

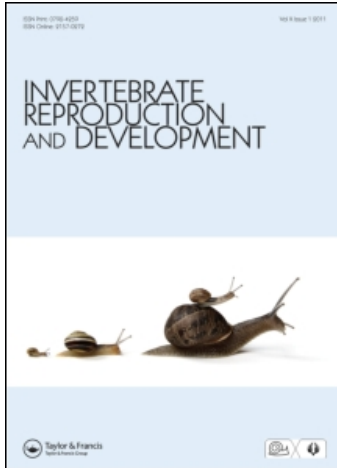
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## Allocation of nutrients during the reproductive cycle of *Ophidiaster ophidianus* (Echinodermata: Asteroidea)

Joana Micael<sup>a\*</sup>, Armindo S. Rodrigues<sup>b</sup>, Maria C. Barreto<sup>bc</sup>, Maria J. Alves<sup>d</sup>, Malcolm B. Jones<sup>e</sup> and Ana C. Costa<sup>a</sup>

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The reproductive cycle of *Ophidiaster ophidianus* (strictly protected status) from São Miguel Island, in the Azorean Archipelago was studied. The reproductive strategy; the energy allocation of each sex during the reproductive cycle and the nutritional condition of the population were analyzed. Gonadal index (GI) showed a clear seasonal pattern with spawning between August and October but histological examination revealed that gamete release can occur throughout the entire year. The pyloric caeca index (PCI) showed little annual variation but with an inverse relationship with the GI. Allocation of energy to the gonads and to the pyloric caeca reflected the seasonal reproductive strategy of this species. Individuals were able to simultaneously develop gonads, pyloric caeca, and quickly regenerate lost arms. There was a major expenditure of energy by females compared to males but, sexual size dimorphism was not observed. The reproductive pattern observed in *O. ophidianus* combining rich food availability and seawater temperatures characteristic of a temperate zone may be the key to the success of this species in the Azorean oceanic Island.

**Keywords:** biochemical cycle; echinoderm; strictly protected status; temperate zone

### Introduction

The growing global pressures on the collection of echinoderms for various commercial enterprises have put these enigmatic invertebrates under threat (Micael et al. 2009). The extent of the demand on sea stars from commercial fisheries is not documented but there is evidence of an emerging demand for them in Asian food markets. Many echinoderms are also popular in the aquarium and souvenir trades. The fishery for the sea star *Oreaster reticulatus* (Linnaeus 1758), for example, has resulted in its decline in Canadian shallow-water populations (Sloan 1985). Certain countries in the Caribbean Sea, such as Mexico, Jamaica, Trinidad, Venezuela, and others, allow the harvesting of *O. reticulatus* without apparent restrictions, which may increase short-term demand and commerce in other areas once the populations in these countries become diminished (Guzmán and Guevara 2002; Micael et al. 2009). It is well-known that some echinoderm species are important in determining

habitat structure for other species and can represent a substantial portion of the ecosystem biomass. Although the sexual reproductive cycle has been well established for a number of sea stars, there is no information about the reproductive pattern of the North Atlantic temperate zone asteroid species. Asteroid echinoderms have diverse reproductive strategies, including seasonal and continuous breeding periodicity usually related to latitude (Pastor-de-Ward et al. 2007). The seasonal changes of sea temperature are the major environmental factor determining the timing and duration of reproductive activity (Orton 1920). Conand (1993) and Hamel et al. (1993) found that many species of echinoderms undergo an increase in gametogenesis after the winter months, when the water temperature begins to increase. It has also been shown for some echinoderms that photoperiod has a large influence on the initiation of gametogenesis (Bay-Schmith and Pearse 1987; Conand 1993). Whatever the reproductive periodicity

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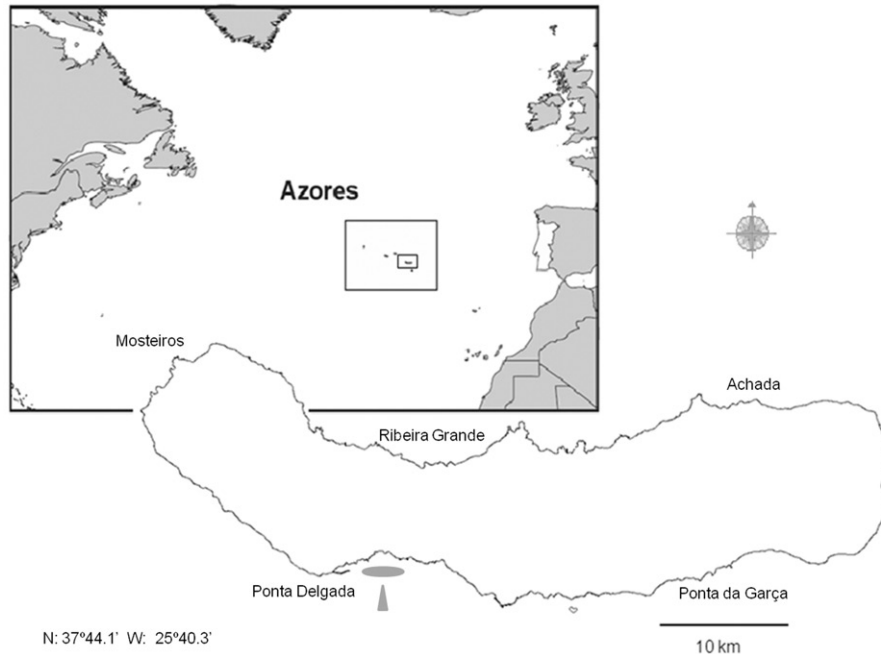


Figure 1. Sampling sites in São Miguel Island, the area is shaded and indicated by the point of the arrow.

(seasonal or continuous), sexual reproduction in asteroids is usually characterized by a distinct pattern of gamete development followed by synchronized spawning period (Chia and Walker 1991), usually entailing major energetic costs (Doughty and Shine 1997; Raymond et al. 2007). Indeed, the energetic requirement of reproduction is so high that echinoderms spend most of the year preparing for it both nutritionally and physiologically (Ferguson 1975; Pastorde-Ward et al. 2007). The changes in gonad and pyloric caeca biochemical components during the reproductive cycle may be used as an indication of the nutritional condition of the population.

