Indian Journal of Geo Marine Sciences
Vol.46 (04), April 2017, pp. 822-829

Reproductive biology of the jellyfish (*Chrysaora* sp.) in the north-western coastal waters of Malaysia (Penang Island)

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Received 05 June 2014 ; revised 11 November 2016

Based on histological, microscopic and macroscopic observations, the highest gonadosomatic index (GSI) value for both sexes of *Chrysaora* sp. was found to be in July. Female GSI value of *Chrysaora* sp. was higher than male. Furthermore, this species showed an asynchronous pattern of reproduction. Spawning and the peak of the reproductive seasons were in July. Maturation in *Chrysaora* consisted of four stages (pre vitellogenic, early vitellogenic, mid vitellogenic and late vitellogenic) in female and three stages (spermatogonia, spermatocytes, and spermatids) in male.

[**Key words:***Chrysaora* sp., gametogenesis, sex ratio, spawning, maturation]

Introduction

Jellyfish is known to have ecological and socioeconomic effects worldwide and is well recognized as a predator species in marine ecosystems [25,26]. Its predation effects have led to changes in species composition and community structure of zooplankton [9] Jellyfish distribution is periodic and apparently unpredictable in nature. Metrological condition, currents, water temperature, pressure, salinity and predation may play a significant role in determining the population size of jellyfish [13]. One of the major jellyfish group along the coastal waters of Penang Island is *Chrysaora* spp. which are known due to their negative impacts on the tourism and aquaculture sectors [30]. However, based on their medical properties this species can also be of economic value. Lal *et al.* [15] explained that useful collagens can be extracted from the nematocyst venoms of *C.quinquecirrha*. Current species are more abundant along the coastal waters of Penang Island based on Sim [30] studies. This may indicate some compelling reasons for the high occurrence. To clarify the reasons for these observations, a comprehensive understanding of the reproductive biology of this species is very important. Several researches have been conducted on the biology and life cycle of different *Chrysaora* spp. but investigations on how the reproductive biology is affected by environmental conditions are limited. For example the biology of *C. quinquecirrha* in Chesapeake Bay has been studied by Cargo and Schultz [3]In another study, the life cycle of *C. fuscescens* from gametes to juvenile stages in the Northeast Pacific Ocean was described by Widmer [32] Condon [5] investigated the effect of low dissolved oxygen on asexual reproduction in *C.quinquecirrha*. However, generally studies on jellyfish reproduction is very limited and more investigation is crucial to clarify its life cycle.

There have been a few documentations on the reproductive biology of jellyfish in other regions of the world. The general aspects of Cnidaria reproduction were described by Campbell [2] and Fautin [10] Eckelbarger and Larson [8] studied the structure of the gonads and oogenesis in the jellyfish. Gametogenesis in *Naustithoe aurea* was described by Morandini and Da Silveira [19].In another study, the reproduction of *N. nomuria* was investigated by Iguchi *et al.* [14] .Corbelli *et al.* [6]

explained the spermatozoon biology and structure in *Carybdea marsupialis* while Rouse [28] studied sperm terminology and inferred reproductive mechanisms in *P. punctata* and *Catostylus mosaicus* in Botany Bay in Australia.

The life history and reproduction of *Aurelia aurita* in relation to ambient environment was studied by Lucas [16] Pitt and Kingsford [22] also investigated the reproductive biology of *Catostylus mosaicus* in Australia. The gonad of *Lychnorhiza lecerna* in the coastline of South America was reported to be gonochoronic while Schiariti [29] reported that it was an asynchronous species. And observed fully developed gametes in the gonad as well as spent follicles that indicated occurrence of primary spawning and recent spawning events. Asexual reproductive mechanisms of *A. aurita* were studied by Vagelli [31]. Growth, reproduction and nutrition of several medusas in natural conditions have been detailed by Zelickman. [33]

Ma [18] investigated the effect of environmental factors on distribution and asexual reproduction of *Moerisia lyosi* and their results showed highest asexual reproduction rate accrued at salinities of 5-20 ‰ at a temperature of 20-29 °C. Furthermore, *C.quinquecirrha* was found to prey heavily on this species and may be the cause of its restricted distribution. Little attention has been paid to the reproductive biology of *Chrysaora* sp. Therefore, this study will provide a comprehensive data on several aspects of reproduction such as gonad maturation cycles, GSI and spawning of this species in the coastal waters of Penang Island in relation to the management of this species which is fast becoming a nuisance and sometimes deadly feature in global waters currently.

