

The reproduction cycle of the sea cucumber *Holothuria (Holothuria) tubulosa* Gmelin, 1791 (Echinodermata Holothuroidea Holothuriidae) in Oran coast, Algeria

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ABSTRACT

The aim of this paper is to present an analytical study of gonad index (GI) and repletion index (RI) of *Holothuria (Holothuria) tubulosa* Gmelin, 1791 (Echinodermata Holothuroidea Holothuriidae). The study is based on biomonitoring data collected from February 2014 to January 2015 in the Oran coast. The gonad index of *Holothuria tubulosa* is analyzed to show the reproductive cycle and its relationship to environmental parameter conditions. Samples of 15 to 20 individuals were collected monthly at three stations on the Oran coast (La Madrague, Cap Carbon, and Ain Franin). The sex ratio of all sampling was found as (female: male) 0.8:1.2 with no significant difference between sites ($X_2 = 2.18$; $df=4$; $p>0.05$) and is also not significant regarding the seasons (spring, summer, autumn, winter) ($X_2 = 3.03$; $df=6$; $p>0.05$). Sea cucumber reproduction occurred in September at Cap Carbon and Ain Franin with values of Gonad index (GI) that are 0.33 ± 0.52 % and 0.22 ± 0.52 % respectively. At the La Madrague site, the spawning occurred in October with a value of GI of 1.49 ± 2.03 %. It was observed that seawater temperature has a positive correlation with Gonad index (GI) and no correlation with Repletion index (RI).

KEY WORDS

Gonad index; *Holothuria tubulosa*; Oran coast; sex ratio; seawater temperature.

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INTRODUCTION

The aspidochirote *Holothuria (Holothuria) tubulosa* (Gmelin, 1788) (Echinodermata Holothuroidea Holothuriidae) is widely distributed in the Mediterranean Sea (Francour, 1990; Coulon & Jangoux, 1993a; Koukouras et al., 2007) where it is an important part of the macrozoobenthos (Mezali et al., 2006; Costa et al., 2014). It lives on rocky substrata, loose sediments, and in sea grass meadows at depths varying between 5 and 100 meters (Ocaña & Tocino, 2005). *Holothuria tubulosa* is a common

species on the Algerian seabed (Mezali, 2008). It is an ecologically important species because of its role in the recycling of organic matter in *Posidonia oceanica* (L.) Delile meadows (Costa et al., 2014) and its stabilization of the bacterial community in the sediments (Amon & Herndl, 1991).

Holothurians have lost pentamerism in their reproductive system (Navarro et al., 2012) and have a single gonad composed of one or two tufts of tubules (Conand, 1989). The gametes are released into the water through gonopore located at the dorsal mesentery (Pérez-Ruzafa, 1984; Conand, 1989).

The gonads tend to fill most of the interior cavity of the body at their peak maturity stage (Pérez-Ruzafa, 1984). In general, holothurians have a seasonal reproductive cycle (Smiley et al., 1991). However, whilst temperate species generally have spawning periods in spring and summer (Cameron & Fankboner, 1986; McEuen & Chia, 1991; Hamel et al., 1993), tropical species may spawn throughout the year (Pearse, 1968; Conand, 1993a; González et al., 2004).

A number of environmental factors may stimulate gonad growth, maturation, and spawning (Fenaux, 1980). For example, sea temperature is an important factor that influences spawning (Fuji, 1960) and the inhibition or slowing down of nutrition (Smith, 1971).

In Algeria, there are few studies on sea cucumbers and their spawning. In this study, we will follow the gonad index and repletion index (reproduction and trophic activity) and the influence of environmental factors on the spawning of the *H. tubulosa* population sampled at three sites on the west coast of Algeria with different locations and substrates.

MATERIAL AND METHODS

Study area

The study was conducted on the Oran coast, in the northwest of Algeria. Three sites were selected. Station 1, La Madrague, $35^{\circ}46'00.58''\text{N} - 0^{\circ}49'33.00''\text{W}$ is located on the west of Cape Falcon, and is a rocky site forming a large bay open to the east. Upstream of this coastal site there are farms that are part of the National Plan of Agricultural Development (PNDA). These farms may affect the coastal marine ecosystem through the use of pesticides, fertilizers and phytosanitary products (Benghali et al., 2006). Station 2, Ain Franin, $00^{\circ}35'46.854''\text{N} - 30^{\circ}768'00''\text{W}$ is located between two headlands forming the large bay of Oran, Cape Ferrat to the north and Cape Falcon to the southeast and 8 km far from Kristel. This station is considered as a low-impact anthropogenic area, but there is presence of a source of sulfuric thermal water. Finally, station 3, Cap Carbon, $35^{\circ}54'6.36''\text{N} - 0^{\circ}20'20.22''\text{W}$ is located near the agglomeration of Arzew. This area is under signif-

icant influence of urban and industrial discharges from the presence of a petrochemical industrial complex (Arzew-Ain El Bia, Oran) as shown in figure 1.