*Ophidiaster ophidianus* (Lamarck 1816) occurs in the western Mediterranean (36°–40°N), the Gulf of Guinea (≈3°N), Canary Islands (≈28°N), Madeira (30°–33°N) and Azores (37°–39°N). Typically, it inhabits subtidal rocky bottoms from 0 to 105 m depth (Clark and Downey 1992) and, in the Azores, in the highest Atlantic latitude where it is found (Clark and Downey 1992) it is the most common sea star on rocky bottoms (Marques 1983). There is a general lack of information concerning the biology of this species, protected under the Habitat Directive (Appendices II) in the Mediterranean Sea Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2007) and which is also considered a vulnerable species in Spain (Catálogo Nacional de Especies Amenazadas 2007). The purpose of this research was to study the reproductive cycle of *O. ophidianus* from São Miguel Island, in the Azorean Archipelago. The focus was designed to provide insights into its

reproductive strategy; to investigate if energy allocation was different between sexes and to understand the nutritional condition of the population at a temperate zone. Information generated about the basic biology of this sea star will provide important insights in a conservation strategy design.

## Materials and methods

### Sampling

At bimonthly intervals from April 2007 to February 2008, *O. ophidianus* was collected randomly by hand (SCUBA diving) from various sites, at a distance of ca. 5 km with the same general conditions (to minimize the impact of sampling) in the shallow subtidal zone of the south coast of São Miguel Island (Azores) (Figure 1). Individuals were transported to the laboratory in solid, inert plastic boxes filled with local seawater, and returned to the sampling site following measurements (see below).

In the laboratory, individuals were narcotized by immersion for 15 min in a 5% solution of chloral hydrate in seawater (Russell 1963); this procedure has no long-term affect on the starfish (Florey and Cahill 1980). For each individual sea star, the length of each arm (normal and regenerating) was measured (from the madreporite to the tip of each arm) using hand-held callipers (to an accuracy of  $\pm 0.1$  mm). Surface water was removed (blotted dry with absorbent paper) and each sea star was wet weighed ( $\pm 1$  mg) using an EW balance (Kern EW 150-3 M). For each individual,

the longest arm was dissected off, and the gonads and pyloric caeca removed and weighed ( $\pm 0.001$  mg) (Precisa 205 SCS, Precisa Instruments).

### Reproductive cycle

The gonads and pyloric caeca were fixed overnight in 4% formaldehyde solution (buffered to pH 7 and stabilized with methanol, PanReac) and then transferred to 70% ethanol. Tissues were dehydrated *via* an ethanol series (70%, 96%, 100%), cleared with xylol, embedded in paraffin wax, sectioned (Microtome – Leica RM2035) at 4  $\mu$ m, and stained with Harris' haematoxylin and eosin Y (Howard and Smith 1983). Sex determination was assessed from histological sections of the gonads under a light microscope (amplification 10 $\times$  – Leica – Leiburluxs). Gonadal growth of *O. ophidianus* was divided into stages of maturity used previously to describe the histology of oogenesis and spermatogenesis of asteroids, and was based on the frequency of cell types, and size and shape of acinus (Byrne 1992; Byrne et al. 1997). To test if the gametogenic condition was homogeneous for all arms, one gonad from each arm of two specimens from each sampling month was examined. Gonad index (GI) and pyloric caeca index (PCI) were calculated as the wet weight of the organ/whole body wet weight  $\times 100$  (modification of Giese 1966; Ventura et al. 1997; Lawrence and Miller 1999; Carvalho and Ventura 2002). Individuals were considered sexually mature when spermatozoa and oocytes were present (Byrne et al. 1997).

### Biochemical analyses

Between April 2007 and February 2008, one of the two excised gonads and pyloric caeca of the sea stars were frozen at  $-20^{\circ}\text{C}$  for biochemical analysis (6 to 8 months later). Only individuals with no regenerating arms were examined. Soluble proteins were measured by the Bradford (1976) method with bovine serum albumin as the standard. Protein concentrations were determined after extraction (0.05 g tissue per 1 mL buffer) in 50 mM Tris-HCL, 0.1% SDS, pH 7.0. Proteins were further solubilized by the addition of Coomassie Brilliant Blue G (S-31). To extract carbohydrates, 0.05 g of the gonads and pyloric caeca were boiled in 5% trichloroacetic acid for 10 min, and soluble carbohydrates were measured by the colorimetric method of Dubois et al. (1956) with glycogen as the standard. Lipid content of the gonad and pyloric caeca was determined using the chloroform-methanol method as modified by Folch et al. (1957). The tissue sample was homogenized in a 20-fold volume of chloroform:methanol (2:1) and then washed with water to remove nonlipid contaminants. Energy values were obtained

by multiplying absolute contents of soluble proteins, soluble carbohydrates and lipids by energetic equivalents [23.651 kJ g $^{-1}$  for protein and 17.163 kJ g $^{-1}$  for carbohydrates (Gnaiger and Bitterlich 1984) and 39.558 kJ g $^{-1}$  for lipids (Brody 1945)].

### Statistical analysis

Differences between male and female arm length and body weight were examined using one-way analysis of variance (ANOVA). A chi-squared goodness-of-fit test ( $\chi^2$ ) test was used to determine if the male: female ratio differed from 1:1 and to test if the gametogenic condition was homogeneous for all arms. Differences in the bimonthly values of GI and PCI were tested using ANOVA and  $\chi^2$ -test was used to verify sex differences of gonadal indices (GIs) and PCIs. Spearman's rank correlation analysis was used to establish any relationship between GIs and PCIs, between biometric variables between sexes (weight, size), and between the GI and environmental variables (temperature, photoperiod). Bimonthly variations of soluble proteins, soluble carbohydrates, and lipids were tested for significance using ANOVA. A  $\chi^2$ -test was used to verify monthly differences between biochemical contents of males and females. Spearman's rank correlation analysis was used to test for the relationship between GIs and PCIs, between biometric variables (weight, size), between the GI and environmental variables (temperature, photoperiod), and biochemical contents of gonads and pyloric caeca.

## Results

### Sex ratio and biometrics

Histological sections confirmed that all individuals sampled were sexually mature. The overall male (57%): female (43%) ratio was not significantly different from 1:1 ( $\chi^2_{3,84} = 2.04$ ,  $p > 0.05$ ), however, there was a 3:1 male bias in December ( $\chi^2_{3,84} = 4$ ,  $p > 0.05$ ) (Table 1).