Materials and Methods

Monthly sampling was carried out for the *Chrysaora* sp. along the coasts of Penang Island specifically at the Centre for Marine and Coastal Studies (CEMACS) in Teluk Aling (5°24′00″N100°14′20″E). (Fig.1). The sampling was performed during the day time, while the sea was calm and jellyfish numbers were high near the seashore at high tide when they come to feed. Two methods were used for sampling namely the push net (scoop nets) for the specimens from the surface and towing methods in deeper waters. Thirty individuals of *Chrysaora* sp. were collected within a period of 12 months beginning from November 2011 to October 2012. All collected individuals were transported to the Biological Laboratory of Universiti Sains Malaysia for analysis.

Figure 1. Sampling site of jellyfish collected along the coast of Penang Island (modified from Google Earth 5.0.11733.9347).

At the laboratory, each individual was placed on a flat surface and total length (TL) total length of umbrella, the nearest 0.5 cm and total weight (TW) to the nearest 1 g were recorded before the specimen was dissected. Jellyfish were dissected individually. The whole gonad including the gonad basis and germinal tubules was removed from each animal, weighed (GW) to the nearest 0.1 g and fixed in 6% buffered formaldehyde in sea waters. The pattern of reproduction was investigated using the gonadasomatic index (GSI) for males and females were calculated separately, using the following equation:

$$GSI = \frac{GW\ (g)}{(BW(g) - GW(g))} \times 100$$

GW= Gonad Weight

BW= Body Weight is total weight (g) with entire gonad

The sex and stages of gonad development were determined by histological examination.

Histological preparation for gonad maturation of jellyfish was carried out at the Anatomy Laboratory, School of Biological Sciences University Sains Malaysia. Males and females of *Chrysaora* sp. were classified to three and four stages of sexual development following the criteria defined by Pitt and Kingsford[22]and Iguchi [14] For histological preparation of tissues, gonads were transferred to alcoholic Bouin's fixative for 4 weeks after which dehydration process was performed through a series of alcohol solutions at 30%, 50%, and 70% allowing 2 h between each change. Samples were then preserved in 70% alcohol. From the tubules of each individual, five to six cross sections of 7 μm thickness were taken to prepare slides by staining with hematoxylin-eosin (H & E). The oocyte diameters of mature jellyfish were measured based on the mean of the short and long axis of the oocytes using a light microscope equipped with a camera.

Since external stimulations play an important role in the reproduction, the rainfall data were collected from Meteorological Department in, Penang Island.

Differences between the biometric parameters (weight) and GSI of males and females were evaluated by Student's *t*-test. A sex ratio was calculated and significance was measured by using a $\chi 2$ test. To determine temporal variability in the gonad index, monthly mean GSI was compared by using single factor ANOVA. The relationship between the reproductive pattern and environmental factors was investigated by correlations between rainfall and GSI data in the area.

Results

A total of 300 jellyfish were collected which comprised of 170 males and 130 females. The monthly sample data showed that the females' bell umbrella length ranged between 100 mm to 210 mm (mean, 140.10±1.61 mm). The total weight was from 73.80 g to 350 g (mean, 160.59±46.90 g). The lengths of bell umbrella for males were 90 mm to 280 mm (mean, 150.03±3.07mm). The total weight varied from 61.70 g to 214 g (mean, 140.31± 2.61g). These results indicated that the weight in females were more than males. During the study period, the average sex ratio (male: female) in *Chrysaora* sp. was 1.42. The number of females was higher than males especially in June (0.52) and July (0.78) and in August the sex ratio was 1.

