# Studies on coral patches along the central west coast of India

A Thesis submitted in partial fulfillment for the Degree of

DOCTOR OF PHILOSOPHY

in Marine Sciences

Goa University

By

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# *Declaration*

As required under the University ordinance **OA-19.8 (v-viii)**, I state that the present thesis entitled "***Studies on coral patches along the central west coast of India***" is the original work carried out by me. I further declare that this work has not been previously submitted and is not concurrently submitted for any other degree at Goa University or any other university or institution.

The literature related to the problem investigated has been cited. Due acknowledgments have been made wherever facilities and suggestions have been availed of.

Afreen

**Place: Goa**

**Date: 25/7/22** **Afreen Hussain**

# *Certificate*

This is to certify that the work presented in this thesis entitled ***"Studies on coral patches along the central west coast of India"*** submitted by Afreen Hussain to the School of Earth, Ocean, and Atmospheric Sciences, Goa University for the degree of Doctor of Philosophy in Marine Sciences, is based on her original work carried out by her under my guidance. The thesis or any part of this work has not been previously submitted for any other degree or diploma in any university or institution.

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***"We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained."***

***-Marie Curie***

Afreen

***Afreen Hussain***

*Date: 25/7/22* *CSIR-National Institute of Oceanography*

*Dedicated to my parents*

*and*

*to the lives lost to the COVID pandemic*

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# 1. Introduction

## 1.1. Coral taxonomy and distribution

Coral reefs are the most diverse, complex, and unique of all the marine ecosystems and are rightfully known as “rainforests of the ocean”. The word ‘coral’ itself is Middle English, derived from Old French, Latin, and Greek (Best, 1999), and was probably based on the reference to the precious Mediterranean red coral (octocoral), *Corallium rubrum* (Linnaeus, 1758; Cairns, 2007). The term *coral* denotes a polyphyletic group for all those Cnidaria belonging to classes Anthozoa and Hydrozoa, having continuous or discontinuous skeleton made up of calcium carbonate (aragonitic or calcitic) or horn-like proteinaceous skeletal elements (Cairns, 2007). According to this definition, the group consists of about 5080 species belonging to seven taxa (Scleractinia, Antipatharia, Octocorallia, Stylasteridae, and Milleporidae, two zoanthids, and three calcified hydractiniids), dominantly, 66% of which are deep water or cold-water corals (ahermatypic), occurring in water deeper than 50 m (Cairns, 2007).

Coral reefs are built by the accumulation of layers of calcium carbonate secreted by organisms like scleractinian or hermatypic corals (aragonite mineral), calcareous macroalgae, and other calcareous marine organisms like bryozoans, foraminifers, and echinoderms (Eyre et al. 2018). To date, almost 845 species of reef-building corals have been identified (Polidoro et al. 2008) with the majority found in the Indo-Pacific region (Fig 1.1).

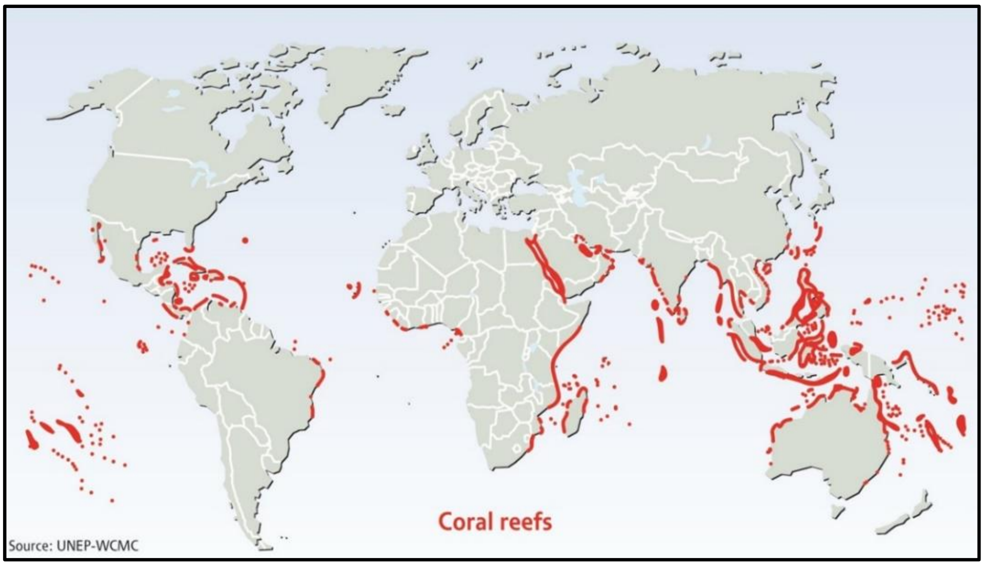


**Fig. 1.1: Distribution of coral reefs around the world (source: UNEP-WCMC).**

Each coral colony in a reef is composed of hundreds to thousands of tiny coelenterates-polyps (Phylum Cnidaria). These small, colourful polyps are the building blocks of coral reefs and play a major role in building the calcium carbonate skeleton of true stony corals by taking the dissolved calcium carbonate from the seawater and hardening it into a hard skeleton of calcium carbonate mineral (aragonite). These skeletons of numerous coral colonies over a geological time scale build coral reefs. Polyps have a unique relationship with symbiotic phototrophic algae-zooxanthellae, which live in coral tissues in high densities (between $0.5 \times 10^6$ to $5 \times 10^6$ cells/$cm^{-2}$; Hoegh-Gulberg & Smith, 1989). This highly sensitive symbiotic relationship between the coral polyp (animal) and the zooxanthellae (plant), which first appeared during the Triassic era (Stanley, 1981; Stanley & Schootbrugge, 2018) is responsible for the evolutionary success of the coral reefs. These symbiotic algae provide corals with 90% of their nutritional requirements (Muscatine & Porter, 1977), using the products of photosynthesis, play a major role in efficient recycling of nutrients (Muscatine & Porter, 1977), leading to faster growth rate and calcification in corals (Pearse & Muscatine, 1971; Bythell, 1988), and provide the corals with their beautiful colours. It is due to this light requirement for photosynthesis, coral reefs are mostly restricted to the oligotrophic, shallow, and sunlit warm waters of the tropics (between 30°N and 30°S latitudes).

## 1.2. Ecological and Socio-economic benefits of corals

Although coral reefs occupy only less than 1% of the benthic environment (Spalding et al. 2014), they are home to more than 25% of all marine life, including over 4000 species of fish (EPA, USA 2008). Coral reefs offer momentous ecological, economic, and societal benefits to millions of people globally, valued at about USD 9.8 trillion per annum (de Groot et al. 2012; Costanza et al. 2014). It is estimated that worldwide, approximately 850 million people reside within 100 km of reefs, and about 275 million people live within 10-30 km from the coast, therefore in the direct vicinity of coral reefs and have a reef-dependent livelihood (Burke et al. 2011). Due to the presence of valuable marine natural products, the coral reefs are also referred to as “medicine chests of the sea” (Coral Reef Alliance, 2020). The anti-inflammatory and anti-cancer properties of the products derived from soft corals such as *Sinularia* have promising potential (Cooper et al. 2014). The naturally occurring calcium in the aragonite mineral of hard corals and calcite of soft corals, when combined with a microporous material-zeolite has shown potential against bone loss in experimental mice and rabbits (Banu et al. 2012; Parizi et al. 2012) and is also a potential candidate for the restoration of dental deformities (Figueiredo et al. 2010). The coral reefs also act as a natural buffer during natural disasters (storms, hurricanes, and tsunami) and play a significant role in shoreline protection by weakening the incoming wave energy by an average of 97% globally, and reducing the wave height by an average of 84% (Ferrario et al. 2014).

## 1.3. Nutrient cycling in coral reefs

It is known that tropical coral reefs are highly productive areas found in low-nutrient waters. This contradiction has been termed the ‘Darwin Paradox’ (Darwin, 1842; Sammarco et al. 1999). This anomalous behaviour can be perhaps explained by two methods: 1) the efficient uptake of nutrients from the surrounding seawater by nitrogen fixation (by cyanobacteria and heterotrophic bacteria), and; 2) the recycling of nutrients by the coral reef organisms (invertebrate host and the holobiont organism; Sivadas et al. 2020).

Nitrogen cycling is a highly orchestrated process between the coral host, symbiotic zooxanthellae, and the coral holobiont, which includes a variety of associated organisms like protozoa, fungi, archaea, and bacteria (Rohwer et al. 2002; Ainsworth et al. 2010; Fig 1.2). Studies have demonstrated the role of coral mucus in coral health, nutrient cycling, and the resilience of the coral holobiont (Rohwer et al. 2002; Knowlton & Rohwer, 2003). Nitrogen cycling microbes appear to be ubiquitous and consistent members of the coral microbiome (Rosenberg et al. 2007; Olson & Lesser, 2013). Symbiodinium density is also controlled by nitrogen levels (Falkowski et al. 1993). Hence, it is believed that nitrogen cycling in the coral holobiont may play a crucial role in the retention and acquisition of nitrogen to sustain photosynthesis in the coral (Rädecker et al. 2015).

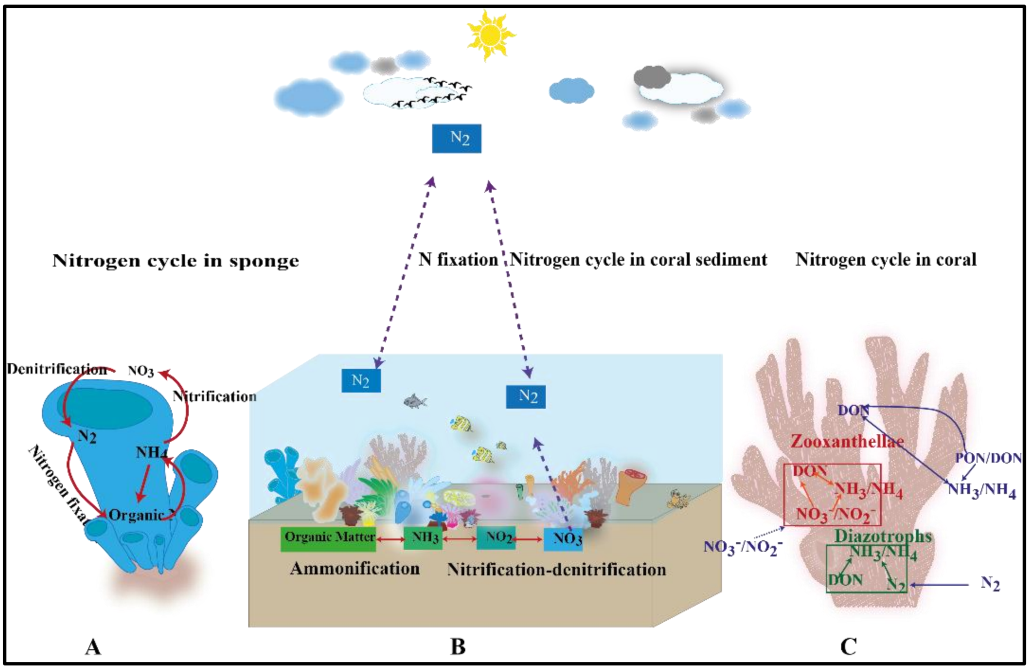


**Fig. 1.2: The nitrogen cycle at important locations in the coral reefs. Sponges (A) take up dissolved nitrogen from the surrounding seawater; the prokaryotes symbiotically associated with the sponges transform the nitrogen (B); symbiotic algae efficiently utilise the inorganic nutrients (DIN) released by the corals and also facilitate the uptake of nitrogen in the form of $NH_4^+$ and $NO_3^-$ from the seawater (C) (adapted from Sivadas et. al. 2020).**

Benthic microalgae or microphytobenthos are major mediators of nutrient fluxes in the reef ecosystems (Heil et al. 2004). Nitrogen cycling in the coral reef sediments is similar to that observed in the benthic sediments and includes nitrogen fixation, ammonification, nitrification, and denitrification (Fig. 1.2). Also, corals actively take up particles and zooplankton as additional nutrients (Lewis, 1993; Houlbrèque & Ferrier-Pagès, 2009).

## 1.4. Threats to coral reefs

Coral reefs are under major threat due to the combined effects of natural and anthropogenic stressors at regional and global scales, primarily from global climate change, increasing levels of $CO_2$, unsustainable fishing practices, coral diseases, and land-based pollution (Bellwood et al. 2004; Gardner et al. 2003; Wilkinson, 2008; Burke et al. 2011; Fig. 1.3). Over the past four decades, there has been an estimated 80 % loss of the coral coverage in the Caribbean (Gardner et al. 2003) and 50 % loss in the Indo-Pacific (Bruno & Selig, 2007). According to the report, Reefs at risk revisited (Burke et al. 2011), 75 % of the global reefs are currently at risk due to global and local stressors and 90% would be lost by 2030. The major coral threats including natural and anthropogenic stressors like thermal stress-induced coral bleaching, ocean acidification, coral diseases, unsustainable tourism, acting at both global and regional levels are discussed here.

**Fig. 1.3: Global and local threats to coral reefs.**

### 1.4.1. Thermal stress and coral bleaching

The year 2015-17 witnessed a global rise of more than 1°C average temperature above the mean pre-industrialisation period's conditions i.e., 1800-1900s (NOAA, 2018; Fig. 1.4). It is reported that nine out of the ten warmest years have been recorded since 2005, with the period 2014-18 as the warmest five years in record and since the 1880s, there has been an increase in global land and ocean temperature at an average rate of 0.07°C per decade (NOAA, 2018). This rise is predicted to continue with an approximately 0.4 – 1.1°C rise in global average temperature by the end of 2025 (IPCC, 2007). Kumar et al. (2016) reported the rate of sea surface temperature (SST) increase in the Arabia Sea as 0.12°C per decade for the period from 1960 to 2009.

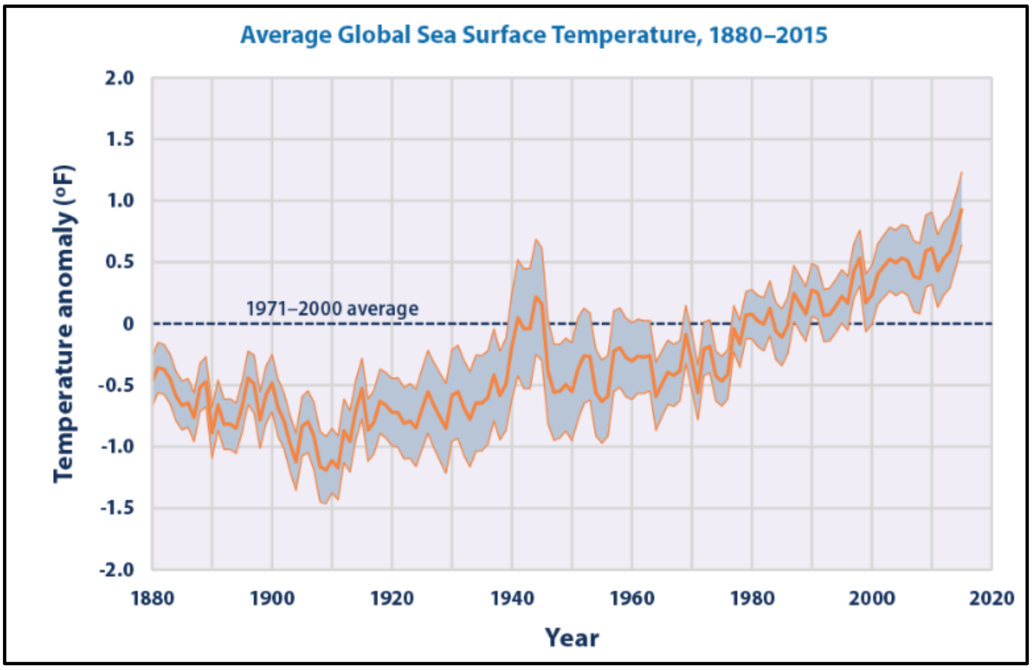


**Fig. 1.4: Average Global Sea Surface Temperature from 1880-2015 (Source: NOAA, 2016).**

The health of coral depends on the delicate relationship between the cnidarian host and the symbiotic intracellular dinoflagellate. It is understood that exposure to elevated temperatures, UV, and other stressors lead to inhibition of algal photosynthesis by disrupting the photosynthetic electron transport chain in the chloroplast, which generates toxic reactive oxygen species (ROS) and hydrogen peroxide ($H_2O_2$) by the oxygen-evolving complex (Weis, 2008; Mehler, 1951; Richter, 1990; Fournier, 2013). These oxyradicals diffuse through the membrane of the symbiont and start accumulating in the coral host's cytoplasm (Down et al. 2002). This increase in ROS is hypothesised to increase cellular damage leading to the expulsion of symbiotic algae (physiological bleaching; Fitt et al. 2001; Fig. 1.5). This release of symbiont algae (source of ROS) is believed to be an adaptive mechanism, to limit the physiological damage (Douglas, 2003; Smith et al. 2005).

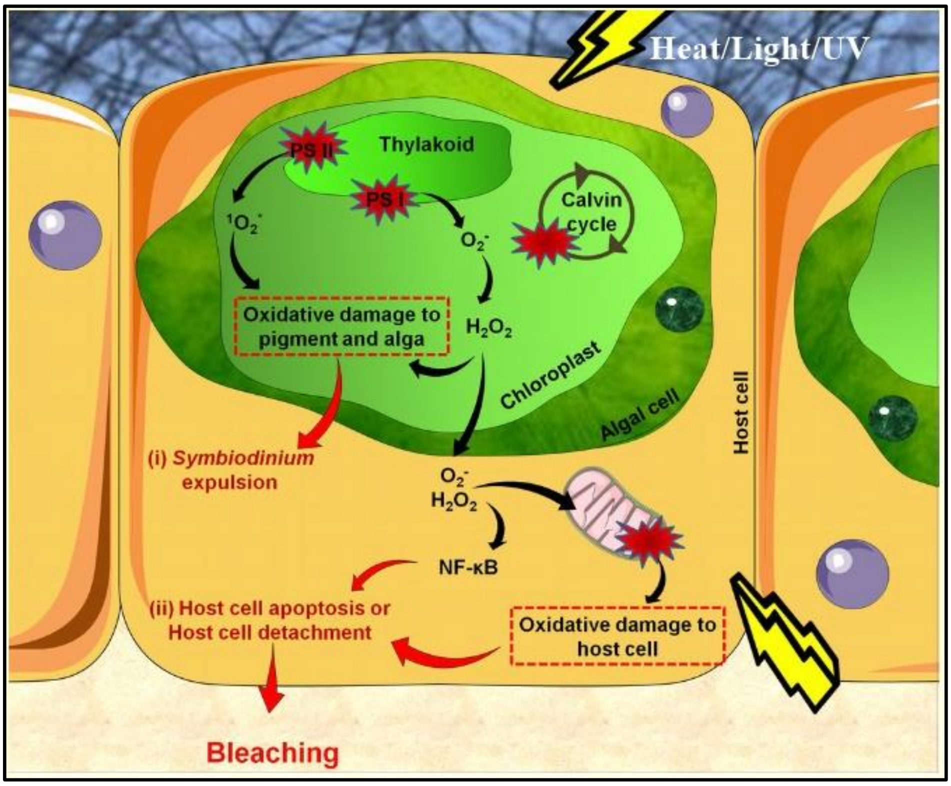


**Fig. 1.5: Steps leading to coral bleaching on exposure to stress (Source: Fournier, 2013).**

However, the cellular and molecular mechanisms leading to the expulsion of algae are poorly understood (Weis et al. 2008; Douglas, 2003). The individual role of both the partners (cnidarian host and symbiotic algal) in bleaching and immune response to environmental stress is poorly recognized. Hawkins et al. (2013) and Dun et al. (2004), state that Nitric oxide (NO) mediates coral bleaching via apoptosis-like cell death however its source (host or symbionts) and mechanism of release is still a highly researched area. Owing to the significance of corals and the looming threat of climate change, it’s highly empirical to understand these cellular events to predict how and whether the corals will adapt to climate change and perhaps provide insights to manipulate the mechanism of symbiosis collapse under changing climatic conditions.

The increase in sea surface temperature during the past few decades has caused unprecedented mass bleaching worldwide, including the three major episodes in 1998, 2010, and 2015/16 (Heron et al. 2016). The 2014–2016 global bleaching event, caused due to the combined effect of global warming and the strong El Nino of 2015–16, was the longest and most extensive on record and led to widespread bleaching and coral mortality (Eakin et al. 2016). Western India started showing early signs of coral bleaching in Oct-Dec 2014 (De et al. 2015) which reached an extensive level during 2015 (Raj et al. 2018; Hussain & Ingole, 2020). In Lakshadweep, coral bleaching began in December 2015 which worsened by April 2016. There have been reports of coral bleaching in all major reefs of India during this period, making it one of the worst bleaching episodes ever recorded.

Even though the corals can recover upon the return of normal conditions, they may undergo mortality if the corals get plagued with diseases during the bleached condition (Berkelmans & Willis, 1999; Eakin et al. 2010; Miller et al. 2009). Decline in corals after bleaching events have long-term ecological, economic, and social impacts. It reduces local fishery production (Rogers et al. 2009; Graham et al., 2006), dents the tourism industry (Pratchett et al., 2011; Mallela et al. 2010; Doshi et al. 2012) as well as leads to changes in benthic habitat (Munday et al. 2008) from coral dominance to macroalgae or sponge dominance (phase shift) (Done 1992; Norström et al. 2009; Pandolfi et al. 2003).

### 1.4.2. Perils of changing ocean chemistry and the resulting ocean acidification.

Because of the interaction between the oceans and the atmosphere, the change in SST can have drastic effects on global climate and is expected to affect the solubility of gases such as the dissolved oxygen and $CO_2$. Over the past 60 years, the annual rate of increase in $CO_2$ concentration has increased by 100 times, reaching a record high of 409.8 ppm in 2019 (Lan et al. 2020; Fig. 1.6).

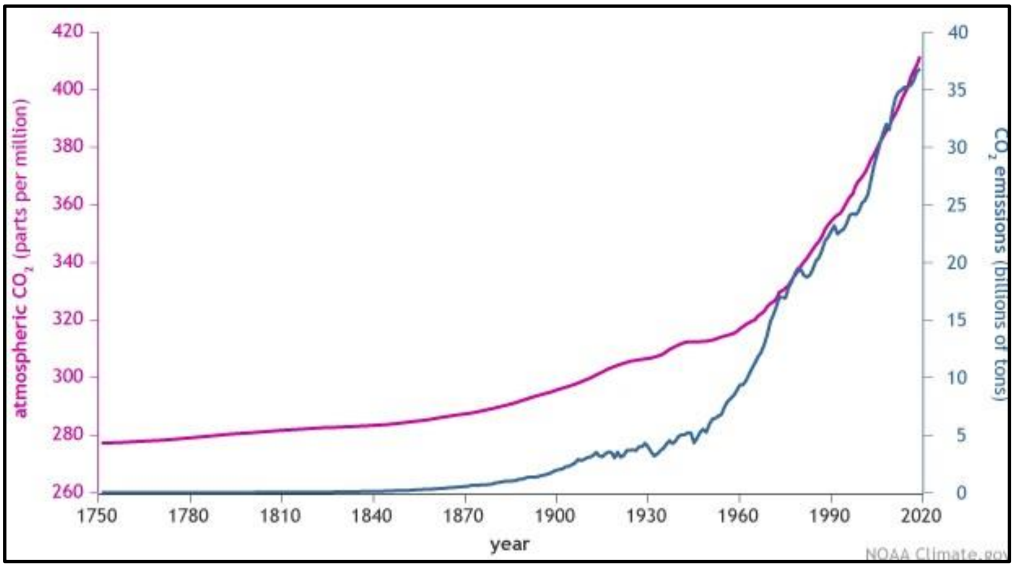


**Fig. 1.6: Increase in $CO_2$ emission and atmospheric $CO_2$ from 1750 to 2019 (Source: NOAA Climate.gov).**

In addition to global warming, the increase in anthropogenic induced $CO_2$ has reduced seawater acidity by and 0.1 pH unit (30% increase in acidity) and depleted seawater carbonate concentrations by ~30 mmol $kg^{-1}$ seawater (IPCC, 2007). Oceanic pH is one of the most important environmental factors controlling the distribution, physiology, morphology, and behaviour of marine invertebrates (García et al. 2018), especially those having calcareous shells. According to Canadell et al. (2007), approximately 25% of the anthropogenically produced $CO_2$ enters the ocean (sink), where it reacts with water to form carbonic acid. This carbonic acid then dissociates to form bicarbonate ions and protons, which further reacts with more carbonate ions to form bicarbonate ions. This, in turn, reduces the carbonate saturation in seawater and depletes the carbonate available for the calcification process in biological systems (corals, bivalves, calcifying algae, sea urchins), leading to erosion of the calcium carbonate skeleton (Fig. 1.7). Many experimental studies have indicated a change in coral physiology with changing pH and aragonite saturation levels. Kleypas et al. (1999) had investigated the change in coral growth with increasing in-situ $CO_2$ levels and observed a 40 % reduction in coral calcification and growth rate on exposure to in-situ $CO_2$ levels of 560 ppm. Fabricius et al. (2011) had observed a marked reduction in coral diversity, recruitment, and complexity of the reef on exposure to ocean acidification scenarios from pH 8.1 to 7.9. Interestingly they observed an increase in coverage of massive coral *Porites* by a factor of two, whereas the coverage of more structurally complex corals reduced threefold. On further exposure to pH less than 7.9 (>1,000 ppm $CO_2$), reef development ceased. It is predicted that by 2050-2100, atmospheric carbon dioxide concentration would exceed 500 parts per million and global temperatures would increase by 2°C (Hoegh-Guldberg et al. 2007). If no strict measures are taken to curb the increase in $CO_2$ emissions as decided in the Paris agreement in 2015 (Ourbak & Tubiana, 2017), the coral reefs with less diversity and poor carbonate accretion will be pushed towards a functional collapse before the corals and the marine organisms could even adapt (Hoegh-Guldberg et al. 2007).

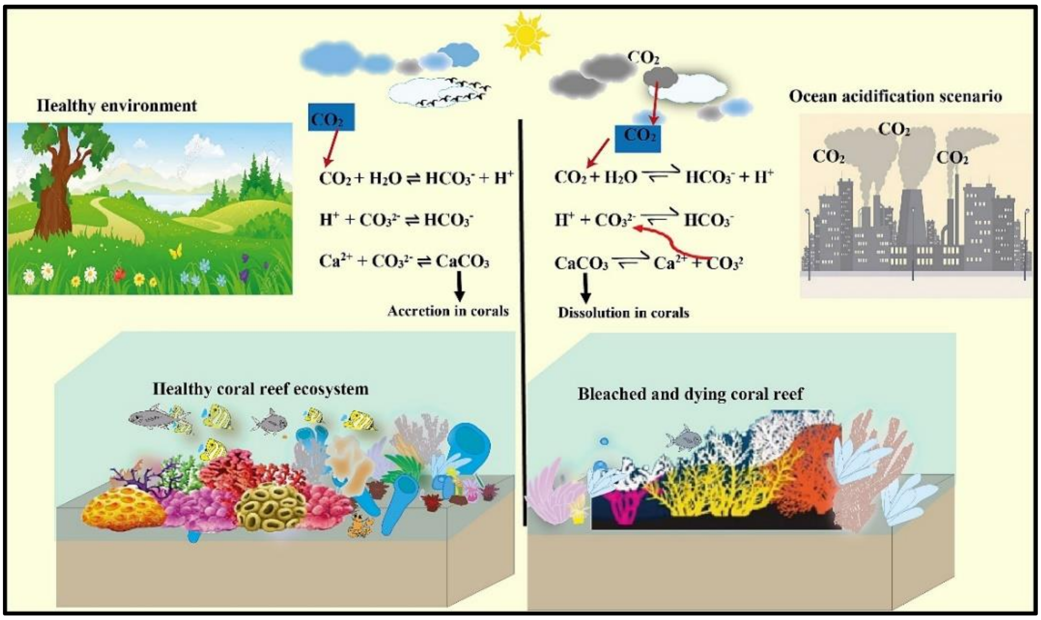


**Fig. 1.7: Reactions leading to ocean acidification and its effect on coral reefs.**

### 1.4.3. Space competitors- sponges and macroalgae

Competition for space among corals is a major process influencing the community structure of reefs by controlling their diversity and abundance pattern (Chadwick & Morrow, 2011; Lang & Chornesky, 1990). Global loss in live coral cover due to episodes of mass bleaching (Hoegh-Guldberg, 2004), disease outbreaks (Harvell et al. 1999), natural disasters like hurricanes (Rogers, 1993), etc. leads to exposed reef substratum which gets colonised by other reef organisms (e.g., sponges, macroalgae, ascidian, and corallimorphs; Chadwick & Morrow, 2011). The siliceous sponge belonging to the genus *Cliona* (Porifera, Demospongiae) are reported as aggressive bioeroders of the coral skeleton (Schönberg et al. 2017; Mote et al. 2019). The siliceous spicules of these Clionaid sponges penetrate and erode the calcium carbonate ($CaCO_3$) skeleton of corals, eventually leading to coral death and causing a phase shift towards sponge-dominated reef (Schönberg et al. 2017; Mote et al. 2019). The aggressive bio-eroding sponge, *Cliona viridis* species complex has been reported to erode the coral reef at an annual rate of greater than 10 kg per meter of sponge area (Schönberg et al. 2000; Schönberg &

Wilkinson, 2001; Achlatis et al. 2019). However, the field of sponge taxonomy in India, and deciphering the mechanism of bioerosion by sponges is still in its nascent stage (Ashok et al. 2018; Mote et al. 2019), and this information is very crucial for reef management and planning under the current scenario (both anthropogenic stress and climate change).

Benthic algae (turf and calcifying algae) play a major role in reef primary productivity and nitrogen-fixation. However, a coral-to macroalgae-dominated phase-shift may be orchestrated by the loss of herbivory, eutrophication, or a combination of both (Done, 1992; Mumby et al. 2016), which can be alleviated by global warming and ocean acidification (Hughes et al. 2010). The detrimental effects of algae on corals may be due to direct or indirect contact. Macroalgae can overgrow on the corals, thus reducing the light required for photosynthesis, harm corals by abrasion and by the release of allelochemicals in both direct and in-direct contacts (McCook et al. 2001; Rasher & Hay, 2010), and can alter the microbial community of the corals (Smith et al. 2006; Morrow et al. 2012). The effects of algae on coral are dependent on several factors like the 1) algal species, 2) size and physical form of the algae, 3) biological characteristics (vegetative or sexual mechanisms of the algae), 4) allelochemicals or secondary metabolites secreted by the algae, and 5) size of the corals (Ferrari et al. 2012; Jompa & McCook, 2003). Ferrari et al. (2012) observed an 18-22 % loss in cover occupied by small coral colonies in competition with macroalgae, whereas no effects were seen in larger colonies of corals. The heightened secondary metabolites production by macroalgae during stressful conditions like ocean warming and ocean acidification is detrimental to corals by increasing coral mortality, reducing recruitment and survival of coral larvae, and reducing the overall complexity of the reefs (Diaz-Pulido et al. 2011; Del Monaco et al. 2017; Campbell et al. 2017). Manikandan & Ravindran (2017) reported algal bloom of

*Caulerpa* spp. in Palk Bay and Gulf of Mannar, India during 2012-14 which led to a loss in live coral coverage. However, given recent bleaching events and ocean acidification, it has become empirical to understand the coral-algal mechanism of algal damage to corals and to design management strategies for algal control.

## 1.5. Nugget of hope: *Aiptasia* as an emerging coral model organism

Despite the growing research in the field of coral-symbiosis relationships, the cellular and molecular mechanisms of bleaching remain poorly understood (Davy et al. 2012; Douglas, 2003; Weis, 2008). Scleractinian corals present logistical difficulties for laboratory investigation as they are costly to maintain, grow slowly, and wild animals have heterogeneous backgrounds making them a hard subject for gene expression studies (Lehnert et al. 2012). These limitations have led to the emergence of *Aiptasia* as a model organism for coral biology studies since it also harbours symbiotic zooxanthellae (Weis et al. 2008; Lehnert et al. 2012). *Aipatsia* is extremely hardy with rapid growth (Hunter, 1984) and reproduces via both sexual and asexual methods, leading to monoclonal strains in the laboratory (Vollmer & Palumbi, 2002) and making it an ideal candidate as a coral model organism for experimental studies.

## 1.6. Reef management

With the complex network of threats, the coral reefs globally are under the looming threat of mass extinction and require an urgent control of $CO_2$ emission, scientific intervention to assist corals to thermally adapt (by assisted evolution, habitat engineering, generation of super corals) (Anthony et al. 2017), encouraging management of resilient/resistant reefs (Hock et al. 2017), and a radical change in governance practices for their conservation (Hughes et al. 2017).

# 1.7. Review of Literature and objectives

## 1.7.1. History of coral reef research in India

India has a coral reef cover of about 5790 km$^2$ (Raghuraman et al. 2013), with unique geography having the Arabian Sea on the west coast and the Bay of Bengal on the east coast. The coral reefs in India are mainly distributed in three major regions- Andaman and Nicobar Islands, mainland India, and the Lakshadweep Islands which are represented by all types of reefs like barrier, fringing, atolls, and patches. The mainland reefs include the Gulf of Kachchh (Gujarat), Gulf of Mannar, and Palk Bay (Tamil Nadu). Besides, these main reefs, scattered coral growth has been reported along with the inter-tidal belts and submerged banks both on the east and west coasts of India (Muley et al. 2002), like intertidal regions of Ratnagiri, Malvan, and Redi in the Maharashtra coast (Qasim & Wafar, 1979), Netrani Islands (Zacharia et al. 2008), Gaveshani Bank (Nair & Qasim, 1978), and submerged bank of Angria Bank located around 60-70 nautical miles west of Vijaydurg, Maharashtra (Ingole, 2017; Fig. 1.8).

The first evidence of corals in India is dated back to the 18th century by Rink (1847), during his surveys of the Nicobar Islands where he gave an account of the deep-sea corals around the Andaman Sea and studied the adverse effects of siltation on corals. This was followed by the majority of the pioneering work conducted by Sudarshan & Mukhopadhyay (1967); Pillai (1967a-f, 1971a, 1971b, 1972); Scheer & Pillai (1974); Reddiah (1977); Sheppard (1987); Pillai & Patel (1988), Pillai & Jasmine (1989) during the late half of the 20th century. Pillai published a set of six papers in 1969, on the coral account of the Gulf of Mannar, which formed the core of coral reef research in India. This was followed by comprehensive work on coral diversity and distribution in Minicoy Atoll, Lakshadweep Islands, where Pillai (1971a) reported 70 species belonging to 26 genera of hermatypic corals. Pillai further conducted extensive research and surveys in the 1970s documenting the coral diversity, distribution, and anthropogenic effects on corals of Gulf of Mannar and Palk Bay (1971b, 1972, 1973, 1975, 1977), where he documented a total of 110 species belonging to 26 genera of hermatypic corals and 7 species of ahermatypic corals. Later, Pillai published his account of the work conducted in Andaman and Nicobar Islands in a series of papers (1977, 1978, 1983), succeeding his joint work with Scheer (Scheer and Pillai, 1974). In a comprehensive study detailing the structure and coral diversity of India, Pillai (1983) reported 199 species belonging to 71 coral genera recorded from, the Gulf of Kutch (37 species, 24 genera), Palk Bay, and the Gulf of Mannar (94 species, 37 genera), Lakshadweep (78 species, 31 genera) and Andaman and Nicobar Islands (135 species, 59 genera). Out of these 199 species, 155 species (40 genera) are hermatypic, whereas 44 species (21 genera) are ahermatypic. This work forms the first comprehensive list of corals from the Gulf of Kutch. The work on the isolated coral patches in the intertidal regions of the West coast of India i.e., Ratnagiri, Malvan, and Redi was initiated by Qasim & Wafar (1979), where they reported sporadic coral colonies. Rodriques et al. (1998) reported three species of hermatypic corals, namely *Porites lutea*, *Favites pentagona*, and *Turbinaria mesenterina* for the first time from Goa. The start of the 21st century witnessed advancements in coral taxonomy by an increase in coral specimen collection led by the Zoological Survey of India (ZSI). Venkatraman et al. (2003) documented 100 new coral records from India taking the tally to 208 species. The majority of the work on corals has been done in Andaman & Nicobar Islands, highlighting the beautiful biodiversity of the islands and exploring pristine parts of the archipelago (Rajan et al. 2010; Raghuraman et al. 2010; Madhan et al. 2010; Mondal et al. 2010). Mondal et al. (2011a-f) in a series of publications in 2011 reported 44 new records across the various Islands of Andaman & Nicobar Islands.

The past two decades led to a significant increase in the knowledge of coral taxonomy, its diversity, distribution, and threat assessment. The current record of corals in India stands at 585 species of scleractinian belonging to 108 genera and 23 families with Islands of Andaman and Nicobar having the highest diversity with 523 species (95 genera), followed by 169 species (46 genera) in the Gulf of Mannar Biosphere Reserve (GoMBR), 165 species (54 genera) in Lakshadweep Islands, and Gulf of Kutch (GoK), Gujarat with 76 species (30 genera; De et al. 2020a; Fig. 1.7, Table 1.1).

Andaman and Nicobar Islands with their vicinity to the coral triangle and rich growth of fringing and barrier reefs host the highest share of unique species of scleractinian corals from India (52.6%, 298 species), followed by GoMBR harbouring 5 % ( 28 species) of unique species, succeeded by the atolls of Lakshadweep Islands hosting 1.2 % (7 species) of unique species and GoK with 0.1 % ( 1 species, *Acanthastrea simplex*) of endemic or unique species, with 6.4 % of the reported scleractinian species from India being common in all the major Indian reefs (De et al. 2020a).

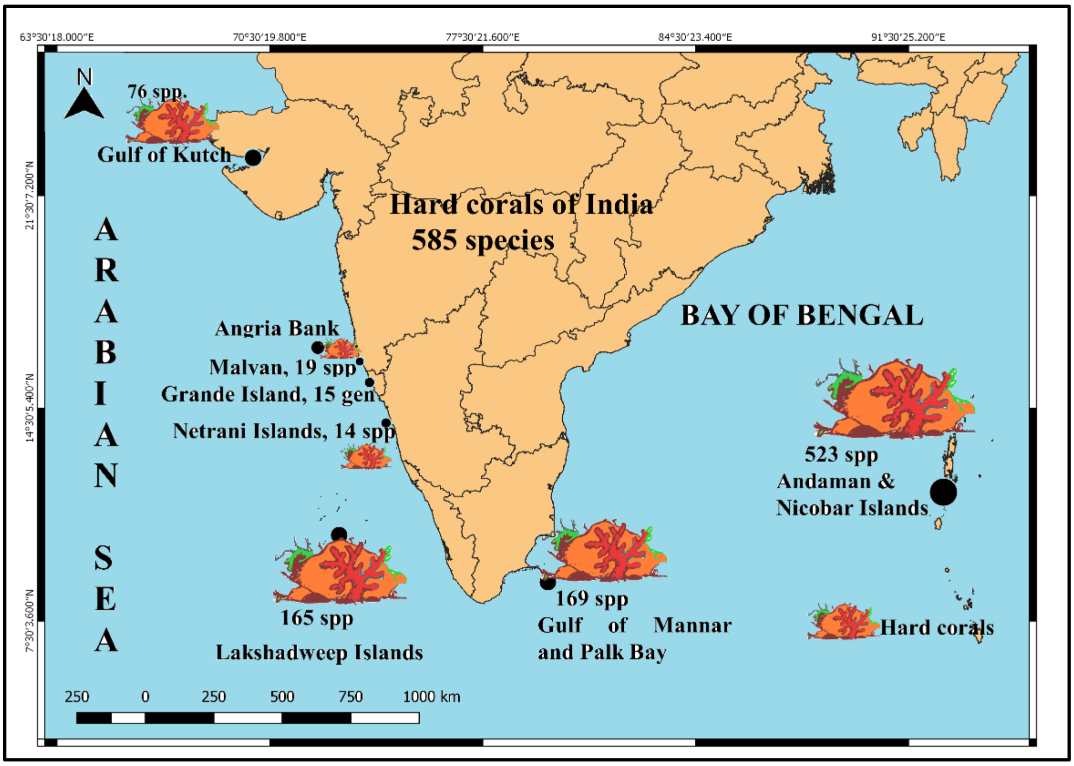


**Fig. 1.8**: **Map of India with the distribution of hard corals.**

**Table 1.1: Abundance of hard coral species from major reefs of India. *Note the data on Grande Island is before the start of this present study. A&N= Andaman and Nicobar Island; GoMBR= Gulf of Mannar Biosphere Reserve; LI=Lakshadweep Islands; GoK=Gulf of Kutch, MMS=Malvan Marine Sanctuary.**

| Location | Species | Genus | Family |
|---|---|---|---|
| **A&N** | 523 | 95 | 23 |
| **GoMBR** | 169 | 46 | 16 |
| **LI** | 165 | 54 | 17 |
| **GoK** | 76 | 30 | 11 |
| **MMS** | 19 | 14 | 8 |
| **Netrani** | 14 | 11 | 7 |
| **Grande Island*** | - | 15 | 11 |
| **Grande Island (Present study)** | 26 | 15 | 10 |

## 1.7.2. Patchy reefs along the central west coast of India

The central west coast of India has scattered coral growth in the intertidal and subtidal regions. The discovery of a submerged coral bank named Gaveshani by the team of CSIR-NIO (Nair & Qasim, 1978), located 100 km west from Mangalore, during the 18th expedition of *RV Gaveshani* was the stepping stone of coral exploration along the central west coast of India. Dredging over the Gaveshani Bank yielded living corals belonging to 5 species namely *Euphyllia fimbriata*, *Leptoseris papyracea*, *Stylocoeniella armata*, *Porites lichen*, and *Cyphastrea serialia*, along with their associated fauna like calcareous algae, serpulids, mollusca, and a specimen of fish (Nair & Qasim, 1978). Qasim & Wafer (1979) further reported coral patches from the intertidal region of Ratnagiri and Malvan. The Grande Island archipelago comprising St. George and Grande Islands located in Goa is a tourism hotspot, yet a relatively understudied coral patch ecosystem. To date, there are only a few published reports on corals, where Rodriques et al. (1998) reported 3 species of scleractinian corals namely, *Porites lutea, Favites pentagona,* and *Turbinaria* *mesenterina*, followed by a recent report on 15 coral genera of hard corals by Manikandan et al. (2016). Patro et al. (2015) reported the prevalence of invasive species of snowflake coral- *Carijoa riisei* from the shipwreck site of Grande Island. Sreekanth et al. (2015) conducted extensive work on fish diversity in the coral reef ecosystem of Grande Island and reported 85 species of fish from the patch reef. Singarayan & Rethnaraj (2016) reported 3 species of azooxanthellate scleractinian corals, namely, *Balanophyllia cumingii, Dentrophyllia indica,* and *Paracyathus profundus* from Grande Island, Goa. The other patchy reef in the Malvan Marine Sanctuary (MMA) located in Maharashtra is the only protected reef along the central west coast of India. The patch reef, located in the core area of the MMS's MPA harbours 74 species of fishes, 73 species of seaweeds, and 12 coral species, as well as 181 other associated flora and fauna species (Parulekar, 1981). This was updated recently by De (2020) with 19 species of hard corals. These patchy reefs along the Central West coast of India provide a natural habitat for numerous benthic organisms and act as breeding, feeding, and shelter ground to a rich diversity of fish species (Sreekanth et al. 2015).

Even though these coral patches are highly important for the local economy, tourism, and fisheries, the management strategies and conservation need a stricter implementation. Information on coral diversity and associated fauna are increasing with more frequent field surveys. These corals are under stress from local and global impacts like high sedimentation rate, thermal stress, coral diseases, unregulated tourism, coral eroding sponge infestation (De et al. 2017; De et al. 2020b; Mote et al. 2019, 2021). However, information is scarce regarding the elements of ecosystem functioning, the impact of local threats, and the associated fauna along with the distribution of coral in these patches. Therefore, there is an urgent need to bridge the gap in the information on the biodiversity of these sites to appreciate their true value, along with the health status of these reefs. The information provided could play a crucial role in designing management strategies and conservation plans for this highly important and vulnerable coral reef ecosystem along the central west coast of India.

With the pressing need for this valuable information regarding the marginal understudied reefs in times of global climate change and increasing anthropogenic pressure, the objectives of the present study are threefold: -

1. To document the diversity of corals.
2. To document the distribution and extent of coral patches in Goa and Malvan.
3. To assess the impact of anthropogenic activities on coral-associated species.

# Chapter 2

## Coral diversity and distribution

### 2.1. Study area

Study area consists of coral patch communities in Grande Island, Goa and Malvan Marine Sanctuary, Maharashtra, along the Central West coast of India (Fig. 2.1). The study area is a highly understudied reef ecosystem with rich bio-ecological and economic significance.

