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Reproductive cycle of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) from the Moroccan western Mediterranean Sea: histology, gonadal index and size at first sexual maturity

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ABSTRACT

The Moroccan Mediterranean coasts are an important habitat for ecologically and commercially valuable species such as the common sea urchin, which is part of the Echinoidea class. A study of the reproductive cycle of *Paracentrotus lividus* was carried out on the coast of Tangier, located in the southwestern Strait of Gibraltar in the western Mediterranean Sea, from January 2021 to February 2022. A total of 840 samples showed that 54.8% were between 50 and 60 mm, and the sex ratio oscillated around 1:1 in most months. Moreover, histological analysis has shown an annual reproductive cycle with a single spawning season from March to July, including six phases. In general, both sexes have synchronous gamete synthesis and release. However, there was no significant variation in the distribution of gametogenic cycle stages between females and males (ANOVA, $P > 0.05$). Furthermore, the gonadal index (GI) recorded its maximum value in April (5.4± 1.43) and the lowest in October (0.4±0.58). while the correlation test indicated a significant relationship between temperature, total chlorophyll and the reproduction cycle of *P. lividus*. Finally, the L50 method showed that the size at first sexual maturity was 34.32 mm for males and 34.94 mm for females. The findings of this research could be helpful for the decision-makers in order to develop a suitable strategy allowing the protection and management of this species.

INTRODUCTION

The sea urchin *Paracentrotus lividus* (Lamarck, 1816) lives on rocky intertidal pools, shallow subtidal reefs and seagrass meadows. They are distributed throughout the Mediterranean coasts and along the Northeast Atlantic, from Northern Ireland to

Morocco, including the archipelagos of the Azores, Madeira and the Canary Islands **(Gharbi *et al.*, 2023)**. This marine invertebrate can be found up to 80 meters below the surface in the intertidal zone. The population density decreases with depth and the highest densities have been found between 0 and 10 meters. **(Gou *et al.*, 2022)**. *P. lividus* often lives in cavities in these intertidal zones, foraging on the rocks using quills and dents **(Baião *et al.*, 2019)**. Sea urchins have a variable diet and their major predators are fish, sea stars, crabs and lobsters **(Prato *et al.*, 2018; Rocha *et al*., 2019)**, although humans are the main predator **(Guinda *et al.*, 2017)**.

Paracentrotus lividus is an important species that plays an ecological role throughout its range by structuring benthic communities and controlling macroalgal abundance, distribution and productivity. In addition, the urchin gonads are composed mainly of somatic cells (nutrient phagocytes) and germinal cells (oogonia and spermatogonia) **(Gharbi *et al.*, 2023)**, which provide a good source of high-quality protein, long-chain polyunsaturated omega-3, micronutrients and poor saturated fats **(Prato *et al.*, 2018; Baião *et al.*, 2019; Gou *et al.*, 2022)**. The urchin represents one of the most harvested and valuable echinoids in many countries **(Guinda *et al.*, 2017; Machado *et al.*, 2019)** and represents a high level of commercial interest due to the highest demand **(Parrondo *et al.*, 2022)**.

Recently, *Paracentrotus lividus* has been frequently used as a bio-indicator of the marine environmental quality **(El-Haimeur *et al.*, 2013; Parra-Luna *et al.*, 2020; Fouad *et al.*, 2021)**. Also, in pharmacological research, the species is considered an excellent model in developmental biology **(Schillaci *et al.*, 2014; Ribeiro *et al.*, 2015; Milito *et al.*, 2022)**. According to the Food and Agriculture Organization (FAO), the fisheries in the Mediterranean Sea have recently experienced a sharp decline **(FAO, 2020)**. This was mainly due to stock overexploitation, recreational fishing practices and illegal catches **(Ouréns *et al.*, 2013; Loi *et al.*, 2015)**. Since 1996, *P. lividus* has been included in the Barcelona Convention's Protocol for the Conservation of Biodiversity as a marine species whose exploitation is regulated **(Ostalé-Valriberas *et al.*, 2022)**. This implies that fishing time, catch quotas and minimum exploitation size should be respected to ensure the sustainability of *Paracentrotus lividus* in the future. Moreover, several countries have already proposed management strategies for *P. lividus* conservation **(Sellem and Bouhaouala-Zahar, 2021)**.

In this context, many of the local populations along the northern parts of the Mediterranean and Atlantic coasts have been studied **(Jacinto *et al.*, 2013; Bertocci *et al.*, 2018*;* Carreras *et al.*, 2020)** . Most studies on the biology of the edible sea urchin have focused on the reproductive cycle **(Vafidis *et al.*, 2019; Ouchene *et al.*, 2021)**, growth **(Lozano *et al.*, 1995)**, biometrics **(Jamila *et al.*, 2018; Nicolau *et al.*, 2022)** and the management dynamics of populations and stocks **(Guinda *et al.*, 2017; Duchaud, 2018).**

In Morocco, the exploitation of sea urchins is restricted to the Atlantic coast, particularly along Casablanca and El Oualidia. However, the exploitation of sea urchins in the Moroccan Mediterranean is nearly non-existent and is confined to scientific reasons. Therefore, given the importance of this species and the increase in demand, temporary prohibition decrees on Paracentrotus have been announced by the Ministry of Agriculture and Maritime Fisheries, for example, the decree n°2096-20 published in July 2020 **(MPM, 2020)**.

The studies on the reproduction of *P. lividus* are limited to the Moroccan Atlantic coast **(Bayed *et al*., 2005; Jouhari, 2014; Ouchene *et al.*, 2021)** and no studies have aimed to investigate the reproduction cycle on the Mediterranean coast, especially the Gibraltar strait. **Fouad *et al* (2021)** reported that the Gibraltar coast is considered a specific environment confronted with many issues such as transformation through urbanization, industry, fishing and recreational activities. Various studies showed that several factors can also influence *Paracentrotus lividus* distribution, parameters such as temperature, salinity, chlorophyll, dissolved oxygen and photoperiod are cited among others **(Duchaud, 2018; Silva, 2022; Santos *et al.*, 2022)**, waves or predation, organic pollution **(Sellem and Bouhaouala-Zahar, 2021)**, overfishing **(Bertocci *et al.*, 2014)**, anthropogenic activities **(Prado *et al.*, 2012)** and the effects of climate change on embryonic development and testicular calcification **(Asnaghi *et al.*, 2014; Bertucci *et al.*, 2022)**. Despite this fact, this species can develop an adaptive mechanism for multiple biotopes if environmental parameters are relatively constant.

This study was conducted to assess the biodiversity and evolution of benthic fauna along the northern Mediterranean coast of Morocco **(Couvray, 2014)**. For this reason, a biological study was performed in Tangier coast. The objective of this study was to determine the reproductive cycle of *Paracentrotus lividus* population using a microscopic study through histological analysis of gonads. Besides, a macroscopic method was used for calculating the gonadal index (GI). In addition, statistical analysis and the correlation of temperature and chlorophyll with GI. Finally, the size of the individual at the first stage of sexual maturity was estimated by the L50 method.

MATERIALS AND METHODS

1. The study area and environmental parameters

The study area is located in the Moroccan Mediterranean Sea, in the Strait of Gibraltar, specifically between the beaches of Ghandouri and Mrisat on the Tangier coast. This strait represents a crucial geographical area, connecting the south of Europe and the north of Africa. Which is between the 35th and 36th parallels of north latitude and the 5^{th} and 6^{th} meridians of west longitude. This region witnesses a mixing of Atlantic Ocean water with water from the Mediterranean Sea **(de Stephanis *et al.*, 2008).**

A straight line between Cape Trafalgar and Cape Spartel bounds the Atlantic Ocean. In addition, the Mediterranean is limited by a straight-line connecting Punta de

Europa (Gibraltar) and Punta Almina (Ceuta). While in the north, it extends from the Gulf of Cadiz to the Bay of Gibraltar (Fig. 1). In terms of topography, the coast is distinguished by a flat character, a rocky coastline and sedimentary deposits (Fig. 2). Generally, two directions dominate Tangier wind regime. The first is being eastern Atlantic origin, which frequently causes precipitation and predominates in the north-east to south-east direction from November to March; while the second derives from the Mediterranean, which often blows in the summer and spring.

