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Study of the Physiological Indices of Sea Cucumber *Holothuria tubulosa* (Gmelin, 1788) of the Oranaise Coast (Algeria)

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Abstract

Sea cucumbers (Holothuroidea) of the order Aspidochirotes constitute a highly visible and diverse group within marine ecosystems. This echinoderm filled with fundamental ecological functions by its activities detritivore and of bioturbation. In the Mediterranean is more precisely in Algerian waters, among the dominant species is the Holothuria tubulosa hence the interest in the study of this species. A total of 170 specimens are harvested at the sites of Sidi lakhdar and Ain Franin during the period October 2013 to October 2014. The analysis concerns the physiological indices, namely the gonad index (GI) and the repletion index (RI), allowing us to determine the reproductive periods and the various stages of the reproductive cycle of Holothuria tubulosa according to the seasons and the temperature and to see the relation between the feeding period and the reproduction period. The results show that sea cucumbers exhibit fluctuations in physiological indices as a function of temperature and seasons.

Key words: Ain Franin; Holothuria tubulosa; gonad index; index of repletion; season; Sidi lakhdar; temperature.

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1. Introduction

Sea cucumbers are broadly distributed Echinoderms in all seas of the world, from intertidal to high seas [1]. Commonly known as sea cucumber or sea cucumber, a fundamental element in the structure of coastal ecosystems in marine Magnoliophytes and in particular ecosystems in *Posidonia oceanica* in the Mediterranean [2,3]. These macroechinoderms constitute an important part of the fishery in the Indo-Pacific region for traditional use and pharmaceutical subsistence [4,5], also used in aquaculture and biomedical research [6]. Sea cucumbers have an important place in the food chain of marine ecosystems at different trophic levels, they are important constituents of benthic fauna, they play a role as depositivores and suspensivores [7], and as bioturbator of sediments by Their feeding behavior [8,9], they stabilize the bacterial community in the sediments [10,1] and convert the detritus to nitrogen compounds thus contributing to primary production [11,12]. In our study we are interested in the follow-up of the reproductive cycle and the nutritional need of *Holothuria tubulosa* widespread on our algerian coasts in order to determine the traits of its biological development by analyzing the evolution of the gonadal index and consequently localize the spawning period of this aspidochirote sea cucumber.

2. Materials and methods

2.1. Choice of study sites

Two sites were taken into account in this study (Figure1), the first site, located 50 kilometers east of the Mostaganem agglomeration (Sidi Lakhdar :Petit Port), whose geographical position is 36°12'40.63"N latitude and 0°23'20.78"E longitude, characterized by a soft substrate rich in photophilic algae with a strong presence of Magnoliophyte *Posidonia oceanica* and is characterized by domestic wastewater discharge located in the Oued Abid to the east is added agricultural pollution from greenhouse fields located near our study site [13]. Conversely, the second site, located about ten kilometers east of Oran (Ain Franin) whose geographical position is 35°46'49.78"N latitude and 0°31'01.51"W of longitude.

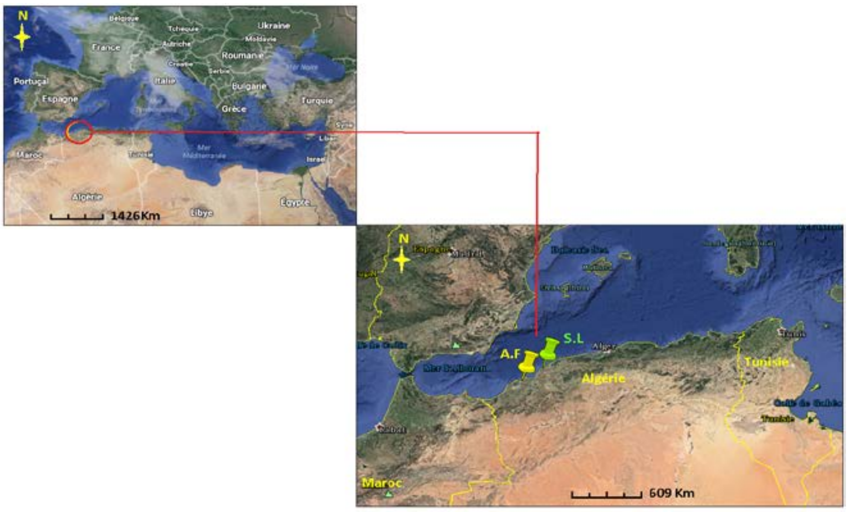

Figure 1: Location of study sites (A.F: Ain Franin, S.L: Sidi Lakhdar)

It is located between the two headlands forming the great bay of Oran, Cap Ferrat in the North and the Cap Falcon south-east and 28 Km from Kristel. This site is characterized by a substrate with pebbles and photophilic algae considered as a zone not impacted because the anthropic action is very little marked [14].