The Gonadosmotic Index (GSI) of *Chrysaora* sp. showed monthly variation. It was therefore apparent that the GSI values changed throughout the year. The highest GSI value in female *Chrysaora* was 19.1 ±3.37% occurring in July while the lowest value was observed in January at 12.19 ±3.91%. In male *Chrysaora* the highest and lowest GSI values also occurred in July and January at 17.37 ±4.89 and 11.4 ±3.87 respectively. GSI value in female was shown to be higher than in male, but however, was not significantly different during the study period (P>0.05). In general, GSIs were correlated with the proportion of matured male and female where GSIs were increased with increasing proportion of mature jellyfish (Fig .2).

The fluctuation of GSI and rainfall showed that there was no correlation between rainfall and spawning seasons during the experimental period from November 2010 to October 2011(Fig .3).The highest GSI values coincided with mature gonads which were found in July.

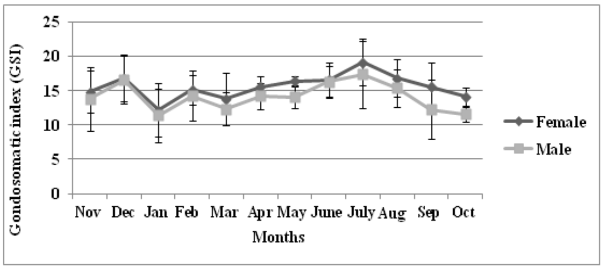

Figure 2: GSI changes in male and female showing the spawning cycle during the study period (November 2011- October 2012**)**.

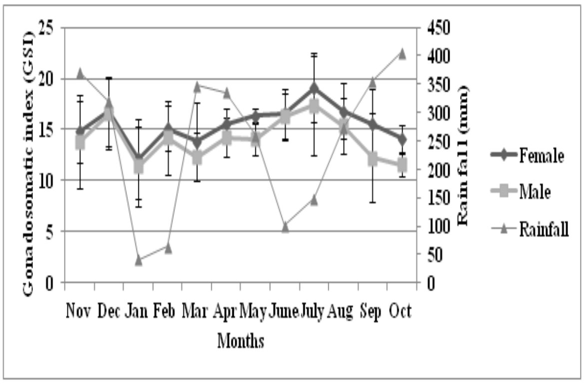

Figure 3: GSI in male and female showing no correlation between rainfall and spawning seasons during the study period (November 2011- October 2012).

The morphological differences of gonad development are summarized as below;

In female, the gonad comprised mostly of storage cells (Fig 4.F1). No traces of sexuality were observed in the mantel. This stage consists of virgin individuals where the reproductive system is elementary and those jellyfish which have completed spawning. The oocyte size was <12 μm. In male, similar to the female gonad, the resting male gonad was characterized by the abundance of connective tissue without germ cells or residual gametes. The spermatogenic follicle did not contain any spermatozoa (Fig5.M1).

Beginning of gametogenesis, the acinus is irregular in shape and is supported by interfollicular connective tissue (Figure 4.F2). Upon development, the core walls are well defined and the interfollicular connective tissue decreased. In the female, this stage is characterized by the presence of variable quantities of developing oocytes. This stage is known as an early vitellogenic stage with the presence of some yolk and occurrence of many oocytes that are still developing. The sizes of the oocytes ranged 12-15 µm and have a circular shape. The nucleus attains an elongated shape with a single nucleolus located in the central part of the nucleoplasma.

In the male, at the early active stage, spermatogenic follicle contains spermatozoa and the acinus look elongated (Fig 5, M2). The germinal epithelium produced spherical spermatogonia. These spermatogonia were located along the internal wall of the acnius in bands of several cells. Spermotocytes consist of spermatid and ripe spermatozoa. Cell diameter decreased and follicle area increased as a result of the accumulation of ripe gametes. At the late active stage, follicle size increased and the number of spermatozoa also increased. Spermatozoa were arranged radially with the tails pointing toward the centre of the follicle.

