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Research Article

Gametogenesis and reproduction cycle of the sea urchin *Echinometra* sp. from the Northern Persian Gulf based on the histology and gonad indices

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Abstract

The sea urchin *Echinometra* sp. is a native species of the Persian Gulf. Despite its great ecological value, the reproductive biology of this animal has not been studied in the Persian Gulf. Therefore, the present study aimed to describe the annual reproductive cycle of this species from the Persian Gulf via gonadal tissue indicators. In the present study, approximately 120 males and females were collected monthly (approximately 10 samples/month) between February 2023 and January 2024 from the tidal zone of the Bushehr coast of the Northern Persian Gulf. The sex of the samples, different stages of sexual maturity, mean gonadal index, gonad coverage area, frequency of oocytes in different stages, and diameter of the oocytes were determined via gonadal tissue sections. Six stages of sexual maturity, including the repair, growth, premature, mature, spawning, and reabsorption stages, were detected in the gonads of the males and females. Only one annual reproductive cycle was identified for this species. In females, this cycle included a period of gametogenic activity (from December to June) and a spawning period of several months (from June to August), followed by a long period of recovery and sexual rest (from August to the end of December). In males, gametogenesis starts in October and lasts until the end of May. This period was followed by the spawning period from June to August and then a short period of recovery and sexual rest (September). It seems that a decrease in seawater temperature stimulates the start of the gametogenic cycle and high seawater temperatures stimulate the release of gametes of *Echinometra* sp. in Bushehr. In general, *Echinometra* sp. has a specific spawning season from late spring to early summer and is not able to spawn throughout the year.

Introduction

Sea urchins are small aquatic animals with hard shells covered with spines that can be found in all oceans worldwide. Approximately 950 species of sea urchins live in the ocean from the intertidal zone to a depth of 80 m. The density decreases with increasing depth, and the highest density occurs between 0 and 10 m depth. Sea urchins move slowly by crawling on tubular feet. These organisms have significant effects on the structure and dynamics of the sub-tidal and shallow populations (Ruppert and Barnes, 1994).

These animals feed on algae. Especially in temperate regions, the high density of sea urchins causes the area to become barren due to excessive feeding on macroalgae that affects the physical and biological structure of the area (Ruppert *et al*., 2004). Therefore, considering the significant impact of sea urchins on the structure of regions, especially temperate regions, obtaining information on the life history of these animals seems essential.

Echinometra sp. has been reported as the most abundant echinoid in the world and is widely distributed in tropical and subtropical regions (Piryaei *et al*., 2018). This species poses significant environmental risks to coral reefs (Siddique and Ayub, 2019). This animal plays an important role in the balance of other species, especially in the shallow areas of the Persian Gulf and Oman Sea, by controlling the abundance and distribution of algae and sea grass (Piryaei *et al*., 2018). Despite the important ecological role of this animal in the Persian Gulf, no studies have been conducted to investigate its reproductive biology.

Seasonal changes in the ecosystem play an important role in regulating the reproduction of echinoids, especially in temperate regions (Gaitán-Espitia *et al*., 2016). Owing to these changes, significant differences in reproductive patterns have been observed even among different populations of the same echinoderm species at various latitudes (Rubilar *et al*. 2005). Therefore, although Siddique and Ayub (2019) previously studied the reproduction of this species from the rocky shores of Bolji in Pakistan, information regarding the reproductive cycle of *Echinometra* sp. populations in the Persian Gulf is limited. Therefore, the present study aimed to describe the annual gametogenesis cycle of *Echinometra* sp. from Bushehr coasts, in the north of the Persian Gulf, by assessing the changes in gonad structure and estimating the mean gonad index and the gonad coverage area throughout the year.

Materials and methods

Sampling

In the present study, approximately 120 male and female *Echinometra* sp. were collected randomly on a monthly basis (approximately 10 samples/month) between February 2023 and January 2024 from the tidal zone of the Bushehr coast (28º 90´N 50º 82´E to 28º 95´N 50º 81´ E), in the Northern Persian Gulf. The samples were fixed in a 10% formalin solution. Environmental parameters such as salinity and temperature were also measured monthly during the sampling time (Figs 1 and 2).

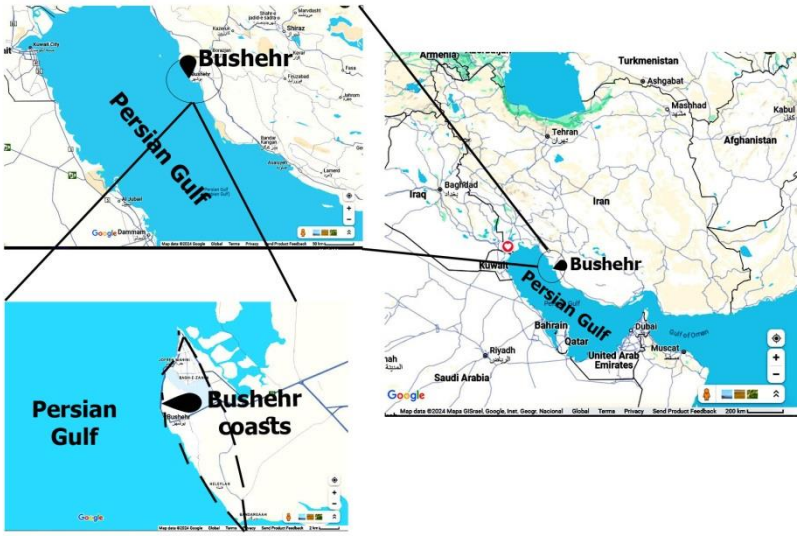

Figure 1: The sampling area on the Bushehr coast in the Northern Persian Gulf.

