Reproductive biology of *Echinometra lucunter* (Echinodermata: Echinoidea) in a northeast Brazilian sandstone reef

EDUARDO J.B. LIMA¹, PAULA B. GOMES² and JOSÉ R.B. SOUZA¹

¹Departamento de Zoologia, Centro de Ciências Biológicas (CCB), Programa de Pós-Graduação em Ciências Área de Biologia Animal, Universidade Federal de Pernambuco (UFPE), Av. Professor Moraes Rego, 1235 50670-420 Recife, PE, Brasil

²Departamento de Biologia, Universidade Federal Rural de Pernambuco (UFRPE), Área de Ecologia Rua Dom Manoel de Medeiros, s/n, 52171-900 Recife, PE, Brasil

Manuscript received on April 2, 2008; accepted for publication on July 22, 2008; presented by ALEXANDER W.A. KELLNER

ABSTRACT

The edible sea urchin *Echinometra lucunter* (Linnaeus, 1758) is a very common species on the sublittoral-midlittoral in Brazilian rocky shores. The aim of this work was to describe the gametogenesis and reproductive strategy of the *E. lucunter* population at Muro Alto beach in the Northeast coast of Brazil from August 2004 to August 2005. A total of 240 specimens were collected on the sandstone reef flat from a tidepool during spring low tides. The overall sex ratio was 1.12:1, without significant temporal variation except in October 2004. First sexual maturity occurred in individuals from a diameter of 20.8 mm. There was not a significant difference in gonad index between females and males during the sampling period. The female’s gonad index variation was associated with a well-defined spawning, corroborated by the histological analysis of the gonads, which demonstrates sex differences of the gamete production. By contrast, the males showed no clear pattern. It is suggested that continuous reproduction with seasonal peaks in the *E. lucunter* population occurs at Muro Alto beach.

Key words: beach rock, Brazil, Echinodermata, reproduction, sea urchin.

INTRODUCTION

*Echinometra lucunter* (Linnaeus, 1758) is a rock-boring species that feeds on algae and encrusting animals, and can be found in coastal waters up to 45 m deep (Tommasi 1966), often inhabiting burrows scraped with its spines and teeth on rocky substrata (Hendler et al. 1995). Its geographical distribution extends south from North Carolina (USA) and the Bermudas to Santa Catarina (Brazil), including the Caribbean and West Central America, and throughout East Africa (Bernasconi 1955, Tommasi 1966, Brito 1968, Hendler et al. 1995). It is the regular echinoid most commonly found on the coral reef formations in Pernambuco State (Brazil), and is used as an indicator of the sublittoral-midlittoral edge (Fernandes et al. 2002).

High-density populations (12–100 urchins/m²) of *E. lucunter* have been implicated in reef damage at various locations; boring behavior alters the community’s physical and biological structure (Hendler et al. 1995, Appana and Vuki 2003). Because of the density-dependent nature of the impact of urchins on some communities, knowledge of their life-history is essential for understanding the ecology of these interactions (Williamson and Steinberg 2002).

Several studies on the taxonomy and/or distribution of the *E. lucunter* have been made for the tropical Atlantic Ocean, such as Rathbun (1879), Bernasconi (1955), Tommasi (1966), Brito (1968), Lima-Verde...
The specimens were fixed via peristome with formaldehyde 4%. Four measurements were taken from each animal, with a digital Caliper (0.01 mm precision): maximum and minimum diameters of the test, test height, and Aristotle’s lantern height. Next, the interradius gonad, which opens on the gonopore from the madreporite, was extracted and fixed with formaldehyde 10% for 72 hours, dehydrated in alcohol series, diaphanized in toluene and embedded in histological parafin. Sections were made perpendicular to the axis of greatest length of the gonad at a thickness of 7μm cut from the middle portion of the blocks, and stained with haematoxylin-eosin (H.E.). The dissected test (spines + washed gut + Aristotle’s lantern) and remaining gonads were put separately into a furnace at 120°C for 48 hours, after which the dry weight was assessed with a 10⁻⁴ g precision balance.