At this stage in female, the acinus was completely mature and ripe (Figure 4. F3). In the female *Chrysaora* this stage is known as the mid vitellogenic or last part of the vitellogenic process. During this stage, the yolk granules were observed throughout oocyte but have not accumulated. The size of the oocytes at this stage achieved 15-50 µm. However, ripe polygonal shape oocytes were free within the follicles. While in the male, the acinus consists of ripe spermatozoa (Figure 5.M3). The spermatogenic cell has released spermatozoa to subgenital sinus. However, the spermatozoa were distributed radially and the diameter that they occupied was greater than the layer of spermatogonia, spermatocytes and spermatids which decrease as gametogensis progressed. In the current study, the milt of the jellyfish was not observed in the specimens collected. The milt may be release in the sea.

Stage IV (mature), is the fully developed stage and in female is known as the late vitellogenic stage and consists of large quantities of accumulated yolk. Acinus was filled with mature postvitellogenic oocytes and the walls of follicles have broken down (Figure 4. F4).Shape is polyhedral due to oocytes crowding the acinus. Free spaces were observed in the follicle and different quantities of oogonia were observed in the follicles. Size of the oocytes in this stages reached to >50 µm. Generally, the size of oocyte increased from stage I to stage IV, with the maximum diameter of oocytes observed in July and a minimum in March.Unfortunately, stages V and VI were not observed in the current investigation.

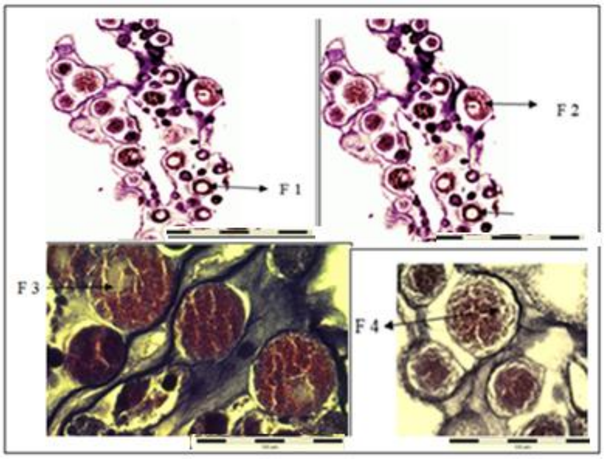

Figure 4. Previtellogenic stage, absence of yolk granules (F_1), Early vitellogenic stage, some yolk granules present (F_2), stained with Haematoxylin and Eosin Mid vitellogenic stage, yolk granules present throughout oocyte, but have not accumulated (F_3) Late vitellogenic stage, large quantity of yolk accumulated (F_4) stained with Haematoxylin and Eosin.

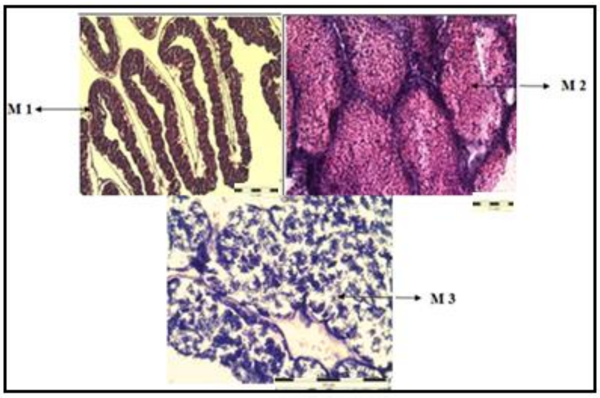

Figure 5. Testis structure in males (M_1); spermatogenic follicle contains no spermatozoa, (M_2); spermatogenic follicle contains spermatozoa, stained with Haematoxylin and Eosin. (M_3); spermatogenic cell has released spermatozoa to subgenital sinus, stained with Haematoxylin and Eosin.