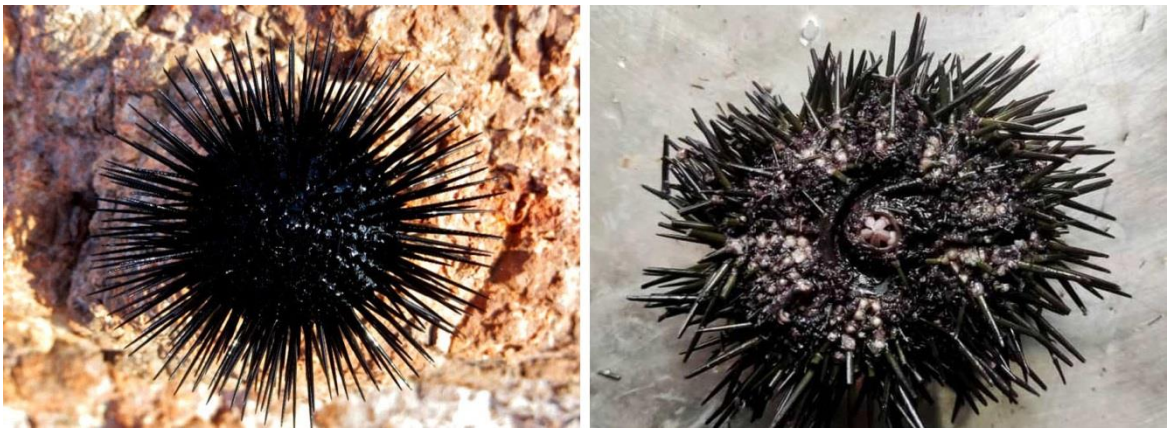

Figure 2: *Echinometra* sp. collected from Bushehr coasts in the Northern Persian Gulf.

Dissection and tissue sampling

First, the tests of individuals were and then the gonads were separated from the sea urchin's body and moved to a 10% formalin buffer solution for 48 hours. The samples were then transferred to a 50% ethanol solution for histological tests. The tissue samples were processed automatically by an Automatic Tissue Processor (model B11, TISSUE-TEK, Tokyo, Japan). During the process, the samples were passed through a series of ethanol alcohols with increasing concentrations (70, 80, 90, and 100%) for dehydration. The samples were subsequently clarified with pure xylene. The samples were then paraffinized and finally blocked. The paraffin blocks containing gonadal tissues were finally cut into 5–6 µm sections via a digital rotary microtome (RM2245, Leica, Wetzlar, Germany). The tissue sections were then stained with hematoxylin and eosin (H&E). Finally, the stained samples were analyzed via an optical microscope (Olympus Bx50) equipped with a Dinolit camera and Dino capture software (FDP2, New Taipei, Taiwan).

Determination of the maturation stages of gonads

The different stages of male and female gonadal development were determined according to the pattern represented by Raposo *et al*. (2023) for other sea urchins:

Stage I (the recovery stage), also called the intercycle stage, refers to the interval between the spawning of the previous cycle and the start of the growth of gonads in the next cycle. This stage is characterized by the presence of small previtellogenic oocytes along the wall of the gonadal acini. Stage II (the growing stage), also called the pre-gametogenesis stage, is characterized by an increase in the number and size of previtellogenic oocytes and a decrease in the abundance of nutritional phagocytes. Stage III (the preadult stage) is characterized by a greater decrease in the number of nutritional phagocytes and an increase in the number and size of vitellogenic oocytes. In Stage IV (the mature stage), the entire lumen of the gonadal acini is occupied by vitellogenic oocytes with no nutritional phagocytes. Some small previtellogenic oocytes are also observed. In Stage V (Ovulation stage), many empty spaces are observed in the gonadal acini due to the release of mature oocytes. The germinal layer is characterized by a line of vitellogenic oocytes (surrounded by trophic tissues). In Stage VI (the reabsorption stage), some remaining vitellogenic eggs are observed in the lumen, ultimately absorbed by phagocytes.

Quantitative histology

Mean gonadal index (MGI)

The mean gonadal index (MGI) is calculated monthly for both males and females alone, as well as in combination, based on the gonad developmental stages via the following formula (Machensen *et al.*, 2011):

$$MGI: (N_{SI}\times 0+N_{S1I}\times 0.33+N_{SIII}\times 0.66+N_{SIV}\times 1+N_{SV}\times 0.66+N_{SVI}\times 0.33)/(_{NSI+S1I+SIII+SIV+SV+SVI})$$

Nx: the number of sea urchins in the x-th stage of gonad development

NSI+S1I+SIII+SIV+SV+SVI: Total number of sea urchins evaluated per month

The GMI ranges from 0 (if all sea urchins are in S0) to 1 (if all sea urchins are in S5).

Gonadal covered area (GCA)

The gonad coverage area (GCA) is the area occupied by the gonad within an area of 7.9 mm^2 at a magnification of ×4. Six different sections from each sample and at least five samples were studied monthly to calculate the GCA. The GCA was then calculated via the following formula (Rodríguez-Jaramillo *et al*., 2008):

$$GCA = (\text{gonad occupation area/total area}) \times 100$$

Frequency of oocytes at different developmental stages

The percentage of oocytes in each developmental stage was calculated in three

regions of the ovary for each female per month.

The diameter of the oocyte at different developmental stages

The diameter of 100 oocytes (with visible nuclei) was measured per female per month (Borisovets *et al*., 2002).

Statistical analysis

Ten tissue sections from each *Echinometra* sp. Individual (at least 6 samples/month) and at least 5 microscopic fields in each section were used for determination of sex, gonad developmental stage, mean gonadal index, gonad coverage area, frequency of oocytes in different maturation stages and diameter of the oocytes. All the data are presented as the means±standard errors (SEs). The normality of the data was controlled via the Shapiro–Wilk test. Statistical differences between the studied parameters in different months were tested via one-way analysis of variance (ANOVA) and Duncan's post hoc test. A significance level of $p>0.05$ was accepted.

Results

Changes in environmental parameters

As shown in Figure 3, no significant changes in water salinity were observed throughout the year. The highest and lowest amounts of 40.2 and 39.52 PSU, respectively, were recorded for the salinity of Iranian waters of sampling sites in the Persian Gulf during the year, which was not significantly different ($p>0.05$). On the other hand, the temperature of the Iranian waters of sampling areas significantly changed throughout the year ($p<0.05$; Fig. 3). The highest and lowest temperatures were measured in August (33.9°C) and February (19°C), respectively.

