Reproductive cycle of the sea cucumber Holothuria (Platyperona) sanctori (Holothuroidea: Echinodermata) in the southwestern Mediterranean Sea: interpopulation variability

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Reproductive cycle of the sea cucumber *Holothuria (Platyperona) sanctori* (Holothuroidea: Echinodermata) in the southwestern Mediterranean Sea: interpopulation variability

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This study is a first contribution on the reproductive biology of *Holothuria (Platyperona) sanctori* from the Algerian coastline in the southwestern Mediterranean. Sampling was conducted at two sites in central Algeria, one (Ain Taggourait, w. Tipasa) where there is little anthropogenic influence and another (Tamentefoust, w. Alger) where there are two major sources of pollution from Oued el Harrach and Oued el Hamiz effluents which contain particulate organic matter. Significant differences in sea cucumber reproduction were observed between the two sites. This could be due to the difference in organic matter noted in the sediment of the two study sites. However, temporal similarities of the gonad index (GI) and the sexual maturity stages suggest that the reproductive cycle of *H. (P.) sanctori* is annual with the main spawning event taking place from June to October. Spawning is of high intensity in the Bay of Bou Ismail (Ain Taggourait) and spread out over time in the Bay of Algiers (Tamentefoust), reflecting an environment sufficiently rich in food to insure reproduction. We also show that gonad maturation of males and females was synchronized. Temperature is the most likely factor influencing the reproductive cycle and spawning. At the study sites, *H. (P.) sanctori* had a winter sexual resting phase, followed by maturation during spring, before spawning in summer. However, there was a slight lag to the start of spawning at the two study sites.

Keywords: holothurians; Aspidochirotida; reproduction; spawning; environmental factors; Algerian basin

Introduction

Aspidochirotid holothurians, commonly known as "sea cucumbers", are major representatives of the benthic macrofauna of the *Posidonia oceanica* habitat of the Mediterranean Sea. This habitat is considered to be one of the most important of Mediterranean coastal areas; within this habitat, holothurians contribute to the recycling of organic matter (Cherbonnier 1958; Francour 1990; Mezali 1998, 2008).

Holothurians belonging to the order Aspidochirotida constitute important commercial fisheries in tropical waters of the western Pacific and Indian oceans (Conand 1990; Purcell et al. 2013). The most important part of the holothurians is the body wall, consumed under different names ("Trepang", "Haisom" and "Iriko") (Conand & Byrne 1993). The dried body wall is marketed as "bêche-de-mer" or "trepang" (Conand 1990; Conand & Byrne 1993). During the last decade, this product has become very lucrative and its importance to fisheries and aquaculture has been widely reported in several FAO publications (Lovatelli et al. 2004; Toral-Granda et al. 2008; Purcell et al. 2010; FAO 2012; Purcell et al. 2012, 2013). At present, holothurians are fished in more than 70 countries around the world, by at least three million

fishermen (Purcell et al. 2013), and most natural stocks are overexploited. In the Mediterranean Sea, holothurians are a marine resource with a very low exploitation, except in Turkey where *Holothuria (Holothuria) tubulosa* (Gmelin 1970), *Holothuria (Roweothuria) poli* (Delle Chaije 1823), and *H. (P.) sanctori* (Delle Chaije 1823) are consumed (Çakli et al. 2004). Turkey actively exports "trepang" to Asia, mainly to Singapore, Hong Kong, and Japan (Çakli et al. 2004; Aydin 2008). However, in Italy, commercial fishermen have recently launched small-scale collections and transformations of holothurians for export to China and Hong Kong (Sicuro & Levine 2011). New fisheries are emerging in areas where holothurians are not part of culinary traditions (Despalatovi et al. 2004). This is the case in the southern Mediterranean Sea and the northeast Atlantic Ocean where holothurian fisheries are developing (González-Wangüemert et al. 2011). In these regions, the new economically important species include *H. (H.) tubulosa*, *H. (R.) poli*, and *H. (P.) sanctori*. These species are most common in the Mediterranean Sea (Mezali 1998, 2008; Ocaña & Sanchez-Tocino 2005). If these species are to be sustainably exploited and eventually cultured in the Mediterranean Sea, studies of their

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systematics, distribution, life cycles, and reproduction are needed (Francour 1990; Sicuro & Levine 2011).

Studies on Mediterranean aspidochirotid holothurians include some taxonomy and phylogeny (Mezali 2008, 2011; Borrero-Pérez et al. 2010) as well as the biology of their reproduction. Some authors have reported observations on spawning (Ocaña & Sanchez-Tocino 2005) and gonad index surveys (Kazanidis et al. 2010). A study of *Holothuria (H.) tubulosa* from the Adriatic Sea (Despalatovi et al. 2004) was limited to observation of the external appearance of the oocytes. Léonet et al. (2009) discovered a new and highly effective oocyte maturation inducer for *in vitro* fertilization of the oocytes of *H. (H.) tubulosa* and *H. (Panningothuria) forskali* collected from Banyuls-sur-Mer (France). In Algeria, detailed studies were carried out on the dynamics of the stock (variation of densities and biomasses) of *H. (H.) tubulosa*, *H. (R.) poli*, *H. (Holothuria) stellati*, *H. (P.) forskali*, and *H. (P.) sanctori* (Mezali 1998, 2008); the biological aspects such as biometrics, growth, and feeding behavior were also detailed (Francour 1990; Mezali & Semroud 1998; Mezali et al. 2003, 2006; Mezali & Soualili 2013). However, aspects of the reproductive cycle and the maturation of the gonads have not been studied from this region of the Mediterranean.

