Expansion behavior, growth and survival of the sea
anemone *Stichodactyla haddoni* (Saville-Kent, 1893)
with anemonefishes in captivity

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ABSTRACT: The sea anemone *Stichodactyla haddoni*’s growth, expansion and survival under captivity at different conditions were studied for a period of 168 days. Each experiment with the same animals was continued after a resting period of 8 weeks in between. The trials were conducted under standardized conditions of water quality and photoperiods. The anemones were kept in two different groups, one with anemonefishes and the other without fishes. The expansion and growth rate in these two groups were assessed at weekly intervals. It was seen that in comparison with anemones made to live in solitude, those allowed to cohabit with anemonefishes showed higher rate of oral disc expansion (p<0.01) and a significant variance in the growth rates and it depends on the species of fish cohabitating with the anemone (p<0.05). Exponential growth rates was shown by anemones cohabitating with native species (fish live with a specific anemone in nature) and this was reversed in anemones lives with non-native species. The growth rates of anemones lives with the presence of anemonefish were 10–15% higher than the anemones lives in solitude. During forced starvation, the survival rate of the anemones living with fish was 80% and the same was 60% in the anemones survived without fish. Chi-Square test confirms the presence of significant association linking the behavior of anemones and anemonefishes in their natural habitats (χ² = 25.019, df (7), p<0.01). Results of this experiment reveal that *S. haddoni* shows enhanced expansion, growth and survival in the presence of anemonefishes.


KEY WORDS: *Stichodactyla haddoni*, anemonefish, growth, survival, captivity.
Introduction

The sea anemone *Stichodactyla haddoni* (Saville-Kent, 1893) inhabits mid tide levels up to depths with sufficient light penetration and prefers to burrow its column into soft sediments (Dunn, 1981; Fautin et al., 2009). Individuals of this species occur in shallow tropical and subtropical seas ranging from the Red Sea through the Indian Ocean up to New Calidonia and from Japan through Singapore to Australia (Dunn, 1981; Fautin, Allen, 1997; Fautin et al., 2009). The Haddon’s anemone has an undulating oral disc, which is broad flat to shallow in shape and it is densely covered with hundreds of slightly tapering tentacles. The yellow-orange coloured area around the mouth is bare. Pedal disc is narrower than the oral disc. The column is white in colour. Pink or purple coloured verrucae can be seen on the column tapering towards the pedal disc (Dunn, 1981; Fautin, Allen, 1997).

Many sea anemones can be kept under various conditions including non-circulating water and also without hosting a fish (Stephenson, 1928; Fautin, Allen, 1997). The growth rate of sea anemones varies between juvenile and adolescents, due to increased feeding behavior (Ashworth, Annandale 1904). Sea anemones may shrink during starvation or stress (Sebens, 1979; Roopin, Chadwick, 2009). The non-reproducing anemones respiration is rated as the main energy sink (Shick, 1991) and the respiration rate depends on temperature and many other factors (Szczebak et al., 2013). For their growth and reproduction, anemones can use the photosynthetic products from zooxanthellae (Achituv, Dubinsky, 1990). Measuring the body size of a sea anemone accurately is difficult as it may hold varying amounts of water in the coelenteron at different times producing errors in wet mass measurements (Stephenson, 1928; Chomsky et al., 2004). The anemone selected for this experiment, *S. haddoni* is one of the ten species of anemones that host anemonefishes (Fautin, Allen, 1997).

