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FULL LENGTH ARTICLE

Aspects on the reproduction of eared horse mussel, *Modiolus auriculatus* (Krauss, 1848) in Red Sea, Egypt

Fatma A. Abdel Razek [a,*], Salah E. Abdel-Gaid [b], Mohamed M. Abu-Zaid [b], Tarek A. Aziz [a]

[a] *National Institute of Oceanography & Fisheries, NIOF, Egypt*
[b] *Faculty of Science, Al-Azhar University, Cairo, Egypt*

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Abstract The reproductive cycle of the eared horse mussel, *Modiolus auriculatus*, was followed for one year from February, 2011 to January, 2012 based on 240 individuals collected from Hurghada, north coast of Red Sea, Egypt. The gonadal maturation of *M. auriculatus* was examined by means of macroscopic and histological preparations of the mantle. Annual cycle with periods of growth, maturity and discharge of gonadic products occurs simultaneously in both sexes. Ripe individuals were observed throughout the year. The largest number of spawning for the individuals occurred from March to June and July for the population. So, it was clear to identify one heavy spawning period for this species. Sexation of *M. auriculatus* did not differ significantly from 1:1. Sexes were distinguishable in all individuals at the ripe stage with a shell length greater than 3.00 cm. A direct positive correlation was observed between gonad index and water temperature.

Introduction

Mussels have been a subject of great interest in recent years due to their increasing commercial importance. Most studies are related to the reproductive characteristics of the species widely distributed in temperate waters. Very little information exists with regard to tropical mussels (Cruz and Villaobos, 1993). Eared horse mussels of mytilidae are widely distributed in the world they occur in several seas of the tropical and subtropical regions. *Modiolus auriculatus* is a sedentary organism and is subjected to a wide range of environmental conditions. The interaction between reproductive processes and environmental changes may assume great importance for the well-being of the population.

Nevertheless, few studies have been carried out on the eared horse mussels *M. auriculatus* in the Red Sea such as on bio-monitors for heavy metal levels (Hamed and Emara (2006); ecology and distribution Fshelson (1971), Oliver (1992) and

* Corresponding author. Tel.: +20 1006620222.
E-mail address: fatma_abdelrazek@hotmail.com (F.A.A. Razek).
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Zuschin and Oliver (2005)). However, information is lacking on the reproduction of *M. auriculatus*.

The aim of the present study was to characterize the gonadal cycle of the mussel *M. auriculatus* in the Hurghada area of the Red Sea of Egypt, to determine the timing of reproductive development and spawning. Analyzing sex ratios and annual trends in gonad index were done. The data obtained provide a basis for the sustainable management of natural populations.

Materials and methods

The reproductive cycle and the gonadal index of *M. auriculatus* in the area of study Map 1 were studied from February 2011 to January 2012 at 3–5 m depth. A total of 240 living specimens of the eared mussel, *M. auriculatus* of a size range from 1.36 cm to 6.02 cm in shell length were collected monthly.

