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Article

Reproductive Traits of Sandbird Octopus, *Amphioctopus aegina* (Gray, 1849) from Mandapam Coastal Waters (Palk Bay), Southeast Coast of India

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Abstract – The sandbird octopus *Amphioctopus aegina* (Gray, 1849) is one of the important octopod species in trawl catches in Mandapam waters (Palk Bay). The reproductive biology of this species from these waters was studied from October 2001 to September 2002. In the majority of months(Jan-June), the sex ratio was biased towards males. The ratios of males to females increased consistently with respect to weight Total weight at first maturity were 78.78g for females and 40.8 g for males. Four maturity stags were recognized for females and two for males. Maturation and spawning occur all year round, with a peak during October and another peak during January-February. In males, no definite seasonal changes were observed in gonadosomatic index (GSI) values. In females there were two peaks in GSI values during October and January-February. For individuals of a DML range of 67-85 mm fecundity varied between 2,962-8,820 oocytes. The average relative fecundity was estimated at 68 to 83 and the average number oocytes per gram of ovary were 488 to 539.

Key words – *Amphioctopus aegina,* maturation, spawning, fecundity, size at maturity

1. Introduction

Cephalopods started gaining importance in India with the development of export market and consequent increased in demand. Production of cephalopods from capture fisheries was estimated to be 12,8692 tons of which octopus constitute 4.39%. Central Marine Fisheries Research Institute (CMFRI 2009). *Amphioctopus aegina* formed 70.7% of the total octopod catches of this Mandapam coastal region (Ignatius 2005). Extensive reviews of cephalopod reproduction are available covering the main features of gamatogensis, maturation, mating and egg laying (Cortez et al. 1995; Gabr et al. 1998; Barratt et al. 2007; Stephen et al. 2008; Alves and Haimovici 2011).

Five reproductive strategies were described for cephalopods based on three features; ovulation pattern, spawning pattern, and whether or not growth occurs between spawning events. The five reproductive strategies defined were (a), spawning once, simultaneous terminal spawning, with synchronous ovulation, monocyclic spawning and absence of growth between egg batches. (b) Spawning more than once including: (i) polycyclic spawning with egg-laying occurring in separate batches during the spawning season and growth occurring between production of egg batches and spawning seasons; (ii) multiple spawning, with group-synchronous ovulation, monocyclic spawning and growth between egg batches; (iii) intermittent terminal spawning, with group-synchronous ovulation, monocyclic spawning and no growth between egg batches; (iv) continuous spawning, with asynchronous ovulation, monocyclic spawning and growth between egg batches (Rocha et al. 2001). Most species of octopus species have simultaneous terminal spawning events that can occur year round or in one or two seasonal peaks. Males have year round maturation and a short life span of less than <2 years (Guerra 1975; Hatanaka 1979; Goncalves 1991; Sanchez and Obarti 1993; Semmens et al. 2004).

Although extensive studies exist regarding the reproductive biology of *O. vulgaris* (Smale and Buchan 1981; Whitakers

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et al. 1991; Silva et al. 2002; Hernanadez-Gracia et al. 2002; Smith and Grifiths 2002), few exist for other species. The present study was undertaken to describe the spawning season, size at first maturity, and fecundity of *A. aegina.* This forms a biological basis for the implementation of possible management measures aimed at sustainable exploitation of this resource. The current rate of exploitation is well below the maximum sustainable yield by operating more number of boats and nets in the study area. (Ignatius and Srinivasan 2006). The aim of this work is to study the reproductive biology of *A. aegina* in the subtropical environment of the continental shelf in Mandapam coastal region, which will allow for a better understanding of the reproductive strategy along the species distribution.

2. Materials and Methods

Specimen collection

Samples were collected from Palk Bay, in the Mandapam region (Lat. 79°10′N; Long. 9°15′E), on the southeast coast of India (Fig. 1). A total of 1,255 specimens (793 males and 462 females) of *A. aegina* were randomly collected over period of a year from October 2001 to September 2002 from commercial fishing trawlers. Because of the annual 45 days closed season for trawling on the southeast coast of India during April 15 to May 30, May data were not available for analysis. The sex ratio of 631 specimens was determined by external examination, (392 males, 239 females), and these were preserved separately for further laboratory studies. They were thoroughly washed, weighed (0.001g) and measured (dorsal mantle length, mm). They were collected Octopus dissected and preserved in 10% formaldehyde for later analysis. Gonads were removed and weighed.

