

# Reproductive biology and energy allocation of the sea star *Cosmasterias lurida* (Echinodermata: Asteroidea) from the Beagle Channel, Tierra del Fuego, Argentina

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**Abstract** Seasonal fluctuations in environmental factors at high-latitude marine regions have a strong influence on the reproductive cycles of echinoderms. The purposes of this study on a population of *Cosmasterias lurida* in the Beagle Channel are to describe the reproductive cycle through histological analyses, to characterize the pattern of gonad and somatic indices, to determine the energy content of different organs and their temporal variation and to establish the pattern of energy allocation. During the study period, from August

2010 to June 2011, four seasonal samplings were performed in the subtidal zone of Ushuaia Bay. Indices (gonad, pyloric caeca and stomach), gonadal histology, organic matter and calorimetric measurements were determined. Gametogenesis begins in February and continues until June when oocyte size increases. The gonadal maximum development and gamete releasing take place between August and December. Temporal variation of energetic reserves in gonads occurs through the variation in gonad mass, with a GI major peak and energy content during August. Stomach and pyloric caeca show their maximum energy accumulation during June, while decrease of organic matter of the body wall takes place from August to December. The population of *C. lurida* from Beagle Channel suggests an annual reproductive cycle with two maturation events and the influence of water temperature on maturation and releasing of gametes. Different patterns of energy allocation to gonads between sexes were observed, with a greater energy investment in females than in males. Pyloric caeca, stomach and body wall were proposed as energy reservoirs for gonadal maturation and spawning.

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**Keywords** Sea star · *Cosmasterias lurida* · Beagle Channel · Reproductive biology · Energy allocation

## Introduction

High-latitude marine ecosystems present important seasonal fluctuations (Clarke 1987; Almandoz et al. 2011). As a consequence, several environmental factors such as sea water temperature, photoperiod, water salinity, food availability, pluvial precipitation and lunar cycle have a strong influence on the reproductive cycles of echinoderms (Pearse and Eernisse 1982; Pearse et al. 1986; Bouland and

Jangoux 1988; Byrne 1992; Byrne et al. 1997; Stanwell-Smith and Clarke 1998; Pearse and Bosch 2002; Benítez-Villalobos and Martínez-García 2012). Gonadal development is closely related with nutrient acquisition (Lawrence 1987a) although ingested energy must be partitioned among organism growth and maintenance as well (Lucas 1996). This allocation may vary between sexes as well as during the reproductive cycle (Lawrence and McClintock 1994; Carey 1999). Gamete production in high- and mid-latitude echinoderms occurs during a short period of the year and entails a high demand of energy, generating a potential conflict in energetic resource allocation between somatic growth and reproductive activities (Calow 1984). It is assumed that selective pressures allow both processes can occur at submaximal rates for organism's survival.

Pyloric caeca are nutrient reserve organs contained in asteroid arms, and the size and concentration of lipid present are indicative of the energy reserves of an individual (Giese 1966; Lawrence 1987b). Some asteroids show an inverse relationship between gonadal and pyloric caeca cycles of variation in mass given the transference of material towards the developing gonads (Scheibling 1981; Chia and Walker 1991), which changes the proximal composition of both organs during the reproductive cycle (Barker and Xu 1991). Though many energetic studies have been carried out in different asteroid species (Barker and Xu 1991; Miller and Lawrence 1999; Pastor-de-Ward et al. 2007), they were made using energetic equivalents from biochemical composition (Brody 1945), whereas the calorimetric method has been scarcely used in asteroids and, as it is a direct method, the amount of energy in the sample of interest is determined by its complete combustion in a calorimetric bomb (Lucas 1996).

*Cosmasterias lurida* (Philippi 1858) is an asteroid that inhabits the subtidal zone from 0 to 650 m depth (Clark and Downey 1992) and presents a continuous distribution from the platform of the Argentine Sea (35°34'S, 52°48'W) to the Chilean coast (41.5°S), including South Georgia and the Islas Malvinas (Hernández and Tablado 1985; Romanelli pers com). *Cosmasterias lurida* presents generalist feeding habits, indirect development, releases its gametes into the sea and produces high amounts of planktotrophic larvae. The diet of *C. lurida* includes mainly molluscs, crustaceans, ascidians, fish and brachiopods; it is a common predator of intertidal and/or subtidal rocky shores in Tierra del Fuego, and feeds mainly on *Mytilus chilensis*, *Pareuthria plumbea*, *Aulacomya atra* and *Trophon gerversianus* (Gordillo and Archuby 2012; Pérez pers com); *M. chilensis* and *A. atra* are important commercial resources for Beagle Channel region. In the Beagle Channel is a major predator in the forests of *Macrocystis pyrifera* and preys according to the abundance of local preys (Castilla

and Paine 1987). Studies about reproductive biology of *C. lurida* have been carried out in northern Patagonia (Pastor-de-Ward et al. 2007), and ecological aspects were studied in relation to the trophic range of the sea star (Vasquez and Castilla 1984; Castilla and Paine 1987; Adami and Gordillo 1999). Also, secondary metabolites and main sterols have been isolated from *C. lurida* (Seldes and Gros 1985; Maier et al. 1993; Roccatagliata et al. 1994; Maier et al. 1998). However, caloric content by direct calorimetric methods has not been determined for this species.

In marine environments, the knowledge of latitudinal variation in life-history patterns of a species from different areas of its geographical range is important for understanding the reproductive adaptations to habitat (Sastry 1970; Strathmann 1985). The present study provides information about the reproductive cycle and the pattern of energy allocation in a population of *C. lurida* at its southernmost limit of their distribution range.

The objectives of this study on a population of *C. lurida* in the Beagle Channel are: (a) to describe the reproductive cycle through histological analyses and to characterize the pattern of gonad and somatic indices and (b) to determine the energy content of different organs and their temporal variation over 1 year in relation to the reproductive cycle, and to establish the pattern of energy allocation.

It is hypothesized that there exists a seasonal variation of energy allocation related to the reproductive cycle of *C. lurida* from Beagle Channel.

