Reproductive cycle of the sea cucumber *Holothuria tubulosa* (*Holothuroidea: Echinodermata*) in the Southwestern Mediterranean Sea

Mezali K.[1*] ; Soualili D. L.[1,2]

[1]*Departement des Sciences de la Mer et d'Aquaculture, Faculté des Sciences de la Nature et de la Vie, UMAB, Université de Mostaganem, Mostaganem, Algeria - Email: mezalikarim@yahoo.fr*

[2]*CNRDPA, Bou Ismaïl, Algeria*

SUMMARY

Aspidochirotid holothurians, commonly known as "sea cucumbers", are conspicuous component of Posidonia oceanica meadows of the Mediterranean Sea, where they are reported as generally large sized and rather abundant. They actively contribute to the functioning of the Posidonia meadow by recycling dead organic matter in the sediments layer, which confers them a major ecological role. This study is a first contribution on the reproductive biology of Holothuria tubulosa (Gmelin, 1790) collected from the South-Western Mediterranean, especially from the Algiers coastal area. The sampling was conducted in Ain Taggourait station (w. Tipasa) which is an unpolluted area under little anthropogenic influence. The temporal evolution of the gonad index (GI) and the sexual maturity stages suggest that the reproductive cycle of H. tubulosa is annual and marked by a main spawning taking place from June to august. We also show the synchronization of the maturation of gonads in both sexes of this species. The influence of temperature is the most likely factor influencing its reproductive cycle and the spawning of H. tubulosa. The general outline of the gametogenesis of H. tubulosa shows a winter sexual resting phase, followed by maturation in spring and spawning at the end of summer until a middle of autumn.

Keywords: *Holothurians, Aspidochirotida, H. tubulosa, reproduction, spawning, maturity, gonad index, Posidonia oceanica, Algerian basin.*

INTRODUCTION

Aspidochirotid holothurians, commonly known as "sea cucumbers", are major representatives of the benthic macrofauna of the *Posidonia oceanica* habitat of the Mediterranean Sea ([1]; [2]). Within this habitat, holothurians contribute to the recycling of organic matter ([3]; [4]; [5]; [6]). Few studies were devoted to their life cycle ([7]; [8]; [9]; [10]). *Holothuria tubulosa* the most common species, and the "best known" species in the Mediterranean Sea, is not one species, but two cryptic species that have not been previously recognized or even suspected ([11]). Studies on the reproduction of *H. tubulosa* are anecdotic. The study carried out by [8] in the Adriatic Sea was limited to observation of the external appearance of the oocytes. [12] discovered a new and highly effective oocyte maturation inducer for *in vitro* fertilization of the oocytes of *H. tubulosa* collected from Banyuls-sur-Mer (France). In Algeria, aspects of the reproductive cycle have been studied only for *Holothuria (Platyperona) sanctori* ([10]). The interest in *H. tubulosa* comes from the abundance of this species in Algerian shallow waters, especially in unpolluted areas such as Ain Taggourait.

The present study examines the macroscopic and microscopic aspects of gonad maturation of *H. tubulosa* to determine its reproductive cycle at Ain Taggourait site.

MATERIALS AND METHODS

Sampling sites

Ain Taggourait site (36°36.608′N, 002°36.995′E) was selected as a reference site for the study of the reproduction of *H. tubulosa*. This site is located at Bou Ismail Bay (Figure 1). It is more open bay, and is much more exposed to the winds coming from the northeast and northwest sector, which generate swells of high amplitudes ([13]). It is more distant from the industrialized area, which reduces the impact of industrial and domestic pollution.

Underwater observations revealed that at Ain Taggourait site the algal coverage and the *P. oceanica* meadow are dense ([10]).