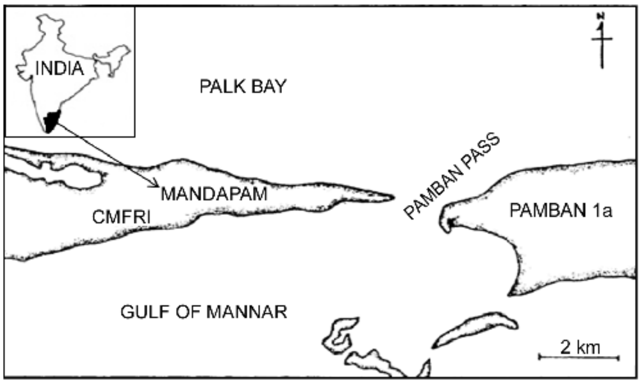

Fig. 1. Map showing the study area (Mandapam region, Palk Bay)

Data analysis

Sex ratio

The sex ratio was calculated for each month and also for each 4-10 cm. The homogeneity in the distribution of males and females was tested using the Chi-square test.

Growth

The length-weight relationships were calculated separately for females, males and then for population as a whole.

$$G = \frac{\ln(W_2 - \ln W_1)}{t_2 - t_1} \times 100 = \%\ \text{of body weight/day}$$

Where,

G - growth; W - weight

Maturity stages

Maturity stages were determined by visual identification of characteristics in the preserved reproductive organs and derived from scales used by (Buckley 1976; Mangold 1987; Whitaker et al. 1991; Alvarez Perez and Haimovici 1991; Smith and Griffiths 2002; Smith et al. 2006). Each scale was expanded and modified for *A. aegina* accordingly. The female maturity stages were categorized as follows in this Table 1.

Males were classified as mature or immature based on the presence or absence of spermatophores in the Needham's sac.

Table 1. Female maturation stage in *A. aegina*

Maturity stage	Morphological characters	Ova size
Stage I, Immature	Ovary small and white	<1 mm
Stage II, Maturing	Ovary granular with small ova, clearly visible medium-sized uniformly whitish ova, but very few of them reticulated.	<1 mm, 1-2 mm
Stage III, Mature	ovary is large and occupies more than half of mantle cavity, loose oocytes are present in the ovary	<1 mm, 1-2 mm, 2-6 mm
Stage IV, Spent	ovary is flaccid with few loose Oocyte	<1 mm, 1-2 mm, 2-6 mm, 6-15 mm

Gonadosomatic index (GSI)

For calculating the GSI, the weight of the reproductive organs (males: testis, Needhams sac and penis, and females: ovary and oviducal complex), and the total body weight of each animal were measured. The GSI was calculated using the following formula (Otero et al. 2007);

$$\text{GSI \%} = \frac{\text{Weight of gonad (g)}}{\text{Total body weight (g)}} \times 100$$

Size at first maturity

To estimate the mean lengths at 50% maturity for males and females, a logistic function was fitted to the proportion of the mature individuals by size class using a non-linear regression of the following function:

$$P = 1/\ \{1 + \exp\ [-a/r\ (L - Lm)]\}$$

(SPSS software was also used for the present work)

where P is the proportion of mature specimens in each size class, r (- slope, b) is a parameter controlling the shape of the curve and Lm is the length at which 50% of specimen attains maturity. The Lm = a/r where 'a' is the intercept.

Fecundity

Fecundity can be defined as the egg potential, or the number of eggs which are about to be released for fertilization, and which can develop into offspring. For estimating fecundity, the total weight of the ovaries was estimated and three samples were taken from each ovary. The number of eggs in each sample was counted after estimating the sample weight. The total number of eggs was calculated from the known weight of the samples and the full ovary as follows (Bensam 1999):

Total number of eggs in ovary = (no. of eggs in the sample/weight of the sub sample) * weight of the ovary

The correlation coefficient (r) was calculated (Snedecor and Cochran 1967) to find out the relationship between fecundity and the mantle length, total body weight and ovary weight.