More recently, Navarro, García-Sanz, and Tuya (2013), Navarro, García-Sanz, Barrio, et al. (2013) studied the size structure, the abundance, the feeding and movement patterns of *H. (P.) sanctori* in the Canary Islands (Atlantic Ocean). In their study on three sites at Gran Canaria, Navarro, García-Sanz, Barrio, et al. (2013), following studies by Tuya et al. (2006), compared the characteristics of the substratum and found higher densities of *H. (P.) sanctori* in sites with a hard substratum which offer a daily refuge for this nocturnal species and for the cryptic juveniles. The reproductive cycle of *H. (P.) sanctori* was determined by Navarro et al. (2012) at Gran Canaria, but without anthropogenic impact investigation. The gonad index and a combination of macro and microscopic analysis of the gonads showed a typical temperate species reproduction pattern with a summer spawning. The authors showed that the first maturity for this species occurred at a size of 201–210 mm.

The interest in *H. (P.) sanctori* comes from the abundance of this species in Algerian shallow waters, especially in unpolluted areas. This species has two polymorphic and ecotypic forms, one that is brown in color and the other characterized by white spots on the bivium (Mezali 2011; Mezali & Francour 2012; Mezali forthcoming 2013). The neighbor-joining and Bayesian consensus trees using the 16S rDNA show no differences between the two morphotypes. They are genetically the same and thus represent one species (Mezali 2008,

2011). *Holothuria (P.) sanctori* has a thick body wall and could be exploited by a fishery.

The present study examines the macroscopic and microscopic aspects of gonad maturation of *H. (P.) sanctori* to determine the variability of its reproductive cycle at two sites, focuses on the effects of anthropogenic impact on the reproduction and compares the differences between the two populations in relation to the degree of potential pollution (organic matter rate) and the predominance of the major ecological factors such as hydrodynamism, according to the previous studies by Maouche (1987) and Braik (1989).

Materials and methods

Sampling sites

The two selected sites are different. The first site (36°48.379′N, 003°13.696′E) is located near Tamentefoust beach at Cap Matifou (eastern end of the Bay of Algiers located near Bordj El-Bahri previously called "La Pérouse") (Figure 1). The site of Tamentefoust, situated in a half closed creek has a low hydrodynamism because it is well protected by the Cap Matifou from the dominant winds originating from northeast and west-south-west sector (Maouche 1987). It is characterized by overgrazed rocks with sparse shoots of *P. oceanica* beds in a state of regression (Semroud 1996). According to Bachari-Houma (2009), the Tamentefoust region is known for its industrial and urban development which exposes it to pollution. This site is characterized by the influence of water discharge mainly via the Oued el Hamiz and Oued el Harrach (water discharge in the rainy period of $1000\,\mathrm{m}^3\mathrm{s}^{-1}$) (Bachari-Houma 2009) (Figure 1) which brings particulate material, and terrestrial particulate organic matter. The Bay of Bou Ismail where the second site of Ain Taggourait (previously called "Bérard") is located (36°36.608′N, 002°36.995′E) (Figure 1) has pollution levels much lower than in the Bay of Algiers (PAC 2005). Bou Ismail Bay is located in a more open bay, and is much more exposed to the prevailing winds coming from the northeast and northwest sector, which generate swells of high amplitudes (Braik 1989). It is more distant from the industrialized area and in addition, urban development of the region is less than that around the Tamentefoust area, which reduces the impact of industrial and domestic pollution. Underwater observations revealed that at Tamentefoust site the algal coverage and the *P. oceanica* meadow are denser when compared with the Tamentefoust region. Ain Taggourait was selected as a reference site for the study of the reproduction of *H. (P.) sanctori*.

