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Determining the level of parental care relating fanning behavior of five species of clownfishes in captivity

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Clownfishes ***Amphiprion sebae, A. clarkii, A. percula, A. ocellaris and Premnas biaculeatus*** were reared in captive condition and their parental care activities were studied. Role of both male and female in parental care and terminating in successful hatching of eggs studied is described. Site selection, clutch formation, spawning, guarding, mouthing and fanning of the eggs were quantified. ***A. sebae*** exhibits more effective parental care for longer durations. The time spent on fanning was longer in ***A. sebae*** and shorter in ***A. clarkii.*** Fanning frequency was higher in ***A. percula*** but lower in ***A. clarkii.*** In the five species the fanning duration and frequency were positively correlated to hatching success. Gradation of dissolved oxygen levels adjacent to the clutch area due to the fanning behavior is discussed. Relation between body weight and the fecundity, fanning duration and frequency with hatching success are documented.

Key wards: Clownfish, Parental care, Spawning, Fanning, Hatching success

Introduction

With smaller burly size and brilliant colors, the ornamental clownfishes are in great demand. Hence breeding of marine clownfishes in captivity may to ensure their conservation in nature and fetch additional income to coastal folk. In the aquarium trade, the clownfish are predominant and have precious value. Clownfish exhibit protoandrous hermaphrodism, which is denotes first male and the most common mode of their reproduction is egg deposition on a secure substrate, usually in close proximity to the anemone, with a distinct type of clutching behavior. Eggs are termed as demersal and result in relatively larger fry. According to many studies, the effort a male expends on parental care is positively correlated with the certainty of paternity[1-3], but this is not always the case[4,5]. In clownfish males most often provide care for the eggs, when care at all is provided[6]. In the clownfish the male cares for the eggs by fanning and guarding them until they hatch, which takes about 6 to 10 days. Instinct parental care to protect their eggs and young ones during the vulnerable periods is of great value. Aim of the present study is to document the parental care exhibited by five different species of clownfish to understand their behavior during the incubation period.

Materials and Methods

Description of the clownfishes

Among the different marine ornamental fishes, clownfishes are ranked as one of the most popular attractions all over the world. Their small size, hardness, pretty colour features, high adaptability to live in captivity, acceptability of artificial diet, their fascinating display behavior and symbiotic relationship with sea anemones are the important features. The clownfishes belong to the subfamily, Amphiprioninae and the family, Pomacentridae; 30 species are recorded, of which one of the selected clownfish belong to the genus ***Premnas*** and others to namely belong to ***Amphiprion sebae***, ***A. clarkii***, ***A. percula***, ***A. ocellaris***.

Sebae clownfish [***Amphiprion sebae*** (Bleeker, 1853)]

Size of the fish, Male, 7.5 and Female, 11.5 cm (Fig. 1a)

Dark brown to blackish with two white bars, the mid body bar starting slight backwards and extending on to rear part of dorsal fin. Snout, breast and belly often yellow orange, tail yellow.

Clark's clownfish [***Amphiprion clarkii*** (Benneff, 1830)]

Size of the fish, Male, 7.0 cm and Female, 9.0 cm (Fig. 1b)

Usually black with variable amount of orange on head, ventral parts and fins, three milky white bars on head, body, base of caudal fin, transition between darker body and bar across caudal fin base usually abrupt, caudal fin whitish yellow edges but sometime yellow.

Percula clownfish [***Amphiprion percula*** (Lacepede, 1802)]

Size of the fish, Male, 6.4 cm and Female, 7.5 cm (Fig. 1c)

Bright orange with three white bars, the middle with forward projecting bulge, bars often outlines with black that varies in width. Although ***A. percula***'s vibrant colours are eye catching, it is easily confused with ***A. ocellaris*** (false clownfish). However, one can distinguish the two by counting the number of dorsal-fin spines. The ***A. percula*** usually has 10 dorsal-fin spines, while ***A. ocellaris*** usually has 11 and also, the latter never has thick black margins outlining the fins.

False clownfish [***Amphiprion ocellaris*** (Cuvier, 1830)]

Size of the fish, Male, 6.5 cm and Female, 8.0 cm (Fig.1 d)

Normally bright in orange colour with three white bars, the middle one with forward projecting bulge, bars have narrow black margins or disappeared. It is really identical, but never a thick black margin around the white bars.

Maroon clownfish [***Premnas biaculeatus*** (Bloch, 1790)]

Size of the fish, Male, 7.5 cm and Female, 10.5 cm (Fig. 1e)

Bright red to brownish-red in colour with three relatively narrow white or grey bars, all fins same colour as body, usually with a long spine in both opercula. Male is brighter red than female with brilliant white stripes and the female bars generally grey, but can be switched rapidly to white if fish is motivated.

Species acclimatization in captive environment

The study was carried from June 2008 to February 2009 in Faculty of Marine Sciences marine ornamental hatchery. Six numbers in each of the selected species of the clownfishes and three numbers in each species of the anemones (***Stichodactyla mertensii*** and ***Heteractics gigantica***) were procured from the fish suppliers from Kolathur, Chennai. They were transported to the hatchery and accommodated in separate conditioning tanks. After the pair formation, the potential couple of each species was shifted to separate spawning tanks (1000 l capacity) along with the host anemone. The tank was provided with live rock, dead corals and shells to mimic those of the natural habitat. The fishes were fed daily at 9:00-9:30 a.m., 1:30-2:00 and 6:00-6:30 p.m. with boiled oyster and prawn meat and anemones fed with prawn meat daily ones. After two months, fishes were started spawning.

Behavioral observation

Behavior of the fishes to clean the substratum, lay eggs, guard the eggs was recorded and hatch out was documented. Every fanning bout was taken to start, when the male's pectoral fin began to ventilate the egg clutch, and when the male stopped fanning and began to swimming around. The fanning duration was estimated since laying till hatching of fry. Fanning duration (minutes), frequency (times/hour) and hatching success (%) of each clutch of each species were obtained by using stop watch and the data were recorded from continues observation during the morning, afternoon, evening and dusk periods in a day before feeding. The means of fanning duration, frequency and hatching success were calculated for each species. Hatching success was estimated by dividing the number of hatchling from the initial number of eggs in a clutch. Effect of dissolve oxygen and its changes in the vicinity area of the eggs was estimated in each species by using D.O. meter (Singapore ECOSAN).

Results

Fanning time

Before making the clutch, the parents of ***A. sebae*** cleared the so called clutch area by removing algae on the substratum. Generally, the shape of the clutch area was round or oval. Eggs were deposited in a clutch and attached to the substratum with sticky fluid. Clutch diameter ranged from 4.5 cm to 8.5 cm and the number which increased with size of the female. Fecundity ranged between 600 and 1500 eggs and the male care of the eggs by ventilating continuously by pectoral fins; the female also took the responsibility but occasionally.

The longest and shortest duration of fanning by the male were 1.43 minutes and 0.43 minutes (one way ANOVA $\boldsymbol{F}_{8,39}= 3.31$, $\boldsymbol{p}<0.05$) (Figure 2a). In the case of female, maximum and minimum fanning duration were 0.92 minutes and 0.03 minutes respectively (one way ANOVA $\boldsymbol{F}_{8,39}= 13.81$, $\boldsymbol{p}=$ NS) (Figure 2b).

