

Seasonal changes in the reproductive cycle and condition index of the surf clam *Mactra stultorum* (Mollusca: Bivalvia) in the Gulf of Gabes, Tunisia

by

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Abstract

The reproductive biology of *Mactra stultorum* from the Gulf of Gabes was investigated monthly during a one-year period (2017). This study is the first study examining its reproductive biology during one-year in the south of Tunisia. The overall samples presented a balanced sex ratio, with males dominating among smaller individuals (< 22 mm) and females predominating in larger size classes (> 34 mm). The obtained total sex ratio (F:M) was equal to 1.03:1. The size at first maturity ($SL_{50\%}$) was 20.43 and 22.10 mm for males and females, respectively. The seasonal variations assessed through macro- and microscopic characteristics in the gonads of both sexes indicated a clearly defined annual reproductive cycle with a principal spawning peak during summer and a resting period during December and January. The reproduction of *M. stultorum* was strongly influenced by fluctuations in the seawater temperature, as reflected through the temporal variation in the gonadal cycle, condition index (CI) and tissue weight rate (TiWR). If managed properly, *M. stultorum* is expected to meet the domestic market demand through artisanal fishery. The adoption and implementation of rules, such as limiting the size of clams, is required to protect this new exploitable fishery resource.

Key words: *Mactra stultorum*, reproductive cycle, sex ratio, size at maturity, condition index, Gulf of Gabes, Tunisia

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1. Introduction

Macra stultorum (Linnaeus 1758), family Mactridae, is a benthic species of bivalve mollusc inhabiting sandy areas of the lower infra-littoral zone. It has a wide geographical distribution ranging from Norway in the north of Europe, to Senegal in West Africa, as well as the Mediterranean and Black Sea (World Register of Marine Species; <https://www.marinespecies.org>). *M. stultorum* is also extensively utilized as seafood and raw material for manufacturing feed at various aquaculture farms (Hou et al. 2006). Despite its abundance, this clam is an unexploited species that has not yet been commercialized for the Mediterranean market.

Even with the increasing importance of shellfish commercialization, available information on the biology of commercially viable species is insufficient. Also, despite the key ecological importance of invertebrates species, their fisheries often occur without assessment, monitoring and ecological surveys (Solis et al. 2021, Stiepani et al. 2023). In Tunisia, commercial bivalve fisheries constitute a cultural, social and economic resource for numerous coastal communities.

In Tunisia, the shellfish commercialization has so far focused only on the clam *Venerupis decussata* (Linnaeus 1758) which is extensively harvested from natural populations and is economically important in terms of employment and exportation. New initiatives in the shellfish fisheries sector include diversification into other exploitable species. Therefore, further research on the reproductive cycle of commercially viable species is required to implement management measures aiming to promote the sustainable exploitation of the shellfish resources. Due to expanding fisheries, the Laboratory of Fisheries Sciences has recently begun new projects dealing with the reproductive biology of potential shellfish production areas in southern Tunisian waters. Among these shellfish species, the surf clam *M. stultorum* is one of the most abundant bivalve species along the Tunisian coasts.

In areas where the resource exists, the clam *M. stultorum* has often attracted considerable research attention because of its economic potential. From the published literature available, some works have dealt mainly with diverse aspects of the clam from the North of Tunisia including its occurrence (Charef et al. 2012), biology and biochemistry (Chetoui et al. 2018, 2019) and genetics (Chetoui et al. 2012, Chetoui 2016). However, no data are available for *M. stultorum* on the reproductive biology from the South of Tunisia. What is known about this species is limited to the

contribution of Derbali et al. (2021) focusing on the species distribution.

The clam *M. stultorum* is particularly abundant in the south of Tunisia and appropriate information on the reproductive biology is required to better manage its exploitation and propose regulatory measures by the fisheries authorities (e.g. to establish closed seasons for fishing). Understanding its biology is thus crucial to specify its present status in the southern Tunisian waters. This study aims to report for the first time information about *M. stultorum* from Gulf of Gabes, by evaluating *M. stultorum*'s reproductive cycle, sex ratio and size at sexual maturity. This work also represents the first step for any future work and may be a valuable research reference about shellfish species.