Field Sampling

The study is based on 12 sampling months from February 2014 to January 2015. From 15 to 20 sea cucumbers were collected each month by scuba divers at a depth of up to 10 m, at the three study areas. Temperature, salinity, and pH of the surface water were measured using a Multiparameter (HANNA-HI9829) instrument. At the laboratory, each animal was dissected along the ventral surface and eviscerated. The gonads and the digestive tract (intestine and digestive contents) were recovered, drained and weighed with electronic scales (0.001 g precision). All gonads were fixed in 10% formalin for microscopic observations using Microscope: Leica DM 2000. The mean Gonad (GI) and mean Repletion indexes (RI) were calculated using the ratio between the gonad weight or intestine weight (GW, IntW) and the total weight of the gutted body (GBW) by the formula (Asha & Muthiah, 2008; Gaudron et al., 2008; Kohler et al., 2009).



Figure 1. Study area: Oran coast, NW-Algeria.

$$\text{Gonad index (GI)} = \frac{GW}{GBW} \times 100$$

$$\text{Repletion index (RI)} = \frac{IntW}{GBW} \times 100$$

Data analysis

The collected data are computed and analyzed with IBM SPSS 20 software. The sex ratio study was tested by chi-square. The differences in both indexes (GI and RI) between sites, months and seasons were tested through a one-factor ANOVA test and the Tukey test. The relations between GI, RI and environmental factors were determined using the Pearson Correlation Analysis.

RESULTS

By using the macroscopic and microscopic observations, the gonads of 640 *H. tubulosa* were sexed as 321 females, 259 males, and 60 unidentified individuals. The sex ratio (female:male) is 0.8:1.2. The sex ratio at La Madrague (female:male) is 1.25:0.79, at Cap Carbon is 1.2:0.83 and at Ain Franin is 1.27:0.78. By chi-square test, the difference in sex ratio compared to sites is not significant ($X_2=2.18$; $df=4$; $p>0.05$) and also not significant

compared to the seasons (spring, summer, autumn, winter) ($X_2= 3.03$; $df=6$; $p>0.05$) (Table 1).

The study was performed on 640 samples collected during sampling campaign. The collected samples allowed us to determine the changes in the Repletion index (RI) and the Gonad index (GI). The averages of repletion and gonad indexes are characterized by a monthly change which is greater for the repletion index (Table 2).

The monthly average repletion index gives the most variations which were the maximum 76.56 ± 14.13 % in October and the minimum 31.47 ± 20.95 % in September at Cap Carbon. For the Ain Franin site the maximum is 97.67 ± 21.26 % in March and the minimum is 22.55 ± 6.22 % in September (Fig. 2). For the La Madrague site, the repletion index is different from the previous sites, the minimum is 20.97 ± 6.40 % in February and the maximum is 81.77 ± 13.47 % in October (Fig. 2).

	Chi-square X_2	df	P
sites	2.18	4	0.702
seasons	3.03	6	0.805

Table 1. The difference in sex ratio compared to sites and seasons by Test Chi-square.

Area		Feb 2014	Mar	Apr	May	Jun	Jul	Agu	Sep	Oct	Nov	Dec	Jan 2015
MA	GI	0.94 (0.56)	1.26 (0.44)	3.58 (2.15)	1.69 (1.11)	16.27 (8.32)	15.86 (8.40)	22.85 (20.71)	14.52 (9.33)	1.49 (2.03)	2.18 (2.09)	0.23 (0.36)	0.2 (0.36)
	RI	20.97 (6.40)	24.32 (10.42)	39.28 (12.76)	35.40 (12.96)	66.67 (12.17)	67.03 (20.21)	64.84 (21.82)	52.27 (9.83)	81.77 (13.47)	72.63 (16.79)	48.84 (25.15)	49.82 (24.74)
C.C	GI	0.53 (0.51)	1.61 (1.22)	1.16 (0.84)	12.57 (4.71)	13.92 (4.39)	9.31 (6.43)	8.79 (6.35)	0.33 (0.52)	0.73 (0.94)	0.81 (0.86)	1.86 (1.79)	1.24 (1.16)
	RI	56.81 (20.32)	51.44 (14.36)	40.48 (15.29)	49.54 (13.54)	49.78 (14.55)	38.55 (13.10)	35.85 (8.67)	31.47 (20.95)	76.56 (14.13)	73.47 (18.46)	73.96 (16.98)	59.11 (11.35)
AF	GI	1.05 (1.33)	1.54 (1.3)1	6.30 (4.40)	4.69 (3.38)	10.75 (3.41)	9.82 (5.67)	10.61 (4.30)	0.22 (0.52)	0.39 (0.55)	1.47 (2.89)	1.5 (1.32)	1.55 (1.19)
	RI	80.47 (19.66)	97.67 (21.26)	67.17 (16.12)	65.33 (27.69)	61.46 (14.36)	42.12 (15.24)	51.63 (19.27)	22.55 (6.22)	68.59 (20.60)	63.31 (20.65)	68.55 (19.02)	61.26 (12.77)

Table 2. Monthly mean evolution of physiological index of *Holothuria tubulosa* in the study area. MAD: La Madrague. CC: Cap Carbon. AF: Ain Franin. Expressed % \pm (standard deviation).

