Gonadal development of the holothurian Holothuria polii (Delle Chiaje, 1823) in spawning period at the Aegean Sea (Mediterranean Sea)

Ege Denizi’nde (Akdeniz) Holothuria polii (Delle Chiaje, 1823) türü deniz hyııranın yumurtlama dönünideki gonad gelişimi

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Abstract: The Mediterranean Sea cucumbers including Holothuria polii has become commercially important in international trade due to the high demand of consumers from Far East countries. Sea cucumbers fisheries is a valuable income for the regional fishermens but natural stocks are endangered by overfishing in recent years. Fisheries regulations and aquaculture studies are important precautions for preserving natural stocks. All these efforts are based on reproduction biology of this species which slightly differed among regions. In this study, the reproduction biology including morphological characteristics, gonadosomatic index and gonad development stages of H.polii at the eastern coast of Aegean Sea (İzmir, Turkey) were investigated during the reproduction (spawning) period from July to October 2018. Gonads and gonad sections of 60 sea cucumbers (120.60 ± 19.56 g) have been observed by macroscopic and microscopic inspections. Three gonadal development stages have been identified by histologic observations as mature (III), spawning (IV) and post-spawning stage (V) in gonadal tubules. Results indicate that both female and male gonads are at mature and spawning stage in July and all gonads are at post-spawning stage in October. The mean gonad weight was 12.53 ± 1.33 g at the beginning of the spawning period (July) and 1.87 ± 0.58 g at the end of the spawning period (October). Gonadosomatic index decreased from 17.53 ± 0.02% (July) to 3.37 ± 0.01% (October) after spawning. The gonadosomatic index, gonad weight, and spawning were related to the seawater temperature. According to this study, the spawning period of H.polii at the eastern coasts of the Aegean Sea starts in July and completes ends in October. As a result, the data related to the reproduction biology in spawning period of H.polii would guide stock management and artificial breeding of this species under controlled conditions.

Keywords: Mediterranean, sea cucumber, gonadal development stage, reproduction biology, gonadosomatic index, Holothuria polii

INTRODUCTION

Sea cucumbers, distributed in almost all seas of the world with approximately 1711 species (WoRMS, 2018). They feed on detritus such as diatoms, cyanophytes, macroalgae, crustaceans, bivalve shells, sponge osciıles and nematodes accumulated on the seafloor (Selbachir and Mezali, 2018) and transform matter and energy by processing organic nutrients in the benthic ecosystems (Purcell et al., 2016). They stimulate nutrient conversion, sediment mixing, bioturbation and microalgae growth in marine sediment due to their feeding and movement patterns (Wolkenhauer et al., 2010). In addition to their ecological importance, the body wall of cucumber is valuable seafood that is particularly demanded by Asian consumers. Moreover, bio-extracts obtained from sea cucumbers are used in the production of pharmaceutical, nutraceutical and cosmetics products (Purcell, 2014). Approximately ten thousand tons of dried sea cucumbers are subject to international trade (Purcell et al., 2013), which means that approximately 200 million sea cucumbers are collected each year from the marine ecosystem (Purcell et al., 2016). The high price and economic value of sea cucumber bring the danger of overfishing and the depletion of its stocks. Today, natural stocks of sea cucumbers are
threatened by overfishing in many parts of the world. Less known sea cucumber species that are not consumed regionally are gaining commercial importance in international trade due to the decrease in trade amount of high-value species (Sellem et al., 2017).

*Holothuria polii* (Delle Chiaje, 1823), a member of Holothuroidea class of Echinodermata phylum, is widely distributed in soft sandy sediments and sea meadows of the sublittoral zone at the Mediterranean coasts of Algeria, Croatia, Egypt, France, Italy, Spain, Tunisia and Turkey. *H. polii* are mainly collected by the fishermen of Turkey and the other Mediterranean countries. Mediterranean sea cucumbers, including *H. polii*, are exported in dried and frozen form to Far East countries at prices of approximately 19 to 48 USD per kg (TURKSTAT, 2019) and became an important source of income for fishermen in the region. Due to the increasing economic importance of sea cucumbers, fishing arrangements, restocking and aquaculture efforts are required to ensure the sustainability of natural stocks (Toscano et al., 2018). Researches on the breeding biology of *H. polii* could have several applications (Rakaj et al., 2019). Although some pioneering research on the aquaculture of *H. polii* has resulted successfully (Rakaj et al., 2019; Tolon, 2017) but there is still scarce information on spawning period and gonadal development which are needed for breeding under controlled conditions.

