



Study on the Gonadosomatic Indices of Sea Urchin *Echinometra mathaei* in Persian Gulf, Iran

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ABSTRACT

The gonad developments of 217 sea urchins (*Echinometra mathaei*) were monitored monthly from March to September 2014 in the intertidal zones of Bandar Lengeh in the Persian Gulf. The numbers of males, females, unsexual samples were counted for 71, 126 and 20, respectively. Females were totally 1.77 times heavier than males. Wet weights of gonads were nearly 8.25% of the mean total wet body weights. Only females were observed by gonad wet weights more than 12 g. The gonad wet weights of females (3.74 ± 3.26) was heavier than males (3.46 ± 2.63). March to August was the spawning months, as a U-shaped trend by August (mid-summer) as its concave curve and it means that the most stores of gonads is in spring. The operational sex ratio (OSR) of *E. mathaei* was female-biased and was not significantly different during the study. The monthly adult sex ratio (ASR) was significantly different. It means that the sex ratio has no effects on OSR. Moreover, the gonadosomatic indices GSI 1, GSI 2, and GSI 3 were 8.2 ± 0.6 , 4.1 ± 0.3 and 9.4 ± 0.9 , respectively. GSIs did not showed differ significantly in females; while in males, they were different. It was also obvious difference for males in spring and summer. All three indices were higher in the spring than summer (for example for GSI 2: 1.6 (August) ≤ 7.3 (March)).

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Authors' Contributions

MK was the main investigator; he designed the study and laboratory experiment, collected data, statistically analysis and interpreted them and wrote the manuscript. EK and NAB revised the manuscript. HZ statistically checked the results. All of the authors read and approved the final manuscript.

Key words

Gonadosomatic index, Operational sex ratio, *Echinometra mathaei*, Sea urchin.

INTRODUCTION

Sea urchins are found widely in tropical and subtropical marine zones, although they can also be found in other places like Antarctic (for example *Sterechinus neumayeri*, Klinger *et al.*, 1997). They have often been overfished in several countries in the last few decades (Siikavuopio, 2009). Their gonads, also known as roe or uni (Arafa *et al.*, 2012) are popular luxury seafood in Asian, Mediterranean and some other countries (Unuma *et al.*, 2002). The right color of the gonad is an important marketing factor (Lawrence, 2013) so that color measurements have been standardized. For example, *Paracentrotus lividus* (Lamarck) is preferred when it has yellow gonads which have a caviar-like appearance and a bittersweet flavor (Robbins *et al.*, 1990).

In the Persian Gulf region the local urchins are not eaten locally. Moreover, they have been neglected and little research has been done, especially on *Echinometra mathaei* (Keshavarz, 2016). These are the dominant urchin found in patches along the rocky coasts of this area between the average low tide and a maximum depth of 10 m (McClanahan and Muthiga, 2013).

Echinometra mathaei is widely distributed species. It scrapes surfaces in the process of grazing (Coppard and Campbell, 2006). It has been studied because of its significant ecological role in coral reef environments like other species of the genus *Echinometra*. It was reported to have densities of 0.1 to 100 individuals m⁻² in a limited area of New Caledonia in the South Pacific (Dumas *et al.*, 2007). At high densities, their bioerosion role has been considered to be a limiting factor for the growth and survival of coral reef ecosystems (Bronstein and Loya, 2014).

Since sea urchins have separate sexes and fertilization is external, they release their gametes into the sea at spawning time via 5 visible gonopores on their external shells (Keshavarz, 2016). The growth and gonad size of sea urchins in primarily rocky coastal regions are affected by the abundance and species of algal foods (Walker *et al.*, 2006). For example, growth and gonad production of *Strongylocentrotus nudus*, which is commercially harvested in northern Japan, are greatest in kelp beds, followed by furoid beds and small perennial algal turf beds such as those formed by the red alga *Chondrus ocellatus*, and least in *Crustose coralline* dominated "barren" beds (Agatsuma, 1997; Sano *et al.*, 2001; Agatsuma *et al.*, 2005a; Nakabayashi *et al.*, 2006).

Various gonadosomatic indices have been used to

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describe the nature of the annual cycles of a variety of marine invertebrates. These measurements, particularly when used in conjunction with histological techniques, have generally been used in characterizing the annual reproductive cycles of a number of species including urchins (Moore, 1934).

