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Reproduction and population structure of the sea cucumber Holothuria tubulosa in the Dardanelles Strait, Turkey

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Abstract

This study investigated *Holothuria tubulosa*, Gmelin 1791 in the Dardanelles Strait from April 2013 to March 2014, in order to outline the morphological characteristics, reproductive patterns and the relationship between population characteristics and environmental parameters. 15 to 30 individuals of this species were sampled monthly at three stations. There was a negative allometry between length and weight, with gutted weight being the most reliable measurement for this species. The reproductive patterns of the species were identified for the first time for examples from the Turkish coast. By macroscopic examination of the gonads, the smallest sizes (gutted length) were measured as 8.4 and 8.1 cm for the female and male, respectively. The sex ratio (female: male) was calculated as 1: 1.1 with differences between seasons. Reproduction of sea cucumbers occurred between August and September after Gonadosomatic Index (GSI) values reached their maximum in July. The species was found at up to a 10 m depth with a population density of 0.21 / m^2, which was rather low compared to previously reported values for Mediterranean populations of this species. There was a high positive correlation between population density and GSI of the species. The highest population density was observed where the largest sea grass meadows are found.

Keywords: *Holothuria tubulosa*, gonadosomatic index (GSI), population structure, Dardanelles Strait.

Introduction

Holothuria tubulosa Gmelin, 1791 is among the most common holothurian species, widely distributed in the Mediterranean Sea and the Atlantic Ocean (Tortonese & Vadon, 1987; Koukouras *et al*., 2007). Besides their commercial value, holothurians are important deposit and filter feeders and play significant roles in recycling nutrients, stimulating microalgae growth, mixing upper sediment layers (MacTavish *et al.,* 2012) and refreshing sea water (Işgören-Emiroğlu & Günay, 2007).

Many studies regarding *H. tubulosa* have been carried out in the Mediterrranean (especially the Adriatic and the western Mediterranean). They have focused on population dynamics (Gustato *et al.,* 1982; Bulteel & Jangoux, 1989; Bulteel *et al.,* 1992; Simunovic & Grubelic, 1998; Simunovic *et al.,* 2000); reproduction (Pladellorens & Subirana, 1975; Bulteel *et al.,* 1992; Despalatovic *et al.,* 2004); feeding ecology (Massin & Jangoux, 1976; Amon & Herndl, 1991; Coulon & Jangoux, 1993; Mezali *et al.,* 2003); and fisheries (Simunovic & Grubelic, 1998). However, findings regarding Aegean Sea populations are limited to the geographical and bathymetric distribution (Koukouras & Sinis, 1981; Koukouras *et al.,* 2007), population density and biometrics (Kazanidis *et al.,* 2010) and the reproduction of this species (Kazanidis *et al.,* 2014). Although a few studies have been carried out related to the population (González-Wangüemert *et al.,* 2014) and fisheries (Aydın, 2008) of *H. tubulosa* on the North Aegean coast of Turkey, there is still little information with regard to life strategies, reproduction and distribution of this species in the Marmara Sea and Dardanelles Strait.

In Turkey, holothurians have been commercially exploited along the coast of Aegean Sea since 1996. The sea cucumber fishery industry in Turkey increased rapidly between 2010 and 2012, reaching a total production of 555 tons in 2012 (González-Wangüemert *et al.,* 2014), mainly exported to Asian countries. Regulations were subsequently implemented for the management of sea cucumber fisheries along Turkish coasts. Closed fishery seasons were established and applied from 1st June – 30th October between 2012 and 2016. Other Turkish coastal areas, with the exception of the South Aegean coasts, were designated as areas closed to sea cucumber fisheries between 2012 and 2016 (Anonymous, 2012; González-Wangüemert *et al.,* 2014).

Since it provides water exchange between the Aegean and the Marmara Sea, the Dardanelles Strait is an important transit point between different habitats for marine organisms (Kanarska & Maderich, 2008). A bidirectional current system, different sea floors and coastal structures are formed along the Dardanelles Strait due to the varying water density of these seas (Beşiktepe *et al*., 1994). The

current state of natural stocks and the effects of environmental parameters on stocks in Dardanelles Strait need to be well documented to provide sufficient information for sustainable sea cucumber fisheries.

The present study was designed to describe the morphological characteristics, characterize the reproductive patterns and examine the relationship between population characteristics of *H. tubulosa* and environmental parameters in the Dardanelles Strait.