It can be noted that the highest average index of repletion is recorded at Ain Franin where the environment is rich in flora and fauna and with the presence of *Posidonia oceanica* meadows. This site is influenced by sporadic pollution, while the Cap Carbon site is under the influence of industrial pollution (Chahrour et al., 2013). The site of La Madrague presents a stony substrate with some clumps of *Posidonia* K.D. Koenig and it is under the influence of an agricultural pollution (Benghali et al., 2006).

The monthly variations of average gonad index are summarized in figure 3. For Cap Carbon and Ain Franin sites, respectively, the minimum values of 0.33 ± 0.52 % and 0.22 ± 0.52 % were recorded in September, while maximum values of 13.92 ± 4.39 % and 10.75 ± 3.41 % in June. On the other hand, for the La Madrague site, the average gonad index minimum value of 0.2 ± 0.36 % is registered in January and the maximum value is 22.85 ± 20.71 % in August.

The maximum values of the gonad index were recorded in June and August, while during the winter season it is slowly decreasing. This decrease in GI_m means (see Figs. 2, 3) that the gonad development is in pre-reproduction or spawning (Tanaka, 1958; Spirlet et al., 2000). These declines in GI_m denote the existence of an autumn season spawning (September and October), as shown in figure 3.

The statistical study by ANOVA test shows no significant differences between GI_m and the three sites ($df=2$; $F=0.62$; $P>0.05$) in contrast to the monthly and seasonal variation, GI has a significant difference ($df=11$; $F=5.02$; $P<0.05$) ($df=3$; $F=18.59$;

$P<0.05$). Whereas for the RI, the difference is insignificant compared to sites, months and seasons. In this study, the microscopic observations confirm the spawning period of the population at the three sites. The tubules of the females and the males almost resorbed especially in the cold months as has been observed by Conand (1993a). In the hot months the gonads are more mature and prepare to spawn. This behavior was also reported by Navarro et al. (2012).

The females and males gonads are composed of 4 stages of maturity in the whole of the year.

Figures 4–9 shows the microscopic and macroscopic observations of female gonads of *H. tubulosa*. Stage I (recovery stage), in this case, it was not observed.

Stage II. Growing (Figs. 4, 5): tubule with early-vitellogenic oocytes and thick tubule walls.

Stage III. Mature (Fig. 6): tubule with mid-vitellogenic oocytes.

Stage IV. Partly spawned (Figs. 7, 8): tubule with vitellogenic mature oocytes. The mature oocytes with a nucleus, germinal vesicle and follicular epithelium.

Stage V. Spent (Fig. 9): spawned tubule with the presence of relict oocytes and with pointed tubule end.

The microscopic observations of male gonads of *H. tubulosa* are depicted in figures 10–15. Stage I (recovery stage), in this case, it was not observed.

Stage II. Growing (Figs. 10, 11): thick tubule with spermatozoa and growing tubule longitudinal fold.

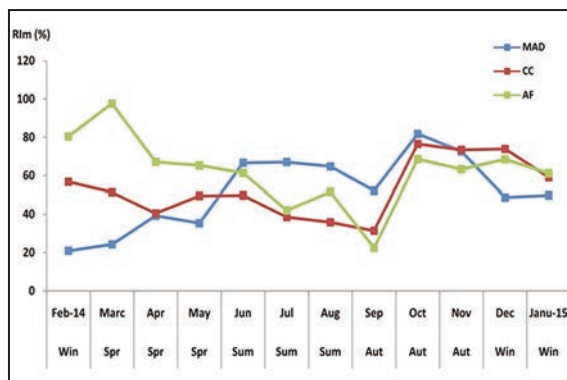


Figure 2. Monthly mean evolution of repletion index *Holothuria tubulosa* in the area. MAD: La Madrague. CC: Ca Carbon. AF: Ain Franin.