2.2. Sampling methods

Each month around 20 specimens are taken from scuba diving from October 2013 to October 2014 by hand, randomly, in the shallow waters (≤ 4 meters) of the study sites. The samples are placed in a tank containing water from the ambient medium. The temperature measurements are carried out in situ using an Aladin 2G precision + 0.5 ° C diving computer.

2.3. Laboratory Treatment

Measurements are carried out on each individual according to the [15, 16]. The fresh samples are weighed one by one and then dissected along the body (from the anus to the mouth). The gonads are recovered drained as well as the digestive tract; each compartment is weighed separately to determine the fresh weight of the milks of the digestive tract and of the total weight of the animal eviscerated.

To determine the reproductive periods and stages of the reproductive cycle of the species, and the relationship between feeding and gonadal development, two physiological indices are calculated, namely the gonadal index (GI) and repletion index (RI) [17, 18, 19, 20].

Several methods have been used to obtain an index of cyclic variation in the organs of marine invertebrates. All are based on relative variations that are comparable to each other. For this study, the method chosen is that of the ratio of the wet weight of the organs (gonads, intestines) to the total wet weight of the eviscerated animal. For each individual, the gonadal index (GI) and the repletion index (RI) are calculated as follows: (**gonadal index GI= fresh weight of the gonads / weight of the eviscerated specimen ×100, and for the repletion index RI = weight of the intestines / weight of the eviscerated specimen × 100** [21, 22, 23, 24, 26, 27, 28, 29].

3. Results and discussion

In a population of *Holothuria tubulosa*, the determination of the fluctuations in the weight of the digestive contents and that of the gonads allows, on the one hand, an approach to the trophic study of this marine invertebrate, a determining element in the structure and evolution of phytocenoses benthic activity [30, 31, 32, 33] and to specify the gonad activity.

The mean indexes of repletion and gonadal are characterized by a monthly evolution which is much more marked for the index of repletion (Table 1).The latter shows significant variations, the maximum of which is 46,65 ± 1,04 (july) and the minimum 6,36 ± 1,09 (december) of the sea cucumbers of Sidi Lakhdar, for the Ain Franin site the maximum (30,44 ± 1,71) (march) and the minimum (20,43 ± 1,88) during the month of august.

In terms of the gonadal index, the highest value for february is recorded for Sidi Lakhadar (7.69 ± 1.53) and the lowest for july with 0,55 ± 0,24, while for individuals of Ain Franin this index is 6,35 ± 1,80 (october) and 0,53 ± 0,09 (april).

Table 1: Monthly averages of the physiological indices of *Holothuria tubulosa* (Gmelin, 1788) expressed (g) ± *(standard deviation)*. SL: Sidi Lakhdar ; AF : Ain Franin ; RI_{av} : average Repletion Index ; GI_{av} : average Gonad Index.