Operational sex ratio (OSR) defined by Emlen and Oring (1977) as the proportion of sexually mature males divided by the total number of sexually mature adults (Emlen and Oring, 1977; Kvarnemo and Ahnesjö, 1996) is a major factor influencing the intensity of sexual selection (Clutton-Brock and Parker, 1992) may help elucidate sex differences during the life-history of a population (Kokko *et al.*, 2012). For example, in a population where 50% of males remain unmated, 50% mate once (Kokko *et al.*, 2012). Any changes in OSR usually leads to increased competition for mates among all members of the more abundant sex, while members of the other sex may have a greater opportunity to exercise mate choice (Emlen and Oring, 1977).

The objective of the present study was to study the OSR and gonadosomatic indices of *E. mathaei* in the Bandar Lengeh area of the Persian Gulf.

MATERIALS AND METHODS

Study area

The study area was located in Bandar Lengeh, Iran, a small inlet along a rocky shore in the northern part of the Persian Gulf (26°32'28"N, 54°52'28"E; water depth: 0.5–1 m), covered with a high density of dominant macroalgae species of *Sargassum wightii* and *Padina antillarum*, from March to September, 2014. Due to the location of the studied area on subtropical zone, the first three months (March, April, May) were considered as spring while the next four months (June, July, August, September) were considered as summer season.

Laboratory measurements

Thirty one random size (height= [2.60 to 40.35 mm]) samples of urchins were collected individually each month using a handy stainless steel forceps in the intertidal zone during low tides of spring tides. They were transported in fiber tanks of sea water with continuous aeration by a portable aquarium air pump (Boyu D-200) of a 1.5V-waterproof battery as fresh *E. mathaei* to a marine biology laboratory in Hormozgan University. It is useful to mention that the temperature of sea water during the total sampling months were 22.46 to 34.05 °C.

Before their dissections, every urchin was placed on a paper towel for about 1–2 min to eliminate any surface water, and the attached algae and crushed seashells of the

urchins were discarded. The total wet weight of the animal was measured using a portable electric stainless steel balance [model: the professional digital table top scale (weighing scale 0.01 - 500 g, 0.01 g precision)]. After measuring the weight, the dissection started. The spines of the urchins were removed by a slim pointed tip Lodestar stainless steel tweezers, L605014; the peristome was cut using a scalpel; the Aristotle's lantern was removed using forceps, and finally the coelomic fluid was removed using a one-milliliter sterile Q Ject Ultra insulin syringe. Two small cuts were made on the sample shell by using a scissors to divide the shell into two parts. The contents of the digestive system were removed and the urchin rinsed several times using sterile natural sea water. Then, the gonads were separated from the shell using small forceps, and kept inside pre-weighed 10 cc glass sterile vials. The wet weights of the gonads were obtained. And finally, sex was determined by observing a small piece of gonad using the easily observed color differences between males and females.

Important indices

As mentioned previously, OSR is one of the indicators on the study of sexual behaviors of animals that can be defined as follows (Emlen and Oring, 1977):

$$OSR = \frac{\text{Sexually mature males}}{\text{Total number of sexually mature adults}} \dots \dots \dots (1)$$

The above formula was used to determine the monthly trend of male adults in compared with the general adult population and specify monthly differences. In this study, the sex ratio (males/females) was also calculated monthly.

$$\text{Sex ratio} = \frac{\text{Males}}{\text{Females}} \dots \dots \dots (2)$$

Moreover, the Gonadosomatic Indices (GSIs) were calculated for each individual (Martínez-Pita *et al.*, 2008; Lozano *et al.*, 1995) as equation 3 to 5, and then monthly and seasonally means and standard deviations were also investigated:

$$GSI\ 1 = \frac{\text{Wet Weight of Gonads}}{\text{Body Wet Weight}} \times 100 \dots \dots \dots (3)$$

$$GSI\ 2 = \frac{\text{Dry Weight of Gonads}}{\text{Body Dry Weight}} \times 100 \dots \dots \dots (4)$$

$$GSI\ 3 = \frac{\text{Wet Weight of Gonads}}{\text{Body Wet Weight} - \text{Wet Weight of Gonads}} \times 100 \dots \dots \dots (5)$$

