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Abundance of anemone fishes in North Bay Island and mass culture of live food organisms for their larval rearing

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Abstract

Understanding the transect survey for abundance of anemone fishes and other living organisms is important to asses reef associated fish diversity in North Bay island. The percentage distribution of 10 different substratum from the disturbed, semi-disturbed and undisturbed areas was recorded during the survey in North Bay islands during November 2009 to April 2010. The survey observations reveal that the fishes were the dominant groups followed by mollusks, lobsters and octopus. There are 5 different anemone fishes were collected during the transect survey and their distribution is more in undisturbed area. We are standardizing the different mass culture techniques for production of phytoplankton and zooplankton for the nutritional source for the anemone fish larvae. Monitoring the water quality parameters and culture the phytoplankton and zooplankton used in different culture media with 2 adjustment studies like with and without salinity adjustment. The results of this experiment indicate that zooplankton was rich in protein and fat content and it will be used as high nutritional source for feeding fish larvae.

Keywords: Anemone fishes, line transect survey, phytoplankton, zooplankton, live feed, North Bay Island

INTRODUCTION

Andaman and Nicobar Islands is the largest archipelago in the southern reaches of Bay of Bengal, situated 14° to 16° north and 92° to 94° east. It comprises of about 572 islands, islets and rocky outcrops. The total coastline of the Islands is 1962 km, which accounts for 25% of the country's coastline and encompasses 28% of the total Indian Exclusive Economic Zone (Rao *et al.* 2000, Rajaram and Nedumaran 2009). There are about 106 protected areas in these Islands, of which 96 are designated as Wildlife sanctuaries, 9 declared as National Parks and a Biosphere reserve in Great Nicobar (Reddiah 1977). These Islands are considered a veritable repository of diversified flora and fauna because of the richness of the existing gene pool. Marine fishes have an important role in the food chain of the Marine Ecosystem. Marine ornamental fishes are said to have association with the coral seas (Herre 1941). Since the coral reef environs can provide with a wide variety of ecological niches for shelter as well as food, extremely rich and varied animal communities with great diversity of species can thrive here. About 400 species of ornamental fishes belonging to 175 genera and 50 families are reported in Indian waters (Satheesh 2002). Ornamental marine organisms can be divided into four distinct components: stony corals, black coral and precious corals, other live invertebrates such as soft corals, anemones, crustaceans and echinoderms and fishes. Anemone fishes are commonly found ornamental fishes in many islands of Andaman. The coral reef ecosystem of Andaman and Nicobar islands harbours a variety of colourful marine ornamental fishes which have high demand in the international marine aquarium trade and is proved to be an ideal area for ichthyological biodiversity (Sale and Douglas 1984). The brightly coloured members of the pomacentrid subfamily Amphiprioninae are well known for their commensal relationship with large sea anemones (Allen 1972 and 1980). The popularity of clown fish among the aquarists is due to their small size, hardy nature, attractive colour, and adaptability in captivity and interesting behaviors' display in association with anemone. In recent years, there has been a surge in the trade of tropical ornamental fishes and at the same time indiscriminate exploitation has also led to negative repercussions on coral reef ecosystem (Godwin and Fautin 1992). Keeping in view of the importance of anemone fish species in aquarium trade as well as its availability in these islands, there is an urgent need to develop suitable techniques to enhance the culture technology of these species in this island to safe guard the natural population. The present study has been undertaken with an objective to record the distribution and abundance of anemone fishes in the North Bay islands and to standardize the mass culture techniques of phytoplankton and zooplankton for successful implementation of brood stock development, spawning and larval rearing of anemone fishes.