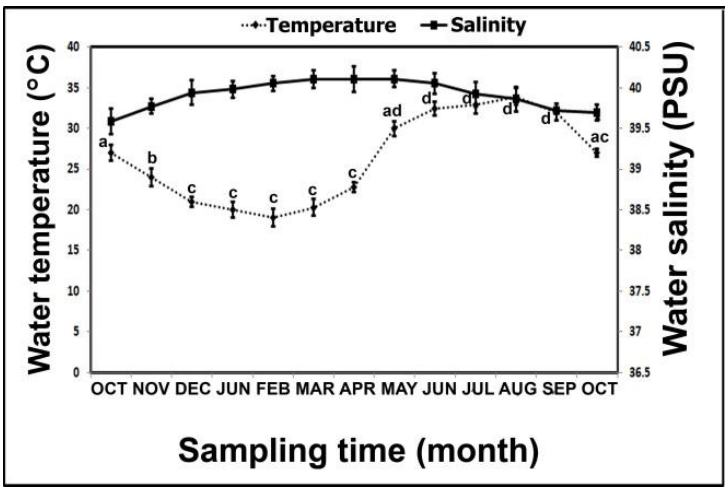

Figure 3: Monthly changes in water temperature and salinity in the sampling area along the Bushehr coast. Lowercase letters indicate significant differences between different months.

Gonad tissue changes during the maturation cycle

Gonadal tissue was not observed in immature sea urchins. The gonads of sea urchins were very small in the recovery stage. In adult *Echinometra* sp., a 5-lobed gonad (with 5-radial symmetry) was observed attached to the body wall on the oral surface. Each of the five gonad lobes consisted of several spherical or oval acini

(Figs. 5 and 6). The parenchyma of *Echinometra* sp. gonads consisted of two cell types: sex cells and storage cells (also known as nutritional phagocytes or NP cells) (Fig. 4 A, B). NP cells store nutrients such as proteins, carbohydrates, and lipids that are necessary for gamete development. NP cells can also engulf (phagocytose) unused sex cells after spawning in each cycle.

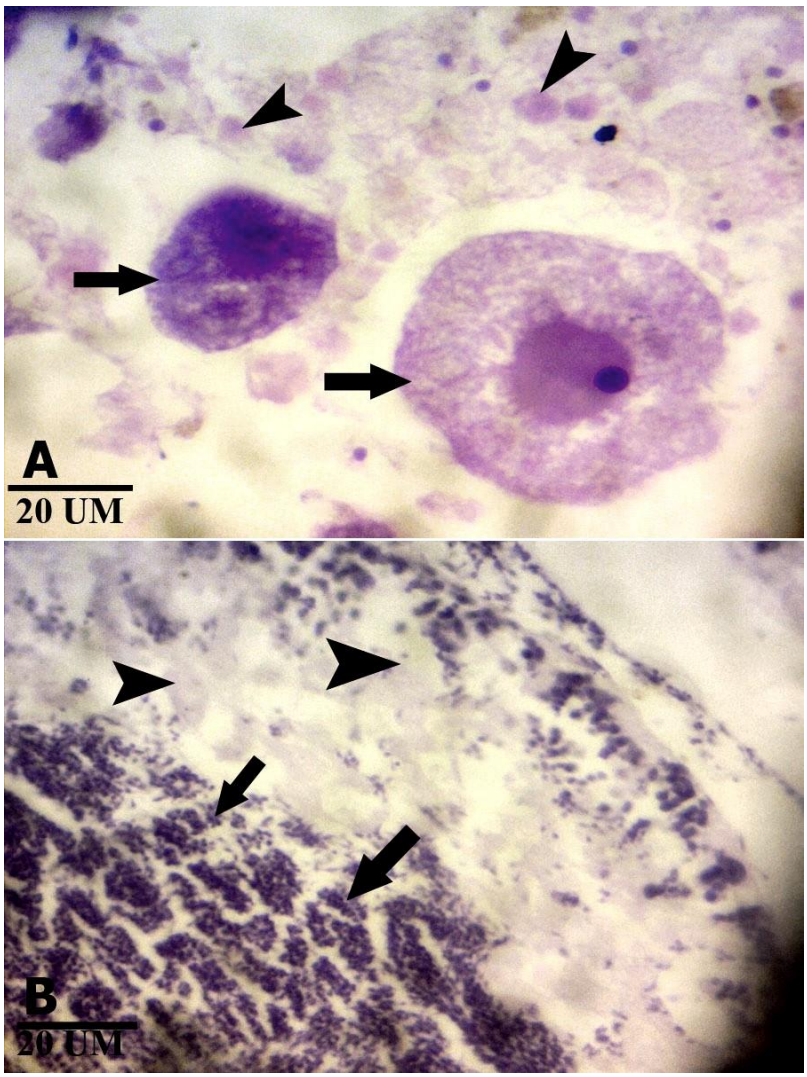

Figure 4: The female (A) and male (B) gonads in *Echinometra* sp. from the Northern Persian Gulf (Bushehr); black arrows: sex cells, black arrowheads: nutritional phagocytes; H&E: ×2900.

Gonad tissue structure during the sexual maturation cycle

Six maturation stages were detected in the female and male gonads of *Echinometra sp.* according to Raposo *et al*. (2023). Tables 1 and 2 and Figures 5 and 6 show the tissue changes in the female and male gonads of *Echinometra sp.* from the Persian Gulf during the annual reproductive cycle.

Annual reproductive cycle and frequency of gonadal maturation stages

Based on the changes in gonad tissue between male and female *Echinometra sp.*, only one annual reproductive cycle was identified. The female reproductive cycle

included a period of gametogenic activity (from December to June) and a spawning period of several months (from June to August), followed by a long period of reabsorption and recovery (from August to the end of December). In males, gametogenesis continued from October to the end of May. This period was followed by a spawning period from June to August and a short period of reabsorption and recovery (September).

Table 1: Different stages of the annual reproductive cycle of female gonads in *Echinometra* sp. the Northern Persian Gulf (Bushehr).