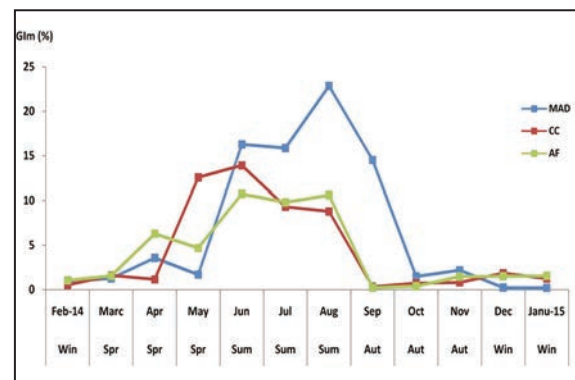
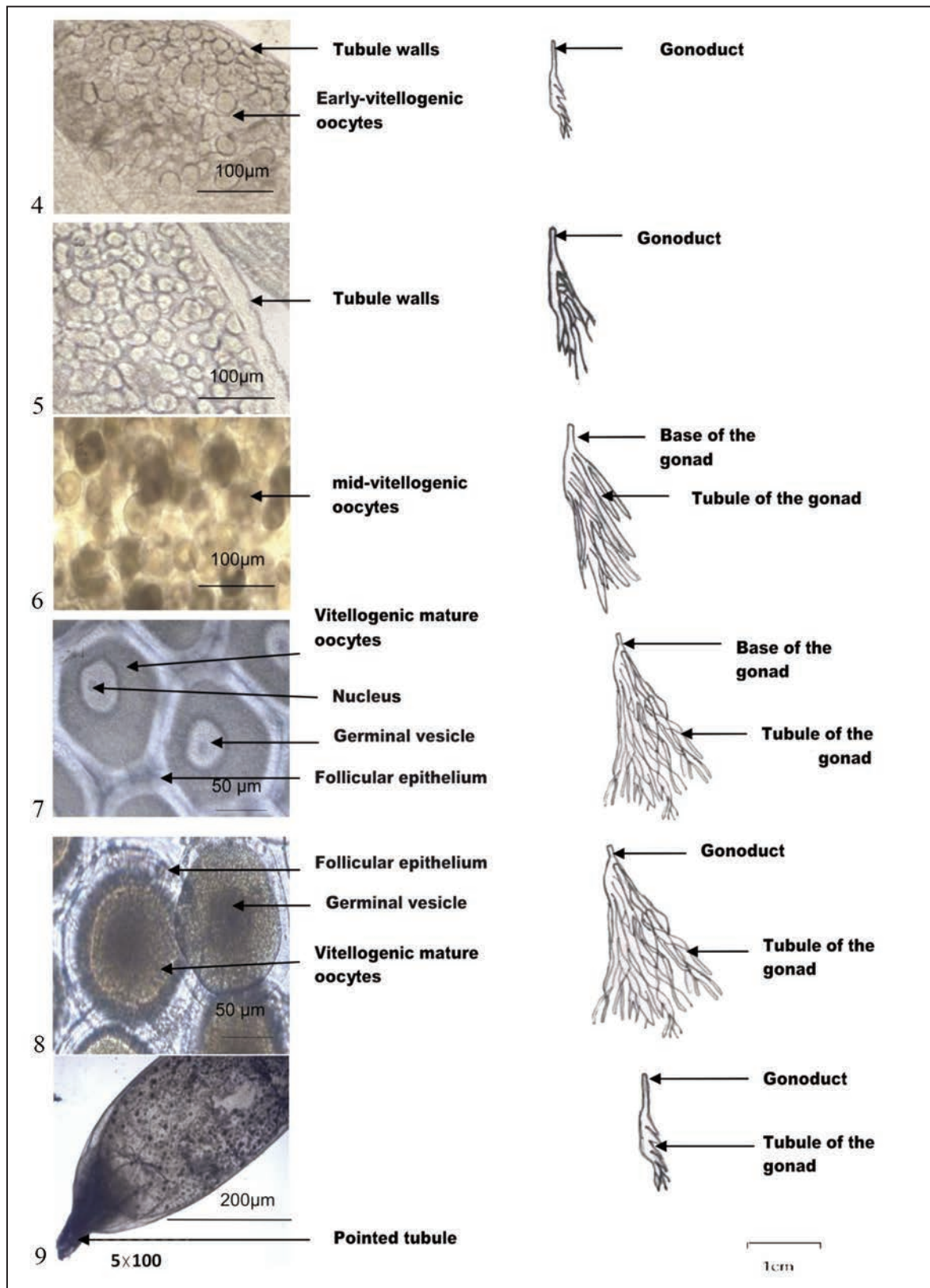
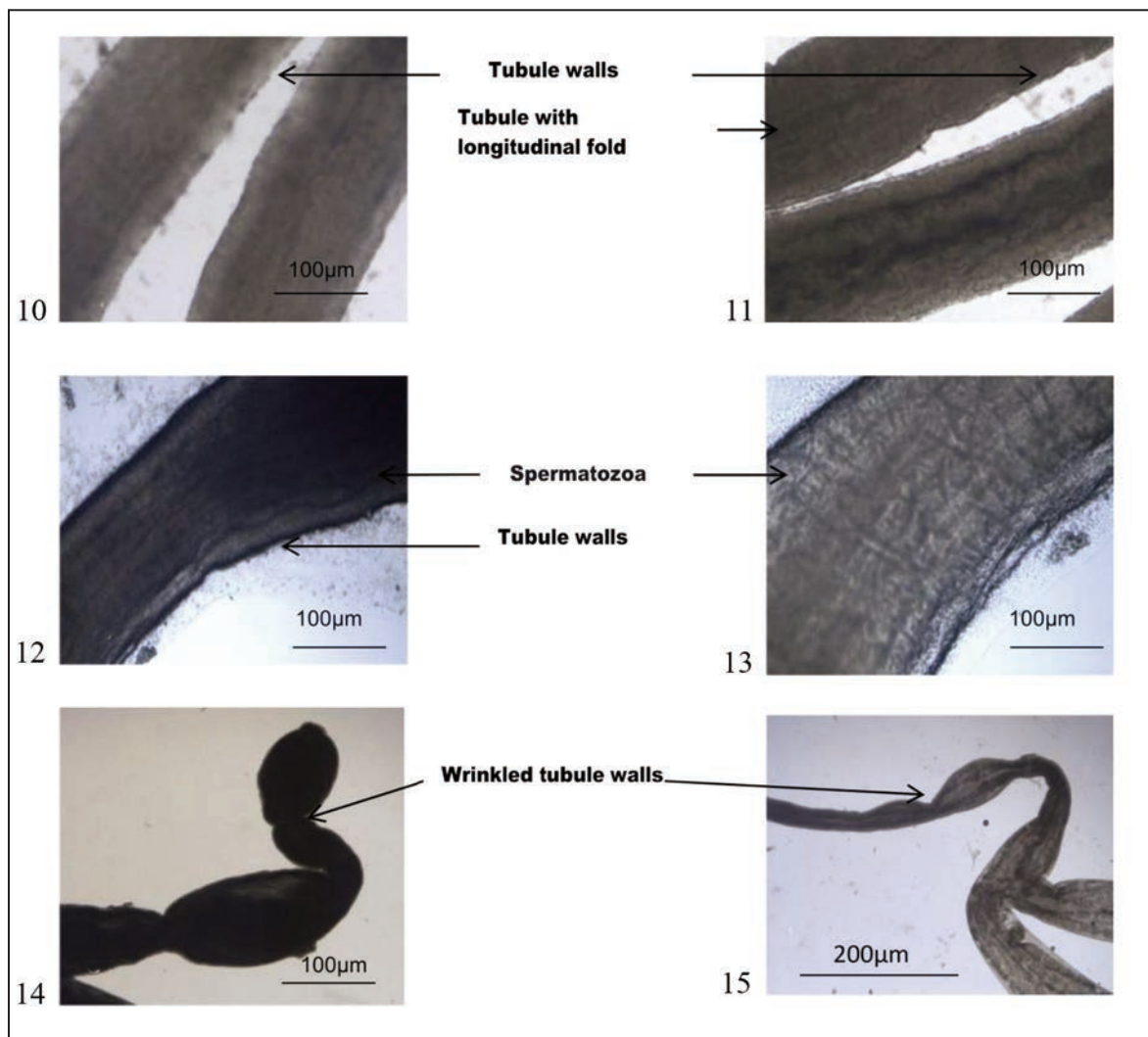


