

Reproductive biology of the sea cucumber *Holothuria sanctori* (Echinodermata: Holothuroidea)

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SUMMARY: The reproductive biology of the sea cucumber *Holothuria sanctori* was studied over 24 months (February 2009 to January 2011) at Gran Canaria through the gonad index and a combination of macro- and microscopic analysis of the gonads. *Holothuria sanctori* showed a 1:1 sex ratio and a seasonal reproductive cycle with a summer spawning: the mean gonad index showed a maximum (3.99 ± 0.02) in summer (June-July) and a minimum (0.05 ± 0.04) between late autumn (November) and early spring (March). Females had significantly wider gonad tubules than males. First maturity occurred at a size of 201 to 210 mm, a gutted body weight of 101 to 110 g and a total weight of 176 to 200 g. *Holothuria sanctori* shows a typical temperate species reproduction pattern. These results could be useful for managing current extractions of *H. sanctori* in the Mediterranean and in case a specific fishery is started in the eastern Atlantic region.

Keywords: *Holothuria sanctori*, sea cucumber, holothurians, reproduction, life-cycle, maturity, Canary Islands.

RESUMEN: BIOLOGÍA REPRODUCTIVA DEL PEPINO DE MAR *HOLOTHURIA SANCTORI* (ECHINODERMATA: HOLOTHUROIDEA). – Se estudió la biología reproductiva del pepino de mar *Holothuria sanctori* durante 24 meses (Febrero de 2009 a Enero de 2010) en la isla de Gran Canaria, mediante el índice gonadal y una combinación de análisis macro y microscópicos de sus gónadas. *Holothuria sanctori* presentó una relación de sexos de 1:1 y un solo ciclo reproductivo anual con desove en los meses estivales: el índice gonadal medio presentó un máximo (3.99 ± 0.02) en verano (Junio-Julio) y un mínimo (0.05 ± 0.04) entre finales de otoño (Noviembre) y primeros de primavera (Marzo). Las hembras presentaron túbulos gonadales significativamente más anchos que los de los machos. La primera madurez tuvo lugar con una talla de 201-210 mm, un peso eviscerado de 101-110 g y un peso total de 176-200 g. *Holothuria sanctori* presenta un patrón de reproducción típico en especies de zonas templadas. Estos resultados pueden ser útiles para gestionar las capturas actuales de *H. sanctori* en el Mediterráneo y en el caso de que una pesquería específica empezase en la región oriental del Atlántico.

Palabras clave: *Holothuria sanctori*, pepino de mar, holoturia, reproducción, madurez, Islas Canarias.

INTRODUCTION

Holothurians, known worldwide as “sea cucumbers”, are marine organisms found in almost every marine environment of the world. Ecologically, holothurians play an important role due to their benthic bioturbation activity (Hyman 1955, Pérez-Ruzafa 1984, Richmond *et al.* 1996, Uthicke 1999, Mangion *et al.* 2004). Many fisheries are, however, overexploiting the stocks of these echinoderms as a result of the large demand for food and medicine purposes

(*beche-de-mer* or *trepang*) in Asian markets (Sloan 1985, Richards *et al.* 1994, Conand 1989, 1997, Herero-Pérezrul *et al.* 1999, Guzmán *et al.* 2003, Toral *et al.* 2008, Purcell 2010). A sustainable exploitation of this resource necessarily requires a description of the reproduction cycles of the different species that may potentially be exploited to implement a harvest season and a closure during the spawning (Guzman *et al.* 2003, Abdel-Razek *et al.* 2005, Muthiga 2006, Toral and Martínez 2007, Gaudron *et al.* 2008, Kohler *et al.* 2009). Estimation of size at first matu-

rity (Conand 2006a, 2008) is also necessary to limit capture sizes, which has a biological justification in maximizing the yield per recruit, allowing individuals to spawn before harvest. However, studies dealing with the reproduction of holothurians have usually been implemented when a fishery has already started, or even when the populations of a specific species are overexploited.

Holothuria sanctori (Delle Chiaje 1823) is a sea cucumber distributed in the eastern Atlantic, including the Biscayan Gulf and Portugal, the Azores, Madeira, the Savage Islands, the Canary Islands, Cape Verde and Santa Helena (Entrambasaguas 2008). In the Mediterranean, this holothurian is a common species and is occasionally consumed and used as fishing bait (Entrambasaguas 2008). In the Canary Islands, as in most of the eastern Atlantic, there is currently no specific sea cucumber fishery. However, the number of Asian businessmen asking at fishermen's associations and harbours about the peculiarities and abundances of holothurians found across the archipelago is increasing alarmingly (author's pers. obs.). In order to have reliable data before a possible start of a sea cucumber fishery, it would be ideal, at least, to describe the reproductive biology of the most common sea cucumber found in the archipelago: *Holothuria sanctori*. This species usually lives on rocky bottoms, from 0 to 70 m depth, particularly in high-complexity reef habitats (Espino *et al.* 2006, Tuya *et al.* 2006). *Holothuria sanctori* has a markedly nocturnal behaviour, hiding in crevices during daylight (Pérez-Ruzafa 1984, Pérez-Ruzafa and Marcos 1987). As many other species from the Holothuroidea class, this is a dioecious species and does not have sexual dimorphism (Pérez-Ruzafa 1984).

The reproductive system of sea cucumbers has lost the pentamerism associated with other echinoderms. Holothurians have a single gonad consisting of one or two tufts of tubules (Conand 1989). The gametes are released into the water through a gonopore located at the dorsal mesentery (Pérez-Ruzafa 1984, Conand 1989). The gonads tend to fill almost the entire interior body cavity at their maximum maturity stage (Pérez-Ruzafa 1984). In general, the pattern of reproduction of sea cucumbers displays a seasonal cycle (Smiley *et al.* 1991). Temperate species generally have discrete spawning periods in spring and summer (Cameron and Fankboner 1986, McEuen and Chia 1991, Hamel *et al.* 1993), while tropical species reproduce for longer periods throughout the year (Pearse 1968, Ong Che and Gomez 1985, Conand 1993).

In this study, we aimed to investigate the reproductive biology of *H. sanctori* at the island of Gran Canaria. Specifically, we studied the annual reproductive pattern through the gonad index, and created a macro- and microscopic scale of the different maturity stages of female and male gonads in order to assess the sex ratio of the species and the size and weight at first maturity.