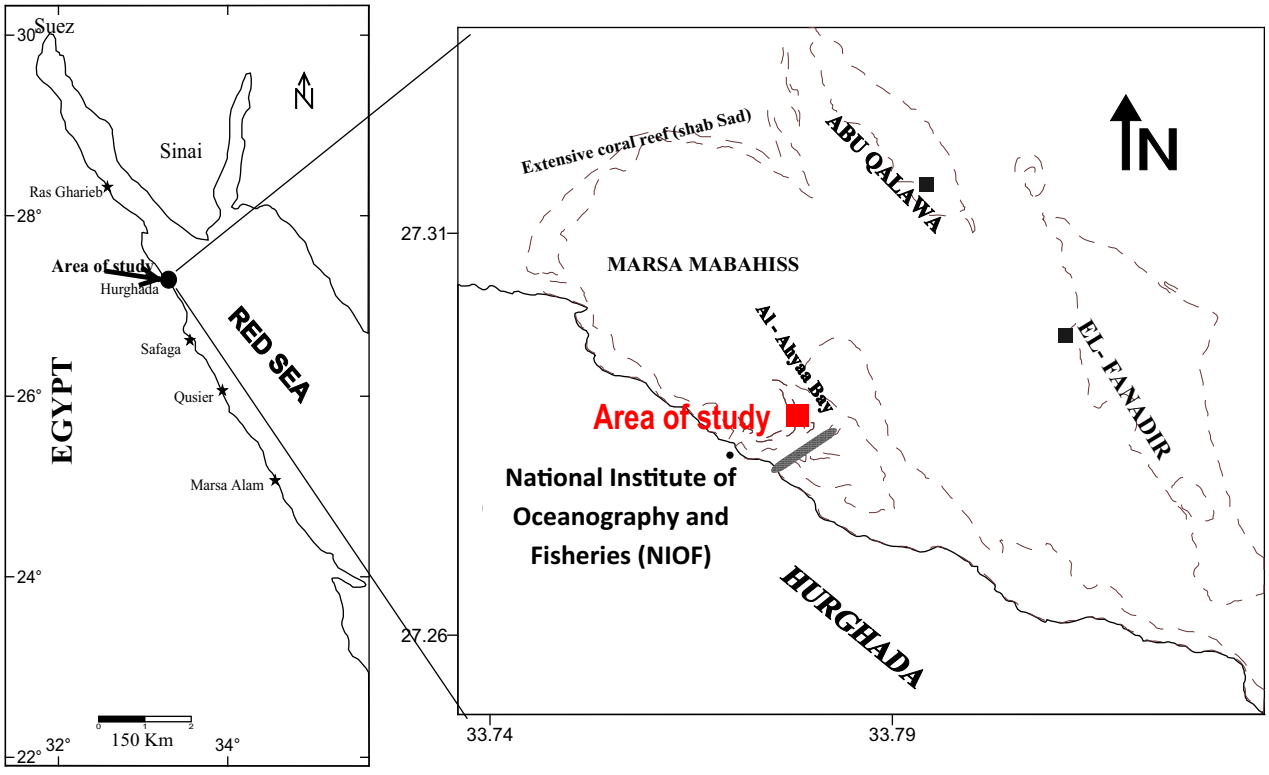
Collection was done by hand and mussels were freshly examined. Each individual of the mussel was first cleaned from the extraneous bio-fouling organisms such as external parasites by scraping it from the ventral and dorsal shells. Lengths of collected specimens were measured to the nearest 0.1 mm using vernier calipers.

Histological analysis

After dissection of the mussel, the soft body was removed from the shell, the mantle and gills were then folded back to expose the body of the mussel adjacent to the foot. From macroscopic observation male gonads appear whitish in color and change to a creamy fluid during the spawning season, while sexually matured ripe females have dark yellow to orange gonads. Gonads tissue surrounded the digestive gland, and in the late ripe phase, extended into the mantle. Gonads were separated from the tissue and immediately fixed in Bouin's fixative according to Lowe et al. (1982), washed with 50% ethanol, and stored in 70% ethanol until processing. Tissue samples were dehydrated in an ascending alcohol series, cleared in Xylene and embedded in paraffin wax. Several sections (5–7 m) were cut, the tissue sections were deparaffinized with toluene, rehydrated with water, and then stained with hematoxylin and counterstained with eosin Bancroft and Stevens (1982). Each section was examined at 100× magnification using a compound microscope (compound microscope Xs_2 – 107 T) and photographed with a Digital camera (solution Disk TP 6031od, processing for software ver. 3.2). The stained gonad preparations from each mussel were classified into different developmental stages. The classification consisted of six main stages early development$^{(3)}$, late development$^{(4)}$, ripe$^{(5)}$, spawned$^{(2)}$, spent$^{(1)}$ and resting$^{(0)}$ according to Walker and Power (2004).

A description of the gonad histological stages is shown in Table 1. The mean gonad index (MGI) values were obtained for each sampling month and for both sexes to estimate the proportion of early and late developing, ripe, spawning, spent and resting individuals.

The mean Gonad index (MGI) for each sample is then determined by multiplying the number of each stage by the numerical ranking of the stage and dividing the sum of these products by the total number of individuals in the sample. The index can vary from zero, if the entire population is spent or resting, to five when fully developed and ripe, (Gray et al., 1996).



Map 1 Sampling area of *M. auriculatus* in Hurghada, Red Sea.

Table 1 Histological characteristics of six gonadal stages in *M. auriculatus*.

