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Population dynamics and reproduction of *Holothuria tubulosa* (Holothuroidea: Echinodermata) in the Aegean Sea

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The Atlanto-Mediterranean holothurian Holothuria tubulosa *is among the conspicuous benthic invertebrates in the shallow sublittoral zone. It is an edible species, harvested at the Aegean Sea and utilized as fishing bait. Considering the lack of information for the Aegean populations, a one-year survey, based on monthly or semimonthly samples, was carried out focusing on population structure, allometric relationships and reproductive status of* H. tubulosa *stocks in Pagasitikos Gulf. Population density varied around 9.93 individuals/100 m². This value was rather low compared with other studied Mediterranean populations of the species, possibly due to the scarcity of seagrass meadows in the area studied. All measured biometric characters showed high plasticity, and all the examined morphometric relationships followed negative allometry indicating a change to the shape of the animal's body as it grows. Holothurians' length was a moderate predictor of biomass since r values reached 60%; in contrast a very strong relation was observed between drained and gutted weight. Size–frequency distribution analysis was unimodal with the exception of spring where a second mode of larger sized individuals appeared. The gonadosomatic index showed a single spawning season per year, in late summer; accordingly the reproductive cycle of the species showed a clear annual pattern which was highly correlated with the seasonal variations of temperature.*

Keywords: population structure, allometry, reproduction, sea-cucumber, eastern Mediterranean, Aegean Sea

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INTRODUCTION

Holothuria tubulosa Gmelin, 1788 is among the most common holothurian species (phylum Echinodermata, class Holothuroidea, order Aspidochirotida, family Holothuriidae) widely distributed in the Mediterranean Sea and the Atlantic Ocean (Tortonese & Vadon, 1987; Koukouras *et al.*, 2007). The species lives on various bottom types from the surface waters down to 100 m depth commonly forming dense populations in the shallow sublittoral seagrass meadows (Massin & Jangoux, 1976; Tortonese & Vadon, 1987; Bulteel *et al.*, 1992; Simunovic & Grubelic, 1998). *Holothuria tubulosa* is an epibenthic deposit feeder selectively ingesting superficial sediment and feeds on non-living detritus and associated microorganisms (Massin & Jangoux, 1976; Amon & Herndl, 1991). Thus, the species contributes to the recycling of bottom detritus (Bulteel *et al.*, 1992; Coulon & Jangoux, 1993) having an important ecological role as it has been also reported for various congeneric holothurians (Uthicke 1999, 2001a, b).

Holothuria tubulosa is exploited in the Mediterranean either as fishing bait or as a food source; various countries export the species to Japan, where it is consumed as a gastronomic delicacy (Tortonese & Vadon, 1987; Simunovic & Grubelic, 1998). On the other hand, the species is also studied for its bioactivity with promising results for the treatment of inflammatory disorders (Herencia *et al.*, 1998). In the Aegean Sea *H. tubulosa* is commercially exploited in some Turkish areas (Cakli *et al.*, 2004; Aydin, 2008), in contrast to Greece, where the species is not included in the explored benthic invertebrates (Chintiroglou *et al.*, 2005). Still divers harvest it by hand, or using hooks, while it is also randomly collected by trawls as by-catch from deeper waters. The species is processed and used as bait in long-line fisheries; accordingly a severe depression of populations has been reported mostly in the southern part of the Aegean (Vafidis *et al.*, 2008).

Several aspects of the biology of this species have been studied, such as 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; Aydin, 2008). However, all the above information has been derived from the Adriatic and the western Mediterranean *H. tubulosa* populations; relevant data for the Aegean populations are missing and the existing information is limited to the geographical and bathymetric distribution of the species (Koukouras & Sinis, 1981; Koukouras *et al.*, 2007).

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Taking into account all the above, the present work aims at studying the population dynamics of *H. tubulosa* in the Aegean Sea, focusing on the assessment of population structure, allometric relationships and reproductive status of its natural stocks.

