



RESEARCH ARTICLE

Artificial Reproduction of Octocoral (*Plumarella flabellata*)

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ARTICLE HISTORY

Received: November 18, 2011

Revised: December 08, 2011

Accepted: January 15, 2012

Key words:

Gonad

Histology

Persian Gulf

Plumarella flabellata

Sexual reproduction

ABSTRACT

The reproductive biology of Octocoral (*Plumarella flabellata*) in artificial habitats located in Northwest of Persian Gulf, Iran was studied by histological examination of gonad development. Various sizes of colonies were collected to determine the relationship between colony size and sexual maturity. The colonies smaller than 15 cm height did not contain gonads. Moreover, *P. flabellata* was a hermaphrodite species with gonads developed along with mesenteries. No planulae were observed in the samples investigated. Therefore, *P. flabellata* in Iran is most likely a broadcast spawner. The gonads were at the same developmental stages in each season. The results showed that spawning of *P. flabellata* probably occurred in July-August which is consistent with the suitable environmental conditions such as higher water temperature and long day-light. The present study indicated that *P. flabellata* exhibits a remarkably high reproductive potential, which contributes to its dominance in the artificial reef in Northwest of Persian Gulf.

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To Cite This Article: Heidari F and N Salamat, 2012. Artificial reproduction of octocoral (*Plumarella flabellata*). Pak Vet J, 32(3): 403-407.

INTRODUCTION

Understanding of the organism's reproductive biology is important to know about maintenance and regeneration of populations. Information about sexuality, reproductive style, sex ratio and seasonality of reproduction in species of concern are critical to establish better policies for the management, preservation and retrieval approaches of besmirched regions.

The octocorals represents a morphologically and geographically diverse group of cnidarians and its reproductive biology is poorly known in most species. Persian Gulf in particular along with many other regions with plentiful soft corals, has received little consideration.

Investigation on the life history of Octocorallia reveals that a variety of reproductive models are adopted by different species (Ben-Yosef and Benayahu, 1999). There are two basic types of sexual reproduction in octocorals including brooding and broadcast spawning. Coral species with brooding reproduction typically display extended or continual cycles of breeding (Benayahu, 1991). On the contrary, broadcast spawning soft corals have short, seasonal and synchronized spawning periods (Shlesinger *et al.*, 1998).

The effect of selective pressures on the reproductive tactics of octocorals is still unclear; however, it seems that

each of brooding and broadcast spawning may bestow specific reproductive benefits particularly in different ecosystems. Many octocorals with broadcast spawning contribute in synchronized crowd spawning events, a policy which may cede ecological benefits in reef environments by reducing predation pressure on newly-spawned gametes (Ben-Yosef and Benayahu, 1999). Such extremely harmonized spawning events are crucial to reproductive success in broadcast spawners, as they increase fertilization rates (Lasker *et al.*, 1996). Among brooder spawning, synchronicity possibly is not critical because eggs are preserved either inside the polyps or on the surface of colony until fertilization (Coma *et al.*, 1995). Moreover, in octocorals, large oocytes are often associated with species which have non-feeding larvae (Edwards and Moore, 2009; Sun *et al.*, 2010; Excoffon *et al.*, 2011) and most planulae observed appear to be lecithotrophic (Hwang and Song, 2007; 2012).

The *P. flabellata* (Versluys, 1906) (family Primnoidae, phylum cnidarian), whip like soft coral, is the most abundant inhabitant of artificial underwater structures in Persian Gulf. The present investigation was conducted to study the sexual reproduction in *P. flabellata* including reproduction mode, sexual maturity and oocyte and spermatozoa size along with the reproduction period.

MATERIALS AND METHODS

Study site: This study was conducted on 6 year artificial habitat in the Northwest Persian Gulf, Iran (29° 54' N; 49° 17' E), where a rich population of *P. flabellata* exists (Fig. 1). A relatively large number of colony samples were collected seasonally by SCUBA diving at a depth between 12-20 m from June 2009 to March 2010.

