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Reproductive Cycle of the Sea Urchin *Echinometra mathaei* (Echinodermatidea: Echinoidea) in Bostaneh, Persian Gulf, Iran

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Abstract: This study documents the annual reproductive cycle of the sea urchin *Echinometra mathaei* on the South coast of Iran in Persian Gulf, Bostaneh, from April 2006 through May 2007. At each sampling date, one gonad from each urchin was selected for histological assessment and after the removal of one gonad, the remaining four were dried to a constant weight for determination of gonad index. Sea urchins from *E. mathaei* populations had shown an annual gametogenic cycle, the timing of which was temporally and spatially consistent. The histology of the gonads revealed six different maturity stages grouped in three main reproductive phases: recovering and growing, premature and mature, partly spawned and spent. Male urchins had mature gametes for a longer period than females, possibly an adaptive strategy that increases the probability of fertilization. Premature and maturing stages occurred from late winter to early summer. Spawning occurred during early summer to late summer. Recovering urchins were observed from late summer to early winter. Gonad indices showed a peak in weight from late winter to late-spring (3.72±2.3 for females; 4.25±2.5 for males; Mean±SE) then a decline for both sexes. There was a peak in the proportion of spawning from late winter to early summer that correlated with the observed peaks in gonad indices and gametogenic cycles. The demographic implications of this reproductive pattern are that new recruits can enter the population through synchronous spawning during the breeding period and smaller haphazard spawning events at other times of the year and reproduction is seasonal and cycle mediated by seawater temperature, day length and feeding activity.

Key words: *Echinometra mathaei*, reproductive cycle, gametogenesis, Persian gulf, sex ratio, gonad index

INTRODUCTION

Sea urchins have a major impact on the structure and dynamics of shallow subtidal communities (Williamson and Steinberg, 2001; Williamson *et al*., 2000). In temperate regions, high densities of urchins often denude large areas of macro-algae and can even create barrens (Woods *et al*., 2008), thus dramatically altering the community's physical and biological structure (Leinaas and Christie, 1996). Because of the density-dependent nature of the impact of urchins in temperate communities, knowledge of their life-history is important for understanding the ecology of these interactions. Reproduction in temperate echinoides is characteristically an annual cycle, with predictable cellular stages of gonadal development and spawning for a species can vary among localities and years (Byrne, 1989; Walker *et al*., 2005; Brooks and Wessel, 2004) and gametogenesis is considered to be controlled principally by seasonal changes in photoperiod (Walker and Lesser, 1998; Olave *et al*., 2007) lunar cycles (Rahman1 and Uehara, 2004; Roepke *et al*., 2005; Atkinson *et al*., 2003), temperature (Leahy *et al*., 1978; Beltrán *et al*., 2007), diet (Russell, 1998) and density (Wahle and Peckham, 1999), water temperature (Byrne, 1990; Pearse and Cameron, 1991). Reproduction in echinoids is generally well-documented due to the ease of obtaining mature gametes (Byrne, 1989; Walker *et al*., 2005; Brooks and Wessel, 2004) and knowledge of their gametogenesis and spawning has played an integral role in a range of commercial applications, including the development of toxicity tests (Dinnel and Stober, 1987) and echinoculture (Walker and Lesser, 1998; Olave *et al*., 2007). Despite the wealth of information on the reproduction periodicity of all urchins in worldwide, there are no studies of reproduction for families in Iran and Persian Gulf. One Echinometridae *Ehinometra mathaei* commonly occurs in shallow waters of Persian Gulf and Oman sea in Iran.

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To further understand the role of larval recruitment in the ecology of host-plant use by *Echinometra mathaei* in particular patterns in the availability of larvae. It is essential to understand the reproductive cycle of these urchins. This study aimed to determine the variability in the pattern of gonadal growth and the timing of spawning activity. In this study, histological examination of gonad development, the gonad index, sex ratio of the urchins were documented.

MATERIALS AND METHODS

Study site and collection of urchins: The reproductive cycle of *E. mathaei* was studied at Bostane harbor 26°31'S, 54°39'E at the West head of lenge harbor, Iran. Bostane harbor is a mosaic of habitats typical of shallow subtidal areas in temperate South of Iran.

Urchins between 3 and 6 cm in diameter were collected by hand of haphazardly. then they were taken back to the laboratory at the Mashhad University. Since *E. mathaei* elongate along the oral-aboral axis as they grow larger (Steinberg, 1995), the size of each urchin was measured with Vernier calipers to the nearest millimeter as the maximum oral-aboral diameter. Animals were sorted into their sexes in the laboratory at each sampling time, resulting in an uneven number of males and females. Apart from the gonopore of males being slightly longer and more slender than those of females-an observation almost impossible to distinguish whilst diving unless the animal is about to spawn-there is no obvious external sexual dimorphism between males and females. Therefore, the sex of each urchin was determined by examining the colour of the gonads of the freshly dissected specimens and any gametes that had been released. No urchins with any visible injury were used in this study.