The sampled locations are located in the Gibraltar Strait and are influenced by the entry of Atlantic waters **(Ibghi *et al.*, 2022)** . Previous research has shown a strong relationship between sea urchins and algae **(Piazzi and Ceccherelli, 2019)**. In the study area, many species of macroalgae were identified and divided into four classes: *Bangiophyceae* (2 species), *Floridaophyceae* (36 species), *Phaeophyceae* (17 species) and *Ulvophyceae* (12 species) **(Kazzaz and Riadi, 1998; Adama *et al.*, 2021)**.

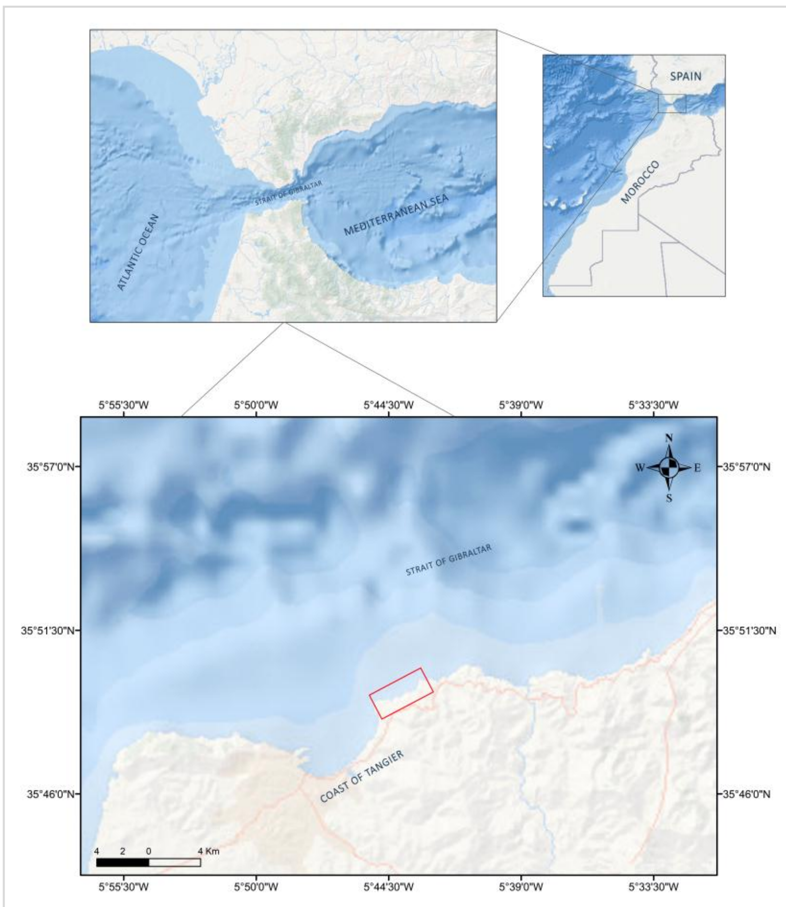


Fig 1. Geographic location of the study area.

Fig 2. Sampling site of *Paracentrotus lividus*.

2. Sampling methods

In this study, biological sampling of sea urchins was conducted monthly from January 2021 to February 2022. The collection required a snorkeling dive at 0–2 meters [40]. The samples were collected at low tide. According to a scientific sampling protocol, all individuals were collected by hand with gloves in a random manner, sorted, rinsed gently with seawater and then transported directly to the laboratory in coolers at +4 °C with seawater to preserve individuals in a fresh condition **(Rouane-Hacene *et al.*, 2018)**. During the sampling period, seawater temperature and total chlorophyll content were measured in situ using the multiparameter (AAQ-RINKO, AAQ-176, Japan).

$$FSR = \frac{NF}{TNI} \times 100 \qquad 1)$$

$$MSR = \frac{NM}{TNI} \times 100 \qquad 2)$$

Each month, sixty individuals of *P. lividus* were sampled and analyzed throughout the study period. Thirty were used for histological analysis and thirty for gonadal index determination. The morphometric parameters, diameter (D) and thickness (E), of the sea urchin test without spines, were measured with a caliper (precision: 1 mm), while the weight was taken with a balance (precision: 0.01 g) of each individual.

3. Sex ratio

The sex of *Paracentrotus lividus* was determined after dissection of the individual and collection of the gonads, which were examined with a binocular loupe. In addition, the texture and color of the gonads were observed. Subsequently, sex identification allowed us to calculate the sex ratio of males and females using the following equations: (1) and (2) **(P.M. Santos *et al.*, 2022)**.

Noting that, FSR: Female Sex-Ratio; MSR: Male Sex-Ratio; NF: Number of females; NM: Number of males; TNI: Total number of individuals.

4. Gonadal index

The test of each individual was opened with a cutter and dissected to allow precise extraction of the gonads. Then the gonadal tissue was completely removed by cutting the specimen around the mouth and extracting the gonadal mass nearest the head. The weight of the gonads was measured using a balance (accuracy: 0.01 g).

Monthly variations in gonadal development were measured by calculating the gonadal index (GI) for each specimen. The gonadal index (GI) was calculated with the following equation (3) **(Cirino *et al.*, 2017)**.

$$GI = \frac{DW}{D3} \times 100 \qquad 3)$$

With DW is dry weight of gonads (g); D is the diameter of the test (cm).

5. Histology

Thirty specimens were collected monthly from January 2021 to February 2022 and for each individual, 2–3 gonads were prepared for histological analysis according to the classical histological methodology. At the microscopic scale, oocyte size, sperm abundance and nutritional phagocyte accumulation were used as indicators during the reproductive cycle. The process followed is described below.

Table 1. Procedure of the histological study of the gonads of *Paracentrotus lividus*.

Step	Process	
1	Putting the cassettes in the Davidson-provided fixing solution.	Fig. 3 (A)
2	After three days, drying the gonads in graded ethanol (70% to 100%) using an automated technique (LEICA TP1020).	Fig. 3 (B)
3	Insertion of 2-3 gonads into histocyte inclusion cassettes and addition of paraffin at +12° C (LEICA EG1150H).	Fig. 3 (C)
4	block cooling (gonads & paraffin) at -30° C (LEICA EG1130).	Fig. 3 (D)
5	Cooling the blocks for around 24 hours at -4°C.	
6	Creation of two histological slices of 2 µm thickness (LEICA MICROTOME).	Fig. 3 (E)
7	Placing the histological sections in a water bath set at 40°C.	
8	Assembling the portions on the microscopic slides.	
9	Drying the blades in an oven at 60°C for 12 hours.	Fig. 3 (F)
10	Staining of sections with hematoxylin.	
11	Apply Roti® Histokitt (Roth) to the slides and allow them to dry.	
12	sing toluene to clean the portions.	Fig. 3 (G)
13	Using a light microscope, examine the gonad slides to determine sex. (40× and 100× magnification) and photographing with a digital camera via software (Toupveiw).	Fig. 3 (H)

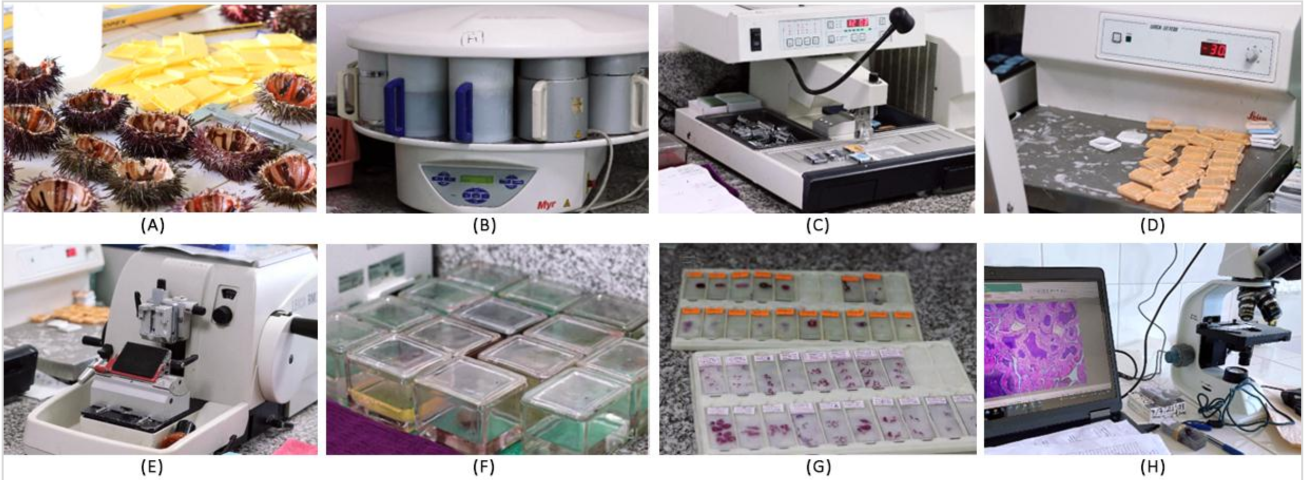


Fig 3. Pictures of the main histological steps of the sea urchin *Paracentrotus lividus*. a: cell fixation, b: dehydration; c: Inclusion; d: block cooling; e: histological cutting; f: coloration step; g: microscopic slide prepared; h: microscopic observation and photography.

