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Reproductive biology in the starfish *Echinaster* (*Othilia*) *guyanensis* (Echinodermata: Asteroidea) in southeastern Brazil

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ABSTRACT. *Echinaster* (*Othilia*) *guyanensis* Clark, 1987 is an endangered starfish distributed throughout the Caribbean and Atlantic Ocean. Even though it has been extensively harvested, little is known about the biology and ecology of this starfish. Here, we examine reproduction seasonality in *E.* (*O.*) *guyanensis*. Individuals were collected monthly for one year, including four complete lunar phases. The gonad index (GI) was calculated to determine annual and monthly reproductive peaks. Gametogenesis stages were also determined. Sex ratio was 1:1.33 (M:F). Gonadosomatic index, body weight, central disc width and arm length were similar for both sexes. Gonads were present in all animals with arm length greater than 36.2 mm. Lunar phase was not associated with *E.* (*O.*) *guyanensis* reproduction. GI and gametogenesis patterns suggest that starfish have an annual reproductive peak with spawning during autumn months (March to May).

KEY WORDS. Brazil; reproduction; gametogenesis; gonad index; lunar periodicity.

Starfish, Asteroidea, have a wide variety of reproduction strategies, which may be seasonal or continuous. Annual reproduction has been demonstrated for several species of starfish, including those in *Echinaster* Müller and Troschel, 1840 from the central Atlantic Ocean (Ferguson 1975) and Gulf of Mexico (Scheibling & Lawrence 1982). Reproduction patterns in *Echinaster* spp., including reproductive effort and spawning period, may be influenced by environmental factors such as temperature, hydrodynamics and abundance of food (Scheibling & Lawrence 1982).

While the annual reproductive cycle in *Echinaster* spp. from the central Atlantic is well documented, little is known about the reproduction of species from the south Atlantic (Carvalho & Ventura 2002). *Echinaster* (*Othilia*) *guyanensis* Clark, 1987 is as starfish found from southern Central America to the state of Espírito Santo (southeastern Brazil) (Hopkins *et al.* 2003).

Echinaster (*O.*) *guyanensis* is economically important, being harvested in great quantities to make handicrafts. Harvesting has caused drastic population declines (Hadel *et al.* 1999), which has generated concerns. The species is considered endangered in Brazil, where collecting is prohibited by law (Normative Instruction number 5, 21 May 2004, Ministério do Meio Ambiente).Little is known about its biology, especially reproduction. Here we examine how reproduction in *E.* (*O.*) *guyanensis* varies throughout the year and with respect to lunar cycles, and help the authorities to use that information to develop conservation strategies in Brazil.

MATERIAL AND METHODS

Samples were collected in Pedra da Sereia (20º20'6.39"S, 40º16'43.59"W), an emergent rocky formation near the beach line of the northern spit of Praia da Costa, Vila Velha, Espírito Santo (Martin *et al.* 1996). The region is classified as tropical humid and water temperatures ranged from 25.5 – to 23.5ºC and salinity 35.5-33.0. Rocks offer a variety of microhabitats where several species may settle, including *E.* (*O.*) *guyanensis*. A total of 180 specimens were collected (collection license number 17260-1). Starfish were collected monthly from December 2007 to November 2008. Ten individuals were collected from a depth of 0-4 m each month, always in the first moon quarter.

Specimens were identified following Hopkins *et al.* (2003) and with the assistance of Carlos Eduardo Rezende Ventura (Museu Nacional/UFRJ). Arm length was measured with a caliper. Gonads were removed and weighed and the whole animals were weighed to 0.01 g. Gonad index – GI (gonad wet weight/body wet weight x 100) was calculated for each individual (Pastor-de-Ward 2007).

Mean GI was calculated for the population on a monthly basis. GI values were subsequently grouped by season (spring, summer, autumn and winter). GI values of the population sampled were compared among seasons using the Kruskal-Wallis Anova ($p < 0.05$) and the non-parametric Tukey test ($p < 0.05$) (Zar 1999).

