CHAPTER 5
SETTLEMENT OF ASCIDIAN LARVAE

Like a shipwreck or a jetty, almost anything that forms a structure in the ocean, whether it is natural or artificial over time, collects life.

Sylvia Earle
INTRODUCTION

Ascidians are considered as macro bio-foulers and contribute as a main constituent of macrofouling community (Venkat et al., 1995; Meenakshi, 2010; Rocha et al., 2009) in coastal ecosystems and cause a great economic loss every year by settling on the mussels of the mussel culture, ship hulls, pontoons etc. (Rocha et al., 2009; Lambert, 2003, 2005). Beside the natural habitat i.e. reef environment, hard rocks, stones, roots and leaves of sea weeds etc., submerged man-made structures like ship hulls, piers, pilings, buoys, harbour installations, materials used for aquaculture, ropes etc. are used as the substratum for the ascidians for carrying out their settlement (Meenakshi, 2010). Being bio-foulers, they invade to non-native places and become the global threat to marine biodiversity (Molnar et al., 2008).

The concept of native species signifies that the species found in the area where it evolves to its present time. The species mostly recorded beyond its historic native range are known as non-indigenous/ non-native/ exotic/ alien species, as, per the explanation of non-native species was coined by the Executive Order- 13112 of 3rd February 1999 of NOAA (NOAA, 1999). However, non-indigenous species can be encountered as invasive species when it ‘threatens the diversity or abundance of native species or the ecological stability of infested waters or commercial, agricultural, aquacultural, or recreational activities dependent on such waters’ (NOAA, 1999). Until it becomes invasive species, the non-indigenous species are not harmful like coho, Chinook and pink salmon are known as non-indigenous species to Great Lakes but not considered as invasive species as they are not harmful to the other native species and the other natural activities of that surroundings (NOAA, 1999). It is proved that the invasive species has the capability to shift their niche from realized niche to the fundamental niche (Tingley, 2014) and the native species residing in that niche may be omitted due to the resource partitioning according to the Gause’s Principle. Now-a-days, the record of non-indigenous species is frequent across the globe as the global shipping movements have been increasing in a rapid way (Lambert, 2002; Lambert and Lambert, 2003; Tamilselvi et al., 2011). The incidence of non-indigenous species in any zone is the consequence of bio-invasion, found almost every part of world and are becoming the hazard factor for the endemic fauna (Shenkar and Swalla, 2011). 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 are visible in world’s ocean in association with mostly algae, octocorals, annelids, molluscs, barnacles, shrimps, bryozoans, sea urchins, sea stars, ascidians, fishes etc. These animals are effortlessly settled on the ship hulls, pantoons etc. blowout to other non-native area. Bulleri and Airoldi (2005) concluded that the artificial marine structures accelerated the distribution of several non-indigenous species world-wide. Andaman and Nicobar Islands are surrounded by the channels, straits and seas, the inter-island transportation and communication as mostly dependable 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 settlement pattern of ascidians are studied carefully across the globe (Goodbody, 2003; Shenkar, 2008; Rocha et al, 2009) along with the settlement of other groups (Bailey-Brock, 1989; Wendt, 1989; Hatcher, 1998; Bowden et al., 2006; Perkol-Finkel and Benayhu, 2005; Perkol-Finkel et al., 2006; Brown and Swearingen, 1998; Qiu et al., 2003, Watson and Barnes, 2004; Knott et al., 2004; Brown, 2005). Seasonal variation of settlement were conducted by several researchers to conclude the variably of larval availability 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 non-indigenous ascidians (Murugan and Ramaswamy, 2003; Holt and Cordingley, 2011; Cahill et al., 2012). From the last three decades, emphasis on the settlement of the faunal group on the Artificial Reef have been carrying out (Bailey-Brock, 1989; Bowden et al., 2006; Brown and Swearingen, 1998; Shenkar, 2008) 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. Venkat et al. (1995) studied the bio-fouling community at the New Mangalore Port. Swami and Chhapgar (2002) made settlement of ascidians at Mumbai harbor. Sahu et al. (2011) in Kalpakkam coast, southeast India and Swami et al. (2011) at the Mumbai Jetty are worth mentioning. The present study attempted to collect the data on the settlement pattern of the ascidian larvae
seasonally, depth wise and on different substratum in two different areas i.e. natural reef and ship wreck to understand the fouling pattern.

**AIMS AND OBJECTIVES**
- Seasonal variation in settlement pattern according to depth and substrate
- Substrate specificity to determine Invasive Alien Species (IAS) aggregation
- Substrate specificity according the depth

**STUDY AREAS**
To study the settlement pattern of ascidians on different substrates as well as different depths, two sites were selected. The first selected study area was Pongibalu (Lat: 11°30.958’N; Long: 92°39.201’E) of South Andaman. It was studied due to presence of natural reef while the artificial reef (ship wreck) at North Bay (Lat: 11°43.006’N; Long: 92°45.465’E) (Fig. 1) was selected as second study area for the experimental set up.