The biological material was sampled at both sites using scuba diving at depths ranging from 3 to 10 m. For each sample, the sea water temperature was recorded
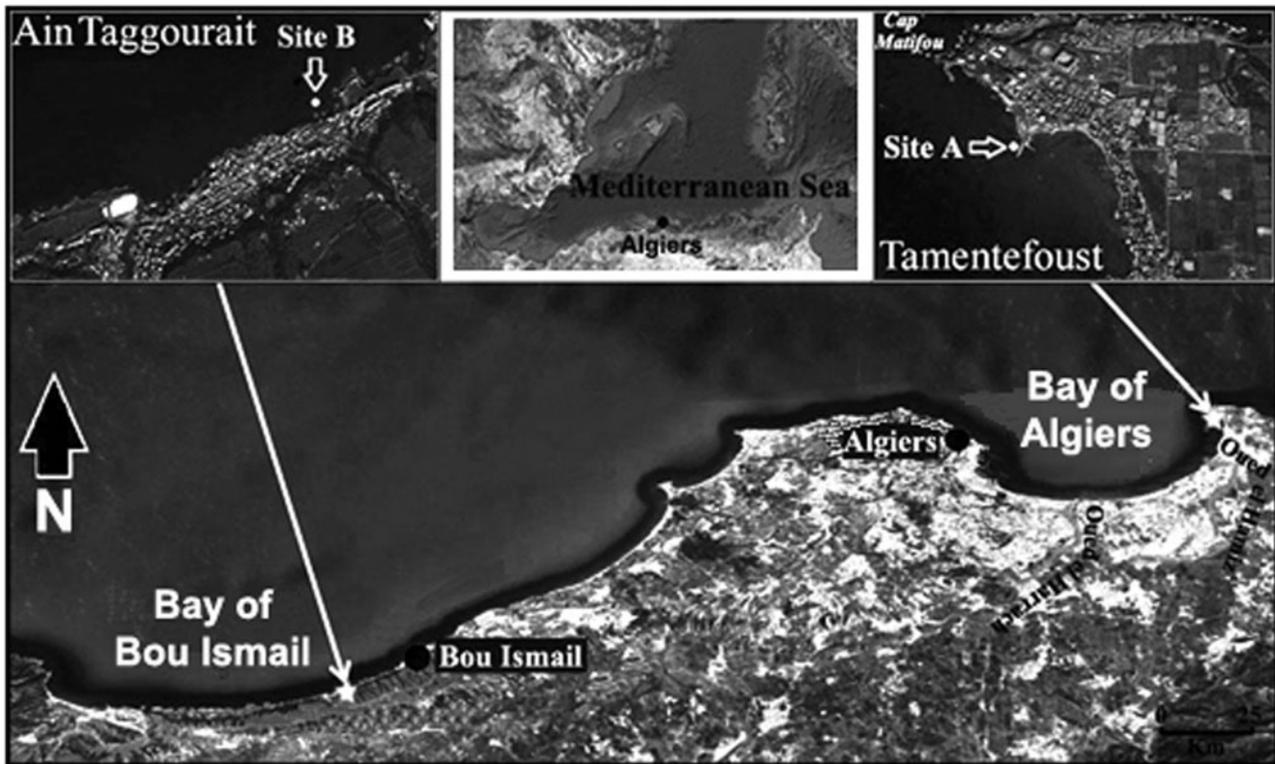
Figure 1. Location of the two sampling sites. Site A: Tamentefoust site located in the Bay of Algiers: Site B: Ain Taggourait site located in the Bay of Bou Ismail (© 2012 Google earth).

using a SUNTO GEKKO dive type computer (precision 0.5 °C). The holothurian samples were kept in a tank filled with sea water until processing in the laboratory.

Biometric and reproductive parameters

The sampled holothurians were immersed in seawater with MgCl$_2$ solution (10–15 g/l) until total relaxation (approximately 10–15 min later). The total anesthetized length (*aL*) was measured (+/−0.5 cm) using a Ribbon meter. The length was measured dorsally from the mouth to the cloaca. Also, the thickness of the Bivium (*tB*) and Trivium (*tT*) (+/−0.1 mm) was measured using a sliding caliper. Subsequently, specimens were dissected by longitudinal incision on their ventral side along the interradial. When possible, sex determination was done macroscopically, the ovary was usually yellow or orange red, and the testes whitish. During the resting stage, sex differentiation was not easy and required microscopic observation.

The gonad was weighted (drained weight, *Wg* ± 0.01 g) and then fixed in aqueous Bouin's solution for 24–48 h. Subsequently, the samples were rinsed in successive baths of ethanol and preserved in ethanol (70%)

for histology according to the protocols of Martoja and Martoja-Pierson (1967) and Bensalem-Bendjelloul (1998). All the internal organs of an individual were removed to obtain the eviscerated body weight (*eBW* ± 0.01 g). Therefore, the *eBW* included the weight of the body wall, the longitudinal muscles, and the buccal bulb (Conand 1990; Kazanidis et al. 2010).

Approximately, 15 adult individuals were sampled each month from each site. Two approaches were used to determine the reproductive cycle of *H. (P.) sanctori*: (1) gonad index (GI) survey by sex and site and (2) histological study to allow the temporal monitoring of the microscopic stages of gonad maturity. The GI was calculated by the following formula (Conand 1981, 1993; Ramofafia & Byrne 2001; Asha & Muthiah 2008; Gaudron et al. 2008; Kohler et al. 2009):

$$\text{GI} = \frac{wg}{eBW} \times 100$$

For the microscopic analysis, the conventional topographic dyes Mann-Dominici (1894–1905 *in* Bensalem-Bendjelloul 1998) and Trichrome on one time (Gabe & Martoja 1957) were used for the determination of the microscopic stages of maturity. They were based on histology and on those of Tanaka (1958), Conand (1981,

1990) and Navarro et al. (2012): Stage I: sexual resting (resting or indeterminate tubules); Stage II: recovery and maturing (increasing tubules); Stage III: mature (ripe tubules); Stage IV: spawning (partly emptied tubules); and Stage V: spent (empty tubules).

