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INFLUENCE OF BROODER DIET AND SEASONAL TEMPERATURE ON REPRODUCTIVE EFFICIENCY OF CLOWNFISH *AMPHIPRION SEBAE* IN CAPTIVITY

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Abstract

An attempt was made to study the influence of seasonal temperature and different feed (boiled clam meat, trash fish, live *Acetes* sp. and formulated feed) on reproductive efficiency of the clownfish *Amphiprion sebae* in captivity using brackish water. The fishes were segregated as two groups and accommodated in separate tanks as pairs. First group fed with different feeds for evaluating their breeding efficiency and second group is to find the influence of surrounding temperature on their reproduction. *A. sebae* spawned at regular interval in relation to seasonal temperature and higher spawning frequency (2.67 times month^{-1}) was observed in summer and spring months (28.7±0.72ºC). Spawning frequency was found low (2 times month^{-1}) during winter (25.3±0.76 ºC). Higher reproductive efficiency [number of eggs laid (260±14.6 eggs nest^{-1})] was observed when they had fed live *Acetes* spp than other feeds such as clam meat (196±12.9 eggs nest^{-1}), trash fish (151±8.9 eggs nest^{-1}) and formulated feed (105±6.9 eggs nest^{-1}). According to the present study, it is revealed that, the maximum number of spawning was observed in the summer and spring months and the brooder fishes showed higher reproductive efficiency, when they fed with live *Acetes* sp.

Keywords: *Amphiprion sebae*, Brackish water, Fecundity rate, Biochemical composition, Spawning rate

Introduction

Marine ornamental fishes play an important role in the interior decoration due to the recent developments in aquarium technology. The hobby of marine aquarium keeping has gained momentum among people all over the world. Coral reefs are one of the most productive ecosystems that support over 4000 species of fish, 800 species of reef building corals, a great number of other invertebrates and sponges (Gopakumar, 2006). More than 50 reef fish families with nearly 175 genera and about 400 species are distributed in the Indian seas and the relation between ornamental fishes and corals are intricate. Global imports on 'Marine, fresh water fish and invertebrates' in 2007 has been valued at US$ 327 million. The value of the fish and invertebrates of marine origin in this trade has increased from 9 million US$ in 2003 to reach almost 29 million US$ in 2007 (Tissera, 2010).

Clownfishes belong to the family Pomacentridae, one of the largest groups of reef fishes, inhabiting tropical and sub-tropical seas and members of this family incorporate 29 genera and 350 species under four sub families (Allen, 1991). There are 29 species of clownfishes with two inter generic hybrids between *Amphiprion* and *Premnas* (Allen *et al*., 2008; Mebs, 2009). The increasing demand for marine ornamental fish due to the recent developments in aquarium keeping has resulted in an over exploitation of the natural stock and consequent destruction of reef areas (Alava and Gomes, 1989). Controlled spawning through temperature manipulations to bring about gonadal maturation has been successful with several temperate species. Temperature is one of the most important physical factors influencing fish growth, body composition and energy budget (Ruyet *et al*., 2004). When temperature is low, growth rates, feeding rates and metabolic rates are suppressed; whereas elevated temperatures correlate with an increase in growth up to an optimum point above which thermal stress occurs (Baum *et al*., 2005). At the same temperature, fish growth and energy budget are mostly affected by nutrition factors (Sun *et al*., 2006, 2007). Plama *et al*. (2008) studied the growth parameters of adult sea horse *H. guttulatus*, fed with three frozen diets (shrimps, mysids and adult *Artemia*) and concluded that the differences in protein content may be responsible for differential growth rates. But the studies on influence of temperature and brooder diet on reproductive efficiency are not abundant in the clownfish; an attempt was made to study the spawning frequency and fecundity rate in *Amphiprion sebae* using brackish water.

