J. Mar. Biol. Ass. India, **49** (1) : 58 - 64, January - June 2007

Influence of lunar rhythm on spawning of clown anemonefish *Amphiprion percula* under captive condition in Andaman and Nicobar islands

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Abstract

The influence of moon phases on the reproduction of the clown anemonefish *Amphiprion percula* from Andaman and Nicobar islands was investigated during January 2002 to September 2005 for the first time in India. Out of the 1281 fishes examined, the female to male ratio of 1: 2.1 observed for the entire period indicated the predominance of males. The males were also smaller due to protandric sex conversion. The influence of lunar phases and water temperature on the weight of gonad in different size groups of the species was assessed. The gonado somatic indices (GSIs) showed a peak during January in females and males whereas the lowest value in the former was observed in July and in the latter during June. The reproductive pairs collected from the wild were reared in captivity and fed with gonad of green mussel, prawn and egg mass of fishes six times daily @ 20% of the body weight in split doses. Apart from these, live feeds such as L type rotifer, enriched with mixed micro algae isolated from Andaman waters, and *Artemia* nauplii and adults were also provided. With these feeding schedule under aquarium conditions spawning in *A. percula* was achieved on an average of 2.3 ± 0.3 month^{-1} throughout the year. Each fish laid 112 to 557 eggs between 0600 and 1545 hrs in 7 to 15 days interval with an annual number of 2500 to 13500 eggs. In all the pairs, significantly higher percentage of spawning was observed 1-5 days after the full moon (I quarter) and new moon (III quarter) than the IV quarter and II quarter which indicated that the spawning is related with lunar periodicity. During the warmer period of the year, a maximum of 4 spawnings month^{-1}pair $^{-1}$ were obtained indicating that water temperature ranging from 26.1 to 33.0ºC also had an influence on the frequency of spawning under controlled conditions.

Keywords : Breeding, gonadosomatic index, spawning, lunar rhythm, *Amphiprion percula*

Introduction

The anemonefish *Amphiprion percula* belonging to the family Pomacentridae and subfamily Amphiprioninae are one of the most popular attractions in the marine ornamental fish trade and always stays as ground pillar in this industry. Nearly 50 species of these taxonomically widely distributed tropical marine fishes show lunar spawning rhythms (Johannes, 1978). Spawning frequency and periodicity in clownfishes have also been strongly correlated to lunar cycles (Allen, 1972; Ross, 1978; Fautin and Allen, 1997) whereas in more temperate regions spawning occurs only during the warmer summer months (Bell, 1976; Ochi, 1985; Richardson *et al.,* 1997). Clownfish, in aquaria have been found to breed year round with an increase in the number of spawning during spring and summer months (Alava and Gomes, 1989; Hoff, 1998) whereas the spawning of *A. melanopus* is strongly influenced by lunar rhythm and recorded two spawning per lunar month in a year (Ross, 1978). The spawning, the larval settlement and the growth in *A. clarkii* (Ochi, 1986) and in *A. latezonatus* and *A. akindynos* (Richardson *et al.,* 1997) were also found to be influenced by the lunar periodicity under natural condition. Many studies have been conducted among clown fishes on the reproductive behaviour, nest preparation, spawning pattern and parental care from the wild (Allen, 1972; Bell, 1976; Ochi, 1986; Richardson *et al.,* 1997; Fautin and Allen, 1997) whereas studies pertaining to the hatchery conditions are limited (Hoff, 1998; Gordon and Bok, 2001). In the present study, emphasis has been given on documenting the sex ratios and influence of lunar phase and water temperature on gonadosomatic indices in the natural habitat, and the spawning pattern, frequency and periodicity in spawning and time of spawning under hatchery conditions for a period of forty five months. This study is

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the first report on the lunar periodicity in spawning of *A. percula* under captive conditions in India.