Entire studies on the reproductive biology of sea cucumber species clearly show that reproductive dynamics differ according to the geographical regions (Bulteel et al., 1992; Costelloe, 1988; Despalatović et al., 2004; Despalatović et al., 2003; Fajardo-León et al., 2008; McEuen, 1988; Navarro et al., 2012; Tuwo and Conand, 1992; Valls, 2004). The effect of water temperature that determines the spawning period is prominent in the temperate zones like the Mediterranean, where seawater temperature significantly varies between regions (Conand, 1981; Costelloe, 1988; Despalatovic et al., 2004; Tuwo and Conand, 1992). Considering the limited mobility of sea cucumbers, it is important to define the region-specific reproductive biology including gonadal development and spawning periods required for aquaculture and stock management studies.

This study aims to determine the morphological characteristics, gonadosomatic index and gonadal development stages of *H. polii* distributed on the eastern coast of the Aegean Sea (Mediterranean) during the reproduction (spawning) period. Thereby, findings derived from the study would be a good reference in the literature concerning microscopic observations of Mediterranean sea cucumber *H. polii*’s reproduction biology. Moreover, the data related to the reproduction biology in spawning period of *H. polii* would beneficial in both stock management and artificial breeding of this species under controlled conditions.

**MATERIAL AND METHOD**

*H. polii* individuals were sampled from the Urla (38°21'55.42"N; 26°46'8.88"E) coasts of the Gulf of Izmir (Turkey), at the Aegean Sea. The dense *H. polii* stocks and prohibition of fishing were the main reasons for the selection of this location (Figure 1).
Two samplings were carried out in July and October 2018, when the seawater temperature at the selected region reached their highest and the lowest level within the year. In order to determine the sampling period, 10-year monthly average seawater temperatures in Urla (İzmir) region and daily seawater temperature data covering the working period were obtained from the data of Urla Mendirek Station of General Directorate of Meteorology of Turkey (MGM, 2018). In addition, seawater temperatures were recorded with a dive type thermometer (Mares Puck Pro) in each sampling.

H. polii adults were hand-picked from a depth of 2-4 m by scuba diving. The collected sea cucumbers were immediately brought to Ege University Fisheries Faculty, Urla Research Unit Laboratories (38°21'48.46” N; 26 ° 46'14.95” E). Samples kept in an ice-molded tank during transfer to prevent them from ejaculating viscera due to stress. Total 60 sea cucumbers, mean wet weight of 120.60 ± 19.56 g were selected from a group of adult individuals in the study. Sea cucumbers were dissected by longitudinal incision on their ventral side. Total length (TL), total weight (TW), gutted body weight, (GBW) and gonad weight (GW) were measured for each sea cucumber (Conand, 1981). The gonadosomatic index (GSI) to determine the level of gonadal development was calculated according to the following formula (Asha and Muthiah, 2008; Conand, 1981; C. J. M. B. Conand, 1993; Ramofafia et al., 1995):

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GSI = \frac{GW}{GBW}
\]

where GSI=Gonadosomatic index; GW=Gonad weight and GBW= Gutted body weight

Gonads were removed and fixed in 10% buffered neutral formalin solution for 24 hours. Gonad samples were dehydrated in graded alcohol series according to Roberts and Ekman (2012). Sections from the samples embedded in paraffin were cut at 5-6 µm by Rotary Microtome Device (Leica RM2125 RTS), cleared in xylene and stained with hematoxylin-eosin. Gonad tissues inspected and pictured under a phase-contrast microscope (Olympus CX-31). The gonadal development stages were identified according to Despalatovic et al. (2004) as Stage I: recovery (resting or indeterminate tubules); Stage II: growing (increasing tubules); Stage III: mature (ripe tubules); Stage IV: spawning (partly emptied tubules); and Stage V: post-spawning (empty tubules). Sex of the individuals was determined by microscopic observation of the gonads.

**Statistical analysis**

Data are presented as mean ± standard error of the mean unless otherwise stated. The homogeneity of variances was tested (Levene test) and, whenever necessary, the log transformation log (x + 1) was used (Zar, 1996). Differences between TL, TW, GBW and GW values of male and female individuals were tested by ANOVA and t-test. The average values of the GSI by sex were analyzed by a Mann and Whitney test. Pearson test was used to analyze the existing correlation between the GW, GSI, and temperature. Mean GSI differences between the sampling periods were analyzed by t-test. SPSS v.24 software package was used for all statistical analysis.

