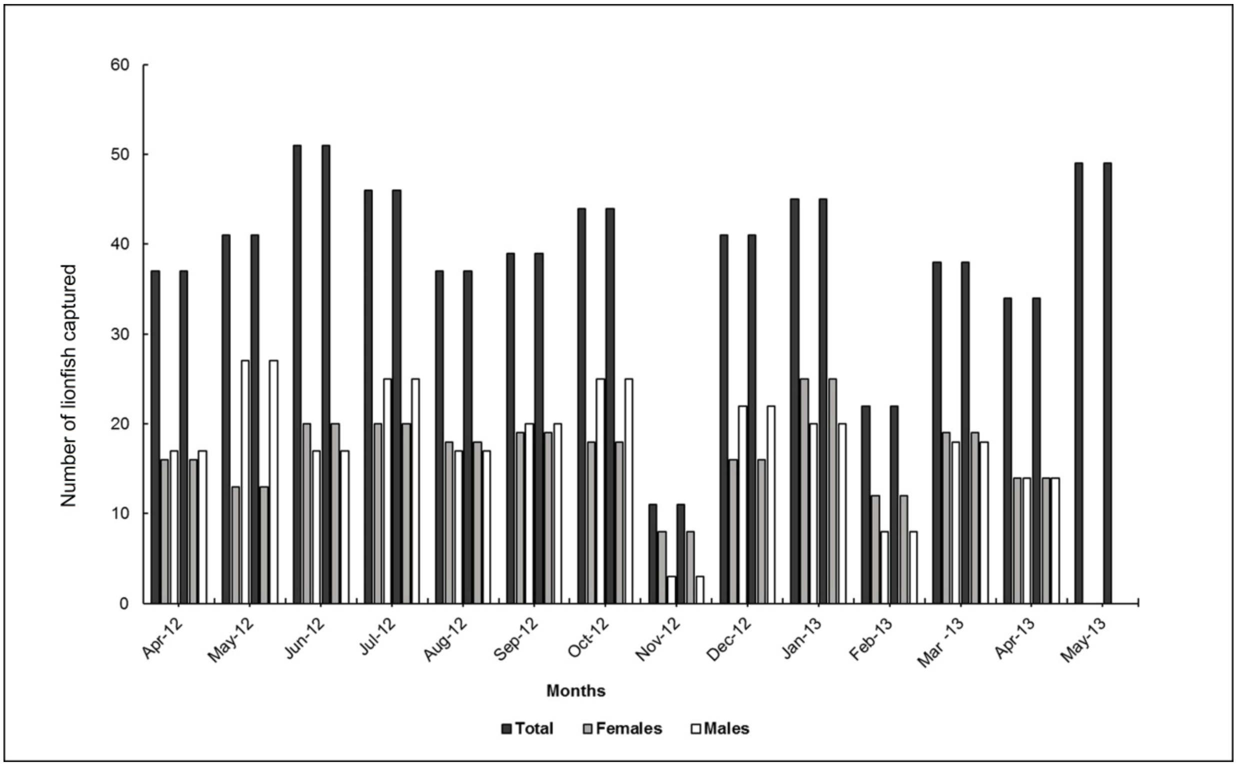

Figure 2. Number of lionfish captured during the sampling period. Baconao Biosphere Reserve, south-eastern coast of Cuba. April 2012 to May 2013.

The size structure by sex revealed that females were mostly in the 22-31 cm size range, while males dominate from 31 cm onward (Fig. 3). In this regard, significant differences in size between males and females were found (U Mann-Whitney: U = 18161.00 [L_T], U =13157.50 [M_T]; $p< 0.001$).

Breeding season. The female GI values show a small decrease from April to June 2012 (4.5 to 2.8) and from March to May 2013 (5.6 to 3.9), which indicates reproductive activity. Males and undifferentiated were not considered since they did not show significant variation (Fig 4 A). Opposite trends were observed between the hepatosomatic index (I_H) and the body fat index (I_F) (Figs. 4 B y C), as well as between each of these two indices with I_G, during spring and summer; also, significant differences were observed in this indices throughout the study period (X^2 (I_H)= 82.693, $p< 0.001$; X^2 (I_F)= 54.818, $p< 0.001$).

