Reproductive biology of *Ophiocoma aethiops* and *O. alexandri* (Echinodermata: Ophiuroidea) from Estacahuite Bay, Oaxaca, Mexico

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ABSTRACT: The reproductive biology of *Ophiocoma aethiops* and *O. alexandri* was analyzed based on monthly time-series samples from May 2009 to April 2010 at Estacahuite Bay, Oaxaca, in the Mexican tropical Pacific Ocean. We evaluated the relationship between the reproductive biology of both species and variations of sea-bottom temperature, salinity, pluvial precipitation, photoperiod (light hours) and chlorophyll. Sex ratio, gonadosomatic index (GSI), actual fecundity and oocyte size were determined. Monthly variations of the environmental factors were compared with variations of GSI. Both species are gonochoric ophiuroids and the ratio of males to females (1:1) was not significantly different throughout the year. There were significant differences in GSI values between the 2 species, but temporal variation patterns were similar. Gametogenesis showed synchrony among males and females and 4 stages were recognized for both sexes. Spawning occurred in females of *O. aethiops* from May to November and from April to December in *O. alexandri*. Main spawning for males of both species occurred from May to December, although there was always availability of mature spermatozoa. Mean oocyte size was 32 to 58 µm for *O. alexandri* and 47 to 63 µm for *O. aethiops*. Mean ± SD fecundity was 3.03 ± 1.72 million vitellogenic oocytes per female for *O. aethiops* and 1.61 ± 0.99 million for *O. alexandri*. The reproductive patterns of *O. aethiops* and *O. alexandri* are influenced by the local oceanic dynamics, as has been reported for other echinoderm species in the area, but they also have a high genetic (endogenous) element, because the observed patterns resemble those determined for the Panamian populations of these 2 species.

KEY WORDS: Echinoderms · Brittle stars · *Ophiocoma* · Reproductive cycle · Fecundity · Histology · Mexican Pacific · Environmental factors

INTRODUCTION

For a number of marine invertebrates, reproductive biology is one of the most important traits in their life history (Stancyk 1979, Avila-Poveda 2004, Benítez-Villalobos 2005, Grange 2005, Wong et al. 2011), and research indicates that it changes with latitude and according to environmental factors (Pearse 1968, Bower et al. 2002, Guest et al. 2005, Mercier & Hamel 2009). In marine invertebrates, large spatial scales generate diverse reproductive patterns (Roy et al. 1998, Ramirez-Llodra 2001, Bower et al. 2002, Avila-Poveda & Baqueiro-Cardenas 2009, Benítez-Villalobos & Díaz-Martínez 2010).

Studies on the reproductive biology of ophiuroid species have been carried out mainly in high latitudes and deep waters (Ramirez-Llodra 2001, Williams & Bentley 2002, Falkner & Byrne 2003, Tominaga et al. 2004, Grange 2005, Soong et al. 2005, Byrne et al. 2008). In those investigations temperature fluctuation has been considered as one of the most important physical factors that affects periodicity of the reproductive cycle in temperate waters; whereas, in tropical environments where isothermal conditions prevail, the influence of other physical and chemical factors, such as salinity, photoperiod, tides and currents, is more important (Giese & Pearse 1974, Stancyk 1979, Böttger et al. 2004). Stephenson (1934) indicated that in temperate waters, a minimal fluctuation in temperature could induce the organism to spawn during the warm months. Nevertheless, Pearse (1968) and Giese & Pearse (1974) established that seasonality in reproductive cycles of marine invertebrates tends to be stronger as the latitude increases.

A few studies regarding reproductive biology have been carried out for the ophiuroid genus *Ophiocoma*, mainly in the Atlantic and Caribbean regions (Hendler 1975, 1979, 1991). Those works show that different species exhibit variations in their reproductive cycles, which are closely related to the latitudinal gradient, and that the most important factor producing those variations is the difference in the rainfall season among localities.

Ophiocoma aethiops and *O. alexandri* are the only species of this genus reported for the American Pacific Ocean, and they constitute an important percentage of biomass in benthic and coral reef ecosystems, playing key roles in the dynamics of those communities (Hendler 1979). Even though geographic distribution of the 2 species ranges from the Gulf of California (~31° N latitude) to Ecuador (~3° S latitude, including the Galápagos Islands), the reproductive biology of *O. aethiops* and *O. alexandri* has been studied only in Panama (~8° N latitude) (Hendler 1979). This fact highlights the necessity to increase the knowledge of the reproductive cycles of these species and their relationship with environmental factors. The purpose of this study was to analyze the reproductive biology of *O. aethiops* and *O. alexandri* in Estacahuite Bay, Oaxaca, Mexico (~15° N latitude) and to determine the relationship between their reproductive patterns and variations of environmental factors, i.e. sea-bottom temperature, salinity, pluvial precipitation, photoperiod and chlorophyll.