MATERIALS AND METHODS

Study area

The study area was Pagasitikos Gulf located in the western Aegean Sea, north of Evia Island. It is a semi-enclosed shallow water basin (mean depth is 69 m) connected with the western Aegean Sea and the North Evoikos through the narrow (5.5 km) and relatively deep (80 m) Trikeri Channel (Petihakis *et al.*, 2005). No major rivers discharge in the wider area; still nutrients produced by intense agriculture enter the system through a network of small torrents, while urbanization and industrialization has strongly affected the area, mostly in the northern part of the Gulf, where the city of Volos is located (Petihakis *et al.*, 2005). The basic physical, chemical and biological parameters of the water column have been studied since 1975. Water masses are cold and homogeneous in winter (12.5°C) and highly stratified in the warm season of the year (27.4°C) following the seasonal pattern of atmospheric warming at this temperate region. Salinity exhibits some fluctuations (32–38 psu) with values increasing with depth due to the inflow of low salinity surface waters from the Aegean Sea (Triantafyllou *et al.*, 2001). Unlike other semi-enclosed gulfs, and besides the significant nutrient inputs, Pagasitikos Gulf has been characterized as meso-oligotrophic undergoing periods of P or N limitation (Petihakis *et al.*, 2005).

Field sampling—processing of samples

Preliminary surveys along the coastline of Pagasitikos Gulf, revealed the occurrence of the species *Holothuria tubulosa* at Kato Gatzea in depths from 3 to 8 m; accordingly one sampling station was set (N39°18′457″E 23°05′869″). The sea bottom consists of soft substratum with a sparse meadow of the seagrass *Zostera marina*, up to a maximum depth of 10 m. The sediment is composed of sand fractions and silt. Samples were collected from June 2007 to July 2008, on monthly or semimonthly basis, by scientists using SCUBA diving, in depths up to 10 m. In order to estimate the abundance of *H. tubulosa* transect methods (5 randomly replicated belt transects of 2 × 10 m each) were employed (Uthicke, 1997); from these data the population density was assessed as the number of individuals per 100 m². Twenty to twenty-five specimens larger than 3 cm were randomly collected by hand, measured in the field for total length (Lt) using a tapeline (0.1 cm precision), with care to avoid body contraction, and then put into individual plastic bags (Despalatovic *et al.*, 2004). Surface seawater temperatures were recorded at the study site at each sampling.

All the collected *H. tubulosa* individuals were transferred to the laboratory, relaxed and dissected. The total drained weight (W) following the opening of the body and the removal of coelomic fluid, the gonad weight (Wg), and the gutted weight (Wgt) following removal of gonads, alimentary canal and respiratory trees (Conand, 1981) were recorded with an electronic scale to the nearest mg. The length of the uncoiled digestive tract (Ld) and the width of the peripharyngeal calcareous crown (Pc) (Bulteel *et al.*, 1992) were also determined for each holothurian. These measurements were taken with a digital Vernier caliper to the nearest 0.1 mm.

The removed gonads were macroscopically examined in order to classify the *H. tubulosa* individuals to sexes and then fixed in 9% formaldehyde (Despalatovic *et al.*, 2004).

Table 1. ANOVA results for the effects of time on the biometric variables and the gonadosomatic index (GI) of *Holothuria tubulosa*. Significant differences in bold.

ANOVA results for temporal effects (df = 14)						
	Mean ± SD	Min	Max	MS	F	*P*
Lt (cm)	29.80 ± 6.34	13.80	49.40	170.607	4.99	**<0.01**
W (g)	218.33 ± 84.79	69.50	535.47	44720.4	8.23	**<0.01**
Wgt (g)	108.46 ± 35.10	39.48	226.38	5328.48	5.12	**<0.01**
Ld (cm)	82.70 ± 19.93	32.00	147.00	1724.34	5.14	**<0.01**
Pc (mm)	52.30 ± 6.02	36.00	81.00	179.681	6.08	**<0.01**
GS	15.20 ± 21.36	3.00	91.19	2234.46	16.43	**<0.01**

SD, standard deviation calculated across all *H. tubulosa* specimens; Min, minimum; Max, maximum; Lt, total length; W, drained weight; Wgt, gutted weight; Ld, length of the uncoiled digestive tract; Pc, width of the peripharyngeal calcareous crown.