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Materials and methods

Broodstock development

The present study was carried out from January 2007 to May 2008. Clownfish *Amphiprion sebae* (50 nos.) and host sea anemones *Stichodactyla haddoni* (20 nos.) were procured from the ornamental fish supplier (Chennai) during January 2007 and transported to the hatchery of the department [Centre of Advanced Study in Marine Biology, Annamalai University, India]. The fishes (60-70 mm length, 5-8 g weight) and anemones were quarantined for fifteen days and were shifted to three ton capacity cement tank (acclimatization tank) and reared for a period of three months. Pair formation occurred during this period and 12 pairs were selected for the experiments and transferred into individual 400-l fibreglass tanks (1.2 x 0.6 x 0.6 m) along with its host anemone. Among them, eight pairs were maintained to study the influence of seasonal temperature and remaining four pairs for determining the effectiveness of brooder diet on reproduction. Interior of these tanks were ash in colour and were initially filled with UV treated brackish water. A locally made underwater filter was fixed in each of these tanks. The pairs have 65-75 mm length and 8-14 g weight at the time of introduction into the spawning tank. Each brooder tank was provided with white tiles, dead coral pieces and live rocks as substratum for egg deposition. Each pairs maintained to determine the spawning frequency, were assigned a code number as A-1 to A-8.

Influence of temperature

Eight pairs of clownfish *Amphiprion sebae* were maintained to predict the influence of seasonal temperature on spawning frequency. Temperature was recorded daily by mercury centigrade thermometer with 0.5^0C accuracy. During the study period, the water quality parameters were maintained at optimum (salinity 23 ± 1 ‰, pH 7.9 ± 0.1 and D.O. 6.3 ± 0.2 mg l^{-1}). The photoperiod of each tank was maintained as 12 L: 12 D by 40-W fluorescent tube light. Salinity, pH, D.O. and light intensity were monitored by salinity meter (ECOSCAN, Singapore), pH pen (EUTECH, Singapore), D.O. probe (ECOSCAN, Singapore) and Lux meter (LUTRON, LX-101, Taiwan) respectively. The fishes were fed with live *Acetes* spp. thrice a day (0900 h, 1300 h and 1700 h) at 5% of its body weight. The water exchange has been done at 20% of tank volume once in a week.

Assessment of brooder feed

Four experimental feeds such as boiled clam meat, trash fish, live *Acetes* sp. and formulated feed were selected to evaluate the reproductive efficiency of broodstock. Each experimental set up have a pair of fishes with its host anemone and there was no specific difference among four set ups in terms of length and weight of fishes, size and shape of tanks and the environmental conditions provided in captivity. During this period, the fishes were fed thrice (0900 h, 1300 h and 1700 h) a day at 5% of its body weight. Aeration was temporarily suspended at the time of feeding. The uneaten feeds and faeces were siphoned out from the tank one hour after feeding to avoid the feed spoilage and deterioration of water quality and 20% of water has been exchanged once in a week. The anemones were fed with shrimp meat. For the comparison of reproductive efficiency of pairs reared with four different feeds, the eggs count was monitored.

The proximate composition (protein, lipid and carbohydrate) of four feeds used was estimated before starting the experiments. Protein, carbohydrates and lipids were determined according to the Lowry method, Colorimetric method and Lipid extraction method proposed by Lowry *et al*. (1951), Dubois *et al.* (1956) and Bligh and Dyer (1959) respectively.

Fecundity

Generally, the nest area was oval in shape and the area was calculated by using the formula proposed by Robertson *et al.* (1988) as modified by Hoff (1996).

$A = \delta R_1 \times R_2$

Where,

A = Area, δ = 3.14, R_1 = radius (maximum), R_2 = radius (minimum)

The total number of eggs per clutch was estimated by counting all eggs in 1 cm^2 and then multiplying with deposition area.

Statistical analysis

The spawning data were statistically analyzed using analysis of variance (ANOVA) and Pearson's coefficient of correlation.

Result and Discussion

The fishes were used to spawn between 0900 h to 1300 h. Spawning started with cleaning of the substratum at which the eggs to be laid which lasted for about an hour. The colour of newly laid eggs was varied from bright orange to yellow. 3^{rd} day onwards, they started to become black and on 7^{th} to 8^{th} day they became silvery due to the glowing eyes of the developing larvae inside the egg capsule. The fecundity was initially less and gradually increased. The interval between successive spawning of a pair varied between 9 to 21 days. The male fishes had average 73±2 mm length, 11±1 g weight and the female had 81±4 mm length, 17±1 g weight at the time of first spawning. The pair A 1 had spawned 30 times excluding the month December as an average of 2.7

times month^{-1}. The pair A 3 produced the lowest number of nests (18 spawning) (Fig. 1). The health condition and size of the fishes were also made a key role in the increased and decreased spawning and fecundity rate. Because the female fishes of the pair, A 3 and A 4 were infected by 'Pop eye' during rainy season.