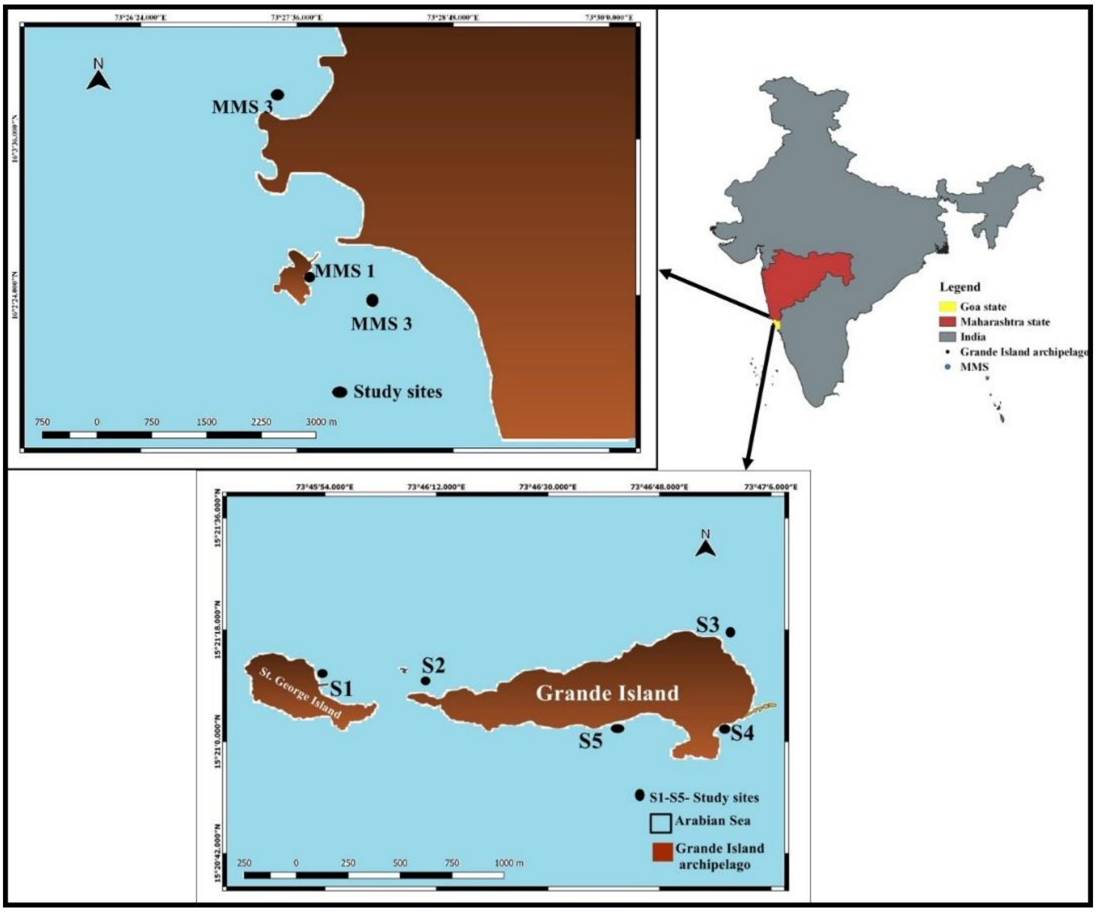


**Fig. 2.1: Map showing study sites in Malvan and Goa.**

### 2.1.1. Grande Island archipelago, Goa

#### Geography, ecology, and economic importance

Grande archipelago (15.352°N, 73.773°E) located some 18 km from the coast of Goa (near Marmagoa port) in the Konkan coast of India. The archipelago encompasses biological and ecological sensitive patchy coral reef ecosystem in the Eastern Arabian Sea (Chary et al. 2020; Sreekanth et al. 2015; Hussain & Ingole, 2020). The Island is surrounded by the inflowing water from Mandovi and Zuari rivers (Chary et al. 2020). The archipelago consists of twin islands namely, a smaller Sao Jorge Island, and the larger, Ilha Grande or Grande Island (Hussain & Ingole, 2020), mainly composed of granite and laterite rocks. The rocky wall of the island extends into the sea, forming submerged rocks encircling the island and serve as a substratum for coral growth. The island's southern shore has an old shipwreck (Suzy's Wreck), which has formed an artificial reef extending up to 14m deep (Sreekanth et al. 2019) and is a popular dive spot.

Coral growth has been observed till the depth of 14 m; however, the shallower sites (6-8) harbour a higher abundance and diversity of corals (Manikandan et al. 2016; Hussain & Ingole 2020; Chary et al. 2020). The patchy coral growth with small expanses of the fringing reef, interspersed with rocky outgrowths and sandy bottom is a highly understudied reef ecosystem with only a few published studies on corals and fish (Hussain & Ingole, 2020). Rodriques et al. (1998) conducted the first study on coral growth and reported three species of scleractinian corals namely, *Porites lutea*, *Favites pentagona*, and *Turbinaria mesenterina*. This was followed by a recent study by Manikandan et al. (2016) who recorded 15 genera of hard corals. At the same time, Patro et al. (2015) reported the presence of invasive species of snowflake coral, *Carijoa riisei* from the shipwreck site of Grande Island. Sreekanth et al. (2015) conducted extensive work on fish diversity in the coral reef ecosystem of Grande Island and reported 85 species of fish from the patch reef. Singarayan & Rethnaraj (2016) reported three species of azooxanthellate scleractinian corals, namely, *Balanophyllia cumingii*, *Dentrophyllia indica*, and *Paracyathus profundus* from Grande Island, Goa.

Tourism is the main source of economy for the coastal state of Goa, owing to the presence of historical architecture, rich history, exquisite cuisine, tropical beaches, and rich marine life (Ministry of Tourism report, Goa). The last decade saw an exponential increase in tourist inflow to Goa rising from 3 million in 2012 to 9 million in 2019 (Fig 2.2) (Dept. of Tourism, Govt. of Goa). Marine ecotourism was initiated in Goa way back in the 1980s, with some local anglers ferrying tourists from Dona Pula Jetty on boats with dolphin watch and Grande Island trips (WWF, 2019).

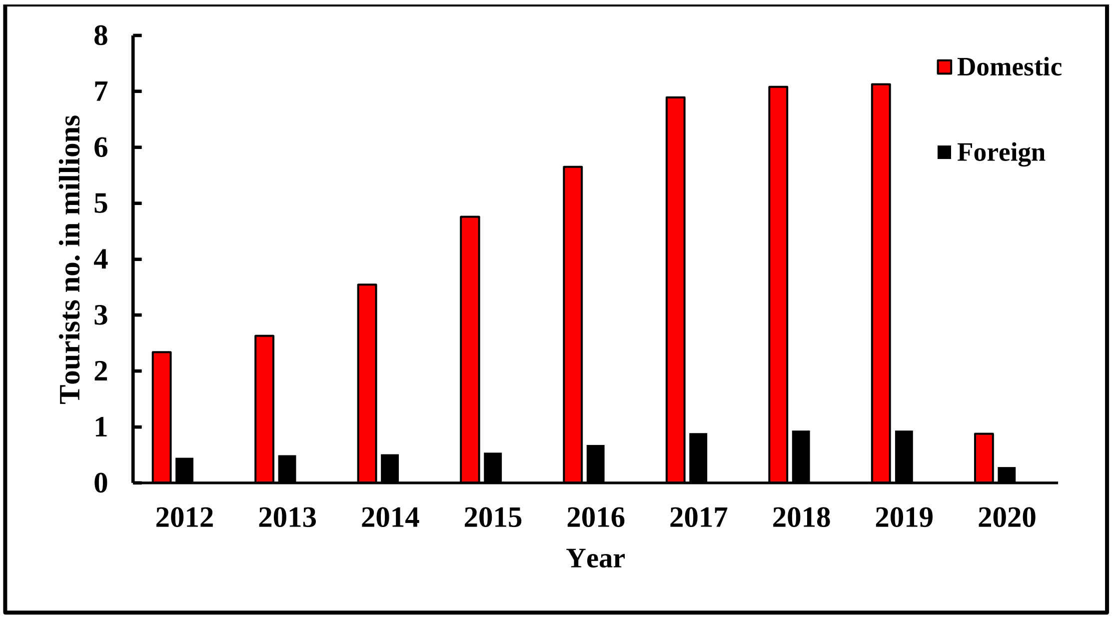


**Fig. 2.2: Domestic and foreign national tourists' inflow in Goa from 2012 to 2020 (till August) (Source: https://www.goatourism.gov.in/tourist-arrival-statistics/).* 2020 saw a fall due to COVID pandemic.**

Over the years, with the increase in global tourism trends, the ecotourism industry in Grande Island witnessed a significant rise owing to the popularity of SCUBA diving and snorkelling activities (WWF, 2019). With more than 16 diving spots including shipwrecks, fringing reefs, and safe spots with a smooth sandy bottom, Grande Island has become a hub of dive certification training (SSI and PADI) in mainland India, operated by more than a dozen of dive schools in Goa. It is estimated that during the peak season (November-March), more than 700 boats operate bringing more than 3000 dive tourists annually to Grand Island, boosting the local economy (WWF, 2019).

## Climatic conditions in Goa

Being a tropical coastal state, Goa experiences a hot and humid climate with heavy rainfall during southwest monsoon (June-September). The maximum temperature reaches 33° C during summers and minimum temperature averages around 25° C during the winter months (December-February; IMD, Goa; Fig. 2.3).

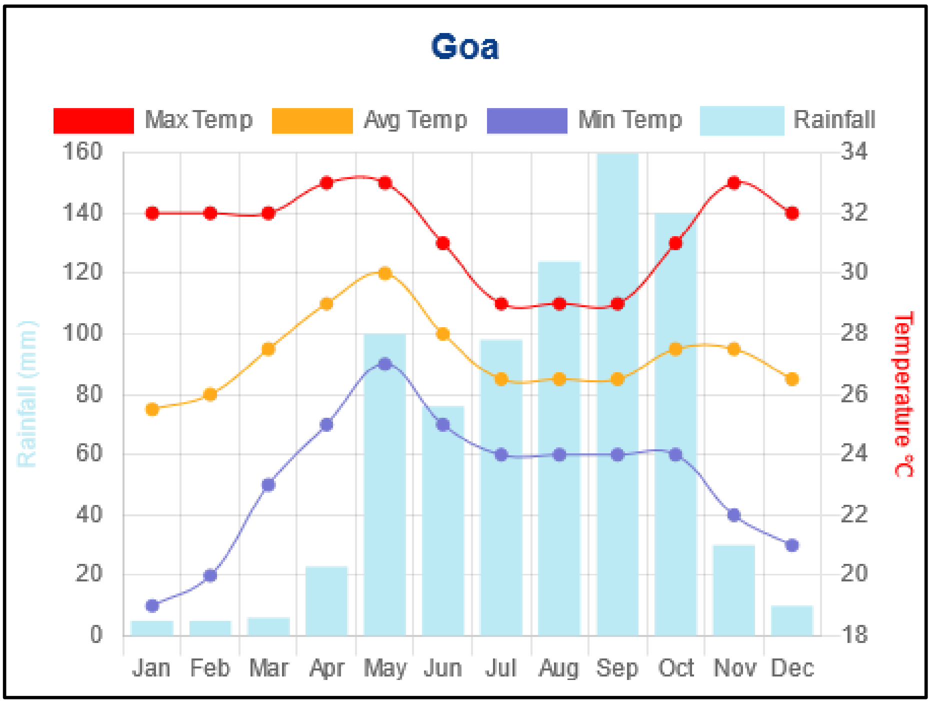


**Fig. 2.3: Monthly variation in temperature and rainfall in Goa (Data source: IMD, Goa).**

## 2.1.2. Malvan Marine Sanctuary

### Geography, ecology, and economic importance

Malvan Marine Sanctuary (MMS), a marine biodiversity hotspot is located in the Malvan Taluka of Sindhudurg district of the western state of Maharashtra from 16° 00’ 00” N to 16° 05’ 00” N latitudes and 73° 25’ 00” E to 73° 30’ 00” E longitudes (ICMAM, 2001). Owing to the rich marine biodiversity, it was declared a marine sanctuary on 13th April 1987, under the Wildlife (Protection) Act (1972), with a total area of 29.12 $km^2$ of Malvan coastal waters (core zone - 3.182 $km^2$ and buffer zone -25.94 $km^2$; ICMAM, 2001). MMS is situated along the coast of Malvan city which encompasses the famous 'Sindhudurg Fort' built by emperor Chhatrapati Shivaji Maharaj in the 16th century (ICMAM, 2001). The fort gives the MMS a historical significance and adds to the tourism industry in the Sanctuary. Malvan coast is part of the Sahyadri range of the Western Ghats and extends to meet the Arabian Sea.

MMS is rich in various marine habitats such as coral reefs, mangroves, sandy beaches, seaweed bed, and, rocky and intertidal belts (SAC, 2012). The rocky coastline is comprised mainly of sedimentary rocks, which are soft and prone to wind and wave erosion (Neenu, 2020). Erosion leads to the formation of crevices and cracks in the rocks which serve as shelter, feeding and breeding ground of a rich diversity of marine fauna (Neenu, 2020). Malvan town is bound by three creeks, namely, Karli, Kolamb, and Kalavli. Malvan coast consists of granites and gneiss with a few gneissic interruptions (SAC, 2012). Towards the North-East, the buffer zone of the MMS includes an area of 50 m from the shore near the Malvan port, whereas, in the south, the buffer zone extends up to Mandal Rock (Neenu, 2020). In the west, the buffer zone includes the area up to Malvan rock, and the core zone encompasses the area including Sindhudurg Fort, Padmagad Island and a stretch of submerged and exposed rocks (Neenu, 2020). This rocky stretch is home to shallow patchy coral reefs in the MSS, which are less diverse and severely damaged (Hussain et al. 2016;

De et al. 2015; De, 2020). The marine sanctuary also encompasses popular sandy beaches such as Tarkarli, Wayari, Chivala, Chowpatty Dandi and, Malvan (Neenu, 2020). The rocky patches and corals play a role in combatting the tidal force along the Malvan coast. (ICMAM, 2001).

The patch reef, located in the core area of the MMS's MPA harbours 74 species of fishes, 73 species of seaweeds, and 12 coral species, as well as 181 other associated flora and fauna species (Parulekar, 1981). This was updated by De (2020) with addition of 18 species of hard corals. Other notable fauna includes cetacean like the blue whale rays, sperm whale, Indo- Pacific finless porpoise, Indian Ocean humpback dolphins, spinner dolphin, Bryde's whale, and seven species of sea snakes (UNDP, 2011; Jog et al. 2015; Patil et al. 2016; Rao & Muralidharan, 2019).

The marine sanctuary contributes significantly to the local economy owing to it being a major fish-landing centre (Munjal, 2019), and as a popular ecotourism destination. Traditional forms of fishing like line, hooks, gillnets trawl nets, and Rampan are used by the fishermen community (UNDP, 2011). Cultivation of crops like mango, coconut, kokum, cashews, Areca nut, and other spices adds to the income. According to the survey by UNDP in 2010, Malvan has a total of 2897 fishers, and 5739 people dependent on fishing-related jobs (UNDP, 2011). Besides marine fishery, eco-tourism is the fastest growing sector in the region. The historical Sindhudurg fort and the Maratha Museum are a big tourist draw in the region. Over the past two last decades, there has been an exponential rise in the number of tourists visiting Malvan owing to the fort, SCUBA and other water sports activities around the fort (Port authority, Malvan) (Fig. 2.4). According to the UNDP report (2011), estimated annual revenue from tourism in Malvan is around $2.5 million sustaining more than 3000 people.

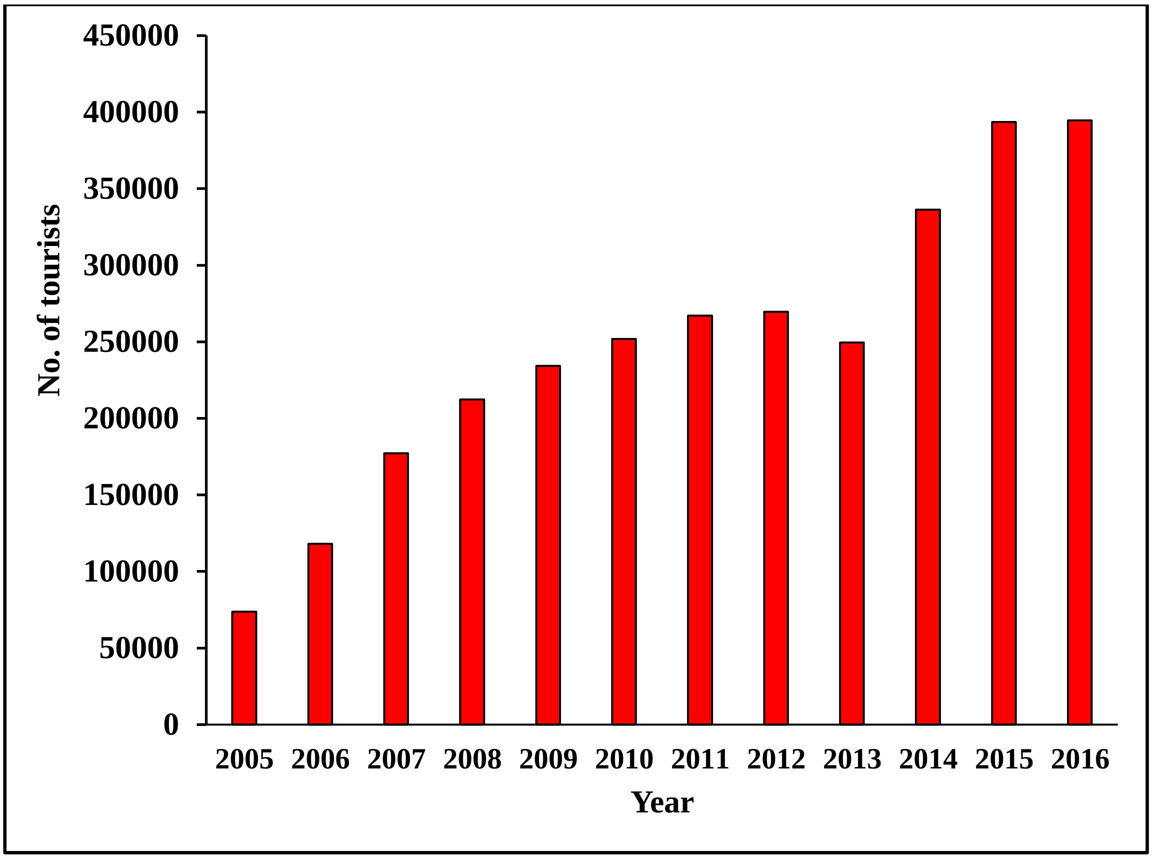


**Fig. 2.4: Increase in number of tourists taking the ferry the boat from Malvan jetty to visit Sindhudurg fort and for water sports activities from 2005-2016 (Data: Port authority, Malvan).**

## Climatic conditions in Malvan

Being a coastal area, Malvan doesn't experience large diurnal or seasonal variation in the temperature and can be generally classified as warm and humid (Munjal, 2019).

April is the hottest month where the maximum temperature touches 33. 8°C, whereas December is the coolest month with a minimum temperature around 16.3°C (Munjal, 2019). The relative humidity is very high (86 to 90%) during the southwest monsoon (June-September) and stays around 57% during winter and summer monsoons (Neenu, 2020).

# 2.2. Diversity of corals and coral distribution mapping

## 2.2.1. Materials and methods

### 2.2.1.1. Study sites

In the present study, five study sites were selected all around Grande Island, archipelago and 2 study sites in MMS to assess the diversity of corals in the study area (Fig. 2.1, Table 2.1). The sites in Grande Island were separated by a distance of ~150 m from each other to cover the entire archipelago.

**Table 2.1: Location, depth and bottom characteristics of study sites in Goa and Malvan. Site S1-S5 represent study sites in Grande Island, whereas, sites. MMS 1-3 denote sites in Malvan Marine Sanctuary (MMS).**

| **Study Sites** | | **Latitude** | **Longitude** | **Depth (m)** | **Type of Substratum** |
|---|---|---|---|---|---|
| **Grande Island, Goa** | S1 | 15°21'9.84"N | 73°45'54.40"E | 2-4 | Rocky |
| | S2 | 15°21'14.29" N | 73°45'58.21"E | 6-8 | Rocky &sandy |
| | S3 | 15°21'7.72"N | 73°47'4.47"E | 3-5 | Sandy |
| | S4 | 15°21'0.59"N | 73°47'1.73"E | 5-7 | Rocky & sandy |
| | S5 | 15°20'58.63" N | 73°46'24.59"E | 8-10 | Rocky |
| **MMS, Malvan** | MMS1 | 16° 2'30.67"N | 73°27'41.37"E | 2-3 | Rocky |
| | MMS2 | 16°3'54.58" N | 73°27'26.09"E | 5-7 | Rocky & sandy |
| | MMS3 | 16º 02'24.3"N | 73º28'13.8"E | 8-10 | Rocky & sandy |

In Malvan, the sites were selected along a distance gradient of 500-1000 m, two sites covering the severely anthropogenically disturbed area around the Sindhudurg fort, and another far off near the Rock Garden. All the sites were primarily composed of rocky substratum, followed by dead coral, rubble, and absence of branching *Acropora* corals due to high wave exposure (Qasim & Wafar, 1979).

### 2.2.1.2. Underwater survey

Monthly underwater surveys were conducted in the study sites from 2015 to 2018 during the diving season (November-March). A 10 m long line transect (English et al., 1997) and 20 X 2 m (belt transect), was laid in triplicate, perpendicular to the shore, separated by a distance of ~50 m using Self Contained Underwater Breathing Apparatus (SCUBA) diving to assess coral diversity and the benthic community structure for habitat mapping, respectively. All the coral colonies and substratum (rock, rubble, sand, macroalgae, turf algae, invertebrates) falling within the belt transects were enumerated, density calculated, and digital photographs and videography were done using a Nikon AW130 (14 Mega Pixels) and a GoPro Hero4 (12 Mega Pixels) camera. Coral colonies were identified to lowest identifiable taxonomic level according to Veron (2000) and Venkataraman et al., (2003), and standardized using the World Register of Marine Species (WoRMS Editorial Board 2017) and corals of the world database (http://www.coralsoftheworld.org). Locations were mapped with a Garmin GPSMAP 78S and the distances between the sites were determined. Habitat was marked using the tool GPS mapping in Nikon AW130 and maps created using the software tools of ArcGIS and QGIS using benthic habitat information.

### 2.2.1.3. Statistical analyses

Cluster analysis and nMDS ordination based on Bray-Curtis similarity on the basis of data in habitat composition obtained from transects were conducted in Primer (v6) to check the similarity between the sites based on a various substratum percentage (coral, macroalgae, turf algae, crustose coralline algae) sponge, rock, rubble, and sand).

## 2.2.2. Results and discussion

### 2.2.2.1. Diversity of corals

A total of 26 species of hermatypic corals belonging to 10 families, and 15 genera, as well as 4 species of ahermatypic corals belonging to 4 families were identified from the study area (Grande Island and MMS) during the present study (Table 2.2; Fig. 2.5-2.7). Grande island had a higher diversity of hermatypic corals with 25 species, compared to the 15 coral species recorded from MMS. Following is the description of the coral species recorded in the study area.

**Phylum CNIDARIA** Hatschek, 1888

**Class ANTHOZOA** Ehrenberg, 1834

**Subclass HEXACORALLIA** Haeckel, 1896

**Order SCLERACTINIA** Bourne, 1900

**Family ASTROCOENIIDAE** Koby, 1890

**Genus** *Stylocoeniella* Yabe & Sugiyama, 1935

- **Species** *Stylocoeniella guentheri* (Bassett-Smith, 1890)

**Common name:** Thorn coral

**Description:** Small encrusting colonies with widely spaced corallites. The two cycles of 6 septa each are of unequal size.

**Colour:** The colour varies from pale to dark greenish-brown, mostly with white polyps.

**Habitat:** Usually found in shallow, turbid reefs.

**Distribution**: Found in coral reefs across the Indo-Pacific.

**Occurrence:** Uncommon

**IUCN Red List of Threatened species status:** Least concern

## Family COSCINARAEIDAE

Benzoni, Arrigoni, Stefani & Stolarski, 2012

**Genus** *Coscinaraea* Milne Edwards & Haime, 1848

- **Species** *Coscinaraea monile (Forskål, 1775)*

**Description:** Colonies range from foliaceous to encrusting to dome shaped. Calices are usually monocentric, up to 7 millimetres diameter, or meandroid. Even septa with thick walls. Septa are thick, with fine serrations.

**Colour:** Usually greenish, tan to pale brown.

**Habitat:** Diverse, covering most of the reef environments.

**Distribution**: Common in western Indian Ocean.

**Occurrence**: Common.

**IUCN Red List of Threatened species status:** Least concern

## **Family DENDROPHYLLIIDAE** Gray, 1847

**Genus** Turbinaria Oken, 1815

- **Species** *Turbinaria mesenterina* (Lamarck, 1816)

**Common name:** Disc coral

**Description:** Colonies are composed of highly convoluted unifacial lamellae, arranged in whorls. Convolutions varies with depth and sunlight availability, and the colonies are more upright tor tiered on upper reef slopes, whereas they become more horizontal in deeper waters. Corallites are tightly spaced crowded, slightly exsert, with vase shaped, tubular or protruding shape, and average 2.5 millimetres in diameter.

**Colour:** Colour is usually is greyish-brown or greyish-green white polyps.

**Habitat:** Most dominant in shallow turbid reefs.

**Distribution:** Recorded from Indo-Pacific reefs. Reported from all major reefs of India.

**Occurrence**: Common

**Remarks**: *T. mesenterina* is a dominant species in my study area, forming monospecific areas of whorl like *T.* mesenterina.

**IUCN Red List of Threatened species status:** Vulnerable

- **Species** *Turbinaria frondens* (Dana, 1846)

**Common name / Synonym:** Cup coral, *Gemmipora frondens* Dana, 1846 (original combination, basionym).

**Description:** The species has large unifacial 'fronds', arranged in the shape of a vase. Small cup-shaped colonies develop into large colonies with unifacial fronds and may become contorted. Corallites on the upper surface (dorsal side) range from immersed to long and tubular, whereas, they are absent on the lower surface (ventral side). Corallites are thicker than those of *T. mesenterina*, averaging 3.5 mm.

**Colour:** Colour is usually uniform greenish-brown.

**Habitat:** Usually found in shallow and rocky forereefs.

**Distribution:** Found in coral reefs across the Indo-Pacific.

**Remarks:** The species was found in rocky reef sites arranged as convoluted whorls and was less prevalent than *T. mesenterina***.**

**Genus** *Tubastraea* Lesson, 1830

- **Species** *Tubastraea coccinea* Lesson, 1830

**Common name:** Orange cup coral

**Description:** *T. coccinea* species is an invasive, ahermatypic (non-reef building) coral. Identifying feature is the orange-coloured tentacles that are extended during night.

**Colour:** Bright orange coloured.

**Habitat:** Inhabits shaded, protected areas such as shipwrecks and can be found in cold deep waters also (Global Invasive Species Database, 2021)

**Distribution:** It has been reported from all the continents except Antarctica. (Global Invasive Species Database, 2021).

**Remarks:** Introduced species, known to compete and affect the native invertebrate fauna.

### **Family FUNGIIDAE** Dana, 1846

**Genus** *Podabacia* Milne Edwards & Haime, 1849

- **Species** *Podabacia crustacea* (Pallas, 1766)

**Common name:** Bracket coral

**Description:** Colonies are attached, laminar or encrusting, unifacial up to 1.5 m across or maybe tiered. A distinguishable central corallite is the identifying feature.

**Colour:** Septo-costae cream coloured; colonies are tan to dark brown.

**Habitat:** Present in most of the reef habitats.

**Distribution:** Indo-Pacific and South Pacific distribution.

**Occurrence:** Uncommon

**Remarks:** Low abundance in the study area, with just a few isolated colonies.

**IUCN Red List of Threatened species status:** Least concern

### **Family MERULINIDAE** Verrill, 1865

**Genus** *Cyphastrea* Milne Edwards & Haime, 1848

- **Species** *Cyphastrea serailia* (Forskål, 1775)

**Common name:** Lesser-knob coral

**Description:** Colonies range from massive or encrusting to columnar, and may have a smooth or hillocky surface. A set of 12 primary septa with similar sized rounded corallites. Distinguished from other similar species by not having alternating costae.

**Colour:** Colour varies from grey to brown to cream.

**Habitat:** Present in all reef habitats.

**Distribution:** Widely present in Indo- Pacific and South Pacific reefs.

**Occurrence:** Common

**Remarks:** Low abundance in the study sites.

**IUCN Red List of Threatened species status:** Least concern.

**Genus** *Dipsastraea* de Blainville, 1830

- **Species** *Dipsastraea lizardensis* (Veron, Pichon & Wijsman-Best, 1977)

**Common name:** Knob coral, synonym is *Favia lizardensis*.

**Description:** Massive, encrusting round colonies with conical corallites. Widley spaced septa, which are irregular. Poorly developed paliform lobes.

**Colour:** Colour varies from mottled grey, green to dark brown with pale calices.

**Habitat:** Reef back margins.

**Distribution:** Widely distributed from western Indian Ocean to Indo-Pacific.

**Abundance:** Common

**Remarks:** The species was more abundant in rocky sites as compared to sandy sites in the present study.

**IUCN Red List of Threatened species status:** Near threatened

- **Species** *Dipsastraea matthaii* (Vaughan, 1918)

**Common name/ Synonym:** *Favia matthaii* Vaughan, 1918 (original combination, basionym).

**Description:** Encrusting, massive colonies. The species has closely packed circular corallites. Septa are thick, ragged wit teeth and well developed paliform lobes forming a crown.

**Colour:** Ranges from light brown to tan or grey.

**Habitat:** Shallow reefs.

**Distribution:** Found throughout Indo-Pacific reefs up to Coral Sea.

**Abundance:** Common

**Remarks:** Low abundance found in reef sites in Grande Island.

**IUCN Red List of Threatened species status:** Critically endangered.

- **Species** *Dipsastraea veroni* (Moll & Best, 1984)

  **Common name/ Synonym:** *Favia veroni* Moll & Best, 1984 (original combination, basionym).

  **Description:** Encrusting, sub-massive, oval colonies. Corallites are monocentric and placoid with poorly developed paliform lobes. Septa has sharp saw-like serrations.

  **Colour:** Colour varies from brown to greenish - tan.

  **Habitat:** Reef back margins.

  **Distribution:** Widely distributed from the western Indian Ocean to Indo-Pacific.

  **Abundance:** Common

  **Remarks:** The species was more abundant in rocky sites as compared to sandy sites in the present study.

  **IUCN Red List of Threatened species status:** Near Threatened.

**Genus** *Favites* Link, 1807

- **Species** *Favites flexuosa* (Dana, 1846)

**Common name / Synonym:** *Astraea flexuosa* Dana, 1846 ((original combination, basionym).

**Description:** Sub-massive colonies, which are hemispherical shaped. Corallites with shared margin are deep and angular. Prominent septa with conspicuous teeth. Paliform lobes are weakly developed.

**Colour:** Varies from brown to green to tan. Oral disc and wall have contrasting colours.

**Habitat:** Present in wide range of reef environments and rocky foreshores.

**Distribution:** Common in Indo-Pacific reefs.

**Abundance:** Common

**Remarks:** Common in rocky shallow reef sites in the present study area.

**IUCN Red List of Threatened species status:** Near Threatened

- **Species** *Favites halicora* (Ehrenberg, 1834)

**Common name:** Larger star coral

**Description:** Massive, encrusting colonies with irregular or hillocky surface. Corallites have thick walls that tend to become subplacoid. Paliform lobes may be visible.

**Colour:** Varies from pale yellow to greenish-brown.

**Habitat:** Present in shallow reef environments.

**Distribution:** Present thought Indo-Pacific from Red Sea in west India Ocean to Kiribati islands in Pacific Ocean.

**Occurrence:** Uncommon.

**Remarks:** Present in rocky shallow reef sites in the present study area.

**IUCN Red List of Threatened species status:** Near Threatened

- **Species** *Favites pentagona* (Esper, 1795).

  **Common name/ synonym:** *Madrepora pentagona* Esper, 1795 (original combination, basionym).

  **Description:** Colonies are sub-massive to encrusting, may form irregular columns. Corallites are angular with thin walls. Paliform lobes are developed and visible.

  **Colour**: Living colonies are brightly coloured, usually brown or red with a very distinct green coloured oral disc.

  **Habitat:** Mostly found in shallow reef environments.

  **Distribution:** Widely distributed in the Indo-Pacific reefs up to the coral sea.

  **Occurrence:** Common

  **Remarks:** Brown coloured sub-massive species was common in most of the reef sites in the present study area.

  **IUCN Red List of Threatened species status:** Least concern

- **Species** *Favites complanata* (Ehrenberg, 1834)

  **Description:** Massive colonies with slightly angular corallites. Thick-walled rounded corallites. Large columella and weakly developed paliform lobes.

  **Colour:** Living colonies are usually brown with green oral discs.

  **Habitat:** Most of the reef environments.

  **Distribution:** Distributed throughout the Indo-Pacific.

  **Occurrence:** Common at times

  **Remarks:** Common in all reef sites in the study area (Grande Island and MMS).

  **IUCN Red List of Threatened species status:** Near Threatened

- **Species** *Favites spinosa* (Klunzinger, 1879)

**Description:** Small, rounded, encrusting colonies with deeply seated and angular corallites. Widely spaced, straight septa that are usually arranged in two alternating orders. Poorly developed paliform lobes. Distinguishing features is the prominent teeth with ragged margins in the septa.

**Colour**: Contrasting colour of wall (off-white) and centre (dark).

**Habitat**: Found in wide array of reef environments.

**Distribution:** Indo-Pacific reefs from Red Sea to Kiribati islands in Pacific Ocean.

**Occurrence:** Uncommon

**Remarks:** Low abundance in rocky reef sites in the present study.

**IUCN Red List of Threatened species status:** Vulnerable

**Genus** *Goniastrea* Milne Edwards & Haime, 1848

- **Species** *Goniastrea retiformis* (Lamarck, 1816)

**Common name/ synonym:** *Astraea retiformis* Lamarck, 1816 (original combination, basionym)

**Description:** The colonies range from sub massive to flat or columnar in shape. Corallites range from four to six sides with alternating long and short septa. Well-developed paliform lobes.

**Colour:** Colour ranges from uniform cream to pale brown, or greenish brown.

**Habitat:** Usually a common species in intertidal habitats.

**Distribution:** Widely distributed in Indo-Pacific from western Indian Ocean to Pacific reefs.

**Occurrence:** Common

**Remarks:** Dominant sub-massive species in shallow reef sites in the present study area.

**IUCN Red List of Threatened species status:** Least concern

## **Family PLESIASTREIDAE** Dai & Horng, 2009

**Genus** *Plesiastrea* Milne Edwards & Haime, 1848

- **Species** *Plesiastrea versipora* (Lamarck, 1816)

**Common name/synonym:** *Favia versipora* (Lamarck, 1816) (previous combination).

**Description:** Colonies are encrusting, massive to sub-massive, with widely spaced, monocentric and placoid, rounded corallites (2-4 mm diameter). Paliform lobes visible in a circle around the small columellae. Tentacles are short, alternating in two sizes and maybe extended even during the day.

**Colour:** Colour varies from cream to pale brown or green.

**Habitat:** Usually found in shaded and protected reef areas.

**Distribution:** Widely distributed in Indo-Pacific from western Indian Ocean to Pacific reefs.

**Remarks**: Massive greenish colonies up to 1.5 m were observed in the study area. Usually found near protected reef areas like that wall overhangs.

**IUCN Red List of Threatened species status:** Least concern

## **Family PORITIDAE** Gray, 1840

**Genus** Porites Link, 1807

- **Species** *Porites lichen* (Dana, 1846)

**Common name:** Hump coral.

**Description:** Colonies form flat laminae or plates. Corallites are arranged in irregular rows, and are separated by low ridges.

**Colour:** Varies from brown to yellowish green or mustard.

**Habitat:** Usually found in reef slopes and lagoons.

**Distribution:** Widely distributed ranging Indo-Pacific Eastern Pacific reefs from Red Sea to Samoa Islands.

**Occurrence:** Common and abundant.

**Remarks:** Dominant species in the study area and prone to bleaching.

**IUCN Red List of Threatened species status:** Least concern

- **Species** *Porites lutea* Milne Edwards & Haime, 1851

**Common name**: Hump coral / Pore coral

**Description:** Massive, hemispherical or helmet-shaped colonies, which can grow up to 4 metres. However, in areas of high sedimentation, they tend to become thick and columnar with a smooth surface. Distinguishing feature is the at the septal triplet is fused to form a trident and calices are smaller with thick walls, as com other species in the same genus.

**Colour:** Varies from brown to yellow and is usually brighter in shallow waters.

**Habitat:** Protected areas of reef like lagoons, and back reef.

**Distribution:** Widespread in Indo-Pacific reefs from Red sea to Coral Sea.

**Occurrence:** Common

**Remarks:** Formed big colonies in both shallow and deeper sites in the present study area.

**IUCN Red List of Threatened species status:** Least concern

**Genus** *Goniopora* de Blainville, 1830

- **Species** *Goniopora columna* Dana, 1846

**Common name:** Anemone coral

**Description:** Colonies are columnar, sub-massive colonies with uniform corallites and large columellae. Diameter of calices varies from 3 to 5 mm. Irregular and perforated septa. Living polyps vary in size and can be large (up to 10 cm) and elongated.

**Colour:** Colour ranges from brown, yellow to green.

**Habitat:** Common in lagoons, sandy and sheltered areas. Found in depth between 2-15 m.

**Distribution:** Common in Indo-Pacific from Red Sea to Fiji. Most common Goniopora in Arabia Sea and Indian Ocean in general.

**Occurrence:** Common

**Remarks:** It is a dominant species in the sandy, deeper reef sites of both Grande Island, Goa.

**IUCN Red List of Threatened species status:** Near Threatened

- **Species** *Goniopora djiboutiensis* Vaughan, 1907

**Common name/synonym:** Anemone coral, *Goniopora pulvinella* Wells, 1954 (synonym).
**Description:** Colonies range from submassive to mostly short thick columns. Prominent dome-shaped columellae divided into six parts, with each part divided into a pattern of deltaic four seta. Polyps have distinguishing whitish large oral cones.
**Colour:** Colour ranges from pale or dark brown to green.
**Habitat:** Turbid waters.

**Distribution:** Widespread in Indo-Pacific reefs from Red Sea to Coral Sea.

**Occurrence:** Common

**Remarks:** Solitary, sporadic, white colonies in deeper sites with sandy bottom in Grande Island, Goa.

**IUCN Red List of Threatened species status:** Least concern

- **Species** *Goniopora stokesi* Milne Edwards & Haime, 1851

**Common name/synonym:** Anemone coral.

**Description:** Colonies can be free-living or attached in the shape of hemispherical or may have short thick columns. Columellae are broad and irregular. Small satellite colonies often occur embedded in the living tissue of parent colonies. Polyps are of mixed sizes, the larger being elongate.

**Colour:** Usually varies from pale brown to green with green tips of tentacles.

**Habitat:** Usually found attached on soft substrates and is free living.

**Distribution:** Indo-Pacific distribution.

**Occurrence:** Uncommon

**Remarks: It w**as found in sites with sandy bottom in the study area.

**IUCN Red List of Threatened species status:** Near Threatened.

### **Family SIDERASTREIDAE** Vaughan & Wells, 1943

**Genus** *Pseudosiderastrea* Yabe & Sugiyama, 1935

- **Species** *Pseudosiderastrea tayamai* Yabe & Sugiyama, 1935

**Common name:** False pillow coral

**Description:** Colonies range from encrusting to submassive and are dome -shaped, up to 16 cm across. Corallites are monocentric, ceroid, and polygonal in shape. Evenly spaced septa usually fused with each other and form fan-like groups. Distinguishing feature is the presence of fine saw-like teeth and distinct white corallite walls.

**Colour:** Colour ranges from brown to pale grey.

**Habitat:** Turbid, shallow waters, usually attached to bare rock.

**Distribution:** Widespread in Indo-Pacific reefs from Red Sea to Coral Sea.

**Occurrence:** It is a cryptic species attached to barren rock and is uncommon in occurrence.

**Remarks:** Small, encrusting colonies were found attached to rocks in most of the study sites in the present study.

**IUCN Red List of Threatened species status:** Near Threatened

**Genus** *Siderastrea* de Blainville, 1830

- **Species** *Siderastrea savignyana* Milne Edwards & Haime, 1849

**Common name:** African pillow coral.

**Description:** Colonies range from encrusting to low mounds and can reach up to one metre across. Corallites are polygonal. Around 10-15 septa are fused together with the columella in the form of fan-like groups. Presence of fine ridges along the corallite walls is the distinguishing feature.