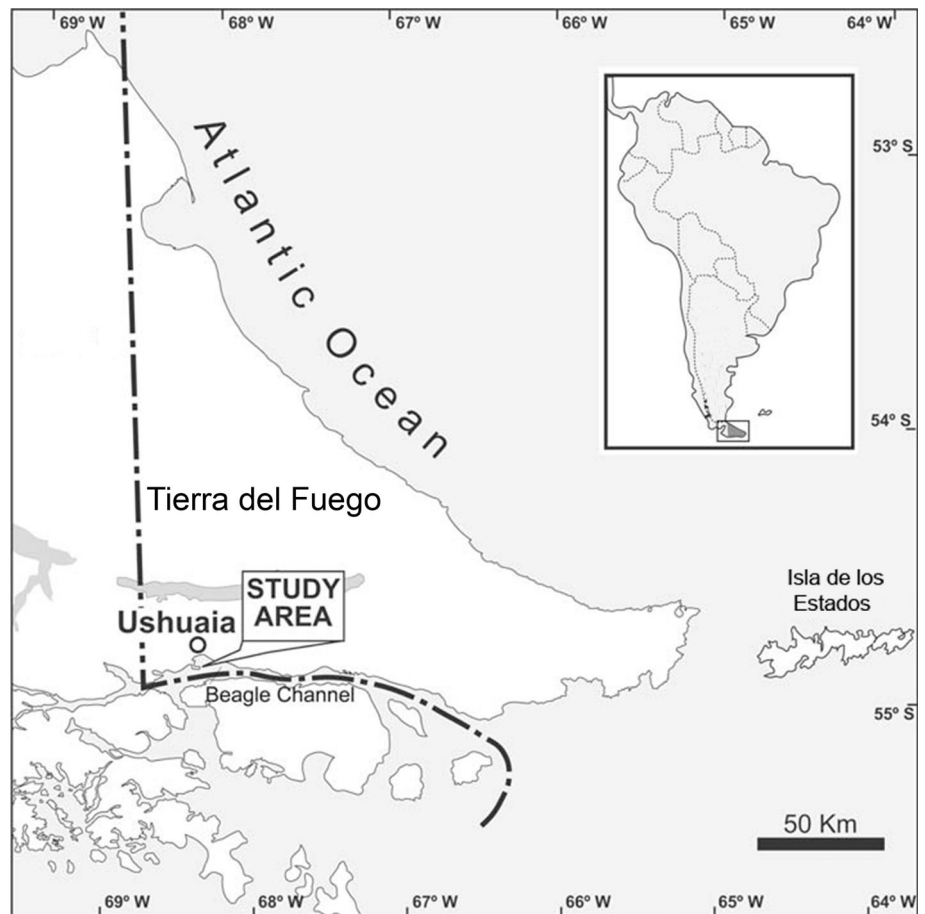
## Materials and methods

### Study site, sampling and environmental factors

During the study period, from August 2010 to June 2011, asteroids of *C. lurida* were collected in four seasonal midday samplings (August,  $n = 30$ ; December,  $n = 30$ ; February,  $n = 28$ ; June,  $n = 30$ ; austral winter, spring, summer and autumn, respectively) from the subtidal zone (2–4 m depth) of Ushuaia Bay, Beagle Channel (54°50'06.08"S, 68°16'26.72"W; Fig. 1) by SCUBA diving. Individuals were transported to the laboratory (approximately 30 min from collection site) and kept in plastic tanks (50 × 70 × 36 cm) containing air-saturated sea water (30 ‰ salinity) at 7 °C for 24 h. Asteroids were let to drip over a paper towel for approximately 1 min before whole animal wet weight was recorded (scale Sartorius CP225D ± 0.01 g).

Sea water temperature was measured (±0.1 °C) with a digital thermometer, and the monthly daylight average (hours of daylight per 24 h) was provided by the Servicio de Hidrografía Naval (<http://www.hidro.gov.ar>).

**Fig. 1** Sampling area in the subtidal zone of Ushuaia Bay, Beagle Channel, Tierra del Fuego, Argentina



### Somatic and gonadal indices

All individuals were anesthetized by immersion in MS-222 (ethyl 3-aminobenzoate methanesulfonate acid salt, 98 %, Aldrich) 1 % in sea water, 45 min (O'Neill 1994), before dissection. Body walls, gonads, pyloric caeca and stomach were dissected, weighted separately ( $\pm 0.01$  g) and stored at  $-20$  °C for calorimetric determinations, except one gonad of each specimen which was fixed in Bouin's solution for histological studies. The indices (gonad, pyloric caeca and stomach), expressed as percentages, were calculated as:

$$[\text{Organ wet weight (g)}/\text{Total wet weight (g)}] \times 100.$$

### Organic matter of body wall determination

The organic content of body wall was measured by ashing the samples (one arm plus 1/5 of the central disc from each asteroid, 10 individuals per sampling) for 24 h at 450 °C to complete calcination. Result was expressed as percentage of total dry mass:

$$\text{Organic Fraction (\%)} = 100 - \text{Ashes (\%)}$$

$$\text{Ashes (\%)} = [(P1 - P)/(P2 - P)] \times 100,$$

where  $P1$  = crucible plus ash weight (g),  $P2$  = crucible plus fresh sample weight (g) and  $P$  = empty crucible weight (g).

### Histological analyses

#### *Oocyte number, diameter, size distribution and frequency of gametogenic stages*

One gonad of each specimen was fixed in Bouin's solution over 12 h and dehydrated in a graded ethanol series. Tissue was embedded in liquid paraffin (Paraplast<sup>®</sup>), sectioned at 5–7  $\mu\text{m}$  (microtome Leica RM2125RT) and stained with Groat's haematoxylin and eosin (Pérez 2009). Sections were examined microscopically, sex was identified and each individual was assigned to a gametogenic stage based on Pastor-de-Ward et al. (2007) and Pérez et al. (2010). Additionally, in females, a quantitative study of oocyte types was added to establish the gonadal stages. Digital images of gonad sections were taken with a digital camera attached to an Olympus BX40 microscope and analysed using Micrometrics SE Premium 4 software. Three microscopic fields were examined (100 $\times$ ; NA: 0.25; Area:

0.378 mm<sup>2</sup>) per female slide. Oocyte number and oocyte diameter ( $\mu\text{m}$ , largest axis of oocytes with nucleus) were measured in each slide. Means of oocyte diameter and oocyte number were calculated per field.

In females, six gametogenic stages were determined considering histological observations, oocyte sizes frequencies and gonadal index: growth (G), premature (P), mature (M), partially spawned (PS), partially spawned with recovery (PSR) and remature (RM). In males, five stages were described based on the gonadal index and frequency of cell type, size and shape: growth (G), premature (P), mature (M), partially spawned with recovery (PSR) and remature (RM).

### Calorimetric determinations

#### *Gonad, stomach and pyloric caeca energy density and energy content*

A section of gonads, stomach and pyloric caeca of each individual was dried in an air-circulating oven at 60 °C to constant weight. Stomachs were rinsed with sea water before drying. After dry mass was obtained, samples were ground and pellets were made with a press (Parr model 2812). The pellets were burned in a micro-bomb calorimeter (Parr model 1425) to complete combustion (Pérez et al. 2008; Boy et al. 2009). The values obtained were corrected for ash and acid content and expressed as kJ/g ash-free dry mass (energy density ED, kJ/g AFDM). Benzoic acid calibrations were done periodically. The total energy content (EC) of gonads, pyloric caeca and gut was calculated as the product between ED and the total dry weight of each organ (g).

### Statistical analyses

The sex ratio of the total individuals sampled was analysed with a Chi-square test (Zar 1984) to verify a 1:1 male/female proportion (Lawrence 1987a). The Pearson linear correlations were used to test relationships between gonadal index (GI) and environmental parameters: photoperiod (daylight hours) and water temperature (°C).