Reef Cnidarians enjoy multiple benefits through endosymbiotic micro algal dinoflagellates and macrosymbionts as fish, mollusks and crustaceans (Venn et al., 2008; Randall, Fautin, 2002; Patzner, 2004; Chadwick et al., 2008; Roopin et al., 2011). Sea anemones that host the anemonefishes is also harbor zooxanthellae as a rule (Dunn, 1981) that supply the host with energy rich photosynthetic compounds for respiration, growth and reproduction (Steen, 1988; Achituv, Dubinsky 1990; Whitehead, Douglas,
Expansion behavior, growth and survival of the sea anemone *Stichodactyla haddoni* (2003). It has been reported that anemones survive and grow better in the wild, when they harbor anemone fishes as symbionts (Porat, Chadwick-Furman, 2004; Holbrook, Schmit, 2005; Huebner et al., 2012). Although anemone biology is not understood completely, it is seen that metabolic energy deficiencies can cause a decrease in poly biomass. Thus, the body size of an anemone may not be directly related to poly age, but be related closely to its nutritional history (Stephenson, 1928; Roopin, Chadwick, 2009). Current study reports reveal that an increase in the expansion behavior, growth and rate of survival of the host anemone in captivity in the presence and absence of anemone fishes.

**Materials and Methods**

**Experiment design**

Totally, 45 nos. of sea anemone, *S. haddoni* and 60 nos. of anemone fishes (*Amphiprion polymnus, A. nigripes, A. perideraion, A. ocellaris, A. frenatus, A. sebae, A. clarkii, A. sandaracinos, A. melanopus* and *Premnas biaculeatus*) were procured from the traders at Chennai, India. The information obtained from the supplier reveal that the fishes and anemones had been collected from the coral archipelago of Indian waters. The animals were transported to a closed system hatchery at the Centre of Advanced Study in Marine Biology, Annamalai University. They were packed in polythene bags with water and oxygen and carefully acclimatized to the rearing system, before releasing to the experimental set-up.

Every two days, food remnants and other accumulated wastes were siphoned and 10% of water change was carried out. A canister filter (Eheim, Germany) was placed on inside the tank to maintain the water quality. Temperature 27 ± 1 °C, salinity 26 ± 0.5 psu, dissolved oxygen 5.5 ± 0.5 ml l–1 and pH 7.5 ± 0.2 were maintained in all tanks uniformly during the whole period of the study. The photoperiod was maintained at 13L & 11D with 18–20 m mol–1 sec–1 (CW Fluorescent bulb (200 W). The animals were fed daily with prawn and muscle meat along with frozen fish. The anemones and fishes selected for the study were of similar size to ensure the accuracy of the observations.

A Vernier caliper calibrated with an error of 0.1mm was used to measure the diameter of the oral and pedal discs. The diameter of the oral disc was taken as measure of expansion and the pedal disc was calculated as measure of growth rate (Porat, Chadwick Furman, 2004; Brace, Quicke, 1986).

**Growth measurements of solitary anemones**

Healthy anemones (N=15, Size =15.4 cm (SE ± 1.3) were transferred to the separate experimental tanks holding 500 liters (150 x 60 x 60 cm) of conditioned sea water. Tanks were marked S1 to S15. The diameters of the oral and pedal discs were taken at weekly intervals, throughout the 8-week of study period.

**Growth measurements of anemones hosting fish**

Ten species of paired anemonefishes were introduced to the tanks with individual anemones separately. The introduced pairs took few minutes to hours to be acquainted with the new host. The average body length of the introduced anemone was 15.4 cm (SE ± 1.34 cm). The diameter of the oral disc was measured in these anemones at weekly intervals throughout the study period. Based on the association of a fish with the anemone in wild, the fishes were categorized as native and nonnative species in association with the anemone (Allen, Fautin, 1997; Rema, Madhu, 2007).

**Survival of anemones in both groups**

Once the growth experiment was concluded, the set up was continued in order to study the effect of anemonefishes on their survival with the anemone. Anemones in both the groups were allowed to starve and fed them, once in a week. The solitary group had 15 anemones in individual tanks. The hosting group had 30 anemones in the group with pairs of different species of anemonefishes in their individual tanks. Release of excess mucus, shrinking, wide-
open mouth and strong decaying smell were taken as signs of death in the anemones. The relative number of anemones that did not die and decayed was taken as surviving number of anemones to calculate the percentages of survival.

