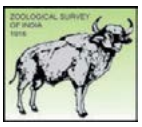
ISSN (Online) : 2581-8686
ISSN (Print) : 0375-1511

Rec. zool. Surv. India: Vol. 123(iS2)/707-716, 2023
DOI: 10.26515/rzsi/v123/i2S/2023/172599



Substratum preferences of Ascidians in Natural and Artificial Reef Environment, Andaman and Nicobar Islands

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Abstract

Ascidians are one of the significant bio-fouling organisms causes great economic loss, as they grow on offshore shellfish and finfish culture system, ship hulls, pontoons, jetties, buoys etc. This study carried out to estimate the fouling preferences of ascidians in variation with depth, season, and substrate at Pongibalu (natural reef) (11°30.958'N; 92°39.201'E) and North Bay (artificial reef) (11°43.006'N; 92°45.465'E) of Andaman and Nicobar Islands. Panels (concrete, glass, ceramic and metal) of 30 X 20 cm^2 were placed at the depth of 10 m and 20 m from January 2015 to December 2015 by SCUBA diving. The data collection was made in every four months interval. Altogether 35 species of ascidians belonging to five families were settled on the panels including 12 species under the family Didemnidae. A total of 29 species of ascidians were recorded from Pongibalu whereas, only nine species were recorded from the North Bay during the study period. It is interesting to note that, *Pyura lanka* was found on the settlement panels only, instead of reef areas of Pongibalu; similarly, *Symplegma brakenhielmi* and *Symplegma rubra* were observed on the panels at North Bay although they were not observed in the reef areas of North Bay during the study period. Both *P. lanka* and *S. brakenhielmi* is considered as cryptogenic in nature and status of *S. rubra* is yet to be established. Among four types of settlement panels, concrete and ceramic panels showed significant coverage of ascidian settlement at both experimental stations. It was observed during the study that the panels of natural reef area showed the higher diversity, species richness, lesser dominance, and lesser coverage. Whereas, panels of artificial reef areas showed lesser diversity and species richness, and higher dominance (90.20%) of three species coverage which indicates an early sign of species invasion.

Keywords: Biofouling, Substrate specificity, Natural reef, Artificial reef, Ascidians

Introduction

Fauna under class Ascidiacea is commonly known as sea-squirts. This exclusively marine sessile faunal community has free-swimming larval stage. These larvae are commonly known as ascidian tadpole larva. After few minutes to several hours of swimming these larvae find suitable substratum to settle and metamorphose into adults. Ascidians are considered as macro bio-fouler and have significant role in macrofouling community (Cheng *et al.*, 2022; Palaniswamy *et al.*, 2018; Meenakshi, 2010; Rocha *et al.,* 2009; Venkat *et al.*, 1995) of coastal ecosystems. These macro-biofoulers cause a great economic loss every year by settling on the mussels of the mussel culture, ship hulls, pontoons etc. (Giangrande *et al.*, 2023; Rocha *et al.,* 2009; Lambert and Lambert, 2003, Lambert, 2005). Beside the natural habitat *i.e.* reef environment, hard rocks, stones, roots, sea grasses, sea weeds etc., the submerged man-made structures like ship hulls, piers, pilings, buoys, harbour installations, materials used for aquaculture, ropes etc. are preferred as the substratum for the ascidians for carrying out their settlement (Meenakshi, 2010). Being bio-foulers, they invade into non-native places

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Article Received on: 10.04.2023 Accepted on: 17.06.2023

and become the global threat to marine biodiversity (Micael *et al.*, 2022; Molnar *et al.*, 2008). Bio-invasion is a rising ecological issue and represents a solemn risk to marine as well as terrestrial biodiversity, existence of native species and economy (Jaffarali et al., 2014). Non-indigenous species e.g., algae, octocorals, annelids, molluscs, barnacles, shrimps, bryozoans, sea urchins, sea stars, ascidians, fishes etc. are visible in world's ocean. These animals are effortlessly settled on the ship hulls, pontoons etc. and are spread to other non-native area.