Seasonal variations in GI, energy density and energy content of gonads ( $ED_G$  and  $EC_G$ ) and organic content of body wall (OC) were analysed using a nonparametric test (Kruskal–Wallis), given that the assumptions required by the parametric test (normality and/or homogeneity of variances) were not reached. Differences between sexes were tested using paired *t* test (Zar 1984; Sokal and Rohlf 1995) or Mann–Whitney nonparametric test when necessary. Variations in pyloric caeca and stomach indices (PCI and SI), energy density and energy content of these organs ( $ED_{PC}$ ,  $EC_{PC}$  and  $ED_S$ ,  $EC_S$ ) were analysed using a two-

way ANOVA (month and sex as factors). Seasonal differences in the mean oocyte number and mean oocyte diameter per field were tested using a one-way ANOVA. The assumptions of normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test) were tested, and transformations were applied when necessary. Significant differences were analysed by Tukey multiple comparisons test (Sokal and Rohlf 1995).

All statistical analyses were performed with Statistica 7.1 and GraphPad InStat 3.01.

## Results

### Sex ratio, environmental factors and gonadal index

A total of 45 females (39.8 %), 67 males (59.3 %) and 1 hermaphrodite (0.9 %) (Fig. 2) were determined from histological analyses. All individuals were adults, given the presence of mature gonads. The sex ratio did not differ significantly from a 1:1 proportion (Chi-square test;  $\chi^2 = 1.80$ ;  $df = 1$ ;  $P = 0.1793$ ).

Daylight varied between 7.3 h in June and 17.4 h in December (Fig. 3a). The increase in daylight was followed by an increase in sea water temperature. Monthly mean temperatures were lowest between June and September (about 5 °C) and highest in February (10 °C) (Fig. 3a). GI varied significantly throughout the year (Fig. 3b; Kruskal–Wallis;  $H = 74.67$ ;  $P < 0.0001$ ), with higher values in June–August than in December–February (Dunn test;  $P < 0.001$ ). A negative correlation was observed between temperature and gonadal index (GI) ( $n = 4$ ;  $r = -0.95$ ;  $P = 0.0471$ ), whereas the correlation between photoperiod and GI was not significant ( $n = 4$ ;  $r = -0.89$ ;  $P = 0.1053$ ).

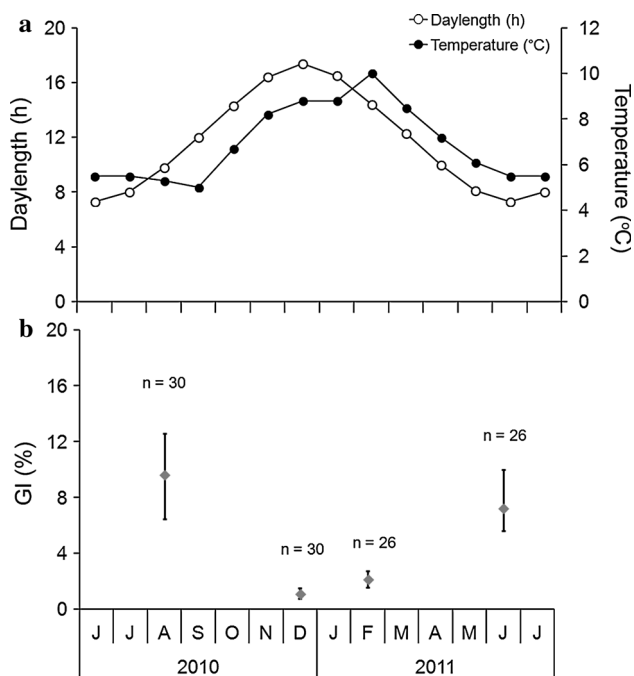
### Somatic and gonadal indices

GI varied significantly among months (Fig. 4a, b; Kruskal–Wallis;  $H = 74.67$ ;  $P < 0.0001$ ), with higher values in June–August than in December–February (Dunn test;  $P < 0.001$ ). In June, males' GI was significantly higher than females' (*t* test,  $t = 3.03$ ;  $df = 24$ ;  $P = 0.0058$ ).

For stomach (SI) and pyloric caeca (PCI) indices (Table 1), the interaction between month and sex was not significant (two-way ANOVA;  $F = 0.56$ ;  $df = 3$ ;  $P = 0.6404$  and  $F = 0.71$ ;  $df = 3$ ;  $P = 0.5504$ , respectively). Also, both indices did not vary significantly throughout the year (two-way ANOVA;  $F = 1.01$ ;  $df = 3$ ;  $P = 0.3938$  and  $F = 2.19$ ;  $df = 3$ ;  $P = 0.0931$ , respectively) nor between sexes (two-way ANOVA;  $F = 1.75$ ;  $df = 1$ ;  $P = 0.1887$  and  $F = 0.32$ ;  $df = 1$ ;  $P = 0.5752$ , respectively).



**Fig. 2** Histological sections of *C. lurida* gonads: **a** female, **b** male, **c** hermaphrodite. Scale bar **a**, **b** 100  $\mu\text{m}$ , **c** 50  $\mu\text{m}$ . *Vo* vitellogenic oocyte, *Sd* spermatids, *Sz* spermatozoa



**Fig. 3** **a** Monthly means of sea water temperature and daylight in Ushuaia Bay ( $54^{\circ}50'06.08''\text{S}$ ,  $68^{\circ}16'26.72''\text{W}$ ), Beagle Channel, **b** gonadal index [GI (%) median  $\pm$  25th and 75th percentiles] of *C. lurida* from the Beagle Channel (August 2010–June 2011)

### Organic matter of body wall

The organic matter of body wall (OM, Fig. 4c, d) varied significantly among months (Kruskal–Wallis;  $H = 23.34$ ;  $P < 0.0001$ ), with lower values in December than in June and August (Dunn test;  $P < 0.001$ ). No significant differences were found between sexes (Mann–Whitney;  $P > 0.05$ ).

### Histological analyses

#### Oocyte number and diameter

From a total of 45 females, 135 microscopic fields were analysed (three fields/female). Mean number oocytes (per

microscopic field; Table 2) presented significant variations among months (one-way ANOVA;  $F = 55.19$ ;  $df = 3$ ;  $P < 0.0001$ ). The highest number of oocytes was found in December–February (Tukey test;  $P < 0.001$ ). Likewise, the mean diameter of oocytes (per microscopic field; Table 2) varied significantly among months (one-way ANOVA;  $F = 72.19$ ;  $df = 3$ ;  $P < 0.0001$ ). Highest values of oocyte mean diameter were reached in August and lowest values during December (Tukey test;  $P < 0.05$ ).

