Distribution and Diversity of Reef Associated Organisms in the Andaman Islands

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by

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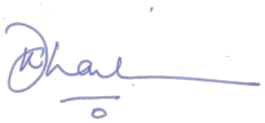
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Certificate

This is to certify that this dissertation entitled Distribution and Diversity of Reef Associated Organisms in the Andaman Islands towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Harshal Patil at CES, IISc Bangalore under the supervision of Kartik Shanker, CES, IISc Bangalore, during the academic year 2017-2022.



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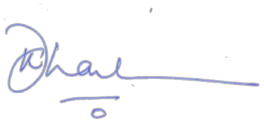
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This thesis is dedicated to my father and uncle

Declaration

I hereby declare that the matter embodied in the report entitled "Distribution and Diversity of Reef Associated Organisms in the Andaman Islands " are the results of the work carried out by me at the CES, IISc Bangalore under the supervision of Kartik Shanker and the same has not been submitted elsewhere for any other degree.



Kartik Shanker



Harshal Patil

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Abstract

The sensitivity of coral reef ecosystems to natural and anthropogenic stresses makes them vulnerable to changes such as global warming. As a result, coral reef ecosystems need to be prioritized for conservation action. Thus, monitoring their health is essential to understanding the impact of human activities on the ocean. However, documenting marine biodiversity is logistically challenging, and in addition, many taxa may be difficult to identify in the field. To overcome these challenges, novel methodologies like ARMS (Autonomous Reef Monitoring Structures) provide a standardized protocol to monitor reef ecosystems worldwide. This study aimed to explore the cryptic biodiversity of the Andaman Islands using the ARMS methodology, the first such initiative in the Indian subcontinent. This project aimed to study the differences in the colonization of sessile assemblages at two sites with different anthropogenic pressures and sedimentation rates. In addition, we aimed to examine the differences in colonization patterns within the units, where each category of plates provides a different habitat for the sessile assemblages. In total, ten ARMS units were recovered and analyzed using photographic analysis. I used photographic analysis to document the occurrence of different species on the plates and units; the percentage cover for the six most commonly observed taxa was determined using Coral Point Count Excel extension software (CPCe). The results showed that the inter-site differences in sedimentation and anthropogenic stresses did not induce a change in the colonization patterns of the observed taxa. On the other hand, the plate orientation (PO) and the spacer type (ST) resulted in distinct colonization patterns. Sessile suspension feeders dominated most of the bottom-facing plates, whereas polychaete tubes and algae dominated most of the top-facing plates. Although the contrast in the colonization was apparent for the top and bottom surfaces, there was large variation in cover across all plates. In conclusion, different ARMS 'microhabitats' revealed colonization preferences of the observed taxa for surface orientation, irrespective of the site locations.

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Chapter 1

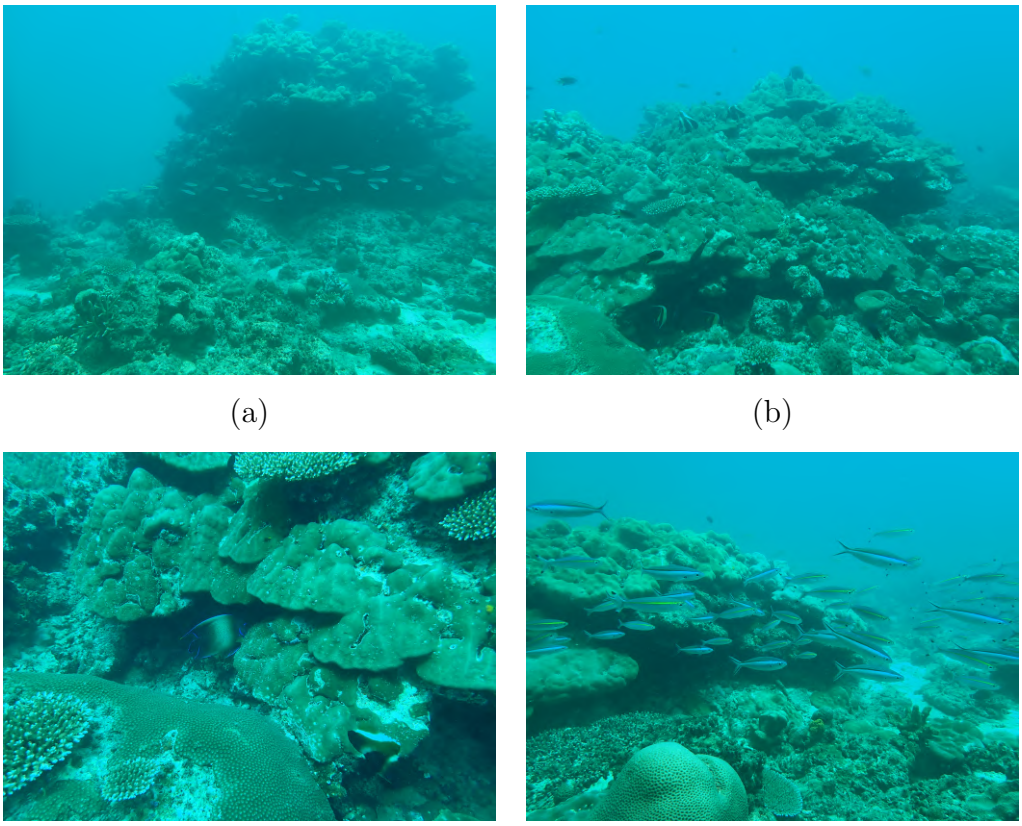
Introduction

1.1 Background

Coral reefs are among the world's most biodiverse and productive ecosystems (Marjorie, 1997) (Knowlton and Jackson, 2001). Coral reef ecosystems account for approximately a quarter of all marine biodiversity despite covering only 0.2 percent of the total seafloor area (Knowlton et al., 2010). These ecosystems play a unique role in the lives of coastal and island people and are an important asset for major coastal cities (Burke et al., 2011). Coral reefs provide a wide range of environmental services and human benefits, including fisheries, coastal protection, tourism, recreation, and medicine, in addition to their incredible biodiversity (Costanza et al., 1997) (Moberg and Folke, 1999) (Cesar et al., 2003) (Teh et al., 2013). Reef ecosystems are thought to be particularly susceptible to changes in environmental conditions (Hoegh-Guldberg et al., 2008) (Hughes et al., 2017). In the absence of immediate conservation action, about 30 % of corals have vanished since the early 1980s, and up to 90% of coral reefs may be lost in the next few decades (Hoegh-Guldberg, 2005) (Hoegh-Guldberg et al., 2018) (Hughes et al., 2018). As a result, coral reefs are widely considered one of the top conservation priorities globally due to their extraordinary biodiversity and complex ecological functions. In addition, global anthropogenic stresses have a drastic impact on the health of these ecosystems (Dight and Scherl, 1997) (Hourigan et al., 2017).

Coral polyps (primarily scleractinians) construct coral reefs by accumulating their calcareous exoskeleton on a substrate (Neder et al., 2019) (Goreau and Goreau, 1959). Coral

polyps can build massive reefs over generations, which provides shelter for many marine organisms due to their structural complexity (Graham and Nash, 2012). Although most of our current knowledge of coral reefs comes from studies in nearshore and coastal areas, coral reefs can be found in various sites far from human habitation areas (D'agata et al., 2016) (Jones et al., 2018). These sites include coral reef habitats on seamounts and submarine ridges and coral reef habitats around remote and uninhabited islands (Buhl-Mortensen et al., 2017) (Rogers, 2008). Although the utility (fishing, tourism, recreation, and coastal protection) of these remote coral reef ecosystems is small compared to their nearshore counterpart, they are essential biodiversity hotspots (Rogers, 1999) (Gove et al., 2016).



(a) (b)

(c) (d)

Figure 1.1: Photographs displaying the structural complexity of reefs near Senior kindergarten (SKG), Wandoor

Coral reefs ecosystems are known for their biodiverse community structure. The structural complexity of the reefs is one of the major reasons behind their biodiverse ecosystems

(Gratwicke and Speight, 2005). Intricately built reefs provide large substrates for the colonization of benthic communities. Reef structure also offers a wide range of habitats, from overhangs to porous substrates (Richardson et al., 2017) (Yanovski et al., 2017). This range of habitats creates chances for the colonization of organisms with various niche preferences. As a result, different ecological functional groups can colonize the same reef with varying surroundings (Rosenzweig, 1995) (Allouche et al., 2012). The presence of diverse functional groups in an ecosystem boosts its structure and function. Unless whole functional groups are removed from the system, ecosystems with high biodiversity are more likely to preserve their ecosystem services than ecosystems with limited biodiversity (Bellwood et al., 2003). Understanding biodiversity patterns can help predict how species diversity and community composition respond to environmental changes (Gaston, 2000).

Overall, the habitat structure of coral reefs is a balance of processes such as bio-erosion, competition, predation, and cohabitation. As reef builders, hard corals are essential for coral reef structural complexity. Corals exhibit a symbiotic relationship with algae called zooxanthellae, where the algae provide corals with nutrition and corals shelter the algae. Since zooxanthellae are sensitive to temperature, rise in water temperature directly affects corals and their reef-building potential (Berkelmans and van Oppen, 2006) (Iwasaki et al., 2016). Depletion in reef-construction rates may result in additional loss of habitat complexity of the reefs (Graham et al., 2015).

Human activities such as fishing, pollution, recreation, transportation, and coastal development put coral reefs under stress, and have led to rapid changes in oceanic health worldwide. Average ocean water temperatures are increasing, and as a result, marine ecosystems are affected adversely. In particular, the rise in the sea temperature directly impacts reef structures and results in coral bleaching (Hoegh-Guldberg et al., 2017). They are especially vulnerable to ocean warming and acidification. These factors are detrimental to corals, promoting ecological interactions (including the loss of consumer functional groups) that favor algae and faster-growing invertebrates over hard corals.

Urban environments provide opportunities for introducing invasive species into the natural environments through activities like shipping, recreational boating, aquaculture, and sewage transfers (Davidson et al., 2010). These activities are also linked with disturbance from pollution, including heavy metal and oil discharges. Such disruptions can enhance invasion success by periodically removing already established communities and providing

'new' substrates for species settlement (Crooks et al., 2011). Human activities near coastal sites might change the entire predator-prey dynamics of the benthic ecosystems. In addition, human-mediated non-indigenous species (NIS) can cause considerable harm to marine ecosystems. Monitoring these changes thus becomes essential (Salimi et al., 2021).

1.2 Novel methodologies for reef monitoring

Coral reef ecosystems are susceptible to temperature, acidity, and salinity stresses. They can act as indicator systems for global change because of their value and sensitivity. Using conventional methods, sampling, and experimenting in coralline habitats has limitations and could harm the natural ecosystem. Hence, novel ways to study these fragile ecosystems are necessary (Hedley et al., 2016).

Long-term reef monitoring efforts can provide essential data to help coastal communities as they depend heavily on the reef ecosystems (Obura et al., 2019). Monitoring efforts can also help marine management authorities track reef health and the economic and social repercussions of degradation. Hence, monitoring programs that assess the threats related to the reef ecosystems are necessary to ensure stable socio-economic conditions for reef associated communities. Aggregate data from local to global levels is essential to feed into the scale of governance processes that might influence global trajectories of long-term coral reef sustainability.

1.2.1 Coral reef monitoring programs

In order to monitor reef health, there are currently several programmes working on assessing reef ecosystems worldwide. The scale and methodologies of these programmes vary extensively, ranging from photographic surveys of local seafloor to the satellite monitoring of reefs across the globe. This wide range of monitoring efforts ensures the assessment of various threats to the reef ecosystems. Listed below are a few ongoing reef monitoring programs working at different scales:

- Global Coral Reef Monitoring Network (GCRMN)

- Atlantic and Gulf Rapid Reef Assessment (AGRRA)
- Caribbean Coastal Marine Productivity Program (CARICOMP)
- Coral Reef Degradation in the Indian Ocean (CORDIO)
- Commission de l'Ocean Indien (COI)
- Mesoamerican Barrier Reef System – Synoptic Monitoring Program (MBRS SMP)
- Florida Keys National Marine Sanctuary Program (FKNMSP)
- NOAA Fisheries Pacific Islands Fisheries Science Center Coral Reef Ecosystem Division (CRED)
- RECON (Reef Condition) of the Ocean Conservancy

One such innovation is the Autonomous Reef Monitoring Structures (ARMS) (Pearman et al., 2019), an artificial structure of parallelly stacked PVC plates. These structures mimic the complex reef and benthic surfaces and act as standardized 3D collectors of marine life. Using standardized ways such as ARMS makes it possible to monitor and compare the diversity of coral habitats worldwide.