Apart from the natural substrates, artificial marine structures have accelerated the distribution of several non-indigenous species world-wide (Bulleri and Airoldi, 2005). The settlement pattern of ascidians is studied carefully across the globe (Goodbody, 2003; Shenkar, 2008; Rocha *et al*, 2009) along with the settlement of other groups i.e., rock oyster, tube worms, bryozoans, crustose coralline algae, sponges, corals etc. (Bae *et al*., 2022; Bowden *et al*., 2006; Perkol-Finkel *et al*., 2006; Perkol-Finkel and Benayhu, 2005; Brown, 2005; Watson and Barnes, 2004; Knott *et al*., 2004; Qiu *et al*., 2003; Bailey-Brock, 1989; Wendt, 1989; Hatcher, 1998; Brown and Swearingen, 1998). Seasonal variation of settlement was conducted by several researchers to conclude the availability of larva and their settlement depending on the changing physical parameters (Shenkar, 2008). It was also tried to establish the eradication method for these bio-foulers as well as for non-indigenous ascidians (Palaniswamy *et al*., 2018; Cahill *et al*., 2012; Holt and Cordingley, 2011; Murugan and Ramaswamy, 2003). From the four three decades, emphasis on the settlement of the faunal group on the artificial reef has been carried out (Bae *et al*., 2022; Shenkar, 2008; Bowden *et al*., 2006; Brown and Swearingen, 1998; Bailey-Brock, 1989) to understand the process of marine habitat restoration (Perkol-Finkel and Benayahu, 2007). The studies on settlement on depth and orientation have been carried out (vertical and horizontal) to understand the species composition in different depth and the larval settlement nature (Shenkar, 2008). In India, studies were conducted by several researchers to understand the settlement pattern of ascidians, viz., studies on bio-fouling community conducted New Mangalore Port (Venkat *et al.,* 1995), Mumbai harbor (Swami and Chhapgar, 2002; Swami *et al.*, 2011), Kalpakkam coast of southeast India (Sahu *et al.*, 2011). As, Andaman and Nicobar Islands are surrounded by the channels, straits and seas, the inter-island transportation and communication is mostly dependent on shipping services along with small boat/dinghy services for the inhabitant of those islands. The records of bio-fouling as well as non-indigenous species are important to prevent invasion in these area as a part of conservatory measures. The objective of the present study was to collect the data on the settlement pattern of the ascidian larvae to understand their substratum preferences (concrete, glass, ceramic and metal) seasonally and depth wise from two different areas *i.e.,* natural reef (Pongibalu) and shipwreck (North Bay).

Material and Methods

Study Area: Two sites were selected to study the settlement pattern of ascidians on different substrates as well as different depths. The first selected study area was Pongibalu (Lat: 11°30.958'N; Long: 92°39.201'E) of South Andaman. It was studied because of the presence of natural reef. While the artificial reef (ship wreck) at North Bay (Lat: 11°43.006'N; Long: 92°45.465'E) (Figure 1) was selected as second study area. Pongibalu is situated at South Andaman region of Andaman and Nicobar Islands and located in the periphery of Mahatma Gandhi Marine National Park. The depth of the area varies between 1.3 to 41m. The area is predominantly covered by hard corals. The diversity of the hard corals is greater up to the depth of 18m while scanty cover of the reef habitat is recorded up to 35m. Shipwreck is situated at North Bay, South Andaman region and it supports the formation of artificial reef in this area. As the main harbor of Andaman and Nicobar Islands is nearby, the settlement in this area is influenced by the nearby natural reef as well as through the larvae dispersed from the ballast water.

