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RARE DATA ON A ROCKY SHORE FISH REPRODUCTIVE BIOLOGY: SEX RATIO, LENGTH OF FIRST MATURATION AND SPAWNING PERIOD OF *ABUDEFDUF SAXATILIS* (LINNAEUS, 1758) WITH NOTES ON *STEGASTES VARIABILIS* SPAWNING PERIOD (PERCIFORMES: POMACENTRIDAE) IN SÃO PAULO, BRAZIL

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ABSTRACT

This study presents data on the reproduction of *Abudefduf saxatilis*, a rocky shore inhabitant at the northern coast of São Paulo State. A total of 73 individuals were collected using hooks and baits. They were measured, weighed and dissected, sex and maturation stage were analysed, first macroscopically, then part of the material was taken for microscopical confirmation. Visual censuses were also done for underwater observation of egg's presence. Results showed equivalence of males and females in the population, first maturation occurring between 101 and 115mm of total length, spawning period occurs from November to February for *Abudefduf saxatilis* and October to January for *Stegastes variabilis*. Reproductive period for *A. saxatilis* was positively related to air temperature and thermic amplitude, but the environmental clue most likely to influence this rhythm is photoperiod. Transects with visual census of males guarding eggs were also a reliable tool for finding reproductive period in these demersal, egg-guarder species.

RESUMO

Esse estudo apresenta dados sobre a reprodução de *Abudefduf saxatilis*, uma espécie habitante de costões rochosos no litoral norte do estado de São Paulo. Os peixes foram coletados com anzol e isca num total de 73 indivíduos, foram medidos, pesados, dissecados, seu sexo e maturidade gonadal foram analisados, primeiro macroscopicamente, depois parte desse material foi levado para confirmação microscópica. Censos visuais também foram feitos para observar a presença de ovos. Os resultados apontam para uma equivalência entre o número de machos e fêmeas na população, a primeira maturação ocorre entre 101 e 115 mm de comprimento total, a desova ocorre entre os meses de novembro a fevereiro em *Abudefduf saxatilis* e outubro a janeiro em *Stegastes variabilis*. O período reprodutivo de *A. saxatilis* está positivamente relacionado à temperatura do ar e amplitude térmica, mas o indicador ambiental mais provável de influenciar o ritmo reprodutivo é o fotoperíodo. Os censos visuais feitos com a transecção mostraram-se uma ferramenta confiável para encontrar o período de desova nessas espécies demersais que guardam seus ovos.

Descriptors: Pomacentrid, Damselfish, Reproductive period, Visual census, Reproduction, São Sebastião, Sergeant major.
Descritores: Pomacentrídios, Donzela, Período reprodutivo, Censu visual, São Sebastião-SP, Sargento.

INTRODUCTION

Pomacentridae (Perciformes: Labroidei) are marine fishes, inhabitants of hard rocky or reef substrates, restricted to shallow regions. This family's major diversity spot is located in South Pacific, actually with circumtropical distribution (Nelson, 1994). Commonly known as damselfishes, pomacentrids are represented in Brasil by 14 species (Moura *et al.*, 2005), divided into four genus. *Abudefduf saxatilis* belongs to Pomacentrinae subfamily (Nelson, 1994).

Abudefduf saxatilis is distributed around the world in tropical and subtropical latitudes, they occupy coral reefs and rocky shore substrates. During their non-reproductive phase this species, wich is commonly named sergeant major, forms feeding groups that spend the day foraging over the rocks

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(Emery, 1973). Although it is believed that this species is originally from the Western Atlantic, populations found in Red Sea, Indian Ocean and Pacific are expected to be conspecifics (Randall, 1968; Hoese & Moore, 1977). When reproducing, males assume nuptial colours, darker and bluish, and defend a territory near the bottom, where oocytes are to be deposited (Breder & Rosen, 1966; Albrecht, 1969; Prapas *et al.*, 1991). Egg laying occurs in pairs and the father stays by the territory, caring for the eggs during the embryonic development. Besides the demersal and addesive eggs, after hatching larvae can be found in ichthyoplankton samples (Prapas *et al.*, 1991).

There are not many papers focussing on reproductive aspects of reef species due to the difficulty in collecting them (Privitera, 2002). Lack of knowledge on this issue reduces the capacity of developing scientifically based projects for preserving reef fish fauna (Pajuelo *et al.*, 2003). If conservation policy's aim is to maintain these species for future generations, whether as a consumption resource or not, then this policy should begin by comprehending and enhancing the species reproduction. Although species of this family are often used in ecological studies related to reproductive behaviour and natural history, data on its sex ratio, length of first maturation and reproductive period are not abundant on literature.