2. Materials and methods

2.1. Study area

The southern coast of Sfax located in the Gulf of Gabes, represents the major shellfish production area of Tunisia (Fig. 1). The climate is that of the arid and semiarid Mediterranean, largely influenced by its gentle topography and maritime exposure (Chamtouri et al. 2008). Both the wide and shallow continental shelves are topographically regular. The bottom slopes gradually toward the sea, with a depth equal to 60 m at 110 km off the coast (Ben Othman 1973). The substrate in the inshore zones, covered in some areas by the seagrass *Cymodocea nodosa* (Ucria) Ascherson and *Zostera noltei* Hornemann, is mostly silty sand (Derbali et al. 2016). The climate is dry (average annual precipitation 210 mm), due to the effect of hot southerly winds (Sirocco). The tide is semidiurnal, with a high tide of +1.60 m and a low tide of +0.30 m in the spring tide (Zaghden et al. 2014).

2.2. Field sampling and analysis

Approximately, 90 specimens of the clam *M. stultorum* from the sandy areas of Skhira were sampled each month between January and December 2017. During each sampling period, the seawater temperature and salinity were recorded at the time of the clams collection using a multi-parameter kit (Multi 340 i/SET). The shell length (SL, mm; SL: maximum distance along the anterior–posterior axis) was measured using a digital caliper (precision of 0.01 mm) and the total weight (TW, g) by a top-loading digital balance (precision of 0.001 g). For all sampled individuals, the soft tissues were carefully removed

from the shells and washed using distilled water. Both the soft tissues and shells were weighted before and after putting in an oven at 60°C for 48 hours using a top-loading digital balance (precision of 0.001 g).

The condition index (CI) reflects physiological and bio-energetic changes in an organism (Prgić 2019). Among bivalves, different methods for CI calculation exist. According to Walne (1976), CI was calculated using the formula:

$$CI = 100 \times \frac{DTiW}{DSW}$$

where DTiW is the dry soft tissue weight and DSW is the dry shell weight. On the opposite side, Ruessler et al. (2011), Dabrowska et al. (2013) as well as Riisgård et al. (2014) suggest other methods which represent the ratio of the dry soft tissue weight and shell length described by the following formula:

$$CI = 100 \times \frac{DTiW}{SL}$$

The tissue weight rate was obtained with the equation proposed by Choi & Chang (2003):

$$TiWR = 100 \times \frac{TiW}{TiW + SW}$$

where TiW = the soft tissue weight and SW is the shell weight.

The reproductive activity of *M. stultorum* was specified by (1) investigating the gonad macroscopic appearance and (2) microscopic examination of the sexual products smears (maturity grading given below). The shell valves were parted and a subjective estimation of gonad volume made. The visceral mass was torn off and smears of the visceral wall with attached gonad were investigated at 100 X magnification. The sex of each individual was identified. Each specimen was sexed, and staged on the basis of the gonad colour, gonad size, and presence or absence of mature cells (see stage description below). The sex ratio (Females:Males; later F:M) and the ratio of mature individuals in 1-mm size classes were computed. A logistic curve was fitted to the data to determine the size at which half of the individuals ($SL_{50\%}$) were sexually mature. The quasi-Newton algorithm for non-linear least square estimation of function parameters was applied to the data according to the following equation:

$$P = \frac{1}{1 + e^{-a(SL - SL_{50})}}$$

where P is the proportion of mature individuals; a is the estimated parameter (slope of the curve); SL is the shell length corresponding to the proportion (P);