MATERIALS AND METHODS

Sampling and measurements

About 20 individuals of each of the 2 species of brittle stars, *Ophiocoma aethiops* and *O. alexandri*, were haphazardly collected by SCUBA diving every month for one year between May 2009 and April 2010, from a depth interval of 3 to 15 m in Estacahuite Bay, Oaxaca (15° 40′ 5.57″ N, 96° 28′ 53.61″ W), off the Pacific Coast of southern Mexico. All individuals were narcotized by leaving them in seawater at 2°C for 24 h before dissection (Lincoln & Sheals 1979). Later, individuals were fixed in 10% neutral formalin saline solution in seawater for 12 h and preserved in 70% ethanol (Grange et al. 2004, Avila-Poveda et al. 2009). The damp-dry weight of the disc without arms (DW, ±0.001 g) was recorded (Fenaux 1970, Hendler 1979). The 10 gonads of every specimen were dissected out by opening the aboral side of the disc, and then the damp-dry weight of total gonads (GW, ±0.001 g) was recorded. The total gonad volumes (GV, ±0.001 ml) were measured by the displacement of fluid method until a hydrostatic equilibrium was reached, using a variation of the Mohr-Westphal balance (Scherle 1970, Nagy & Pieri 1975).

During each monthly collection, salinity was measured using a waterproof multiparameter meter (HANNA HI 9828), while temperature was recorded at 1 h intervals using a pendant data logger (HOBO) permanently attached to the bottom. Data for pluvial precipitation (rainfall), photoperiod (light hours) and chlorophyll were obtained monthly from online databases (www.wunderground.com/ and http://oceancolor.gsfc.nasa.gov/, respectively). Monthly means were calculated for every parameter recorded.

Histological procedure and microscopy examination

Preserved gonads were dehydrated in a series of graded ethanol solutions (2 h baths with 2 changes for each solution of 70, 96 and 100% ethanol), cleared in Citrisolv (45 min baths with 3 changes), infiltrated in Paraplast paraffin with a 56°C melting point (1 h baths with 4 changes) and embedded in Paraplast paraffin with the aid of an embedding machine. Serial sections were cut at 7 μm thickness using a manual rotary microtome (LEICA RM2145), later mounted on glass slides and stained with the routine Harris hematoxylin-eosin regressive method (HHE₂: Luna 1968, Howard & Smith 1983). Slide

examinations were performed at visual magnifications of 30×, 100× and 400×, and digital images were achieved with a digital camera Logitech HD ProWeb-Cam C910 mounted on a microscope (Olympus CX21) and stored in computer in graphic format. Adobe Photoshop CS2, v.9.0, was used to produce the artwork for the figures.

Reproductive traits

Gonad developmental stages (GDSs) for each sex were defined by matching the most outstanding histological features throughout the development process of the gonad (i.e. frequency, size and features of gametes, presence/absence of phagocytes, as well as thickness and presence/absence of a gonadal wall of fibrous networks) with the GDS used for other ophiuroid species (Cuénot 1888, Selvakumaraswamy & Byrne 1995, Stewart & Mladenov 1995, Falkner & Byrne 2003, Tominaga et al. 2004, Borges et al. 2009). For both sexes 4 stages were designated: gametogenesis, mature, spawning and post-spawning.

Sex ratio (male:female) was calculated annually, and deviation from the expected ratio of 1:1 was tested with the chi-square (χ^2) test (Sokal & Rohlf 1995). A general gross anatomy description of the gonad was performed. The gonadosomatic index (GSI) for each species and sex was calculated monthly as: GSI = (GW/DW) × 100.

Fecundity was quantified as the number of vitellogenic oocytes per female (actual fecundity) from the mean volume of oocytes and the GV in each female (Ramirez-Llodra 2001, 2002, Ramirez-Llodra et al. 2002). Oocyte size of at least 100 oocytes per female per species was measured through sections of the nucleolus (Ramirez-Llodra et al. 2002), using the following tool: Feret's diameter measurement (i.e. the longest distance between any 2 points along the selection perimeter, also known as maximum caliper; Walton 1948) with the image analysis package ImageJ 1.41 (National Institutes of Health).

Statistical analysis

General data were expressed as mean ± SE. Statistical differences in GSI were tested using 2-way ANOVA (sex and month as factors), while differences in fecundity for females were tested using ANOVA. Relations among temperature, salinity, pluvial precipitation, photoperiod and chlorophyll (independent variables) with the reproductive cycle of *Ophiocoma aethiops* and *O. alexandri* represented by GSI (dependent variables) were assessed through a step-by-step multiple linear regression analysis. Statistical analyses were carried out using the computer program STATISTICA 6 and SigmaStat 3.5. Statistical significance was accepted at p < 0.05.