This paper intends to present some parameters for the reproductive variables of *Abudefduf saxatilis*, such as sex ratio, length of first maturation and reproductive period by means of three methods: a) dynamics of gonadal maturation; b) GSI (gonadosomatic index); and c) visual census counting the number of males caring for the eggs. This information may be used as a basis for future studies on reproduction of this species, as well as explaining its reproductive needs.

Material and Methods

Individuals collected for reproductive biology analyses were caught with fishhooks size 12 to 18, light lead, and bamboo rod and nylon string number 20. Salted shrimp was used as bait. Collection areas were Barequeçaba Point (23°49'53" S; 045°25'53"W), da Praia Grande Point (23°49,3'S; 45°24,6'W), Moleques Light House (23°49,7'S; 45°24.7'W) and Pier of Ilhabela ferryboat (23°49,1'S; 45°22,1'W). Fished animals were maintained during fishery and carried to the laboratory in plastic recipients filled with salt water. Species were anesthetized till death using high concentrations of benzocaine, about 100 parts per million.

Before dissection the animals were weighed with 1g precision scale and its total length was measured with 1mm precision. A cut was made on the left lateral flank, so as to expose the abdominal cavity. A macroscopic analysis of the gonads permitted access to the sex and maturation stage, based on the classification proposed by Vazzoler (1996), which is: A - Immature; B – In maturation; C - Mature; and D-Gamet laying. Each gonad was weighed in an analytical scale down to 0,0005 g, tagged, preserved in 4% buffered formalin-water solution and processed for histology.

Coloration used was hematoxiline-eosine (HE). Material was included in paraffin and cut in 5 µm wide slices. Each slide was analysed under microscope and reclassified for its sex and maturation stage, as suggested in Dias *et al.* (1998) to compare macroscopic and microscopic observations. Histological preparations were made for three gonads of each sex in each maturation stage to confirm the precision of macroscopic observations and for all ovaries in C stage.

Sex ratio was observed in percentile of males and females based on 74 specimens. Individuals with gonads too small to be diagnosed were defined as "unidentified" (U). Sex ratio was compared through χ^2 method with expected ratio of 1:1, and with expected ratio equal to the general one found in the samples for each stage of gonadal development. Sex distribution in length classes was observed in an attempt to test differences in size between the sexes.

The mean length of first maturation (L_{50}) was identified by data of pooled gonad maturation stages (A=juvenile; B+C+D=adults) and total length as the size in which half of the population was starting maturation or was already mature. The mean size of the already mature population was also estimated (L_{100}). Values obtained graphically were analysed qualitatively due to the low sampling. A curve was adjusted by hand to point L_{50} value.

The reproductive period was estimated under four techniques: gonado-somatic index, maturation dynamics of the ovaries assessed by a maturity stage scale, condition factor and visual census to search for males guarding eggs. The three initial techniques gathered information on females only. Gonado-somatic relation (GSR), refers to the percentile proportion of the animal body represented by the gonad. Its value may be obtained by the following formulae:

$$GSR_1 = \frac{Wg.100}{Wt} \qquad GSR_2 = \frac{Wg.100}{Wb}$$

Wg = Gonad weight
Wt = Total weight
Wb = Body weight, *i.e.* total body weight minus gonad weight

Since the ovary has an important and variable influence on the total weight of the animal along the reproductive cycle, the difference between

GSR_1 and GSR_2 must present major values in the period when the ovaries contribute the most to the individuals' total weight, the period prior to laying the oocytes. Thus, the differences between these two relations were plotted in graphics along the months, evidencing as reproductive period that related to the loss of ovaries weight, due to the spawning process (Dias *et al.*, 2005).

The dynamics of gonads maturation is a good tool to indicate the time in which females are capable of reproducing. Months with great number of females with mature oocytes, followed by months with females with empty gonads are clues to the oocyte laying period, mainly if the macroscopic condition of this ovary may be confirmed by microscopic analysis (Dias *et al.*, 1998). The maturity stages of females were recorded each month, according to Vazzoler (1996), counting out the immature gonads in stage A, since their sex could not be identified macroscopically.