Maturity stage	Male	Female
Early developing (3) stage	The testis contains small sized tubules; stem cells and spermatogonia (SPG) residing in the follicle wall (FW) with few spermatocytes (SPC) could be observed (plate, 1A)	The follicle wall was thick and small primary oogonia (OG) could be seen as well as developing oocytes (OC) that were attached to the follicle wall (plate, IIA)
Late developing stage (4)	The tubules had noticeably exhibited a gradual increase; most of the testicular lobules became filled with cysts at different stages of spermatogenesis. The lumen of the follicle is gradually filled up with spermatogonia which will develop into spermatocytes (SPC). Similarly, spermatocytes and spermatids (SPT) filled the follicle lumen. While the follicle wall becomes thinner, SPC are condensed in a dark band beneath the layer of spermatogonia and are attached to the follicle wall (plate, IB)	The beginning of yolk deposition, oogonia grow along the wall of the follicle and are pear shaped (plate, IIB)
Ripe Stage (5)	This stage is found all the year round. The diameter of the seminiferous tubules increased and became filled with cysts of germ cells at all spermatogenic stages. SPT are transformed into spermatozoa with free active tails. Spermatozoa, which are observed in the center of the lumen, are with larger quantities of mature spermatids (Plate IC)	This stage was represented all the year with an actively developing ovary. The follicle wall is rounded & swollen. Ripe free oocytes are resident & accumulate in the lumen of the follicle. They are mostly mature oocytes that are not tightly packed (plate IIC)
Spawning stage (2)	The diameter of the testicular tubules attained their maximum width. Follicles are enlarged as the spermatozoa continuously increase in number and this gives rise to the spawning condition. A few resorptive phagocytic cells have appeared. Part of spermatozoa had been released, the central lumen appear to be more or less empty (plate, ID)	The free oocytes gradually fill the lumen of the follicle and the follicle wall is broken with a corresponding reduction in a number of earlier stages of oogonia. A characteristic feature of the ova at this stage is their polygonal shape which results from the pressure exerted by the follicle wall (plate, IID)
Spent stage (1)	This stage is marked by almost total cessation of spermatogenic activity and also by an increase in the inter lobular space of the connective tissue. Some gametes remain unreleased in the lumen. Although phagocytes unreleased spermatids and residual mass, the sex can still be identified as male by the presence of the unresorbed gametes. The follicle has shrunken with isolated pockets of spermatids (plate, IE)	The volume of the follicle is reduced and regressive or collapsed but not all are empty, because some free oocytes which are small and not well developed remained. The sex can still be identified by the presence of the unresorbed gametes. Follicle walls are shrunken and broken into debris. Some phagocytes with unreleased oocytes are observed (plate, IIE)
Resting stage (0)	The sex cannot be identified and gonads in males are less active after spawning; only the connective tissue is observed in this degenerative state. Some tissues are known as stage (0) of indeterminate or inactive gonad where the follicles are collapsed and reduced in volume (plate, IF)	The sex can be recognized and the gonad is inactive after spawning; with connective tissue in this degenerative state. It is noted here also that, there are no yolk nuclei visible in the cytoplasm of the oocytes as ova (plate, IVE&F)

Size at first sexual maturity of both sexes was determined for the studied species by estimating the percentage of all the maturity stages and plotting the shell length against the percentage of ripe individuals in each length group. For fitting the regression relationship, the Least Square method was used.

A Chi-Square goodness of fit was applied for sex ratio analysis. Measurements of environmental variables such as monthly water temperature, pH, salinity and photoperiod in surface water at the time of sample collection were done using a Hydrolab model: HI9829.

Results

Classifications of the gonad developmental stages

Results confirmed that, no hermaphrodite individuals were recorded after examination of the gonad tissue of all samples of *M. auriculatus*. Sequences of spermatogenesis in males and oogenesis in females based on histological changes in the gonad condition are described in Table 1.

Gametogenic cycle and spawning

In the present study, there appeared to be no difference in gametogenesis or spawning between males and females. The higher values of the mean gonad index, (4.7) were attained in February with highest percentage of ripe individuals (70%) as shown in Fig. 2. The main spawning period started in March, April and May but some spawning continued until June and July, Fig. 1. After the marked spawning period, the mean gonad index (MGI) fell to a minimum of 0.7 and 0.8 for males and females, respectively Fig. 2 when most of the individuals were in the resting conditions during August and September, (86% in males and 85% & 64% in females) Fig. 1. In October, the gonad index slowly increased again with