1.3 Autonomous Reef Monitoring Structures (ARMS)

ARMS have proven to be an effective way of assessing and monitoring our ever-changing oceans. Sessile and motile marine organisms settle and colonize the plate surfaces over time. The top and bottom of ARMS plates offer different inhabitable conditions and generally manifest differences in organismal colonization patterns. These differences may elucidate niche partitioning trends and species-wise habitat preferences.



Figure 1.2: ARMS unit installed at Hanging garden (HG), Wandoor

The ARMS protocol follows a standard methodology to ensure optimal results. Initially, the ARMS units are assembled in their 3D form. This is followed by their deployment at the desired location. They are left there for 1-3 years to provide shelter for local marine flora and fauna. These units are then retrieved and disassembled. The motile fauna captured in and around the plates is photographed individually and vouchered. These specimens are then stored in ethanol for DNA barcoding. The disassembled ARMS plates are photographed, and the sessile fauna is scraped off the plates, blended, and the combined samples are analyzed via metabarcoding.

1.3.1 ARMS structure

Each ARMS unit comprises nine 22.5 cm x 22.5 cm PVC plates stacked vertically parallel to each other, held in an arrangement via stainless steel hardware, with spacers creating a gap of 1.27cm between them. The resulting three-dimensional structure is designed to mimic hard bottom marine substrates. The entire structure is affixed to the seafloor attached to a

35cm x 45cm PVC base plate.

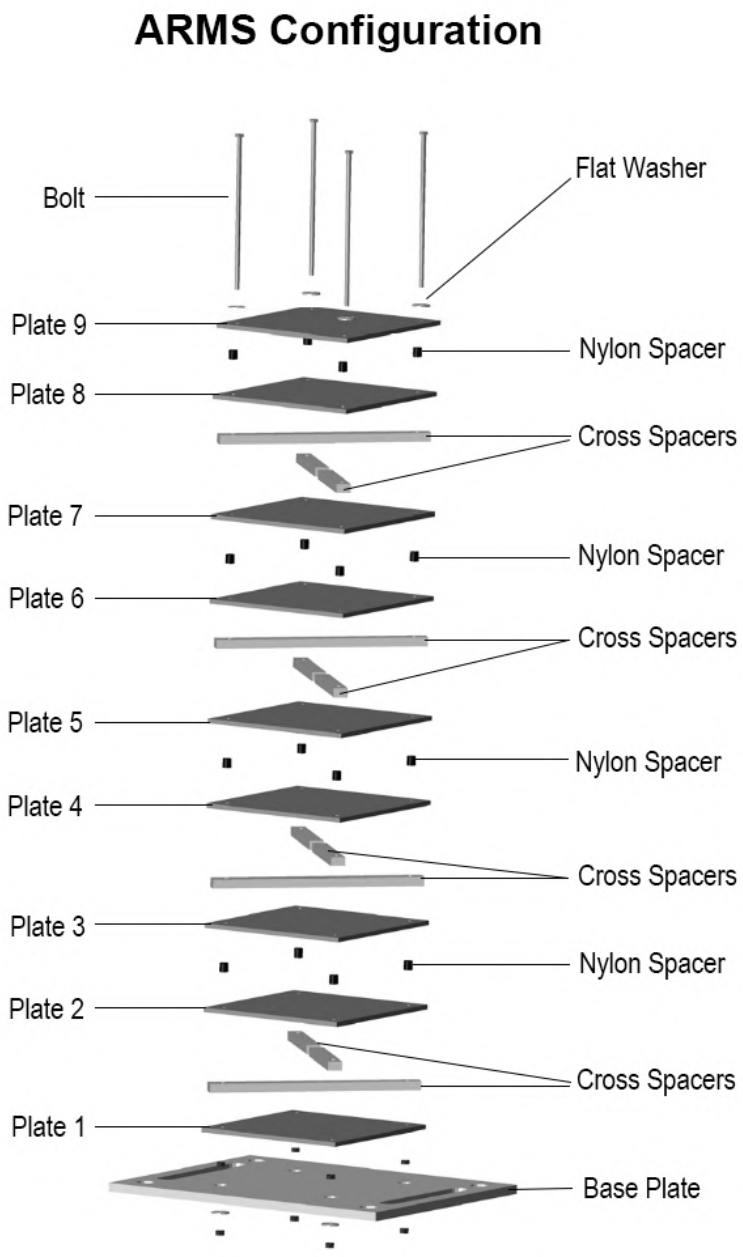


Figure 1.3: ARMS Components & Structure

Figure 1.3 provides a schematic of ARMS components and assembly. Plates in a unit are denoted by numbers 1 to 9, where plate 1 is closest to the base plate, and plate 9 is the topmost. Each plate has a top and bottom surface, except plate 1 (bottom being attached to the base plate). For hereon, we will denote each surface with a combination of its plate number and orientation (e.g., 1T, 5B, 8T, etc.). There are 17 surfaces with colonization in each unit, of which only 9T has access to direct sunlight.

As it can be seen in Figure 1.3, plates are held 1.27cm apart with alternate nylon and cross spacers. Four nylon spacers are positioned near each corner to allow plates to be horizontally parallel without touching each other. This arrangement allows water and nutrients to flow freely between the plates. On the other hand, cross spacers divide the space between the plates diagonally, creating four enclosed compartments to allow colonization. This arrangement restricts the water and nutrient flow between the plates. Since the nylon and cross spacers are placed between alternate plates, odd-numbered plates have cross spacers on top and nylon spacers at the bottom. In contrast, even-numbered plates have nylon spacers on top and cross spacers at the bottom. From hereon, we will denote the plate faces adjacent to cross spacers as closed, and plate faces adjacent to nylon spacers as open. This segregation leads to differences in sedimentation, water flow, exposure to predators, and availability of sunlight. In addition to the differences between locations, we aimed to test whether the differences in abiotic and biotic factors prompted by the arrangement of spacers cause a change in the colonization patterns of the taxa.

1.4 The Andaman Islands

The coral reefs near the Andaman and Nicobar Islands are among the richest coral formations in the Indo-pacific region (Vinithkumar et al., 2008); therefore, the Andaman Sea is considered a biodiversity hotspot. Increased industrialization, excessive fishing, and natural calamities have had severe impacts on the natural ecosystems near the Andaman Islands (Majumdar et al., 2019) (Thomas et al., 2017). Monitoring these alterations is essential to conserve the rich marine biodiversity of the islands.

The 2004 tsunami caused minor to severe damage to coral reefs in the Andaman and Nicobar Islands, with around 40,000 hectares of reef area devastated (Patterson et al., 2005). The most common cause of damage was debris, such as logs, washing off the coast and suffocat-

ing corals. However, the most evident repercussions were caused by the region's tectonic movements, which resulted in the permanent deformation of many reefs. In certain regions, like Mahatma Gandhi Marine National Park, these reefs are now covered by several metres of water, allowing additional coral growth and improved reef development. The reefs of the South Andaman Islands have sunk by nearly a metre, and efforts to erect seawater barriers to preserve residences, paddy fields, and other infrastructure have resulted in extensive siltation, which could harm the adjacent coral reefs.

The natural habitat of the Andaman Islands is comparatively less influenced by human activities due to its distance from the mainland. However, the introduction of invasive species by human-mediated transport can be highly detrimental to the indigenous habitats of the islands (Airoldi et al., 2015). This study can provide important insights into the spatio-temporal dynamics of various constituents of Andaman's native marine ecosystem and provide early warnings of invasive forms in the long-term.

1.5 Aims and objectives

Our study aims to provide a quantitative analysis of fauna inhabiting the coralline environments of the Andaman Islands. Due to the lack of extensive research on these ecosystems, our knowledge on the subject is limited. The rationale behind this study is to get insights into the cryptic biodiversity of the coral ecosystems of the Andaman Islands, using novel methodology such as ARMS, the first such initiative in the Indian subcontinent.

Hence, the objectives of this study are (1) To study the diversity and distribution of the reef associated fauna across different locations in the Andaman Islands using ARMS; (2) To study the colonization trends of ARMS inhabiting sessile species, including top-bottom surface colonization and differences in colonization at different deployment locations.

Objective 1: As this is the first study in India involving ARMS, we aim to collect information about indigenous and invasive marine species inhabiting the Indian waters. The information will include the entire range of organisms settled on ARMS, which further will be identified using photographic and laboratory analysis, followed by categorization in different taxonomic groups.

Objective 2: The colonization trends can provide insights into the mechanisms of niche

partitioning on benthic surfaces. These patterns may help us understand the impact of biotic and abiotic factors on the colonization of marine organisms. This being the first ARMS initiative in India, the marine diversity and colonization patterns would be the first to be observed in Indian waters.

Chapter 2

Methodology

2.1 Study site

The deployment sites, Wandoor (11.5944° N, 92.6196° E) and Havelock (11.9761° N, 92.9876° E), were chosen to highlight the differences in anthropogenic pressures. Wandoor is a village surrounded by Mahatma Gandhi Marine National Park and Lohabarrack Crocodile Sanctuary. Due to its proximity to urban settlements, the Wandoor site has a higher degree of perturbance. Boating, docking, fishing, shipping activities, and sewage transfers might contribute to the increased sedimentation near the deployment site (VishnuRadhan et al., 2015). On the other hand, Havelock (Swaraj Dweep) is an island northeast of Port Blair. Havelock is known for its rich biodiversity of coral reef ecosystems and clear waters (Sarkar and Sarkar, 2015). With almost no industrial activity nearby, Havelock is economically driven by tourism. Diving is the major component contributing to the sedimentation near the deployment site.

2.1.1 ARMS deployment

In January 2020, twelve ARMS units were deployed in two different coralline areas, at both sites. The location of each site is illustrated in Figure 2.1. In Wandoor, six units (three each) were installed at two different locations, Hanging garden (HG1, HG2, HG3) and Senior

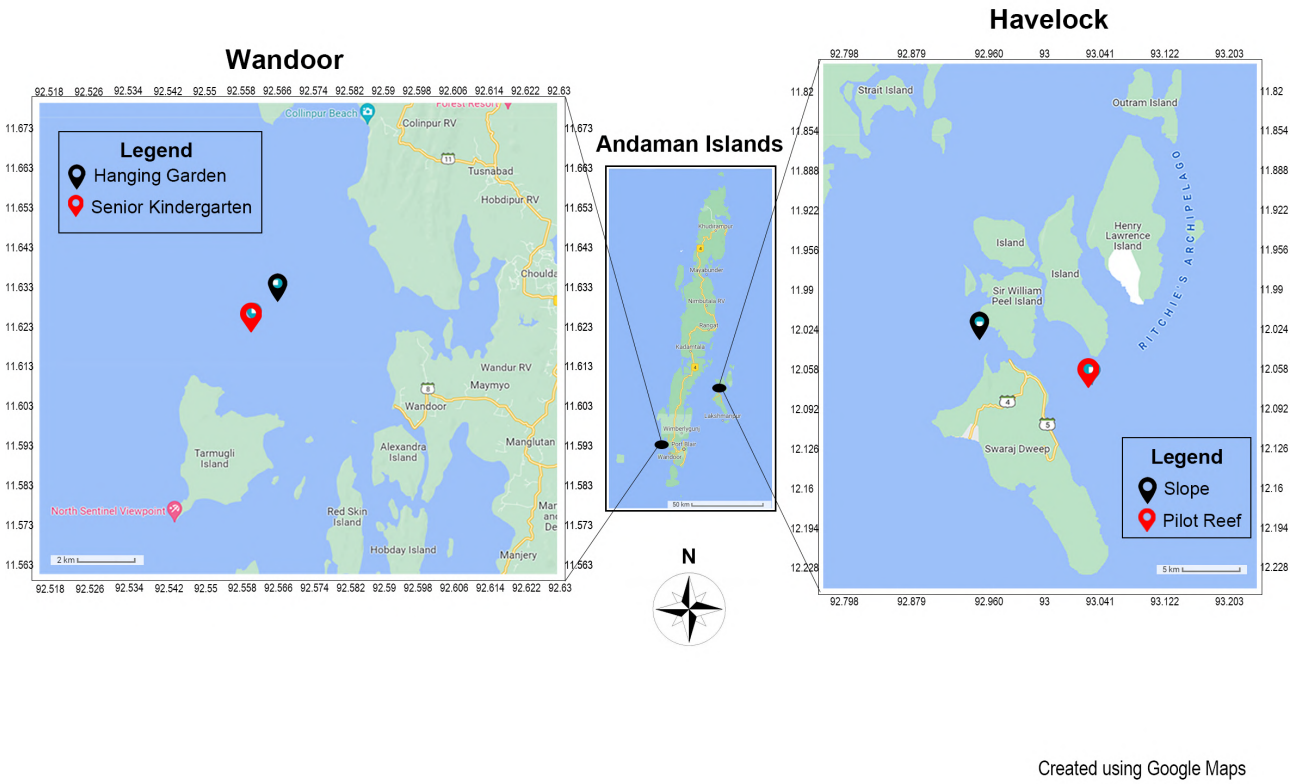


Figure 2.1: Map illustrating the deployment sites

kindergarten (SKG1, SKG2, SKG3). In Havelock, three replicates each were installed at Pilot (PLT1, PLT2, PLT3) and Slope (SLP1. SLP2, SLP3). The ARMS units were anchored in spaces within the reef structure to avoid damage to living coral formations. Units were carefully affixed to the seafloor at a depth of approximately 10m. Distance between the units at each location was around 8-12m.