Data analysis

The null hypotheses of no significant differences among temporal samplings in the mean values of the estimated biometric characters (i.e. Lt, Ld, Pc, W, Wgt and Wg) of the holothurian were tested with one-way ANOVA. Prior to the analyses, the homogeneity of variance was tested by Cohran's test and, when necessary, data were log-transformed. The Fisher's LSD test was used for *post-hoc* comparisons.

Size–frequency distributions were estimated seasonally, on the basis of gutted weight, in order to study the population structure (Conand, 1981; Bulteel *et al.*, 1992). The distributions were calculated per 20 g size-classes, and the modal was identified applying the Battacharya and the NORMSEP analysis using the FISAT software package (Gayanilo & Pauly, 1997).

Morphometric relationships were estimated using power function ($Y = aX^b$ which is solved as $\text{Log}Y = \text{log}a + b\text{Log}X$) applying a linear regression analysis (Zar, 1984). Weight measurements were used to estimate the gonadosomatic index (GS = Wg/Wgt) expressed as the ratio of gonad weight to gutted weight (Shiell & Uthicke, 2006), in order to study the reproductive status of *H. tubulosa*. GS was also tested for temporal differences with ANOVA. A Chi-square test was applied to determine whether sex-ratio deviates from 1:1.

RESULTS

Holothuria tubulosa individuals were found in shallow waters (6–8 m depth) and mostly in unvegetated sediments. Its population density ranged from 5 (September 2007) to 20 (July 2007) individuals /100 m² with an overall mean of 9.93 ± 3.28 individuals/100 m².

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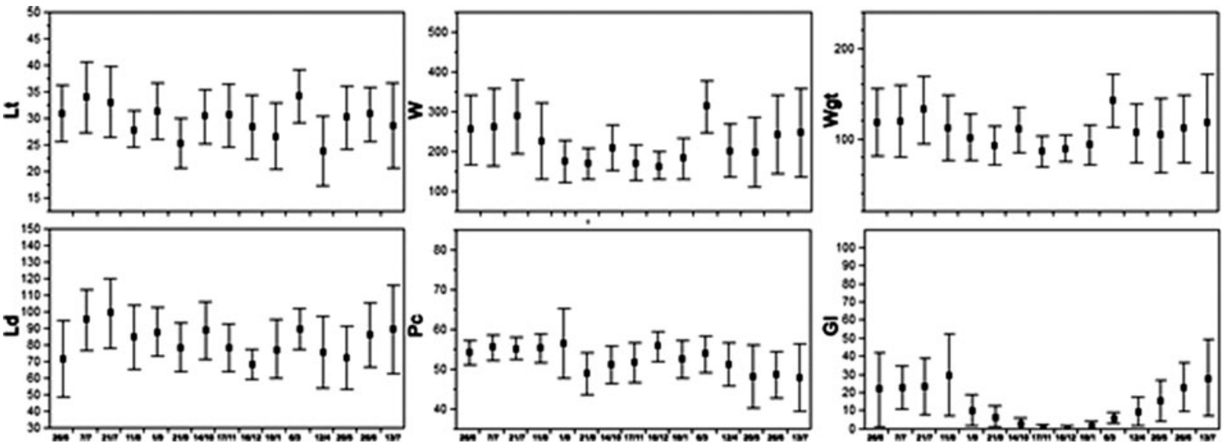

Fig. 1. Temporal variability of the measured biometric characters and the gonadosomatic index (GI) of *Holothuria tubulosa* population in Pagasitikos Gulf.

Overall, 314 specimens of *H. tubulosa* were collected and measured. All the estimated biometric characters showed significant temporal variations (ANOVA results; Table 1). These variations produced a rather complicated pattern (Figure 1) revealing a high morphological plasticity of *H. tubulosa* population. In general, holothurians were larger in size in the warm season of the year, at least considering W and Wgt.