3. Results

Sex ratio

Out of 1,255 specimens examined (3.3 to 8.7 cm dorsal mantle length), there were 793 males and 462 females. The overall ratio of males to females was 1.71: 1.00, which was significantly different from a 1:1 ratio ($\chi^2 = 87.9$, $P>0.05$). The proportion of males was more in the samples collected and the details were given in the Table 1. There were significantly more males in all months except June and July, indicating a male biased population. Analysis of sex ratio based on mantle length revealed that the proportion of males to females increases as weight increases (Table 2).

Growth

The length-weight relationships were calculated separately for females, males and then for population as a whole. Based on the data and calculation the following length-weight relationships were found: $W=0.6697\times DML^{2.3516}$ (r=0.95) for males, $W=0.6906\times DML^{2.4053}$ (r=0.97) for females and $W=0.6758\times DML^{2.3846}$ (r=0.96) for all specimens analyzed. Analysis of covariance did not indicate significant differences in

Table 2. Monthly sex ratio of *Amphioctopus aegina* from October 2001 to September 2002 at Mandapam region of Palk Bay

Months	No. of Octopus examined	Male	Female	Proportion of male	χ^2 test
Oct	185	120	65	1.85	*
Nov	149	98	51	1.92	*
Dec	56	36	20	1.8	*
Jan	43	30	13	2.31	*
Feb	110	78	32	2.44	*
Mar	68	48	20	2.4	*
Apr	100	62	38	1.63	*
May	--	--	--	--	--
Jun	205	115	90	1.28	ns
Jul	150	86	64	1.34	ns
Aug	66	42	24	1.75	*
Sep	123	78	45	1.73	*

Significant (P<0.05) departs from 1:1 are indicated by *; ns - not significantly differ from 1:1 via chi square test

Table 3. Sex ratio of different groups of *Amphioctopus aegina* based on dorsal mantle length from October 2001 to September 2002 at Mandapam region of Palk Bay

Size groups (DML cm)	No. of Octopus examined	Male	Female	Proportion of male	χ^2 test
3.0-3.4	54	28	26	1.08	ns
3.5-3.9	80	44	36	1.22	ns
4.0-4.4	92	53	39	1.36	ns
4.5-4.9	109	68	41	1.66	*
5.0-5.4	158	103	55	1.87	*
5.5-5.9	188	124	64	1.94	*
6.0-6.4	183	123	61	2.02	*
6.5-6.9	119	75	44	1.7	*
7.0-7.4	54	35	19	1.84	*
7.5-7.9	52	33	18	1.83	*
8.0-8.4	98	64	34	1.88	*
8.5-8.9	68	43	25	1.72	*

Significant ($P<0.05$) departs from 1:1 are indicated by *; ns - not significantly differ from 1:1 via chi square test

length weight relationship by sex.

Seasonal occurrence of maturity stages

The monthly evolution of maturity stages of males and females by percentage throughout the sampling period is given in Fig. 2. Mature females were found in all months. Immature ovaries were more prevalent during March-June, and the maximum percentage of mature ovaries was recorded during the months of October, January and February. No spent animals were recorded during the study.

More than 50% of the males are found to be mature throughout the year. Highest values were found from September-November and January-March. From the monthly evolution of maturity stages, could be concluded that the reproductive season of *A. aegina* occurred throughout the year. Greater reproductive activity occurred from July to February with a primary peak spawning activity during January-February followed by a secondary peak in October.

Gonado Somatic index (GSI)

Gonado Somatic index is used to monitor the breeding activity in a species. Analysis of gonadosomatic index for *A. aegina* showed annual changes especially in the case of females. Figure 3 shows the monthly changes in the gonadosomatic index for males and females respectively. Females showed a significant increase in the GSI from July to February, reaching a peak in October and another peak in January-February, coinciding with the major proportion of mature females. Gonado Somatic index showed a more or less consistent trend throughout the year. Two small peaks were produced in March and September coinciding with the major proportion of mature males. The range of GSI values obtained for different maturity stages of females were; Stage I: 0.023-2.98; Stage II: 3.71-5.89 and Stage III: 8.081-19. 39. In males, the mean GSI values were 1.76±0.21 (immature) and 2.95±0.69 (mature).

Size at first maturity

The smallest mature specimen collected in the sample was 4.8 cm DML in females and 3.6 cm DML in males. All the males were immature up to 3.4 cm mantle length. The percentage maturity gradually increased from 3.5-3.9 cm and 100% mature animals were found above 7.5-7.9 cm DML. The size at first maturity was estimated at 5.70 cm DML for males (Fig. 4).

