

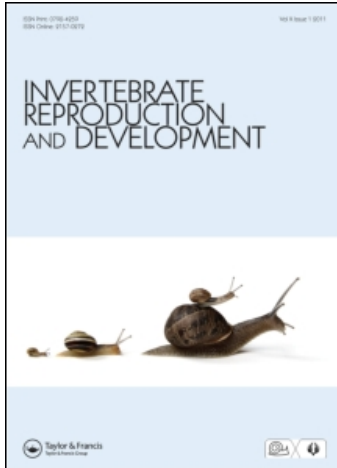
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## Reproductive cycle of the sea cucumber *Psolus patagonicus* Ekman 1925, off Mar del Plata, Buenos Aires, Argentina

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The reproductive cycle of *Psolus patagonicus* Ekman 1925 was studied. The species exhibits an annual reproductive cycle with one reproductive period in austral summer (February–March). During spring and summer, females showed the highest gonad-index (GI) values, while the GI values of males did not vary significantly throughout the year. Males had higher GI values than females. Maximum oocyte diameters ranged from less than 300 µm in spring to 900 µm in summer. In February, there is an increase in phytoplankton bloom for the bottom community in which *P. patagonicus* inhabits. The annual range in water temperature is small (6–7°C). Spawning coincided with increase in available food rather than the increase in water temperature.

**Keywords:** Holothuroidea; Dendrochirotida; Psolidae; brooding; spawning

### Introduction

*Psolus patagonicus* Ekman 1925 is a member of the order Dendrochirotida, inhabiting the Argentine Sea from 38°S offshore of Mar del Plata to Ushuaia (54°S), and the Pacific Ocean from Los Chonos archipelago to Cabo de Hornos (Bernasconi 1941; Pawson 1964, 1969; Hernández 1981; Lancellotti and Vasquez 1999; Bremec et al. 2000).

Holothurians are dioecious, but a clear sexual dimorphism is missing. Sex can be determined only from gonad squash mounts and histological preparations or in the late stage of oogenesis when large oocytes become macroscopically recognizable (McEuen and Chia 1991; Sewell 1992; Hamel et al. 1993; Guzman et al. 2003). In brooding species, females can also be identified during the incubation period (Giménez and Penchaszadeh 2010).

The single gonad is composed of simple elongated tubules and is located in the anterior part of the coelom (Hyman 1955; McEuen 1988; McEuen and Chia 1991). The reproductive cycle of various species of Dendrochirotida has been studied in terms of annual variations of gonad compared to body weight throughout the year (Rutherford 1973; Costelloe 1985; Hamel et al. 1993; Guzman et al. 2003).

Brooding behavior has been described for many temperate and Antarctic holothurians (McEuen and Chia 1991; Sewell 1994; Hamel et al. 2007), and in

*P. patagonicus* embryos are incubated in the ventral sole from February to October (Giménez and Penchaszadeh 2010).

This article describes the reproductive cycle of *P. patagonicus* off Mar del Plata, Argentine Sea, based on the gonad index (GI) between months and the histological examination of gonad development. Relationships between environmental factors and reproduction are analyzed.

The reproductive cycle in *P. patagonicus* contributes to the general knowledge in this population. This information can be used to develop management strategies because of the close relationship with the commercial exploited scallop *Zygochlamys patagonica*.

### Material and methods

Individuals of *P. patagonicus* ( $N = 254$ ) were separated from the scallop *Z. patagonica*, collected by the FRV “Capitán Cánepa” of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP). Samples were taken randomly from November 1999 to February 2001 by dredging (2.5 m mouth opening, 10 mm mesh size at the cod end) at depths between 100 and 110 m in the “Reclutas” bank zone (39°24'S; 55°56'W) of the Argentine Sea. At each sampling, depth and temperature (with a CTD SBE 911) were measured. Animals were separated from their

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substratum, fixed in 5% formalin–seawater, and preserved in 70% ethanol. There is a voucher on the Museo Argentino de Ciencias Naturales (37574 – MACN).