Visual censuses were accomplished during SCUBA dives from July 2002 to March 2003 over rocky shores of Ponta do Baleeiro (23°49'43"S; 045° 25'16"W) and Ponta do Jarobá (23°49'38" S; 045°25'50"W) (Fig. 1). They were conducted along a transect in which a rope marking every meter was extended between two points 32 m apart. The diver swam along this transect slowly and constantly, counting the number of *A. saxatilis* males caring for eggs within the visual field. The same method was applied to *Stegastes variabilis* to obtain this species reproductive period. Although two species of this genus, *Stegastes variabilis* and *S. fuscus* hardly differentiated during dives, have been recorded for the area, , the reproductive period seems to be related to *S. variabilis*, since it was the only species collected within testimony specimens. Results were presented monthly in Table 2 for absolute frequency of occurrence.

RESULTS

Sex ratio for *Abudefduf saxatilis* showed tendency for a major number of males than females. Though Chi-square test revealed no significant difference between 1:1 ratio and the values observed, p value was surprisingly close to alpha (χ^2=3,689). Male and female temporal distribution (Fig. 1a) did not indicate significant differences from 1:1. Nevertheless, chi-square test does not work precisely with values below five. No difference in size between sexes was found (Fig. 1b) nor statistically identified by Mann-Whitney test (U= 680,5 P= 0,634).

Few individuals in stage A were collected (13 out of 74), whereas length class, that separates youngs from adults had only two individuals. Hence, a statistical analysis of the first maturation length would not be precise enough. Smallest mature individuals were101 mm long and the largest immature one measured 115 mm. L_{50} was placed between 114 and 123 mm, while L_{100} was above 133 mm (Fig. 2) by a curve adjusted by hand. *Abudefduf saxatilis* reaches maturation at about 80% of its total length, around 150 mm.

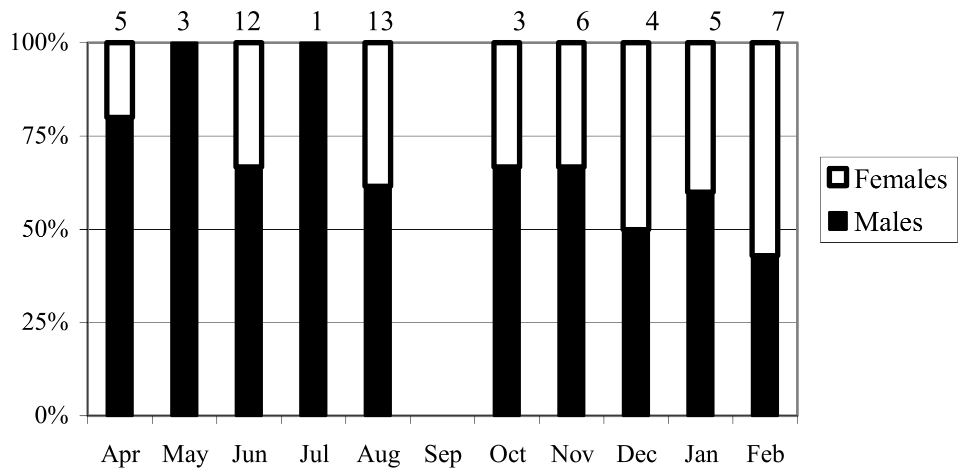

Fig. 1a. Monthly sex ratio of adult individuals. Values on top of each column indicate corresponding sample.

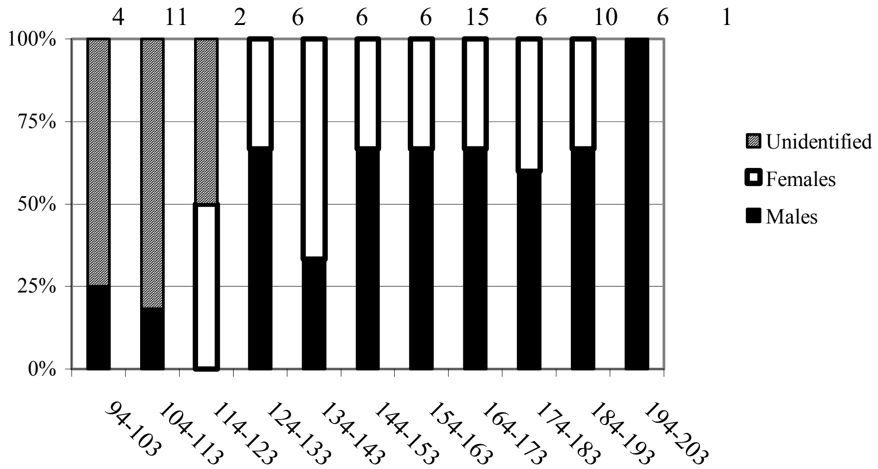

Fig. 1b. Sex ratio per length classes (including individuals with gonads unable to be identified visually). Values on top of each column indicate corresponding sample.