**RESULTS**

Sex was primarily determined by macroscopic observations based on gonad color. The tubules of the female gonads are usually yellow or orange, and male gonads are pale white (Figure 2). However, to make a complete gender determination, reproductive cells in the gonads of all H. polii individuals were examined and the gender was confirmed by microscopic observations.

![Figure 2. Mature gonads of female (A) and male (B) H. polii adults](image-url)
Total of 60 samples (28 male and 32 female) were inspected in this study. Gender ratio did not show significant difference from 1:1 distribution according to chi-square test results ($x^2=0.133$; df=1; $p=0.72$). Average length (TL) of sampled sea cucumbers was $14.14 \pm 1.83$ cm ($13.99 \pm 1.66$ cm male; $14.29 \pm 2.03$ cm female). There was no significant difference between the genders in terms of mean lengths (t-test; $p = 0.66$; $p>0.05$). The average weight (TW) of the sampled sea cucumbers was $120.60 \pm 19.56$ g ($120.81 \pm 17.30$ g male; $120.38 \pm 22.21$ g female). There was no significant difference between female and male mean weights (t-test; $p = 0.95$; $p>0.05$). The mean gutted body weight was $64.41 \pm 12.08$ g ($67.30 \pm 9.85$ g male; $61.53 \pm 13.69$ g female). There was no significant difference between male and female individuals in terms of gutted body weight values (t-test; $p = 0.20$; $p>0.05$) (Table 1).

| Period          | Male gonad weight (g) | Female gonad weight (g) | Mean gonad weight (g) |
|-----------------|-----------------------|-------------------------|-----------------------|
| Male            | $120.81 \pm 17.30$    | $67.30 \pm 9.85$        | $92.66 \pm 12.25$     |
| Female          | $120.38 \pm 22.21$    | $61.53 \pm 13.69$       | $84.46 \pm 14.93$     |
| Mean            | $120.60 \pm 19.56$    | $64.41 \pm 12.08$       | $98.47 \pm 13.74$     |

The mean gonad weight of all samples was $12.53 \pm 1.33$ g ($11.81 \pm 2.16$ g male gonads; $13.34 \pm 1.56$ g female gonads) at the beginning of the spawning period (July). The mean gonad weight at the end of the spawning period (October) was determined as $1.87 \pm 0.58$ g ($2.13 \pm 0.93$ g male gonads; $1.64 \pm 0.76$ g female gonads). There was no significant difference in gonad weights between genders in both sampling periods (ANOVA; July $p=0.87$; $p>0.05$) (ANOVA; October $p=0.99$; $p>0.05$). However, significant differences were found between the mean gonadosomatic index in July and October in both male and female individuals (t-test; $p=0.00$; $p<0.05$) (Table 3).

| Period          | Male (%) | Female (%) | Mean (%) |
|-----------------|----------|------------|----------|
| July 2018       | $13.99 \pm 1.66$ | $120.81 \pm 17.30$ | $92.66 \pm 12.25$ |
| Female          | $14.29 \pm 2.03$ | $120.38 \pm 22.21$ | $84.46 \pm 14.93$ |
| Mean            | $14.14 \pm 1.83$ | $120.60 \pm 19.56$ | $98.47 \pm 13.74$ |

There is a significant difference between the monthly seawater temperature means of the sampling area during the study period (ANOVA; $p=0.00$; $p<0.05$). Gonadosomatic index (Pearson; $p = 0.77$) and gonad weights (Pearson; $p = 0.92$) of the sampled sea cucumbers were positively and significantly correlated with the seawater temperature at the studied location (Figure 3). Gonad weight and gonadosomatic index mean values were the highest in July parallel with the highest mean seawater temperature ($25.80 \pm 0.95$ °C), while they decreased significantly in October when the mean seawater temperature dropped to $19.81 \pm 1.17$ °C.

**Microscopic observations of the gonads**

Morphological and histological examination of gonads tissues revealed three gonadal development stages in male and female individuals. Mature (III) and spawning stages (IV) were determined in gonads of female and male individuals in July samples, while gonads of both genders were in post-spawning stage (V) in October. Since the aim of the study was to determine the developmental stages of female and male *H. polii* gonads during the spawning period, Stage I (recovery; resting or indeterminate tubules) and Stage II (growing: increasing tubules) phases were not observed.