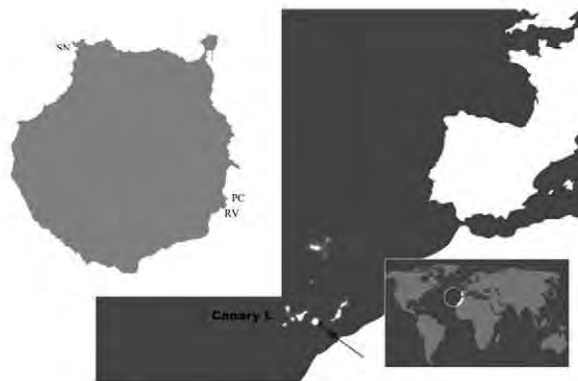


FIG. 1. – Situation of the study area in the eastern Atlantic (right) and study sites in Gran Canaria island (left).

MATERIALS AND METHODS

Area of study and sampling

This study was carried out between February 2009 and January 2011 at Gran Canaria, Canarian Archipelago, Spain (28°N, eastern Atlantic Ocean) (Fig. 1). Three sites between 3 and 10 m depth were selected: Risco Verde (RV) (27°51'25.16"N, 15°23'15.90"W), Playa del Cabrón (PC) (27°52'14.84"N 15°23'2.31"W) and Sardina del Norte (SN) (28°9'9.87"N, 15°41'53.18"W). Three collections in each season (winter, spring, summer and autumn), evenly spaced by about 25 to 35 days, were carried out at each site. On each sampling occasion, about 5 to 10 individuals were randomly collected by SCUBA-divers at night with the help of underwater lamps. A minimum separation of 10 m was left between adjacent individuals (Dart and Rainbow 1976). Each individual was introduced in a zip-loc bag and, once out of the water, immersed in a plastic container with sea water and anaesthesia (MgCl₂). In the lab, all individuals were kept at 4°C for 12 to 24 hours. During the first 12 months (February 2009-January 2010) all three sites were sampled, whereas during the last 12 months (February 2010-January 2011) only PC and RV were sampled because of logistic limitations.

Gonad index

Animals were taken out of the fridge the following day after collection, and left for 2 to 3 minutes in a plastic tray to drain. The total length (TL) was measured, from mouth to anus, with a metric tape to the nearest 0.5 cm. Each individual was then weighed with an electronic scale to the nearest 0.01 g (TW). The ventral part of each animal was dissected and the entire gonad was removed, including the germinal tubules, and then towel-dried and weighed to the nearest 0.01 g (GW). Following removal of the internal organs and excess of coelomic fluid, the gutted body weight (GBW) was determined to the nearest 0.01 g. The gonad index was subsequently calculated using

the ratio between the gonad weight and the GBW through the formula (Conand 1981, 1993b, Ramofafia *et al.* 2001, Asha and Muthiah 2008, Gaudron *et al.* 2008, Kohler *et al.* 2009):

$$GI = \frac{GW}{GBW} \times 100$$

All gonads were fixed using 10% formalin and stored for later macroscopic and microscopic analysis (Shiell and Uthicke 2005).

Macro and microscopic analysis of gonad tubules

Maturity stages were established according to the physical characteristics of the tubules (length, diameter, colour and branching). We used the following maturity stages (modification by Shiell and Uthicke 2005 of Conand 1981):

- Stage I: Resting or indeterminate tubules.
- Stage II: Growing tubules.
- Stage III: Mature tubules.
- Stage IV: Partly spawned tubules.
- Stage V: Spent tubules.

The gonad of each animal was placed on a Petri dish and 10 to 15 tubules were randomly selected from the tubular base. The length and diameter of each tubule was measured using an electronic calliper, to the nearest 0.01 mm (Hamel *et al.* 1993); the colour and number of branches was also annotated. A cross section of 3 to 5 tubules was cut using a scalpel and later analysed with light microscopy to determine the sex and maturity stage of the gonad. In females, the diameter of 30 oocytes was also measured using an ocular micrometer (Conand 1989, 1993a, b, Harriott 1985, Tuwo and Conand 1992). Photographs were taken with a digital camera attached to the light microscope and processed with Qimaging software.

Size and weight at first maturity

First sexual maturity was defined as the size (LT₅₀) or gutted body weight (GBW₅₀) at which the gonads of 50% of the individuals were mature. It was determined by plotting the percentage of individuals with mature gonads against size or GBW classes (Conand 1981). The size was categorized into 10-mm classes and the GBW into 10-g classes (Toral and Martínez 2007). Additionally, the total weight at first maturity (TW₅₀) was calculated following the same procedure, but with 25-g weight classes.

Statistical analysis

Differences in the GI between sites, years, annual seasons and months within each annual season were tested through a four-way permutation-based ANOVA. The design included “Season” as a fixed factor (winter, spring, summer and autumn), while “Month”, “Year” and “Site” were considered as random factors.

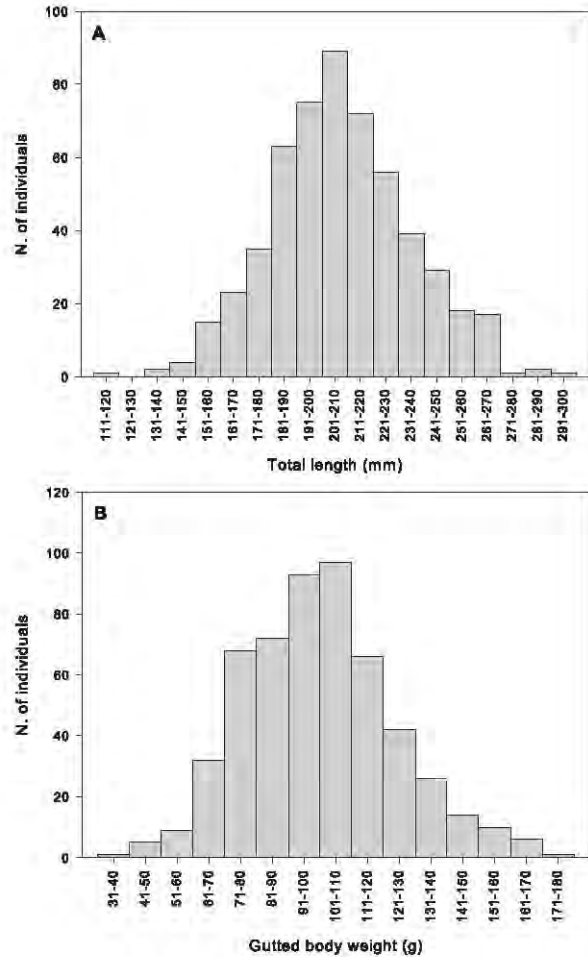


FIG. 2. – Size (A) and gutted body weight (B) distribution of *H. sanctori* at Gran Canaria island, n=542.

