Reproductive biology of the starfish *Pharia pyramidatus* (Echinodermata: Asteroidea) from the Mexican tropical Pacific

FRANCISCO BENÍTEZ-VILLALOBOS AND MARITZA MARTÍNEZ-GARCÍA

Instituto de Recursos, Universidad del Mar, Carretera a Zipolite Km 1.5, Puerto Ángel, Oaxaca, México C.P. 70902

The starfish Pharia pyramidatus *is a tropical species of high ecological importance, mainly because of its role as generalist predator. Nevertheless, there are no studies about its reproduction. This study analysed the reproductive biology of* P. pyramidatus *based on monthly time-series samples from April 2008 to March 2009 at Isla Montosa in the Mexican tropical Pacific. The gonad index (GI), and pyloric caecum index (PCI) were determined and the results were corroborated by histological examination of the gonads.* Pharia pyramidatus *is a gonochoric asteroid and the ratio of males to females was not significantly different throughout the year. Gametogenesis showed high synchrony among males and females and five stages were recognized for both sexes. Spawning occurred from July to September. Maximum oocyte size was ∼150 μm and mean fecundity was 7.3 ± 1.2 millions of oocytes per female. GI and PCI showed seasonal variations and an inverse relationship from May to August. Sea-bottom temperature and photoperiod showed a direct relationship with GI, and apparently constitute the proximate causes of the seasonal reproductive pattern observed in* P. pyramidatus, *while the highly seasonal rainfall and strong upwelling probably act as ultimate causes.*

INTRODUCTION

Starfish species belong to the phylum Echinodermata and class Asteroidea, and about 2100 extant species are currently known (Pawson, 2007), of which the majority is known to be dioecious or gonochorics, and they reproduce sexually with external fertilization (Hyman, 1955; Lawrence, 1987; Chia & Walker, 1991; Hendler *et al.*, 1995; Lawrence & Herrera, 2000). Although there are still inquiries about the factors that control reproduction of starfish, in a number of studies some answers have been found, providing evidence on the close relationship between the reproductive strategy and environmental, physiological and phylogenetic factors. Farmanfarmaian *et al.* (1958) described the reproductive cycles of four species of asteroids from the coast of California using the gonad and pyloric caecal indices, which showed an inverse relationship indicating the translocation of nutrients from pyloric caeca to the gonads. A number of studies have been carried out with asteroids in different parts of the world (see Chia & Walker, 1991; Georgiades *et al.*, 2006 for most of them; also Benítez-Villalobos *et al.*, 2007; Pastor de Ward *et al.*, 2007; Benítez-Villalobos & Diaz Martinez, 2009), revealing different patterns in gonad and pyloric caecum development, but most of them resembling the results obtained by Farmanfarmaian *et al.* (1958).

Seasonality of ecosystems increases gradually from the tropics to highly seasonal regions of the North and South Poles. This pattern mainly follows the annual cycle of solar energy received, and it is exacerbated in the marine environment by the oceanic ice in winter (Priddle *et al.*, 1986). This fact is important in regulating the reproductive activity of echinoderms, because some dissimilarity has been found in the patterns of reproductive periodicity, which reflect the seasonal differences of the environment they inhabit (Byrne *et al.*, 1997; Lessios, 2003). It has been observed that species from the poles or high latitudes show a notable seasonality in reproduction (Stanwell-Smith & Clarke, 1998), which decreases in species from mid-latitudes (Carvalho & Ventura, 2002; Rubilar *et al.*, 2005) and is not evident in tropical species (Pearse, 1968; Guzmán & Guevara, 2002).

The reproductive biology of asteroids has been widely documented for different locations around the world, however, publications on tropical shallow-water species are scarce, and they have been carried out mainly in the Indo-Pacific and Caribbean.

This paper presents an analysis of the reproductive biology of *Pharia pyramidatus*, a starfish belonging to the family Ophidiasteridae. It is a species from shallow tropical waters, it is distributed from California to Peru as well as the Galápagos Islands, inhabiting rocky and sandy bottoms and associated with coral reefs (Caso, 1943).

Pharia pyramidatus is considered a generalist predator, which preys mainly on molluscs, crustaceans, algae (Palma-Ruíz, 2003), sponges, hydroids and bryozoans (Glynn & Wellington, 1983), and it also has been reported as corallivore, mostly feeding on species of the genus *Pocillopora* (Dana & Wolfson, 1970). The ecological importance of *P. pyramidatus* is related to the role that it plays in the trophic network as a predator of other populations,

Corresponding author:
F. Benítez-Villalobos
Email: fbv@angel.umar.mx

1409

contributing significantly to the flux of matter and energy of the ecosystems in which it inhabits, especially in those highly productive ecosystems like coral reefs.

Economically, *P. pyramidatus* has become relevant in recent years, as it is among the species of marine invertebrates with the most important demand in Mexico for use as ornaments. The commercial extraction of *P. pyramidatus* started in the mid-1960s, but according to the Mexican National Institute for Fisheries, an historical record of catches does not exist (Piña-Espallargas, 2004).

In light of the growing rate of extraction of species for ornamental purposes, it is necessary to improve the knowledge about this potential resource. Also the analyses of the reproductive patterns of *P. pyramidatus* will contribute to knowledge about its reproductive strategy as well as the importance of the environmental factors that generate those patterns, which become very significant in the context of the relative rapid change that the ecosystems are currently undergoing, especially those of great ecological value like coral reefs, where *P. pyramidatus* stands as a key species for the control of other populations.

MATERIALS AND METHODS

Sampling

About twenty individuals of *Pharia pyramidatus* were haphazardly collected by SCUBA diving monthly between April 2008 and May 2009, from a depth interval of 3–15 m on the Isla Montosa reef community (15°45′48″N 96°04′57″W), on the Pacific Coast of southern Mexico. During each monthly collection, salinity and pH of the water were measured using a waterproof multiparameter meter, and temperature was recorded at one hour intervals using a pendant HOBO® data logger, permanently attached to the bottom. Data of pluvial precipitation and photoperiod (light hours) were obtained from the weather centre of the Water National Commission (CNA) located in Puerto Angel, Oaxaca. Monthly means were calculated for every parameter recorded.

Gonad general structure and gametogenic cycle

All individuals were narcotized by leaving them in seawater at 2°C for 24 hours before dissection. The whole animals were damp-dried and weighed to the nearest mg. The major radius (R: centre of disc to the tip of the largest arm) and minor radius (r: centre of disc to the interradius) were measured for every individual to the nearest mm. The gonads and gut caeca of the five arms were dissected out by opening the oral side of the disc along each radius. Each organ was damp-dried and weighed separately to the nearest mg, and subsequently fixed in Bouin's solution and stored in 70% ethanol.

The gonad index percentage (gonads weight/total body weight ×100) and pyloric caecum index percentage (caeca weight /total body weight ×100) were calculated for each individual. These data were summated for each sample, and the mean and 95% confidence limits calculated in order to detect seasonal reproduction.

