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Reproductive activity of three sea cucumber species *Holothuria forskali*, *Holothuria sanctori* and *Holothuria tubulosa* in WSW Alboran Sea, M'diq Bay, Morocco

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ABSTRACT

Sea cucumber is one of the resources under fishing pressure, which needs more knowledge and studies to manage its sustainability. This study was conducted to provide necessary information on the reproduction cycle, maturity evolution, size at first maturity and other parameters of *Holothuria forskali*, *Holothuria sanctori*, and *Holothuria tubulosa* in the M'diq Bay. The three species showed variability in the morphometric measurements with superiority for *H.sanctori* and *H.tubulosa*. The potential spawning period and the gonad index "GI" peaks were different from one species to another, with a larger spawning period and an important GI percentage for *H. sanctori*. The first maturity size was above 17.50cm and more than 250g for the three species. The environmental factors and GI correlation varied among species under study. Furthermore, the percentage of potential individuals assumed in the spawning period did not manifest a correlation with the environmental factors for the three species. This work can provide a strong baseline for further studies in this region, introducing an important contribution to the management of the studied species in fisheries and acting as an essential opening for the three sea cucumbers farming in near geographical areas.

INTRODUCTION

The irrational exploitation or overharvesting represents one of the unreasonable human impacts on marine resources. The last decade has witnessed a decline in marine captures **(FAO, 2018)**. Sea cucumber, as one of the invertebrates, is experiencing an exponential fishing pressure. From 1950 to 2017, regardless of the species (*Apostichopus japonicus*, *Holothuroidea* and *Cucumaria frondosa*), the capture increased by around 1140 % **(FAOSTAT, 2019)**, which necessitates effective solutions to relieve or reduce the pressure on this resource.

In Morocco, sea cucumber fishing is not a distinct fishing category in the national reports; it has been declared in the Echinoderms class. Moreover, the statistics of this class were not published until 2010. Between 2010 and 2018, the captured quantity was averagely about 50 tons and reached a maximum of 112 tons in 2015 **(ONPstatistics, 2019)**.

Thus, the Moroccan government has suspended sea cucumber fishing after 2011 and has been devoting remarkable effort to control the illegal fishing of these species. On many occasions, the concerned authorities announced that they arrested some interested IUU (Illegal, Unreported and Unregulated) sea cucumber quantity. In the same context, the Moroccan government declared its willingness to preserve this resource, adopt concrete measures to ensure sustainable preservation and exploitation and provide the procedures that optimise the resource recovery, such as restocking programs for the threatened areas.

To be in line with these factors, it is necessary to have a clear idea about the reproductive activity of these species, which is the key to their fishery management and aquaculture development, either in a general context or in a specific area. Therefore, the lack of data about sea cucumber species in the Moroccan Mediterranean Sea reveals a serious need for scientific research in this field.

Therefore, this study was organized to contribute to the knowledge of three sea cucumber species *Holothuria forskali, Holothuria and Holothuria sanctori* reproduction cycle, maturity characteristics, and the possible temporal variations. Furthermore, this study can be a support for the management of the three species in the studied area on the Moroccan Mediterranean coast or worldwide.

MATERIALS AND METHODS

General description

The sea cucumbers have a large habitat distribution **(Slater & Chen, 2015)**. In the studied area, the three species are found in relatively different microhabitats; *H. sanctori* is found in rocky bottom with the presence of detritus, sand and small seaweed. *H. tubulosa* is found in fine gravel bottom mixed with sand, near to seaweed. This species, unlike the two others, can be found in greater depth (more than 15 m). Furthermore, both the two previous species can co-exist in the same area being unequal. *H. forskali* is generally observed near rocky habitats covered with fine sand and slight detritus; this species is rarely found near the previous ones.

Generally, the studied species were collected at a depth between two and eight meters. Prior to data acquisition, several sampling campaigns were conducted to allow observing 15 to 20 collected *H. sanctori* or *H. tubulosa* specimens, and about 10 *H. forskali*.

Considering the prohibition set by the Moroccan government, which is conditioned by authorisation delivered by the Moroccan National Institute of Fisheries Research “INRH”, 10 individuals of each species represented the acceptable number to proceed in the sampling. It is worth noting that, the small individuals were not permitted to be collected, and generally, the sampling was executed in the three middle days of each month; from February 2018 to Mars 2019.

Morphometric measurements

The harvested individuals were transported to the laboratory and taken out of the water for 5 to 7 minutes to evacuate the internal water **(Sun *et al.*, 2012)**. Afterwards, a massage was performed on the individual entire body, by rolling it slowly and gently (hand-rubbing)

(Yingst, 1982; Laboy-Nieves & Conde, 2006) to stimulate the contraction of the longitudinal muscle bands and get the maximum contracted form.Then, the morphological data were determined; total length (Tl), total weight (Tw) (named also Dried weight Dw), internal organs' weight (IOw), gonad weight (Gw) and gutted body weight (GBw) (named also eviscerated wet weight (EW)). Moreover, 1cm from the middle of the gonad tubule was observed under a reversed microscope or the microscope. As described by **Liu *et al.* (2015)**, the gonad index (GI) was calculated by dividing the gonad weight (Gw) over the GBw.

$$GI(\%) = \frac{Gw}{GBw} \times 100$$

Length-Weight Relationship

The length-weight relationship was calculated using the following equation of **(Pauly,1984):**

$$GBw = aTl^b$$

Where, "a" is the intercept and "b" is the slope.

Maturity stages and first maturity size/weight

The individual's maturity was classed on six categories based on a combination between **Conand (1981)** and **Ramofafia *et al.* (2000)** maturity stage categories. I. Immature, II. Recovery/Resting, III. Growing, IV. Mature, V. Partly-spawned, and VI. Spent/Post Spawning.