It is useful to mention that for achieving dry weights

of each individual on equation 4, samples were put inside a 60 °C oven for 24 hours, and then they were weighted as “primary dry weights”. This step has repeated continuously until a fixed unchanged weight named “Dry Weight” was found for each individual sample in compare with previous step. It was found useful to measure and analyze the weight of the gonads (Feng *et al.*, 2014) by equation 6, where “GMC” is gonad moisture content:

$$GMC = \frac{\text{Wet Weight of Gonad} - \text{Dried Weight of Gonad}}{\text{Wet Weight of Gonad}} \times 100 \dots \dots (6)$$

Statistical analysis

Statistical analysis was done using different software programs suitable for windows as the operating system: IBM SPSS statistics 32-bit version 22.0.0.0, MINITAB Inc. 814-238-328 version 11.12 32-bit, Microsoft office Excel (2007) and MATLAB R2014a (8.3.0.432) 64-bit, here after SPSS, MINITAB, Excel, MATLAB, respectively.

The gonad weights and total wet weights distributions were calculated by using SPSS (Mean ± Standard Deviation). The data outputs were also checked by Skewness to see whether the data was symmetric (-2≤Standard Error of Skewness≤2) or asymmetric (-2> Standard Error of Skewness>2).

The data checked for the homogeneity of the variance and normal distribution in SPSS. OSR, sex ratio and gonad indices were compared by one-way ANOVA. Chi-squared test and Tukey’s HSD were used to determine significant differences.

Finally, MINITAB was used to calculate the gonad moisture content index (GMC) for the two genders, Quartiles (Q₁(x_i) and Q₃(x_i)), Interquartile Range (IQR = Q₃(x_{i1}(x_i)), and Range (R= Maximum (x_i) – Minimum (x_i)), where x_i refer to males, females, or total data regards to equation 6.

RESULTS

Out of 217 *E. mathaei*, 71 were males, 126 were females and the genders of 20 of samples were not obvious, because they were small size without any gonad. The largest sample was 103.77 g while the smallest one was only 0.07 g.

Table I represents the most frequent data of each parameter and if they are symmetric by calculating Skewness. Multi-high frequencies were seen for wet weights of gonads. Figure 1 presents the distribution of the measured weights for all samples by considering their genders.

Figure 2 shows the monthly OSR of *E. mathaei* in October (0.46± 0.106), and April (0.11± 0.106). The adult

sex ratios are significantly different between September and other months (P<0.05). There was significant deviation at overall sex ratio of *E. mathaei* from 1:1 (Table II) without any hermaphrodites samples.

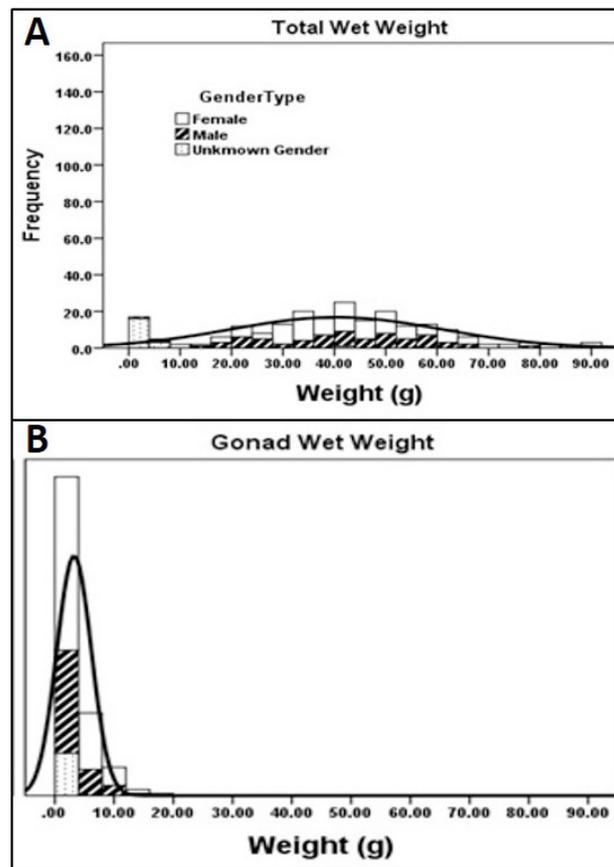


Fig. 1. Distributions of weights of 217 *E. mathaei* samples for the studied period (March–September, 2014) due to their genders. A, Total wet weight (40.38±20.592); B, Wet weight of gonads (3.13±2.725).

Table I.- The weight of sea urchin and their gonads of *E. mathaei*, derived by SPSS analysis.