Starfish are not externally sexually dimorphic. Sexes were identified after analysis of histological sections of the gonads of each animal. For sex identification and to describe gametogenesis, a small piece of gonadal tissue was dehydrated, embedded in paraffin wax, sectioned (7 µm), mounted and stained with hematoxylin and eosin. Sexes were compared with respect to weight, disc diameter, arm length and GI using Student's *t* test ($p < 0.05$). To describe gametogenesis, 24 starfish (12 of each sex) were used from each season. Gonad development was described in three stages, based on the frequency of cell types, size and shape of acinus, with adaptations, BYRNE *et al.* (1997) and PASTOR-DE-WARD *et al.* (2007). Frequency of gametogenesis stages were compared seasonaly for each sex.

All starfishes collected were included in the sex-ratio estimation using tested by chi-square (ZAR 1999).

Samples were sorted into size classes (mm) following Sturge's rule (VIEIRA 1991). Eight size classes were defined, for which relative frequency (%) and median GI were calculated. During the monthly collections, additional individuals were collected (arm length < 21 mm) to estimate body size at the onset of sexual maturity.

Sampling was conducted in four complete moon phases every three months in the second month of the season (February, May, August, and October/November 2008). Five individuals were collected at each phase (new, first quarter, full and last quarter) during these months. Mean GI were calculated and the variation in terms of moon phase was assessed using the Kruskal-Wallis Anova and the non-parametric Tukey test ($p < 0.05$).

Sea water temperature and salinity were measured during the 12-month sampling period, using a multiparameter YSI 85, and compared with regressions in terms of the variation in GI to detect patterns.

RESULTS

Although water temperature varied monthly, it did not seem to vary seasonally. By contrast, salinity varied seasonally, and was greatest in summer and lowest in autumn and winter (Fig. 1).

The GI in both sexes was greatest in March and May and lowest in August and September. The GI peaked mainly when temperatures were high and salinity declined (Figs 1 and 2). The GI was greatest in autumn, and was similar and lower during the other seasons (Fig. 3).

Gametogenesis stages were [classified into] growing, mature and spent. 1) Growing – female: primary oocytes were present due to vitellogenesis and remained attached to the acinus wall, surrounded by nutritive phagocytes; male – massive proliferation of the germinal epithelium and spermatozoa began to accumulate in the lumen of the acinus. 2) Mature – female: ovaries were filled with large polyhedral closely packed eggs, nutritive phagocytes were absent or formed a pale, thin network around the small oocytes; male: a dense mass of spermatozoa accumulated in the lumen of the acinus. 3) Spent – female: ovaries were heterogeneous and contained primary oocytes clustered on the acinus wall, some ovaries had numer-

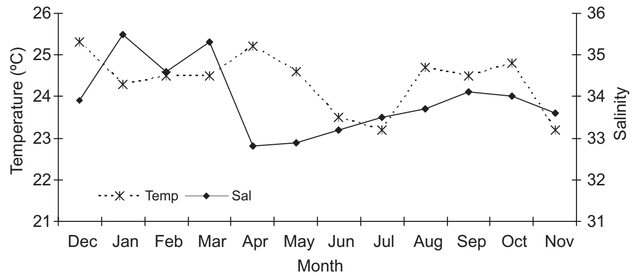

Figure 1. Water temperature and salinity during our study (December, 2007 to November, 2008) of *Echinaster* (*Othilia*) *guyanensis* in southeastern Brazil. Each point represents one day.

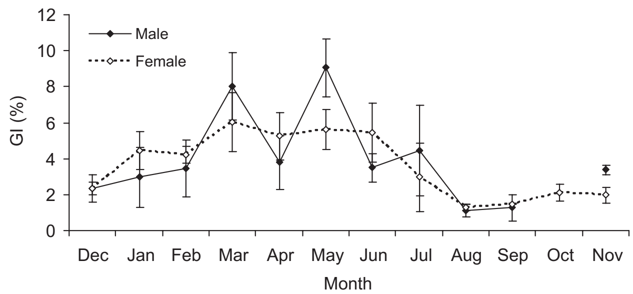

Figure 2. Monthly variation of the gonad index (GI) of males and females of *Echinaster* (*Othilia*) *guyanensis* collected in southeastern Brazil (Dec., Jan., Fev., Mar., Apr., Jun., Ago., Sept. n = 5 females and 5 males; May., Jul., n = 6 females and 4 males; Oct. n = 10 females; Nov. n = 4 females and 6 males).