**Colour:** Colour varies from pale tan to dark centres with distinct with white corallite walls.

**Habitat:** Shallow reef environment; colonies maybe buried in sand.

**Distribution:** Distributed from Red Sea to Coral Sea.

**Occurrence:** Uncommon

**Remarks:** Encrusting colonies were present in rocky sites with sediment.

**IUCN Red List of Threatened species status:** Least concern.

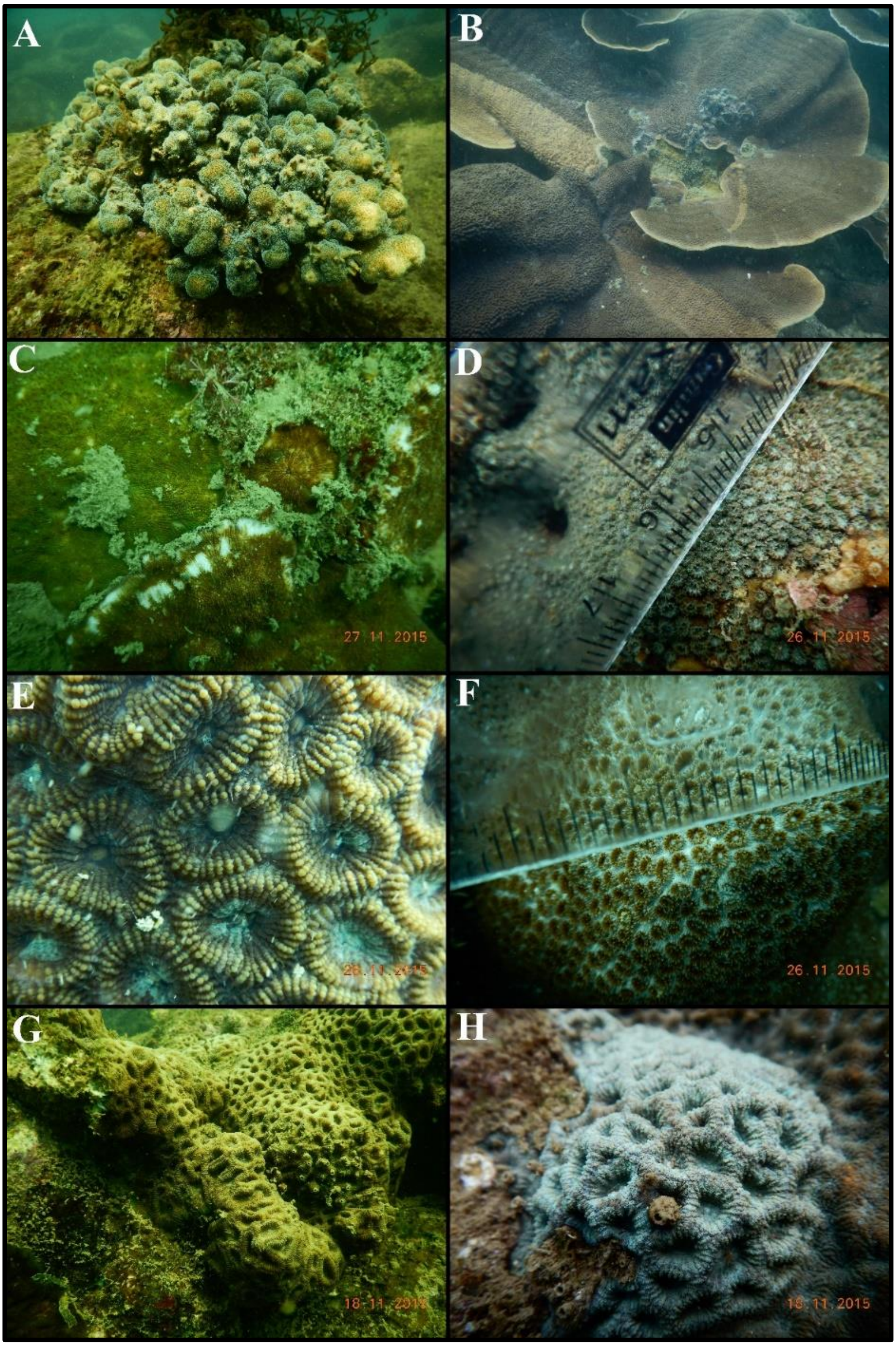


**Fig. 2.5: Scleractinian (hermatypic) corals from the study area. A) *Stylocoeniella guentheri;* B) *Turbinaria mesenterina;* C) *Podabacia crustacea;* D) *Cyphastrea serailia;* E) *Dipsastraea lizardensis;* F) *Dipsastraea matthaii;* G) *Dipsastraea veronii;* H) *Favites flexuosa.***

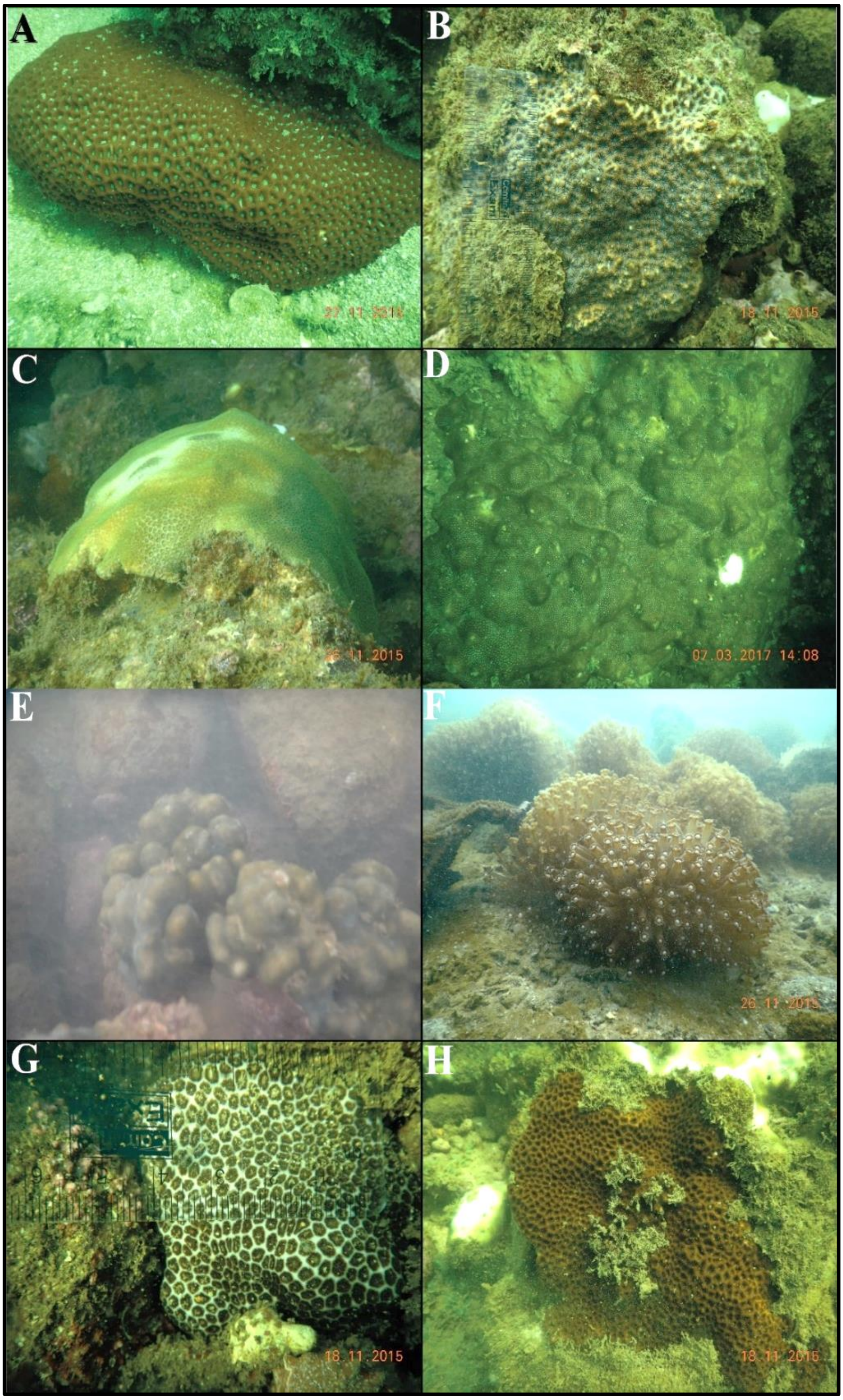


Fig. 2.6: Scleractinian (hermatypic) corals from the study area. A) *Favites pentagona;* B) *Favites spinosa;* C) *Plesiastrea versipora;* D) *Porites lichen;* E) *Porites lutea;* F) *Goniopora columna,* G) *Pseudosiderastrea tayamai;* H) *Siderastrea savignyana*

## OCTOCORALS/AHERMATYPIC CORALS

**Phylum CNIDARIA** Hatschek, 1888

**Class ANTHOZOA** Ehrenberg, 1834

**Subclass OCTOCORALLIA** Haeckel, 1866

**Order ALCYONACEA** Lamouroux, 1812

**Suborder HOLAXONIA** Studer, 1887

**Family ACANTHOGORGIIDAE,** Gray, 1859

**Genus** *Acanthogorgia* Gray, 1857

- **Species** *Acanthogorgia ceylonensis* Thomson & Henderson, 1905

**Common name/Synonym:** *Acalycigorgia ceylonensis* (changed combination).

**Description:** Colonies are fan-shaped, with a prominent basal attachment disc. Main branch arising from the stalk, reaches up to two-third of the colony. The main branch gives rise to alternate branchlets in opposite direction. The stalk portion can be around 1 mm, whereas the branchlets are finer, from 0.4-0.6 mm. Calyces are small, conical or tubular in shape with a crown of spines giving them a bristle like appearance.

**Colour:** The colony observed here was blue in colour.

**Habitat:** It has been documented from shallow as well as deep waters.

**Distribution:** First reported from Ceylon (Sri Lanka) and subsequently reported from Malay Archipelago and the Arabian Sea.

**Remarks:** Present in deeper reef sites with sandy bottom in Grande Island.

**Genus** *Psammogorgia* Verrill, 1868

- **Species** *Psammogorgia flabellum* (Pallas, 1766)

**Common name/Synonym:** *Heterogorgia flabellum* (Pallas, 1766)

**Description:** Colonies are fan-shaped (flabellate), with a robust stalk. The stalk divides into branches, which reaches the middle of the colony and further divided into branchlets (Thomas & George, 1986). Main branches are flattened and the branchlets curve out and maybe grown at a right angle connecting to the laminar plane. The network of branches and branchlets gives it a fan-like or mesh-like appearance. Can grow up to 100 cm in height and 80 cm laterally.

**Colour:** Alive colonies are dull brown in colour and turn pale white when dead.

**Distribution:** Indo-Australian waters.

**Remarks:** It was present in high numbers in deeper sites with sandy bottom in the study area.

### **Suborder CALCAXONIA** Grasshoff, 1999

### **Family ACANTHOGORGIIDAE** Gray, 1859

**Genus** *Ellisellidae* Gray, 1859

- **Species** *Junceella juncea* (Pallas, 1766)

**Common name:** Red Sea whip, whip coral.

**Description:** Colonies are unbranched and whip like and diameter varies from 3 to 7 cm (Sivaleela 2015). The surface of the colony has small clubs (0.005mm). Single stalk with small papillated calyces and small polyps.

**Colour:** Bright red.

**Distribution:** Tropical Indo-Pacific.

**Remarks:** This species of soft coral was prevalent in the study area (both Grande Island and MMS) in areas with deeper waters and sandy substratum.

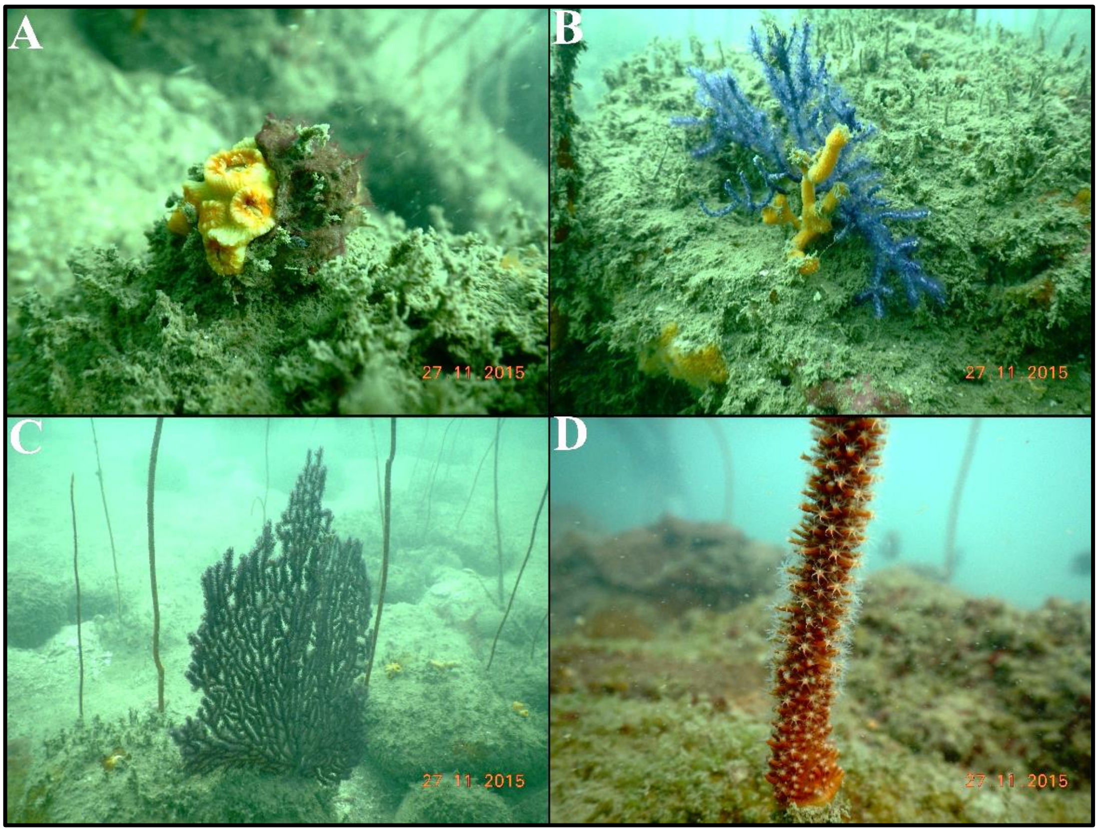


**Fig. 2.7: Ahermatypic corals from the study area, A) *Tubastraea coccinea;* B) *Acanthogorgia ceylonensis;* C) *Psammogorgia flabellum;* D) *Junceella juncea.***

**Table 2.2: Presence /absence (1/0) of coral species, both hermatypic and ahermatypic recorded from study sites across Grande Island, Goa and Malvan Marine Sanctuary, during the present study.**

| Coral species | Grande Island, Goa | | | | | Malvan Marine Sanctuary | | |
|---|---|---|---|---|---|---|---|---|
| **Hermatypic corals** | **S1** | **S2** | **S3** | **S4** | **S5** | **MMS1** | **MMS2** | **MMS3** |
| 1.*Stylocoeniella guentheri* (Bassett-Smith, 1890) | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 2.*Coscinaraea monile* (Forskål, 1775) | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| 3.*Turbinaria mesenterina* (Lamarck, 1816) | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| 4.*Turbinaria frondens* (Dana, 1846) | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| 5.*Podabacia crustacea* (Pallas, 1766) | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| 6.*Cyphastrea serailia* (Forskål, 1775) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 7.*Dipsastraea lizardensis* (Veron, Pichon & Wijsman-Best, 1977) | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 8.*Dipsastraea matthaii* (Vaughan, 1918) | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 9.*Dipsastraea veroni* (Moll & Best, 1984) | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| 10.*Favites complanata* (Ehrenberg, 1834) | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| 11.*Favites flexuosa* (Dana, 1846) | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 12.*Favites halicora* (Ehrenberg, 1834) | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| 13.*Favites pentagona* (Esper, 1795) | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 14.*Favites spinosa* (Klunzinger, 1879) | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| 15.*Goniastrea retiformis* (Lamarck, 1816) | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 16.*Plesiastrea versipora* (Lamarck, 1816) | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |

Contd.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *17.Porites lichen* (Dana, 1846) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| *18.Porites lutea* Milne Edwards & Haime, 1851 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *19.Goniopora columna* (Dana, 1846) | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| *20.Goniopora djiboutiensis* (Vaughan, 1907) | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| *21.Goniopora stokesi* (Milne Edwards & Haime, 1851) | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| *22.Goniopora pedunculata* Quoy & Gaimard, 1833) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *23.Pseudosiderastrea tayamai* (Yabe & Sugiyama, 1935) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 |
| *24.Siderastrea savignyana* (Milne Edwards & Haime, 1849) | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| *25.Leptastrea* sp. (Milne Edwards & Haime, 1849) | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 |
| *26.Pavona* sp. (Lamarck, 1801) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Ahermatypic corals** | | | | | | | | |
| *1.Tubastraea coccinea* (Lesson, 1830) | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 |
| *2.Acanthogorgia ceylonensis* (Thomson & Henderson, 1905) | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| *3.Psammogorgia flabellum* (Pallas, 1766) | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| *4.Junceella juncea* (Pallas, 1766) | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| **Total** | **21** | **22** | **11** | **28** | **21** | **12** | **9** | **11** |

Family Merulinidae had the highest diversity with 10 representative species followed by the family Poritidae with five species (Table 2.2). The presence of *Dipsastraea matthaii* and *Favites spinosa*, critically endangered and vulnerable species (IUCN Red List), respectively noted only in Grande Island and not in MMS. Another vulnerable species *Turbinaria mesenterina* was a dominant species in both Grande Island and MMS. This indicates the significance of these reefs' communities and points towards better sustainable management to protect these reefs with endangered and vulnerable coral species. There are very few published reports on corals from the Grande Island. Rodriques et al. (1998) reported three species of scleractinian corals namely, *Porites lutea*, *Favites pentagona*, and *Turbinaria mesenterina*, followed by fifteen genera of hard corals reported by Manikandan et al. (2016) including, *Acanthastrea*, *Coscinarea*, *Dendrophyllidae*, *Favia (=Dispastraea)*, *Favites*, *Goniastrea*, *Goniopora*, *Leptastrea*, *Montastrea*, *Plesiastrea*, *Pocillopora*, *Porites*, *Pseudosiderastrea*, *Siderastrea*, and *Turbinaria*. Out of these previously reported genera, *Acanthastrea* and *Pocillopora* were two genera not encountered in the present study and three genera including *Stylocoeniella*, *Podabacia*, and *Cyphastrea*. Patro et al. (2015) reported the prevalence of invasive species of snowflake coral-*Carijoa riisei* (soft coral) from the shipwreck site of Grande Island. Singarayan and Rethnaraj (2016) further reported three species of azooxanthellate scleractinian corals, namely, *Balanophyllia cumingii*, *Dentrophyllia indica*, and *Paracyathus profundus* from Grande Island, Goa. The present study reports additional three species of soft corals namely, *Acanthogorgia ceylonensis*, *Psammogorgia flabellum*, and *Junceella juncea* and one more species of azooxanthellate scleractinian coral namely, *Tubastraea coccinea* from Grande Island, taking the tally to four species of both soft corals and azooxanthellate scleractinian corals (Table 2.2).

De (2020), reported eighteen species of hermatypic and one species of ahermatypic coral (*Tubastrea coccinea*) from MMS. However, three species of hermatypic corals namely, *Porites compressa, Bernardpora stutchburyi,* and *Favites melicerum* were not recorded during the present study. This could be possibly due to difference in the selection of the study sites within the MMS. *Junceella juncea* was the soft coral species noted from MMS during the present study, taking the tally to two species of soft corals. (Table 2.3)

**Table 2.3: Comparative coral species count between Grande Island and MMS.**

| **Coral species** | **Grande Island** | | **MMS** | |
|---|---|---|---|---|
| | **Previous** | **Present study** | **Previous** | **Present study** |
| **Hermatypic** | 15 genera (Manikandan et al. 2016) | 25 species | 18species (De, 2020) | 14 species |
| **Ahermatypic** | 1 species (Patro et al. (2015) | 3 species | 1 (De, 2020) | 2 species |

**Table 2.4: Species richness (S) and Shannon diversity (H') based on presence of coral species in various study sites in Grande Island (S1-S5) and Malva Marine Sanctuary (MMS1-MMS3), during the present study.**

| **Sites** | **S** | **H'(loge)** |
|---|---|---|
| **S1** | 21 | 3.05 |
| **S2** | 22 | 3.09 |
| **S3** | 12 | 2.45 |
| **S4** | 28 | 3.33 |
| **S5** | 21 | 3.05 |
| **MMS1** | 11 | 2.39 |
| **MMS2** | 10 | 2.30 |
| **MMS3** | 11 | 2.39 |

Overall, coral species diversity is low in the central west coast of India as compared with other major coral reefs of India. The reef communities in the central west coast of India are characterized by the absence of branching corals, possibly due to high wave surge, turbidity, and the monsoonal influx of seawater (Nair and Qasim, 1978). In the present study, deeper reef sites with a rock and sandy bottom had coral diversity as compared to the shallower sites. Species richness and Shannon diversity index, H'(loge) were higher in reef sites of Grande Island as compared to MMS (Table 2.4).

### 2.2.2.2. Community structure and habitat distribution mapping

Analysis of community structure based on a percentage of various communities (live coral, rubble, macroalgae, turf algae, crustose coralline algae, rock, and sand) obtained from transects revealed the highest similarity of around 90 % in the two deeper sites of MMS, and similarity of around 80 % in the two sites with higher coral diversity in Grande Island (Fig. 2.8).

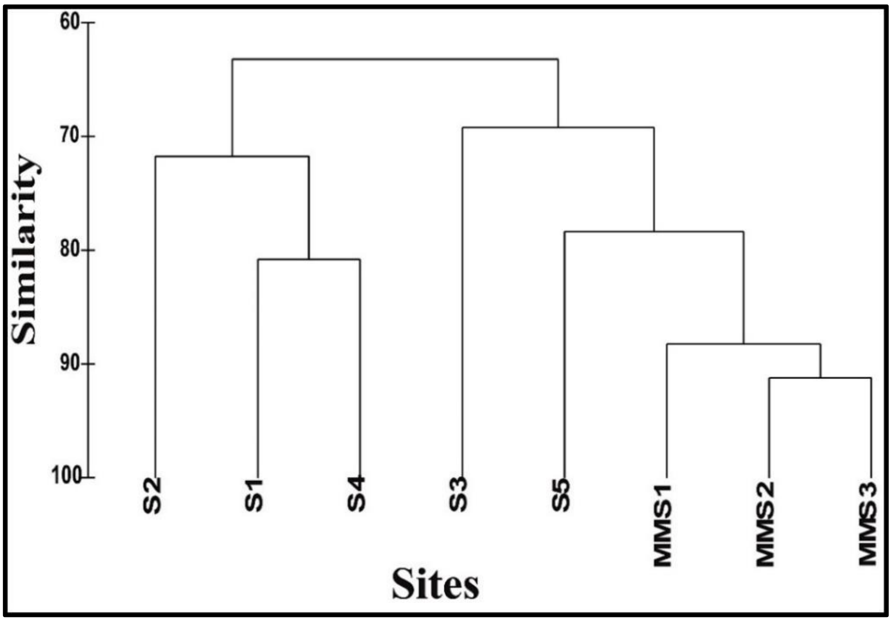


**Fig. 2.8: Cluster analysis of study sites-based community structure, where S1-S5 are sites in Grande Island, Goa, and MMS1-MMS3 are sites in Malvan Marine Sanctuary, Maharashtra.**

Habitat distribution mapping revealed that the shallow and rocky reef sites had a higher abundance of small encrusting corals like *Porites*, *Favites*, *Siderastrea*, and *Pseudosiderastrea*, whereas the deeper sites had a sandy bottom and were dominated by colonies of *Turbinaria* and *Goniopora* (Fig. 2.9-2.10). It was also noted that the rocky sites had a higher distribution of benthic fauna like sea urchins and sea cucumbers. The soft corals were present in deeper sites with a sandy substratum (Fig. 2.9).

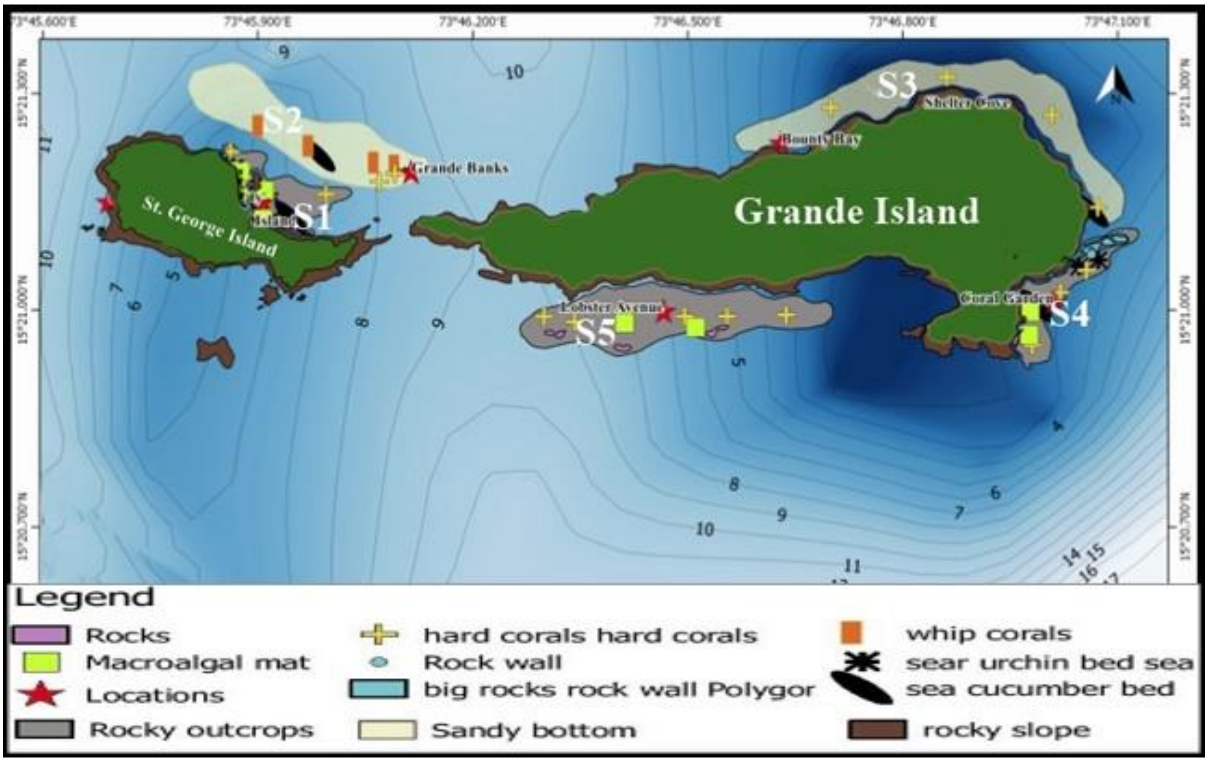


**Fig. 2.9: Habitat distribution map at Grande Island, Goa, where S1-S5 are study sites.**

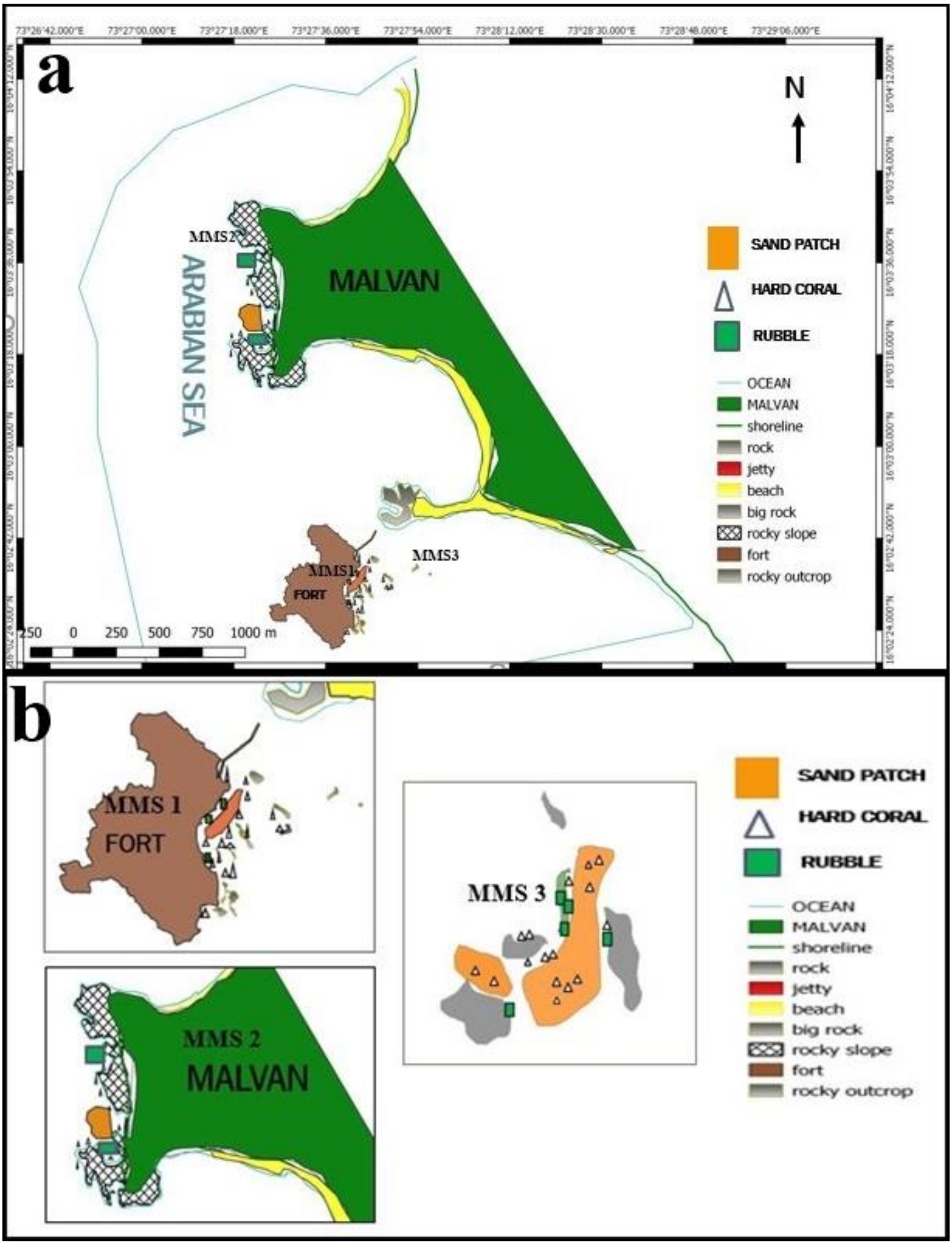


**Fig. 2.10: Habitat distribution map at MMS, a) MMS with all the sites; b) distribution mapping at the various sites in MMS.**

Habitat mapping also revealed that site 1 and site 4 in Grande Island had the highest percentage of live coral (Fig. 2.11), whereas in MMS live coral coverage was similar in all the three sites (Fig. 2.12). Based on the habitat composition mapping, site 1 and site 4 formed one cluster by applying nMDS ordination, whereas all the three reef sites in MMS formed one cluster (Fig. 2.14).

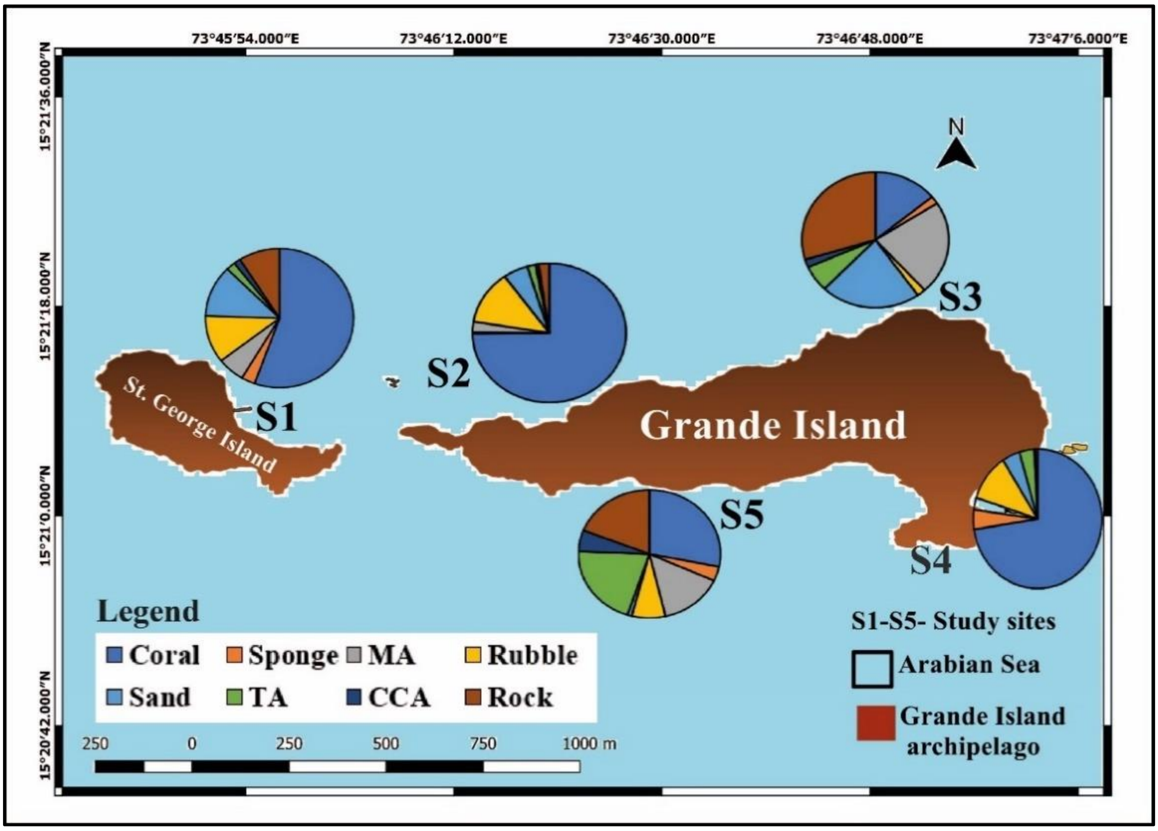


**Fig. 2.11: Map representing various substratum percentage (pie charts) across the study sites from Grande Island.**

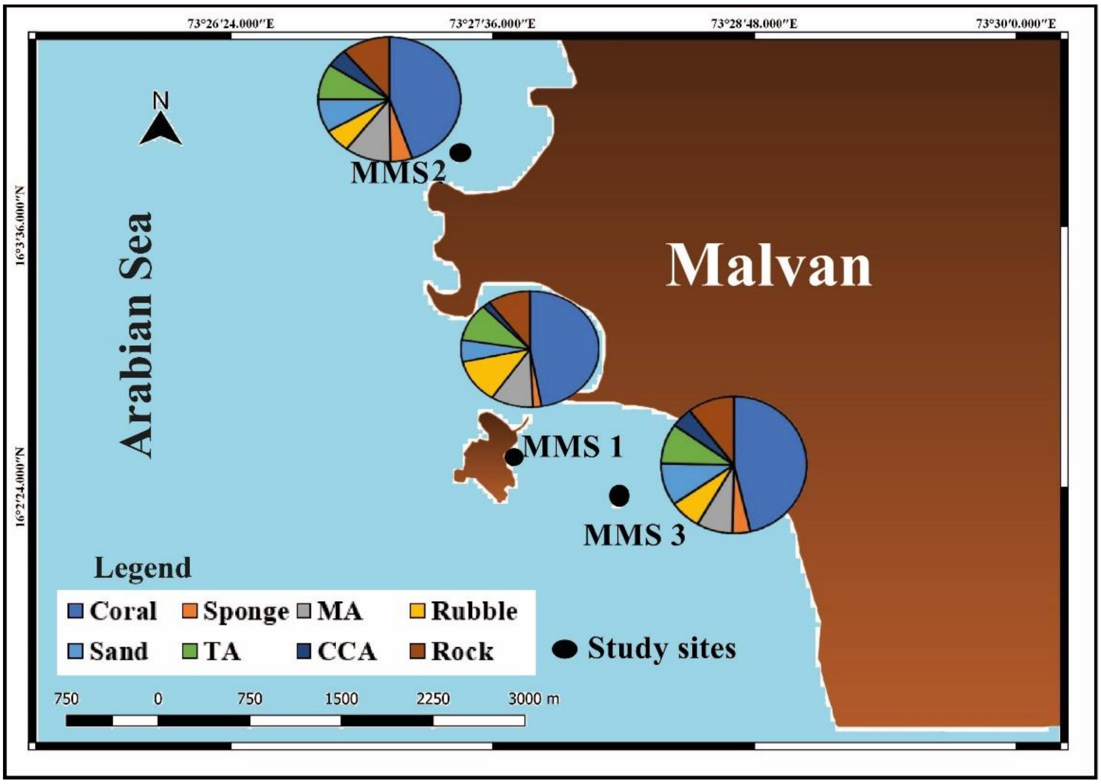


**Fig. 2.12: Map representing various substratum percentage (pie charts) across the study sites from Malvan Marine Sanctuary.**

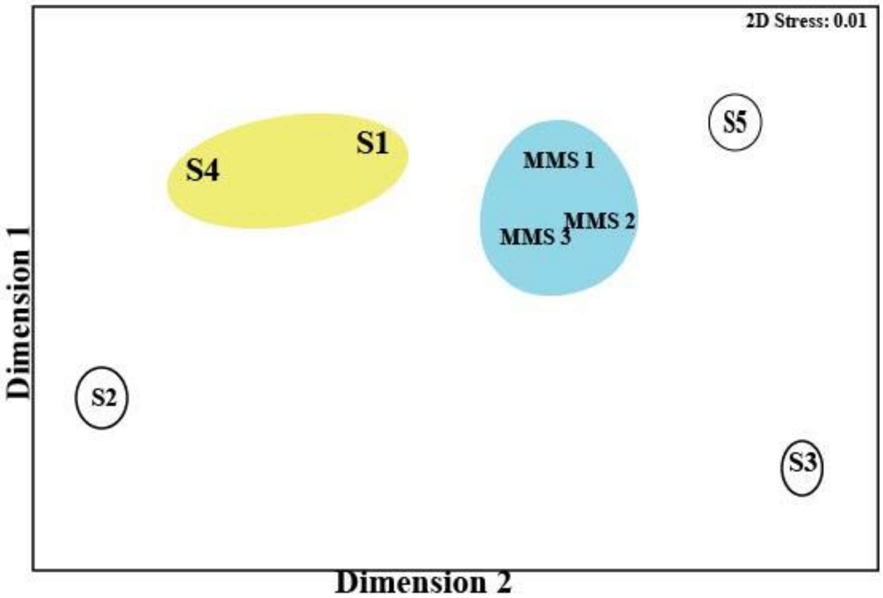


**Fig. 2.13: nMDS ordination of study sites based on Bray-Curtis similarity at 80% resemblance, where S1-S5 are sites in Grande Island, Goa and MMS1-MMS3 are sites in Malvan Marine Sanctuary, Maharashtra.**

## 2.3. Conclusion

An account of the biodiversity of reefs is crucial to appreciate the true beauty of the reefs. The present study has updated the diversity of corals from the highly understudied reefs of the central west coast of India. The presence of critically endangered and vulnerable coral species (IUCN red list) in the study area indicates the significance of these reef communities and points towards better sustainable management practices to protect them. Habitat distributing mapping revealed that the sites with a sandy bottom can be used for boat anchoring and for SCUBA related tourism activities.

# *Chapter 3*

## Biological indicators of reef health- Sea slugs

### 3.1. Introduction

Heterobranch sea slugs are an exquisite and highly sensitive group of shell-less, colourful, and soft-bodied molluscs which are also an indicator to study climate change and a source of natural products. They comprise of more than 6000 species worldwide (Yonow, 2008). They were previously grouped within '*Opisthobranchia*', which is now considered paraphyletic, although it is still considered as an "informal group" among the *Heterobranchia* (Wägele et al. 2014). To avoid the use of their misnomer, Yonow (2015), suggested the usage of the term 'sea slugs' to refer to this diverse group of marine animals. With an evolutionarily divergent lineage, this group of gastropods developed some unique adaptations to compensate for shell protection and slow movement (Marin, 2019). Being easy prey, they evolved defence strategies like chemical secretions, coloration for camouflage, warning, mimicry, and nematocysts for prey acquisition (Gosliner, 2001; Wägele & Klussmann-Kolb, 2005; Greenwood, 2009; Aguado & Marin, 2007; Haber et al. 2010; Putz et al. 2010). Sea slugs have recently gained interest in natural product research (Avila, 1995; Dean & Prinsep, 2017) owing to the presence of bioactive compounds in them. Ecologically they are an environmental indicator taxon due to their short life cycles (generally less than 12 months), high food and microhabitat specificity (Rudman & Willan, 1998), and their response to changing oceanographic conditions (Nimbs & Smith, 2016, 2018). Some species harbour symbionts that are useful microbes

(Zan et al. 2019) and many acquire chloroplasts from plants for in-house energy production (Rumpho et al. 2000; Pierce et al. 2015). Most of the species are exclusively benthic with some exceptions like *Scyllaea pelagica* which is attached to the floating masses of *Sargassum* (Hickman, 1973).

Compared to other regions of the world, the research on Indian sea slugs has been limited to isolated samplings in selected locations (Apte & Desai, 2017). Up to date, approximately 611 species of heterobranch sea slugs belonging to 183 genera, 62 families have been reported in 174 publications from India (BNHS, Unpublished Data). Sea slug diversity and distribution are well documented in some regions of Western India, such as Gujarat and Lakshadweep Islands, with 95 and 106 species recorded, respectively; (Vadher et al. 2020). On the other hand, the regions of Maharashtra, Goa, Karnataka, and Kerala coasts remain almost unexplored. The state of Goa has the most deficient records with only a few previous reports, wherein *Dendrodoris goani* was described by Rao & Kumary (1973). This was followed by the work of Jagtap et al. (2009) where they reported and described *Elysia bangtawaensis* based on specimens found in a mangrove. Apte & Desai (2017) reported 18 new records and a recent report by WWF-India (2019) included three additional records.

The beautifully coloured sea slugs with exquisite patterns attract underwater macro photography enthusiasts (Nimbs & Smith 2016), aiding in collecting information about their occurrence and diversity at the diving spots (Mehrotra & Scott, 2016). It is highly likely that the diverse habitat of Goa ranging from sub-tidal areas to rocky intertidal pools offers a much higher diversity of sea slugs than is presently known. Comprehensive documentation of sea slugs from Goa, will not only increase knowledge at these understudied sites but also help in identifying ecologically sensitive areas which would be crucial in aiding management plans. The information on distribution patterns will serve as a proxy for detecting shifts in distribution due to changing environmental parameters (Wassmann et al. 2011). Therefore, the purpose of this study is to amalgamate data on the sea slug diversity and distribution across the state of Goa.

## 3.2. Materials and methods

Sea slugs have a wide distribution ranging from habitats like rocky shores, coral reefs, sandy shores, mudflats, seagrasses, and mangroves (Apte & Desai, 2017). Because of their cryptic nature, low abundance, and high variance in distribution, survey methods were deployed in habitats ranging from coral reefs to rocky shores (rock pools, ledges, and crevices) and sandy bottom. Manual samplings were conducted across the following three habitats (Fig. 1.1).

1) The reefs of Grand Island, Goa (Lat. 15° 21' 01.73" N; Long. 73° 46' 58.68" E), with the aid of SCUBA during pre-monsoon (February-May) and post-monsoon (October-January) of 2016-18. A total of 55 dives, deploying benthic transects were carried out to assess the biodiversity of these patch reefs over three years. These shallow patch reefs are dominated by macroalgae during Feb-April (Shetye et al. 2020) and have scattered growth of hard corals interspersed with rocky outcrops and sandy patches with an average depth of 5-6 m (Hussain & Ingole, 2020).

2) Various rocky shores along the coastline of Anjuna, Vagator, Marmugoa, Bambolim, and Betul in Goa, over a five days survey, carried out in December 2018.

3) Bottom trawl conducted in Zuari estuary.

Digital photographs were taken of live specimens in their natural habitat using Nikon AW130; specimens were measured and then wherever possible were preserved in 99% ethyl alcohol for further taxonomic examination. Wherever possible, the egg cases were also noted and photographed for identification. The samples collected were deposited in the museum of the Bombay Natural History Society (BNHS). Since the holotype sample of *Dendrodoris goani* is missing, we dissected the animal to confirm the identification based on the internal anatomy (digestive and reproductive system) as described by Rao and Kumary (1973). The sea slugs were identified with key references such as Rao & Kumary (1973); Apte (2009, 2012); Gosliner et al. (2008, 2015), Venkataraman et al. (2015); and Apte & Desai (2017). Taxonomy was according to the World Register of Marine Species (WORMS).

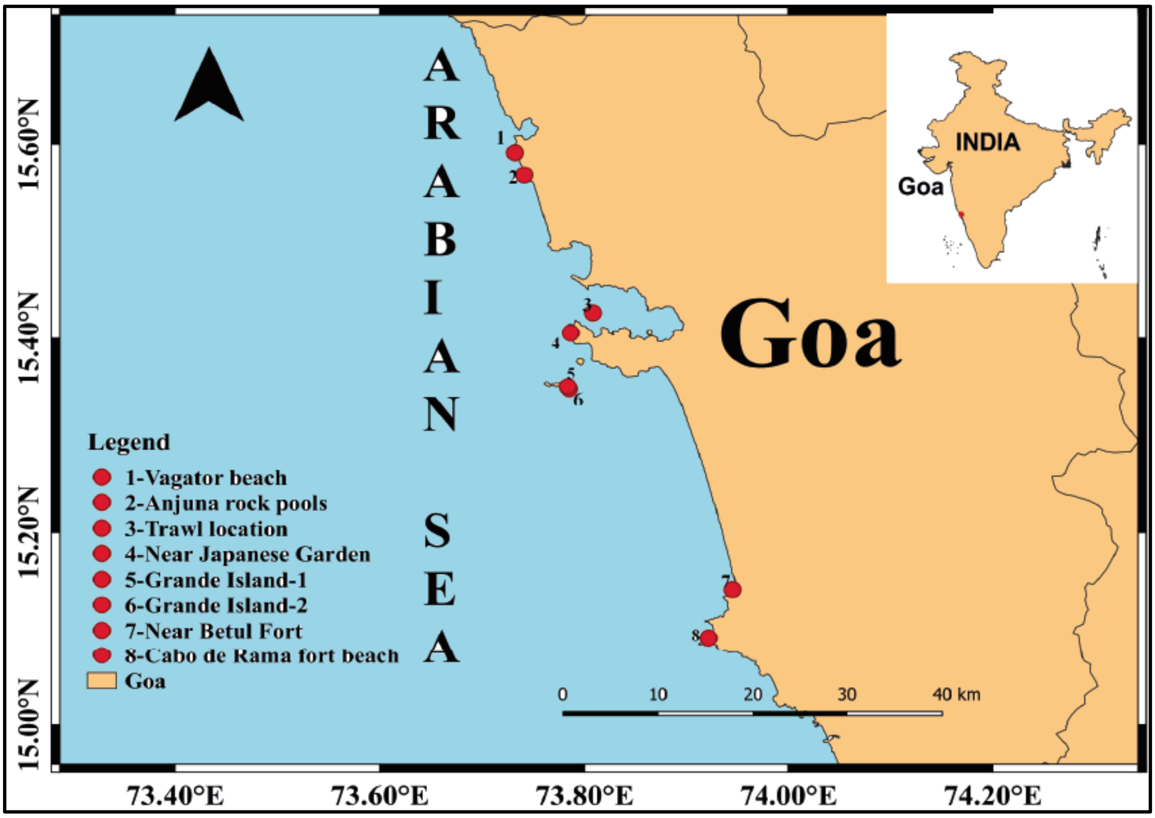


**Fig. 3.1: Map of Goa showing sampling sites from North to South Goa.**

## 3.3. Results

This study reports 15 species of sea slugs belonging to 14 families, which are new records to Goa, including *Dendrodoris goani*, been reported almost half a century after its original description, while *Hallaxa albopunctata*, a new record from India. The following is the description of 15 species recorded in our study with the museum voucher numbers (BNHS-Opistho-number). The date of collection, size of the animal as well as the shape of the egg mass (if recorded) is also mentioned in the description.