Figure 3. Monthly mean evolution of gonad index of *Holothuria tubulosa* in the area. MAD: La Madrague. CC: Cap Carbon. AF: Ain Franin.



Figures 4–9. Microscopic and macroscopic observations of female gonads of *Holothuria tubulosa*.



Figures 10–15. Microscopic observations of male gonads of *Holothuria tubulosa*.

Stage III. Mature (Figs. 12, 13): tubule with complete spermatozoa.

Stage IV. Partly spawned (Fig. 14): tubule with wrinkled and partly spawned.

Stage V. Spent (Fig. 15): tubule with wrinkled wall and completely spent.

Figures 16–24 show the effect of environmental conditions on gonad index and repletion index, where it can be seen that the temperature is the most important factor in the reproduction and feeding. For the three sites, the spawning period is between September and October where the temperature is around 19–20 °C. For the repletion

index, the highest means are in the autumn season for two sites (La Madrague and Cap Carbon). For Ain Franin, on the other hand, it is during the spring season.

According to Spirlet et al. (2000) and Shpigel et al. (2004), an increase in temperature may increase ingestion and absorption efficiency and stimulate the growth and biological activities of sea cucumbers. In the autumn season, *H. hawaiiensis* Fisher, 1907 ingests more sediments than during the rest of the year to allow the growth of his sexual organs and the expulsion of gametes (Dar & Ahmad, 2006). Also the previous results confirm that the temperature is the most important factor affecting

the reproduction of *H. tubulosa* (Navarro et al., 2012; Dereli et al., 2016; Bahiri et al., 2017).