Analysis of data

The standard statistical tool SPSS version number 16.0 (Norusis, 2009) was used for carrying out the statistical analysis. ANOVA, Chi-Square and Multiple Regression tests were carried out to determine the significance of the correlation between different species of anemone fishes and the wellbeing of the anemones, namely their expansion, growth and survival.

Results

Under the experimental conditions, the expansion rate of the solitary anemones showed variance between individuals. In comparison, a significant increase (ANOVA F\textsubscript{29.210} = 11.27, p<0.01) of the oral disc was observed in anemones hosting fishes. The expansion showed a steady increase, once the acclimation of the resident fish with the host was completed (Fig. 1). The maximum increase of 12.83 ± 2.5 cm was seen, when the anemone was hosting \textit{A. polymnus}. Anemones cohabitating with \textit{A. sandaracinos} and \textit{P. biaculaetus} showed an oral disc increase next to those with \textit{A. polymnus}, which was up to 8.83 ± 0.28 cm and 4.83 ± 1.25 cm respectively. The effect of cohabitating fish such as \textit{A. melanopus}, \textit{A. frenatus}, \textit{A. perideraion} and \textit{A. nigripes} has influenced to increase the size of the anemone’s oral disk up to 1.56 ± 0.02 cm, 1.02 ± 0.02 cm, –7.5 ± 3.04 cm and –12 ± 5.29 cm respectively. The influence of \textit{A. sebae}, \textit{A. clarkii}, \textit{A. sandaracinos} and \textit{A. ocellaris} showed no variations among themselves in increasing the diameter of the oral disc of the anemones. However, considerable variation was seen (Table 1) among other species as

Fig. 1. Expansion rate of oral disc diameter (cm) in \textit{Stichodactyla haddoni} in the presence native inhabited and non-inhabited anemonefish.

Рис. 1. Увеличение диаметра орального диска у \textit{Stichodactyla haddoni} в условиях присутствия и отсутствия рыб-клоунов.
Table 1. Analysis of variance (ONEWAY ANOVA) to determine variation between anemonefish and its host anemone expansion rate.

Таблица 1. Анализ отклонения (дисперсионный анализ), определяющего зависимость между видом рыбы-клоуна и ростом морских анемонов.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fish species</th>
<th>Mean / Среднее</th>
<th>Standard deviation (SD±)</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Amphiprion polymnus</em></td>
<td>28.85&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Amphiprion sebae</em></td>
<td>15.87&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Amphiprion clarkii</em></td>
<td>15.62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Amphiprion sandaracino</em></td>
<td>18.68&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Amphiprion frenatus</em></td>
<td>10.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Amphiprion ocellaris</em></td>
<td>18.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Amphiprion nigripes</em></td>
<td>13.69&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Amphiprion percula</em></td>
<td>7.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Amphiprion melanopus</em></td>
<td>21.69&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><em>Premnas biaculeatus</em></td>
<td>17.90&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2.11</td>
<td>2071.18</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* significant at the level of 1%  
* значимость 1%  
<br>

*a, b, c, d, e, f, g, and h superscripts determines significance at 5% level based on DMR test.

a, b, c, d, e, f, g, and h индекс определяющий значимость 5%, основную на DMR тесте.

Fig. 2. Multiple regression analysis of sea anemone growth rate.


Рис. 2. Множественный регрессионный анализ роста морских анемонов.  
confirmed by the Duncan Multiple Range Test (DMRT).

