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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

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 hıyarının yumurtlama dönemindeki 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 fishermen but natural stocks 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 gonadal development stages of *H.polii* at the eastern coast of Aegean Sea (Izmir, 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 completely 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*

Öz: *Holothuria polii* türünün de dahil olduğu Akdeniz'de yaşayan deniz hıyarları uzak doğu ülkeleri tüketicilerinin yoğun talebi karşısında uluslararası ticarette önem kazanmışlardır. Son yıllarda deniz hıyarı avcılığı bölgedeki balıkçılar için iyi bir gelir kaynağı olmakla birlikte aşırı avcılık nedeniyle doğal stokları tehlike altına girmiştir. Bu anlamda av düzenlemeleri ve yetiştiricilik çalışmaları doğal stokların korunmasında önemli önlemlerdir. Bu girişimlerin tümü türün bölgeler arasında farklılıklar gösteren üreme biyolojisini temel almaktadır. Bu çalışmada, Ege Denizi'nin doğu kıyılarındaki (İzmir, Türkiye) *H.polii* türü deniz hıyarlarının morfolojik özellikleri, gonadosomatic indeksi ve gonad gelişim evrelerini içeren üreme biyolojisi, Temmuz-Ekim 2018 arasındaki üreme (yumurtlama) döneminde incelenmiştir. Bu kapsamda, ortalama ağırlıkları 120,60 ± 19,56 g olan 60 deniz hıyarının gonad ve gonad kesitleri makroskobik ve histolojik incelemelerle gözlenmiştir. Yumurtlama dönemindeki *H.polii* ergin bireylerinin gonad tübüllerinin histolojik incelemesinde olgun (III), yumurtlama (IV) ve yumurtlama sonrası (V) olmak üzere üç gonad gelişim evresi tanımlanmıştır. Elde edilen sonuçlar her iki cinsiyetteki deniz hıyarı gonadlarının Temmuz ayında olgun ve yumurtlama aşamasında, Ekim ayında ise yumurtlama sonrası aşamada olduğunu ortaya koymaktadır. Türün yumurtlama dönemi başında (Temmuz) ortalama gonad ağırlığı 12,53 ± 1,33 g iken, yumurtlama dönemi sonunda (Ekim) 1,87 ± 0,58 g olarak kaydedilmiştir. Gonadosomatik index ise %17,53 ± 0,02 (Temmuz)'den yumurtlama sonrası aşamada (Ekim) %3,37 ± 0,01'e düşmüştür. Gonadosomatik indeks, gonad ağırlığı ve yumurtlama döneminin deniz suyu sıcaklığı ile doğru yönlü ve önemli bir etkileşim içinde olduğu saptanmıştır. Bu çalışma sonucuna göre, *H.polii* türü deniz hıyarının Ege Denizi'nin doğu kıyılarındaki yumurtlama dönemi Temmuz ayında başlayıp, Ağustos ve Eylül aylarında yoğun olarak devam etmekte ve Ekim ayında tamamen son bulmaktadır. Sonuç olarak türün yumurtlama dönemindeki üreme biyolojisine ilişkin elde edilen veriler hem stok yönetiminde hem de yetiştiricilik çalışmalarında yol gösterici niteliktedir.

Anahtar kelimeler: Akdeniz, deniz hıyarı, üreme biyolojisi, gonad gelişim evreleri, gonadosomatik indeks, *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 ossicles and nematodes accumulated on the seafloor (Belbachir 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; Despalatovic 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).