Recruitment size at reproduction. The size at first maturity of females was 26.2 cm (a= 11.406; b=0.4352); for males, it was 30.07 cm (a=10.94; b=0.36). The smallest mature female was 17.5 cm; the smallest male, 17.9 cm (Fig. 5).

DISCUSSION

Mean total length of the fish caught during this study is similar to the mean value reported in the reefs of Santa Marta, Colombia (24.8 cm; González *et al.*, 2011). On the other hand, Froese and Pauly (2018) reported a maximum length of 35 cm, which is lower than the length of the largest fish caught in this study (42.2 cm), and similar to the maximum length reported by Baker *et al*. (2004) in North Carolina (43 cm). The observations in this work highlight the presence of small fish at shallow depths and larger fish at deeper depths, suggesting segregation according to size across the water column, which opens up a new line of research for future studies. Similarly, the predominance of females in sizes between 15-28 cm at a depth around 25 m suggests a possible bathymetric segregation by sex, as males >30 cm in length prevail beyond 30 m depth, as observed in Turks and Caicos Islands, Bahamas, and in Roatán, Honduras (Claydon *et al.*, 2012; Babour *et al.*, 2010; Biggs & Olden, 2011).

Variations in I_G values suggest an asynchronous reproductive cycle, which is consistent with the findings reported by Morris (2009) in waters of North Carolina, South Carolina, and the Bahamas. This reproductive profile partially explains the rapid dispersal, invasion, and colonization of lionfish in the Caribbean and Atlantic, as a result of the favorable environmental conditions (Morris, 2009; Morris *et al.*, 2011). These include the presence of abundant food for adults, ensuring gonad development at a proper temperature (27.6 °C) (García, 1989) in the study area, known to have a positive effect on reproduction in this species. This temperature is well above the temperature limiting the development of this species, as determined by Kimball *et al.* (2004) (feeding stops at 16.1 °C; 10 °C is lethal). A factor closely related to temperature is photoperiod, also showing little variation in tropical latitudes; both factors contribute to constant food availability (https://searchworks.stanford.edu/view/1077428).