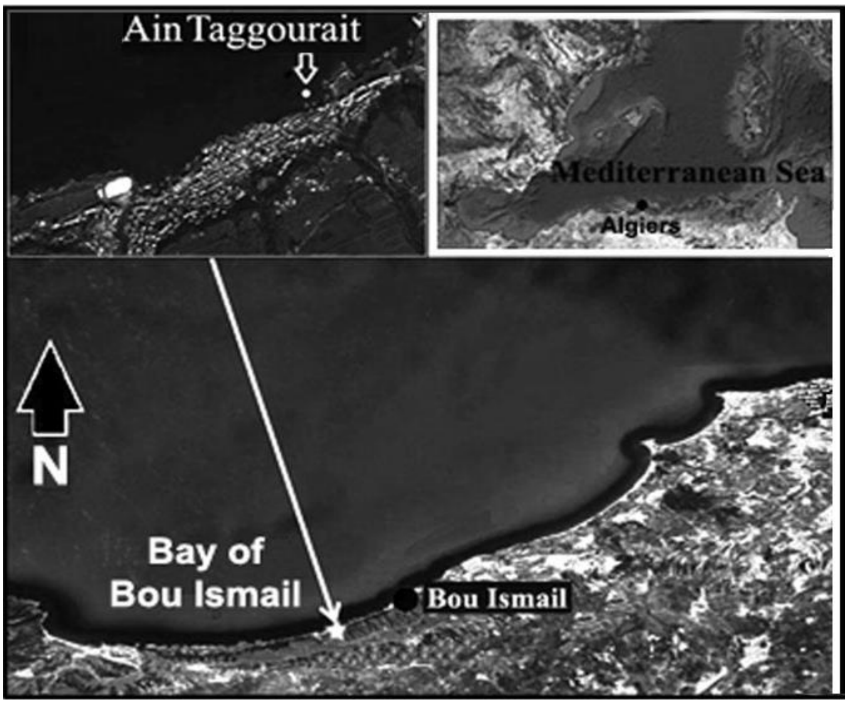

Figure 1. Location of the sampling site (© 2012 Google earth)

The biological material was sampled using scuba diving at depths ranging from -3 to -10 m. For each monthly sample, the sea water temperature was recorded using a SUNTO GEKKO dive type computer (precision 0.5 °C). Individuals of *H. tubulosa* collected in the field were kept in a tank filled with sea water until processing in the laboratory.

Reproductive parameters

The sampled holothurians were measured (+/−0.5 cm) dorsally from the mouth to the cloaca using a Ribbon meter. Subsequently, specimens were dissected by longitudinal incision on their ventral side along the interradial. When possible, sex determination was done macroscopically, the ovary was usually yellow or orange red, and the testes whitish. The gonad was weighted (drained weight, Wg ± 0.01 g) and then fixed in aqueous Bouin's solution for 24–48 h. Subsequently, the samples were rinsed in successive baths of ethanol and preserved in ethanol (70%) for histology according to the protocols of [14] and [15]. All the internal organs of an individual were removed to obtain the eviscerated body weight (eBW ± 0.01 g). Therefore, the eBW included the weight of the body wall, the longitudinal muscles, and the buccal bulb ([16]; [17]). Approximately, 15 adult individuals were sampled each month during two years (from march 2010 to march 2012). Two approaches were used to determine the reproductive cycle of *H. tubulosa*: (1) gonad index (GI) survey and (2) histological study to allow the temporal monitoring of the microscopic stages of gonad maturity. The GI was calculated by the following formula ([18]; [19]; [20]; [21]; [22]:

$$\mathbf{IG} = \frac{Wg}{GBW} \times 100$$

Wg = weight of the gonad ; GBW= Gutted body wall.

For the microscopic analysis, the conventional topographic dyes Mann-Dominici (1894–1905 in [15] and Trichrome on one time ([23]) were used for the determination of the microscopic stages of maturity. They were based on histology and on those of ([24]; [25];

[16]) and [9]: Stage I: sexual resting (resting or indeterminate tubules); Stage II: recovery and maturing (increasing tubules); Stage III: mature (ripe tubules); Stage IV: spawning (partly emptied tubules); Stage V: spent (empty tubules).
Once the homogeneity of variances had been verified, differences in the values of the GI by seasons and years were conducted using a two-factor ANOVA ($p < 0.05$). The average values of the GI were analyzed by a Mann and Whitney test. A Spearman test was used to analyze the existing correlation between the GI and temperature. All statistical analyses were performed using STATGRAPHICS and STATISTICA 7.