Materials and methods

The reproductive rhythm of *A. percula* was investigated using specimens collected during October 2002 to September 2004 from North Bay, Marine Hill, Coriyaghat, Chidiyattappu, North Wandoor, South Wandoor and Mayabunder of Andaman Islands (Latitude 10º 48' N and 13º 15' N and longitude 92º 12' E and 93º 57' E). A total of 1281 fishes (females n=419 and males n=862) were analyzed using the Chi-square Test to find out the contribution of male and female in the population. In order to study the sex ratio, the fishes were grouped into 5 mm size class. To asses the variations in the gonadosomatic indices, a total of 695 adult females and 694 males were observed during different lunar phases. Seven to ten individuals of length 75 to 99 mm, body weight 11.3 to 14.7 g for females and 55 to 69 mm length, 5.1 to 7.8 g body weight for males were collected every lunar day. The weight of the individual fish and their gonad were noted to the nearest milligram after wiping with a blotting paper. The gonadosomatic index was calculated separately for both sexes as suggested by June (1953) and Yuen (1955) and its relationship to the body weight was analyzed through Pearson correlation. The influence of lunar phases on gonadosomatic indices in different size groups of male and female was studied through ANOVA wherein the factors were compared using Duncan's Multiple Range Test (DMRT). The seawater temperature was measured three times a day (8 am, 12 pm and 6 pm) and its average was taken to study the effect of temperature on the gonadosomatic indices using Pearson correlation.

Seven pairs of *A. percula* were collected from the coral reefs of Andaman and Nicobar islands, and transported to the Marine Research Laboratory (MRL) of Central Agricultural Research Institute (CARI), Andamans. Each pair stocked in 250 l perspex aquaria was assigned a code number (Ap1 to Ap7) and the water was recirculated through bucket filters. All the pairs were fed six times daily@ 20% of the body weight in split doses with green mussel (*Perna viridis*) gonad, prawn and egg mass of fishes, and live feeds such as L type rotifer *Brachionus plicatilis* 500 to 750 numbers ml^{-1}, enriched with mixed micro algae (*Chlorella salina*, *Nannochloropsis oculata* and *Pavlova lutheri* (50-60x10^6 cells ml^{-1}) isolated from Andaman waters, newly hatched *Artemia salina* nauplii (10 to 25 numbers ml^{-1}) and its adult form (3 to 5 numbers $ml^{-1}day^{-1}$). The natural seawater was drawn directly from the intertidal zone of the sea adjacent to the laboratory and stored in 15000 liters capacity filtration tank fitted with under-gravel sand filters and was allowed to settle for 24 hours. After this, the water was filtered through nine series of filtration tanks (1000 l capacity) before being taken to the hatchery. Throughout the study period the water quality parameters remained within the acceptable limits with *p*H ranging from 8.01 to 8.14, NH_{4+} /$NH_{3,}$ NO_2 values at 0 mg per l, NO_3 level below 6 mg l^{-1}, water temperature between 26.1 and 33.0ºC and dissolved oxygen 4.2 to 5.8 ml l^{-1}. All the experimental tanks were maintained in the hatchery where transparent roofing was provided to get an incident light intensity of 2500 to 3000 lux as the sea anemones required sunlight for their better survival under laboratory condition. Since ambient light entered the hatchery through transparent roofing, a 12 h light: 12 h dark photoperiod during new moon phase and 12 h moon light during the full moon phase was maintained. Daily one–third water was exchanged with filtered sea water. In each broodstock tank, a rough surfaced white ceramic tile (30cm l x 24cm b) was provided near to the sea anemone for egg deposition. All the pairs were observed twice daily (morning and late afternoon) for the record of spawning. The date of spawning, full moon and new moon, time of spawning, number of eggs laid at each spawning, time gap between successive spawning and water temperature were recorded from January 2002 to September 2005 to study the influencing factors on spawning of these fishes under captive conditions. All data were analyzed through SPSS statistical package version 14.0.

Results

Sex ratio: The observed sex ratios in each month and among different size groups of 1281 fishes (females n=419 and males n=862) were tested against an expected ratio of 1:1 using Chi square Test. The 1: 2.1 sex ratio obtained for the entire period of investigations indicated the predominance of males over females in the natural population mainly on account of protandry (Table 1). The analysis of sex ratio in different size groups revealed a preponderance of males in length groups 20.0 – 24.9 to 50.0 – 54.9 mm and no females were observed in the size groups from 20.0 to 39.9 mm. The length frequency distribution showed that the females were of larger average size than the males, although there was an overlap in the length distribution of the sexes. The mean size at sex reversal from male to female appeared to be in the level of 55.0 mm. In the present study, the number of females in the length group 55.0- 59.9 mm onwards was greater than males and all fishes were females in the size group 80.0 –84.9 to 95.0 –99.9 mm groups (Table 2).