METHODOLOGY

Survey of Ornamental fishes: North Bay Island is located eastward to Port Blair and it is one of the islands having rich reef biodiversity (Figure 1). The Bay is almost triangular in shape and endowed with sedentary massive splendid corals and fragile marine biota. Survey has been conducted in 3 selected sites, which are approximately 200 m away from the shore. Totally 10 transects (Tr) has been conducted out of three selected sites. Transect 1 to 4 were from disturbed areas where there has been high degrees of human activities because of heavy tourist inflow like boat movement, snorkeling, fishing, SCUBA diving etc. In site 2, three transect survey was conducted which was semi disturbed area, where not much human activities was carried out, but only boats are playing through that areas. Site 3, three transect survey were conducted which is undisturbed area. The objective was to compare and record the distribution of anemone fishes in all above mentioned conditions. Anemone fishes were collected using scoop net and brought back to laboratory. Along with fishes specific sea anemones were also collected. Fishes were precisely identified using standard field works (Rajan *et al.* 1992, Rao *et al.* 2000, Rajaram *et al.* 2007, FAO Identification sheets and FISHBASE database). Their total length (TL) and Total weight were measured for morphometric analysis. Fish were classified according to species and grown in glass tanks with their specific host sea anemone. They are fed with rotifers twice a day.

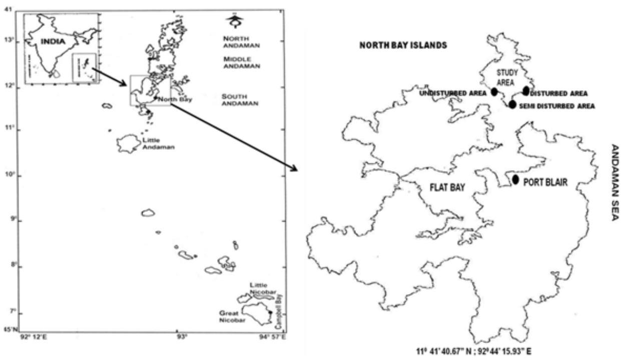

Figure 1: Map of the study area, the North Bay Island

Mass culture of phytoplankton: Phytoplankton mass culture was treated in two phases namely with and without salinity adjustments. Four numbers of cultured tanks were arranged in continuous pattern to keep the cultures of the *Chlorella* species in the Marine research Laboratory, Fisheries division of Central Agricultural Research Institution, Marine Hill.

Treatment of phytoplankton culture: The Walne's media and three types of various doses of fertilizers were used and treated to the cultured *chlorella* sp. The Walne's media was treated with four different compositions namely Solution: A, B, C and D. The solution A contains $FeCl_2$ (1.3 g), $MnCl_2$ (0.36 g), H_3BO_3 (33.4 g), EDTA[(b)] (45 g), Sodium di-hydrogen orthophosphate (20 g), Sodium nitrate (100 g) which are make up into 1 litre with distilled water. The solution B contains $ZnCl_2$ (4.2 g), Cobalt chloride (4 g), Ammonium molybdate (1.8 g) and copper sulphate (4.0 g) which are make up into 1 liter with distilled water. The Solution C contains Vitamin B_1 (0.8 g) make up into 800 ml with distilled water and Solution D contains Vitamin B_{12} (0.4 g) which make up into 800 ml distilled water. The four different composition of the Medias are poured in the tanks were left alone for one day. *Chlorella* culture was inoculated to each of the tanks at the rate of 1:20. Aeration was provided in plenty for enhanced growth of *Chlorella sp.*, Water quality parameters such as pH, salinity, temperature, turbidity and dissolved oxygen (DO) were monitored critically every day. *Chlorella sp.*, Collected on daily basis and their optical density (OD) is determined by Spectrophotometer at 540 nm. To correlate the optical density with the plankton population, plankton number and its corresponding optical density was found out and a trend of relationship was established.

Treatment of zooplankton culture: The rotifers (*Brachionus sp.*) were collected by the zooplankton net (BSC no 25) from the Sippighat Brackish water regions. The salinity ranges around 29 ppt. Samples were subsequently transferred to the sampling bottles and brought back to the laboratory. The collected Zooplanktons are treated with five different compositions namely T_0, T_1, T_2, T_3, T_4. Whereas T_0: Algae as feed for zooplankton, T_1: Low dose of organic matter, T_2: Medium dose of organic matter, T_3: High dose of organic matter and T_4: Very high dose of organic matter. Mass culture of *Brachionus sp.*, was done by using two ways: using *Chlorella* as feed and using a vibrant mixture of organic manures consisting of Green nut oil cake: poultry manure: cow dung manure: pig manure in the ratio 1:1:1:1. Five numbers of cleaned plastic FRP tanks were (500 l) used in this experiment. 300 litre of filtered sea waters and the different doses of manures are poured in all the tanks. Sufficient aeration was provided in all tanks soon after the inoculation. Water quality parameters were measured every day. Five liter of zooplankton rich water was collected from five different regions of tanks. Samples thus collected were allowed to pass through zooplankton filtered net and collected in glass bottles. They were preserved in 5% formalin. One ml of concentrated rotifer sample was taken in Sedgwick-Rafter counter chamber and the number of *Brachionus* sp., was counted under microscope.