“Month” was nested within “Season” and the other three factors (“Season”, “Year” and “Site”) were crossed with each other. In order to obtain a balanced design and therefore reduce a Type I error, we decided to rule out data from the SN site, and therefore to include only data from PC and RV during 2 years in the analysis. The number of samples selected was n=5; if monthly samples had n>5, values were selected randomly for the analysis. GI data were fourth-root transformed to accomplish for homogeneity of variances, which was tested before the analysis via the Levene test. As data transformations did not render homogeneity of variances, we decided to decrease our level of significance from the conventional 0.05 to a 0.01 level to reduce a Type I error (Underwood 1981, 1997). The analysis was based on Euclidean distance dissimilarities (Anderson 2001) and the number of permutations for the analysis was 4999. Mean male and female tubule lengths and diameters were compared with a *t* test, while differences in mean GI between sexes were analysed with a Mann-Whitney test. The sex-ratio was tested for a theoretical 1:1 relation with a chi-square (χ^2) test.

TABLE 1. – Results of four-way ANOVA testing for differences in the GI of *H. sanctori* among seasons, years, sites and months within seasons.

| Factor | df | S.S. | M.S. | Pseudo-F | P | Differences |
|-----------------------------|-----|-----------|-----------|----------|--------|-----------------|
| Season | 3 | 35.343 | 11.781 | 4.0835 | 0.0018 | Winter < Summer |
| Year | 1 | 8.5252E-2 | 8.5252E-2 | 0.2832 | 0.9192 | |
| Site | 1 | 3.9524 | 3.9524 | 24.977 | 0.0004 | |
| Month(Season) | 8 | 7.9152 | 0.9894 | 1.8657 | 0.1108 | |
| Season x Year | 3 | 2.9507 | 0.98358 | 1.3749 | 0.3284 | |
| Season x Site | 3 | 3.2053 | 1.0684 | 2.6917 | 0.077 | |
| Year x Site | 1 | 4.9512E-2 | 4.9512E-2 | 0.88371 | 0.3758 | |
| Month(Season) x Year | 8 | 3.5949 | 0.44936 | 8.0204 | 0.0034 | |
| Month(Season) x Site | 8 | 0.88779 | 0.11097 | 1.9807 | 0.1756 | |
| Season x Year x Site | 3 | 0.92032 | 0.30677 | 5.4754 | 0.0242 | |
| Month(Season) x Year x Site | 8 | 0.44822 | 5.6028E-2 | 0.45253 | 0.8812 | |
| Residual | 192 | 23.772 | 0.12381 | | | |

RESULTS

Gonad index

A total of 542 *H. sanctori* were sampled; 222 (40.96%) did not show any visible gonad. From these 222 individuals, 105 were recorded in winter, 41 in spring, 9 in summer and 67 in autumn. By sites, 106 (47.75%) of them were recorded at PC, 65 (29.28%) at RV and 51 (22.97%) at SN.

The mean size (TL) of *H. sanctori* was 209.85 ± 27.53 mm (modal size = 205 mm) (Fig. 2a). The sizes ranged from 115 to 300 mm, but individuals smaller than 150 mm and larger than 270 mm were relatively scarce (Fig. 2A). The mean GBW ranged between 34.42 and 175.76 g (Fig. 2B), with a mean GBW of 100.06 ± 23.61 g.

The maximum values of the gonad index for males and females were recorded in June and July, during the summer season, while during winter the gonads were slowly shrinking until they completely disappeared; differences between summer and winter were statistically

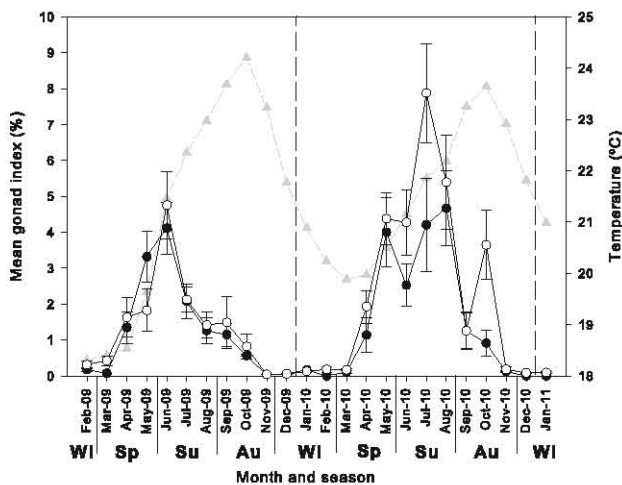


FIG. 3. – Mean monthly gonad index of males and females of *H. sanctori* at Gran Canaria and mean sea water temperature through time. The seasons are represented as: Wi, Winter; Sp, Spring; Su, Summer; and Au, Autumn. Males: full circles. Females: empty circles. Temperature: grey triangles. The error bars are the standard error of means. Mean GI from Feb-2009 to Jan-2010 were pooled from three sites, while mean GI from Feb-2010 to Jan-2011 were pooled from two sites.

significant (four-way ANOVA: “Season”, $P=0.002$; Table 1, Fig. 3). These significant differences between seasons were not consistent for “Year” and “Site” factors (four-way ANOVA: “Season” x “Year” x “Site”, $P=0.0242$; Table 1). The RV site showed significantly higher mean GI values than the PC site (ANOVA: “Site”, $P=0.0004$; Table 1). Females reached slightly higher GI values than males throughout the study; however, this difference was not significant (Mann-Whitney U test, $U=234$, $P=0.270$).

Sex-ratio, size and weight at first maturity

Of the 320 animals with visible gonads, 7 of them could not be sexed. Of the 313 sampled individuals, 165 were females and 148 males. The sex ratio was not significantly different from a 1:1 relation (chi-square test, $\chi^2=0.92$, $P>0.05$). The size at first maturity (TL_{50}) was between 201 and 210 mm (Fig. 4A), while the gutted body weight at first maturity (GBW_{50}) was between 101 and 110 g (Fig. 4B). The total weight at first maturity was between 176 and 200 g (Fig. 4C).