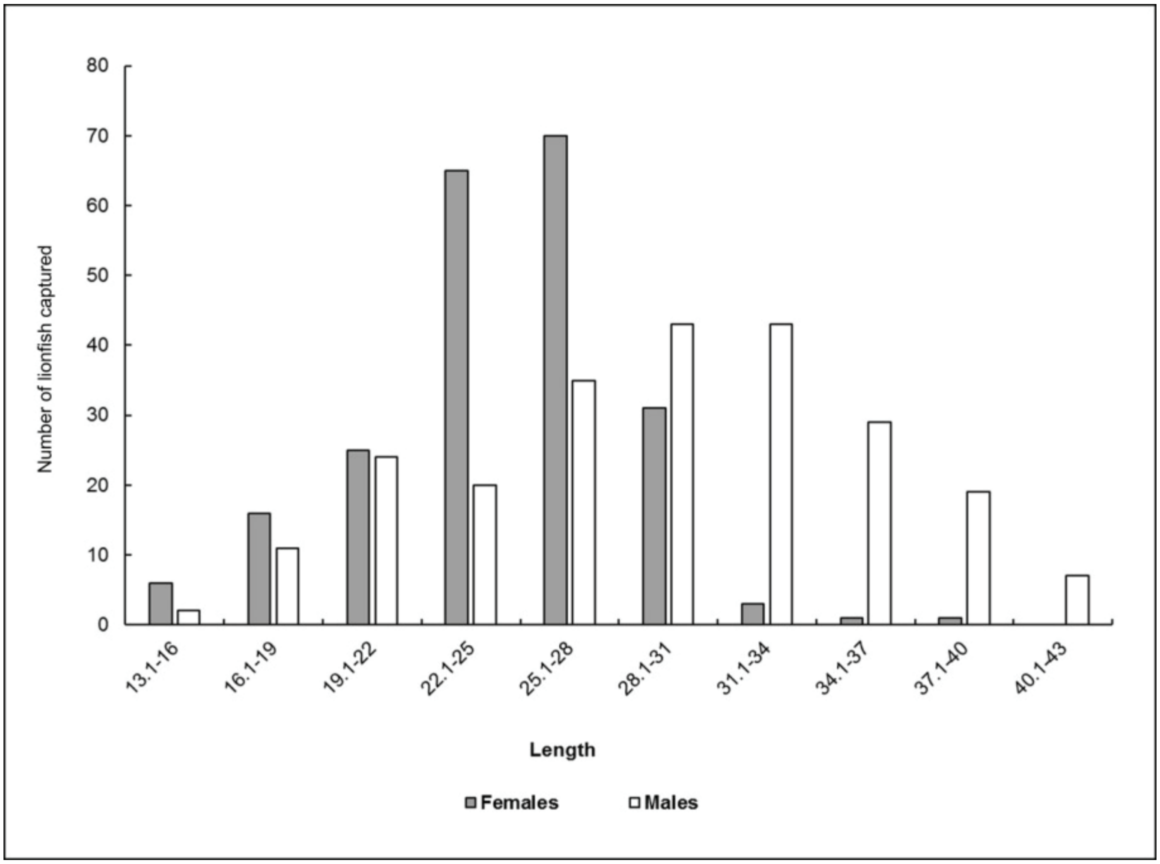

Figure 3. Distribution of sexes by size class. Baconao Biosphere Reserve, south-eastern coast of Cuba. April 2012 to May 2013.

The peak in reproductive activity in winter was reported previously in Florida waters by Ruiz-Carus *et al*. (2006). When these spawning seasons is compared with reports for the natural range of lionfish in the southeast coast of India in August (Priyadharsini *et al*., 2013), an important difference of 4-7 months emerges, which may be an adaptive response of this species to conditions in Cuban waters.

The opposite trends of I_H and I_F values during the months when lower I_G values were recorded may be explained by the accumulation of reserves, mainly lipids and vitellogenin, the yolk precursor stored in oocytes during vitellogenesis (Love, 1970; Van Bohemen *et al*., 1981). In this study, I_G and I_H apparently do not follow an opposite trend, with both decreasing as a result of reproduction. This is assumed to happen as a consequence of a surplus energy reserve, thus allowing lionfish not to deplete its reserves. This is supported by the fact that lionfish is iteroparous, so the liver does not play a major role, contrasting with species inhabiting in limiting environments that impose the need to store reserves to cope with starvation periods (Love, 1970; Van Bohemen *et al*., 1981; Saborido-Rey, 2008). To determine whether this statement is actually true would require performing bioenergetic studies. The stability of CF throughout the study period regardless of the reproductive stage reflects favorable conditions for lionfish, i.e., abundant food and absence of predators, allowing them to maintain a constant physiological performance throughout the whole year (Morris *et al*., 2011).

As regards the size of recruitment to the reproductive stock, the sizes at first maturity observed in the present study (26.2 cm for females and 30.07 cm for males), are larger than those by reported by Morris (2009) for North and South Carolina, Bahamas, and the Philippines, i.e., a size of first maturity of 17.5 for females and 10 cm for males in pooled samples of the three locations. The smallest female with reproductive activity reported in this study (17.5 cm) is similar to the size reported by Morris (2009) (17.2 cm), but differs from values reported by this same author for lionfish from North and South Carolina (15.8 cm) and the Bahamas (9.8 cm). In the case of males, the values reported by Morris (2009) for North (13.2 cm) and South Carolina (10.5 cm), the Bahamas and the Philippines (10 cm) differ from those recorded in the present study (17.9 cm). These differences may be explained as a response to fishing pressure by reaching maturity at smaller sizes.

