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†Acclimation and growth of hatchery produced false clown *Amphiprion ocellaris* to natural and surrogate anemones

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Abstract

Anemonefishes live unharmed among the tentacles of sea anemones. Among the tested natural sea anemones, *Heteractis magnifica, H. crispa* and *Stichodactyla gigantea* were found as suitable hosts for acclimation and rearing of hatchery produced false clownfish *Amphiprion ocellaris* under captive conditions and the maximum settlement (100% on 6th day) was obtained by using *H. magnifica*. To study the acclimation time and behaviour, *A. ocellaris* were allowed to associate with surrogate anemone prior to being reared with natural anemone *H. magnifica*. The average acclimation time in surrogate and natural anemone was 6472.3 and 102.4 minutes respectively in comparison with 18.7 minutes for those fish that were allowed to orient to the surrogate anemone prior to being with natural anemone *H. magnifica.* The study revealed that exposure of *A. ocellaris* to surrogate anemone resulted in substantial decrease subsequently in the time of acclimation to a natural sea anemone. This result shows that the fish is responsible for developing its own protection from sea anemone's mucous during acclimation. There was considerable difference in growth among the juveniles as well as between the individuals reared in natural and surrogate anemones. Higher growth was obtained in fishes acclimated to natural anemones followed by surrogate anemone, whereas fishes reared without sea anemone showed less growth. This difference could be attributed to the social inhibition of growth of subordinate late settlers by frequent attack from the dominant early settlers. The present study also concludes that in the absence of natural host-specific sea anemones, the surrogate anemone may be used as a substitute to rear fishes under captive conditions.

Keywords: *Amphiprion ocellaris*, sea anemones, acclimation, cnidae, symbiotic relationship

Introduction

Anemonefishes belonging to the genus *Amphiprion* and *Premnas* are known to have proclivity to live in association with sea anemones. Under the condition of close proximity and high density on coral reef, a whole range of interspecific interactions occur often leading to stable symbiotic relationship between vertebrates and invertebrates (Allen, 1975; Fricke and Fricke, 1977). This symbiotic relationship occurs with various behavioural processes such as acclimation during which an anemone fish becomes protected from the cnidae (nematocysts and spirocysts) of its host sea anemones and whatever change occurs during acclimation lay in the surface mucous coat of fish and not in the anemone (Mariscal, 1970a,b,c, 1971, 1972). To understand this process, two hypotheses have been suggested *viz*., (a) an anemone fish might be either coating or complexing on its surface with anemone-derived chemical compounds, thus masking its own stimuli for cnida discharge, or (b) during acclimation an anemone fish might be qualitatively or quantitatively altering its own mucous coat in response to visual, tactile or chemical cues from the sea anemone (Mariscal,1970a, 1971). There are also suggestions that during acclimation, the anemone fish picks up species specific anemone compounds for their protection by "impregnation" (Schlichter, 1976) and that mucus coat produced by

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fish for their life protection also acts as an inert physical barrier and is lacking in chemical compounds that might trigger cnida discharge (Lubbock, 1979a,b; 1980a,b, 1981; Brooks and Mariscal, 1984). Apart from this, various studies reported that mucous coat is produced during aggregation behaviour (Ross, 1978), species specific partnership (Miyagawa, 1989; Fautin, 1991), as a chemical stimulant (Murato *et al.,* 1986) and as resistance to the toxin secreted by sea anemone (Mebs, 1994), and for its growth (Ochi, 1986). With this backdrop, experiments were designed to test the above hypotheses for which surrogate anemones were used, and hatchery produced juveniles of *Amphiprion ocellaris* were allowed to associate with surrogate anemones for specific period of time. After exposure to the surrogate anemones, the same fishes were introduced to a natural anemone *Heteractis magnifica* and the acclimation time was compared to those of control fish that had been introduced directly to natural sea anemones. In addition to these, the influence of these association to the growth of anemone fishes were assessed under captive conditions.

Materials and Methods

The hatchery produced anemonefish, *A. ocellaris* of standard length 36 to 45 mm (as measured from the anterior-most point of the upper jaw to the middle of the line of flexure at the caudal fin base: Allen, 1975) were used for symbiotic experiments in the Marine Hatchery of CMFRI, Kochi. They were fed with mixed diet consisting of *Artemia* nauplii (20-25 numbers/ml) and minced prawn meat three times a day (9.45 am, 12.45 pm and 03.45 pm) at 15% of body weight.

