Recovery status of sea anemones from bleaching event of 2010 in the Andaman waters

Anemones are closely related to scleractinian corals as they are similar in structure. However, anemones lack a hard skeleton formed of calcium carbonate and are not colonial. Like scleractinian coral polyps, the tentacle of anemones contains single-celled symbiotic dinoflagellate called 'zooxanthellum', which imparts the brown colour. These symbionts living within the cells of anemone are essential to the host as they are responsible for producing high-energy substance (glucose) through photosynthesis, that leaches into the host tissue and forms an important dietary supplement[1–3]. Hence, the growth of anemone depends on their relationship with its symbiotic alga. Besides the symbiotic relation with zooxanthellae, anemones from the genera *Heteractis* and *Stichodactyla* are also well known for mutualistic relationship with clownfish, *Amphiprion* sp. Even though the anemones are equipped with microscopic stinging cells called nematocysts located at the end of their tentacles, the associated clownfishes secrete special mucus substance to avoid stinging by their host[4]. In addition to their ecological significance, sea anemones are reported to produce many biologically active polypeptides and proteins, like green fluorescent protein, which are used as pharmacological and biomedical tools and are of commercial importance[5].

Middle and South Andaman Islands have a fairly good fringing reef ecosystem, particularly the North Bay, Chidiyatappu, Ross and Havelock Islands. These reefs are dominated by *Porites lutea*, *Porites nigrescens* and *Acropora* spp. The appearance of corals or part of them in white (bleaching) is known to be associated with stress, which may be induced by sudden increase of sea-surface temperature (SST). The bleaching is caused by the expulsion of the symbiotic algae, zooxanthellae from coral polyps. Coral bleaching was observed in Andaman Islands from April to July 2010. During this period, almost 74% of live corals appeared to be in bleached condition at North Bay, Chidiyatappu, Ross and Havelock Islands (Figure 1). SST rose from 30.5°C to 34°C (April to May 2010) within the bays of Port Blair during this period. Rainfall data of the India Meteorological Department (IMD)[6] have shown that due to delayed onset of the southwest monsoon, the summer period got extended beyond the normal cycle and caused rise in SST of study area. Moreover, the reason for this unusual SST observed in the summer of 2010 has been reported to be a combination of El Niño followed by La Niña, which was caused by climate change[7]. The SST data[8] of the National Environmental Satellite, Data and Information Service of NOAA (NESDIS) have shown 1–2°C increase above the normal level in the Andaman and Nicobar (A&N) Islands during 1 April to 24 May 2010. There were three bleaching events reported in the A&N Islands prior to this current event, i.e. in 1998, 2002 and 2005 (ref. 9).

Sea anemones of these reef ecosystems have also undergone bleaching similar to coral bleaching. The extent of anemone bleaching and percentage of recovery were estimated following Line Intercept Transect method[10] on the GPS-fixed transect coordinates. During the study period, 100% of the anemones were recorded in bleached condition. Three different species of anemone, *Heteractis magnifica*, *Heteractis aurora* and *Stichodactyla* sp. hosting clownfishes were found to be bleached in all the three places studied from April to July 2010 (Figure 2 and Table 1). It was also observed that there was slight reduction in the clownfish numbers between the 2010 bleaching event and its corresponding recovery period. Similarly, there was significant reduction in clownfish numbers due to complete disappearance of several sea anemone species during the 1998 bleaching event reported[11] from Sesoko Island, Japan. It was also reported that such bleaching event could affect the ability of the clownfish to detect chemical signals essential for locating their alternate anemone homes, which will ultimately affect their survival[11]. However 100% recovery of *H. magnifica* in the North Bay and 80% recovery of *Stichodactyla* sp. at Chidiyatappu were