The classification of gametogenic development stages were modified from Tavares and Borzone (2006), which were described in results. The size at the onset of sexual maturity was estimated from the histological analysis of 45 specimens (10–30 mm in maximum diameter of the test) collected in October 2004, during period of maximum maturity in the adult population.

The gonad index (GI) was calculated monthly, and expressed as a percentage: GI = [dry weight of 4 gonads/dry weight of dissected test – (gonads + gut content)] × 100, modified from MacCord and Ventura (2004). The non-parametric Kruskal-Wallis test (H) was used to compare the gonad index of sexes and throughout the year. Spearman’s correlation coefficient (r_s) was applied to establish the relationship between the gonad index and the morphological parameters (maximum and minimum diameters of the test, test height, and Aristotle’s lantern height; n = 195) for both sexes as gonad index for males and females were not significantly different. Chi-square test (χ²) was used to test differences in the sex ratio (Siegel 1975). All analyses were done with BioEstat 3.0 software (Ayres et al. 2003), using a 5% significance level.
RESULTS

ENVIRONMENTAL PARAMETERS

The temperature of the tidepool water varied between 28°C (July-August 2005) and 37°C (April 2005); and the salinity, between 35 (June 2005) and 39 (November-December 2004, March-April 2005).

SEX RATIO

Of 195 adults specimens collected, 103 (52.82%) were females and 92 (47.18%) were males. The overall sex ratio in the population of *E. lucunter* was 1.12:1, without significant difference between sexes ($\chi^2 = 0.62; df = 1, p = 0.431$). Furthermore, there was no significant variation from a sex ratio of 1:1 in monthly samples of urchins, except in October 2004 ($\chi^2 = 5.40; df = 1, p = 0.020$).

HISTOLOGY OF THE GONADS

Based on previous authors (Tavares and Borzone 2006), five stages were used to characterize the process of gametogenesis in *E. lucunter*:

**Proliferative stage:** gonad tubes were filled with nutritive phagocytes (storage cells), and small numbers of primary sex cells (oocytes in females, spermatocytes in males) were present along the tube wall. In females, the nutritive layer has attained its maximum thickness and oogonia were visible as intensely stained cells near the tube wall. In the early development of this stage, spermatocyte columns were sometimes observed in males, with a basophilic layer of spermatogonia and primary spermatocytes lining the tube wall. Few mature gametes (ova or spermatozoa) were found in the center of the tube among nutritive phagocytes.

**Premature stage:** the abundance of nutritive phagocytes decreased and the first mature gametes (ova or spermatozoa) began to accumulate in the lumen. Nutritive phagocytes were closed between tube wall and lumen. In females, nutritive phagocytes occupied a peripheral position. In males, most of the columns of developing spermatocytes extended from the tube wall into the lumen. Spermatozoa accumulated within the lumen among the few remaining nutritive phagocytes.

**Mature stage:** most of the lumen was occupied by mature gametes, and nutritive phagocytes were reduced to a thin layer along the tube wall. In females, most of the ovarian lumen was filled by mature gametes with polygonal shape that exhibited a great number of cortical granules at their surface. Few primary oocytes were found and nutritive phagocytes were located towards the periphery along the tube wall. In males, spermatozoa often appeared to swarm in strands. The tubes were filled with a dense mass of spermatozoa in the lumen. In some male gonads, a straight band of primary cells were still present but nutritive phagocytes were absent.

**Depletion stage:** the lumen was emptied as mature gametes were shed but not yet replaced to any great extent by nutritive phagocytes. In females, some relict oocytes/ova may be present in the lumen, where a layer of nutritive phagocytes began to grow. Besides, in males, there were many small spaces between the masses of spermatozoa and spermatocytes, as in the masses of spermatozoa.

**Resting stage:** the lumen of the tubes contained many nutritive phagocytes and unspawned residual ova/spermatozoa.

GAMETOGENIC CYCLE

Gametogenic development stages in females of *E. lucunter* were presented in Figure 1A. The proliferative stage was recorded briefly, focusing on the period between January and May 2005, when 9 to 18% of specimens showed immature sexual cells in their gonads. The premature stage was recorded only in May 2005 (44.5%). However, from August to October 2004 the majority of females (60–92%) were in the mature stage. Spawning started in early December 2004 and lasted until June 2005, with a peak from February to April 2005 (72–100%). The resting stage was identified in all months, except February and April 2005.