Table 1. Number of males, females and unidentified per stage of gonadal development (ME).

ME	Male	Female	Unidentified	n
A	0	0	13	13
B	27	5	0	32
C	11	18	0	29

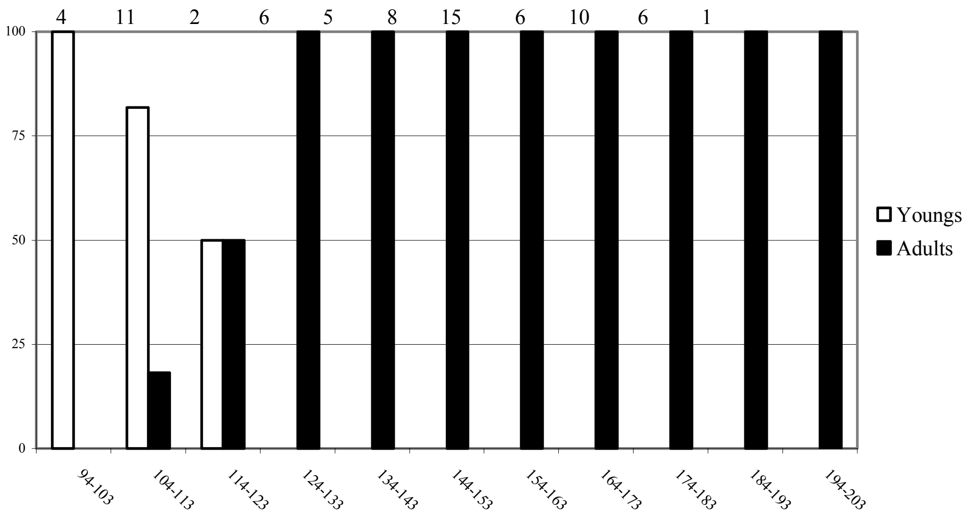

Fig. 2. Proportion of Youngs (Y) and Adults (A) per length class .Values on top of each column indicate corresponding sample.

Maximum mean values of GSR were found in November while values from December and January decreased, indicating spawning activity (Fig. 3). The proportion of females at different macroscopic stages of classification varied along the sample period (Fig. 4). Young immature females were collected in June and from October till December, and mature ovaries were also present in different proportions during all the period, except in April. Females in spawning process were not identified using macroscopic gross classification.

Males guarding eggs were found in visual censuses along the transects from November to February (Table 2). Likewise, other dives performed in the same time of the year confirm this reproduction period.

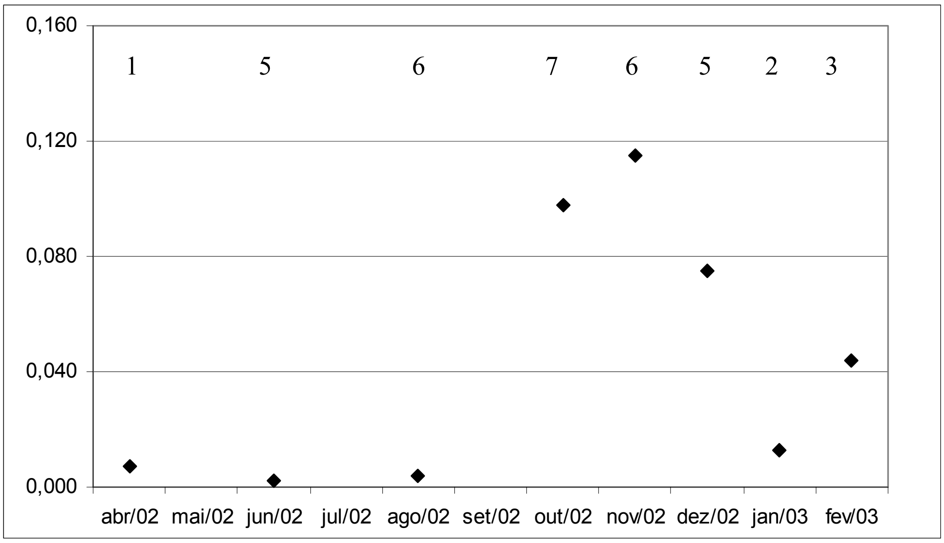

Fig. 3. Dynamics of ΔGSR along one year. Values on top of each dot indicate corresponding sample.