RESULTS

Reproductive biology

A total of 367 individuals of *H. tubulosa* were sampled in Ain Taggourait site. During our sampling, 18 individuals lacking gonads were found in the fall and spring. To understand the variability of the reproductive cycle of *H. tubulosa* at the studied site, the gonad index data of males and females were pooled and the percentages of individuals at stage V examined (Figure 2).

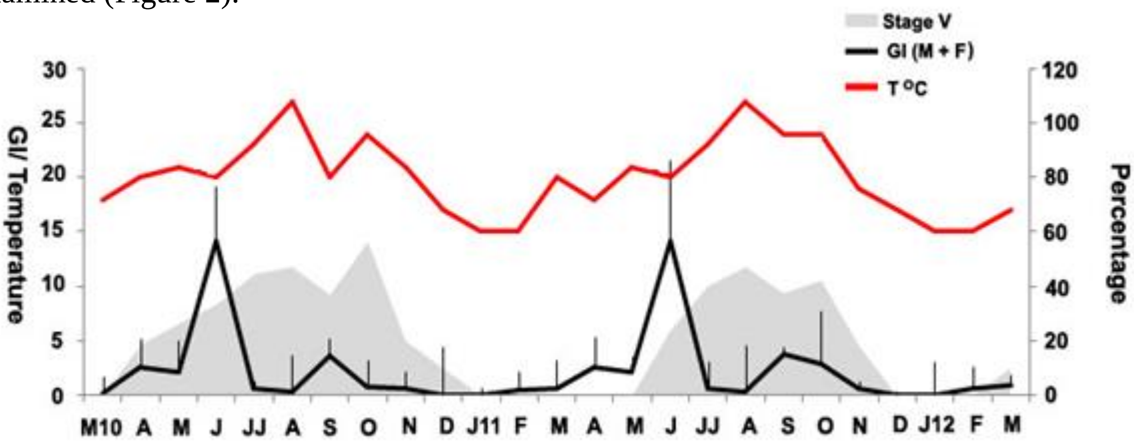

Figure 2. Monthly gonad index means of *H. tubulosa* (pooled sex), monthly mean temperature, and the percentage of individuals observed at stage V (post-spawning) at the site of Ain Taggourait. GI: Gonad Index; M: Male; F: Female; T: Temperature.

At the studied site, the reproductive cycle was marked by a period of gonad growth in early spring, followed by two gamete emissions, the first in the spring and the second massive in summer. Both marked by the fall in the gonad index (Figure 2). Moreover, the spawning spread till the end of fall. The period of sexual resting in the winter was marked by the low GI values. Furthermore, the main and massive spawning of *H. tubulosa* was confirmed by the presence of individuals in stage V (post-spawning) (Figure 3) and the low GI values in the fall.
However, we noted that a period of a high temperature rise during the two years at the site of Ain Taggourait (22–24.5 °C in 2010 and 20–23 °C in 2011) coincides with the period where the GI of *H. tubulosa* decreases (Fig. 2). The correlation between the evolution of the GI and the temperature is significantly confirmed for Ain Taggourait site ($p > 0.01$).