Materials and Methods

Study Area

The study area was the Dardanelles Strait, close to the eastern Aegean Sea. The Dardanelles is a long, narrow and shallow strait which has an approximate length of 62 km from Gallipolli to the Aegean Sea exit (Figure 1). The width of the strait varies from 1.2 km to 7 km with an average width of 4 km and an average depth of 55 m (Ünlüata *et al.,* 1990).

The Dardanelles Strait plays an important role in water exchange through the Mediterranean Sea and Black Sea systems (Kanarska & Maderich, 2008). It has a bidirectional current system between the Sea of Marmara and the Aegean Sea (Beşiktepe *et al.,* 1994) which influences water temperature and salinity in the Dardanelles Strait. In addition, the water undercurrent entering from the Aegean Sea strikes the Nara Cape (the narrowest point of the Strait) and 40% of this water rises and mixes with the surface current from the Marmara Sea and returns to the Aegean Sea. This movement increases the salinity level of water between the Nara Cape and the Aegean Sea (Ünlüata *et al.,* 1990; Polat & Tuğrul, 1996; Cociasu *et al*., 1997; Türkoğlu *et al.,* 2004).

Sampling was carried out at three stations in the Dardanelles Strait , all of which have a sandy bottom structure, and two which have *Zostera marina* and *Posidonia oceanica* meadows (Stations 2 and 3) (Ateş *et al.*, 2014). Station 1, Gelibolu, 40.367778° N, 26.6325° E is close to the Marmara Sea; Station 2, Umurbey, 40.2525° N, 26.548056° E is in the central region of the Dardanelles Straits; Station 3, Dardanos 40.073333° N, 26.352778° E is near the Aegean Sea (Fig. 1).

Field Sampling

Samples were collected monthly by SCUBA divers at a depth of up to 30 m from April 2013 to March 2014. Population density was estimated by the use of quadrat plots, where each quadrat consisted of a metal frame covering an area of 1 m^2 (1 m x 1m). Overall, 60 quadrats were used at each sampling and the individuals present in each quadrat were identified and counted (Mezali *et al.,* 2006). *Holothuria polii* was also present in the sampling stations during the underwater observation. The population density of *Holothuria tubulosa* was ultimately expressed as individuals per square m.

Between 15 and 30 specimens were randomly collected by hand at each station. Total length (TL_s) was measured *in situ* using a tapeline (0.1 cm precision), taking care to avoid body contraction, and specimens were placed into individually labeled plastic bags (Despalatovic *et al.,* 2004; Kazanidis *et al.,* 2010). After sampling, the total weight (TW_s) was measured on the boat with a digital scale (0.1 g precision). Samples were then transferred to the laboratory. Sampling could not be performed in December 2013 since no individual was present at the Station 1 and 2. At each sampling, the dissolved oxygen, pH and temperature of the surface water were measured with a multiparameter probe (WTW Multi 3420 Model).

The total weight (TW), gutted weight (GW) and gonad weight (G_oW) of each individual was obtained with electronic scales (0.01 g precision) in the laboratory (Conand, 1981; Kazanidis *et al.,* 2010). The gutted length (GL) of each individual was also recorded with a tapeline (0.1 cm precision). The removed gonads were macroscopically examined to classify individuals according to sex (Despalatovic *et al.,* 2004). In order to determine the weight loss before commercial processing, the boiled-dried weight (BDW) was measured after boiling the samples for 20 minutes and drying them at room temperature (Çakli *et al.,* 2004) in all individuals obtained from the first three months' sampling.

The GSI (%) was calculated for females and males as the gonadal weight divided by the total weight multiplied by one hundred (Hoareau & Conand, 2001; Guzmán *et al.,* 2003; Kazanidis *et al.,* 2010).

Data Analysis

The homogeneity of variances was tested (the Kolmogorov-Smirnov and Levene's test) and, whenever necessary, the log-transformation log (x + 1) was used. One way analysis of variance (ANOVA) was used at the 5% significance level to determine differences among the mean results from the different stations. The Tukey-HSD test was applied when it was significant. A chi-square test was used to assess if the sex ratio differed between stations and seasons. A regression analysis was used to examine morphometric relations (length and weight) in situ, on the surface and in the laboratory. The relations between physical parameters and GSI and population density were determined using the Pearson Correlation Analysis (Zar, 1996). All statistical analyses were carried out using Microsoft Excel and the IBM SPSS 21 Software package (SPSS® Inc., Chicago IL, USA).

Results

Physicochemical parameters of sea water

The mean physical parameters of sea water from the three sampling sites in Dardanelles Strait are summarized in Table 1.