In the case of ***A. clarkii,*** the clutch diameter varied between 3.5 - 4.5 cm and the fecundity ranged 500-800 eggs in each spawning. Its fecundity was lesser than ***A. sebae*** but more than that of ***A. percula*** and ***A. ocellaris***. The maximum and minimum fanning durations by the male were 1.12 minutes and 0.39 minutes respectively (one way ANOVA $F_{9, 28}$= 23.43, ***p***= NS) (Figure 3a) and in the case of the female, it was 0.7 minutes and it was also noticed that in few occasion there were no fanning for an hour (one way ANOVA $F_{9,28}$= 3.94, p<0.05) (Figure 3b).

In ***A. percula,*** the parents made a clutch which was oval in shape and the eggs were deposited and attached to the substratum with the sticky end. The clutch area diameter varied from 3.5-5.0 cm and the fecundity ranged between 300-500 eggs. The male was fanning the eggs continuously but the female did it occasionally. The maximum and minimum fanning duration by the male were 1.62 minutes and 0.37 minutes respectively (one way ANOVA $F_{8,40}$= 8.69, ***p***=NS) (Figure 4a) and in the case of female, the maximum and minimum fanning duration in total

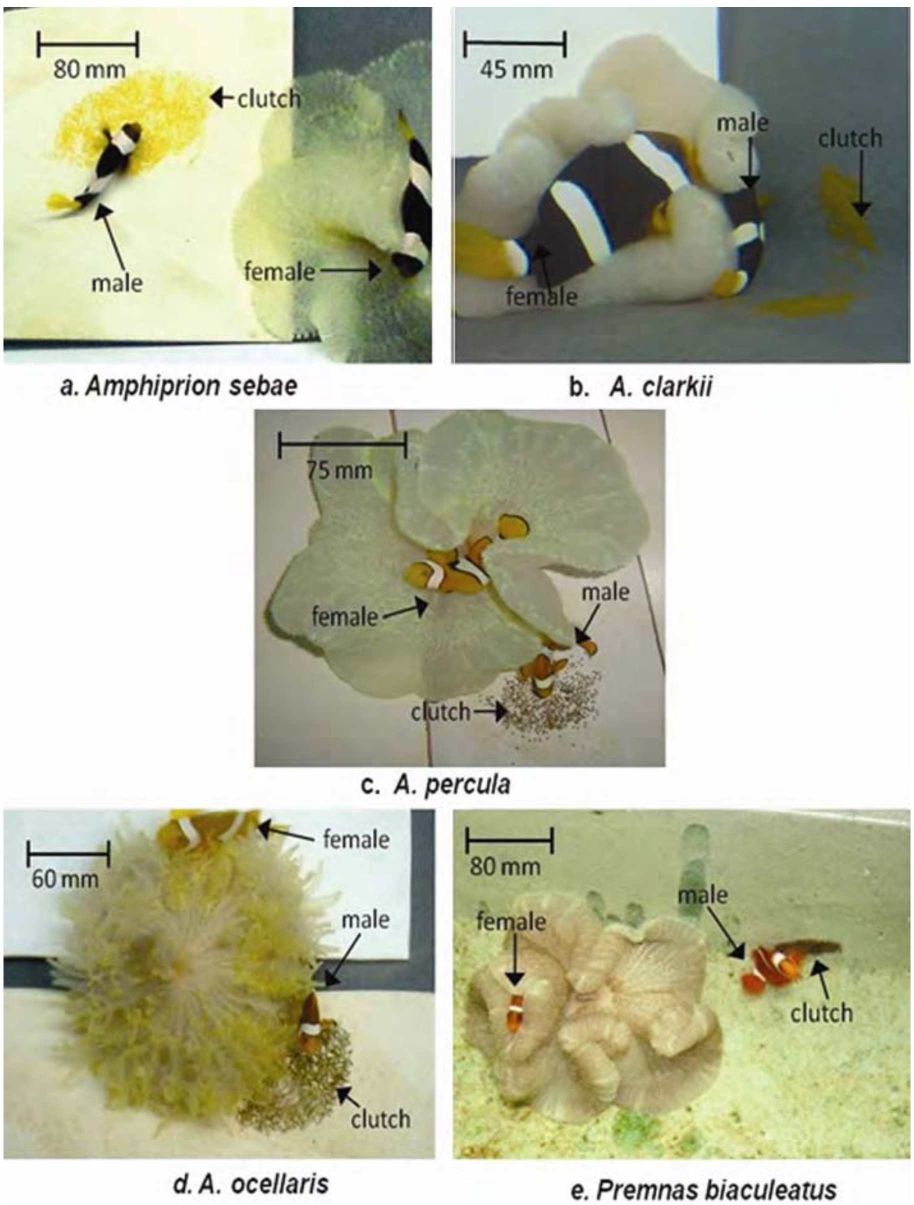

Fig. 1—Different species of clownfish with deposited eggs a. ***Amphiprion sebae***, b. ***A. clarkii, A. percula, A. ocellaris*** and e. ***Premnas biaculeatus***.

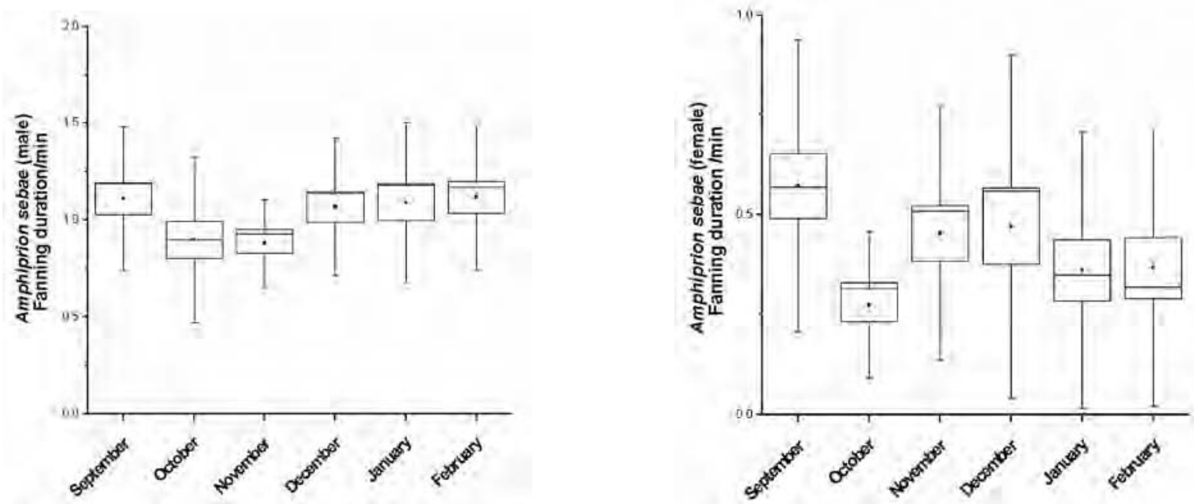

Fig. 2—Fanning duration (Sep. - Feb.) in ***A. sebae*** (2a) male and (2b) female (Error bar - SE, □ - SD and ■ - mean).