Following **Conand (1981)**, the size and/or the GBw at first sexual maturity were defined at the level where 50% of the individuals are mature, and this percentage is extrapolated on the TL and the GBw and used to determine consecutively the LT50 and GBw50.

Regarding the necessity of a practical measure that can be used by fisherman, we examined the first sexual maturity next to the total weight (Tw50) instead of GBw50. This measure was used in **Navarro *et al.* (2012)**, while it can provide a reasonable baseline for fishery management of these species and determine their minimal harvesting weight in this area.

The spawning month can refer to the month(s) when the individuals have the potential to spawn; this can be determined based on the percentage of individuals reaching stage IV or above.

As described by **Tuwo and Conand (1992)**, stage VI resorption can take several months. For that, the spawning months in our study were determined based on the individuals reaching stages IV and V and surpassing 50 % of the sampled population.

Environmental data

For the study period, the monthly average sea temperature (ST) was calculated dependng on real data (1.5m below sea level). The chlorophyll-a concentration (Chl-a) was evaluated using the Copernicus Marine Environmental Monitoring Service (CMEMS) (http://marine.copernicus. eu/faq/cite-cmems-products-cmems-credit/?idpage=169) which has a spatial resolution of 0.042degree x 0.042degree (around 4 Km).

Furthermore, for the photophase duration, it was determined on basis of the data provided by sunrise and sunset website (timeanddate.com).

Statistical analysis

Data are presented as mean ± standard error (SEM). ANOVA test used to examine the differences in Tw, Tl, GBw, and GI within months.

The deviation percentage from the population mean ($dev\% = \frac{|\bar{X}|-\mu}{\mu} \times 100$) known also as percent deviation, was calculate to express the stability of some value.

A chi-square test was applied to examine the species population difference from the sex ratio 1:1 and/or not 1:2.

The length-weight relationship was used to determine whether the growth of each sea cucumber population was isometric or allometric **(Pauly, 1984)**. A linear regression analysis was applied to determine the Length-weight relationship coefficient (a and b), the significance of the regression was assessed with the F-statistic.

Pearson's correlation was used to determine the relation between Gw and GBw before spawning. Furthermore, the same statistic test was performed to evaluate the correlation of the maturity evolution and the GI next to the Tw, where the Tw was classed in 25g categories.

The GI and the percentage of potential individuals to spawn (IV and V) monthly means were tested for correlation and cross-correlation next to the study period monthly mean photophase, temperature and Chl-a.

Statistical analysis were performed using RStudio 1.2.1335 (**RStudio Team, 2018**). the used packages were Kruskal.test (**Package stats version 3.6.0; Hollander & Wolfe, 1973**), shapiro.test (**Package stats version 3.6.0; Royston, 1982**), ggplot2 (**3.1.1; Wickham, 2016**), ggpubr (**0.2; Kassambara, 2018**), tseries (**0.10-47; Trapletti & Hornik, 2019**) and devtools (**2.0.2; Wickham *et al.*, 2019**).

RESULTS

Morphometric measurements

Generally, for the morphometric parameters (Tw, Tl, GBw) (Fig. 1), *H. sanctori* and *H. tubulosa* showed higher values compared to those recorded for *H. forskali*. Similarly, the Tw mean was 261.16±64.61g and 260.07±83.22g for the 1^{st} & 2^{nd} species, respectively, which is higher than the third species value by around 21.71% (213.60±63.01g).

The total length mean was respectively 18.79 ±2.82 cm and 19.10±3.74 cm; these numbers are higher than the one recorded for *H. forskali* by around 13.87% (Tl =16.64±3.52 cm).

The gutted body weight for the three species is around 38% of the Tw (for *H. sanctori* is 37.77%, *H. tubulosa* is 39.21% and *H. forskali* is 37.88%). The GBw was higher in the first two species by about 20.58% compared to the 3rd species; The GBw values were 162.56±41.69 g, 158.09±48.43 g, and 132.68±34.73 g, respectively for the 3 species..