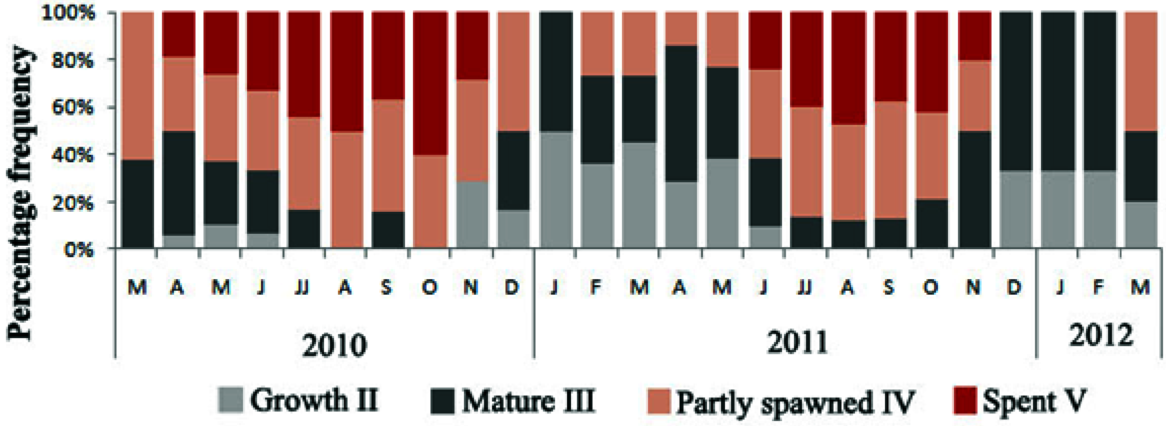

Figure 3. Maturity stages of the tubules of *H. tubulosa* gonads (sexes pooled) at the site of Ain Taggourait from March 2010 to March 2012. Histograms show percentages of individuals in one of the four gametogenic stages.

DISCUSSION

Holothuria tubulosa is a gonochoric species without external sexual dimorphism. The sex can be determined macroscopically by the appearance and color of the gonads ([8]; [9]). In this study, the gonad index of *H. tubulosa* shows that the reproductive cycle of this species is annual, with spawning starting in the spring, peaking in the summer, and ending in the fall, marked by maximum values of the GI in early summer and the minimum values at the end of the autumn. The maximum values of the GI were obtained in summer (June to July) (Figure 2). The mean GI obtained at Ain Taggourait is higher than that obtained for other species like *H. sanctori* in the Canary Islands where the individuals were smaller ([9]). A good similarity of the temporal change of the GI and the stages of maturation of the gonads has been reported for various aspidochirotid holothurians ([18]; [26]; [17]). Therefore, a GI survey is a reliable method for describing the reproductive cycle. In general, holothurians have an annual reproductive cycle ([24]; [27]; [28]; [29]; [30]; [31]; [18]; [32]; [33]; [34]); although semi-annual cycles have been described by some authors ([27]; [18]) or even the presence of a continuous reproduction throughout the year ([27]) which can occur especially in the tropical regions. In the studied site the changes in the GI coincided with that of the sea water temperature and/or photoperiod (Spearman test, $p > 0.01$) and the gonad development seemed to be induced by the gradual warming of the water. This suggests that if temperature and/or photoperiod are important factors in reproductive timing, they work in an absolute (threshold) pattern rather than in a relative one ([35]; [36]).
Moreover, the presence of stage II throughout the sampling period, with the exception of the summer-autumn (July to October), suggests that gonad maturation occurs throughout the year with a slowdown during the winter (figure 3). The main spawning is massive in the summer–autumn and coincides with the elevation of the sea water temperature (Spearman test, $p < 0.05$); an average temperature rise of 2 °C between June and July was observed during the two years (22–24.5 °C in 2010 and 20–23 °C in 2011) and coincides with the decrease of the GI and the presence of a high percentage of individuals of *H. tubulosa* with partially spawned gonads. However, during the two years (27 and 28 °C, respectively, in August 2010 and 2011), a high temperature rise coincided with the period where individuals of *H. tubulosa* had a minimum GI value and were in the post-spawning stage (figure 3). The slight decline of the GI in the spring could be explained by a partial spawning by precociously mature individuals due to temperature values which seem to favor a rapid maturation of the gonads.