RESULTS AND DISCUSSION

Lines transect survey in percentage distribution of different substratum: Data pertaining to percentage distribution of different substratum recorded during the line transect survey off North Bay Island was illustrated in Tables 1-2 and Figure 2. In this study data on nature of substratum were estimated from 10 number of transects from three selected sites, namely disturbed, semi-disturbed and un-disturbed area in the North Bay island. The survey results shows that the coral reef was the dominant substratum in semi disturbed area (69%), however, there were not much difference in the percentage contribution from undisturbed (44.8%) and disturbed area (45.6%).

Table 1: Percentage distribution of different substratum recorded during the line transect survey of North Bay Island

Substratum	***Disturbed area***				***Semi disturbed area***			***Undisturbed area***			***Mean (±SE)***
	Tr 1	***Tr 2***	***Tr 3***	***Tr 4***	***Tr 5***	***Tr 6***	***Tr 7***	***Tr 8***	***Tr 9***	***Tr 10***	
Rock	17.03	12.00	14.44	27.69	8.45	7.81	6.89	6.66	0.00	4.34	10.53±2.45
Rubble	0.00	4.66	0.00	0.00	0.00	0.00	0.00	12.38	10.20	7.82	03.50±1.55
Sand	8.14	10.00	0.00	0.00	0.00	0.00	2.41	9.52	15.30	14.78	06.01±1.98
Dead coral	19.25	0.00	19.44	13.84	0.00	2.18	0.00	5.71	0.00	7.82	06.82±2.52
DC +Sn	0.00	13.33	5.55	7.17	0.00	0.00	0.00	8.57	15.30	0.00	04.99±1.88
DC + Sn +MA	11.11	23.33	10.55	0.00	4.22	11.87	18.27	19.04	26.53	21.73	14.66±2.70
Sn + MA	0.00	0.00	0.00	0.00	7.04	0.00	0.00	0.00	0.00	0.00	00.70±0.07
Soft coral	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.08	0.00	00.40±0.40
Coral reef	44.44	36.66	50.00	51.28	80.28	78.12	72.41	38.09	28.57	43.47	52.33±5.78

Sand =Sn, Dead coral =DC, Marine Algae =MA, Soft coral=SC, Tr 1-10 =Transect 1-10

Table 2: Average percentage distribution of different substratum from disturbed area, semi-disturbed, undisturbed area during the line transect survey of North Bay

Substratum	***Disturbed area***	***Semi-disturbed***	***Undisturbed area***
Rock	17.79±3.46	10.24±0.45	5.31±1.95
Rubble	1.17±1.16	0.00	7.68±1.31
Sand	4.54±2.64	1.74±0.80	10.34±1.84
Dead coral	13.14±4.56	3.83±0.72	4.34±2.33
DC +Sn	6.52±2.74	0.00	6.38±4.42
DC + Sn +MA	11.25±4.77	11.41±4.06	19.68±2.18
Sn + MA	0.00	1.76±2.34	0.00
Soft coral	0.00	0.00	1.02±1.36
Coral reef	45.60±3.32	69.10±2.34	44.81±4.35