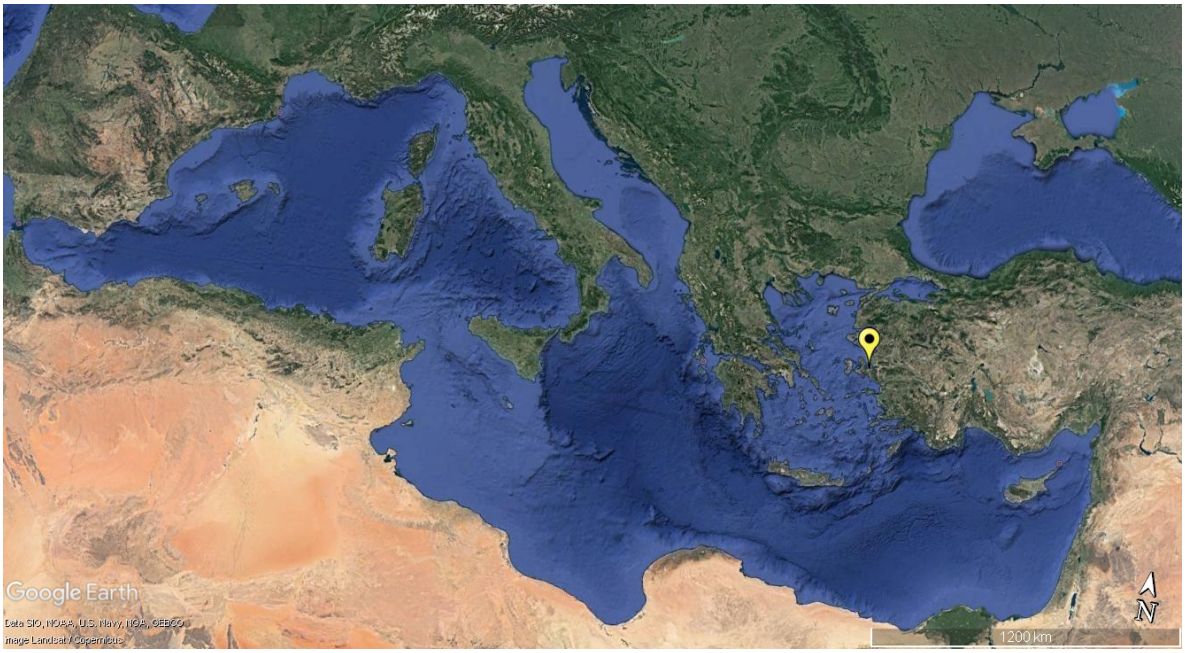

Figure 1. Sampling area in Gulf of Izmir, Aegean Sea (Mediterranean) (38°21'55.42"N; 26°46'8.88"E) (Google Earth, 2019)

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) :

GSI= 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.

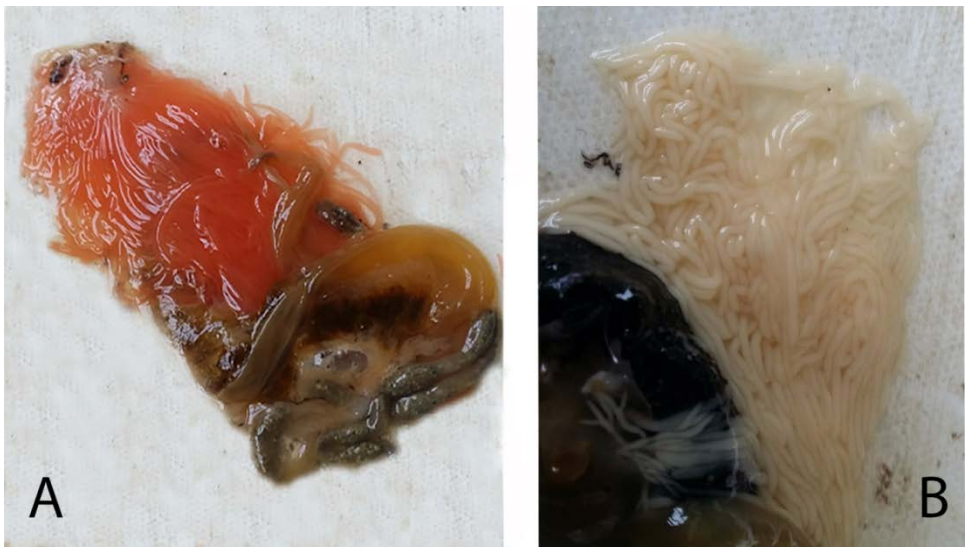

Figure 2. Mature gonads of female (A) and male (B) *H.polii* adults

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 ± 1.83 cm (13.99 ± 1.66 cm male; 14.29 ± 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 ± 19.56 g (120.81 ± 17.30 g male; 120.38 ± 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 ± 12.08 g (67.30 ± 9.85 g male; 61.53 ± 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).

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

	TL (cm)	TW (g)	GBW (g)
Male	13.99 ± 1.66^{a}	120.81 ± 17.30^{b}	67.30 ± 9.85^{ab}
Female	14.29 ± 2.03^{a}	120.38 ± 22.21^{b}	61.53 ± 13.69^{ab}
Mean	14.14 ± 1.83	120.60 ± 19.56	64.41 ± 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)

The mean gonad weight of all samples was 12.53 ± 1.33 g (11.81 ± 2.16 g male gonads; 13.34 ± 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 ± 0.58 g (2.13 ± 0.93 g male gonads; 1.64 ± 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 gonad weights of *H.polii* individuals in July and October in both genders (t-test; p=0.00; p<0.05) (Table 2).