2.1.2 ARMS retrieval

In April 2021, one unit from each location at the Wandoor site was retrieved, i.e., HG1 and SKG1. Further retrievals were delayed due to a surge in COVID-19 cases. All the remaining units were retrieved between December 2021 and February 2022, barring one unit from each

site, i.e., HG3 and SLP3, which could not be found. Table 2.1 provides detailed information of the site coordinates, and the dates of deployment and retrieval. The standard operating procedure for ARMS retrieval, disassembly, and sample preservation was followed as per (Leray and Knowlton, 2015))

		Site	Coordinates	Date of Deployment	Date of Retrieval
HG	**HG1**	Wandoor	11°37' 59.9" N 92°33' 58.1" E	30/01/2020	20/04/2021
	HG2	Wandoor	11°27' 59.9" N 92°33' 58.1" E	30/01/2020	24/12/2021
SKG	**SKG1**	Wandoor	11°37' 25" N 92°33' 30.7" E	30/01/2020	23/04/2021
	SKG2	Wandoor	11°37' 25" N 92°33' 30.7" E	30/01/2020	28/12/2021
	SKG3	Wandoor	11°37' 25" N 92°33' 30.7" E	30/01/2020	30/12/2021
PLT	**PLT1**	Havelock	12°01' 27.7" N 93°02' 29.4" E	08/02/2020	17/02/2022
	PLT2	Havelock	12°01' 27.7" N 93°02' 29.4" E	08/02/2020	19/02/2022
	PLT3	Havelock	12°01' 27.7" N 93°02' 29.4" E	08/02/2020	20/02/2022
SLP	**SLP1**	Havelock	12°03' 30.8" N 92°57' 37.4" E	10/02/2020	14/02/2022
	SLP2	Havelock	12°03 30.8 N 92°57 37.4 E	10/02/2020	14/02/2022

Table 2.1: Site coordinates, and the date of deployments and retrievals for respective units

2.2 ARMS pre-retrieval protocol:

2.2.1 Materials

- 10% Sodium Hypochlorite solution, Drinking water, Kimwipes

2.2.2 Procedure

- A day before retrieval, 25L of 10% Sodium Hypochlorite solution (22.5L water+2.5L bleach) was prepared.
- All the non-sterilized equipment needed for retrieval was soaked in the prepared bleach for 20 minutes.
- Bleach cleaned tools were then rinsed with water and placed on a table to dry.
- Dried tools were kept upside down and were covered with kimwipes to avoid contamination by air.

2.2.3 Chemicals

- 95% Ethanol (500mL preparation, 475mL 99.99% Ethanol+25mL molecular grade water)
- Salt-saturated DMSO solution (2L preparation: 1L liquid EDTA+500mL molecular grade water+500ml DMSO. 300gm NaCl was added to the solution and the solution was kept overnight for stirring.)
- 70% Ethanol (500mL preparation: 350mL 99.99% Ethanol+150mL distilled water.)

2.3 ARMS retrieval protocol

2.3.1 Materials

- Icebox, Recovery crate, Plastic bucket, Jerrycan, Ropes, Electronic bubblers, Dive Knife, Hammer, Chisel, 40-micron mesh, 40-micron plankton net

2.3.2 Procedure

- The icebox and jerrycan were filled with seawater using a plankton net on the boat. Operating bubblers were placed in the water for continuous aeration.
- Before retrieval, photographs of the ARMS unit were taken underwater.
- As shown in Figure 2.3 a, all the plates were covered with the 40-micron mesh to capture the motile fauna. The mesh was tied to the unit using a thin rope.
- The crate was placed on the unit, and the anchoring system was removed from the seafloor.
- As shown in Figure 2.3 b, the crate was tied to a strong rope, and the whole system was brought to the surface.
- The unit was then placed upside down (base plate facing upward) in the icebox, filled with filtered seawater that was kept aerated till the post processing in the lab.

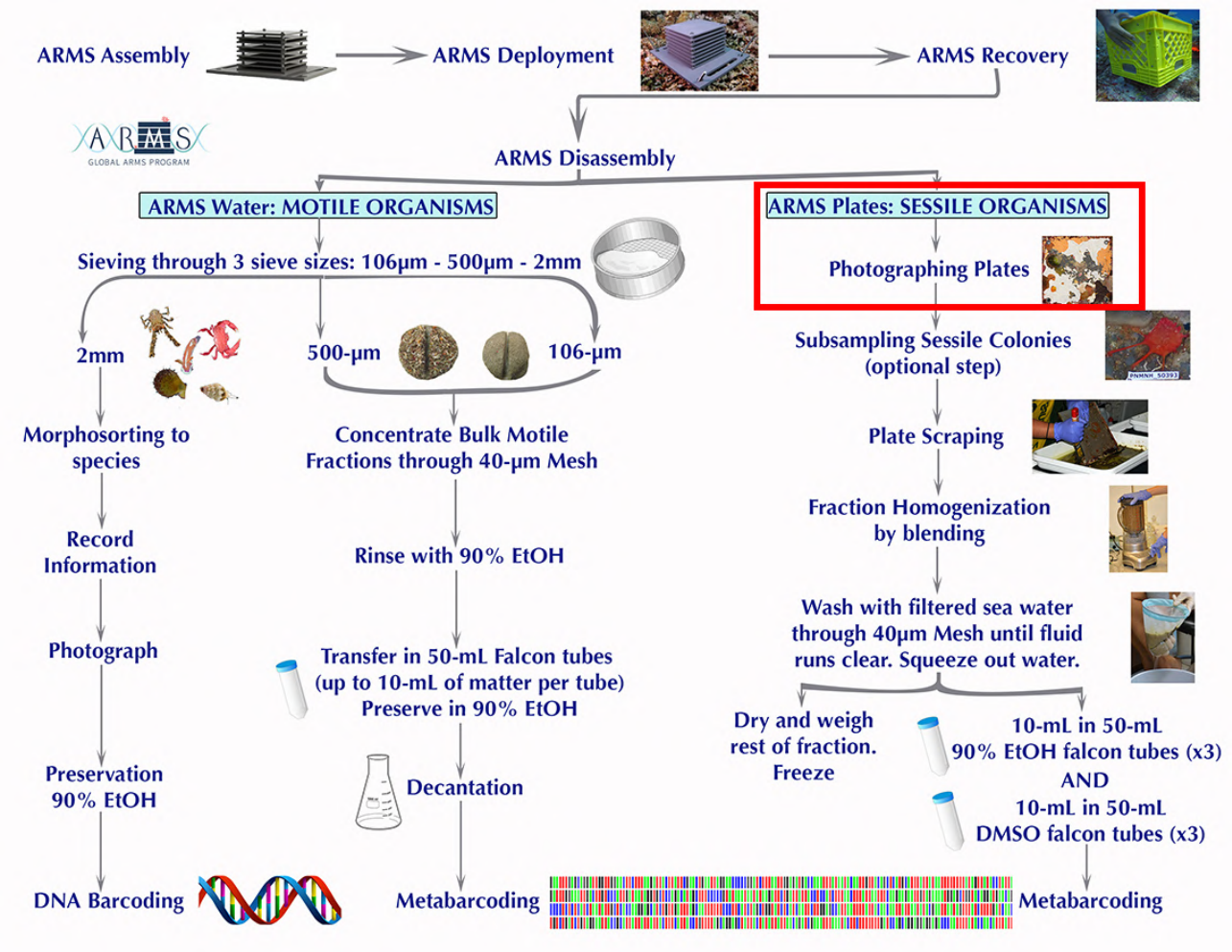


Figure 2.2: ARMS Protocol Schematic

- The ARMS containing icebox and jerrycan were then brought back to the lab at the field base.

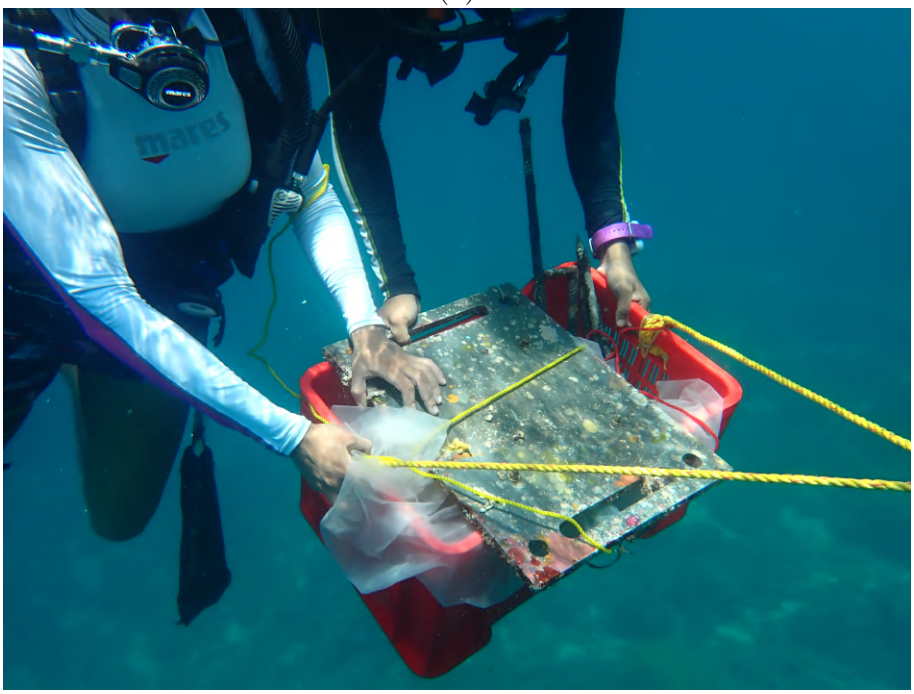
2.4 ARMS post-retrieval protocol

2.4.1 Materials

- Plastic bin containing seawater and a tray, Bubblers, Wrenches, Tweezers, Squeeze bottles, Long and laboratory gloves, Zip ties, 2ml-5ml-50ml vials, 2mm-500microns-



(a)



(b)

Figure 2.3: Photographs displaying the retrieval protocol for ARMS unit at Senior kindergarten (SKG), Wandoor

105microns sieves, Mixer grinder

2.4.2 Procedure

- The unit was brought back safely from the field to the lab, where the 40-micron mesh was removed, and the unit was disassembled using wrenches.
- Each plate was tagged with a zip tie to mark the plate number and orientation (top/bottom). Labeled plates were then placed in the plastic bin containing seawater.
- Bubblers were placed inside the bin to keep the water aerated.
- Motile fauna from the icebox was collected on 2mm sieve and stored in 95% Ethanol filled 2mL, 5mL, and 50mL vials based on size.
- After photography (Refer to 2.5.1), subsamples of unique specimens were taken and stored in 2mL vials in 95% Ethanol solution.
- After subsampling, the seawater in the containers was sieved through 2mm, 500 microns, and 105 microns sieves. All the collected fractions were labeled and stored in 95% Ethanol filled 50mL vials.
- The sessile fauna colonized on the plates of the entire ARMS unit was scraped off and blended using a mixer grinder.
- Half of the blended sessile fraction was stored in 95% Ethanol solution, and the other half was stored in salt-saturated DMSO solution.
- All the collected samples were stored in a -20 degree freezer. DNA barcoding would be carried out for motile fauna while the sessile and sieved fraction through 500 and 105 microns sieves would be analyzed using DNA metabarcoding.