Symbiotic relationship: Anemones such as *Cryptodendrum adhesievum, Entacmaea quadricolor*, *Heteractis aurora, H. crispa, H. magnifica, H. malu, Macrodactyla doreensis*, *Stichodactyla gigantea, S. haddoni* and *S. mertensii* were purchased from aquaria and pet shops. All anemones were acclimatized in 500 liter perspex tanks, and were fed with *Artemia* nauplii and pieces of shrimp muscle after 24 hours of transportation. One specimen of each of these anemones was released in 500 liter perspex tank and acclimatized for one week. Subsequently, 25 numbers of *A. ocellaris* were released into the same tank and seven replicates were maintained. Temperature, pH, and salinity were maintained at the optimum level of 25-27° C, 7.8-8.2 and 30-35‰ respectively. The experimental fish and anemone were kept on a 12:12 day-night cycle. All the experimental tanks were daily cleaned with cotton and magnetic tank cleaner to avoid settlement of slimy coat, and faecal matter and removed the unspent feed. The fishes were observed up to 12 hours per day during day time for one week to find out the settlement. Cnida discharge capabilities of anemones were tested following the methodology suggested by Brooks and Mariscal (1984).

Surrogate or artificial anemones: Surrogate anemones of different colour and shapes were purchased from the local market of Kochi in order to analyze the acclimation process. After introduction of surrogate anemone in 500 l tanks, the fishes were also introduced in the same manner as in the case of live anemone. The fishes were allowed to associate with the surrogate anemone at least for 2 days and in some cases as long as 10 days and noted the acclimation time. After the fish had been with the surrogate anemone, they were transferred to the tank with the real anemone (*H. magnifica*) and the acclimation time were recorded and compared.

Growth of anemone fishes: Hatchery produced *A. ocellaris* (length: 10 ± 0.5 mm) of the same clutch were used for growth study with natural anemones (*H. magnifica*), surrogate anemones and without anemone. The fishes were reared in 100 L perspex tanks, and in each tank 10 individuals/100 l were stocked. Five replicates were maintained for each experiment. Temperature, salinity, dissolved oxygen and pH were measured every day and maintained to their suitable range and 25% of water was exchanged once in a week. The fishes were fed with chopped prawn and fish egg mass at the rate of 15% of body weight at an interval of every 3 hours during day time. At every 15^{th} day, standard length and weight were taken. The experiment was carried out for 10 months.

Statistical analysis: Durbin–Watson D Statistic, Post Hoc test of occurrence and Fisher's Least–

Significant- Difference Test were used for symbiotic relationship and growth studies. The influence of natural and surrogate anemone on the acclimation time of *A. ocellaris* was tested with Pearson Correlation Matrix. All data were analyzed with SYSTAT VERSION 7.0.1.

Results

Symbiotic host anemones: Among the 10 anemones tested for a period of one week, settlement of *A. ocellaris* occurred with *H. magnifica, S. gigantea* and *H. crispa* (Table 1). Since these fishes have resting tendency during night hours, the observations on the rate of occurrence were carried out up to 12 hrs per day during day time. On introduction of fishes into anemones, they approached and hovered over the anemones and tentacles of non-specific anemones were found to be shrunk during the time of their touching. The number of visits of fishes to all anemones was less on the first day whereas from the second day onwards highly significant variation ($p < 0.01$) was noticed between three anemones and the rest of the anemones. Among the three anemones, the variation in occurrence was found to be less after 60 hrs. After the 4th day, the introduced fishes started settling with *H. magnifica, H. crispa* and *S. gigantea.* The maximum settlement was noticed with *H. magnifica*.

Acclimation time: The start of acclimation was established from the time when the fish made intentional contact with the anemone. Intentional contact was the time when the fish made a move directly toward the tentacles of the anemone. Incidental contact of fishes with the anemone that appeared to be accidental also occurred when a fish was investigating its surroundings shortly after introduction into the experimental tank. The criterion used to determine completion of acclimation was that contacts by the fish caused no apparent adhesion. Usually when the tentacles adhered to the surface of the fish, the fish behaved as if stung, retreating quickly to free itself from the tentacles. Thus, if no tentacular adhesion occurred, it was assumed that the cnidae were not discharging and the fish was acclimated. When introduced to the surrogate, the fish approached and hovered over the surrogate and even nibbled at tentacles as fish normally do with a natural anemone (Fig. 1). The general behavior of the fish associated with the surrogate anemone was similar to that with the natural anemone. However,

