



International Journal of
**Zoological
Research**

ISSN 1811-9778



Academic
Journals Inc.

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Studies on Spawning in Clownfish *Amphiprion sebae* with Various Feed Combinations under Recirculating Aquarium Conditions

Olivia J. Fernando, K. Raja and T. Balasubramanian
Marine Research Aquarium, CAS in Marine Biology, Annamalai Nagar-608 002, India

Abstract: *Amphiprion sebae* kept in the recirculating Marine Research Aquarium spawned a maximum of 0.8 times per month throughout the year. This is the first spawning record from a recirculating aquarium environment. Generally the spawning of *A. sebae* has been described from fresh seawater by various authors. The present study mainly observes the maintenance of brooders, their pairing and spawning in recirculating aquarium environment. Among the 54 adult fishes collected from the natural environment, three pairs are formed in the aquaria. The parental care of egg guarding male was observed. Three pairs are fed with different feed i.e., prawn meat, fish meat and a combination of prawn, fish and clam meats. The spawning frequency was observed to be moderately high (0.8/month) when fed with the mixed feed, whereas a frequency of 0.5 and 0.6 per months was observed when fed with prawn, fish meats respectively. The maximum clutch size (911) was observed also in mixed feeds and it was minimum (395) when the pairs were fed with fish meat alone. The increase in the frequency of spawning was during summer months compared to other months.

Key words: *Amphiprion sebae*, marine ornamental fish, spawning in aquaria, spawning with various feeds

Introduction

Nowadays, the marine ornamental fish industry is developed day by day. Conroy (1975) valued the world retail trade in fish keeping at only US \$ 250 million in 1970's. A rapid increase in the harvest of marine species for home aquaria occurred in the 1980s (Andrews, 1990) and exports have continued to increase in the 1990s (Vallejo, 1990). According to Bassleer (1994) world wholesale trade in ornamental fish is worth about US\$ 900 million. But after two decades, Devenport (1996) estimated the total world retail trade including live fish, associated equipments and accessories at US\$ 7,000 million and the retail trade in live products at US\$ 3,000 million. These dramatic developments of this industry can be directly affecting the wild population of the marine ornamental fishes. The overexploitation resulting in depletion of certain species of ornamental fishes (Wood, 1985) has presented aquaculture with an opportunity to enter a new market and provide a consistent supply of marine aquarium fish to an increasing market through the development of captive breeding programmes.

The family Pomacentridae, particularly the clownfish species of the genus *Amphiprion*, represents the most important group of captive-bred marine species. They are also considered a good reference fish for scientific research, especially for nutritional studies and the determination of spawning the eggs and larval quality (Delbare *et al.*, 1995). The spawning of clown fishes in wild appears to be varying with latitude. Clownfish species occurring in tropical waters spawn throughout the year (Allen, 1972; Ross, 1978), whereas in more temperate regions spawning occurs only during the warmer summer months (Bell, 1976; Moyer, 1980; Ochi, 1985; Richardson *et al.*, 1997). However, in temperate

clownfish species, the correlation between spawning periodicity and moon phase becomes much weaker (Richardson *et al.*, 1997). Generally, the reproductive cycle of fishes in the aquarium environment is very often absent or weak. This is mainly due to the low water quality conditions in such environments. As a result, fish become entrained by the simulated temperatures and lighting regimes imposed by the aquarist and reproductive activity changes accordingly (Kohler *et al.*, 1994). Clownfish, in aquaria, appear to breed throughout the year (Alava and Gomes, 1989; Hoff, 1996), with an increase in the number of spawning during the spring and summer months (Hoff, 1996). In the present study, an advanced filtration system such as protein skimmer and ozoniser was not used and the recycling is mainly by the basic filters.

Materials and Methods

Fifty four numbers of adult (6 to 8 cm total length) *A. sebae* were collected from Gulf of Mannar, Southeast coast of India and transported to the Marine Research Aquarium. They are divided into three groups in three different aquarium tanks. Pairs of clownfish were each placed in 2000 L concrete (floor with tiles) glass mount aquaria and connected to a 40,000 L partially recirculating system. During the course of the study pairs were fed twice a day. After the pairing in aquarium tanks they were observed twice daily, in the morning and late afternoon, for evidence of spawning. There is no 100% exchange of water with natural seawater. However, the evaporation and seepage are compensated with natural seawater. The natural seawater is drawn directly from the mouth of a nearby estuary and transported in plastic tanks to the Marine Research Aquarium at a distance of 25 km. The aquarium was incorporated with filters and daily water exchange rate is 10% in each experimental aquaria. The entire recirculating system is showed in Fig. 1. Aquarium floors are covered with crushed sea shells and spawning decor was provided in the form of earthen pots and 50 mm PVC pipes.