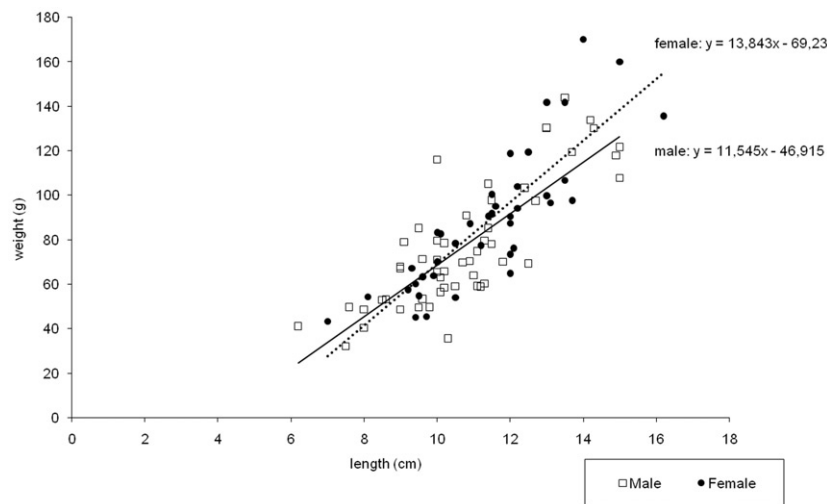
Males and females had similar arm length (Table 1) ( $\chi^2_{3,84} = 0.232$ ,  $p > 0.01$ ) but females were significantly heavier at each length ( $\chi^2_{3,84} = 8.261$ ,  $p > 0.01$ ) (Figure 2). There was a significant positive linear relationship between body wet weight and arm length for males ( $r_s = 0.623$ ,  $p < 0.05$ ) and females ( $r_s = 0.872$ ,  $p < 0.05$ ). There was also a significant positive linear relationship between gonad weight and arm length for males and females ( $r_s = 0.566$ ,  $p < 0.05$ ,  $r_s = 0.293$ ,  $p < 0.05$ , respectively).

### External gonad morphology

The wide morphological variation of the gonads, both in males and females, did not enable macroscopic distinction between sexes, in any of the described

Table 1. Bimonthly biometrics of *O. ophidianus* collected to describe the reproductive cycle. ( $n_2$  – Number of individuals with regenerating arms).

		Male						Female						
		$n$	$n_2$	Length	SE	Weight	SE	$n$	$n_2$	Length	SE	weight	SE	$N$
2007	April	8	2	9.9	±0.80	79.74	±16.33	9	1	11.17	±1.90	94.46	±30.54	17
	June	8	3	8.84	±0.99	50.27	±5.33	7	1	9.82	±1.67	60.00	±14.49	15
	August	11	2	13.8	±0.94	118.42	±20.71	5	2	13.94	±2.01	135.12	±25.00	16
	October	8	3	10.78	±1.11	74.76	±13.33	8	1	11.23	±1.36	83.03	±24.15	16
	December	12	2	9.88	±1.65	62.23	±22.58	4	0	11.20	±1.49	86.99	±18.82	16
2008	February	7	0	11.01	±0.75	73.64	±16.56	8	1	11.51	±1.40	82.64	±14.98	15
Total		54	12	11.57	±1.04	76.51	±15.81	41	6	11.48	±1.64	90.37	±21.33	95
SE		–		±2.10	–	±28.22	–	–		±1.87	–	±30.72	–	–

Figure 2. *Ophidiaster ophidianus* weight and length distribution. Males' regression (solid line):  $y = 11.545x - 46.915$ ; Females regression (dotted line):  $y = 13.843x - 69.23$ .

gametogenic stages (see below). No pattern was found between gonad coloration and sex, between the number of branches in the gonad and sex, or between the development stage of the gonad and gonad coloration or number of branches in the gonad.

### Gonad and pyloric caeca indexes

*Ophidiaster ophidianus* showed a clear seasonal periodicity in the GI index (ANOVA, male  $F_{(2,438)} = 31.492$ ,  $p < 0.001$ ; female  $F_{(2,409)} = 39.692$ ,  $p < 0.01$ ) For each sex, relatively low values from February to June (winter/spring) increased to a maximum in October (summer/autumn), followed by a decline from October to December (winter) (Figure 3). Monthly GIs values were not significantly different for males and females ( $\chi^2_{3,841} = 0.131$ ,  $p > 0.01$ ). The PCI showed little annual variation (one-factor ANOVA, male  $F_{(2,404)} = 1.984$ ,  $p = 0.098$  and

female  $F_{(2,503)} = 0.901$ ,  $p = 0.492$ ), and there were no significant differences between males and females throughout the year ( $\chi^2_{3,841} = 3.238$ ,  $p > 0.05$ ). However, there was a significant inverse relationship between the seasonal patterns of the gonad and pyloric caeca indices ( $r_s = 0.123$ ,  $p < 0.05$ ), with the maximum GI coinciding with lowest PCI values (Figure 3).

Individuals with regenerating arms (Table 1), only considered in this analyses, had similar GIs compared with intact individuals ( $U_{101} = 46$ ,  $p < 0.001$ ). There were no significant differences in the GI between the arms of the two individuals from each sampling month ( $\chi^2_{3,841} = 0.274$ ,  $p > 0.05$ ).

### Gonad development

Testes and ovary growth of *O. ophidianus* was divided into four stages of maturity: I – Growing

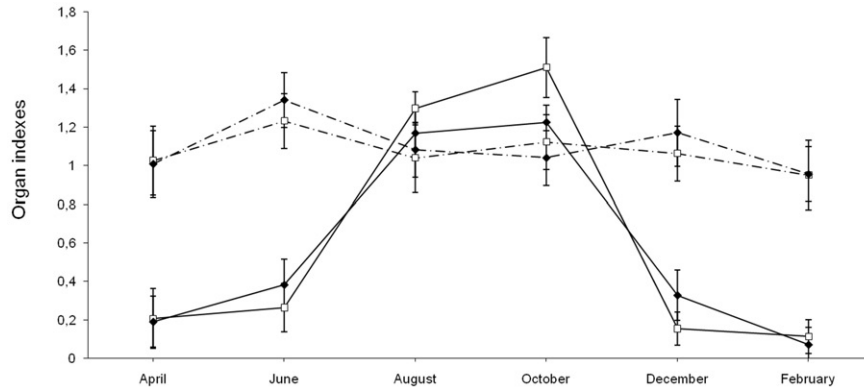


Figure 3. *Ophidiaster ophidianus* bimonthly variation of gonadal (GI – continuous line) and pyloric caeca indices (PCI – interrupted line) from April 2007 to February 2008 (mean  $\pm$  SE). Males shown as black circles, females as white squares.

phase; II – Mature phase, III – Partly-spawned phase and IV – Post-spawned and recovering phase.