Mean growth rate of the anemones hosting with fish varied significantly between individuals depending on the species of fish hosted by them (ANOVA, $F_{9, 210} = 2071.18, p<0.001$) the growth rate was observed as 35-40% increase from the initial size. The rate of growth was significantly associated with the fact whether the anemones were hosting the fish or not (Table 2). Multiple regression analysis of the growth rate of the anemones showed an exponential character for the combinations of anemone fish and anemones for the particular species (Fig. 2). It should also be noted that an exponential decrease of growth was seen in *A. nigripes* and *A. perideraion* associated anemones. The anemones, which were not hosting any fish showed a growth rate around 20–25% against 35–40% showed by the anemones hosting with fishes. Furthermore, multiple regression analysis on growth rate of host anemone with residents like *A. polymnus* ($R^2=0.87, t = -9.19, p<0.001$), *A. sebae* ($R^2=0.51, t = -4.33, p<0.05$), *A. clarkii* ($R^2=0.67, t = -5.95, p<0.001$), *A. sandaracinos* ($R^2=0.31, t = -2.44, p<0.05$), *A. frenatus* ($R^2=0.84, t = -9.31, p<0.001$), *A. ocellaris* ($R^2=0.81, t = -9.28, p<0.001$), *A. melanopus* ($R^2=0.62, t = -2.48, p<0.05$) and *P. biaculeatus* ($R^2=0.71, t = -5.36, p<0.001$) showed an exponential increase. An exponential growth decrease was recorded in *A. nigripes* ($R^2=0.69, t = 12.3, p<0.001$) and *A. perideraion* ($R^2=0.65, t = 12.5, p<0.001$) (Fig. 2).

In the survival of anemones, it was seen that those who didn’t increased in size or shrank, they were ultimately died. During a period of 8 weeks starvation, 80% of the anemones hosting fish survived as against 60% of the survival in non-hosting group (Fig. 4). In Chi Square analysis, the survival rate of the hosting group of anemones was seen to be 54.1% and the non-
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Table 2. Chi-square analysis to find out the significant relationship between growth rate of anemone with native and non-native resident fishes.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fish species</th>
<th>Native степень сродства к анемонам</th>
<th>Non-Native степень сродства к анемонам</th>
<th>Chi-square value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Amphiprion polymnus</em></td>
<td>683 (32.2%)</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Amphiprion sebae</em></td>
<td>380 (17.9%)</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Amphiprion clarkii</em></td>
<td>373 (17.6%)</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Amphiprion sandaracinos</em></td>
<td>–</td>
<td>445 (24.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Amphiprion frenatus</em></td>
<td>260 (12.3%)</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Amphiprion ocellaris</em></td>
<td>–</td>
<td>447 (24.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Amphiprion nigripes</em></td>
<td>–</td>
<td>295 (16.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Amphiprion percula</em></td>
<td>–</td>
<td>161 (8.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Amphiprion melanopus</em></td>
<td>–</td>
<td>492 (26.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><em>Premnas biaculeatus</em></td>
<td>424 (20.0%)</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at 1% level  
* значимость 1%  
Values within () bracket shows the column value  
Значение в скобках () показывает долю в процентах

hosting group, it was 45.1%. The presence or absence of a hosted fish and the ability of the anemone to survive in the starving period showed a strong association ($\chi^2 = 25.019$, df(7), $p<0.01$). It was seen that the survivability of the sea anemone, *S. haddoni* during starvation was enhanced by the presence of hosted anemone fish. Further, the rate of growth of the sea anemone *S. haddoni* was seen to be higher, when hosting the species of natural choice (that it prefers to host in nature). However, the survival depended on the availability of hosted fish with no particular preference to the fish species.

Discussion

The mutual relationship between anemone fish and their host anemones in coral reefs has been known for a limited species (Fautin, Allen, 1997). It is seen that the relationships between 30 species of anemone fishes of the genera *Amphiprion* and *Premnas* and 10 anemones belonging to the order Actiniaria (Fautin, Allen, 1997; Allen et al., 2010). The fish cannot survive in nature without safety of the tentacles of the hosting anemones (Mariscal, 1970; Allen, 1972; Fautin, Allen, 1997). However, both co-
habitants can survive in aquaria (Fautin, Allen, 1997).