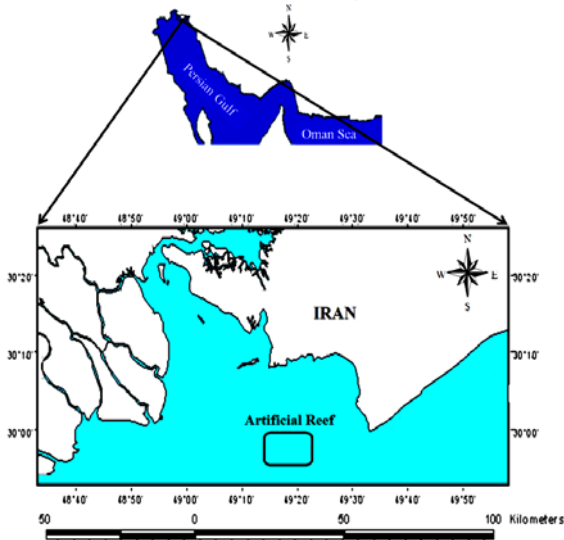


Fig. 1: Map of study site on the artificial habitat of Northwest Persian Gulf, Iran.

Sexual reproduction: All samples were fixed in 10% formalin for a week and preserved in 75% ethyl alcohol afterwards. To determine the relationship between colony size and sexual maturity, colonies of various sizes were randomly selected for histological analysis. After standard dehydration by graded alcohols series, samples were embedded in paraffin wax (melting point 57 to 60°C). It was ascertained that thinner sections were more adequate for obtaining better histological results; therefore serial 5 µm sections were prepared for gonad examination. The sections then were stained with hematoxyllin and eosin and examined under light microscope Dino Lit lens with Dino Capture software. Colonies were considered sexually mature if oocytes or sperm sacs were visible under the microscope. The variation in the gamete size was plotted with temperature to evaluate if the spawning time was related with the temperature increase in early summer. The diameters of the oocytes and sperm sacs were also measured to determine the size frequencies of gonads. The percentages of polyps containing gonads were also plotted against time. The samples were studied for planula or embryo within the colonies to determine mode of reproduction.

Data analysis: The statistical analysis was made using SPSS 15. Normality of the data was tested with Kolmogorov-Smirnov test prior to analysis. Differences in gamet's size between the seasons were tested by ANOVA. In addition, regression value presenting the relationship between the gamet's size and temperature are presented in Fig. 2A, B.

RESULTS

Mode of reproduction: The soft coral *P. flabellata* was observed to be a hermaphroditic, since both female and male gonads were observed in the same sample (Fig. 3A). Female and male gonads developed along the mesenteries within the polyp cavities (Fig. 3B). A layer of mesoglea surrounded the sexual cells and stained strongly blue. The mesoglea thickened in the oocytes as they developed. The absence of embryos or planula stages inside the gastric cavities of all examined specimens suggested a broadcast spawning mode of reproduction in *P. flabellata* of Persian Gulf (Iranian waters).

Fecundity: Large number of oocytes and sperm sacs was observed in mature samples during sampling periods. The number of sperm sacs was more than number of oocytes per polyp (Fig. 4A). The number of gametes differed in different colonies depending on the size of colony. The number of gonads per polyp was determined by its polyp cavity size. More than 20 gametes were presented in each slide of mature polyp.

Size at the time of sexual maturation: Study of 104 colony samples from different groups with various size (<15 cm, 15-20 cm, >20 cm height) indicated that all specimens smaller than 15 cm are sexually immature (Fig. 5). The length of the smallest polyp with gonad was at least 16 cm. Most of the large colonies (>20 cm) were mature and just a small percentage of them didn't have gonads.

Spawning: Measurement of the oocytes and the sperm sacs size showed a noticeable synchronization in the reproductive state of different polyps within a colony (Fig. 4B). The early gametes appeared in September 2009 and continued to grow up to March 2010 (Table 1). Mature oocytes and sperms reached the maximum size of 225.39 ± 23.26 and 231.74 ± 33.08 µm in diameter, respectively in June (late spring). There was a significant difference in the gonads diameter between June and other months (ANOVA, $P < 0.05$, Table 1). No significant differences were found in the diameter of gonads of *P. flabellata* among the other months.

Table 1: Mean (±SD) of gonads diameter in *P. flabellata*

	Oocyte size(µm)	Sperm sac size(µm)
June	225.39 ± 23.26^a	231.74 ± 33.08^a
September	97.25 ± 21.35^b	102.29 ± 19.87^b
December	112.28 ± 17.87^b	118.08 ± 11.32^b
March	141.95 ± 13.96^b	149.81 ± 26.59^b

The seasons with the same letter did not differ significantly at $P < 0.05$.