#### Testes development

Generally, *O. ophidianus* testes have the two-sac structure typical of male asteroid gonads. In some individuals however, more than two branches in the gonad were observed. Separation of the stages of *O. ophidianus* testes maturity was based upon the stage of the spermatogenic cycle: spermatogenic condition of the germinal layer, the amount of spermatozoa in the lumen, and the development of the haemal layer (Figure 4).

**Growth phase.** This stage was clearly identified by a massive proliferation of the germinal layer. In growing testes, columns of spermatocytes form a basophilic layer and the spermatozoa start to migrate from the germinal epithelium to the lumen of the acinus. With the accumulating mass of spermatozoa, the testes assume a filled appearance and the acinar wall appeared thin until spawning (Figure 4a).

**Mature phase.** This stage is characterized by the densely-packed accumulation of spermatozoa in the lumen of acinus, forming a dense mass (Figure 4b). Columns of spermatocytes may be present in the germinal layer.

**Partly-spawned phase.** In partly-spawned testes, the spermatozoa are less densely packed, with spaces in the acinar lumen created by gamete release (Figure 4c). A gap between the peripheral spermatogenic layer and the central mass of sperm is usually present.

**Post-spawned and recovery phase.** This stage, characterized by spent testes, was identified by shrunken

gonad walls and an almost empty lumen. A pale meshwork of nutritive phagocytes could be found around the periphery, some relict spermatozoa were found. The cells of the germinal epithelium were also evident (Figure 4d). In recovering testes, basophilic columns of spermatocytes were present along the thick testis wall, the germinal layer became folded and a few relict sperm may be present and the nutritive phagocytes formed a meshwork across the ascinus. Gonads were very small and testes were initiating a new cycle. Spermatic columns were evident along the thick testis wall and the geminal layer became folded.

#### Ovary development

Typically, *O. ophidianus* ovaries had the two-sac structure characteristic of female asteroid gonads, although some females had more than two branches. The maturity stages were characterized by the number, size, position, and staining properties of the oocytes, the width of the ovary wall, the extent of development of the haemal layer, and the presence of phagocytes and cell debris. Pre-vitellogenic oocytes are basophilic. Vitellogenic oocytes become darker as the yolk is deposited. Late-vitellogenic oocytes are intensely dyed (Figure 5).

**Growth stage.** In growing ovaries, small previtellogenic oocytes (range size: 0.01–0.075 mm; SE = 0.012) were common in the acinar wall and were surrounded by nutritive phagocytes associated with cell debris and flocculent material derived from lysed oocytes. Relict oocytes may persist for a long period. Some oocytes showed a tear shape. Advanced oocytes were detached from the wall and some had a central location and showed a dramatic increase in oocyte diameter (Figure 5a).