**Observations on the gonads of female *H. polii* by development stages**

Stage III (Mature): The gonad wall was thin in this stage. The development of oocytes was completed and the presence of previtellogenic oocytes was not observed in the germinal layer, but the number of vitellogenic eggs increased (Figure 4A).

Stage IV (Spawning): The gonad wall was still thin in this stage. Gaps were detected in the tubules for easy ovulation (Figure 4B).

Stage V (Post-spawning): After spawning, a large migration of phagocytic hemocytes from thick-walled phagocytic gonadal tubules and gametes remaining in the resorption process occurred in the gonads. Tubules were empty, flaccid and wrinkled. Large gaps are visible in the gonads (Figure 4C).

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**Table 1.** Morphological observations on the *H. polii* specimens

| Period | TL (cm) | TW (g) | GBW (g) |
|--------|---------|--------|---------|
| Male   | $13.99 \pm 1.66$ | $120.81 \pm 17.30$ | $67.30 \pm 9.85$ |
| Female | $14.29 \pm 2.03$ | $120.38 \pm 22.21$ | $61.53 \pm 13.69$ |
| Mean   | $14.14 \pm 1.83$ | $120.60 \pm 19.56$ | $64.41 \pm 12.08$ |

TL: Total length (cm); TW: Total wet weight (g); GBW: Gutted body weight (g); n=60

Data with different superscripts in columns indicate significant differences from each other ($p<0.05$).

**Table 2.** Mean gonad weights of *H. polii* within the reproduction period

| Period     | Male gonad weight (g) | Female gonad weight (g) | Mean gonad weight (g) |
|------------|-----------------------|-------------------------|-----------------------|
| July 2018  | $11.81 \pm 2.16$      | $13.34 \pm 1.56$        | $12.53 \pm 1.33$      |
| October    | $2.13 \pm 0.93$       | $1.64 \pm 0.76$         | $1.87 \pm 0.58$       |

Data with different superscripts in columns indicate significant differences from each other ($p<0.05$).

The mean gonadosomatic index of all specimens was $17.53 \pm 0.02%$ at the beginning of the spawning period ($15.97 \pm 0.03%$ male; $19.30 \pm 0.02%$ female). It was decreased to $3.37 \pm 0.01%$ at the end of the spawning period ($3.70 \pm 0.02%$ male; $3.08 \pm 0.01%$ female). There was no significant difference in gonadosomatic index between genders in both sampling periods (ANOVA; July $p=0.74$; $p>0.05$) (ANOVA; October $p=0.99$; $p>0.05$). However, a significant difference was found between the mean gonadosomatic index in July and October in both male and female individuals (t-test; $p=0.00$; $p<0.05$) (Table 3).
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Figure 3. Mean seawater temperature versus (a) gonad weight and (b) gonadosomatic index of H. polii

Figure 4. Microscopical characteristics of gonadal stages of the females. (A) mature stage; (B) spawning stage; (C) post-spawning stage. Scale bars: A, B, 50 µm, C, 100 µm.

Microscopic observations on gonads of male H. polii by development stages

Stage III (Mature): Lumens have reached their maximum diameter and were filled with seminal fluid. Histologically, the walls of the lumens were smooth and thin, with germinal folds. The lumen of the spermatozoa tubules looks like a mature testicle. Spermatogenesis formation was accelerated and apparent. Gonad wall was thin (Figure 5A).

Stage IV (Spawning): The expulsion of gametes from the gonad began, the gonad wall thickened. Histologically, the walls of the tubules are smooth and thin, with germinal folds. Cells and tubules found in early spermatogenesis stages were characterized by mature spermatozoa (Figure 5B).

Stage V (Post-spawning): After resorption gonad was almost empty (Figure 5C).
DISCUSSION

Gonadal development stages of *H. polii* in spawning period were evaluated in this study to fully identify the gonadosomatic index, gonad size and morphological properties of both sex in a wild population from İzmir (Turkey) coasts of the Aegean Sea. Results derived from the study indicate the occurrence of three development stages in gonads. Mature and spawning stages observed at the beginning of the spawning period, and only post-spawning stage at the end.