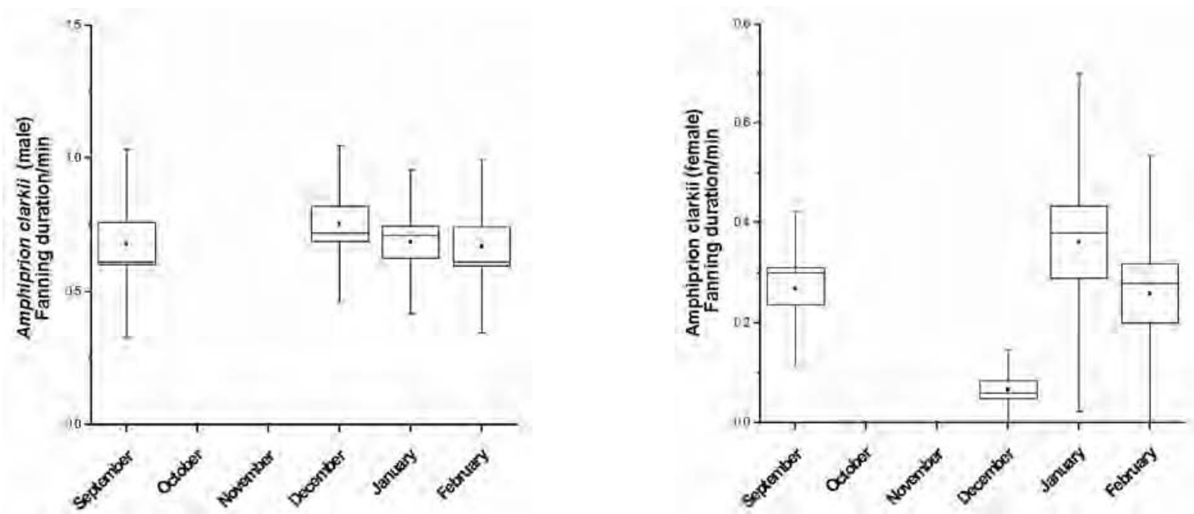

Fig. 3—Fanning duration (Sep. - Feb.) in ***A. clarkii*** (3a) male and (3b) female (Error bar - SE, □ - SD and ■ - mean).

fanning period were 0.88 minutes and 0.06 minutes respectively (one way ANOVA $\boldsymbol{F}_{8,40}$= 26.20, ***p***= NS) (Figure 4b).

In ***A. ocellaris***, the parents made the clutch diameter varied from 3.5-4.5 cm. Fecundity of this fish ranged between 200-500 eggs. Here, both the male and female parents fanned vigorously. Male was involved in mouthing the clutch and removing the unfertilized and dead eggs. The maximum and minimum fanning done by the male were 1.67 minutes and 0.37 minutes (one way ANOVA $\boldsymbol{F}_{8,39}$= 3.47, ***p***<0.05) (Figure 5a) and that of the female were 0.92 minutes and 0.05 minutes respectively (one way ANOVA $\boldsymbol{F}_{8,39}$= 6.00, ***p***<0.05) (Figure 5b).

In ***P. biaculeatus,*** the clutch area diameter was 4-5 cm. Fecundity of this fish ranged from 300-600 eggs. Mostly, the male was caring the eggs and the female spent maximum time with the sea anemone. The maximum and minimum fanning made by the male were 1.67 minutes and 0.39 minutes respectively (one way ANOVA $\boldsymbol{F}_{9,35}$= 1.88, ***p***= NS) (Figure 6a). In the case of the female, maximum and minimum fanning duration were 0.92 minutes and 0.01 minutes respectively (one way ANOVA $\boldsymbol{F}_{9,35}$= 37.16, ***p***= NS) (Figure 6b).

Fishes of the genera, ***Amphiprion*** and ***Premnas*** have obligate associations with anemones and lay their eggs on the hardy substratum under the shelter of the anemone's stinging tentacles (Wilkerson, 1998).

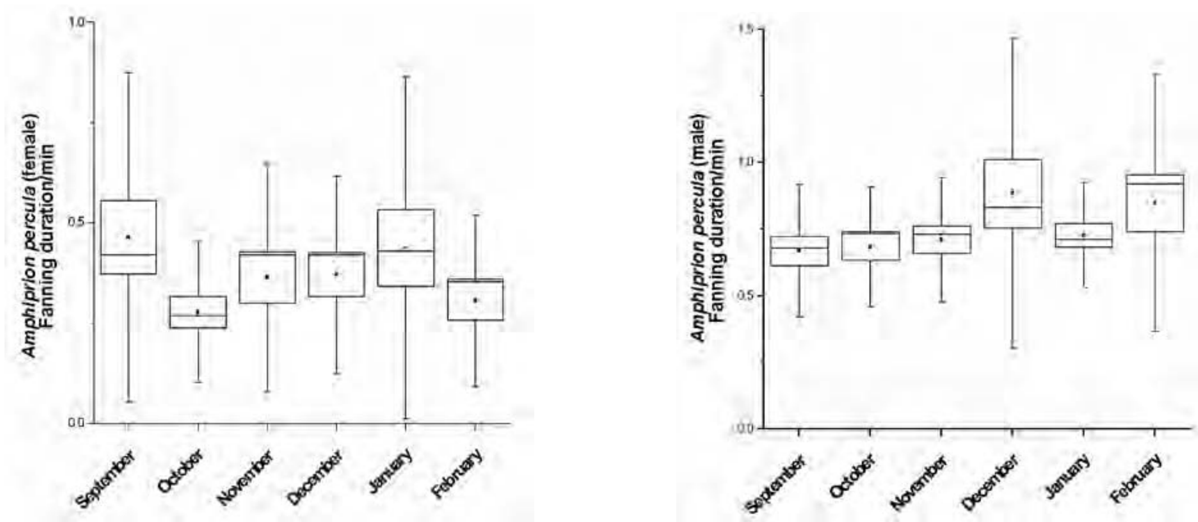

Fig. 4—Fanning duration (Sep. - Feb.) in ***A. percula*** (4a) male and (4b) female (Error bar - SE, □ - SD and ■ - mean).

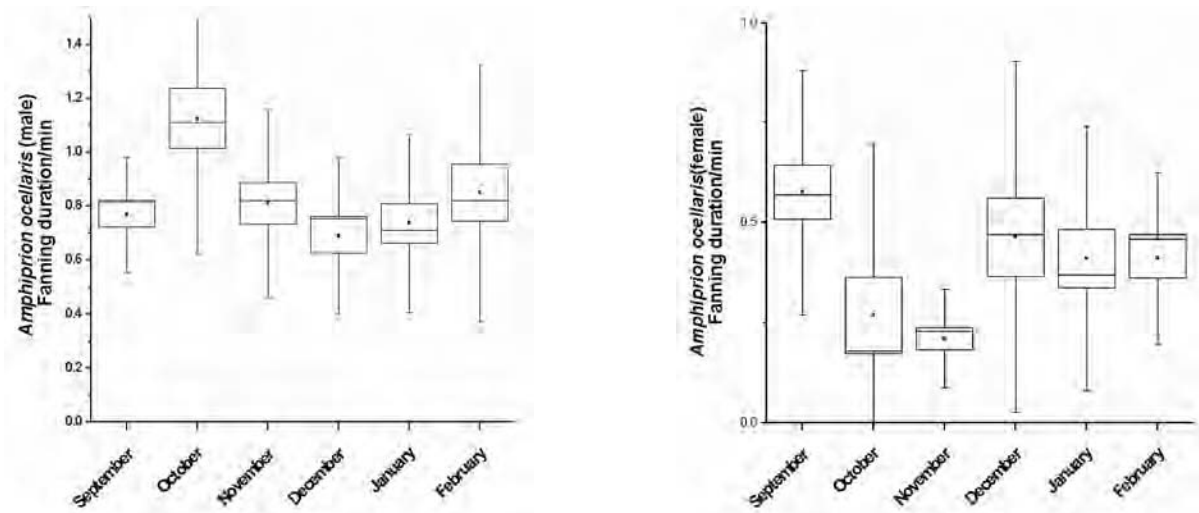

Fig. 5—Fanning duration (Sep. - Feb.) in ***A. ocellaris*** (5a) male and (5b) female (Error bar - SE, □ - SD and ■ - mean).