Experimental Design: *In situ* studies on settlement pattern of ascidians were carried out at Pongibalu and North Bay at the depth of 10m and 20m during January 2015 to December 2015 by using four artificial substrates (30 × 20 cm^2) such as Concrete, Glass, Ceramic and Tin panels. All the panels were kept in vertical manner for the settlement of ascidians (Figs. 2). Panels were replaced by the new panel and the old panels with settlement were collected in every 4 months interval. Before collecting the panels, it was observed carefully with a hand lens for tiny ascidians which may have been missed or damaged during the collection. *In-situ* digitization was made by Canon G15 and Canon 1X Mark II with marine Pack. Collected panels were brought to the laboratory and examined to assess the settlement of small ascidians also. After measuring the settlement coverage, all the specimens were scrapped from the panels and defecated and narcotized with the help of magnesium sulphate and menthol crystal

respectively. All the narcotized specimens were preserved in 4% formaldehyde-seawater solution. Preserved specimens were dissected under the microscope (Labomed CZM4) and digitization of detailed taxonomical characters was carried out under Leica M205A DFC 500 stereo zoom microscope to record the species composition in an individual panel. Species were identified in conjunction with Kott (1985, 1990, 2001), Monniot and Monniot (2001) and Renganathan (1984). Dominance of the species in a particular panel is measured by its settlement coverage. Coverage of the ascidian settlement was measured under the microscope. The total settlement coverage of each species was calculated in the following manner:

Results and Discussion

A total of 35 species of ascidians belonging to 5 families were settled on the experimental panels (Table 1). Among them, 29 species of ascidians were recorded from Pongibalu, whereas only 9 species were recorded from the North Bay during the period of study (Table 1 & Figure 3). The maximum number of species was documented from Pongibalu area at the depth of 10m (22 species) and the least number of species were documented from the North Bay at the depth of 20m (5 species) (Table 1). Among them, *Didemnum molle, Didemnum psammathode, Phallusia arabica, Herdmania pallida, Pyura sacciformis, Rhopalaea bilobata, Eusynstyela latericius, Halocynthia spinosa* and *Styela canopus* were common species in Andaman and Nicobar Islands. It is interesting to note that although *Pyura lanka* was not recorded from the reef area of the Pongibalu, the species was found on the settlement panels. Similarly, *Symplegma rubra* and *Symplegma brakenhielmi* were observed on the panels at North Bay although they were not observed in the reef area of North Bay during the study period.

In North Bay, the total 34.96% of ascidian settlement was recorded on all four panels at the depth of 20 m at North Bay during May to August 2015 while no settlement was recorded at the depth of 10 m at North Bay during September to December 2015 (Figure 4). While in Pongibalu, the maximum (12.63%) of ascidian settlement was recorded at the depth of 20m during May to August 2015 whereas minimum (0.70%) coverage was documented at the depth of 10m during January to April 2015 (Figure 5). In North Bay, highest percentage of cover of 90.20% was observed on concrete panel with a composition of 3 species during May to August 2015 at the depth of 20m while lowest (0.02%) was found on ceramic panel with a colony of a single species during January to April, 2015 at 10m depth (Figure 4).

Among the four types of substrate panels, concrete and ceramic panels showed significant cover of ascidian larval settlement at both the study sites. In Pongibalu, the highest settlement coverage of 37.14% was recorded with 8 species at a depth of 20m during May to August 2015 on glass panel. The lowest (0.25%) settlement with 4 species was found on ceramic panel during January to April 2015 at a depth of 10m (Figure 5).

The four panels viz. concrete, glass, ceramic and tin were selected to conduct the settlement study and observe the difference between the settlement patterns on each panel. Concrete materials are used for the construction of jetties and other permanent structures. Andaman and Nicobar Islands is a tourist destination where glass bottom boats are used to show the magnified coral reef areas of Andaman and Nicobar Islands and they also can be prone to fouling. Hence, glass panels are used as substratum of ascidian settlement. Ceramic panels were used to compare the settlement world-wide as several researchers generally use ceramic panels to study settlement pattern. Tin panel is used as a metallic sheet which can be comparable to the metallic structures like ship hulls etc. Earlier studies also used acrylic panels (Swami and Chhapgar, 2002; Bowden *et al.*, 2006), recycled PVC (Perkol-Finkel and Benayahu, 2007) and wood (Sahu *et al.*, 2011) materials for settlement.