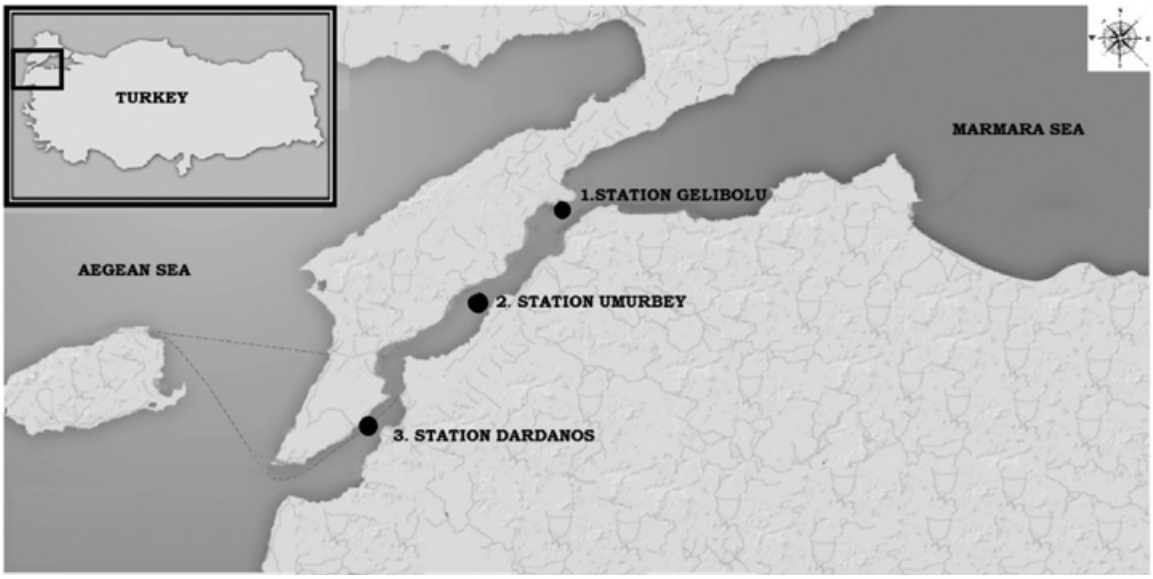

Fig. 1: Study area (Dardanelles Strait) and sampling stations: 1. Gelibolu, 2. Umurbey and 3. Dardanos.

In terms of temperature, pH and dissolved oxygen, there were no differences between stations. As regard to salinity, Station 3 had higher values than Stations 1 and 2. The differences between the mean salinity in the stations were statistically significant ($p < 0.05$) (Table 1).

Morphometric relations and biometric characters

Overall, 598 specimens of *H. tubulosa* were collected in the Dardanelles Strait. Gutted weight (GW) values ranged from 6.1 to 127 (49.9 ± 0.81) g and gutted length (GL) values ranged from 5.6 to 30.3 (15.5 ± 0.16) cm, respectively. Mean values of GL and GW for *H. tubulosa* increased sequentially between the stations in the order 1 < 2 < 3. The differences between the mean GL and GW in the different stations were statistically significant ($p < 0.05$) (Table 2).

The mean GW of the species increased between June and August, then decreased until January, then rose again until March, when the totality of the samples was evaluated. Station 3 displayed the highest values, whereas Station 1 showed the lowest in all sampled periods except for January (Fig. 2).

The GL of *H. tubulosa* individuals showed multimodal size-frequency distribution in all stations, ranging from 6 cm to 21 cm at Station 1, from 9 cm to 24 cm at Station 2 and from 10 cm to 30 cm at Station 3. For pooled GL, the highest value was found in the 16 cm size group. The dominant size groups were determined as 13 cm (Station 1), 16 cm (Station 2) and 17 cm (Station 3) (Fig. 3).

A negative allometry (b: 1.0617 – 1.67) was obtained for all length – weight relations (Table 3). Correlations between total length (TL_s) and total weight (TW_s, TW) ranged between 0.24 and 0.61. Highest correlations (0.70; 0.72 and 0.82) were obtained between gutted weights (GW) and gutted lengths (GL).

Sex ratio and spawning period

By means of macroscopic observation, the gonads of 598 *H. tubulosa* were sexed as 146 male (minimum GL: 8.1 cm), 133 female (minimum GL: 8.4 cm) and 317 unidentified, giving a sex ratio of 1 : 1.1 respectively. The differences in the sex ratios between stations were not significant ($\chi^2 = 0.80$; df = 2; $p > 0.05$) while they were significantly different across the seasons (spring, summer, autumn, winter) ($\chi^2 = 9.63$; df = 3; $p < 0.05$).