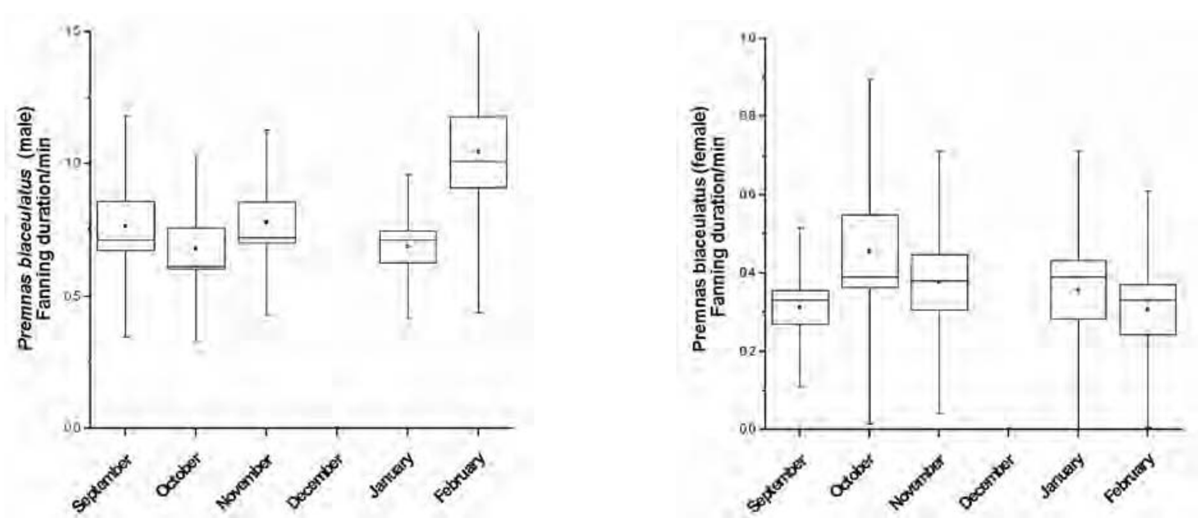

Fig. 6—Fanning duration (Sep. - Feb.) in ***Premnas biaculeatus*** (6a) male and (6b) female (Error bar - SE, □ - SD and ■ - mean).

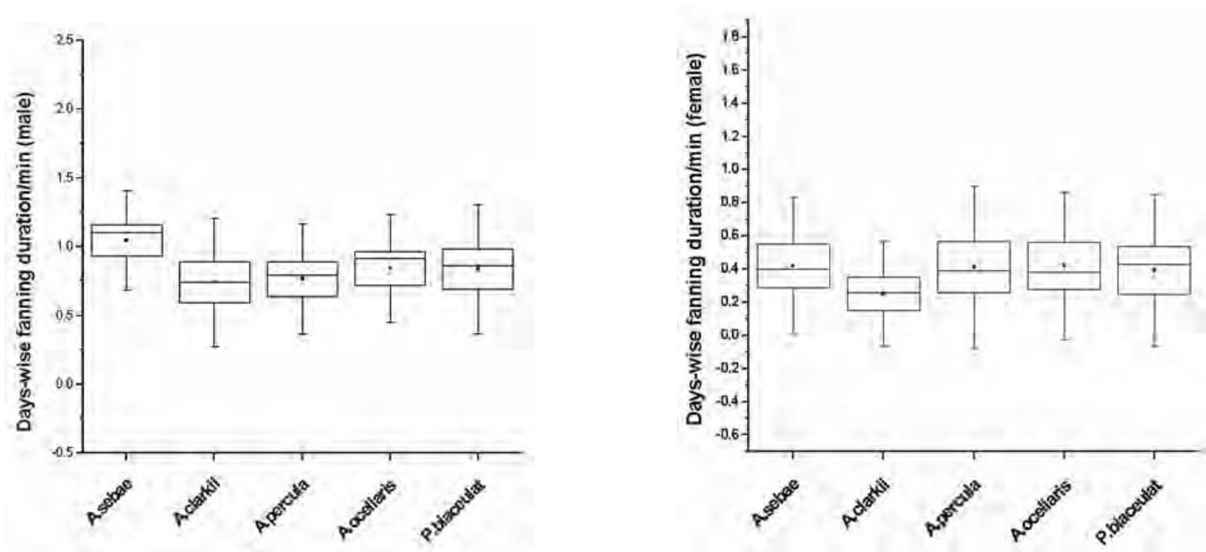

Fig. 7—Average fanning duration monthly variation (Sep. - Feb.) in five clown fishes (7a) male and (7b) female (Error bar - SE, □ - SD and ■ - mean).

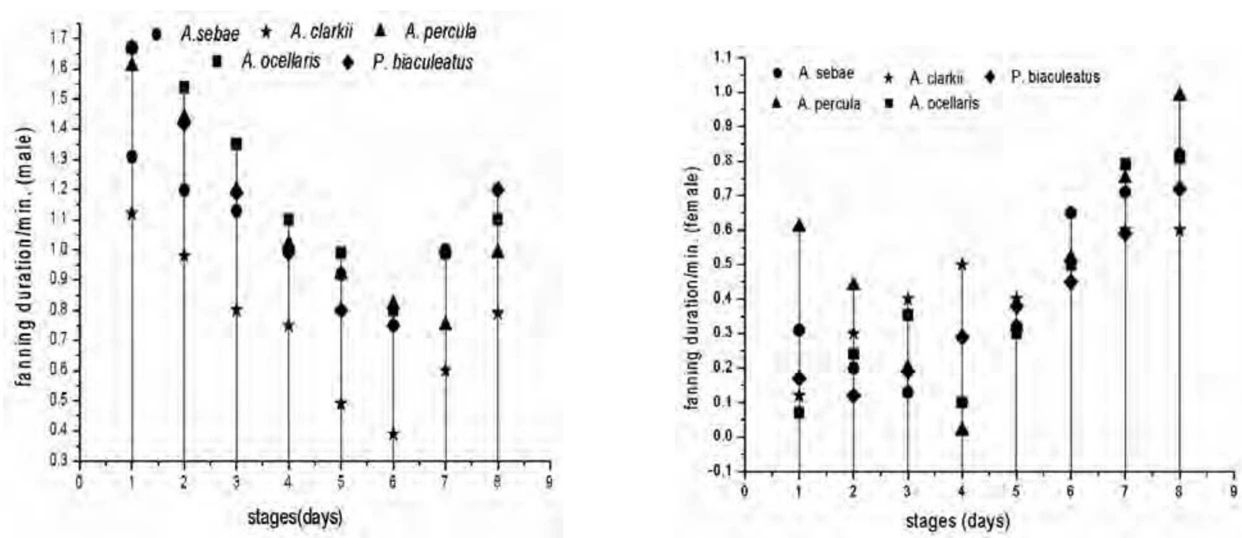

Fig. 8—Comparing fanning duration with incubation period (stages days wise) in five clown fishes (8a) male and (8b) female.