Reproduction and gonadal development in holothuroids are depending on water temperature. Gonadal development reaches to the highest level in the periods when the water temperature also reaches the highest levels, and spawning occurs (Conand, 1981; Costelloe, 1988; Tuwo and Conand, 1992). Morphological examinations of the gonads in this study revealed that gonadosomatic indexes and gonad weights of the sampled sea cucumbers were high in July when the water temperature reached the highest value of the year. Seasonal changes in water temperature by regions may cause changes in the reproduction period of sea cucumbers. In the study covering the northern part of the Aegean Sea, Bardanis and Batjekas (2018) reported that spawning for *H. polii* occurred between July and August and that the gonads had gone through the recovery stage from September. The rapid decrease in water temperature from September is effective in the early termination of the spawning period. In the southern regions of the Mediterranean, Slimane-Tamacha et al. (2019) and Sellem et al. (2017) reported that the gonad development was completed in May with the effect of earlier warming in the summer period and the ovulation started in June and ended in September. In the current study, the post-spawning stage observed in some individuals revealed that ovulation started in July, extensively continued in August and September, and ended completely in October at Eastern coasts of the Aegean Sea. However, not only the temperature but also many other environmental factors such as sediment types, feeds, water quality etc. are known to affect the sea cucumbers gonadal development in the nature. Therefore, the effects of these factors on gonad development should be examined in detail in future studies.

In terms of gonad weights and gonadosomatic indexes, there were no significant differences among the gender in both periods which means that gonad development is synchronous in *H. polii* male and female. The mean gonadosomatic index calculated in this study is 17.53%, which is above the gonadosomatic indexes calculated in previous studies during the same periods of the year. Bardanis and Batjekas (2018) observed the highest gonadosomatic index by 14% in late June and Sellem et al. (2017) observed 16% in May. The highest values of gonadosomatic index, which is an important indicator of gonad development, do not vary excessively among the regions, but the periods in which they reach these highest values may be different.

Studies on reproduction biology of economic sea cucumber species that are widely distributed in the Mediterranean like *H. polii* are important for the sustainability of natural stocks, which are endangered due to overfishing.
This study reveals that the reproductive period and gonadal development of this species differ even between the close geographic locations. The future studies on reproduction biology of sea cucumbers will guide the region-specific conservation measures and stock enrichment efforts suitable for the species.

REFERENCES

Asha, P. & Mudhiah, P.J.A.I. (2008). Reproductive biology of the commercial sea cucumber Holothuria spinifera (Echinodermata: Holothuroidea) from Tuticorin, Tamil Nadu, India. 16(3), 231-242. DOI: 10.1007/s10499-007-9140-z

Bardanis, E. & Batjakas, I., 2018). Food preferences of four aspidochirotid holothurians species (Holothuroidea: Echinodermata) inhabiting the Posidonia oceanica meadow of Mostaganem area (Algeria). SPC Beche-de-mer Information Bulletin, 38, 55-59.

Belbachir, N.-E. & Mezali, K. (2018). Reproductive biology of sea cucumbers from Mediterranean Sea grass beds. Marine Ecology. 13(1), 53-62. DOI: 10.1111/1439-0485.12003.99

Conand, C. (1981). Sexual cycle of three commercially important holothurian species (Echinodermata) from the lagoon of New Caledonia. Bulletin of Marine Science, 31(3), 523-543.

Conand, C.J.M.B. (1993). Reproductive biology of the holothurians from the major communities of the New Caledonian Lagoon. Marine Biology, 116(3), 439-450. DOI: 10.1007/BF00350561

Costelloe, J. (1988). Reproductive cycle, development and recruitment of two geographically separated populations of the dendrochirote holothurian Aslia lefevrei (Echinodermata). Marine Biology, 99(4), 535-545.

Despalatović, M., Grubelić, I., Šimunović, A., Antolić, B. & Žuljević, A. (2004). New data about reproduction of the holothurian Holothuria forskali (Echinodermata) living in geographically different places. Fresenius Environmental Bulletin, 12(11), 1345-1347.