Reproductive Cycle

To describe the reproductive cycle, during monthly sampling from November 2011-October 2012, three hundred (300) of specimens were examined. No hermaphrodite specimens were found and this species was detected as an asynchronous.The highest and lowest development of mature gonad was observed in July (95.34%) and November (21.78%) respectively (Figure 6 and Table 1). Female weight showed no correlation with oocytes diameter during the study period (November 2011- October 2012) and, all medusae were observed to become ripe, irrespective of size even the small sized ones (Fig 7).

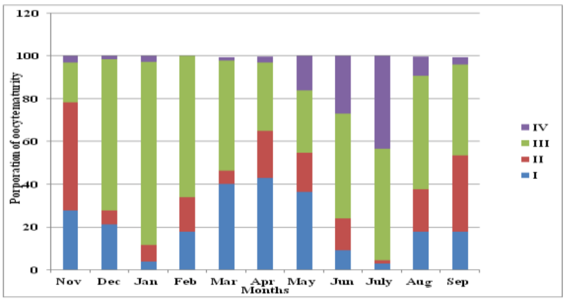

Figure 6. Proportion of oocyte in *Chrysaora* sp. during one year of sampling according to gonad development stages (I: immature, II: Develop, III: Mature and IV: Ripe).

Table 1: Proportion of gonad females developmental stages according to monthly sampling.

	I (Immature) Pre vitellogenic stage	II (Develop) Early vitellogenic stage	III (Mature) Mid vitellogenic stage	IV (Mature) Late vitellogenic stage	Mature (III+IV)
November	27.72	50.49	18.81	2.97	21.78
December	21.31	6.55	70.49	1.63	72.12
January	3.88	7.76	85.5	2.91	88.41
February	17.71	16.28	66.01	0.00	66.01
March	40.22	6.06	51.51	1.65	53.16
April	43.05	21.75	31.94	2.77	34.71
May	36.55	18.27	29.03	16.12	45.15
Jun	9.01	14.92	49.01	27.04	76.05
July	3.01	1.55	51.93	43.41	95.34
August	17.71	20	53.11	8.81	61.92
September	17.85	35.71	42.28	3.50	45.78
October	55.76	7.69	30.79	5.71	36.5

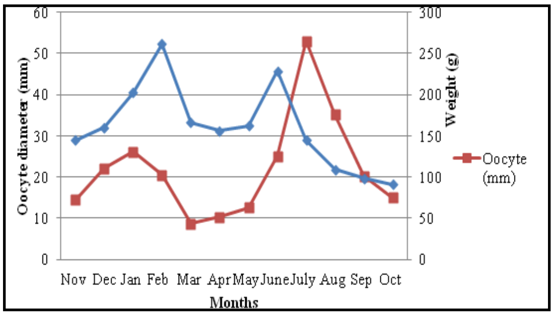

Figure 7: Female weight showing no correlation with oocyte diameter during the study period (November 2011- October 2012).

Discussion

The gonadosomatic index (GSI) values for both sexes of *Chrysaora* sp. along the coasts of Penang Island revealed that the likely start of the spawning season was in July. There was no apparent correlation with rainy season and the number of jellyfish occurrence during the rainy season decreased. Unfortunately, to this date there was no information on the spawning activities of tropical jellyfish to compare with the current results. Contrary to this investigation, many studies in vertebrate animals such as fish in tropical regions have reported high correlation between rainy season and spawning. For example Muchlisin [20] reported that in *Rasbora tawarensis* the spawning during the wet season was higher than dry season.

While in invertebrate animals, such as sea cucumber in tropical countries, Dissanayake and Stefansson [7] did not observed relationship between rainfall and GSI. However, the results of the current study were comparable with findings in other marine invertebrate animals. Based on the above it is suggested that there was no evidence to support the role of rainfall in reproductive period of the tropical jellyfish. Food abundance, temperature and salinity, photoperiod and age are considered as the major factors affecting reproduction cycles .[14]