Similar observation was recorded in all our five species. The average fanning duration of male anemone fishes viz., ***A. sebae***, ***A. clarkii***, ***A. percula***, ***A. ocellaris*** and ***P. biaculeatus*** have mean (± S.D.) viz. 1.32 (±0.13), 0.65 (±0.34), 1.10 (±0.31), 1.20 (±0.28), 1.19 (±0.32) minutes (one way ANOVA $F_{4,29}= 4.70$, $p<0.05$) (Figure 7a) and in the case of female were the mean (± S.D.) 0.80 (±0.22), 0.30 (±0.29), 0.65 (±0.19), 0.62 (±0.28) and 0.56 (±0.31) minutes respectively (one way ANOVA $F_{4,29}= 2.93$, $p<0.05$) (Figure 7b). In the present study, fanning duration also illustrated with the incubation period, which is refers to their everyday fanning ability on the clutch. In case of male, fanning on first three were more compare to fourth to sixth days and again increased fanning on the days of seventh to up to hatching days (Figure 8a). In case of female, it has linear relations and gradually increased from the first day to till before hatching (Figure 8b).

Trends in the fanning mechanism have revealed that the parents are adjusting their fanning timing in response to oxygen availability. In the present study, it was observed that ***A. sebae, A. percula*** and ***A. ocellaris*** bred throughout the six months period at regular intervals and fanned their eggs normally. In the case of ***A. clarkii***, it did not spawn for two months period and ***P. biaculeatus*** did not breed for one month. The average fanning duration of the male fishes during the study period (two way ANOVA $\boldsymbol{F}_{5,29}=1.21$, $\boldsymbol{p}=$ NS and $\boldsymbol{F}_{4,29}=3.68$, $\boldsymbol{p}<0.05$) were mean (±S.D.) 1.32 (±0.13) minutes in ***A. sebae***, and in ***A. clarkii*** 0.54 (±0.49) minutes.

Generally ***A. clarkii*** spent maximum time spent along with sea anemone and showing less parental care and hatching duration also more compare to ***A. sebae.*** In ***A. percula***, ***A. ocellaris*** and ***P. biaculeatus*** mean (± S.D.) fanning times were 1.10 (±0.31), 1.20 (±0.28), 1.07 (±0.57) minutes respectively. In the female fish of ***A. sebae***, ***A. clarkii***, ***A. percula***, ***A. ocellaris*** and ***P. biaculeatus***, the mean (±S.D.) fanning times were 0.80 (±0.22), 0.30 (±0.29), 0.65 (±0.19), 0.62 (±0.28) and 0.56 (±0.31) minutes respectively (two way ANOVA $\boldsymbol{F}_{5,\ 29}=1.75$, $\boldsymbol{p}=$ NS and $\boldsymbol{F}_{4,29}=3.37$, $\boldsymbol{p}<0.05$).

Fanning frequency

In the present study, the fanning frequency indicating cares taking capacity of parents and the development of eggs for successful hatching. The average fanning frequency of male clownfishes viz. ***A. sebae***, ***A. clarkii***, ***A. percula***, ***A. ocellaris*** and ***P. biaculeatus*** was mean (±S.D.) 40.18 (±5.45), 42.68 (±3.48), 35.75 (±6.33), 42.00 (±4.37), 39.02 (±4.47) / hrs (one way ANOVA $\boldsymbol{F}_{4,29}=2.70$, $\boldsymbol{p}<0.05$) (Figure 9a) and in the case of females, the average fanning frequency was mean (±S.D.) 5.33 (±1.57), 6.85 (±0.55), 5.23 (±4.09), 5.5 (±0.72) and 5.68 (±2.84) / hrs respectively (one way ANOVA $\boldsymbol{F}_{4,29}=0.47$, $\boldsymbol{p}<0.05$) (Figure 9b). Present finding also revealed that fanning frequency with the incubation period, which is refers to their everyday number of fanning time on the clutch/hr. In case of male, frequency are first three days more then gradually decreased fourth to sixth days and again increased fanning on the days of seventh to up to hatching days (Figure 10a). In case of female, fanning frequency gradually increased from the first day to before hatching (Figure 10b). Among the five species, ***A. percula*** and ***A. ocellaris*** took more care of their eggs and ***A. clarkii*** showed lesser care than others. In the present study, fanning duration, fanning frequency and hatching success varied with varying species (Table 1 and 2).

Hatching success

The fanning time and frequency correlated well with the hatching success of individuals, revealing the fact that the hatching of eggs depends on the fanning and mouthing of the parent clownfish. The average hatching success during the study period varied with varying species viz. ***A. sebae, A. clarkii, A. percula, A. ocellaris and P. biaculeatus*** were mean (± S.D.) 87.18 (±6.83), 46.64 (±36.45), 84.34 (±5.35), 87.47 (±11.34), 87.47 (±31.92) % (one way ANOVA $\boldsymbol{F}_{4,29}=3.84$, $\boldsymbol{p}<0.01$) respectively (Figure 11).

Effect of Dissolved oxygen

Present finding also sustaining that the differences between pre-fanning dissolved oxygen and post-fanning dissolved oxygen variation were measureed, which signifying cares captivating ability of parents and the development of eggs for successful hatching. The average increased dissolved oxygen lavel in

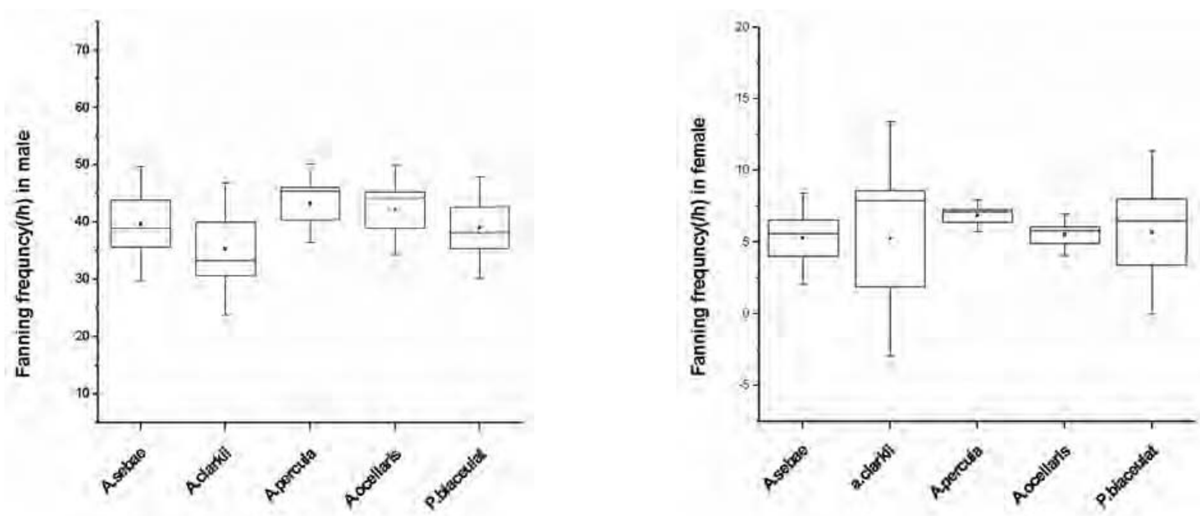

Fig. 9—Comparing fanning frequency in five clown fishes (9a) male and (9b) female (Error bar - SE, □ - SD and ■ - mean).