Macro- and microscopic analysis of gonad tubules

Tubules mainly grew (Stage II) from December to March-April; growing was less accentuated until June. The gonads reached their maturity (Stage III) between April-May and October, and started spawning (Stage IV) from June to October. From here on, most of the tubules were completely spent (Stage V) until January (Fig. 5).

Physical characteristics of gonad tubules followed the seasonal changes of the GI. Male tubules were slightly, but not significantly (t test, $t=0.113$, $P=0.910$) longer than female tubules (Fig. 6A). However, female tubules were significantly wider than male tubules (t test, $t=-2.578$, $P=0.013$) (Fig. 6B). The length and diameter of tubules increased from Stage II to Stage III and slowly decreased in Stages IV and V (Table 3). This decrease is observable in the tubule walls in both sexes, as it begins to shrink and finally the tubule finishes with a “baggy” or wrinkled appearance (Fig. 8). The tubule diameter decrease makes the tip of the tubules appear more “pointed” than in Stage III (Fig. 7).

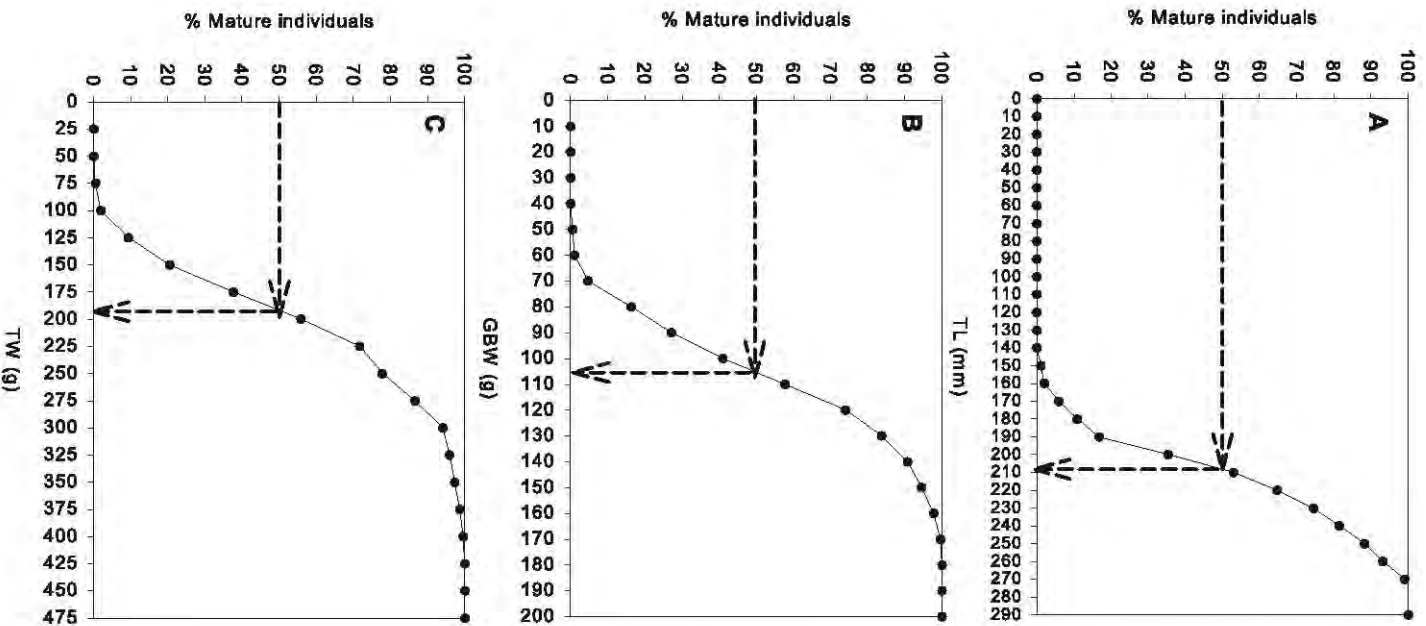


Fig. 4. – Size (A), gutted (B) and total (C) weight at first maturity of *H. sanctori*. TL, total length; GBW, gutted body weight; TW, total weight.

Tubule coloration also changed throughout the year, depending on the stage of maturity. Females showed translucent and light pinkish colours during the growing phase (Stage II), while orange or even reddish dominated during maturity (Stage III). During and after spawning (Stages IV and V), the coloration changed from brownish to translucent, frequently spotted with

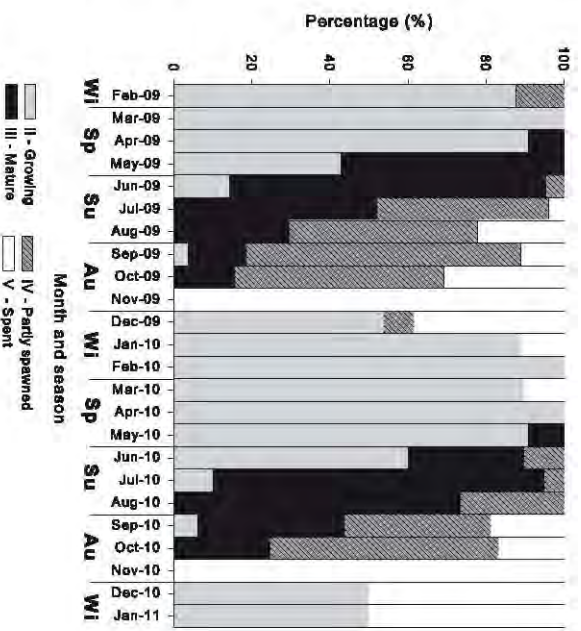


Fig. 5. – Maturity stages of *H. sanctori* at Gran Canaria from February 2009 to January 2011.

TABLE 2. – Mean oocyte diameter for stages of maturity II, III, IV and V of *H. sanctori* at Gran Canaria.

| Stage | N | Minimum | Maximum | Oocyte mean diameter (µm) | SD |
|-------|-----|---------|---------|---------------------------|-------|
| II | 490 | 5 | 200 | 57.76 | 41.10 |
| III | 540 | 45 | 200 | 152.68 | 14.39 |
| IV | 230 | 120 | 235 | 165.53 | 15.55 |
| V | 80 | 110 | 235 | 183.58 | 19.18 |

dark orange spots (phagocytes). Males had translucent and salmon colours while growing (Stage II), more whitish and beige colours during maturity (Stage III) and finally brown and translucent colours, again, during and after spawning (Stages IV and V) (Table 3).