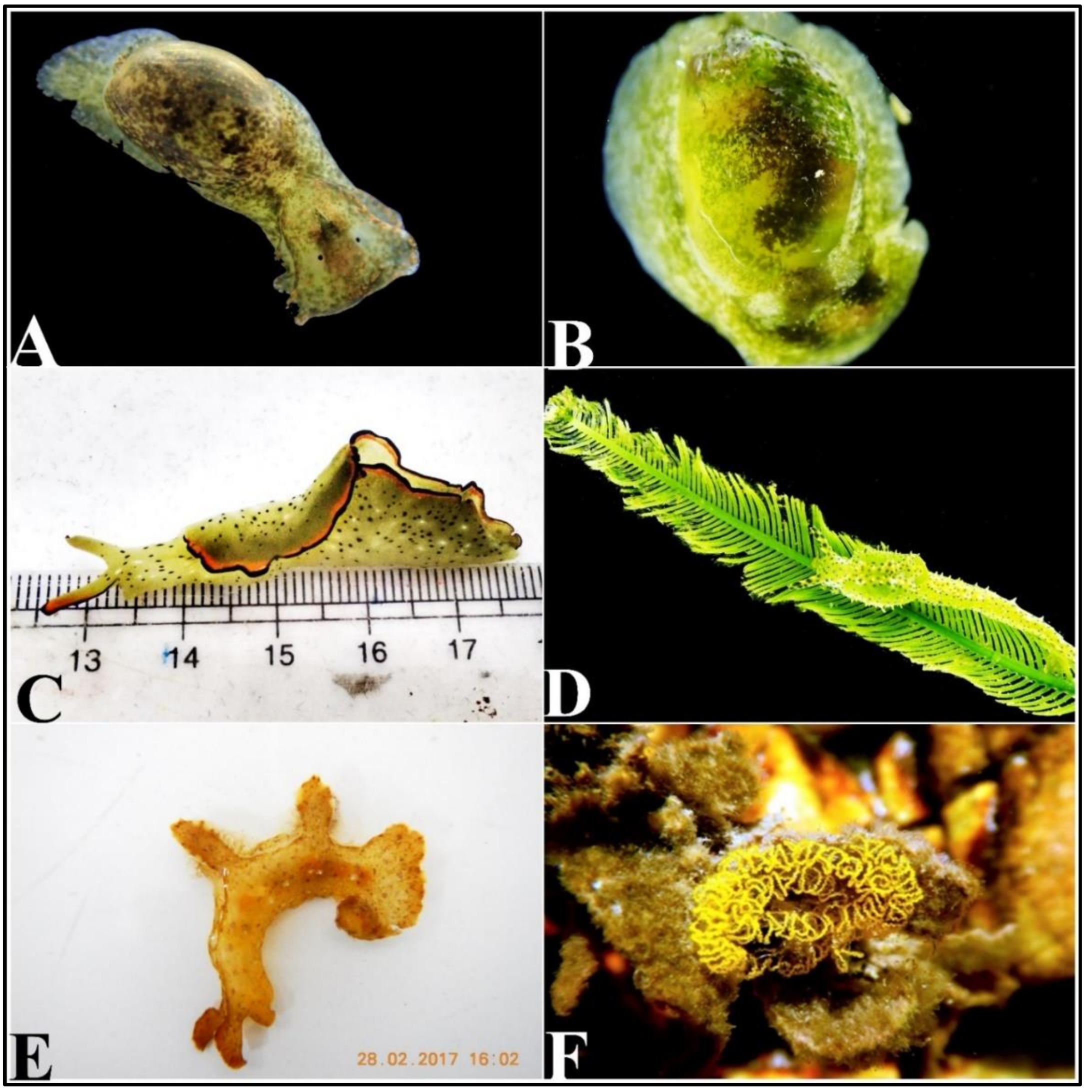


**Fig. 3.2: Live photographs of: A) Haloa *pemphis*; B) *Smaragdinella sieboldi*; C) *Elysia ornata;* D) *Oxynoe viridis;* E) & F) *Scyllaea pelagica* and its egg (Hussain et al. 2022).**

### Family HAMINOEIDAE Pilsbry, 1895

### Genus *Haloa* Pilsbry, 1921

### *Haloa pemphis* (Philippi, 1847)

(Fig. 3.2A)

*Material examined:* Specimens were collected from two locations.

BNHS-Opistho-1608 (Voucher no. [V. no.]), Location-Vagator (Goa), 15°35'28.41"N, 73°43'55.64"E, Date of collection- 19-Dec-2018, Habitat- Rockpools, 1 specimen (specimen.), Length (*L)* = 25 mm. BNHS-Opistho-1617 (V. no.), Location-Anjuna (Goa), 15°34'5.22"N, 73°44'27.78"E, Date of collection-20-Dec-2018, Habitat- Rockpool, 1 specimen., *L* = 18 mm.

*External morphology:* Shell visible, round to oval with mottled green colour. Cephalic shield deeply bilobed, eyes closely spaced. Parapodia rounded, slightly overlapping. Rhinophores short, brown coloured.

*Ecology:* Herbivorous, commonly found eating filamentous green algae, but sometimes also *Ulva* sp.

*Distribution in India:* Maharashtra (Oskars & Malaquias, 2019) and Goa (present study).

*Global distribution:* Australia, Japan, Red Sea-Djibouti, South Madagascar, Mozambique.

*Remarks:* This species is found in shallow rocky waters (Oskars & Malaquias, 2019) laying sausage-shaped egg mass which appears like a coil of yellow beads packed into a transparent sheath.

### Genus *Smaragdinella* A. Adams, 1848

### *Smaragdinella sieboldi* (A. Adams, 1864)

(Fig. 3.2B)

*Material examined:* Specimens were found across four locations.

BNHS-Opistho-1618 (V.no.), Location 1-Vagator beach (Goa), 15°35'28.41"N, 73°43'55.64"E, Date of collection- 19-Dec-2018, Habitat- Tide pools, 1 specimen., *L* = 18 mm;

BNHS-Opistho-1612 (V. no.), Location 2- Anjuna beach (Goa), 15°34'5.22"N, 73°44'27.78"E, Date- 20-Dec-2018, Habitat- Tide pools, 1 specimen., *L* = 12mm.

Location 3- Mormugao beach (Goa), 15°24'19.44"N, 73°47'11.09"E, Date of collection- 22-Dec-2018, Habitat- Tide pools, 1 specimen., *L* = 17 mm (specimen not collected).

Location 4- Canaguinim /Betul (Goa), 15° 8'27.41"N, 73°56'40.31"E, Date of collection- 23-Dec-2018, Habitat- Tide pools, 1 specimen., *L* = 15 mm (specimen not collected).

*External morphology:* Body ground colour is greenish-brown with white patches. Eyes present laterodorsally and on the cephalic shield. Parapodium overlapping the shell partially. Foot wide, white-coloured, anterior margin straight while posterior margin is circular. Shell large, low profile, transparent, and yellowish-brown with a narrow spiral lamella.

*Ecology:* This species feeds by scraping fine mats of blue-green algae on rocks and other hard substrates.

*Distribution in India:* Ratnagiri, Maharashtra [Melvill & Abercrombie, 1897: As *Smaragdinella (Glauconella) andersoni* (Nevill, 1871)], Mandapam, Gulf of Mannar (Sundaram et al. 1969: as *Smaragdinella* sp. CMFRI – M. 160 and Rao & Sundaram, 1972), Maharashtra (Maharashtra Gazetteers).

*Global distribution:* Indo-West Pacific, Japan, South China Sea-Vietnam (Chaban & Chernyshev, 2016), and the Red Sea.

*Remarks:* Members of this species can be easily located by sighting bright, yellow-coloured egg cases laid in shallow pools/ crevices/ dead oyster beds/ polychaetes tubes made up of sand grains. A detailed study of material from type locality by Chaban & Chernyshev (2016) suggested the presence of a narrow spiral lamella as a key diagnostic feature of *S. sieboldi*.

### Family PLAKOBRANCHIDAE Gray, 1840

### Genus *Elysia* Risso, 1818

### *Elysia ornata* (Swainson, 1840)

(Fig. 3.2C)

*Material examined:* BNHS-OPISTHO-1632 (V. no.), Location- Grande Island (Goa), 15°21’01.10” N, 73°47’02.15” E, Date of the collection- 18-Feb-2017, Habitat- Coral reef at 5 m depth, 1 specimen., *L* = 12 mm.

*External morphology:* Body translucent green in colour with numerous white, orange, and black spots, visible black parapodial margin, and a submarginal margin with a yellow and orange band. Rhinophores short with similar white and orange bands.

*Ecology:* This species feeds by sucking the sap of *Bryopsis* sp. (Jensen, 1992).

*Distribution in India:* Widespread.

*Global distribution:* The Caribbean and Indo-West Pacific: New Caledonia, French Polynesia, Australia, Florida, Indonesia, Réunion Island, Dubai, South China Sea, Red Sea, Bahamas, Malaysia, Singapore, Japan, Taiwan, Saudi Arabia, Papua New Guinea, Philippines, Hawaii, American Samoa.

*Remarks: E. ornata* is a part of the *Elysia grandifolia-ornata* species complex which has posed many taxonomic difficulties. Further integrative molecular taxonomy is required to decipher the members of this species complex.

### Family OXYNOIDAE Stoliczka, 1868(1847)

### Genus *Oxynoe* Rafinesque, 1814

### *Oxynoe viridis* (Pease, 1861)

(Fig. 3.2D)

*Material examined:* Specimens were found in two locations.

BNHS-OPISTHO-1604 (V. no.), Location 1-Vagator beach (Goa),15°35'28.41"N, 73°43'55.64"E, Date of collection- 20-Dec-2018, Habitat- Rockpool, 1 specimen., *L* = 25 mm.

Location 2-Grande Island (Goa), 15°21’15.00” N, 73°45’58.36” E, Date of collection- 07-Mar-2017, Habitat- Coral reef at 6 m depth, 1 specimen., *L* = 32 mm (specimen not collected).

*External morphology:* Body slender with a long and tapering tail, covered with pointed fleshy papillae, ground colour varies from dark to light green with scattered blue spots and a white band in the longitudinal centre. Shell bubble-shaped, partially enclosed with parapodial flaps. Rhinophore tips cream coloured.

*Ecology:* The species feed on algae (*Caulerpa* sp.)

*Distribution in India:* Andaman and Nicobar Island, Gujarat, Kerala, Lakshadweep, Andhra Pradesh, Tamil Nadu, and Goa (present study).

*Global distribution:* South Africa, Tanzania, Madagascar, Hawaii, Society Islands.

*Remarks:* These sacoglossans are found camouflaged within members of macroalgae *Caulerpa* spp. and in times of danger, they can shed their long muscular foot which can later regenerate.

### Family SCYLLAEIDAE

### Genus *Scyllaea* Linnaeus, 1758

### *Scyllaea pelagica* (Linnaeus, 1758)

(Fig. 3.2E, F)

*Material examined:* BNHS-OPISTHO-1631 (V. no.), Location- Grande Island (Goa), 15°20’50.86” N, 73°47’06.53” E, Date of collection- 28-Feb-2017, Habitat- Found attached to floating *Sargassum* in coral reef area.12 specimen., adult, *L* = 22-28 mm

*External morphology:* Body dorso-ventrally flattened with two pairs of dorso-lateral lobes, background colour yellowish brown with some brown mottling, small scattered white markings throughout, and brilliant blue spots along the flanks. Rhinophores are enclosed within two flattened rhinophore sheaths. The inner surface of the dorso-lateral lobes is lined with fine dendritic gills for respiration. Skin is smooth except for a few small, scattered conical tubercles on the sides of the body.

*Ecology:* This species is known to graze on hydroids living on *Sargassum* rafts, a shared habitat of both organisms (Hickman, 1973).

*Distribution in India:* Lakshadweep, Tamil Nadu, Andhra Pradesh, and Goa (this study).

*Global distribution:* Caribbean Sea, Costa Rica, Cuba, Gulf of Mexico, Mediterranean Sea, North Atlantic, Spain, Japan, France, Turkey, USA, Bahamas.

*Remarks:* The species is found in association with drifting *Sargassum* rafts where it lays its spiral, jelly coated egg mass.

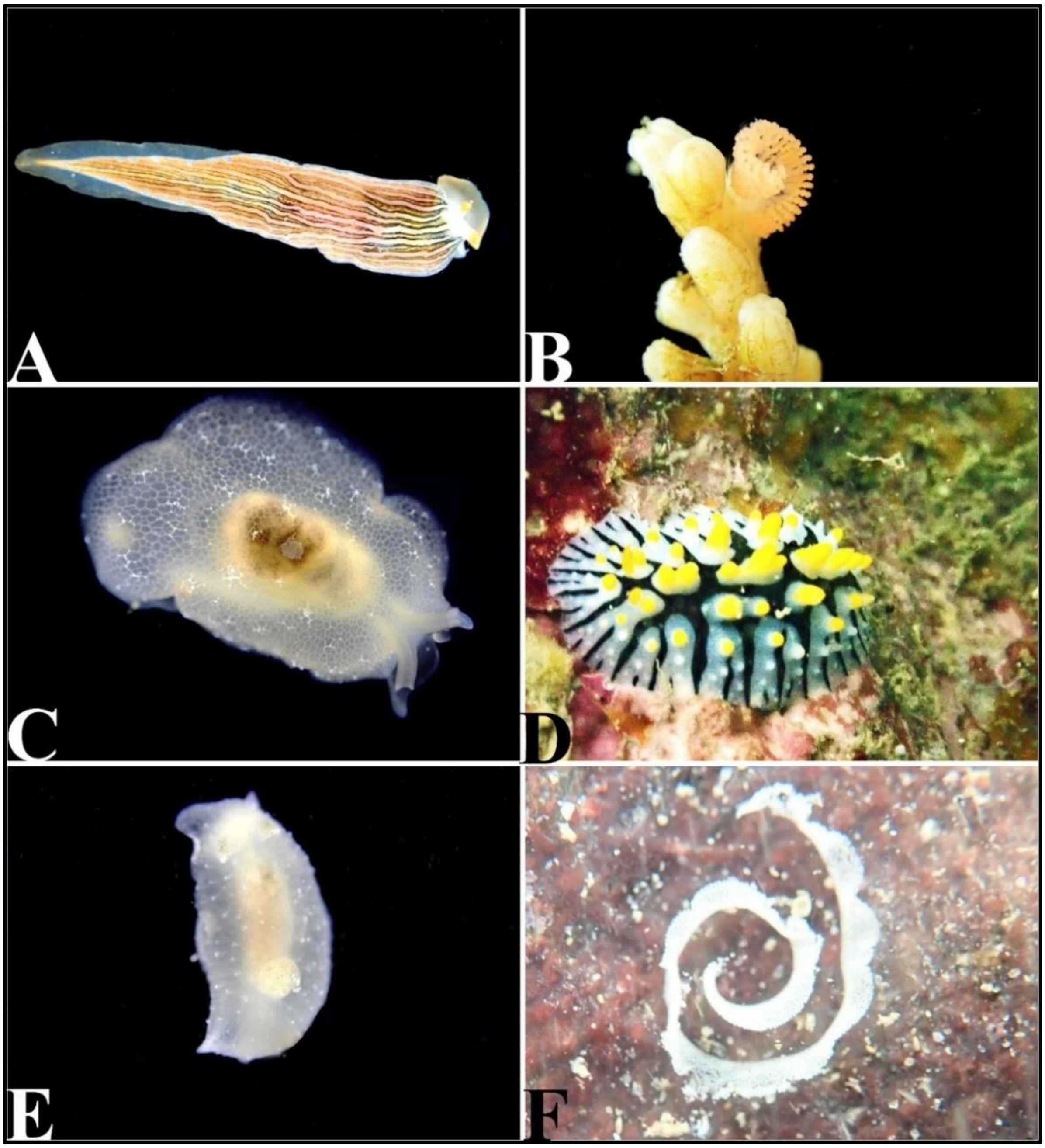


**Fig. 3.3: Live photographs of A) & B) *Pleurophyllidiella paucidentata* and its egg case on *Carijoa* polyp; C) *Berthella stellata*; D) *Phyllidia marindica*; E) & F) *Hallaxa albopunctata* and its egg case (Hussain et al. 2022).**

### Family ARMINIDAE Iredale & O’Donoghue, 1923 (1841)

### Genus *Pleurophyllidiella* Eliot, 1903

### *Pleurophyllidiella paucidentata (*O’Donoghue, 1932)

(Fig. 3.3A, B)

*Material examined:* 3 specimens collected from the same location, BNHS-OPISTHO-1611, 1615, 1616 (V. no.), Location- Anjuna beach (Goa), 15°34'5.22"N, 73°44'27.78"E, Date of collection- 20-Dec-2018, Habitat- Rockpool, 1 specimen., *L* = 32 mm.

*External morphology:* Body colour yellow or orange with white speckles, whitish to pale pinkish ridges bearing median black broad lines. Eyespot is visible at the base of ridged rhinophores. Rhinophores white except 1/4th distal part coloured orange. The frontal part of the head between rhinophores has a black patch.

*Ecology:* This species feeds voraciously on *Carijoa rissei*, an azooxanthellate octocoral.

*Distribution in India*- Pamban, Pamban Bridge, Gulf of Mannar (O’ Donoghue, 1932: 8 specimens), Maharashtra, and now Goa (present study).

*Global distribution:* Only reported from India so far.

*Remarks:* It has been observed that the organism hangs freely with the help of foot, either to feed or escape predation.

### Family PLEUROBRANCHIDAE

### Genus *Berthella* Blainville, 1824

### *Berthella stellata* (Risso, 1826)

(Fig. 3.3C)

*Material examined:* BNHS-OPISTHO-1607 (V. no.), Location- Anjuna beach (Goa), 15°34'5.22"N, 73°44'27.78"E, Date of collection- 20-Dec-2018, Habitat-Tide pool, 1 specimen., *L* = 22mm.

*External morphology:* Body elongate, translucent, ground colour whitish to pale ochre with scattered white spots and irregular markings. Skin rough with a minute irregular honeycomb pattern. Viscera and shell are visible. Mantle margin wraps all over the foot. Gills white, twelve pairs of alternately arranged

lamellae. Rhinophores joined medially, folded, protruding under the anterior edge of the mantle. Head is indistinct, eyes large, and visible from the mantle.

*Ecology:* The species is always found under boulder filed or in tidal pools at meso to the infralittoral zone.

*Distribution in India:* Known from Lakshadweep, Gujarat, Ratnagiri, and now Goa (present study).

*Global distribution:* Red Sea, Australia, Mexico, South Africa, Indo-West Pacific.

*Remarks:* These slugs are commonly called side-gilled slugs. Recent work by Ghanimi et al. (2020) provided evidence that the globally distributed *B. stellata* is a part of a species complex. Further work is needed to decipher the Indian Ocean species complex.

### Family PHYLLIDIIDAE Rafinesque, 1814

### Genus *Phyllidia* Cuvier, 1797

### *Phyllidia marindica* (Yonow and Hayward, 1991)

(Fig. 3.3D)

*Material examined:* Photographic record (No specimen collected); Location- Grande Island (Goa), 15°21’15.00” N, 73°45’58.36” E, Date- 03-Feb-2020, Habitat- Coral reef at 5 m depth, 1 specimen., *L* = 20mm.

*External morphology:* Body ground colour varies from black, blue to yellow with several laterally running black stripes intertwined with pairs of white stripes running from the centre to the edge of the mantle. These white stripes have several white and yellow tubercles on them. The dorsal surface has a median black longitudinal ridge with raised yellow tubercles.

*Ecology:* Feeds on sponges and can take in the toxins from the sponge to use for its defence.

*Distribution in India:* Tamil Nadu, Lakshadweep, Andaman, and the Nicobar Islands, and now Goa (present study).

*Global distribution:* Thailand, Western Australia, Eastern Africa, Maldives.

*Remarks:* First record from the mainland West coast of India. Endemic to the Indian Ocean.

### Family ACTINOCYCLIDAE O'Donoghue, 1929

### Genus *Hallaxa* Eliot, 1909

### *Hallaxa* cf. *albopunctata* (Gosliner & S. Johnson, 1994)

(Fig. 3.3E, F)

*Material examined:* BNHS-OPISTHO-1620 (V. no.), Location- Anjuna beach (Goa), 15°34'5.22"N, 73°44'27.78"E, Date- 20-Dec-2018, Habitat-Tide pool, 1 specimen., *L* = 21 mm.

*External morphology:* Body ovoid, dorso-ventrally flattened, ground colour varies from translucent white to cream with scattered white spots. Rhinophore bulbous, with seven lamellae, branchial plume same colour of rhinophores, simple unipennate with eight gills.

*Ecology:* All members of the genus *Hallaxa* are known to feed exclusively on sponges.

*Distribution*: First record of the species from India. So far was only reported from its type locality- Durban, South Africa (Gosliner, 2008). This study confirms its distribution in the West Indian Ocean. It was found attached to the bottom of a boulder in a tidal pool at Anjuna beach, North Goa.

*Remarks: Hallaxa albopunctata* has cryptic colouration and a sponge-like appearance. White coloured egg case was found attached under the surface of a boulder (Fig. 3.3F).

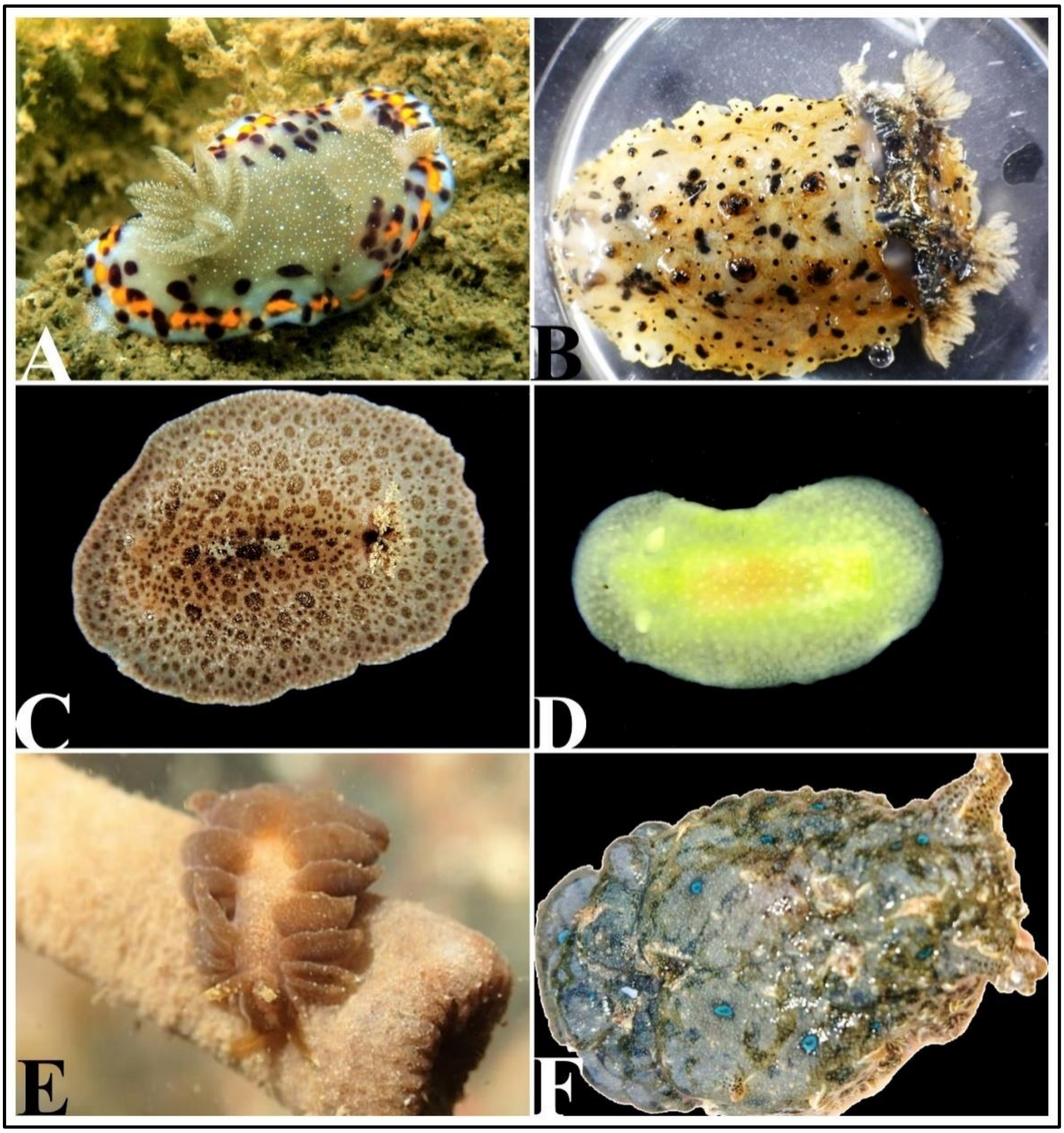


**Fig. 3.4: Live photographs of A) *Goniobranchus bombayanus*; B) *Dendrodoris goani*; C) *Sebadoris fragilis*; D) *Doriopsis granulosa*; E) *Aeolidiopsis palythoae;* F) *Bursatella leachii* (Hussain et al. 2022).**

### Family CHROMODORIDIDAE Bergh, 1891

### Genus *Goniobranchus* Pease, 1866

### *Goniobranchus bombayanus* (Winckworth, 1946)

(Fig. 3.4A)

*Material examined:* Location-Grande Island (Goa), 15°21’01.10” N, 73°47’02.15” E, Date of collection-28-Feb-2017, Habitat- Coral reef at 6 m depth, 1 specimen., *L* = 16 mm; Photographic record (specimen not collected).

*External morphology:* Body oblong, base colour whitish with an orange-coloured line made of overlapping spots present along the mantle margin, purple spots present along the margin of the orange line and few in the centre, the colour becomes darker towards the centre due to visceral mass. Rhinophores with 20-22 lamellae, retractable in pockets. Seven simple, pinnate gills on the dorsal side with a simple gill pocket.

*Distribution*: Known only from India so far from Ratnagiri, Mumbai Maharashtra (Winkworth, 1946; Bhave & Apte, 2011 as *Chromodoris naiki*), Gujarat (Apte et. al. 2010), and Tamil Nadu (Valdés Mollo & Ortea, 1999; Apte & Desai, 2017: as *Chromodoris naiki*), and Goa (Present study).

*Remarks:* This species was first described from Bombay, India, hence, the species name *‘bombyanus’*.

### Family DENDRODORIDIDAE O’Donoghue, 1924

### Genus *Dendrodoris* Ehrenberg, 1831

### *Dendrodoris goani* (Rao & Kumary, 1973)

(Fig. 3.4B)

*Material examined:* BNHS-OPISTHO-1633 (V. no.); Location- Zuari estuary, Vasco da Gama (Goa), 15°25’35.50” N, 73°48’29.06” E, 09-Feb-2017, Habitat- Sandy bottom at 25 m depth, 2 specimens, $L$ =50 and 80 mm.

*External morphology:* Body larger, soft, ovate with the ground colour varying from a light brown, dorsal surface covered with black spots and brownish-black papillated tubercles, some of which mid- dorsally are larger and more raised than the sub-marginal ones. Mantle margin undulating and light-yellow coloured. The posterior end of the dorsal surface has five pairs of pale brown, tripinnate gills which are retractile under stress. Rhinophores short and stout, dark brown coloured and are retractile. The foot on the ventral side has an undulating margin and is pale brown.

*Internal anatomy*: As to confirm the species internally, we dissected the specimen of the *D. goani* and part of the digestive system observed was similar to the description by Rao & Kumary (1973). The digestive system was marked by the absence of the radula and the odontophore, as observed by Rao & Kumary (1973; Fig.4.5). The hermaphroditic reproductive system was well developed with accessory glands. Very few dendrodorids including *D. goani, D. nigra, and D. elongata*. have accessory glands - vestibular gland (Brodie, 2004) The reproductive system seen here is in the consensus of Rao & Kumary (1973; Fig.4.6).

*Ecology:* Feeds on sponges as evident by the examination of the gut and faecal content (Rao & Kumary, 1973).

*Distribution:* Currently recorded only from Goa, India.

*Remarks:* This species was first described by Rao & Kumary, (1973) in Goa, India, and since has not been reported from elsewhere in the world. As the holotype specimen from the museum is missing, we designate this dissected specimen as the neotype.

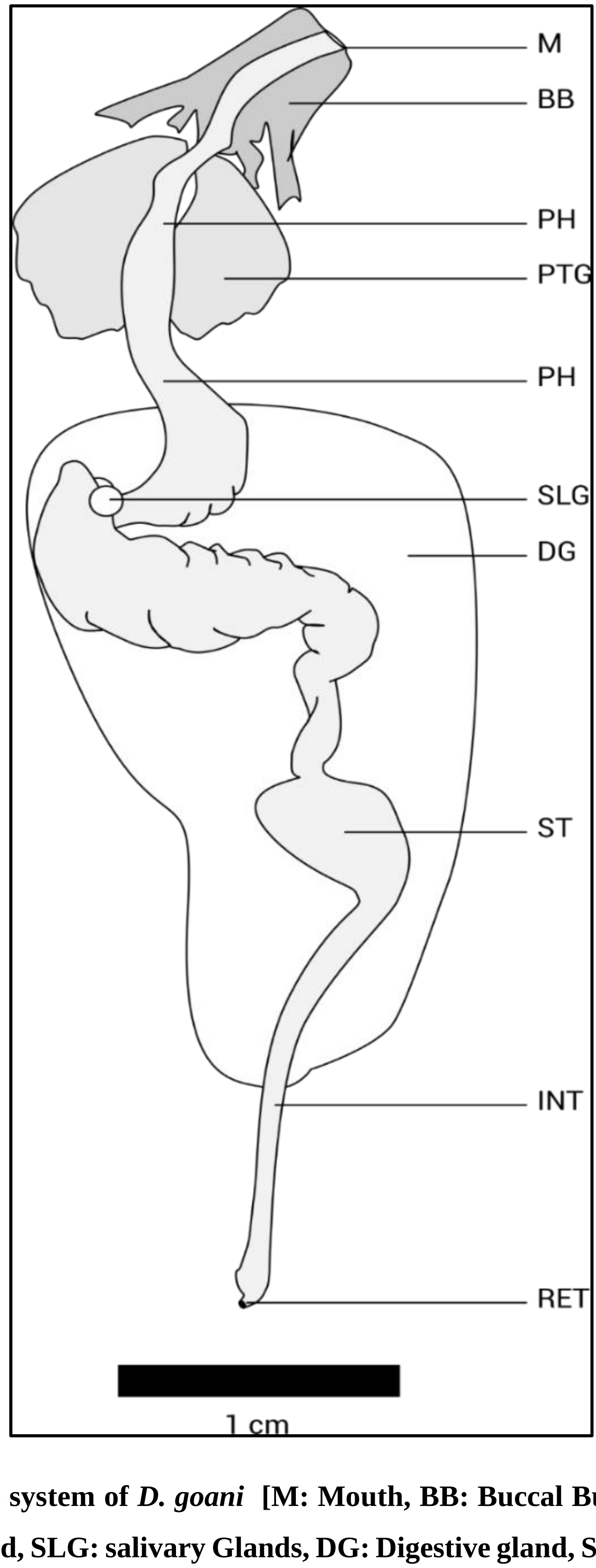


**Fig. 3.5**: **Digestive system of *D. goani* [M: Mouth, BB: Buccal Bulb, PH: Pharynx, PTG: Ptyline gland, SLG: salivary Glands, DG: Digestive gland, ST: Stomach , INT: Intestine, RET: Rectum].**

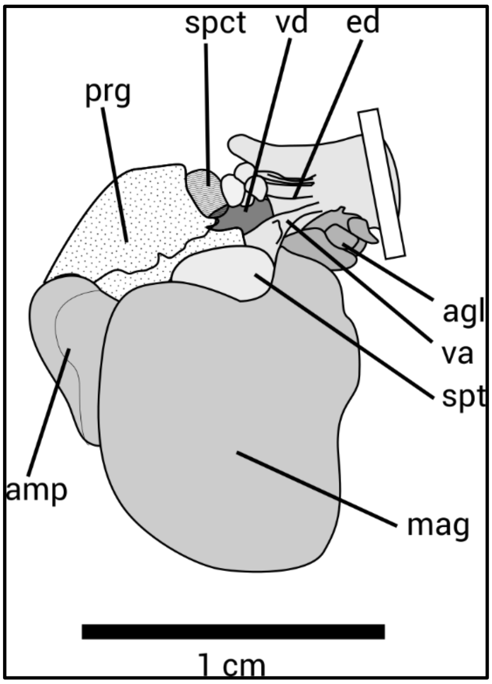


**Fig. 3.6: Reproductive system of *D. goani* [prg: Prostate gland, speciment: Spermatocyst, vd: Vas deference, ed: Ejaculatory duct, agl: accessory/vestibular gland, va: Vagina, spt: Spermatheca, mag: Muco-albuminous gland, amp: Ampulla].**

### Family DISCODORIDIDAE Bergh, 1891

### Genus *Sebadoris* Marcus & Marcus, 1960

### ***Sebadoris fragilis*** (Alder and Hancock, 1864)

(Fig. 3.4C)

*Material examined:* BNHS-OPISTHO-1614 (V. no.), Location- Anjuna beach (Goa), 15°34'5.22"N, 73°44'27.78"E, Date of collection- 20-Dec-2018, Habitat- Tide pool, 1 specimen., *L* = 30 mm.

*External morphology:* Body, large, dorid-form with distinct brown mottling on the foot. Rhinophores are lamellate with 28-30 lamellae, brown mottled with white colour. Gills tripinnate.

*Ecology:* The species is known to feed on sponges.

*Distribution in India*- Ratnagiri (Bhave & Apte, 2011), Gulf of Mannar (Tamil Nadu), (Rao & Kumari, 1974), (Andhra Pradesh), (Alder & Hancock, 1864).

*Global distribution:* Red Sea Djibouti, Gulf of Aden, Tanzania, Madagascar, Réunion Islands, Mauritius, Seychelles, Chagos Archipelago, Sri Lanka, Thailand, Vietnam, South China Sea, Hong Kong, Taiwan, Okinawa, Philippines, Indonesia, Papua New Guinea, Solomon Islands, Marshall Islands, Samoa, Fiji, New Caledonia, Australia,

*Remarks:* Yellow rosette-shaped egg mass about 40 mm in diameter. The species can autotomize large parts of the mantle or sometimes the entire mantle skirt if disturbed repeatedly.

### Family DORIDIDAE Rafinesque, 1815

### Genus *Doriopsis* Pease, 1860

### ***Doriopsis granulosa*** (Pease, 1860)

(Fig. 3.4D)

*Material examined*: BNHS-OPISTHO-1605(V. no.), Location-Anjuna beach (Goa), 15°34'5.22''N, 73°44'27.78''E, Date of collection- 20-Dec-2018, Habitat- Tide pool, 1 specimen., $L$ = 18 mm.

*External morphology:* Body dorso-ventrally flattened; background colour yellow with brown spots encircling blunt uniformly sized tubercles present dorsally. Gills, six, tri-pinnate, transverse; gill openings covered with a mantle flap. Rhinophores simple, lamellate with smooth stalks. Foot narrower ventrally rounded at the tail and grooved anteriorly, smooth, yellow. Mouth small, bulbous without oral tentacles.

*Ecology:* This species is always found associated with a yellow mat-forming sponge.

*Distribution in India:* Ratnagiri, Lakshadweep (Apte & Desai, 2017), and Goa (present study).

*Global distribution:* Widely distributed across the Western Indian Ocean (Madagascar, Seychelles, Réunion Island) to Western Pacific and Central Pacific (Marshal Islands, Hawaii).

*Remarks:* Egg case is in the shape of a flat ribbon, without undulations; circularly laid on substratum in anticlockwise direction with 4-5 circles.

## Family AEOLIDIIDAE

### Genus *Aeolidiopsis* Pruvot-Fol, 1956

### *Aeolidiopsis palythoae* (Gosliner, 1985)

(Fig. 3.4E)

*Material examined:* BNHS-OPISTHO-1610 (V. no.), Location-Anjuna beach (Goa), 15°34'5.22"N, 73°44'27.78"E, Date of the collection- 20-Dec-2018, Habitat-Tide pool, 1 specimen., $L$ = 25 mm.

*External morphology:* Body broader and elongated with visible brown venations which are specialized ramifications of the digestive glands where zooxanthellae are housed. Rhinophores papillated with blunt tips. Both rhinophores and oral tentacles are brown at the base and pale towards the tips. Cerata 6-8 mm long, transversely placed in a single row, translucent, and filled with brown matter from digestive glands along with zooxanthellae. Foot extends laterally with blunt, rounded anterior corners.

*Ecology:* Always found in association with zoanthid sea anemone-*Palythoa* sp. and ingests symbiotic zooxanthellae from feeding on the mucus of the zoanthid.

*Distribution in India:* Ratnagiri (Maharashtra), Gujarat, and Goa (present study)**.**

*Global distribution:* South Africa

*Remark:* The animal can shed its cerata on disturbance. It has a greenish-brown coloured egg case, similar to that of mucous secreted by *Zoanthiniaria and* laid mostly on the *Zoanthiniaria*.

## Family APLYSIIDAE

### Genus *Bursatella* Blainville, 1817

### ***Bursatella leachii*** **(**Blainville, 1817)

(Fig. 3.4F)

*Material examined:* Photographic record (specimen not collected). Location-Bambolim beach (Goa), 15°26'59.10"N, 73°51'6.51"E, Date of collection- 10-Mar-2017, Habitat-Washed ashore on a sandy beach, 1 specimen., *L* = 100 mm.

*External morphology:* Body greenish-brown with dark brown blotches and blue round spots scattered over the dorsal surface; skin covered with numerous long, branching fleshy papillae giving the organism its ragged appearance. Rhinophores long, retractile present on the head with small, vilified edges.

*Ecology: Bursatella leachii* primarily feeds by grazing on cyanophytes and diatom mats or films found on sandy, muddy, and other benthic surfaces.

*Distribution in India:* Maharashtra; Tamil Nadu, Andaman, and Nicobar Island, and Goa (present study).

*Global distribution:* Circumtropical, i.e., found in warm waters around the world.

*Remarks:* A thin shell is present in juveniles but absent in adults and it has large, spaghetti-like benthic egg masses that are usually orange, yellow, green, or brown.

## 3.4. Discussion

At present, there is limited knowledge on the diversity, distribution, and occurrence of sea slug species from Goa. This study, therefore, reports 15 new records of sea slug species and also a re-sighting of an old lost species (*Dendrodoris goani)* that was collected in the 1970s (Rao & Kumary, 1973) from the coastal state of Goa (Table 4.1). Interestingly, just like in the present study, Rao & Kumary also reported *D. goani* as a trawl by-catch near the Dona Paula Bay, indicating their preference for the sandy bottom. Since the original holotype specimens of *D. goani* from the 1973 collection is not traceable and might be missing, hence the new collection is crucial to fill the information gap and can act as a baseline for studying the distribution and biogeography of this species in India. Specimens of *Elysia* spp. were frequently observed in Grande Island, Goa during the underwater surveys, suggesting good reef health conditions. *Scyllaea pelagica* was sighted in two consecutive years (2017-18) during March, attached to the floating rafts of *Sargassum*. This confirms their seasonality and dependence on *Sargassum* (Hickman 1973). *Elysia ornata* and *Oxynoe viridis* of the Sacoglossans group were found in the rock pools as well as in the patchy coral reefs. The genus *Elysia* has posed many taxonomical difficulties and has been placed under complexes like *Elysia marginata-grandifiola* and *Elysia tomentosa-expansa* complex. *E. ornata* (Swainson, 1840) was first described from the Caribbean and has been consistently synonymized with Indo-Pacific, *E. marginata,* and *E. grandiofiola* (Jensen, 1992). However, recent integrative taxonomic work by Krug *et al.* (2013) revealed that the *E. marginata-grandifolia* complex contained four candidate species in the Pacific, all distinct from each other and *E. ornata.* Similarly, the *Elysia tomentosa-expansa* complex with large parapodial flaps and feeding exclusively on *Caulerpa* spp. (except *for E.pratensis*) has at least 6 morphotypes, synonymized with each other. Recently, Bharate et al. (2020) described two new similar colour and pattern looking *Cratena* species (family: Facelinidae) from western India, based on an integrative molecular phylogenetic and morphological approach. Deciphering the individual species of these complexes in Grande Island, would require extensive sample collection from different localities followed by anatomical and integrative molecular taxonomy. The first Indian record of the ragged sea hare, *Bursatella leachi* was from Pulicat Lake, east coast of India (Sethi et. al. 2015), and since then has been sporadically spotted in Andaman & Nicobar Islands and Maharashtra coast (west coast, India) (Apte and Desai, 2017). The present study confirms the distribution of this true pantropic (Bazzicalupo et al. 2018) sea hare species from the Goa coast.

Loss of habitat due to increasing anthropogenic activities like dredging, unsustainable coral reef tourism, coastal pollution, coral bleaching, ocean warming, and introduction of invasive species (Rehitha et al. 2010, Goddard et al. 2013, Hussain & Ingole, 2020, De et al. 2020; Goddard et al. 2016, Molnar et al. 2008) is an increasing threat to the associated marine biodiversity, including sensitive sea slugs. Over the past decade, recreational SCUBA diving activity has increased in the patchy reefs of Grande Island, Goa boosting the local economy, which in turn put the sensitive reefs and the associated marine life at stress (WWF-India, 2019; Hussain & Ingole, 2020). Nimbs & Smith (2016) reported poleward shifts in the distribution of seven species of Heterobranch sea slugs from the subtropical east Australian coast, a climate change hot spot. Some Heterobranch sea slugs are considered invasive (Giacobbe & De Matteo, 2013) which are mostly distributed through the ballast water (Barry et al. 2008) or attached to the bryozoans on the ship’s hull (Ravinesh et al. 2017). However, the information on invasive sea slugs from India is very scanty with few previous records like the invasive umbrella slug- *Umbraculum umbraculum* from Tuticorin, Southeast coast of India (Sankar et al. 2011), invasive winged thecacera- *Thecacera pennigera* (Montagu, 1813) from Kerala, South West coast of India (Ravinesh et al. 2017), and the non-indigenous euryhaline aeolid sea slug *Tenellia adspersa* from the southeast coast of India (Dhanya et al. 2017). Due to the lack of information on the presence and distribution of heterobranch sea slugs from Goa, it is difficult to assess which species in our study are endemic or invasive and to pinpoint their source. Therefore, there is a need to assess the diversity and distribution patterns of these species.

**Table 3.1. Sea slug species collected from Goa region during the present study.**

| S.No. | Species | Habitat |
|---|---|---|
| *1.* | *Haloa pemphis* (Philippi, 1847) | Intertidal rocky reef, in dense algal mass. |
| *2.* | *Smaragdinella sieboldi* (A. Adams, 1864) | Found intertidally in crevices, dead oyster shells, empty polychaetes tubes. |
| *3.* | *Elysia ornata* (Swainson, 1840) | Rocky reef with algal mat. |
| *4.* | *Oxynoe viridis* (Pease, 1861) | Rocky reef (attached to *Caulerpa sertularioides*) |
| *5.* | *Scyllaea pelagica* (Linnaeus, 1758) | *Sargassum* raft. |
| *6.* | *Pleurophyllidiella paucidentata* (O'Donoghue, 1932) | Attached to azooxanthellate octocoral *Carijoa rissei.* |
| *7.* | *Berthellastellata* (Risso, 1826) | Under boulder - tidal pool; Rocky reef. |
| *8.* | *Phyllidia marindica* (Yonow and Hayward, 1991) | Rocky reef |
| *9.* | *Hallaxa*cf. *albopunctata (*Gosliner & Johnson, 1994) | Under boulder - tidal pool; rocky reef. |
| *10.* | *Goniobranchus bombayanus* (Winckworth, 1946) | Rocky reef. |
| *11.* | *Dendrodoris goani* (Rao & Kumary, 1973) | Sandy bottom (Subtidal). |
| *12.* | *Sebadoris fragilis* (Alder and Hancock, 1864) | Under boulder - tidal pool; Rocky reef. |
| *13.* | *Doriopsis granulosa* (Pease, 1860) | On Soft coral - *Carijoa* sp. |
| *14.* | *Aeolidiopsis palythoae* **(**Gosliner, 1985) | On zoantharian - *Palythoa* sp. |
| *15.* | *Bursatella leachi* **(**Blainville, 1817) | Washed on the shore |

## 3.5. Conclusion

This study contributes to increasing the knowledge of Heterobranch sea slug diversity of Goa; however, it is still very low as compared to their diversity from India (611 species), and Western India (264 species). Goa has a similar habitat ranging from sub-tidal areas to rocky intertidal pools as other coastal regions like Ratnagiri and Malvan, therefore, there is a very high likelihood of finding more sea slug species with an increase in intra-annual surveys and more long-term monitoring from this region. This study also sheds light on the biodiversity of the patchy reef of Grande Island and points towards its protection as an ecologically sensitive area. This study would also act as a baseline for the diversity of seas slugs in Goa, to identify the ecologically sensitive spots, which in turn would help to monitor the invasion of Heterobranch sea slugs in the future as well as to assess the shift in the distribution pattern of these highly sensitive sea slug species to climate change.

# *Chapter 4*

# Climate change-induced coral bleaching

## 4.1. Introduction

Coral reefs are under major threat due to the combined effects of natural and anthropogenic stressors at regional and global scales (Bellwood et al. 2004; Gardner et al. 2003; Wilkinson et al. 2008; Burke et al. 2011). An increase in ambient temperature above the coral's tolerance level for several weeks leads to thermal stress. Consequently, the symbiotic zooxanthellae are expulsed causing coral bleaching (physiological bleaching; Berkelmans & Willis, 1999; Fitt et al. 2001; Reaser et al. 2000; Glynn & D croz, 1990). The increase in sea surface temperature during the past few decades has caused unprecedented mass bleaching worldwide, including the three major episodes in 1998, 2010, and 2015/16 (Heron et al. 2016).