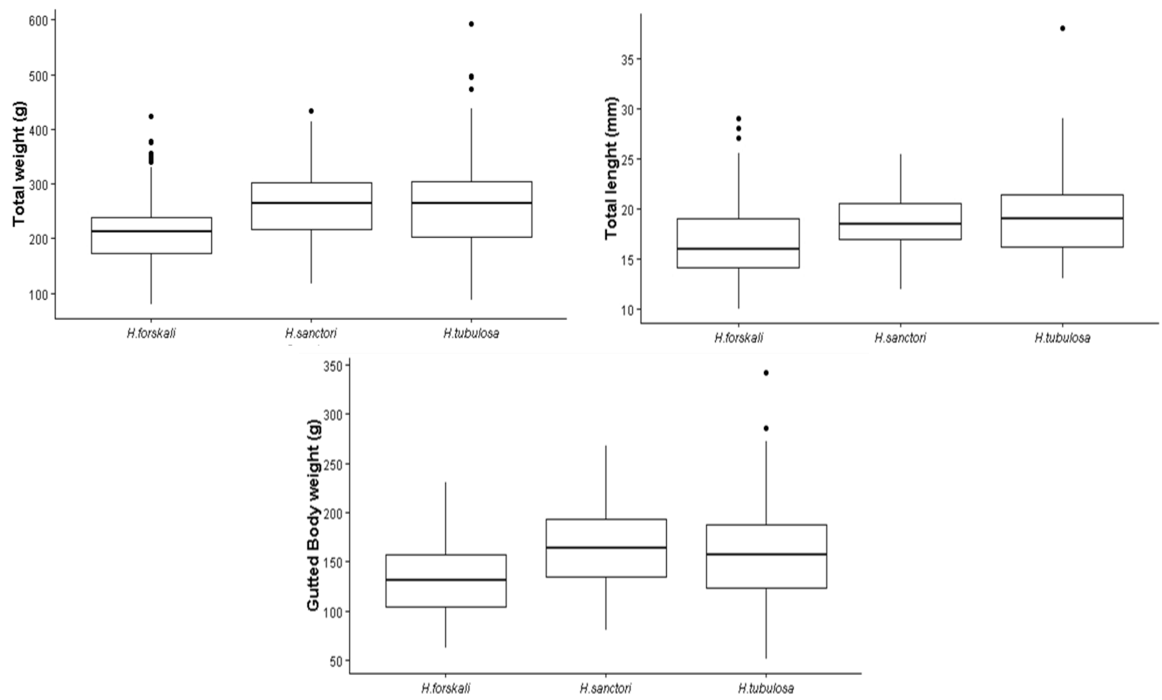

Fig. 1. Box-whisker plots of *Holothuria forskali*, *H. sanctori*, *H. tubulosa* morphometrics characteristics (Total weight (Tw), Total length (Tl), Gutted Body weight (GBw)) (n=140).

The ANOVA test (Table 1) demonstrates a significant difference between months for the three species characteristics, except for *H. sanctori* Tw where there was no significant difference between months. Furthermore, for the *H. forskali* GI, kruskal test shown a significant difference between months (Chi square = 74.396, $p = 1.23x10^{-10}$, d.f = 13).

Table 1. ANOVA test results for Morphometric measurements next to Month variation.

(d.f = 13)

'*' p <0.05, '**' p <0.01, '***' p <0.001 and 'ns' non-significant.

Species	Elements	F	P	Significant level
H.forskali	Tw	4.497	$2.94x10^{-06}$	***
	Tl	5.69	$4.07x10^{-8}$	***
	GBw	2.36	0.0074	**
H.sanctori	Tw	1.427	0.156	Ns
	Tl	1.882	0.038	*
	GBw	1.873	0.0393	*
	GI	9.776	$7.34x10^{-14}$	***
H.tubulosa	Tw	2.835	0.00134	**
	Tl	2.76	0.00176	**
	GBw	2.317	0.00862	**
	GI	9.558	$1.41x10^{-13}$	***

Total weight (Tw):

In general, the monthly Total weight (Tw) mean (Fig. 2) is characterised by a fluctuation. For the *H. forskali*, a maximum month value was recorded in March 2018 (293.10±82.96g) and a minimum value was in December (158.72 ±51.65g). In *H.sanctori* case, the lower value was recorded in March 2018 (210.30±55.05g) and the highest mean value was observed in June 2018 (291.10±77.81g). *H.tubulosa* had a high value in May 2018 (361.80±70.59g) and a lower one in December (185.67±57.42g).

Total length (Tl):

The monthly Tl mean (Fig. 3) have relatively a stable variation, a low standard deviation. For *H. forskali*, the maximum Tl mean was 21.10±4.06 cm and the minimum Tl mean was 13.93±1.75 cm, recorded consecutively in March 2018 and November 2018. *H.sanctori*, maximum Tl mean 20.45±2.36 cm was recorded in February 2018 and the minimum Tl mean 17.49±2.31 cm was recorded in November 2018. *H.tubulosa* maximum Tl mean 22.99±2.92 cm was recorded in February 2019 and the minimum Tl mean 16.90±2.33 cm was recorded in July 2018.

Gutted Body weight (GBw):

The GBw (Fig. 4) is characterised by a variable modality for each species. For *H. forskali*, the percent deviation (*dev%*) did not surpass 9.51% in 42.86% of the studied period (in 3 months did not exceed 2.17% and for the same duration it was between 6.57% and 9.51%) and was around 13.50% for 50% of the studied months. Based on these findings, we can assume that the *H.forskali* population's GBw have relatively a stable deviation from the general mean, where if we exclude the maximal *dev%*, the GBw extent is between 134.28±10,96 and 153.73±29,04 g. The unique maximal deviation percentage was recorded in December 2018 (106.74± 24,53 g).

The *H.sanctori* GBw variate between 134.30±38.21 and 190.62±29.72 g. Precisely, the *dev%* in 50% of the studied period did not exceed 6.79%, in three months was between 8.27% and 11.76% and for four months was between 14.61% and 17.38%. As a general view, we can describe that this species was in majority near to the population mean ($\mu\pm\sigma$=162.56±41,69g).

For *H.tubulosa*, the *dev%* did not exceed 7.08% (between 0.74% and 7.08%) in 57.14% of the studied period, it did not exceed 15.61% (between 11.69% and 15.61%) in 3 months and exceed 20.89% (between 20.89% and 27.90%) in 21.43% of the studied period. So generally, the GBw are not distributed in a homogeneous way and the extent goes from 121.50±28.48g calculated for December 2018 to 202.20±38.75g calculated for May 2018.

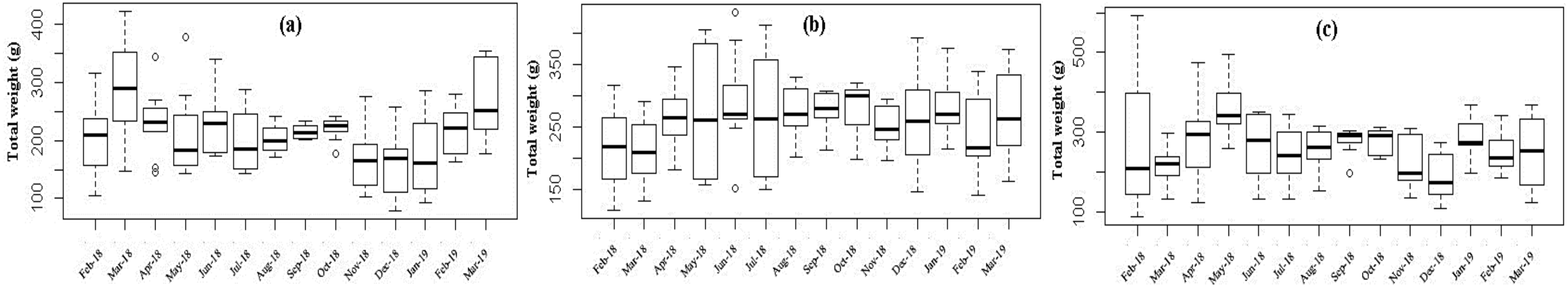

Fig. 2. *Holothuria forskali* (a), *H.sanctori* (b), *H.tubulosa* (c) (n=140) Total weight (Tw) temporal variation.