Tubule branching did not vary between sexes during the different stages of maturity, although there was a slight increase in the number of branches in both sexes between the growing stage (II) and the maturity stage (III) (Table 3).

In female gonads, the mean diameter of oocytes increased as the gonads were maturing (Table 2). During the growing period (Stage II), some oocytes grew faster than others, creating a bi-modal size distribution of oocytes, with relatively small oocytes (20-40 µm) and medium oocytes (70-150 µm) in the same tubule (Table 3 and Fig. 7). In the maturity phase (Stage III), the oocyte diameter was more uniform, with sizes between 140 and 160 µm (Table 3). The relatively large size of oocytes boosted the external diameter of the tubule considerably, so oocytes had a more robust and compact shape than in Stage II. In Stage IV, some tubules appeared almost empty, only with “relict” oocytes of 200 to 235 µm. In Stage IV we also found tubules partially spawned and others still not spawned, which also generated bi-modal oocyte size distributions (Table 3). Finally, in Stage V, all tubules were totally spent with

TABLE 3. – Macroscopic and microscopic characteristics of *H. sanctori* gonads at each of the stages of sexual maturity.

| Stage of maturity | Sex | N | Tubule characteristics Length±SD Diameter±SD (mm) | Branching | Colour | Macroscopic morphology | Microscopic characteristics |
|-------------------|-----|-----|------------------------------------------------------------|-----------|-------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| II | ♀ | 630 | 26.75±15.80 | 0.61±0.41 | Translucent to light pink | Tubules smaller than in Stage III, very thin and short. | Early and mid-vitellogenic oocytes. Two modal size classes: 20-40 µm and 70-150 µm. Very thick tubule walls. Very thick tubule walls with clearly marked longitudinal fold. Spermatozoa and spermatocyte areas distinguishable. |
| Growing | ♂ | 340 | 40.52±21.91 | 0.52±0.26 | Translucent to salmon | Tubules smaller than in Stage III, very thin and short. | Very thick tubule walls with clearly marked longitudinal fold. Spermatozoa and spermatocyte areas distinguishable. |
| III | ♀ | 540 | 50.91±16.49 | 1.39±0.37 | Intense orange to red | Wide, long and densely packed tubules, occupying almost the whole interior cavity of the animal. | Abundant mature oocytes at late stage of vitellogenic (140–160 µm), with nucleus, germinal vesicle and follicular epithelium. Very thin tubule walls. |
| Mature | ♂ | 460 | 61.22±17.30 | 0.86±0.26 | White to light beige | Long tubules packed with spermatozoa. | Tubules completely packed with spermatozoa. Thin tubule walls. |
| IV | ♀ | 240 | 27.61±11.33 | 0.83±0.47 | Light orange to pink | Both wide and narrow tubules. Lengths smaller than in Stage III. | Two modal size classes of oocytes: 145–175 µm and 185–205 µm, presence of some phagocytes, wrinkled walls and pointed tubule ends. |
| Partly spawned | ♂ | 500 | 33.44±13.45 | 0.51±0.21 | Beige to translucent white | Both wide and narrow tubules. Lengths smaller than in Stage III. | Some tubules with pointed ends, wrinkled walls and patchy spermatozoa. |
| V | ♀ | 240 | 16.58±10.80 | 0.22±0.17 | Brown-green to orange blotches | Very thin and short tubules. Completely empty. | Some relict oocytes left (160–210 µm), large number of phagocytes and extremely wrinkled tubule walls. |
| Spent | ♂ | 180 | 12.31±4.90 | 0.13±0.08 | Brown-green to translucent with orange blotches | Very thin, short and wrinkled tubules. Completely empty. Some of them with rolled up ends. | Extremely wrinkled wall, almost empty tubules, only relict spermatozoa patches. Large number of phagocytes. |

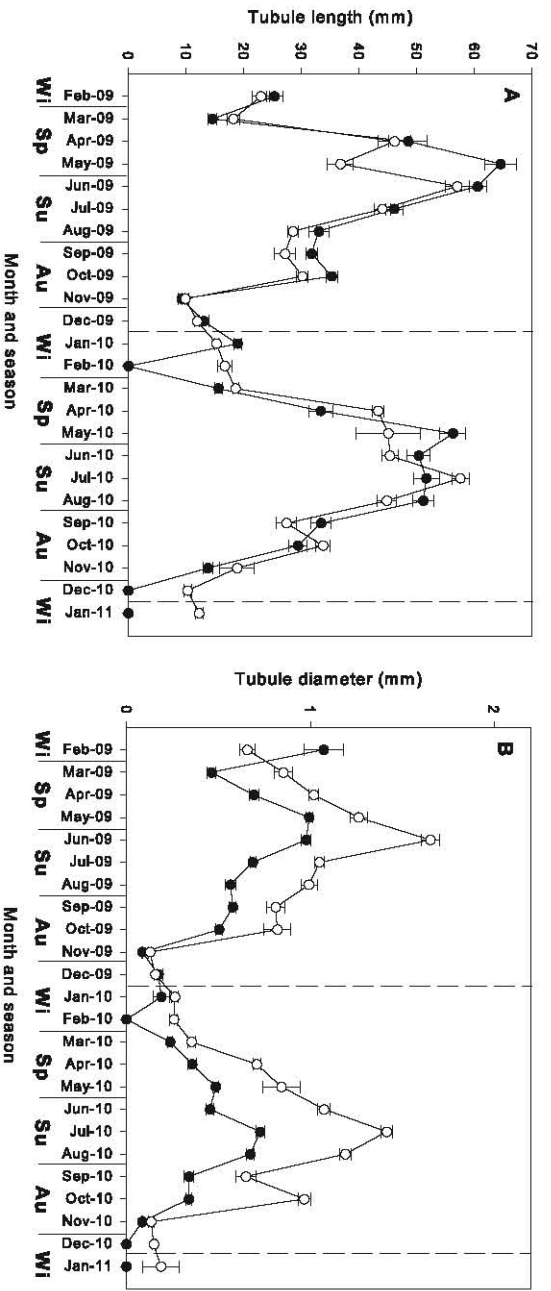


Fig. 6. – Monthly mean length (A) and diameter (B) of *H. sanctori* gonad tubules at Gran Canaria. The seasons are represented as: Wi, Winter; Sp, Spring; Su, Summer; and Au, Autumn. Males: full circles, Females: empty circles. The error bars are the standard error of means. Mean GI from Feb-2009 to Jan-2010 were pooled from three sites, while mean GI from Feb-2010 to Jan-2011 were pooled from two sites.