The volumes of the gonads were measured to the nearest 0.001 ml by the displacement of fluid method, using a variation of the hydrostatic balance of Mohr–Westphal (Scherle, 1970). The gonads were processed in paraffin wax, sectioned at 7 µm and stained with haematoxylin and eosin.

Five stages in gonad development were described, based on the frequency and characteristics of gametes, presence of phagocytes and cell remains, as well as thickness and shape of the gonad wall. Gonad stages were based on the developmental scales used with other asteroid species (McClary & Mladenov, 1989; Byrne *et al.*, 1997; Carvalho & Ventura, 2002; Rubilar *et al.*, 2005).

Oogenesis and spermatogenesis were described using the sections. At least 100 oocytes per gonad, sectioned through the nucleolus, were measured (Feret diameter) using the image analysis package Image J. Oocyte size–frequency diagrams were constructed to identify evidence of seasonal reproduction. To compare the oocyte size–frequency data from different samples, the data were plotted into 20 µm classes. Two slides per male were prepared to identify the developmental stage of sperm.

Fecundity was quantified as the number of vitellogenic oocytes per female (actual fecundity), from the mean volume of oocytes and the volume of the ovary in each female. Oocyte volume (OV) is calculated assuming a spherical shape [$OV = (4 \times \pi \times R^3)/3$] and averaged. We estimated the volume of the gonadal fluid that solidifies during fixation from ten slides in ten females as it was proposed by Ramirez-Llodra *et al.* (2002) and concluded that it occupies approximately 15% of the ovary. This percentage was extracted from the total gonad volume.

Fecundity was estimated as follows:

$$F_{vo} = V_g/V_{vo} + (V_{pvo} \times P).$$

where V_g = volume of the gonad, V_{pvo} = mean volume of a previtellogenic oocyte, V_{vo} = mean volume of a vitellogenic oocyte, P = ratio between previtellogenic and vitellogenic oocytes: $P = N_{pvo}/N_{vo}$, N_{pvo} = number of previtellogenic oocytes counted on a subsample of 100 oocytes per gonad, N_{vo} = number of vitellogenic oocytes counted on a subsample of 100 oocytes per gonad.

Sex-ratio was tested by the Chi-square (χ^2) test and statistical differences in gonad index (GI) and pyloric caecum index (PCI) were tested using two-way analysis of variance (ANOVA) (sex and month as factors), and differences in fecundity for females were tested also using ANOVA. In order to know the relationship between salinity, temperature, pluvial precipitation and photo-period (independent variables) and the reproductive cycle of *P. pyramidatus* represented by GI and PCI (dependent variables), we performed a step by step multiple lineal regression analysis.

RESULTS

Sex-ratio and gonad morphology

Pharia pyramidatus did not show sexual dimorphism and it was confirmed that it is a gonochoric asteroid as not one of the 225 examined organisms was hermaphrodite. The ratio of males to females did not differ significantly, showing a 1:1 proportion throughout the year. The total number of individuals sexed was 218, with 107 males and 111 females (χ^2 = 0.073, $P > 0.05$). The gonads were located in the coelomic

cavity at the actinolateral zone underneath the pyloric caeca, and showed a serial arrangement, forming two rows of individual clusters (acini) running along every arm and separated by the ambulacral groove. Every acinus stayed attached to the lateral plates of the body wall by means of individual short gonoducts that conducted to their respective gonopore at the lateral papular zone.

The number of acini varied depending on the length of the arm, the largest specimens, with major radius (R) about 16 cm long, presented up to 26 clusters, whereas the smallest (R ≈ 8 cm) showed between 10 and 12 clusters. All the acini were equally small in an individual at the growing stage, but at the mature stage, the size of the acini was largest at the proximal zone and decreased gradually towards the distal zone, filling the coelomic cavity that was not occupied by the pyloric caeca (about 60%).

The microscopic analysis allowed for differentiation of the structural components of the gonad wall. Similarly to the majority of asteroids, ovaries and testes of *P. pyramidatus* possessed three layers of tissue: the inner and outer sacs and the genital coelomic sinus (perihaemal sinus) that separate them (Figure 1).

The outer sac was constituted by two layers of epithelial tissue separated by a layer of connective tissue, which showed a variable width. Conformation of the external layer of epithelium changed according to the gonad developmental phase, being formed by flat, cubic or cylindrical cells. The inner sac contained the haemal sinus, which was clearly visible during the active phase of the germinal epithelium when gametogenesis was activated.

Ovary development

GROWING STAGE

There was a predominance of previtellogenic oocytes with basophilic cytoplasm that stained dark purple with haematoxylin. The outer sac appeared extended and thin; the inner sac looked very thin and had a reduced perihaemal sinus. As this phase advanced, vitellogenic oocytes predominated and they had an eccentric nucleus and acidophilic cytoplasm that stained light pink with eosin. The gonad wall appeared thin and there were visible epithelial cells, the perihaemal sinus was occasionally visible (Figure 2A).
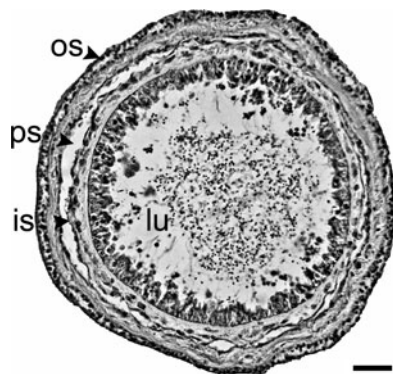

Fig. 1. Transverse section of testis showing the general arrangement of the tissues in the gonad wall. is, inner sac; lu, lumen; os, outer sac; ps, perihaemal sinus. Scale bar = 30 μm.
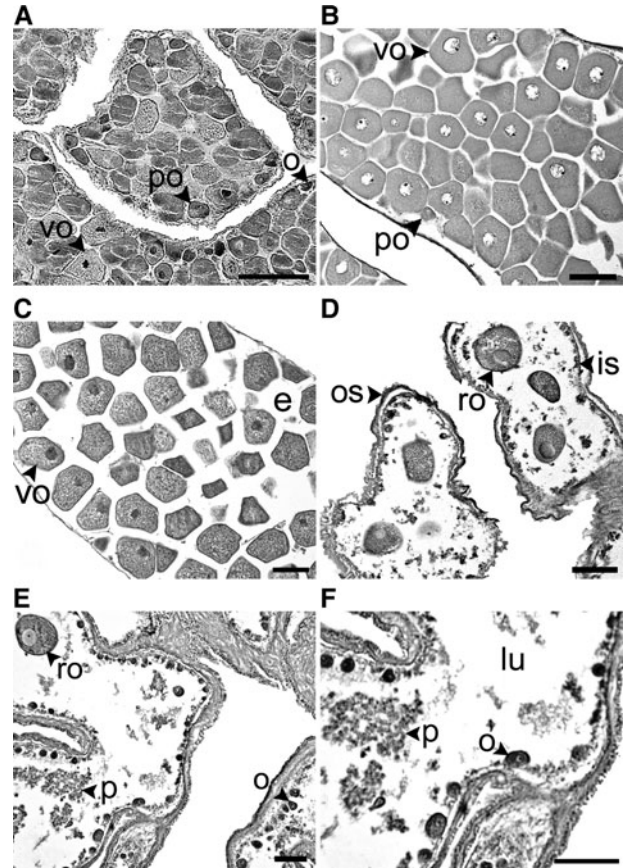