RESULTS

Sex ratio and gross anatomy of gonads

Ophiocoma aethiops and *O. alexandri* did not show sexual dimorphism, and it was confirmed that they are gonochoric ophiuroids, since none of the 427 organisms examined histologically (202 of *O. aethiops* and 225 of *O. alexandri*) showed ovotestes, i.e. they are not hermaphrodites. The ratio of males to females did not differ significantly and showed a 1:1 proportion throughout the year ($\chi^2 = 0.99$, p = 0.3270) for *O. aethiops* and ($\chi^2 = 1.44$, p = 0.2008) for *O. alexandri*.

The macroscopic anatomy of the gonad was that of a sac (i.e. thin and transparent epithelium), which contained a number of smaller oval sacs, herein called follicles. Those small sacs were whitish in males and varied from pink to purple in females. Occasionally the sacs had 1 or 2 ramifications. Every gonad was located at both sides of the bursal slits, constituting 10 discrete units that could be identified in the disc of an individual.

All gonad development stages were found in the 427 examined organisms (202 of *Ophiocoma aethiops* and 225 of *O. alexandri*) throughout the year, but for males of both species, the gametogenesis and mature stages were histologically similar; consequently both stages were represented together in the reproductive cycle (see Fig. 6). Sporadically one developmental stage concurred with the previous or the subsequent stage within isolated follicles of the same gonad and/or within gonads of the same individual. In general, microscopic analysis allowed the histological differentiation of the structural components through the development process of the gonad and the sexual cells, i.e. gametes, and presence/absence of phagocytes, as well as thickness and presence (only during post-spawning) or absence of a gonadal wall of fibrous networks (Figs. 1 to 4). On the other hand, the ovaries (Figs. 1 & 2) and testes (Figs. 3 & 4) of both species are sheltered by a follicular wall consisting of 2 layers of tissue: the internal and external sacs and the genital coelomic sinus that separates them. The external sac constitutes the visceral peritoneum,
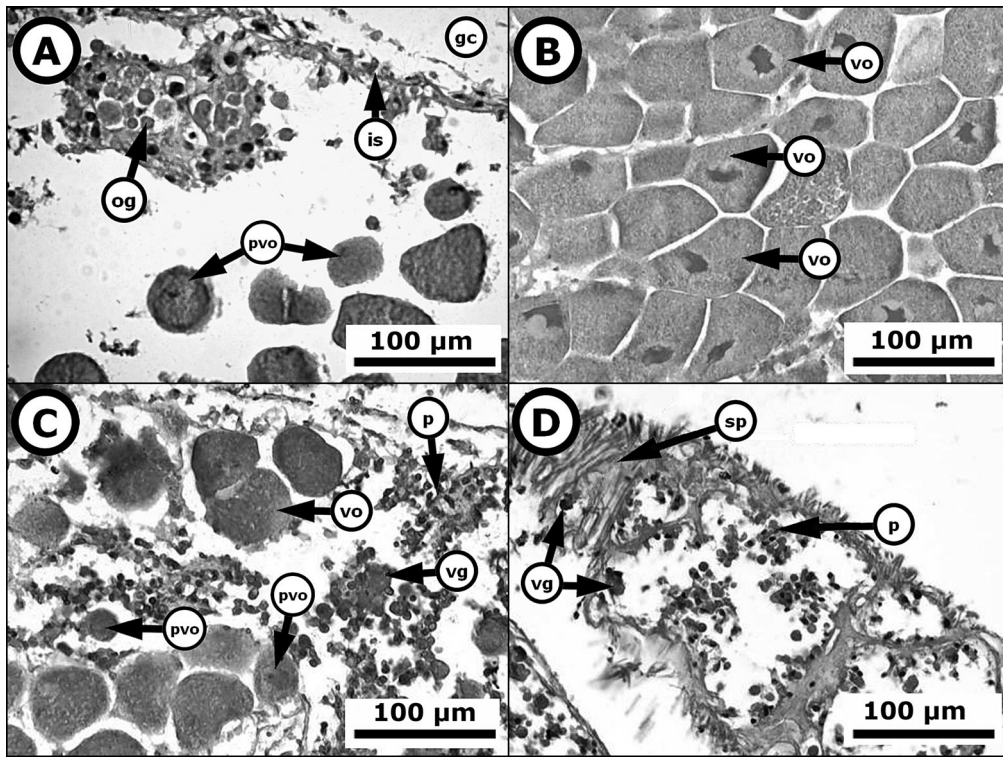
Fig. 1. *Ophiocoma aethiops*. Light microscopy of the histology of the ovaries: (A) gametogenesis, (B) mature, (C) spawning, (D) post-spawning. gc: genital coelom; is: internal sac; og: oogonia; p: phagocytes; pvo: pre-vitellogenic oocyte; sp: spicules; vg: vitellin granule; vo: vitellogenic oocyte
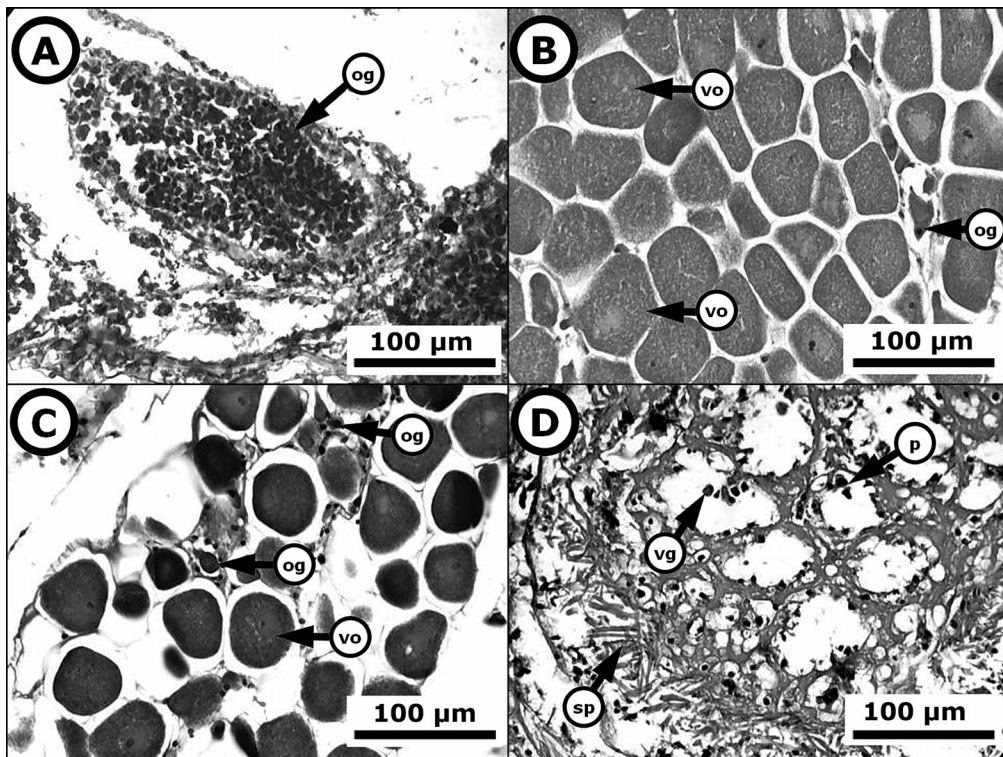