During the study period, totally the 8 pairs recorded 209 spawning (Fig. 1). The pairs were began to spawn at different times, thus the unspawned months were excluded from the data. Increased spawning rate (2.67 times month^{-1}) had shown during the summer and spring months (28.7±0.72ºC) and decreased spawning rate was found low (2 times month^{-1}) during winter months (25.3±0.76 ºC) that was illustrated in Figure 2. The increment in the spawning rate was significant (ANOVA, $p < 0.05$) and positively correlated with temperature.

Figure 1. Annual spawning of brooders

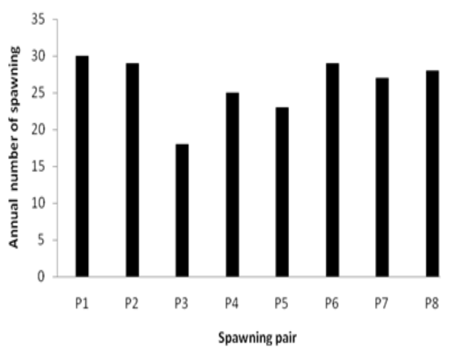
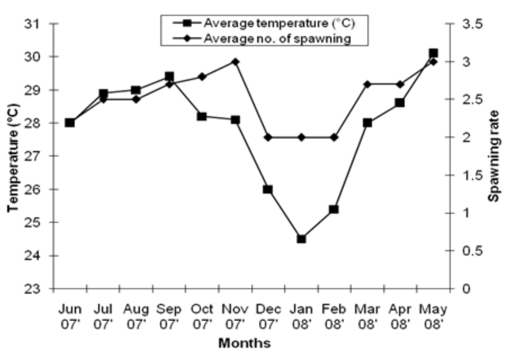


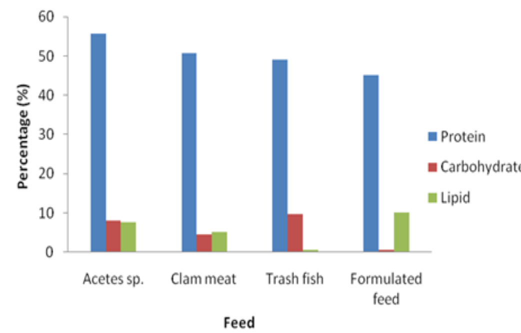
Figure 2. Spawning frequency in relation to temperature



Reproductive efficiency of brooders

Reproductive efficiency was specifically higher in *A. sebae* fed with live *Acetes* sp. when compared to other feeds provided. It was measured in terms of the number of eggs laid by parents. The fishes reared with live *Acetes* sp., clam meat, trash fish and formulated feed, laid 260±14.6, 196±12.9, 151±8.9 and 105±6.9 eggs respectively. The evaluation of proximate composition of four feeds revealed that the *Acetes* sp. have higher amount of protein (55.66%) when compared to that of clam meat (50.8%), trash fish (49.12%) and formulated feed (45.02%). The amount of carbohydrate and lipid in *Acetes* sp., clam meat, trash fish and formulated feed were 7.94% and 7.56%; 4.53% and 5.04%; 9.6% and 0.5%; 18.13% and 10% respectively (Fig. 3).

Figure 3. Proximate composition of feeds



In the present study, the fishes showed increased spawning (2.67 times month^{-1}) during summer and spring months, but showed decreased spawning (2 times month^{-1}) in winter. Alava and Gomes (1989) and Hoff (1996) had already reported that an increase in the number of spawning during summer months. Madhu and Madhu (2007) observed that the water temperature is also an influencing factor for the continuous spawning in clownfish similar to the present study. Increased spawning frequency in Amphiprioninae has been attributed to the shorter time taken for the incubation of embryos at higher water temperature (Bell, 1976; Hoff, 1996; Richardson *et al*., 1997; Madhu and Madhu, 2007). It has also been suggested that larval and juvenile fitness may be enhanced at warm water temperature due to higher growth rates (Moyer, 1980). In the present study, seven to eight days was the incubation period. Wilkerson (2001) also reported that, the surrounding temperature should be 27^{0}C for the normal incubation period. Variation in temperature influence the eggs to take either more or less time to hatch out.