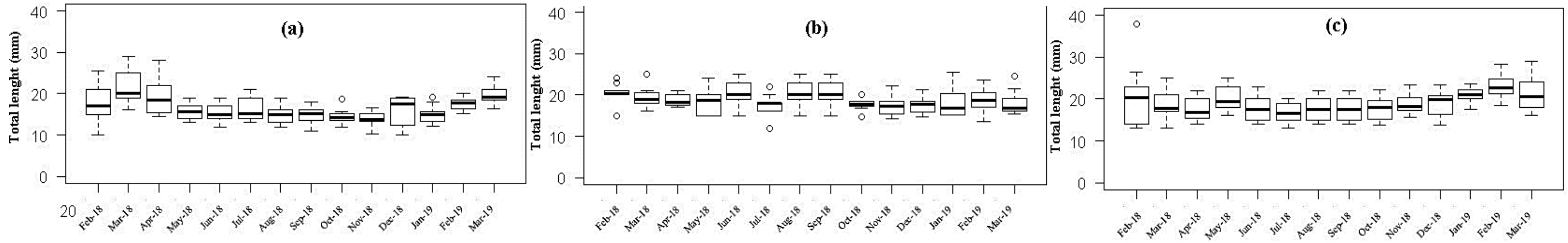

Fig. 3. *Holothuria forskali* (a), *H.sanctori* (b), *H.tubulosa* (c) (n=140) Total length (Tl) temporal variation.

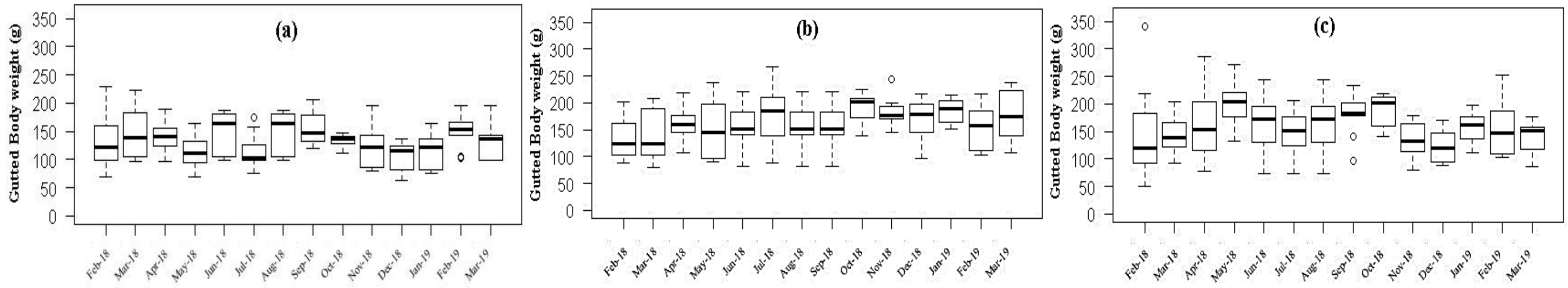

Fig. 4. *Holothuria forskali* (a), *H.sanctori* (b), *H.tubulosa* (c) (n=140) Gutted body weight (GBw) temporal variation.

Length-Weight Relationship:

The Length-Weight relation illustrates; general inferiority of the slope "b" then three, which mean that generally, the three species are allometric (*H. tubulosa* b=0.0406, a=12.686; *H.sanctori* b=0.017, a=16.042; *H.forskali* b=0.046, a=10.465). The F-test present that *H. tubulosa* adj R^2=0.271, p=2.708×10^{-11}; *H.sanctori* adj R^2=0.056, p=0.003; *H.forskali* adj R^2=0.204, p=1.259×10^{-08}.

GBw and Gw correlation:

The Pearson test show that the GBw and Gw manifest a significant correlation for *H. sanctori* (Cc= 0.31, p-value=0.003) and *H. tubulosa* (Cc= 0.481, p-value= 1.398e-05) and not a significant correlation for *H. forskali* (Cc= 0.064, p-value=0.622).

Maturity stages and Gonad Index:

Maturity:

For the three species, the Gw may have different values inside the same maturity stage (Fig. 5). The mature stages (stage IV, V and VI) for *H. sanctori* are presented throughout the year. For *H.tubulosa* these stages are absent during September 2018 and January 2019. Moreover, for *H.forskali* they are absent in August and September 2018.

The spawning months for *H. forskali* are March, October and December. Where the value of March 2018 was 60% and 2019 was 90%, for the other months it was 50%.

For *H.sanctori* the spawning months are April "60%", June "90%", September "70%", October "90%" and February "50%". The difference between 2018 and 2019 can be explained by the percentage of recovery/resting individuals, which means the ones collected in February 2018 had spawned at the beginning of the month, otherwise, the collected ones in 2019 are in stage IV and V.

The *H.tubulosa* spawning months are in June and October where the values are consecutively 80% and 70%.

The Gonad Index "GI":

H. forskali GI is characterised by three peaks in March, July, and October. The peak in March was both in 2018 and 2019, which are consecutively 12.77±13.63% and 15.37±7.03%. The other peaks were 8.99±13.55% in July and 15.63±4.15% in October. The GI was almost nil in May, June and August. Excluding the foregoing months (the peaks and the values near to zero), the Monthly GI mean was between 1.86% and 7.89%.