The gonads identification was determined according to the classification, developed and described in detail in the identification key **(Byrne, 1990a).**

5.1 Females case

- Phase 1 (Recovery): Females cycles begin with the buildup of reserve chemicals in the vegetative tissues. Inclusions (droplets) are seen.
- Phase 2 (Growth): Phase 2 (Growth): The oocytes are turned into primary oocytes. The nourishing tissue is still highly formed, although less thick than in phase 1. The number of nutritive phagocytes falls modestly. Pre-vitellogenic oocysts surrounded by nutritional phagocytes stay mostly connected to the follicular wall, but some separate from the acinar wall.
- Phase 3 (Premature): Females exhibit increasing cellular growth along the acinous wall. The pedunculated oocyte is only kept in the acinar wall by gravity and it migrates to the center during maturity, continues to develop and expand and accumulates nutrient reserves and nutritive cells over time.
- Phase 4 (Maturity): The gonads reach maturity. The ovule consumes all of the light in the acinus, leaving little nutritive tissue. Mature, large ovules are generally more or less polygonal. These ovules can be buried. The vitellogeneses is still functioning and the previtellogenesis oocytes are still present.
- Phase 5 (Partially developed): The spaces become more visible to the light as time passes and the eggs are discharged. The germ layer is made up of a sequence of previtellogenic eggs that are surrounded by nutritional tissue. These ovules migrate to the lumen's center before being ejected again following maturation.
- Phase 6 (Spent): the remaining oocytes and some vitellogenic oocytes are reabsorbed by phagocytes in the lumen. However, nutrient-scavenging cells continue to replace all germ cell absorption.

5.2 Males case

- Phase 1 (Recovery): Small follicles define this stage. The lumen is occupied by nutrient phagocytes and unfilled sperm. The onset of spermatogenesis may be seen around the acinar wall.
- Phase 2 (Growth): Spermatogonia cells change into spermatids and the testes start to grow. This makes spermatogenesis happen faster as columns of cells move into the nourishing tissue.
- Phase 3 (pre-maturity): Sperm cells proliferate and dominate the lumen's center, while nutritional phagocytes congregate in the acinar wall. The first sperm emerges toward the end of the row.
- Phase 4 (Maturity): Spermatozoa proliferate and fill the whole lumen. Nutritive phagocytes, on the other side, create a thin coat and disperse around the acinar membrane.
- Phase 5 (partially developed): Low sperm concentration and the nutritional phagocytes phagocytose the remaining spermatozoa that aren't expelled. It regains significance when it shows oviposition voids and nutritional tissue begins to occupy the majority of the testicle visible under the microscope.
- Phase 6 (Spent) Presence of a coating of important phagocytic cells. Some spermatozoa remain in the lumen and are consumed by phagocytes.

6. Size at first sexual maturity

Maturation represents the final step in the process of transforming primordial sex cells into mature, fertile cells. The measure used to determine this size is L50, which is the size at which 50% of the individuals collected are mature.

After having analyzed the 420 samples collected for the histological study. The size at first sexual maturity was estimated using sea urchin diameter and sexual maturity phases. The proportion of mature individuals in each size class was estimated by establishing the maturity threshold from stage III. The symmetric sigmoid logistic model is used for the graphical representation **(Ouchene *et al.*, 2021)**

$$P = \frac{1}{1 + e^{-(a+b*LT)}} \qquad 4)$$

P, percentage of mature individuals by size class; LT, total length (mm), “a” and “b”, constants.

The parameters “a” and “b” are obtained by a logarithmic transformation of the expression (1) which makes it possible to have a linear equation (5):

$$-ln\left(\frac{(1-P)}{P}\right) = a + b * LT \qquad 5)$$

With L50 = - a/b.

7. Multivariate statistical analysis

In this study, multivariate statistical analysis was performed using IBM SPSS Statistics 25. The relationship between temperatures, total chlorophyll, gonadal index and GI as well as the correlation between the GI of females and males were evaluated using Pearson's correlation test. In addition, an ANOVA test was performed to compare the gametogenic cycles of both sexes. In addition, the Python programming language was used to develop an algorithm for calculating size at first sexual maturity.

A correlation coefficient of +1 indicates a perfect relationship between the variables; -1 means an imperfect relationship. Again, the variables move in opposite directions; a value of zero means that there is no relationship between the variables. An R-value between 0.5 and 0.7 is considered a moderate correlation, while a significance level of $p = 0.7$ is considered a high correlation. knowing that $p < 0.05$ represents a significant difference between the variables, while $p > 0.05$ shows non-significance **(Azhari *et al.*, 2023)**. The map was created using ArcGIS software (ver. 10.8).

RESULTS

1. Diameter test classes

All 840 individuals measured indicated the existence of a distinct group of individuals. The majority of the total specimens (61.55%) had a diameter greater than 50 mm (±2.39), which represents the minimum size reference for conservation (MCRS). The diameters of the *P. lividus* urchins were distributed as follows: The size class 50–60 mm (±2.7) represented the highest proportion with 54.76%, followed by the size class 40–50 mm (±4.07) with 15.96% and the size class 60–70 mm (±2.09) with 6.78%. 10.83% had a diameter of 30–40 mm (±2.71). While, individuals smaller than 30 mm (±1.8) accounted for only 3.09%.

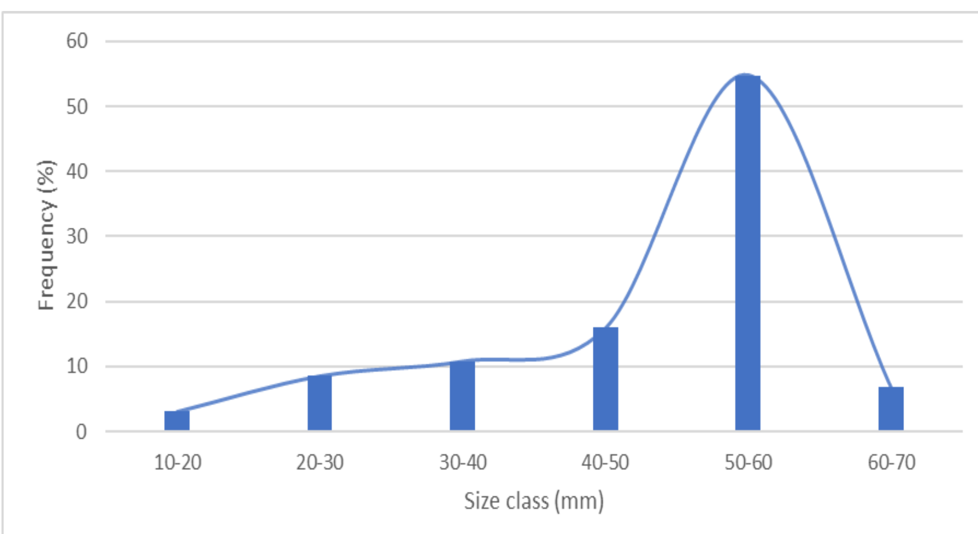


Fig 4. Demographic structure of *Paracentrotus lividus*.