#### Oocyte size distribution

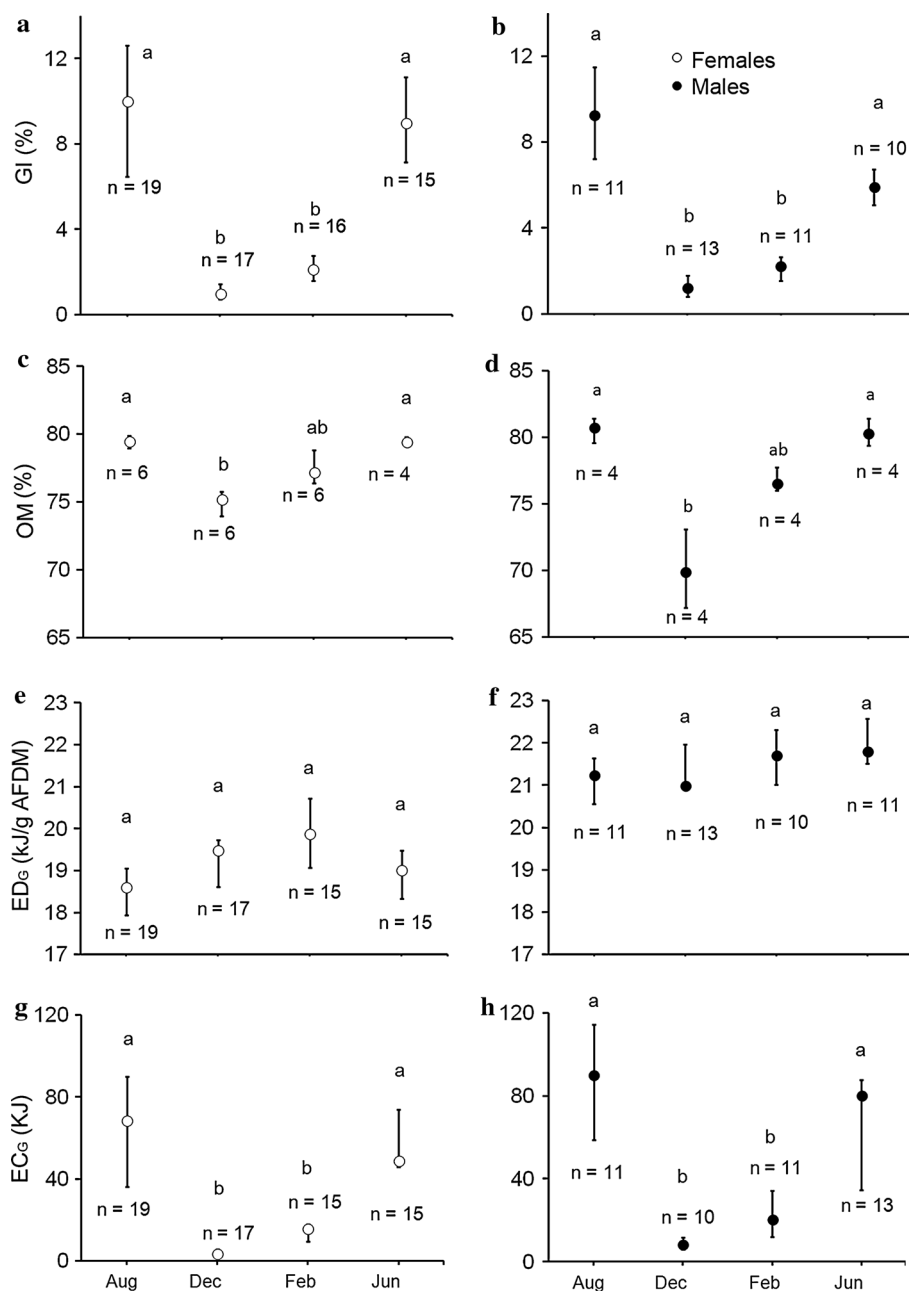
Small oocyte sizes ( $61.14 \pm 11.15 \mu\text{m}$ ) together with high oocyte number ( $80.64 \pm 18.50$ ) were observed in December. Towards February and June, there was an increase in oocyte diameter (from  $80.63 \pm 14.20$  to  $108.56 \pm 10.86 \mu\text{m}$ ) together with a decrease in frequencies and in the proportion of small oocytes (from  $58.17 \pm 19.95$  to  $26.42 \pm 7.23$ ). The highest proportion of largest oocytes ( $121.64 \pm 7.10 \mu\text{m}$ ) and lowest oocyte number occurred during August ( $25.09 \pm 4.43$ ) (Fig. 5; Table 2).

#### Frequency of gametogenic stages

Different stages of gonadal development during the study period were observed. For females, six gametogenic stages were present (Fig. 6): growth (G), premature (P), mature (M), partially spawned (PS), partially spawned with recovery (PSR) and remature (RM). For males, five stages were observed (Fig. 7): growth (G), premature (P), mature (M), partially spawned with recovery (PSR) and remature (RM).

In August, a high percentage of females were in mature stage (M; 72.7 %), while some of them were in partially spawned (PS; 18.2 %) and partially spawned with recovery (PSR; 9.1 %) stages. In December, more than 90 % of females were found in remature stage (RM) and only 7.7 % in PSR stage. During February, 70 % of females presented

**Fig. 4** Seasonal variation of **a**, **b** gonadal index [GI (%) median  $\pm$  25th and 75th percentiles]; **c**, **d** organic matter from body wall [OM (%) median  $\pm$  25th and 75th percentiles], **e**, **f** gonad energy density [ED<sub>G</sub> (kJ/g AFDM) median  $\pm$  25th and 75th percentiles], **g**, **h** gonad energy content [EC<sub>G</sub> (kJ) median  $\pm$  25th and 75th percentiles] in *C. lurida* from the Beagle Channel. Statistical differences between months are indicated with different letters above the bars



ovaries in growth stage (G), 20 % in RM and 10 % in premature (P) stages. In June, 100 % of the gonads were found in P stage (Fig. 8a).

During August, males were found mostly in premature (P; 50 %) and mature (M; 44.4 %) stages and only 5.6 % of them in partially spawned with recovery (PSR) stage. The highest percentage of PSR males were found in December (76.5 %) along with a few individuals in remature stage (RM; 23.5 %). In February, 75 % of male gonads were in growth (G) stage and the remaining 25 % in PSR stage. By June, 53.3 % of males had reached M stage, while 40 % were found in P stage and only 6.7 % were in G stage (Fig. 8b).