Despalatović, M., Grubelić, I., Simunović, A., Antolic, B. & Žuljević, A. (2004). Reproductive biology of the holothurian Holothuria tubulosa (Echinodermata) in the Adriatic Sea. Journal of the Marine Biological Association of the United Kingdom, 84(4), 409-414. DOI: 10.1093/miba/50.4.003.2651

Fajardo-León, M., Suárez-Higuera, M., del Valle-Manríquez, A. & Hernández-López, A. (2008). Biología reproductiva del pepino de mar Parastichopus parvimensis (Echinodermata: Holothuroidea) de Isla Natadidad y Bahá Tortugas, Baja California Sur, México. Ciencias Marinas, 34(2), 165-177. DOI: 10.7773/cmm.34/2.1334

McEuen, F. (1988). Spawning behaviors of northeast Pacific sea cucumbers (Holothuroidea: Echinodermata). Marine Biology, 98(4), 565-585. DOI: 10.1007/BF00391548

MGM. (2018). 1970-2018 Ayına göre Ege Denizi Deniz Suyu Ort. Sıcaklıklar (°C). Retrieved on 10.12.2018, Orman ve Su İşleri Bakanlığı Meteoroloji Genel Müdürlüğü. http://212.174.109.9/FILES/resmi/istatistikler/Ege-DenizSuyu-Sicakliklari.pdf

Navarro, P.G., García-Sanz, S. & Tuya, F. (2012). Reproductive biology of the sea cucumber Holothuria sanctori (Echinodermata: Holothuroidea). Scientia Marina, 76(4), 741-752.

Purcell, S.W. (2016). Ecological roles of exploited sea cucumbers. Oceanography and Marine Biology: An Annual Review, 54, 367-386. DOI: 10.1080/00207291.2015.1034657

Purcell, S.W., Conand, C., Uthicke, S. & Byrne, M. (2016). Artificial reproduction of Holothuria polii: A new candidate for aquaculture. Aquaculture, 498, 444-453. DOI: 10.1016/j.aquaculture.2016.08.069

Ramofola, C., Gervis, M. & Bell, J. (1995). Spawning and early larval rearing of Holothuria atria. South Pacific Commission Bêche-de-mer Information Bulletin, 7, 2-6.

Roberts, J.M. & Ekman, D.J.S. (2012). The reporting of anal cytology and histology samples: establishing terminology and criteria. 9(6), 562-567. DOI: 10.1071/SH10140

Sellem, F., Graja, S. & Brahmi, Z. (2017). Données biologiques et valeur nutritive de la paroi d’Holothuria polii (Delle chiave, 1823) d’Hédé (Holothuroidea aspidochirotida) des îles Kerkennah (golfe de Gabès, Tunisie). Bulletin de l’Institut National des Sciences et Technologies de la Mer de Salammbô. 44, 139-146.

Stilman-Tamach, F., Souaili, D.L. & Mezali, K. (2019). Reproductive biology of Holothuria (Roweothuria) polii (Holothuroidea: Echinodermata) from Oran Bay, Algeria. SPC Beche-de-mer Information Bulletin, 39, 47-53.

Tolon, T. (2017). Efect of salinity on growth and survival of the juvenile sea cucumbers Holothuria tubulosa, Holothuria forskali and Holothuria polii (Delle chiave, 1823) in Mediterranean Sea (Echinodermata: Holothuroidea) within subtropical seagrass beds. Journal of the Marine Biological Association of the United Kingdom, 97(11), 1345-1347.

Turkstat. (2019). Sea Cucumber Trade. Available from Turkish Statistics Institute Foreign Trade Statistics Retrieved on 14.06.2019, https://biruni.tuik.gov.tr/disticaretapp/

Tuvo, A. & Conand, C. (1992). Reproductive biology of the holothurian Holothuria forskali (Echinodermata). Journal of the Marine Biological Association of the United Kingdom, 72(4), 745-758. DOI: 10.1017/S0025315400060021

Valls, A. (2004). Natural spawning observation of Holothuria tubulosa. SPC Beche-de-mer Information Bulletin, 19, 40.

Wolkerhauer, S.-M., Uthicke, S., Burnide, C., Skeves, T. & Pitcher, R. J. J. o. t. M. B. A. o. t. U. K. (2010). The ecological role of Holothuria scabra (Echinodermata: Holothuroidea) within subtropical seagrass beds. Journal of the Marine Biological Association of the United Kingdom, 90(2), 215-223. DOI: 10.1017/S0025315409990518

WoRMS. (2018). Holothuria Linnaeus, 1758. Retrieved from: http://www.marinespecies.org/aphia.php?p=taxdetails&id=123456, (2018)

Zar, J.J.L. (London). (1996). Biostatistical analysis--Prentice--Hall International.