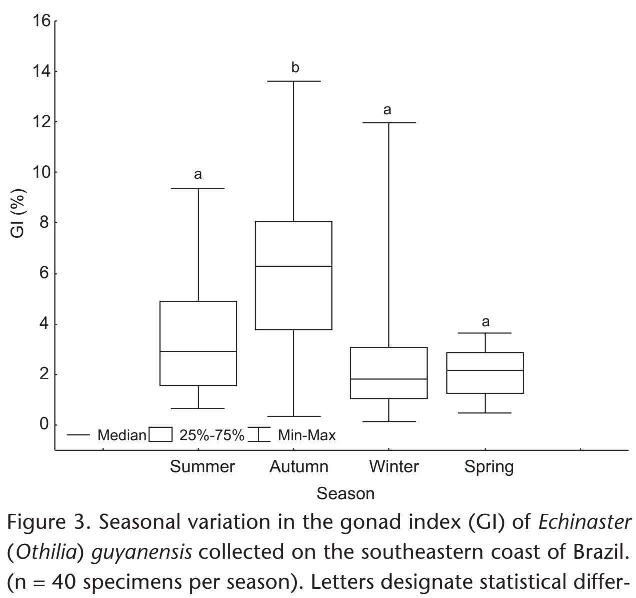

Figure 3. Seasonal variation in the gonad index (GI) of *Echinaster* (*Othilia*) *guyanensis* collected on the southeastern coast of Brazil. (n = 40 specimens per season). Letters designate statistical differences (different letters) as indicated in the non-parametric Tukey test ($p < 0.05$).

ous relict eggs, and others had abundant nutritive phagocytes with a few relict eggs; male: lumen was almost empty in testes, although with relict spermatozoa. Gonad walls became shrunken with a pale network of nutritive phagocytes.

In summer, 50% of males and 60% of females were mature and~ 30% of gonads were in the growing stage (Fig. 4). In autumn, mature male and female frequencies increased to 85%. For both sexes, spent gonads comprised 60% of all gonads in the winter and 75% in the spring.

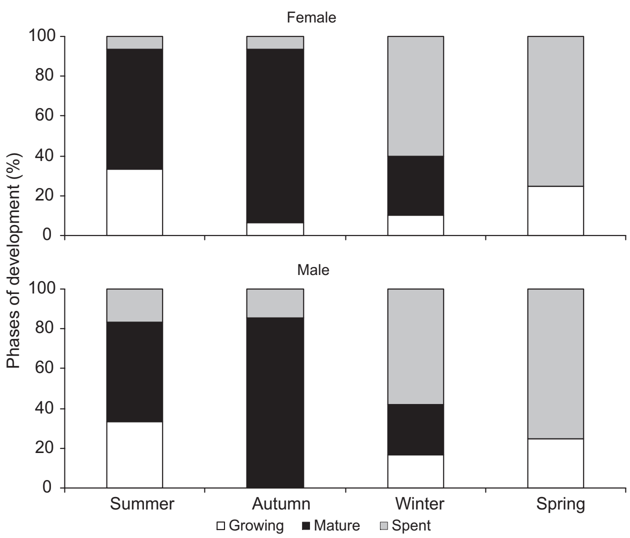

Figure 4. Seasonal variation of gonadal development phases of *Echinaster* (*Othilia*) *guyanensis* collected in southeastern Brazil (n = 12 males and 12 females per season).

Median GI was similar among moon phases at 4.57, 2.55, 2.69 and 3.85% in new, first quarter, last quarter and full moon phases (Fig. 5). Thus, the moon apparently does influence spawning in *E.* (*O.*) *guyanensis*.

A total of 176 starfish were used to estimate sex ratio, of which 58 were males, 77 females, 34 juveniles (without gonads) and seven adults were also undetermined. Males and females were approximately equally abundant, with the male:female sex ratio at 1.00:1.33 ($\chi^2 = 0.465909$; p > 0,05). Size (morphology) and GI were also similar between the sexes (Tab. I).