## Calorimetric determinations

### Gonad energy density and energy content

The energy density of gonads (ED<sub>G</sub>; Fig. 4e, f) did not vary significantly among months (Kruskal–Wallis;  $H = 6.96$ ;  $P > 0.0733$ ), but females showed higher values than males (August:  $t$  test;  $t = 6.48$ ; February:  $t$  test;  $t = 2.98$ ; June:  $t$  test;  $t = 7.81$ ; December: Mann–Whitney;  $U = 30.00$ ;  $P < 0.05$ ), whereas the energy content of gonads (EC<sub>G</sub>; Fig. 4g, h) varied significantly among months (Kruskal–Wallis;  $H = 69.55$ ;  $P < 0.0001$ ), being higher in June–

**Table 1** Seasonal variation of somatic indices (mean  $\pm$  SD), stomach and pyloric caeca energy density and content (mean  $\pm$  SD) in *C. lurida* from the Beagle Channel

Month	Stomach			Pyloric caeca		
	SI (%)	ED <sub>S</sub> (kJ/g AFDM)	EC <sub>S</sub> (kJ)	PCI (%)	ED <sub>PC</sub> (kJ/g AFDM)	EC <sub>PC</sub> (kJ)
August						
Female ( <i>n</i> = 11)	1.32 $\pm$ 0.24 <sup>a</sup>	20.38 $\pm$ 1.19 <sup>a</sup>	11.49 $\pm$ 3.52 <sup>a,c</sup>	13.65 $\pm$ 5.75 <sup>a</sup>	24.97 $\pm$ 0.82 <sup>a</sup>	215.21 $\pm$ 95.70 <sup>a</sup>
Male ( <i>n</i> = 19)	1.30 $\pm$ 0.32	20.24 $\pm$ 0.74	10.54 $\pm$ 4.73	12.62 $\pm$ 3.59	25.23 $\pm$ 1.53	189.91 $\pm$ 77.46
December						
Female ( <i>n</i> = 13)	1.39 $\pm$ 0.28 <sup>a</sup>	20.25 $\pm$ 1.13 <sup>a</sup>	11.31 $\pm$ 6.69 <sup>a</sup>	11.05 $\pm$ 6.16 <sup>a</sup>	25.27 $\pm$ 1.14 <sup>a</sup>	234.58 $\pm$ 240.50 <sup>a</sup>
Male ( <i>n</i> = 16)*	1.39 $\pm$ 0.20	20.72 $\pm$ 0.66	9.07 $\pm$ 3.95	11.56 $\pm$ 3.75	25.41 $\pm$ 1.32	146.47 $\pm$ 77.97
February						
Female ( <i>n</i> = 10)	1.46 $\pm$ 0.28 <sup>a</sup>	19.96 $\pm$ 1.15 <sup>a</sup>	12.53 $\pm$ 4.44 <sup>a,c</sup>	12.38 $\pm$ 3.18 <sup>a</sup>	25.06 $\pm$ 1.00 <sup>a</sup>	219.58 $\pm$ 127.36 <sup>a</sup>
Male ( <i>n</i> = 16)	1.26 $\pm$ 0.41	20.98 $\pm$ 1.08	12.54 $\pm$ 4.19	11.20 $\pm$ 2.46	25.41 $\pm$ 1.18	209.56 $\pm$ 107.86
June						
Female ( <i>n</i> = 11)	1.49 $\pm$ 0.26 <sup>a</sup>	21.04 $\pm$ 0.60 <sup>b</sup>	13.76 $\pm$ 3.36 <sup>c</sup>	10.35 $\pm$ 2.63 <sup>a</sup>	25.76 $\pm$ 1.66 <sup>b</sup>	174.01 $\pm$ 46.89 <sup>a</sup>
Male ( <i>n</i> = 15)	1.41 $\pm$ 0.25	21.76 $\pm$ 1.04	13.73 $\pm$ 5.83	9.24 $\pm$ 2.10	26.76 $\pm$ 1.95	191.49 $\pm$ 119.58

Statistical differences between months are indicated with different letters

SI stomach index, PCI pyloric caeca index, ED energy density, EC energy content

\* For pyloric caeca *n* = 17

**Table 2** Seasonal variation of oocyte number, oocyte diameter ( $\mu$ m) and gonadal index (GI) in *C. lurida* females from the Beagle Channel (mean  $\pm$  SD)

Month	Oocyte number		Oocyte diameter ( $\mu$ m)		GI (%)
	Mean $\pm$ SD	Range (min–max)	Mean $\pm$ SD	Range (min–max)	
August ( <i>n</i> = 11)	25.09 $\pm$ 4.43 <sup>a</sup>	18.67–32	121.64 $\pm$ 7.10 <sup>a</sup>	113.12–137.05	9.34 $\pm$ 2.93 <sup>a</sup>
December ( <i>n</i> = 13)	80.64 $\pm$ 18.50 <sup>b</sup>	38–109.33	61.14 $\pm$ 11.15 <sup>b</sup>	48.59–91.17	1.52 $\pm$ 1.06 <sup>b</sup>
February ( <i>n</i> = 10)	58.17 $\pm$ 19.95 <sup>c</sup>	37.67–98.33	80.63 $\pm$ 14.20 <sup>a,b</sup>	56.49–99.46	2.31 $\pm$ 1.09 <sup>b</sup>
June ( <i>n</i> = 11)	26.42 $\pm$ 7.23 <sup>a</sup>	17.67–40	108.56 $\pm$ 10.86 <sup>a,b</sup>	81.25–122.60	5.83 $\pm$ 2.03 <sup>a</sup>

Statistical differences between months are indicated with different letters

August than in December–February (Dunn test;  $P < 0.001$ ). In December, EC<sub>G</sub> was significantly higher in females than in males (Mann–Whitney;  $U = 46.00$ ;  $P = 0.006$ ).

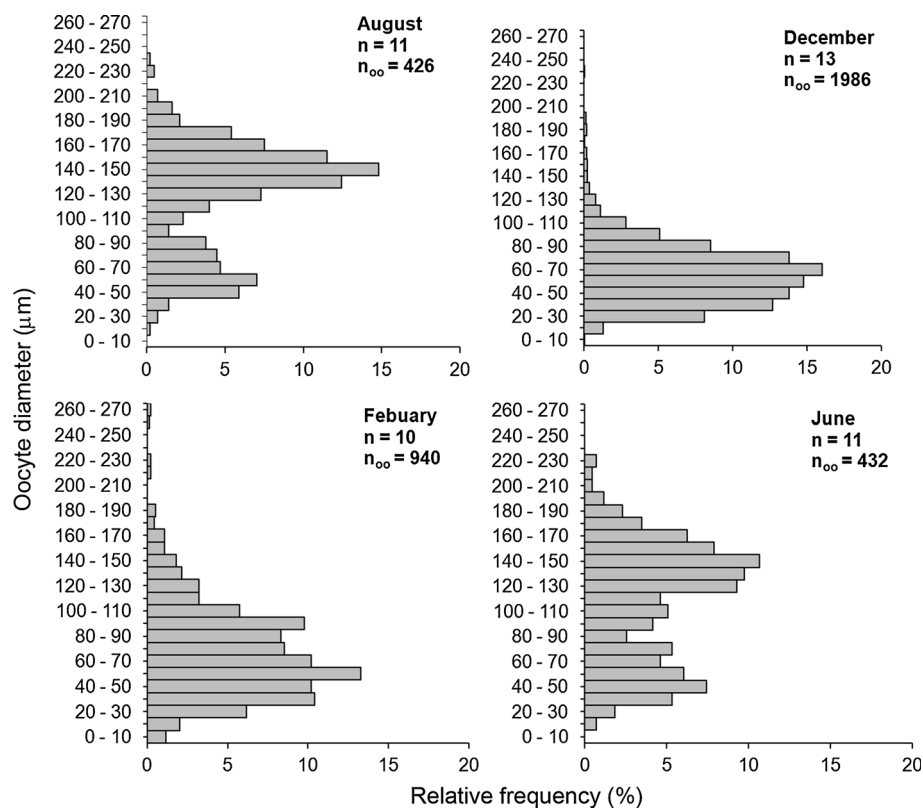
#### Stomach and pyloric caeca energy density and energy content