The male cycle showed no annual temporal pattern (Fig. 1B). The depletion stage was recorded, except in May 2005, with high frequency value (25–100%). On the other hand, the resting stage occurred in most months, with peaks in January, March and June 2005, when 50 to 75% of specimens displayed this stage.

ONSET OF SEXUAL MATURITY

Most of the 45 specimens examined (10–30 mm in maximum diameter) did not have ripe sex cells (73%).

*An Acad Bras Cienc* (2009) **81** (1)
Fig. 1 – Monthly variation of the relative frequency (%) of each gametogenic development stage of *Echinometra lucunter* during the sampling period at Muro Alto beach, Pernambuco, Brazil. (A) Females; (B) Males. Values indicate the total number of specimens analyzed per month.

Ripe sex cells were absent from all males of *E. lucunter* smaller than 20.8 mm and from all females smaller than 21.4 mm.

**Gonad Index**

In the both sexes GI average changed synchronously in a well-defined annual pattern, reaching maximum values in dry season (September to October 2004), decreasing from the beginning of rainy season (March 2005), and rising again in late rainy season (August 2005) (Fig. 2). The gonad index of females was not significantly different from males, during the sampling period at Muro Alto beach (*H* = 0.02; *df* = 1; *p* = 0.898). But the monthly variation of GI for females and males was significant (*H* = 72.93; *df* = 12; *p* < 0.0001, respectively).

In relation to gametogenic development stages, the highest female GI values were found in the mature stage (3.72%), and the lowest value was found in the depletion stage (1.18%). The males’ GI values presented a lower range, from 1.49% in resting stage to 2.72% in proliferative stage (Fig. 3).

*An Acad Bras Cienc* (2009) 81 (1)
REPRODUCTIVE BIOLOGY OF *Echinometra lucunter*...

Fig. 2 – Monthly variation of the mean gonad index (GI) of *Echinometra lucunter* for both females and males during the sampling period at Muro Alto beach, Pernambuco, Brazil (Vertical bars indicate: ± SD, standard deviation).

Fig. 3 – Mean gonad index (GI) of *Echinometra lucunter* for both females and males in different gametogenic development stages at Muro Alto beach, Pernambuco, Brazil (Vertical bars indicate: ± SD, standard deviation). (♀) a single specimen.

Spearman’s correlation, based on a sample of 195 urchins, showed relatively low correlation coefficients between GI and morphological parameters at Muro Alto beach (Table I).

DISCUSSION

*Echinometra lucunter* presented a marked reproductive cycle in the local population as in some other regions and other regular echinoid populations (McPherson 1969, Crapp and Willis 1975, Lessios 1981, Cameron 1986, Lessios 1991, Lamare et al. 2002, Tavares et al. 2004). There was an equal sex ratio and no significant differences between male and female gonad indices. The gametogenic cycle of females was annual, and the cellular processes during gametogenesis between males and females were asynchronous.

The overall sex ratio of *E. lucunter* did not differ from other gonochoric species of echinoids (Crapp and
TABLE I

Spearman’s correlation coefficient between gonad index (GI) and morphological parameters of *Echinometra lucunter* at Muro Alto beach, Pernambuco, Brazil, August 2004 to August 2005.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>( r_s )</th>
<th>( p )</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum diameter of the test ( \times ) GI</td>
<td>-0.254</td>
<td>0.0003</td>
<td>195</td>
</tr>
<tr>
<td>Minimum diameter of the test ( \times ) GI</td>
<td>-0.315</td>
<td>0.0001</td>
<td>195</td>
</tr>
<tr>
<td>Test height ( \times ) GI</td>
<td>-0.199</td>
<td>0.0053</td>
<td>195</td>
</tr>
<tr>
<td>Aristotle’s lantern height ( \times ) GI</td>
<td>-0.319</td>
<td>0.0001</td>
<td>195</td>
</tr>
</tbody>
</table>

GI = gonad index; \( r_s \) = Spearman’s correlation coefficient; \( p \) = probability; \( n \) = number.