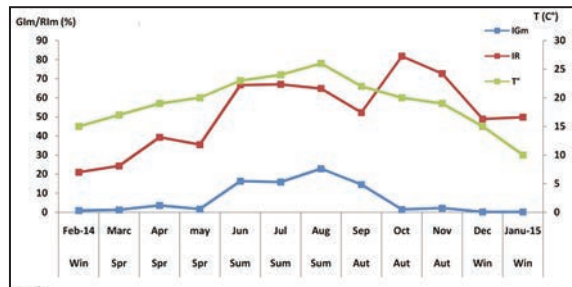
The average physical seawater parameters of the three sampling sites in Oran coast are summarized in Table 3.

There is no significant difference between the stations, the temperature, and pH. However, there is a significant difference between salinity and the study sites.

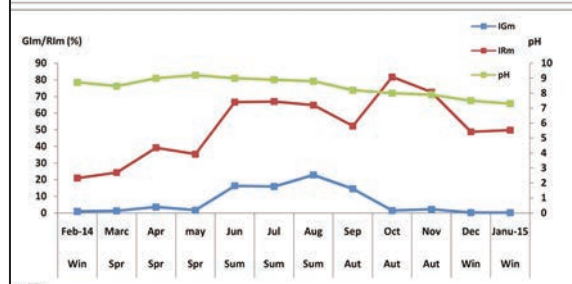
The temperature has a positive relationship with gonad index ($p < 0.01$) as shown by the Pearson correlation analysis, presented in Table 4, and no correlation between temperature and index repletion. The pH has a positive correlation with gonad index ($p < 0.05$) and also has a positive relationship with the temperature ($p < 0.01$).

DISCUSSION

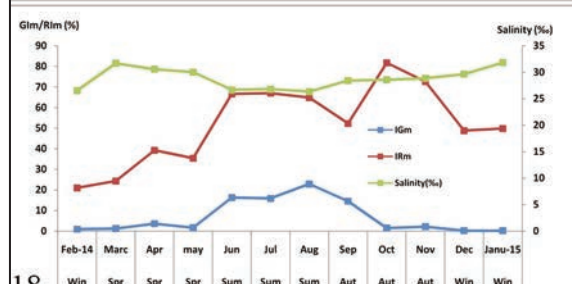
The sex ratio obtained from *H. tubulosa* in this study shows a significant difference in the relationship 1:1. In most sea cucumbers of the Aspidochirotrida order the sex ratio usually coincides with a balanced relationship 1:1 (Ramofafia et al., 2001; Rasolofonirina et al., 2005; Asha & Muthiah, 2008). Despite some species show a slightly unbalanced ratio, with more males than females, or vice versa (McPherson, 1965; Navarro et al., 2012), some sea cucumber species have an unbalanced ratio of 1:2 or 2:3 due to fishing pressure affecting the population (Shiell & Uthicke, 2006; Muthiga et al., 2009). In Algeria, sea cucumbers are not exploited, thus it is assumed that



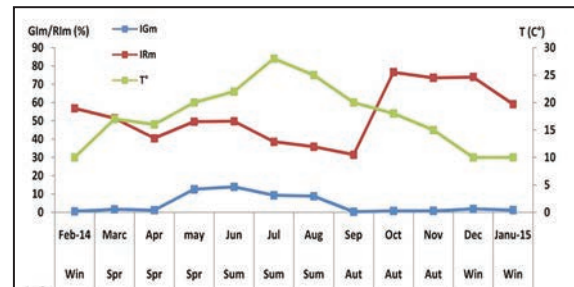
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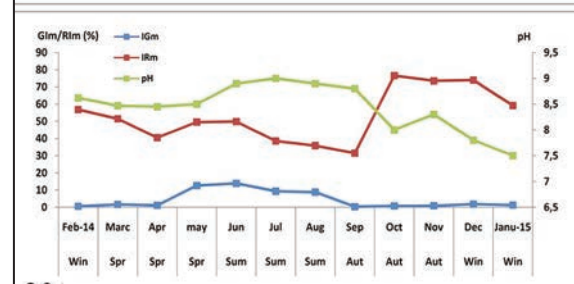
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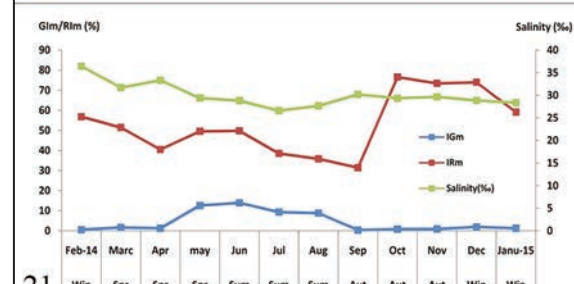
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Figures 16–18. Monthly mean gonad index and repletion index of *Holothuria tubulosa* at La Madrague. Mean seawater temperature (Fig. 16), pH (Fig. 17) and salinity (Fig. 18).

Figures 19–21. Monthly mean gonad index and repletion index of *Holothuria tubulosa* at Cap Carbon. Mean seawater temperature (Fig. 19), pH (Fig. 20) and salinity (Fig. 21).

there is another important factor that affects the balance of the sex ratio. This sex ratio unbalance can be explained by the pollution or the hydrodynamics which often favors the reduction of the populations as a response to stress.