In comparison with the artificial reef at North Bay, the species richness was higher on the settlement panels in natural reef area of Pongibalu, as the low species diversity was observed at the experimental site of the North Bay and higher species diversity was observed at the natural reef area. Due to this phenomenon, the release of ascidian larvae of several reef associated species were abundant in Pongibalu and they also preferred all panels to settle on it. The species composition was very low in North Bay, and species dominance was higher on the settlement panels. The similar trend of settlement was observed on the shipwreck. Although the species assemblage was higher in Pongibalu in comparison with North Bay, the percentage of settlement was lesser in Pongibalu than in North Bay. In Pongibalu, due to the natural reef area, the settlement panels contained other reef associated faunal communities such as sponges, soft corals, sea anemones, hydrozoans, bryozoans, polychaetes, bivalves, barnacles along with less sediments and algae. However, in North Bay settlement panels contained few bivalves with enormous

quantity of sediments and algae. In Pongibalu, *Rhopalaea bilobata* was observed during every season from both depths. *Herdmania pallida* was observed during a single season from both depths of North Bay. Among the documented species from the settlement panels, 6 species namely, *Ascidia sydneiensis*, *Phallusia Arabica*, *Styela canopus*, *Didemnum psammatodes*, *Herdmania momus* and *Herdmania pallida* were reported as non-indigenous to Indian waters. Among these, *H. pallida* showed settlement on the concrete panels from North Bay and 7 individuals were found from the same panel. However, the *Symplegma viride*, *Symplegma rubra* and *Didemnum* sp.3 made maximum settlement cover but their status regarding indigenous or non-indigenous were not evaluated till now from Indian waters. Ascidian cover on the panels at Pongibalu was maintained almost a stable value during two seasons except during May to August 2015; whereas, the settlement on glass panel was high at the depth of 20m. However, no settlement was observed during January to April 2015 and September to December 2015 on the glass panels at the depth of 10m. While in North Bay, settlement cover was not stable and varied seasonally and no settlement was observed during several seasons on several panels from both depths although mostly no settlements were observed at the depth of 10m. while, at the depth of 20m in North Bay, concrete panels always showed massive settlement of ascidians, although the cover was always made by a single species only. During September to December 2015 ceramic panels displayed the higher settlement than the concrete panel.

Irrespective of the depth, season and locality, settlement of ascidians is found to be more on average on the concrete panel. During present study, the settlement or percentage of cover was found same on average in most of the seasons from most of the panels at Pongibalu, while ascidian settlement was displayed by a maximum percentage of the panels at North Bay at the depth of 20m. Only a few small crabs were noticed on the settlement panels when the ascidian settlement was not observed on those panels. The present study reported maximum ascidians density during the month of June with two species only. The study made by Swami and Chhapgar (2002) revealed settlement of 12 species of ascidians and documented the highest settlement during September from Tidal basin. Ascidian diversity was found highest by Sahu *et al.* (2011) during March-April on weekly panels at Kalpakkam coastal waters. They also recorded maximum species richness from South break waters during February and March and the highest settlement was recorded during the month of February. As Andaman and Nicobar Islands have prolonged rainy season it may not affect the settlement of ascidians throughout the year unlike other researchers have documented (Swami and Chhapgar, 2002; Sahu *et al.*, 2011) form the mainland, India.

Table 1: Ascidian species recorded from the settlement panels area and depth-wise

Sl. No.	Taxa	Pongibalu		North Bay	
		10m	20m	10m	20m
	Phylum CHORDATA Haeckel, 1874				
	Subphylum TUNICATA Lamarck, 1816				
	Class ASCIDIACEA Blainville, 1824				
	Order APLOSOBRANCHIA Lahille, 1886				
	Family DIDEMNIDAE Giard, 1872				
1	*Didemnum* sp.1		•		
2	*Didemnum* sp.2	•			
3	*Didemnum* sp.3	•	•		•
4	*Didemnum* sp.4	•			
5	*Didemnum psammatodes* (Sluiter, 1895)		•		