All females, < 4.4 cm DML were immature At 6.5-6.9 cm DML there was a sudden increase in maturity, and by 8.5 cm nearly 94% of females were mature The size at first maturity was estimated at 7.17 cm DML for females (Fig. 5). The weight at first maturity was estimated as 78.78 g for females and 40.78 g for males. The parameters of the logistics model (a, b) and the regression coefficients (r) obtained for male and female are shown in Table 3.

Fecundity

Fecundity varied from 2,962-8,820 numbers in the individuals of mantle lengths ranged from 67-85 mm. The mean fecundity of *A. aegina* estimated from these samples was 5,646 numbers.. Average relative fecundity was estimated

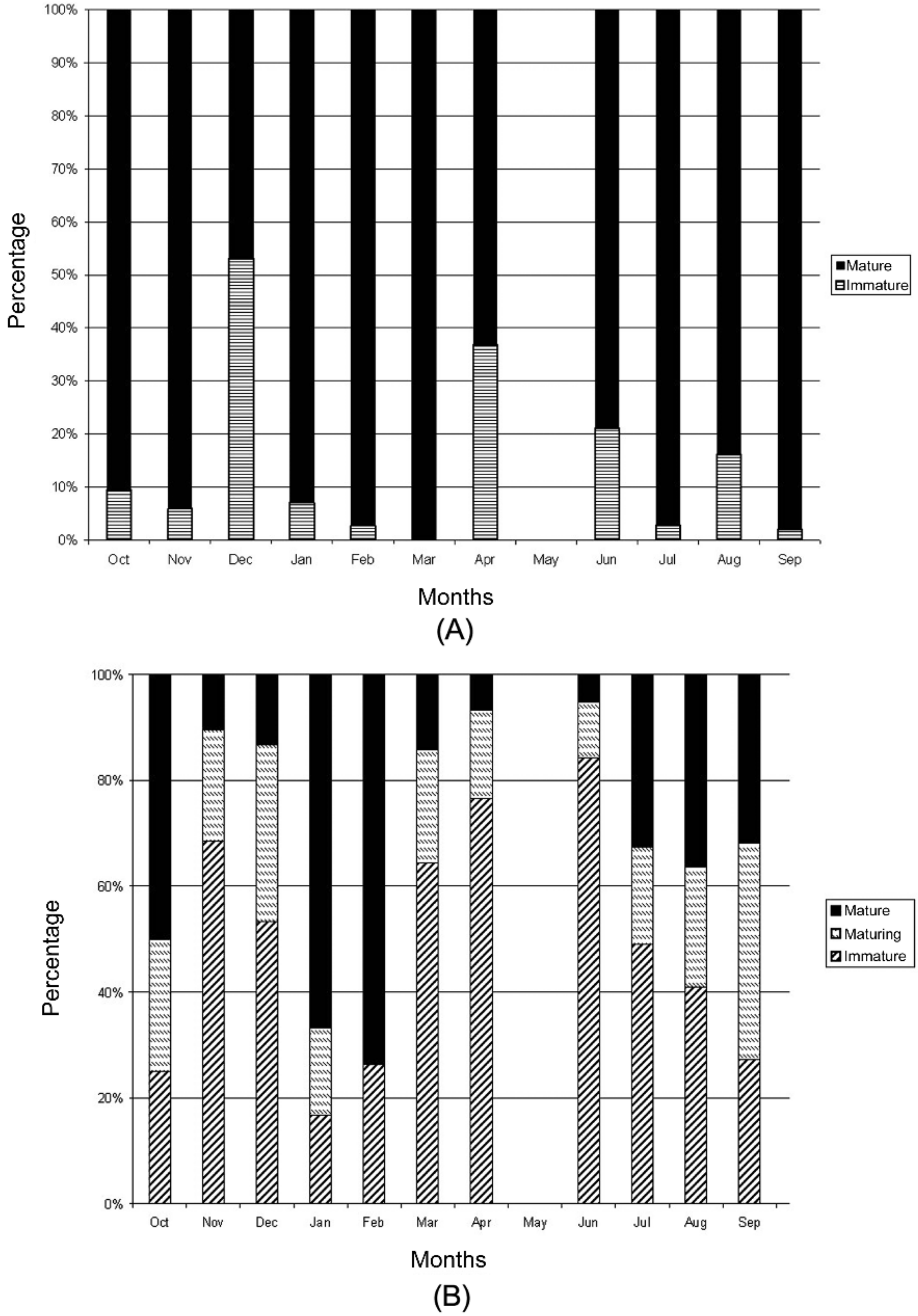

Fig. 2. Monthly percentage of different maturity stages of (A) male and (B) female *Amphioctopus aegina* during October 2001-September 2002