In a population of *H. tubulosa*, the determination of the fluctuations of the digestive contents weight and the gonads is an essential approach to the trophic and spawning study of this marine invertebrate. It allows, primarily, to determine the structure and the evolution of benthic phytocenosis (Nedelec, 1982; Verlaque & Nedelec, 1983a, b) and secondly to clarify the gonadal activity.

The average repletion index shows maximum values in October and March and minimum values in September and February. Dar & Ahmad (2006)

observed that *H. atra* Jaeger, 1833 in the Red Sea has the highest average weight and the lowest average percentage of sediments in spring. They concluded that at this time of the year the most individuals are at the stag, which precedes the maturation and are preparing for the period of reproduction, from June to July. They also observed (Dar & Ahmad, 2006) that the highest mean sediment weights were recorded in summer when individuals need sediments to contract and expel their gametes.

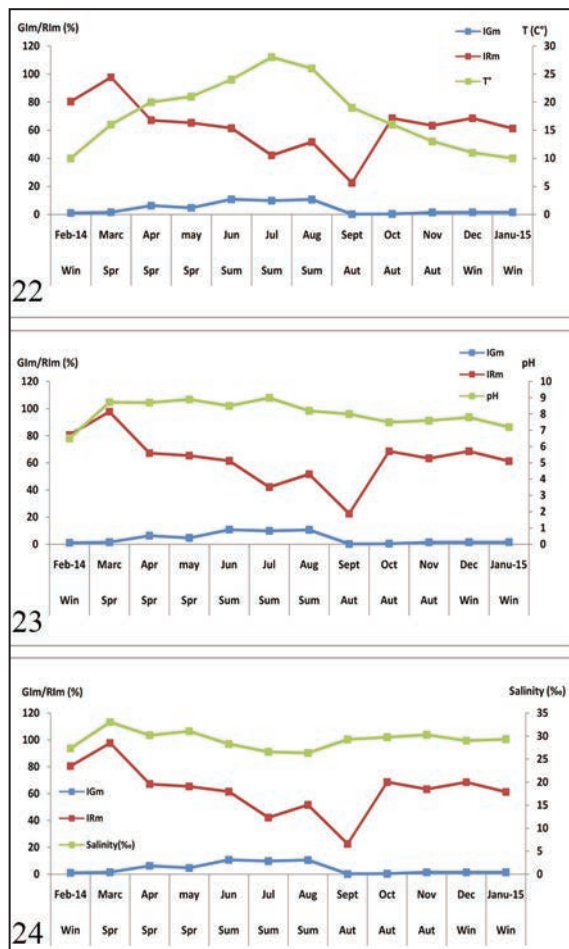
Feeding behavior and feeding mechanism of sea cucumbers during the different seasons are related to the stage of sexual maturation of the animal (Dar & Ahmad, 2006). The sea cucumbers often show an annual cycle of reproduction as observed by other studies (Navarro et al., 2012; Mezali et al., 2014; Dereli et al., 2016).

The study shows that GI_m values increased during the year, and the spawning peak is registered when the GI values are low in October for the La Madrague site and in September for the other two sites (Ain Franin and Cap Carbon), while GI values have their maximum between June and August. The field observations show and confirm that spawning occurs in shallow waters during the summer in the Mediterranean populations (Valls, 2004; Moosleitner, 2006; Andrade et al., 2008). The results of our study coincide with those of the work performed in the regions of the Dardanelles Strait (Dereli et al., 2016) and of the Aegean Sea (Kazanidis et al., 2010; Kazanidis et al., 2014) and other regions (Despalatović et al., 2004; Navarro et al., 2012) (see Table 5 with *H. forskali* Delle Chiaje, 1823 and *H. sanctori* Delle Chiaje, 1824).

For many species of echinoderms, the spawning is extended over a long period, but the reproduction season can be shortened when the environmental conditions are not favorable for a short period (Giese, 1987; Chia & Walker, 1991; Pearse & Cameron, 1991).

The correlation was observed between GI_m of *Holothuria tubulosa* and the sea water temperature of the Oran coast. This result confirms previous studies that claim that the temperature is the most important factor affecting reproduction of *H. tubulosa* (Despalatović et al., 2004; Kazanidis et al., 2010) and other congeneric species (Conand, 1981; Tuwo & Conand, 1992; Despalatović et al., 2003).

The seasonal variations of seawater temperature and photoperiod are common parameters correlated



Figures 22–24. Monthly mean gonad index and repletion index of *Holothuria tubulosa* at Ain Franin. Mean seawater temperature (Fig. 22), pH (Fig. 23) and salinity (Fig. 24).