The sex ratio was defined as the ratio of the number of males to the number of females.

Statistical analyses

Once the homogeneity of variances had been verified, differences in the values of the GI between the two sites by seasons, sites, years, and sexes were conducted using a four-factor ANOVA ($p < 0.05$). Sites and years are treated as random. Also, comparison of the means between sites of the following parameters: *aL*, *eW*, *tB*, *tT*, and *GI* was applied, using a *t*-test ($p < 0.05$). The average values of the GI by sex were analyzed by a Mann and Whitney test. To check the balanced distribution between males and females 1:1, we used a Chi-square test (χ^2). A Spearman test was used to analyze the existing correlation between the GI and temperature. All statistical analyses were performed using STATGRAPHICS and STATISTICA 7.

Results

Environmental conditions of the two studied sites

The two studied sites differ in a range of variables. Tamentefoust is a polluted area with harbor activities. This marine area under the influence of the Algiers metropolis catches the highest pollution flow of the Algerian coasts (PAC 2005) with 100 thousand tonnes y^{-1} of organic chemical compounds, 175 thousand tonnes y^{-1} of suspended matter, 1.5 thousand tonnes y^{-1} of nitrogen, and 4000 tonnes y^{-1} of phosphorus (Larid 2003). In 2004, 46 beaches of the Bay of Algiers were prohibited for swimming. The bottom sediment of the site of Tamentefoust is characterized by a high rate of fine fraction (55.13%) (Mezali 2004). Organic and inorganic pollutants from anthropogenic activities increase the organic matter in the sediment. Previous studies (MATE 2005; Bachari-Houma 2009; Mezali & Soualili 2013) have shown a higher metallic and domestic pollution at Tamentefoust than at Ain Taggourait which is considered as a more pristine site. The situation of Ain Taggourait halfway between the Oued Mazzafran in the eastern part of the Bay of Bou Ismail and the Oued Nador in its western part (approximately 10 nautical miles of distance from the mouths of two Oueds) makes it relatively less affected by the continental contributions. Furthermore, the building of the dam of Boukourdene (w. Tipasa) has tremendously dropped the output flow of the Oued Nador.

Biometry and reproductive biology

A total of 582 *H. (P.) sanctori* were sampled in the central region of Algeria, including 385 individuals collected in the Bay of Bou Ismail (Ain Taggourait), and 197 in the Bay of Algiers (Tamentefoust). During our sampling, 27 individuals lacking gonads were found in the fall and spring at Ain Taggourait and only in the spring at Tamentefoust.

Holothuria (P.) sanctori had an average anesthetized length of 225 ± 38.90 mm at Ain Taggourait and 248 ± 47.10 mm at Tamentefoust. During the survey, adults with sizes and weight ranging from 155 to 330 mm and 33.40 to 121.28 g at Ain Taggourait and 150 to 360 mm and weight between 34.80 and 175 g at Tamentefoust were collected (Table 1; $p < 0.05$). The thickness of the bivium and the trivium was significantly greater at Tamentefoust than Ain Taggourait (Table 1; $p < 0.05$). *Holothuria (P.) sanctori* of Tamentefoust is significantly bigger and larger than *H. (P.) sanctori* of Ain Taggourait.

The number of males and females collected from the two sites was 180 and 189, respectively, at Ain Taggourait, and 90 and 96 at Tamentefoust. The sex ratio calculated for *H. (P.) sanctori* at the two sites (1.05:1 at Ain Taggourait and 1.06:1 at Tamentefoust) was not significantly different from 1:1 (Chi-square test $\chi^2 = 0.01$, $p > 0.05$). From the gonad index calculated, the maximum values in males and females were obtained in June to July at Ain Taggourait and in May at Tamentefoust (Figure 2). However, in the spring (March 2011), another high value was recorded, although lower than the summer maximum value obtained during the two years at the two sites. During the late fall and winter (October to January), the GI reached its minimum value (Figure 2). According to the four-factor ANOVA (Table 2), there was no significant difference between year ($F_{(1, 547)} = 1.00$; $p = 0.12$). However, the variability noted between the summer and winter values was significantly different ($F_{(3, 547)} = 67.50$; $p < 0.001$). The value of the GI of Ain Taggourait was significantly higher than that obtained at Tamentefoust ($F_{(1, 547)} = 29.00$; $p < 0.001$). The higher values of the GI of females compared to the males throughout the period of this study were not significantly different ($F_{(1, 547)} = 0.50$; $p = 0.48$).