Sl. No.	Taxa	Pongibalu		North Bay	
		10m	20m	10m	20m
6	*Didemnum cuculliferum* (Sluiter, 1909)	•			
7	*Tridemnum* sp.	•			
8	*Lissoclinum* sp.1		•		
9	*Lissoclinum* sp.2	•			
10	*Diplosoma* sp.1		•		
11	*Diplosoma* sp.2	•			
12	*Leptoclinides* sp.	•	•		
	Family POLYCITORIDAE Michaelsen, 1904				
13	*Eudistoma* sp.	•			
	Family CLAVELINIDAE Forbes and Hanley, 1848				
14	*Clavelina robusta* Kott, 1990		•		
	Family DIAZONIDAE Seeliger, 1906				
15	*Rhopalaea bilobata* Mondal *et al.*, 2017	•	•		
	Order PHLEBOBRANCHIA Lahille, 1886				
	Family ASCIDIIDAE Herdman, 1882				
16	*Ascidia sydneiensis* Stimpson, 1855			•	
17	*Phallusia arabica* Savigny, 1816		•		
	Order STOLIDOBRANCHIA Lahille, 1887				
	Family STYELIDAE Sluiter, 1895				
18	*Polycarpa aurita* (Sluiter, 1890)	•			
19	*Polycarpa* sp.			•	
20	*Styela canopus* Savigny, 1816	•		•	
21	*Cnemidocarpa areolata* (Heller, 1878)	•			
22	*Botrylloides violaceus* Oka, 1927	•	•		
23	*Eusynstyela latericius* (Sluiter, 1904)	•			
24	*Symplegma rubra* Monniot, 1972				•
25	*Symplegma viride* Herdman, 1886				•
26	*Polyandrocapa* sp.		•		
	Family PYURIDAE Hartmeyer, 1908				
27	*Pyura vittata* (Stimpson, 1852)			•	•

Sl. No.	Taxa	Pongibalu		North Bay	
		10m	20m	10m	20m
28	*Pyura sacciformis* (Drasche, 1884)	•	•		
29	*Pyura lanka* Herdman, 1906	•			
30	*Pyura* sp.		•		
31	*Microcosmus exasperatus* Heller, 1878	•		•	
32	*Microcosmus* sp.	•			
33	*Herdmania momus* (Savigny, 1816)	•			
34	*Herdmania pallida* (Heller,1978)	•		•	•
35	*Halocynthia spinosa* Sluiter, 1905	•			
	Total number of species	22	13	6	5
	Total number of genera	16	10	6	4
	Total number of families	8	7	3	3