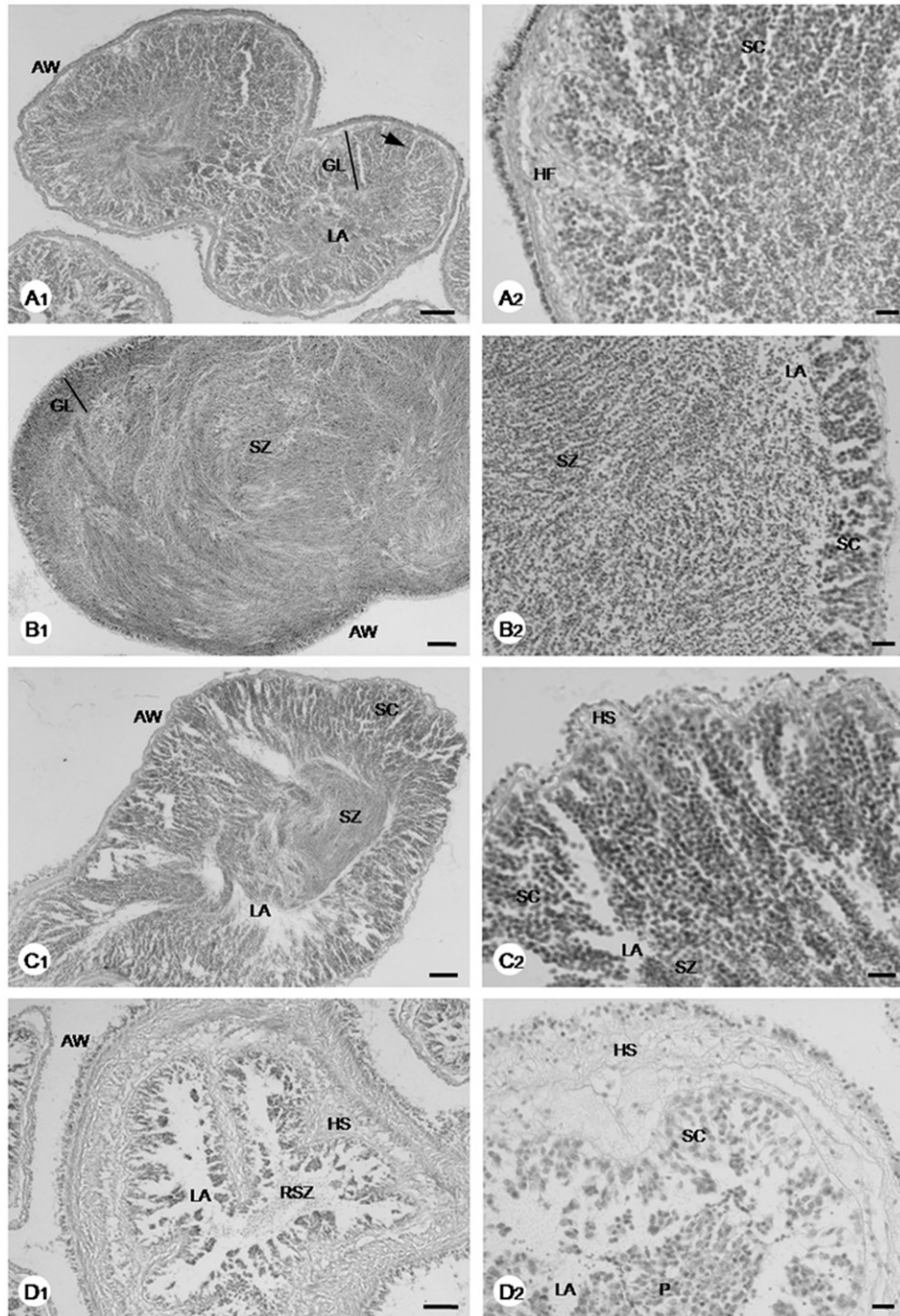


Figure 4. Male gonadal development stages: a – Growth phase; b – Mature phase; c – Partly-spawned phase, and d – Post-spawned and recovery phase (1 – scale = 50  $\mu$ m; 2 – scale = 10  $\mu$ m). AW – Acinar wall; GL – Germinal layer; HS – Hemal sinus; NP – nutritive phagocytes; LA – Lumen of acinus; RSZ – relict spermatozoa; SC – spermatocyte column; SZ – spermatozoa.

*Mature stage.* Prespawning (mature stage) ovaries were characterized by an abundance of mid- and late-vitellogenic oocytes (range size: 0.023–0.120 mm; SE=0.024). They were eosinophilic staining light pink. The lumen was filled with large oocytes and the ovary wall was distended. Previtellogenic oocytes could be observed along the germinal layer. Nutritive phagocytes were absent or formed a pale, thin meshwork around the small oocytes (Figure 5b).

*Partly-spawned stage.* Partly-spawned ovaries of *O. ophidianus* were similar to mature ovaries in containing an abundance of large oocytes (range size: 0.035–0.093 mm; SE=0.033). Nevertheless, ovaries were loosely packed, with space existing between ova due to gamete release. Ova were round and centered in the acinus. In some ovaries, released oocytes were replaced through the breeding season by oocytes of different stages of development, while others had small

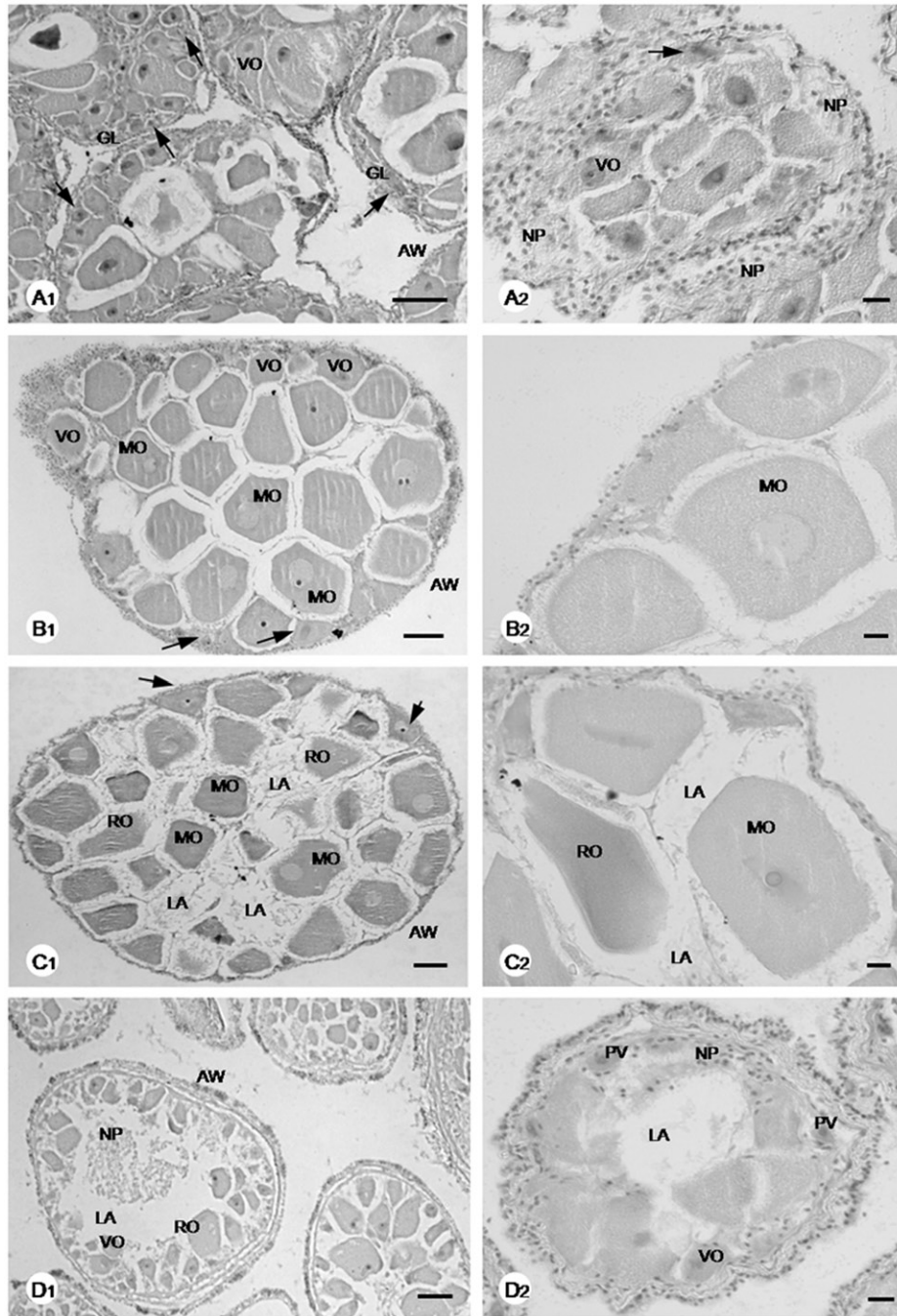


Figure 5. Female gonadal development stages: a – Growth phase; b – Mature phase; c – Partly-spawned phase, and d – Post-spawned and recovery phase (1 – scale = 50  $\mu$ m; 2 – scale = 10  $\mu$ m). Arrow and PVO – previtellogenic oocyte; AW – Acinar wall; VO – vitellogenic oocytes; MO – mature oocytes; NP – nutritional phagocyte; RO – relict ova.

primary oocytes in the acinar walls as described in the growth phase (Figure 5c).