2. Sex-ratio

The general sex ratio of *P. lividus* showed that there was no statistically significant deviation from 1:1 from January 2021 to February 2022 and no hermaphroditism was observed. The monthly comparison between males and females shows that, with the exception of February 2021, males outnumbered females with a ratio of 1.23. Thereafter, in the months of July and November 2021, the sex ratio experienced a significant drop and females became dominant in the samples, with a ratio of 0.11 and 0.3 respectively. Subsequently, the sex ratio has been increasing again, reaching a ratio of 1 in February 2022, indicating that the number of males and females was similar. It is noted that no cases of hermaphrodism have been observed.

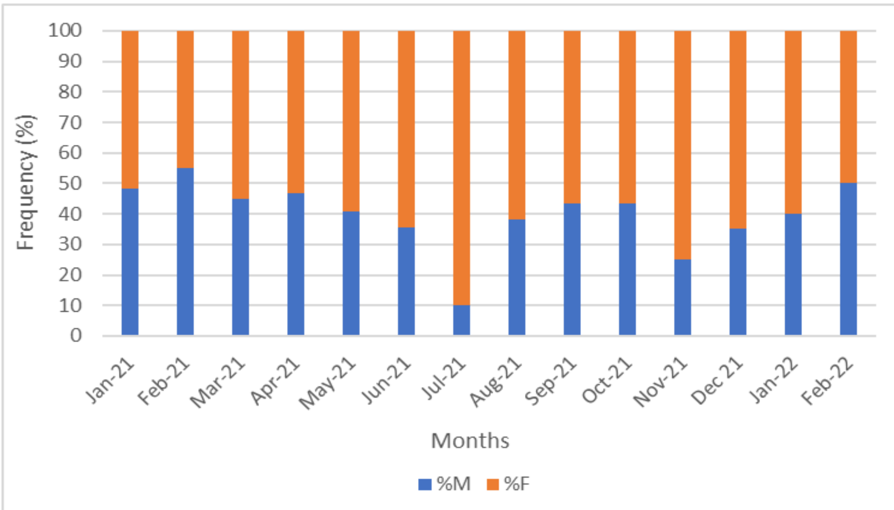
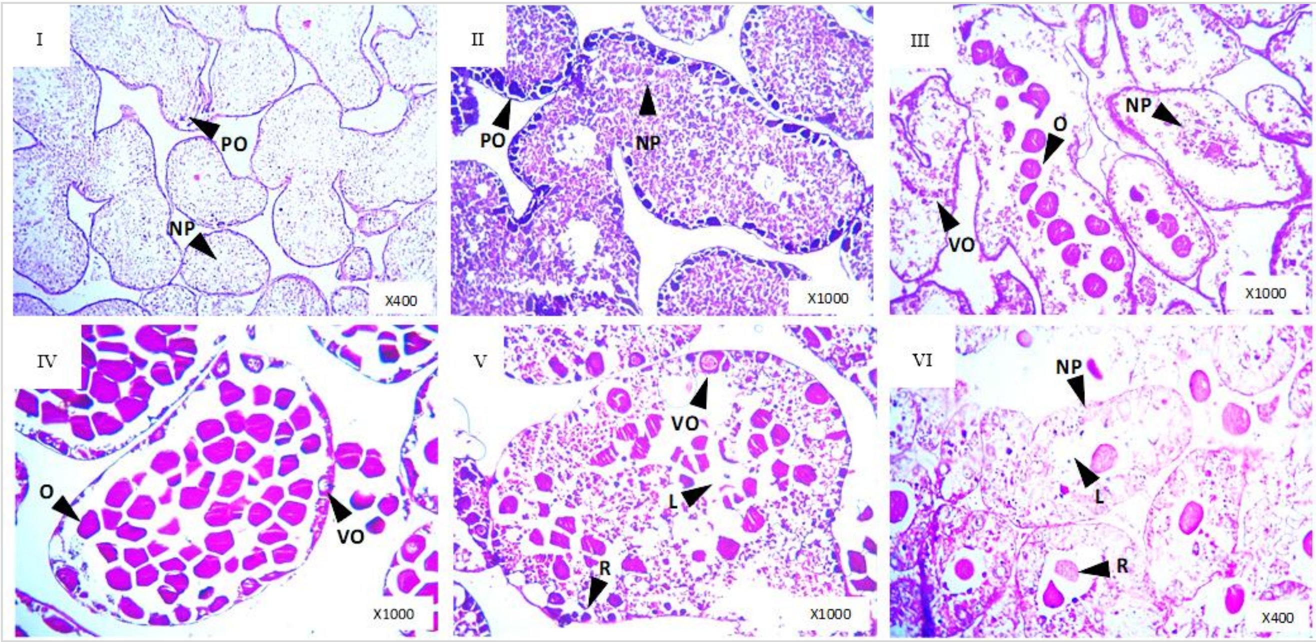


Fig 5. Evolution of sex ratio of *Paracentrotus lividus* during the reproductive cycle.

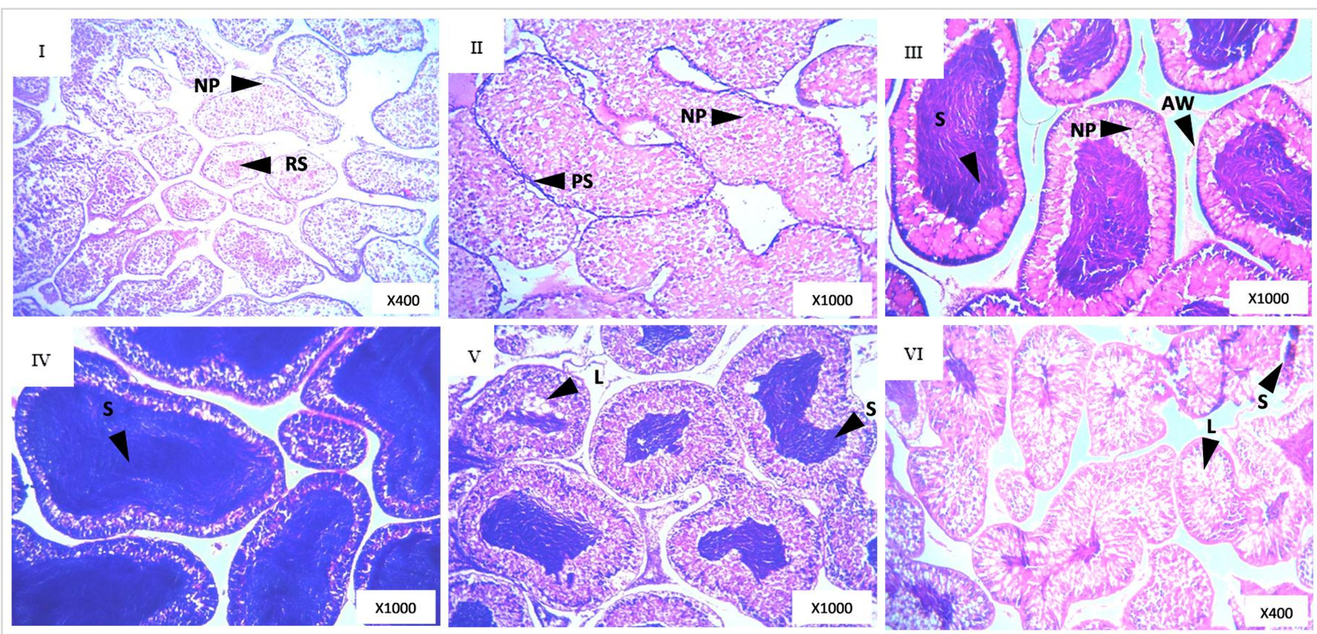
Table 2. Sex distribution in the population of *Paracentrotus lividus*.