Stage	Female
Stage I (Recovery stage)	Gonadal acini were empty of sex cells and mainly composed of connective tissue. The number of NP cells increased at the end of this stage (Fig. 5 A)
Stage II (Growing stage)	The appearance and increase in the number and size of previtellogenic oocytes, from the margins to the lumen of the gonadal acini (Fig. 5 B, C)
Stage III (Premature stage)	The continued increase in the number and the size of vitellogenic oocytes and their movement to the center of gonadal acini along with the decrease in the number of NP cells (Fig. 5 D)
Stage IV (Mature stage)	Gonadal acini contained many vitellogenic oocytes in the center and previtellogenic oocytes with a small number of NP cells in the periphery (Fig. 5 E)
Stage V (Spawning stage)	Release of vitellogenic oocytes from gonads. previtellogenic oocytes surrounded by NP cells were visible on the germinal layer of acini (Fig. 5 F)
Stage VI (Reabsorption stage)	Vitellogenic oocytes were released from the gonads and only a small number of them remained in the center of the acini, which would be gradually absorbed by NP cells (Fig. 5 G)

Table 2: Different stages of the annual reproductive cycle of male gonads in Echinometra sp. the Northern Persian Gulf (Bushehr).

Stage	Male
Stage I (Recovery stage)	Gonadal acini were empty of sex cells and mainly composed of connective tissue. The number of NP cells increased at the end of this stage (Figs. 5 A)
Stage II (Growing stage)	The start of gonadal growth and acceleration of spermatogenesis. Gonadal acini consisted of NP cells in the lumen surrounded by the slight layer of germ cells (Fig. 6 B, C)
Stage III (Premature stage)	Abundant sperm in the center of gonadal acini were surrounded by NP cells at the periphery (Fig. 6 D)
Stage IV (Mature stage)	Gonadal acini filled with sperm were surrounded by a thin layer of NP cells (Fig. 6 E)
Stage V (Spawning stage)	Decreased sperm density in the center of gonadal acini due to their release. NP cells were visible in the margin of acini (Fig. 6 F)
Stage VI (Reabsorption stage)	Gonadal acini without sex cells. Gradual absorption of remaining sperms in the center of gonadal acini by NP cells (Fig. 6 G, H)

As the water temperature decreased, 80 to 90% of the female *Echinometra sp.* collected from September to December were in the recovery stage. The female *Echinometra sp.* gradually entered the growing stage in January, and 74% of the

female samples collected in January were in the growing stage. By the end of March, 65% of the female samples were in the growing and premature stages, and 35% were mature. By May, almost all the females had matured. The female samples collected from June to the beginning of September had spawned completely or partially. The highest percentage of mature female *Echinometra sp.* was observed in May when the temperature increased to 30 °C (Fig. 7A). The spawning started at the same time as the water temperature increased. The reproductive cycle of the males followed a slight pattern. Gametogenesis in male *Echinometra sp.* started in October at the same time as the water temperature decreased. Despite the early start of gametogenesis, males like females matured in April and May at the same time as the temperature increased and spawned at the same time as females did in June and July (Fig. 7B).