Sex ratio: Biases in the ratio of sexes have been documented for a number of species of marine invertebrates (McCartney and Lessios, 2004; Otway, 1994; Kelaher and Cole, 2005; Van Dover *et al*., 1999) and vertebrates (Sadovy and Shapiro, 1987; Gillanders, 1995). Such ratios have been used as broad scale diagnostic indicators of the mode of reproduction (gonochoristic or hermaphroditic) of a particular population, with hermaphroditic populations typically having uneven adult sex ratios (Geyer and Palumbi, 2003; James *et al*., 2007; Sadovy and Shapiro, 1987). Chi-squared goodness-of-fit tests were used to see if the overall proportion of males and females deviated significantly from 1:1 and if the monthly proportion of males and females deviated significantly from this ratio over time.

Histology of the gonads: Gonads collected from *E. mathaei* monthly from Apr. 2006 to Feb. 2007 were processed for histological assessment of the cellular events of gametogenesis. At each sampling date, one gonad from each urchin was selected at random and preserved in Bouin 's for 48 h, then stored in 70% ethanol. Fixed gonads were dehydrated with graded ethanol (from 70 to 100%) and embedded in paraffin. After embedding, the wax blocks were trimmed and left on an ice slurry for approximately 10 min to facilitate sectioning. Three replicate sections of 7 μm thickness, approximately 50 μm distance from each other, were then cut from the middle portion of these blocks using a microtome (Microtom HM 325). These sections were mounted on slides, dried overnight in a 37°C oven and stained with Mayer's haematoxylin and eosin (H+E). Coverslips were mounted with Entlan mountant and the slides were allowed to dry in a fume hood for at least 48 h before examination. The clearest of the three sections was analysed (Williamson and Steinberg, 2001; Williamson *et al*., 2000).

All slides of gonad sections were examined under light microscope for sex determination and photographed using a Zies model digital camera. Maturity stages were classified according to earlier studies of echinoid Gametogenesis (Byrne, 1990; Spirlet *et al*., 1998) based on oocyte size (females), thickness of peripheral spermatocyte layer (males). Since the ovaries aren't at a similar stage of maturity through the year, ovary sections were classified according to changes in different maturity stages were recognised after histological analysis. The different stages were recovery, growing, premature, mature, partially spawned, spent that 50 lumen per individual counted. For analysis the first three groups were combined into one category developing ovaries and were analysed along with the mature ovaries and partially spawned ovaries.

A classification system for testes was based on the different stages. In this study testes were classified according to the change in relative abundance of the different stages of maturity of 50 testes per individual. Here, testes at different stages of maturity were placed into six categories recovering testes; growing testes; premature testes; mature testes; partially spawned testes; and spent testes. For analysis the first three groups were combined into one category developing testes and were analysed along with the mature testes and partially spawned testes.

A pilot study was done with samples collected on 12 Jan 2007 to determine whether all of the five gonads per individual and different locations within each gonad were in a similar gametogenic state. Here, all gonads from three

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females and three males were preserved divided into three locations (top, middle and bottom), depending on the distance of the sample on the gonad from the gonophore sectioned and analysed separately for each sex.

Data on the two developmental groups that comprised the bulk of cells in the ovaries and testes (i.e., developing and mature stages) were analysis.

Prior to analysis, Coch-ran's tests (Zigler *et al*., 2005) were used to examine homogeneity of variances ($p>0.05$). For each sex, the three locations within the gonads were compared separately with a two-factor nested analysis of variance, with urchin and location nested within urchin as random factors. If location was not significant in this analysis, it was pooled and data on the different replicates compared with a two- factor nested analysis of variance, with urchin and gonad nested within urchin as random factors.

These analysis confirmed that gametogenesis is homogeneous throughout each gonad and among gonads of individual urchins. Consequently, only one section from the middle portion of one gonad per individual was examined thereafter. The maturity stage of each gonad was compared using a one-factor analysis of variance to determine if the proportion of cells within the stages varied significantly over time. Due to unequal sample sizes within each developmental group, Cochran 's tests could not be done and assumptions of normality and homogenecity of variance for each data set were checked using residuals as for the onset of sexual maturity and transformations made where appropriate. A posteriori pairwise comparisons (Tukey's test) tested at $p = 0.05$ were used to determine the location of differences among the treatment means after analysis of variance.