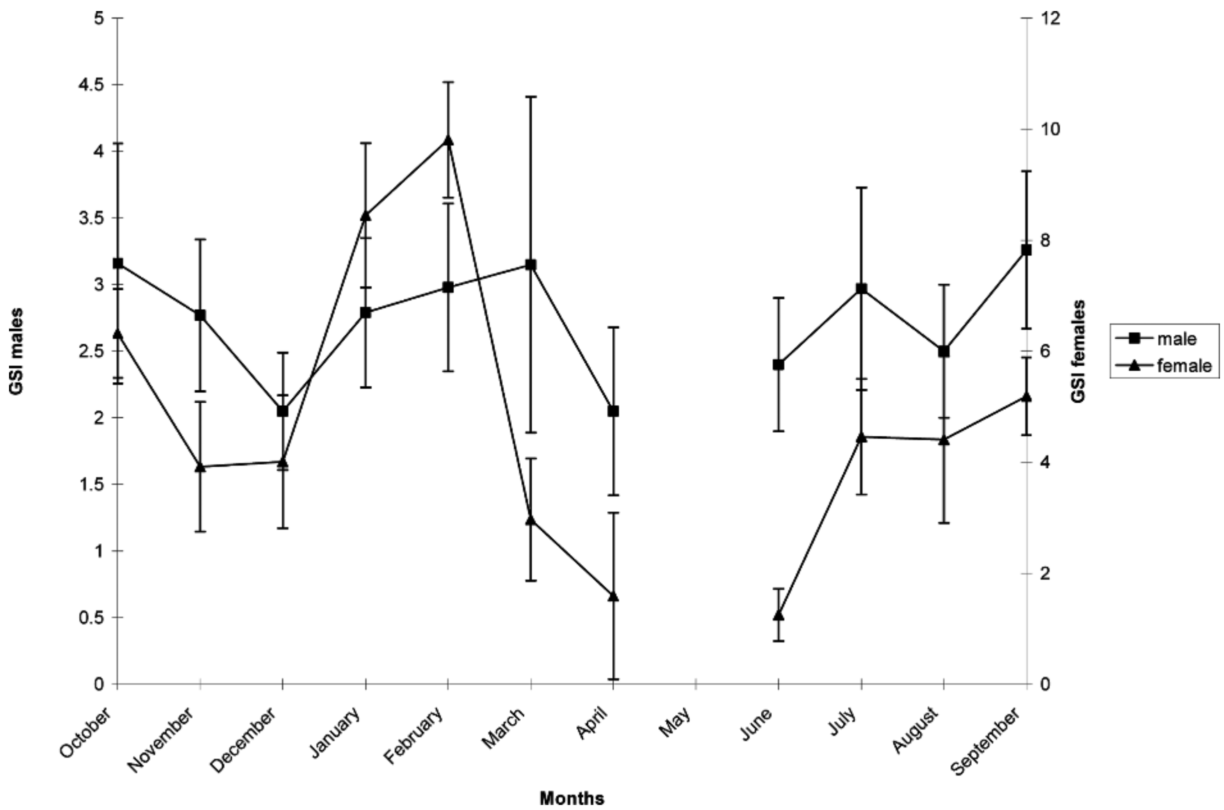

Fig. 3. Monthly variations of the gonadosomatic index (GSI±SD) in male and female *Amphioctopus aegina*. Vertical lines indicate the standard deviation (±)