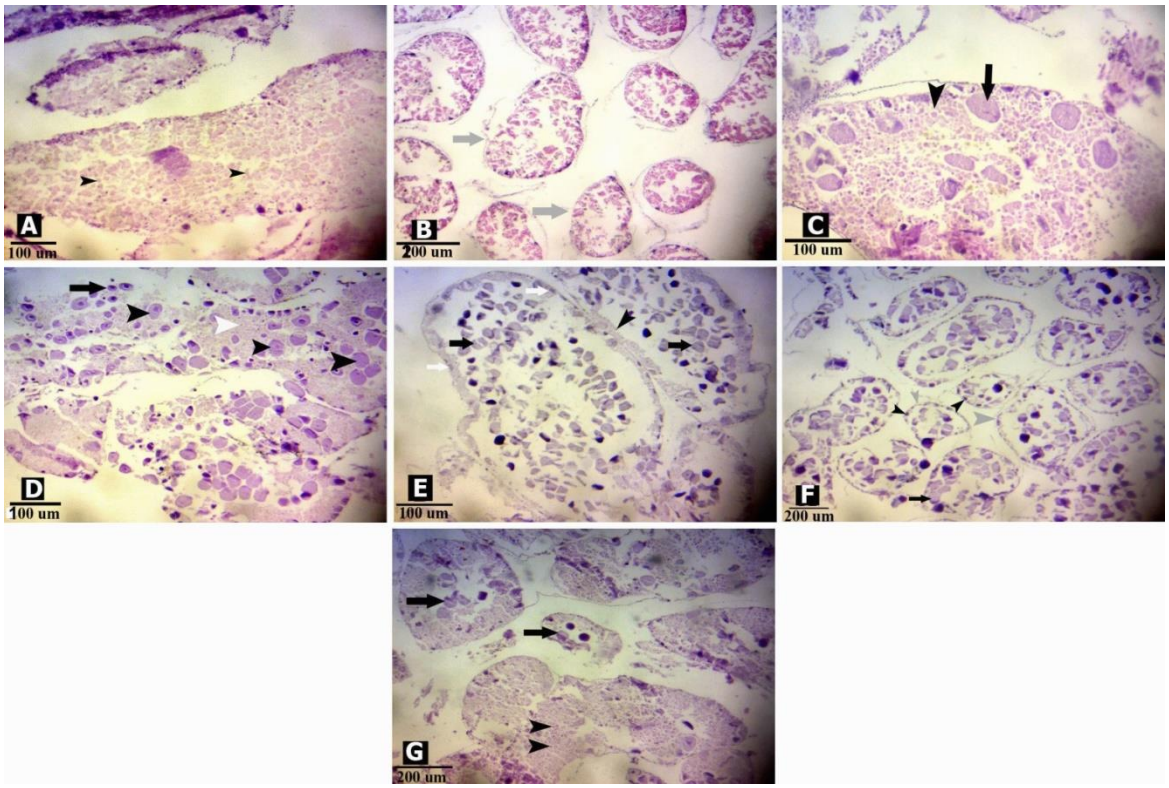

Figure 5: The tissue structure of female gonads in *Echinometra sp.* from the Northern Persian Gulf (Bushehr); A: In the recovery stage (stage I), black arrowheads: nutritional phagocytes; B and C: At the beginning (B) and end (C) of the growing stage (stage II); gray arrow: gonadal acini, black arrow: sex cells, and black arrowhead: nutritional phagocytes; D: In the premature stage (stage III), black arrows: vitellogenic oocytes, black arrowheads: previtellogenic oocytes, and white arrowheads: nutritional phagocytes; E: In the mature stage (stage IV); black arrows: vitellogenic oocytes, white arrows: previtellogenic oocytes, and black arrowheads: nutritional phagocytes; F: In the spawning stage (stage V); gray arrowheads: germinal layer, black arrows: vitellogenic oocytes, black arrowheads: previtellogenic oocytes, and white arrowheads: nutritional phagocytes; G: In the reabsorption stage (stage VI); black arrows: vitellogenic oocytes, and black arrowheads: nutritional phagocytes; A, C, D, E: H&E: ×725; and B, F, G: H&E: ×290.

Mean gonadal index (MGI)

The monthly changes in MGI exhibited similar patterns in female and *male Echinometra sp.* In addition, a significant positive correlation (Rs=0.7; p=0.001) was observed between the total MGI and

temperature. The highest amount of MGI (male, female, and total MGI) was recorded in June and May at the same time as male and female maturation, with its peak in June. On the other hand, the lowest amount of MGI (male, female, and total MGI) was recorded from August to December (p<0.05; Fig. 8A).

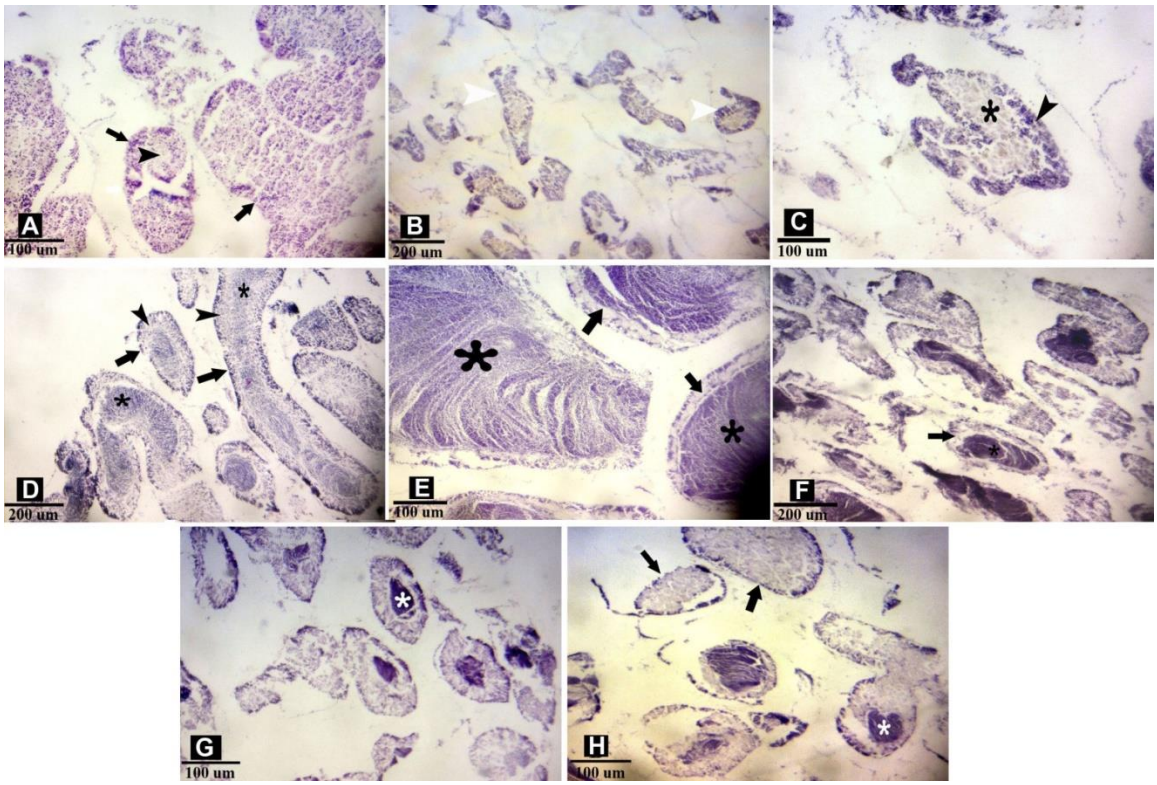

Figure 6: The tissue structure of male gonads in *Echinometra sp.* from the Northern Persian Gulf (Bushehr); A: in the recovery stage (stage I): black arrowhead: nutritional phagocytes, black arrows: gonadal acini; B and C: in the growing stage (stage II): white arrowheads: gonadal acini, black arrowheads: sex cells, and black stars: nutritional phagocytes; D: in the premature stage (stage III): black stars: sperm, black arrowheads: nutritional phagocytes, and black arrows: gonadal acini; E: in the mature stage (stage IV): black stars: sperm, black arrows: nutritional phagocytes; F: in the spawning stage (stage V): black stars: sperm, and black arrows: nutritional phagocytes; G and H: in the reabsorption stage (stage VI): white stars: sperm, black arrows: discharged gonadal acini. ; A, C, D, E: H&E: ×725; B, F, G, H: H&E: ×290.

Gonadal coverage area (GCA)

GCA followed a similar pattern in male and female *Echinometra sp.* during different gonad developmental stages. The highest amount of GCA was recorded in mature male and female *Echinometra sp.* in May (95±5.3% in females and 97±5% in males) as the temperature increased. The lowest amount of GCA was measured in both sexes in the recovery stage from September to October (p<0.05; Fig. 8B). The amount of GCA in different gonad developmental stages followed the next pattern in both sexes: IV>V≥VI> III>II ≥I

Monthly changes in oocyte maturation stages and oocyte size

The gonads of female *Echinometra sp.* had no sex cells in the recovery stage from September to late December. With the growth of gonads in female *Echinometra* sp., the number of sex cells in the gonad

connective tissue gradually increased in late December.