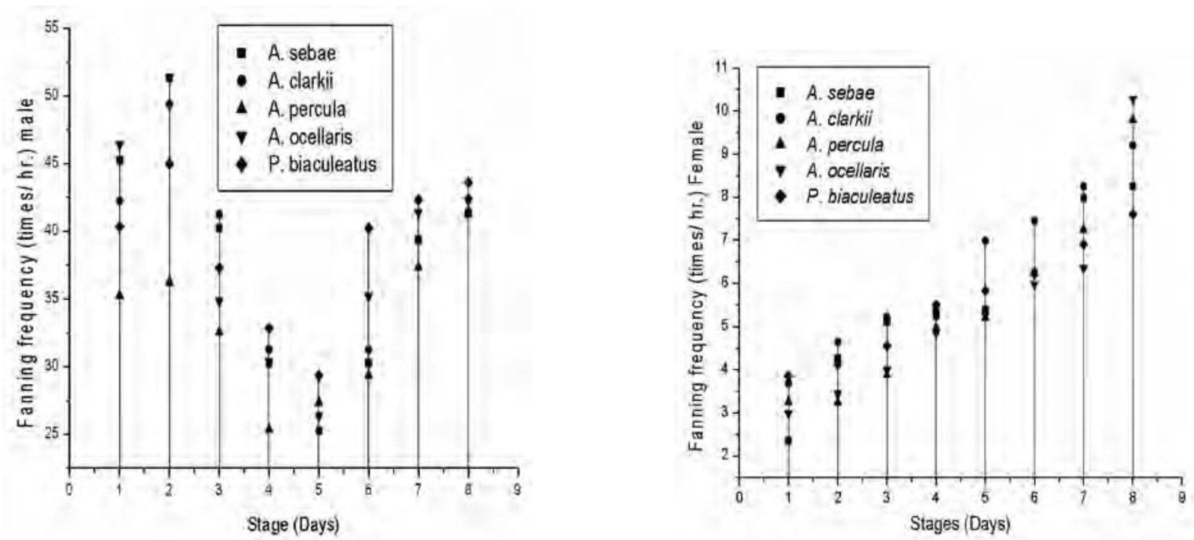

Fig. 10—Comparing fanning frequency with incubation period (stages days wise) in five clown fishes (10a) male and (10b) female.

Table 1—Variable measured in male and female with this five anemone fishes fanning paternity

Sl.No.	Species	Degree of paternity (fanning) Duration (min.)	Frequency (time/h)	Hatching success (%)
1.	***A.sebae***			
	Male	1.3 (± 0.13)	40.2 (± 5.45)	87.2 (± 6.83)
	Female	0.8 (± 0.22)	5.3 (± 1.57)	
2.	***A.clarkii***			
	Male	0.5 (± 0.34)	35.8 (± 6.33)	46.6 (± 36.45)
	Female	0.3 (±0.29)	5.2 (± 4.09)	
3.	***A.percula***			
	Male	1.1 (± 0.31)	42.7 (± 3.48)	84.3 (± 5.35)
	Female	0.7 (± 0.19)	6.9 (± 0.55)	
4.	***A.ocellaris***			
	Male	1.2 (± 0.28)	42.0 (± 4.37)	87.5 (± 11.34)
	Female	0.6 (± 0.28)	5.5 (± 0.72)	
5.	***P.biaculeatus***			
	Male	1.1 (± 0.32)	39.0 (± 4.47)	87.5 (± 31.92)
	Female	0.6 (± 0.31)	5.7 (± 2.84)	

All values given are means (± SD) from each pair in all five cultured anemone fishes.

Table 2—Variable measured in male and female with this five anemone fishes fanning observation

Sl.No.	Species	Ob/D	Ob/I	Ob/H	TOB
1.	***A.sebae***				
	Male	9	5	11	99
	Female	9	5		
2.	***A.clarkii***				
	Male	9	5	5	45
	Female	9	5		
3.	***A.percula***				
	Male	9	5	12	108
	Female	9	5		
4.	***A.ocellaris***				
	Male	9	5	13	117
	Female	9	5		
5.	***P.biaculeatus***				
	Male	9	5	7	63
	Female	9	5		

Ob/D- observation/day, Ob/I- observation/ incubation, Ob/ H- observation / no of hatching, TOB- total number of observation

clownfishes tanks viz. ***A. sebae***, ***A. clarkii***, ***A. percula***, ***A. ocellaris*** and ***P. biaculeatus*** were mean (±S.D.) 1.71 (±0.05), 0.46 (±0.06), 1.04 (±6.33), 0.96 (±0.07), 0.68 (±0.38) mg/lit (one way ANOVA $F_{4,29}$= 7.02, p<0.05) (Figure 12). The dissolved oxygen depends on days wise developmental stages involved fanning in selective male species of male clownfishes viz. ***A. sebae***, ***A. clarkii***, ***A. percula***, ***A. ocellaris*** and ***P. biaculeatus*** were mean (±S.D.) 1.06 (±0.18), 0.74 (±0.24), 1.09 (±0.30), 1.19 (±0.29), 1.13 (±0.31) mg/lit (one way ANOVA $F_{4,39}$= 3.46, p<0.01) (Figure 13). The average increased dissolved oxygen depends on days wise developmental stages involved fanning in selective male species of female clownfishes viz. ***A. sebae***, ***A. clarkii***, ***A. percula***, ***A. ocellaris*** and ***P. biaculeatus*** were mean (±S.D.) 0.40 (±0.28), 0.43 (±0.16), 0.48 (±6.31), 0.39 (±0.28), 0.36 (±0.21) mg/lit (one way ANOVA $F_{4,39}$= 3.36, p<0.01) (Figure 14).

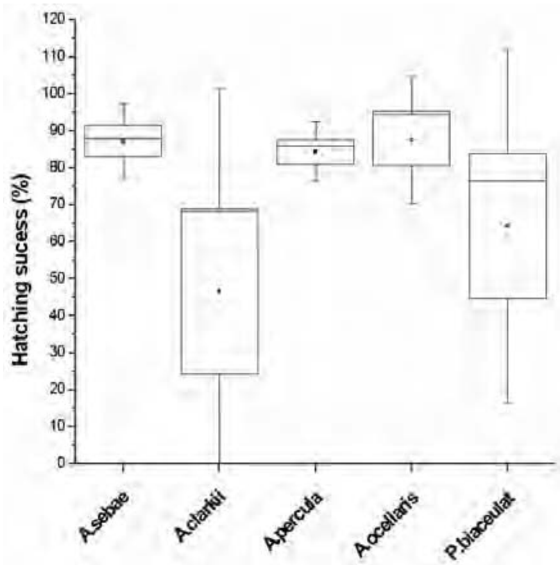

Fig. 11—Hatching success in five clown fishes (Error bar - SE, □ - SD and ■ - mean).