Length/length, weight/length, width/length and weight/weight relationships followed negative allometry considering the drained and the gutted weight, the length of the body and of the digestive tract, as well as the width of the peripharyngeal crown (Table 2). A strong relationship was established only between gutted weight/drained weight. In most relationships the correlation coefficient had moderate values varying around 0.6.

Gutted weight–frequency distribution analysis was unimodal in autumn and winter consisting of medium-sized individuals (Figure 2). In both summers one main mode and a minor second one were detected at 120g and 210g, respectively; in spring multimodality appeared clearly as the *H. tubulosa* population was constituted by two size-groups at 100g and 175g (Figure 2).

Holothuria tubulosa is a gonochoristic species. In total, 26.75% of the collected holothurians were males, 26.11% females and 47.13% had undifferentiated gonads; thus the sex of almost half of the specimens remained undetermined. In the cold season of the year, from November up to April, all *H. tubulosa* individuals had undifferentiated gonads, while in the warm season the portion of female and males was almost equal. Overall, the sex-ratio of the studied population did not significantly deviate from 1:1 ($\chi^2 = 5.88$, df = 9, $P > 0.05$). The GS of *H. tubulosa* showed significant temporal variations ($F = 16.43$ $P < 0.01$), with the minimum values recorded in November up to January and the maximum in June–July, coinciding with the gradual buildup of the gonad (Figure 3). GS followed a clear annual pattern and strongly correlated with seawater temperature (Spearman rank-correlation 0.81 $P < 0.01$).

DISCUSSION

Holothuria tubulosa is widely distributed in the sublittoral zone of the Aegean Sea, being most frequently found in shallow depths, usually down to 10 m (Koukouras & Sinis, 1981). In the present study the surveys covered 30 m depth, but the species was restricted up to 8 m depth with densities varying temporally around 0.1 individuals/m^2. This value was rather low, since about 3.77 individuals/m^2 have been reported from a dense *Posidonia oceanica* meadow in similar depths from the Gulf of Naples, with a significant decrease according to depth reaching only 0.34 individuals/m^2 in 30 m depth where the meadow was sparser (Coulon & Jangoux, 1993). In the latter area, earlier studies reported a density of 0.17 to 0.34 individuals/m^2 in various habitats (Gustato *et al.*, 1982). In the Adriatic *H. tubulosa* density

Table 2. Morphometric relationships of the *Holothuria tubulosa* population in Pagasitikos Gulf. Asterisk (*) denotes statistically significant values, $P < 0.01$.

Relation	Model	a	b	*t*	r	
W/Lt	$W = a\,Lt^b$	6.87 ± 1.294	1.005 ± 0.076	−26.25*	0.60	Allometry (b < 3)
Wgt/Lt	$Wgt = aLt^b$	6.71 ± 1.244	0.810 ± 0.065	−33.69*	0.58	Allometry (b < 3)
Ld/Lt	$Ld = aLt^b$	10.21 ± 1.185	0.612 ± 0.050	−7.76*	0.56	Allometry (b < 1)
Pc/Lt	$Pc = aLt^b$	25.64 ± 1.096	0.209 ± 0.027	−29.29*	0.40	Allometry (b < 1)
Wgt/W	$Wgt = aW^b$	1.57 ± 1.086	0.786 ± 0.016	−13.37*	0.94	Allometry (b < 1)
Ld/W	$Ld = aW^b$	6.69 ± 1.142	0.467 ± 0.025	−21.32*	0.72	Allometry (b < 3)
Pc/W	$Pc = aW^b$	23.33 ± 1.086	0.151 ± 0.015	−56.6*	0.48	Allometry (b < 3)
Ld/Wgt	$Ld = aWgt^b$	6.50 ± 1.156	0.542 ± 0.031	−14.77*	0.70	Allometry (b < 3)
Pc/Wgt	$Pc = aWgt^b$	20.84 ± 1.086	0.197 ± 0.018	−44.61*	0.52	Allometry (b < 3)
Pc/Ld	$Pc = aLd^b$	19.95 ± 1.114	0.219 ± 0.024	−32.54*	0.45	Allometry (b < 1)

N, number of individuals examined equals 314; a, intercept; b, slope; *t*, *t*-test value; r, correlation coefficient.