For *H.sanctori* GI monthly mean was comprised between 1.24±1.57% and 23.12±13.45%, except in November where the GI was near zero (0.20±0.21%). In four months, the GI reached a value above 10%; in April GI=11.17±5.24%, June GI= 23.12±13.45%, September GI= 17.03±8.63% and I October GI=15.82±2.79%.

The *H.tubulosa* GI monthly mean was around zero in two months (November and December). From May 2018 to October 2018, GI monthly mean were above 11.25%. For the rest of the studied period, the values were comprised between 1.94 and 9.06 %.

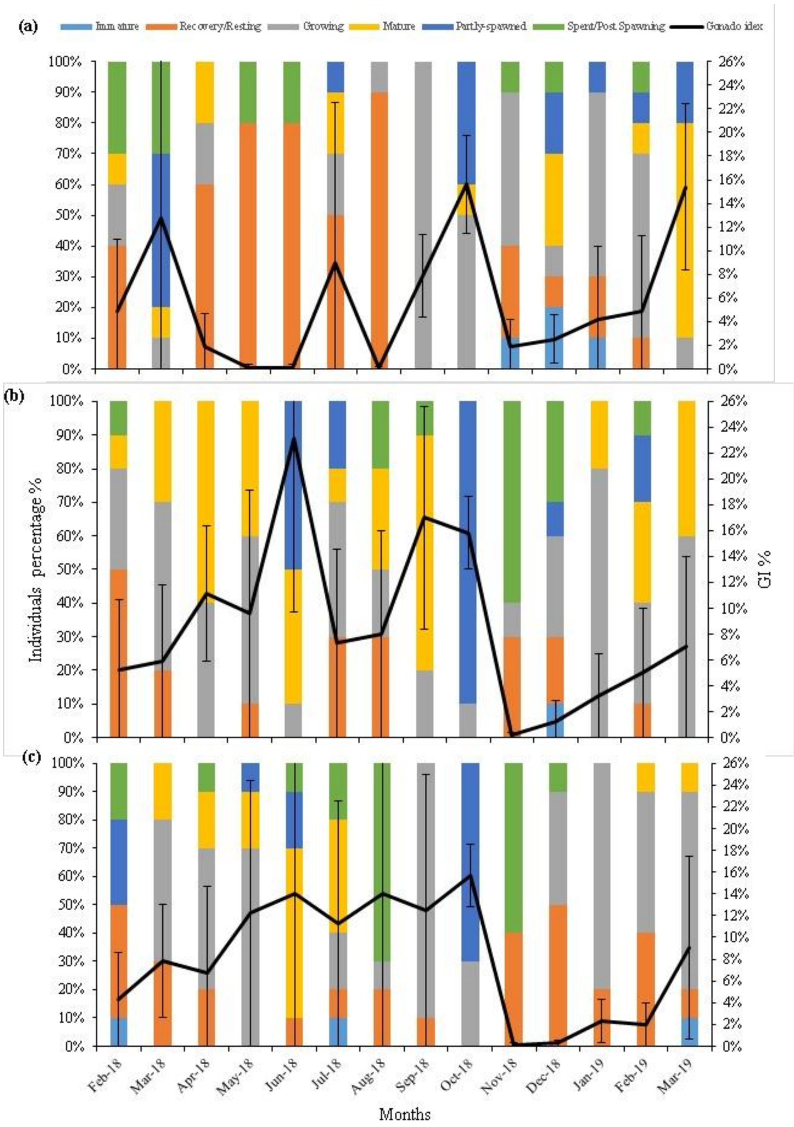

Fig. 6. Maturity stages evolution and the Gonad Index Monthly mean ±SEM for (a) *Holothuria forskali*, (b) *H.sanctori* and (c) *H.tubulosa*.

First maturity size/weight and sex ratio:

The size/weight at first maturity may vary from species to another. The Total weight at the first maturity Tw_{50} was above 250 g for the three species (*H. forskali*, Tw_{50}=250g; *H.sanctori*, Tw_{50}= 275g; *H.tubulosa*, Tw_{50}=312 g). Otherwise, the Total length at the first maturity Tl_{50} was almost similar for the three species (*H. forskali*, Tl_{50}=18.12cm; *H.sanctori*, Tl_{50}= 17.50 cm; *H.tubulosa*, Tl_{50}=18.10 cm) (Supplementary materials).

After excluding the unknown sex character, the Chi-square test was performed to examine each species sex ratio. For *H. forskali* sex ratio was significantly different from 1:1 (df=1, χ^2=16.667, p= 4.456×10^{-5}) and did not differ significantly from 1:2 sex ratio (df=1, χ2=0.75, p=0.386). Concerning *H. sanctori* and *H. tubulosa* both populations did not differ significantly from the 1:1 sex ratio (*H. sanctori*, df=1, χ2=3.03, p=0.082 and *H. tubulosa*, df=1,□ χ2=0.21, p=0.65).

Maturity and the GI:

Pearson's, correlation test shown a positive correlation between the Maturity and the GI, where generally the Maturity increase when the GI increase (*H. forskali*, R=0.64, p= 0.015; *H.sanctori*, R=0.79, p=0.001; *H. tubulosa*, R=0.94, p= 1.2 10^{-7}).

Spawning period and Environmental factors:

The Photophase monthly mean manifest a sinusoidal fluctuation with a maximum in June (14.52 ±0.05 hrs) and a minimum in December (9.80±0.05 hrs) (Fig. 6a). The sea temperature monthly mean curve follows a bell-shaped distribution with a maximum value recorded in September (21.797±0.493 C°), followed by August (21.771±0.707 C°) and a minimum recorded in February (Feb 2018, 15.187±0.181 C°) (Fig. 6b). The chlorophyll-a showed the first pick in March 2018 (0.509±0.095 mg/m^3) followed by a progressive decrease until achieving a minimum value on July 2018 (0.091±0.009 mg/m^3) succeeded by a gradual increase to reach a maximum value on February 2019 (0.547±0.050 mg/m^3) (Fig. 6c).