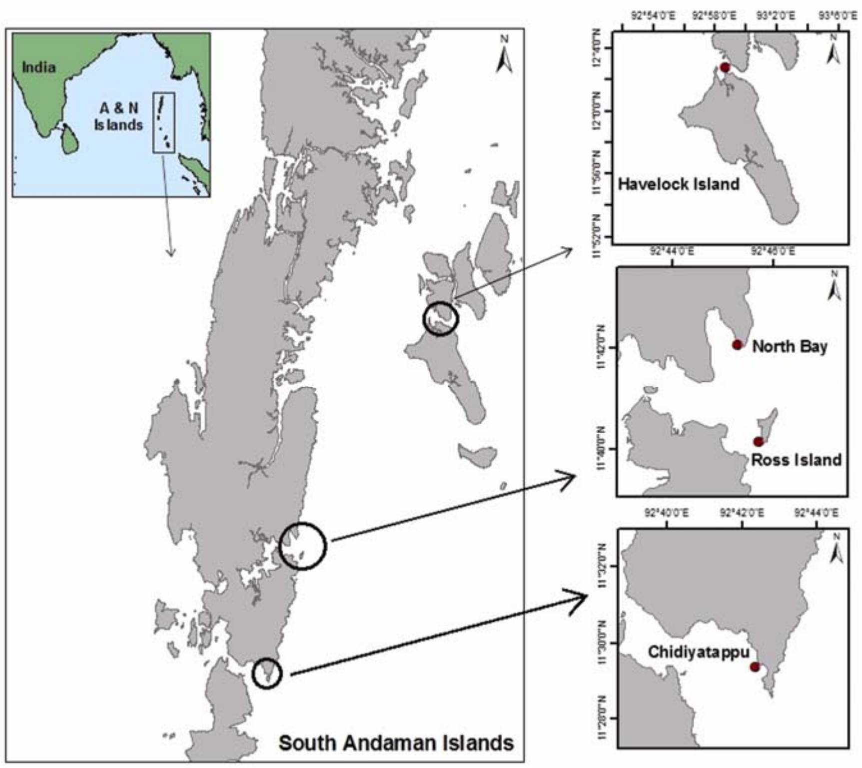

Figure 1. Study sites at South and Middle Andaman Islands.

Table 1. Status of sea anemone and associated clownfish during the bleaching (July 2010) and recovery (January 2011) periods

Location	Anemone species	Cover (%)	Crown width (cm)	Bleaching (%)	Recovery (%)	Number of clownfish
North Bay 11°42′13.96″N, 92°45′06.14″E	*Heteractis magnifica*	0.5	35	100	100	*Amphiprion ocellaris* – 3
Havelock 12°02′51.77″N, 92°58′46.27″E	*H. magnifica*	0.7	40	100	100	*A. ocellaris* – 4
Ross Island 11°40′15.79″N, 92°45′37.21″E	*Heteractis aurora*	0.5	25	100	100	*Amphiprion* sp. – 1
Chidiyatappu 11°29′40.50″N, 92°42′36.40″E	*Stichodactyla* sp.	1.25	25	100	80	*Amphiprion clarkii* – 3

Figure 2. Bleached and recovery of sea anemones in the Andaman waters. ***a***, Bleached anemone, *Heteractis magnifica* at Ross Island during July 2010. ***b***, False clownfish, *Amphiprion ocellaris* on bleached *H. magnifica* at North Bay during July 2010. ***c***, Partially recovered *Stichodactyla* sp. at Chidiyatappu during December 2010. ***d***, Recovered *H. magnifica* at North Bay during January 2011.

recorded during a subsequent study made in December 2010.

The mean SST observed during the recovery period (December 2010–January 2011) was 29.5°C and the transparency was in the range 8–10 m. NESDIS[8] has shown that SST observed was 1°C less than the normal SST during December 2010 at South Andaman Islands. These conditions have helped the bleached species to recover to their previous state. During this recovered (healthy) condition, *Stichodactyla* sp. with the disc width of 25 cm along with 3 of each *Amphiprion clarkii* and *H. magnifica* with the disc width of 35–40 cm along with four numbers of *Amphiprion ocellaris* were recorded.