Parameter	Weight of Wet Gonads (g)	Total Weight (g)
Mean ± SD	3.3 ± 2	40 ± 21
Skewness	1.4	0.083
Symmetric/Asymmetric	Asymmetric	Symmetric
Most frequent data	0.00–0.83, 2.51–3.34	35.0–45.0

Table III shows gonadosomatic indices separated by season and gender without including unsexual samples. The numbers of female, male and unsexual samples were

59, 31 and 7 in spring and 67, 40 and 13 in summer, respectively. Tukey’s test showed that in spring (March-May) there were no significant differences between GSI 1 and GSI 3 while in summer (June-September) it was found no significant differences among three gonadosomatic indices (GSI 1, GSI 2, GSI 3) ($p>0.05$).

Figure 2 also represents monthly changes in the gonadosomatic indices of *E.mathaei* of Bandar Lengeh. It is clear that maximum and minimum GSI 1 occurred in March and August, respectively. GSI 2 declined March to August. Maximum of GSI 3 is March, and GSI 3 gradually decreases until August. Broadly speaking, all 3 gonadosomatic indices decreases from April to August, and there is a slight increase in September.

A 3D scatter gonadosomatic indices of females and males are shown in Figure 3. In spring, gonadosomatic indices of females scattered more than summer (Fig. 3A). This feature can also be observed in males (Fig. 3B). Although in summer, gonadosomatic indices of females more scattered in compare with males.

Table IV shows that the mean of GMC has no significant differences between populations. In females, IQR for GMC were lower than the males. Since GMC is the ratio of the difference between wet and dry weight to wet weight, R as a difference between maximum and minimum of GMC shows lower values in males in compare with females which shows that less moisture of

gonads of males in compare with females.

Table II.- Binomial test analysis for the sex ratio of on *E. mathaei*.

	No.	Observed prop.	Test prop.	Asymp. Sig. (2-sided)
Females	126	64.14	0.5	0.157
Males	71	30.86		
Total	197	100		

Table III.- The calculated GSI from March to September, 2014.

		GSI 1	GSI 2	GSI 3
Season	Spring (March-May)	11 ± 6 ^a	5.5 ± 3 ^b	13 ± 7 ^a
	Summer (June-September)	3.6 ± 3 ^b	2.0 ± 2 ^b	3.8 ± 3 ^b
Gender	Female (126 samples)	7.7 ± 5 ^a	4.0 ± 3 ^a	10 ± 8 ^b
	Male (71 samples)	8.6 ± 6 ^a	4.3 ± 4 ^b	8.7 ± 6 ^a
Total		8.2±0.6 ^a	4.1 ± 0.3 ^b	9.4 ± 0.9 ^a

The different superscript alphabets above numbers in each rows show significant differences between rows ($p<0.05$).