Even though the corals have the capability to recover upon the return of normal conditions, they may undergo mortality if the corals get plagued with diseases during the bleached condition (Berkelmans & Willis, 1999; Eakin et al. 2010; Miller et al. 2009). Declines in corals after bleaching events have long-term ecological, economic, and social impacts. It reduces the local fishery production (Rogers et al. 2009; Graham et al. 2007), dents the tourism industry (Pratchett et al. 2011; Mallela et al. 2010; Doshi et al. 2012) as well as leads to changes in benthic habitat (Munday et al. 2008). The timing of the bleaching peak varies between different ocean basins and hemispheres but it generally occurs during midsummer. Near the equator, there are two additional potential bleaching "seasons" following the equinoxes (NOAA, 2013).

Remote sensing-derived data has been widely used for coral reef management, monitoring, and research, as they have proven to be cost-effective, cover a large area, and are easily disseminated (Mumby et al. 2004; Green et al. 1996). Predictive models based on remote sensing data enable scientists and reef managers to forecast future conditions and mitigate potential reef damage (Hedley et al. 2016). In the last few decades, the National Oceanic and Atmospheric Administration (NOAA)-Coral Reef Watch (CRW) Program has been successfully delivering global thermal stress products via its web-based Decision Support System (DSS) (Brandt et al. 2013; Eakin et al. 2009, 2010; Liu et al. 2003, 2006, 2012 and 2013). CRW launched a daily global 5 km product suite in May 2014 (Liu et al. 2014). This product is based on the NOAA/NESDIS operational daily 5 km geostationary and polar-orbiting blended night-only SST analysis (Liu et al. 2014). The satellite-derived SST forms the core of products such as HotSpot, Degree Heating Week, Bleaching Alert Areas, and Satellite Virtual Stations (Liu et al. 2014). The Hotspot technique forms the basis of the "Degree Heating Week" (DHW) product, which is a measure of accumulated heat stress over an area over the past three months (Strong et al. 2006). One DHW is equivalent to one week of HotSpot staying at 1°C or 0.5 weeks of HotSpot at 2°C above the thermal threshold of any given area. The thermal threshold temperature is defined as 1°C above the maximum monthly mean (MMM) temperature (Glynn & D Cruz 1990), where MMM is the mean temperature of the warmest month in a seasonal cycle. This DHW product indicates which reefs are at risk of bleaching and CRW integrates the information from HotSpot and DHW into the Bleaching Alert Area product (Liu et al. 2008). This product provides a 95% cover of all coral reefs in the world and summarises the available data into the presence or absence of bleaching, stress level, and severity of bleaching (Table 4.1).

**Table 4.1. NOAA Coral Reef Watch stress level based on HotSpot and DHW (Degree Heating Week) techniques.**

| **Stress level** | **Definition** | **Potential bleaching intensity** |
|---|---|---|
| | | |
| No stress | HotSpot ≤ 0 | *No bleaching* |
| Bleaching Watch | 0< HotSpot <1 | |
| Bleaching Warning | 1≤ HotSpot and 0< DHW < 4 | *Possible bleaching* |
| Bleaching Alert Level | 1≤ HotSpot and 4≤ DHW < 8 | *Bleaching likely* |
| Bleaching Alert Level | 1≤ HotSpot and 8≤ DHW | *Mortality likely* |

An early forecast of coral bleaching events is becoming an effective tool for the management of coral reefs and provides sufficient time for planning the assessment and monitoring of the ecological impacts of coral bleaching (Maynard et al. 2009). This led to the development of CRW's four-month coral bleaching Thermal Stress Outlook product in Feb 2015. With recent advancements in 5 km products with higher spatial resolution, CRW developed 212 experimental 5 km Regional Virtual Stations. South Asia has 10 such stations including the Gulf of Kutch and Western India (NOAA: CRW, 2013). The Thermal Stress Gauge uses these Virtual Stations to provide information regarding observed bleaching status as well as to forecast the bleaching.

Although India has a coral reef cover of about 5790 km$^2$ (Raghuraman et al. 2013), research on the long-term impact of bleaching and specific management strategies is alarmingly scarce (De et al. 2017). Scattered coral growth has been reported along with certain inter-tidal belts and submerged banks both on the east and west coasts of India (Muley et al. 2002). The Grande Island archipelago (15°21' N and 73°46' E) comprising St. George and Grande islands located in the eastern Arabian Sea is a relatively understudied coral patch ecosystem. To date, there are only three published reports on hard corals (Rodrigues et al. 1998; Manikandan et al. 2016; Hussain & Ingole, 2020) and one on reef fishes (Sreekanth et al. 2015). The coral reefs at Grande Island provide a natural habitat for numerous benthic organisms as well as breeding, feeding, and shelter ground to a rich diversity of reef fish and shellfish species (Sreekanth et al. 2015).

Therefore, following the bleaching alerts issued by CRW, the present study was conducted to survey the coral patches and assess whether the CRW prediction can be used in the future to assess the coral reef bleaching along the Indian coast. If the predictions of CRW are correct, one would expect to find severe bleaching of corals in the study area. Therefore, objectives of the present study are (1) to examine the extent and severity of coral bleaching and compare it with CRW bleaching products for validation in the study region (virtual station-Western India), (2) to assess differential bleaching and bleaching susceptibility in different coral species, and 3) to assess the changes in the benthic community after the bleaching event in terms of sponge and macroalgae prevalence.

## 4.2. Materials and methods

### 4.2.1. Study area

For the present study, two sites - a shallow zone and a deeper zone (Fig. 4.1) - were selected near St. George Island, at a distance of ~150 m from each other. Site 1, the shallow zone (2-4 m), was located at 15.352°, 73.770° to 15.354°, 73.763° comprising rocky substrate dominated by Porites spp. Site 2, the deeper zone (4-8 m) was located at 15.353°, 73.770° to 15.355°, 73.764° comprising mixed rocky/sandy substrate dominated by *Turbinaria* sp. and *Goniopora* spp. interspersed with whip corals. These patchy reefs in Western India are characterized by high turbidity and the absence of branching corals and have been reported to be susceptible to diseases (Hussain et al. 2016).

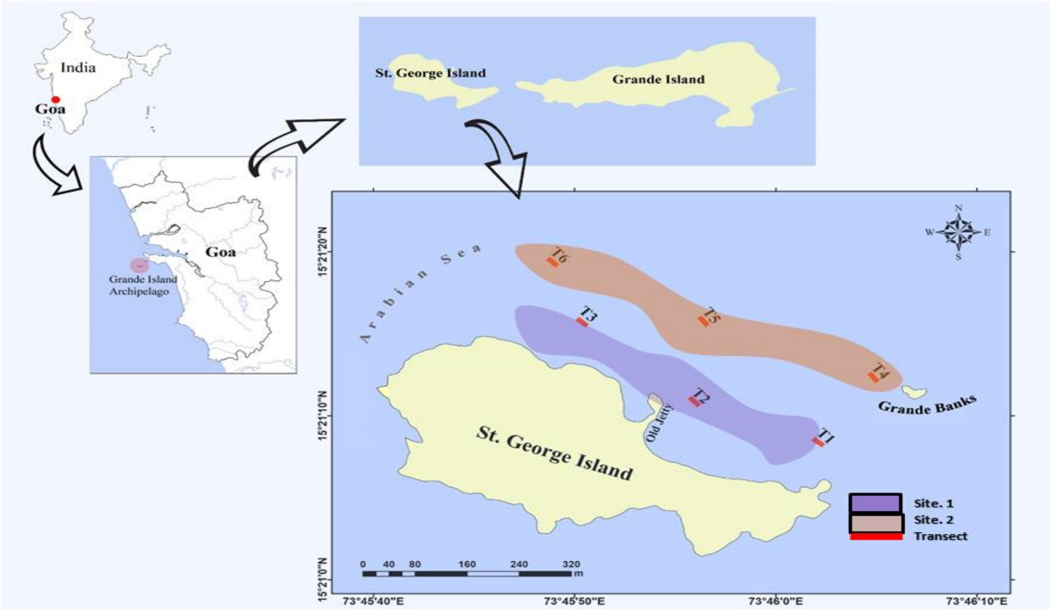


**Fig. 4.1: Study sites for bleaching monitoring with transects at St. George Island, Goa.**

### 4.2.2. Underwater survey for bleaching assessment

As part of the ongoing coral and associated biodiversity study of CSIR-National Institute of Oceanography, Goa, field surveys were conducted between October 2014 and April 2016 in Grande Island using SCUBA with line transects (10 m, triplicate) and belt transects (20 X 2 m, triplicate) to study the coral composition in the study area and the number of colonies bleached was also counted. CRW NOAA issued bleaching threats and alerts on 2$^{nd}$ June for the period of Oct-Nov 2015. Therefore, an intensive field survey and examination was conducted in November 2015, to estimate the severity and extent of bleaching across different genera. Following the method of Hill & Wilkinson (2004), belt transects of 20 m X 2m were laid in triplicate at both sites. The transects were laid parallel to the shore along the water depth contour of 2-4 m in site 1 and 4-8 m in site 2 at a distance of ~100 m from each other. All the coral colonies falling within the belt transect were enumerated and digital photographs and videography were done using a Nikon AW130 (14 Mega Pixels) and a GoPro Hero4 (12 Mega Pixels) camera. Coral colonies were identified to genus level according to Veron (2000) and standardized using the World Register of Marine Species (WoRMS Editorial Board, 2017). A qualitative assessment of “beaching severity” was done for each colony following an index developed by Hill & Wilkinson (2004). The following categories were used: (0) no bleaching evident; (1) partially bleached (surface/tips); or pale but not white; (2) completely bleached, (3) bleached with partial recent mortality defined by Marshall & Baird (2000) as an absence of living tissue with minimal algal overgrowth, and 4) complete recent mortality. The coordinates were obtained using a Garmin GPSMAP 78S and the distances between the sites were calculated.

### 4.2.3. Benthic community change monitoring

Sponge infestation over coral colonies was monitored in the deeper station (site 2) in Grande Island for three years from 2015-17 by deploying a triplicate belt transect (10m X 2) and quantifying the percent prevalence of bio-eroding sponge *Cliona thomasi* various coral colonies. To monitor the long-term effect of coral bleaching on reef benthic communities, triplicate 10 m long line transects, each with three 1 X 1 m quadrats (divided into 16 small cells) were laid in the deeper station (station 2) of Grande Island from 2015-2017. Underwater observation and photography were done of each cell of the quadrat, seasonally (pre and post-monsoon), percentage of live coral, dead coral, and rubble, macroalga, turf algae, and other substrates (sand, rock, sponge) was noted for each quadrant to assess the phase shift in coral reefs.

### 4.2.4. Climatology and bleaching alerts

Regional-scale accumulated thermal stress (DHW), and HotSpot values were obtained from the United States NOAA Coral Reef Watch’s daily 5 km coral bleaching thermal stress product. In western India, 5 km - Bleaching Thermal Stress Gauge was used to monitor coral bleaching four months in advance (http://coralreefwatch.noaa.gov/vs/gauges/western_india.php). These gauges are based on CRW's 5 km 7-day maximum bleaching alert area composite product. Five years of real-time daily SST data obtained from AVHRR was retrieved from the CSIR-NIO Live access server to study the climatology of the area (http://las.nio.org).

### 4.2.5. Data analyses

The average percentage of colonies of each genus falling under each bleaching class was calculated per transect. This was done to eliminate the chance of the abundance of coral colonies in each transect influencing the pooled averages per transect. Mean bleaching in the study area was the prevalence of bleached corals i.e., the average percent of colonies bleached in terms of number. The average prevalence of sponge infestation was calculated by taking the average of the triplicate transects assessing the sponge encrustation on various coral genera. Further, the average percentage of various benthic substratum (live coral, rubble, macroalgae, and turf algae) was calculated per transect by averaging the data obtained from triplicate 1m quadrats.

### 4.2.6. Statistical analyses

One-way ANOVA was performed to assess the significance of the difference in bleaching response among different coral genera followed by the Tukey post-hoc test. Mann-Kendal test was conducted to assess the trend analysis of SST for five years (2011-15) in the study region. All the statistical analyses were performed using Statistica 2.0.

## 4.3. Results and discussion

The 2014-2017 global bleaching event, caused due to the combined effect of global warming and the strong El Nino of 2015-16, was the longest and most extensive on record (NOAA) and led to widespread bleaching and coral mortality (NOAA, 2018; Fig.4.2).

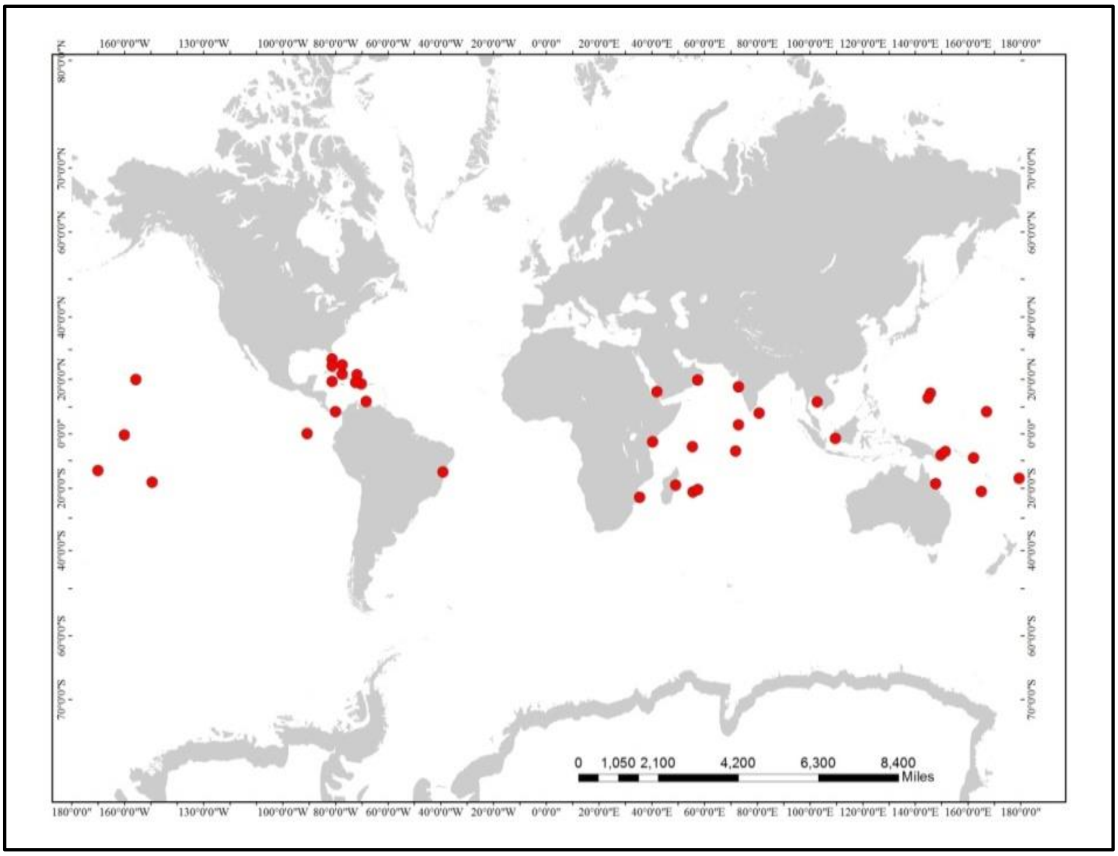


**Fig. 4.2: Map showing bleached reefs during 2014-2016 (Data source: Gomez et al. 2016). The red circle indicates (●) bleached sites.**

Western India started showing early signs of coral bleaching in Oct-Dec 2014 (De et al. 2015) which reached an extensive level during 2015 (Diraviya Raj et al. 2018 and this study). In Lakshadweep, bleaching began in December 2015 which worsened by April 2016 (Table 5.2). There have been reports of coral bleaching in all major reefs of India during this period, making it one of the worst bleaching episodes ever recorded (Table 4.2).

**Table 4.2 Coral bleaching in Indian reefs during 2014-2016.**

| Region | Time | Extent of bleaching | Method used | Source |
|---|---|---|---|---|
| **Malvan Marine Sanctuary, Maharashtra** | Oct-Jan 2014 | ~Mean 15% | *Belt transects (50 m X 2 m); percent incidence* | De et al. 2015 |
| **Malvan Marine Sanctuary, Maharashtra** | December 2015 | ~75% | *Belt transects (20 X 2 m); percent incidence* | Diraviya Raj et al. 2018 |
| **Lakshadweep Islands** | Bleaching started in Dec 2015, worsened in April 2016 | - | *Percent incidence* | Dasgupta 2016 |
| **A reef in Kavaratti Island, Lakshadweep** | Dec 2015-April 2016 | 87 % of individuals | - | |
| **Vaan Island, Gulf of Mannar** | March, 2016 | 9.7% of live corals | *Percent incidence* | http://www.thehindu.com/news/national/tamil-nadu/Climate-change-triggers-coral-bleaching/article14238730.ece |
| **Koswari Island, Gulf of Mannar** | | 12.1% of live corals | - | |
| **Kariyachalli Island, Gulf of Mannar** | | 10.4% of live corals | - | |
| **North Bay, Andaman** | April 2016 | - | *Method not described* | Mohanty et al. 2017 |
| **Gulf of Mannar** | Upto May, 2016 | Mean 33% | *Belt transects (20 X 4m); Percent incidence* | Patterson et al. 2017 |
| **Palk Bay** | Upto May, 2016 | Mean 12% | - | |
| **Grande Island, Goa** | Nov-Dec, 2015 | Mean 50.5 % | *Hill & Wilkinson (2004)* | Present study |

### 4.3.1. Temperature data

Five years plot of the SST in the study area showed a rise in temperature over the years 2011-2015 (Fig. 4.3). The Mann-Kendal test showed a significant positive trend (p =< 0.0001). The analysis was conducted at a 95% confidence level (alpha=0.05). The average temperature of 2015 (28.98 °C) was 0.54 °C higher than in 2011 (28.38 °C). During 2015, the average SST during January-March was low (28.38°C), which began rising during April and reached the highest in May. The average SST during the summer months (April-June) was recorded as 29.63 °C. With the onset of the southwest monsoon, the temperature started dropping and reached an average of 28.58 °C during the period of July-September. Post- monsoon period (October-December) again showed a rise in SST, reaching an average of 29.34 °C. The current bleaching was recorded during this period of accumulating heat stress and rising SST.

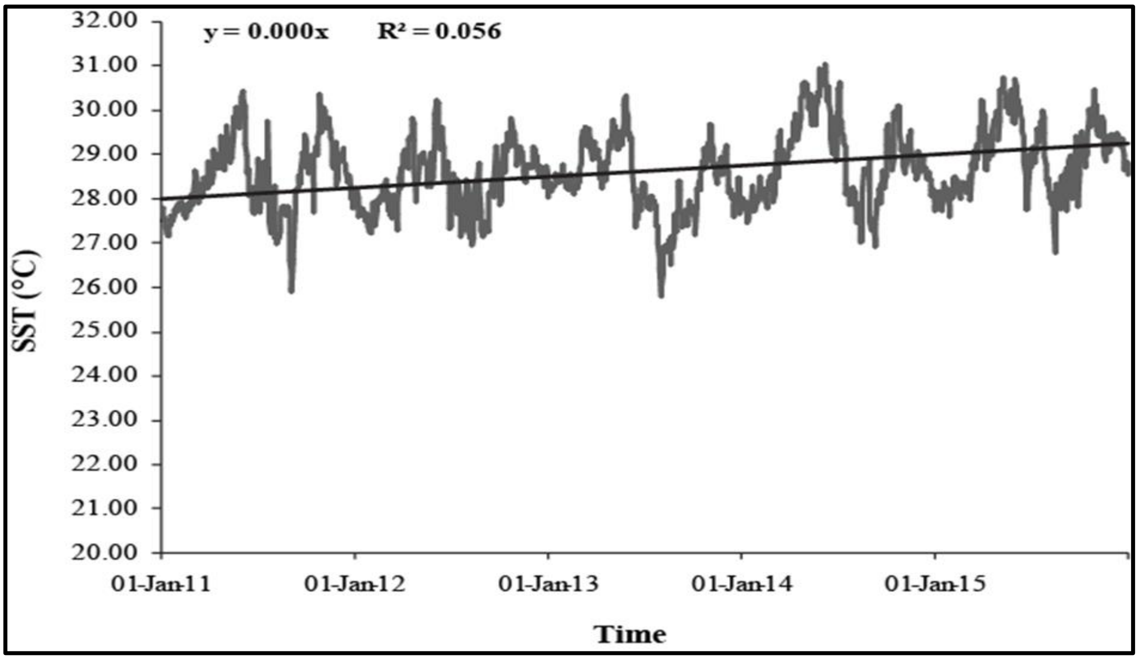


**Fig. 4.3: Graph showing the temporal SST trend at the study site.**

### 4.3.2. Bleaching prevalence

Surveys during October 2014 revealed mild, 6%, and 4 % bleaching in site 1 and 2 respectively; 7 % and 5.34 % in site 1 and 2 respectively in April 2015 (Fig. 4.4) which increased to 51 % in site 1 and 50 % in site 2 during November 2015. Surveys during April 2016 revealed alive colonies of *Porites* undergoing recovery with mean bleaching of 7% and 8 % in site 1 and 2 respectively (Fig. 4.4) and an increase in turf algae, macroalgal, and rubble composition from mean 17.88 % in November 2015 to mean 35.83% in April 2016 in the study area (pers. observation).

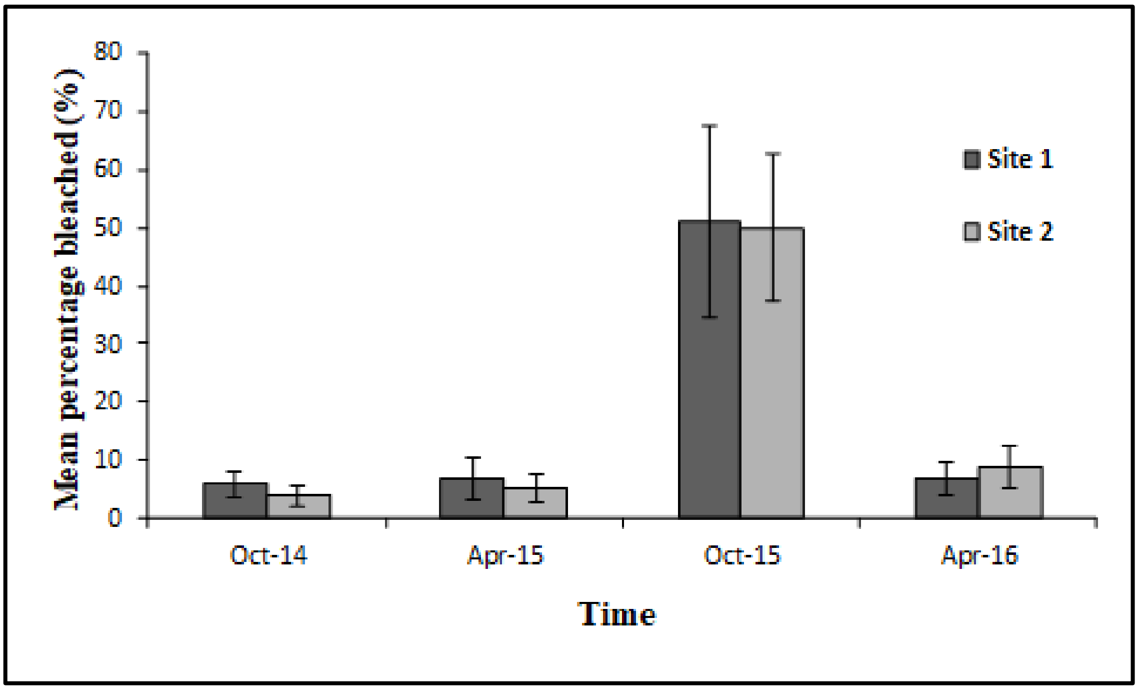


**Fig. 4.4: Mean percentage (%) of bleached corals (Mean ± SD) at sites 1 and 2 from October 2014 to April 2016 (Hussain & Ingole, 2020).**

### 4.3.3. Benthic community structure

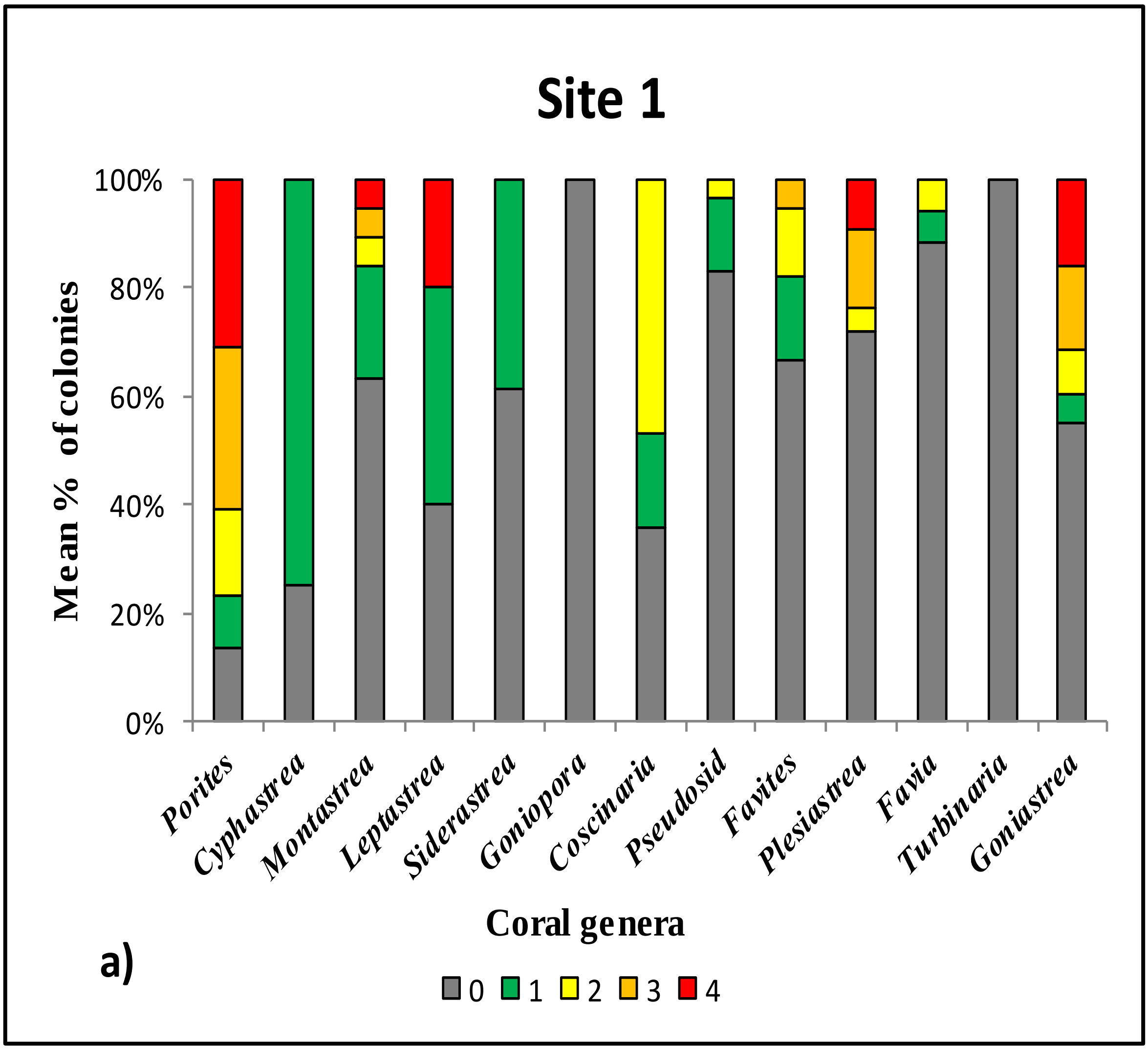
Line intercept transects during October 2015 revealed that *Porites* is the most dominant genus in site 1 (15%) whereas, site 2 is dominated by *Turbinaria* colonies (30.62%) followed by *Goniopora* (21.75%) (Table 4.3). Site 1 had a higher percentage of rock and rubble. Site 2 had intermittent sandy patches with whip corals. Macroalgal and encrusting sponge infestation were higher in site 1.

**Table 4.3. Percentage composition of different benthic communities in the study area.**

| | **Average % cover** | |
|---|---|---|
| **Substratum** | **Site 1** | **Site 2** |
| **Rocks** | 9.33 | 2.25 |
| **Sand** | 12.33 | 5.25 |
| **Rubble** | 11.00 | 12.5 |
| **Sponge** | 3.00 | 0.25 |
| **Turf algae** | 2.00 | 1.75 |
| **Macroalgae** | 6.00 | 2.50 |
| ***Porites*** | 15.00 | 2.50 |
| ***Siderastrea*** | 4.50 | 0.25 |
| ***Favites*** | 8.67 | 1.5 |
| ***Plesiastrea*** | 4.67 | 1.75 |
| ***Goniastrea*** | 1.67 | 5.00 |
| ***Dipsastraea*** | 1.77 | 1.13 |
| ***Leptastrea*** | 0.83 | 0 |
| ***Psuedosiderastrea*** | 0.83 | 0.5 |
| ***Coscinaria*** | 3.50 | 4.25 |
| ***Cyphastrea*** | 1.83 | 1.00 |
| ***Montastrea*** | 2.67 | 0.50 |
| ***Goniopora*** | 1.00 | 21.75 |
| ***Turbinaria*** | 9.40 | 30.62 |
| **Octocorals** | 0.00 | 4.75 |
| **Total** | 100 % | 100 % |

### 4.3.4. Differential bleaching

For Site 1, *Porites* and *Coscinaria* were most susceptible to bleaching, *Leptastrea, Montastrea, Cyphastrea,* and *Goniastrea* moderately so, and remaining genera relatively resistant (Fig. 4.5a, 4.6). In contrast, in Site, 2, *Porites*, *Cyphastrea*, *Montastrea*, and octocorals were most susceptible and *Goniastrea* and *Coscinaria* moderately so with remainder relatively resistant (Fig. 4.5b, 4.6). *Turbinaria* spp. was the least affected coral in both the sites and was in a relatively healthy state.



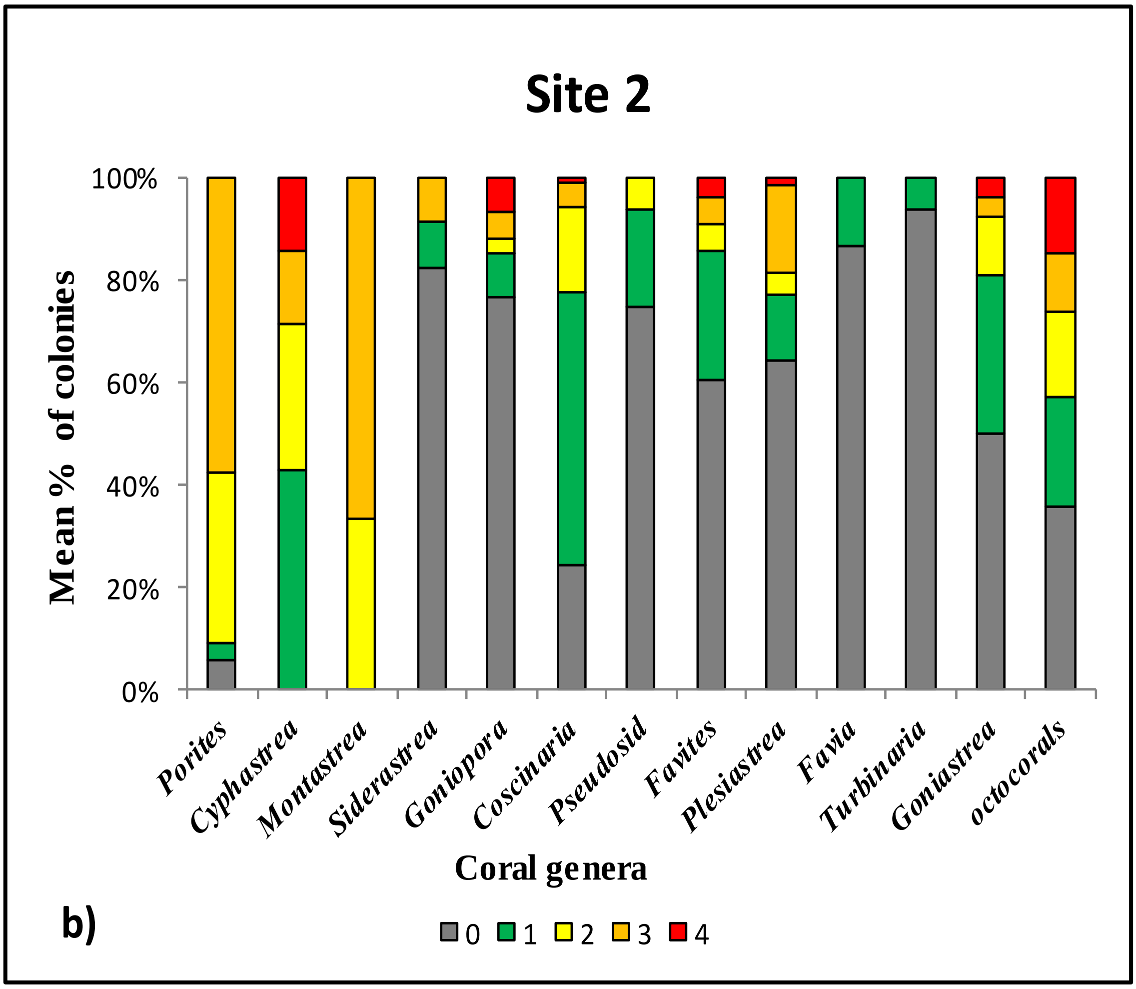


**Fig. 4.5: Mean percentage (%) of corals at site 1-(a) and site 2-(b) in various classes of bleaching severity, where (0) no bleaching evident; (1) partially bleached (surface/tips); or pale but not white; (2) completely bleached, (3) bleached with partial recent mortality, and 4) complete recent mortality (Hussain & Ingole, 2020).**

Coral bleaching severity varies in space and time as a consequence of the severity of thermal stress (Kleypas et al. 2008), irradiance (Dunne & Brown, 2001), symbionts clade (Baker et al. 2004; Berkelmans & Oppen, 2006) species composition (Marshall & Baird, 2000), local factors like turbidity and cloud cover (Mumby et al. 2001), and thermal history of the area (Thompson & Woesik, 2009).

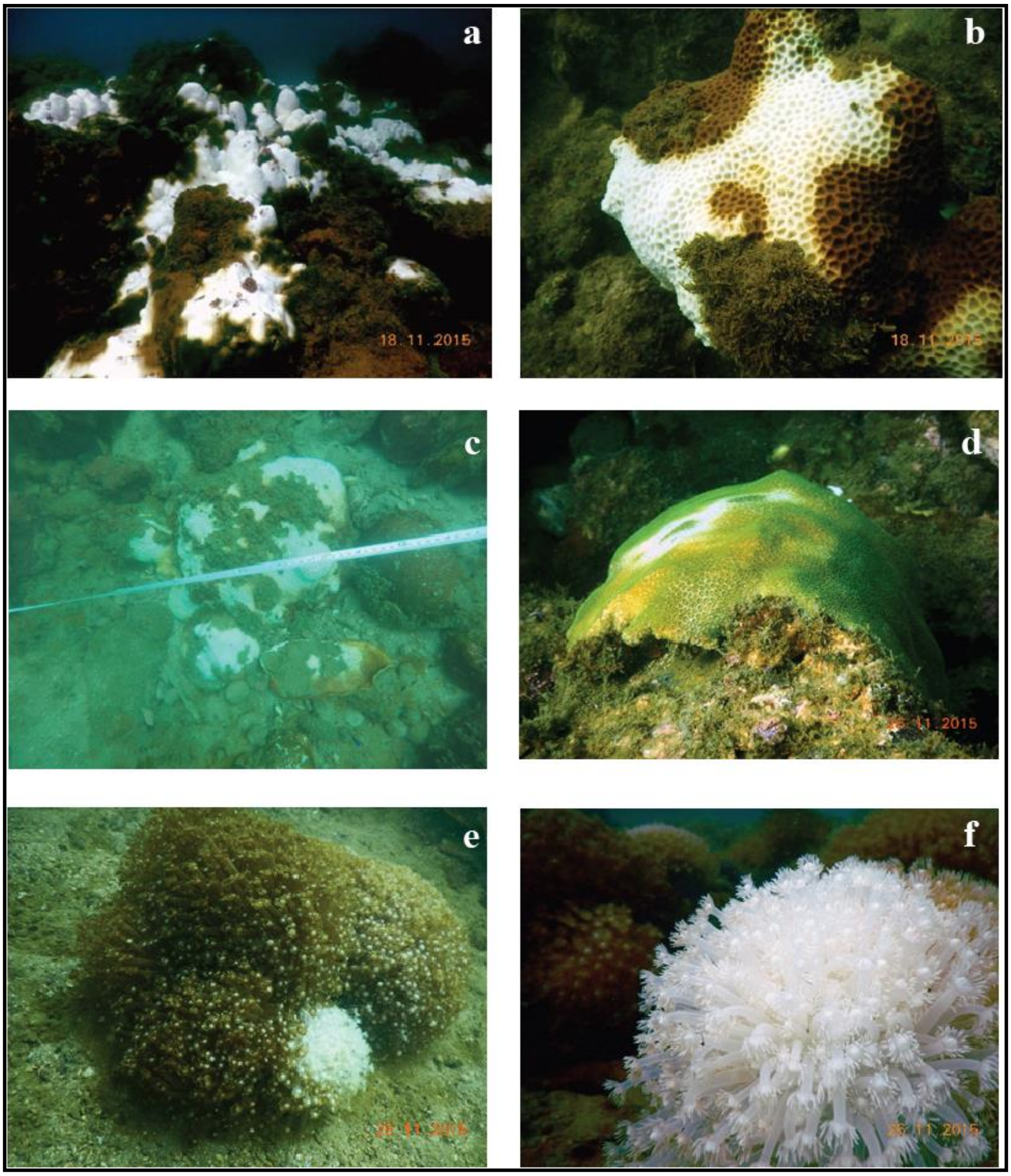


**Fig. 4.6: Bleached corals during 2015 bleaching event; a) completely bleached *Porites* sp. in site 1. b-c) bleached *Favites* sp. d) partially bleached *Plesiastrea* sp. e-f) partially and completely bleached *Goniopora* colonies in site 2 (Hussain & Ingole, 2020).**

### 4.3.5. Bleaching susceptibility

The average percentage of bleached colonies varied among the different coral genera (Fig. 4.7). *Porites* spp. was observed to be most susceptible to bleaching (19.8% n=55; Fig. 4.7). This was followed by *Goniopora* spp. (16.5 %, n= 13) and *Coscinaria* spp. (13.3% n= 37). The foliose coral *Turbinaria* spp. and slow-growing corals like *Dipastraea* spp. and *Leptastrea* spp. were observed to be less susceptible to bleaching. One-way ANOVA revealed a significant difference in bleaching amongst the various coral genera (p=0.002) in terms of percent colonies bleached. Tukey HSD post-hoc test revealed that Porites was significantly more bleached *Cyphastrea* (p=0.018), *Montastrea* (0.011), *Pseuodsiderastrea* (p=0.015), *Dipsastraea* (p=0.008), *Turbinaria* (p=0.006) and *Leptastrea* (p=0.006).

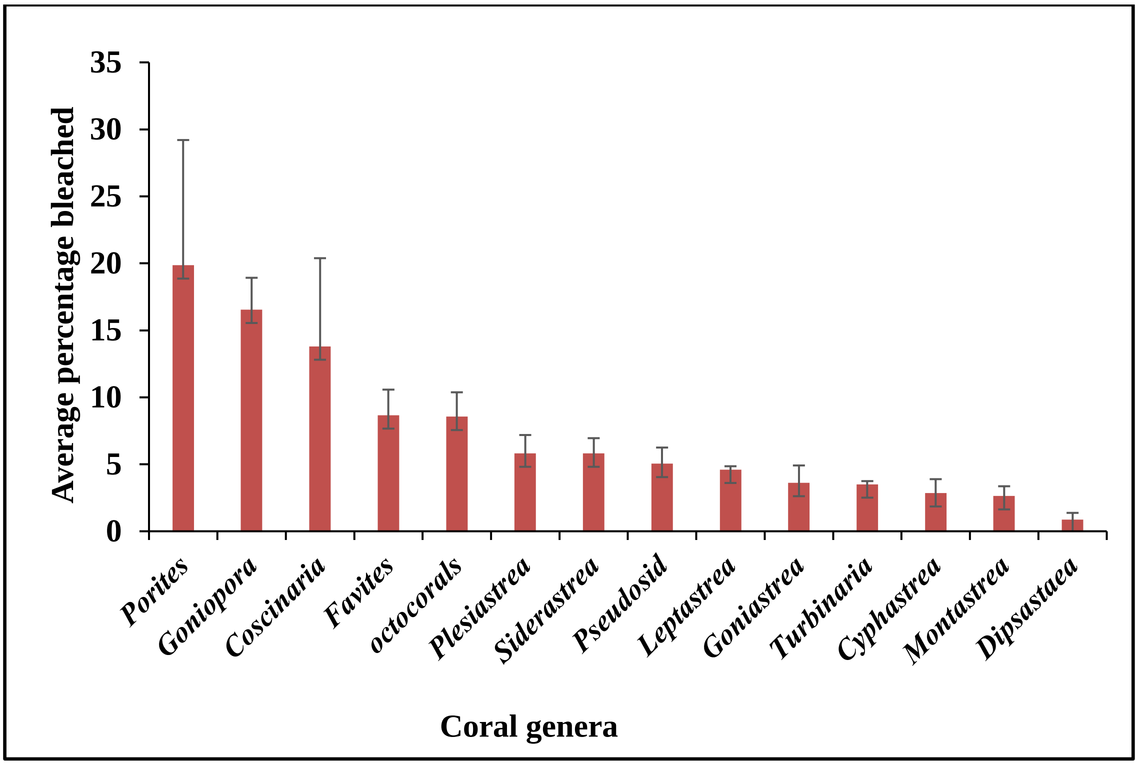


**Fig. 4.7: Bleaching susceptibility (Mean + SD) in different genera at St. George Island. (*Pseudosid= Pseudosiderastrea*).**

Coral bleaching severity varies in space and time as a consequence of the severity of thermal stress (Kleypas et al. 2008), irradiance (Dunn & Brown, 2001), symbionts clade (Baker et al. 2004; Berkelman & Oppen, 2006) species composition (Marshall & Baird, 2000), coral morphology (Loya et al. 2001) and local factors like turbidity and cloud cover (Mumby et al. 2001) and thermal history of the area (Thompson & Woesik, 2009). Loya et al. (2001) found that small colonies of acroporids survived better than the larger colonies of acroporids after mass bleaching in Okinawa. Similar results were seen in the Java Sea (Hoeksema, 1991) and the Mediterranean Sea (Shenkar et al. 2005). In this study, *Porites* was severely affected which is also the most dominant genus in the turbid shallower site. *Porites* are otherwise considered to be highly resistant species to bleaching (Guest et al. 2012). However; Joshi et al. (2014) observed delayed recovery in *Porites* after the 2002 bleaching event in the Gulf of Kutch. It is possible that the corals had bleached during the heat stress of summer (May-June) of 2015 and were undergoing recovery but were hit by another round of bleaching episodes in Oct-Nov 2015. As highlighted by Hughes et al. (2017), the critical point for coral reef recovery is the frequency of recurrent disturbances and the availability of sufficient time for the corals to recover and reassembly of mature coral assemblages.

It was interesting to note that the colonies of *Cyphastrea* and *Montastrea* underwent higher bleaching in the deeper site as compared to the shallow site. This contravenes the general expectation that corals in the shallow area would have higher thermal stress leading to more severe bleaching as compared to deeper corals. The general belief of depth being a coral refuge has been recently challenged by the work of Neal et al. (2013), Bongaerts et al. (2009); Penin et al. (2007), where they observed more severe bleaching in deeper corals as compared to shallow corals. Neal et al. (2013) recorded strikingly higher coral bleaching at 10 m depth in the colonies of *Siderastrea siderae* and

*Stephanocoenia michelini* as compared to the colonies at 1-3 m at Bocas del Toro, Panama and concluded this anomaly due to higher temperature, Hotspot and DHW in the deeper waters. It has also been postulated that this could be due to variation in coral-symbiont association in conspecific corals at different depths (Gleason & Wellington, 1993; Rowan & Knowlton, 1995). *Turbinaria mesenterina* colonies recorded here were least susceptible to bleaching and were in a healthy state and corroborates with Marshall & Baird (2000) who stated that the genera *Turbinaria* are highly resistant to bleaching. Differential bleaching susceptibility amongst different coral taxa may lead to ecological changes by affecting the potential of species to adapt to thermal stress. This may lead to a natural section of the more resilient taxa (Baird & Maynard, 2008). The recovery time of fast-growing corals and good colonizers like *Turbinaria* after extirpation is usually 10-15 years (Connell et al. 1997, Kayanne et al. 2002, Gilmour et al. 2013), but the replacement of long-lived species like *Porites* may take decades (Hughes et al. 2017). This progressively leads to a phase shift in coral assemblage structure, as it has been observed in severely bleached reefs of the Northern Great Barrier Reef (Hughes et al. 2017) where reefs previously dominated by *Acropora pulchra* which underwent massive bleaching in 1998 are still dead and have transitioned into muddy rubble dominated area.