Gonad index: The Gonad Index (GI), which measures the relative changes in weight in the gonads over time, was used on urchins as early as 1934 (Shpigel *et al*., 2004; Fernandez and Boudouresque, 2000) and is based on the assumption that maturation and breeding coincide with maximum gonad weight. Since, the size of urchins may influence the GI (Roepke *et al*., 2005; Barbaglio *et al*., 2007), the size range of urchins used was restricted to 30-60 mm.

Urchins were dissected, their gonads removed and the five gonads and the rest of the urchin were dried separately at 60°C to a constant weight for a minimum of 3 days. On several dates these urchins were also used for histological analysis. Thus, after the removal of one gonad, the remaining four were dried to a constant weight and the gonad weight extrapolated to an approximation of the dry weight for each urchin.

A pilot study showed that there was no significant difference between the dried weight of four gonads that had been extrapolated to include the weight of the 5th gonad and the dried weight of the five gonads for the same urchin (Williamson and Steinberg, 2001; Williamson *et al*., 2000). After drying, the ratio of total gonad weight to total dry weight for each urchin was used to calculate the GI.

RESULTS

Sex ratio: The overall sex ratio of *E. mathaei* did not deviate significantly from 1:1 (169 males, 153 females; $\chi^2 = 0.5$, df = 1, p = 0.403) (Table 1). Similarly, there was no significant deviation from a sex ratio of 1:1 in monthly samples of urchins from April 2006 to February 2007 ($\chi^2 = 7.114$, df = 11, p = 0.790). No hermaphrodites were observed.

Histology of the gonads

Cellular events of gametogenesis

For females: Histological examination of the ovaries of *E. mathaei* revealed that eggs in various stages of development were usually present (Fig. 2A).

Stage 1: Recovery: The appearance of the first previtellogenic oocytes, defines the recovery stage. This stage happens either at mid-late or late spent stage. Figure 1A shows a recovering ovary at the mid-late spent stage where some relict ova are still present at the centre of the lumen and are being resorbed by phagocytes while previtellogenic oocytes differentiate along the acinal wall. Most of the ovaries (76%) recovered at this stage of development, while, the rest recovered only when all the ova have been resorbed, that is, after late spent stage (Fig. 1A). The diameter of the previtellogenic oocytes range from 10 to 30 μm.

Stage 2: Growing: This stage is characterised by growth in size of the previtellogenic oocytes due to the onset of vitellogenesis. The growing ovaries contained numerous vitellogenic oocytes that are still in contact with the acinal

Table 1: Analysis for the sex ratio on sea urchin for females and males of *E. mathaei*

		N	Mean	SD	Minimum	Maximum
Descriptive statistics						
All		322	1.52	0.500	1	2
		Category	N	Observed prop.	Test prop.	Asymp. sig. (2-tailed)
Binomial test						
All	Female	1	153	0.48	0.50	0.403a
	Male	2	169	0.52		
	Total		322	1.00		

a: Based on z approximation

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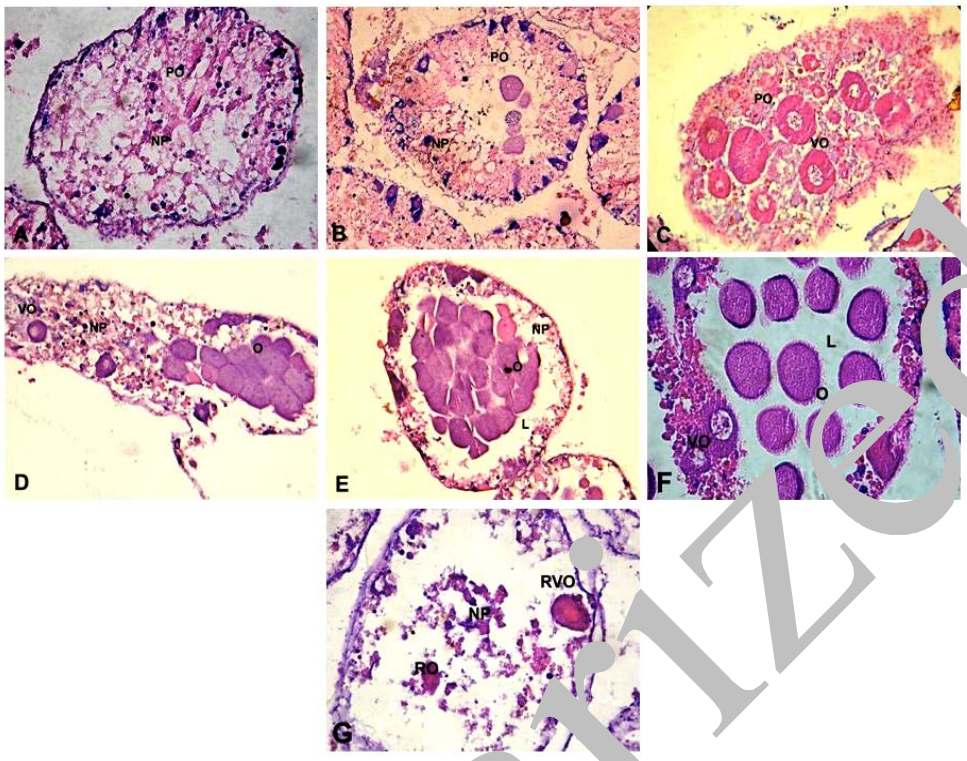