Willis 1975, Williamson and Steinberg 2002). In fact sex ratio estimated for *E. lucunter* at contrasting habitats was not significantly different for two Brazilian populations: sandstone reef at Muro Alto beach (this study) and rocky shores on the Galheta Island, on the southern coast of Brazil (Y.A.G. Tavares, unpublished data). Besides the overall 1:1 sex ratio, the great variability in the monthly sex ratio suggests that the individuals would be randomly distributed, and a higher monthly sample is advisable.

The population of *E. lucunter*, at Muro Alto, showed one spawning period at the dry season (October 2004 to February 2005). However, the reproduction data was obtained for a single year and this does not justify the assumption of gametogenesis cycle. Other populations of *E. lucunter* presented one or two periods of spawning. In agreement with this, in Barbados (West Indies) *E. lucunter* differed in their periodicity, spawning occurring once a year in high wave-energy habitats and twice a year in low-wave habitats (Lewis and Storey 1984). On the other hand, the Caribbean echinoid population of *E. lucunter* exhibits mixed patterns of reproductive periodicity. *E. lucunter* in Fort Randolph (Panama) reaches a peak of gonad content in October, probably spawns until December, and starts building up its gonads subsequent to that. There may be a secondary spawning right after March. In Maria Chiquita (Panama), on the other hand, if there is a seasonal trend, it is much less pronounced (Lessios 1981). This species is also reported to spawn between August and October, in Puerto Rico (Cameron 1986), and to attain a peak in gonad index during June and July, in Florida Keys (McPherson 1969).

Ventura et al. (2003) also found significant geographical variation in the gametogenic stages in *E. lucunter* populations on two Brazilian habitats, suggesting that spawning time may be longer in populations from coral reef than in those on the rocky shore. In a southern Brazilian beach, the spawning period of *E. lucunter* occurred during October and November (Tavares et al. 2004).


Different gametogenic development stages were found between females and males suggesting a reproductive asynchrony in *E. lucunter*. This asynchrony had been observed in other regular echinoids of continuous reproduction, such as *Echinometra mathaei* (Blainville, 1825) and *Lytechinus variegatus* (Lamarck, 1816) (Pearse and Phillips 1968, A.O.R. Junqueira, unpublished data). However, according to Lima et al. (2006), if the spawning occurs over the entire year, with variation in the monthly frequency of reproducing females and conspicuous periods of higher reproductive activity, then continuous reproduction with seasonal peaks may be applied. In the same manner, *E. lucunter* females and males probably do not release all of their gametes at once. Males presented many gaps in the mass of spermatozoa (depletion stage) that were observed in all months (except May 2005), indicating that spermatogenesis appears to occur faster than oogenesis, as observed by MacCord and Ventura (2004) with *Cassidulus mitis* Krau, 1954.
The first sexual maturity of *E. lucunter* occurred in individuals with a diameter of at least 20 mm. McPherson (1969) found also different sizes at sexual maturity in two species of *Echinometra*: *E. lucunter* males reaching maturity from 18 mm and females from 21 mm in diameter, whereas in *E. viridis* Agassiz, 1863 maturity was reached in individuals from about 15 mm diameter for both sexes.

Gametogenesis and intra-gonadal nutrient storage are linked in sea urchin reproduction (Walker et al. 2006). The *E. lucunter*’s GI displayed two contrasting results: males and females presented the same variation in GI, but only females showed a synchrony between GI and the gametogenic development stages. *E. lucunter*’s females seem to need a certain amount of energy for oogenesis. Thus, the allocation of resources, energy, and materials for growth, reproduction, or maintenance probably differ for both sexes. Starr et al. (1993) found that the phytoplankton abundance may also modulate the onset of spawning in echinoids. Indeed, the area presents a high quantity of material suspended at the mouth of the estuary, adjacent to Muro Alto beach that is influenced by rainfall (which has a seasonal variation) and tides, causing an increasing variation in phytoplanktonic production (Koening et al. 2003). Harmelin et al. (1981) associated the high density of echinoids to the seasonal nutrient availability on the French Mediterranean coast, as a consequence of domestic pollution. Finally, the asynchrony of *E. lucunter* between males and females depends on the seasonality of resources.