Fig. 2. *Ophiocoma alexandri*. Light microscopy of the histology of the ovaries: (A) gametogenesis, (B) mature, (C) spawning, (D) post-spawning. og: oogonia; p: phagocytes; sp: spicules; vg: vitellin granule; vo: vitellogenic oocyte
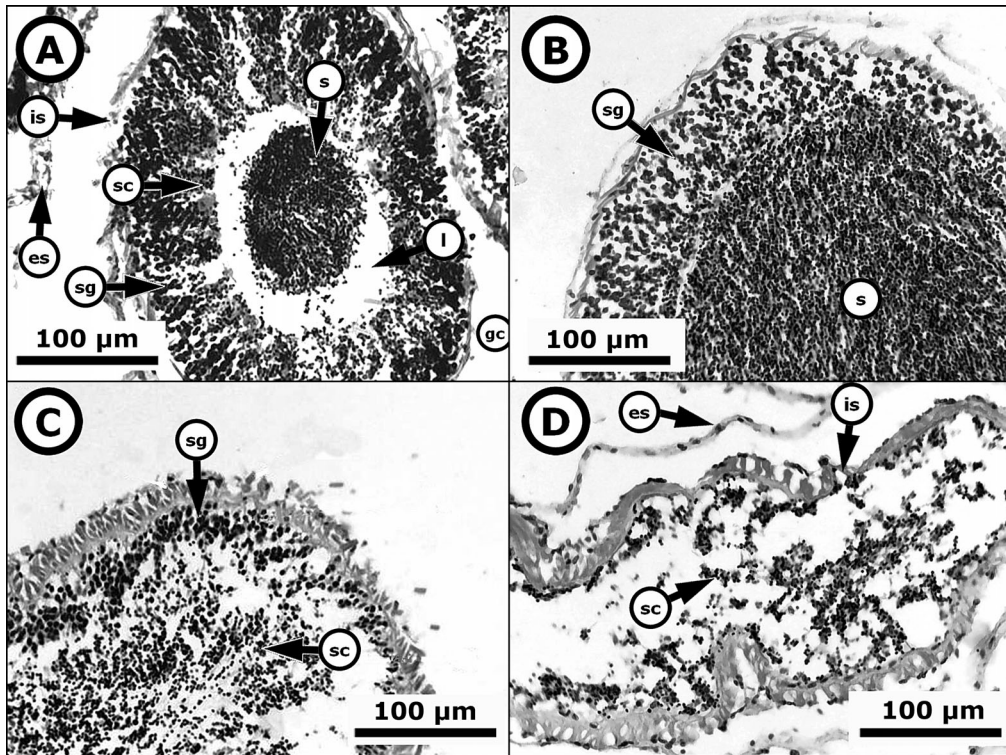
Fig. 3. *Ophiocoma aethiops.* Light microscopy of the histology of the testes: (A) gametogenesis, (B) mature, (C) spawning, (D) post-spawning. es: external sac; gc: genital coelom; is: internal sac; l: lumen; s: spermatozoa; sc: spermatocytes; sg: spermatogonia
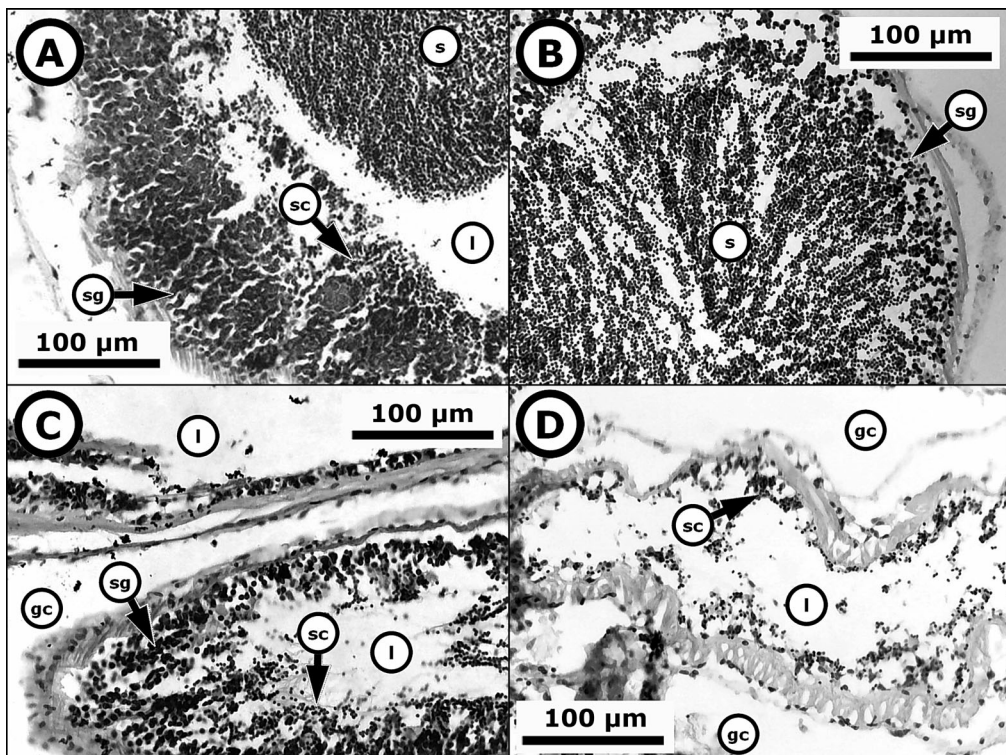