Fig. 1: Histology of ovaries (A-F) of Echinometra. (A) Recovering satge, (B) Growing stage, (C) Late growing stage, (D) Premature stage, (E) Mature stage, (F) Partly spowned stage and (G) Spent. (x400X). L: Lumen; Np: Nutritive phagocytes; O: Mature ovum; Po: Previtellogenic oocyte; Ro: Relict ovum; Rvo: Relict vitellogenic oocyte; Vo: Vitellogenic oocyte

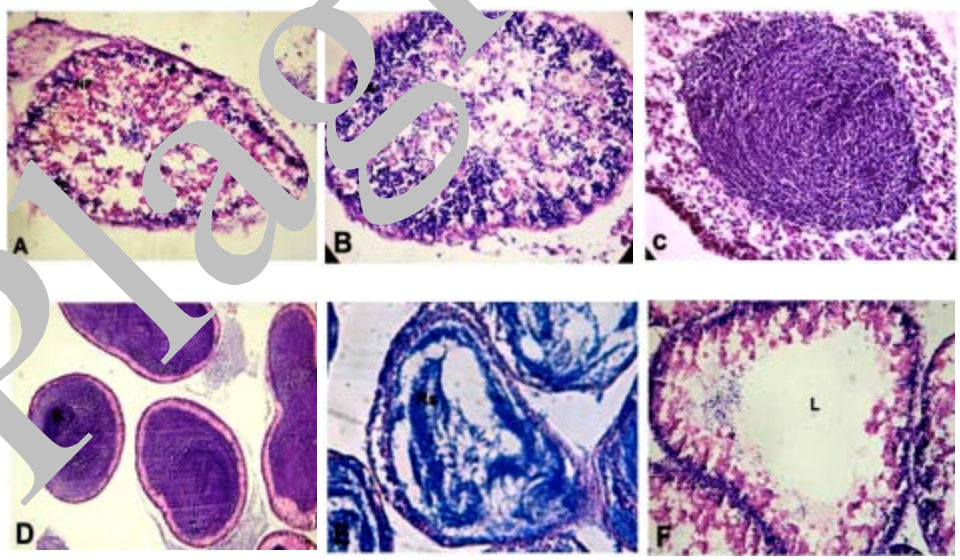

Fig. 2: Histology testes (A-F) of *E. mathei,* showing the six maturity stages. (A) Recovering (x400X), (B) Growing (x400X) (C) Premature (x400X), (D) Mature (x100X), (E) Partlyspowned (x400X) and (F) Spent (x400X). Cs: Column of spermatocyte; L: Lumen; Np: Nutritive phagocytes; Rs: Relict sperm; S: Sperm; Sg: Spermatogonia

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wall and are surrounded by the nutritive phagocytes (Fig. 1B). The size of these vitellogenic oocytes ranges from 30 to 60 μm in diameter. Some previtellogenic oocytes may still be present along the acinal wall and the lumen is still filled by nutritive phagocyte.

Stage 3: Premature: In premature ovaries, vitellogenesis is a continual process and oocytes of all stages are present. Vitellogenic oocytes continue to grow and as they become bigger, they get detach from the acinal wall to migrate in the centre of the acini (Fig. 1C). Fully grown vitellogenic oocytes undergo maturation and the resulting ova accumulate in the lumen, which is now devoid of any nutritive tissues.

Stage 4: Mature: The ovarian lumen is filled with densely packed ova and most of the nutritive tissues have been used up, leaving a thin layer at the periphery of the acini (Fig. 1D). The mature ova have a small nucleus and measure from 100 to 150 μm in diameter. Vitellogenic oocytes are still present in the germinal layer indicating that vitellogenic activity is not completed.

Stage 5: Partly spawned: Partly spawned ovaries are characterised by the presence of ova that are less densely packed than mature ovaries (Fig. 1E). Vacated spaces are observed in the acini, indicating partial release of ova. In the germinal layer, nutritive tissues are still present as a thin layer surrounding vitellogenic oocytes. These oocytes continue their development and subsequently replace the ova as they are shed.