2.5 Photographic analysis

The standard ARMS protocol consists of molecular analyses (DNA barcoding and metabarcoding). Considering the time constraints for this study, an alternative method for quantifying the diversity and distribution of coralline organisms became necessary. Therefore, I

used photographic analysis to quantify the distribution of visually identifiable organisms on the plates. Figure 2.2 depicts the ARMS protocol, highlighting the photographic analysis in relation to the main ARMS protocol. I only included sessile fauna attached to the plates for photographic analysis. Hence, this study's results and analysis are based on colonization patterns of sessile fauna on ARMS plates.

Photographic analysis has proven to be a cost-effective method for identifying marine substrates involving minimal laboratory analysis (David et al., 2019). Since the specimens are visually identified in photographic analysis, accuracy is limited while identifying organisms at lower taxonomic levels. If time permits, we aim to compare the results of photographic and molecular analyses.

2.5.1 ARMS photography

Materials

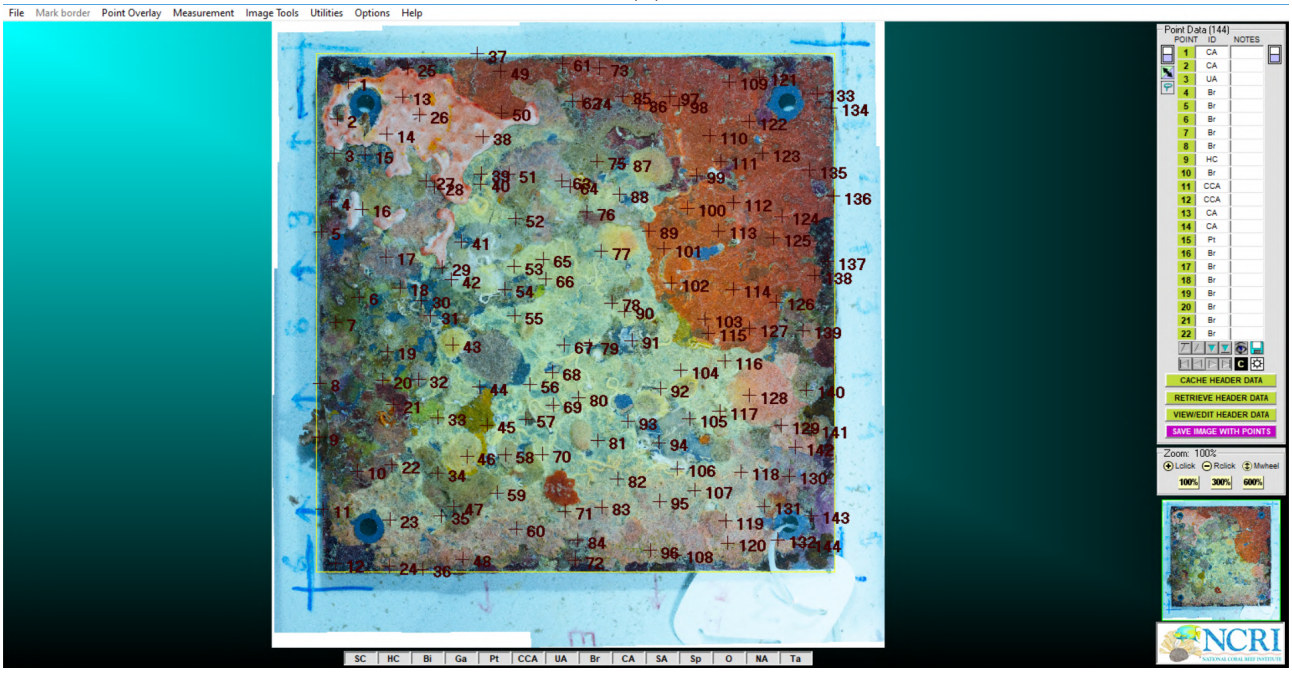
- Photo tray, Nylon spacers, Camera with 100mm lens, Camera tripod, Gloves

Procedure

- The photo tray was filled with filtered seawater. 4 nylon spacers were kept in the tray to hold the plates horizontally.
- Each plate was kept in the photo tray, and a photograph of the entire plate was taken.
- For high-resolution images, the plate was photographed in sections (grid of 3x4) such that there would be a 15%-20% overlap between the pictures.
- The same procedure was repeated for every plate's top and bottom surfaces.
- All the images were stitched to obtain the final high-resolution image of every plate.
- The colonization on the plates was analyzed using CPCe (Coral Point Count with Excel extensions) software to find the percentage cover of each taxon.



(a)



(b)

Figure 2.4: Images describing CPCe Protocol a) Code file directory b) Point distribution.

2.5.2 CPCe

The CPCe program is a Windows-based software that provides a tool for determining coral cover using transect photographs (Kohler and Gill, 2006). A specified number of spatially random points are distributed on a transect image, and the features underlying the points are user-identified. Every point is assigned to a code from the code file that denotes a single specimen. The software quantifies the percentage cover of that specimen on the given plate surface.

Procedure

- A code file for all the observed taxa was created (Refer to Figure 2.4 a)).
- High-resolution stitched images of all the plates were selected, and borders were marked.
- A stratified random point generation method was used to distribute 144 points (12 rows, 12 columns, one random point per square) per plate (Refer to Figure 2.4 b)).
- Each observed specimen under the points was identified and marked with a code.
- The same procedure was repeated for every plate's top and bottom surfaces in all the ARMS units.
- All of the data was extracted into Excel spreadsheets.

2.5.3 Advantages and disadvantages of photographic analysis

Molecular analyses like DNA barcoding and metabarcoding provide a way to analyze the cryptic biodiversity of marine ecosystems. But the time and cost involved with such methods are greater than photographic analysis. As per ARMS protocol, DNA metabarcoding is carried out for the sessile fraction of an entire ARMS unit. The end result provides information about the diversity of micro and macrofauna in a single unit. Due to time and cost constraints, it becomes challenging to conduct molecular analyses for studying individual plate colonization. Photographic analysis can overcome this hurdle as it enables us to explore the diversity of macrofauna for individual plates. Factors such as exogenous DNA contamination

hinder the results of molecular analyses. But photographic analysis is relatively unaffected by it, as the appearance of specimens does not change significantly. Photographic analysis can also reveal hidden patterns in co-occurrence and avoidance of various taxa on an artificial substrate.

On the other hand, photographic analysis has several limitations—the most important being its inability to study microfauna. Since microfauna is too small to be detected by human eyes, visual identification limits the ability to explore the cryptic biodiversity of marine ecosystems. Identification accuracy would decrease for lower taxonomic categories. The lack of baseline data on visual identification of marine fauna adds up to the difficulties in photographic analysis. Expertise in marine fauna becomes necessary for visual identification.

2.6 Statistical analysis

The percentage cover for different taxa for each plate (top and bottom) was extracted into Excel spreadsheets from CPCe. The topmost surfaces in all the units, i.e., 9T, have an unknown algal, bryozoan, or sponge cover. Since these have not been identified for any of the units, all the data points from the topmost surfaces are excluded from the analysis for now. All analyses were conducted in the R statistical software (version 4.1.3).

The percentage cover data was converted to proportion data to perform the statistical analysis. Plates were divided based on the site, and the data points were used to plot a bar graph, indicating the mean proportional cover with standard error between the locations. As the data constitutes proportional cover, I used quasibinomial GLMs with logit link for the majority of my analyses. Quasibinomial GLM relaxes the assumption of Bernoulli trial proportions and allows for any proportional data. In addition, the observed data were over-dispersed, i.e., the variance and mean of the data are comparatively similar. Since binomial GLM can not be used for over-dispersed data, I used quasibinomial GLM.

I tested for the differences in proportional cover of taxa using a GLM contrasting taxa proportion cover with algal proportion cover within the sites. I have reported the respective likelihood ratio estimates for each taxa in each site (Table 3.6).

I tested the effect of plate orientation and spacer type separately for each taxon (six

different models). I carried out quasibinomial GLMs contrasting closed plate cover to the open plate cover and bottom plate cover to the top plate cover for separate taxa. Again, I have reported likelihood ratio estimates and uncertainty for the model (Table 3.9).

I used non-metric multi-dimensional scaling (NMDS) to compare the composition of sessile taxa across sites and plates. NMDS portrays the clusters of data formed based on the dissimilarity and distance matrices. I used vegan library in R and Bray-Curtis distance matrix to plot NMDS graphs. The Bray-Curtis distance matrix was chosen because it provides a comparative measure between communities considering both abundance and species diversity, giving a reliable measure of comparison between the taxa. PERMANOVA (one way) was used to test the differences in composition across sites, orientation and flow (Anderson, 2017). The function adonis2 from vegan was used. I ran 999 permutations for each model. From hereon, plate orientation is denoted as PO or orientation, and spacer type is denoted as ST or flow.

Chapter 3

Results

3.1 Diversity of ARMS colonizing fauna

3.1.1 Motile fauna in ARMS

	HG1	HG2	SKG1	SKG2	SKG3
Crab	3	9	5	2	3
Fish	2	1	3	3	0
Flatworm	1	1	1	0	0
Gastropod	3	4	12	8	14
Shrimp	4	3	5	2	2
Polychaete	6	2	4	5	4
Starfish	2	1	2	0	0
Nudibranch	0	1	0	1	0
Crustacean	0	3	0	7	4
Brittle star	0	1	0	1	4
Limpet	0	1	0	0	1
Bivalve	0	1	0	2	0
Sea urchin	0	0	0	0	0

Table 3.1: Number of different motile species belonging to the respective taxa, observed in the Wandoor units

A wide range of motile taxa were observed in the ARMS retrievals at both sites (Table 3.1 and 3.2). Most of the taxa occurred irregularly even in the units at the same site. The

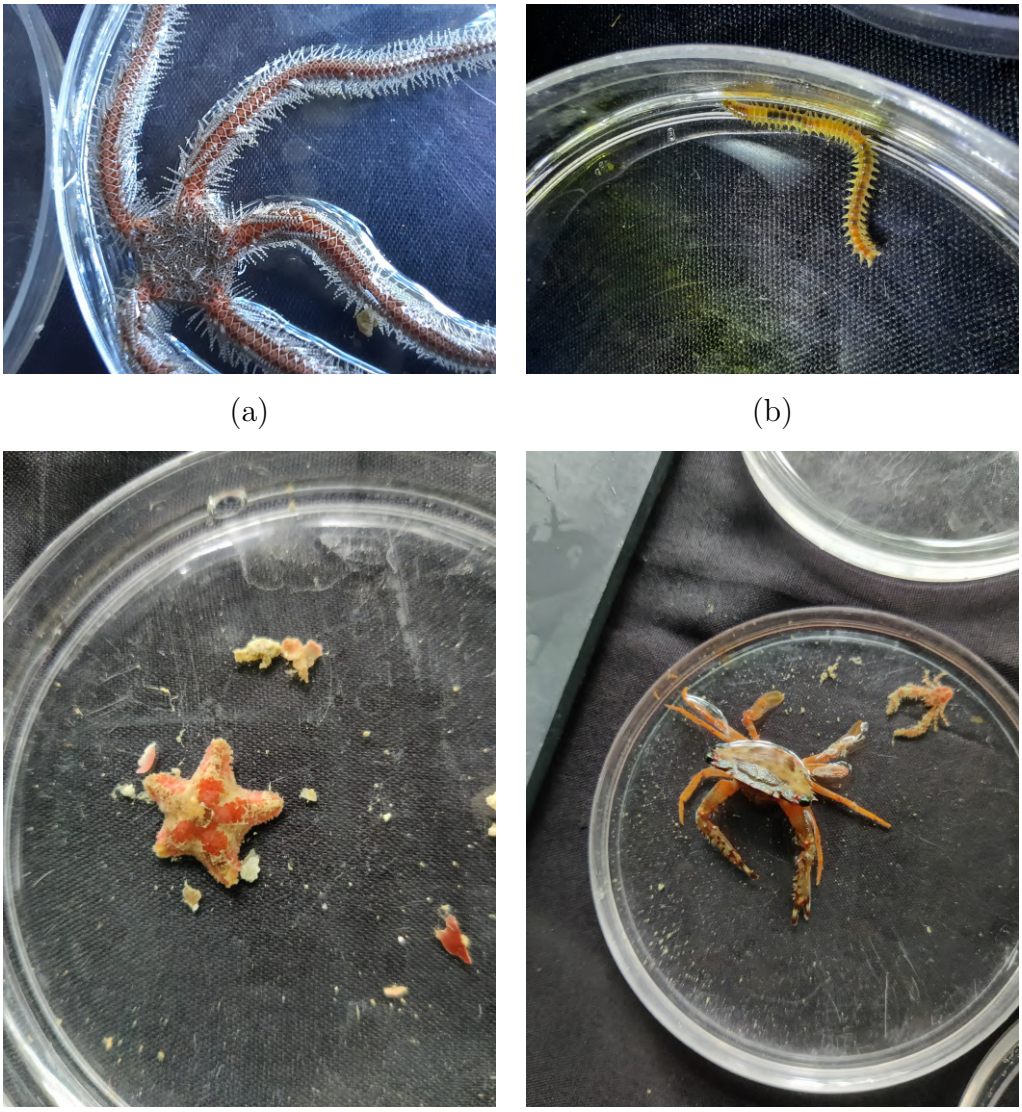
motile fauna detectable by human eyes were separated and vouchered with a 2mm sieve. A number of different species belonging to various taxa were found in the ARMS units at the Wandoor and Havelock site respectively (Table 3.1 and 3.2). A brief overview of the motile fauna observed in the ARMS units is as follows.