Fig. 4. *Ophiocoma alexandri.* Light microscopy of the histology of the testes: (A) gametogenesis, (B) mature, (C) spawning, (D) post-spawning. gc: genital coelom; l: lumen; s: spermatozoa; sc: spermatocytes; sg: spermatogonia

whereas the internal sac constitutes the gonadal tunica.

Reproductive cycles

In females of *Ophiocoma aethiops*, spawning was observed from May to November with frequencies above 40%. The post-spawning stage appeared during August (17%) and was mainly observed from November to February with the highest frequency in December (100%). The gametogenesis stage occurred from January to March with the highest frequency in February (80%). The mature stage occurred from June to September (40 to 11%) and from January to April with more than 67% mature (Fig. 5A).

The cycle of *Ophiocoma alexandri* was slightly different from that of *O. aethiops*. Spawning was observed from May to December with the highest frequency above 65%, and also during April with 33%. Post-spawning occurred during October to November and in February with the highest frequency in November (64%). The gametogenesis stage occurred in May (25%), but was observed mainly during Jan-uary (100%) to February. The mature stage occurred during June and July and from February to April, with frequencies above 63% during March and June (Fig. 5B).

In males, it was evident for both species that spawning occurs throughout the year, with the highest frequencies occurring from May to October (>70%). Post-spawning was present from October to January in *Ophiocoma aethiops,* and from October to February in *O. alexandri*, and the highest frequency occurred in December (>60%) for both species. Gametogenesis and mature stages were observed in *O. aethiops* during December and from February to April, whereas for *O. alexandri* these stages occurred from May to July, during October, and from January to April, and the highest frequency was apparent in February and/or March (~60%) for both species (Fig. 6).

Gonadosomatic index

The GSI of each sex per species displayed a similar fluctuation pattern between the same sexes of the 2 species throughout all the months of the study, and tended to increase together, but it was more pro-
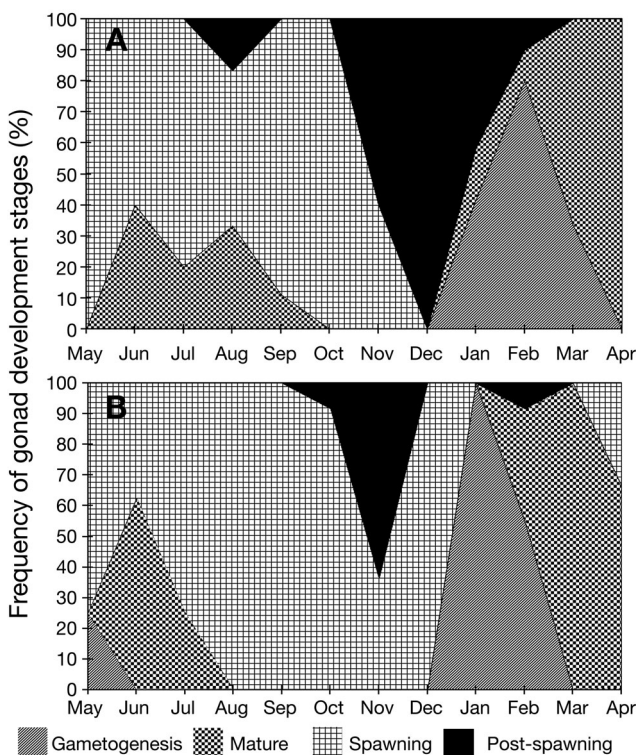

Fig. 5. *Ophiocoma aethiops* and *O. alexandri*. Female gametogenic cycle of (A) *O. aethiops* and (B) *O. alexandri* from May 2009 to April 2010 at Estacahuite Bay, Oaxaca, Mexico
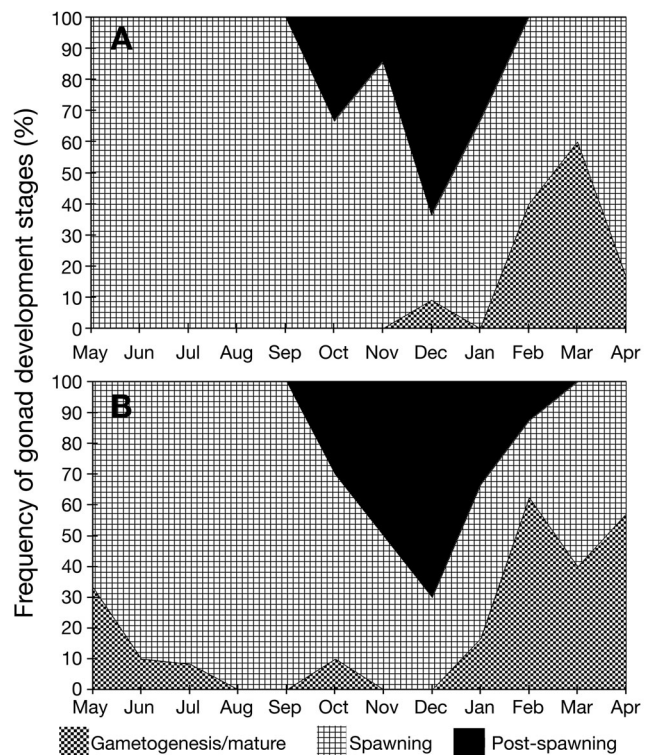

Fig. 6. *Ophiocoma aethiops* and *O. alexandri*. Male gametogenic cycle of (A) *O. aethiops* and (B) *O. alexandri* from May 2009 to April 2010 at Estacahuite Bay, Oaxaca, Mexico

nounced and higher for *Ophiocoma alexandri* than for *O. aethiops*, although slightly more pronounced in males than in females (Fig. 7). In females and males, maximum average values were found around September and during April, suggesting a synchronism between males and females.