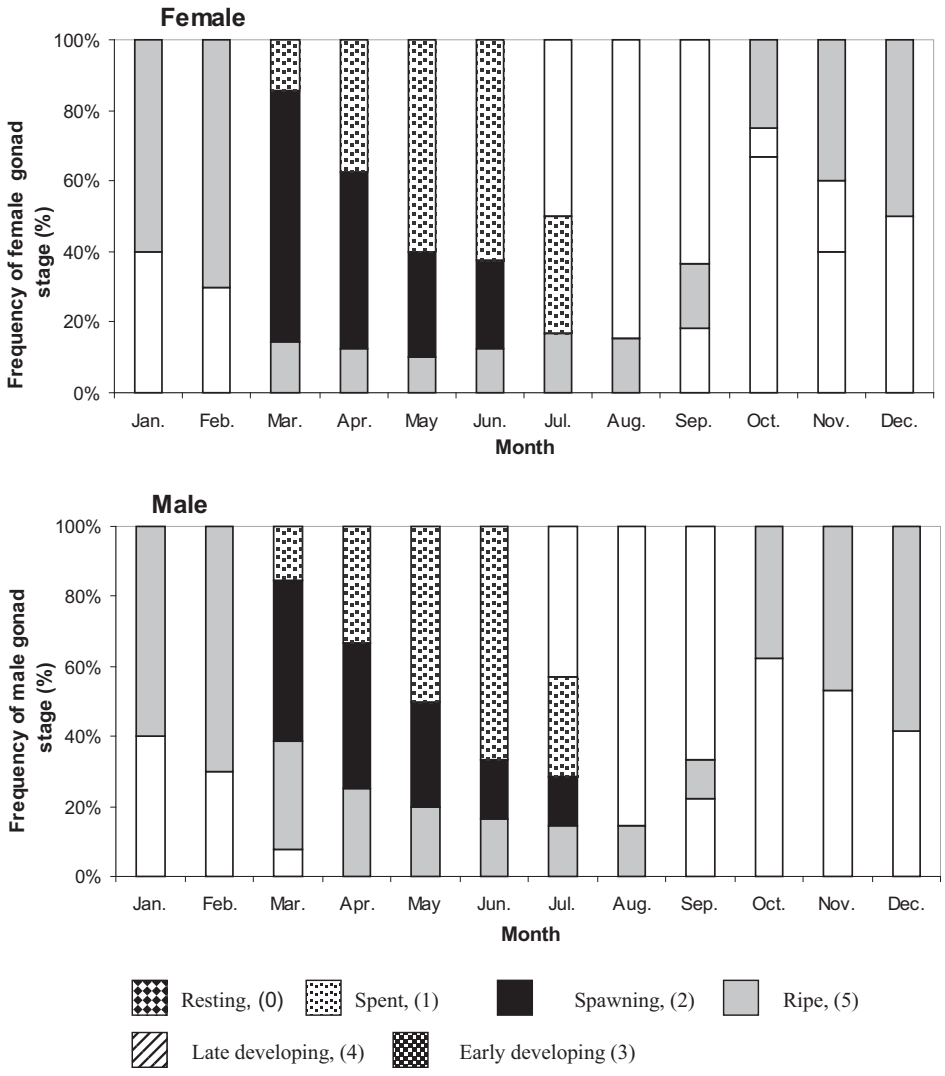


Figure 1 The relation frequency of different gonad stages of *M. auriculatus* females and males over the study period.

most of the individuals investigated in early developing stage as 63% & 67% in males and females respectively, followed by late developing individuals 42% in males and 50% in females, by December as in Fig. 1.

In conclusion the macroscopic and histological study reveals that the annual cycle with a period of growth, maturity and discharge of gonadic products occurs simultaneously in both sexes (Plates I and II).

Ripe individuals were observed throughout the year. The largest number of ripe individuals occurred from December, January and February. Spawning occurred from March to June, July and it was possible to identify one heavy spawning period for *M. auriculatus* population in the present studied area (Fig. 1).

Sex ratio

Out of 240 individuals examined, 108 (45%) were females and 132 (55%) were males. In the sample the proportion of females in all size classes did not differ significantly from 1:1. Male and female gonads were distinguishable in all size classes studied (starts with average shell length 1.5 cm) during the present study.

The size at first sexual maturity in *M. auriculatus* as observed from the examination of the histological results revealed that the first appearance of a fully mature female with a large number of mature eggs inside the ovary was at 2.7 cm shell length. Meanwhile, the case in males was at 2.9 cm in shell length. The calculated minimum biological sizes of both sexes as in Fig. 3 were 2.4 cm and 2.1 cm for females and males respectively. These results confirm that, less than these sizes, no records for ripe individuals can be found in the population of *M. auriculatus*.