- Crab & Shrimp: Phylum- Arthropoda, Decapod crustaceans.
- Flatworm: Phylum- Platyhelminthes, Non-segmented soft bodied invertebrates.
- Gastropod: Phylum- Mollusca, Soft bodied organisms covered with a single coiled shell.
- Polychaete: Phylum- Annelida, Segmented body with parapodia in each segment.
- Starfish, Brittle star & Sea urchin: Phylum- Echinodermata, A central disk and generally five arms with radial symmetry.

	PLT1	PLT2	PLT3	SLP1	SLP2
Crab	4	3	6	3	4
Fish	2	1	1	1	2
Flatworm	0	0	0	0	0
Gastropod	8	3	4	0	2
Shrimp	2	4	16	5	2
Polychaete	5	2	3	4	0
Starfish	0	0	0	0	0
Nudibranch	0	0	4	0	0
Crustacean	10	2	1	9	3
Brittle star	1	3	1	2	0
Limpet	0	0	0	0	1
Bivalve	1	1	3	1	2
Sea urchin	0	0	1	0	0

Table 3.2: Number of different motile species belonging to the respective taxa, observed in the Havelock units

The motile fauna found in the ARMS units (Figure 3.1) were stored in 95% ethanol and refrigerated until the laboratory analysis. DNA barcoding would be carried out for the motile fraction obtained by 2mm sieving, while DNA metabarcoding would be carried out for the motile fraction obtained by 105 and 500 micron sieving.



(a) (b)

(c) (d)

Figure 3.1: Representative photographs of a few motile fauna [a) Brittle star b) Polychaete c) Starfish d) Crab] observed in the ARMS units

3.1.2 Sessile fauna in ARMS

Six major sessile taxa colonized most of the ARMS plates (Table 3.3). It can be seen that ascidians and molluscs colonized a comparatively smaller number of surfaces. A brief

	Wandoor	Havelock
Algae	80	80
Ascidian	57	32
Bryozoan	80	80
Mollusc	42	36
Polychaete tube	78	80
Sponge	77	80

Table 3.3: The number of surfaces (out of 80 for each site) with the occurrence of the respective taxa

overview of the sessile fauna colonizing the ARMS plates is as follows.

- Ascidian: Phylum- Chordata, Characterized by tough outer polysaccharide "tunic".
- Bryozoan: Phylum- Bryozoa, Marine colonial invertebrate with individual zooids.
- Sponge: Phylum- Porifera, Porous bodies allowing water circulation.
- Algae: Phylum- Rhodophyta, Photosynthetic coralline algae.
- Mollusc: Phylum- Mollusca, Soft bodied bilaterally symmetrical segmented animals.
- Polychaete tube: Byproduct of polychaete dwelling and tube formation. Covered high surface area on almost every plate. Not a living entity, but denoted as a taxon to calculate the percentage cover on the ARMS plate.

The aggregate percentage cover occupied by each category on the ARMS units at both sites varied (Table 3.4). Sponge and coral cover was higher in the Havelock units, whereas the ascidian cover was higher in the Wandoor units. The "Unoccupied" category represents the data points that overlapped with the spacers, tags or empty surfaces. These points were not included in the analysis of cover (Section 3.2). Representative photographs of the major sessile fauna observed on the ARMS plates are provided (Figure 3.2).

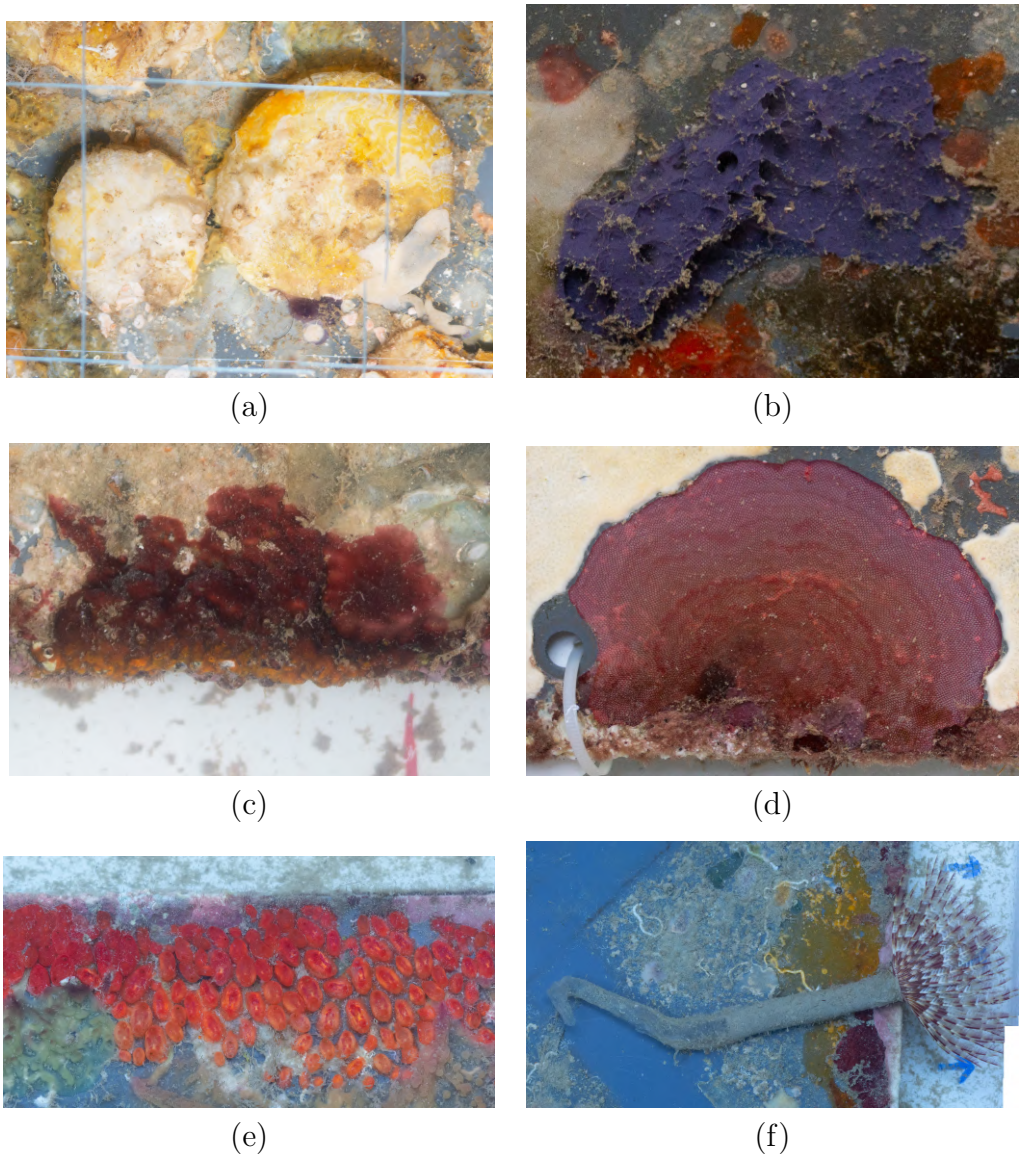


Figure 3.2: Representative photographs of the major sessile fauna [a) Mollusc b) Sponge c) Algae d) Bryozoan e) Ascidian f) Polychaete tube] observed in the ARMS units

	All units	Wandoor units	Havelock units
Coral	0.38	0.22	0.53
Mollusc	2.77	2.55	3.00
Polychaete tube	25.72	23.51	27.91
Algae	14.45	16.34	12.58
Bryozoan	20.12	23.33	16.95
Ascidian	5.28	7.90	2.69
Sponge	30.50	24.99	35.94
Other	0.16	0.14	0.17
Unidentified	0.63	1.03	0.23
Unoccupied	16.01	16.50	15.51

Table 3.4: Aggregate percentage cover occupied by respective categories on the ARMS units

3.2 Colonization trends of sessile fauna on ARMS

3.2.1 Inter-site differences in colonization

Analysis of taxa cover across sites

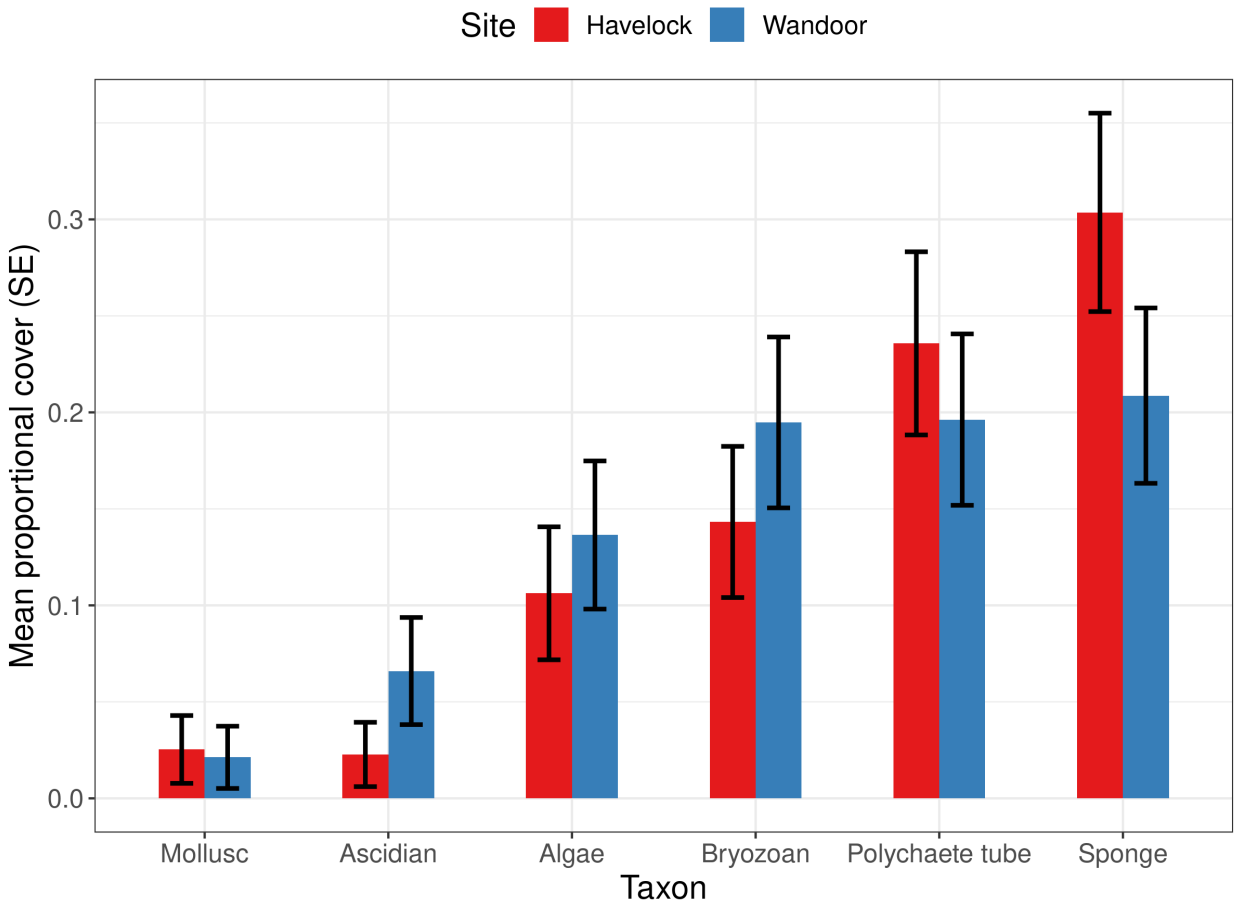


Figure 3.3: Bar graphs representing the mean proportional cover and standard error of taxa cover on all the plates at both sites.