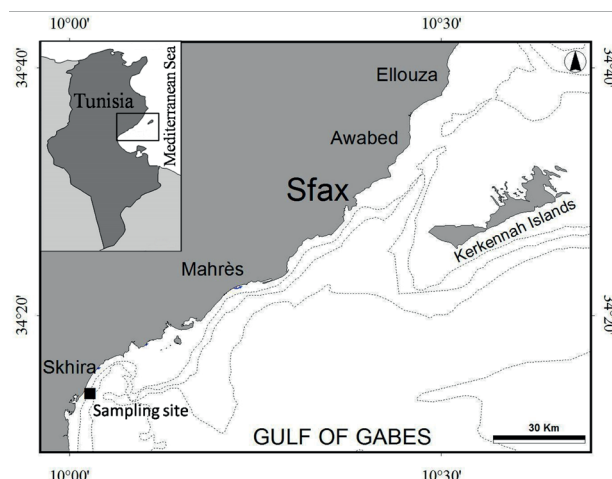


Figure 1

Map of the study area. The location of sampling transects is indicated

SL_{50} is the total length of 50% mature clams. Samples were classified into five stages according to the scale modified from Wolowicz (1987). This scale could be adapted to *Cerastoderma glaucum* (Derbali et al. 2009a), *Polititapes aureus* (Derbali, 2022) and *Macra stultorum* gathered from the southern Tunisian coasts. These species have the same gonadal development and gametogenesis activity in this area.

- Stage I: undifferentiated, beginning of gametogenesis; gonads were not observed and sex has not yet been differentiated.
- Stage II: developmental; gonads start to develop in the foot tissue and around the digestive gland, and sex is distinguishable.
- Stage III: gonads, formed by a compact tissue, were developed: white-cream streamed line in males and yellow cream granular isles in females.
- Stage IV: reproductive stage characterized by inflated and ripe gonads.
- Stage V: gonads appear spent and visceral mass becomes flaccid.

2.3. Statistical analysis

The comparison of clam size (SL and TW) was performed with a Z-test. Then and after \log_{10} transformation of data, the null hypothesis with no difference between the slopes of the SL vs TW relationship was verified with a Student's t-test



(Zar 1996). Statistically significant deviations from a balanced sexual proportion equal to 1:1 were evaluated using the χ^2 test. ANOVA followed by the Tukey/post hoc test (Zar 1996) was employed to test for differences among the reproductive variables (CI and TW) observed between months. The results were illustrated as means (\pm 95% confidence interval) and the significance level threshold used in the tests was set to 0.05.

3. Results

3.1. Sex ratio

Overall, 477 females (43.5%), 462 males (42.2%) and 157 individuals of undetermined sex (14.3%) were identified. Specimens of the two sexes showed a broad size range in terms of their SL (10.15–43.60 mm) and TW (0.22–13.28 g). Generally, females were larger and heavier (31.95 ± 0.41 mm and 6.74 ± 0.20 g, respectively) than the males (31.20 ± 0.51 mm and 6.19 ± 0.20 g, respectively) (Z-test, $p < 0.05$). Individuals with undetermined sex appeared in samples taken in December and January (at the resting phase or in the gametogenesis initial stage).

The overall sex ratio (F:M = 1.03:1) was not significantly divergent from parity (F:M = 1:1; χ^2 test, $p > 0.05$). To detect the changes of the sex proportion as a function of specimen size, data was classified into length classes of 6-mm intervals (Fig. 2). The small individuals (< 22 mm SL) were largely males and a statistical balance was observed in both sexes in the intermediate size classes (22–34 mm SL), while females predominated in the larger size classes (> 34 mm SL) (χ^2 test, $p < 0.05$).

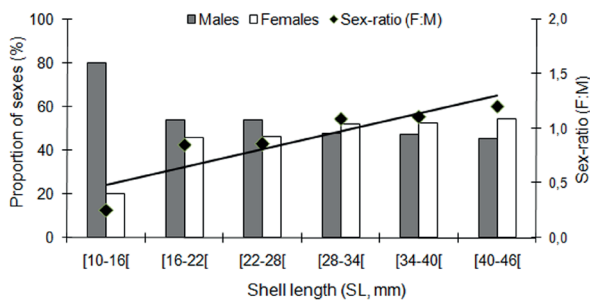


Figure 2

Mactra stultorum: Variation of sexual proportion (%) and sex ratio (F:M) according to individuals size (SL, 6 mm classes). The dotted line illustrates an unbiased sexual proportion (F:M = 1:1). * Size classes with unbalanced sex ratio (χ^2 test, $p < 0.05$).