Throughout the study period water quality parameters ranged within accepted limits and when required it was balanced immediately by 50% of water exchange. The overall pH ranged from 8.01 to 8.35 and salinity from 33 to 36‰. The Ammonia was measured at 0.01 to 0.09 mg L⁻¹ and NO₃ levels were below 4 mg L⁻¹. Water temperature was maintained by means of centralized air-conditioner and the data was collected from the aquarium in broodstock system ranged from 26 to 32°C. There is

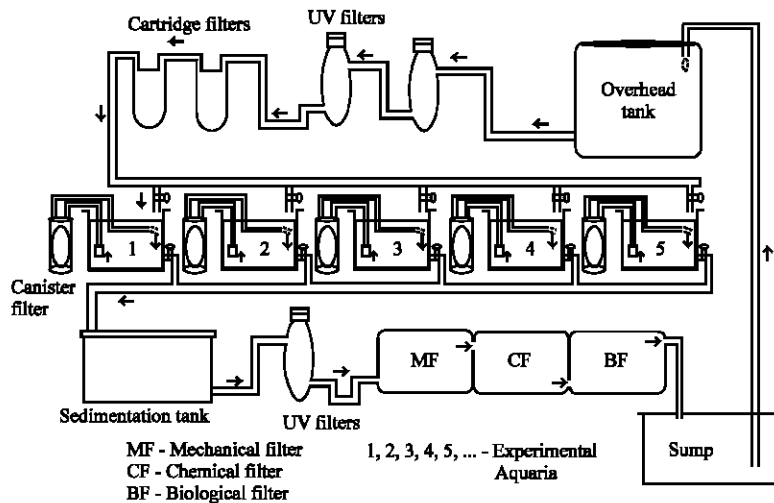


Fig. 1: Technical view of the recirculating system of the present aquaria

no natural light entering the aquaria. Supplementary artificial lighting was provided by cool white fluorescent tubes situated 30 cm above each tank and set on a 10 h light: 12 h dark cycle. Under these experimental conditions, the clown fish behaviors of pairing, spawning and clutch size characters are observed.

Results and Discussion

In the present study spawning frequency in relation to various feeds was observed. The overall spawning records are showed in Table 1. The duration was calculated, when the first spawning occurred in the tanks. The total numbers of eggs are also counted approximately and given in Table 2.

Research on spawning and clutch characteristics of marine ornamental fishes is still relatively in its earlier stages. Ignatius *et al.* (2001) have provided data on the spawning behavior of *A. sebae* from Indian waters. It is not extensive whereas during the present study a 15 months study was undertaken. Data on spawning was collected from March 2005 to June 2006. During the period of pair formation twenty three female fishes died. Among these, 13 fishes died due to the fighting behavior of brooder fishes within the tank. This behavior is common in other *Amphiprion* species that is observed by Hoff (1996). He noted that when pairing formed in a tank, the pair fishes tend to stay together and chase others from specific areas and eventually attempt to destroy others. In the present study the affected fish could be easily identified by its fin damage and when it always stands in the corner of the tank. This chasing behavior sometime occurred vigorously resulting in mortality. The other ten fishes may have died due to weak immune system with regard the change in water quality and environment. After the pair formation in three tanks the remaining twenty five fishes are removed from that tank. However, not all the three pairs provided data for the duration of the study as pairs began to breed at different times and an individual from each of two pairs died after their 4th and 5th spawning. This mortality may be due to their feed. The dead pairs are fed with the same type of feed i.e., prawn meat and fish meat respectively throughout the study period. But it was noticed that in the third pair fed with a mixed feed spawned more frequently (Table 3).

Year-round spawning for captive Amphiprionae at Instant Ocean Hatcheries (IOH) in Florida is reported (Hoff, 1996), with *A. akallopisos* spawning an average of 2.4 times per month (Table 3). Year-round spawning is also recorded for wild populations of tropically occurring *A. chrysopterus*, *A. perideraion* (Allen, 1972) and *A. melanopus* (Allen, 1972; Ross, 1978). In contrast, low water temperatures and more unstable environmental conditions of temperate regions resulted in spawning occurring only during the summer months (Richardson *et al.*, 1997). In the present study also more spawning occurred during summer compared to other months. Wild populations of *A. clarkii* from

Table 1: The spawning records of *A. sebae* in marine research aquarium

Pair code	No. of spawning events during the present study	No. of months of fish survival after their first spawning
ASF	4	6
ASP	5	7
ASM	11	15
Total	19	28

ASF- *Amphiprion sebae* fed with fish meat only, ASP-*A. sebae* fed with prawn meat only, ASM-*Amphiprion sebae* fed with mixed feed i.e., prawn, fish, clam meats