The findings in this study suggest that the lionfish, *P. volitans*, shows an asynchronous reproductive cycle characterized by high gonadal index values during the breeding season and an inverse trend of I_H and I_F, suggesting that the liver and body fat store nutrients for use in reproduction.

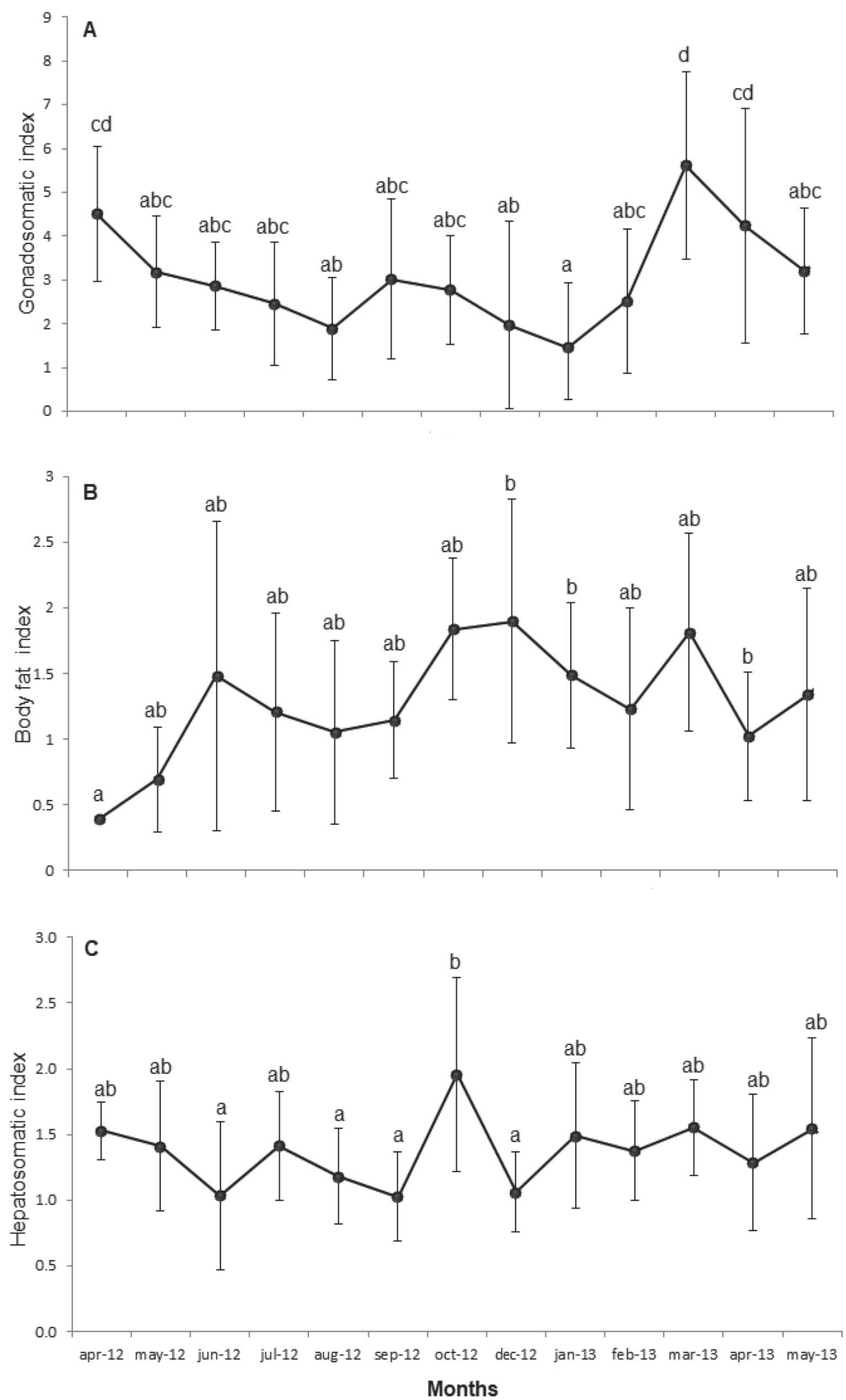

Figure 4. Monthly variation of mean values A) Gonadosomatic index (I_G); B) Body fat index (I_F), C); Hepatosomatic index (I_H), of the lionfish population sampled in Baconao Biosphere Reserve, south-eastern coast of Cuba. April 2012 to May 2013.