Months		***Oct 2013***	***Nov***	***Dec***	***Jan***	***Feb***	***Mar***	***Avp***	***May***	***Jun***	***Jul***	***Aug***	***Sept***	***Oct 2014***
S,L	***RI_{av}***	**10, 34**	**9,41**	**6,36**	**13,72**	**52,53**	**27,03**	**34,33**	**37,67**	**42,54**	**46,65**	**34,98**	**23,87**	**14,54**
		(3,76)	*(2,23)*	*(1,09)*	*(2,04)*	*(2,91)*	*(1,71)*	*(1,34)*	*(1,14)*	*(2,04)*	*(1,04)*	*(1,14)*	*(3,04)*	*(2,94)*
	GI_{av}	**4,07**	**4,01**	**5,90**	**4, 10**	**7,69**	**1,27**	**0,98**	**0,57**	**0,66**	**0,55**	**2,65**	**5,43**	**5,09**
		(0,19)	*(0,22)*	*(0,18)*	*(1,24)*	*(1,53)*	*(0,65)*	*(0,07)*	*(0,04)*	*(0,34)*	*(0,24)*	*(0,94)*	*(1,64)*	*(2,43)*
A,F	***R_{av}***	**28,74**	**26,26**	**21,73**	**20,43**	**24,86**	**30,44**	**23,34**	**25,32**	**23,87**	**22,77**	**21,60**	**20,50**	**20,56**
		(1,18)	*(1,76)*	*(2,09)*	*(1,94)*	*(1,91)*	*(1,71)*	*(1,44)*	*(2,43)*	*(3,12)*	*(1,90)*	*(1,88)*	*(2,90)*	*(2,60)*
	GI_{av}	**0,56**	**0,66**	**3,98**	**4 ,01**	**2,23**	**2,74**	**0,53**	**0,54**	**1,54**	**1,23**	**2,67**	**4,32**	**6,35**
		(0,29)	*(0,32)*	*(1,18)*	*(1,24)*	*(1,53)*	*(1,65)*	*(0,09)*	*(0,10)*	*(0,97)*	*(0,60)*	*(0,90)*	*(1,01)*	*(1,80)*

The mean maximum values recorded for the repletion index are observed during spring and summer, while the gonad index records the minimum values during the same seasons for both sea cucumber populations.

Monitoring of GI_{av} fluctuations revealed one or two laying periods, which is represented by a sharp fall in gonad index values within the same sea cucumber population. If the spawning of aspidochirotes occurs at different periods of the year depending on the species, sea cucumbers often show an annual reproductive cycle [34, 35, 36, 37, 38, 39, 40, 41, 16, 42] although semi-annual cycles [43, 25] or even continuous breeding activity throughout the year [43] are also frequent, especially in tropical regions. Sea cucumbers usually spawn in summer [25, 44, 45], with some exceptions such as winter spawn observed in *H. whitmaei* and *H. tubulosa* [24, 46].

In *H. tubulosa*, mean physiological indices fluctuate from one sample to another. These physiological variations in Echinoderms may be related to meteorological periods (bad or good weather), and consequently lead to a loss of synchronism in the feeding and thus in the maturation of the gonads, where it is observed that some Individuals are in the feeding phase, while others are in the fasting phase [47, 48, 49, 50].

In the Echinoderms, the evolution of the index of repletion decreases during the fasting phase, whereas it evolves inversely in phase of nutrition. Thus, the feeding rate is low when the gonads are highly developed, this is the case observed in *Holothuria tubulosa* in two sampling sites (Ain Franin and Sidi Lakhdar).

According to [51], when gametogenesis is complete, the need for food is less important, energy is needed than for maintenance of growth. Increased gonadal weight may be due in part to increased water in the gonad, but especially to progression of gametogenesis [52].

Reference [53] refers to three stages of gametogenesis; The season of gonad development, the egg-laying season and finally the lethargic season of development. The analysis of variance with respect to the two sites indicates a significant difference For the GI_{av} (F_{obs} = 11,13> F_{th} = 4,26 with P < 0.05) and for the IRm (F_{obs} = 70,45 < F_{th} = 4,26 with P < 0.05) between the two populations.

The evolution of the gonad index shows strong seasonal variations (Figures 2 and 3). In Echinoderma, the feeding rate is low when the gonads are highly developed [54, 55, 56] with this observation.

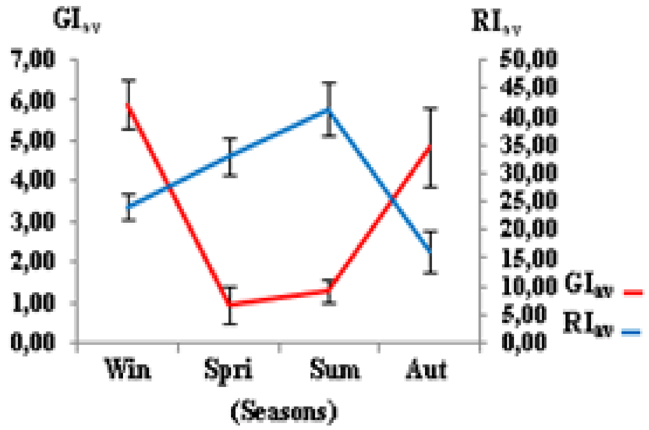

Figure 2: Average Seasonal variation physiological index (m g/cm3) of Holothuria tubulosa (Gm elin,1816) of Sidi Lakhdar