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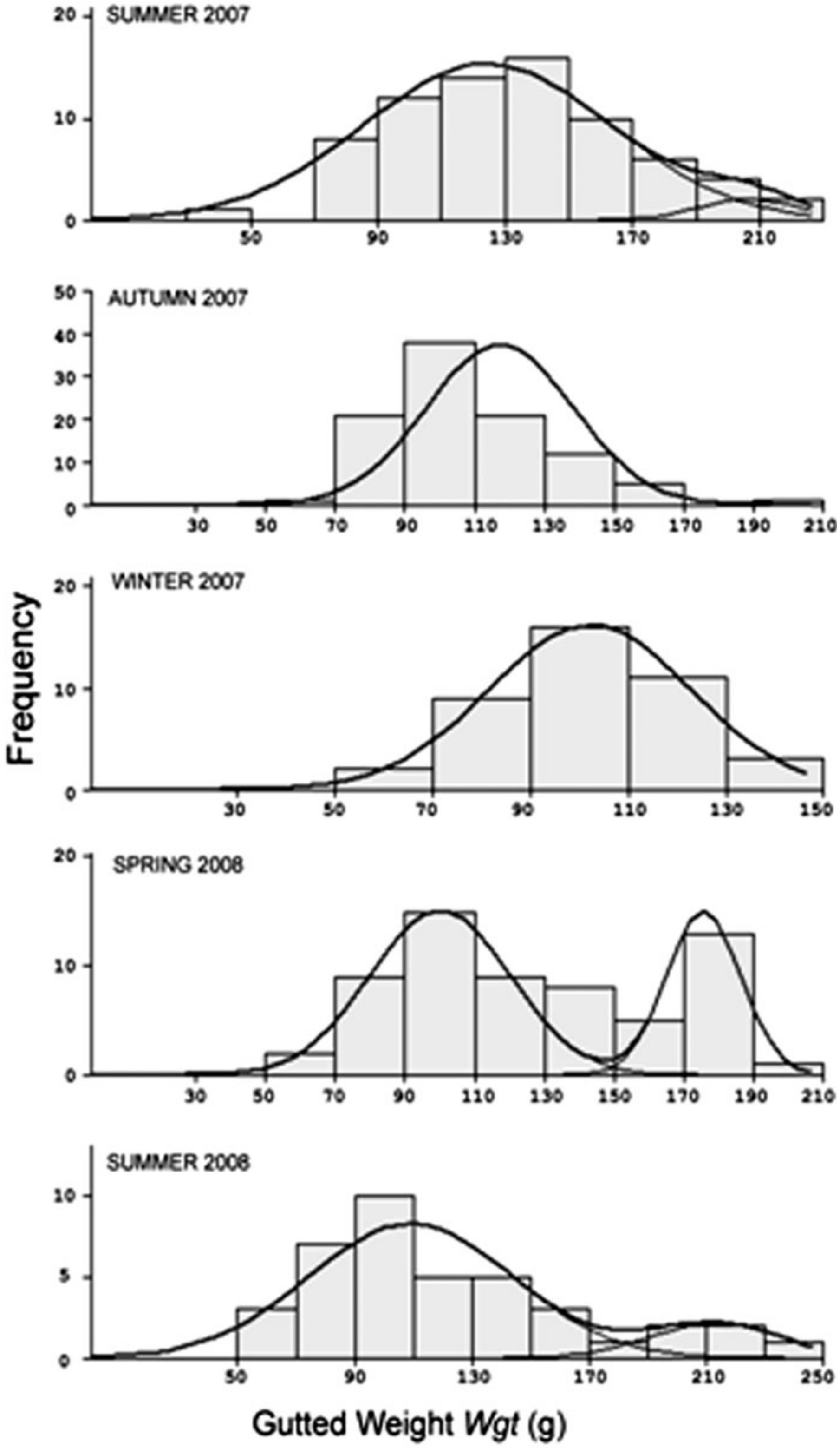