For all stations, individuals with an unidentified sex were dominant during the cold periods (October-April).

Table 1. The comparison of physical parameters at stations in the Dardanelles Strait (mean ± standard deviation).

Parameters	Stations		
	1	2	3
Temperature (°C)	17.63±1.85	17.79±1.76	17.92±1.38
Salinity (‰)*	24.10±0.43	24.68±0.51	30.33±0.49
pH	8.44±0.03	8.49±0.04	8.41±0.03
Oxygen (mg/l)	10.06±0.52	11.01±0.55	10.04±0.40

(*Station 3 is significantly different ($p < 0.05$) from the others)

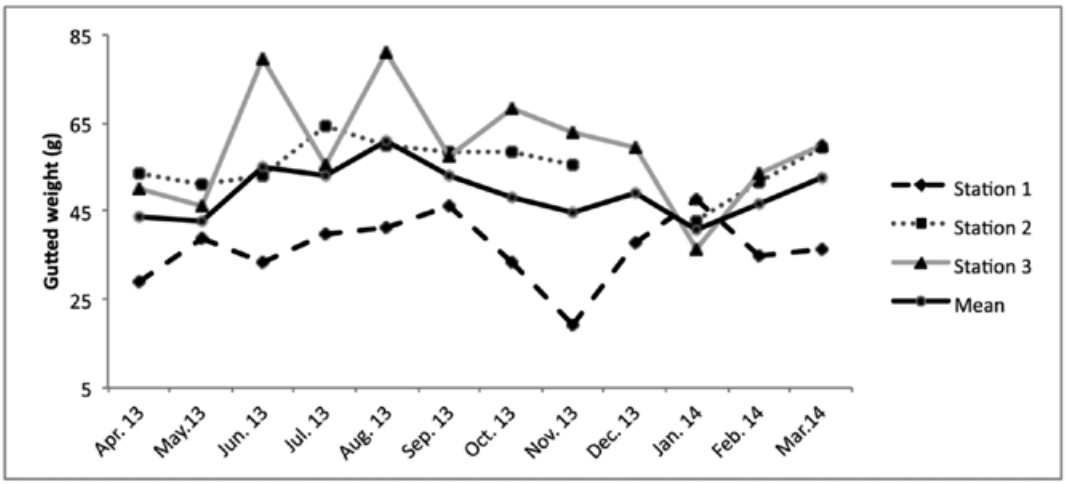

Fig. 2: Monthly changes in mean gutted weight of *H. tubulosa* in the Dardanelles Strait.

Table 2. Comparison of the population parameters of *H. tubulosa* in the Dardanelles Strait (mean ± standard deviation). (GSI_F: Gonadosomatic index of female; GW: Gutted weight; GL: Gutted length; PD: Population density; n: number of individuals).

Parameters	Stations		
	1	2	3
GL (cm)*	12.8 ± 2.93	16.2 ± 0.24	17.7 ± 0.25
GW (g)**	36.0 ± 0.97	55.9 ± 1.17	58.9 ± 1.38
GSI_F**	11.68 ± 1.64	22.02 ± 3.51	20.42 ± 2.29
PD (n/m^2)*	0.17 ± 0.025	0.06 ± 0.015	0.04 ± 0.068

(*Significant difference ($p < 0.05$) shown between all stations; **Significant difference ($p < 0.05$) shown between Stations 1 and 3).

In the warmer months (May-September), female (F) individuals were dominant in August while males (M) were dominant in September. The sex ratio (F:M) varied between 0.7 and 3.6 and reached the maximum value in September when the values increased significantly at all stations (Station 1: 1.5; Station 2: 3.0; Station 3: 6.0). The GSI of females and males increased from May to July and decreased from August to November. Only one annual spawning peak was observed. This occurred between August and September after maximum GSI values were reached in July. Surface water temperature increased from April and reached the highest value (24.4 °C) in September. Similar to the temperature trend, GSI values decreased from August until January (9.5 °C) and then rose again until March (Figure 4).