*Post-spawned and recovering phase.* The inner part of the ovaries in this stage were heterogenous. In some ovaries, the lumen was empty or contained debris from relict oocytes. There were very few vitellogenic oocytes in the lumen. Phagocytes were abundant around the

periphery and concentrated around the relict material. The cells lining the germinal layer were evident. In other ovaries, there was an abundance of small primary oocytes lining the ovary wall. These oocytes included pre- and early vitellogenic oocytes. Oogonial proliferation was evident in the germinal layer of recovering ovaries. Early vitellogenic oocytes were closely associated with the ovary wall. Nevertheless, there were still some ovaries that apart from relict

oocytes contained large oocytes to be released or reabsorbed (Figure 5d).

**Gametogenic cycle**

Histological analyses of male and female gonadal sections indicated a continuous pattern of gametogenesis in *O. ophidianus* from São Miguel Island, with all gonadal development stages (4) observed in each sampling month; the exception was that no mature female stage was detected in October. In February, 44% of male and 47% of female gonads were in the *Growth stage*, the *Mature stage* dominated between April and early June (males – 48%, females – 31%) and the *Partly-spawned stage* dominated between late June and early October (males – 23%, females – 26%). Around December, 64% of male and 58% of female gonads were in the *Post-spawned and recovery phase* (Figure 6).

**Biochemical cycle**

Biochemical contents of male and female gonads differed significantly throughout the year (ANOVA,

Soluble protein – male  $F_{(4,034)}=92.889$ ,  $p < 0.001$ ; female  $F_{(4,034)}=39.692$ ,  $p < 0.001$  Soluble carbohydrate – male  $F_{(4,073)}=80.291$ ,  $p < 0.001$ ; female  $F_{(4,073)}=79.877$ ,  $p < 0.001$ ; Lipid content – male  $F_{(4,196)}=93.924$ ,  $p < 0.001$ ; female  $F_{(4,196)}=94.010$ ,  $p < 0.001$ ), increasing until August, which corresponds to the *Mature* and *Partly-spawned stages* and decreasing until December, which corresponds to the *Post-spawned and recovering phase* (Figure 7).

The biochemical contents of the pyloric caeca were stable all year (ANOVA, soluble carbohydrate – male  $F_{(4,037)}=1.636$ ,  $p < 0.001$ ; female  $F_{(4,037)}=1.208$ ,  $p < 0.001$ ; lipid content – male  $F_{(4,043)}=0.4949$ ,  $p < 0.001$ ; female  $F_{(4,043)}=1.1062$ ,  $p < 0.001$ ); the exception was the protein content (ANOVA, male  $F_{(4,043)}=85.533$ ,  $p < 0.001$ ; female  $F_{(4,043)}=73.512$ ,  $p < 0.001$ ) which was raised between June and December, corresponding to the *Partly spawned, Post-spawned and recovery stages* and decreased until February, corresponding to the beginning of the *Growth stage* (Figure 7).

There were no significant differences between the biochemical contents of males and females (Protein