Months	**Total number**			**Rate ± IC**		**Sex-ratio (F:M)**	**X^2 Cal**
	Females	**Males**	**Total**	**females**	**males**		
Jan-21	31	29	60	51.67	48.33	1: 0.9	-
Feb-21	26	32	58	44.83	55.17	1: 1.2	-
Mar-21	33	27	60	55.00	45.00	1: 0.8	-
Apr-21	32	28	60	53.33	46.67	1: 0.9	-
May-21	32	22	54	59.26	40.74	1: 0.7	-
Jun-21	38	21	59	64.41	35.59	1: 0.6	-
Jul-21	53	6	59	89.83	10.17	1: 0.1	-
Aug-21	37	23	60	61.67	38.33	1: 0.6	-
Sep-21	34	26	60	56.67	43.33	1: 0.8	-
Oct-21	34	26	60	56.67	43.33	1: 0.8	-
Nov-21	45	15	60	75.00	25.00	1: 0.3	-
Dec 21	39	21	60	65.00	35.00	1: 0.5	-
Jan-22	36	24	60	60.00	40.00	1: 0.7	-
Feb-22	30	30	60	50.00	50.00	1: 1	-
TOTAL	500	330	830	60.24	39.76	1: 0.66	11.4

3. Description of gonadal histology



(A)



(B)

Fig 6. The different stages of embryonic development of *Paracentrotus lividus* Females (a) and males (b): phase I. recovery; phase II. growth; phase III. premature; phase IV. maturation; phase V. partially produced and phase VI. spent.

NP: Nutritive phagocytes. PO: Previtellogenic oocytes. O: Ova. VO: Vitellogenic oocytes. R: Relict ova. L: Lumen. RS: Relict spermatozoa. PS: Primary spermatocytes. S: Spermatozoa. AW: Acinal wall.

4. Gametogenic cycle

During this study, the analysis of histological sections showed that the same gonads can contain different stages but with different proportions; this asynchrism is observed both in males and females. However, gametogenesis is synchronized in both sexes, while no significant differences were found (ANOVA. $P > 0.05$). In this population, the gametogenic cycle begins in August, with the frequency of individuals in gametogenic activity (premature and growth) increasing until October, reaching its higher proportion of 52% in the same month. The individuals continue their gametogenic activity forward and most individuals become mature from March to May, when they reach 50% of the total number of individuals. The appearance of partially produced and spent phages begins in April and reaches its peak in July (70%). The *P. lividus* could be described as a summer spawning species with a single peak towards the end of the summer season. The distribution of the six phases during the gametogenesis cycle was relatively approximate: the recovery phase was observed in 22% of all individuals followed by the growth phase in 20%. While the prematurity phase was the least observed with 12%. (Fig. 8) shows the stages distribution between males and females.

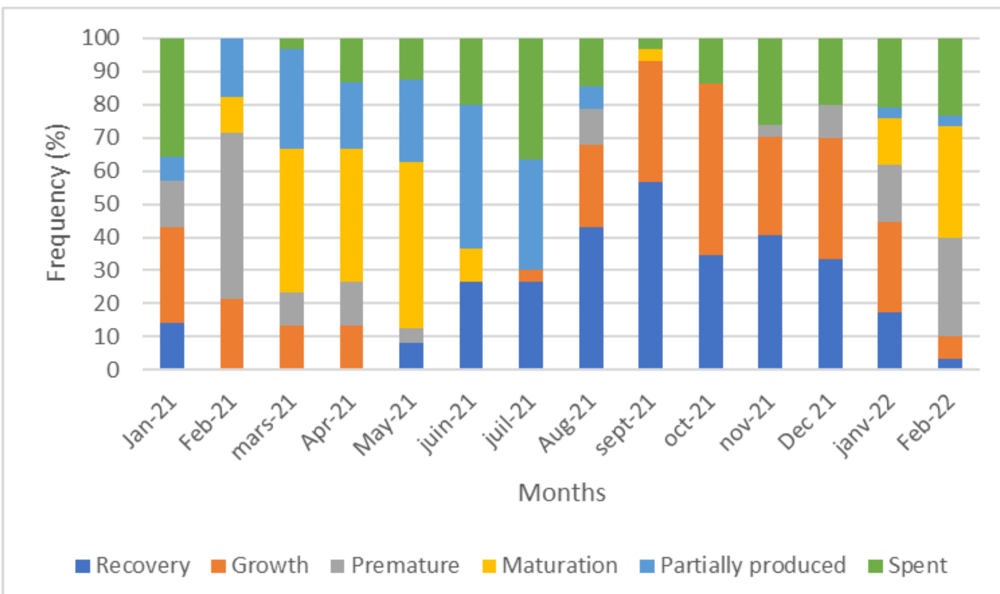
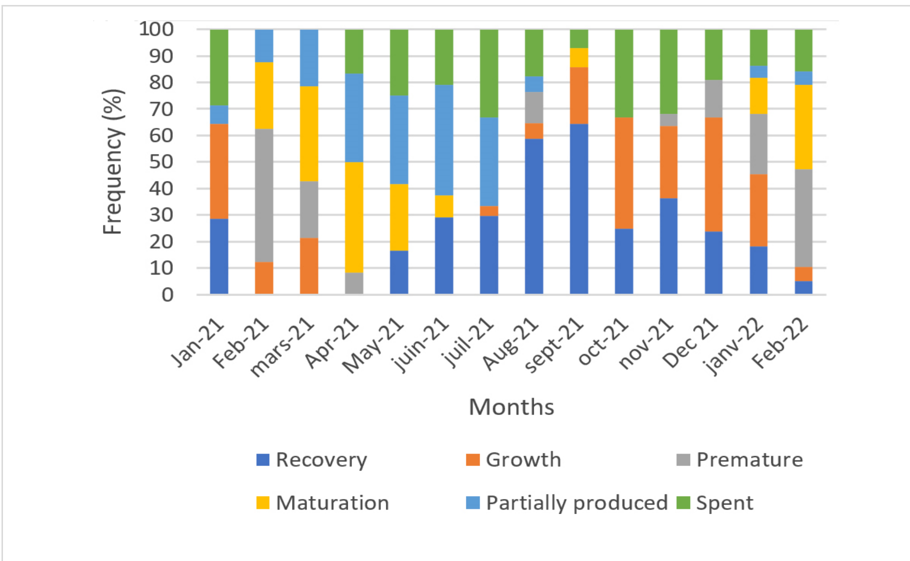
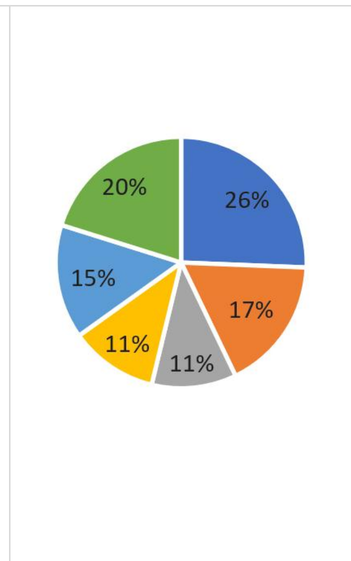


Fig 7. The annual gametogenic cycle of *P. lividus* shows the frequencies of maturity stages in sea urchin gonadal sections from the Tangier coast. The numbers above each column indicate the urchin frequency. The pie charts represent the average annual proportion of gonadal maturity stages of samples during January 2021 to February 2022.

The distribution analysis of gonadal cycle stages between the two sexes showed a rapid growth for males in January 2021. By February, embryonic development was continuing and 50% of males and females were approaching maturity (the premature stage). From March, the number of individuals in the mature phase increased, reaching 36% in females and 50% in males. The maximum value of this premature stage was recorded for males in May at 75%, while for females it was 42% in April. For both sexes, the maturity stage starts to decrease until it finishes at the end of June. Then, in July, all males become mature (Maturation, partially produced and spend), while mature females represent only 67%. It is also observed that females begin the recovery phase of the new cycle before males. The beginning of the second cycle in 2022 shows a slight acceleration in pace from 2021. The analysis of the gametogenesis cycle shows that the most dominant phase in females is the recovery phase (26%). followed by the spent phase (20%). The growth phase occurs most frequently in males (27%) and the maturation phase is second (19%). In general, the analysis of the gonadal cycle in both sexes showed an important similarity.