Table I. Morphological measurements (mean ± standard deviation) of *E. (O.) guyanensis*. No sexual dimorphism was found (all comparisons p > 0.05).

Variable	Male (n = 43)	Female (n = 48)
Body wet weight (g)	12.3 ± 0.8	12.5 ± 0.5
Central disk diameter (mm)	16.9 ± 0.3	17.3 ± 0.3
Arm length (mm)	36.3 ± 0.8	37.4 ± 0.8
GI (%)	4.0 ± 0.5	3.8 ± 0.3

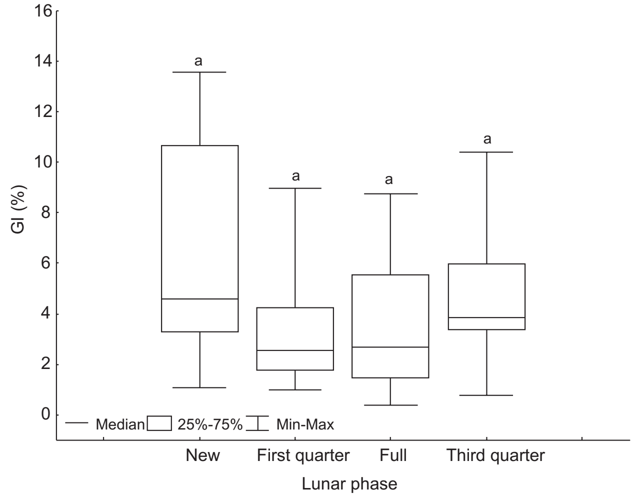

Figure 5. Variation in the gonad index (GI) of *Echinaster* (*Othilia*) *guyanensis* collected in southeastern Brazil (n = 20 specimens per phase) according to the moon phase. Letters designate statistical differences (different letters) as indicated in the non-parametric Tukey test (p < 0.05).

Arm length varied between 21.0-51.3 mm and animals were grouped into eight arm length classes (Fig. 6). The greatest relative frequency was in the class 32.4-36.2 mm (23%), followed by 28.6-32.4 mm (19%). The lowest relative frequency (3%) was the largest class (47.6-51.4 mm). The greatest GI was in animals > 36.2 mm and the lowest GI in the three smallest size classes. Starfish with arm length < 20.01 mm did not have gonads. Fifteen percent of the individuals with arm length between 24.80-28.60 mm had gonads, while 100% of the starfish with arm length > 36.20 mm had gonads.

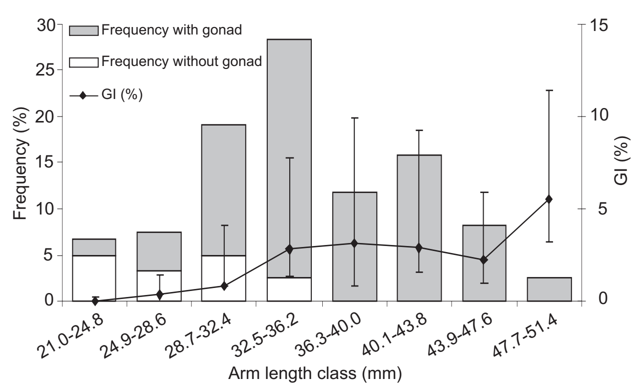

Figure 6. Median gonad index distribution (%) (upper error bar = 75% and lower error bar = 25%), relative frequency and relative frequency of animals with and without gonads of the several arm length class of *Echinaster* (*Othilia*) *guyanensis* collected in southeastern coast of Brazil (n = 120; 54 males and 66 females).

DISCUSSION

To better determine timing and duration of the breeding season of a starfish, [both] the GI method and histological examinations should be used (Pastor-de-Ward *et al.* 2007). Gonad index values were greater in autumn and lower in the spring. Histological examinations agreed with GIs: gametogenesis in both sexes started in summer and spawning occurred in autumn. The spring was characterized as a recovery period with most gonads classified as "spent."