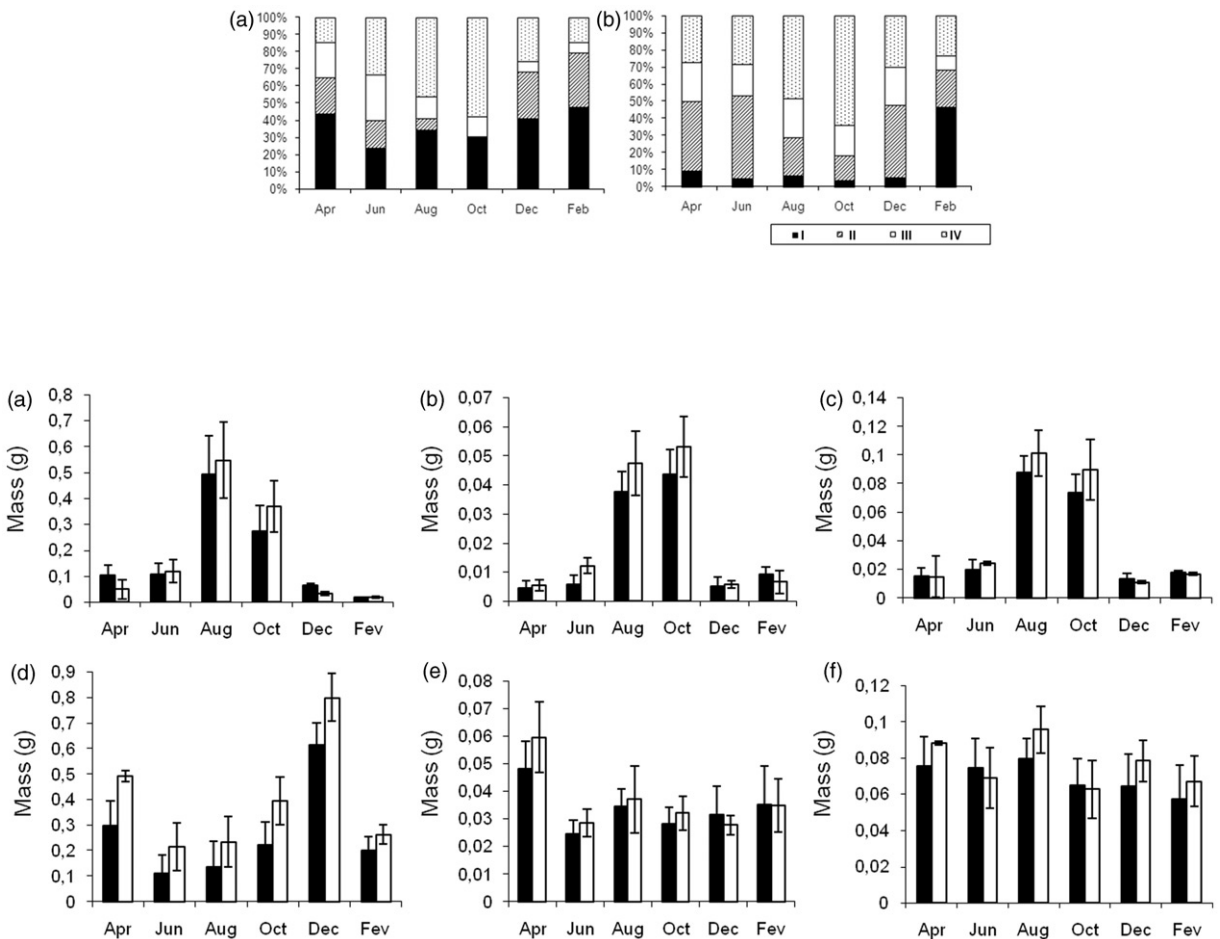


Figure 7. *Ophidiaster ophidianus*, bimonthly biochemical composition of the gonads: protein (a), carbohydrate (b), and lipid (c) contents, and the pyloric caeca: protein (d), carbohydrate (e), and lipid (f) contents. Males are in black, females are in white (mean ± SE). Vertical axis represents biochemical content per total gonad weight (g).

content, gonad  $\chi^2_{3,84} = 0.086$ ,  $p > 0.05$ ; pyloric caeca  $\chi^2_{3,84} = 0.503$ ,  $p > 0.05$ ; Carbohydrate content, gonad  $\chi^2_{3,840} = 0.009$ ,  $p > 0.05$ ; pyloric caeca  $\chi^2_{3,840} = 0.004$ ,  $p > 0.05$ ; Lipid content, gonad  $\chi^2_{3,840} = 0.007$ ;  $p > 0.05$ ; pyloric caeca  $\chi^2_{3,840} = 0.009$ ,  $p > 0.05$ ). Protein, carbohydrate, and lipid content of the gonads of males and females increased in parallel with the increase in ovarian and testis mass. Prior to spawning, the total content of all three biochemical components in the gonads were lower in males than in females, but after spawning the protein, carbohydrate, and lipid contents were similar in the two sexes. There was a negative correlation between protein and carbohydrate content of both organs for males and for females (Protein content,  $r_s = -0.429$ ,  $p < 0.05$ , for each sex and carbohydrate content, male  $r_s = -0.600$ ,  $p < 0.05$ ; female  $r_s = -0.143$ ,  $p < 0.05$ ); nevertheless, in males, there was a positive correlation between lipid content of both organs ( $r_s = 0.486$ ,  $p < 0.05$ ) and in females, the correlation between lipid content of gonad and pyloric caeca was not significant ( $r_s = -0.029$ ,  $p < 0.05$ ).

During the sampling period, the main biochemical component in the gonad and pyloric caeca of both sexes was soluble protein. The percent of biochemical content of the gonads varied inversely to the biochemical content of male and female pyloric caeca. Changes in the protein and lipid composition of the gonads and the pyloric caeca were translated into statistically significant differences in energetic content between sexes, being higher in females (gonad protein  $\chi^2_{3,840} = 40.912$ ,  $p > 0.05$ ; pyloric caeca protein  $\chi^2_{3,840} = 43.230$ ,  $p > 0.05$ ; gonad lipid  $\chi^2_{3,840} = 6.201$ ,  $p > 0.05$ ; pyloric caeca lipid  $\chi^2_{3,840} = 7.823$ ,  $p > 0.05$ ). There were no significant sexual differences between the energetic content of both (gonad  $\chi^2_{3,840} = 1.786$ ,  $p > 0.05$ ; pyloric caeca  $\chi^2_{3,840} = 1.987$ ,  $p > 0.05$ ).

## Discussion

*Ophidiaster ophidianus* is a gonochoristic species and, as with the majority of echinoderm species, there is no external sexual dimorphism. The equal sex ratio of *O. ophidianus* is consistent with the pattern found for other Atlantic echinoderm species [e.g., *Echinaster* sp. – Eastern Gulf of Mexico (Scheibling and Lawrence 1982)]. However, there was a male bias in December which coincides with a higher number of males in the *Post-spawned and recovering phase*. In some asteroids, sexual maturity depends on size (Mead 1900; MacGintie and MacGintie 1949) but, all individuals of *O. ophidianus* sampled in this study were sexually mature (smallest diameter recorded was 6.2 cm).

GI data indicated that spawning occurred between October and December, while the histological study revealed that gamete release, although more intense in

that period, can occur throughout the entire year. Also, as shown by the histological examinations, there was a high frequency of pre-vitellogenic cells in each sampling month, indicating a continuous production of primary oocytes, as found, for example in *Cosmasterias lurida* (Philippi 1858) (Pastor-de-Ward et al. 2007). Although it was evident that excess oocytes were produced, resulting in the presence of relict oocytes after spawning, the rationale underlying excess oocyte production, a feature reported for several asteroids, is not understood (Pearse 1965; Worley et al. 1977; Walker 1982). Abortive oocytes may serve as a local nutrient source for oogenesis, with phagocytes playing a central role in recycling vitellogenic material (Pearse 1965; Walker 1982). In São Miguel Island, *O. ophidianus* has a seasonal reproductive pattern characterized by three major events: (1) the onset of gonadal recovery around December, (2) the intense proliferation of gametes around May, as females enter a period of intense vitellogenesis and males enter a period of increased spermatogenesis, and (3) the onset of major spawning activity around September. Synchronous release of gametes within a population has the advantage of increasing chances of successful fertilization (Olive 1992).

The pyloric biochemical cycle is usually interpreted as an accumulation of reserves during a period of sexual inactivity; these reserves being utilized later by the gonads during gametogenesis (see Giese 1966 and Lawrence and Lane 1982 for reviews). However, working with the seastar *Luidia clathrata* (Say 1825), Lawrence (1973) reported that gonadal development could occur without a decline in the reserves of the pyloric caecum. Although an inverse relationship between GI and PCI is very common in asteroids (Giese 1966; Lawrence 1973; Oudejans et al. 1979; Byrne 1992; Ventura et al. 1997), not all seastars with seasonal reproduction display it (Carvalho and Ventura 2002), especially if food is abundant during the entire year (Lawrence 1987). From April 2007 to February 2008, *O. ophidianus* there was little variation in the PCI even though there was a clear reproductive cycle, providing further evidence to support the likelihood of constancy of food availability over the study period. Although not very pronounced, the inverse relationship between pyloric caeca and gonad indices in *O. ophidianus* suggests a transfer of nutrients from the pyloric caeca to the gonads during gametogenesis, with caeca reserves supporting gametogenetic development. In most echinoderms (and marine invertebrates in general), somatic growth decreases or ceases during periods of gonadal growth (Lawrence 1987) as a consequence of the limited food available to support both processes (Lawrence 1987). Nevertheless, when individuals are well fed they are able to simultaneously develop gonads, pyloric caeca and quickly regenerate the lost arms (Alves et al. 2002). In the Azores,

*O. ophidianus* individuals with regenerating arms had similar gonadal indices to intact individuals, suggesting that regenerating individuals are also able to invest in the production of gametes.