Clownfishes are protracted spawners and produce one nest per month or less in the wild due to the presence of predators and disturbances from other fishes, but in captivity most of the pairs spawn a minimum of 11 months a year, regardless of the species due to the high nutritional brooder diet (Gopakumar, 2006). In the present study, the overall spawning frequency of all the pairs were 2.5 nests month^{-1}. But in wild tropical condition, *Amphiprion melanopus* spawned an average of 1.6 nests month^{-1} (Ross, 1978), *Amphiprion chrysopterus* and *Amphiprion perideraion* spawned 0.7 nests month^{-1} (Allen, 1972), *A. clarkii* spawned 0.5 nest month^{-1} (Ochi, 1985, 1989) (Table 1). There is a higher spawning frequency in captivity (average 2.2± 0.8 times $month^{-1}$) as compared with wild (Gordon and Bok, 2001). This is may be the good water quality, sufficient nutritious feeds and free from predators.

Table 1. Comparison of fecundity and spawning frequency in captivity and wild

Species	Spawning/ year	Nests/month	Eggs/nest	Annual number of eggs/pair	References
Wild condition					
A. melanopus	19.8	1.6	200-400	7,200	Ross (1978)
A. chrysopterus	8.5	0.7	~ 400	3,000-5,000	Allen (1972)
A. perideraion	8.5	0.7	300-700	2,000-4,000	Allen (1972)
A. clarkii	7.0	0.6	1,000-2,500	8,000-17,500	Bell (1976)
A. clarkii	5.5	0.5	1,600-5,400	11,000-15,000	Ochi (1985), Ochi (1989)
A. akindynos	6.7	0.6	700-5,025	2,810-26,890	Richardson *et al*. (1997)
A. latezonatus	8.0	0.7	800-3,870	10,470-33,140	Richardson *et al*. (1997)
Captive condition					
A. percula	14.0	1.2	67-649	804-7,788	Hoff (1996)
P. biaculeatus	20.7	1.7	146-986	1,752-11,832	Hoff (1996)
A. clarkii	34.6	2.9	435-981	5,220-11,772	Hoff (1996)
A. ephippium	25.9	2.2	225-869	2,700-10,428	Hoff (1996)
A. frenatus	27.2	2.3	309-551	3,708-6,612	Hoff (1996)
A. melanopus	21.3	1.8	172-339	2,064-4,068	Hoff (1996)
A. ocellaris	24.8	2.1	168-313	2,016-3,756	Hoff (1996)
A. akallopisos	29.0	2.4	212-392	2,544-4,704	Hoff (1996)
A. sebae	30.0	2.7	~105-289	~1,260-3,468	Present study

In fishes, the broodstock nutrition is one of the poorly understood research area and unpredictable brooder performance are often considered as a bottleneck for successful mass scale production of juveniles (Izquierdo *et al*., 2001). The nutritional requirements particularly for brooders are mostly unknown. In the present study, the proximate composition analysis revealed that, *Acetes* sp. showed high level protein (55.66%) content than the other feed. The difference in protein content of the feed has been influenced to the variation in reproductive performance and growth rate of broodstock (Murugan *et al*., 2009). In the present study it was also revealed that the protein content of the diet plays an important role in influencing the reproductive efficiency of the brooder. However, these assumptions are based on the proximate composition of diets.

Conclusion

The present study revealed that the maximum number of spawning, 2.67 times $month^{-1}$ was observed in the summer and spring months (28.7±0.72ºC). So, normally it is easier to carry out the breeding works in tropical waters rather than temperate regions. Meanwhile, higher reproductive efficiency (260±14.6 eggs $nest^{-1}$) was found, when the brooder fishes were fed with live *Acetes* sp. However, the increasing pressure on natural populations of coral reef animals due to their expanding popularity in the aquarium trade has stimulated interest in developing culture techniques for marine ornamental fishes. The breeding of clownfishes has fascinated by many aquarists and now a day some of the species were successfully bred and reared under captive conditions. Thus the breeding of marine ornamental fishes will lead to reduce the stress associated with destructive fishing practices from the wild habitat and can save the fragile coral reef ecosystem from degradation.

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