Gametogenesis also indicates synchronous spawning in males and females. The greatest advantage of a seasonal reproduction cycle is the synchronous and syntopic release of male and female gametes, which increases the probability of fertilization (Carvalho & Ventura 2002, Raymond *et al.* 2007, Bos *et al.* 2008). Annual reproductive cycles have been reported for many species of Asteroidea in different locations (Scheibling & Lawrence 1982, Chen & Chen 1992, Guzman & Guevara 2002) yielding results consistent with ours. The reproductive peak for this group usually occurs usually between autumn and winter (Tab. II).

Reproduction in *E.* (*O.*) *guyanensis* does not follow a lunar pattern. The main explanation for this observation is that the reproduction pattern of the species is annual and non-continuous. Normally, spawning is strongly influenced by lunar phases in species whose spawning pattern is continuous, as already observed for several Echinoidea (Coppard & Campbell 2005, Muthiga 2005). The effect of lunar phases on the spawning of Asteroidea has been observed only for *Protoreaster nodosus* Author, (Linnaeus, 1758)in captivity (Scheibling & Metaxas 2008).

The equal sex ratio in *E.* (*O.*) *guyanensis* is also present in *Oreaster reticulatus* Linnaeus, 1758 (Guzman & Guevara 2002) and *Cosmasterias lurida* Philippi, 1858 (Pastor-de-Ward *et al.* 2007). Equal sex ratios are common in species with sexual reproduction. Furthermore, equivalent numbers of males and females during spawning seem to confer anadvantage in marine environments, which have dispersal rates that cause gamete loss. One exception is *Allostichaster capensis* Perrier, 1875, with a sex ratio of 1:420. This species reproduces by binary fission (Rubilar *et al.* 2005), which precludes the need for a balanced sex ratio.

Females in *Archaster typicus* Müller & Troschel, 1840 (Run *et al.* 1988) and *P. nodosus* (Bos *et al.* 2008) invest more on reproduction and have greater GI values than males. This result was contrary to our data, were no difference was observed between GI of males and females of *E.* (*O.*) *guyanensis*. The similar reproduction pattern between males and females obtained in this study were consistently to several Asteroidea species with GI < 5% (review in Run *et al.* 1988).

Eight size classes were defined in terms of the variation in arm length. GI results reveal that the investment in reproduction is greater in animals with arm length > 32.4 mm. In smaller size classes, GI was always near 0%. In the three largest classes some individuals had darker gonads, which indicates aging, as in other echinoderms (Agatsuma *et al.* 2005). The 36.2-40.0 mm size class was the smallest class with all individuals having gonads, and therefore the smaller size classes tended to be juveniles.

We suggest that management for the conservation of the species should include the collection of only animals with arm length > 44 mm (around 10% of the population). We also recommend that collecting during autumn and winter be avoided because that is when reproduction takes places. If these two suggestions are followed, we predict that populations will recover.

Table II. Reproductive seasons of some representative starfishes.

Species	Reproductive Season	Region	Source
Allostichaster capensis (Perrier, 1875)	Spring	Patagonia, Argentina	Rubilar *et al.* (2005)
Anasterias minuta Perrier, 1875	Autumn	Patagonia, Argentina	Gil & Zaixson (2007)
Archaster typicus Müller & Troschel, 1840	Winter	Taiwan	Run *et al.* (1988)
Asterina stellifera (Möbius, 1859)	Winter	Southeastern Brazil	Carvalho & Ventura (2002)
Coscinasterias tenuispina (Lamarck, 1816)	Winter	Southeastern Brazil	Alves *et al.* (2002)
Protoreaster nodosus (Linnaeus, 1758)	Autumn	Indo-Pacific	Bos *et al.* (2008)
Cosmasterias lurida (Philippi, 1858)	Autumn	Patagonia, Argentina	Pastor-de-Ward *et al.* (2007)
Echinaster echinophorus (Lamarck, 1816)	Autumn Winter	Florida, USA	Atwood (1973)
Echinaster sp. 1	Autumn	Gulf of Mexico	Scheibling & Lawrence (1982)
Echinaster sp. 2	Autumn	Gulf of Mexico	Scheibling & Lawrence (1982)
Echinaster sp.	Autumn	Florida, USA	Ferguson (1975)
Echinaster (O.) guyanensis Clark, 1987	Autumn Winter	Southeastern Brazil	Present study

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