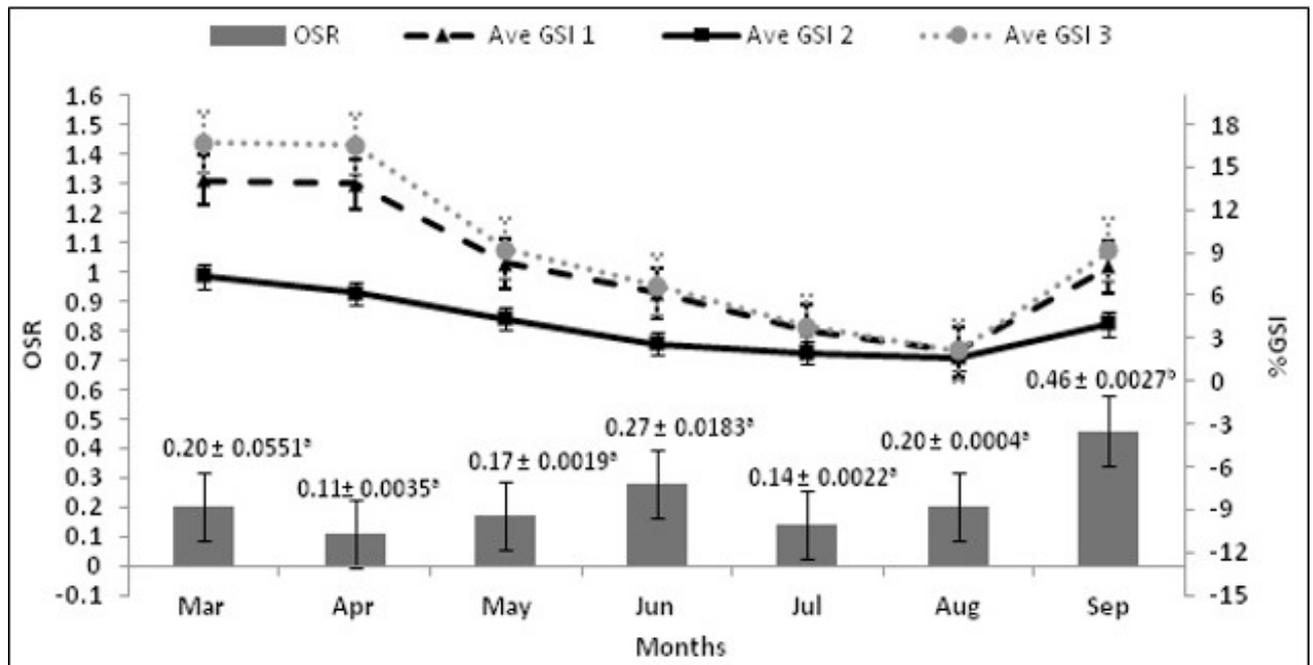


Fig. 2. OSR (Mean ± Standard Deviation) and the gonadosomatic indices of *E.mathaei* from March to September, 2014. The different alphabet above numbers as a superscript show significant differences between months on samples ($p<0.05$).

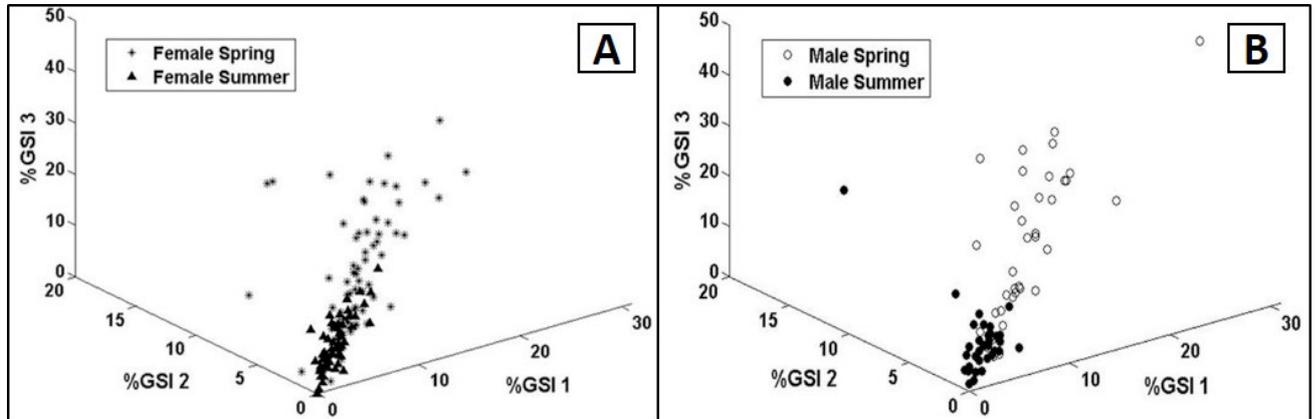


Fig. 3. The three %GSIs shown on one a 3D plot for females (A) and males (B) in two seasons of spring (March- May) and summer (June-September).

Table IV.- The gonad moisture contents index (GMC) for the two genders of *E. mathaei*.

Parameters	No.	Mean \pm SD	Q1	Q3	IQR	R
Male	71	74 \pm 10	70	81	11	57
Female	126	75 \pm 11	72	80	7.6	91
Total	217	68 \pm 24	70	80	10	99