Mean ($\pm$SD) GSI values of males and females combined were 13.66 $\pm$ 6.57 for *Ophiocoma aethiops* and 19.88 $\pm$ 12.72 for *O. alexandri*. Monthly variations of GSI throughout the sampling year were statistically significant for both species ($F = 9.999$ and 16.151, p < 0.001, for *O. aethiops* and *O. alexandri*, respectively). There were also significant differences in variations of GSI between species for the sampling period ($F = 4.255$, p < 0.001). The observed differences were the result of values from July to December and April (Tukey's test: July q = 3.656, August q = 5.012, September q = 8.816, October q = 5.530, November q = 2.952, April q = 4.542, p < 0.05).

The comparison of GSI between sexes did not show significant differences in *Ophiocoma alexandri* ($F =$ 0.395, p = 0.957), whereas for *O. aethiops* there were significant differences ($F = 3.032$, p = 0.002). Those differences were determined for values from June, September, October and April (Tukey's test: June q = 3.014, September q = 4.082, October q = 4.098, April q = 2.978, p < 0.05).

Fecundity and oocyte size

Actual mean ($\pm$SD) fecundity was 3.03 $\pm$ 1.72 million eggs female^{-1} for *Ophiocoma aethiops* and 1.61 $\pm$ 0.99 million eggs female^{-1} for *O. alexandri*. Fluctuations in fecundity values throughout the year showed significant differences for both species ($F = 4.837$, p < 0.001 for *O. aethiops*, and $F = 2.067$, p = 0.038 for *O. alexandri*). There were significant differences also when fecundity of both species was compared ($F = 1.926$, p = 0.047), which occurred in May and June (Tukey's test: May q = 3.023, June q = 6.398, p < 0.005). Apparently, fecundity is related to the size of the organisms, as individuals of *O. aethiops* are larger (~35 mm disc diameter) than those of *O. alexandri* (~25 mm disc diameter).

Mean diameter of oocytes oscillated between 47 and 63 µm for *Ophiocoma aethiops* and from 32 to 58 µm for *O. alexandri*, and vitellogenic oocytes were present during the entire sampling period. Oocyte size class of 10 to 40 µm had a permanent stock along the year. Oocytes within the size class of 40 to 70 µm for *O. alexandri* and 40 to 80 µm for *O. aethiops* were present during the entire sampling period, except in December. Size classes larger than 70 and 80 µm for *O. alexandri* and *O. aethiops*, respectively, did not exceed 5 % in any month.

Reproductive traits versus environmental factors

The multiple regression analysis of GSI pooled for both sexes of *Ophiocoma aethiops* with respect to the environmental factors was performed in 3 steps, resulting in the following model:

$$GSI = -44.31 + 0.475PP + 0.326PH \qquad (1)$$

where PP represents pluvial precipitation and PH is photoperiod. The model showed that the total explained variance over the GSI, in terms of the selected environmental factors, was 53.7 % ($R^2 = 0.537$, R = 0.732, p multiple < 0.05), indicating that the variables pluvial precipitation and photoperiod are correlated 73.2 % with GSI.
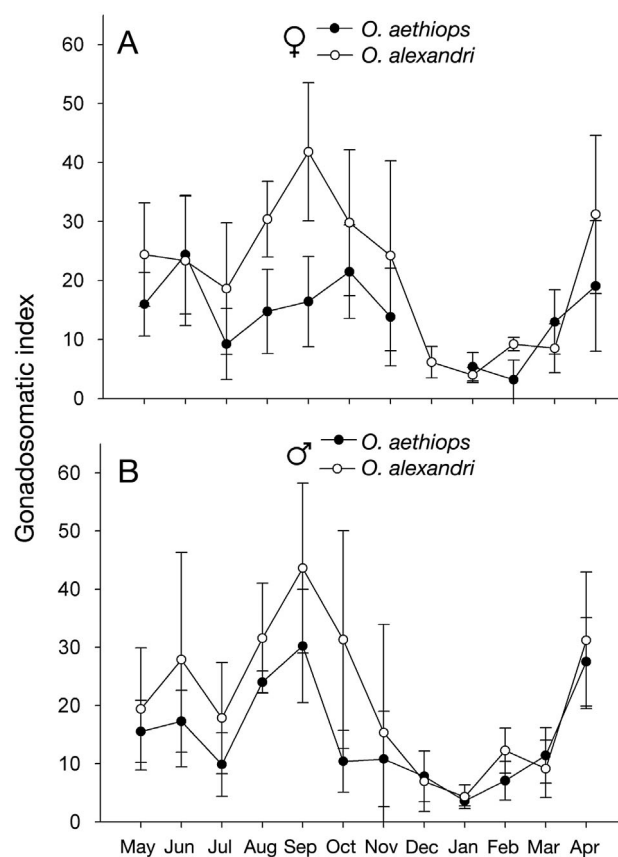