Fig. 2. Light histology of ovaries. (A) Growing stage: ovary showing the distribution of oocytes, the small oocytes remain at the periphery and the vitellogenic oocytes are accumulated in the lumen; (B) mature stage: mature ovary filled with vitellogenic oocytes; (C) spawned stage: ovary showing empty spaces due to gamete release; (D) spent stage: ovary almost empty and contracted with relict oocytes; (E) recovery stage: ovary containing cellular debris and phagocytes; (F) detail of recovery ovary with oogonia proliferating in the germinal epithelium. e, empty space; is, inner sac; lu, lumen; o, oogonia; os, outer sac; p, phagocytes; po, previtellogenic oocyte; ro, relict oocytes; vo, vitellogenic oocytes. Scale bar = 100 μm

MATURE STAGE

Ovaries were filled with agglomerated vitellogenic oocytes (110–130 μm diameter) resulting in an oval shape of the oocytes located at the lumen, while the ones at the periphery showed an almost rectangular shape. A pale thin meshwork of tissue surrounded each oocyte. Very scarce previtellogenic oocytes and oogonia remained. The gonad wall showed the thinnest and smoothest appearance of the entire reproductive cycle (Figure 2B).

SPAWNED STAGE

This stage was characterized by the presence of empty spaces in the lumen of the gonads, which evidenced the liberation of full-grown gametes. Oocytes with maximum development predominated in the ovaries. There were a few early previtellogenic oocytes and some atresic oocytes. The gonad wall remained thin but it started to show slight undulations (Figure 2C).

SPENT STAGE

All ovaries appeared almost empty and contracted, containing relict full-grown oocytes, most of them atresic or not well

developed. In the areas where most of the occytes were present, the gonad wall remained thin, whereas in those areas where the lumen was almost empty, the outer sac started widening and undulating, the perihaemal sinus was evident, and the first phagocytes were observed (Figure 2D).

RECOVERY STAGE

There still existed relict oocytes with a cracked appearance and diffuse nuclei in the gonads. As the amount of cell remains increased, a proliferation of phagocytes was observed forming irregular agglomerations in the lumen of gonads. The gonad wall showed the thickest appearance of the entire reproductive cycle, the outer sac showed many undulations and the inner sac had a smooth appearance. Oogonia started to proliferate from the germinal epithelium (Figure 2E, F).

Testis development

GROWING STAGE

Spermatogonia ($\sim$4.2 μm) proliferated and they were organized in columns and through mitosis produced spermatocytes ($\sim$3 μm), which extended to the lumen forming colonettes. Spermatids ($\sim$2 μm) differentiated from the apices of the colonettes and gave rise to spermatozoa ($\sim$1.4 μm), which concentrated in the lumen of the gonads. The gonad wall appeared thin, the inner sac was extended and the epithelial cells were visible (Figure 3A, B).

MATURE STAGE

Almost the entire lumen was occupied by gametes ready to be spawned. The different layers of the gonad wall were not distinguishable. Spermatozoa were highly abundant and compressed so that they had the appearance of a purple uniform tissue, surrounded by a thin layer of spermatogenic columns (Figure 3C).

SPAWNED STAGE

A number of empty spaces with the appearance of fine striations were visible among the dense mass of spermatozoa. The inner and outer sacs were visible again with slight undulations, as well as the perihaemal sinus (Figure 3D).

SPENT STAGE

The testes appeared contracted and the lumen was almost empty. There were a few relict spermatozoa and the first phagocytes appeared. The outer sac started to thicken and showed undulations; a wide perihaemal sinus was visible (Figure 3E).

RECOVERY STAGE

The outer sac formed ample undulations and the perihaemal sinus diminished noticeably. Phagocytes at the periphery of the lumen proliferated and dispersed centrally, and were progressively abundant until most of the cell remains were eliminated. At the end of this stage the outer sac diminished in undulations, the perihaemal sinus expanded notably and the first spermatogonia were visible (Figure 3F).

Reproductive cycle

In females, the growing stage was observed from January to June. In January, 73% of females were at this stage and the
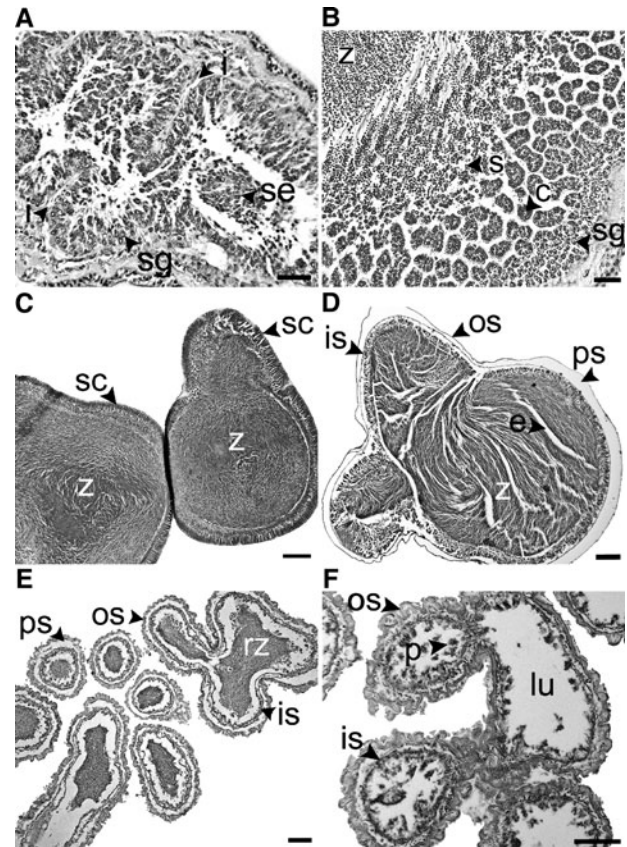

Fig. 3. Light histology of testes. (A) Growing stage: testis showing the invaginations of the germinal epithelium in the lumen; (B) detail of growing testis; (C) mature stage: testis filled with spermatozoa and thin spermatogenic columns; (D) spawned stage: testis showing empty spaces due to gamete release; (E) spent stage: contracted testis with residual spermatozoa; (F) recovery stage: proliferation of phagocytes in the lumen. c, colonette; e, empty space; I, invagination; is, inner sac; lu, lumen; os, outer sac; p, phagocytes; ps, perihaemal sinus; rz, residual spermatozoa; s, spermatids; sc, spermatogenic columns; se, spermatocytes; sg, spermatogonia; z, spermatozoa. Scale bars: A–B = 25 μm; C–F = 100 μm.

most dominant size interval of oocyte was 30–50 μm. The percentage of females in the growing stage grew progressively to 91% in April, which coincided with a high abundance of previtellogenic (30–70 μm) oocytes followed by early vitellogenic (70–110 μm) oocytes. During May and June, 100% of females were mature, and most oocytes were in early vitellogenic stages. In July–August, 30% of females were at the mature stage and the dominant size interval of oocytes was 110–130 μm, followed by the adjacent intervals. Also in this period, 50% of females started spawning and continued until September. During the spawning period, the dominant size intervals of oocytes were similar to those of the previous stage. The spent stage was observed first in July–August in 20% of females, during September, it increased to 38% and decreased in October to 25% of females. During October, a noticeable change was observed in oocyte size intervals because oogonia (10–30 μm), which had remained in low numbers increased visibly, followed by a few relict full-grown oocytes (90–110 μm). The recovery stage was recorded in October in 75% of females, during November and December all females were at this stage and in January the frequency decreased to 27%. During this period, the oocyte size distribution was very similar; oogonia were the most

dominant frequency, followed by previtellogenic oocytes (30–50 μm) and practically no vitellogenic oocytes (Figures 4A & 5).