Table 1. *Month-wise Chi square test for* A. percula *(pooled data* 2002 to 2004)

Months	Male	Female	Chi sq.
Oct.	50	18	15.06**
Nov.	49	26	7.053**
Dec.	62	19	22.83**
Jan.	73	36	12.56**
Feb.	87	37	20.16**
Mar.	81	41	13.11**
Apr.	66	35	9.515**
May	113	47	27.23**
Jun.	78	43	10.12**
Jul.	71	41	8.036**
Aug.	89	44	15.23**
Sep.	53	22	12.81**

** Significant = p<0.01

Table 2. *Sex ratio in different size groups of* A. percula

Length- Group (TL in mm)	Number of fishes		% females
	Female	Male	
20.0-24.9	0	74	0
25.0-29.9	0	87	0
30.0-34.9	0	120	0
35.0-39.9	0	152	0
40.0-44.9	11	145	7.05**
45.0-49.9	24	124	16.22**
50.0-54.9	25	97	20.49**
55.0-59.9	40	18	43.1**
60.0-64.9	49	15	76.56**
65.0-69.9	52	12	81.25**
70.0-74.9	46	6	88.46**
75.0-79.9	52	5	91.23**
80.0-84.9	48	0	100
85.0-89.9	35	0	100
90.0-94.9	36	0	100
95.0-99.9	8	0	100
Sex Ratio	F : M = 1 : 2.1		

** Significant = p<0.01

Influence of moon phases on GSI: The gonado-somatic index (GSI) varied from 1.74 to 2. 28 in the females and 0.62 to 1.64 in males with peak in January during the period of study (Table 3). In both sexes, the low values were observed during June to September. The values showed an increasing trend from September onwards. As the GSI values were obtained for all months during the study period, it was concluded that the spawning occurs throughout the year in Andaman waters whereas frequency in spawning was slightly reduced during the period of southwest monsoon. Correlation of gonad index on body weight of male and female was not significant (p<0.01). On the other hand the lunar phase showed statistically significant influence on gonad index in phase I and III of the moon (p <0.01) than the other two phases (IV &II). The gonad indices increased from the full moon quarter and reached the lowest values in the second quarter. The mean sea water temperature in the wild did not display a defined seasonal change and the water temperature during the study period ranged between 26.4 to 33.9º C. A comparison between mean gonad index and mean seawater temperature showed no significant relationship (p <0.05).

Influence of lunar rhythm and temperature on captive spawning: Under laboratory condition, 537 spawning were recorded during the period of study. However, not all the seven pairs provided data for the entire duration as they began to breed at different months and the male partner of one breeding pair (Ap7) died one month before completion of study. Hence percentages of the number of pairs reproductively active at that time were considered for data analysis. Under aquarium conditions through feed management, seven pairs spawned with an average of 2.3 ± 0.3 month^{-1} throughout the period of study. During the warmer period of the year, a maximum of 4 spawning month^{-1} pair $^{-1}$were obtained (Table 4). The Pearson correlation coefficient between sea water temperature and frequency of laboratory spawning was significant (r =0.834, p<0.01). During a period of 12 months in the present study, the breeding pair (Ap2) spawned 33 times in the year 2004 and maximum spawning (4 times) was observed in March. The pairs Ap1, Ap3 and Ap6 also spawned 2 to 4 times in March during the entire period of study. The frequency of captive spawning also showed significant relationship with water temperature (p<0.01) and exhibited increased spawning frequency during the warmer months throughout the study period. The time gap between successive spawning varied from 7 to 15 days intervals with lowest during months where 4 spawning were achieved (Fig.1). In all the cases, the

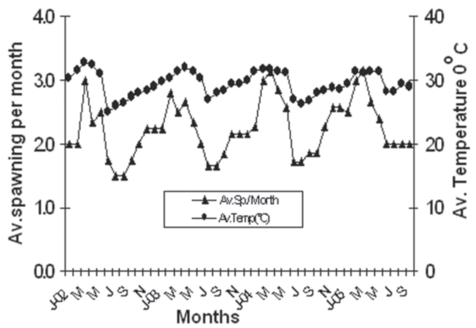

Fig. 1. Average spawning per month and sea water temperature (2002-05)

Table 3. *Gonadosomatic indices (GSIs) of female and male of* A. percula *during 2002-2004 (pooled data). Values for males in parenthesis*