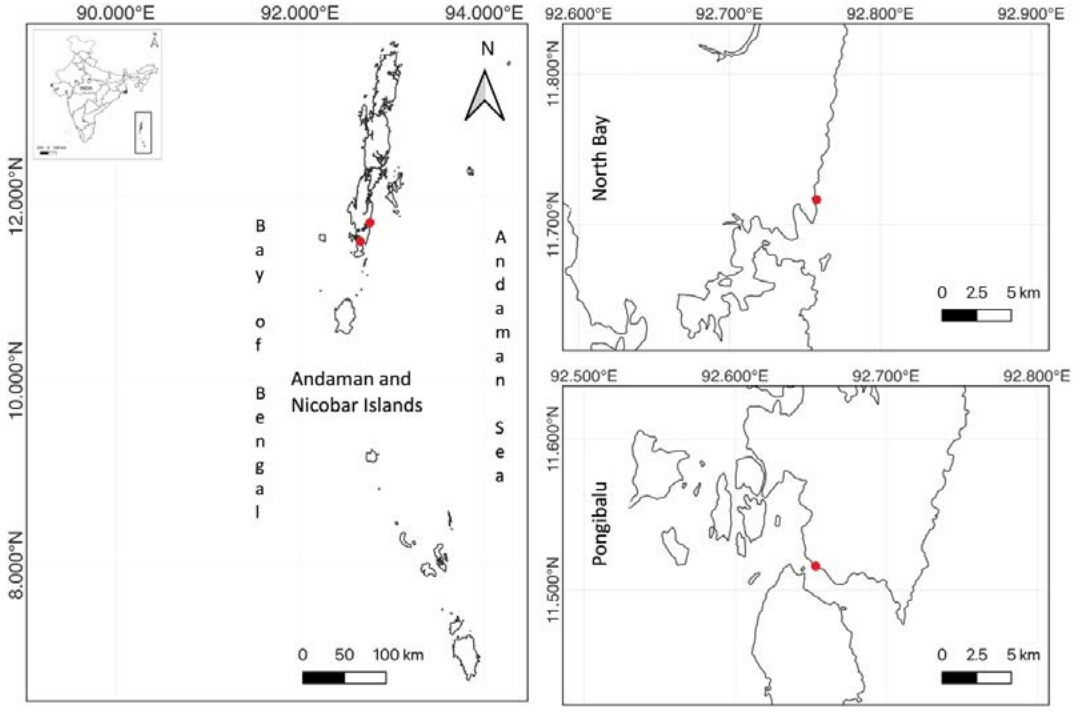

Figure1: Study Area of Andaman and Nicobar Islands

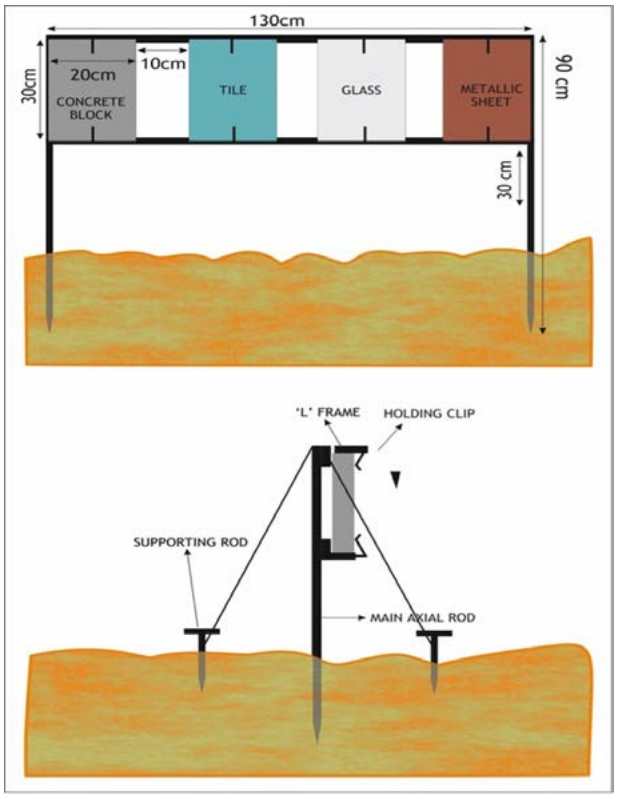

Figure 2: Schematic diagram of experimental set up

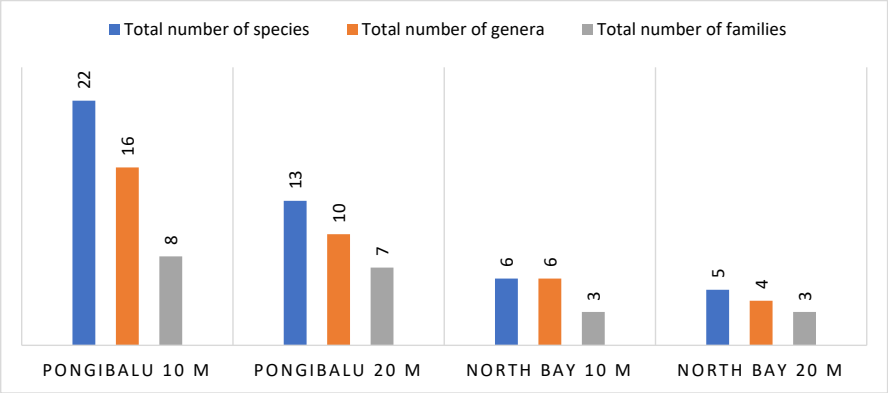

Figure 3: Species composition at the 10m and 20m Depth at Pongibalu and North Bay