In males, the growing stage also started in January with a frequency of 40%. During February and March the frequency of males in this stage increased, and during April and May it was observed in the totality of organisms. In June 75% of males were mature while the rest were still in the growing stage. The highest intensity of spawning was observed during July and August, when testes of 83% of males were at this stage. During September, 21% of individuals were still spawning and during October, 14% of males still presented a considerable portion of full-grown gametes ready to be liberated. During September 36% of males were at the spent stage, the same percentage was observed in October, and in November the highest frequency was recorded with 43% of males at this stage. The percentage of males at the recovery stage in September, increased slightly during October and November. During December the gonads of the totality of males were at this stage (Figure 4B).

Gonad and pyloric caecum indices

From January ($\dot{x} = 1.6$; SE = 1.1; N = 20), and through the following months, a constant increase was observed in GI values for females, indicating that ovaries were at the growing stage. The highest value was reached in July–August ($\dot{x} = 10.4$; SE = 4.5; N = 20), when organisms initiated spawning. In September there was a high variability in GI values ($\dot{x} = 7.7$; SE = 4.5; N = 20) as a result of spawning, characterized by the presence of organisms both mature and totally spawned. At the end of the spent stage

Fig. 4. Gametogenic cycle of *Pharia pyramidatus* from April 2008 to May 2009. (A) Females; (B) males.

(October), GI reached the lowest values ($\dot{x} = 0.31$; SE = 0.1; N = 20). From October to December, values were low, coinciding with the recovery stage (Figure 6A).

In males values of GI also started to increase from February ($\dot{x} = 0.3$; SE = 0.1; N = 20) until July–August when it reached a peak ($\dot{x} = 7.1$; SE = 2; N = 20). In September it dropped abruptly ($\dot{x} = 1.6$; SE = 0.1; N = 20) as a consequence of the spawning and followed this trend until December, when the lowest value was recorded ($\dot{x} = 0.2$; SE = 0.1; N = 20) (Figure 6B). There were significant differences between months (ANOVA, $F_{(10,196)} = 29.13$, $P < 0.001$) and sexes (ANOVA, $F_{(1,196)} = 40.43$, $P < 0.001$), with females possessing the highest and most variable values (Figure 6).

From April to May 2008, for both sexes, a slight increase in PCI values was recorded, reaching a monthly mean of around 8, but in June it decreased ($\dot{x} = 5.0$; SE = 2.4; N = 20 for females and $\dot{x} = 4.8$; SE = 3.0; N = 20 for males). This trend continued until October, when PCI reached the lowest values ($\dot{x} = 3.8$; SE = 1.9; N = 20 for females and $\dot{x} = 4.0$; SE = 1.2; N = 20 for males). From November and through the subsequent months, values increased gradually until April–May, when the maximum values were reached for females ($\dot{x} = 12.6$; SE = 3.1; N = 20) and males ($\dot{x} = 15.0$; SE = 3.5; N = 20). There were no significant differences between sexes (ANOVA, $F_{(1,196)} = 0.451$, $P > 0.05$), but there were differences between months (ANOVA, $F_{(10,196)} = 34.1$, $P < 0.001$). The general GI showed a negative relation ($\beta = -0.25$) with the general PCI (Figure 6).

Fecundity

Actual mean ($\pm$ SE) fecundity of *Pharia pyramidatus* was 7.3 ± 1.2 million of eggs per female. Fluctuations in fecundity values behaved similarly to those of the GI values in females. From April to June, an increase was observed in fecundity, reaching a maximum of 18.5 ± 1.7 millions of eggs. Starting in August, the values decreased continuously until October (0.28 ± 0.1 million). During November and December, no vitellogenic oocytes were recorded; therefore actual fecundity was zero for both months. In January, fecundity was 1.5 ± 0.2 million, and the values increased until April–May (7.5 ± 0.9 million). There were significant differences in monthly mean values of fecundity (ANOVA, $F_{(8,74)} = 10.95$, $P < 0.001$).

Environmental variables

The multiple regression analysis of GI with respect to the environmental variables was performed in two steps, resulting in the following model:

$$GI = -50.63 + 0.632\,P + 0.396\,T$$

The model showed that the total explained variance over the GI, in terms of the selected environmental variables was 86.95% ($R^2 = 0.8695$, R = 0.9325, P multiple < 0.05), indicating that the variables photoperiod (P) and bottom water temperature (T) are correlated 93.25% with GI (Figure 7). The first variable that went into the model was P providing 77.36% of the total explained variance, whereas at the second step T contributed to 9.6%.
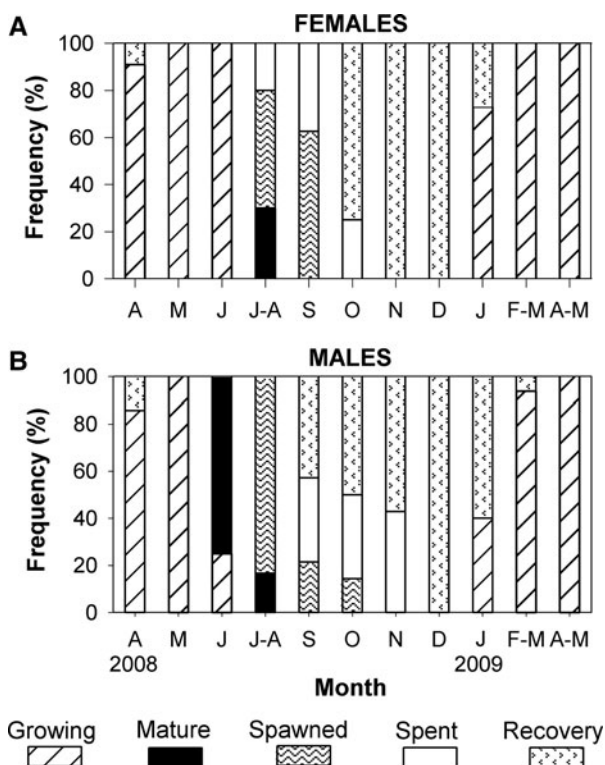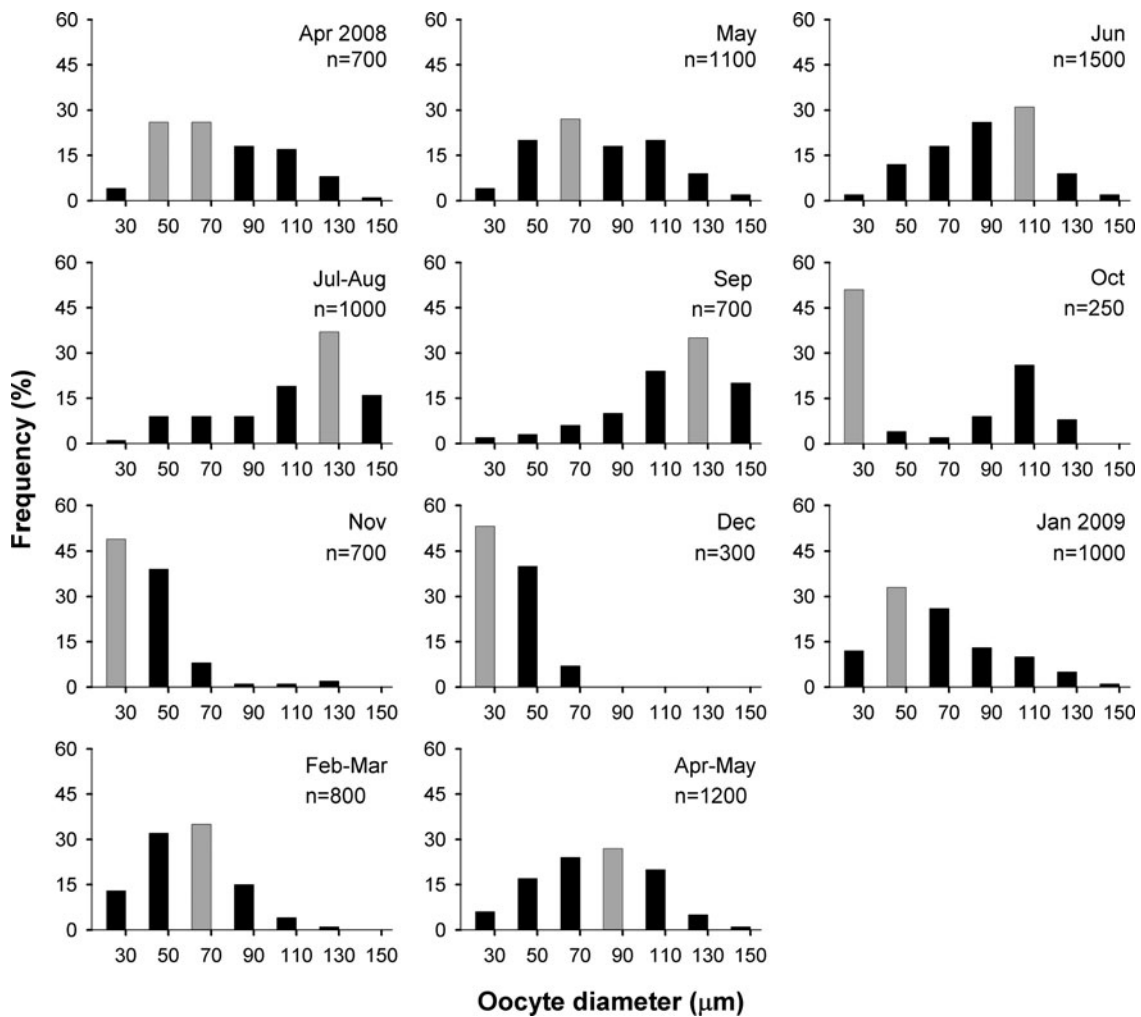
Fig. 5. Oocyte diameter frequency distribution of *Pharia pyramidatus* (April 2008–May 2009). Grey bars are modal size.

Regarding the PCI the analysis was performed in two steps, resulting in the following model:

$$PCI = 15.77 - 1.1\ T + 0.675\ P$$

This model showed that the total explained variance in terms of the selected environmental variables was 68.16% ($R^2 = 0.6816$, $R = 0.8256$, P multiple < 0.05). The variables T and P showed a multiple correlation value of 82.56% with the PCI (Figure 7). At the first step, the total explained variance was 40.32% as a result of the variable T and at the final step the variable P contributed to 27.84%.

Although pluvial precipitation was not considered as a significant variable by the model, when a simple lineal regression analysis was performed, the result showed that this variable had a significant correlation ($R = 0.80$, $R^2 = 0.64$, $P < 0.01$) with GI (Figure 7B).

DISCUSSION

As it has been reported for other species (Kim, 1968; Rubilar *et al.*, 2005), the maturity stage of the gametogenic cycle of *P. pyramidatus* was the shortest and the growing stage was the most extensive, because it included vitellogenesis, which

is considered the longest and most energetically expensive process of ovogenesis (Ramirez-Llodra, 2002). The spawning period is highly variable and it can last two (Chen & Chen, 1992; Rubilar *et al.*, 2005), three (Kim, 1968; Stanwell-Smith & Clarke, 1998), four (Scheibling, 1981; Tyler & Pain, 1982; Byrne *et al.*, 1997) or up to five months (Carvalho & Ventura, 2002), according to the degree of seasonality of the species and synchrony of the population.