Stage 6: Spent: Ovaries in the early spent stage are characterized by a thin layer of Nongerminal nutritive phagocytes lining the acinal walls (Fig. 1F). Unspawned ova and few vitellogenic oocytes largely occupy the lumens. Both ova and vitellogenic oocytes are destined to be resorbed by the Phagocytes. Resorption of germinal cells happens throughout the spent stage no that the lumens of late spent ovaries are completely deprived of any Germinal cells and fill with dense meshwork of nutritive phagocytes.

For males: Testis development in *E. mathaei* was divided into six stages (Fig. 2), based on the basophilia of the nucleus in developing stages and the presence of spermatogenic columns and spermatozoa. These stages were used to describe the histology of spermatogenesis. Spermatids could not be discerned in any of the stages with the light microscope but were assumed to be present.

Stage 1: Recovering: Recovering acini were small, with a basophilic layer of spermatogonia and primary spermatocytes lining the acinal wall (Fig. 2A). The lumen of the acini contained nutritive phagocytes and unspawned spermatozoa. The beginnings of spermatogenic columns were sometimes observed.

Stage 2: Growing: Growing testis contained conspicuous columns of spermatocytes, projecting towards the lumen (Fig. 2B). These columns contained spermatogonia at the base, with primary and secondary spermatocytes and spermatozoa at the tips. At this stage the spermatocyte layer was fairly thick, with nutritive phagocytes still in the center of the lumen.

Stage 3: Premature: This stage contained columns of developing spermatocytes that extended from the acinal wall into the lumen (Fig. 2C). Spermatozoa accumulated within the lumen among the few remaining nutritive phagocytes.

Stage 4: Mature: Mature testes were filled with a dense mass of spermatozoa in the lumen (Fig. 2D). Columns of developing sperm were often absent and nutritivephagocytes were located towards the periphery along the germinal epithelium. Spermatozoa often appeared to swarm in strands.

Stage 5: Partly spawned: Partly spawned acini were similar to mature acini but the sperm was less concentrated and spaces due to spawning were evident (Fig. 2E). Some of the acini appeared to have spawned before others within a gonad and mature acini and partly spawned acini were often present next to each other. Swarming of spermatozoa were easily visible near spaces where spawning had occurred.

Stage 6: Spent: Testes at the spent stage are characterised by the presence of a developing layer of nutritive phagocytes along the acinal walls. In early spent testes, relict spermatozoa are still present in the centre of the acini and are being resorbed by the phagocytes. Late spent testes are devoid of any germinal cells and the acini are filled with dense meshwork of nutritive phagocytes. At the spent stage, no spermatogonial proliferation is observed (Fig. 2F).

Gametogenic cycles: The pilot study for females showed that the proportion of developing ovaries and mature ovaries were not significantly different throughout a single urchin (Table 2a, b) and between the five gonads of a single gonad (Table 2c, d), although the proportion of developing ovaries and mature ovaries were significantly different between individual urchins. The homogeneous nature of developing cells throughout a single gonad and

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Table 2: Analysis of variances for the pilot study on gonadal developmental stages for females and males of *E. mathaei*

Source	df	MS	F	p-value
Female				
(a) Developing ovaries				
Urchin	2	40.16	25.81	0.000000***
Location	6	0.78	0.50	0.804095^{NS}
Error	36	1.56		
(b) Mature ovaries				
Urchin	2	39.27	32.42	0.000000***
Location	6	0.98	0.81	0.571002^{NS}
Error	36	1.21		
(c) Developing ovaries				
Urchin	2	40.16	35.43	0.000000***
Gonad	12	2.22	1.96	0.066521^{NS}
Error	30	1.13		
(d) Mature ovaries				
Urchin	2	39.27	38.41	0.000000***
Gonad	12	1.57	1.53	0.166946^{NS}
Error	30	1.02		
Male				
(e) Developing testes				
Urchin	2	35.47	11.32	0.000154***
Location	6	2.58	0.82	0.559871^{NS}
Error	36	3.13		
(f) Mature testes				
Urchin	2	39.47	32.89	0.000000***
Location	6	1.44	1.20	0.32828^{NS}
Error	36	1.20		
(g) Developing testes				
Urchin	2	35.47	13.19	0.000078***
Gonad	12	3.97	1.48	0.188260^{NS}
Error	30	2.69		
(h) Mature testes				
Urchin	2	39.47	38.61	0.000000***
Gonad	12	1.77	1.73	0.110050^{NS}
Error	30	1.02		

(a), (b), (e) and (f) ANOVAs comparing different locations within each urchin; (c), (d), (g) and (h) ANOVAs comparing different gonads within gonads; Cochran's tests showed all data to be homogeneous, therefore all data were untransformed, ***p<0.001, NS: Not Significant

between the five gonads of each urchin validated present procedure of sectioning and analyzing the middle portion of only one gonad per female urchin. The oogenic cycle of *E. mathaei* showed annual temporal pattern (Fig. 1) at Persian Golf.