Months	Sample size (Nos)	Average body weight (g)	Av.weight of ovary and testis (g)	GSI
Oct.	50(55)	11.55 (7.23)	0.24 (0.1)	2.1 (1.44)
Nov.	51(48)	13.75 (7.15)	0.30 (0.1)	2.19 (1.46)
Dec.	62(60)	14.0 (6.19)	0.30 (0.1)	2.17 (1.58)
Jan.	58(55)	13.19 (6.79)	0.30 (0.1)	2.28 (1.64)
Feb.	59(55)	13.46 (6.83)	0.30 (0.11)	2.23 (1.62)
Mar.	54(67)	13.25 (7.04)	0.30 (0.12)	2.25 (1.63)
Apr.	59(68)	13.86 (6.94)	0.30 (0.1)	2.15 (1.41)
May	67(56)	13.80 (6.73)	0.29 (0.06)	2.10 (0.92)
Jun.	60(68)	12.83 (5.32)	0.23 (0.03)	1.80 (0.62)
Jul.	60(54)	12.53 (5.41)	0.22 (0.05)	1.74 (0.82)
Aug.	53(53)	12.23 (6.00)	0.23 (0.06)	1.86 (1.00)
Sep.	62(55)	12.00 (6.07)	0.23 (0.07)	2.0 (1.20)
Total	695(694)			

Table 4. *Monthwise breeding frequency of seven pairs of* A. percula *under laboratory* condition (January 2002 to September 2005)

Pair Code	Years	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Av.Spa. ±S.D
Ap1	2002	2	2	4	3	3	2	1	1	1	1	3	1	2 ± 1.04
	2003	2	3	2	3	3	2	2	1	2	2	2	2	2.2 ± 0.58
	2004	3	3	3	3	2	2	2	2	2	2	3	3	2.5 ± 0.52
	2005	2	3	2	2	2	2	2	2	2				2.1 ± 0.33
	2002	-	-	2	2	3	2	2	2	2	3	2	2	2.2 ± 0.42
	2003	3	3	3	3	3	2	2	2	2	3	3	2	2.6 ± 0.51
Ap2	2004	3	3	4	3	3	2	2	2	2	3	3	3	2.8 ± 0.62
	2005	3	3	3	2	-	-	-	-					2.8 ± 0.5
	2002		2	3	2	2	1	2	2	2	2	2	3	1.9 ± 0.54
	2003	2	3	3	3	2	3	2	2	2	2	2	2	2.3 ± 0.49
Ap 3	2004	2	3	3	3	2	2	2	2	2	2	2	2	2.3 ± 0.45
	2005	2	3	4	3	2	2	2	-					2.6 ± 0.79
	2002	-	-	-	-	-	-	-	-	-	-	-	-	
	2003	-	-	2	2	2	2	2	2	2	2	2	3	2.1 ± 0.32
Ap4	2004	2	3	3	3	3	1	2	2	2	2	3	3	2.4 ± 0.67
	2005	3	4	3	3	3	2	2	2					2.8 ± 0.71
	2002	-	-	-	-	2	2	1	1	2	2	2	3	1.9 ± 0.64
	2003	2	2	2	2	2	2	2	2	2	2	2	2	2 ± 0.6
Ap5	2004	2	3	3	2	2	2	1	2	2	2	2	2	2.1 ± 0.51
	2005	3	3	3	3	2	2	2	-					2.6 ± 0.53
	2002	-	-	-	-	-	-	-	-	-	-	-	-	
	2003	-	-	-	-	-	-	-	-	-	-	-	-	
Ap6	2004	2	2	3	3	3	1	1	2	2	3	3	3	2.3 ± 0.78
	2005	2	2	4	3	3	2	-	-					2.7 ± 0.82
	2002	-	-	-	-	-	-	-	-	-	-	-	-	
	2003	-	3	2	2	2	2	1	1	1	2	2	2	1.8 ± 0.6
Ap7	2004	2	4	3	3	3	2	2	1	1	2	2	2	2.3 ± 0.87
	2005	-	-	-	-	-	-	-						

- No spawning

Fig.2. A pair of *A percula* with egg cluster deposited on the tiles at the final stage of spawning

spawning occurred between 0600 and 1545 hrs and lasted for one to one and a half hour. Each pair deposited 112 to 557 eggs (Fig. 2). During 6 to 7 days of incubation both the parents carefully looked after the embryos. The percentage of spawning showed relationship with lunar phase under laboratory condition (Table 5). In all the pairs, higher percentage of spawning was obtained 1 to 5 days after the full moon (I quarter) of lunar period followed by new moon (III quarter), IV quarter and II quarter *($p<0.05$)* and these indicate that the spawning in this species is highly correlated with lunar periodicity in Andaman waters.