DISCUSSION

Sea urchins' gonads have a positive potential for presentation of a valuable sea food and therefore the culture of sea urchin can be very important and lead to job creation and income for local people. In the discussion of sea urchin growing, gonad weights compared to the total weight of the body is of great importance. In Bandar Lengeh, according to Figure 1A, in the total weights less than 12 g, the gender was mostly unknown. Higher than 12 g, the gender was known. The highest frequency of the total weights referred to 40-44 g (both female and males). It was seen that the frequency of females was more than males in the total weights more than 70 g (70-92 g). It means that the females were heavier than males in studied samples. Figure 1B also shows the gonad weight was less than 16 g. Mostly, the frequency of the gonad wet weight is less than 10 g. Table I shows obviously that the mean wet weights of gonads was nearly 8.25% of the mean total body weight. The most frequent wet weights of gonads (female frequencies: 80, male frequencies: 60, unknown sex frequencies: 20) were observed in weights less than 4 g. A sharp drop of frequency was observed in gonad wet weights of 5-12 g. Only females were observed in gonad wet weights of 12-16 g. By looking carefully at Figure 1,

it can be understand that total wet weight distribution is wider than gonad weight distribution. Briefly, the gonad wet weight distribution is not normal.

The GSIs as indicators are particularly helpful in identifying days and seasons of spawning, if some reasonable numbers of adults' gonads collected each month over a year. In our study, the spawning period was determined March to August (Fig. 3). In Kenyan coral reef lagoons, effects of seasons on the reproduction of the Indo-Pacific echinoid *Echinometra mathaei* were studied by Muthiga and Jaccarini (2005) in 3 stations. In Kanamai station as an example, which is equivalent to GSI1 (equation 3) of the current study, a roughly U-shaped continuous trend with a minimum value of GSI can easily be seen in mid-summer (July-August) during different years (1986-1987, 1992-1994). The same rhythm is also exists in our studied months (Fig. 3, the dash line) by August as its concave curve. Although our studied months (March-September) do not cover the whole year but a slight increase can be seen in September (Fig. 3). This carefully proves that our studied months corresponded with the study of Muthiga and Jaccarini (2005). Moreover, on study of *Paracentrotus lividus*, the gradual maturation of gonads' peak was seen in April. In *P. lividus* (Tomšić *et al.*, 2010), the range of GSI1 was 1.33 (September) \leq GSI1 \leq 4.83 (April), while we have found it between 2.05 (August) and 14.09 (March). In another study of *P. lividus* (Murillo-Navarro and Jimenez-Guirado, 2012), the monthly variation of GSI1 peak value was produced in February and the lowest values were observed from October to January. In the study of Fabbrocini and D'Adamo (2010a), it was found more than 90% of GSI1 in *P. lividus* occurred on April without any decrease

Table V.- *Echinometra mathaei* spawning season.

Location	Spawning months	References
Bandar Lengeh, Iran	March to August	Current study
Zanzibar Island, Tanzania	June to September	Bronstein and Loya (2014)
S.E. Honshu, Japan	January to September	Kobayashi (1969)
Minatogawa, Japan	May to December	Fujisawa and Shigei (1990)
Sakurajima (Kagoshima Bay), Japan	June to September	Fujisawa and Shigei (1990)
Shirahama (Kii Pen), Japan	July to August	Fujisawa and Shigei (1990)
Seto, Japan	July to August	Onoda (1936)
Sesoko Island, Japan	September to October	Arakaki and Uehara (1991)
Hawaii	January to December	Kelso (1971)
Gulf of Suez	July to September	Pearse (1969)
NW Red Sea	January to December	Pearse (1969)
Diani, Kanamai and Vipingo, Kenya	January to May & November to December	Muthiga (1996), Muthiga and Jaccarini (2005)
Eastern coast South Africa	January to March	Drummond (1995)
Rottneest Island	January to December	Pearse and Phillips (1968)

until summer-time (50%). In the study of Bronstein and Loya (2014) on the coral reefs of Zanzibar, of *E. mathaei* reached an annual peak during August–September and was followed by a drop in the GSI value in October, indicating a single annual spawning event. GSI values reached their annual minimum from March through May, after which GSI build up was observed (Bronstein and Loya, 2014). Table V represents a very good comparison of spawning months of our study with other studies of *E. mathaei* in different locations. Again, it is valuable to remind that different factors effect spawning months, and in different locations through a year, their months' variations are reasonable.