3.2. Size at first maturity

The relationship between the percentage of mature *M. stultorum* and total SL for both sexes is shown in figure 3. The variations of the proportion of mature individuals demonstrate that, at a shell length (SL) < 13 mm, no mature individual was recorded, and that at a size (SL) > 24 mm all individuals were mature. The results revealed that size at first maturity ($SL_{50\%}$) was equal to 20.43 mm for males, 22.10 mm for females and 21.28 mm for both sexes combined (Table 1). The sizes at first maturity corresponding to 25, 50 and 75% mature *M. stultorum* ($SL_{25\%}$, SL_{50} and $SL_{75\%}$) are summarized in Table 1.

Table 1

Mactra stultorum. Size at first maturity corresponding to being 25, 50 and 75% mature (L_{25} , L_{50} and L_{75}) in the Gulf of Gabes (Tunisia)

Parameters	Males	Females	Combined sexes
α	0.987	1.138	1.025
R^2 (correlation)	0.993	0.997	0.998
L_{50} (mm)	20.43 ± 0.19	22.10 ± 0.13	21.28 ± 0.11
L_{25} (mm)	19.32	21.13	20.21
L_{75} (mm)	21.54	23.07	22.35

3.3. Environmental parameters

Changes in water temperature in the study area ranged from 12.2°C to 14.5°C in winter and from 24.1°C to 25.6°C in summer. The highest values were recorded in July (25.6°C), whereas the lowest values were recorded in January (12.2°C). Salinity measurements showed an annual fluctuation between 29.2 in winter and 45.5 in summer, as shown in figure 4.

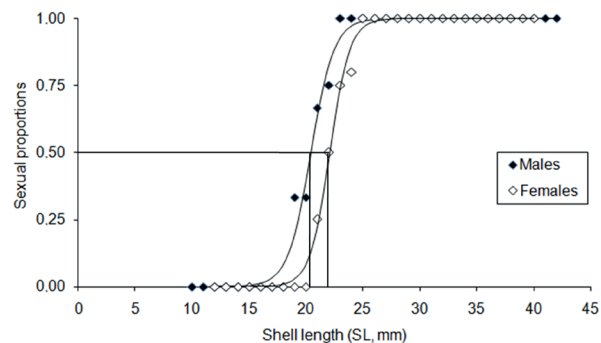


Figure 3

Mactra stultorum: Relationship between percentage of mature individuals and total shell length

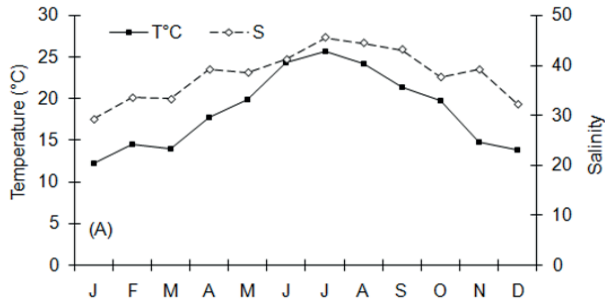


Figure 4

Mactra stultorum: Monthly variations in water temperature and salinity recorded during the study period (2017) in the Skhira region (south of Tunisia)

3.4. Condition index and tissue weight rate

Monthly changes in the CI and TiWR of the clam *M. stultorum* are shown in figure 5. The CI calculated by different methods varied over the monitoring period. A major peak occurred in May in specimens with high proportions of ripe gonads. The minimum CI values recorded in January, February and March were significantly different from those recorded in the other months of the present study (ANOVA, Tukey/*posthoc*, $p < 0.05$). This index afterward showed a tendency to decrease in summer and remained constant in autumn months. The monthly changes in the TiWR were similar to those of the CI.