Table 2: The maximum, minimum and mean (SD) clutch sizes of *A. sebae* in the present study

Pair code (*)	Maximum	Minimum	Mean (±SD)
ASF (4)	613	395	483 (100.22)
ASP (5)	712	396	578 (125.93)
ASM (11)	911	500	694 (132.43)

(*)- Number of spawning

Table 3: Comparison of spawning records of clownfish pairs at Instant Ocean Hatchery (Hoff, 1996) and the present study

Species	Months	Total spawning	Average/month
<i>A. akallopisos</i>	29	70	2.41
<i>P. biaculeatus</i>	59	102	1.73
<i>A. clarkii</i>	52	150	2.88
<i>A. ephippium</i>	64	138	2.16
<i>A. frenatus</i>	56	127	2.27
<i>A. melanopus</i>	58	103	1.78
<i>A. ocellaris</i>	64	132	2.06
<i>A. percula</i>	55	64	1.16
<i>A. polymnus</i>	29	52	1.79
<i>A. sebae</i>	15	11	0.73
(In the present study)	7	4	0.57
	6	5	0.83



Fig. 2: The spawning and parental care of clown fish *A. sebae* in Marine Research aquarium

Miyake-jima and Shiko-ku Island spawned 7.0 and 5.5 times per year, respectively (Bell, 1976; Ochi, 1985, 1989), while *A. clarkii* in captivity spawned 34.6 times per year (Hoff, 1996). There is no particular spawning data in natural and captive environment of *A. sebae* (Fig. 2).

In the present study the clutch size and annual fecundity is less compared to the natural environment. The environmental factors are important in modifying the reproductive behavior and biology of the anemonefish (Moyer, 1980; Richardson *et al.*, 1997). Hoff (1996) reported increased spawning frequency during spring and summer months in captive Amphiprionae at Instant Ocean Hatchery (IOH). Broodstock at IOH and Oceanic Research Institute (ORI) hatchery, were exposed to ambient light in addition to artificial light and depended to a certain degree on solar heat to maintain water temperatures. Consequently, photoperiod and water temperatures fluctuated with the seasons. Increased spawning frequency in Amphiprionae has been attributed to the shorter time taken for incubation of embryos at higher water temperatures (Bell, 1976; Hoff, 1996; Richardson *et al.*, 1997). It has also been suggested that larval and juvenile fitness may be enhanced at warm water temperatures due to higher growth rates and a reduction in temperature related stress (Moyer, 1980). During the present study it could not be discerned whether water temperature or photoperiods were factors influencing spawning frequency. Fluctuations in water temperature in the aquarium had no correlation with spawning frequency of clownfish. Perhaps temperatures in the present aquarium did not drop to the level where embryo incubation time is increased and consequently spawning frequency reduced. It is, therefore, postulated that the increase in photoperiod or light intensity associated with summer may play more of a role in increasing the number of spawning at this time. Moyer (1980) who observed the influence of photoperiod on spawning of wild clownfish *A. clarkii* at Miyake-jima

suggested that diminishing light and not water temperature restricted their breeding season. Hoff (1996) believes light intensity and photoperiod were not major factors influencing spawning frequency at IOH. Data showed that clownfish at IOH consistently spawned more frequently during December than the other months, even though December experienced the shortest day in terms of photoperiod and lowest light intensity. In the present study clownfish are exposed to ambient light, but it is likely that the surrounding lights may prevail any effect on their reproductive cue. But most of the spawning was recorded during the full or new moon times. This could account for the different spawning frequencies at different moon phases.

Gardon *et al.* (1998), Johnston *et al.* (2003) and Sales and Janssens (2003) are pioneer researchers regarding the nutrition studies i.e., the effect on growth and survival of larvae of clownfishes and other ornamental fishes. Studies on influence of nutrition on ornamental fish are hampered by the lack of suitable measurement other than growth (Johnston *et al.*, 2003) whereas in the present study, the effect of feed on the spawning frequency was observed. It was observed that the adult *A. sebae* could be weaned onto a mixed feed with a significant spawning record compared to other weaning experiments. According to Pannevis (1993) it was also proved that the ornamental fish can exhibit a very consistent preference for one diet over another when two or three diets are offered simultaneously.

Although this study was a small-scale pilot study, it is hoped that it would throw light on the interactions between environmental factors and spawning frequency and periodicity in *A. sebae* maintained under recirculating aquarium conditions. Further trials should be conducted using larger numbers of broodstock and their larval rearing commercial basis and focus specifically on synthetic seawater.

Acknowledgments

We thank the authorities of Annamalai University for allowing use of Marine Research Aquarium in the present study.

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