Fig. 1. Juveniles of *A. ocellaris* settled in surrogate anemone

Table 1. Settlement (%) of *Amphiprion ocellaris* with different sea anemones

Sea anemones	Days of acclimation						
	1	2	3	4	5	6	7
Heteractis magnifica	21.9	48.4	91.7	95.6	97.3	100	100
Stichodactyla gigantea	18.1	36.3	89.7	95.3	97.9	98.3	100
Heteractis crispa	10	30.3	52.1	78.3	88.3	98.6	99.3
Heteractis aurora	4	7.6	2	1.1	0	0	0
Stichodactyla mertensii	1.9	2	1.6	0	0	0	0
Entacmaea quadricolor	1.6	2	1.9	0	0	0	0
Stichodactyla haddoni	1.6	0	0	0	0	0	0
Macrodactyla doreensis	1.3	0	0	0	0	0	0
Heteractis malu	1.3	0	0	0	0	0	0
Cryptodendrum adhesievum	1.6	0	0	0	0	0	0

the fish did not bathe among the tentacles of surrogate anemones as they do among the tentacles of the natural anemone. In addition, when the fishes were introduced to surrogate, all fishes showed gregarious tendency in the corner of the tanks before entering into the surrogate anemone.

The acclimation time of *A. ocellaris* with natural and surrogate anemones are shown in Table 2. The average acclimation time with surrogate and natural anemone was 6472.3 and 102.4 minutes respectively in comparison to 18.7 minutes for those fish that were allowed to orient to the surrogate anemone prior to being with natural anemone *H. magnifica*. The experienced fishes with artificial anemones showed that subsequent acclimation time with natural anemones were significantly shorter ($p < 0.01$). After exposure to the surrogate, the acclimation behavior of the fish to natural anemone seemed to be slightly different and the fish quickly approached and made contact with the anemone and substantial decrease in the subsequent time of acclimation to a natural sea anemone (Fig. 2).

Growth of anemone fishes: Growth rate was different among fishes acclimatized in natural (*H. magnifica*) and surrogate anemones and in fishes reared without sea anemone. Mean monthly growth rate of fishes reared with natural and surrogate anemones showed an increase during initial three months and in subsequent months average growth rate was slower. In the case of fishes without sea anemone, all the fishes showed less growth during the entire period. Higher growth was observed in fishes acclimated to natural anemones (Fig. 3).

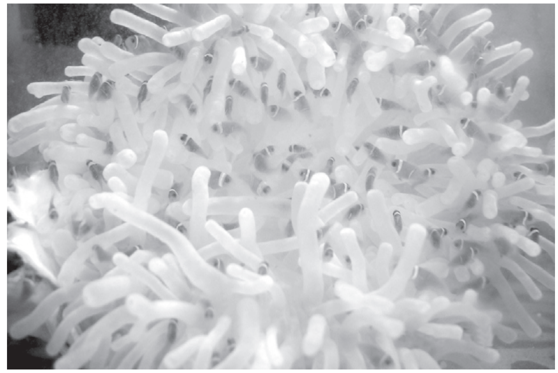

Fig. 2. Juveniles of *A. ocellaris* settling in natural anemone *H. magnifica* after exposure to surrogate anemone

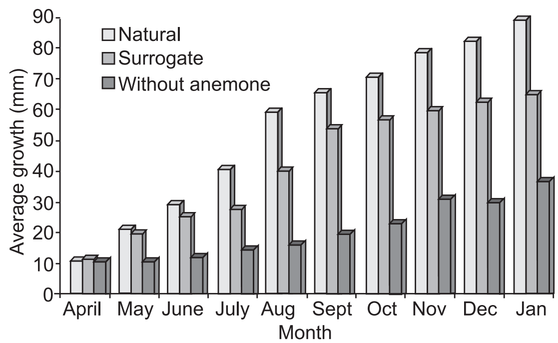

Fig. 3. Influence of animones on growth of *A. ocellars*

Table 2. Comparison of acclimation time of *Amphiprion ocellaris* with different sea anemones

Natural anemone		Surrogate anemone		Natural anemone (after experience with surrogate)	
No. of fish	Time (min)	No. of fish	Time (min)	No. of fish	Time (min)
2	145	5	2880	9	32
1	111	4	5760	2	21
4	78	6	8640	3	12
6	56	7	10080	3	6
8	148	5	14400	6	7
12	159	3	4320	7	23
3	89	2	5760	5	34
9	64	8	2880	4	45
2	82	5	5678	6	2
3	92	5	4325	5	5
Mean acclimation time ± SD	102.4 ± 36.7		6472.3 ± 3609.6		18.7 ± 14.7