Population density

Although the surveys covered a depth of up to 30 m in the Dardanelles Strait, the species was not found deeper than 10 m. The population density (PD) of *H. tubulosa* in the sampling stations in the Dardanelles Strait was estimated as 0.21 individuals / m^2. The mean values of PD at stations followed the sequence 2 < 1 < 3, which was significant ($p < 0.05$) (Table 2). The peak in population density was estimated to occur in July and the lowest value was estimated to be in December at all three stations. No individuals were found in Station 1 and 2 in December. Also, an increase between April and July and a decrease between August and September (especially at Station 3) were observed at all three stations (Fig. 5).

Effects of physical parameters of sea water on H. tubulosa

Temperature had a positive relation to GSI_F and GW, as shown with the Pearson Correlation Analysis. Dissolved oxygen had a negative relation to GW and a strong negative relation to GSI_F. A strong negative relation was determined between pH and PD (Table 4). As seen in Table 4, GSI_F had a strong positive relation to PD. In addition, a positive relation was identified between GSI_F and GW. A strong positive relation was also detected between GW and GL.

Discussion

In this study, gutted weight was the most consistent biometric feature for the estimation of population dynamics of sea cucumbers, as also declared by previous studies (Conand, 1981; Bulteel *et al.,* 1992; Tuwo & Conand, 1992; Despalatovic *et al.,* 2004; Kazanidis *et al.,* 2010; Gonzalez-Wangüemert *et al.,* 2014). This

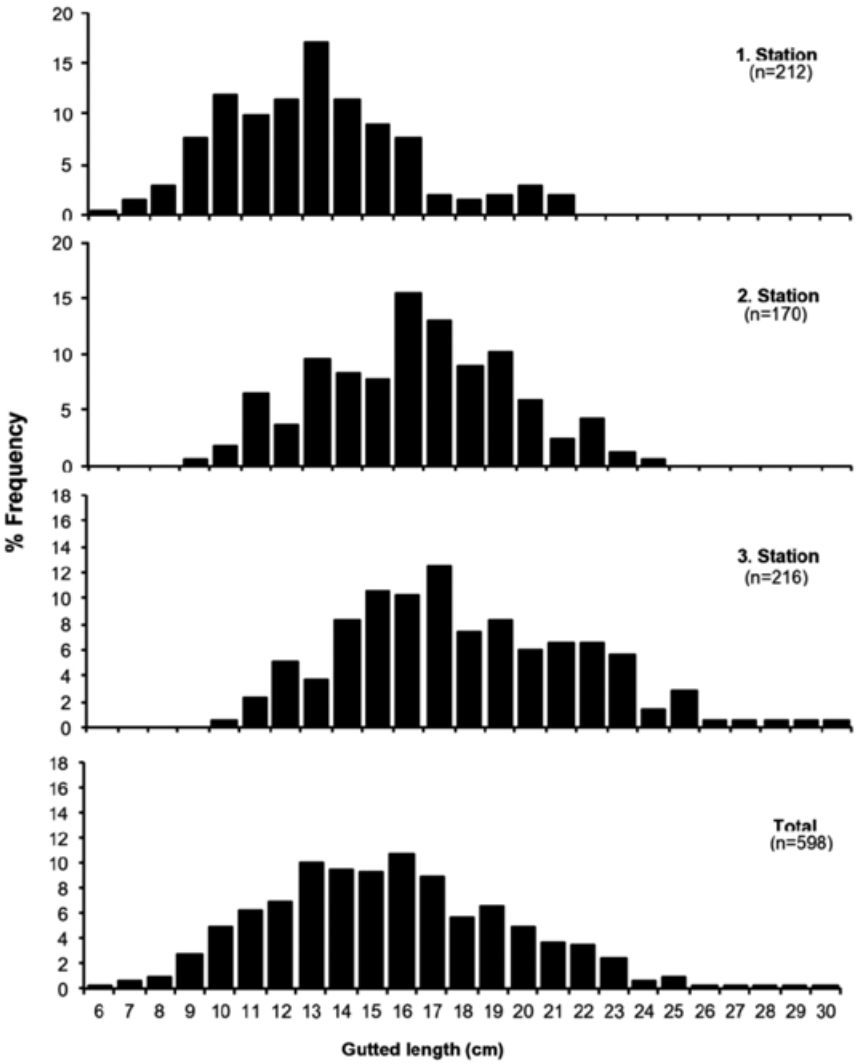

Fig. 3: Gutted length frequency of *H.tubulosa* in the Dardanelles Strait.

is mainly because sea cucumbers empty their coelomic fluid and strain under stressful conditions, thus the total lengths and total weights of the animals differ significantly during measurements under water and on the surface. Moreover, the best correlations ($R^2 = 0.70$; 0.72 and 0.82) determined between gutted weight and gutted length support the above argument.