Fig. 2. Modal progression analysis of *Holothuria tubulosa* size–frequency data (thick line, NORMSEP method based on previous results of Battacharya method).

estimated from trawl surveys, ranged from 0.33 to 492.72 individuals/km² according to the sediment composition and depth (Simunovic *et al.*, 2000). Thus, the density of the animal populations seems to be highly variable according to the habitat type and depth. The bottom type in the study area consists of a sparse *Z. marina* meadow interspersed with silt sediments, which seems not to be the preferred habitat of the species.

Holothuria tubulosa showed a high plasticity level of the measured biometric characters, mostly with respect to the length. This plasticity can be interpreted by taking into account the general body form of the animal, which is soft

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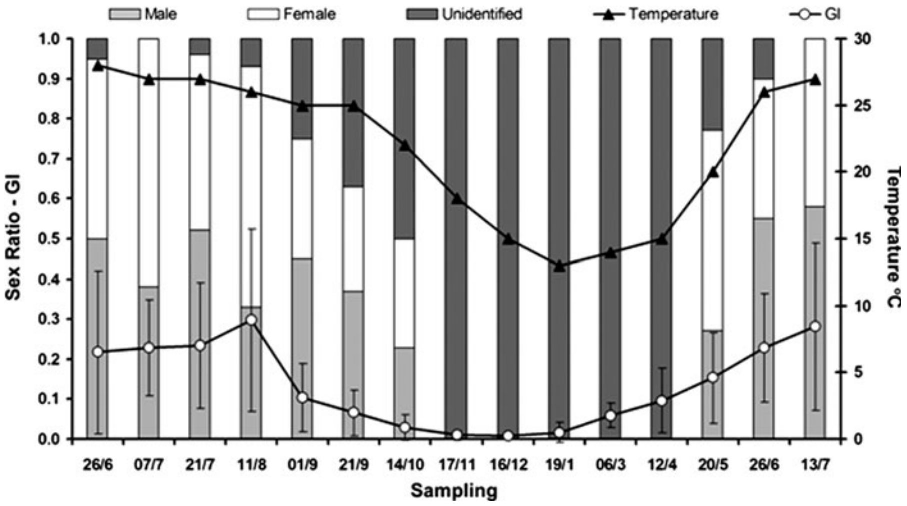

Fig. 3. Temporal variability of temperature (T), sex-ratio and gonadosomatic index (GI) of *Holothuria tubulosa* population in Pagasitikos Gulf.

with strong transversus and longitudinal muscles. Thus, the estimation of body length and width is subjected to increased error, since it is very difficult to obtain a complete relaxation of the body muscles (Conand, 1981). In our case body length measurements were performed underwater and care was taken to avoid body contraction (Despalatovic *et al.*, 2004); nevertheless we cannot exclude methodological artefacts from the observed variability. Therefore, weight measurements seem to be more reliable for estimating holothurians population dynamics, as stated by various authors (Conand, 1981; Bulteel *et al.*, 1992; Tuwo & Conand, 1992).

All the examined morphometric relationships followed negative allometry. Such a pattern, also reported from other Mediterranean populations (Bulteel & Jangoux, 1989; Bulteel *et al.*, 1992), indicate a change in the shape of the animal's body as it grows, since the relative volume growth rate is lower than the linear one. However, most of these relationships had a rather low determination coefficient probably for the same reasons as stated above. Similar results have been reported from other congeneric species attributed to the 'variability of biological material' (Conand, 1981). Holothurians' length appeared to be a moderate predictor of biomass. In contrast a very strong relation was observed between drained and gutted weight, supporting the utility of the latter parameters in population dynamic studies.