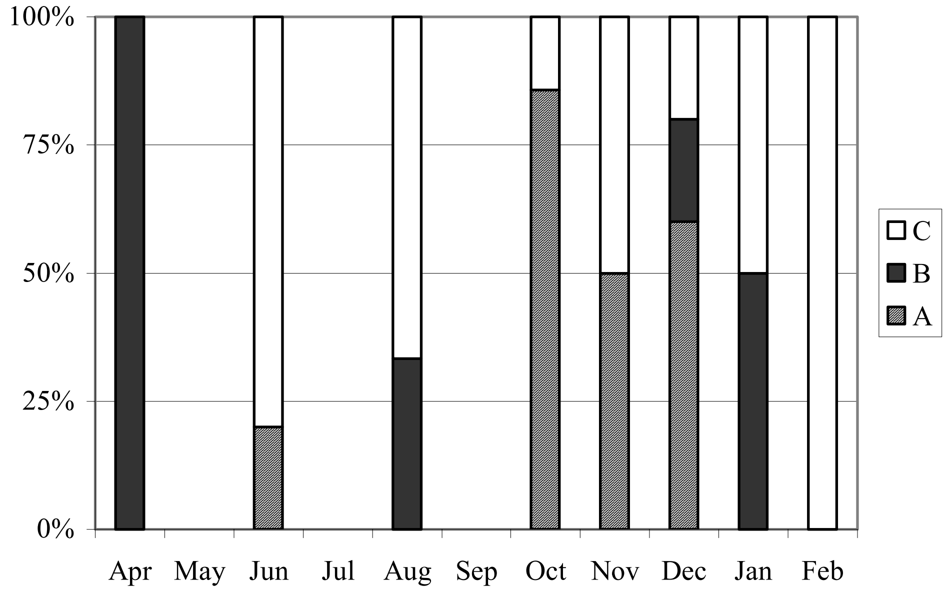

Fig. 4. Gonad maturation stage along one year. Sample for each month is the same as that shown in Fig. 2.

Table 2. Number *Abudefduf saxatilis males.* Adult keeping a territory (ad. terr.) and males guarding eggs (egg) along transects.

		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
A. saxatilis	ad. terr.	0	0	0	0	3	3	9	4	3
	egg	0	0	0	0	5	1	3	11	0

Comparing the reproductive period with the corresponding environmental characteristics observed it coincides with the warmest months of the year, for water or air temperature. Likewise, thermal amplitude and daylength also coincide with the reproductive period. Pearson's analysis did not indicate linear correlations between these factors and reproductive period. Spearman's test was also applied for non-linear correlation. Factors most likely to show any influence on reproduction (air temperature, sea temperature, daylength and thermal amplitude) were correlated to ΔGSR (Table 3). Hence, correlations were found between ΔGSR and ΔTair, Tair and daylength, and number of males with eggs and daylength (Table 4).

Table 3 – Spearman correlation coefficient values (CC) obtained by ΔGSR for physical factors and reproductive period: probability (P) and number of samples (n).

Δ GSR	Δ T air	T sea	T air	Daylength
CC	0,470	0,349	0,457	-0,585
P	0,0239	0,101	0,0285	0,0035
n	23	23	23	23

Table 4. Spearman correlation coefficient values (CC) obtained by transects, for physical factors and reproductive period: probability (P) and number of samples (n).

Transects	Δ T air	T air	T sea	Daylength
CC	0,490	0,318	0,148	0,778
P	0,217	0,438	0,720	0,005
n	10	10	10	10

Discussion

Sex ratio was slightly deviated to the predominance of males. During the reproductive period males are spending more energy with egg or territory protection, increasing its need for food. On the other hand, they should not leave the territory and the spawning for too long in search of food, or it may be taken by other males or destroyed by predators of eggs. This makes any source of food close to the territory a potential meal, including the bait. This was also proved with the main deviations from sex ratio 1:1 in reproductive months. Deviations on 1:1 sex ratio are not to be commonly expected, as it would represent a disadvantage for the individuals producing more of one sex on the availability of partners for its brood (Hamilton, 1967).