### 4.3.6. Sponge infestation after the mass bleaching event

Sponge surveys revealed a higher prevalence of sponge after the bleaching event as observed during 2015-17. The highest infestation was noted in *Turbinaria* colonies, which is the also most dominant coral genera in the study site (Fig. 4.8- 4.9). Interestingly, *Turbinaria* corals were resilient to bleaching in the study site (Fig. 4.7), but were more prone to sponge infestation, probably due to their foliose morphology, which provides a higher surface area for the sponge to spread.

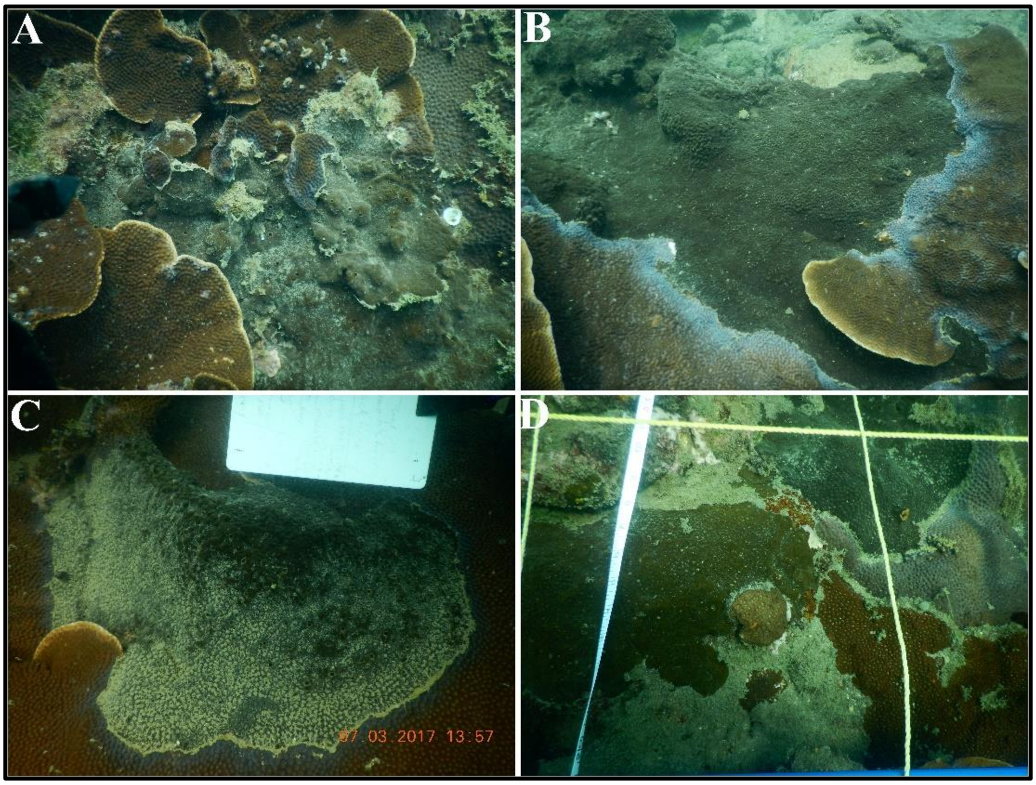


**Fig. 4.8: Sponge infestation in Grande Island, Goa. A-C): *Cliona thomasi*, a bio-eroding sponge infestation on *T. mesenterina* colonies. D) Sponge infestation on other corals like *Favites* and *Goniastrea*.**

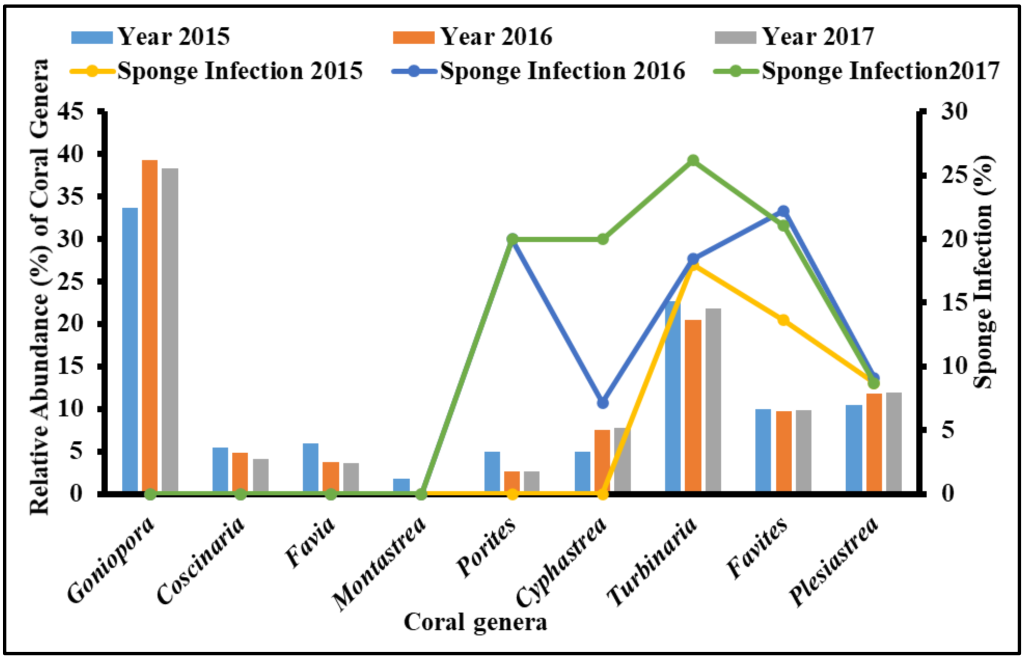


**Fig. 4.9: Sponge infestation in the various coral genera in the study site (Mote et al. 2021).**

### 4.3.7. Benthic community changes after the mass bleaching event.

The reef witnessed seasonal changes in benthic communities which were exacerbated by the bleaching event. With a continuous decline in the percentage of live coral, a sharp increase in the coverage of macroalgae and rubble was observed from 2015-17 (Fig 4.10-4.12).

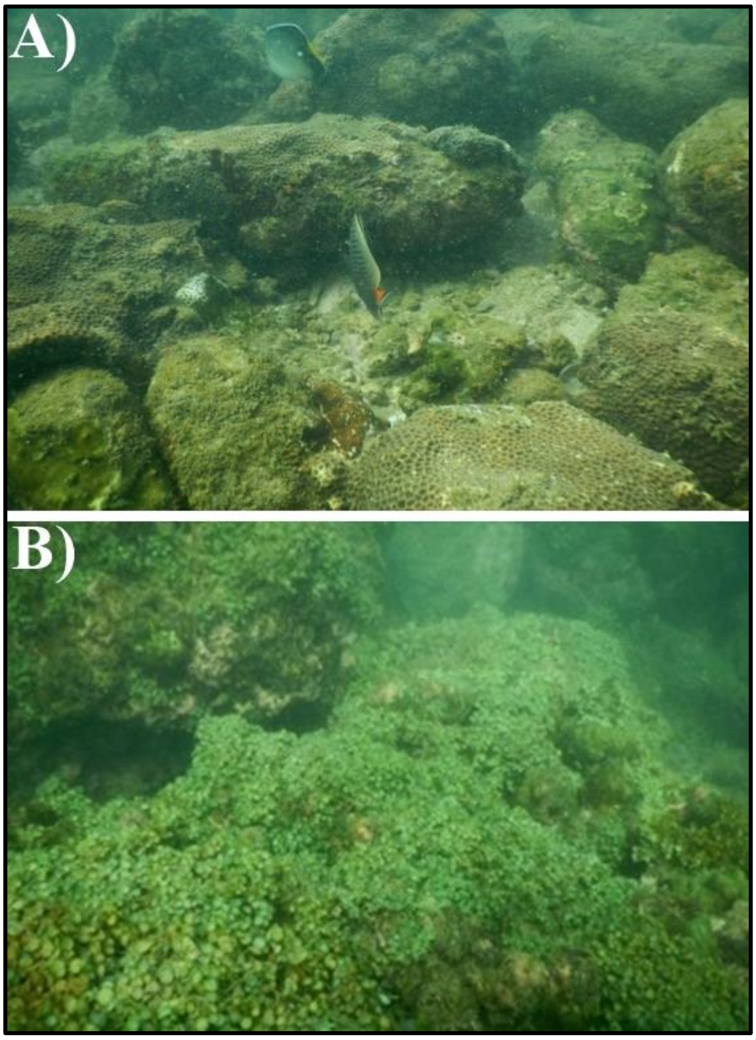


**Fig. 4.10: Increase in macroalgal coverage after the bleaching event, A) reef area during pre-bleaching (April 2015), B) reef area post-bleaching event (April 2017).**

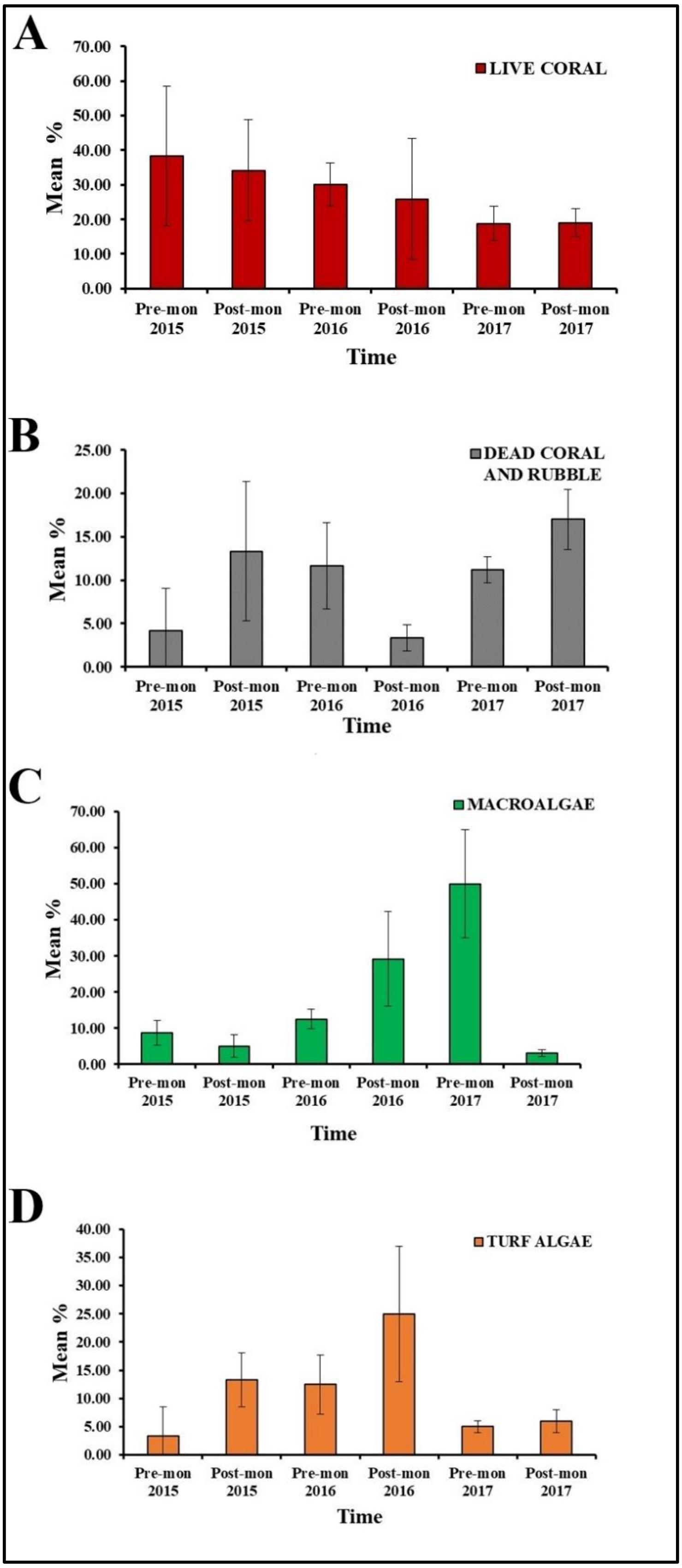


**Fig. 4.11: Benthic community changes after the mass bleaching event from 2015-17 in benthic communities such as A) Live coral, B) Dead coral and rubble, C)**

**Macroalgae, and D) Turf algae (Pre-mon = Pre-monsoon period and Post-mon =Post monsoon).**

This can be explained by the increase in the percentage of rubble after the coral mortality observed during the bleaching event of 2015. As the corals are dying, they get infiltrated by space competitors like sponge and macroalgae leading to a subtle phase shift in the reef from coral dominance to sponge and macroalgae dominated reefs (Hughes et al. 2017). Long-term studies are needed in the study area to observe the changes in coral community structure over time.

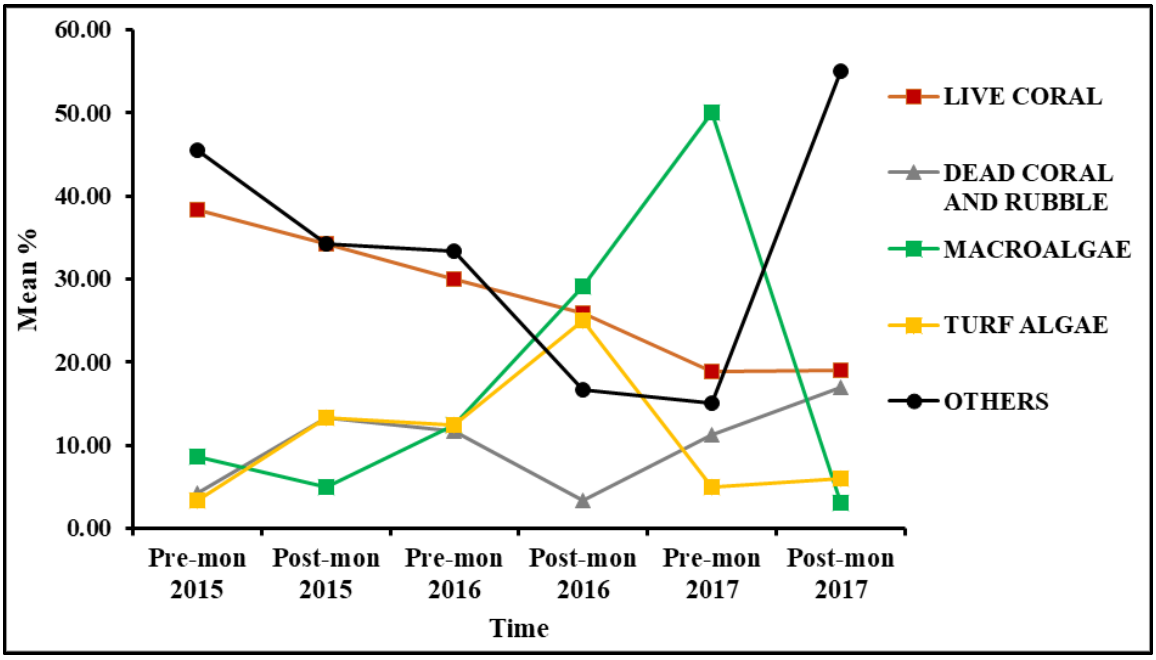


**Fig. 4.12: Changes in the benthic community from 2015-17 in terms of percentage coverage of various substratum (Pre-mon = Pre-monsoon period and Post-mon =Post monsoon).**

### 4.3.8. CRW's bleaching products for the study area

Coral bleaching thermal stress (60% Probability) for Sep-Dec 2015 (Fig. 4.13) revealed that western India including the Gulf of Kachchh was at alert level 1 stress.

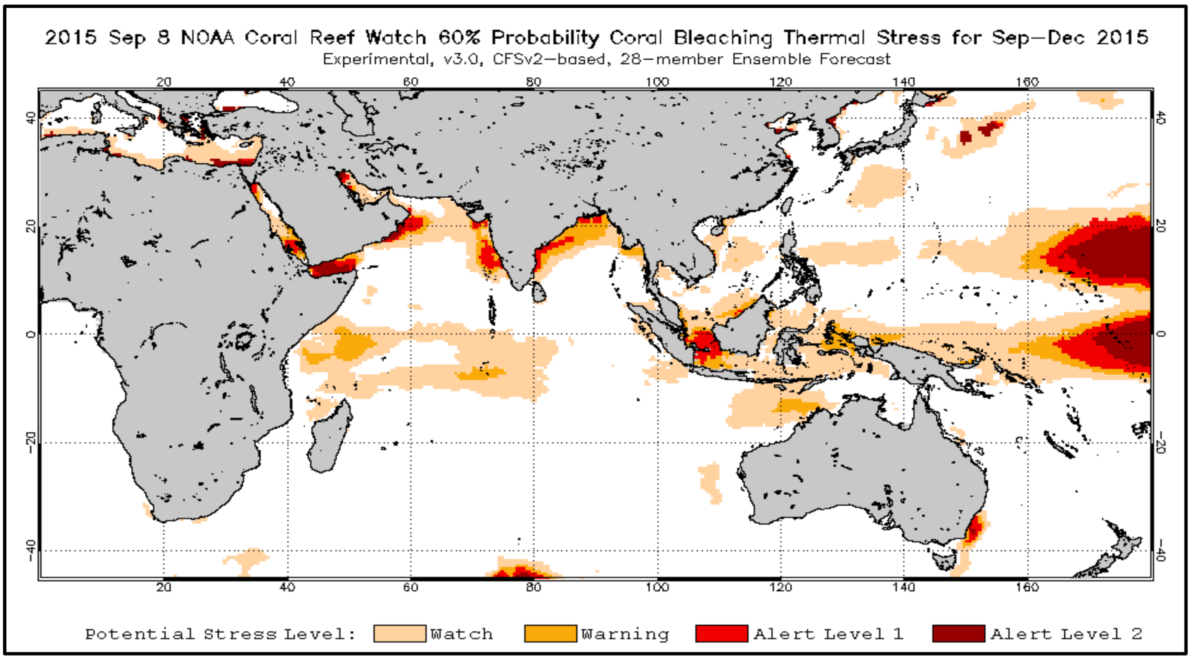


**Fig. 4.13: CRW's 60% probability of coral bleaching thermal stress (Source: NOAA CRW).**

Western India Satellite bleaching alert in June 2015 indicated that western India (Virtual station) was under an alert level 2 (Fig. 4.14a). The outlook product for September 2015 shows accumulating stress in the study area and from week 5 to 12 the study area was under an alert level 1 (Fig. 4.14b).

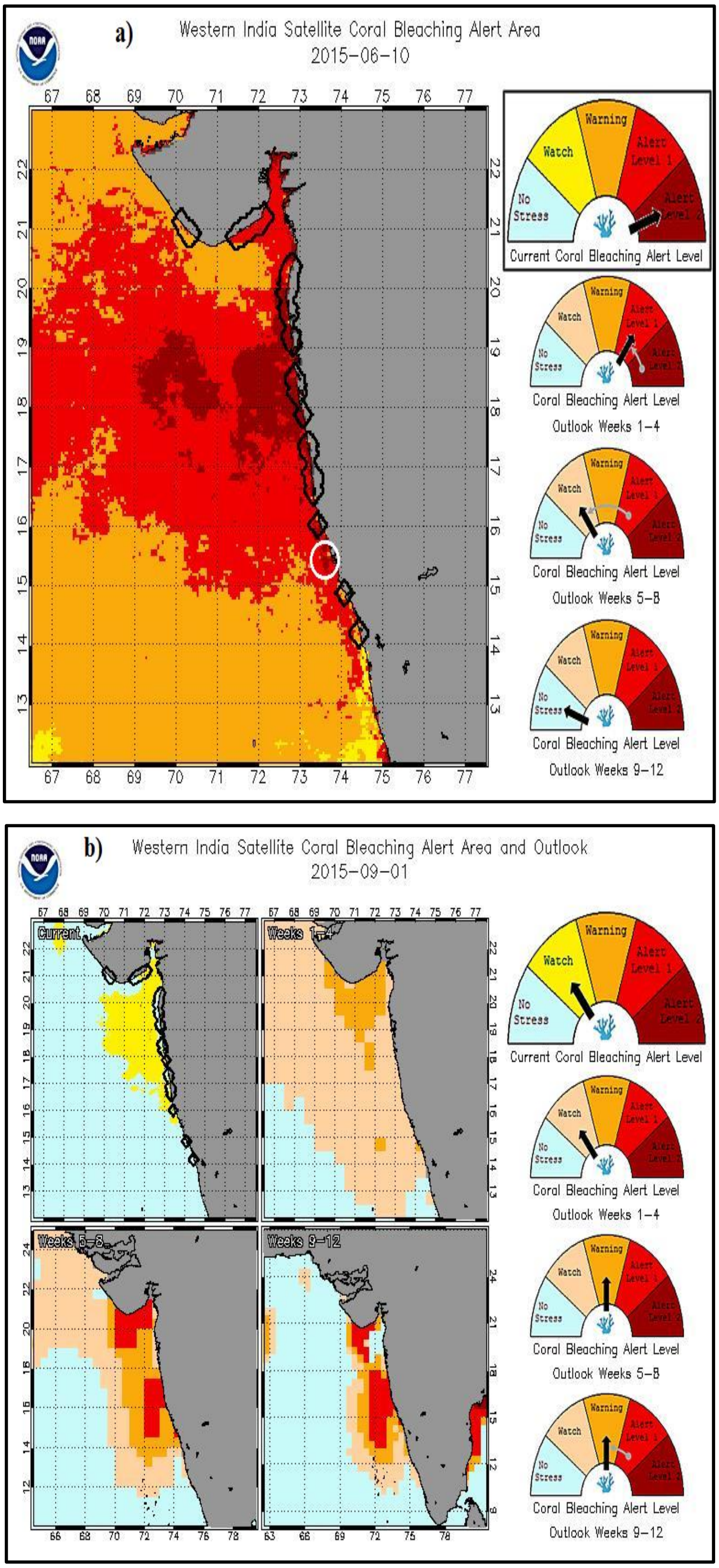


**Fig. 4.14: a) Western India Satellite bleaching alert area. ○ denotes study area, and b) Alert area and outlook product (Source: NOAA CRW).**

The virtual station (Western India) time series graph for 2 years period (2014-15) gives information regarding changing DHW with changes in SST (Fig. 4.15).

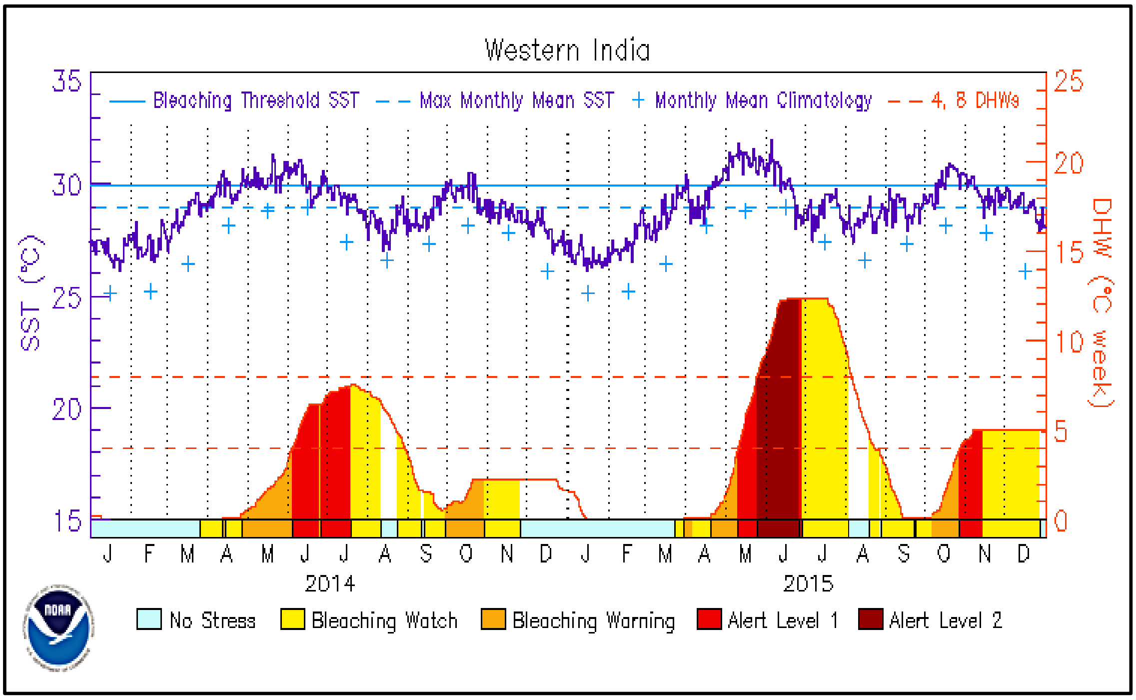


**Fig. 4.15: CRW's time-series graph representing the change in thermal stress and subsequent bleaching alert for the Virtual Station (Western India) Source: NOAA CRW.**

It revealed that during June-July 2014, the DHW had crossed the DHW threshold values of 4- degree Celsius-weeks triggering alert level 2. However, during the summer of 2015, the DHW started accumulating and crossed the threshold values of 8-degree Celsius-weeks triggering alert level 2. With the fall in temperature, DHW also started to decrease during the post-monsoon period of 2015 before again accumulating and crossing the threshold in Oct-Nov 2015 putting the area under stress level 1. It could be possible that corals had already bleached during May-June 2015 and were on the course of recovery during monsoon (July-September, 2015) but they were again hit by a heatwave weakening the corals further and leading to widespread bleaching and mortality in Oct-Dec 2015.

Fig. 4.16 denotes the trend in bleaching observations (mean %) in western India with the changing DHW. During the post-monsoon of 2014, the bleaching was and 5 % in St. George Island. During Pre-monsoon 2015, average bleaching in St. George Island was low (6.17%) with few affected colonies.

With accumulating DHW crossing the threshold in post-monsoon 2015, the bleaching also became widespread with ~50.5% mean bleaching. With a drop in DHW and SST post-December 2015, average bleaching of 7.9 % was observed during April 2016 in this study area with *Porites* undergoing bleaching recovery.

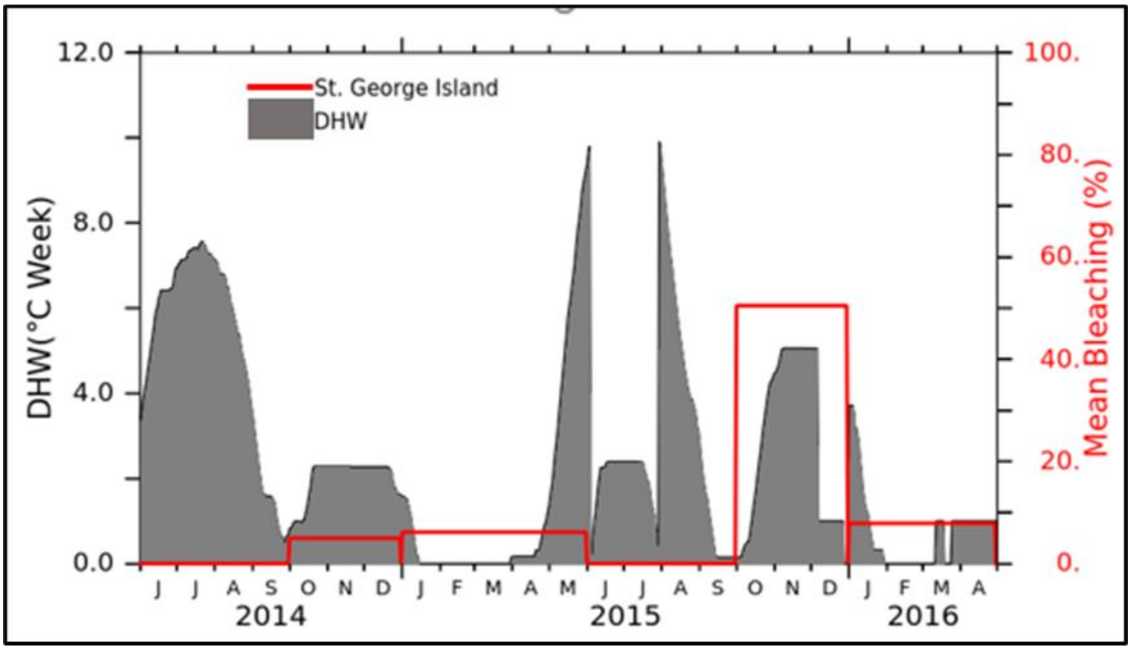


**Fig. 4.16: Trend in bleaching observed (mean %) in the western Indian coast with the changing DHW (Hussain & Ingole, 2020).**

## 4.4. Conclusion

The results of the present study replicate the predictions of CRW products and can be a good tool for future coral reef bleaching monitoring in the study region. The marked differences in bleaching susceptibility amongst various coral taxa with the observed increase in the sponge, macroalgae, and rubble prevalence here imply a possibility of community phase shift towards a reef with sponge and macroalgae dominance with successive disturbances and illustrate the need for long-term monitoring of the coral community. Additionally, these findings imply a need for a comprehensive study of local depth-stratified temperature records which would improve our understanding of the observed pattern of coral bleaching response in the coral community at different depths.

# *Chapter 5*

## Coral diseases- Skeletal tissue growth anomalies

### 5.1. Introduction

The past four decades have witnessed an estimated 80% loss of the coral coverage in the Caribbean (Gardner et al. 2003) and 50% loss in the Indo-Pacific (Bruno & Selig, 2007). According to the report, Reefs at risk revisited (Burke et al. 2011), 75% of the global reefs are currently at risk due to global and local stressors and 90% would be lost by 2030. Coral diseases are considered to be one of the major factors for the global decline of living coral cover and for the shifts in coral community structure (Harvell et al. 1999; Rosenberg & Loya, 2004; Patterson et al. 2002). A case of the potential damage caused by coral diseases is best exemplified by the successive disease outbreak in the Caribbean leading to ecological phase-shift from coral-dominated to the algal-dominated ecosystem (Aronson & Precht, 2001; Sutherland et al. 2004). Another example is the white band disease outbreak on *Acropora palmata* and *Acropora cervicornis*, which led to an estimated 95% loss in coral colonies in the Florida Keys during the 1980s (Vollmer & Kline, 2008).

So far, 18 coral diseases have been identified of which the causative agent is known only for 6 diseases (4 bacterial, 1 viral, and 1 fungal) (Ritchie, 2006; Rosenberg et al. 2007). Geiser et al. (1998) isolated the fungus *Aspergillus sydowii* responsible for the mass mortality of sea fan corals (*Gorgonia ventalina*) in the West Indies. A breakthrough in understanding coral diseases came with the identification of the bacteria *Vibrio shiloi* as the causative agent of bleaching in scleractinian coral *Oculina patagonica* in the Mediterranean Sea (Rosenberg & Falkowitz, 2004). The dearth in the knowledge of the disease etiology and pathogenesis is due to our poor understanding of the complex interaction between the coral host, pathogen, and the environment.

The success of coral reef depends on the complex yet a delicate network of interactions between the coral host, the algal symbiont, and the plethora of symbiotic members of the coral holobiont including bacteria, viruses, archaea, fungi, and endolithic algae (Rohwer et al. 2001, 2002; Olson et al. 2009; Ritchie et al. 2006). Coral hosts distinct bacterial populations in 3 habitats namely, 1) the surface mucous layer (SML), 2) coral tissue comprises of the epidermis and gastrodermis layers, and 3) the $CaCO_3$ skeleton. It is estimated that the mucopolysaccharide-rich surface mucus layer harbours $10^5$ – $10^6$ bacterial colony forming units per ml (Ducklow & Mitchel, 1979; Koren & Rosenberg, 2006), which is much greater than the bacterial count in the surrounding seawater by 100-1000-fold (Rosenberg et al. 2007). It was also observed that the abundant bacterial species in coral mucus (SML) are distinct from the abundant species in coral tissue, even though the total bacterial counts are similar (Bourne & Munn, 2005; Koren & Roserberg, 2006). This specific association is further highlighted by the presence of similar bacterial communities in the same coral species, even when separated by geographic distance (Rosenberg et al. 2007). The endolithic community of $N_2$ fixing cyanobacteria in the coral skeleton is estimated to fulfil 50 % of the nitrogen needs of the coral (Ferrer & Szmant, 1988) and may satisfy major nutrient requirements during periods of bleaching. The high density of archaea (>$10^7$ cells per $cm^2$ of coral surface area) does not seem to have a specific association with corals and the information on their biological role is also very scanty (Rosenberg et al. 2007). The research on the diversity and functional role of coral viruses is still in its nascent stage with difficulties in techniques for culturing marine viruses. The shift in the equilibrium of this complex coral holobiont can compromise coral's health, leading to the onset of diseases

(Bourne et al. 2009). Therefore, there is an urgent need to understand the diversity, function, and community structure of the members of the corals holobiont (Bourne et al. 2009).

A combination of standard biomedical tools like veterinary sciences, immunology, ecology, and molecular genetics along with new emerging tools is essential for understanding the etiology and pathogenesis of coral diseases (Bourne et al. 2009). Culture-free methods like metagenomics, transcriptomics, and microarrays have shown success in understanding the dynamics of the coral holobiont. For example, it was only recently that Thurber et al. (2008) with the aid of metagenomics and real-time PCR approach reported the generation of herpes-like viral particles in *Porites compressa* on exposure to heat stress. Sunagawa et al. (2009) successfully applied the tool of high-density 16S rRNA approach to document the changes in bacterial community associated with Caribbean coral *Montastrea faveolata* showing signs of white plague disease type II.

Growth anomaly is a poorly understood disease affecting corals all over the Indo-Pacific and Caribbean (Gateno et al. 2003; Harvell et al. 2007; Work et al. 2008). Unfortunately, due to the lack of consistency in the description and unknown causative agents, there is a lack of understanding of this disease, which has led to confusion in coral disease literature (Work & Aeby, 2006). Coral ‘tumors’ or skeletal tissue growth anomalies (STAs) are spherical or irregular-shaped growths on the coral skeleton. They are characterized by their overgrown corallites (Burns et al. 2011) and easily recognized by their characteristic shape, size, and by their lack of normal zooxanthellae pigmentation (pathological bleaching) (Cheney, 1975; Bak, 1983). The affected tissue has fewer polyps, reduced zooxanthellae (Domart-Coulon et al. 2006; Cheney, 1985; Peters et al. 1986), reduced fecundity (Stimson, 2011). Tumor formation is an energy-draining process as observed by depletion of lipid storage in the affected corals and reduction in the growth of healthy parts of the affected colony rendering the corals more prone to bleaching (Yamashiro et al. 2001).

Globally STAs have been reported on hard corals ranging from Hawaiian Islands (Squires, 1965), Palmyra Atoll, Central Pacific (Williams et al. 2011), Heron Island of the Great Barrier Reef, Australia (Haapkyla et al. 2010), the Philippines (Kaczmarsky & Richardson, 2011), Indo-Pacific (Aeby et al. 2011), Hawaii (Stimson, 2011 and the Gulf of Oman (Coles & Seapy, 1998). This study is the first report of STAs from the Indian coast.

Despite being reported from various coral regions, STAs' etiology has not been fully understood. Some of the hypotheses suggest STA formation may be a result of genetic or environmental factors or a combination of both (Coles & Seapy, 1998). It has been reported that some corals develop tumors in response to an attack by foreign organisms including crustaceans, trematodes, polychaetes (Wielgus et al. 2002), algae (Morse et al. 1981), and endolithic skeleton-boring filamentous fungi (Le Campion-Alsumard et al. 1995). According to Peters et al. (1986) and Coles & Seapy (1998), ultraviolet radiation-induced cell damage is a potential mechanism leading to growth anomalies in corals. The severity may be related to exposure to environmental stressors such as light, high levels of sedimentation (Riegl et al. 1996), turbidity, seasonal temperature extremes (Peters et al. 1986), poor water quality, and high nutrients (Bruno et al. 2003). There is immense potential to apply the emerging molecular genetics tools and culture-free methods to understand the mechanisms by which the environment and the pathogen interact with the corals leading to the formation of coral tumors.

This study investigates the STAs from the foliose coral *Turbinaria mesenterina* (Hexacorallia: Scleractinia: Dendrophyllidae) which dominates the shallow coral communities of the Central West Coast of India. STAs have been an understudied coral disease in India; therefore, our work aims to improve the understanding of STAs of *Turbinaria* corals. The objectives of the present work were three-fold, 1) To describe the gross morphology of skeletal tissue growth anomalies (STAs) in the foliose coral *Turbinaria* *mesenterina* observed in our study area, 2) To estimate the prevalence and distribution of these STAs in MMS, and 3) To investigate the difference in bacterial communities between healthy and STA infected *Turbinaria mesenterina* colonies using a metagenomics approach.

## 5.2. Tumor characterization

### 5.2.1. Study area- Malvan Marine Sanctuary (MMS)

The MMS is a Marine Protected Area (MPA) and nearshore coral reef, with an area of 29.122 km$^2$ located between 16° 02’N−16° 03’ N and 73° 25’ E−73° 29’ E (Fig. 5.1) – the only protected reef along the Central West coast of India.

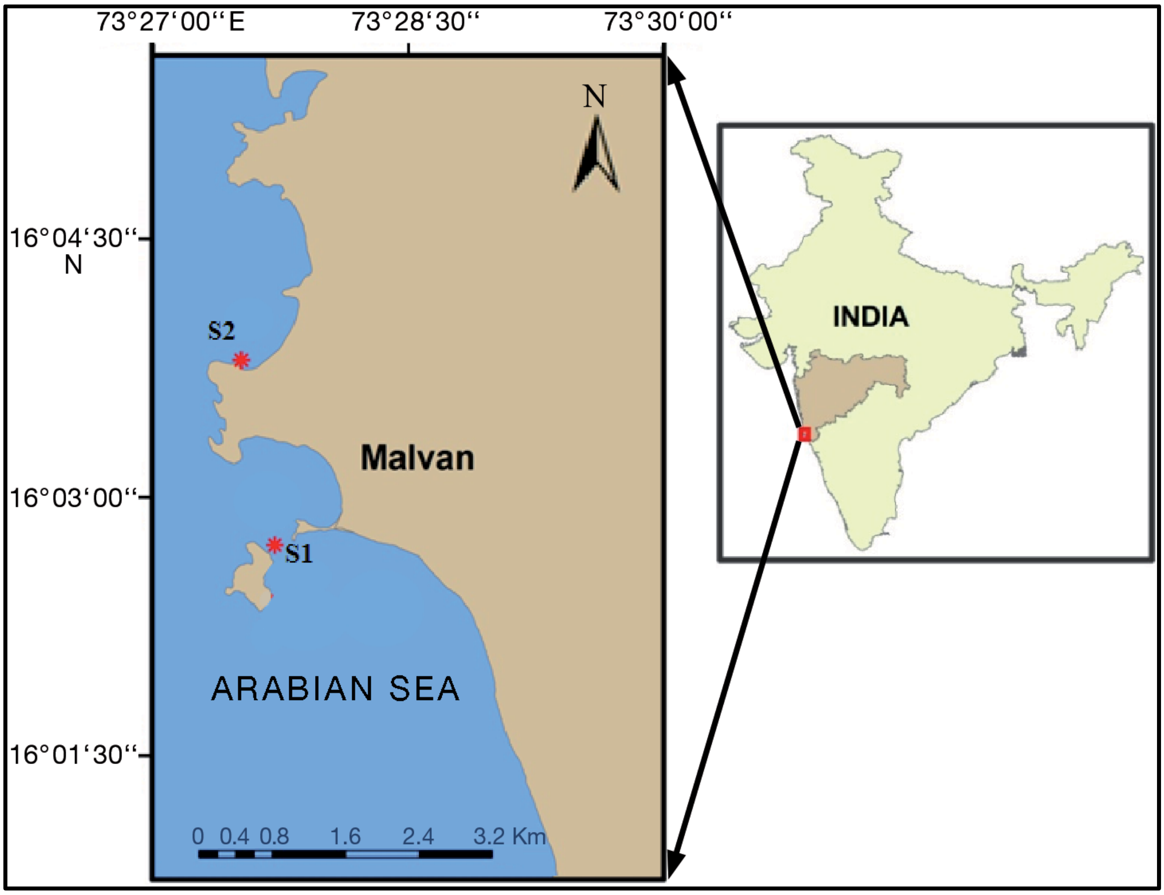


**Fig. 5.1. Location of Malvan Marine Sanctuary. S1 & S2: Study Sites 1 & 2, respectively (Hussain et al. 2016).**

The Grande Island archipelago (15˚ 21’ N and 73˚ 46’ E) comprising St. George and Grande islands located in the eastern Arabian Sea is a relatively understudied coral patch ecosystem.

Two sites were selected randomly along a distance gradient of 2 km in each study area to study the prevalence of STAs. Both the study areas are composed of rocky substratum followed by dead coral rubble, absence of branching *Acropora* corals due to high wave exposure (Qasim & Wafar, 1979), annual bleaching events (De et al. 2015), abundant macroalgae, and the presence of fewer herbivores (pers. obs.). Depth ranges between 1.5-4m and 5-7m in the MMS (sites 1 and 2 respectively).

### 5.2.2. Underwater survey

Surveys were conducted in MMS during March 2015 with the aid of SCUBA. At each site of MMS, Belt Transects (Hill & Wilkinson, 2004) (50m X 2m) was laid in triplicate, separated by a distance of ~50 m, perpendicular to the shore, along the depth contour of 2-4 m in site 1 and 4-7 m in site 2. All the *T. mesenterina* colonies falling within the Belt transect were enumerated underwater as well as close digital photographs were taken. The distribution and prevalence of STAs on *T. mesenterina* in both sites were recorded. We documented (a) the diameter of the colony; (b) the number of growth anomalies per colony and (c) the size of the growth anomalies on *T. mesenterina* colonies. STAs on *T. mesenterina* were also recorded with digital photographs and videography, using Nikon AW120 (14 Mega Pixels) and GoPro Hero4 (12 Mega Pixels) underwater camera. The growth anomalies on *T. mesenetrina* corals were described morphologically following Work & Aeby (2006). Percent coral cover was calculated using Line Intercept transect (LIT) (English, 1997) at both sites.

### 5.2.3. Statistical analysis

A chi-squared test of independence, using Statistica 13, was used to analyze the difference between the prevalence of STAs in Sites 1 and 2 of MMS. The relation between *Turbinaria* colony diameter and the number of tumors in Site 1 of MMS was analyzed by Spearman rank-order correlation analysis using Statistica 13.

### 5.2.4. Water sample analysis of MMS

Water samples were collected in Niskin bottles from both the sites and Total Suspended Solids (TSS) and nutrient analysis was done. 1 litre of water sample was filtered through a 0.22 µm polycarbonate filter (pre-weighed) and the TSS was estimated by measuring the weight of matter retained on the filter. The essential dissolved nutrients like Nitrate-$NO_3$, Nitrite-$NO_2$, and Phosphate-$PO_4$ were analyzed using a Skalar SAN ++ Continuous Flow Analyser.

## 5.3. Metagenomics study of coral tumors

### 5.3.1. Study area for metagenomics study

Grande Island (15°20’12.98’N, 73°46’19.74’E) is a shallow patchy reef located around the west coast of India, forming breeding, feeding, and shelter grounds for a diversity of fishes (Sreekanth et al. 2015). The study site is dominated by *Turbinaria mesenterina* colonies interspersed with rocky patches. The marginal reef is subjected to daily anthropogenic pressure (tourists and boats), high sedimentation, exhibits low pH and productivity during the northeast monsoon (Shetye et al. 2020), and witnesses recurrent bleaching episodes (Hussain & Ingole, 2020).

### 5.3.2. Coral mucus sample collection

Surface mucus layer from three healthy-looking and tumor-infected regions of three *T. mesenterina* colonies, at least 5 m apart from each other were collected in-situ, using a sterile 5 ml needleless syringe. Each mucus sample containing a syringe was kept in a separate ziplock bag in an icebox till reaching the laboratory. Mucus samples were spun at 4000 rpm (4°C) for 5 mins in a sterile microfuge tube, seawater was decanted and the remaining viscous mucus samples were stored at - 20°C until DNA extraction.

### 5.3.2.1. DNA extraction and V3 library preparation

Frozen Coral mucus samples were homogenized using steel beads in TOMY Microsmash. Homogenized mucus samples were treated with 0.3 ml Lysozyme (50mg/ml in 4%PVPP) and incubated at 37°C for 30 minutes. Samples were lysed in Standard lysis solution (2% sodium dodecyl sulphate, 1% β-ME, 50Mm EDTA9 pH8.0), 150 mM Tris.Cl, pH8.0, 1M Boric acid) with proteinase K (20µl, 20mg/ml) at 56°C for 1 hour. Residual RNA was removed by incubating the mixture with RNase at 65°C for 20 minutes. The lysate was centrifuged to remove debris. The supernatant was subjected to Phenol: Chloroform: isoamyl alcohol precipitation. Metagenomic DNA was precipitated by the standard Isopropanol precipitation method. DNA pellet was re-suspended in 10mM Tris.Cl (pH8.0). The quantification and quality of the genomic DNA was assessed using Nanodrop2000 (Thermo Scientific, USA), Qubit (Thermo Scientific, USA), and agarose gel electrophoresis.