Discussion

Bivalves are known to undergo an annual reproductive cycle that involves gametogenesis followed by either a single or several spawning events Crnčevič et al. (2013). Data on the reproductive characteristics obtained in this study shows that in the Red Sea *M. auriculatus* has one spawning peak per year, which occurs during spring-early summer months and that female

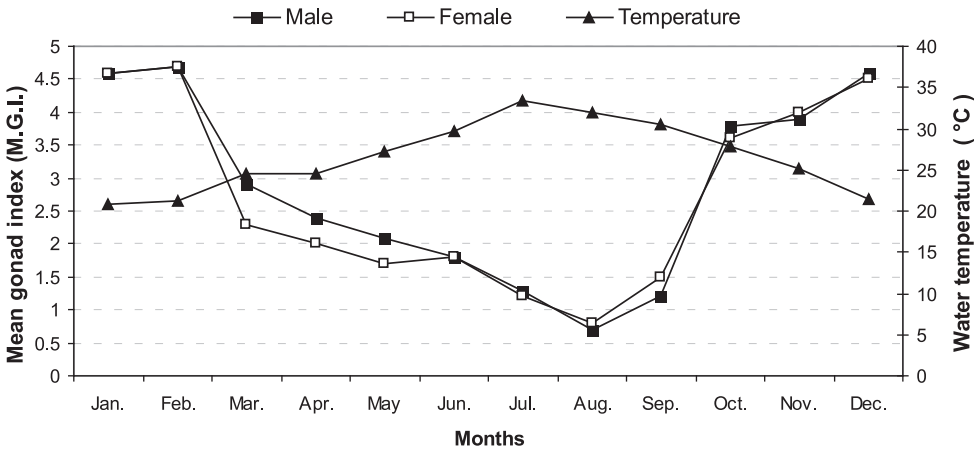


Figure 2 Mean gonad index (MGI) of *M. auriculatus* males and females in relation to water temperature over the study period.

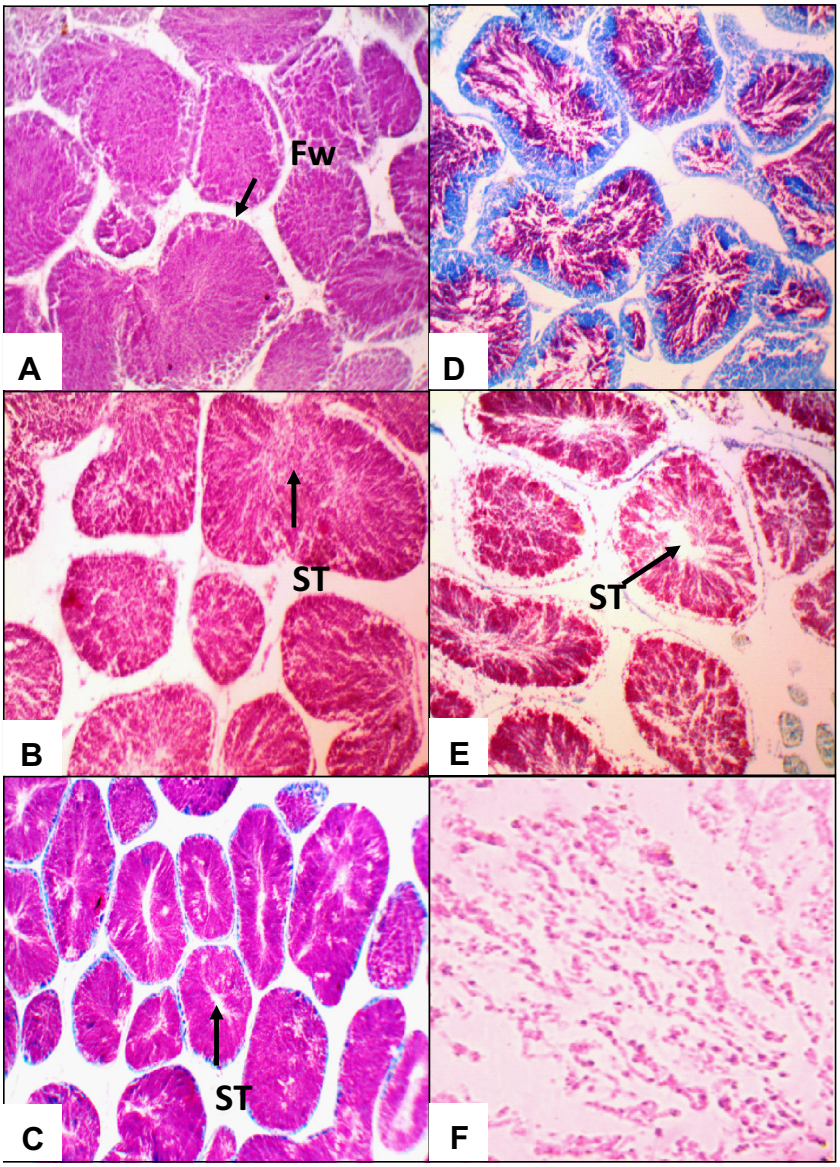


Plate I Photomicrographs of gonads at different stages in the annual cycle of *M. auriculatus* male (H-EX100). (A) Early developing stage, (B) late developing stage, (C) Ripe, (D) spawning, (E) spent and (F) resting. (FW, Follicle walls, St Somniferous tubules).