Area	T°(C)	pH	Salinity (‰)*
La Madrague	19.16±4.26	8.42±0.66	28.85±1.89 ^a
Cap Carbon	17.58±5.85	8.44±0.45	30.03±2.56 ^b
Ain Franin	17.83±5.94	8.05±0.73	29.22±1.83 ^a

Table 3. The comparison of physical parameters at stations in Oran coast (mean ± standard deviation). *: Significant difference between salinity and sites (Madrague and Cap Carbon. $p < 0.05$; Cap Carbon and Ain Franin. $p < 0.05$).

with reproductive synchrony (Booolootian, 1966; Giese & Pearse, 1974). Although, some echinoderms appear to show more prolonged or continuous spawning throughout the year in tropical climate regions (Harriott, 1985).

In literature, some studies show the existence of a close relationship between temperature and reproduction of *Actinopyga mauritiana* (Quoy & Gaimard, 1834) in the island of Guam (Conand & Sloan, 1989; Conand, 1993a, b). In fact, in the Mediterranean Sea, another study suggests that the spawning would be induced when the water temperature is between 13–20 °C (Fenaux, 1980). In our study, it is clear that the beginning of spawning depends on the sea temperature in the populations

of the three sites (Figs. 4a, 5a, 6a). The starting point of the spawning of this echinoderm is much more induced by the sudden increase of sea temperature due to salinity and pH. There are other factors favoring the induction gametic, such as turbulence (Pedrotti, 1993; Soualili, 2008), the phytoplankton blooms (Pedrotti, 1993; Starr et al., 1993), the hydrodynamics (Soualili, 2008; Dermeche, 2010; Navarro et al., 2012) and the photoperiod (Starr et al., 1993; Spirlet et al., 1998).

The effect of sea temperature and light variations on sea cucumber feeding activity is not extensively documented in the literature. Coulon & Jangoux (1993b) estimated that a sea cucumber population can filter from 6 to 13 kg of sediments according to temperature and body size. Due to this activity, the sea cucumbers alter the stability of the seabed (Massin, 1982; Coulon & Jangoux, 1993b; Dar & Ahmad, 2006).

CONCLUSIONS

In this study, it has been able to estimate and evaluate the spawning period of *H. tubulosa*. The sex ratio was found as (female: male) 0.8:1.2 with no significant difference between sites and seasons (spring, summer, autumn, winter). The spawning period was between September and October in the west coast of Oran. The peak spawning is reg-

	T°(C)	pH	Salinity (‰)	GI	RI	station	Months
pH	0.569**						
Salinity (‰)	0.015	0.176					
GI	0.712**	0.370*	-0.131				
RI	-0.232	-0.242	-0.220	0.018			
Station	-0.101	-0.194	0.027	-0.173	0.243		
Months	-0.224	-0.339*	-0.148	-0.137	0.206	0.000	
Seasons	0.563**	0.195	0.057	0.246	0.053	0.000	0.194

Table 4. Correlation between physical parameters of areas, physiological index (GI, RI), months and seasons.

*: significantly different ($p < 0.05$). **: significantly different ($p < 0.01$).

istered where the values of GI are low in October for the La Madrague site with values of 1.49 ± 2.03 %, and in September for the other two sites of Cap Carbon and Ain Franin, with values of 0.33 ± 0.52 % and 0.22 ± 0.52 % respectively, while the GI values have their maximum between June and August.

The temperature of seawater seems to play a crucial role in the release of gametes.

Other studies are also needed on this coast and generally in all the Algerian coasts in order to obtain more information (density, mortality, and longevity).

Species	Location	Methods	Reproductive pattern	Spawning period	Reference
<i>H. tubulosa</i>	Oran coast, Algeria	GI	Annual	Sept-Oct	Ower study
<i>H. tubulosa</i>	Oran coast, Algeria	GI	Annual	Mar- Apr-Jul and Oct	Bahiri et al., 2017
<i>H. tubulosa</i>	Dardanelles Strait, Turkey	GI	Annual	August and September	Derlie et al., 2016
<i>H. tubulosa</i>	Pagasetikos Gulf, western Aegean Sea, Greece	Histological examination	Annual	Summer	Kaznidis et al., 2014
<i>H. tubulosa</i>	Adriatic Sea, Croatia	Histological examination	Annual	Jul- sept	Despalatovic et al., 2004
<i>H. tubulosa</i>	Costa Brava, the north east coast of Spain	Field spawning observation		June and July	Valls, 2004
<i>H. tubulosa</i>	Ischia Island, Italy	Field spawning observation	Annual	August-sept	Bulteel et al., 1992
<i>H. forskali</i>	Britain	Histological examination and GI	Annual	Apr- June	Tuow & Conand, 1992
<i>H. forskali</i>	Adriatic Sea	Histological examination	Annual	Jun-August and Dec	Despalatovic et al., 2003
<i>H. sanctori</i>	Gran Canaria, Spain	GI, macro and microscopic analysis of the physical characteristics of tubules.	annual	Jun-August	Navarro et al., 2012

Table 5. Characteristics of Holothurian species reproduction. Location, methods used and spawning period.

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