Fig. 5 shows the seasonal changes in the mean diameters of sperm sacs and oocytes of *P. flabellata*. Moreover, corresponding water temperature, plotted in each sampling date, illustrates the relationship between temperature and gonadal development. This indicated that spawning of *Plumarella* spp. probably occurred in early summer (Jul-Aug), when the water possibly had the highest temperature in the year. The regression value ($P < 0.01$) indicate that the diameter of oocytes and sperm sacs has a direct relation with temperature ($R^2 = 0.686$ and $R^2 = 0.695$ for oocytes and sperm sacs, respectively).

DISCUSSION

The present study provided important information about approximate spawning time of the soft coral, *P. flabellate*. Spawning of *P. flabellate* from Iranian waters probably occurs in July-August. Long day-light and high temperature of sea water in summer may possibly act as inducing factors for *P. flabellate* to spawn. Coral reproduction studies have disclosed that corals may respond to several cues to spawn such as tidal or lunar

cycles, photoperiod, temperature, etc. (Penland *et al.*, 2004). Previous studies on the time of other species spawning in other regions showed that the spawning mostly occurred during summer (Linares *et al.*, 2008). Higher temperature of water in summer may be more favorable for raising the survivorship of the recruiting new coral larvae. Hence it is possible that most soft and hard corals have adapted to spawn in summer to stay away from the lower water temperature which may be unfavorable for coral growth and the early development of their recruits.

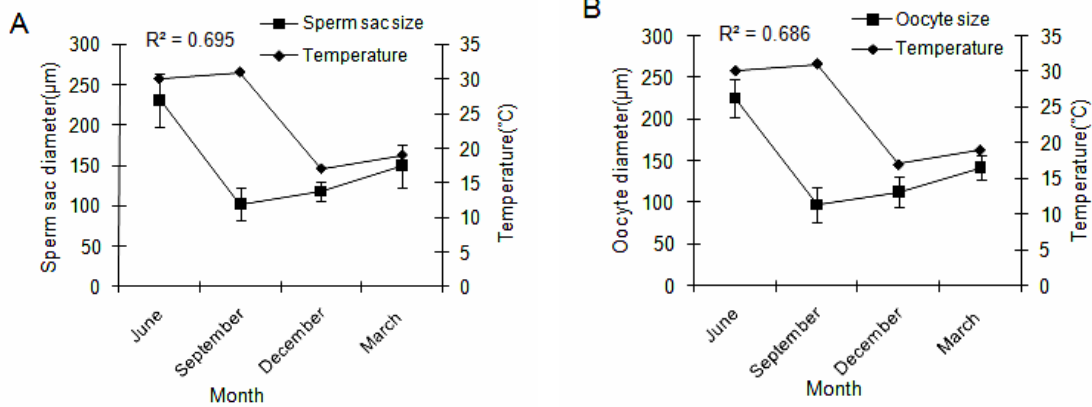


Fig. 2: Changes in (A) sperm sacs and (B) oocytes size (µm in diameter) in *P. flabellate* during the sampling period. The corresponding seawater temperature at each sampling date is also presented (Spawning of *P. flabellate* likely occurred in early summer) (mean±SD)