Sand =Sn, Dead coral =DC, Marine Algae =MA, Soft coral=SC, Tr 1-10=Transect 1-10

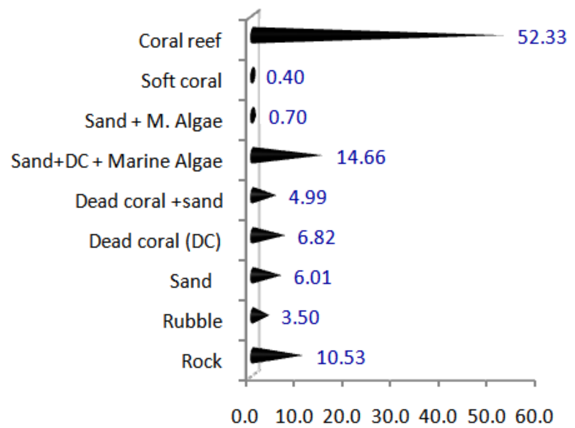

Figure 2: Percentage distribution of different substratum recorded during the transect survey

Distribution of anemone fishes: From the data obtained from the ten transects, it could be accomplished that fishes were the dominant group in all transects that were studied (Figure 3).

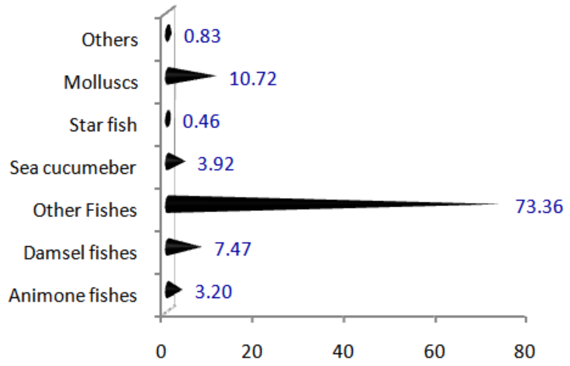

Figure 3: Percentage distribution of anemone fishes and other living organisms recorded during the transect survey from different locations of North Bay

A total of 80.9 % was constituted alone by fishes and in those anemone fishes represents only about 7.5%. Based on the overall percentage contribution, anemone fishes placed third. The second dominant group in the study area was the molluscs (10.7%). Anemone alone also constituted about 3.2% in the study area. It is generally well established facts that anemone fishes and anemone exhibit a symbiotic relationship and in the present study, it was observed that percentage contribution of anemone fishes was slightly more than double to the anemone. There were five anemone fishes observed during the survey and among them *Amphiprian akallopisos* was the dominant species (5.3%) followed by *Amphiprian percula* (1.4%). Total percentage contribution of anemone fishes in undisturbed area transect was 15.7% where as in disturbed area was 4.4% and semi disturbed area was 2.3% (Table 3).

Table 3: Average percentage distribution of anemone fishes from disturbed area, semi-disturbed, undisturbed area during the line transect survey of North Bay

Substratum	***Disturbed area***	***Semi-disturbed***	***Undisturbed area***
Amphiprian percula	2.342±1.06	0.563±0.08	0.835±0.11
Amphiprian ephippium	0.829±0.29	0.714±0.11	0.000
Amphiprian akallopisos	0.947±0.40	0.590±0.06	15.752±0.84
Eprion biaculatus	0.316±0.18	0.359±0.10	0.139±0.13
Amphiprion clarkii	0.000	0.054±0.05	0.000

The result shows that anemone fishes prefer undisturbed environment for their growth and reproduction. So the ratio between anemone fishes and anemone were almost double. In overall the condition of coral reefs in terms of coral cover and reef fish abundance in marine managed areas was considerably better than in open access areas. A reduction in destructive fishing activities, overfishing and eutrophication, undermining the recovery of the reefs was indicated by an increase in the number of carnivorous fish such as groupers, snappers and jacks, which are target species for local fisheries (Ardiwijaya *et al.* 2007). A number of other studies have demonstrated that the abundance of the fish community is higher in protected areas (Alcala 1988, Russ and Alcala 1989, Alcala and Russ 1990, Polunin and Roberts 1993, Watson and Ormond 1994).