The histological analysis showed that females of *P. pyramidatus* reached the maturity stage slightly later than the males, and remained mature for a longer period as it has been reported for other asteroid species (Byrne *et al.*, 1997). Nevertheless, gametogenesis in males and females occurred simultaneously and both sexes underwent a massive spawning during approximately three months (July–September). Hence, individuals of the population showed a high synchronicity, which is essential for spawning, in order to guarantee reproductive success (Mercier & Hamel, 2009). In contrast with *P. pyramidatus*, Pearse (1968) found that gametogenesis of *Linckia laevigata* was very asynchronous among individuals, and suggested that some individuals were reproductively active all year, at least when the populations were near the equator. This fact is interesting because *L. laevigata* is another tropical starfish closely related to *P. pyramidatus* as it belongs to the family Ophidiasteridae; however, their reproductive patterns are dissimilar.
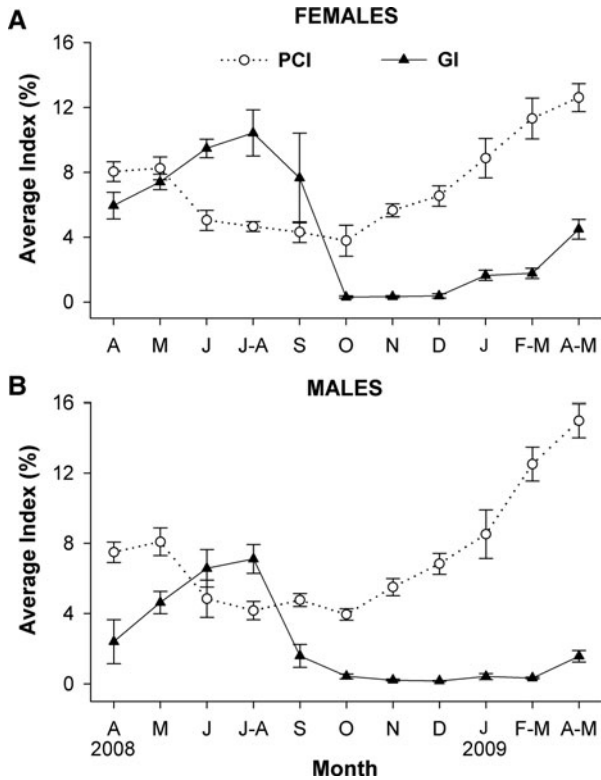
Fig. 6. Monthly variation of gonad index (GI) and pyloric caecum index (PCI) of *Pharia pyramidatus.* (A) Females; (B) males. Vertical bars indicate standard error.
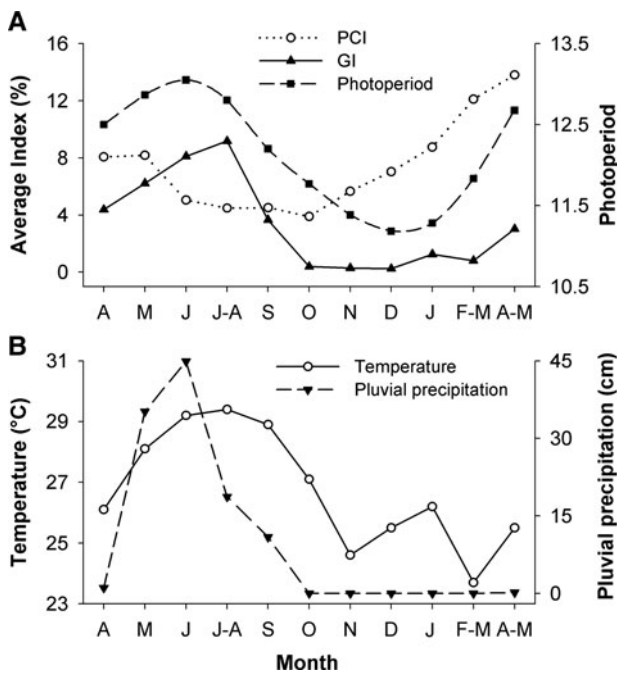

Fig. 7. Monthly reproductive changes compared to environmental variables. (A) Mean values of gonad index (GI) and pyloric caecum index (PCI) of total population of *Pharia pyramidatus* compared with photoperiod; (B) monthly variation of bottom seawater temperature and pluvial precipitation.

Production of eggs of *P. pyramidatus* is shown to be seasonal rather than continuous, evidence that the females must concentrate their energy in order to make use of it in a single annual generation of numerous eggs. Giese & Pearse (1974) mentioned

that precise breeding seasons may be important to provide larvae or juveniles with favourable environmental conditions. Baker (1938) recognized two causes of reproductive seasonality: (1) exogenous factors which serve as clues to synchronize the cycles; and (2) environmental conditions that exert selective (evolutionary) pressure, favouring for a species one season for reproduction over others. Baker (1938) distinguished these as the proximate and ultimate causes, respectively.

Photoperiod and sea temperature were parameters of high importance for the dynamics of reproduction of *P. pyramidatus* and apparently they constitute the proximate causes that generate the seasonal pattern observed, synchronizing the reproductive cycles. The statistical analyses suggested that photoperiod was the most significant variable and the gonadic maturation coincided with the increasing of day length, as it was recorded with *Oreaster reticulatus* (Scheibling, 1981). In our study the stage of gonad reabsorption coincided with the months when the days were shorter, whereas the period of spawning occurred during the longest days. The apparent photoperiodic regulation of gametogenesis and gonadal growth in the case of *P. pyramidatus* coincides with what was observed for other asteroids at different latitudes (Pearse & Eernisse, 1982; Pearse & Beauchamp, 1986; Pearse & Walker, 1986; Pearse & Bosch, 2002; Georgiades *et al.*, 2006). Byrne *et al.* (1997) provided evidence that photoperiod regulates the gametogenic cycle of *Asterias amurensis*. Furthermore the authors compared reproduction of *A. amurensis* in Japan and Tasmania at similar latitudes, showing that gametogenesis of the northern and southern populations was six months out of phase. For this reason, it is important to carry out similar studies with *P. pyramidatus* in order to discern if the photoperiod has the same effect on populations of this tropical species at different latitudes.

Fluctuations in sea temperature generally act as a clue for the spawning or particular reproductive behaviours such as aggregations. For *P. pyramidatus*, the maximum values of sea temperature coincided with the spawning, and it is probably the external signal for that process. In another study carried out in eastern Canada, the spawning of *Asterias vulgaris* corresponded with a noticeable increase in sea temperature (Raymond *et al.*, 2007). Also the gonad maturation and spawning of the tropical asteroid *Oreaster reticulatus* occurred during the season of highest sea temperatures (Scheibling, 1981).

Ultimate causes usually have to do with survival of offspring, and little is known about them, making them difficult to demonstrate, but most cycles are timed so that the production of larvae or juveniles must be synchronized with periods favourable for feeding or survival (Giese & Pearse, 1974). On the coast of Oaxaca, there is a remarkable difference between dry (mean pluvial precipitation 0 mm) and the rainy (mean pluvial precipitation 800–1200 mm) seasons. The rainy season includes the period from May to September and this highly seasonal rainfall produces an important increase in availability of nutrients for larval food, through extensive runoffs from the main streams in the zone (especially the Copalita River). Furthermore, during the rainy season, the movement of tropical storms and hurricanes generate pulses of winds that propagate along the coast (Lavín *et al.*, 1992; Barton *et al.*, 1993; Trasviña & Barton, 1997), and probably the resultant currents favour the retaining of larvae near shore. On the other hand, between November and April, strong wind jets called 'Nortes' blow perpendicularly to the coast and generate upwelling in the Gulf of Tehuantepec.

This specific oceanographic dynamic probably affects the reproductive pattern of marine organisms, carrying larvae offshore, and it is likely a condition that has been selected against. Consequently, the strong seasonal cycles of heavy rain and dry season, as well as the strong winds and the resulting upwelling in autumn–winter, are likely to constitute the ultimate causes of the reproductive seasonality of *P. pyramidatus*.

In this work GI and PCI showed an inverse relationship from May to August (Figure 6), which indicates a transfer of nutrients from the pyloric caeca to the gonads, as it has been documented by a number of authors for other species (Mauzey, 1966; Rao, 1966; Kim, 1968; Crump, 1971; Jangoux & Vloebergh, 1973; Ferguson, 1975a, b; Barker, 1979; Harrold & Pearse, 1980; Scheibling, 1981; Chia & Walker, 1991; Carvalho & Ventura, 2002; Rubilar *et al.*, 2005; Georgiades *et al.*, 2006). However, during five months the two indices showed no inverse relationship, probably because the available energy was abundant enough in order to allow an increase in the PCI even though gametogenesis was occurring in the gonads.