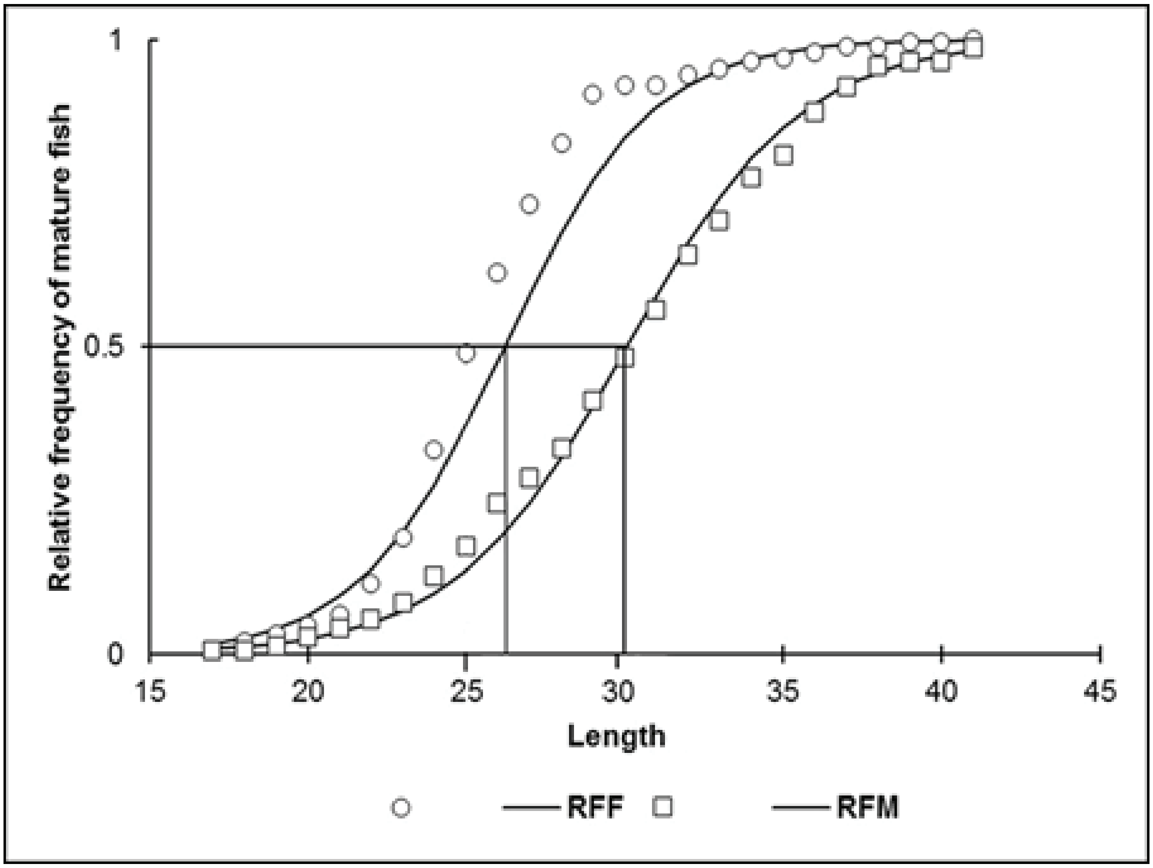

Figure 5. Size at first maturity of the lionfish population. RFF: Relative frequency of females, RFM relative frequency of male, Circles and squares are observed data. Lines are refer to data estimated by equation in Baconao Biosphere Reserve, south-eastern coast of Cuba. April 2012 to May 2013.

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