The pooled mean gutted weight (GW) of *H. tubulosa* in the Dardanelles Strait was measured as 49.9 ± 0.81 g which was considerably lower than in other studies. For instance, Gonzalez-Wangüemert *et al.* (2014) reported a GW of 88.78 ± 30.93 g on the Turkish coast of the North Aegean Sea (where a sea cucumber fishery was in operation between 2007 and 2012), Kazanidis *et al.* (2010) reported 108.46 ± 35.10 g on the Greek coast of the North Aegean Sea (where sea cucumber fishing is not yet developed) and Vafeiadou *et al*. (2010) reported 61.89 ± 23.25 g in the South Aegean Sea (where *H. tubulosa* has been over-exploited). These differences could be explained by local environmental conditions (e.g. higher food availability in some areas) and/or different pres-

Table 3. Morphometric relations (length, weight) of *H. tubulosa* (TL_s: Total length (sea); GL: Gutted length; TW_s: Total weight (sea); BDW: Boiled-dried weight; GW: Gutted weight; TW: Total weight).

Relation	n	Formula	r^2
Length - Length Relations			
TL_s – GL	532	$GL = 0.4366 \times TL_s + 7.7193$	0.33
Weight - Weight Relations			
TW_s - BDW	226	$BDW = 0.0194 \times TW_s + 2.0111$	0.24
GW - BDW	226	$BDW = 0.1334 \times GW - 0.8991$	0.72
TW_s - TW	532	$TW = 0.6206 \times TW_s - 0.6111$	0.61
TW_s - GW	598	$GW = 0.1942 \times TW_s + 15.944$	0.50
TW - GW	532	$GW = 0.3239 \times TW + 15.363$	0.82
Length - Weight Relations			
TL_s – TW_s	532	$TW_s = 7.6684 \times TL_s^{1.0617}$	0.52
TL_s - GW	532	$GW = 2.0645 \times TL_s^{1.0839}$	0.53
GL - GW	598	$GW = 0.9249 \times GL^{1.4393}$	0.70
GL – TW_s	598	$TW_s = 7.2196 \times GL^{1.1422}$	0.43
GL - TW	532	$TW = 1.0089 \times GL^{1.67}$	0.67
TL_s - TW	532	$TW = 2.3659 \times TL_s^{1.288}$	0.52

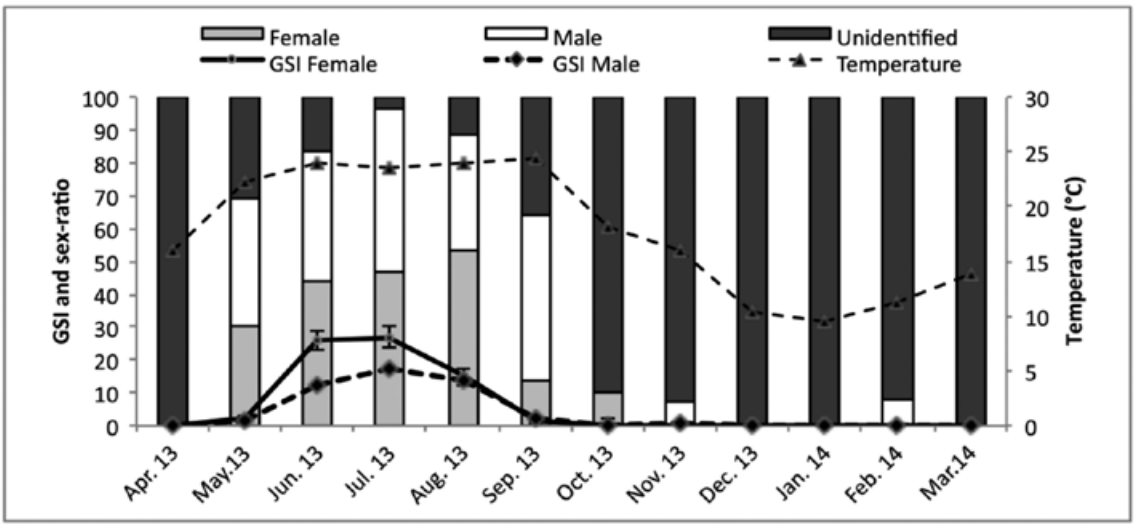

Fig. 4: Monthly changes of temperature and gonadosomatic index (GSI) for female and male *H. tubulosa*. The proportions of male and females are included for each month.