Four stages of maturity (growth stage II, mature stage III, partial spawning IV, and post-spawning V) were observed in the tubules of male and female of *H. (P.) sanctori* gonads (pooled sex). These were used to clearly follow the seasonal changes at both sites (Figure 3).

Ain Taggourait site

During the winter period, the proportion of individuals observed at stage II was at its peak in December

Table 1. Biometry of *H. (P.) sanctori* sampled in the two study sites.

	AinTaggourait			Tamentefoust			
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	*p*
aL (mm)	225 ± 38.90	155	330	248 ± 47.10	150	360	*0,006*
eBW (g)	76.90 ± 18.37	33.40	121.28	132.82 ± 7.86	34.80	175	*0,0001*
tB (mm)	04.08 ± 3.07	03.50	09.40	05.76 ± 3.04	04.00	10.20	*0,0412*
tT (mm)	02.69 ± 1.95	02.50	06.35	04.46 ± 2.33	00.59	09.79	*0,0016*

Notes: *aL*: anesthetized length; *eBW*: eviscerated body weight; *tB*: thickness of the Bivium; *tT*: thickness of the Trivium. Min: Minimum value; Max: Maximum value. Differences between biometrics data of *H. (P.) sanctori* at AinTaggourait and Tamentefoust sites were tested by Student's *t*-test at 95% of confidence. SD: standard deviation.
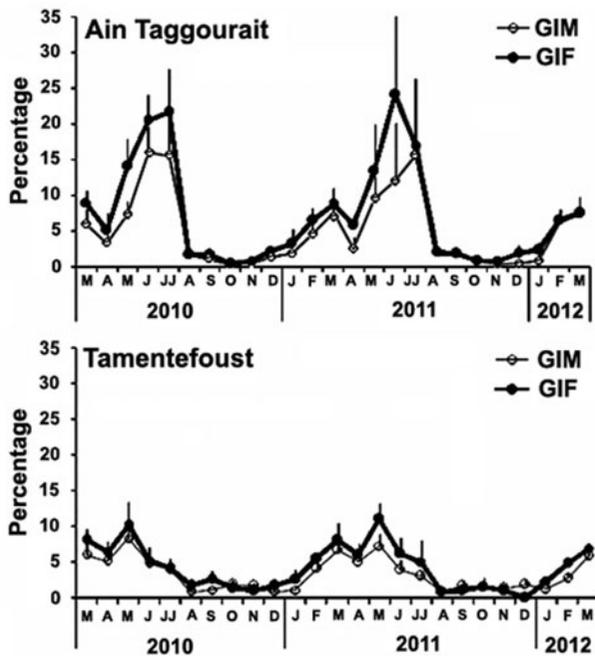

Figure 2. Temporal variation of the gonad index of *H. (P.) sanctori* by sex and at the two study sites. GIM: Gonad Index Male; GIF: Gonad Index Female.

(Figure 3). The percentage of individuals observed in stage III was higher than 50% during spring (Figure 3). Stage IV was observed from April to August marked by the presence of a maximum number of individuals at this stage in July. Stage V was observed from June to October, but the maximum percentage was observed in August (Figure 3).

Tamentefoust site

Stage II was observed during the period of sampling even in summer. However, the maximum number of individuals at stage II was observed in winter (Figure 3). Stage III was seen during all seasons except winter, marked by a maximum of individuals observed in stage III in April (Figure 3). However, individuals in stage IV were observed as for stage III throughout the period of sampling but with a maximum of individuals partly spawning in August (Figure 3). Stage V was observed from April to November with a maximum in August and September (Figure 3).

To understand the variability of the reproductive cycle of *H. (P.) sanctori* at both sites, the gonad index data of males and females were pooled and the percentages of individuals at stage V per site examined (Figure 4). At both sites, the reproductive cycle was marked

Table 2. Results of the ANOVA four factors to test differences in gonad index of *H. (P.) sanctori* by seasons, sites, year, and sex.

Factors	DF	SS	MS	Pseudo-*F*	*p*-value	Differences
Seasons	3	7541.23	2513.74	67.5	10^{-4}	Winter−summer
Sites	1	1080.02	1080.02	29.00	10^{-4}	Spring−summer
Years	1	117.84	58.92	1.00	0.20	Spring−fall
Sex	1	18.476	18.47	0.50	0.48	
Seasons × years	3	168.429	84.21	2.15	0.11	
Seasons × sites	3	1127.61	1127.61	30.25	10^{-4}	
Years × sites	1	215.95	107.793	2.13	0.12	
Sex × years	1	6.86	6.86	0.13	0.71	
Residual	547	20370.1	37.2397			
Sum corrected	554	29355.6				