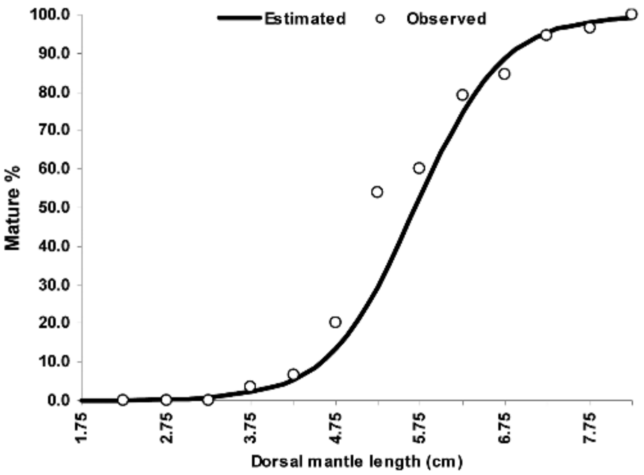

Fig. 4. Maturity ogive corresponding to dorsal mantle length for male *Amphioctopus aegina*

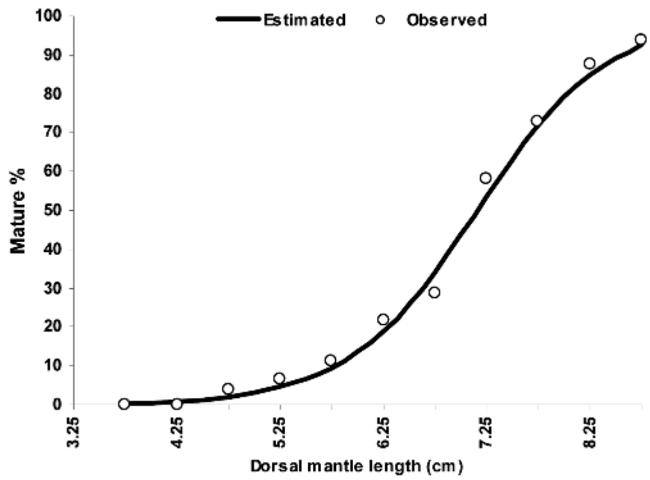

Fig. 5. Maturity ogive corresponding to dorsal mantle length for female *Amphioctopus aegina*

at 68±15.9, numbers and the average number of oocytes per gram of ovary was estimated as 488±51.9. The length of spermatophores in males ranged from 2.7-3.8 cm. Figure 6 shows the relationship between size (DML), body weight (BW) and ovary weight (OvW) with total fecundity.

Table 4. Parameters of the logistic model (a, b) for weight and length and regression coefficient for male and female *Amphioctopus aegina*