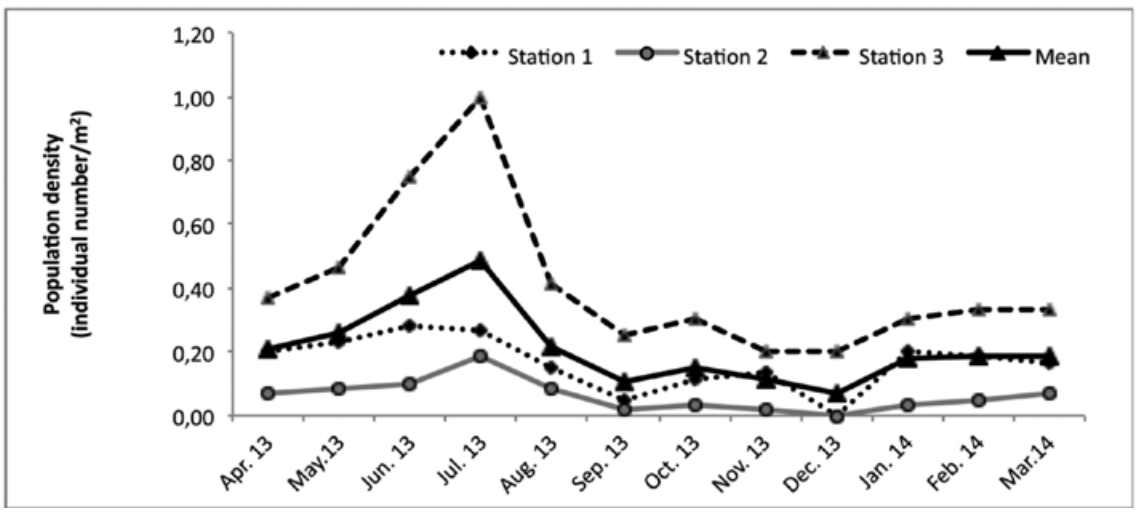

Fig. 5: Population density (number of individuals / m^2) of *H. tubulosa* in the Dardanelles Strait.

sures on fisheries as explained by Gonzalez-Wangüemert *et al.* (2014).

The mean GW of *H. tubulosa* at stations followed the sequence 1 < 2 < 3, and the differences between the GW in stations were significant ($p < 0.05$). The existence of significant differences in weight among localities could be due to food availability. It has been reported that there are differences in mean gutted weight of sea cucumber populations along the Turkish coast of the Aegean Sea (Gonzalez-Wangüemert *et al.,* 2014), and the *H. tubulosa* population displays superior growth performance in areas containing high organic inputs (Vafeiadou *et al.,* 2010).

The mean values of GW of the species in the Dardanelles Strait increased in summer, then decreased throughout winter and then rose again in spring. The weight loss of *H. tubulosa* from summer to autumn and subsequent weight gain until summer in Greek coasts has been reported by Kazanidis *et al.* (2010). The seasonal changes in GW in the present work could be related to feeding and reproduction activities in the life cycle of the species. Bulteel *et al.* (1992) also observed that small individuals are distributed in shallow waters whilst the larger individuals migrate to deeper zones during the grow-out period. However, large-sized individuals migrate to shallow waters (above the thermocline 15 to 20 m) when the water temperature increases during the summer period (Bulteel *et al.,* 1992; Mezali *et al.,* 2006). Ocaña & Sanchez-Tocino (2005) have also reported that spawning of the species was observed in the Alboran Sea when the water temperature ranged from 24 to 25 ºC in August.

A correlation was observed between the GSI of *H. tubulosa* and the water temperature in the Dardanelles Strait, which confirms previous findings that temperature

Table 4. Correlations between physical parameters of stations and population parameters of *H. tubulosa* (T: Temperature; S: Salinity; O: Dissolved oxygen; GSI_F: Gonadosomatic index of female; GW: Gutted weight; GL: Gutted length; PD: Population density).