(A)



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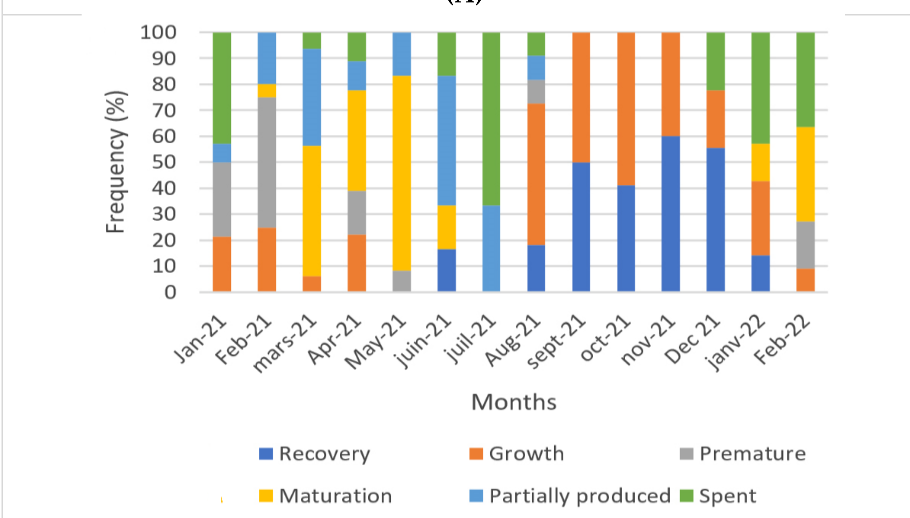
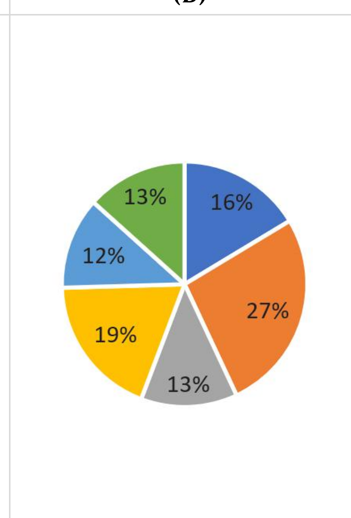


(C)



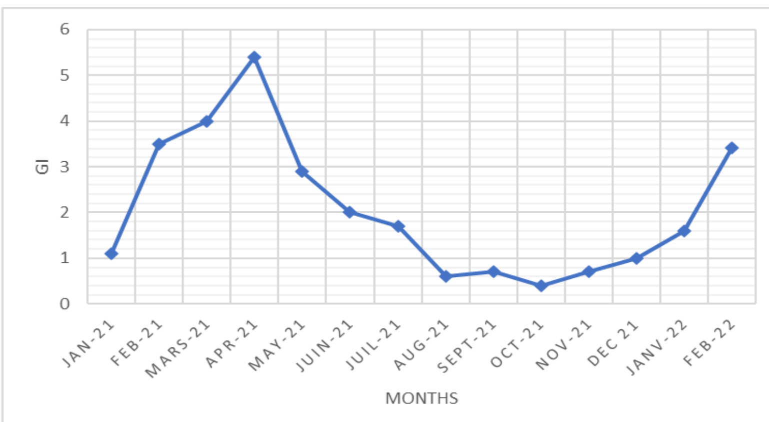
(D)

Fig 8. Females **(A)** and males **(B)** of the *Paracentrotus lividus* population from Tangier (on the northwest coast of Morocco) at different stages of sexual maturity. proportion of gonadal stages in females (C) and males (D) between January 2021 and February 2022, during January 2021 to February 2022.

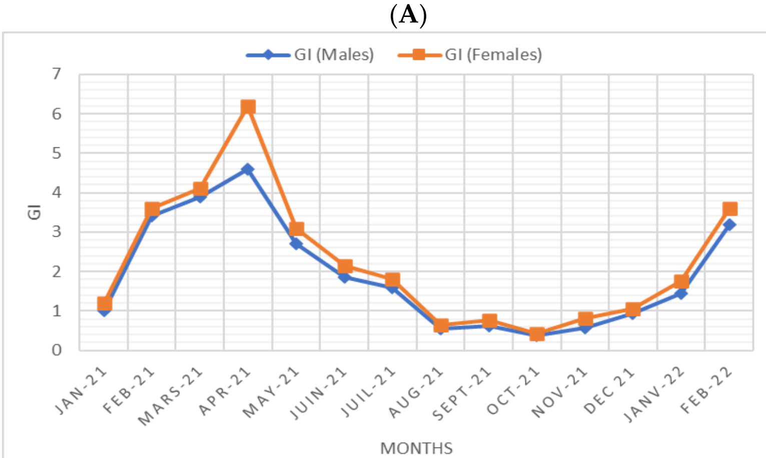
5. Gonadal index

For gonadal index calculation, 420 sea urchin specimens were collected from January to February 2022. The sea urchin diameter ranged from 17 to 69.5 mm without spines, with an average of 5.48 cm. The total weight of the individual has varied from 21.83 g to 109.75 g showing an average of 62.48 g.

The calculation of the gonadal index showed a progressive increase from January 2021 to April, where it registered a maximum value (5.4; ±1.77), then it started to decrease progressively, registering its minimum value between August and November (between 0.4 and 0.77, ±0.12). The GI began to rise again in December (from 1 to 3.4; ±1.27) in February 2022, roughly at the same rate as in early 2021. The gonadal index evolves in the same trend in males and females, with a moderate difference in April, when females have a slightly higher gonadal index (6.2), while the GI in males was 4.6. The Pearson correlation coefficient of the gonadal index in both sexes was significant ($R^2 = 0.982$; N = 550; $p > 0.05$), hence no significant differences were identified.



(**A**)



(**B**)

Fig 9. (A) Variation in gonadal index of all individuals of *P. lividus*; **(B)** Variation in gonadal index for each sex of *P. lividus*.

Seawater temperature monitoring during this study revealed two distinct seasonality in the temperature trend. Between January and May 2021, the lowest temperature values were recorded (<20 °C). These temperatures coincided with the highest values of the GI. This index began to decline at the end of July, reaching its lowest values between June and October, coinciding with a significant temperature increase (>20 °C). This period seems to coincide with a peak in the spawning period. Thereafter, a decrease in temperature below 20 °C was recorded between November and February (from 19.7 to 16.9 °C), accompanied by a significant increase in GI (from 0.7 to 3.4).

Concerning the chlorophyll concentration, the first five months of 2021 saw a significant chlorophyll concentration exceeding 0.3 mg/m^3. From the beginning of June, a remarkable decrease was observed at 0.19 mg/m^3 and it remained around 0.2 mg/m^3 until November, when it increased to the value of 0.3 mg/m3. Whereas, the maximum value of 0.45 mg/m3 was recorded in February 2022.

The correlation between temperature and gonadal index showed a moderately negative correlation (R^2 = -0.56) indicating that the temperature has an inverse impact on the gonadal index's evolution. On the other hand, the Pearson correlation test showed a positive correlation of 0.59 between the evolution of chlorophyll and the gonadal index. Indicating that chlorophyl stimulates the reproduction cycle of sea urchins.

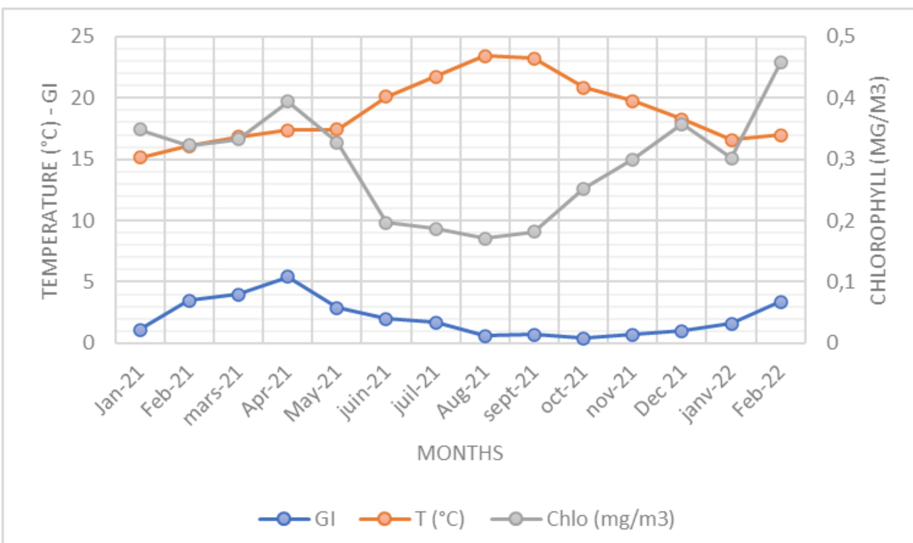


Fig 10. Monthly variation in the gonad index (GI) of *Paracentrotus lividus* as a function of seawater temperature and chlorophyll. Results are expressed as monthly mean ± SD (n = 30).