Gonads containing all stages of developing ovaries and mature ovaries were observed throughout the study in few months (Fig. 1). There was significant difference in the proportion of developing ovaries [recovery ovaries (One-factor ANOVA F11, 121 = 36.896, p = 0.00) and growing ovaries (one-factor ANOVA F11, 121 = 34.134, p = 0.00), premature ovaries (one-factor ANOVA F11, 121 = 49.0607, p = 0.00)], the proportion of mature ovaries significantly increased during spring (one-factor ANOVA F11, 121 = 110.654, p = 0.00), partly spawned (one-factor ANOVA F11, 121 = 19.994, p = 0.00), spent (one-factor ANOVA F11, 121 = 95.294, p = 0.00).

All analysis in the pilot study for males showed a significant difference in the proportion of developing testes and mature testes between individual urchins (Table 2e-h), which may have been due to the low number of replicate urchins in the study. Although there was a significant interaction in the proportion of developing testes between urchin and location, location was not significant (Table 2e), suggesting that testes were homogeneous throughout a gonad. Moreover, the proportion of mature testes throughout a gonad was not significantly different (Table 2g). The proportion of both developing testes and mature testes were not significantly different between the five gonads within an individual (Table 2g, h). Therefore, sectioning and analyzing the middle portion of one gonad per male urchin gave a valid estimate of the gametogenic cycle at that time for an individual.

The spermatogenic cycle of *E. mathaei* at Persian golf showed a distinct temporal pattern in the relative abundance of the different stages of testis maturity (Fig. 4). Recovering testes, Growing testes, premature testes were present throughout the year (Fig. 4).

Mature testes and spent testes were present in low numbers for 6 months of the year (from August to February (Fig. 4). There was a significant difference in the proportion of recovering testes, growing testes, premature testes throughout the year. [recovering testes (One-factor ANOVA F = 11, 77 = 5.734, p = 0.00) growing testes (one-factor ANOVA F = 11, 77 = 9.47, p = 0.00) and premature testes (One-factor ANOVA F = 11, 77 = 9.399, p = 0.00)] the proportion of mature testes significantly increased during spring (One-factor ANOVA F = 11, 77 = 16.75, p = 0.00).

This, along with the significant increase in the proportion of partially spawned testes from April to October (one-factor ANOVA F11, 77 = 17.055, p = 0.00), indicates that spawning of males, occurring from spring to late-summer (Fig. 3, 4).

Gonad index: Gonad Index (GI) values of females at Bostaneh revealed a strong temporal effect (one-factor ANOVA F = 26.716, df = 11, p-value = 0.00<0.05) (Fig. 5a). GI peaks are in Apr. 2006 to June 2006 and then there is a decrease in gonad index. This suggests that spawning occurred in July to October.

GI values for males also revealed a temporal effect (one-factor ANIOVA F = 39.114, df = 11, p-value = 0.00<0.05) (Fig. 5b).

GI peaks are in April to June 2006 and then there is a decrease in GI and these suggests that spawning in male occurred in July.

This suggests that onset of spawning in male is almost synchronus. Comparison of the GI values of both sexes showed a significant Pearson correlation coefficient (rs = 0.608, n = 153, p<0.00), indicating some synchrony between the sexes. When sexes were pooled, values for GI were similar among month (One-factor ANOVA F = 2731.055, df = 11, p-value = 0.00<0.05) (Fig. 5c).

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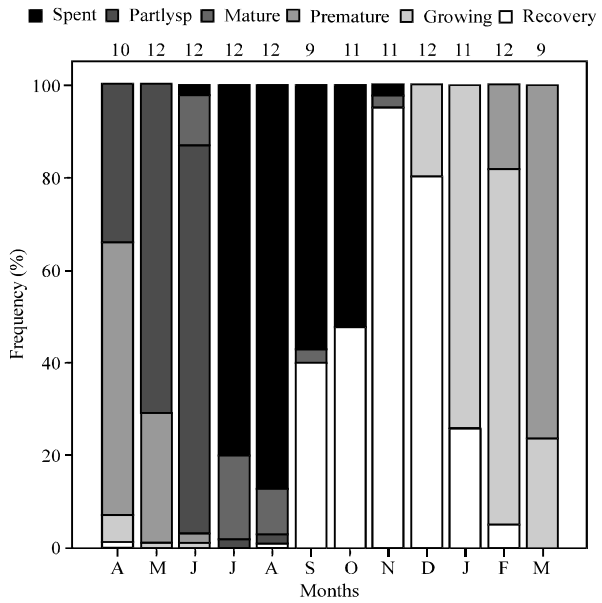