Notes: DF: degree of freedom; SS: sum of squares; MS: square average. F: measure the separation between all classes.
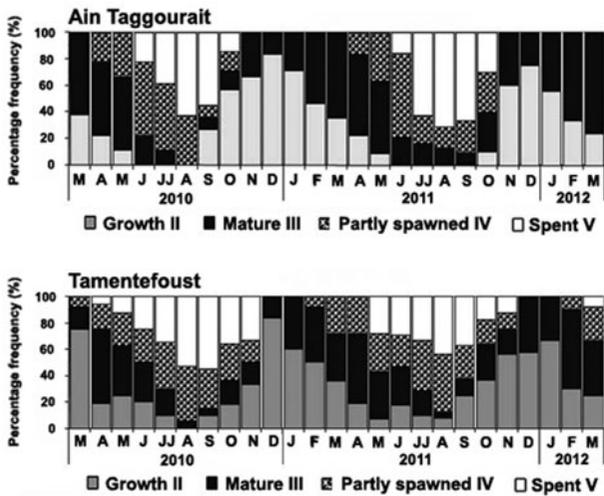
Figure 3. Maturity stages of the tubules of *H. (P.) sanctori* gonads (sexes pooled) at the two study sites from March 2010 to March 2012. Histograms show percentages of individuals in one of the four gametogenic stages.

by a period of rapid gonad growth in early spring, followed by two gamete emissions, the first of low intensity in the spring and the second massive and principal in summer, both marked by the fall in the gonad index (Figure 4).

The period of sexual resting in the winter at the two study sites was marked by the low GI values (Figure 4). Furthermore, the main and massive spawning of *H. (P.) sanctori* was confirmed by the presence of individuals in stage V (post-spawning) and the low GI values in the
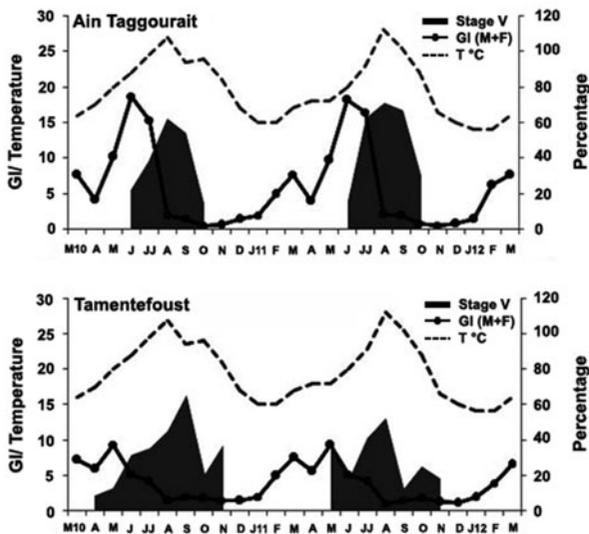

Figure 4. Monthly gonad index means of *H. (P.) sanctori* (pooled sex), monthly mean temperature, and the percentage of individuals observed at stage V (post-spawning) at the two study sites. GI: Gonad Index; M: Male; F: Female; T: Temperature.

fall. A significant difference of intensity of spawning was detected between the two sites ($p < 0.05$). However, we noted that a period of a high temperature rise during the two years at the two sites (22–24.5 °C in 2010 and 20–23 °C in 2011) coincides with the period where the GI of *H. (P.) sanctori* decreases (Fig. 4). The correlation between the evolution of the GI and the temperature is significantly confirmed for Ain Taggourait site but not for Tamentefoust site (Spearman test ($p < 0.01$ at Tamentefoust) and ($p > 0.01$ at Ain Taggourait)).

Discussion

Characteristics of the two sites of the southwestern Mediterranean

In this study, we considered the reproductive cycle of two populations of *H. (P.) sanctori* in two sites of the southwestern Mediterranean; one site as representative of human-altered conditions (Tamentefoust) and the other of more pristine conditions (Ain Taggourait). According to previous studies by Mezali et al. (2003) and Mezali (2008), the granulometric and chemical (organic matter) characteristics of bottom sediment in which we find the deposit feeder *H. (P.) sanctori* show differences between Tamentefoust site (bay of Algiers) and Sidi Fredj site (located in the bay of Bou Ismail near Ain Taggourait). A high rate of fine fraction is found at Tamentefoust (55.13%, Mezali 2004). This could be related: (1) to the exposition of the site included in a semi-closed area in the bay of Algiers where hydrodynamism is low in contrast with the Ain Taggourait site which is situated in an open bay with high hydrodynamism; and (2) to the organic matter concentration (3.18%, Mezali and Soualili 2013), higher than at Ain Taggourait site (2.03%, K. Mezali unpublished data). In fact, the rate of organic matter increases with the importance of the smallest sediment particles (that mean that the ratio "volume-surface" is very important in promoting the adhesion of organic matter). Furthermore, Plotieau et al. (2013) suggested that the nutritional value of the fine sediments would be higher than that of the coarse sediments because of their higher number of nutritive micro-organisms.

Interpopulation variability in biometry, and comparison with one Atlantic population

According to Harmelin et al. (1981), holothurian species are very sensitive to pollution, and can be considered as good benthic bioindicators. The difference in mean densities of *H. (H.) tubulosa* between an unpolluted station (Sidi-Fredj, situated at the bay of Bou Ismail, 0.95 individual.m^{-2}) and the polluted station (Tamentefoust, 0.40 individual.m^{-2}) (Mezali 2008) confirms the conclusion of Harmelin et al. (1981).