The differences in mean proportional cover between sites seem to vary differently for each taxon with high standard errors (Figure 3.3, Table 3.5).

Molluscs showed inconsistent results for all the units. The average proportional cover of

molluscs at both sites was low and highly variable. Mollusc proportional cover was higher for Havelock units.

Polychaete tubes displayed high mean cover with high standard deviation for both sites. The mean and standard deviation are quite similar, which indicated that the data had high variation from the mean. Polychaete tube proportional cover was higher for Havelock units.

Algae showed consistent cover for all the units, where mean proportional cover and standard deviation of the data was moderate. Algae proportional cover was higher for Wandoor units.

Taxa	**Site**	**Mean proportional cover**	**Standard error**
Algae	Havelock	0.10625125	0.03445321
Algae	Wandoor	0.1364625	0.03837976
Ascidian	Havelock	0.02274	0.01666692
Ascidian	Wandoor	0.06596875	0.02775267
Bryozoan	Havelock	0.14322375	0.03916483
Bryozoan	Wandoor	0.194795	0.04427893
Mollusc	Havelock	0.025345	0.01757222
Mollusc	Wandoor	0.02126	0.01612762
Polychaete tube	Havelock	0.2357625	0.04745768
Polychaete tube	Wandoor	0.19626125	0.04440478
Sponge	Havelock	0.303645	0.05141069
Sponge	Wandoor	0.20867875	0.04543291

Table 3.5: Table representing the mean proportional cover and standard error of taxa cover on all the plates at both sites.

Bryozoans displayed moderate-high mean proportional cover and standard deviation for both sites. Bryozoan proportional cover was higher for Wandoor units.

Similar to molluscs, ascidians displayed high standard deviation and low mean proportional cover for both sites. The standard deviation was greater than the mean proportional cover, indicating the inconsistency in proportional cover data. Ascidian proportional cover was notably higher for Wandoor units.

Sponges displayed high mean proportional cover and moderate-high standard deviation. Sponge proportional cover was notably higher in the Havelock units compared to the Wandoor units.

Analysis of change in composition across sites

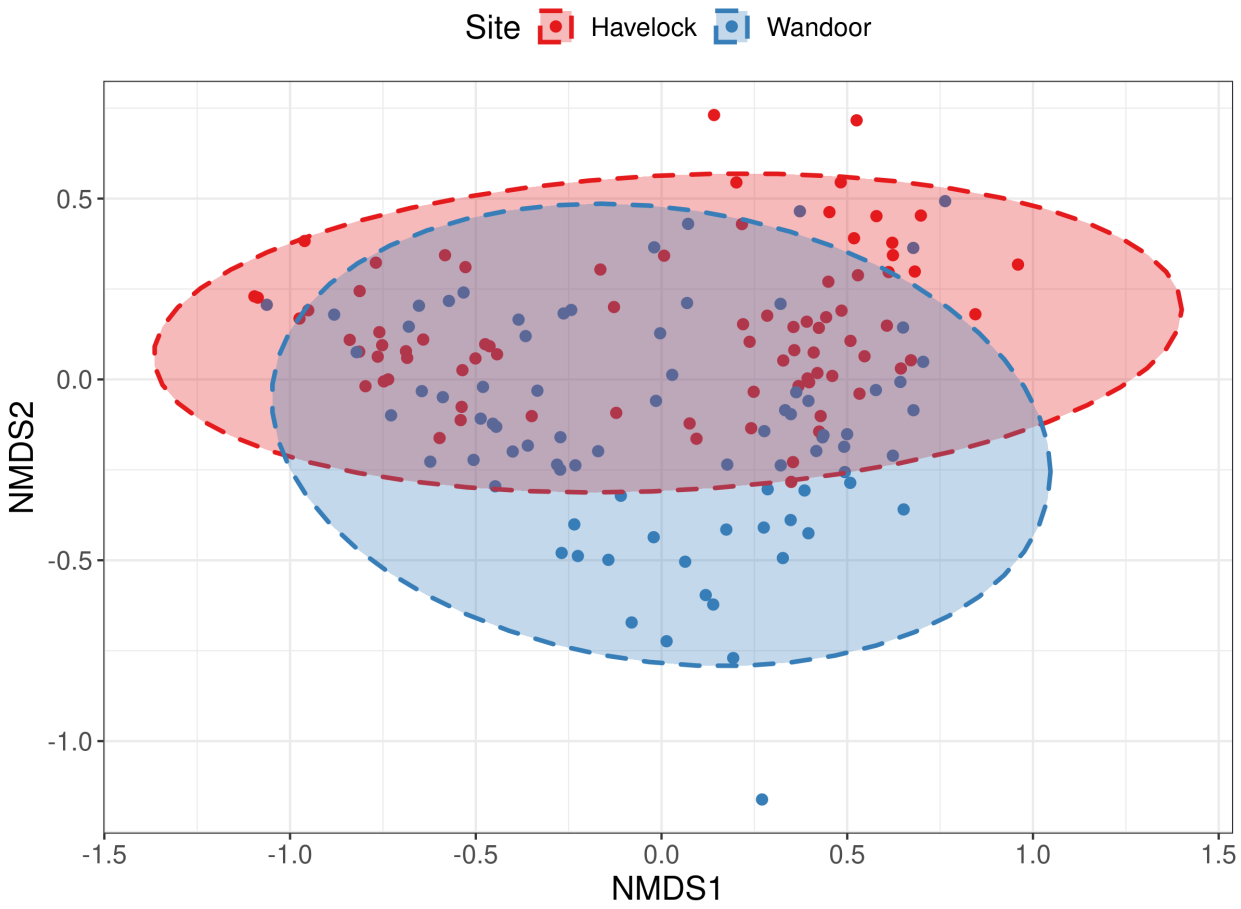


Figure 3.4: NMDS plot representing the compositional differences in colonization between sites.

The output of the GLM based on the inter-site differences in colonization provided odds ratio as an estimate of contrast in mean cover between the taxa. Algal cover was used as a contrast variable for both site (see Table 3 for the GLM output for various taxa at both sites). Except for bryozoans at the Havelock site, the p values for the rest of the categories are less than 0.05. This indicates that the difference between the algal cover and cover of

Site	Term	Estimate	Standard Error	Statistic	p value
Wandoor	Intercept	0.158	0.111		
Wandoor	Taxa-Ascidian	0.447	0.189	-4.27	**2.38e-5**
Wandoor	Taxa-Bryozoan	1.53	0.146	2.91	**3.78e-3**
Wandoor	Taxa-Mollusc	0.137	0.285	-6.95	**1.19e-11**
Wandoor	Taxa-Polychaete tube	1.55	0.146	2.98	**3.05e-3**
Wandoor	Taxa-Sponge	1.67	0.145	3.54	**4.43e-4**
Havelock	Intercept	0.119	0.142		
Havelock	Taxa-Ascidian	0.196	0.327	-4.99	**8.60e-7**
Havelock	Taxa-Bryozoan	1.41	0.190	1.80	7.30e-2
Havelock	Taxa-Mollusc	0.219	0.313	-4.85	**1.69e-6**
Havelock	Taxa-Polychaete tube	2.59	0.176	5.42	**9.59e-8**
Havelock	Taxa-Sponge	3.67	0.171	7.58	**1.83e-13**

Table 3.6: GLM table representing the model for colonization of taxa comparing the differences between sites.

rest of the taxa is significantly high at both sites (except bryozoan cover at the Havelock site).

The 2D NMDS plot for composition between sites shows that the overlap between compositions of observed taxa between both sites is high (Fig 3.4). This shows that the colonization at both sites for observed taxa shows considerable similarity despite the differences detected using a GLM.

The differences in colonization of taxa between sites was significant ($p = 0.014$) but the low PERMANOVA R-squared value (0.02) implies that the proposed model does not explain the variation in cover data for the site variable (Table 3.7)

	Df	Sum of Squares	R^2	F	P(F)
Site	1	0.4866	0.02715	4.4093	**0.014**
Residual	158	17.4375	0.97285		
Total	159	17.9241	1.0000		

Table 3.7: PERMANOVA table representing the significant values for proportional cover between sites.

3.2.2 Colonization differences due to Orientation and Flow

Analysis of taxa cover with orientation and flow

The mean cover and standard error of taxa cover on bottom and top plates with closed and open surfaces varied considerably (Figure 3.5, Table 3.8). Flow is analogous to the spacer type (closed for cross spacers and open for nylon spacers) and it represents whether the flow of water and nutrients is allowed between the plates.

Molluscs appeared to occupy a higher cover on the bottom plates than the top plates. This trend was visible at both sites. In addition, mollusc colonization was observed abnormally high in two particular units, i.e., HG1 and SLP1, compared to other units. Molluscs showed uniformly high variation for all four categories. The peak mollusc colonization occurs at the Open-Bottom plates at both sites. The rest of the categories displayed low mollusc cover.

Polychaete tubes dominated the top plate cover but displayed negligible colonization on the bottom plates. This trend was visible at both sites. Polychaete tubes showed low-

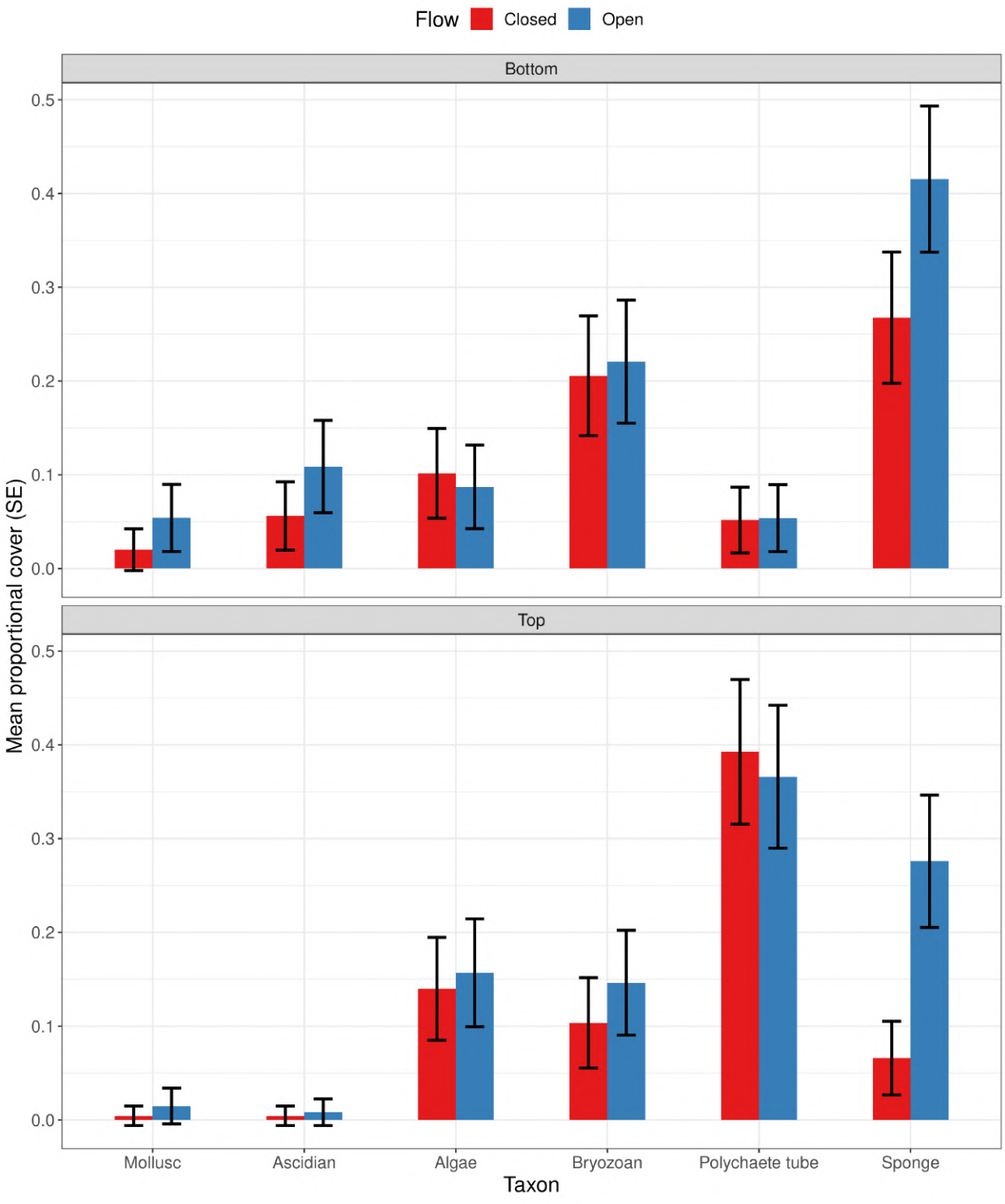


Figure 3.5: Bar graphs representing the mean proportional cover and standard error of taxa cover on bottom and top plates with closed and open surfaces.

moderate standard deviation for bottom plates and high standard deviation for the top plates for both sites. No significant difference between the flow was observed for bottom and top plates.