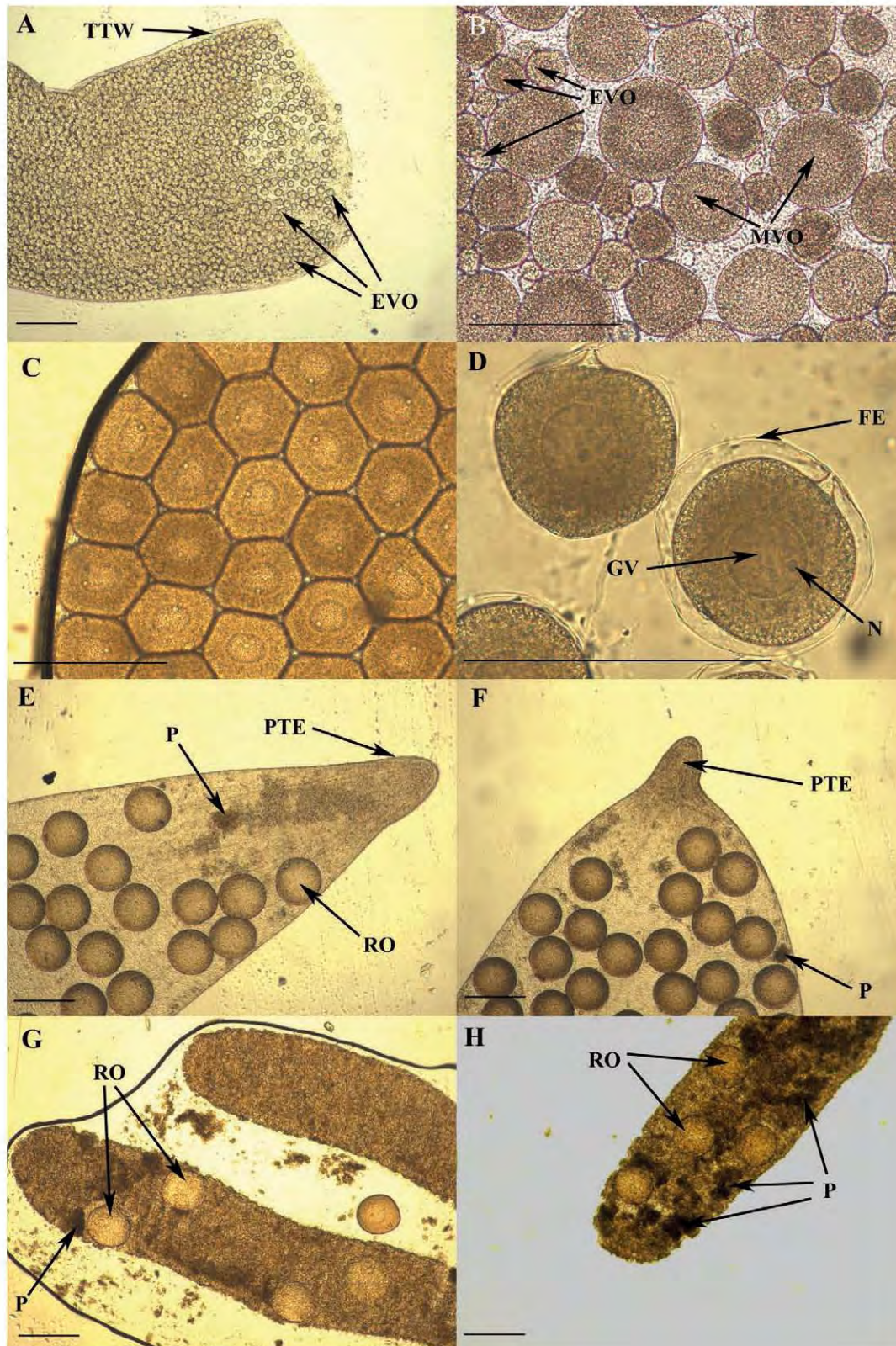


FIG. 7. – Light micrographs of female *H. sanctori* stages of maturity. A, growing tubule with early vitellogenic oocytes (EVO) and with thick tubule walls (TTW); B, tubule with early (EVO) to mid-vitellogenic oocytes (MVO); C, tubule completely full of late vitellogenic mature oocytes; D, mature oocytes, with nucleus (N), germinal vesicle (GV) and follicular epithelium (FE); E-F, partly spawned tubules with presence of relict oocytes (RO), phagocytes (P) and with pointed tubule end (PTE). G-H, totally spent tubules with phagocytes (P) and few relict oocytes (RO). Scale bars: 250 μ m; A-B, stage II; C-D, stage III; E-F, stage IV; G-H, stage V.