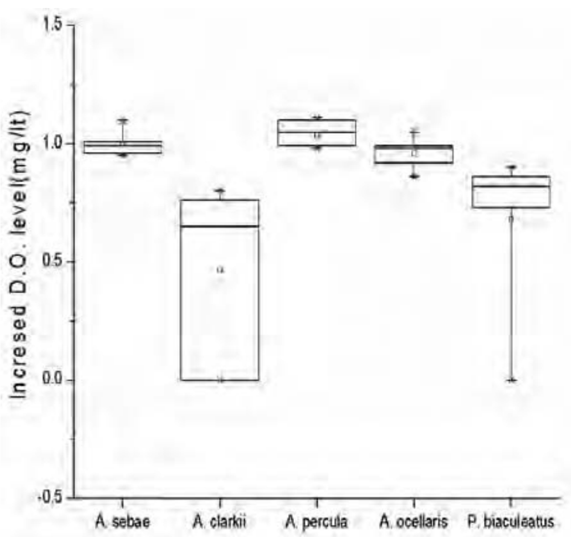

Fig. 12—Increased Dissolved oxygen (D.O.) after fanning in selective species (Error bar - SE, □ - SD and ■ - mean).

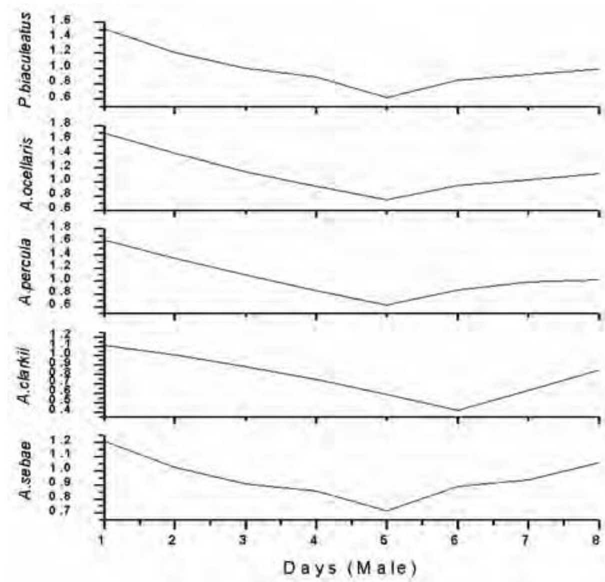

Fig. 13—Increased Dissolved oxygen (D.O.) depends on days wise developmental stages involved fanning in selective male species.

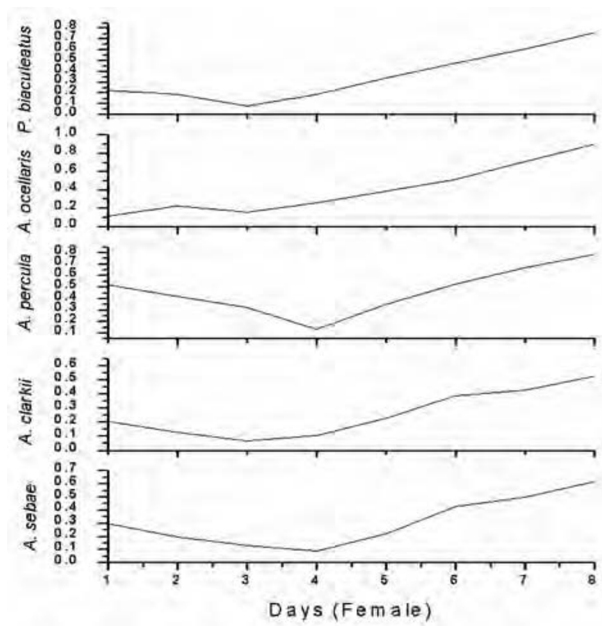

Fig. 14—Increased Dissolved oxygen (D.O.) depends on days wise developmental stages involved fanning in selective female species.

Total body weight

The relation between number of eggs and total body weight involved fanning in selective female species are also studied in the same experiment. This different weight of fishes and their relation with the fecundity were variables (one way ANOVA $\boldsymbol{F}_{4,64}$= 1.21, $\boldsymbol{p}$<0.01) (Figure 15). In ***A. sebae*** maximum weight was observed, compare to ***P. biaculeatus, A. clarkii, A. ocellaris*** and ***A. percula***, where average body weight was 15±2.89, 14±3.11, 13±3.34, 12±2.5 and 12±2.0 gm, respectively. The average egg laying capability of the fishes in the present study are 1200, 400, 350, 400 and 800 number per spawning in ***A. sebae***, ***A. clarkii***, ***A. percula***, ***A. ocellaris*** and ***P. biaculeatus.*** The total body weight of the female and male shows the relation with the fanning time (one way ANOVA $\boldsymbol{F}_{4,64}$= 3.36, $\boldsymbol{p}$<0.01) (Figure 16 & 17) and fanning frequency (one way ANOVA $\boldsymbol{F}_{4,64}$= 3.36, $\boldsymbol{p}$<0.01) (Figure 18 & 19).

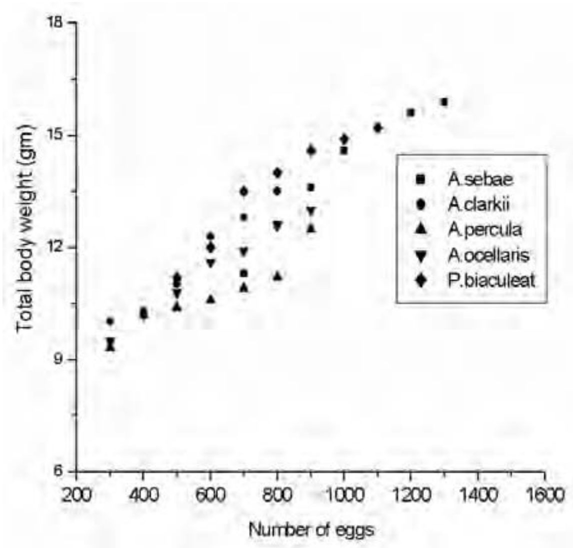

Fig. 15—Relation between number of eggs and total body weight (gm) involved fanning in selective female species.

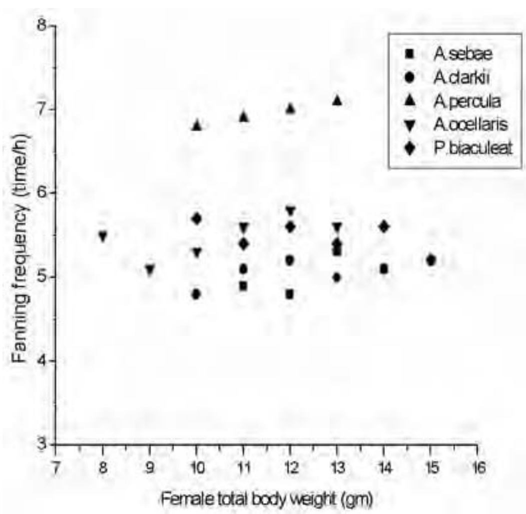

Fig. 16—Relation between female total body weight (gm) and Fanning frequency (time/h).

Discussion

Parental care consists of fanning and mouthing the eggs, in addition to oxygenation and removal of metabolic wastes[7]. Mouthing would remove dead eggs and clean the live ones[7]. Further timing of fanning has been negatively correlated with oxygen concentration within fish clutches throughout development[8-13].