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

Period	Male gonad (g)	Female gonad (g)	Mean gonad weight (g)
July 2018	11.81 ± 2.16^{a}	13.34 ± 1.56^{a}	12.53 ± 1.33^{a}
October 2018	2.13 ± 0.93^{b}	1.64 ±0.76^{b}	1.87 ± 0.58^{b}

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 ± 0.02% at the beginning of the spawning period (15.97 ± 0.03% male; 19.30 ± 0.02% female). It was decreased to 3.37 ± 0.01 % at the end of the spawning period (3.70 ± 0.02% male; 3.08 ± 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).

Table 3. Gonadosomatic index of the *H.polii* during the reproduction period

Period	Male (%)	Female (%)	Mean (%)
July 2018	15.97 ± 0.03^{a}	19.30 ± 0.02^{a}	17.53 ± 0.02^{a}
October 2018	3.70 ± 0.02^{b}	3.08 ± 0.01^{b}	3.37 ± 0.01^{b}

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

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 ± 0.95 ºC), while they decreased significantly in October when the mean seawater temperature dropped to 19.81 ± 1.17 ºC.

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 vitellogenetic 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 observed in the gonads. Tubules were empty, flaccid and wrinkled. Large gaps are visible in the gonads (Figure 4C).

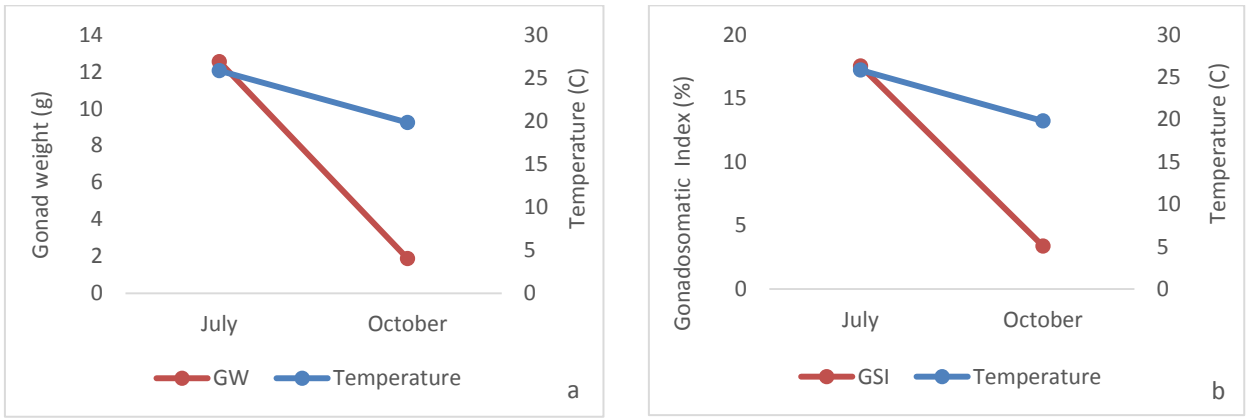

Figure 3. Mean seawater temperature versus (a) gonad weight and (b) gonadosomatic index of *H.polii*