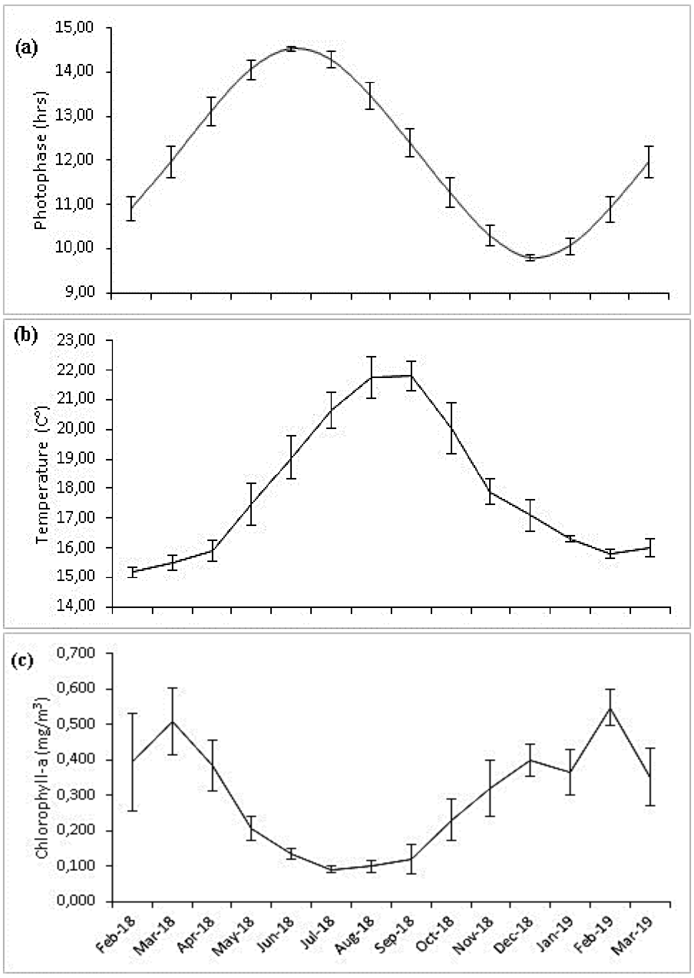

Fig. 7. Environmental factors monthly mean ±SEM: (a) Photophase duration (hrs), (b) Sea temperature (C°) and (c) Chlorophyll-a (mg/m^3)

No correlation was between *H. forskali* monthly GI and the other environmental factors. A positive correlation showed between *H. sanctori* monthly GI and Photophase (Pp) (R=0.630) and a negative one with the Chl-a (R=-0.559). For *H.tubulosa* monthly GI, a positive correlation with Pp (R=0,761) and Temperature (R=0,660) was showed and a negative correlation with Chl-a (R= -0,735) (Table 2).

Table 2. The three species environmental factors and Gonad Index (GI) correlation test.

	Pp	**T**	**Chl-a**	***GI H. forskali***	***GI H. sanctori***	***GI H. tubulosa***
Pp	1					
T	0,448	1				
Chl-a	-0,656	-0,884	1			
GI H. forskali	-0,167	-0,033	0,157	1		
GI H. sanctori	0,630	0,481	-0,559	0,062	1	
GI H. tubulosa	0,761	0,660	-0,735	0,232	0,794	1

For the three species, no correlation was shown between the percentage of the individuals that are potential to spawn and the environmental factors (Table 3).

Table 3. Individuals percentages reached stage IV and V Correlation test next to the environmental factors.

	Pp	**T**	**Chl-a**	***H. forskali* maturity**	***H. sanctori* maturity**	***H. tubulosa* maturity**
Pp	1					
T	0,447	1				
Chl-a	-0,650	-0,886	1			
H.forskali maturity	-0,232	-0,332	0,388	1		
H.sanctori maturity	0,472	0,363	-0,332	-0,004	1	
H.tubulosa maturity	0,476	0,167	-0,322	0,018	0,652	1

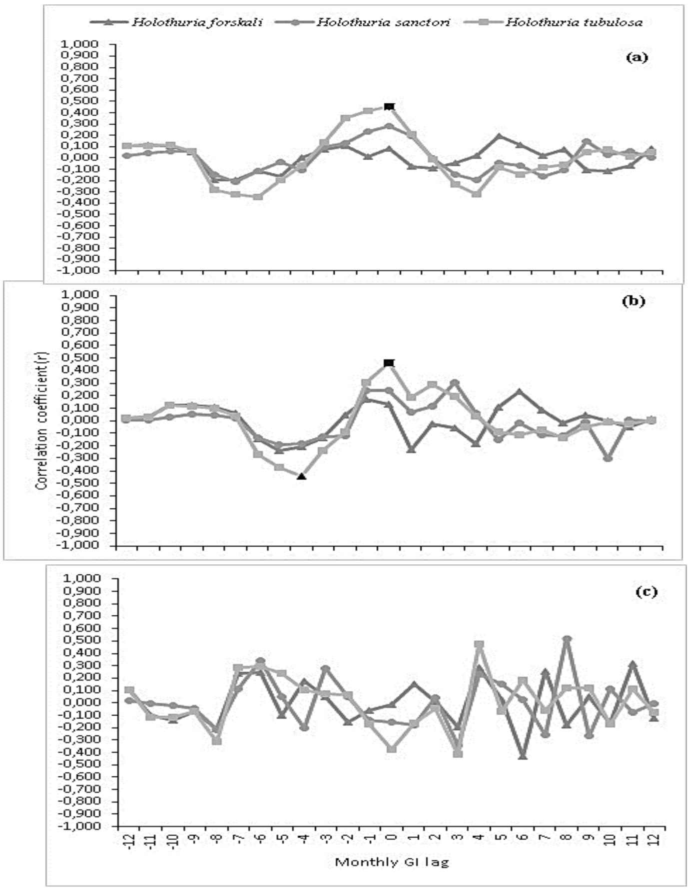

Fig. 8. Cross-correlation function (CCF) between Gonad Index and (a) Photophase duration ,(b) Temperature,and (c) Chlorophyll-a.Black triangle indicate significant correlation and the black square symbol indicate the points close to the significant correlation.