Although algae displayed higher colonization on the top plates than on the bottom plates in general, the mean proportional cover for both orientation was comparable. The standard deviation of proportional cover was moderate for all four categories.

Bryozoans showed mixed colonizing patterns but occupied more cover at the bottom surfaces in general. The standard deviation of proportional cover was moderate-high for all four categories.

Ascidians erratically displayed higher colonization on the bottom surfaces. Minimal ascidian proportional cover was seen on the top plates. The standard deviation was low for top plates, whereas high standard deviation was observed for bottom plates. Similar to molluscs, ascidian cover also peaked at the Open-Bottom surfaces irregularly for both sites. The rest of the categories displayed low ascidian cover.

Similar to bryozoans, sponges showed mixed colonizing patterns but occupied more cover at the bottom surfaces for both sites. The standard deviation was high for top plates and moderate-high for bottom plates. Sponges displayed continuously low proportional cover for Closed-Top plates at both sites. The rest three categories constantly showed high cover, where Open-Bottom plates occupied the highest cover at both sites. Sponges showed notably higher mean cover for open surfaces on both top and bottom plates, which shows their affinity to the water flow between the plates.

Taxa	Orientation	Flow	Mean proportional cover	Standard error
Algae	Bottom	Closed	0.1015625	0.04776179
Algae	Bottom	Open	0.0871525	0.04459735
Algae	Top	Closed	0.1397625	0.05482448
Algae	Top	Open	0.15695	0.0575145
Ascidian	Bottom	Closed	0.0560725	0.03637594
Ascidian	Bottom	Open	0.108855	0.04924571
Ascidian	Top	Closed	0.0043325	0.01038476
Ascidian	Top	Open	0.0081575	0.0142223
Bryozoan	Bottom	Closed	0.2055575	0.06389515
Bryozoan	Bottom	Open	0.2206525	0.06556771
Bryozoan	Top	Closed	0.1034725	0.04815754
Bryozoan	Top	Open	0.146355	0.05588721
Mollusc	Bottom	Closed	0.020135	0.022209
Mollusc	Bottom	Open	0.0539925	0.0357342
Mollusc	Top	Closed	0.0043325	0.01038476
Mollusc	Top	Open	0.01475	0.01906072
Polychaete tube	Bottom	Closed	0.0517375	0.03502168
Polychaete tube	Bottom	Open	0.0538125	0.03567797
Polychaete tube	Top	Closed	0.3925325	0.07720925
Polychaete tube	Top	Open	0.365965	0.07616341
Sponge	Bottom	Closed	0.26753	0.06999245
Sponge	Bottom	Open	0.4152775	0.07791375
Sponge	Top	Closed	0.0659725	0.03924924
Sponge	Top	Open	0.2758675	0.07066906

Table 3.8: Table representing the mean proportional cover and standard error of taxa cover with orientation and flow.

Analysis of change in composition with orientation and flow

The output of the GLMs based on orientation and flow provided odds ratio as an estimate of contrast in mean cover between the taxa on different surfaces. Bottom plate cover was used as a contrast variable for top plate cover, whereas closed surface cover was used as

Taxa	Term	Estimate	Standard Error	Statistic	p value
Mollusc	Intercept	0.0199	0.299		
Mollusc	Flow Open	2.90	0.338	3.14	**2.00e-3**
Mollusc	Orientation Top	0.249	0.368	-3.78	**2.20e-4**
Polychaete tube	Intercept	0.0581	0.160		
Polychaete tube	Flow Open	0.918	0.124	-0.690	4.91e-1
Polychaete tube	Orientation Top	11.0	0.164	14.6	**3.91e-31**
Algae	Intercept	0.104	0.0669		
Algae	Flow Open	1.01	0.0716	0.183	8.55e-1
Algae	Orientation Top	1.67	0.0730	7.04	**5.66e-11**
Bryozoan	Intercept	0.243	0.0880		
Bryozoan	Flow Open	1.23	0.106	1.98	**4.92e-2**
Bryozoan	Orientation Top	0.527	0.108	-5.94	**1.78e-8**
Ascidian	Intercept	0.0596	0.181		
Ascidian	Flow Open	2.04	0.223	3.21	**1.61e-3**
Ascidian	Orientation Top	0.0693	0.400	-6.67	**4.20e-10**
Sponge	Intercept	0.300	0.112		
Sponge	Flow Open	2.75	0.132	7.68	**1.60e-12**
Sponge	Orientation Top	0.381	0.131	-7.35	**1.03e-11**

Table 3.9: GLM table representing the models for colonization of taxa with factors like orientation and flow. This table represents six models (one for each taxon).

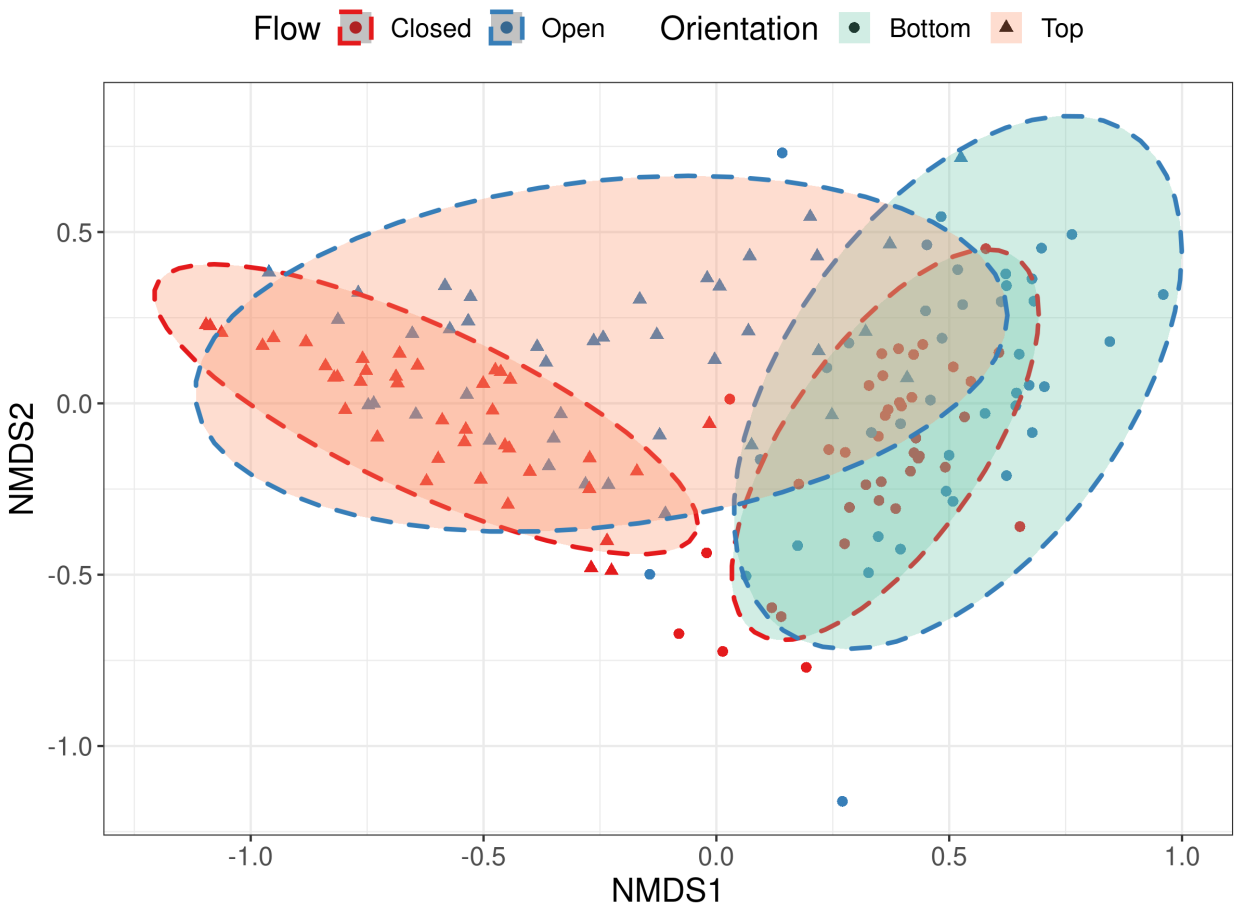


Figure 3.6: NMDS plot representing the compositional differences in colonization on top and bottom plates with closed and open surfaces.

a contrast variable for open surface cover (see Table 3.9 for the GLM output for various taxa at different surfaces). Except for polychaete tubes and algae on the open surfaces, the remaining taxa showed significant differences for flow and orientation (p value less than 0.05). This indicates that the top and bottom surfaces showed significantly different cover for every taxa, whereas open and closed surfaces showed significantly different cover for ascidians, bryozoans, sponges and molluscs. Flow did not have a significant impact on the proportional cover of polychaete tubes and algae.

The 2D NMDS plot for composition of every taxa on open and closed surfaces of top and bottom plates shows a clear distinction in composition of bottom and top plate colonization (Figure 3.6). This can be observed by the low overlap between bottom and top plate ellipses.

Closed top surface ellipse had a negligible overlap with both open bottom and closed bottom ellipses. This indicates that the compositional similarity between closed top surfaces and both bottom surfaces is negligible. On the hand, compositional similarity between both bottom surfaces appears to be quite high as the overlap between the ellipses is significant. Data for both top surfaces is comparatively more scattered.

Orientation explained a significant amount of variation in cover data (PERMANOVA R-squared = 0.42, p = 0.001); flow was also significant but had explained a low amount of variation (PERMANOVA R-squared = 0.06, p = 0.001)(Table 3.10).

	Df	Sum of Squares	R^2	F	P(F)
Orientation	1	7.5738	0.42255	129.269	**0.001**
Flow	1	1.1517	0.06426	19.657	**0.001**
Residual	157	9.1986	0.51320		
Total	159	17.9241	1.00000		

Table 3.10: PERMANOVA table representing the significant values for proportional cover at surfaces with varying orientation and flow.

Chapter 4

Discussion

It is essential to monitor ecosystems to track their responses to global change, and devise actions that can guide conservation and management. ARMS are being used globally as a standardized method for monitoring reef associated fauna. Since this is the first study involving ARMS in the Indian subcontinent, we aimed to get new insights into the marine biodiversity and distribution patterns of coral ecosystems in India. The Andaman Islands were chosen for ARMS deployment due to their highly biodiverse coral ecosystems which are under threat for both local and global stressors. The broad aim of the study was to generate a baseline for the diversity and distribution of marine fauna in reefs of the Andamans Islands towards a long term monitoring programme.

ARMS are designed to mimic the structural complexity of coral reefs. The distinct 3D structure of the ARMS units was expected to prompt differences in settlements for various taxa. It was hypothesized that the top and bottom plates of the ARMS would acquire contrasting colonization due to the surface orientation. The top plates resemble the horizontal surfaces provided by reefs in natural systems, whereas the bottom surfaces resemble overhangs in reef structures. In addition, two types of spacers used to assemble the ARMS provide different conditions for water flow. We expected to observe differences in sessile assemblage patterns due to variable water flow conditions on different surfaces.