3.5. Seasonal gonadal development

Mactra stultorum showed an annual reproductive cycle in the Gulf of Gabes. Gonads began to develop around the digestive gland. During the maturation period, the gonads reached a great size and almost disappeared at the lowest reproductive activity.

There were variable proportions of all stages of gametogenesis in *M. stultorum* males and females throughout the year of investigation (Fig. 6). Results showed a the large proportion of the target specimens spawned in summer months with a major peak in July, but that spawning could continue, at a reduced intensity, during the remaining period of the year. Gametogenesis started in January. Rapid gametogenesis (stage II) was then recorded for both sexes in February, March and the beginning of April, resulting in a great distension of the visceral mass. In April, most males and females reached gonad maturity development (stage III and IV) (85% and $\approx 77\%$, respectively). After this time, the frequencies of ripe individuals of both sexes maintained at high levels in

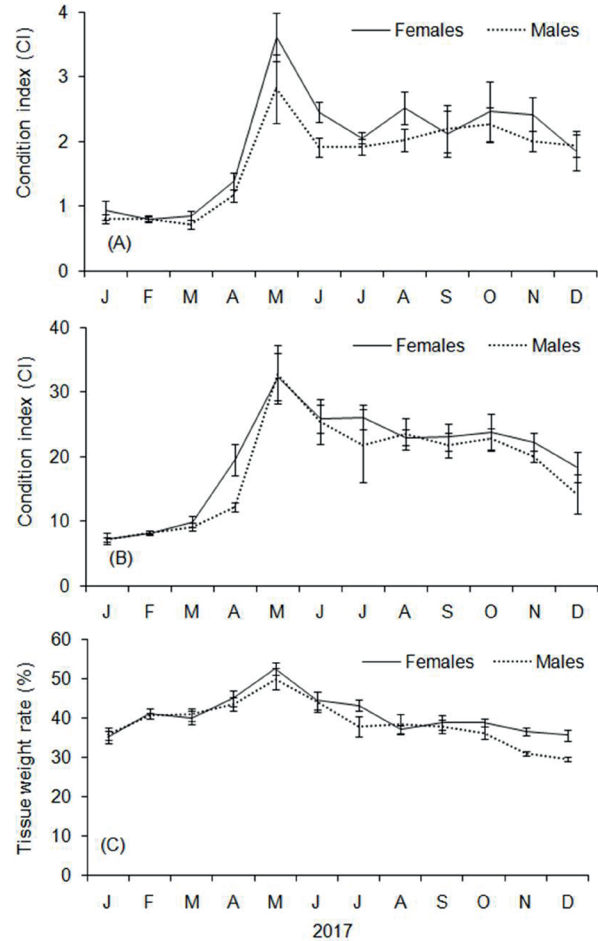


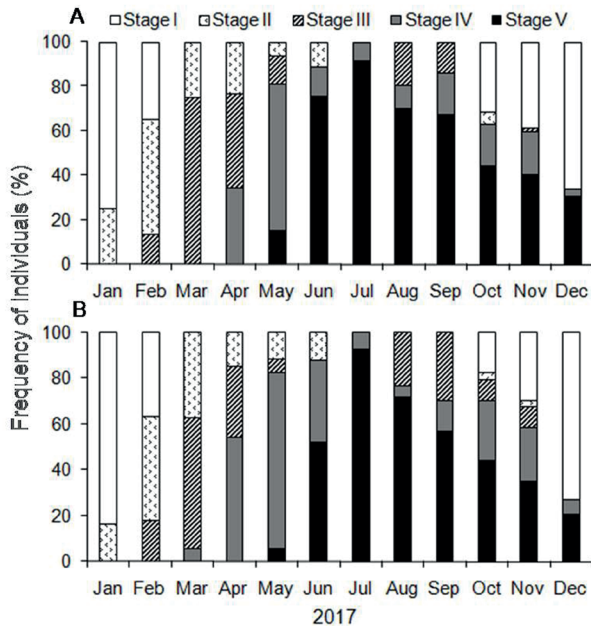
Figure 5

Mactra stultorum: Monthly variations in the condition index A (supplemented with data for shell length) and B, and tissue weight rate as percentage of total weight (C) in the Gulf of Gabes (Tunisia). Values expressed as mean \pm IC. The number of females and males are 477 and 462, respectively.