Fig. 3: Annual gametogenic cycle of female *E. mathaei*. Histograms show the relative frequencies of maturity stages of the ovaries in histological sections of gonads of urchins from Persian Gulf (Bostaneh). Numbers above each column show the number of urchins from which the frequencies were estimated

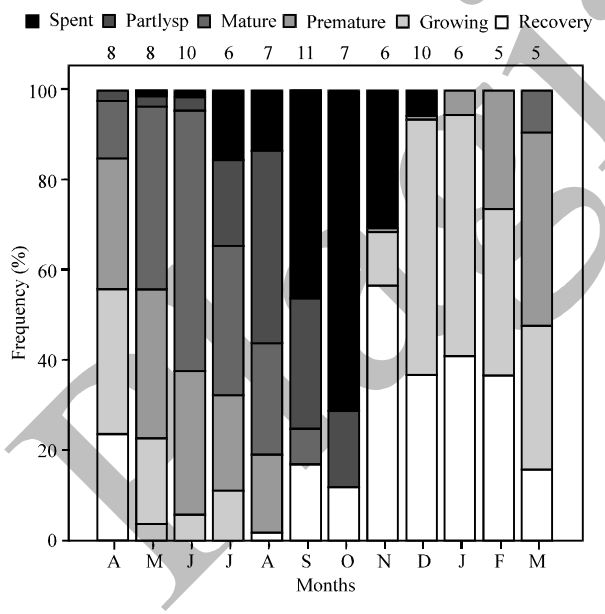
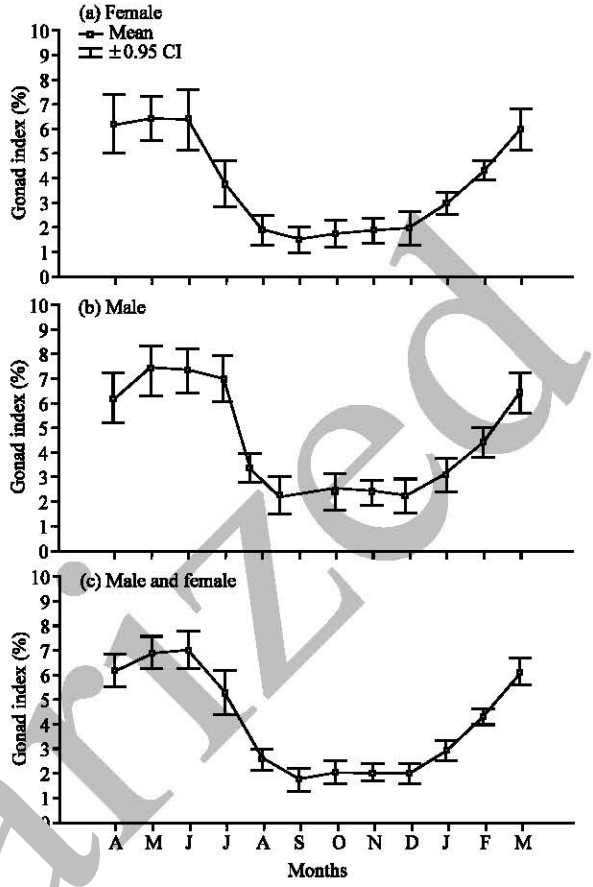


Fig. 4: Annual gametogenic cycle of male *E. mathaei*. Histograms show the relative frequencies of maturity stages of the testes in histological sections of gonads of urchins from Persian Golf (Bostaneh). Numbers above each column show the number of urchins from which the frequencies were estimate



5: Mean±SE gonad index for a female, b male and c male and female *E. mathaei* from Persian Gulf (Bostaneh) pooled over time. The number of replicates for each date and sex ranged from 15-18, with an average of 16

DISCUSSION

Echinometra mathaei have a defined breeding period during spring and early summer, There is an equal sex ratio, no significant differences in male and female gonad indices and an annual reproductive cycle (Akiyama *et al*., 2001; James *et al*., 2007). Similarly, the cellular processes during gametogenesis and high interindividual gametogenic variability are generally consistent with other temperate echinoids with an annual gametogenic cycle (Meidel and Scheibling, 1998; Meidel and Yund, 2001).