Total body length was measured with a 0.1 mm Vernier caliper. Gonads were dissected, weighed with a Mettler balance (precision = 0.0001 gr), and fixed for subsequent histological analysis.

The GI (=100 × gonad weight/body weight) was determined monthly for males and females. These values were analyzed statistically by parametric (ANOVA) and non-parametric (Kruskal–Wallis and Dunn's) tests (significance level:  $p < 0.05$ ). The total number of adult specimens analyzed was 254.

Fixed gonads were dehydrated, embedded in paraffin and Leica Historesin® embedding media, and sectioned with a Leitz microtome (10 μm sections) and a Leica RM2155 rotary microtome (5 μm sections), respectively, and then stained in hematoxylin and eosin.

Slides were examined and photographed with a Zeiss AxioImager Z1 light microscope. The sex ratio was analyzed with a Chi-squared test. The oocytes with visible nuclei were classified into three categories based on diameter (<300 μm, 300 to <600 μm, and 600–900 μm). The presence or absence of spermatozoa in the testis was established.

## Results

As in other holothurians, the sexes in *P. patagonicus* are separate, a clear external sexual dimorphism is missing, and the gonads are made of tufts of elongate tubules. In *P. patagonicus* males possess longer tubules than females. Mature gonads were present in individuals larger than 14 mm, and those individuals were selected for the reproductive study. The sex ratio did not deviate significantly from 1:1 ( $\chi^2 = 0.01$ ,  $df = 1$ ,  $p < 0.92$ ) corresponding to 129 males and 125 females.

The bottom water temperature ranged between 6°C and 7°C. A small increase (from 6.2°C to 6.7°C) was observed from April to June (Figure 1).

For males, the GI varied between 6 and 13, and for females between 1 and 4 (Figure 2a and b). Males showed an increase in GI from December to June and a decrease from September to November, followed by another increase in December (Figure 2a). However, there were no significant differences among months ( $F_{(1,13)} = 1.78$ ;  $df = 12$ ;  $p = 0.06$ ).

For females neither homoscedasticity nor normality could be fulfilled; Kruskal–Wallis method ( $H_{(1,13)} = 49.92$ ;  $df = 11$ ;  $p < 0.05$ ) revealed some significant differences and a *post hoc* test was carried out (Dunn's test) (Table 1) among months were evaluated for multiple comparisons (Figure 2b). The difference in female GI between November 1999 and January 2000

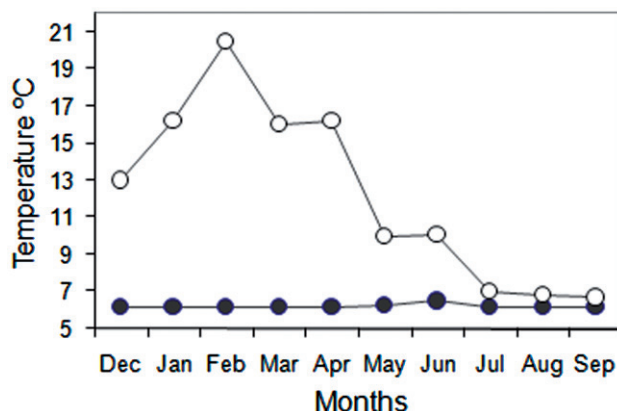


Figure 1. Temperature water in the study area. Open circles, bottom water temperature at 100–110 m depth. Solid circles, surface temperature.

was significant, as was the difference between March and May 2000. From May to October 2000, no significant differences were observed, except in August. There were significant differences between October and both November and December, but not between November and December.

Mature sperm in males were observed in February and March 2000 (Figure 3b) as well as in November and December 2000 (Figure 3d). During the rest of the annual cycle, the testis showed active spermatogenesis, but mature spermatozoa were lacking (Figure 3a and c).