**Pongibalu**
It is situated at South Andaman region of Andaman and Nicobar Islands and located in the periphery of Mahatma Gandhi Marine National Park. It is situated at Macpherson’s Strait which connects Bay of Bengal and Andaman Sea. The depth of the area varies between 1.3 to 41m. The area is predominantly covered by scleractinian corals. The diversity of the scleractinian corals are greater upto the depth of 18m while scanty cover of the reef habitat is recorded up to 35m. The live cover of the scleractinian corals is between 21.34-33.75% (unpublished data T. Mondal).

**North Bay**
It is situated at Andaman Sea region of South Andaman region of shipwreck supports the formation of artificial reef in this area. Due to presence of the good settlement of scleractinian corals with the live cover of 12.45-18.5% (unpublished data T. Mondal) in reef areas and healthy diversity of corals, it is one of the most preferred destinations for the tourists for SCUBA diving.
MATERIAL AND METHODS

*In situ* studies on settlement pattern of ascidians were carried out at Pongibalu and North Bay at the depth of 10 m and 20 m during January 2015 to December 2015 by using four artificial substrates (30 × 20 cm²) such as Concrete, Glass, Ceramic and Tin panels. All the panels were kept in vertical manner for the settlement of ascidians (Figs. 2 & 3). 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 miss or damage 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 of 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). One way ANOVA was applied to measure the differences in settlement in different seasons on different panels. Factorial ANOVA was also performed to understand the significant variation between the settlement variations. The used factors were: season, depth and area. Natural reef areas and artificial reef areas of the experimental set-up were also studied to correlate the settlement of ascidians.
Fig. 2: Schematic diagram of Experimental set up
Fig. 3: a & b. Settlement panels at the depth of 10 m at Pongibalu; c & d. Settlement panels at the depth of 20 m at Pongibalu; e & f. Settlement panels at the depth of 10 m at North Bay; g & h. Settlement panels at the depth of 20 m at North Bay (a, c, e & g. Initial deployment; b, d, f & h. After settlement)
RESULTS

A total of 35 species of ascidians belong 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). The maximum number of species was documented from Pongibalu area at the depth of 10 m (22 species) and the least number of species were documented from the North Bay at the depth of 20 m (5 species) (Table 1). Among them, *Didemnum molle*, *Didemnum psammatodes*, *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, though *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 reported in the reef area of North Bay during the study period.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Taxa</th>
<th>Pongibalu</th>
<th>North Bay</th>
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<tbody>
<tr>
<td>1.</td>
<td><em>Didemnum</em> sp. 1</td>
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<tr>
<td>2.</td>
<td><em>Didemnum</em> sp. 2</td>
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<td>3.</td>
<td><em>Didemnum</em> sp. 3</td>
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<td>4.</td>
<td><em>Didemnum</em> sp. 4</td>
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<tr>
<td>5.</td>
<td><em>Didemnum psammatodes</em> (Sluiter, 1895)</td>
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<td>6.</td>
<td><em>Didemnum cuculliferum</em> (Sluiter, 1909)</td>
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<tr>
<td>7.</td>
<td><em>Trididemnum</em> sp.</td>
<td></td>
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<tr>
<td>8.</td>
<td><em>Lissoclinum</em> sp. 1</td>
<td></td>
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<tr>
<td>9.</td>
<td><em>Lissoclinum</em> sp. 2</td>
<td></td>
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<tr>
<td>10.</td>
<td><em>Diplosoma</em> sp. 1</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>11.</td>
<td><em>Diplosoma</em> sp. 2</td>
<td></td>
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</tr>
<tr>
<td>12.</td>
<td><em>Leptoclinides</em> sp.</td>
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<td>13.</td>
<td><em>Eudistoma</em> sp.</td>
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<tr>
<td>14.</td>
<td><em>Clavelina</em> robusta* Kott, 1990</td>
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<tr>
<td>15.</td>
<td><em>Rhopalaea</em> bilobata* Mondal, Raghunathan and Mondal, 2017</td>
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<td>*</td>
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<tr>
<td>16.</td>
<td><em>Ascidia sydneiensis</em> Stimpson, 1855</td>
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17. *Phallusia arabica* Savigny, 1816
18. *Polycarpa aurita* (Sluiter, 1890)
20. *Styela canopus* Savigny, 1816
21. *Cnemidocarpa areolata* (Heller, 1878)
22. *Botrylloides violaceus* Oka, 1927
23. *Eusynystyla latericius* (Sluiter, 1904)
25. *Symplegma viride* Herdman, 1886
27. *Pyura vittata* (Stimpson, 1852)
28. *Pyura saciformis* (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