Our results match those obtained for *H. (P.) sanctori* on the same site ([10]) and of the Canary Islands by [9] where maximum reproductive activity was observed during the warm months and the minimum activity (resting) during the cold months. The absence of gonads in the winter (cold season) in some specimens is explained by the tubular resorption after spawning ([18]). This is the case for *H. tubulosa* of the Ain Taggourait site with a few individuals having gonads completely resorbed after the spawning observed in 2010. Similar observations were made by [8] for *H. tubulosa* from the Adriatic Sea and by [19] for *H. fuscogilva* and *Actinopyga mauritiana*. However, the presence of a few mature *H. tubulosa* at the end of the spring at Ain Taggourait site seems to confirm the start of an early spawning in spring for some individuals.
There are still few data on the reproductive cycle of *H. tubulosa* or the other aspidochirotid holothurians. [7] presented the reproductive cycle of *H. tubulosa* off Ischia Island (Italy) and [17] in the Aegean Sea. Moreover, the effect of temperature on gametogenesis has been well studied in holothurians where it was reported that the sea water temperature could influence gonad growth by acting directly on food availability (appearance of the phytoplankton bloom) ([36]; [37]; [9]). The data collected for *H. tubulosa* do not allow testing this hypothesis. However, this species displays an annual reproductive cycle with spawning of high intensity in the Bay of Bou Ismail (Ain Taggourait).
However, from the study of the reproductive cycle of *H. tubulosa* from the Mediterranean southwestern basin (Algerian coast), it appears that spawning begins in June and ends around October. Considering that holothurians larvae settle in the benthos after about one month [12–17 days for *Holothuria scabra* ([38]); 22–27 days for *Isostichopus fuscus* ([39]) and 20 days for *Apostichopus japonicus* ([40]], we can assume that the recruitment of juveniles of *H. tubulosa* occurs from the end of the summer (August) as reported by [41]. The collected data suggest that temperature is the most likely factor in the onset of spawning of *H. tubulosa* and the determination of its reproductive cycle, as is the case for other aspidochirotid holothurians species ([25]; [31]; [8]).:

REFERENCES

[1] Harmelin, JG, Bouchon, C, Hong, JS. 1981. Impact de la pollution sur la distribution des échinodermes des substrats durs en Provence. *Téthys*. 10:13–36.
[2] Coulon, P, Jangoux, M. 1993. Feeding rate and sediment reworking by the holothuroid *Holothuria tubulosa* (Echinodermata) in a Mediterranean seagrass bed off Ischia Island, Italy. *Marine Ecology Progress Series*. 92:201–204.
[3] Cherbonnier, G. 1958. Faune marine des Pyrénées orientales Echinodermes. Vol. 2. France: Université Paris. Laboratoire Arago. University Paris VI; p. 1–17.
[4] Francour, P. 1990. Dynamique de l'écosystème à *Posidonia oceanica* dans le parc national de Port Cros: Analyse des compartiments matte, litière, faune vagile, échinodermes et poissons. *Thèse de doctorat*. Paris: Université Pierre et Marie Curie; p. 373.
[5] Mezali, K. 1998. Contribution à la systématique, la biologie, l'écologie et la dynamique de cinq espèces d'holothuries aspidochirotes (*Holothuria (H.) tubulosa, H. (L.) polii, H. (H.) stellati, H. (P.) forskali* et *H (P.) sanctori*) de l'herbier à *Posidonia oceanica* (L.) Delille de la Presqu'île de Sidi-Fredj. *Thèse de Magister*. Institut des sciences de la mer et de l'aménagement du littoral. p. 192 [in French].
[6] Mezali, K. 2008. Phylogénie, systématique, dynamique des populations et nutrition de quelques espèces d'holothuries aspidochirotes (Holothuridea: Echinodermata) inféodées aux herbiers de Posidonies de la côte Algéroise. *Thèse de doctorat d'état*. Université des Sciences et de la Technologie Houari Boumediene. 228 p.
[7] Bulteel, P., Jangoux, M., Coulon, P. 1992. Biometry, bathymetric distribution, and reproductive cycle of the holothuroid *Holothuria tubulosa* (Echinodermata) from Mediterranean Sea grass beds. *Marine Ecology*. 13:53–62.
[8] Despalatovic, M, Grubeli, I, Šimunovic, A, Antoli, B, Žuljevic, A. 2004. Reproductive biology of the holothurian *Holothuria tubulosa* (Echinodermata) in the Adriatic Sea. *Journal of the Marine Biological Association of the UK*. 84:409–414.