	T	S	O	pH	GSI_F	GW	GL
S	**-0.976*** **						
O	**-0.836*** **	**0.790** **					
pH	-0.344	0.239	0.441				
GSI_F	**0.648***	-0.538	**-0.704** **	**-0.647***			
GW	**0.582***	-0.501	**-0.586***	-0.214	**0.624***		
GL	0.527	-0.456	-0.531	-0.017	0.537	**0.927*** **	
PD	0.525	-0.418	-0.496	**-0.748** **	**0.865*** **	0.280	0.180

is the most significant factor affecting the reproduction of the *H. tubulosa* (Despalatovic *et al.,* 2004; Kazanidis *et al.,* 2010) and other congeneric species (Conand, 1981; Tuwo & Conand, 1992; Despalatovic *et al.,* 2003). For this reason, the GSI parameter was used for the description of the reproductive cycle of *H. tubulosa* in the Dardanelles Strait as suggested by Kazanidis *et al.* (2010). Our study demonstrated one annual spawning peak between August and September after maximum GSI values were reached in July. This period was supported for the Aegean Sea (Kazanidis *et al*., 2010, 2014) and Mediterranean populations of sea cucumbers (Bulteel *et al*., 1992; Despalatovic *et al*., 2004) by GSI measurements. Field observations also confirmed that spawning occurs in shallow waters in the summer months in Mediterranean populations (Valls, 2004; Moosleitner, 2006). In this study, a very strong correlation was also determined between GSI_F and population density (PD). PD increased in parallel to the increase of GSI_F during the May and July in all stations.

The female-male ratio of *H. tubulosa* in the Dardanelles Strait was calculated as 1:1.1 with some difference across the seasons. This was slightly different from other studies in which an equal ratio (1:1) (Despalatovic *et al*., 2004; Kazanidis *et al.,* 2010, 2014) or a predominance of females (Simunovic & Grubelic, 1998) were reported. Male individuals were dominant especially in September at all stations (Station 1: 1.5; Station 2: 3.0; Station 3: 6.0) while females were dominant in August only. The sexual distribution of this species as observed in the present study could be related to reproduction characteristics but this hypothesis needs to be tested by further studies.

The population density of *H. tubulosa* was estimated as 0.21 individuals / m^2 down to 10 m depth, although the surveys were carried out to 30 m depth in the Dardanelles Strait. This was higher than at the Greek coasts of the North Aegean Sea, where a study conducted in the Pagasitikos Gulf, in a sparse *Z. marina* meadow interspersed with silt sediments (Kazanidis *et al.,* 2010), found a density of 0.1 individuals / m^2. The population density of *H. tubulosa* varies according to the habitat type and depth (Simunovic *et al*., 2000; Kazanidis *et al.,* 2010). Coulon & Jangoux (1993) reported 3.77 individuals / m^2 in the Gulf of Naples in a dense *P. oceanica* meadow, and this decreased with increasing depth, achieving only 0.34 individuals / m^2 at a depth of 30 m where the meadow was sparse. Bulteel *et al*. (1992) have reported that this species grows more efficiently in areas of low water turbulence where *Posidonia* shoots are scattered and detrital food is more easily accessible. In nearshore waters off the island of Elba, Italy, this species was found to be less abundant at 12-13 m than at either 6-7 m or 23-24 m, and this difference was attributed to the higher water current (Reinthaler & Scheiblauer, 1998). Thus, the low population density of the species in the Dardanelles Strait can be related to intense water turbulence arising from surface and deep currents (Beşiktepe *et al.,* 1994).

In this study, the differences between the mean PD among the stations were found to be significant ($p < 0.05$). These differences are thought to result from the presence or absence of seagrass meadows, and from differences in food abundance and salinity. *Zostera marina* and *Posidonia oceanica* meadows were observed at Stations 2 and 3 in this study, as was also reported by Tuncer *et al.* (2007) and Ateş *et al.* (2014). Organic inputs were reported at Station 3 by Ateş *et al.* (2014). That the highest GW values (except in January) were recorded at this station also supports this idea. In addition, the salinity values at Station 3 were higher compared to other stations, as was reported by Polat & Tuğrul (1996), Cociasu *et al.* (1997) and Türkoğlu *et al*. (2004).

In conclusion, continuous monitoring of *H. tubulosa* populations is necessary due to increased fishing pressure (Kazanidis *et al.,* 2010), since the processed products of the species have high profit margins in the Asian market (Simunovic & Grubelic, 1998; Çakli *et al*., 2004; Aydin, 2008; Vafidis *et al.,* 2008; Gonzalez-Wangüemert *et al.,* 2014). In this investigation, some essential parameters of the population dynamics of *H. tubulosa* were estimated and assessed and the spawning period of the species was determined for the first time along Turkish coastal areas. However, the spawning time of species should be confirmed with comprehensive histological studies, as pointed out by Kazanidis *et al.* (2014). Further studies are also required in this and other regions of the Mediterranean Sea in order to obtain more detailed data especially regarding the life-history (e.g., recruitment, growth, mortality and longevity) of *H. tubulosa*.

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