### 5.3.2.2. 16S rRNA library preparation and sequencing

16S rRNA gene amplicon library preparation and sequencing were performed at the Genotypic Technology Pvt Ltd, Bangalore, India. Bacterial V3 hypervariable region of the 16S rRNA gene were amplified using the specific targeting primers 341F (CCTACGGGAGGCAGCAG) and 518R (ATTACCGCGGCTGCTGG). PCR amplification was performed using 20 ng of genomic DNA which was amplified using HiFi HotStart PCR Kit (KAPA, Biosystems, USA) using 0.2uM of forward and reverse primer. PCR amplification was done using an initial denaturing step of 95 °C for 3 min, followed by 26 cycles at 98 °C for 20 sec, 60 °C for 15 sec, and 72 °C for 30 sec, and then a final elongation step of 72 °C for 5 min Illumina MiSeq 16S rRNA libraries were generated following a standard protocol (Illumina). The libraries were validated using for quality check, and samples were sequenced using the 2 × 300 paired-end chemistry (MiSeq Reagent Kit).

#### 5.3.2.3. Data pre-processing and quality check

The Illumina MiSeq V3 paired-end reads were demultiplexed using the bcl2fastq tool and quality verified using FastQC (Andrews, 2010). Raw reads were processed using Cutadapt for adapters and low-quality bases. The high-quality reads with primer sequence were stitched using Fastq-join (Aronesty, 2013). These stitched reads were considered for further analysis using QIIME (Caporaso et al. 2010) pipeline. The query sequences were clustered using the UCLUST method (Edgar, 2010). The taxonomy of these clusters was assigned based on >=97% sequence similarity against the curated chimera free16s rRNA database (Greengenes7 v 13.8) (DeSantis et al. 2006). This results in the generation of a biome file which was taken ahead for further advanced analysis and visualization. The NGS sequencing services were provided by Genotypic Technology Pvt Ltd, Bangalore, India.

#### 5.3.2.4. Statistical analysis for bacterial communities

Alpha diversity of all the samples was conducted using different matrices i.e Shannon, Simpson, chao1, and observed species. This was followed by plotting rarefaction curves for all the samples (total number of distinct species versus the number of sequences sampled), stacked column bar plots (R package, ggplot2), and heat maps using R package-NMF (Gaujoux & Seoighe, 2012).

A two-tailed student's t-test was performed to check whether there is a significant difference among the values of alpha diversity indices. Furthermore, beta diversity was assessed with Principal coordinate analysis (PCoA) in Primer v7 (Clarke and Gorley, 2015). The differences in the bacterial communities between the healthy and diseased coral were tested using PERMANOVA with the Bray Curtis test in PRIMER v7. SIMPER analysis was performed to calculate the contribution of each bacterial community to the dissimilarity within and between samples in the PRIMER v6. This was followed by One-way ANOVA to check for a significant difference in communities between healthy and diseased corals and was further confirmed by the post-hoc Tukey test. ANOSIM analysis (analysis of similarity) was performed to check for statistically significant differences between the bacterial community composition between the samples.

### 5.3.2.5. Functional profiling based on bacterial community composition

This study further explored the taxonomic-based predictive functions of bacterial communities (OTU taxonomy and abundance) between healthy and diseased corals samples using the ‘metabolism by phenotype’ function in the METAGENassist webserver (Arndt et al. 2012). The data on the functional role was represented in the form of bar plots.

## 5.4. Results

The substrate was observed to be dominated by macroalgae-covered rocks and dead corals. *Porites spp*. was the most dominating coral followed by *Turbinaria spp.* in both the study sites (Table 5.1).

**Table 5.1. Percent coral cover (%) in site 1 and site 2. CCA- Crustose coralline algae.**

| | **% cover** | |
|---|---|---|
| **Substratum** | **Site 1** | **Site 2** |
| Macroalgae | 24.20 | 16.48 |
| Turf algae | 10.56 | 9.22 |
| CCA | 1.84 | 4.76 |
| *Porites* spp. | 15.00 | 17.76 |
| *Favites* spp. | 9.44 | 7.80 |
| *Plesiastrea* spp. | 6.32 | 5.80 |
| *Turbinaria* spp. | 13.9 | 13.46 |
| *Siderastrea* spp. | 1.52 | 0.12 |
| *Coscinaria* spp. | 1.10 | 0.10 |
| Sand | 6.08 | 11.72 |
| Rock | 10.04 | 12.78 |
| **Total** | **100** | **100** |

### 5.4.1. STA morphology and prevalence in MMS

Growth anomalies on *T. mesenterina* appeared as distinct globular, pigmented to partially bleached masses of the coral skeleton (Fig. 5.2A). The growth anomalies were described with morphological diagnosis (Table 5.2). Approximately 30% of the growth anomalies were infiltrated by filamentous algae (Fig. 5.2A). In ~25% of cases, the growth anomalies had grown and coalesced together (Fig. 5.2B). Tissue damage, where the bare coral skeleton was covered with algae and with a total loss of polyps (Fig. 5.2C), was prevalent in ~70% of the colonies with growth anomalies. The distribution of the growth anomalies was mostly towards the periphery of the colonies, extending towards the rim. Colonies were damaged by a high amount of sedimentation at both sites (Fig. 5.2C). STA prevalence on *T. mesenterina* was 71% in Site 1 and 40% in Site 2 (Table 5.3). Chi-square test of independence to determine the difference between the prevalence of STAs at Sites 1 and 2 revealed no significant difference in prevalence ($\chi2$ =2.84, p = 0.0919, df = 1) between sites. Spearman rank-order correlation analysis showed a significant positive relationship between *T. mesenterina* colony diameter and number of tumors for Site 1 (n = 15, Spearman R = 0.708, p = 0.0031). Another growth abnormality in the form of distinct pillar-like skeletal growths (Fig. 5.2D) with fewer polyps was observed in 4 colonies of *Turbinaria* spp. (ending in small branches). Normal colonies consist typically of unifacial horizontal plates or sometimes vertical growths. The prevalence of this abnormality on *Turbinaria* was 8.33 and 20% at Sites 1 and 2, respectively, and did not differ significantly between the 2 sites ($\chi2$ = 0.93, p = 0.3360, df = 1. TSS analysis of water samples from November, 2014 to March 2015 revealed high levels (Table 5.4) and nutrient analysis also revealed higher than optimum levels of nitrate and phosphate in the water sample (Fig. 5.3).

**Table 5.2: Description of skeletal tissue growth anomalies (STAs) on *T. mesenterina* (method adapted from Work & Aeby 2006). See also Fig. 5.2A−D in the present study (Hussain et al. 2016).**

| ***Turbinaria mesenterina*** | | |
|---|---|---|
| **Description** | **Distribution of STAs** | Multifocal to coalescing |
| | **Location on colony** | Peripheral |
| | **Edges** | Indistinct |
| | **Margins** | Undulating, serpigious |
| | **Shape** | Circular to irregular |
| | **Relief** | Nodular to exophytic |
| | **Size** | Small |
| | **Number** | 1 to 50 |
| | **Colour** | Brown, purple to bleached |
| | **Texture** | Rugose |
| **Morphological diagnosis** | **Extent** | Mild to moderate |
| | **Time** | Chronic, subacute process |
| | **Lesion** | Discolouration, reduced number of polyps, tissue loss, infiltrated by filamentous algae |
| | **Structures affected** | Skeleton, polyps |

**Table 5.3: Prevalence of STAs on *T. mesenterina* found in the MMS. Tum.= Tumor, Prev.=Prevalence, Max.= Maximum, and Min.= Minimum (Hussain et al. 2016).**

| Site | Location | Depth (m) | No. of *Turbinaria* colonies | Colony size (cm) | | No. of colonies with tumors | Tum. size (cm) | | Tum. prev. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Max. | Min. | | Max. | Min. | (%) |
| 1 | 16°03’786.4” N<br>73°27’725” E | 5-6 | 24 | 35 | 2 | 17 | 3 | 0.2 | 71 |
| 2 | 16°03’84” N<br>73°27’43.3” E | 6 | 10 | 200 | 20 | 4 | 2 | 0.3 | 40 |

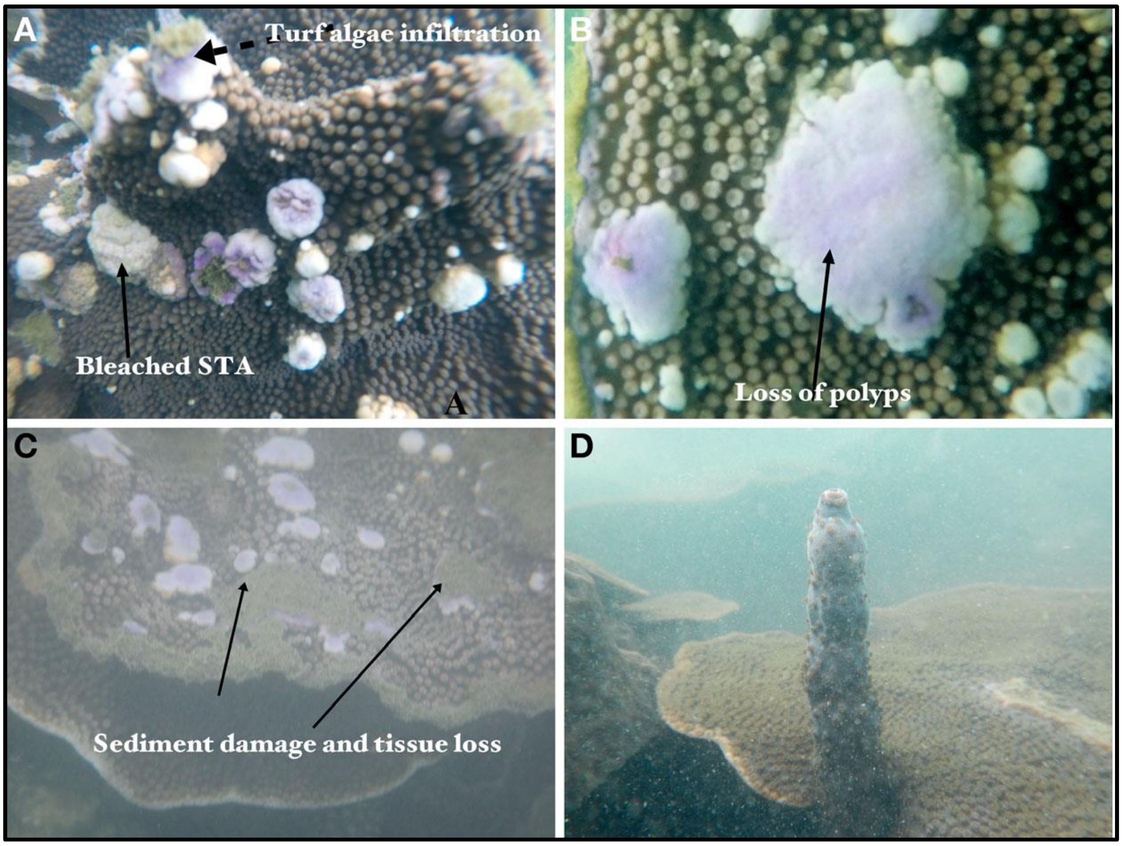


**Fig. 5.2: A−D. Skeletal tissue growth anomalies (STAs) on *Turbinaria mesenterina.* (A) Note multifocal distribution of variably sized nodular growth overlaid by unpigmented tissues (bereft of polyps?) (solid arrow) some of which are ulcerated and overgrown with turf algae (dashed arrow). (B) An enlarged STA, showing loss of polyps. (C) A**

**colony with signs of sediment damage and tissue loss. (D) Pillar-like atypical growths on *Turbinaria* spp. colonies (Hussain et al. 2016).**

**Table 5.4: TSS analysis from bottom water samples**

| Month | SITE | TSS (mg/L) |
|---|---|---|
| Nov, 2014 | 1 | 13.61 |
| | 2 | 20.39 |
| Jan, 2015 | 1 | 18.81 |
| | 2 | 48.22 |
| March, 2015 | 1 | 22.38 |
| | 2 | 26.95 |

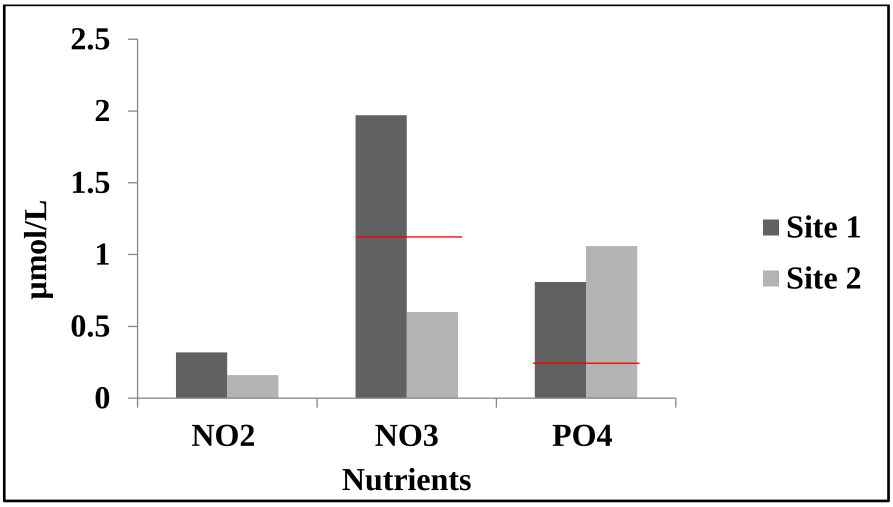


**Fig. 5.3: Nutrient analysis of water sample in MMS. (—) indicates optimum limit.**

### 5.4.2. V3 sequencing and bioinformatics analysis.

For healthy coral tissue, the filtered sequences were obtained in the range of 164950-332144, which were further assigned to 1156-1734 OTUs. Similarly, the diseased tissue had 50298-150168 filtered sequences, further assigned to 930-1425 OTUs (Table 5.5). Alpha diversity indices showed higher diversity and richness in diseased samples than the healthy samples. Shannon’s diversity index determined for the STA affected coral (D) and healthy coral (H) were 6.48±2.44 and 5.90±0.150, respectively (Table 5.6). The rarefaction curve showed high taxonomic coverage for all the samples (Fig. 5.4). Multidimensional ordination by Principal coordinate analysis (PCoA) also showed higher variation within the diseased sample group (Fig. 5.5). Nucleotide sequences have been submitted to the NCBI database under Bio project ID- PRJNA706525.

**Table 5.5: Sequencing read summary across the coral mucus samples where, H1-H3 are Healthy, whereas, D1-D3 are Diseased.**

| Sample ID | H1 | H2 | H3 | D1 | D2 | D3 |
|---|---|---|---|---|---|---|
| **Total pair ends read** | 467221 | 252681 | 510541 | 105362 | 182629 | 69072 |
| **Processed reads** | 279929 | 164950 | 332144 | 87916 | 150168 | 50298 |
| **Total Identified rRNA sequences** | 229161 | 141648 | 250473 | 67091 | 129124 | 34037 |
| **Total OTUs Picked** | 1734 | 1156 | 1694 | 1425 | 930 | 1157 |

**Table 5.6: Alpha diversity indices summary across the samples where, H1-H3 are Healthy, whereas, D1-D3 are Diseased.**

| Sample ID | H1 | H2 | H3 | D1 | D2 | D3 |
|---|---|---|---|---|---|---|
| **Shannon Diversity** | 6.08 | 5.79 | 5.84 | 7.35 | 3.72 | 8.36 |
| **Simpson Index** | 0.97 | 0.96 | 0.96 | 0.95 | 0.63 | 0.99 |
| **Chao 1** | 1871.62 | 1543.43 | 2006.76 | 1784.86 | 1321.02 | 1361.30 |
| **Observed Species** | 1475 | 1154 | 1437 | 1425 | 930 | 1157 |

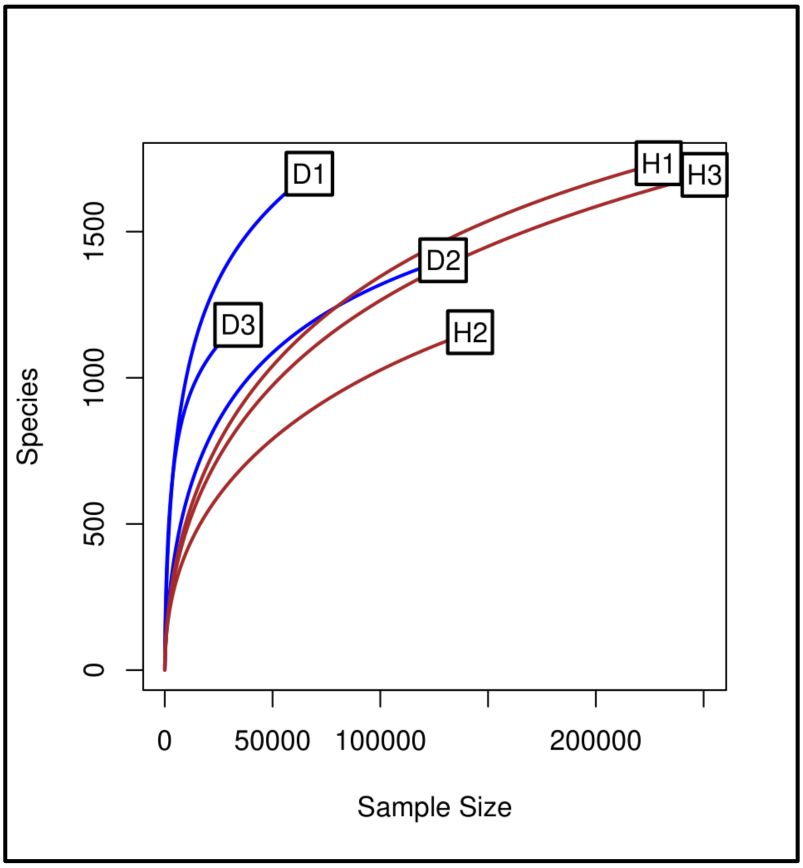


**Fig. 5.4: Rarefaction curve of triplicate samples of each healthy and STA affected *T. mesenterina* corals. H=Healthy coral, D=Diseased coral.**

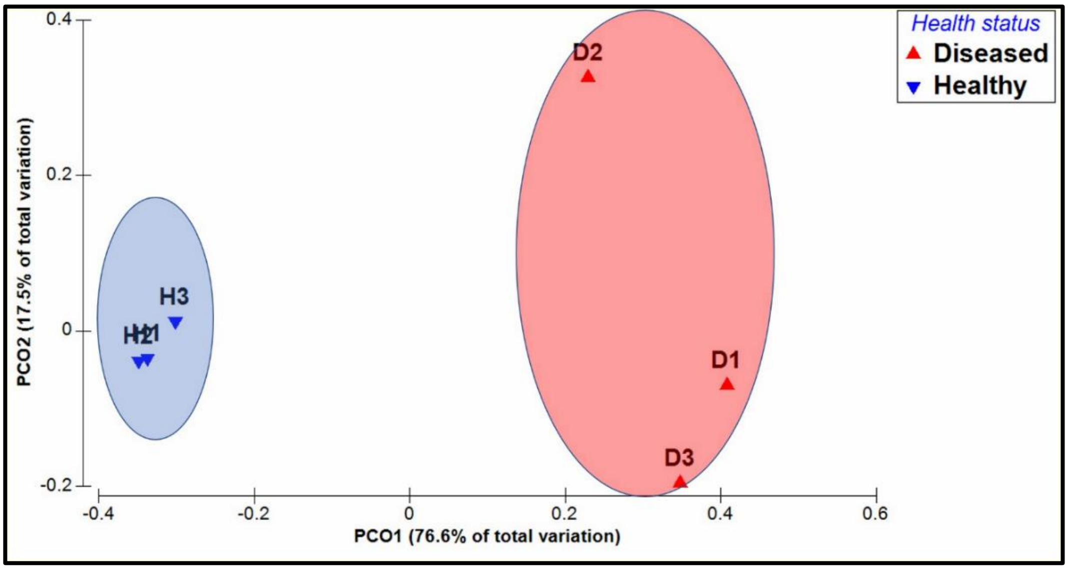


**Fig. 5.5: PCoA plot for healthy and STA affected coral samples based on weighted unifrac distance matrix.**

Sequences were classified into 30 phyla, 87 classes, and 542 genera for the healthy samples, and 31 phyla, 95 classes, and 584 genera for the diseased samples. The phylum Proteobacteria represented 55.0% (SD±4.07) of all analysed sequences obtained from the healthy corals, followed by Bacteroidetes (41.9%, SD±4.17), whereas the diseased sample was dominated by Proteobacteria 51.64% (SD±23.9), followed by Planctomycetes (17.09%, SD±9.87), Cyanobacteria (12.6%, SD±10.65), and Actinobacteria (10.3 %, SD±5.45) (Fig. 5.6). The beta-diversity of diseased samples was significantly different in the healthy samples at phyla level (PERMANOVA; p = 0.02, Permutation N: 999). This was further confirmed by analysis of similarity (ANOSIM; where Global R =1, p= 0.010). Based on the box and whisker plots, phyla Bacteroides and Planctomycetes were the major contributors to the differences between the healthy and diseased corals (Fig. 5.7).

The bacterial communities associated with the healthy coral were dominated by members of the Flavobacteria (42%, SD±5.06), followed by Alphaproteobacteria (32.4%, SD±1.98), and Gammaproteobacteria (22.44%, SD±4.17) at class level, whereas diseased samples exhibited higher diversity and were dominated by members of Gammaproteobacteria (44.28%, SD±2.87), followed by Planctomycetia (16.67%, SD±9.6) at class level (Fig. 5.8). Among Proteobacteria, the subgroup Alphaproteobacteria (32.42%, SD±1.97) was dominant in healthy tissues, whereas Gammaproteobacteria (44.28%, SD±2.87) was dominant in the STA affected tissues (Fig. 5.9).

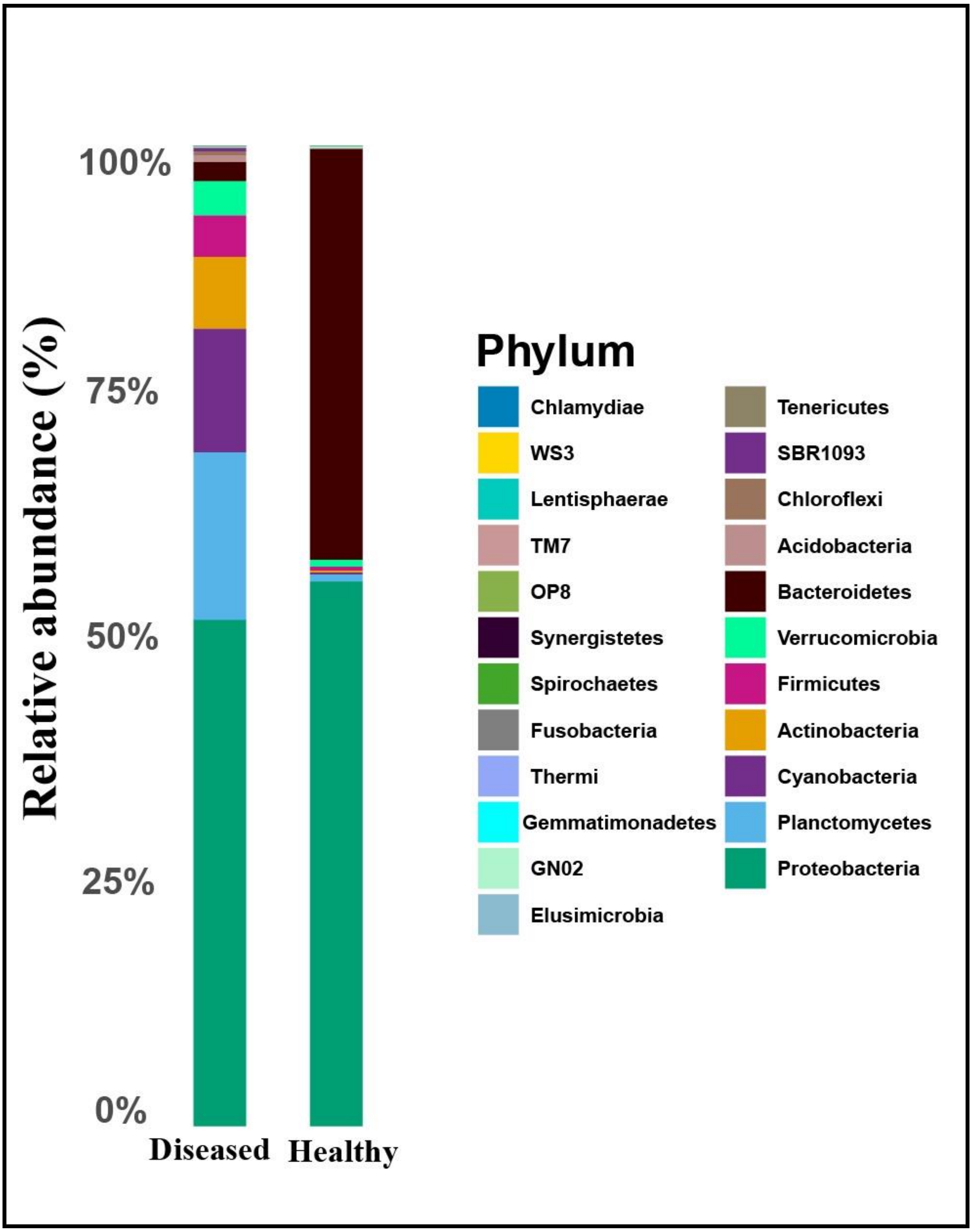


**Fig. 5.6: Bar plot representing relative abundance (%) of bacterial communities of diseased (D) and Healthy corals (H) at the phylum level.**

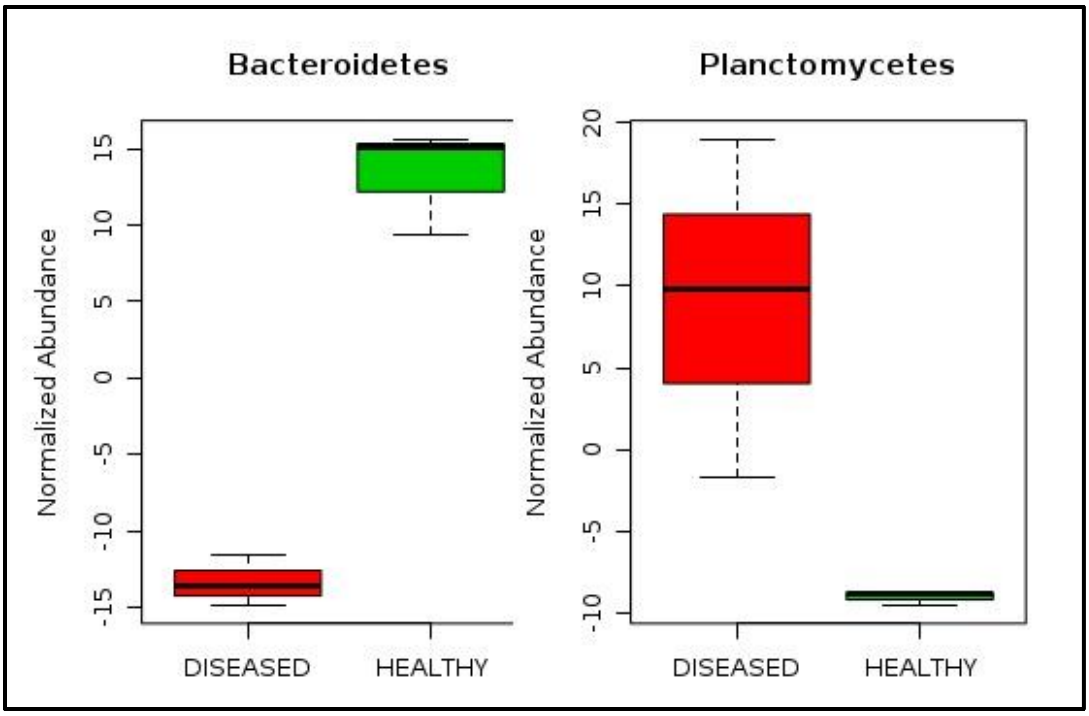


**Fig. 5.7: Box and whisker plot showing the phyla contributing to the major differences between the healthy and diseased samples.**

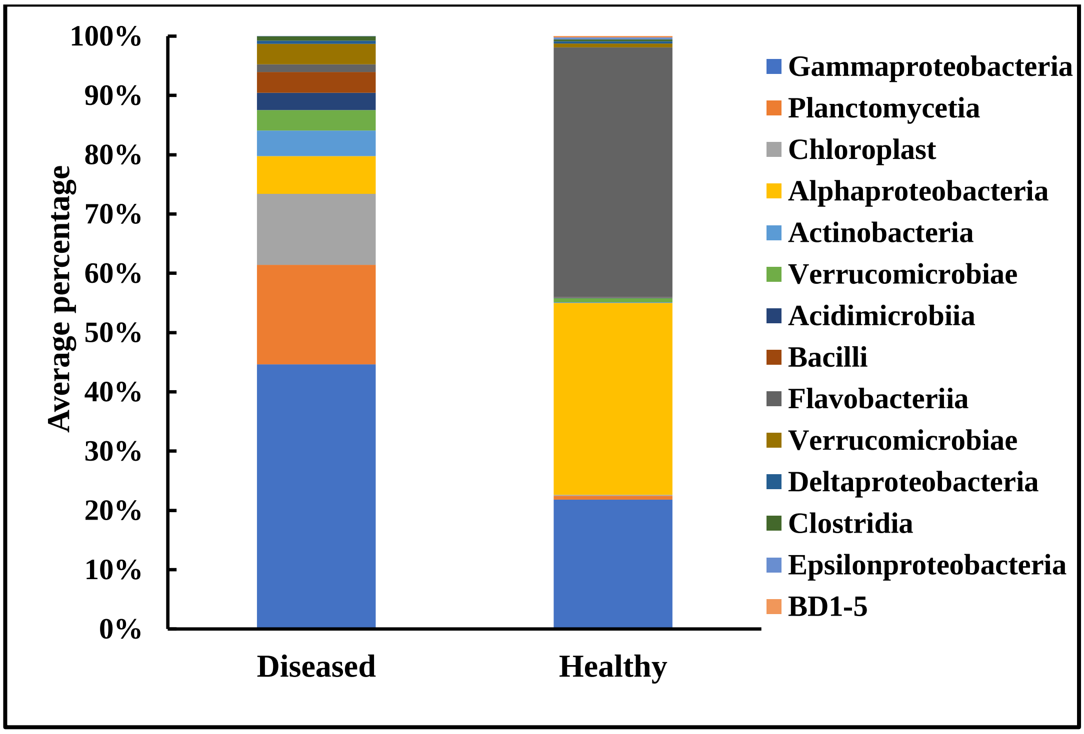


**Fig. 5.8: Bar plot representing average percentage (%) of top 10 abundant bacterial communities of both diseased and healthy corals at class level.**

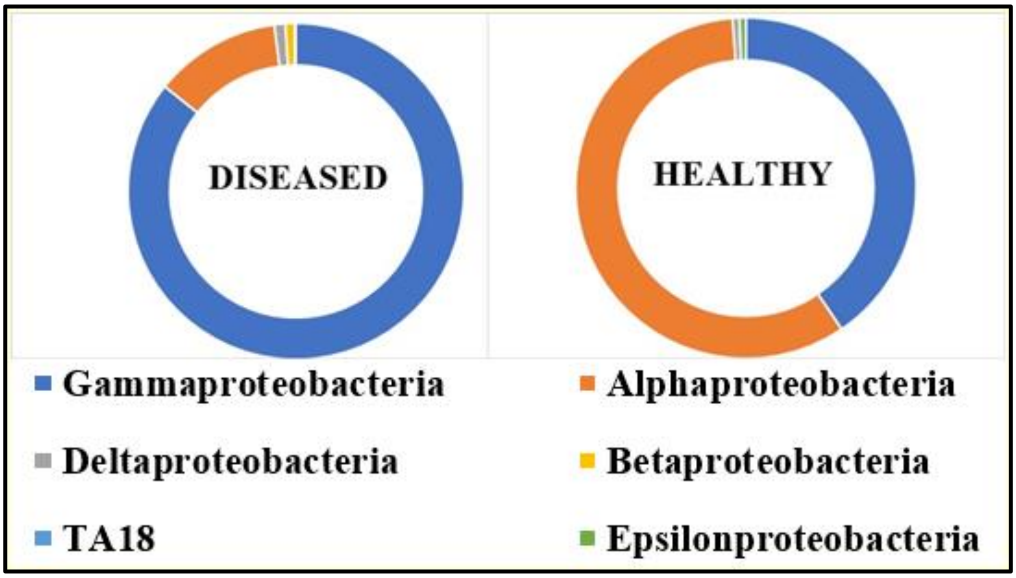


**Fig. 5.9: Pie Charts representing the percentage of classes amongst proteobacteria between diseased and healthy corals.**

SIMPER analysis with Bay Curtis resemblance conducted at family level, revealed average 64.59 % dissimilarity between the two groups (Table 5.7). This was followed by one- way ANOVA of the top 20 families contributing to 50 % of the dissimilarity, followed by post-hoc Tukey test revealed Flavobacteriaceae, Rhodobacteraceae, Pseudoalteromonadaceae, Idiomarinaceae, Alteromonadaceae, Oleiphilaceae, Vibrionaceae to be significantly higher in healthy corals whereas, family Pirellulaceae, Nocardioidaceae, Planctomycetaceae, Verrucomicrobiaceae, and koll13 to be significantly higher in the diseased samples (Table 5.6). Other families found to be significantly higher in diseased coral as compared to healthy corals included Mycoplasmataceae (p=0.019), Shewanallaceae (p= 0.024), Peptostreptococcaceae (p=0.028), and Mycobacteriaceae (p=0.038). Students' T-test between healthy and diseased corals revealed genera like *Mycobacterium*, *Shewanella*, *Enterococcus*, *Roseiclus*, *Planctomyces*, *Bacillus*, *Luteobacter*, *Victoria*, and *Verrumicrobium* to be significantly higher in diseased corals (p<0.05), as also evident in the heatmap (Fig. 5.10).

**Table 5.7: Summary of SIMPER analysis with Bray-Curtis dissimilarity, showing the average abundance, average similarity (Av. dissim), contribution (contrib%), and cumulative percent (Cum.%) of the top twenty bacteria contributing to the dissimilarity between diseased and healthy colonies of *T. mesenterina* at family level.**

| Family | Average Abundance (Diseased) | Average Abundance (Healthy) | Average diss. | Contrib % | Cum.% |
|---|---|---|---|---|---|
| **Average dissimilarity = 64.59 %** | | | | | |
| Flavobacteriaceae | 1.08 | 5.46 | 3.72 | 5.75 | 5.75 |
| Rhodobacteraceae | 1.74 | 5.6 | 3.27 | 5.07 | 10.82 |
| Pseudomonadaceae | 6.24 | 2.73 | 2.98 | 4.61 | 15.43 |
| Streptophyta Unclassified | 3.44 | 0.24 | 2.71 | 4.2 | 19.63 |
| Pirellulaceae | 3.79 | 0.76 | 2.56 | 3.97 | 23.6 |
| Cryomorphaceae | 0.22 | 2.98 | 2.34 | 3.63 | 27.22 |
| Pseudoalteromonadaceae | 0.22 | 2.5 | 1.94 | 3 | 30.22 |
| Unidentified | 0.12 | 1.74 | 1.37 | 2.12 | 32.34 |
| Nocardioidaceae | 1.38 | 0.04 | 1.14 | 1.76 | 34.1 |
| Idiomarinaceae | 0.07 | 1.38 | 1.11 | 1.72 | 35.82 |
| Xanthomonadaceae | 1.32 | 0.04 | 1.08 | 1.68 | 37.49 |
| Alteromonadaceae | 0.18 | 1.45 | 1.08 | 1.67 | 39.17 |
| Bacillaceae | 1.39 | 0.17 | 1.04 | 1.61 | 40.77 |
| Planctomycetaceae | 1.52 | 0.35 | 0.99 | 1.54 | 42.31 |
| Oceanospirillaceae | 0 | 1.1 | 0.94 | 1.45 | 43.76 |
| Verrucomicrobiaceae | 1.85 | 0.82 | 0.87 | 1.34 | 45.1 |
| Oleiphilaceae | 0 | 0.98 | 0.83 | 1.29 | 46.39 |
| Mitochondria | 1.06 | 0.11 | 0.81 | 1.25 | 47.64 |
| Vibrionaceae | 0.07 | 0.96 | 0.76 | 1.18 | 48.82 |
| koll13 | 1.02 | 0.15 | 0.73 | 1.14 | 49.95 |
| Staphylococcaceae | 1.03 | 0.22 | 0.69 | 1.07 | 51.02 |

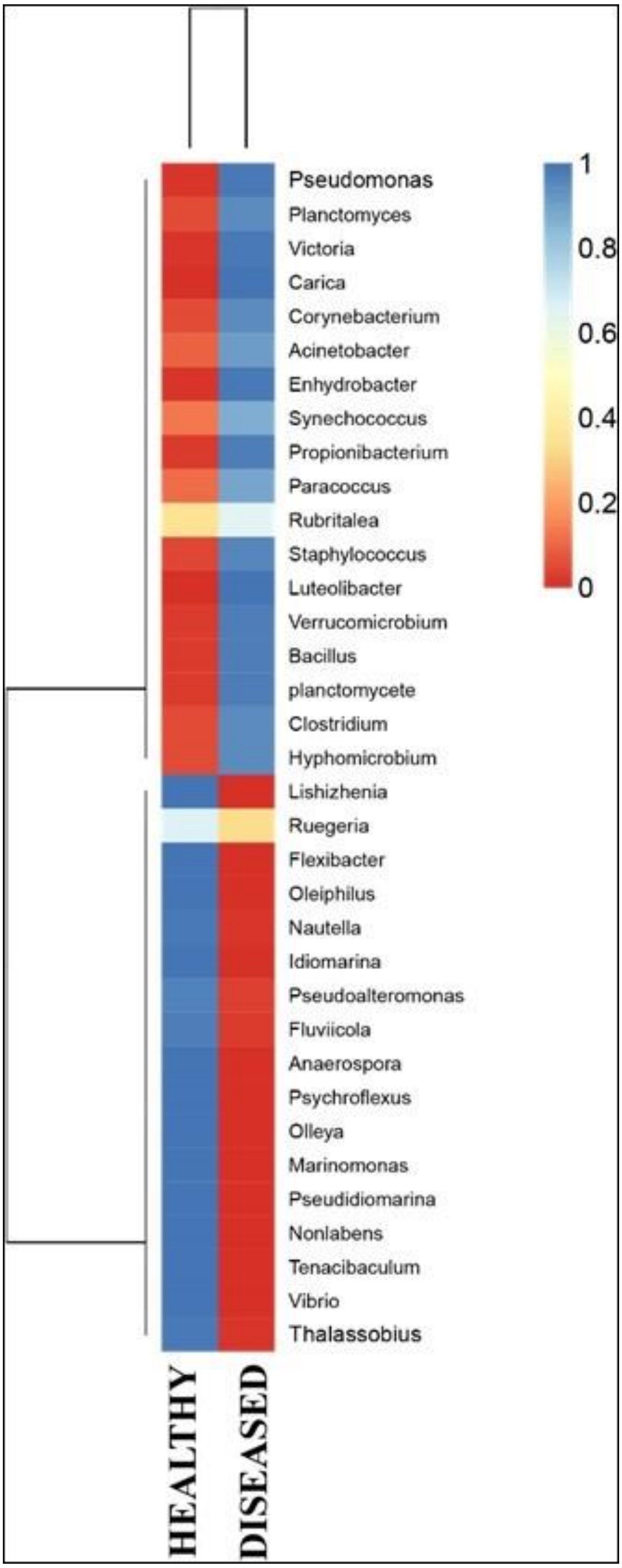


**Fig 5.10: Heatmap analysis showing clustering of the genera from healthy and diseased samples. Colours represent the correlation between the samples.**

### 5.3.2. Functional annotation using METAGEassist

To further understand the putative functional differences between the associated bacterial communities, taxonomy-based functional profiling was applied. The bacterial communities associated with STA-affected corals revealed higher functional roles, indicating a higher functional diversity in diseased samples (Fig. 5.11). Functions such as ‘chitin degradation’, ‘nitrogen fixation’, ‘streptomycin production’, ‘sulfate reducer’, ‘sulfur oxidizer’, and ‘xylan degrader’ were more abundant in the diseased samples, whereas ‘nitrate reducer’ and ‘sulfide oxidizer’ were higher in the healthy samples.

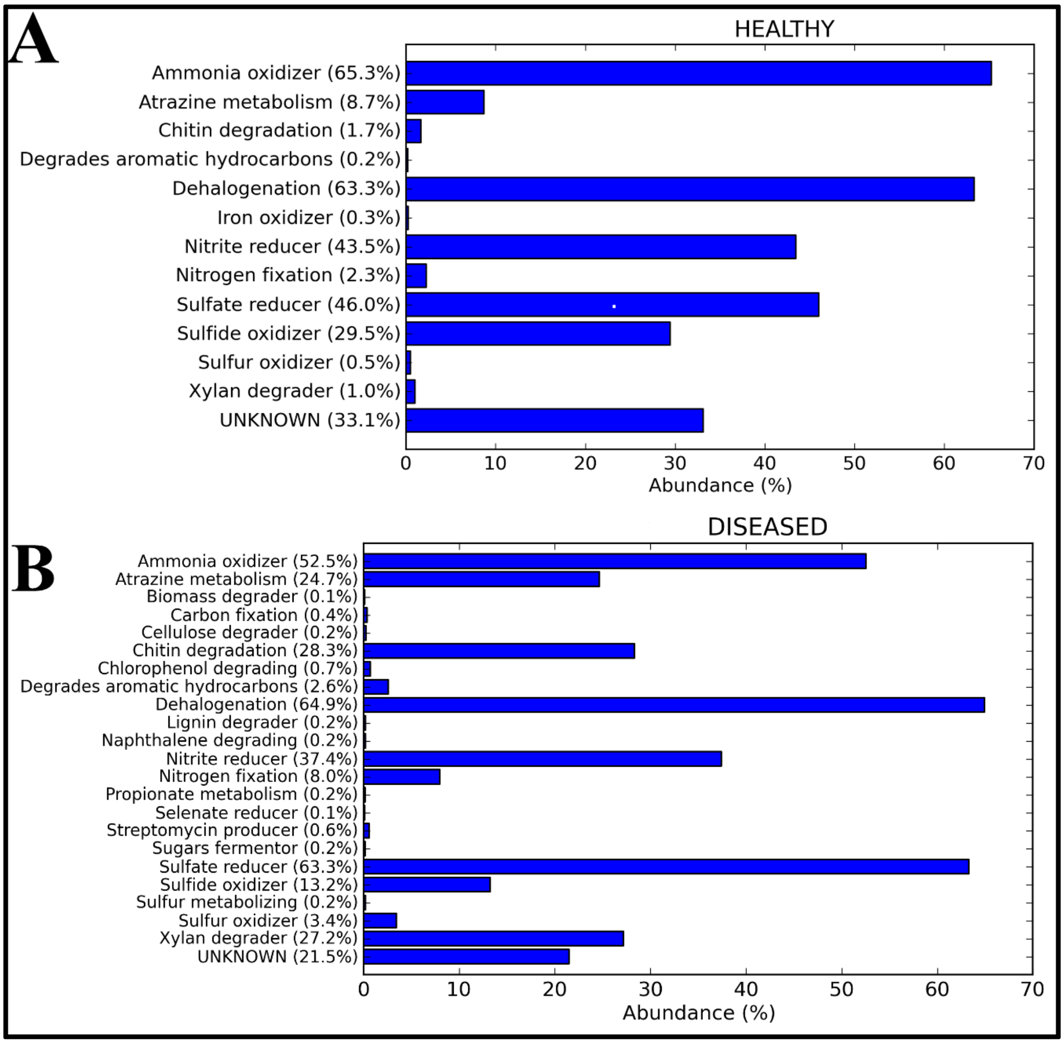


**Fig. 5.11: Taxonomy-based functional profiling of bacterial communities isolated from the mucus of A) Healthy and B) STA affected (Diseased) corals.**

## 5.4. Discussion

STAs have been reported in around 16 the Caribbean and 24 Indo-Pacific scleractinian corals, reviewed by Sutherland et al. (2004). Amongst them, acroporids are the most susceptible to developing growth anomalies (Cheney, 1975; Bak, 1983; Peters et al. 1986; Coles & Seapy, 1998; Yamashiro et al. 2001). The present study is the first description of STAs from Indian corals. The growth anomalies were recorded in both the sites of MMS but the frequency and severity were higher in Site 1. Site 2 is located away from the fort and is mainly a traditional fishing area. A possible reason for this could be the fact that site 1 is closer to the Sindhudurg fort and is the site of the main tourist attraction for recreational activities such as SCUBA diving and snorkelling (about 10 tourist boats operate on an hourly basis with approx. 15-20 tourists per boat during peak season (per. obs.). The movement of boats, tourists, and all related activities could be generating a stressful condition for the sensitive corals making them more susceptible to diseases and physical damage. Atypical morphology in the form of 2 ‘arms’ has been observed in a *Turbinaria peltata* colony (Chou & Ng, 2010), but there have been no previous reports of tumor-like growth anomalies in *Turbinaria*, globally. Therefore, this study provides the first evidence of STAs for the *Turbinaria* genus and is the first descriptive study of STAs from Indian corals. The STAs of T*urbinaria* recorded here show similar characteristics to growth anomalies observed in other genera. The STAs observed here are globular and bleached and, in ~25% cases, had coalesced together, which agrees with the description of growth anomalies in *Acropora valenciennessi* (Coles & Seapy, 1998). Similarly, Gateno et al. (2003) and Work et al. (2008) characterised growth anomalies with few or no polyps which is consistent with the trend observed in this study. The size of the coral colony was the most significant predictor of the prevalence of STAs in Site 1. Our results agree with the previous description by Loya et al. (1984), who suggested that large colonies had more STAs per unit mean colony diameter as compared to smaller colonies, and larger colonies are more susceptible to external damage. Domart-Coulon et al. (2006) also reported larger colonies of *Porites compresssa* with higher numbers of STAs. The healthy parts of the colony were seen to undergo damage, which agrees with Peters et al. (1986) who showed that GAs in *Acropora palmata* are progressive, leading to death of the surrounding normal tissue. Skeleton growths on *Turbinaria* spp. (Fig. 5.2D) could be a response to sediment accumulation. This coral with horizontal plates or lamellae is known to concentrate sediments in the centre of the colony (Riegl et al. 1996). This could lead to damage and make corals more susceptible to diseases and bleaching. Analysis of water samples revealed high TSS values (site 1-22.38 mg/L, site 2- 26.94 mg/L). Generally, chronic sediment deposition rates greater than 10 mgcm-$^{2}$day-$^{1}$ have adverse effects on corals (Rogers et al. 1990). According to Couch et al. (2008), nutrient enrichment can modify host coral immunity by favouring the growth of microbial associates increasing the severity and prevalence of diseases. In the present study, the nutrient levels were higher than the optimum levels suggested by Bell (1992) which could have impaired host immunity and/ or increased pathogen virulence making the corals more susceptible to STAs.