The mean size and weight of *H. (P.) sanctori* at Tamentefoust are significantly higher than those measured at Ain Taggourait (Table 1, $p < 0.05$). This could be due to the availability of OM in the polluted site of Tamentefoust (Mezali 2004; Mezali & Soualili 2013). Accordingly, the thickness of the trivium and the bivium of *H. (P.) sanctori* of Tamentefoust are higher compared to Ain Taggourait (Table 1, $p < 0.05$).

In their study of three *Holothuria* species from three sites at Gran Canaria Island, Navarro, García-Sanz, and Tuya (2013) noted that *H. (P.) sanctori* was the most abundant species, with more than one individual.m^{-2}; their modal length 181 mm was much smaller than at the two sites of the present study. Small specimens < 150 mm were not collected at the Mediterranean sites, these were also very rare at the Atlantic study sites with only nine individuals under 100 mm during the whole study (Navarro, García-Sanz, & Tuya 2013). In fact, the abundances and size distributions did not show any seasonal changes in both studies; this probably, as in many other holothurians, is owing to a very discrete recruitment, despite a seasonal sexual reproduction (Mezali & Semroud 1998). Nevertheless, more research effort might provide a better knowledge of the juvenile recruitment and their possible migration. The highest values of biomass/density ratios recorded in summer by Mezali et al. (2006) for *H. (H.) tubulosa* in the site of Tamentefoust (bay of Algiers) and the site of Sidi-Fredj (bay of Bou Ismail) indicate migration of adult individuals towards the shallow part of *P. oceanica* meadows for reproduction during this period, when food availability and water temperature increase. Furthermore, it should be noted that *H. (P.) sanctori* has a nocturnal behavior in feeding and random movements (Navarro, García-Sanz, Barrio, et al. 2013) which can explain the population stability.

Reproductive cycle of H. (P.) sanctori

Holothuria (P.) sanctori is a gonochoric species without external sexual dimorphism. The sex can be determined macroscopically by the appearance and color of the gonads (Despalatovi et al. 2004; Navarro et al. 2012). The sex ratio of populations at the two sites was approximately 1:1, similar to the results of Navarro et al. (2012). This is also observed in many other aspidochirotid holothurians, although the sex ratio in some species is slightly unbalanced (Simunovic & Grubelic 1998; Shiell & Uthicke 2005).

In this study, the gonad index of *H. (P.) sanctori* in the Bay of Bou Ismail and the Bay of Algiers shows that the reproductive cycle of this species is annual, with spawning starting in the spring, peaking in the summer, and ending in the fall, marked by maximum values of the GI in early summer and the minimum values at the end of the summer. The lower gonad index obtained for

the population at the Tamentefoust site suggests that *H. (P.) sanctori* has continuous spawning there, which does not allow it to reach the maximum values found at Ain Taggourait (76.9 ± 18.37 g; Table 1). It should also be noted that the Tamentefoust site is polluted by organic matter (PAC 2006; Mezali & Soualili 2013). The presence of organic matter throughout the year seems to favor the rapid restoration of the gonads, which induces a more extended spawning, but with less intensity compared with *H. (P.) sanctori* of Ain Taggourait. The maximum values of the GI in males and females were obtained in summer (June to July), especially at Ain Taggourait (Figure 2). This period is the same at the Canary Islands (Atlantic Ocean). The mean GI obtained at Ain Taggourait is higher than that obtained for this species in the Canary Islands where the individuals were smaller (Navarro et al. 2012). A good similarity of the temporal change of the GI and the stages of maturation of the gonads has been reported for various aspidochirotid holothurians (Conand 1993; Shiell & Uthicke 2005; Kazanidis et al. 2010). Therefore, a GI survey is a reliable method for describing the reproductive cycle. In general, holothurians have an annual reproductive cycle (Tanaka 1958; Harriott 1985; Cameron & Fankboner 1986; Ong-Che 1990; Smiley et al. 1991; Tuwo & Conand 1992; Conand 1993; Chao et al. 1993, 1995; Foster & Hodgson 1995); although semi-annual cycles have been described by some authors (Harriott 1985; Conand 1993) or even the presence of a continuous reproduction throughout the year (Harriott 1985) which can occur especially in the tropical regions. In the two study sites, the changes in the GI coincided with that of the sea water temperature and/or photoperiod (Spearman test, $p < 0.01$ at Tamentefoust and $p > 0.01$ at Ain Taggourait) and the gonad development seemed to be induced by the gradual warming of the water. This suggests that if temperature and/or photoperiod are important factors in reproductive timing, they work in an absolute (threshold) pattern rather than in a relative one (Pearse & Pearse 1986; Hopper et al. 1998).