<table>
<thead>
<tr>
<th>Total number of species</th>
<th>22</th>
<th>13</th>
<th>6</th>
<th>5</th>
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<tbody>
<tr>
<td>Total number of genera</td>
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<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Total number of families</td>
<td>8</td>
<td>7</td>
<td>3</td>
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In North Bay, the maximum (34.96%) ascidians cover was recorded 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 (Fig. 4) while in Pongibalu, the maximum (12.63%) of ascidian cover was recorded at the depth of 20 m during May to August, 2015 whereas minimum (0.70%) coverage was documented at the depth of 10 m during January to April, 2015 (Fig. 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 20 m during May to August 2015 on glass panel. The lowest (0.25%) settlement with 4 species was found in ceramic panel during January to April 2015 at a depth of 10 m (Fig. 5).
Fig. 5: Depth, season & plate wise percentage cover of ascidians at Pongibalu (number of colony patches or solitary individuals present on each panel is presented in parantheses)
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 20 m while lowest (0.02%) was found on ceramic panel with a colony of a single species during January to April, 2015 at 10 m depth (Fig. 6).

![Figure 6: Depth, season & plate wise percentage cover of ascidians at North Bay (number of colony patches or solitary individuals present on each panel is presented in parantheses)](image)

On the basis of two factor ANOVA on settlement substrate and season for both the depth of 10 m and 20 m at Pongibalu, the value was not significant as the P value was 0.266 and 0.397 (P > 0.05). They were significant in single factor ANOVA also as P > 0.05 for both the depths. There were no significant variation in the larval
settlement of ascidian between both the depth of 10 m and 20 m at Pongibalu as \( P > 0.05 \).

On the basis of two factor ANOVA on settlement substrate and season of North Bay at both the depth of 10 m and 20 m, the value was not significant as the \( P \) value was 0.265 and 0.299 (\( P > 0.05 \)). They were not significant in single factor ANOVA also as \( P > 0.05 \) for both the depth. However, single factor ANOVA showed significant variation in settlement pattern of ascidian larvae at the depth of 20 m at North Bay as the \( P \) value was 0.043 (\( P < 0.05 \)). There was also a significant difference observed between both the 10 m and 20 m depths at the North Bay as the \( P \) value was 0.05 (\( P = 0.05 \)).

**DISCUSSION**

The four panels viz. concrete, glass, ceramic and tin were selected to conduct the settlement study to 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 tourists destination for global population where glass bottom boats are used to show the magnified reef areas of Andaman and Nicobar Islands and they also can be prone to fouling, ceramic panels were used to compare the settlement of world-wide as several researchers generally use ceramic panels to study settlement pattern and tin used a metallic sheet which can be comparable to the metallic structures like ship hulls etc. Few more studies also used acrylic (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 because of the low species diversity of the experimental site of the North Bay on the shipwreck and higher species diversity of the reef area. Due to this, the release of ascidian larvae of several reef associated species were plenty in Pongibalu and they also preferred the panels to settle on it. The species composition was very low in North Bay, and species dominance was higher on the settlement panels display the similar trend of settlement as 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 the depths but from North Bay only *Herdmania pallida* was observed during a single season from the both depth. 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. There species were previously recorded as non-indigenous from Indian waters (Jaffarali et al., 2014; Jhimli et al., 2015). Among these, *H. pallida* shown 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. Surprisingly non-indigenous species *Phallusia nigra* which was reported from North Bay with high density might be due to navigational channel exist at this area. Ascidian cover on the panels at Pongibalu was maintained almost a stable value during two seasons except during May to August, 2015 when the settlement on glass panel was high at the depth of 20 m. However, no settlement was observed during January to April, 2015 and September to December, 2015 on the glass panels at the depth of 10 m. 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 the depths although mostly no settlements were observed at the 10 m. At the depth of 20 m 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. The number of species recorded from artificial reefs was also supported by Shenkar (2008), as in her experiment she recorded only 8 species from the settlement panels.

During present study, it was observed that the settlement rate was lesser in horizontal panel than in vertical panel and the settlement of the colonial ascidians was observed on the cryptic side of the horizontal panel and supported the experiment
made by Shenkar (2008). It was denoted that the highest percentage of ascidian cover on the settlement plate was recorded spring and autumn however, during spring only 3 species while during autumn 5 species were recorded (Shenkar, 2008; Perkol-Finkel and Benayahu, 2007). During present study, the settlement or percentage of cover was found higher in most of the seasons from most of the panels at Pongibalu while, ascidians cover a maximum percentage of the panels at North Bay at the depth of 20 m and 10 m except some small crabs no other faunal group were noticed when the ascidian settlement was nil. Sahu et al., (2011) observed the ascidian diversity was peak during March-April on weekly 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 12 species of ascidians and documented the highest coverage during September from Tidal basin and recorded maximum species richness from South break waters during February and March and the highest cover was documented 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 like other researchers have documented (Swami and Chhapgar, 2002; Sahu et al., 2011) form the mainland India. The present study pioneers the species specific settlement pattern in Andaman and Nicobar Islands to assess the settlement patter of the reef associated ascidians along with the observation of non-indigenous species of ascidians on the basis of season, substrate and depth.