Table 3. Correlations of Pearson between IG. Temperature and Chlorophyll

		GI
T	Correlation of Pearson	-0.556
	Bilateral	0.019
Chlo	Correlation de Pearson	0.588
	Bilateral	0.006

6. Size of the first sexual maturity

In order to determine the size at first sexual maturity of the various populations of *P. lividus* at our study site, 420 individuals (17 to 69.5 mm) were collected and analyzed between January 2021 and February 2022. The results showed that the size at first sexual maturity has reached 34.32 mm for males. While for females this size was about 34.94 mm (Fig. 11). These results indicate that the sizes at first sexual maturity were significantly smaller than the legally established minimum conservation reference size (MCRS) of 50 mm test diameter **(Kassila *et al.*, 2018)**.

By comparing the L50 values of both sexes, it is noticed that the L50 of females was slightly higher than that of males. This result indicates that males reach sexual maturity before females. Statistical analysis showed that there was no significant difference between the sexes regarding L50.

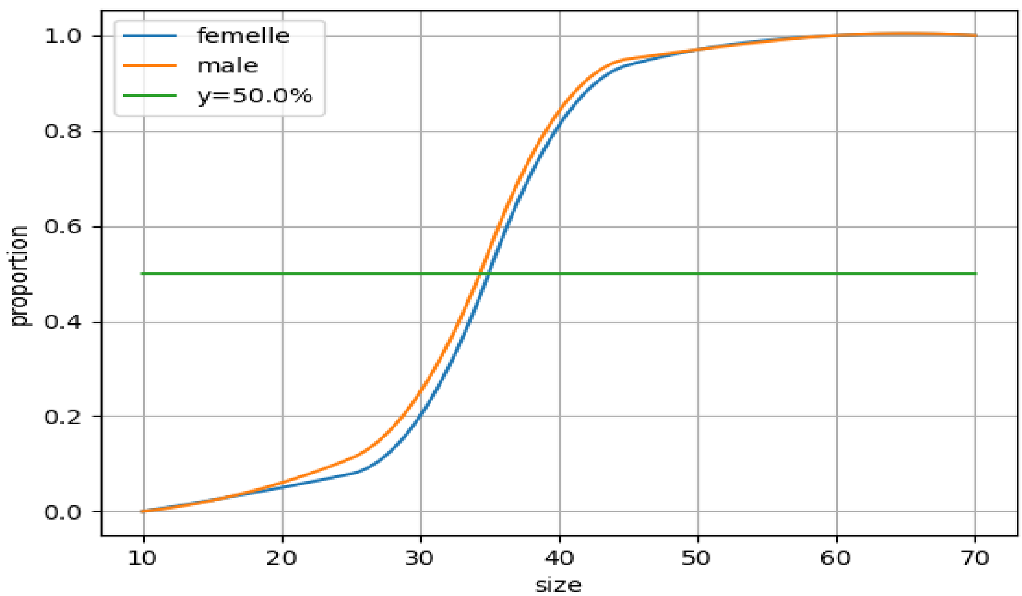


Fig 11. Size at first sexual maturity (L50) of *Paracentrotus lividus* populations of the Tangier coast

DISCUSSION

The large diameter of the sampled *Paracentrotus lividus* indicates the existence of a single cohort which can be explained by the lack of exploitation in our study area. Indeed, **El Jouhari *et al.* (2011)** studied the size distribution of sea urchins in the Sidi Bouzid area located in the Moroccan Atlantic and reported that the exploitation of sea urchins could contribute to the appearance of two different cohorts. This single cohort can also be explained by the fact that the population of our study area is not very old (the maximum size is 66 mm). According to Levitan (1998), the appearance of two different cohorts of sea urchins could be attributed to the age of the sea urchin **(Levitan, 1988).** The diameter test class analyses showed a high frequency, with 68% of individuals having a size larger than 50 mm. Compared to other studies, in the Atlantic Ocean area, specifically in the Aoufiste area near Agadir, only 10% of the total number of individuals collected was higher than 50 mm. The intensive fishing activities might generate a reduction in size classes due to the selective harvesting of the larger individuals **(Kassila *et al.*, 2018).**

In general, the sex ratio is balanced at 1:1 in this study. The results are in accordance with other studies on *Paracentrotus lividus* showing a balanced sex ratio. For example, Galicia Spain **(de Pesca, 1995)**, El-Jadida-Safi region, Morocco **(Jouhari *et al.*, 2014)** and on the west coast of Portugal **(Machado *et al.*, 2019)**. However, other studies have shown a sex ratio imbalance in favor of females, such as in Algeria **(Guettaf *et al.*, 2000)**. or variation over time in Portugal **(Rocha *et al.*, 2019)**. Differences in growth and mortality could explain the dominance of one sex over the other. In addition, this dominance could also be influenced by the difference in gonad maturation after spawning between males and females **(McPherson, 1965)**. This variation in the sex ratio can be related to environmental factors and also to the individual's maturity state. This behavior of adopting this strategy of sexual distribution is meant to ensure the survival and sustainability of the species **(Jouhari *et al.*, 2014).**

After analyzing histological sections, six developmental phases were identified in both males and females. Similarly to other studies carried out in the south Atlantic of Morocco **(Ouchene *et al.*, 2021),** in the Mediterranean sea **(Sánchez-España *et al.*, 2004a; Loi *et al.*, 2015; Elakkermi *et al.*, 2021)** , in the Adriatic Sea **(Fabbrocini and D'Adamo, 1816)** and on the west coast of Ireland **(Byrne, 1990a)**. The cycle did not present any resting phases. During this study, a synchronism was found in gametogenesis and the spawning period between females and males.

Histological observation of *Paracentrotus lividus* gonads showed that the reproductive cycle starts with gonadal development, which occurred from August during the last summer and autumn. Nutritive phagocytes are replaced by germinal and gametic structures while the number of sex cells increases. This was confirmed by the study carried out in Bostaneh. Persian Gulf. where *Paracentrotus lividus* populations are dominated by premature and mature individuals in winter and early spring with a clear change in the role of the nutritive phagocytes from nutrient storage to nutrients transfer for the gametes development **(Mahdavi SHahri *et al.*, 2008)**. Furthermore, Raymond (1987) suggests that the low temperatures trigger a cue for the completion of vitellogenesis **(Raymond and Scheibling, 1987)**.

Our study reported a continuous process with complete gonadal restitution after one spawning episode in June-July, without a resting period. The sea urchin has a prolonged reproductive cycle year-round, which gives this species a high reproductive capacity. A prolonged spawning season with one major spawning was also reported for *P. lividus* in Annaba Bay in Algeria **(Amri *et al.*, 2017)**, Tunisia Bay **(Sellem and Guillou, 2007; Arafa *et al.*, 2012),** North Atlantic of Morocco, Agadir **(Jouhari *et al.*, 2014)** Rabat **(Bayed *et al.*, 2005)** and Salobrea in the Mediterranean of Spain **(Murillo-Navarro and**

Jimenez-Guirado, 2012). On the other hand, other studies conducted in the Mediterranean have indicated the presence of two spawning periods, such as the investigations carried out on the beaches of Algeria and Marsa **(Guettaf *et al.*, 2000)** and Oran **(Belkhedim *et al.*, 2014)** which showed the presence of two spawning periods during an annual cycle (Table 4). This reproductive strategy characterized by a prolonged spawning period may be advantageous for the species since it may ensure a continuous 'input' of settling juveniles over many months. Thereby spreading reproduction risk over time **(Moura *et al.*, 2008)**.