This behaviour of the two indices coincided with the period of more intense effect of the 'Nortes' which generate upwellings in the Gulf of Tehuantepec and cause an important increase in primary production in the entire region of the Mexican south Pacific (Lavín *et al.*, 1992), providing a high availability of energy for all biological processes, in this case storage of energy in the pyloric caeca and gametogenesis in the gonads of *P. pyramidatus*. The intensity of the event varies from year to year and consequently so does the increase in available energy, which probably caused the pattern observed in the values of the PCI, as the results showed that the PCI was nearly twice as large in April–March of 2009 than in the same period in 2008.

When the features of the study site were first analysed it was supposed that *P. pyramidatus* would exhibit patterns characteristic of a continuous reproductive cycle because the fluctuations of temperature and photoperiod are apparently minimal and the availability of food is theoretically constant. However, the results of this study showed the opposite, because even though the variation of temperature (5.7°C) and photoperiod (1.9 hours) at Isla Montosa are relatively narrow, the statistical analyses showed that those variables were highly correlated (93%) with the cycle of the GI and they probably constitute the proximate causes. Although the study site of this asteroid species corresponds to a tropical locality, the local oceanic processes such as upwellings in the Gulf of Tehuantepec between November and April, and a remarkable difference between the rainy and dry seasons determine seasonal oceanographic regimes. Those regimes act as ultimate (evolutionary) causes, which influence the biological processes of the communities, determining the seasonal reproductive patterns observed in *P. pyramidatus*.

According to the reproductive features of *P. pyramidatus* (high fecundity, small eggs, relatively large adults and seasonality of gametogenesis), it is evident that the reproductive strategy of this species includes a larva with planktotrophic development, as is reported in other asteroid species studied in other parts of the world (Tyler & Pain, 1982; Ramirez-Llodra *et al.*, 2002).

ACKNOWLEDGEMENTS

REFERENCES

Baker J.I. (1938) The evolution of breeding seasons. In De Beer G.R. (ed.) *Evolution: essays on aspects of evolutionary biology presented to Professor E.S. Goodrich on his seventieth birthday.* London: Oxford University Press, pp. 161–177.

Barker M.F. (1979) Breeding and recruitment in a population of the New Zealand starfish *Stichaster australis* (Verrill) (Echinodermata: Asteroidea). *Journal of Experimental Marine Biology and Ecology* 41, 195–211.

Barton E.D., Argote M.L., Brown J., Kosro P.M., Lavín M., Robles J.M. R., Smith L., Trasviña A. and Velez H.S. (1993) Supersquirt: dynamics of the Gulf of Tehuantepec, Mexico. *Oceanography* 6, 23–30.

Benítez-Villalobos F. and Díaz-Martínez J.P. (2009) Reproductive patterns of the abyssal asteroid *Styracaster elongatus* from the NE Atlantic Ocean. *Deep-Sea Research I* 57, 157–161.

Benítez-Villalobos F., Díaz-Martínez J.P. and Tyler P.A. (2007) Biología reproductiva del asteroideo de mar profundo *Henricia abyssicola* en el Atlántico NE. *Ciencias Marinas* 33, 49–58.

Byrne M., Morrice M.G. and Wolf B. (1997) Introduction of the northern Pacific asteroid *Asterias amurensis* to Tasmania: reproduction and current distribution. *Marine Biology* 127, 673–685.

Carvalho A.L.P.S. and Ventura C.R.R. (2002) The reproductive cycle of *Asterina stellifera* (Möbius) (Echinodermata: Asteroidea) in the Cabo Frio region, southeastern Brazil. *Marine Biology* 141, 947–954.

Caso M.E. (1943) *Contribución al conocimiento de los Asteridos de México.* Instituto de Investigaciones Marinas y Costeras. Available at http://www.invemar.gov.co/zsibficha2.jsp?clave=60.55.3382&phyl=&niv= &esp=null&nombre=Phariapyramidata&phylum=60&nomphylum= ECHINODERMATA (accessed 8 November 2008).

Chen B.Y. and Chen C.P. (1992) Reproductive cycle, larval development, juvenile growth and population dynamics of *Patiriella pseudoexigua* (Echinodermata: Asteroidea) in Taiwan. *Marine Biology* 113, 271–280.

Chia F.S. and Walker C.W. (1991) Echinodermata: Asteroidea. In Giese A.C. and Pearse J.S. (eds) *Reproduction of marine invertebrates. Volume 6. Echinoderms and lophophorates.* New York: Academic Press, pp. 301–353.

Crump R.G. (1971) Annual reproductive cycles in three geographically separated populations of *Patiriella regularis* (Verrill), a common New Zealand asteroid. *Journal of Experimental Marine Biology and Ecology* 7, 137–162.

Dana T. and Wolfson A. (1970) Eastern Pacific crown of thorns starfish populations in the lower Gulf of California. *Transactions of the San Diego Society of Natural History* 16, 83–90.

Farmanfarmaian A., Giese A.C., Boolootian R.A. and Bennett J. (1958) Annual reproductive cycles in four species of west coast starfish. *Journal of Experimental Zoology* 138, 355–367.

Ferguson J.C. (1975a) The role of free amino acids in nitrogen storage during the annual cycle of a starfish. *Comparative Biochemistry and Physiology* 51A, 341–350.

Ferguson J.C. (1975b) Fatty acid and carbohydrate storage in the annual cycle of *Echinaster*. *Comparative Biochemistry and Physiology* 52A, 585–590.

Georgiades E.T., Temara A. and Holdway D.A. (2006) The reproductive cycle of the asteroid *Coscinasterias muricata* in Port Phillip Bay, Victoria, Australia. *Journal of Experimental Marine Biology and Ecology* 332, 188–197. doi: 10.1016/j.jembe.2005.11.014.

Giese A.C. and Pearse J.S. (1974) Introduction: general principles. In Giese A.C. and Pearse J.S. (eds) *Reproduction of marine invertebrates. Volume I*. New York: Academic Press, pp. 1–49.

Glynn P.W. and Wellington G.M. (1983) *Corals and coral reefs of the Galapagos Islands*. Berkeley, CA: University of California Press, 330 pp.

Guzmán H.M. and Guevara C.A. (2002) Annual reproductive cycle, spatial distribution, abundance, and size structure of *Oreaster reticulatus* (Echinodermata: Asteroidea) in Bocas del Toro, Panama. *Marine Biology* 141, 1077–1084. doi: 10.1007/s00227-002-0898-2.

Harrold C. and Pearse J.S. (1980) Allocation of pyloric caecum reserves in fed and starved sea stars, *Pisaster giganteus* (Stimpson): somatic maintenance comes before reproduction. *Journal of Experimental Marine Biology and Ecology* 48, 169–183.

Hendler G., Miller J.E., Pawson D.L. and Kier P.M. (1995) *Sea stars, sea urchins and allies: echinoderms of Florida and the Caribbean*. Washington and London: Smithsonian Institution Press, 390 pp.