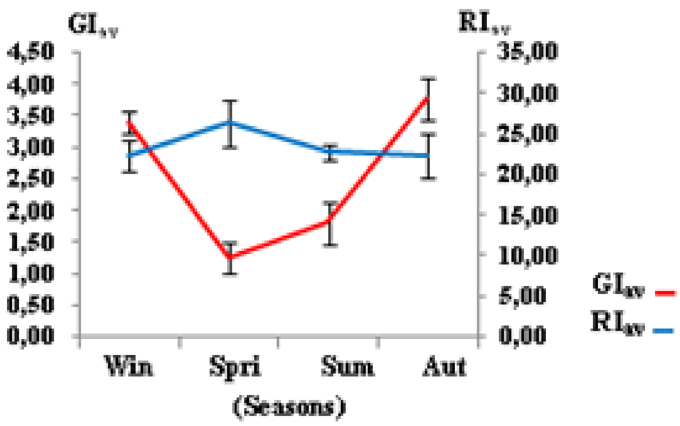

Figure 3: Average Seasonal variation physiological index (m g/cm3) of Holothuria tubulosa (Gm elin,1816) of Ain Franin

4. Effect of temperature on the gonad index

The shape of the curve (Figures 3 and 4) shows an increase in the gonad index with respect to the rejection index, so GI evolves inversely with respect to RI, thus the highest values of RI_{av} s 'Recorded in winter (41,39 ± 4,83) for Sidi Lakhdar and 26,37 ± 2,99 for the site of Ain Franin, on the other hand the GI_{av} recorded low values of around 1,29 ± 0,97 and 1,27 ± 1,04 respectively.

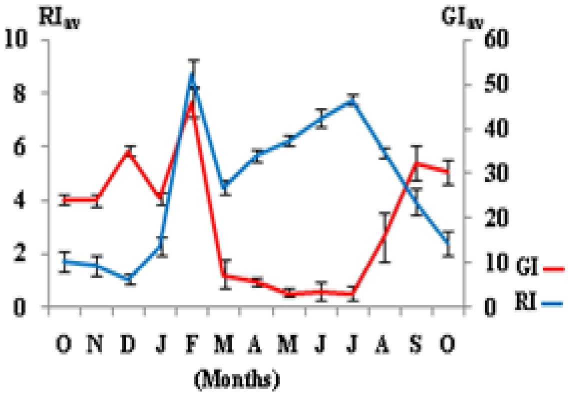

Figure 2: Evolution of the average onthly physiological index (m g/cm3) of Holothuria tubulosa (Gm elin,1816) of Sidi Lakhdar

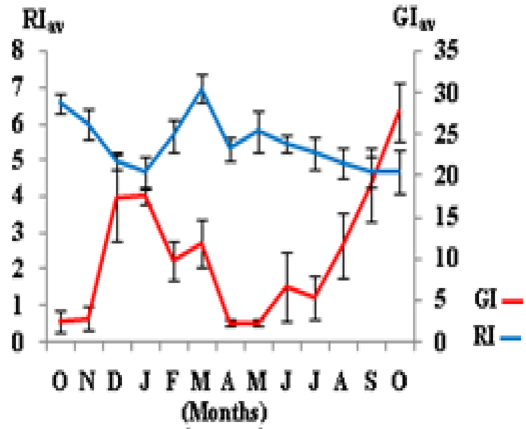

Figure 2: Evolution of the average onthly physiological index (m g/cm3) of Holothuria tubulosa (Gm elin,1816) of Ain Franin

The analysis of the physiological indexes with respect to the seasons was analyzed by an analysis of the variances which indicates a significant difference for the GI_{av} (F_{obs} = 3,01 > F_{th} = 2,16 with P < 0.05), and the analysis of the physiological indexes with respect to the seasons was analyzed by an analysis of the variances which indicates a significant difference for the GI_{av} (F_{obs} = 3.01> F_{th} = 2,16 with P < 0.05), and a difference not significant for IRm (F_{obs} = 1,53 < F_{th} = 2,16 with P < 0.05). The significant difference in GI_{av} may be due to distribution, abundance of the species in the medium and the difference in composition of the substrate. Generally in echinoderms the increase of the repletion index is intended to provide the energy needed for sexual gametes for eventual maturity and therefore subsequent spawning, then the more the sea cucumber feeds the more it develops these gonads.