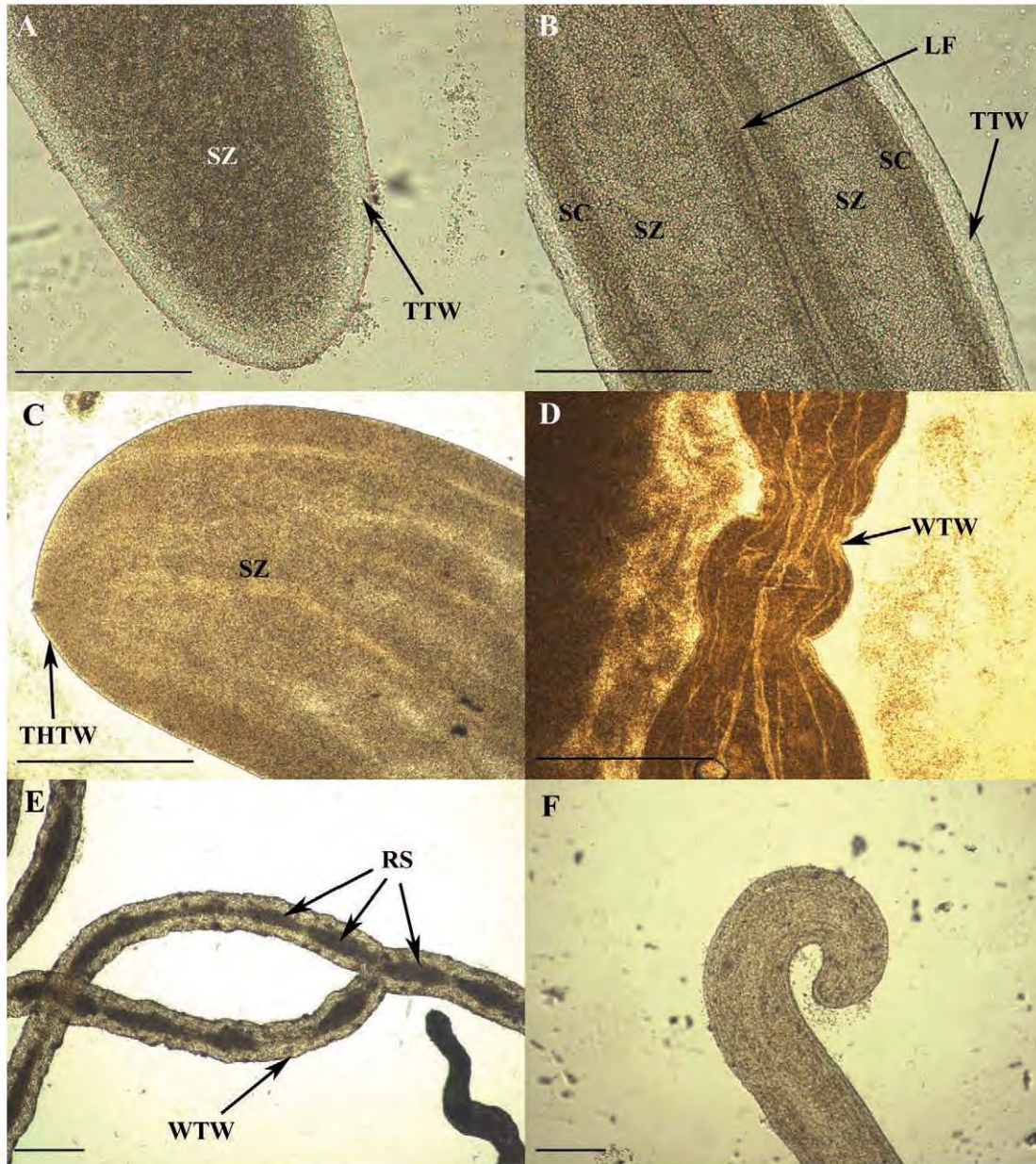


FIG. 8. – Light micrographs of male *H. sanctori* stages of maturity. A, growing tubule with spermatozoa (SZ) and with thick tubule walls (TTW). B, growing tubule with longitudinal fold (LF) surrounded by spermatozoa (SZ) and spermatocytes (SC) at both sides of it; C, tubule completely full of spermatozoa (SZ) and thin tubule walls (THTW); D, partly spawned tubule with wrinkled tubule wall (WTW) and marked folds; E, completely spent tubule showing wrinkled tubule walls (WTW) and relict spermatozoa concentrations (RS) in lumen. F, detail of a completely spent tubule with rolled up end. Scale bars: 250 μ m; A-B, stage II; C, stage III; D stage IV; E-F, stage V

the exception of very scarce “relict” or residual oocytes with maximum sizes (235 μ m) surrounded by phagocytes (Tables 2 and 3).

DISCUSSION

Though *H. sanctori* is a common species in the Mediterranean Sea and the adjacent Atlantic, data about its biology and reproduction are absent; as a result, any attempt to compare our results with other populations is difficult. Therefore, our data were compared with

those of other similar species of the *Holothuria* genus or with other species from the *Aspidochirotida* order in other regions of the world.

Gonad index

Sea cucumbers often show one annual reproductive cycle (Tanaka 1958, Conand 1993a, b, Harriott 1985, Cameron and Fankboner 1986, Ong Che 1990, Smiley *et al.* 1991, Tuwo and Conand 1992, Chao *et al.* 1993, 1995), although semi-annual cycles (Harriott

1985, Conand 1993b) or even continuous reproduction activity throughout the year (Harriott 1985) are also frequent, particularly in tropical regions. In Gran Canaria, *H. sanctori* followed one single annual cycle, as a typical temperate sea cucumber species (Sewell and Bergquist 1990, Sewell 1992, Tuwo and Conand 1992, Hamel and Mecier 1996): the maximum reproductive activity was observed in warm months and the minimum activity (resting) in cold months.

The lack of gonads during winter (cold months) is due to the resorption of tubules after the spawning of gametes into the water column (Conand 1993a). The resorption and total absence of gonads during the resting phase of maturation in *H. sanctori* means that the tubule recruitment model (TRM) for ovarian development in holothurians (Smiley and Cloney 1985, Smiley 1988, 1994, Smiley *et al.* 1991) may not be applicable to this species. This case is not rare, as there are many other sea cucumber species that do not follow the TRM, such as *H. leucospilota* (Ong Che 1990), *H. atra* (Chao *et al.* 1994), *H. tubulosa* (Coulon 1994), *H. fuscogilva* and *A. mauritiana* (Ramofafia and Byrne 2001). In fact, and according to Sewell *et al.* (1997), in the genus *Holothuria*, evidence for the TRM is provided only by some individuals of some species at some geographical locations.

Currently, there are no data about the reproduction cycles from other sea cucumber species inhabiting the Canary Islands, such as *Holothuria mammata* and *Holothuria arguinensis*, which share the same habitat as *H. sanctori* (Tuya *et al.* 2006). The only observation of spawning individuals belonging to *H. mammata* at Gran Canaria was recorded on August (author's pers. obs.). This could suggest that *H. mammata*, like *H. sanctori*, reproduces during the warm months in Gran Canaria. These observations can indicate that reproduction cycles of *H. sanctori*, *H. mammata* and *H. arguinensis* may be synchronized and they spawn simultaneously, as other echinoderms do (Pearse *et al.* 1988). However, further studies should focus on the reproduction of these two other common sea cucumber species to confirm this hypothesis.

The differences in oceanographic characteristics of each site, such as wave exposure, temperature and hydrodynamic regimes, may have influenced the differences in the GI detected between sites.