May (77% males and 66% females). Spawning activity started in late May and continued until December for both sexes.

Some individuals became mature (rose promptly) and the recorded ratio from September to November of mature males was ($\approx 23\%$) and females (19%). The main gonad activities and spawning periods throughout the year were roughly synchronous between the two sexes and were apparently triggered by the rising seawater temperature. For both sexes, the resting phase started in late December and continued until January. This stage occurred at the period of the lowest seawater temperature in winter.



**Figure 6**

Mactra stultorum: Relationship between the monthly frequency distribution of gonadal development stages in females (A) and males (B) during the study period (2017)

4. Discussion

The present study reports for the first time the current status of the surf clam *Mactra stultorum* from the Gulf of Gabes (South of Tunisia) in terms of its reproductive biology. Though it is one of the most important components of the benthic fauna, knowledge of the reproductive cycle of *M. stultorum* in Tunisian waters is relatively insufficient. What is known about this species is limited to the contributions of Chetoui et al. (2018) conducted in the North of Tunisia. In the present study, the overall sex ratio is balanced, but the sex ratio varies in relation to the SL size classes. Males dominated among smaller individuals, while females predominated in the larger size classes. Similar observations have been reported in other bivalve species like the golden carpet shell *Polititapes aureus* from the Sfax coasts (Derbali 2022) and the cockle *Cerastoderma glaucum* from Tunisia (Derbali et al. 2009a). In contrast, in the clam *Ruditapes decussatus* from the same study area, the sex ratio does not vary with age. Ansell (1961) had the same conclusion after studying *Chamelea striatula*. Generally, females are more common in older populations of gonochoristic molluscs (Fretter & Graham 1964). The overall sex ratio (F:M = 1.03:1, $p > 0.05$) was not significantly divergent

from that of parity. Similar records were reported also for *M. stultorum* from the north coast of Tunisia (Chetoui et al. 2018).

Investigations on the size at sexual maturity revealed that males matured earlier at 20.43 mm SL compared to females at 22.1 mm SL, as is generally the case of many clams (Derbali et al. 2009a,b; Derbali et al. 2016). No data are available for a comparison with the surf clam *M. stultorum*. For another bivalves' species, *R. decussatus* reached maturity at 24.08 for males, against 24.76 mm SL for females (Derbali et al. 2016) and *P. aureus* became mature at 18.07 mm and 20.20 mm SL for males and females, respectively (A. Derbali, unpublished data). Elsewhere, Chung et al. (1987) reported after studying *Mactra chinensis* in Korea that the first sexual maturity of males and females was over 50% among those individuals ranging from 35 mm to 39 mm SL, and 100% in those over 50 mm SL.

The reproductive cycle of mollusc bivalves usually can be described as follows: a progressive development from a condition where the gonads were indistinguishable, then differentiating the gonad (gametogenesis), to spawning (with partial or total release of gametes), and a return to one of these earlier stages (Boyden 1971). In the present study, a similar gonad maturation cycle was found for the surf clam *M. stultorum*. Very rapid gametogenic development (stages II, III and IV) was then recorded for both sexes in the spring months (March, April and May), resulting in a great distension of the visceral mass, after a short resting phase during December and January, which culminated in spawning in summer. In fact, the clam population displayed a clearly defined annual reproductive cycle with a principal peak during summer. In comparison, Chetoui et al. (2018) clarified that the reproductive cycle of the *M. stultorum* population from the northern part of Tunisia showed a prolonged reproductive cycle with a major peak of spawning in late summer and an inactive stage during autumn. Elsewhere, similar conclusions were reached by Qiaozhen & Qi (2013) for *Mactra veneriformis* from the north coast of Shandong (China). On the contrary, different trends were reported for *Spisula solida* (Portugal) and *Lutraria philippinarum* (Philippines) which have an annual reproductive cycle with two spawning peaks (Joaquim et al. 2008, Bantoto & Ilano 2012). Overall, the continuous reproduction may be due to the highest nutritional value and more suitable seawater temperature for gametogenesis.