Temperature and salinity variation result in changes in activity and metabolism which can affect growth and reproduction [18]. Purcell [26] reported that the asexual production of *C. quinquecirrha* was affected significantly by salinity. Ribes [27] observed that the timing of gonadal development of *Eunicella singularis* (Cnidaria: Octocorallia) in cold temperature areas occurred in late spring and summer which are periods of higher resource availability while in tropical countries presence of phytoplankton bloom can provide the metabolic energy required for gametogenesis. Unfortunately, no data is available for phytoplankton bloom which should be incorporated in future studies of this type. According to Chuah[4] jellyfish blooms can be affected by nutrient concentration. He also reported the highest and the lowest concentration of nitrates in the Straits of Malacca was in October and July respectively and did not find any relationship between metrological parameters and blooming of jellyfish. This correlated well with the present data where the month of July was detected as the peak of spawning. Based on the above it is suggested that nutrient concentration was inversely correlated to spawning peaks; low concentration, high jellyfish occurrence.

Microscopic method was used to determine the sex in *Chrysaora* sp. By comparing the shape and texture of the gonad under a microscope, it was possible to sex -differentiate sexually mature individuals. The sexes of immature male and female of *Chrysaora* sp. could not be determined throughout the year if gametes have not started to develop. These results were comparable with Pitt and Kingsford [22] and Pope [24] who also used microscopic methods to distinguish between sex in *Catostylus mosaicus* and *Craspedacusta sowerbyi* respectively. It must be noted that the population of *Chrysaora* sp. is dioecious having male and female organ in separate and distinct individuals. The sex ratio of this species can be very unequal and may vary throughout the year. This study showed that the number of male medusa were more than female during most of the year. However, during the peak of the spawning season in July the number of female increased but was still lower than males. In a previous study, Pitt and Kingsford [22] reported the sex ratio in *Catostylus mosaicus* was 1:1 within a period of one year. In another study, Lucas and Lawes [16] also observed a 1:1 ratio in *A. aurita*. Brewer [1] reported that a sex :ratio of greater female:male in *Cyanea* sp. Pope [24] reported that the medusae population of *Craspedcusta sowerbi*in North America in 1977 was predominantly female while during 2001 and 2002 male predominated. He suggested that sample sizes may have influenced the results. Therefore, he recommended sampling be conducted at various times during the season with more specimens to effectively study the dynamics and importance of sexual reproduction in the species.

Grandcourt[12]explained sex ratio may be different according to the time of year or due to the selectivity of fishing gear. However, in the current study two methods were used to collect 30 specimens of *Chrysaora* every month. Thus, the variation may be related to the number of specimens collected. Fautin[10]suggested another alternative explanation: this species is a protandrous and starts life as male then changed to female and therefore led to the statistical variation observed. More study is necessary to address these questions. This study revealed, based on the gonadal development and the oocyte size, as well as the ovary and microscopic observation that *Chrysaora* sp. is an asynchronous species (different stages were observed in the gonad during the studied year). Furthermore, female weight showed no correlation with oocyte diameter during the study period (November 2011-October 2012). Therefore, all medusae become ripe even those of small size (Fig 7). This data is in agreement with, Lucas and Lawes [16] who reported asynchronous oogenesis and different size at maturity for *A. aurita.* They suggested that continued somatic and reproductive growth was in competition for the assimilated food. This was particularly observed in *Chrysaora* population, when gonad index displayed monthly changes. Ohtsu [21] in another study on *N. nomuria* around Oki Island reported that gonad maturation had no direct relation to size of medusae but may be accelerated when the medusae is injured. Maturation not only occurred in the gonads in situ in isolated case such as in set-nets or trawl nets but maturation may also be induced in their separated the gonads to support fertilization. In addition he reported that the chance of fertilization was high in warm temperature and may be very low when the temperature went down to less than 10°C.Based on this study the presence of the gonad all the year round along the Penang coast suggested that gametogenesis was overlapping, possibly due to the stable and uniform tropical environment.