The energy density of stomach (ED<sub>S</sub>; Table 1) did not show an interaction between month and sex (two-way ANOVA;  $F = 1.73$ ;  $df = 3$ ;  $P = 0.1652$ ), but it did vary significantly among months (two-way ANOVA;  $F = 6.96$ ;  $df = 3$ ;  $P = 0.0003$ ), with highest values in June (Tukey test;  $P < 0.05$ ), and also between sexes (two-way ANOVA;  $F = 7.87$ ;  $df = 1$ ;  $P < 0.006$ ). The ED<sub>S</sub> of males was significantly higher in June than in August (Tukey test;  $P < 0.05$ ). Likewise, the energy content of stomach (EC<sub>S</sub>; Table 1) interaction between month and sex was not

significant (two-way ANOVA;  $F = 0.34$ ;  $df = 3$ ;  $P = 0.7963$ ), and significant differences were observed among months (two-way ANOVA;  $F = 2.99$ ;  $df = 3$ ;  $P = 0.0342$ ) with higher values in June than in December (Tukey test;  $P < 0.05$ ). No significant differences were found between sexes (two-way ANOVA;  $F = 0.77$ ;  $df = 1$ ;  $P = 0.3823$ ).

Energy density of pyloric caeca (ED<sub>PC</sub>; Table 1) interaction between month and sex was not significant (two-way ANOVA;  $F = 0.50$ ;  $df = 3$ ;  $P = 0.6844$ ). The differences were attributable to months (two-way ANOVA;  $F = 3.69$ ;  $df = 3$ ;  $P = 0.0144$ ) but not to sex (two-way ANOVA;  $F = 2.63$ ;  $df = 1$ ;  $P = 0.1076$ ). The ED<sub>PC</sub> was highest in June (Tukey test;  $P < 0.05$ ). Energy content of pyloric caeca (EC<sub>PC</sub>; Table 1) did not show an interaction between month and sex (two-way ANOVA;  $F = 0.47$ ;  $df = 3$ ;  $P = 0.7058$ ) and did not vary significantly among months

**Fig. 5** Oocyte size frequencies of *C. lurida* from the Beagle Channel;  $n$  = number of females;  $n_{oo}$  = number of oocytes



(two-way ANOVA;  $F = 0.69$ ;  $df = 3$ ;  $P = 0.5615$ ) nor between sexes (two-way ANOVA;  $F = 1.25$ ;  $df = 1$ ;  $P = 0.267$ ).