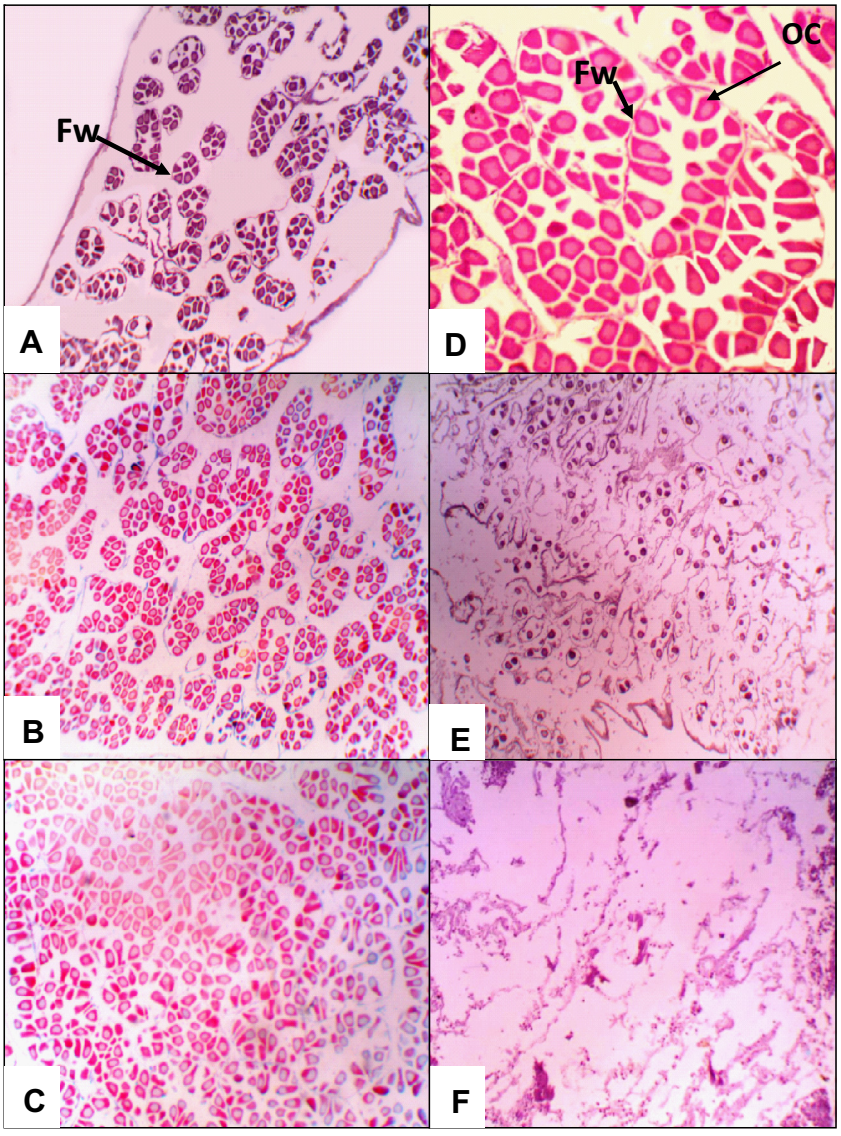


Plate II Photomicrographs of gonads at different stages in the annual cycle of *M. auriculatus* female (H-EX100). (A) Early developing stage, (B) late developing stage, (C) Ripe, (D) spawning, (E) spent and (F) resting. (FW, Follicle walls, OC oocytes).

and male gonad developments are synchronous which is similar as the species of Glycymerididae, as in *G. glycymeris* Crnčevič et al. (2013).

Generally, spawning in bivalves is either continuous or seasonal Caballos-Vazquez et al. (2000). The present horse mussel *M. auriculatus* showed an annual reproductive cycle which involves a prolonged period of gametogenesis. Also, there was a rather extended phase of the annual cycle with ripe individuals in the study population. When most individuals were in spent condition by July there was another part of the population which was still ripe (during August & September). This extended breeding season seems to be an essential condition for successful reproduction Eversole (1989) and Mladineo et al. (2007).