According to the current study, the highest GSI value in female *Chrysaora* was 19.1±3.37% in July while minimum value was 12.19± 3.91% which was observed in January. In male *Chrysaora*, the highest and lowest amount of GSI was 17.37 ± 4.89 in July and 11.4 ±3.87 in January respectively (Figure 2). This indicated that GSI values varied throughout the year. Female GSI values of *Chrysaora* sp. were higher than male. The GSI approach is one of the simplest techniques that could be used to explain gonad development. The high correlation of GSI with number of matured female and male could apply to estimate the peak spawning season. To date no information has been reported in jellyfish. The current results are comparable with other marine invertebrates. Dissanayake and Stefansson [7] in their study on sea cucumber reported the value range of GSI in female was higher than male with the range and the highest value of GSI observed during spawning season. This agreed with the present findings. Another best method, for examining reproductive cycles in animals is the measurement of the oocytes [12]. The highest and lowest development of mature gonad was observed in July (95.34%) and November

(21.78%) respectively (Fig 6 and Table 1). However, the current results showed that there were several individuals with mature gonads during the year. This suggested that reproduction is continuous throughout the year or asynchronously several times during the year but having the peak in July.

Maturation stages in *Chrysaora* based on the present results consisted of four stages (previtellogenic, early vitellogenic, mid vitellogenic and late vitellogenic) in female and three stages (spermatogonia, spermatocytes, and spermatids) in male. Iguchi [14] observed six stages for female and three stages for male in *N. nomuria*. In the current study, none of the specimens could be identified as having spent ovaries. One explanation is that oocytes and spermatozoa of F5-F6 and M3 are thought to flow into the sea immediately through the subgenital sinus which is connected to the outside of the medusae by a gasterovascular cavity. Thus, they could not be observed.

Maturation stages derived from observation of gonad tissues was strongly related to maximum oocyte diameters. This parameter could be easily measured and is a useful index for spawning and maturation in female *Chrysaora* unlike maturation stages. In addition, it increased as the gonads developed as shown in the size frequency distribution of oocytes (Figure 7). Furthermore, maximum oocyte diameter was observed when the gonad developed. This is in agreement with this study where maturation stages were correlated with oocyte diameters. The same is comparable with Iguchi [14] who worked on *N. nomuria*. Unfortunately, up to date no data on *Chrysaora* sp. in another location of the world is available for comparison. Consequently, reproductive biology and culture of jellyfish provide many experiments on husbandry of medusae especially *Chrysaora* sp. regeneration, sexuality and variation that previously were difficult to explain. Pope [24] explained that information regarding variation in jellyfish is important as often it parallels the changes in the rate and type of variation in a population and therefore an indication of environmental stress. It is also beneficial to have a complete picture of the species ecology or its environment. In fact, if the trend and changes in the type of variation could be correlated with environmental factors, the information could be applied to better understanding of adaptation, reproduction or other aspects of the species. One of the short comings of the present study is the unpredictability of the jellyfish natural occurrence and also the difficulty to culture the medusae to maturity during captivity. However, the findings of the present study may be significant in drawing a more complete picture of the ecology of the species and its environment. Thus, if trend and changes in type of variation could be correlated with environmental factors, and the information could be applied for better understanding on adaptation, reproduction or other aspects of the species, management strategies could be more effectively implemented. Consequently, the results of the current study could clarify some unanswered questions and also prepare fundamental information for the biology of this species in tropical waters but which is generally applicable to the management of the different species of jellyfish. In addition, the results of current study provided a good reference for other marine invertebrates that spawn in the water column, i.e. these strategies are not restricted to jellyfish. Thus, the data has wide potential application and is certainly an important contribution to the reproductive study of gelatinous zooplankton.

Conclusion

The spawning and peak reproductive season of *Chrysaora* sp. was in July and the male were the predominant sex during the year while only during the reproductive season the number of female increased. This species is classified as a group of asynchronous spawner. Generally, the structure of the gonad and the gametogenesis process were similar to those described for other medusae such as *Catostylus mosaicus* and *N. nomuria*. Results of this study will be useful for management strategies.

Acknowledgements

Funding support for this research was provided by the Universiti Sains Malaysia Grant (1001/PPANTI/843085). USM PGRS grant number which the authors acknowledge with gratitude.

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