[9] Navarro, PG, García-Sanz, S, Tuya, F. 2012. Reproductive biology of the sea cucumber *Holothuria sanctori* (Echinodermata: Holothuroidea). *Scientia Marina*. 76:741–752.

[10] MezalI, K., Soualili, D. L., Neghli, L., Conand, C., 2014. Reproductive cycle of the sea cucumber *Holothuria (Platyperona) sanctori* (Holothuroidea: Echinodermata) in the southwestern Mediterranean Sea: Interpopulation variability. *Invertebrate reproduction and development.* Vol. 58(3): 179-189.

[11] Mezali, K., Francour, P., 2012. Les holothuries aspidochirotes de quelques sites des côtes algériennes: révision systématique et relations phylogénétiques. *Bulletin de la Société Zoologique de France*, 137(1-4): 177-192.

[12] Léonet, A, Rasolofonirina, R, Wattiez, R, Jangoux, M, Eeckhaut, I. 2009. A new method to induce oocyte maturation in holothuroids (Echinodermata). *Invertebrate Reproduction & Development*. 53:13–21.

[13] Braik, D. 1989. Etude de la dynamique sédimentaire devant Bou Ismail: sédimentologie, morphologie, problème d'érosion du littoral et aménagement. Thèse de Magister. Institut des sciences de la terre; p. 174.

[14] Martoja, R, Martoja-Pierson, M. 1967. Initiation aux techniques de l'histologie animale. Paris: Masson; p. 1–345.

[15] Bensalem-Bendjelloul, M. 1998. Techniques histologiques: théorie et pratique. Office des Publications Universitaires, Alger, p. 1–109.

[16] Conand, C. 1990. The fishery resources of Pacific island countries Part 2: Holothurians. FAO Fisheries Technical Paper no. 272. Rome: FAO; p. 1–143.

[17] Kazanidis, G, Antoniadou, C, Lolas, AP, Neofitou, N, Vafidis, D, Chintiroglou, C, Neofitou, C. 2010. Population dynamics and reproduction of *Holothuria tubulosa* (Holothuroidea: Echinodermata) in the Aegean Sea. *Journal of the Marine Biological Association of the United Kingdom*. 90: 895–901.

[18] Conand, C. 1993. Reproductive biology of the holothurians from the major communities of the New Caledonian Lagoon. *Marine Biology*. 116:439–450.

[19] Ramofafia, C, Byrne, M. 2001. Assessment of the "tubule recruitment model" in three tropical aspidochirote holothurians. *S.P.C. Bêche-de-mer Information Bulletin*. 15:13–16.

[20] Asha, PS, Muthiah, P. 2008. Reproductive biology of the commercial sea cucumber *Holothuria spinifera* (Echinodermata: Holothuroidea) from Tuticorin, Tamil Nadu, India. Aquaculture International. 16:231–242.

[21] Gaudron, S, Kohler S, Conand, C. 2008. Reproduction of the sea cucumber *Holothuria leucospilota* in the Western Indian Ocean: biological and ecological aspects. *Invertebrate Reproduction & Development*. 51:19–31.

[22] Kohler, S, Gaudron, S, Conand, C. 2009. Reproductive biology of *Actinopyga echinites* and other sea cucumbers from Reunion Island (Western Indian Ocean): a contribution for a regional management of the fishery. *WIOJMS*. 8:97–111.

[23] Gabe, M, Martoja, M. 1957. Une coloration trichrome en un temps sans différenciation. *Bulletin Microbiolgie Appliquée*. 7:60–63.

[24] Tanaka, Y. 1958. Seasonal changes occurring in the gonad of *Stichopus japonicus*. *Bulletin of the Faculty of Fisheries Hokkaido University*. 1:29–36.

[25] Conand, C. 1981. Sexual cycle of three commercially important holothurian species (Echinodermata) from the lagoon of New Caledonia. *Bulletin of Marine Science*. 31:523–543.

[26] Shiell, G, Uthicke, S. 2005. Reproduction of the commercial sea cucumber *Holothuria whitmaei* (Holothuroidea: Aspidochirotida) in the Indian and Pacific Ocean regions of Australia. *Marine Biology*. 148:973–986.