Allocation of nutrients to the gonads and to the pyloric caeca reflected the seasonal reproductive strategy of *O. ophidianus*. In contrast to the relative stability of the PCI, the biochemical composition and energetic content of the pyloric caeca changed markedly during gametogenesis, in both males and females.

Between December and February (the beginning of the gonad maturation *Growing stage*), there was a decrease in protein levels in the pyloric caeca, when gonad demand for structural material (e.g., amino acids) was high, confirming the importance of proteins in the reproductive cycle. Higher carbohydrate values (Figure 7) found in the pyloric caeca in October coincides with the beginning of the gonadal *Post-spawned and recovering phase*. As carbohydrates are used, typically, for short-term storage of energy from food intake (Pearse and Giese 1966), changes in carbohydrate levels may reflect recent energy expenditures or feeding activity. Lipids were stored in higher quantities than carbohydrates both in gonads and in pyloric caeca (Figure 7). Boolootian (1963) stated that lipids are important in the economy of many echinoderms and are stored early in their reproductive cycle for later use in reproductive processes and, as lipids are a concentrated energy source, they are preferred over carbohydrates for long-term storage (Greenfield et al. 1958; Pearse and Giese 1966). It was clear that during gonadal maturation, an increase in energetic requirements occurred (Figure 7). The major expenditure of

energy by females compared to males (Figure 7) may be due to the need of nutrients for the yolk formation of the oocytes. If the reproductive effort is higher for one sex than the other, we could expect that it could cause differences between sexes in the energy available for somatic growth and provoke changes in growth rates leading to a change in the sex ratio with increasing body size. Nevertheless, as mentioned previously sexual size dimorphism was not observed in *O. ophidianus* individuals.

In asteroid species, inducement of gametogenesis, gamete maturation and/or spawning may be triggered by environmental factors such as seawater temperature and photoperiod (Giese and Pearse 1975). The average day length in São Miguel Island, established from Beck (1968), ranged from a monthly mean of 9.4 h in winter to 14.5 h in summer (Figure 2). Seawater temperatures from 2005 to 2008 were obtained from the satellites NOAA12, NOAA16, NOAA 17 and NOAA18 (data available at: <http://oceano,horta,uac.pt/detra/temperatura.php>) and showed similar seasonal patterns with a minimum of 16°C in early spring and a maximum of 22°C in late summer (Figure 8). Gamete release started after the maximum annual temperature in September and terminated a couple of months before the minimum winter temperature was reached. Also, in the Azores, by early June, the various plankton populations characteristic of spring have reached their peak in biomass (Marra 1989). August is the month of greatest water column stability, and both August and September are the months when the summer plankton community species reaches its maximum (Colebrook 1984). In September, there is some evidence for a fall

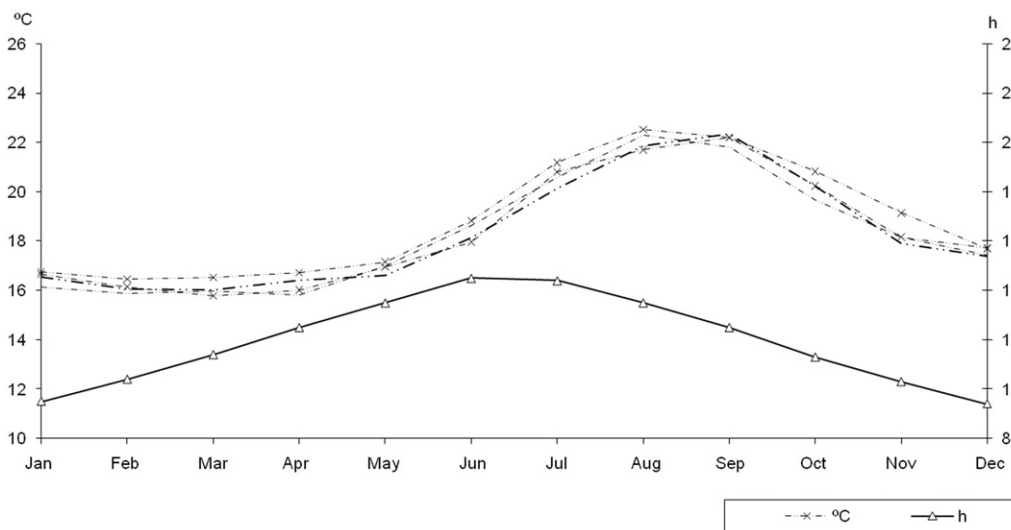


Figure 8. Monthly variation of day length and seawater temperature at São Miguel Island. Seawater temperature data correspond to a 4 years period (each interrupted line), between 2005 and 2008. Advanced Very High Resolution Radiometer (AVHRR) images were capture by satellites NOAA12, NOAA16, NOAA17, and NOAA18 and processed with Terascan software. Data available at <http://oceano,horta,uac.pt/detra/temperatura.php>. Day length at São Miguel Island was obtained from Beck (1968).

bloom as stratification declines (Woods 1988). The advantage in having a cue, like temperature, which coordinates the production of gametes and/or the release of them, is the maximization in the rate of fertilization and the minimization of wasted gametes (Hamel and Mercier 1999).

The reproductive pattern observed in *O. ophidianus* such as a long reproductive season, autumn reproductive peak and efficient energy allocation for reproduction, coupled with rich food availability and seawater temperature characteristics of a temperate zone may be the key for the success of this species in the Azorean oceanic Island. However, only further studies on population from other geographic zones with confirm to contrast these findings.

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