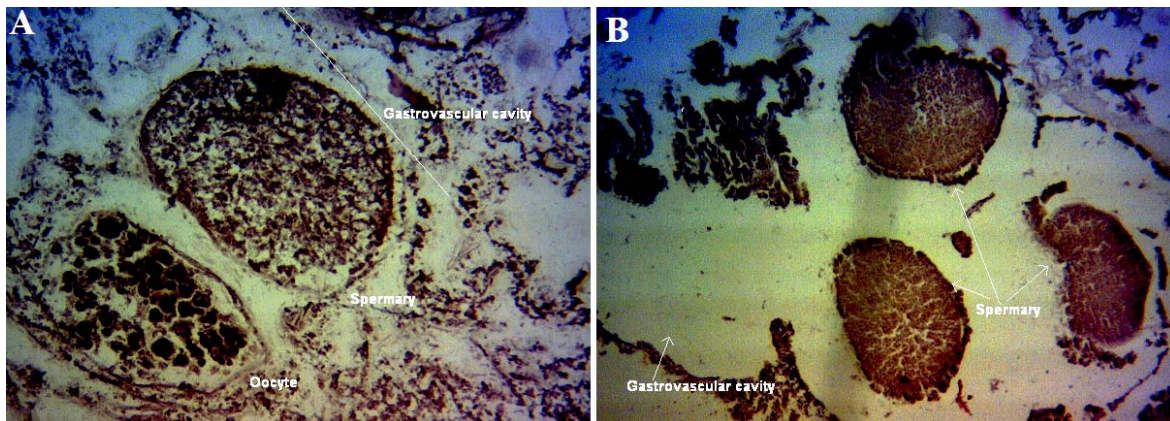


Fig. 3: Cross sections of gonads of *P. flabellate* from Persian Gulf. (A) Spermary and oocyte are found in a single polyp (H&E; ×10) (B) Gonads developed within the polyp cavities (H&E; ×4).

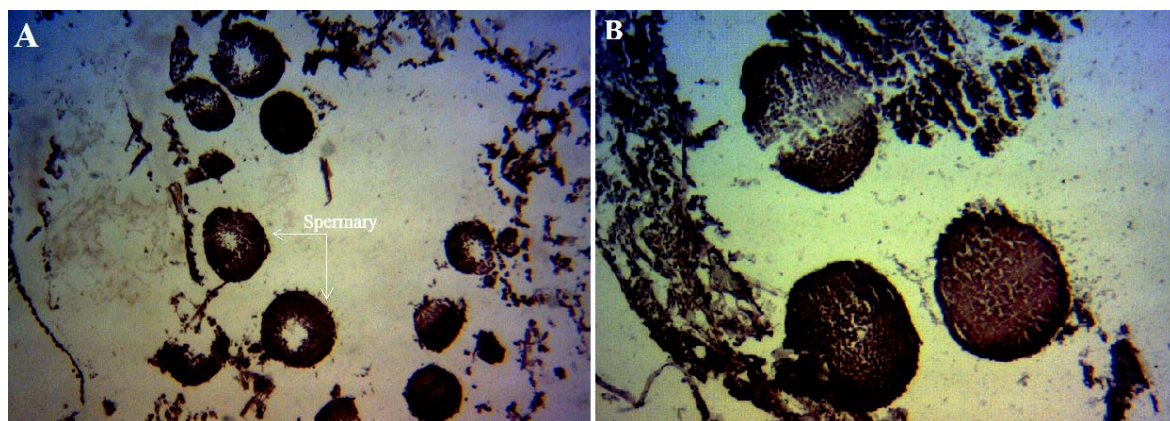


Fig. 4: (A) High number of sperm sacs per polyp (H&E; ×4) (B) Sperm sacs in the same stage of development stage was found in the same polyp (H&E; ×10)

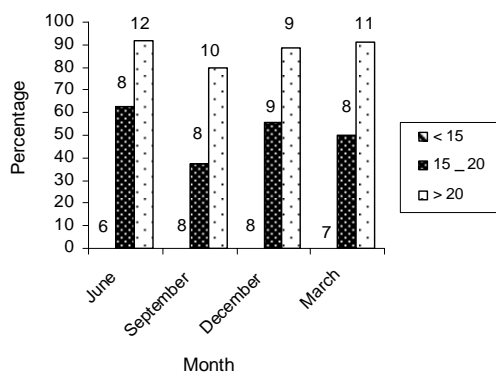


Fig. 5: Percentage of *P. flabellata* colonies containing mature gonads in 2 size groups. (All colonies smaller than 15 cm contain no gonad).

Octocorals vary in age and size at first reproduction (Coma *et al.*, 1995; Ribes *et al.*, 2007). Within a species, the size of colony is a good indicator of reproductive maturity (Gutierrez-Rodriguez and Lasker, 2004). The present study indicated that *P. flabellata* starts to reproduce only after gaining a considerably large size colony (more than 15 cm height). It shows that young colonies of *P. flabellata* utilize energy for growth rather than for reproduction. This characteristic enables the coral to initially arrest spaces on the reef, and then reproduce at older age.