Fig. 7. *Ophiocoma aethiops* and *O. alexandri*. Monthly mean values ($\pm$SD) of GSI for (A) females and (B) males of both species from May 2009 to April 2010 at Estacahuite Bay, Oaxaca, Mexico (December data for females of *O. aethiops* missing)

Regarding GSI pooled for both sexes of *Ophiocoma alexandri*, the analysis was performed in one step, resulting in the following model:

$$GSI = -119.93 + 0740\,T \qquad (2)$$

where T represents temperature. This model showed that the total explained variance over the GSI was 54.7 % with a correlation value of 74 % between temperature and GSI ($R^2 = 0.547$, $R = 0.740$, p multiple <0.05).

DISCUSSION

According to the histological analysis, GSI and fecundity, we concluded that *Ophiocoma aethiops* and *O. alexandri* exhibit a semi-continuous reproductive cycle and that females have a long spawning season, which oscillates between April and December. Males showed availability of mature sperm throughout the year, and with almost continuous gametogenesis, except for a few months when the gonads temporally cease the process and the postspawning stage is noticeable.

Because the development of ovaries was variable, some females showed eggs with synchronic development, whereas in others it was asynchronic. The sporadic concurrence of previous or subsequent developmental stages within gonads of the same individual occurred in females that were clearly classified in one of the stages, providing evidence that for these species, the start of every developmental stage may possibly differ by at least 1 mo, especially at the start of spawning.

Spawning for some ophiuroid species is short and highly synchronous, whereas for other species it is characterized by a relatively long spawning period followed by a shorter period of low activity or absence of gamete release (Falkner & Byrne 2003). In the present study, both species conformed to the second pattern, as the spawning period lasted approximately 7 mo and variations in mean fecundity indicated a strong pulse of gamete release during which time the number of oocytes decreased abruptly, whereas during the following pulses the decrease was less conspicuous.

The synchrony of spawning may involve diverse physical and biological factors related to signals in the environment, among which endogenous mechanisms of biological clocks can be found (Soong et al. 2005). Consequently, several species in tropical regions show a single reproductive season during the year, and in some species with an extensive geographical distribution, reproduction may occur throughout the year in populations close to the equator, whereas it may be seasonal in those located at different latitudes (Giese & Pearse 1974).

Giese & Pearse (1974) mentioned that the duration of breeding seasons might be important to provide larvae or juveniles with favorable environmental conditions. Baker (1938) recognized 2 causes of reproductive seasonality: (1) exogenous factors, which serve as clues to synchronize the cycles, and (2) environmental conditions that exert selective (evolutionary) pressure, favoring one season for reproduction over others for a given species. Baker (1938) distinguished these as proximate and ultimate causes, respectively.

Models regarding fecundity establish that if food is scarce, the reproductive success decreases for species in which their strategy involves small egg size, whereas it increases when the egg size is larger. However, when availability of food is high, the reproductive success is high even for species with a small egg size (McEdward & Miner 2003, Grange et al. 2004). Regarding the 2 ophiuroids we studied, which have an apparent planktotrophic strategy with a high number of small eggs similar to other species previously studied (Selvakumaraswamy & Byrne 2000), food levels are sufficient to satisfy the requirements of larvae and juveniles. We make this conclusion because the spawning season does not coincide with peaks of chlorophyll (increases in primary production) and there is no significant correlation between spawning (represented by GSI) and chlorophyll values. We conclude that the larvae of *Ophiocoma aethiops* and *O. alexandri* do not depend on pulses of primary production for survival. This observation agrees with that of Hendler (1979), who reported a spawning period similar to the one determined in the present study for Panamanian populations of these 2 species. Moreover, Hendler (1979) also compared the spawning timing with increases in primary production and found that there was no correlation.

In spite of this, the statistical analysis revealed a significant correlation between reproductive cycle (GSI) and temperature, photoperiod and pluvial precipitation; the amount of explained variance is lower than 60 %, which suggests that those environmental variables are not determinant for the cycle. When our results were compared with those of Hendler (1979) for Panama, it was evident that the reproductive cycles of the populations coincide, which led us to conclude that the reproductive patterns of *Ophiocoma aethiops* and *O. alexandri* are not determined by local dynamics, as otherwise occurs with other

echinoderm species in the area (Benítez-Villalobos & Martínez-García 2012). Consequently, we propose that the general pattern of reproduction has a high genetic (endogenous) element, because different populations of these ophiuroids living at dissimilar latitudes show very comparable reproductive patterns. Local dynamics of the environmental factors probably adjust those patterns to a short-term particular scale, with possible inter-annual fluctuations.

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