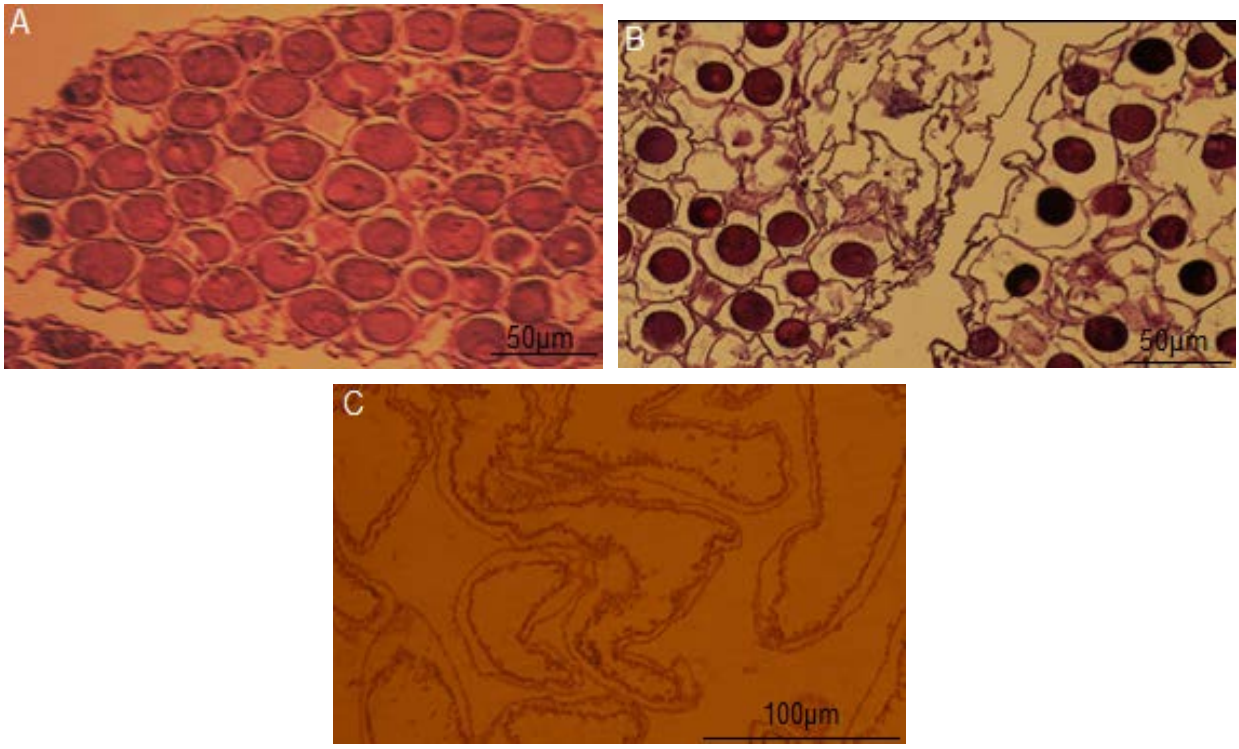

Figure 4. Microscopical characteristics of gonadal stages of the females. (A) mature stage; (B) spawning stage; (C) post-spawning stage. Scale bars: A, B, 50 µm, C, 100 µm.

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).

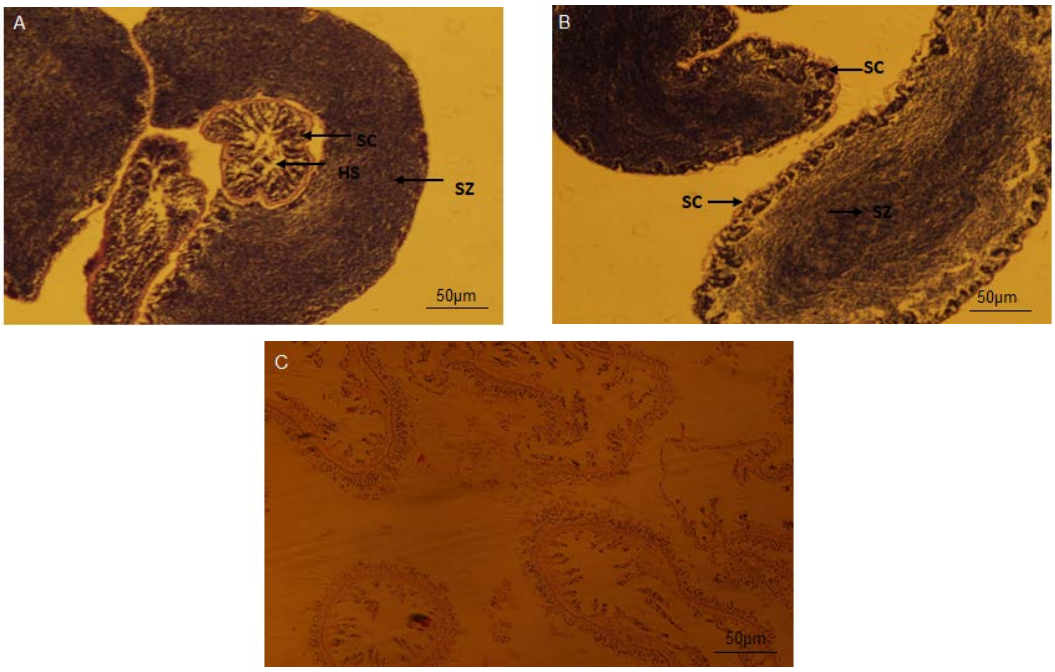

Figure 5. Microscopical characteristics of gonadal stages of the males. (A) Mature stage; (B) spawning stage; (C) Post-spawning stage. HS, haemal sinus; SC, spermatocyte columns; SZ, spermatozoa. Scale bars: 50 µm

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 postspawning 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 Batjakas (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 Batjakas (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.

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