The factors that influence the timing of the reproductive cycle of *Echinometra*, however, are not well understood (Muthiga, 1996; Muthiga and Jaccarini, 2005). Pearse (1974) suggested that *E. mathaei* might have a restricted spawning period in the higher latitudes and continuous spawning throughout the year closer to the equator where the environmental factors, especially temperature, are presumed more stable. It was found that different complicated factors effect spawning time of *Echinometra*, which were mainly environmental parameters as temperature, salinity, light, availability of food, the population density, wave-swept areas or even pH in different studies. The seasonal reproductive pattern of *E. mathaei* on the Kenyan coast is closely correlated to seawater temperatures, which are influenced by the

monsoons (McClanahan, 1988). Temperature variations may be the key factor in *E. mathaei* rather than an absolute temperature minimum or threshold for spawning (See Byrne, 1990; Siikavuopio *et al.*, 2006, 2008) for further information). Carballeira *et al.* (2011) tested embryolarval development (ELD) of sea urchin and its fertilization by a bioassays attempt to find out an optimum range of salinity (15-40.5 psu) with two species of Atlantic sea urchin: *Arbacia lixula* and *Paracentrotus lividus*. It was discovered wider salinity range for *A. lixula* (29-35.5 psu) than for *P. lividus* (29-33psu). Lessios (1981) postulated that salinity could act as a proximate cue for controlling the timing of spawning as it coincided with increased salinity for *E. lucunter* and *E. viridis* at Fort Randolph, Caribbean. Light is another factor which effects spawning time (*e.g.* Iliffe and Pearse, 1982). Temporal and spatial variability of reproductive conditions of sea urchins is closely related to trophic conditions (Scheibling and Hatcher, 2007). Fabbrocini and D'Adamo (2010b) have tested the effects of food on gametogenesis of *Paracentrotus lividus* and found that starvation significantly affected gametogenesis, whereas developing gametes were always observed in fed animals, whose GSI had doubled by the end of the four-week trial. For example, growth and gonad production were lower in beds dominated by *D. divaricata* and *Laurencia* spp. than in beds of small algae without defense chemicals (Agatsuma *et al.*, 2005b). Moreover, the fertilization ability of gametes from starved urchins was significantly

lower (Fabbrocini and D'Adamo, 2010b). Even more, availability of food for the larvae may be also an important factor in controlling spawning. Spawning occurs just prior to the peak of phytoplankton concentrations (Lessios, 1981) (See also Azad *et al.*, 2011; Bayed *et al.*, 2005). Lessios (1981) showed that spawning synchrony is tighter in sparsely populated populations of *E. viridis* and less important in densely populated populations of *E. lucunter* (See also Muthiga and Jaccarini, 2005). Spawning also occurs in *E. lucunter* during a discrete period from July to October in wave-swept areas, and occurred during several periods in populations living in calm waters (Lewis and Storey, 1984). pH, (e.g. Moulin *et al.*, 2011), the farm effluents, organic pollution (Cook and Kelly, 2007) and contamination of heavy metals (e.g. Bayed *et al.*, 2005) can also effect spawning time.

As it is evident, the cause of spawning in any region is faced with extremely complex parameters. Therefore, the comparison of results with other previous observations (the same as Table V) is not completely right and the details of how spawning occurs need to be checked. We have separated GSI values due to gender and seasons. Table III represents our calculated data of GSIs. The GSIs showed the higher values in spring, when Acinal wall of gonads were filled of both nutritive phagocytes and mature gametes. In summer, there is no significant difference between the indices spatially GSI1 (equation 3) and GSI2 (equation 4), then the most stores of gonads is in spring. Figure 3 is also confirmed this claim that after April, the amount of storage gonads is reduced sharply so that in August (mid-summer) it reaches its minimum value. This may refer to the environmental condition of this area, that spawning season starts at the begging of spring and ends in middle of summer. The indices GSI1 and GSI2 did not differ significantly in females by gender separation, while in males, they were different (Table III) and GSIs scatter graphs of samples (Fig. 3) also showed the obvious difference of males in spring and summer. We have followed the sexual relationships of GSI by OSR. Many species encounter spatial variation of OSR (Rohr *et al.*, 2005). In a male-biased situation, it is often correct to follow the traditional approach of this assumption that finding mates for females are easy (Kokko *et al.*, 2012) and females are selective (Balshine-Earn, 1996). A male-biased OSR can be affected by three major parameters: a) the adult sex ratio (Kvarnemo *et al.*, 1995); b) the sex-specific time that individuals spend outside the mating pool and c) environmental factors (Kokko *et al.*, 2012).