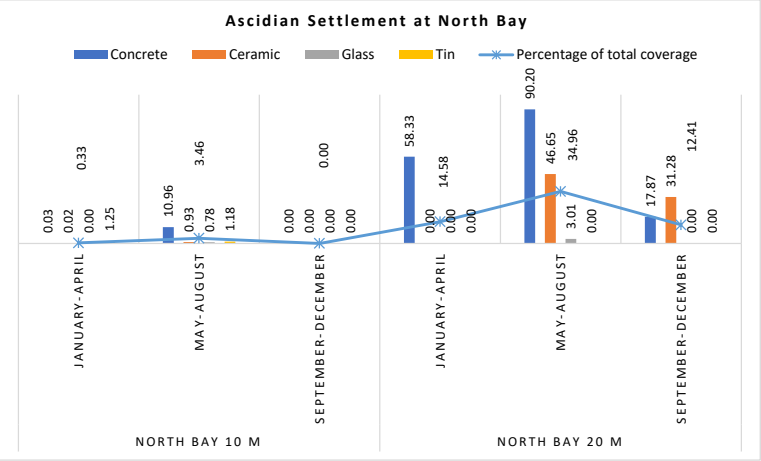

Figure 4: Depth, season and panel wise percentage cover of ascidians at North Bay

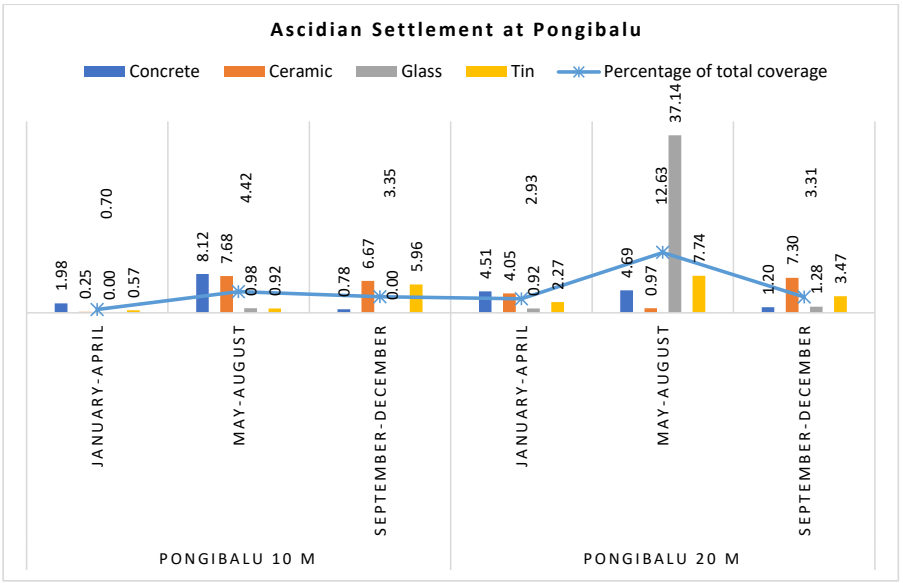

Figure 5: Depth, season and panel wise percentage cover of ascidians at Pongibalu

Conclusion

The present study pioneers the species-specific settlement pattern of ascidians in the Andaman and Nicobar Islands. The study also observes the settlement of the reef associated ascidians along with the non-indigenous species based on season, different substratum, and depth of natural reef and artificial reef habitat. There was significant difference in settlement found between 10m depth and 20m depth of Pongibalu (natural reef), and North Bay (artificial reef). A great variation among the species composition was also recorded depending on the seasons and the depth between natural reef and artificial reef habitat. Beside the natural reef environments, settlement of ascidian was diverse and dominant on concrete substrate along with non-indigenous/cryptic species from both areas. In comparison with Pongibalu (natural reef), North Bay (artificial reef) is prone for fouling by non-indigenous/cryptic species as it serves as a navigational channel.

Acknowledgements

Authors are grateful to the Director, Zoological Survey of India, Kolkata for providing necessary facilities and permission to carry out the work. The supports extended by Department of Environment and Forests, Andaman and Nicobar Administration during field trips are duly acknowledged.

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