The monitoring of fluctuations of the GI_{av} revealed a laying period which is represented by a sudden drop in the values of the gonad index within the same population. The increase in the index of repletion is intended to provide the energy needed for sexual gametes for maturity and therefore subsequent spawning, the more the sea cucumber feeds the more it develops its gonads [57]. According to [25] maturation takes place between september and november and spawning occurs during the months when the temperature begins to rise, a peak of spawning and gametogenesis occurs in spring, which corresponds to the cycle of several aspidochirote holothurians [35, 58, 24, 16, 25], our results are consistent with the sharp fall in GI_{av} for the months of april and july (spring and summer). It is also important to specify that the shape of the gonad-size index relationship can be strongly influenced by the environmental conditions in which echinoderms evolve [56, 59]. Since reproduction is a critical activity in the life cycle of marine invertebrates, survival success and the selection of organisms are mostly related to the latter [60]. In any reproductive cycle, the stimuli are such that the entire population is ready at the same time for spawning [60].

References [61, 62] shows that environmental factors act as stimuli on gonadal growth, maturation and spawning. First, in terms of gonadal growth, it is nutrition, or rather the quantity and quality of nutrition that regulates the gonad volume. This growth does not depend only on the level of gonad growth abundance, but also of the quality of the diet as in *Astrostole scabra* [63]. In a second time, come the biological stimuli which can present themselves as the trigger of an epidemic laying with the presence of sexual products in the environment, or the appearance of a phytoplankton bloom [64, 65, 63]. Finally, it is physico-chemical factors represented mainly by temperature that can have an impact on available food, metabolism, and therefore on growth [63]. Gamogenetic events are controlled by endogenous factors, but synchronization of individuals is probably coordinated by one or more external factors [63, 21].

Effect of temperature on gonad index:

The reproductive cycle of most echinoderms is characterized by temporal, seasonal or other variations, which are believed to be the result of complex interactions between different endogenous and exogenous signals.

Several ecological, chemical and hormonal factors, acting alone or by combined action, direct or indirect, announce, stimulate or modulate a set of reproductive functions, from gametogenesis to gametic expulsion [66].

The mean gonad index can also be influenced by several biotic and abiotic factors such as temperature, biotope and environmental conditions. Falling GI_{av} values reflect the occurrence of egg lakes in the fall and early spring. Generally, laying periods when the study is spread over an annual cycle are in the spring and late summer [67, 68, 69, 70], it is necessary to specify that the annual variation in the egg-laying period.

In sea cucumbers, spawning periods are recorded mainly in spring and even in winter [71]. These may be due to many factors, such as variations in water temperature [72]; But also to variations in the period of appearance of phytoplankton blooms [73].

Knowing that sea cucumbers are detritivores that ingest sediments containing organic matter including bacteria, protozoa, diatoms and matter released by plants and animals.

According to [74,75], an increase in temperature can increase the ingestion and absorption efficiency and stimulate the growth and biological activities of sea cucumbers [76, 77]. According to [78], after studying sexual variations and gonadosomal relationships in *Isostichopus fuscus* (Holothuroidea), sea water temperature is the main trigger for the summer spawning peak.

However, the only notorious environmental change at the end of march, when sea cucumber gametogenesis begins, is the return of photoperiod elongation as seawater temperature has not yet begun to rise. Therefore, this photoperiod may also play a role in gametogenesis [73].

Experiments on sea urchins have shown a correlation between gametogenesis and photoperiod [79]. Thus there is every reason to believe that the photoperiod can synchronize internal phenomena [80].

However, temperature, as well as aggregation of individuals, would be the most likely exogenous factors for stimulating spawning [60]. If we refer to the temperature records recorded during the sampling, they show that for the thirteen months of study, egg laying occurs during the spring rise in water temperature (Figure 6).