The mean gonad index (MGI) of the studied species began to increase in October and reached a maximum in February when the water temperature in the area of sampling was in the range of 20 °C to 22 °C. Thereafter, the MGI values then showed a gradual decrease because of the spawning, with the increase of temperature in the range of 25–28 °C. Chung (2007) reported that the high average values of MGI coincided with gonadal maturity, and the minimal average value following high average values were considered an an indication of spawning.

Accordingly, variations in the MGI showed a close relationship with gonadal development and gonadal activity.

This strongly suggests that water temperature is an important controlling factor in spawning as reported by Gray et al. (1996). Results of this study indicate that *M. auriculatus* is dioecious with the ratio of males to females as 1–2:1 where 55% of the population was males and 45% females. Also the proportion of sexes in all size-classes did not differ significantly for 1:1 and this follows that reported for other tropical mussels Veloz and Martinez, (1967). A similar variation was found

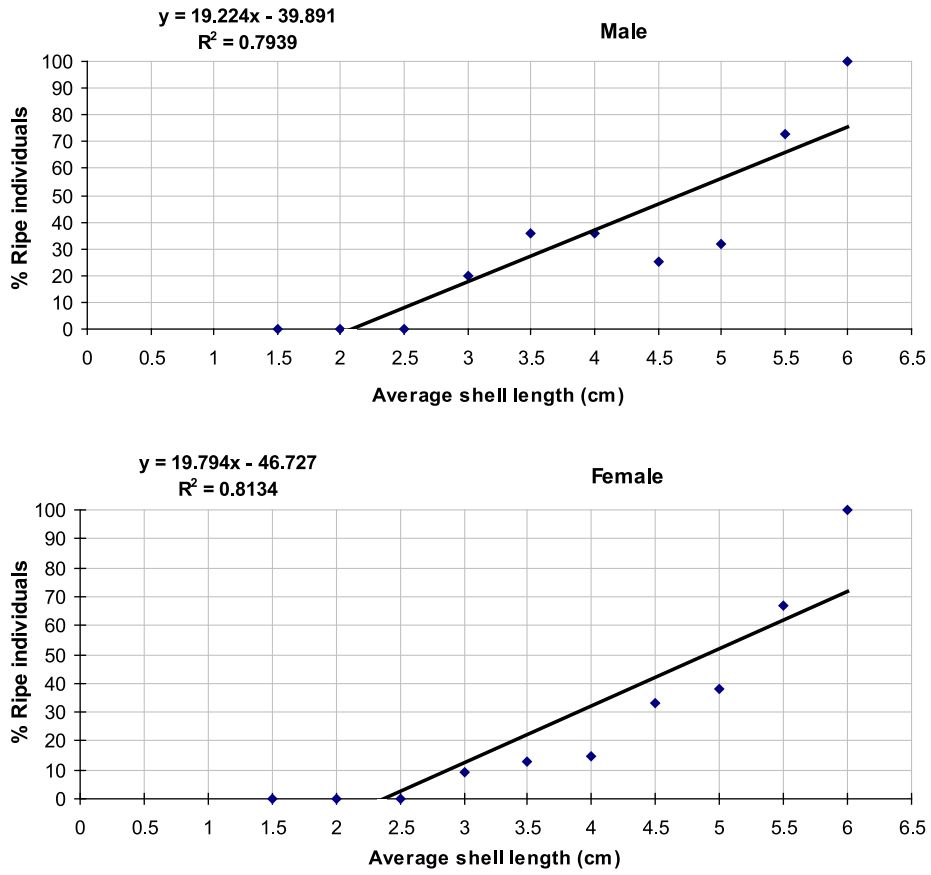


Figure 3 Calculated size at first maturity of *M. auriculatus* males and females over the study period.

with respect to sex ratio, in *M. Barbatus* in the Adriatic Sea with a slight increase in females Mladineo et al. (2007), while *M. modiolus* in the North Irish Sea has an equal sex ratio as reported by Anwar et al. (1990), Sastry (1979) and Peharda et al. (2006) explained this deviation from an equal sex ratio that it is a result mainly of sex reversal. While Seed (1969), reported that such deviations may be related to age or size which can result from sex-specific differences from an equal sex-ratio as a result of growth rate this was also observed in the present study.