Holothuria tubulosa is a continuous deposit feeder ingesting large amounts of sediment (Coulon & Jangoux, 1993) and consequently its weight is strongly influenced by its feeding rate. Therefore, the gutted weight was used to estimate population dynamics as the most consistent biometric variable (Conand, 1981; Bulteel *et al.*, 1992; Despalatovic *et al.*, 2004). Gutted weight–frequency analysis of *H. tubulosa* populations was unimodal, with the exception of: (1) summer, where few large sized individuals constitute a minor second mode; and (2) spring, where a second mode was clearly defined. The size spectra of the population were rather similar among temporal samplings; nevertheless significant differences in the mean value were seasonally detected. From summer to autumn the population studied decreased in size; afterwards weight is increasing towards next summer. The failure to detect a separate mode of juveniles may be due to the sampling method, which is size-selective; individuals measuring less than 3 cm in length are not easily observed and they are occasionally collected. Alternatively, a very fast growth during the first age of the species could explain the scarcity of small-sized specimens (Massin & Jangoux, 1976). Interestingly, prior studies of various aspidochirote holothuroids report few or no juveniles in the populations (Conand, 1981; Tuwo & Conand, 1992; Despalatovic *et al.*, 2004).

A well-marked size distribution related to depth has been reported for *H. tubulosa* populations with small individuals in the shallow and large ones in deeper waters. This pattern has been attributed either to a downward migration of growing individuals or to different growth constraints according to depth (Bulteel *et al.*, 1992). This pattern was not supported by our data since multimodality appeared in shallow depths and not any individual has been recorded beyond 10 m depth and up to 30 m, at each of the 15 sampling attempts. Thus, the two modes observed may represent different age-classes of the studied population, or they may be ascribed to differences in the microhabitat and specifically to the concentration of detritus and microorganisms into the superficial sediment, i.e. food availability, that could influence the growth rate of this slowly moving species implying that similar in size individuals may be of different age (Bulteel *et al.*, 1992).

Holothuria tubulosa is a gonochoristic species with no external sexual dimorphism, in which sex can be macroscopically determined by the appearance and the colour of gonads (Despalatovic *et al.*, 2004). The sex-ratio of the studied population approximated 1:1, conforming to results of similar studies (Despalatovic *et al.*, 2004), while a predominance of females has been reported from a central Adriatic population (Simunovic & Grubelic, 1998). A proportion of individuals with undifferentiated gonads, and thus of undetermined sex, were also caught during the warm period; these were all of small size (below 60 g in gutted weight) implying the presence of a small cohort of juveniles in the studied population.

A good homology between the GS and the stages of the gonads recorded with a histological analysis has been reported for various aspidochirote holothurians (Conand, 1993; Shiell & Uthicke, 2006) and therefore GS seems to be reliable for the description of the reproductive cycle. In Pagasitikos

Gulf, the reproductive cycle of *H. tubulosa*, as manifested by the GS, was annual with one annual spawning event, after mid-summer when maximum GS values occurred, as also reported from other Mediterranean populations (Bulteel *et al.*, 1992). GS variability coincides with sea surface temperature and gonad development seems to be induced by the gradual warming of the water masses in spring. These findings conform to the results of a comprehensive study of the reproductive biology of *H. tubulosa* in the Adriatic, according to which the species spawns once per year during the warm season, from July to September (Despalatovic *et al.*, 2004). These data suggest that temperature is the most likely factor determining the reproduction of the species, as is the case for other congeneric species (Conand, 1981; Tuwo & Conand, 1992; Despalatovic *et al.*, 2003).

Concluding, the *H. tubulosa* population studied was constituted by moderate-sized individuals with most specimens having a drained weight around 220 g. However, the recently induced fishing pressure in several Mediterranean populations and the increased economic profit of the processed product, 'trepang', in the Asian market (Simunovic & Grubelic, 1998; Cakli *et al.*, 2004; Aydin, 2008; Vafidis *et al.*, 2008) necessitate the monitoring of its populations. This became imperative considering that *H. tubulosa* is an ecosystem-engineering species; thus any disruption of its populations could cause cascading effects on the associated community (Amon & Herndl, 1991; Coulon & Jangoux, 1993).

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