Table 5. *Spawning (%) of* A. percula *in the Marine Research Laboratory of CARI*

	Moon phases			
	Full moon		New moon	
Pair code	I qr.	II qr.	III qr.	IV qr.
Ap1	42	11	33	14
Ap2	33	17	30	20
Ap3	33	13	30	24
Ap4	36	17	28	19
Ap5	32	16	27	25
Ap6	27	23	25	25
Ap7	42	15	28	15

Discussion

The protandric hermaphroditism as observed here has been reported in *Amphiprion* species (Allen, 1972; Fautin and Allen, 1997), *A. clarkii* (Hattori,1994), *A. polymnus, A. perideraion, A.sandaracinos, A. frenatus* and *A. ocellaris* (Moyer and Nakazono, 1978), *A. clarkii* and *A. frenatus* (Brusle-Sicard *et al.,* 1994; Nakamura *et al.,* 1994). In Andaman waters, each host anemone usually sheltered a social group of *A. percula* consisting of a sexually active pair of adults and one to three juvenile or sub-adult fishes. A similar social system and female/male ratio were reported in different sea anemone fishes (Fautin, 1991; Fautin and Allen, 1997). In the present study, the female to male ratio of 1:2.1 showing significant preponderance of males can be attributed to the protandric hermaphroditism and social structure of the anemone fishes. The length range at which the males and females attain maturity has been found to vary from species to species. Thus, while the males and females of *A. percula* as found in the present study matured between 20.0 and 54.9 mm, 55.0 and 99.9 mm total length (TL) respectively. The minimum body size (Standard length-SL) of breeding males and females reported were 85 and 90mm in *A. clarkii*, 40 and 85mm in *A. frenatus*,45 and 65mm in *A. perideraion* (Hirose, 1995) and 71 and 91 mm in *A. chrysopterus*, 47 and 56mm in *A. perideraion,* 61and 75mm in *A. tricinctus* (Allen, 1972) respectively.

Although the GSI value varied in different months with peak in January and less values during June to September in males and females, the size of gonad showed no significant relationship with their body weight, suggesting that spawning of *A. percula* occurs throughout the year. Year round spawning has also been observed in *A. perideraion* (Allen, 1972), *A. clarkii* (Bell, 1976), *A. akallopisos*, *A. ocellaris*, *A. bicinctus* (Fricke and Fricke, 1977), *A. frenatus, A. bicinctus* and *A. clarkii* (Moyer and Nakazono, 1978), *A. melanopus* (Ross, 1978), *A. ocellaris* (Juhl, 1992) and *A. akallopisos* (Gordon and Bok, 2001).

During the period 2002 to 2005, under laboratory conditions, all the pairs spawned a minimum of 2 times in the warmer months from January to May indicating that water temperature (26.1 to 33.0ºC) is also an influencing factor for the continuous spawning in *A. percula*. During this period the incubation time was also found shortened. In temperate seas, the reproductive activity is mostly restricted to warmer period of the year (spring and summer) whereas in the tropical seas, spawning is observed throughout the year in relation to lunar phases (Fautin and Allen, 1997). Similar results have also been reported in *Amphiprion* species (Bell, 1976; Hoff, 1998; Richardson *et al.,* 1997). The seasonally varying water temperatures and unpredictable environment in higher latitudes result in the prolongation of incubation period from 6 to 12 days in *A. clarkii* (Bell, 1976). Under such conditions, the time of hatching in the higher latitude would be unpredictable and as a result, may not be synchronized with the phases of the moon or associated tidal patterns (Ochi, 1985; Richardson *et al.,* 1997). On the other hand the spawning periodicity in tropically occurring clown fishes has been strongly correlated with lunar cycles (Allen, 1972; Ross, 1978; Fautin and Allen, 1997). In the present study, the fluctuation in average water temperature between months in the hatchery ranged from 26.1 to 33.0ºC throughout the period. The higher frequency of spawning during the warmer period can be related to the shorter incubation period (7 to 8 days) on account of higher water temperature and it also appears to be an indication of the stability of environmental conditions in the hatchery. The increase in spawning frequency was also reported in captive Amphiprioninae during warmer seasons (Hoff, 1998). Peak spawnings in *A. perideraion* were also recorded in February and April to August in Enewetak Atoll (Allen, 1972; Fautin and Allen, 1997) whereas in *A. clarkii* it was from early June to early October in Japan (Ochi, 1989).