The observed size at first sexual maturity was 2.7 & 2.9 cm for females and males, while the calculated was 2.4 & 2.1 cm respectively. However, the size at first maturity decreases as a consequence of over exploitation, thus it should be monitored periodically to update the regulation if necessary.

References

Anwar, N.A., Richardson, C.A., Seed, R., 1990. Age determination, growth rate, and population structure of the horse mussel *Modiolus modiolus*. J. Mar. Biol. Assoc. U.K. 70, 441–457.

Bancroft, J.D., Stevens, A., 1982. Theory and Practice of Histological Techniques, Second Edition. Churchill Livingstone, New York.

Caballos-Vazquez, B.P., Arellano-Martinez, M., Garcia-Dominguez, F., Villalejo-Fuerte, M., 2000. Reproductive cycle of the rugose pen shell, *Pinna rugosa* Sowerby, 1835 (*Mollusca: Bivalvia*) from Bahia Concepcion, Gulf of California and its relation to temperature and photoperiod. J. Shell Fish Res. 19, 95–99.

Chung, Ey (2007): Oogenesis and sexual maturation in Meretrix lusoria (Roding 1798) (Bivalvia: Veneridae) in western Korea.

Cruz, R.A., Villaobos, C.R., 1993. Shell length at sexual maturity and spawning cycle of *Mytella guganesis* (Bivalvia: Mytillidae) from Costa Rice. Rev. Biol. Trop. 41 (1), 89–92.

Crnčevič, M., Peharda, M., Ezgeta-Balic, D., Pečarevič, M., 2013. Reproductive cycle of Glycymeris nummaria (Mollusca: Bivalvia) from Mail son Bay, Adriatic Sea, Croatia. Sci. Marina 77 (2), 293–300.

Eversole, A.G., 1989. Gametogenesis and spawning in North American clam populations: implications for culture. In: Manzi, J.J., Castagna, M. (Eds.), . In: Developments in Aquaculture and Fisheries Science, Clam Mariculture in North America, 19. Elsevier, Amsterdam, pp. 75–109.

Fshelson, L., 1971. Ecology and distribution of the benthic fauna in the shallow waters of the Red Sea. Vlarine Biol. 10, 113–133.

Gray, A.P., Seed, R., Richardson, C.A., 1996. Reproduction and growth of Mytilus edulis chilensis from the Falkland Islands. Sci. Marina 61 (Suppl. 2), 39–48.

Hamed, M.A., Emara, A.M., 2006. Marine molluscs as biomonitors for heavy metal levels in the Gulf of Suez, Red Sea. J. Marine Syst. 60 (3–4), 220–234.

Lowe, D.H., Moore, M.N., Bony, B.L., 1982. Aspects of gametogenesis of the marine mussel Mytilus edulis L. J. Mar. Biol. Assoc. U.K. 62, 133–145.

Mladineo, I., Peharda, M., Orhanovic, S., Bolotin, J., Pavela-Vrančić, M., Treursič, B., 2007. The reproductive cycle, condition index and biochemical composition of the horse-bearded mussel *Modiolus barbatus*. Helgol. Mar. Res. 61, 183–192.

Oliver, P.G., 1992. Bivalved Seashells of the Red Sea. Christa Hemmen, Wiesbaden and National Museum of Wales, Cardiff, p. 330.

Peharda, M., Mladineo, I., Bolotin, J., Kekez, L., Skaramuca, B., 2006. The reproductive cycle and potential protandric development of the Noah's Ark shell, *Arca noae* L.: implications for aquaculture. Aquaculture 252, 317–327.

Sastry, A.N., 1979. Pelecypoda (excluding Ostreidae). In: Giese, N., Pearse, J.S. (Eds.), . In: Reproduction of Marine Invertebrates, 4. Academic, New York, pp. 113–292.

Seed, R., 1969. The ecology of *Modiolus modiolus* L. (Lamellibranchiata) on exposed rocky shores I. Breeding and settlement. Oecologia 3, 277–316.

Veloz, A., Martínez, R., 1967. Reproducción y desarrollo larval experimental del mejillón comestible de Venezuela Perna perna (L L.1758). Biol. Just. Oceanogr. Univ. Oriente 6, 266–285.

Walker, R.L., Power, A.J., 2004. Growth and gametogenic cycle of the transverse ark Anadara transversa (Say, 1822) in coastal Georgia. Am. Malaco Bull 18 (1–2), 2004.

Zuschin, M., Oliver, P.G., 2005. Diversity patterns of bivalves in a coral dominated shallow-water bay in the northern Red Sea/high species richness on a local scale. Marine Biol. Res. 1, 396–410.