The Cross-Correlation test illustrates a correlation in one point (Fig. 7b), which is between *H. tubulosa* GI and temperature at log -4. (The Black triangle). Furthermore, the same species GI was close to the correlation in two points (Figs. 7 a and b) by around 93% at lag 0 with temperature and with photophase. In spite of that, the test did not show a cross-correlation with the environmental factors, neither for the species GI's or the percentage of the potential individuals to spawn (Figs. 7 and 8).

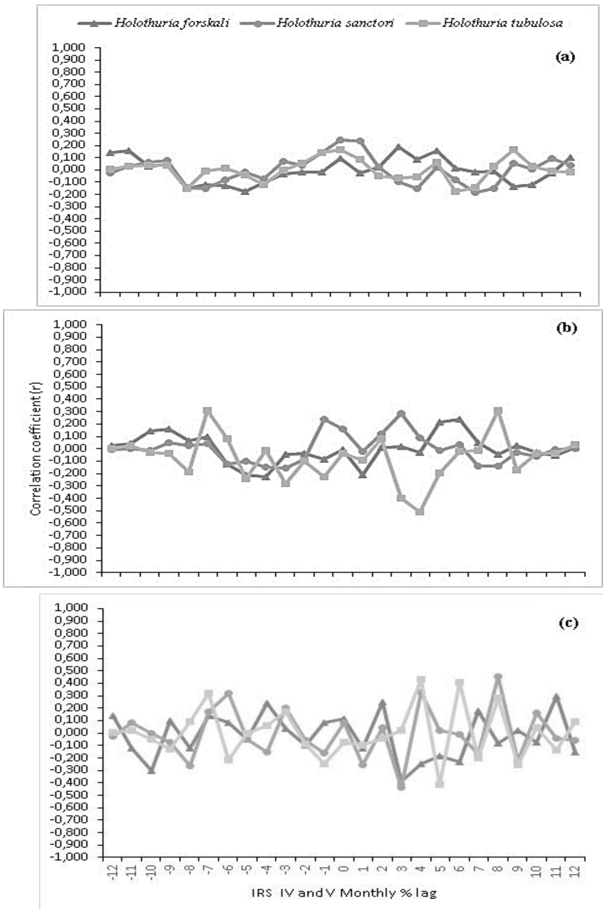

Fig. 10. Cross-correlation function (CCF) analysis between the percentage of individuals reached stage IV and V and (a) Photophase duration ,(b)Temperature, and (c) Chlorophyll-a

DISCUSSION

The three studied species *H. forskali*, *H.sanctori* and *H.tubulosa* showed diversity on the morphometric characteristic. The length-weight relation indicates a similarity for the three-species characterised by a positive allometry that can be explained by the cylindrical form (Vermiform) (**Tuwo and Conand, 1992; Gao and Yang, 2015**) and a positive correlation between the GBw and the Tl that characterises almost all the Holothuroidea class (**Guzmán *et al.*, 2003; Herrero-Pérezrul and Reyes-Bonilla, 2008; Marquet *et al.*, 2017**).

The GBw and Gw did not show a significant correlation for *H. forskali* but a significant correlation for the two other species. This variability can be explained by the species physiology and the growth mechanism, such as, for *H. sanctori* and *H. tubulosa* where the gonad weight is affected by the body growth (**Bueno *et al.*, 2015; Marquet *et al.*, 2017; Ramos-Miranda *et al.*, 2017**).

Maturity and spawning months:

The actual research reveals that the spawning months in the studied region for *H. forskali* are in March, October and December. Comparing that with other regions, as in NE Atlantic coast (SW of Portugal) where **Silva *et al.* (2018)** conducted research during winter and spring months. Their findings indicate that *H. forskali* potentially has a reproductive behave during the winter months and based on gametogenic analysis this species is assumed to spawn in February.
In the Portugal coast (the east coast, Peniche) based on **Santos *et al.* (2016)** findings that was conducted for 10 months assumed that the spawning months (Mature and Partly spawned stages) for females are in November, February, March and April and for Males are from November to June. Based on that, and if we want to calculate the percentage against the examined population (n=137) without making a distinction between the sexes, the higher percentage will be in November, February, March and April with an estimative percentage of 66.42% and for December, January, May and June the percentage are consecutively 53.14%, 43.18%, 46.50% and 33.21%. Based on that, the potential spawning months in **Santos *et al.* (2016)** research are similar to these in our study. For **Tuwo and Conand (1992)** in the Glenan archipelago (Brittany). If we take the pre-spawning individuals as the individuals that reached the stage IV, we can conclude that the spawning months can be from January to March and in November. So generally, *H. forskali* have a spawning period occurring during cold months.

The spawning months for *H. sanctori* revealed to be in February, April, June, September and October. In a near geographical study (Algeria) **(Mezali *et al*., 2014)**, the spawning months in Ain Taggourait can be assumed to be from February to June. However, in Tamentefoust the percentage higher than 50% are in February and April to July.
Furthermore, in the eastern Atlantic (Gran Canaria Island) based on **Navarro *et al.***

(2012) results, the spawning months are from June to October. In the light of that, the actual research reveals some resemblance with both studies, which can refer to the geographical position, making clear that the study zone is situated between the cited areas and can also be coupled with the environmental conditions influenced by the Atlantic Ocean and the Mediterranean Sea.