Mass culture of phytoplankton

Growth and production of mass culture of phytoplankton under different culture media without salinity adjustment: Culture of algae is one of the important and prerequisite steps for successful running of a hatchery operation (Kailasam and Thirunavukarasu 2008). Because the algae constituted an important component as feed for zooplankton and other fish food organisms (Benoit 1964). Walnes medium is one of widely used media used for culture and maintenance of algae. The phytoplankton mass culture was survived longest in Walnes media for about 27 days without any problem and after that the media got crushes. But the other three tanks like different doses of fertilizers the phytoplankton concentrations are crushed very short period than the walnes media. The result shows that Walnes media was the best media for maintaining mass culture of phytoplankton in outdoor location as well as in large scale (Table 4).

Table 4: Growth and production of mass culture of phytoplankton under different culture media

Culture period (days)	***Concentration of phytoplankton (In Optical Density)***			
	Walnes Medium (T_0)	***Low dose of fertilizers (T_1)***	***Medium dose of fertilizers (T_2)***	***High dose of fertilizers (T_3)***
1	0.029	0.037	0.040	0.033
7	0.150	0.124	0.143	0.083
13	0.224	0.175	0.213	0.164
19	0.306	0.201	0.096	0.058
20	0.313	0.150	0.043	*Crushed*
21	0.320	0.080	*Crushed*	
23	0.260	*Crushed*		
27	*Crushed*			

Hence, next best choice will be with the low dose of fertilizer *viz.* di ammonium Phosphate, urea and single super phosphate with 2.0 g, 0.2 g and 0.2 g respectively.

Number of days the mass culture maintained was relatively less, as only once the fertilizer was used. However, if fertilizer dose was repeated on an average 19-21 days in low of fertilizer there might be a chance that there will be a constant growth of the mass culture of phytoplankton. Also other than Walnes medium, next best medium which could be used for mass culture of phytoplankton was the low dose of fertilizer.

Growth and production of mass culture of phytoplankton under different culture media after salinity adjustment: In this method, the observation was clear that maximum growth and survival of phytoplankton was recorded at low dose of fertilizers (T_1) culture media compare to T_2 and T_3. This was followed by medium dose of fertilizers (T_2) and then high dose of fertilizers (T_3). In T_2 and T_3 culture media the phytoplankton got crushed on the same day on 18th day. In case of Walnes media with salinity adjustment, growth and survival of phytoplankton, was not very good compared to other media. The phytoplankton got crushed on the 17th day itself. The maximum growth and survival of phytoplankton observed in the present study, was on the low dose of fertilizers (T_1) with salinity adjustment and phytoplankton could be maintained up to 20th day of culture before getting crushed (Table 5). Thus it could be concluded that both with salinity adjustment or with salinity adjustment low dose of (phosphate) fertilizer could be optimum for growth of phytoplankton and could be maintained for relatively longer period in open outdoor condition without any problem.

Table 5: Growth and production of mass culture of phytoplankton under different culture media after salinity adjustment

Culture period (No. of days)	*Concentration of phytoplankton (in optical density)*			
	Walnes medium (T_0)	*Low dose of fertilizers (T_1)*	*Medium dose of fertilizers (T_2)*	*High dose of fertilizers (T_3)*
1	0.013	0.053	0.047	0.043
5	0.107	0.177	0.140	0.127
9	0.163	0.207	0.183	0.163
13	0.123	0.193	0.093	0.130
17	*Crushed*	0.077	0.027	0.053
18		0.053	*Crushed*	*Crushed*
19		0.033		
20		*Crushed*		

Mass culture of zooplankton

Culture of zooplankton (rotifers) under different organic manure: Feed density has a significant effect on the growth and survival of the fish larvae (Lubzens *et al.* 1997). Rotifers, especially *Brachionus* sp., are used worldwide or in conjunction with other types of food, to rear development stages of marine finfish and crustaceans. The culture technology of rotifers used to be dependent on the empirical skills of technicians and the productivity of rotifers was comparatively low (Kitajima 1983). Hence, monitoring and sustainable production of the live fish feed is an important criteria in hatcheries (Boyd 1982). The Zooplankton culture observation shows that maximum growth and survival of zooplankton was recorded at very high dose of organic manure (T_4). Second highest production was achieved in low dose of manure. The minimum growth of zooplankton (rotifers) was observed in the phytoplankton media. This result showed that the organic manure was a good media for growth of zooplankton when compared to the phytoplankton media. In phytoplankton media 80 l phytoplankton was supplemented in 220 l of sea water for the zooplankton growth. Low production of zooplankton could also be due to insufficient food (phytoplankton) available for the rotifer to grow and multiply (Table 6). Immediately after manure application is highly adverse to aquatic life and it is likely that populations of zooplankton are largely altered due to environmental perturbations. The organic fertilizers may also cause dissolved oxygen problems during the initial decomposition period (Morris and Mischke 1999). Jana and Chakrabarti (1993) suggested that the interval between 2 and 10 days after manure application is a prerequisite for establishing satisfactory environmental conditions for zooplankton production and decomposition process encountered.