Parental care is maintaining the health of a brooder in many organisms such as amphipods[14], crabs[8] and fishes[15]. Rombough[16] found that in the clownfish, ***A. melanopus***, such behavior was necessary because of the semicryptic areas with poor water circulation where the demersal eggs were laid and the boundary

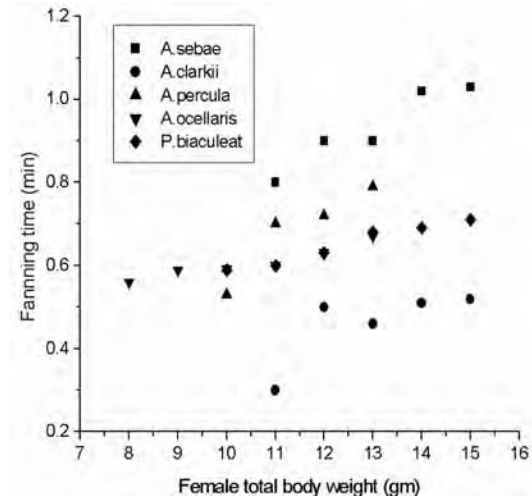

Fig. 17—Relation between female total body weight (gm) and Fanning time (min) involved fanning in selective female species.

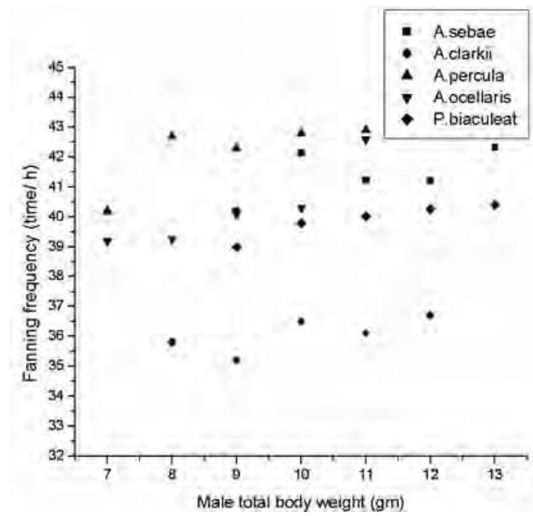

Fig. 18—Relation between male total body weight (gm) and Fanning frequency (time/h).

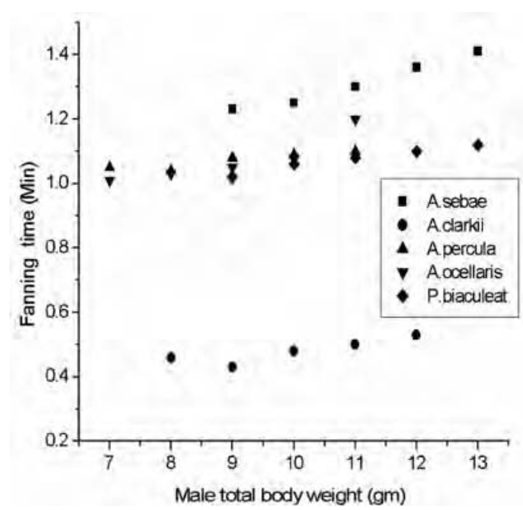

Fig. 19—Relation between male total body weight (gm) and Fanning time (min) involved fanning in selective male species.

layers formed rapidly around the eggs, reducing the transfer of oxygen from the surrounding water to the developing embryos. Males normally spend more time than females in active egg fanning, which is typical of the genus ***Amphiprion***[17-19] and in the present study it was well noticed.

Results have revealed that the eggs in the clutch area developed rapidly when the male fanned properly (Table 3 and 4). This suggests that the male would be able to adjust its paternal care according to the amount of energy and time he puts into the egg clutch. Other studies have also shown that the male is more alert in defending more valuable broods[20]. Thus, with regard to fanning and mouthing, the male with full paternity invests more effort in the clutches than the female.

Many studies have indicated that the females of several fish species prefer to spawn in nests with many eggs[21-27]. In the three-spined stickleback, ***Gasterosteus aculeatus***, the females have been shown to prefer nests with many eggs and this has offered the classical explanation as to why the males engage in egg stealing in this species[22,28-29]. In the present study also the female always prefer nests for spawning and the fecundity is ranged between 200-1500 numbers depends the species and their gonadal maturity. It was also observed that once the spawning was completed, the female used to take rest on the anemone and the male only guarded the egg with more care.

Mass loss of eggs is a consequence of reduced food intake by the male during the paternal care, as caring shortens the time available for food searching[20,24,30-32]. The paternal care in the clownfishes exceeds just fanning and mouthing and there may be some time loss their eggs suddenly by consuming them. This eggivorous characteristic is generally called egg eating behavior which could be due to adverse environmental conditions, physical disturbances or any biological hazard. This egg eating behavior was noticed in the present study and it was more in ***A. clarkii*** due to physical disturbance and was less in ***A. ocellaris.*** Another type of disturbance noticed in the present study was the slow movement of the sea anemone along with the parent fish from the vicinity of clutch area, truly causing a distance between the clutch and parent. Generally, this type of movement is very rare in captivity. In this study, ***A. sebae*** with ***S. mertensii*** moved occasionally.

Table 3—Variable measured in male and female, five anemone fishes fanning observation

Sl. No.	Species	Clutch area(cm)	Fecundity (nos. of eggs/clutch)	Fanning behaviour
1.	***A.sebae***	4.5-8.5	600-1500	Both fanned by pectoral fins
2.	***A.clarkii***	3.5-4.5	500-800	Female very rare. Male using fanning
3.	***A.percula***	3.5-5.0	300-500	Both are actively fanning
4.	***A.ocellaris***	3.5-4.5	200-500	Male and female very active in fanning behaviour
5.	***P.biaculeatus***	4.0-5.0	200-500	Male actively fanned, female occationally

Table 4—Measured in male and female, five anemone fishes in clutch area, fecundity and fanning duration

Sl.No.	Species	CL	F	FD
1.	***A.sebae***			
	Male	4.5-8.5	600-1500	1.3 (± 0.13)
	Female			0.8 (± 0.22)
2.	***A.clarkii***			
	Male	3.5-4.5	500-800	0.5 (± 0.34)
	Female			0.3 (± 0.29)
3.	***A.percula***			
	Male	3.5-5.0	300-500	1.1 (± 0.31)
	Female			0.7 (± 0.19)
4.	***A.ocellaris***			
	Male	3.5-4.5	200-500	1.2 (± 0.28)
	Female			0.6 (± 0.28)
5.	***P.biaculeatus***			
	Male	4.0-5.0	300-600	1.1(± 0.3)
	Female			0.6 (± 0.31)

CL- clutch area (cm), F- fecundity (Nos. of eggs/clutch), FD- fanning duration (Min./ventilation)

Conclusion

In conclusion, the male increase their paternal caring effort as the significance of the hatching successes. Increased fanning time not only increases the hatching success of clownfishes but fanning frequency also play same role in clownfish parental care. Female showed less preference for eggs caring behavior than the male in all the five species. It also revealed that the fanning was an imperative biology of their life cycle and it's a crucial mechanism to develop their eggs and receiving hatching success.

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