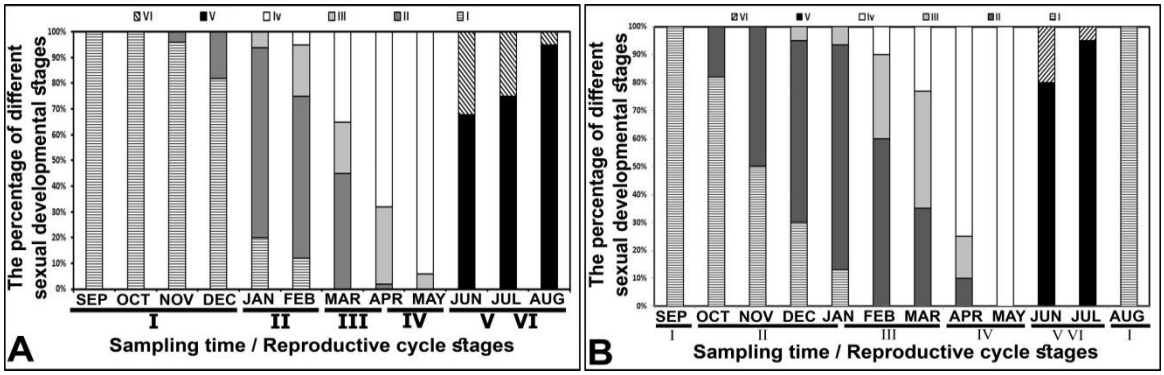

Figure 7: Frequency of gonad developmental stages in female (A) and male (B) *Echinometra sp.* from the Northern Persian Gulf (Bushehr). Recovery stage (I), growth stage (II), premature stage (III), mature stage (IV), spawning stage (V), and reabsorption stage (VI).

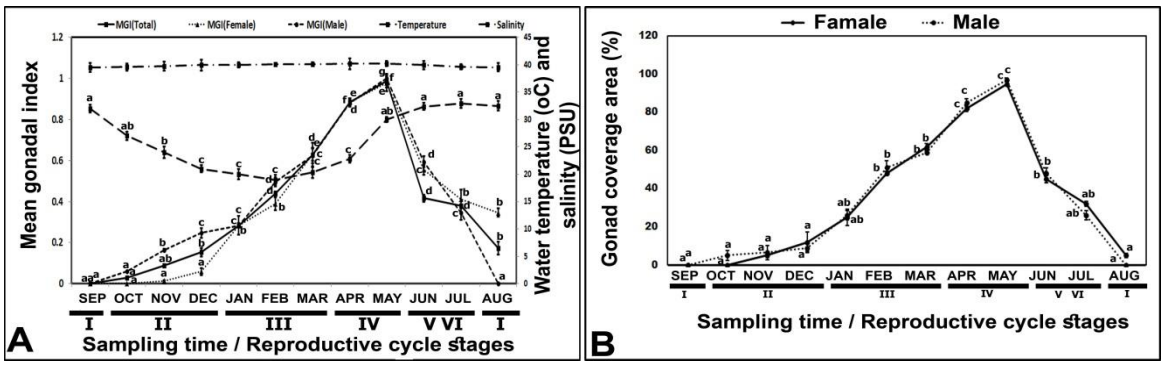

Figure 8: The mean gonadal index (A) and gonad coverage area (B) in male and female *Echinometra sp.* from the Northern Persian Gulf (Bushehr). Recovery stage (I), growth stage (II), premature stage (III), mature stage (IV), spawning stage (V), and reabsorption stage (VI). Lowercase letters indicate significant differences between various months and stages of gonadal development.

Abundant previtellogenic oocytes were visible in the female gonad from late December to mid-March (Figs. 5 and 9 A). The size of the oocytes changed from 37.292±6.5 µm in December to 68.104±11.8 µm in March (Fig. 9 B). The number of vitellogenic oocytes in the female gonads increased from mid-March. Therefore, most of the oocytes were vitellogenic in April and May, and few previtellogenic oocytes were observed in the margins of the gonadal acini (Figs. 5 and 9 A). The size of the vitellogenic oocytes increased from 72.91±15.2 µm at the end of March to 86.57±18 µm in May (Fig. 9 B). The gonads of female *Echinometra sp.* mainly contained vitellogenic oocytes (82.787±18.12 µm mean diameter) during spawning in June and July (Fig. 9 A, B). Gonadal acini were discharged from the oocyte in late August (Fig. 9 A).

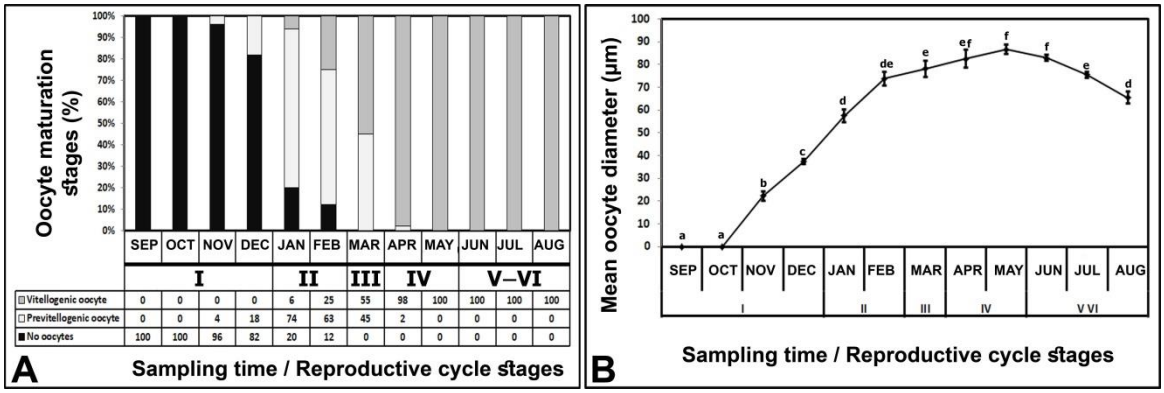

Figure 9: Frequency of oocyte maturation stages and the size of oocytes in different gonad developmental stages in female *Echinometra sp.* from the Northern Persian Gulf (Bushehr). Recovery stage (I), growth stage (II), premature stage (III), mature stage (IV), spawning stage (V), and reabsorption stage (VI). Lowercase letters indicate significant differences between various months and stages of gonadal development.