Hyman L.M. (1955) *The invertebrates: Echinodermata. The coelomate bilateria, Volume IV*. Delhi: CBS Publishers & Distributors, 763 pp.

Jangoux M. and Vloebergh M. (1973) Contribution à l'étude du cycle annuel de reproduction d'une population d'*Asterias rubens* (Echinodermata, Asteroidea) du littoral Belge. *Netherlands Journal of Sea Research* 6, 386–408.

Kim Y.S. (1968) Histological observations of the annual change in the gonad of the starfish *Asterias amurensis* Lüken. *Bulletin of the Faculty of Fisheries, Hokkaido University* 19, 97–111.

Lavín M.F., Robles J.M., Argote M.L., Barton E.D., Smith R.L., Brow J., Kosro P.M., Trasviña A., Velez H.S. and García J. (1992) Física del Golfo de Tehuantepec. *Ciencia y Desarrollo* 18, 97–108.

Lawrence J.M. (1987) *A functional biology of echinoderms*. Baltimore, MD: Johns Hopkins University Press, 340 pp.

Lawrence J.M. and Herrera J. (2000) Stress and deviant reproduction in echinoderms. *Zoological Studies* 39, 151–171.

Lessios H.A. (2003) Reproductive periodicity of the echinoids *Diadema* and *Echinometra* on the two coasts of Panama. *Journal of Experimental Marine Biology and Ecology* 50, 47–61.

Mauzey K.P. (1966) Feeding behavior and reproductive cycles in *Pisaster ochraceus*. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 131, 127–144.

McClary D.J. and Madlenov P.V. (1989) Reproductive pattern in the brooding and broadcasting sea star *Pteraster militaris*. *Marine Biology* 103, 531–540.

Mercier A. and Hamel J.F. (2009) *Advances in marine biology: endogenous and exogenous control of gametogenesis and spawning in echinoderms*. Elsevier. Available at http://www.amazon.com/Advances-Marine-Biology-Gametogenesis-Echinoderms/dp/012374959#reader_012374959X (accessed 10 May 2010).

Palma-Ruíz A. (2003) *Determinación de las especies depredadoras de coral presentes en los arrecifes coralinos del Parque Nacional Huatulco, Oax.: un estudio de campo y gabinete*. Dissertation. Benemérita Universidad Autónoma de Puebla, México, 79 pp.

Pastor-de-Ward C.T., Rubilar T., Díaz-De-Vivar M.E., González Pisani X., Zarate E., Kroeck M. and Morsan E. (2007) Reproductive biology of

Cosmasterias lurida (Echinodermata: Asteroidea) an anthropogenically influenced substratum from Golfo Nuevo, Northern Patagonia (Argentina). *Marine Biology* 151, 205–217.

Pawson D.L. (2007) Phylum Echinodermata. *Zootaxa* 1668, 749–764.

Pearse J.S. (1968) Patterns of reproduction in four species of Indo-Pacific echinoderms. *Proceedings of the Indian Academy of Science B* 68, 247–279.

Pearse J.S. and Beauchamp K.A. (1986) Photoperiodic regulation of feeding and reproduction in a brooding sea star from central California. *International Journal of Invertebrate Reproduction and Development* 9, 289–297.

Pearse J.S. and Bosch I. (2002) Photoperiodic regulation of gametogenesis in the Antartic sea star *Odontaster validus* Koehler: evidence for a circannual rhythm modulated by light. *International Journal of Invertebrate Reproduction and Development* 41, 73–81.

Pearse J.S. and Eernisse D.J. (1982) Photoperiodic regulation of gametogenesis and gonadal growth in the sea star *Pisaster ochraceus*. *Marine Biology* 67, 121–125.

Pearse J.S. and Walker C.W. (1986) Photoperiodic regulation of gametogenesis in a North Atlantic sea star, *Asterias vulgaris*. *International Journal of Invertebrate Reproduction and Development* 9, 71–77.

Piña-Espallargas R. (2004) *La pesquería de especies marinas con fines de ornato en México. El parque marino de Loreto, B.C.S. como estudio de caso*. MSc dissertation. Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional. Available at http://itzamna.bnct.ipn.mx:8080/dspace/bitstream/123456789/1799/1/espallargas.pdf. (accesed 10 November 2010).

Priddle J., Hawes I., Ellis-Evans J.C. and Smith T.J. (1986) Antarctic aquatic ecosystems as habitats for phytoplankton. *Biology Review* 61, 199–238.

Ramirez-Llodra E. (2002) Fecundity and life-history strategies in marine invertebrate. *Advances in Marine Biology* 43, 88–154.

Ramirez-Llodra E., Tyler P.A. and Billett D.S.M. (2002) Reproductive biology of porcellanasterid asteroids from three abyssal sites in the northeast Atlantic with contrasting food input. *Marine Biology* 140, 773–788. doi: 10.1007/s00227-001-0750-0.

Rao K.S. (1966) Reproductive nutritional cycles of *Oreaster hedemanni* Lutken. *Journal of the Marine Biological Association of India* 8, 254–272.

Raymond J.F., Himmelman J.H. and Guderley H.E. (2007) Biochemical content, energy composition and reproductive effort in the broadcasting sea star *Asterias vulgaris* over the spawning period. *Journal of Experimental Marine Biology and Ecology* 341, 32–44. doi: 10.1016/j.jembe.2006.10.030.

Rubilar T., Pastor de Ward C.T. and Díaz de Vivar M.E. (2005) Sexual and asexual reproduction of *Allostichaster capensis* (Echinodermata: Asteroidea) in Golfo Nuevo. *Marine Biology* 146, 1083–1090. doi: 10.1007/s00227-004-1530-4.

Scheibling R.E. (1981) The annual reproductive cycle of *Oreaster reticulatus* (L.) (Echinodermata: Asteroidea) and interpopulation differences in reproductive capacity. *Journal of Experimental Marine Biology and Ecology* 54, 39–54.

Scherle W. (1970) A simple method for volumetry of organs in quantitative stereology. *Mikroskopie* 26, 57–60.

Stanwell-Smith D. and Clarke A. (1998) Seasonality of reproduction in the cushion star *Odontaster validus* at Signy Island, Antarctica. *Marine Biology* 131, 479–487.

Trasviña A. and Barton E.D. (1997) Los 'Nortes' del Golfo de Tehuantepec: la circulación costera inducida por el viento. In Lavín

M.F. (ed.) *Contribuciones a la Oceanografía Física en México, Monografía No. 3, Unión Geofísica Mexicana*, 25–46.

and

Tyler P.A. and Pain S.L. (1982) The reproductive biology of *Plutonaster bifrons*, *Dytaster insignis* and *Psilaster andromeda* (Asteroidea: Astropectinidae) from the Rockall Trough. *Journal of the Marine Biological Association of the United Kingdom* 62, 869–887.

Correspondence should be addressed to:
F. Benítez-Villalobos
Instituto de Recursos, Universidad del Mar
Carretera a Zipolite Km 1.5,
Puerto Ángel,
Oaxaca, México C.P. 70902
email: fbv@angel.umar.mx