In the present study, *A. percula* produced 2.3 ± 0.3 nest month^{-1} and laid 112 to 557 eggs nest^{-1} giving an annual number of 2500 to 13500 eggs pair^{-1} year^{-1}. The spawning showed a decreasing trend from June to October which may be due to the temperature fluctuation during the southwest and the northeast monsoons. Similarly, low spawning due to low water temperature has been reported in *A. akindynos* which spawned 6.7 times year^{-1} with 0.6 nest month^{-1} and 700- 5025 eggs per nest accounting an annual number of 2810 to 26,890 eggs. In *A. latezonatus* 8.0 spawnings in a year with 0.7 nest per month and 800-3870 eggs per nest totaling an annual number of 10470 to 33140 eggs showed that the low water temperatures and unstable environmental conditions of temperate regions may restrict the spawning of anemone fishes to the summer months (Richardson *et al.*,1997). Though reproduction is continuous throughout the year with a monthly spawning rhythm, the gonad showed little growth difference from month to month which may be due to different stages of gametogenic development with little synchrony amongst the individuals, indicating that being a partial spawner, individuals reach maturity and spawning at different times throughout the year in *A. percula* as also reported in *Tripneustes gratilla* (Muthiga, 2005). *A. percula* in the present study showed a reproductive rhythm that coincided closely with changing phases of the moon. Peak spawning was obtained just after the beginning of first quarter (full moon phase) and decreased to minimum in the second quarter. The GSI values also showed the same trend during these lunar phases. It is believed that fluctuations in gonad are influenced by the lunar phase which also coincides with increase in the spawning frequency. Though a slight variation in the spawning occured during the different months, significant variations ($p<0.05$) were noticed in different phases of the moon. This implies that *A. percula* under captive conditions spawns according to lunar rhythms. The moon's influence on the reproductive behavior of temperate marine organisms (Clark, 1925; Korringa, 1947) and in tropically occurring marine fishes (Johannes,1978) has long been recognized. Our laboratory observations indicated that increase in the reproductive activity of *A. percula* coincided with the waxing of the moon. Among the 4 lunar quarters, increased number of spawnings were observed in the lunar quarter: 1 to 5 after the full moon and new moon in the entire period of study. There is a significant influence of lunar phase on the number of spawning under laboratory condition ($p<0.05$). Similar results in increased percentage of spawning over new and full moon in *A. akallopisos* were also recorded (Gordon and Bok, 2001) and *A. akindynos* had more spawnings in the lunar quarters before and after full moon. *A. latezonatus* had more spawning after full moon, before new moon, and after the new moon than the other quarter (Richardson *et al.,* 1997).

Though reproductive activity was high within the whole period of full moon phase (I quarter) and new moon phase (III quarter), there were also few spawnings in the IV and II quarters. The adaptive significance of such spawning behaviour is unclear, but these results can be compared with some of the numerous hypotheses used to explain lunar spawning cycles in marine fishes (Pressly, 1980). Among pomacentrids, especially those with only one spawning peak per month, the lunar spawning cycles is correlated to the fact that moonlight improves the conditions for nocturnal egg care and helps photopositive larvae to avoid benthic predators. The peak spawning activity precedes the full moon by several days, so that hatching occurs when nocturnal moonlight is maximum (Allen, 1972; Moyer, 1975). In the present study the highest spawning activity in *A. percula* was observed from one to five days after full moon which coincided with the time when there was sufficient moonlight just prior to dawn so that parents could provide nocturnal egg care. Thus the study concluded that under captive conditions, the spawning of *A.percula* is influenced by lunar periodicity and water temperature, and most spawning could be expected during the period in the order of full moon (I quarter) followed by new moon (III quarter), IV quarter and II quarter in all the matured breeding pairs under water temperature of 26.1 to 33.0ºC.

Acknowledgements

The authors are highly indebted to Dr R.B. Rai, Director, CARI and its former Director Dr. S.P.S. Ahlawat for the kind support and facilities provided. The authors wish to express their sincere thanks to Dr.D.G.Fautin and

Dr. H. Ochi for providing valuable references. We are grateful to Dr. K. J. Mathew, Former Principal Scientist, CMFRI, Cochin for his valuable suggestions and to Dr. Somy Kuriakose and Dr. J. Jayasankar for the statistical analysis. The authors are also thankful to Mr. Bipul Chandra Ray, Mr. Haldar and Mr. Horan Naskar for their assistance in the filed work. The assistance of all members of the Marine Research Laboratory is highly appreciated.

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Received: 13 March 2007
Accepted: 15 May 2007