Sex ratio is important to the notion of echobiological and genetic balance for species in land and marine ecosystems. It provides information about the

representations of sea urchin males and females (as the proportion of male to female, equation 2), and indicates the gender dominance species in a given population. The adult sex ratio is such as sex-biased mortality (Moore and Wilson, 2002), the primary sex ratio, and differential reproductive investment between the two sexes (Bateman, 1984). In this study, the sex ratio of *E. mathaei*, showed no significant differences in individual males and females by the Chi-square test ($p=0.157$ ($P>0.05$)). It means that there is no preference between males and females as the sex ratio and then no effects on OSR. The same result was found in other studies. No significant differences were found by Bronstein and Loya (2014), from a ratio of 1:1 for *Echinometra* sp. in Zanzibar. Zhao *et al.* (2010) also found no significant differences by the Chi-square test for *Strongylocentrotus intermedius* in China.

In the study of *E. mathaei*, we have found that the adult sex ratios, ASR, had significant differences between months (by ANOVA-test, $P<0.05$) but OSR had no significant differences between months (by ANOVA-test, $P>0.05$). Gianguzza *et al.* (2009) showed that the OSR had no significant differences ($P>0.05$ and $P>0.001$) for *Paracentrotus lividus*, the same as our study.

Therefore, OSR can be affected by two other parameters. The sex-specific time that individuals spend outside the mating pool, are like providing parental care (Kokko and Jennions, 2008) which is not included for a sea urchin, replenishing gamete supplies, regaining body condition (Clutton-Brock and Parker, 1992) or different mating behavior between sexes (Xiao and Kumar, 2004). Environmental factors are such as food supplies (Gwynne and Simmons, 1990) and hunger (Rowe *et al.*, 1994), nesting sites, predators (Gianguzza *et al.*, 2009), density of population (DeRivera, 2003; Gianguzza *et al.*, 2007) or even temperature (which effects on breeding cycle; see Kvarnemo (1994) as described previously. As an example, the females of sea urchin *Paracentrotus lividus* were more vulnerable to the starfish *Marthasteria glacialis* predation at high densities of this starfish during the summer spawning period and therefore OSR is female-biased (Gianguzza *et al.*, 2009). Or another example, if males are required to provide a nutrient high gift before mating (most likely food) then when the available nutrients are high, the OSR will be male biased because there are plenty of nutrients available to provide gifts. However, if nutrients is low, less males will be ready to reproduce, causing the population to have a female biased OSR. (See Gwynne and Simmons, 1990; Kvarnemo and Simmons, 1999). In this study of *E. mathaei*, since the standard definition of OSR is adult males to total adults (equation 1), it is assumed that OSR should be male-biased, but surprisingly

it was found that OSR in this species is female-biased (male: 71; female: 126). It means that adult females are roughly 1.77 times in compare with adult males. In another study on *Paracentrotus lividus*, it was suggested that OSR depends on density, so that, at higher densities, there was a more equal sex ratio whereas at low density sites males of *P.lividus* were significantly more abundant (Gianguzza *et al.*, 2007) and therefore OSR is male-biased. Another male-biased OSR for *Strongylocentrotus franciscanus* was also found at intermediate densities by the study of Levitan (2004). Levitan (2004) mentioned that at low and high densities, OSR is roughly 50% of total for both sexes. It means that the results of our study for *E. mathaei*, in Bandar Lengeh is completely opposite with those two studies of *P.lividus* and *S. franciscanus*. But on the other hand, by regarding to ANOVA-test, we had the same results as Gianguzza *et al.* (2009). Overall, due to no effects of sex ratio on OSR, more studies are required to check the effects of time spending outside mating pool and spatially environmental factors on OSR to understand why *E. mathaei* is female-biased in Bandar Lengeh, Iran.

CONCLUSIONS

In this study, we have collected random size of *E. mathaei* once per month (March – September, 2014), Bandar Lengeh, Iran. Their gonads were tested by two methods of operational sex ratio (OSR) and gonadosomatic indices. We have discovered out *E. mathaei* is female-biased and consequently finding mates for males is convenient. It was also found out March to August, 2014, as spawning months of this species. Since sex ratio has no effects on OSR, therefore, the other factors can affect OSR: 1) the time that individuals spend outside the mating pool; 2) regaining body condition; 3) different mating behavior between sexes; 4) environmental factors, which all are needed continuous studies during years on *E. mathaei* natural conditions of Bandar Lengeh; and finally, 5) different replenishing gamete supplies of females and males that can be more highlighted because of a female-biased OSR in *E. mathaei*.

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Conflict of interest statement

We declare that we have no conflict of interest.

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