In females, growing oocytes were found throughout the year (Figure 4). Previtellogenic oocytes occupied the ovary (Figure 5a–d). From October to March, the ovary showed very active oocyte development, and the wall of the ovary was composed of a simple epithelium of cylindrical cells with granular contents (Figure 5b). This type of ovarian wall was observed during months of rapid oocyte growth (Figure 5a–e).

Previtellogenic oocytes have diameters up to 300 μm. Vitellogenic oocytes range from 300 to 600 μm in diameter (Figure 5a–d and g). In their final growth phase, fully grown oocytes reached 900 μm in diameter (Figure 4), with a large number of yolk granules (Figure 5c). Oocytes larger than 900 μm in diameter were spawned between February and March 2000. Post-spawning individuals were found during March. Unspawned oocytes within the ovary underwent lysis (Figure 5f and g). Remains of unspawned oocytes could be found in the ovary from May to August 2000 and in February 2001 (Figure 4).

Histological sections of specimens collected after the period of maximum spawning indicate that approximately 45% of the remaining oocytes with a nucleus and visible nucleoli showed signs of atresia in the unspawned oocytes (Figure 5f). In February 2001, 35% of the oocytes were degenerating oocytes.

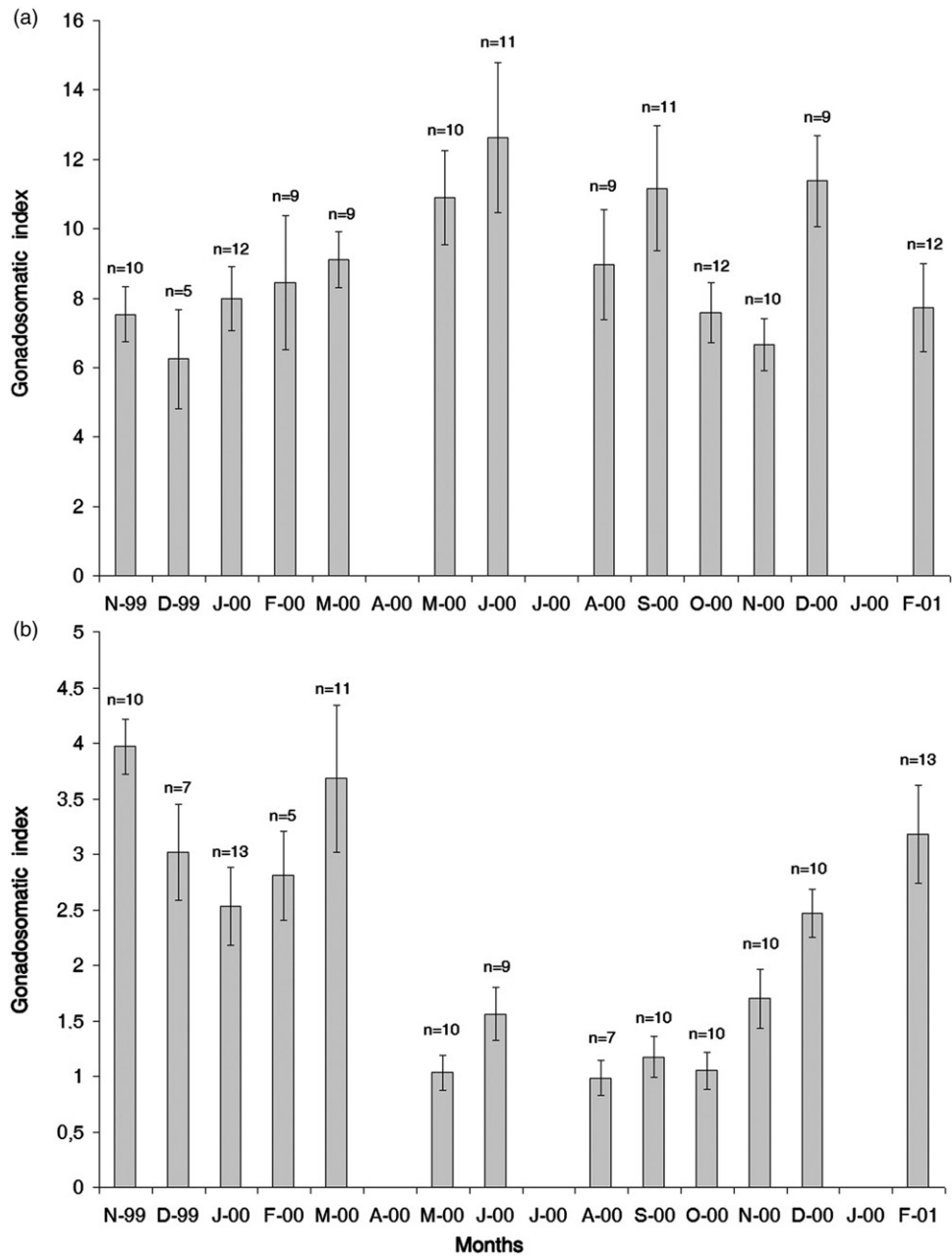


Figure 2. GI from November 2000 to February 2001: (a) adult males and (b) adult females.

Table 1. Dunn's contrast *post hoc* test significant differences found between months.

	June 2000	September 2000	October 2000	December 2000	February 2001
January 2000	NS	NS	NS	NS	-36
February 2000	NS	-31	-4.2	NS	NS
May 2000	-58	-19	-46	NS	NS
June 2000		-16	-43	NS	NS
September 2000			-34	NS	NS
November 2000				-11	NS

Note: NS, no significance.