Fish utilize these habitats for a variety of activities such as feeding, resting and as sites for reproduction (Fautin, Allen, 1997; Arvedlund et al., 2000). Despite their ubiquity, the exact nature of these associations is often not understood well (Holbrook, Schmitt, 2005; Huebner et al., 2012). In addition to their host of preference, fish have also been forced to associate with anemones other than their natural preference in the wild. It is seen that the anemones and fish grow faster in the wild as well as in captive conditions when cultured together (Porat, Chadwick-Furman, 2005). Furthermore, the spawning efficiency of a resident fish was higher, when they were held with sessile host anemones in aquaria (Balamurugan et al., 2013).

The expansion response of the host anemones to their fish symbionts appears to be adaptive, in that it allows them to expose their endosymbiotic zooxanthellae to light in the presence of anemone fish (Porat, Chadwick-Furman, 2004). Anemones could potentially derive direct and indirect nutritive benefits from the fish occupying them. Anemones may directly ingest particles dropped by the fish hosted by the fish or absorb their waste, which could provide sources of regenerated nitrogen (Roopin et al., 2007), sulfur and phosphorus (Godinot, Chadwick, 2009).

It was seen in this study that the expansion of the oral disc was greater when the anemone hosted the fish by its natural choice, rather than when hosting other species. It could be that the innate immunity of the fish to its natural host helps the fish to acclimatize quicker and settle down with them quickly. Roopin and Chadwick (2009) suggested that the typical behavior of the fish in swimming and fanning on the tentacles of the anemone during feeding would expand the oral disc (tentacle crown) more than in the
solitary anemones. Dependence of long-term growth, expansion and survival of *E. quadricolor* depends on both numbers and size of its resident anemone fish depicts the strong benefits of this association (Porat, Chadwick-Furman, 2004). Therefore, the negative expansion of the oral disc would indicate the shrinkage of anemone tentacles from their initial size due to the absence of fanning and dissociation behavior of anemone fish from the host.

Based on the current study, the growth rate of the sea anemones associated with hosted fish (35–40%) showed an increase of 10–15% in comparison with anemones not associated with fish (20–25%). Thus, the anemone fish appear to provide physiological benefits to their host anemones in large part through their contribution of excreted ammonia and act as a primary factor of controlling the zooxanthellae in the host anemones (Roopin, Chadwick, 2009). In times of scarcity of food, host feeding and the anemone’s catabolism are often insufficient to supply the complete nitrogen requirement of the zooxanthellae (Rahav et al., 1989).

During the period of starvation, the solitary anemones *S. haddoni* had a low survival rate of 60% in comparison to the higher survival rate of 80% in the anemones hosting fishes. This suggests that the presence of the fish hosted by the anemone has an influence on the growth and survival of host anemones in captivity. Spotte (1996) and Roberts et al. (1999) suggested that the source of nitrogen could be extremely beneficial as it can be limiting in these systems and anemones may take up even small amounts of nitrogen. The presence of even one adult fish with the anemone may supply sufficient quantities of ammonia and other nutrients (Cleveland et al., 2008; Godinot, Chadwick, 2009; Roopin et al., 2011). Porat and Chadwick-Furman (2004) and Roopin et al (2008) reported that sea anemones that had been maintained without anemone fish for four weeks took up ammonia from enriched water at a faster rate than those that had been kept with anemone fish, which absorbed very little ammonia. Based on this, they concluded that anemone fish provides ammonia to their host anemones and the zooxanthellae they harbor, which enhances the rates of tissue growth and regeneration.

During the period of starvation, the anemones hosting fish had a better chance of obtaining nitrogen than those without any hosted fish. This provided them with a better chance of survival in comparison with the solitary anemones during the starvation period. In this study, we have reached the conclusion as reported in the earlier studies, that the expansion, growth and survival of anemones in captivity is associated with the size, numbers and the species of anemone fish associating with it.

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**References**