	a	b	r^2
Male DML	11.13048	-1.9536	0.9778
Female DML	11.44508	-1.597	0.9731

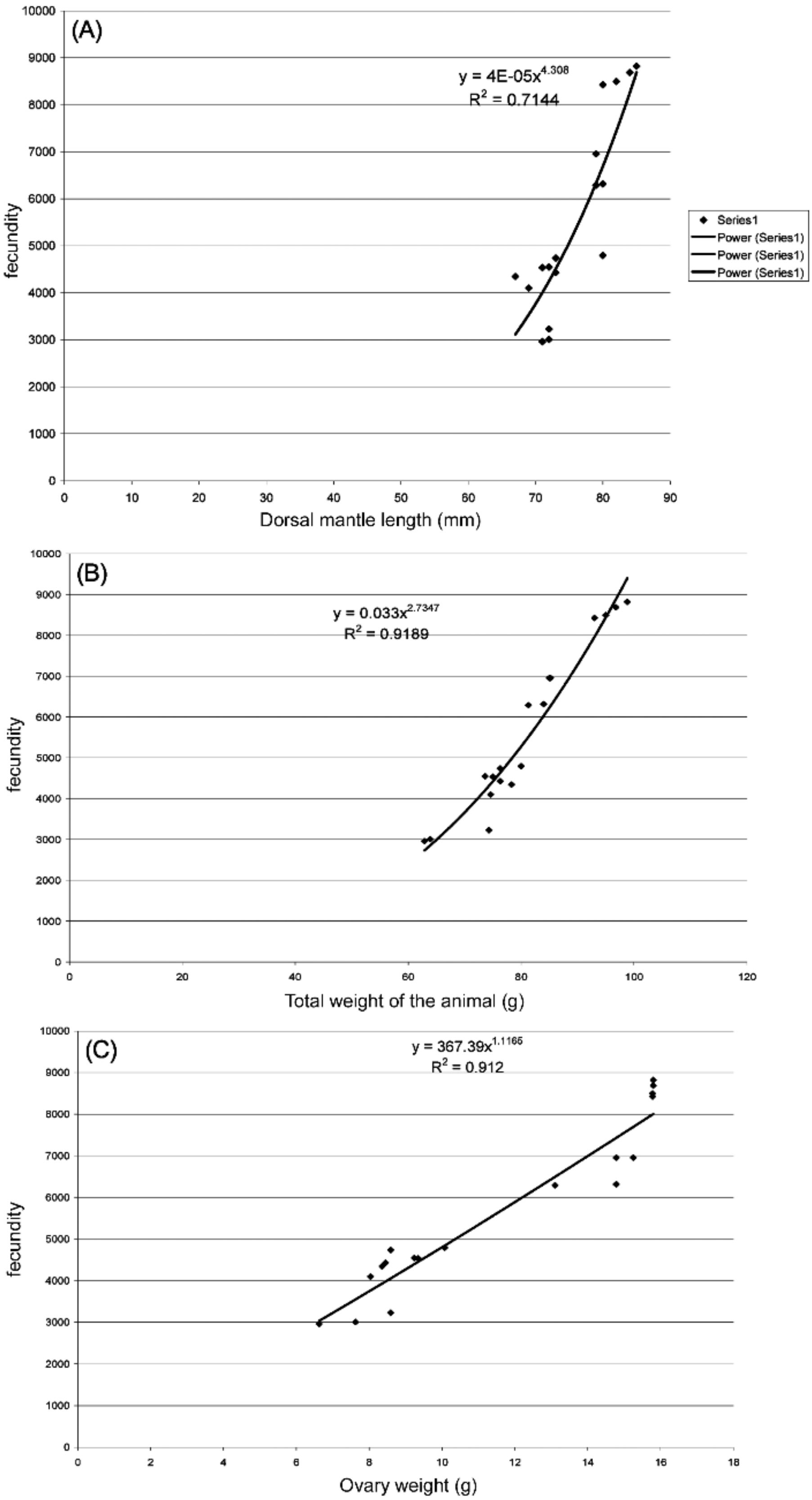

Fig. 6. Relationship between the total fecundity and (A) dorsal mantle length; (B) body weight and (C) ovary weight

4. Discussion

This study reported higher numbers of males than females, and this is consistent with earlier work on field populations of *Octopus* sp. (Mangold Wirz 1963; Hatanaka 1979; Cortez et al. 1995; Hernanadez Garcia et al. 2002; Smith and Griffith 2002; Uriarte and Jara 2009). Males predominated in all months of sampling and the overall sex ratio showed a significant variation from the expected ratio of 1:1. The sex ratio observed in the catches was influenced by various factors like migrating behavior associated with sexual maturation, feeding behavior, post spawning mortality, differences in growth rates of sexes.Mangold Wirz 1963; Mangold 1983a). The different behavioral pattern of male and female *A. aegina* and the fishing method might also have influenced the sex ratio. Observations made on the specimens held in our laboratory, brooding females do not feed during the whole period and spend most of the time in its shelter ventilating and caring festoons which were held close to the body on the aboral side of the broad extensive interbrachial membrane (Ignatius and Srinivasan 2006). This wandering behavior of males make them more vulnerable to capture and the brooding females are less vulnerable to in bottom trawls as they were hiding in the shelters. This is reflected in the sex ratio during months coinciding with peak spawning.

The length weight relationship showed an allometric growth pattern and this too is consistent with earlier work regarding other Octopus species (Cortez et al. 1999; Smith and Griffiths 2002; Otero et al. 2007) Analysis of covariance showed insignificant variations between males and females. Various criteria such as size and colour of the gonad, ova measurements, Gonadosomatic Index were used by various workers in order to classify the maturity condition of the octopods and to determine the spawning season (Silva et al. 2002; Smith and Griffith 2002). Mangold (1987) suggested that beyond the rough distinction of immature, maturing and mature, no classification system could be applied to all cephalopods and to both sexes. In this study the female maturity stages were classified into four stages based on the maturity scale developed by Mangold (1983b) for *O. vulgaris* and found suitable for this species also. The brooding behavior of females and simultaneous terminal spawning behavior of this species were possible reasons for the non-availability of spent specimens of *A. aegina*. Difficulty in collection spent stages has also been reported by Arnold and Williams Arnold (1977), and; Mangold et al. (1993).