We also expected to observe differences in colonization between the sites with varying degrees of anthropogenic stresses and sedimentation. It was hypothesized that the increase in sedimentation and human activities would lead to decrease in overall biodiversity of the

ARMS colonization. Sites with higher perturbance would create additional stress for colonizing the ARMS plates and would thus support less biodiversity.

4.1 Observed colonization trends

The six major taxonomic categories included in the analysis (i.e., mollusc, polychaete tube, algae, bryozoan, ascidian, sponge) showed some patterns in the preliminary analysis. When the plates were divided based on the plate orientation (Orientation), every taxon displayed a distinct colonization pattern with plate orientation. The distinction between the colonization patterns of some taxa (molluscs, ascidians, and sponges) was amplified when plates were separated based on combinations of spacer type and plate orientation (Flow and Orientation).

Bryozoans, ascidians, sponges, and molluscs seem to prefer the bottom surfaces, whereas polychaete tubes and algae generally prefer the top surfaces. With further categorization, sponges exhibited an intriguing pattern where percentage cover dropped drastically for Closed-Top surfaces and was particularly high for the rest of the combinations. Bryozoans showed a similar trend for the units at the Havelock site. Ascidian percentage cover peaked steeply for Open-Bottom surfaces. Except for the topmost surface, crustose coralline algae colonization was primarily observed on the edges of the plates. As a whole, Polychaete tubes clearly dominated the top plate cover in both Havelock and Wandoor units. Sponges showed dominant cover in the Havelock units for bottom plates, whereas bryozoans and sponges shared most of the Wandoor bottom plate cover.

Polychaete tubes are an organic product of polychaete dwelling and tube formation; hence they cover the substrate and make it inaccessible for other marine invertebrates to colonize (Merz, 2015) (Fuirst et al., 2021). High polychaete cover directly implies less potential colonization area for other taxa. Since polychaete tubes dominated the top plate colonization, other suspension feeders like sponges, bryozoans, molluscs, and ascidians appeared to coexist frequently on the bottom plates. The algal cover was mainly observed around the edges of the plates. This pattern could be because of the relatively high light availability around the edges of the plates. Since the direct sunlight reaches the top plates, a higher algal cover could be expected at the top plates, which matches our results.

Since categorization of the plates separates different habitats, one might expect amplified

colonization patterns due to differences in structural complexity. But polychaete tubes, algae, and bryozoans did not show further development in their colonization patterns with categorization (Flow). Although sponges, ascidians, and molluscs appear to show further elaboration in their patterns with categorization (Flow), ascidian and mollusc cover across the units demonstrated large standard deviation. Ascidian and mollusc cover was zero for plenty of the plates. In addition, ascidians and molluscs showed erratic distribution trends for different units. HG1 had a particularly high ascidian and mollusc percentage cover compared to the rest of the units. Since polychaete tubes dominated the top plate cover uniformly, clear segregation between the top and bottom plate colonization was expected. But the results do not appear to follow this trend strictly. For the three units at Pilot (Havelock), top plates showed shared cover dominance of sponge and polychaete tubes.

4.2 Factors influencing ARMS colonization

Two observed trends in this study need to be scrutinized in particular. The first trend is the difference in variance depending on the group of plates analyzed. The second pattern that needs more examination is the segregation in colonization between the top and bottom plates.

4.2.1 Differences between ARMS locations

It is apparent from the results that the inter-site differences in cover do not display any significant pattern. Except for a few exceptions, these differences show similar trends when compared with groups of plates with a similar configuration (e.g., Wandoor Top-Havelock Top, Wandoor Closed Bottom-Havelock Closed Bottom). On the other hand, strong patterns are observed when two different plate configurations are compared irrespective of the site location (e.g., Wandoor Top-Wandoor Bottom, Havelock Closed Top-Wandoor Closed Bottom).

With this observation, the most obvious hypothesis would be that the inter-site differences do not results in a change in colonization patterns for the observed taxa. In contrast, substrate orientation is enough to promote variance in cover in the same unit and between

the units. One of the possible reasons behind this could be the overall low anthropogenic pressure in the Andaman Islands. If the water quality at both sites is comparable, we would expect fewer variations in the settlement patterns. Alternately, the parameters that vary between these sites may not influence colonization. And finally, different factors may have opposing effects, resulting in patterns that are similar.

A similar study can be conducted with sediment traps installed near the deployment sites to test this hypothesis. Another possible reason behind the observation could be the short distance between the sites. More specific results can be expected if the distance between the sites is increased. Installing more units would provide additional data to test the presented hypotheses.

4.2.2 Differences in ARMS microhabitats

The bottom surfaces offer shelter from predation as they resemble overhangs created due to coral reef complexity. These surfaces are relatively less affected by factors like sedimentation and turbidity as the detritus does not settle down on bottom surfaces due to gravity (Kane and Clare, 2019) (Tolhurst et al., 2020). On the other hand, the top surfaces are vulnerable to predators and high degrees of sedimentation. But light availability is higher on the top surfaces, which is a prime factor for the survival of several marine species. Different taxa have different preferences for factors like water and nutrient flow, light availability, predator pressure, and sedimentation (Riisgård and Larsen, 2010). These factors change with spacer type and plate orientation. As a result, we may expect clear segregation between percentage cover for various taxa with the categorization of the plates. Table 4.1 illustrates a rough estimate of variation of biotic and abiotic factors with categorization.

	Closed-Top	Open-Top	Closed-Bottom	Open-Bottom
Sedimentation	High	High	Low	Low
Predator Pressure	High	High	Low	Low
Water Flow	Low	High	Low	High
Space to Colonize	Moderate	High	Moderate	High
Light Availability	Moderate	Moderate	Low	Low

Table 4.1: A rough estimate of the degree of varying biotic and abiotic factors on different sets of plates

Since most of the observed colonizing taxa were sessile invertebrates, the feeding mechanisms can provide important insights into the observed colonization. Suspension feeding is one of the most prominent feeding mechanisms for sessile marine invertebrates (Hentschel and Shimeta, 2008). Suspension feeders that actively create a flow of water to stir up nutritious particles in water are called active suspension feeders (Riisgård and Larsen, 2000). Sponges, bryozoans, ascidians, and bivalves are active suspension feeders. In addition, ascidians, sponges, and bivalves are also filter feeders, a class of suspension feeders that pump water to filter particles from the suspension (Rubenstein and Koehl, 1977).

Deposit feeding is another prevalent feeding mechanism in marine organisms (Lopez and Levinton, 1987). Deposit feeders generally stay near the benthic surface and feed on the detritus on the seafloor. Since the deposit feeders derive their nutrition from sediment, they can be expected to prefer top surfaces more than the bottom. Some polychaetes are known to exhibit deposit-feeding mechanisms (Gordon, 1966). This might explain why polychaete tube cover domination was observed on the top plates.

We may expect higher colonization of deposit feeders on the top plates in the ARMS structure and suspension-feeding sessile organisms on the bottom plates. Particle size and quality, the density of sediment, and water flow determine the nutrition intake of suspension feeders(Riisgård and Larsen, 2010). Although this may vary with the particle capture mechanisms of various taxa, we may expect bottom surfaces to support more sessile invertebrates due to shelter and low sedimentation (Irving and Connell, 2002).

It is clear from the results that polychaete tubes dominated the top cover, and sponge-bryozoan dominance was observed on the bottom surface cover. The driver behind this trend remains in question.

If the sessile invertebrates were the drivers behind this trend, the most obvious hypothesis would be that the predation pressure is the driving force behind this pattern. Sedimentation and predation pressure are the only factors that change significantly with a change in PO (both the factors are at a higher degree on the top surfaces and lower at the bottom, Table 4.1). Now, as inter-site differences in colonization were insignificant, the difference in sedimentation does not seem to induce any change in the percentage cover of observed taxa (here, we assume that there is a significant difference in sedimentation between the sites). As a result, predation pressure driving the sessile invertebrates' colonization trends becomes the most plausible hypothesis according to the observed results. Due to high predation pressure

on the top plates, competition for space becomes scarce, and polychaete tubes occupy the easily available substrate. On the other hand, bottom plates promote fierce space competition among the sessile invertebrates. Sponges and bryozoans show the highest degree of competition for space, whereas ascidians and molluscs show weak competition.

If polychaete tubes were the driving factor behind this trend, we would expect them to be a better competitor for space than the sessile invertebrates. This expectation assumes that tube-forming polychaetes exhibit deposit-feeding as a feeding mechanism. As mentioned earlier, Deposit-feeders obtain nutrition by digesting the sediment particles by feeding on the detritus. Hence, more detritus would mean higher feeding chances for the feeder. As the top surfaces accumulate increased sedimentation over time, we would expect a higher cover of polychaete tubes over time. As a result, competition for space at the top surfaces would fade, and the weak competitors would need to compete for cover on the bottom plates. The assumption here is that the sessile invertebrates have a certain tolerance level for sedimentation, over which they can not obtain any nutrition due to the change in particle size and concentration.

Observing the colonization trend of sessile invertebrates like sponges (and bryozoans to some extent) makes it apparent that they can colonize the top surfaces as long as cross spacers do not restrict the water flow. This contrasts with the aforementioned hypotheses as the factors like sedimentation, predation pressure, and space competition are not considered. Assuming that these sessile invertebrates can compete for top and bottom colonization, we can hypothesize that substrate with a high sedimentation rate and restricted water flow is the only habitat structure that inhibits colonization of these taxa. In this case, sponges followed by bryozoans exhibit the most elevated competition for space on both surfaces. But the unfavorable conditions on Closed-Top plates reduce their percentage cover, resulting in higher polychaete tube cover on the overall top plates.

Other previously published work complements the observed results of this study. (Virgilio et al., 2006) found that sessile invertebrates were more abundant on overhangs than on horizontal surfaces. Organisms like bryozoans and ascidians showed higher cover on overhangs with depth. Interactions between depth and inclination influence sessile assemblages as they alter other physical factors like sedimentation and light. This study found that sedimentation may play a role in shaping sessile assemblages, especially in deeper zones. (Bravo et al., 2020) recorded macroalgae domination on horizontal and vertical surfaces,

suspension-feeding cnidarian domination on the overhangs, followed by filter feeders like sponges. Cavefloor surfaces mostly had bare substrate with a significant cover of colonial ascidians. With depth, algal cover decreases, and filter feeder cover increases on all surfaces. On overhangs, the percentage cover of filter feeders decreased with depth while suspension feeders increased. (Cárdenas et al., 2012) found a negative correlation between sponge and algal abundance, with surface inclination strongly correlating with the distribution patterns of both taxa.

As mentioned in the introduction, we aimed to test whether the spacer and plate arrangement affected colonization. The results show that the trends in colonization are generally amplified when plates are separated based on PO or PO+ST. Since no significant trend is observed within the sites, the colonization trends across the taxa are likely driven by the plate and spacer arrangement more than the site and unit location.

4.3 Conclusion

Since this is the first study involving ARMS in India, we expected to explore the biodiversity of Indian waters through our methodology. However, baseline data on marine ecosystems is still in its initial stage of development in this region. The reason behind the scarcity of data is the lack of research and records on marine systems due to evident challenges related to in-water research. For in-water explorations, obtaining high quantity and quality data covering a wide range of taxa over time is challenging. The issue gets even more complicated when the studies have to be carried out in various regions to examine the similarities and differences between the systems over time. Exploring the marine ecosystems on a deeper level becomes of utmost priority due to their impact on the entire globe and the rapid change they are going through due to human interventions.

The challenges in identifying the wide range of taxa boil down to the lack of expertise on the subject. Even though the contrast in colonization between the plates is clearly visible, it is difficult to differentiate between even the broad taxa. As species colonize the same substrate and compete for spaces, the characteristic features of each taxa become hard to distinguish. Considering the limited knowledge and the lack of baseline data on marine life, it becomes essential to examine it using novel methodologies like ARMS. ARMS approach could prove essential to study marine systems as it uses a two layered protocol to identify marine fauna.

The molecular approach in the ARMS methodology can help delineate the cryptic diversity that may go unnoticed during photographic analysis. The novel photo analysis approach and the unique ARMS structure would provide a potential means to study the reef ecosystems in the future. The results and conclusions could help further the knowledge about the cryptic marine biodiversity of the Andaman Islands.

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