Comparing these results with those of the Bay of Algeria, we can note an important variation, where two spawning periods were recorded, the first from April to May and the second from August to September. This confirms that the determination of the reproductive cycle of the sea urchin in the Mediterranean is very complex, as reported by **(Gianguzza *et al.*, 2013; Ouréns *et al.*, 2013).** This difference can be explained by the fact that the Gibraltar area is characterized by a higher degree of variability related to environmental parameters, showing a significant variability between Tangier and M'diq, located in the Moroccan Mediterranean., making this strait a particular ecosystem **(Zaafa *et al.*, 2012)**. By comparing with the Moroccan North Atlantic, we find that the spawning period in Tangier (March to July) is more comparable to that of Rabat **(Bayed *et al.*, 2005)**. While Agadir's spawning period exhibited a greater degree of variation (July–August) **(Jouhari *et al.*, 2014)**. Temperature and phytoplankton bloom may stimulate spawning, according to previous studies **(López *et al.*, 1998; González-Irusta *et al.*, 2010a)** and photoperiod visibility and the lunar period can also impact the spawning period **(Reuter and Levitan, 2010)**. At the same time, other researchers have shown that other conditions, such as population density, may potentially influence genetic variation across populations **(Ouréns *et al.*, 2013)**.

The gonadal index results showed a correlation between the spawning period and the peak of the index; also, the lowest gonadal index values corresponded to the onset of gametogenesis activity in August 2021. In the same way, Byrne (1990) reported that before spawning, there was an increase in GI during the gametogenesis phase. The gonadal index in *Paracentrotus lividus* of Tangier showed a strong similarity between sexes, except in the period from March to May, which corresponds to the spawning period, where this index was more important in females, as on the north Atlantic coast of Portugal, where GI is also in favor of females during some periods **(Rocha *et al.*, 2019).** An important variation compared to the Atlantic population was observed, where the GI reached up to 25 in the Safi region of the Moroccan Atlantic **(Jouhari *et al.*, 2014)**. Whereas the maximum value recorded in Tangier was 5.4, this may be related to the difference in size of gonads between the two populations.

Table 5. Comparison of our *Paracentrotus lividus* spawning season results and other studies in the Atlantic Ocean and Mediterranean Sea.

Country	Location	Period	Number	Spawning season	Methods	Reference
Algeria	Bay of Algiers MED	Jan 1994–Dec 1994	2	Apr-May Aug-Sep	GSI	(Guettaf *et al.*, 2000)
	El Marsa MED	Jan 1994–Dec 1994	2	Apr-Jun Oct-Dec	GSI	(Guettaf *et al.*, 2000))
	Bou Ismail MED	Jan 1994–Dec 1994	1	Feb-Mar	GSI	(Guettaf *et al.*, 2000)
	Port of Oran MED	Jan 2011–Jan 2012	1	Mar-May	GH. PGI	(Belkhedim *et al.*, 2014)
	Gulf of Annaba MED	Feb 2012–Jan 2013	1	Mar-Jun	GSI	(Amri *et al.*, 2017)
Croatia	Bistrina Bay ADR	Aug 2002–Jul 2003	1	Mar-Jul	GSI	(Tomšić *et al.*, 2010)
France	Southern Brittany ATL	Mar 1993–Mar 1995	1	Lay-Aug	GH. GSI	(Spirlet *et al.*, 1998)
Italy	Province of Lecce ION	Jul 2009–Jun 2010	3	Jan-Mar Jul Oct-Nov	GH. GSI. PGI	(Tenuzzo *et al.*, 2012)
Morocco	Tangier MED	Jan 2021 – Feb 2022	1	Mar-Jul	GH. GI	Present study
	Agadir ATL	Mar 2018- Feb 2019	1	Mar-Oct	GH	(Ouchene *et al.*, 2021)
	El Jadida-Safi ATL	Mar 2009-Mar 2011	1	May-Aug	GH. GI	(Jouhari, 2014)
	Rabat and Casablanca ATL	Apr 1999–Apr 2000	1	Mar-Jun	PGI	(Bayed *et al.*, 2005)
Portugal	Carreço (NW) ATL	Nov 2010–Nov 2012	1	Apr-Sep	GH. GI. GSI	(Machado *et al.*, 2019)
	Aljezur (SW) ATL	Nov 2010–Nov 2012	1	Apr-Oct	GH. GI. GSI	(Machado *et al.*, 2019)
	Cabo Raso ATL	May 1999–Jul 2000	1	Mar-Aug	GSI	(Gago *et al.*, 2003)
Spain	Tossa and Cubelles MED	Jun 1992–Aug 1993	1	Mar-Jun	GH. GSI	(Lozano *et al.*, 1995)
	Southern coast MED	May 1999–Nov 2001	1	Mar-Sep	GH. GSI	(Sánchez-España *et al.*, 2004b)
	Bay of Biscay ATL	May 2004–Sep 2005	1	Mar-Sep	GH. GSI	(González-Irusta *et al.*, 2010b)
	Bay of Biscay ATL	Jan 2006–Feb 2007	1	Apr-May	PGI	(Garmendia *et al.*, 2010)
	Salobreña MED	Nov 2008–Oct 2009	1	Mar-Sep	PGI	(Murillo-Navarro and Jimenez-Guirado, 2012)
	Galicia ATL	Jun 2006–May 2008	1	Apr-Jun	PGI	(Ouréns *et al.*, 2013)
	Western coast ATL	May 1986–Aug 1988	1	Jul-Aug	GH. GSI	(Byrne, 1990a)
Turkey	Çanakkale AEG	Feb 2003–Jul 2003	1	Feb-Jul	BC	(Dincer and Cakli. 2007)

Notes. ATL: Atlantic Ocean; AEG: Aegean Sea; ADR: Adriatic Sea; ION: Ionian Sea; MED: Mediterranean Sea; BC: biochemical composition; GH: gonadal histology; GI: mean gonadal index; GSI: gonadoso-matic index; PGI: physiological and gonadal indices

In this study, seawater temperature was chosen as a factor influencing the reproductive cycle of sea urchins. There is a significant relationship between the spawning of this species and the availability of phytoplankton because there is a positive correlation between chlorophyll concentrations and the percentage of mature gonads and a negative correlation between temperature and this percentage. The results of the population structure and gonadal index in females and males were generally close to each other, except in the period from March to May, which corresponds to the spawning period, where it is more important in females, as on the north Atlantic coast of Portugal, where GI is also in favor of females **(Rocha *et al.*, 2019)**. Similar to the findings of the reproductive cycle, which revealed no significant differences between the sexes, the size at first maturity of females and males was approximately equivalent (34.94 mm and 34.32 mm, respectively). In the Agadir research by Ouchène (2021), the L50 showed more variation between sexes (36.52 mm for females and 33.33 mm for males). This difference can be attributed to the difference between the Atlantic Ocean and the Mediterranean Sea, which is mainly due to the physicochemical parameters, variation of nutrients and abundance of algae **(Murillo-Navarro and Jimenez-Guirado, 2012)**, without forgetting also that the *Paracentrotus lividus* population of Gibraltar showed a significant genetic differentiation to the Atlantic population, due to a restricted genetic flow through the geographical border zone imposed by the Strait of Gibraltar **(Duran *et al.*, 2004)**.

CONCLUSION

This study was carried out on the coast of Tangier near the Strait of Gibraltar which is considered a particular ecosystem. The results obtained showed complementarity and congruence between the microscopic method by histology and the macroscopic method by the calculation of the gonadal index (GI) as well as the effects of environmental parameters which showed a strong correlation between the reproductive cycle and temperature and chlorophyll respectively.

Finally, this work aims to provide useful information that can be used to propose other national management measures such as setting up a biological rest during the season when gametes are released (February to July) if this species is exploited and making a national conservation management plan. In addition, *P. lividus* has been the subject of several international breeding studies in order to valorize these resources whether for commercial or ecological reasons. Therefore, these results may be of interest in the aquaculture field.

In perspective, other studies such as expanding the study to the entire Moroccan Mediterranean evaluating the stock and associated species and determining the quality of *Paracentrotus lividus* gonads could be useful in developing a scientific database on the sea urchin in the context of sustainable development and conservation of fishery resources. Overall. The results will support decision-makers in establishing adequate policies to valorize this species and the implementation of precautions necessary for the sustainability of biodiversity in the future.

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