The synchronized gametogenesis development in both sexes almost year-round was one of the most apparent characteristics of the bivalves' species cycle in southern Tunisian waters (Derbali 2011). Here, we can note that the surf clam population develops

gonads all year round in southern Tunisian waters. The same trends were reported earlier for the hen clam *M. chinensis* from Korea (Chung et al. 1987) and for *M. stultorum* (Chetoui et al. 2018) from the north coast of Tunisia. Consequently, differences in reproductive behaviour can be observed within a species in the same geographical area which could arise from environmental variability in the temperature, food availability and salinity, which are involved in defining and controlling the gametogenesis (Lubet 1991, Derbali 2011).

From the obtained findings, the highest reproductive activity in the examined surf clam population was accompanied with the rise of the water temperature and salinity (> 37). The sexual rest occurred in December-January characterized by temperature and salinity decrease (< 33). The variations of salinity between 29 to 33 and 35 to 39 in winter and spring triggered massive spawning in the summer followed by gonadal recovery in the earlier part of autumn. Vázquez et al. (2021) had the same conclusion after studying four commercially important bivalves in Spain. In fact, bivalves are known to show considerable variation in their gametogenic development (Thorarinsdottir 1993). Changes in the gametogenesis and spawning timing within a given species over a latitudinal range can be explained by critical temperatures and salinities reached at various times (Derbali 2011, Vázquez et al. 2021). Jaramillo & Navarro (1995) showed also that spawning can be triggered by combining internal and environmental factors whose interaction varies seasonally, creating annual changes during spawning. Besides, the availability of nutrients could explain the long-lasting ripening and spawning stages (Pouvreau et al. 2000, Derbali et al. 2009b).

From the published literature available, several studies have been conducted on seasonal changes in bivalves' species during the reproductive cycle (Wolowicz 1984; Giese & Pearse 1974; Derbali et al. 2009a, b). According to Ojea et al. (2004) and Peharda et al. (2006), CI is a good descriptor of the reproductive cycle for bivalves' species. It reflects the physiological and bio-energetic changes in the organism (Prgić, 2019). Gosling (1992) reported food availability and changes in the reproduction phases as the most important factors affecting the CI in bivalves. This parameter can give supplementary information about the gametogenic cycle in bivalves. For mactridae species, the fluctuations of CI body weight were reported by Chetoui et al. (2018) for the surf clam *M. stultorum* (northern Tunisia) and by Chung et al. (1987) for *M. chinensis* (Korea). The maximum values of the condition index (CI) and the tissue weight rate (TiWR) were recorded in May, while the lowest ones

were registered in December and January. Our findings confirm these observations. The increase in body weight during the spring and autumn months can be explained by the gonads development. An important decline in body weight was observed immediately after the completed spawning stage. So, this indicates that the reproductive cells were responsible for the fluctuations of body weight for the bivalves.

Overall, the present study provides the first data available on the reproductive biology of the surf clam *M. stultorum* in southern Tunisia by investigating the reproductive cycle, sex ratio and, for the first time, the size at sexual maturity. Similarly to many bivalve species from the Tunisian coast, the reproductive cycle of *M. stultorum* was characterized by a well-defined seasonality, comprising periods of storage, gametogenesis, spawning and resting. This information may be useful to provide a good indication of when individuals are potentially at their most valuable for the commercial sector. Our study will also help to establish a closed season to help to maintain a sustainable fishery. For production purposes, further study is necessary to examine the population dynamics parameters such as recruitment and mortality to accurately monitor the population dynamics of clams and to introduce measures of appropriate fisheries management.

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