The spawning period of *H. sanctori* starts in July and ends around October. Considering that sea cucumber larvae can settle into the benthos in approximately one month (12-17 days in *Holothuria scabra*, Mercier *et al.* 2000; 22-27 days in *Isostichopus fuscus*, Hamel *et al.* 2003; 20 days in *Apostichopus japonicus*, Matsuura *et al.* 2009), we could assume that settlement of *H. sanctori* larvae might be happening during late summer and autumn.

The spawning period of *H. sanctori* coincides with the spawning of the most abundant echinoderm in the Canary Islands, the long-spined urchin *Diadema aff. antillarum* (Hernández 2006, Hernández *et al.* 2006a,

Hernández *et al.* 2010). For both echinoderms, the annual reproductive cycle is clearly related to sea water temperature: gametogenesis occurs when sea water temperature rises, spawning when the maximum sea water temperature is reached, and resorption when sea water temperatures are cooler. Generally, cold waters are associated with a high nutrient availability that triggers phytoplankton blooms; a high nutrient content in the water column therefore favours larval development (Boidron-Metairon 1995) and therefore subsequent settlement of, for instance, sea urchins (Ebert 1983). Indeed, a synchronization of the spawning of echinoderms with phytoplankton availability is considered as an advantageous adaptation (Starr *et al.* 1990). Our study, however, revealed that spawning was not synchronized with phytoplankton availability, which in the Canary Islands is maximized in winter-spring (Hernández *et al.* 2006a). As a result, *H. sanctori* larvae are able to survive and develop in a low-food environment and, like *Diadema aff. antillarum* larvae, are adapted to the oligotrophic waters around the Canary Islands (Hernández 2006, Hernández *et al.* 2010).

Sex-ratio

H. sanctori showed a sex ratio that does not differ significantly from a 1:1 relation. In most holothurians from the Aspidochirotida order, the sex ratio usually coincides with a balanced 1:1 relationship (Cameron and Frankboner 1986, Ramofafia *et al.* 2000, 2001, Rasolofonirina *et al.* 2005, Asha and Muthiah 2008), although some species show a slightly unbalanced ratio with more males than females, or vice versa (Shiell and Uthicke 2005).

Macro- and microscopic analysis of gonad tubules

The maturity of the gonads in *H. sanctori* was similar to that of other sea cucumber species, e.g. *Isostichopus fuscus* at Galápagos, where the mean diameter of oocytes in Stage III of maturity, a commonly used population parameter (Conand 1993), was $153.4 \pm 24.6 \mu\text{m}$ (Toral and Martínez 2007), very close to the $152.68 \pm 14.39 \mu\text{m}$ (mean \pm SD) of *H. sanctori*. These oocyte sizes are within the normal Aspidochirotida order diameters, between 150 and 210 μm (Conand 1993b).

Mean tubule diameters found for *H. sanctori* were similar to those of other species, such as *Holothuria whitmaei*, which has dimensions of $1.04 \pm 0.33 \text{ mm}$ for males and $1.56 \pm 0.86 \text{ mm}$ for females, very similar to the $0.86 \pm 0.26 \text{ mm}$ for males and $1.39 \pm 0.37 \text{ mm}$ for females of *H. sanctori*. Throughout the study, female tubules were wider than male tubules, which is a common pattern for many holothurians (Shiell and Uthicke 2005, Toral and Martínez 2007). However, tubules were slightly longer in males than in females, as occurs in other Aspidochirotida species, such as *H. fuscogilva*, *H. nobilis*, *H. fuscopunctata*, *H. scabra*, *H. atra*, *Actinopyga mauritiana*, *Actinopyga echinites* (Conand

1993b), *H. witmaei* (Shiell and Uthicke 2005) and *H. spinifera* (Asha and Muthiah 2008).

Size and weight at first maturity

Size at first maturity is a common parameter used in almost every fishery of the world to establish a minimum capture size, indicating the size from which 50% of the captured animals are mature or have already gone through a reproductive period. As measuring sea cucumbers precisely is difficult because of their contraction and enlargement movements, absence of skeleton and soft body wall, some authors have suggested the GBW or drained weight at first maturity (Toral and Martínez 2007). We also calculated the total weight at first maturity, which, although not precise because it includes guts, coelomic fluid and water, could be obtained without killing the animal, by weighing them directly on board and letting the fishermen release the animals with less weight than permitted. This measure could avoid extracting immature animals from the population.

The size at first sexual maturity is difficult to compare between studies because different parameters of weight/size are used and correlations between parameters are not always available (Kohler *et al.* 2009). The size at first maturity (TL₅₀) of *H. sanctori* was 201 to 210 mm, which is comparable to that of other species with similar maximum lengths such as *Actinopyga mauritiana* and *Holothuria scabra versicolor* (now *H. lessoni*) (New Caledonia) (Conand 1993b). The GBW₅₀ was 101 to 110 g, which is similar to that of other medium-sized species such as *Actinopyga echinites* (New Caledonia) (Conand 1993b). The TW₅₀ for *H. sanctori* was 176-200 g, which is similar to that of other species such as *H. scabra* and *H. atra* (New Caledonia) (Conand 1993b), although the latter shows considerably higher maximum lengths than *H. sanctori*.

Sea cucumber fisheries management

All the calculated parameters, along with the reproduction cycle data from *H. sanctori*, can be useful to implement a sustainable exploitation of this resource, as it would help to establish a harvest season and a closure during the spawning of animals (Guzman *et al.* 2003, Abdel-Razek *et al.* 2005, Muthiga 2006, Toral and Martínez 2007, Gaudron *et al.* 2008, Kohler *et al.* 2009). The size at first maturity (Conand 2006a, 2008) is also a relevant parameter for managing this type of fisheries, as it helps to limit capture sizes. However, in order to effectively manage these resources, other parameters should also be studied, because the size at first maturity has not always worked suitably for general sea cucumber fisheries management. It is the only possible measure when the fishing gear is a net, but the fishery of sea cucumbers is usually made by hand (mainly in rocky and shallow water species). An alternative management tool could be fishing medium-

size individuals and keeping the larger individuals for breeding, as they usually show higher fecundity than smaller ones. Therefore, we encourage future studies on the reproduction of *H. sanctori* to focus on the fecundity per size distribution, estimating the ideal size to capture.

As sea cucumber fisheries in the eastern Atlantic has not yet started, it is crucial to develop as many biological/ecological studies as possible in order to test and apply effective management measures that avoid overexploitation of this, or other, sea cucumber species.

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