[27] Harriott, VJ. 1985. Reproductive biology of three congeneric sea cucumber species, *Holothuria atra, H. impatiens and H. edulis*, at Heron Reef, Great Barrier Reef. *Marine and Freshwater Research*. 36:51–57.

[28] Cameron, JL, Fankboner, PV. 1986. Reproductive biology of the commercial sea cucumber *Parastichopus californicus* (Stimpson) (Echinodermata: Holothuroidea). I. Reproductive periodicity and spawning behavior. *Canadian Journal of Zoology*. 64:168–175.

[29] Ong-Che, RS. 1990. Reproductive cycle of *Holothuria leucospilota* Brandt (Echinodermata: Holothuroidea) in Hong Kong and the role of body tissues in reproduction. *Asian Marine Biology*. 7:115–132.

[30] Smiley, S, McEuen, FS, Chaffee, C, Krishnan, S. 1991. Echinodermata: Holothuroidea. In: Giese A, Pearse J, Pearse VB, editors. Reproduction of marine invertebrates echinoderms and lophophorates. Vol. 6. Pacific Grove (CA): Boxwood Press; p. 663–750.

[31] Tuwo, A, Conand, C. 1992. Reproductive biology of the holothurians *Holothuria forskali* (Echinodermata). *Journal of the Marine Biological Association of the United Kingdom*. 72:745–758.

[32] Chao, S.M., Chen, C.P, Alexander, PS. 1993. Reproductive periodicity of a tropical dendrochirote holothurian *Phyrella fragilis* (Echinodermata: Holothuroidea) in Taiwan. *Bulletin of the Institute of Zoology, Academia Sinica*. 32:111–119.

[33] Chao, SM, Chen, CP, Alexander, PS. 1995. Reproductive cycles of tropical sea cucumbers (Echinodermata: Holothuroidea) in southern Taiwan. *Marine Biology*. 122:289–295.

[34] Foster, GG, Hodgson, AN. 1995. Annual reproductive cycles of three sympatric species of intertidal holothurians (Echinodermata) from the coast of the Eastern Cape Province of South Africa. *Invertebrate Reproduction & Development*. 27:49–59.

[35] Pearse, JS, Pearse, VB. 1986. Photoperiodic regulation of gametogenesis and growth in the sea urchin *Strongylocentrotus purpuratus*. *Journal of Experimental Zoology*. 237:107–118.

[36] Hopper, DR, Hunter, CL, Richmond, RH. 1998. Sexual reproduction of the sea cucumber *Actinopyga mauritiana* in Guam. *Bulletin of Marine Science*. 63:1–9.

[37] Conand, C, Uthicke, S, Hoareau, T. 2002. Sexual and asexual reproduction of the holothurian *Stichopus chloronotus* (Echinodermata): a comparison between La Réunion (Indian Ocean) and east Australia (Pacific Ocean). *Invertebrate Reproduction & Development.* 41:235–242.

[38] Mercier, A, Battaglene, SC, Hamel, JF. 2000. Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*. *Journal of Experimental Marine Biology and Ecology*. 249:89–110.

[39] Hamel, JF, Ycaza-Hidalgo, R, Mercier, A. 2003. Développement larvaire et croissance des juvéniles de l'holothurie des Galapagos, *Isostichopus fuscus*. *S.P.C. Bêche-de-mer Information Bulletin*. 18:3–8.

[40] Matsuura, H, Yazaki, I, Okino, T. 2009. Induction of larval metamorphosis in the sea cucumber *Apostichopus japonicus* by neurotransmitters. *Fisheries Science*. 75:777–783.

[41] Mezali, K, Semroud, R. 1998. Analyses modales et essai d'estimation des paramètres de croissance, de l'âge et du mois de recrutement de trois espèces d'holothuries aspidochirotes (Holothuroidea: Echinodermata) de la région de Sidi-Fredj (Algérie). *Rapport et PV de la Commission international pour l'Exploration Scientifique de la Mer Méditerranée*. Dubrovnik (Croatia) 35; p. 466–467.