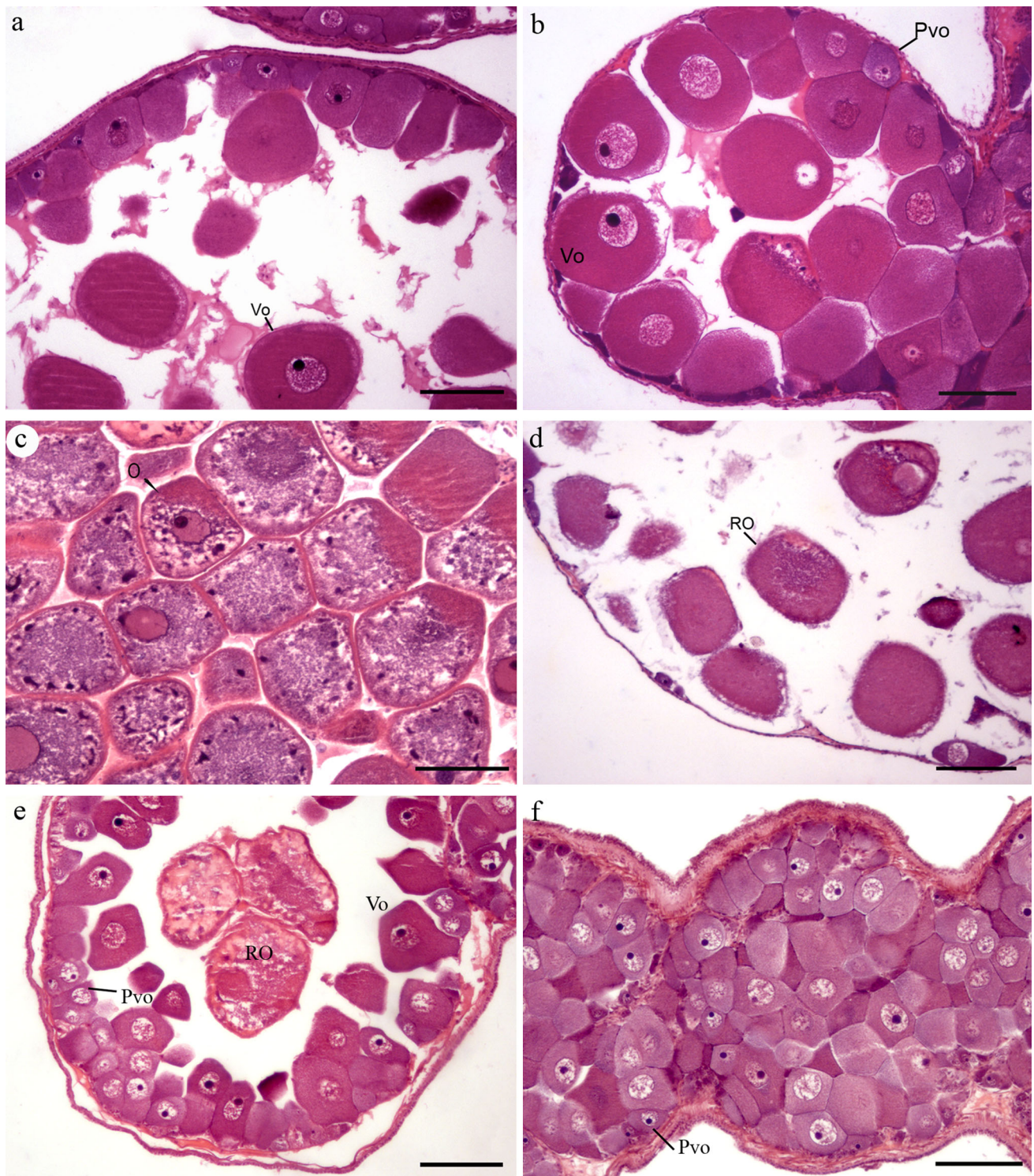
## Discussion

The studied population of *C. lurida* presented a roughly 1:1 sex ratio proportion. This result agrees with the results found for *C. lurida* from Golfo Nuevo, northern Patagonia (Pastor-de-Ward et al. 2007) and also for other asteroid species (Carvalho and Ventura 2002: *Asterina stellifera*; Raymond et al. 2007: *A. vulgaris*; Mariante et al. 2010: *Echinaster guyanensis*). A hermaphrodite *C. lurida* individual was found in the present study (0.9 %) (Fig. 2c). Similar findings have been reported for other asteroids regarding hermaphrodite's occurrence in the population (Pearse 1965; Stanwell-Smith 1997; Grange et al. 2007). Simultaneous hermaphroditism is a phenomenon in which male and female sex organs are found in the same individual. It occurs in most invertebrate phyla, and it is relatively common in echinoderms (Hyman 1955; Lawrence 1987a; McEuen 1987). One of its variants is called "accidental functional hermaphroditism" (Coe 1943), or "casual hermaphroditism" when in typical gonochoric species occasional monoecious individuals appear (Mackie 1984). Due to the low frequency of this particular event in

echinoderms, few studies have been undertaken, and detailed analyses and histological descriptions are lacking (Lawrence 1987a). Coe (1943) suggested that occasional hermaphrodites could be due to abnormalities in hereditary mechanisms of sex differentiation.

The temporal variation in the gonadal index (GI; Fig. 4a, b) and in the frequency of gametogenic stages (Fig. 8a, b) suggests that the studied population of *C. lurida* presents an annual reproductive cycle. Gametogenesis begins in February (austral summer), 2 months after the maximum daylight and together with the highest mean temperature, and continues until June (austral autumn) when oocyte size increases; therefore, the proportion of small oocytes decreases. The gonadal maximum development occurs in coincidence with the increase of photoperiod and the decrease of water temperature. Gamete releasing takes place between August and December, together with increasing daylight hours and water temperature (Fig. 3a, b), and the planktotrophic larvae could coincide with the increase in primary production during December (austral spring) (Almandoz et al. 2011). In August (austral winter), mature (M) is the most representative stage in the population, GI reaches a maximum value and gonads present a low number of sexual cells and larger size than those during other months; gametes spawning begins, so some females are found in partially spawned (PS) and partially spawned with recovery (PSR) stages. December



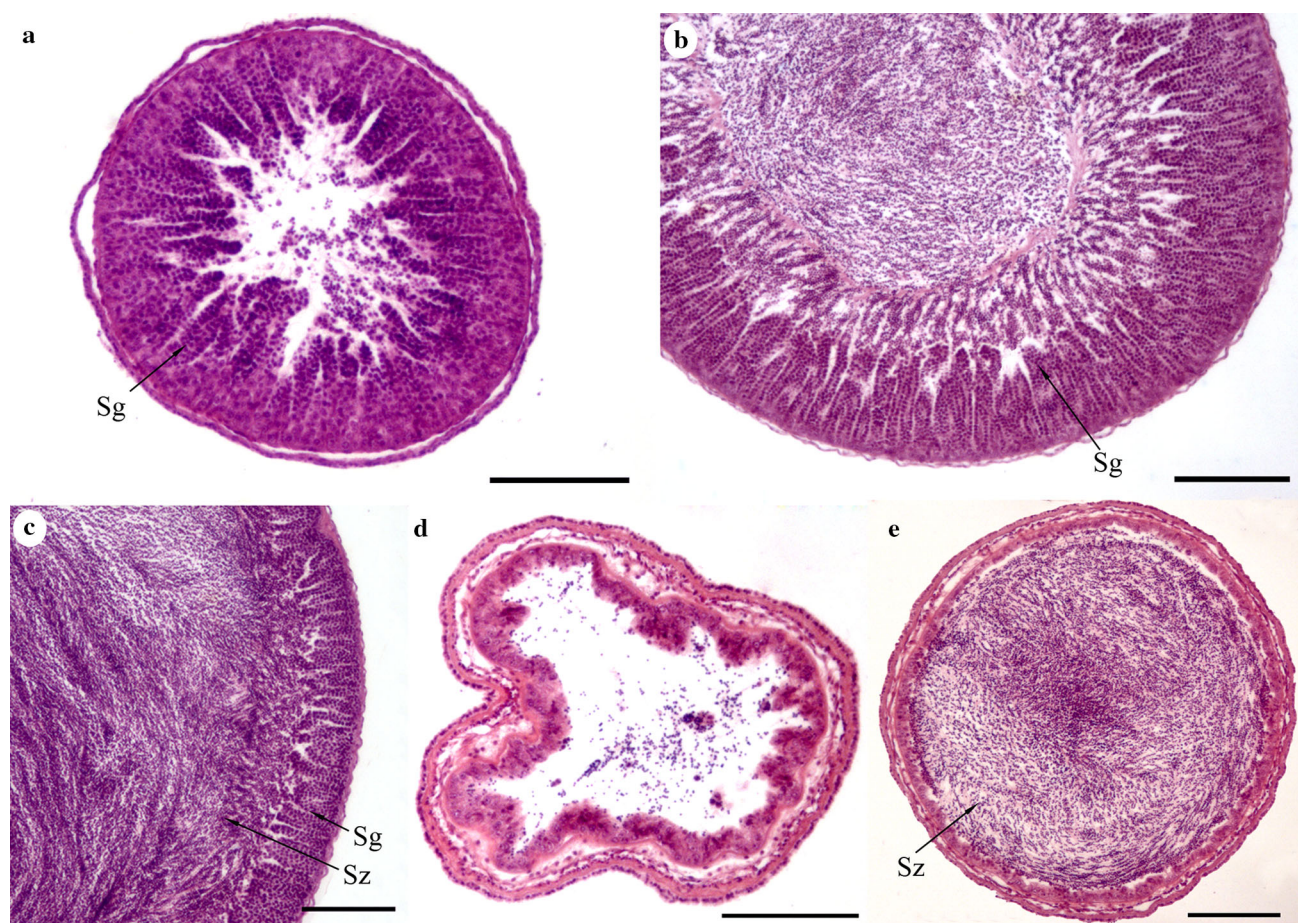


**Fig. 6** Histological sections of the ovary of *C. lurida* from the Beagle Channel showing gonadal stages: **a** growth, **b** premature, **c** mature, **d** partially spawned, **e** partially spawned with recovery and **f** remature.