Discussion

To date, several studies have been conducted on various aspects of the biology of *Echinometra sp.*, such as morphology, population structure, and determination of embryonic stages; however, there is limited information about the annual reproductive cycle of this species in the Persian Gulf. Siddique and Ayub (2019) studied the annual reproduction cycle of *Echinometra sp.* from the Balji coast in Pakistan. However, reproductive biology may differ even between individuals of the same species in the same population or among specimens collected at different latitudes due to the various environmental conditions of different habitats (Sewell and Bergquist, 1990). This species is a gonochoric with two separate male and female sexes. There are several reports on the gonochoric nature of other sea urchin species, such as *Echinometra vannamei* (Villalba Villalba *et al*., 2021) and *Loxechinus albus* (Olivares and Avila-Poveda, 2019).

The results of the present study revealed that seawater temperature is a major factor in the growth and maturation of gonads in *Echinometra sp.*. In the present study, a decrease in the water temperature in January and October stimulated the growth of male and female gonads. By the end of March, most of the collected samples were in the premature stage. By May, almost all the collected female and male samples had matured. Both males and females collected from June to late August (with the maximum temperature) had completely or partially spawned. Like in the present study, Raposo *et al*. (2023) reported that the gametogenic cycle of *Paracentrotus lividus* (from the west coast of Portugal) started with an increase in gonad weight at the same time as the water temperature decreased and ended with spawning and a decrease in gonad weight when the temperature reached its peak. Spirlet *et al*. (1998) reported that the gonad development of sea urchins is affected by the temperature of the sampling area. The relationship between temperature and gonad development has also been confirmed for *Loxechinus albus* in Argentina (Pérez *et al*., 2010). Pérez *et al*. (2010) reported that lower temperatures and short photoperiods stimulate the initiation of gametogenesis.

Seasonal environmental factors, such as water temperature and photoperiod, play important roles in the gametogenic cycle, maturation, and spawning. Both male and female *Echinometra sp.* were in the recovery stage, with no sex cells in the gonads during September, October or November in the present study. According to Raposo *et al*. (2023), the gonads of *P. lividus* were almost empty of sex cells in December 2015 and December 2016. Gonad weight began to increase in *P. lividus* in January 2016, and by February 2016, 80% of *P. lividus* were in the premature stage (Raposo *et al*., 2023). A similar pattern was also reported in the present study. Tavares and Borzone (2015) reported that in seasonal environments, fluctuations in environmental factors may impose strict selective regimes on sea urchins to control their reproduction mode and timing. Accordingly, as observed in the present study, the spawning season coincides with the months when the temperature and photoperiod increase. The main reason is that larvae have more access to food during warm months because of the proliferation of phytoplankton (Starr and Himmelman, 1993). According to Starr and Himmelman (1993) study, the spawning season of echinoids (temperate animals) coincides with an increase in temperature, which is associated with an increase in phytoplankton biomass and, in turn, more food sources for echinoids. Starr and Himmelman (1993) reported that the abundance of phytoplankton may stimulate the initiation of spawning in echinoids. The high density of echinoids is believed to directly relate to nutrient availability. As food increases in the environment, the sea urchin stores nutrients in its nutritional phagocytes to be consumed in late winter and spring, increasing the size of the gonads (Starr and Himmelman, 1993; Tavares and Borzone, 2015).

Based on the results of this study, *Echinometra sp.* spawns once a year in the Northern Persian Gulf (Bushehr). Lima *et al*. (2009) reported only one annual spawning period for *Echinometra lucunter* from the sandy coasts of northeastern Brazil. Other species, such as *Arbacia punctulata* (Hernandez *et al*., 2020), *Arbacia lixula* (Wangensteen *et al*., 2013), and *Echinometra vanbrunti* (Villalba Villalba *et al*., 2021), have been reported to have only one annual spawning period. However, Boudouresque and Verlaque (2007) reported that there may be differences in spawning time and frequency depending on environmental conditions, even for the same species in different years at the same geographic latitude or different geographic latitudes. For example, Raposo *et al*. (2023) reported that *P. lividus* from the west coast of Portugal spawns once a year in June. However, Boudouresque and Verlaque (2007) reported differences of up to four weeks in spawning time, in distinct years, or spawning twice a year for the same population. In the present study, *Echinometra sp.* from the Northern Persian Gulf was investigated over the course of one year. The study of the tissue structure of the gonads of both males and females of this species has provided important information about their reproductive cycle. In the present study, *Echinometra sp.* from the Northern Persian Gulf presented an annual gametogenic cycle that was asynchronous in both male and female

gonads (oocytes and sperm at different developmental stages were observed simultaneously in each sample). This asynchrony has been reported in other sea urchins, such as *E. Lucunter* (Lima *et al*., 2009), *P. lividus* (Raposo *et al*., 2023), *Lytechinus variegatus* (Tavares and Borzone, 2015), *E. vanbrunti* (Villalba Villalba *et al*., 2021), and *Evechinus chloroticus* (Brewin *et al*., 2000). In the present study, the development of gonads occurred almost simultaneously in both sexes. This behavior has already been reported for other echinoids, such as *E. vanbrunti* (Villalba Villalba *et al*., 2021) and *E. chloroticus* (Brewin *et al*., 2000). As observed in the present study, the spawning period in many echinoids is longer in males than in females. This phenomenon has been mentioned as an adaptation strategy to ensure the success of fertilization, especially at the end of the spawning period of females (Villalba Villalba *et al*., 2021).