On the other hand, the larger spawning for males at Muro Alto beach show that release of spermatozoa triggers oocytes’ release. Levitan (2005) reported that generally males spawn before females. For this author, a hypothesis to explain this is that sperm are the cue for triggers oocytes’ release. Levitan (2005) reported that at Muro Alto beach show that release of spermatozoa males depends on the seasonality of resources. Indeed, the area that the phytoplankton abundance may also modulate the onset of spawning in echinoids. Moreover, the asynchrony of *E. lucunter* between males and females was reached in individuals from about 15 mm diameter for both sexes. Starr et al. (1993) found that the phytoplankton abundance may also modulate the onset of spawning in echinoids. Indeed, the area presents a high quantity of material suspended at the mouth of the estuary, adjacent to Muro Alto beach that is influenced by rainfall (which has a seasonal variation) and tides, causing an increasing variation in phytoplanktonic production (Koening et al. 2003). Harmelin et al. (1981) associated the high density of echinoids to the seasonal nutrient availability on the French Mediterranean coast, as a consequence of domestic pollution. Finally, the asynchrony of *E. lucunter* between males and females probably differs for both sexes. Starr et al. (1993) found that the phytoplankton abundance may also modulate the onset of spawning in echinoids. Indeed, the area presents a high quantity of material suspended at the mouth of the estuary, adjacent to Muro Alto beach that is influenced by rainfall (which has a seasonal variation) and tides, causing an increasing variation in phytoplanktonic production (Koening et al. 2003). Harmelin et al. (1981) associated the high density of echinoids to the seasonal nutrient availability on the French Mediterranean coast, as a consequence of domestic pollution. Finally, the asynchrony of *E. lucunter* between males and females depends on the seasonality of resources.

On the other hand, the larger spawning for males at Muro Alto beach show that release of spermatozoa triggers oocytes’ release. Levitan (2005) reported that generally males spawn before females. For this author, a hypothesis to explain this is that sperm are the cue for females to release eggs. The triggering mechanism was observed by Starr et al. (1990, 1992), in some species, including sea urchins. Besides, sex differences in spawning timing of marine invertebrates can be explained on the basis of the differential costs and benefits of spawning out of synchrony with the other sex (Levitan 2005).

ACKNOWLEDGMENTS
We thank to Fábia Lira and Elisângela Dias of the Aggeu Magalhães Research Center (CPqAM/Fiocruz-PE) for their assistance in the laboratory work for the histological procedures. We wish to thank two anonymous referees for suggestion and grammatical revision.

RESUMO
O ouriço-do-mar comestível *Echinometra lucunter* (Linnaeus, 1758) é uma espécie muito comum no infralitoral e mediolitoral do Brasil. O objetivo deste trabalho foi descrever a gametogênese e a estratégia reprodutiva da população de *E. lucunter* na praia de Muro Alto entre agosto de 2004 e agosto de 2005. Um total de 240 espécimes foi capturado de uma peca de maré situada no topo recifal, durante as marés baixas de sizigia. A razão sexual total foi de 1,12:1 sem variação temporal significativa, exceto em outubro de 2004. A primeira maturidade sexual ocorreu em indivíduos a partir de 20,8 mm de diâmetro. Não houve nenhuma diferença significativa no índice gonadal entre fêmeas e machos durante o período de amostragem. A variação do índice gonadal das fêmeas foi associada a um período de desova bem definido, corroborado pela análise histológica das gônadas, que demonstra diferenças sexuais na produção de gametas. No entanto, os machos não apresentaram nenhum padrão. Sugere-se que a reprodução da população de *E. lucunter* na praia de Muro Alto é contínua, com picos sazonais.


REFERENCES

An Acad Bras Cienc (2009) 81 (1)


Rathbun R. 1879. A List of the Brazilian Echinoderms, with Notes on their Distribution, etc. Trans Conn Acad 5: 139–158.


REPRODUCTIVE BIOLOGY OF Echinometra lucunter