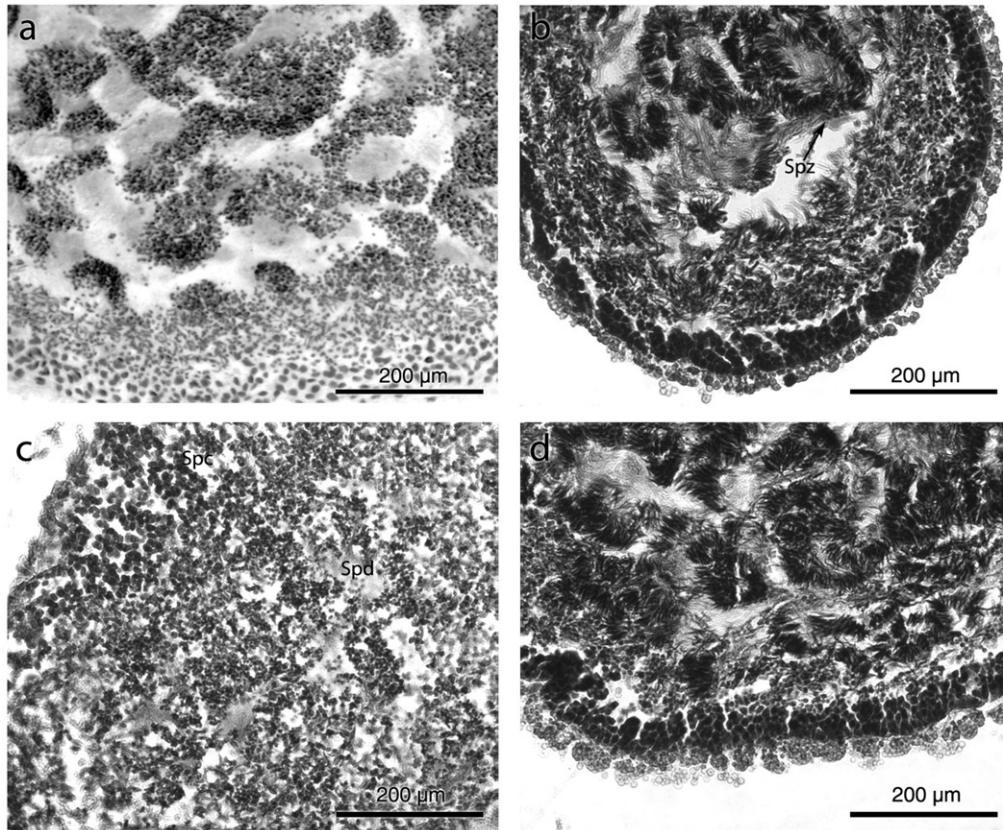


Figure 3. Light micrographs showing histological sections of testes from adult males of *Psolus patagonicus*: (a) active stage of spermatogenesis in the month of May (Spz); (b) testis showing mature spermatozoa (Spz); (c) testis in the month of August showing spermatids (Spd) and spermatocytes; and (d) testis with mature spermatozoa in the month of December.

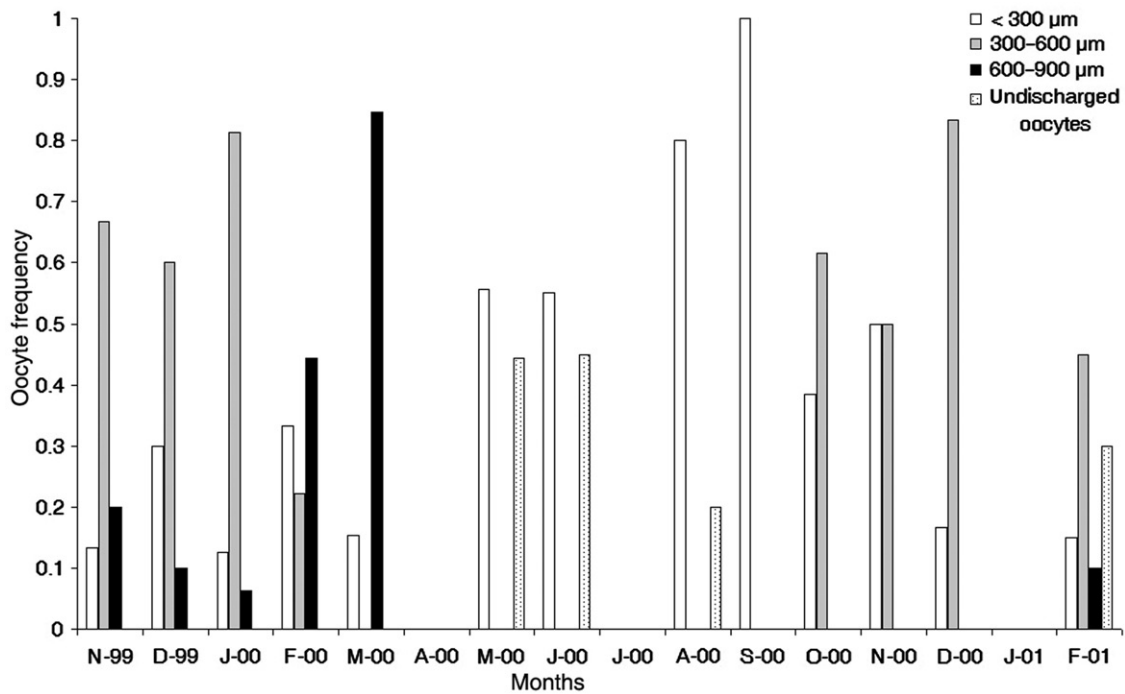


Figure 4. Frequency distribution of oocyte diameters in different size groups (<300 µm; 300–600 µm; 600–900 µm and undischarged oocytes) from November 1999 to February 2001.