Size-dependant reproduction is approximately general in colonial marine invertebrates and may signify a strategy whereby resources are allowed to grow at the peak of reproduction, until a colony reaches a minimal size necessary for beginning of reproduction. By achieving a “threshold” size as quickly as possible, octocorals may diminish the elevated risk of mortality associated with small size of colony (Kapela and Lasker, 1999; Gutierrez-Rodriguez and Lasker, 2004).

The result of the present study revealed that *P. flabellata* in Persian Gulf are hermaphroditic species and both male and female gonads develop along the mesenteries. Moreover, there was no obvious evidence for changes in sexuality of colony. Complete reversal in colony sexuality of Octocorals (between males and female and vice versa) is unknown till now, and the same is true for scleractinian corals (Richmond and Hunter, 1990). It should be noted that sexuality can differ in some species, for example *Heteroxenia elizabethae* is hermaphroditic in Red Sea, but gonochoric in Australia (Benayahu, 1991).

The size of oocytes and sperm sacs appears to vary extensively within individual species at different sites even in polyps with similar size (Gutierrez-Rodriguez and Lasker, 2004). It is still unclear, how much effective factors influence the size of the eggs in many studied species, and this makes it difficult to determine possible ecological significance of oocyte size variation among octocorals.

It has been confirmed that *P. flabellata* produces many small oocytes and sperm sacs per polyp. We suggested that production of so small gametes together with synchronous maturation and egg maturation in short time are characteristics of such species.

In trying to understand brooding and broadcast spawning in corals, several studies have confirmed that reproductive mode and fecundity are related to oocyte

diameter and polyp size (Shlesinger *et al.*, 1998). Similar findings were reported by Pires *et al.* (2009) which suggested that broadcasting corals have a tendency to have smaller eggs and higher fecundity than those which exhibit parental care (brooding) and release large larvae. Generally, brooding species produce few large eggs, which let them to provide supplementary nourishment for each planula. Thus, different reproductive styles may occur in response to energetic restrictions or environmental pressures (Shlesinger *et al.*, 1998).

The absence of developing embryos or brooded larvae in *P. flabellata* polyps suggested that this specie like many tropical octocorals is most likely broadcasting. In these species, quicker embryogenesis raises spat survival and fortunes for successful colony development (Ben-Yosef and Benayahu, 1999). This strategy may play an important role to successful colonization of *P. flabellata* on artificial substrata.

Many studies associated with general descriptions of octocorals reproductive cycles demonstrated that two styles are frequently displayed by many species; tropical, broadcasting spawning octocorals exhibit synchronous maturation and gametes spawning (Permata *et al.*, 2000; Cervino *et al.*, 2003; Fitzsimmons-Sosa *et al.*, 2004); while temperate brooding species have asynchronous gamete development and a prolonged spawning period (Coma *et al.*, 1995; Cordes *et al.*, 2001). As it seems, reproductive cycles are affected by both reproductive strategy as well as environment. In this regard, *P. flabellate*, similar to most broadcast spawning soft corals, have been found to have short and synchronized spawning episodes (Brown and Bythell, 2005).

The fecundity of a coral species can be influenced by several factors. The number of oocytes per polyp may differ in accordance with the thickness of the coenanchyme and the length of the polyps (Benayahu, 1991). In broadcasting species, polyp diameter is significantly associated with the number of oocytes produced per polyp (Shlesinger *et al.*, 1998). We also suggest that the number of gametes per polyp was determined by the size of polyp cavity and the large quantity of gametes per polyps may be caused by their rather rapid development and maturation. The same findings were reported by Dahan and Benayahu (1997).

In conclusion, the frequency of *P. flabellata* on artificial substrates in Persian Gulf may be explained partially by the accessibility of the necessary conditions for successful recruitment. The results of this study also suggested that *P. flabellata* is an opportunistic specie with a notable reproductive potential, causing its dominance in this region. Their short gametogenesis phase and rapid growth support them to be able to colonize quickly in available space and to settle successfully on the reef surface. The present study can therefore contribute to design artificial reefs aimed in attracting rapid colonization by this soft coral.

Acknowledgments: We express our special thanks to R. Waller at the University of Hawaii for her advices during the study. We thank South Iran Aquaculture Research Center for their assistance with specimen collection and acknowledge Khorramshahr Marine Science and Technology University for funding this research.

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