H. tubulosa manifests a potentiality to spawn during June and October. On the other side, the research elaborated by **Despalatović *et al.* (2004)** in the Adriatic Sea (Kastela Bay) demonstrates that during June the examined individuals were in the recovery and the growing stages. Moreover, during October no data were represented. Despite that, the spawning months are considered to be in January, April, July and August.

In Greece, the Aegean Sea central-western region (Pagasitikos Gulf), **Kazanidis *et al.* (2014)** results reveal that the spawning months without distinction between sexes were from June to September. The same months were also reported in Turkey on the Aegean Sea (five sampling station: Ayvalık, Aliağa, Foça, Şakran and İzmir) (**Aydın and Erkan, 2015**).

In a general way, for the three species, the studies demonstrate a similarity in some months with variations that can be based on the thermal profiles, the feeding activity in each region and other bio-physico-chemical conditions, which can correlate with geographical positions.

Gonad index evolution:

The Gonad-Index (GI) as one of the tools that is been used to characterise the reproduction behave of sea cucumber species (e.g.: **Conand, 1981,1993; Gaudron *et al*., 2008; Arsad *et al.*, 2017**) it was widely studied and manifest a large variability.

In our case, *H. forskali* showed a maximum GI in March, July, and October. These months had a similarity to other scientific research. In **Tuwo and Conand, (1992)** study, (without distinction between sexes) the maximum GI was from January to March and from October to December, with values above 13 % in August and September. Furthermore, for **Santos *et al.* (2016)** the maximum values were in February and March, followed by November where the percentage was near to 9% (Both sexes Mean). For **Silva *et al.* (2018)**, the GI peaks were assumed to be during the winter with an important decrease in February.

H. sanctori maximum GI percentages were recorded on April, June, September and October. Based on **Mezali *et al*. (2014)** and without making a distinction between the sexes, the maximum GI in Ain Taggourait were recorded from May to July. However, in Tamentefoust the GI did not exceed 10%, where the maximum values were registered during May, March and April. Same in **Navarro *et al*. (2012)** results, where the GI monthly mean, did not exceed 8% and the maximum values were from June to August.

During May and October *H. tubulosa* have the maximum GI values. For **Kazanidis *et al*. (2010)**, the maximum GI values are from June to August. In **Bulteel *et al*. (1992)** research the maximum GI values are on August and September.

First maturity size/weight and sex ratio:

Generally the size at first maturity can go from 12 cm such as for *Actinopyga echinites* **(Conand, 1982)** to 32.4 cm for *Microthele fuscogilva* **(Conand, 1981),** noting that for an important species number the size at first maturity is above 16 cm (**Conand, 1981; Dissanayake and Stefansson, 2010; Omar *et al*., 2013; Natan *et al*., 2015**).

Furthermore, the weight (dried weight "Dw") at first maturity characterised by a large variation spectrum (**Conand, 1981,1982; Herrero-Pérezrul *et al*., 1999**), based on each species biology, physiology, and reproduction mechanism, etc.

However, for *H. tubulosa* the weight (Dw) at first maturity was estimated to be near to 220g (**Kazanidis *et al*., 2014**), which is lower than our result by around 29%. Moreover, the Tl_{50} for *H. sanctori* was reported to be between 20.1 and 21.0 cm and the Tw_{50} is between 176 and 200g (**Navarro *et al*., 2012**). The Tl_{50} was higher than the one in our findings by about 15% to 20%. In addition, without including the individuals' internal water, the Tw_{50} was lower than the one recorded in our study between 27% and 36%.

The sex ratio for *H. sanctori* and *H. tubulosa* did not differ from 1:1 known as the general H. ns sex ratio (**Despalatović et *al.*, 2004; Dissanayake and Stefansson, 2010; Kazanidis *et al.*, 2010; Omar *et al.*, 2013; Kazanidis *et al.*, 2014; Marquet *et al.*, 2017**). However, for *H. forskali*, the sex ratio was different from the balanced one, which was already reported for this species (**Tuwo and Conand, 1992; Santos *et al*., 2016**). And for other sea cucumber species (**Conand *et al*., 2002; Shiell and Uthicke, 2006; LEE *et al*., 2009; Dolmatov, 2014**).

Environmental factors, GI and spawning months:

Generally speaking, sea cucumber maturity and spawning periods are influenced by temperature, photoperiod and food availability (**Hamel *et al.*, 1993; Hamel and Mercier, 1996; Mercier *et al.*, 2000; Hamel and Mercier, 2005; Mercier *et al*., 2007; Benítez-Villalobos *et al.*, 2013**). In our study, some parameters showed a correlation with the environmental factors and others did not; this can be referred to the complexity and the interaction of the parameters into the reproduction process. In the same way, it is been reported that the environmental factors can be not correlated with the GI, Maturity evolution or spawning period (**Ramofafia *et al*., 2000; Kohler *et al*., 2009; Marquet *et al*., 2017**).

CONCLUSION

As a fact, this study is considered to be the first that describes the reproduction behaviour of *H. forskali*, *H.sanctori* and *H.tubulosa* in the Moroccan Mediterranean coast in a general way and specifically in the M'diq bay. This can offer a baseline for further research, by providing the maturity cycle, the Gonad index variation, and making the light on the Tl_{50} and the Tw_{50}, as effective tools for sea cucumber fisheries management. Furthermore, more researches are invited to determine the relation of the environmental factors next to the reproduction mechanism of the three studied species, to determine the abundance, the spatial distribution and the climate change influence on the reproduction behaviour or other biological factors of these species in this region, or near geographical area.

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