Unlike some species of Pomacentridae, *Abudefduf saxatilis* does not present sexual dimorphism of size. Sex ratio per total length class did not show sex predominance nor have these differences been reported before (Prapas, *et al.*, 1991), although females bigger than males are common in protandric hermaphroditic species as some pomacentrids are, such as clownfishes (Ross, 1978; Devlin & Nagahama, 2002; Buston, 2003).

The collect method with hook and bait did not allow many small individuals to be caught (only 13) which prejudiced the analysis of length of first maturation. First maturation representing the beginning of energy investment in reproduction, instead of growth, occurs when the species grew up to 80% of its maximum length. In *Parma microlepis*, an Australian pomacentrid slightly smaller then *A. saxatilis* (L_{max} = 145 cm), first maturation occurs around 115 mm, with mature individuals from 106 mm, corresponding to the age of five years (Tzioumis & Kingsford, 1999). Non-published data for age of first maturation of *Hypsypops rubicunda* and *Parma victoriae* also report ages between four and six years (Tzioumis & Kingsford, 1999).

Three of the four methods applied for obtaining the reproductive period for *Abudefduf saxatilis*: ΔGSR, dynamic of maturation stage and visual census, agree with November and December. These values are also in accordance with the expected period, once reef species reproductions tend to occur during the hot season in batches or continuously. Other researchers report continuous reproduction along the year for *A. saxatilis* (Albrecht, 1969; Prapas *et al*, 1991), for *Amphiprion melanopus* (Ross, 1978) and for *Abudefduf abdominalis* (Helfrich, 1959). Breder & Coates (1933) found the reproductive period of *Pomacentrus leucoris* between the beginning of spring, end of March, and first half of August in the northern hemisphere; *Chromis dispilus* lays their eggs along the summer continuously (Russell, 1971), and so does *C. multilineata* (Myrberg *et al.*, 1967). These reproductive periods were obtained by behavioural observations during dives, never with anatomical techniques such as the ones applied herein for *A. saxatilis*.

Visual census resulted in reliable data for reproductive period of *Abudefduf saxatilis*, as well as for other species with demersal eggs and parental guard. Visible egg masses and a parent to allow identification of whose eggs are these is enough to allow recognising reproductive period of a species. Though it may be possible that one male guards the eggs of some other male, nest piracy is unlikely to happen among different species (Knapp *et al*., 1995). Transects in a densely populated area allow to quantify males in reproductive activity. It is worth emphasising the importance of shifting the position of the transect between months to avoid its incidence always on the territory of a male more interesting to females, thus, more liable to have eggs, for a longer period than expected for the species.

Biological rhythms have fundamental role privileging individuals with capacity of anticipation of favourable or unfavourable conditions (Marques, 2003). Certain factors that show cyclic patterns and bring any level of interference on the individual may influence a biological rhythm. Since there are differences among characteristics that influence reproduction along the year, the ability to alter physiology and behaviour previously to environmental change would be fundamental for survival and success of the individual (Marques & Menna-Barreto, 2003). So, if *A. saxatilis* is able to synchronise its reproductive cycle with regular environmental factors that indicate the arrival of a season more propitious for reproducing, whatever the environmental clue may be, this species would enhance its capacity of gene propagation.

Among environmental factors investigated, the photoperiod is most likely to operate as a clue for *Abudefduf saxatilis*. However, it is impossible to assure a direct cause-effect relationship between photoperiod and reproduction, it is highly probable that this factor serves as a dragger for the species reproductive rhythm (Marques, 2003). It is important to remember that Pearson's analysis only recognises linear correlation between factors while Spearman's test is more efficient for detecting a correlation between order of values in each factor (Zar, 1999). A slight increase in temperature or photoperiod represents a large increase in factors that point to the reproductive period (*e.g.* gonad development).

According to data on its reproductive autoecology, *Abudefduf saxatilis* presents, characteristics for preserving its offspring at the cost of more reproductive events. Egg laying on the hot periods of the year, when food is abundant, may favour larvae survival. Likewise, hatching at night, period of less predation pressure (McAlary & McFarland, 1993), takes to a higher level of offspring survival.

This study focussed on a species inhabiting rocky shores. Due to the difficulty of collecting them in sufficient number for statistical analysis, few studies were previously done on fish from this environment. Thus, the present study comes to offer basic information of great interest for a group where little is known.

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