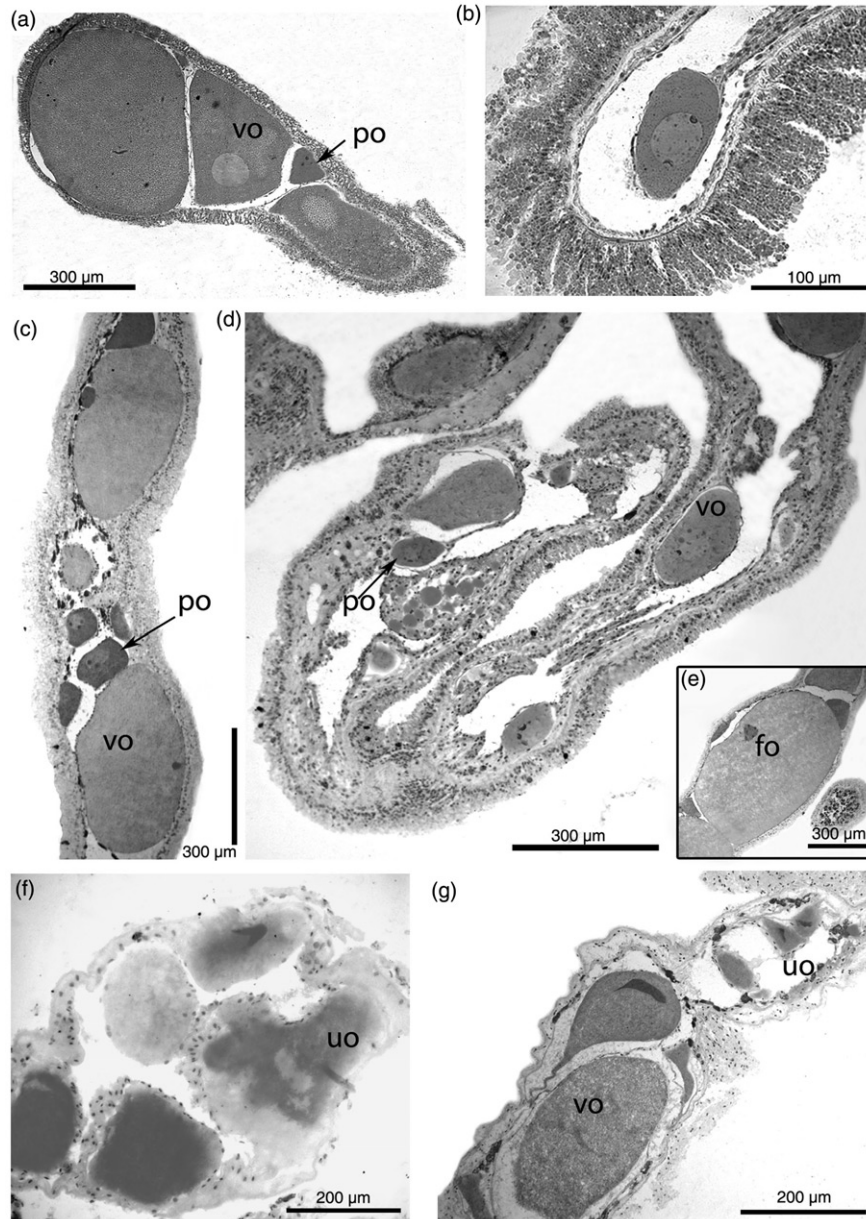


Figure 5. Light micrographs showing histological sections of ovaries from adult females of *Psolus patagonicus*. (a) Ovary showing previtellogenic oocytes (pv) and vitellogenic oocytes (vo) during October. Scale bar 300 µm. (b) Ovary with oocyte in previtellogenic stage. Note the simple cylindrical epithelium with granular contents. (c) Ovary in the month of December showing previtellogenic, vitellogenic oocytes, and scale bar 300 µm. (d) Ovary in the month of March with an elongated wall, after the spawning period. Scale bar 300 µm. (e) Detail of fully grown oocytes (fo) 750 µm in diameter from March. (f) Ovary from the month of June, showing unspawned oocytes (uo). (g) Ovary from August with unspawned oocytes and vitellogenic oocytes.