Like coral bleaching, lack of zooxanthellae also poses a significant risk for the sea anemones which become translucent and bleached; they are also deprived of supplementary nutrients supplied by the zooxanthellae. The protection given by the anemones is necessary for the clownfishes as they are poor swimmers and would not survive without their symbiont (anemone) in open water[11]. At the same time clownfishes provide ammonia as an excretory product to the host anemone, which is in addition to feeding of planktonic organisms, which are necessary for regeneration of zooxanthellae during the recovery period from bleaching[12]. The bleached anemone requires suitable environment with appropriate light conditions and less sedimentation, which would be helpful for it to recover to its previous state. These conditions helped to rebuild the zooxanthellae population and the normal brownish hue over time. This process may take three months or longer[13]. At least five different types of cellular mechanisms of symbiont loss (expulsion, *in situ* degradation, digestion, exocytosis, and apoptosis and necrosis) from anemone host have been reported[14]. A maximum of 100% and a minimum of 80% recovery have been recorded in sea anemones in the present study. Such a high rate of recovery suggests that the present bleaching was due to the elevated SST recorded during the summer months and the type of bleaching was a consequence of simple expulsion of zooxanthellae due to the stress induced by the elevated SST.

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Description and phylogenetic characterization of common hydra from India

Hydra, a freshwater polyp belonging to phylum Cnidaria and class Hydrozoa, is globally distributed except in the Antarctic region and Oceanic islands[1]. Although this organism has been extensively used as a model system in biology, there has been considerable uncertainty over its taxonomy, primarily due to lack of taxonomically distinct features. This created a doubt whether to put different hydra species under the genus *Hydra*, which was first reported by Carl Linné[2], or to follow Schulze's three genera classification[3]. Campbell[1] has classified the genus *Hydra* into four different groups: 'oligactis group' (stalked hydra), 'vulgaris group' (common hydra), 'braueri group' (gracile hydra) and 'viridissima group' (green hydra), based on morphological differences. A recent study based on molecular phylogenetic analysis has shown the reliability of Campbell's system of grouping different hydra species[4]. Hydra types were first studied in India by Annandale[5,6]. A detailed study based on morphological and physiological characters of Indian hydra types was conducted by Prasad and Mookerjee[7]. However, they have refrained from naming any species, except *Chlorohydra* (green hydra) collected from Hyderabad[7]. In India, a local species referred to as *Pelmatohydra oligactis*, is being used as a model system for studying regeneration, pattern formation and development[8–12], but has not been taxonomically described till date. It has so far been referred to as *P. oligactis* based on a personal communication between late L. H. Hyman and late Leela Mulherkar. However, a detailed taxonomic study of Pune hydra ecotype, especially in view of the prevailing principles of hydra taxonomy, has not been carried out. With increasing use of this organism as a model system, it is necessary to describe the taxonomic position and phylogenetic relationship of Indian hydra with other species of hydra.

Polyps collected from a local pond were cultured by standard method[13]. Live polyps were collected randomly from the culture and their body length was measured by placing a graph paper under the glass beaker containing the animals. Hydra at various stages of budding were randomly selected from a mass culture, relaxed by exposure to 2% urethane for 2 min and fixed in 4% paraformaldehyde overnight at 4°C (ref. 14). The pattern of emergence of tentacles was studied with an Olympus SZX16 stereomicroscope. Nematocysts were prepared for observation as described by David[15] and photographed with a Zeiss Axio ImagerZ1.

Total DNA was isolated from 50 polyps by the phenol/chloroform method[16]. Primers reported earlier[4] were used to amplify regions of mitochondrial 16S rRNA gene. PCR product was sequenced and a 379 bp sequence was submitted to GenBank (accession no. GU591886). Mitochondrial 16S rRNA sequences from other hydra species reported recently[17] were used for comparison with the sequence from Indian hydra. Sequence alignments were carried out using ClustalW[18] and cured manually.

Phylogenetic tree was constructed by neighbour joining (NJ) method based on *p*-distance using MEGA 4.0 software[19]. A separate analysis by maximum parsimony (MP) and maximum likelihood (ML) methods was carried out with PAUP* 4.0b10 (ref. 20). The MP analysis was performed with heuristic searches of 100 random additions with characters weighed equally and tree bisection and reconnection (TBR) branch swapping algorithm. The ML analysis was performed with general time reversible (GTR)