Table 6: Mass culture of zooplankton under different organic manure

Culture period (days)	*Organic manures*				
	*T_0**	*T_1***	*T_2**	*T_3***	*T_4***
0 – 12 days	Allow them to grow (gm/l)	Kept for decomposition (gm/L)			
13th days (Initial inoculums/l)	0.3	0.3	0.3	0.3	0.3
30th day	22.00±1.15	28.67±0.7	23.33±0.7	26.67±0.6	33.33±0.7

T_0, Phytoplankton media (80 l phytoplankton); T_1, Low dose (75 g); T_2, Medium dose (150 g); T_3, High dose (300 g); T4, Very high dose (600 g), *, in 220 l water; **, in 300 l sea water*

Proximate composition of phytoplankton and zooplankton: The percentage of moisture content in phytoplankton (80.6%) was higher than zooplankton (72.6%). The percentage of protein and fat content were found to be higher in zooplankton (56.5% & 51.8%) than phytoplankton (51.8% and 8.2%) (Figure 4). Goswami *et al.* (1981) studied the variation in proximate composition of zooplankton from Vengurla to Ratnagiri, west coast of India. They recorded on an average of 13.44 to 45.55% protein and 15.93 to 22.47% lipid in zooplankton. However, there was not much variation in the ash content in phytoplankton and zooplankton. From the results, it was obvious that zooplankton was rich in protein and fat content. Hence it can be concluded that zooplankton forms an excellent source of nutrition for feeding fish larvae. Zooplankton constitutes the largest ecological group organisms in the sea and plays an important role in marine food chain. They feed on phytoplankton and in turn form the food for animals at higher trophic level.

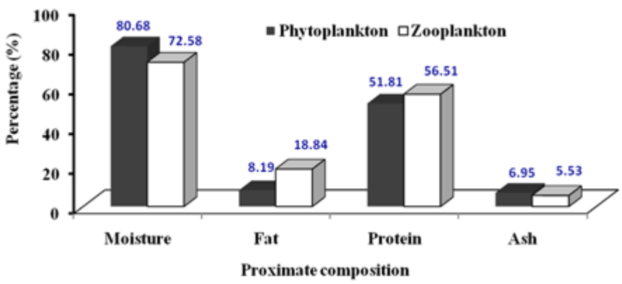

Figure 4: Proximate composition of phytoplankton and zooplankton (%)

Distribution of anemone fishes and other living organism during the transect survey from 10 locations of the three transected sites of North Bay Islands result indicates that fishes were the dominant groups (80.9%) followed by molluscs (10.7%) followed by other like lobsters, octopus and eels. There are 5 anemone fishes were recorded during the survey, among the anemone fishes collected, *Premnas biaculatus* was the biggest one, the average length and weights was 10 cm and 34.3 g respectively. Further, there was very high correlations exist between length and weight relationship of anemone fishes. The relationship between phytoplankton number and optical density shows highly significant (r=0.98). The efficient of different culture media on growth and mass culture of phytoplankton without salinity adjustment indicates that, Walnes media was the best media for maintaining mass culture of phytoplankton in outdoor location as well as in large scale. Rotifers in different organic manure study showed relatively higher dose of organic matter and high dissolved oxygen level facilitates higher production of zooplankton. Proximate composition of phytoplankton and zooplankton results revealed that, zooplankton was rich in protein and fat content it will be used as an excellent sources of nutrition for feeding fish larvae.

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