Discussion

The question of how anemone fishes are able to live unharmed among the tentacles of their host sea anemone remains enigmatic. The key to understanding on how the fishes are protected is based on observation of behavioural process. Two major hypothesis suggested to test the relationship between sea anemones and anemone fishes are that (i) the fish coat itself with anemone's mucus to mask its chemical stimulation for cnida discharge, or (ii) the fish is protected by some alteration in its own mucus coating. The tentacles of unrelated anemones shrink if touched by the fish, which is due to the discharge of cnida compound. Fukui (1973) reported that *A. percula* could not enter the tentacles of anemone *Parsicyonis actinostoloides* with impunity and were massively stung and killed which indicated that *A. percula* have no innate protection from the cnida of the anemone. In the present study, at the start of acclimation, *A. ocellaris* were slightly stung by their symbiotic anemone whereas at the end of acclimation, the fishes were able to move freely among the tentacles of anemone and were not stung even with repeated, forceful contact against the tentacles. However what remains unclear is the nature of change in the fish during acclimation. Schlichter (1976) suggested that anemonefish by coating its body with anemone-derived compounds is, in fact, engaging in a form of macromolecular mimicry, thereby it ceases to be recognized as a potential prey organism by the anemone. Other studies (Lubbock, 1979a,b, 1980a,b, 1981) suggest that during acclimation, the clownfish is picking up significant amounts of anemone's mucus to protect itself from the cnida of anemone. The short duration of acclimation time leads to less coating in *A. ocellaris* which indicates protection from sea anemone pre-exists or is innate as reported in *A. clarkii* (Fukui, 1973; Miyagawa and Hidaka, 1980). In the present study, no fish entered with impunity into anemones immediately on exposure, but could enter into *H. magnifica*, *S. gigantea* and *H. crispa* from 4^{th} day onwards. This may be due to the fact that *A. ocellaris* had no innate protection from the cnidae of anemone as in other anemonefishes. Although *A. clarkii* required only 1-2 minutes of acclimation time to enter into *S. haddoni*, it took 4 days to enter into *Gyrostoma hertwig* (Lubbock, 1979a, 1980b). This is due to the fact that *A. clarkii* does not have the "innate" protection as inferred by Fukui (1973) and Miyagawa and Hidaka (1980). Studies have also shown that the anemone (*Parasicyonis actinostoloides*) stung and killed *A. percula* (Fukui, 1973). It has been demonstrated that the acclimation time of a single *Amphiprion* species to sea anemones may vary inter and intra-specifically from only a few minutes to many hours and even upto a few days as reported by Mariscal (1970a) and Lubbock (1980b). Obviously, the surrogate anemone used in the present study possessed no "anemone" mucus. Thus the results of this study conclude that the fish produces its own protection during acclimation. Further studies are needed to determine the exact nature of this protection, *i.e.*, whether the differences are primarily quantitative or qualitative in nature.

Normally, when disturbed, an anemonefish will retreat as deeply as possible into the tentacles of a living sea anemone, sometimes even going into the mouth of the anemone as reported for *A. bicinctus* and its host anemone (Masry, 1971). Significant reduction of the acclimation time to a natural anemone following association with a surrogate anemone would provide evidence that the fish is capable of altering its own surface coat, presumably as a result of visual or tactile cues received from the surrogate. On the other hand, the observation that acclimation time is significantly different or greater following association with a surrogate would suggest that perhaps a derivative from the natural anemone is responsible for protecting the fish.

Growth was found to be exceedingly variable not only in fish associated with natural anemones but also for individuals with surrogates. This is due to social hierarchy or peck order based on size which usually exists when there is more than one fish per anemone. The growth study shows that socially dominant fish exhibit faster growth to become pairs than subdominant conspecifics and show differential growth. The presence of an adult pair commonly exerts an inhibiting effect (stunting) on the growth of the smaller fish (Fricke and Fricke, 1977; Ross, 1978) whereas growth rate of *A. clarkii* greatly changed due to change in season in wild condition (Ochi, 1986). The early settlers in anemones also showed faster growth than the late settlers which also helped to inhibit the growth of late settlers as reported for *A. clarkii* (Ochi, 1986).

The present study concludes that *H. magnifica, H. crispa* and *S. gigantea* are the befitting hosts for successful rearing of *A. ocellaris* under captive environment and even if the natural host specific anemone is not available, the surrogate anemone can be used as a substitute to rear fishes under captive condition.

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