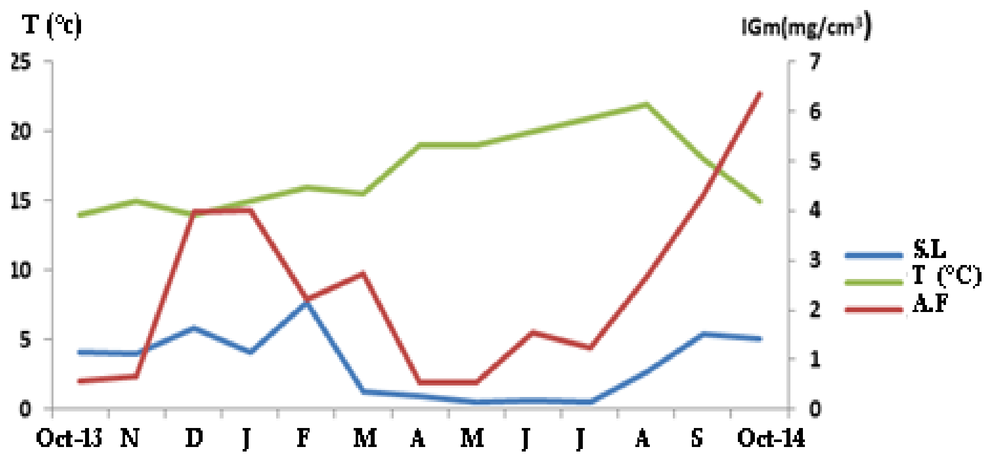

Figure 6: Effect of temperature on the spawning in the *Holothuria tubulosa* (Gmelin, 1816)

Reference [53] suggests temperature as a triggering factor for spawning, not as the achievement of a defined temperature threshold for spawning, but rather as a temperature gap within which spawning is possible. Reference [60] speaks of temperature as a factor inhibiting or slowing down nutrition. Generally, a specific temperature is considered as a regulator of spawning [79, 60]. Temperature is a critical factor influencing gametogenesis but is unlikely to be a single and sufficient factor [60].

Feeding or the level of food reserves coupled with a rise in temperature provides gametogenesis with all the elements essential for its realization. These environmental factors influence and synchronize the reproductive cycle by their intervention at [60].

Many authors assume that several ecological, chemical and hormonal factors, acting alone or by combined action, either directly or indirectly, are expected to stimulate or modulate A set of reproductive functions, from the beginning of gametogenesis to gametic expulsion [66].

Table 2: Intraspecific comparison of reproductive characteristics in *H. tubulosa* and 4 species from temperate regions.Methods used: 1: histological examination; 2: GI; 3: field spawning observations; 4: presence of eggs and embryos in plankton; 5: macro-and microscopic analysis of the physical characteristics of tubules.

Species	Latitude	Methods	GI_{max}	Reproductive activity	Period of spawning	References
Aslia lefevrei	43N	1	—	Annual	Jul-Sep	[82]
	42N	2	—	—	Jul	[83]
	40N	3	Aug	Annual	Aug-Sep	[71]
H.forskali	47N	1&2	Feb-Mar	Annual	Apr-Jun	[39]
	43N	1	—	—	Jun-Aug &Dec	[84]
H .sanctori	28N	2&5	Jun-Jul	Annual	Jun-Aug	[85]
Aslia lefevrei	53N	1&2	Nov-Mar	Annual	Mar-May	[86]
	43N	2&4	Oct-Jan	Annual	Jan-Mar	
Parastichopus parvimensis	37N	3	—	—	May-Jun	[87]
	27N	1&2	Fev-Apr	Annual	Jan-Jul	[73]
H.tubulosa	**59.81''N**	**2**	**Jan-Oct**	**Annual**	**Mar- Jul**	**Present study**
	27.19'' N	**2**	**Dec-Feb**	**Annual**	**Apr - Jul &Oct**	

5. Conclusion

The study carried out on the populations of *Holothuria tubulosa* revealed a relationship between the physiological indices despite the difference in structure of the two substrates. The values of the repletion index allowed characterizing the two populations by a phase of intense (nutritive) activity in march and april (spring) compared to the rest of the seasons.

The lowest level of this index is observed in winter, the monthly variations of the gonad index have revealed that our species has two spawning periods which is represented by a sudden fall of the values of this index within the same population of sea cucumbers.

The temporal increase in temperature during the sampling period also highlights the role of this temperature in the triggering of spawning that is representative in spring and summer. However, there are other environmental factors involved in this physiological phenomenon of the reproduction of this species.

For a better knowledge of the reproductive biology of this Mediterranean species and for an optimization of the resource, it is necessary to study the influence of different trophic and anthropogenic ecological parameters on the evolution and eventual exploitation of this marine resource. It also seems necessary to undertake a granulometric study of the various sediments in order to highlight the existing relationship between the diet and the physiological indices of these macroinvertebrates.

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