In the present study, the morphology of the gonads was similar to that reported for other echinoids. The gonads of *Echinometra sp.* from the Northern Persian Gulf are similar to those of *P. lividus* (Ouchene *et al*., 2021), *L. variegatus* (Tavares and Borzone, 2015), and *E. vanbrunti* (Villalba Villalba *et al*., 2021). Like the gonad tissue structure of *Echinometra sp.* in the present study, Villalba Villalba *et al*. (2021) and Tavares and Borzone (2015) reported that the gonads consisted of five lobes with a radial arrangement in *L. variegatus* and *E. vanbrunti*. Each lobe consisted of numerous acini and each of these was composed of male and female sex cells and nutritional phagocytes (Tavares and Borzone, 2015; Ouchene *et al*., 2021; Villalba Villalba *et al*., 2021). No sexual dimorphism was observed in *Echinometra sp.* in the Northern Persian Gulf. Sexual dimorphism has not been observed in other sea urchin species that have been studied (Brewin *et al*., 2000; Tavares and Borzone, 2015; Ouchene *et al*., 2021; Villalba Villalba *et al*., 2021).

A histological study of the gonads of *Echinometra sp.* revealed six stages, namely, the recovery, growth, premature, mature, spawning, and reabsorption stages, in both males and females. These steps have been mentioned in other sea urchin species, such as *P. lividus* (Ouchene *et al*., 2021) and *Lytechinus variegatus* (Tavares and Borzone, 2015). Byrne (1990) introduced stage I (the recovery stage) as the phase of the reabsorption of gametes that were not released during the spawning stage of the previous cycle. However, in this study, the model introduced by Raposo *et al*. (2023) was used to determine the stages of gonadal development. Reabsorption of gametes that were not released during the spawning stage of the previous cycle and repair of the gonads for the emergence of new gametes during the next cycle are two completely distinct events. They should be considered in two different stages (Raposo *et al*., 2023). As a result, in the present study, as described by Raposo *et al*. (2023), stage I (recovery) was reported as the stage of gonad repair, and stage VI (reabsorption) was reported as the stage of reabsorption of the remaining gametes. Thus, the gametogenic cycle of *Echinometra sp.* starts with the appearance of the first gametes and ends with the resorption of the remaining oocytes/sperm. In many cases,

identifying the stages of recovery and reabsorption is difficult. However, empty spaces and the absence of nutrients in the acini constitute the only way to distinguish the reabsorption stage from the recovery stage.

In the present study, the beginning of the gametogenic cycle in *Echinometra sp.* coincided with the appearance of the first oocytes and spermatocytes in the margins of gonadal acini in late November and October, respectively, for females and males. Ouchene *et al*. (2021) reported that in the *P. lividus* population (from the southern coast of Morocco), the beginning of gametogenic activity coincides with the appearance of the first germ cells, along with the abundance of nutritional phagocytes in late September to October (end of summer or early autumn). Similar results for the *P. lividus* population from the west coast of Portugal were reported by Machado *et al*. (2019).

As mentioned in the present study, stages I, II, and III of the gametogenesis cycle in most studied sea urchin species, such as *P. lividus* (Machado *et al*., 2019; Ouchene *et al*., 2021), *L. variegatus* (Tavares and Borzone, 2015), *E. vanbrunti* (Villalba Villalba *et al*., 2021), and *Evechinus chloroticus* (Brewin *et al*., 2000), occur during autumn and winter and may continue until the end of the spring. Similar to the results of the present study, spawning occurs from April to June in these species. Spawning may continue until the end of August in *E. vanbrunti* (Villalba Villalba *et al*., 2021).

Based on microscopic observations, the gonads of male and female *Echinometra sp.* were subjected to a simultaneous pattern, and all the gonad acini were in the same stages of development in this species. Similar results have been reported in other species, such as *L. variegatus* (Tavares and Borzone, 2015) and *E. vanbrunti* (Villalba Villalba *et al*., 2021).

Like other echinoids, the abundance of NP cells was greater in the early stages of the gametogenic cycle in *Echinometra sp.*. NP cells make the gonad structure more cohesive; for this reason, male and female gonads have more cohesive tissue during the early stages of development. On the other hand, the abundance of mature sex cells and the significant reduction in the number of NP cells, lead to a decrease in cohesion and an increase in the fragility of mature gonads (Raposo *et al*., 2023). A similar result was reported in *P. lividus* by Raposo *et al*. (2023).

In the present study, the mean gonad index (MGI) was used to determine the gonad growth pattern in both male and female *Echinometra* sp.. Owing to the relatively low distribution of MGI between male and female *Echinometra sp.*, this index is recommended to obtain appropriate information about the reproductive cycle. The MGI increased in *Echinometra sp.* during the gametogenic cycle and reached its maximum in late winter and spring. Tavares and Borzone (2015) also reported a significant correlation between MGI and the gametogenic cycle of *L. variegatus*. The mean gonadal index was greater in female *Echinometra sp.* than in males during the reproductive cycle; however, the difference was sometimes insignificant. Tavares and Borzone (2015) reported that female *L. variegatus* had higher mean gonadal

indices than males. The higher MGI in females than in males seems to be due to the greater energy required for synthesizing female gametes, which are produced in greater quantities in a shorter time (Tavares and Borzone, 2015).

Oocyte size is an important factor commonly used in the study of population reproduction. In the present study, a maximum diameter of 86.57±18 µm was measured for *Echinometra sp.* oocytes in stage VI (maturity stage) in May. Raposo *et al*. (2023) reported that the diameter of oocytes increased from 10 to 50 µm in the early stages of the gametogenic cycle to 90 µm in the maturation and spawning stages in *P. lividus*. As reported in *P. lividus* (Raposo *et al*., 2023), small previtellogenic oocytes are visible throughout the gametogenic cycle in the gonads of female *Echinometra* sp..

In conclusion, the present study provides important information about the reproductive biology of *Echinometra sp.* from the Persian Gulf. The indices used in this study, such as the MGI and GCA, are reliable tools for timing the reproductive cycle because the microscopic examination was consistent with these indices. The present study revealed an annual pattern characterized by a spawning period in the summer months. The decrease in water temperature seems to stimulate the start of the gametogenic cycle, and its increase stimulates the release of gametes in *Echinometra sp.* from the Persian Gulf. In the general reproductive pattern of *Echinometra sp.* from the Persian Gulf, maturity was determined by an increasing number of mature sex cells (stages III and IV) from late March to early June. This stage was followed by the spawning period from June to early September and the reabsorption period, which started in September.

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Conflicts of interest

The authors report no declarations of interest.

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