A significant difference of intensity of spawning was detected between the two sites ($p > 0.05$). It is more pronounced at Ain Taggourait compared to Tamentefoust; this can be explained by the availability of food (the organic matter throughout the year) and by the fact that Tamentefoust remains a sheltered site, which allows nutrients to stay longer locally. This is not the case for Ain Taggourait site which is exposed and subjected to a strong hydrodynamism where the inputs of nutrients are marked at the end of spring. Also, this could explain the extending of spawning from May to October in Tamentefoust and from July to September at Ain Taggourait. Moreover, the presence of stage II throughout the sampling period, with the exception of the summer (June to August), suggests that gonad maturation occurs

throughout the year with a slowdown during the winter. The main spawning is massive in the summer and coincides with the elevation of the sea water temperature (Spearman test, $p < 0.05$); an average temperature rise of 2 °C between June and July was observed during the two years (22–24.5 °C in 2010 and 20–23 °C in 2011) and coincides with the decrease of the GI and the presence of a high percentage of individuals with partially spawned gonads. However, during the two years (2010–2011) and at the two sites (27 and 28 °C, respectively, in August 2010 and 2011), a high temperature rise coincided with the period where individuals of *H. (P.) sanctori* had a minimum GI value and were in the post-spawning stage. The slight decline of the GI in the spring could be explained by a partial spawning by precociously mature individuals due to temperature values which seem to favor a rapid maturation of the gonads. Our results match those obtained for *H. (P.) sanctori* of the Canary Islands by Navarro et al. (2012) where maximum reproductive activity was observed during the warm months and the minimum activity (resting) during the cold months. The absence of gonads in the winter (cold season) in some specimens is explained by the tubular resorption after spawning (Conand 1993). This is the case for *H. (P.) sanctori* of the Algiers coast with a few individuals having gonads completely resorbed after the spawning observed in 2010. Similar observations were made by Despalatovi et al. (2004) for *H. (H.) tubulosa* from the Adriatic Sea and by Ramofafia and Byrne (2001) for *H. fuscogilva* and *Actinopyga mauritiana*. However, the presence of a few mature *H. (P.) sanctori* at the end of the spring at both sites seems to confirm the start of an early spawning in spring for some individuals.

There are still few data on the reproductive cycle of *H. (Platyperona) sanctori* or the other aspidochirotid species in the Mediterranean basin. Bulteel et al. (1992) presented the reproductive cycle of *H. (H.) tubulosa* off Ischia Island (Italy) and Kazanidis et al. (2010) in the Aegean Sea. Moreover, the effect of temperature on gametogenesis has been well studied in holothurians where it was reported that the sea water temperature could influence gonad growth by acting directly on food availability (appearance of the phytoplankton bloom) (Hopper et al. 1998; Conand et al. 2002; Navarro et al. 2012). The data collected for *H. (P.) sanctori* do not allow testing this hypothesis. However, this species displays an annual reproductive cycle with spawning of high intensity in the Bay of Bou Ismail (Ain Taggourait) and spread out over time in the Bay of Algiers (Tamentefoust) reflecting an environment sufficiently rich in food to insure reproduction.

Differences in the characteristics of the environment of each site (Ain Taggourait and Tamentefoust), such as wave exposure, the hydrodynamic regime, and water quality, may have influenced differences in the observed changes in GI and the intensity of the spawning between the two sites. However, from the study of the reproductive cycle of *H. (P.) sanctori* from the Mediterranean southwestern basin (Algerian coast), it appears that spawning begins in June and ends around October. Considering that holothurian larvae settle in the benthos after about one month [12–17 days for *Holothuria scabra* (Mercier et al. 2000); 22–27 days for *Isostichopus fuscus* (Hamel et al. 2003) and 20 days for *Apostichopus japonicus* (Matsuura et al. 2009)], we can assume that the recruitment of juveniles of *H. (P.) sanctori* and *H. (R.) poli* occurs from the end of the summer (August) as reported by Mezali & Semroud (1998). The collected data suggest that temperature is the most likely factor in the onset of spawning of *H. (P.) sanctori* and the determination of its reproductive cycle, as is the case for other aspidochirotid holothurians species (Conand 1981; Tuwo & Conand 1992; Despalatovi et al. 2004).

Importance for future management

As the studied populations of *H. (Platyperona) sanctori* at Ain Taggourait and Tamentefoust were composed of adult individuals, it will therefore be necessary to extend the study to smaller young individuals to determine the size at first sexual maturity, to be able to decide a size limit in the context of a future exploitation of this resource in Algerian coastal areas. The recent fishing pressure in several Mediterranean populations and the increased economic benefit of the "trepang" product in the Asian market (Simunovic & Grubelic 1998; Çakli et al. 2004; Aydin 2008; Vafidis et al. 2008) require the monitoring of these holothurian populations. This is important for *H. (P.) sanctori* that represents an engineer species contributing to the balance of the *P. oceanica* ecosystem of the Mediterranean Sea, where the depletion of this species stock would have an impact on the survival and a cascade of consequences on the associated communities (Amon & Herndl 1991; Coulon & Jangoux 1993; Gaudron et al. 2008; Purcell et al. 2013).

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