## Discussion

This study indicates that *P. patagonicus* exhibits an annual reproductive cycle with one reproductive period in late austral summer (February–March). Annual cycles have also been described for some other species of the order Dendrochirotida; for example, *Cucumaria pseudocurata* Deichmann, 1938, reproducing in winter (Rutherford 1973), and *Aslia lefevrei* Barrois, 1882, and *Holothuria forskali* Delle Chiaje, 1823,

reproducing in spring when water temperature increases (Costelloe 1985; Tuwo and Conand 1992). In particular, a breeding season on the austral summer was found for *Actinopyga echinites* (Jaeger 1833) and *Holothuria leucospilota* (Brandt 1835) in La Réunion (21°07'S, 55°32'E) (Gaudron et al. 2008; Kohler et al. 2009).

In *P. patagonicus*, small oocytes (<300 µm) were found in the ovary all year round and intermediate

oocytes (300–600  $\mu\text{m}$ ) were observed from spring to summer. During late spring and summer, when the female GI increases, a histological analysis also revealed the additional presence of large oocytes (600–900  $\mu\text{m}$ ). The maximum oocyte diameter in *P. patagonicus* (900  $\mu\text{m}$ ) is clearly smaller than in *Psolus fabricii* Duben and Koren, 1846, where oocytes can attain diameters up to 1400  $\mu\text{m}$  (Hamel et al. 1993). Conversely, oocytes of *A. lefevrei* and *Stichopus mollis* have maximum sizes of only 600 and 180  $\mu\text{m}$ , respectively (Costelloe 1985; Sewell 1992).

Unlike the situation in *P. patagonicus* with more or less continuous development and growth of oocytes, *P. fabricii* and *H. leucospilota* passes a resting stage after spawning (Hamel et al. 1993; Gaudron et al. 2008). In *S. mollis*, the gonad is completely reabsorbed in both sexes, so that no gonadal tissue exists for five to six months of the year (Sewell 1992).

While in males of *P. patagonicus*, the GI did not change significantly after the registered reproductive event, the GI of females decreased significantly twice, corresponding to a reproductive period during which brooders (February–March) (Giménez and Penchaszadeh 2010).

Mature spermatozoa were observed from November to December of 1999, and in February and March as well as from October to December 2000. Since the GI values did not show any statistically significant variation, the presence of mature spermatozoa was the only indication of reproductive events. In the months between reproductive events, January and May 2000, mature spermatozoa were not found in male tubules. However, various stages of spermatogenesis were present throughout the entire period studied.

Giménez and Penchaszadeh (2010) reported the brooding period from February to October. The results of this study show that the February/March spawning event is related to the beginning of the breeding season.

From April to June 2000, a very slight increase in temperature (about 1°C) was observed, but the GI for both males and females did not show any statistically significant variation over this period. Some environmental factors including temperature and light can influence holothurian spawning, as shown for *A. lefevrei* (Costelloe 1985). A direct effect of temperature and/or photoperiod on the timing of spawning in *P. patagonicus* seems to be little unlikely. The variation in water temperature is low and did not coincide with spawning, and light is virtually absent from the studied locality at 100–110 m depth. There are reports on echinoderms that indicate spawning is triggered by phytoplankton (Himmelman 1975; Starr et al. 1990; Hamel et al. 1993; Gaudette et al. 2006; Himmelman et al. 2008). This may also apply to *P. patagonicus* in the studied Reclutas community. In contrast to what happens during the spring–summer months, winter

phytoplankton concentration is lowest (Schejter et al. 2002). Three brooding species reviewed by McEuen (1987) and *P. patagonicus* studied by Gimenez and Penchaszadeh (2010) develop their brooding periods in winter. The large release of energy from the mother invested in large oocytes confers a great amount of energy to the larvae. This brooding scenario offers protection from predators and provides the energy from the oocyte.

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