Amphioctopus aegina spawns throughout the year with a proportion of mature individuals present almost all months of the study, although this proportion increased at specific times of the year. There are two peaks in spawning activity each during months of October and January-February. A similar behavior has been reported for other species of octopods. As observed in the present study, various workers reported year round spawning activity for octopus species from various other areas (Wodinsky 1972; Van Heukelem 1973; Hatanaka 1979; Smale and Buchan 1981; Hartwick 1983; Whitaker et al. 1991; Hernandez Gracia et al. 2002; Silva et al. 2002; Smith and Griffith 2002; Uriarte and Jara 2009). Although previous information regarding the spawning season of this species in this area is lacking, several authors for various cephalopod species reported similar results of year round spawning with one or two spawning peaks (Rao 1969; Unnithan 1982; Silas et al. 1986) in this area. The peak spawning seasons of this species in Mandapam waters (Palk Bay) coincided with the onset and withdrawal of Northeast monsoon at this area. During this period, water temperature often falls below the normal average of 27ºC, and there are intermittent rains during this season. Among various factors that influence the sexual maturity of octopods, photoperiod and temperature play important roles (Mangold 1983; Forsythe and Hanlon 1988). All these environmental factors along with reduced day hours during this time may influence the gonad maturation and reproduction in this species.

The reproductive cycle of *A. aegina* was reflected by pronounced variations in gonad size. When assessing gonad activity, animals of different sizes are frequently sampled and it is generally assumed that gonad weight depends on animal size and stage of gonad development (De Vlaming et al. 1982). The gonad and the digestive gland increase in size with sexual maturation, and these contribute to why the maximum values of these indices occurred when there was maximum reproductive activity (Mangold 1983b).

The present study showed that the GSI values of females increased from July and reached a maximum value in October, and again in January and February, GSI values were also increased for males during this period. The high GSI values during these months indicated that the maximum gonad activity had taken place, indicating a probable spawning period of the species. Low GSI values during March to July showed a reduction in reproductive activity. In males, no definite seasonal change was observed in GSI values indicating that the males may breed throughout the year. A similar pattern of more or less constant GSI values throughout the year for males and one or two peak GSI values for females in an year, coinciding the spawning season females was observed in other octopods species (Hatanaka 1979; Silva et al. 2002; Smith and Griffith 2002) The size at first maturity for *A. aegina* available in this area was established as 5.70 cm DML for males and 7.17 cm DML for female. Several species of octopods show the same pattern in which males mature at smaller size than females (Hanlon 1983; Hartwick 1983; Mangold 1983, 1987; Cortez et al. 1995; Silva et al. 2002; Avila Poveda et al. 2009; Uriarte and Jara 2009)).

Knowledge of fecundity is extremely important from a viewpoint of successful fisheries management. Most of the authors defined fecundity as the number of ripening eggs in the ovary just before spawning (Norman and Sweeney 1997). In the present study, the average fecundity of *A. aegina* was estimated as 5,690. Chung (2000) estimated the fecundity of this species from Thailand as 2,000-10,000. Norman and Sweeney (1997) estimated the number of ovarian eggs in *A. aegina* as 1,000-10,000 depending upon the size of the animals. The fecundity values showed wide variations within the same species caught from different area. In *O. vulgaris* fecundity varied from 1,00,000-5,00,000 from the samples collected from Thailand. It seems that environmental factors like food availability, its nutritive quality, and other environmental parameters play a role in determining the fecundity of the animal. Saville (1987) concluded that cephalopods have a wide range of fecundity, from 0.6 eggs/g of body weight to 200 eggs/g of body weight for octopus, and from 40-1,000 eggs/g for squids. This will be more reliable in making any comparison the values of fecundity per unit weight of the ovary of the animal. The average number of eggs g^{-1} of ovary was estimated as 488±51.9 and number of eggs per gram of body weight as 68±15.9 numbers. Similar values were reported made by Saville (1987) in various octopus species. The present study provided some important information on the reproductive traits of sandbird octopus, *Amphioctopus aegina* (Gray, 1849) from Mandapam coastal waters (Palk Bay), southeast coast of India which will be useful in developing strategies management and conservation of this resource.

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