Timing of gametogenesis was synchronous between males and females at bostaneh. The sex ratio of *E. mathaei* was consistently 1:1, typical of gonochoristic echinoderms (McCartney *et al*., 2000). Cellular events during gametogenesis occurred uniformly throughout the gonads and included proliferation and growth of sex cells

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in late winter and spring this was accompanied by an apparent change in the role of the nutritive phagocytes from nutrient storage to transfer of nutrients to the developing gametes.

Spermatocytes and oocytes were first observed around the walls of gonad lobes in the in the Jan. 2005 sample between the winter and spring samples the proportion of gonad volume occupied by both germinal tissues and developing gametes increased in all the populations studies.

The sequence of oogenesis in *E. mathaei* was similar to those described for other echinoids: oogenesis started with primary germ cells that developed through several vitellogenic stages and resulted in ova. Pre-vitellogenic and early vitellogenic oocytes were consistently the most abundant types of oocytes present, similar to echinoids such as *Stylocidaris affinis* and *Paracentrotus lividus* (Van Dover *et al*., 1999; Bianchini *et al*., 2007) and such as male maturity of ovaries divided to 6 stages.

Histological analysis and gonad index changes showed that spawning occurred between June and October at bostaneh. Spermatogenesis in *E. mathaei* was similar to other species of echinoids and was classified into the maturity stages typical for echinoids. Most developmental stages of testes were observed in all months. The spermatogenic cycle showed a distinct temporal pattern in the relative proportion of the different maturity stages present. Mature testes and partially spawned testes were present for 6 months, indicating that gametes were released in a distinct temporal pattern, the significant increase in the proportion of mature testes in spring followed by a similar increase in the proportion of partially spawned testes in late spring and early summer, points to increased breeding activity during this time.

Spent testes in late Summer and early Autumn suggests that the production of new sperm in the germinal layer is not continuous. The temporal patterns of spawning observed (Fig. 4) suggest that favourable conditions for spawning occur during spring and early summer.

Consistently higher gonad indices were recorded for *E. mathaei* like other echinoieds, can retain mature gametes for extended periods before spawning (Van Dover *et al*., 1999; Bianchini *et al*., 2007).

The adaptive significance of this ability is that it may allow synchronous spawning independent of annually varying factors (Roepke *et al*., 2006) demonstrated the importance of the spring phytoplankton increase for synchronizing spawning in populations of *Strongylocentrotus droebachiensis* separated by considerable distance.

Also common in studies on other echinoids was the synchrony between the sexes observed here, where a gradual increase in gonad indices in winter, leading to a peak in late winter to late-spring, occurred for both male and female urchins. Since gonad indices generally increase prior to a spawning event and then decrease during the event (Böttger and James, 2002; Wahle and Gilbert, 2002), this data is consistent with the pattern shown by the gametogenic data.

Moreover, the seasonal timing of this peak in the reproductive cycle for male and female *E. mathaei* was similar to many temperate echinoids, both in Australasia (Pearse and Lockhart, 2004; Lee *et al*., 2004; McBridea *et al*., 2004; Robinson *et al*., 2002) and in the same season in the northern hemisphere (King *et al*., 1994; Keats *et al*., 1984).

Urchins typically allocate energy to the gonads justprior to and during gametogenesis. The relationship with the GI and the gametogeniccycle indicates that *E. mathaei* may also do this.

Interestingly, when the GI increased in females the proportion of oocytes in different stages of development remained unchanged, indicating that the ovaries were growing in bulk, but not changing in constitution. Therefore, although females may spawn episodically throughout the year, they are likely to release more ova during spring than at other times of the year due to an increase in the size of the gonads. As the GI for males increased, however, the proportion of growing and premature testes decreased and the proportion of mature and partially spawned testes increased. This suggests that, although the males appeared to be opportunistic spawners through the year, they increased the maturity of their testes in winter and had a large spawning event in spring, releasing proportionally more gametes than females during this time some other endogenous or exogenous cue may affect the spawning of the population. For example, the abundance of phytoplankton in the surrounding water may trigger the onset of spawning as in some echinoids (Starr *et al*., 1990; Cook *et al*., 2007; Inamdar *et al*., 2007; Otero-Villanueva *et al*., 2004).

Spawning may be an extremely localised phenomenon. Proximal cues may stimulate or stress a few individuals within a population to spawn and the gametes or pheromones released by these individuals in turn may initiate a broader spawning response. Such a model for inducing neighbouring conspecifics to spawn has been proposed for echinoids by a number of researchers (Spirlet *et al*., 2001; Daggett *et al*., 2006; Levitan, 2000; Lauzon-Guay and Scheibling, 2007).

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In conclusion, *E. mathaei* has a defined breeding season in spring and early summer, with no ability to reproduce throughout the year. Simple the majority of the population appear to be reproducing during this breeding season.

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