Scale bar 100  $\mu\text{m}$ . *Pvo* previtellogenic oocytes, *Vo* vitellogenic oocytes, *O* ovas, *RO* relict ovas

shows the lowest GI values, the number of oocytes increases and its diameters diminish in relation to August in correspondence with cellular growing and proliferation

during recovery (PSR) and remature (RM) stages. Despite the absence of a GI relative maximum, in spring, a feasible second period of gonad maturity is observed in both sexes



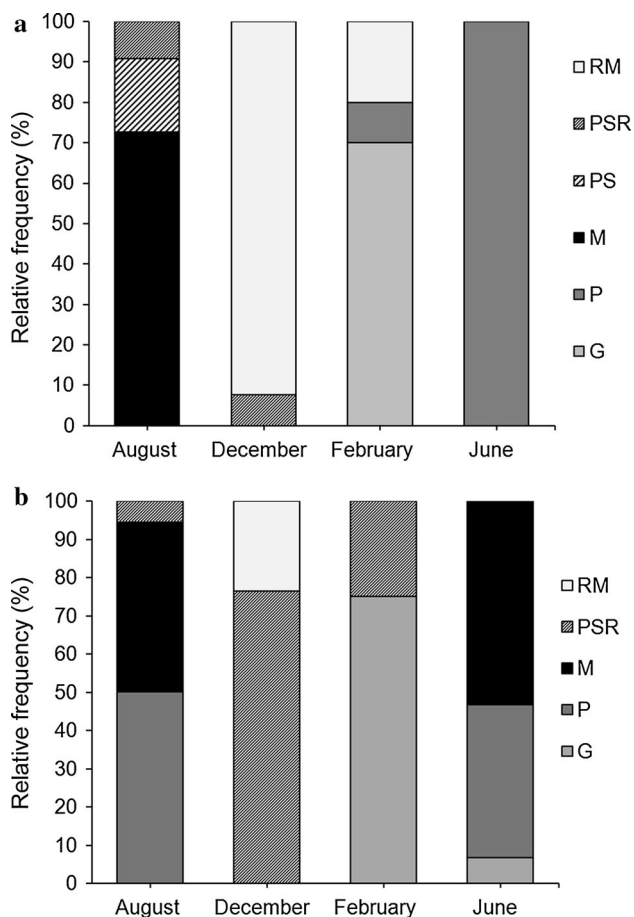
**Fig. 7** Histological section of the testis of *C. lurida* from the Beagle Channel showing gonadal stages: **a** growth, **b** premature, **c** mature, **d** partial spawned with recovery and **e** remature. Scale bar 100  $\mu$ m. Sg spermatogenesis, Sz spermatozoa

since gonads present mature gametes. Different results were found for *C. lurida* from Golfo Nuevo, northern Patagonia ( $42^{\circ}44'S$ ,  $65^{\circ}2'W$ ), where photoperiod is the main extrinsic factor that influences gametogenesis and gamete releasing, gametogenesis for both sexes takes place between June and January, sexual maturity in summer, when GI values are maximum, and spawning occurs in April (Pastor-de-Ward et al. 2007). In *C. lurida* from Beagle Channel, the GI in males was higher than that in females in June, while in the other months sexes did not differ significantly (Fig. 4a, b), unlike other studies in some asteroids, including *C. lurida*, which reported higher GI values in females than in males (Carvalho and Ventura 2002; Pastor-de-Ward et al. 2007; Bos et al. 2008). The GI values found here are about a half of those found in the northern population of the species (about 20 %; Pastor-de-Ward et al. 2007), where there is an unusually abundant food supply consequence of anthropogenic effects. Taking into account that food supply determines the investment in gonad and/or somatic growth (Lawrence and Ellwood 1991), more research is needed to elucidate if there are

energetic constraints on the individuals from Beagle Channel, which cannot allocate more energy to gonadal growth.

A latitudinal pattern is proposed in echinoids where at higher latitudes spawning of gametes is delayed in time (Zamora and Stotz 1992). In accordance to this pattern, gamete releasing in *C. lurida* occurs in April in the north of Patagonia (Pastor-de-Ward et al. 2007) and in August in the Beagle Channel.

Temporal variation of energetic reserves in gonads of *C. lurida* occurs through the variation in gonad mass, with a GI major peak and energy content ( $EC_G$ ) during August (Fig. 4a, b, g, h), while energy density of gonads ( $ED_G$ ) mean values remained constant among months (Fig. 4e, f). Though more studies about the proximal composition of gonads are needed, a possible explanation to this could be the alternation of gametes and accessory cells, which have a small nucleus and a thin cytoplasm. Accessory cells are usually found surrounding growing oocytes, and as oocytes develop, they increase in number (Pain et al. 1982, Tyler et al. 1982). Its function is associated to phagocytosis of



**Fig. 8** Seasonal variation of gametogenic stages of *C. lurida* from the Beagle Channel: **a** females, **b** males. *G* growth, *P* premature, *M* mature, *PS* partially spawned, *PSR* partially spawned with recovery, *RM* remature

unspawned oocytes (Pain et al. 1982). The  $ED_G$  and  $EC_G$  values were higher in ovaries than in testes (Fig. 4e–h). Similar results were found in Subantarctic (Pérez et al. 2010) and Antarctic echinoderms (McClintock and Pearse 1987; McClintock 1989), given the higher content of lipids and carbohydrates in oocytes with respect to cells present in male gonads (Barker and Xu 1991).

Our results suggest that stomach, pyloric caeca and body wall play a role as energy reservoirs to reproduction, supplying the demand of the sexual maturation (with energy stored in stomach and pyloric caeca), and the demand to evacuation (with energy stored in body wall). Stomach and pyloric caeca show its maximum energy accumulation during autumn ( $ED_S$ ,  $ED_{PC}$ ,  $EC_S$ ; Table 1), probably due to an increase in the ingestion rate or a better diet quality, which could be transferred to maturing gonads, whereas the decrease of organic matter of the body wall from winter to spring (Fig. 4c, d) suggests its role to afford the energy demands during the evacuation period. Some authors have already suggested that body wall in echinoderms could act

as a reserve organ to support reproduction and even more in conditions of limited food availability (Giese 1976; Lawrence and Lane 1982; Xu and Barker 1990). In contrast to what it was found here, in the Beagle Channel population, the northern population of *C. lurida* could afford simultaneous somatic and gonadal growth without the contribution of body wall and pyloric caeca, given the highest food supply.

## Conclusion

Results obtained in this study for a population of *C. lurida* from Beagle Channel suggest a reproductive cycle with two maturation events and the influence of water temperature on maturation and releasing of gametes. Different patterns of energy allocation to gonads between sexes were observed, with a greater energy investment in females than in males. Pyloric caeca, stomach and body wall were proposed as energy reservoirs for gonadal maturation and spawning in autumn–winter and early spring. Further energetic studies are required to complement these results. This study provides information for understanding the latitudinal variation in the life-history traits and energy allocation of *C. lurida*.

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