Several studies have highlighted the shifts in microbial communities of scleractinian corals inflicted with various diseases like black band disease (Sato et al. 2010; Miller & Richardson, 2011), white plague disease (Sunagawa et al. 2009; Cárdenas et al. 2012; Roder et al. 2014a), white patch syndrome (Godwin et al. 2012; Wilson et al. 2012; Séré et al. 2013), paling and necrosis (De Castro et al. 2010), yellow blotch (Cervino et al. 2008), dark spot syndrome (Kellogg et al. 2014), white pox diseases (Patterson et al. 2002). However, the reports on variation in bacterial communities in STA-affected corals are scarce and inconsistent (Chiu et al. 2012; Ng et al. 2015; Breitbart et al. 2005; Rajasabapathy et al. 2020). This study is crucial as it is the first comparative metagenomic analysis of bacterial communities between healthy and STA-affected *T. mesenterina* from the eastern Arabia Sea.

A higher alpha diversity (Shannon's diversity index) in diseased corals as compared to healthy corals observed in this study is consistent with previous reports on other scleractinian corals (Ng et al. 2015; Bourne et al. 2008; Reis et al. 2009; Sunagawa et al. 2009). It is believed as the immunity of a diseased coral is compromised, opportunistic bacteria invade and proliferate in the coral, disrupting the homeostasis of the coral holobiont and altering metabolic pathways (Sunagawa et al. 2009; Roder et al. 2014). Proteobacteria was the most abundant group in this study which corroborates with previous work on coral-associated bacteria (Lampert et al., 2006, Chiu et al., 2011, Miller & Richardson, 2010, Thurber et al. 2008, Rajasabapathy et al. 2020). Following Proteobacteria (55.53%), Bacteroidetes (41.9%) were the second most dominant phyla in the healthy corals in the present study. Interestingly, Bacteroidetes were significantly lower in the diseased sample (1.96%) and were replaced by other opportunistic phyla dominated by Planctomycetes (17%) Cyanobacteria (12.6), Actinobacteria (7.32%), Firmicutes (4.2%), Verrucomicrobia (3.5%), and Acidobacteria (0.69%), rendering statistically significant and higher beta diversity in the diseased samples.

Pirellulaes (dominant here) order within Planctomycetes phylum comprises of anaerobic oxidizing bacteria and contribute to the nitrogen cycling by the removal of ammonium (metabolic waste within the coral microbiome) and convert it into nitrites (Mohamed et al. 2010). Studies by Taguchi & Kinzie (2001); D'elia et al. (1983), proposed that the symbiodinium prefers to take ammonium over other forms of dissolved inorganic nitrogen. Rädecker et al. (2015) suggested that this may lead to depletion in the amount of nitrogen required for symbiodinium growth. Planctomycetes are also frequent colonizers of macroalgae and have been reported from almost 30% of all the studies on macroalgae-bacterial community association (Lage & Bondoso, 2014). The high abundance of planctomycetes observed could be due to the presence of bacterial communities infiltrating the diseased corals in association with endolithic turf algae. Cyanobacteria play a major role in nitrogen fixation in the coral reef ecosystem (Lesser et al. 2004) and the process is tightly controlled by environmental conditions (Rädecker et al. 2015). An increase in nitrogen fixation during stress conditions accompanied with high dissolved organic carbon (DOC) may lead to an imbalance in the nutrient availability in corals (Rädecker et al. 2014; Grover et al. 2014). Recent studies have reported that an increase in nitrogen fixation can increase the N:P ratio in corals, stimulate cell division in *Symbiodinium*, and lead to phosphate starvation in the *Symbiodinium* (D'Angelo & Wiedenmann, 2014). This scenario of phosphate starvation destabilizes the thylakoid membrane and eventually causes the breakdown of the coral-algae symbiosis (Wiedenmann et al. 2013). Members of cyanobacteria are also linked to coral diseases, being the causative agent of black band disease across the Caribbean and the Indo-Pacific region (Lopez et al. 2003, 2014) and the present result of a higher count of cyanobacteria in diseased samples is consistent with previous studies (Miller & Richardson, 2011; Hadaidi et al. 2018). Additionally, order Oscillatoriales of the benthic cyanobacterial group observed to be higher in diseased samples here is known to produce allelopathic compounds that inhibit the settlement of coral larvae (Kuffner & Paul, 2004; Kuffner et al. 2006).

Actinobacteria group plays a major role as producers of antimicrobial and anti-fungal compounds in the reef environment (Mahmoud & Kalendar 2016; Blunt et al. 2011) and contrary to the present study, their count is expected to decrease in diseased corals with the loss of beneficial microbes (Reis et al. 2009). However, the present finding agrees with the work of Sweet et al. (2013a), who reported a significantly higher amount of Actinobacteria in corals affected with dark spot syndrome. As hypothesized by Sweet et al. (2013a), the increase in anti-fungal producing Actinobacteria may be a defence mechanism to ward off the fungal pathogens. In agreement with this study, the Firmicutes group has been reported to be significantly higher in corals exposed to stressors like elevated temperatures and increased algal contacts (including turf alga, cyanobacteria, and macroalgae) (Zaneveld et al. 2016; Thurber et al. 2012; Nugues et al. 2004). Other dominant groups in this study like Verrucomicrobia and Acidobacteria have also been documented with coral diseases (Yang et al. 2014; Roder et al. 2014b; Ng et al. 2015).

The result of functional annotation corroborated with the observed changes in bacterial communities between healthy and diseased corals. Major differences were observed in the bacteria associated with sulphur and nitrogen cycling. An increase in ‘sulphide reducing bacteria’ (SRBs) indicate an anoxic condition and SRBs have been primarily implicated in the pathogenesis of black band disease in corals (Richardson, 2004; Glas et al. 2012). SRBs are the source of toxic sulphides in the corals and are involved in the progression of the disease (Carlton & Richardson, 1995; Glas et al. 2012). In the present study, cyanobacteria were more abundant in diseased corals and are reflected by an increased amount of sulphide reducers. Healthy corals had a higher abundance of sulfide oxidizers, which play a role in scavenging harmful reactive oxygen species (ROS) by metabolising dimethylsulfoniopropionate (DMSP) into dimethyl sulfoxide (DMSO) (free radical scavenger) (Sunda et al. 2002; Hadaidi et al. 2017). However, the reduced abundance of sulfide oxidizers in the diseased corals might be leading to the accumulation of harmful reactive oxygen species, promoting disease pathogenesis. The increased amount of ‘nitrogen reducers’ in diseased corals accompanied with high dissolved organic carbon (DOC) (due to dying coral tissue) may lead to phosphate starvation and eventually breakdown the coral-algal symbiosis (Rädecker et al. 2014; Grover et al. 2014; Wiedenmann et al. 2012). The high abundance of ammonia-oxidizing bacteria belonging to order Pirellulales in the diseased samples is consistent with the high ‘nitrogen fixation’ function observed here. A high abundance of ‘chitin degraders’ in diseased corals may indicate immune response against fungal pathogens (Han et al. 2009). Endolithic fungi have been reported to be the causative agent of pearl-like skeletal growth anomalies in *Porites lobata* by Le Campion-Alsumard et al. (1995). They suggested that similar to opportunistic pathogens, stress conditions may increase the frequency of attacks by endolithic fungi in corals.

**Potential coral pathogens?**

A comparatively higher abundance of copiotrophic bacteria including *Mycobacterium*, *Shewanella* observed in the diseased samples is consistent with reports of their higher abundance in niches with high organic matter (Wild et al. 2004a, 2004b). Sweet et al. (2013b) reported *Shewanella* as an opportunistic invader of decaying coral tissue following disease pathogenesis. *Mycobacterium gordonae* observed in diseased samples in this study has been also reported to cause infection in redclaw crayfish *Cherax quadricarinatus* (Davidovich et al. 2019). *Neisseria,* a potential pathogen of Yellow Band Disease (YBD) of Caribbean corals with benthic algae as a reservoir of pathogens (Sweet et al. 2013b) was present exclusively in diseased samples *Serratia marcescens,* a fecal enterobacterium observed in the diseased sample here is an opportunistic necrotizing coral pathogen of white pox disease in *Acropora palmata* (Sutherland et al. 2011). Human fecal indicator bacteria including *Clostridium perfringens* and *Enterococcus* were detected in higher abundance in the diseased samples (Lipp et al. 2002; Faiqoh, 2020). The trend of higher abundance of human fecal indicator bacteria directs towards their accumulation and propagation in the mucus of immune-compromised corals and poses a public health risk in the reef area. *Propionibacterium acnes*, an opportunistic human pathogen causing inflammatory skin condition, acne vulgaris (Dessinioti & Katsambas, 2010), and also play a potential role in the etiology of sarcoidosis (Yasuhara et al. 2002), and prostate cancer (affecting prostate epithelial cells) (Fehri et al. 2011) was in higher abundance in the diseased samples. Coenye et al. (2007) also demonstrated that the biofilm formation in *P. acnes* is linked to resistance to antimicrobial compounds and promotes the production of virulent agents. Another opportunistic biofilm-forming human pathogen, *Staphylococcus aureus* that causes infections like localized skin lesions to more life-threatening infections (Ragle & Bubeck, 2009) was found to be more abundant in the diseased corals. Loss of beneficial microbes like *Pseudoalteromonas* (antibacterial) (Shnit-Orland et al. 2012) and *Marinobacter* (inhibits biofilm formation by the white pox causing *S. marcescens*) (Alagely et al. 2011) in the diseased corals might have increased the corals' susceptibility to opportunistic pathogens.

## 5.5. Conclusion

This study establishes the presence of skeletal tissue growth anomalies in the disturbed marginal reefs of the west coast of India and revealed a salient shift in bacterial communities between the healthy and STA-affected corals. The diseased samples exhibited higher diversity and were dominated by opportunistic phyla like Planctomycetes, Cyanobacteria, Verrucomicrobia, and Acidobacteria. Several human pathogens and copiotrophic coral pathogens were observed in the diseased samples, however, the etiology of the STA could not be established in this study. Functional annotation of the bacterial communities revealed major changes in sulphur and nitrogen cycling in corals and may have a role in disease. The coral tumors could have formed in reaction to environmental stress (UV, heat stress, high nutrients, and sedimentation load), however, the shift in the bacterial community may have aggravated the already immune-compromised coral health and led to changes in metabolic pathways. It would be interesting to further study the histopathology of the affected corals to shed light on the etiology and disease pathogenesis.

# *Chapter 6*

# Impact of anthropogenic activities on corals

## 6.1. Introduction

Coral reefs are under major threat due to the combined effects of natural and anthropogenic stressors at regional and global scales, primarily from global climate change, increasing levels of $CO_2$, unsustainable fishing practices, unregulated tourism, dredging activities, coral diseases, and land-based pollution (Bellwood et al. 2004; Gardner et al. 2003; Wilkinson, 2008; Burke et al. 2011). Coral reef-based tourism provides livelihood to millions worldwide and is one of the fastest growing tourism sectors in the world (Ong & Musa, 2011). Reef-based SCUBA diving has become of the most popular water sports worldwide (Garrod & Gossling, 2012). However, due to lack of proper management and sustainable growth of reef tourism, the conservation of reef life is overlooked. Physical damage caused to the corals by SCUBA divers due to stamping on corals, and physical contact by fins damage the coral skeletons and disrupts the biological process of the corals leading to disruption in growth and sexual reproduction in corals (Zakai et al. 2000). Physically damaged reefs are also more prone to coral diseases and infestation of sponges and macroalgae. Case study by Lamb et al. (2014) on the tourist popular reefs of Koh Tao, Thailand revealed a threefold increase in disease prevalence in reef sites with higher tourists' inflow. Over the last two decades, the increase in tourists in Malvan and Goa for reef related activities has put the vulnerable reef communities under major anthropogenic stress (WWF, 2019). With more than sixteen diving spots including shipwrecks, fringing reefs, and safe spots with a smooth sandy bottom, Grande Island has become a hub of dive certification training (SSI and PADI) in mainland India, operated by more than a dozen of dive schools in Goa. It is estimated that during the peak season (November-March), more than 700 boats operate bringing more than 3000 dive tourists annually to Grand Island, boosting the local economy (WWF, 2019). Over the past two last decades, there has been an exponential rise in the number of tourists visiting Malvan owing to the historical fort, SCUBA and other water sports activities around the fort (Port authority, Malvan). According to UNDP report (2011), estimated annual revenue from tourism in Malvan is around $2.5 million sustaining more than 3000 people. With the increase in recreational SCUBA diving activities, the physical damage to coral reefs due to boat anchoring and divers has led to widespread damage and loss of biodiversity of the reefs. Although these tourism activities play a major role in supporting the economy of Goa and Malvan, there is an urgent need to quantify the damage and devise strategies for sustainable tourism within the carrying capacity of the reefs in the study area.

Coral Damage Index (CDI) is one such tool to identify reef sites with severe physical damage to corals (Jameson et al. 1999). This screening aides in sustainable management of reef's sites at more risk due to physic injury. Severity and extent of physical damage to the corals also depends on the coral's morphology. Branching and plate corals are more susceptible to physical injury as compared to massive and encrusting corals, caused by divers, boat anchors and fishing gear (Ballesteros et al. 2018; Hawkins & Roberts, 1992). However, the recovery rate of some of the branching species like *Acropora palifera* is higher than encrusting corals like *Porites lutea* (Liddle & Kay, 1987). Coral reef classification based on the morphology of the colonies and applying their respective conservation class is another tool to predict coral species richness, presence of rare species and habitat complexity (Edinger et al. 1999). Therefore, the objective of the present study is to apply Coral Damage Index (CDI) with coral conservation classes (morphology based) to classify the sites with high deterioration and possible risk due to direct anthropogenic disturbances.

## 6.2. Methodology

### 6.2.1. Study area

For the present study, five sites in Grande Island, and two sites in MMS were selected to zone them for conservation classes (Fig. 6.1). Site S3, S5, and MMS2 had comparatively lesser tourism activity than sites S1, S2, S4 and MMS1. Sites S1, S3, S5, MMS2 were dominated by encrusting corals (*Porites*, *Favites*), whereas, sites S2, S4, and MMS1 had both encrusting as well foliose corals (*T. mesenterina*).

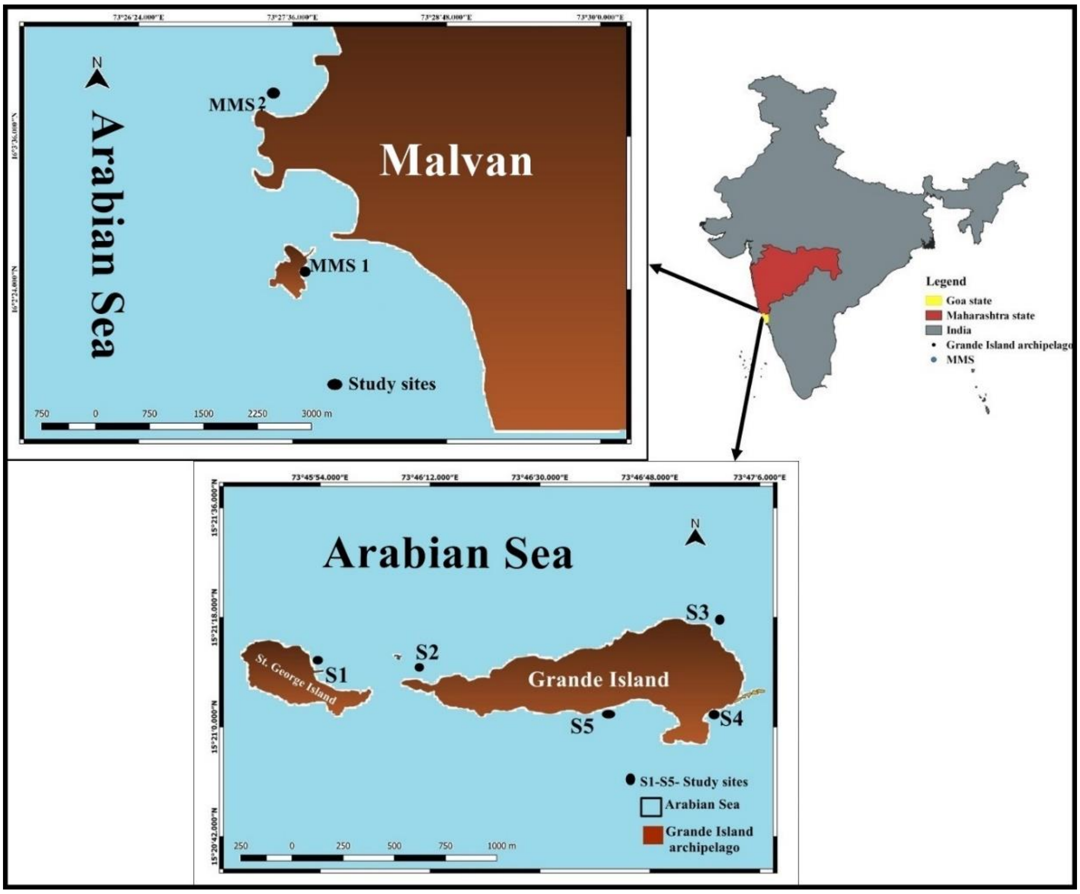


**Fig. 6.1: Map with study sites in MMS and Grande Island, Goa.**

### 6.2.2. Coral damage index

For calculating CDI, triplicate belt transects (20 X 2 m) were laid in in each site, perpendicular to the shore, separated by a minimum distance of 10 m from each other.

Healthy colonies, broken colonies and rubble were counted in each transect and their cumulative percent was calculated. Sites with more than or equal to 4% broken corals were considered as “hot spots” and require urgent conservation efforts (Jameson et al. 1999). CDI was further applied with morphology-based conservation classification of reef sites.

### 6.2.3. Morphology based conservation classification

Following triplicate 20 m line transects in each site, relative abundance of coral colonies falling under different morphology classes like branching, encrusting, foliose and sub-massive was calculated. The percentage of different morphological forms was used assign (r-K-S) and conservation class was assigned for reefs (Edinger et al. 2000). Sites dominated by (>50%) stress-tolerant (S) massive and sub-massive forms were assigned conservation class 01. Reef sites dominated (>50%) competitors (K) foliose and branching non-acroporids) were assigned class 02 and reefs with (>50%) ruderals (r) (the branching *Acropora* spp.) were assigned class 03. Sites with equal proportion of all morphological groups were assigned class 04. Conservation class 01 was defined as a reef dominated by S (stress tolerant) type and can be termed as near-shore polluted reefs. Class 02 is defined as a reef dominated by K competitors comprised by foliose and branching non-acroporids corals. Conservation class 03 is defined as a reef dominated by disturbance–adapted ruderals.

## 6.3. Results and discussion

Widespread damage was recorded in the study area mainly due to unregulated tourism activities like SCUBA and boat anchors dropping on vulnerable corals like *T. mesenterina* (Fig 6.2). Coral Damage Index revealed severe degree of damage in most of the sites, much higher than the critical-cut-off of 4% with sites S1 (16.7%), S2 (15.78%), S4 (17.83%), MMS1 (19.1%), and MMS2 (13.5%, Fig 6.3). Two sites in Grande Island, Goa, S3 (2.75%) and S5 (3.45%) had damage below the critical cut-off, possibly due to low coral diversity and density, as well as lack of tourism activities. Site MMS1 had the highest damage, possibly due to proximity to the Sindhudurg fort which gets hundreds of visitors every day, and is the also the main diving spot in MMS (Hussain et al. 2016; De et al. 2020).

Categorising sites according to the coral morphology revealed that that the sites dominated by foliose corals (conservation class 2) had a greater number of broken colonies as compared to the reef site dominated by massive and sub-massive corals (Class 1). Sites S1, S3 and S5 were dominated by encrusting, stress tolerant (S) corals like *Porites*, whereas Sites S2, S4, MMS1 and MMS2 had a higher abundance of competition adapted (K) *T. mesenterina* colonies (Table 6.1). Interestingly, sites with conservation class 2 had higher Coral Damage Index due to the dominance of competitive (K) species like *T. mesenterina.* They are more susceptible to breakage due to their shape and have also been reported to be having higher prevalence of coral diseases like skeletal tissue growth anomalies (Hussain et al. 2016). Although Site S1 in Grande Island comes under conservation class 1 (S species), it had high CDI, possibly due massive bleaching of *Porites* which turned into rubble after the massive bleaching event of 2015/16 (Hussain and Ingole, 2020). None of the sites in the study area were classified under conservation class 3 due the absence of branching corals (r species) in the study area. Conservation class 2 sites are most susceptible to physical damage by boat anchors and SCUBA divers and therefore require strict regulations for recreational water sports activities.

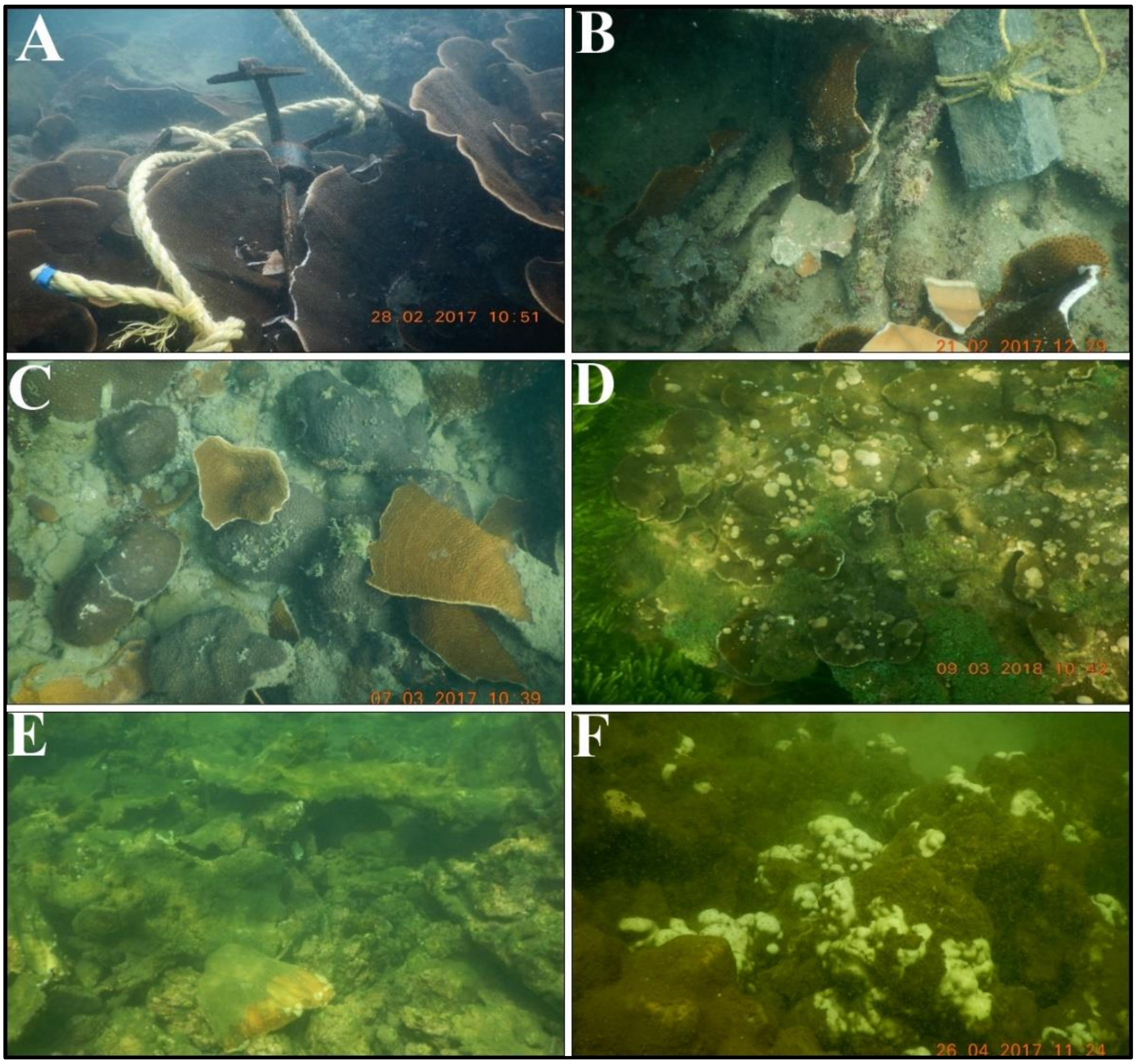


**Fig. 6.2: Damage due to tourism activities in the study area, A) Anchor damage in *T. mesenterina*; B-C) Broken *T. mesenterina* colonies due to anchor drops, D) Disturbed colonies with diseases; E) Rubble in Grande Island, F) Bleached and dead colonies with rubble in MMS.**

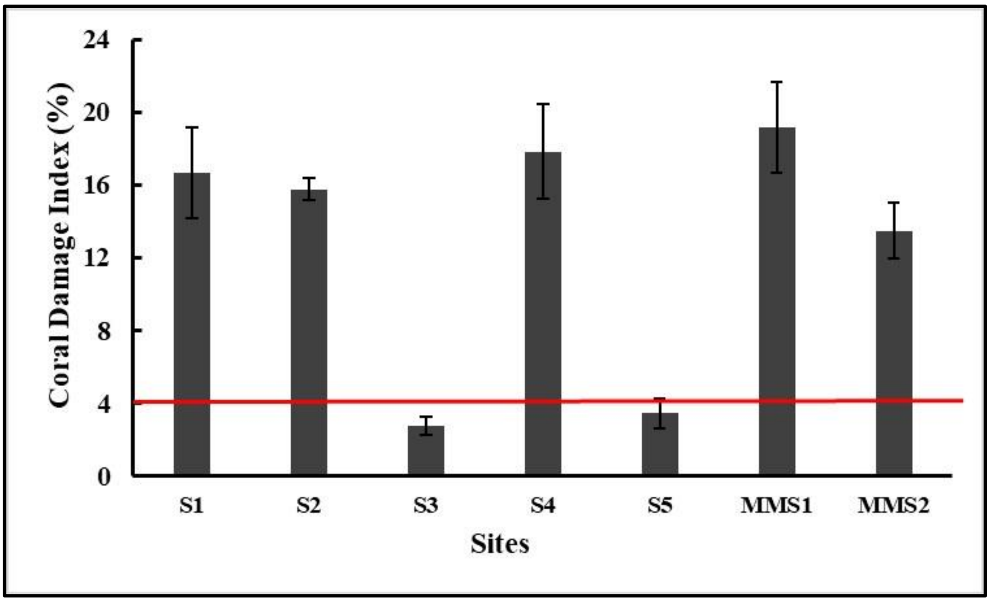


**Fig. 6.3: Mean Coral Damage Index across the study sites (±SD). The red line indicates the critical cut-off of 4 %.**

**Table 6.1: Coral morphology category, conservation class and Coral damage index (CDI) across the study sites (S=stress tolerant; K= Competition adapted, r=ruderals).**

| Site | Morphology category | Conservation class | CDI |
|---|---|---|---|
| S1 | S | 1 | 16.67 |
| S2 | K | 2 | 15.78 |
| S3 | S | 1 | 2.75 |
| S4 | K | 2 | 17.83 |
| S5 | S | 1 | 3.45 |
| MMS1 | K | 2 | 19.2 |
| MMS2 | K | 2 | 13.5 |

There are three probable reasons for high CDI in the study area; 1) high rubble density after the bleaching episodes, 2) unregulated SCUBA diving activities and untrained divers, and 3) damage caused by boat anchors. The fragile *Turbinaria* corals are most susceptible to breakage by divers and anchors. The damage caused by anchors and anchor cables was quantified in Arraial do Cabo Marine Extractive Reserve, Brazil, which is the most popular and visited diving destination in the southwestern Atlantic (Giglio et al. 2017). They found significant damage to benthic organism caused by anchor cable suggested deployment of mooring buoys to mitigate damage caused by anchors. Furthermore, boats and divers in the reef area increase sediment resuspension, increase nutrient load and also release toxic boat paint the coral habitat, as well as discharge harmful petroleum hydrocarbons in the reef waters, reducing the coral complexity as well as the fish communities (Whitfield & Becker, 2014; Hawkins et al. 1999). Exploitation of reef resources beyond the carrying capacity leads to long-term ecological and economical damage (Jameson et al. 1999) and is urgently required to be calculated and implemented in the study area. The unregulated tourism activities in the wake of recurrent bleaching (Eakin et al. 2019, Hughes et al. 2018), events would hinder the recovery process and would lead to coral community phase-shift with widespread reef mortality (Giglio et al. 2020).

## 6.4. Conclusion

The present damage calls for urgent intervention by policy-makers, stakeholders, and management strategists to conserve the reefs along the Central West coast of India. Sites with lower diversity of foliose corals could be used for recreational SCUBA diving activities and mooring buoys should be deployed in the sites to minimise the damage. Also, the number of boats and tourists should be within the carrying capacity of the reefs.

At the local level, the tourists as well as the tour operators should be trained about conservation of coral reefs. Unless, strict immediate actions and regulations are implemented in the Indian reefs, we might not be able to enjoy these wonderful and highly productive marine ecosystems in the coming years.

# *Chapter 7*

# Impact of heat stress on the model coral organism- *Aiptasia pulchella*

## 7.1 Introduction

A healthy relationship between cnidarians (e.g., corals, octocorals, and sea anemones) and the endosymbiotic zooxanthellae is needed for the successful functioning of a coral reef ecosystem. The loss of symbiont attributed to changing environmental conditions like elevated sea surface temperature, ocean acidification, and poor water quality leads to the whitened appearance called 'bleaching' (Berkelmans & Willis, 1999) often resulting in the death of the host. Over the past two decades, coral reefs globally have undergone unprecedented bleaching due to the increase in sea surface temperature, including the three major bleaching episodes of 1998, 2010, and 2015 (Heron et al. 2016) and there has been a rising interest in the field of protein expression in scleractinian corals with these recurrent coral bleaching events.

Organisms can tolerate stress to a certain extent by regulating their gene expression (Xu et al. 2015) and generating molecules like the heat shock proteins (HSPs). HSPs are molecular chaperones commonly used as a biomarker for coral health assessment (Sharp et al. 1997; Bythell et al. 1995; Downs et al. 2000) and are classified by their molecular weight as HSP40, HSP60, HSP70, HSP90, HSP100, and the small HSPs, each with a specific function and intracellular localization (Pockley, 2003). Under normal cellular physiological conditions, HSPs regulate protein homeostasis, sort intracellular protein transport (Lindquist, 1986), and guide assembly of protein complexes (Bukau & Horwich, 1998). Under heat or other environmental stress, HSPs act to reduce the accumulation of damaged proteins (Bozaykut et al. 2014; Balchin et al. 2016) and restore proteolytic homeostasis through their cytoprotective functions (Louis et al. 2017). HSP induction under stress conditions has been reported in various zooxanthellate cnidarians. HSP70 has been observed to be up-regulated in the colonies of *Acropora aspera* during laboratory-induced heat stress experiments (Leggat et al. 2011) and in the aposymbiotic larvae of *Acropora millepora* (Rodrigues-Lanetty et al. 2009). Desalvo et al. 2008 reported increased expression of HSP90 in *Orbicella faveolata* on exposure to thermal stress. Similar results have been observed in colonies of *Porites astreoides* when subjected to heat/light stress (Kenkel et al. 2011). Kenkel et al. 2011 also found a 4- fold increase in the expression of HSP60 in *Porites asteroides* after exposure to heat stress. Similarly, HSP60 has been wildly studied across different animals and is known to provide resistance in adverse temperature conditions (Cheng et al. 1989).

Despite the growing research in the field of coral-symbiosis relationships, the cellular and molecular mechanisms of bleaching remain poorly understood (Davy et al. 2012; Douglas, 2003, Weis, 2008). Scleractinian corals present logistical difficulties for laboratory investigation as they are costly to maintain, grow slowly, and wild animals have heterogeneous background making them a hard subject for gene expression studies (Lehnert et al. 2012). These limitations have led to the emergence of *Aiptasia* as a model organism for coral biology studies since it also harbours symbiotic zooxanthellae (Weis et al. 2008; Lehnert et al. (2012). *Aipatsia* is extremely hardy with rapid growth (Hunter, 1984) and reproduces via both sexual and asexual methods, leading to monoclonal strains in the laboratory (Vollmer et al. 2002). Additionally, *Aiptasia* can be maintained in laboratory conditions in the aposymbiotic state (free of symbionts) for long periods and can be re-infected with symbionts of different strains (Hoegh-Guldberg, 1999; Belda-Baillie et al. 2002; Perez & Weis, 2006). Black et al. (1995) reported the generation of HSP68 and HSP72 in *Aiptasia pallida* after temperature treatment at 35°C as measured by autoradiography. Choresh et al. (2001) recorded an over-expression of HSP60 by *Anemonia viridis* as recognized by the specific monoclonal antibody on exposure to 31° C in the laboratory as well as in tidal pools.

To date, all studies of HSP in cnidarian have focused on molecular aspects or gene expression as measured by tools like PCR or western blot. Although somewhat informative, these studies give no evidence as to where protein expression occurs in the host. Such studies might yield insight as to the physiology of HSP and pathogenesis of conditions like bleaching. Here, the aim was to 1) investigate the histological and cellular changes in *Aiptasia pulchella* under heat stress, and 2) to use immunohistochemistry to localize expression of HSP60 and HSP90 in the host. The results obtained from this comparative study could provide further insights into the cellular and histological basis of coral bleaching in scleractinian corals and provide the foundation for further research about mechanisms of heat tolerance conferred by heat shock proteins in cnidarians.

## 7.2 Materials and methods

### 7.2.1 Animal collection and stock maintenance

Symbiotic *Aipatsia pulchella* individuals were collected from the Coral Restoration nursery at Sand Island, Hawaii and maintained in an incubator in 250 ml small plastic tanks in ASW (30-32 ppt) at 25°C with 20-40 µmol photons $m^{-2}$ $s^{-1}$ of photosynthetically active radiation (PAR) on a 12 h:12 h light: dark cycle. They were fed twice weekly with freshly hatched brine-shrimp nauplii and the water was changed after three hours of feeding. Once animals reached a density of 10 animals per tank, they were transferred to new tanks (5 animals per tank) and were allowed to propagate for two months before final transfer to experimental tanks.

### 7.2.2 Experimental setup

Large-sized looking individuals with oral disk's diameter ~ 1.0 cm (n=3) were transferred to experimental tanks and allowed to acclimatize for a week before the experiment. The experimental setup comprised duplicate tanks each containing three captive-raised that were in culture for at least 60 days and or wild-caught individuals, which were immediately experimented on, for a total of 4 tanks, henceforth called Captive and Wild set, respectively. All the animals were grossly examined before and after the experiment, photographed and the following data recorded: colour (pale or dark), body size based on the diameter of the oral disk (mm) as small (<=3 mm), medium (>3 to <=7 mm) and large (>7 mm) and tentacles (extended or retracted, thick or thin). The animals were preserved in Zinc Formaldehyde solution (Z-Fix, Anatech) diluted 1:4 in seawater at the end of each experiment.

### 7.2.3 Heat stress experiment

The treatment set was exposed to water at 32°C (preheated to 32°C) for 24 hours whereas the control set was maintained at 25°C. All other parameters were kept the same as in the propagation culture. After 24 hr of heat exposure, experimental and control animals were observed and the experiment was terminated by fixing 2 animals per tank.

Fixed animals were processed for histopathology by paraffin embedding, trimmed at 5-µm, and stained with hematoxylin and eosin (H&E).

### 7.2.4 Immunohistochemistry

For immunohistochemistry (IHC), tissues were deparaffinized in xylene and rehydrated in ethanol series (100%, 95%) followed by rehydration water. A panel of the following antibodies was tested on *Aiptasia* tissues using a variety of antigen retrieval techniques: SPA-805 (anti-HSP60), SPA-807 (anti-HSP 60), SPA-810 (anti- HSP 70), SPA- 815 (anti-

HSP 73/HSC 70), SPA-822 (anti-HSP 70/HSC 70), SPA- 840 (anti-HSP90 α) and SPA-842 (ant-HSP 90ß). HSP 90ß and HSP 60 were selected for further studies, because they provided positive reactions whereas all the other antibodies failed to recognize HSP epitopes on IHC. Heat retrieval was done in Tris-EDTA (pH 6) in a steamer for 20 min and cooled for 20 mins. Tissues were incubated with wash buffer (1X PBST) for 1 min, blocked with 3% $H_2O_2$ for 10 min followed by 2% MPBS for 1 hr.  Primary antibody incubation for HSP90ß was done overnight at 4° C with mouse anti-HSP90ß clone K3705 monoclonal antibodies (Enzo cat# ADI-SPA-842) diluted 1:250 in 2 % MPBS. Primary antibody incubation for HSP60 was done overnight at 4° C with mouse anti-HSP60 clone LK-2 monoclonal antibodies (Enzo cat# ADI-SPA-807-E) diluted 1:50 in 2 % MPBS. After washing, tissues were incubated with goat anti-mouse conjugated with HRP (Southern biotech) at 1:1000 dilution in 2% MPBS for 1 hr at room temperature. After washing, colour development was visualized with diaminobenzidine (Dako), and tissues were counterstained with hematoxylin (Vector Labs) before dehydration in reverse alcohol series and clearing with xylene. Tissues were then mounted in cytoseal (Richard-Allan Scientific) with a cover-slip and examined under light microscopy. Positive control for SPA 807 and SPA 842 was human colon cancer tissue and human breast cancer tissue respectively. Experimental negative controls were *Aiptasia* tissue without primary antibody incubation.

## 7.3. Results

### 7.3.1. Morphological changes

Both sets of animals showed a reaction to heat stress in 24 hours (Fig. 1) comprising shrinking and pallor. A more pronounced shrinking was observed in the wild animals than the captive setting.  T-test revealed a significant difference between the mean size of the animals (oral disk diameter), before and after the heat treatment. The significant difference was higher for the wild animals (before versus after treatment) (p= 0.0376) than the captive animals (p=0.0489). There was no significant difference in the mean size of captive animals versus wild animals.

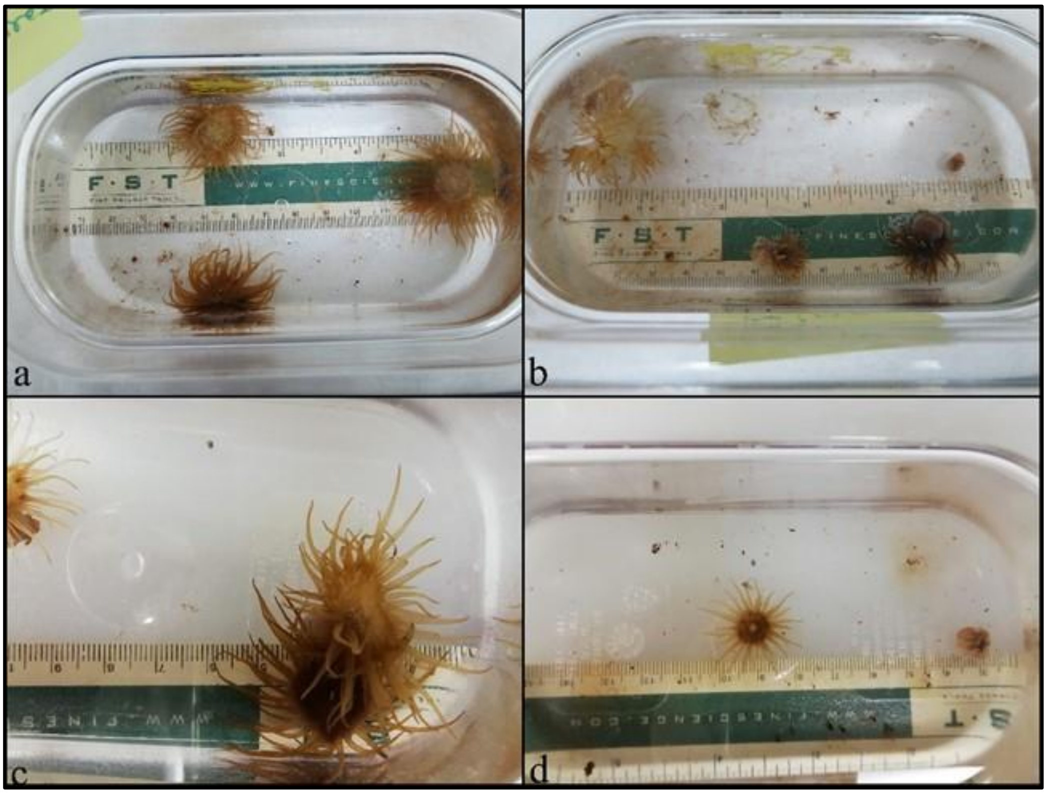


**Fig. 7.1: Morphological differences in *Aiptasia pulchella* before (a, c) and after heat treatment (b, d) in captive (a, b) and wild (c, d) animals.**

### 7.3.2. Histopathological changes

The tissue and cellular structure showed drastic changes between the heat-stressed and control animals in both captive and wild set (Fig. 7.2-7.4). Control animals had the normal architecture of tissue epithelia, mesoglea, and the gastrodermis. The epidermis was thin with stratified columnar epithelium containing basal nuclei and an even distribution of brightly stained spirocysts and mucocytes in control animals of both captive and wild set. (Fig. 7.2A, 7.3A). Mesoglea was thin and distinct (Fig. 7.2A, 7.3A). The gastrodermis layer appeared healthy with cuboidal cells with tiny intercellular spaces (Fig. 7.2A, 7.3A). Tentacles had a clear distinction between the layers of epidermis, mesoglea, and gastrodermis. Epidermis of tentacle had a uniform arrangement of brightly stained spirocysts and the gastrodermis was intact and replete with zooxanthellae. Evenly distributed zooxanthellae in the gastrodermis were round with eosinophilic cytoplasm and prominent nucleus, and had distinct brownish-green accumulation bodies (Fig 7.2B, 7.3B). However, they looked paler in the wild set (Fig. 3B). Mesenterial filaments had an even distribution of spirocysts in the cnidoglandular caps and were densely packed in the wild set. Gonads were brightly stained and had prominent cuboidal supporting cells (Fig. 7.4C, 7.5C).

In contrast, the heat-stressed animals showed loss of tissue architecture in both captive and wild set. Epidermal cells of heat-treated captive animals showed pyknosis and a visible concentration of zooxanthellae towards the distal end of gastrodermis (Fig. 7.2D). The epidermis of wild heat-treated animals showed hypertrophied pyknotic epidermis, with an increase in the number of mucocytes and cleft formation between epidermis and mesoglea (Fig. 7.3D). Tentacles had a disruption in shape with vacuolated epidermis and an increase in mucocytes numbers. Gastrodermis showed dissociation of cells with loss of zooxanthellae and the presence of empty symbiosome (Fig. 7.2E, 7.3E). Zooxanthellae were irregular, pallor, and had a notable distance between symbiont and symbiosome was visible (Fig. 7.2E, 7.3E). However, the response was more drastic in the wild animals with a visible reduction in symbiont density and presence of expelled spirocysts in the gastrodermis (Fig. 7.3E).

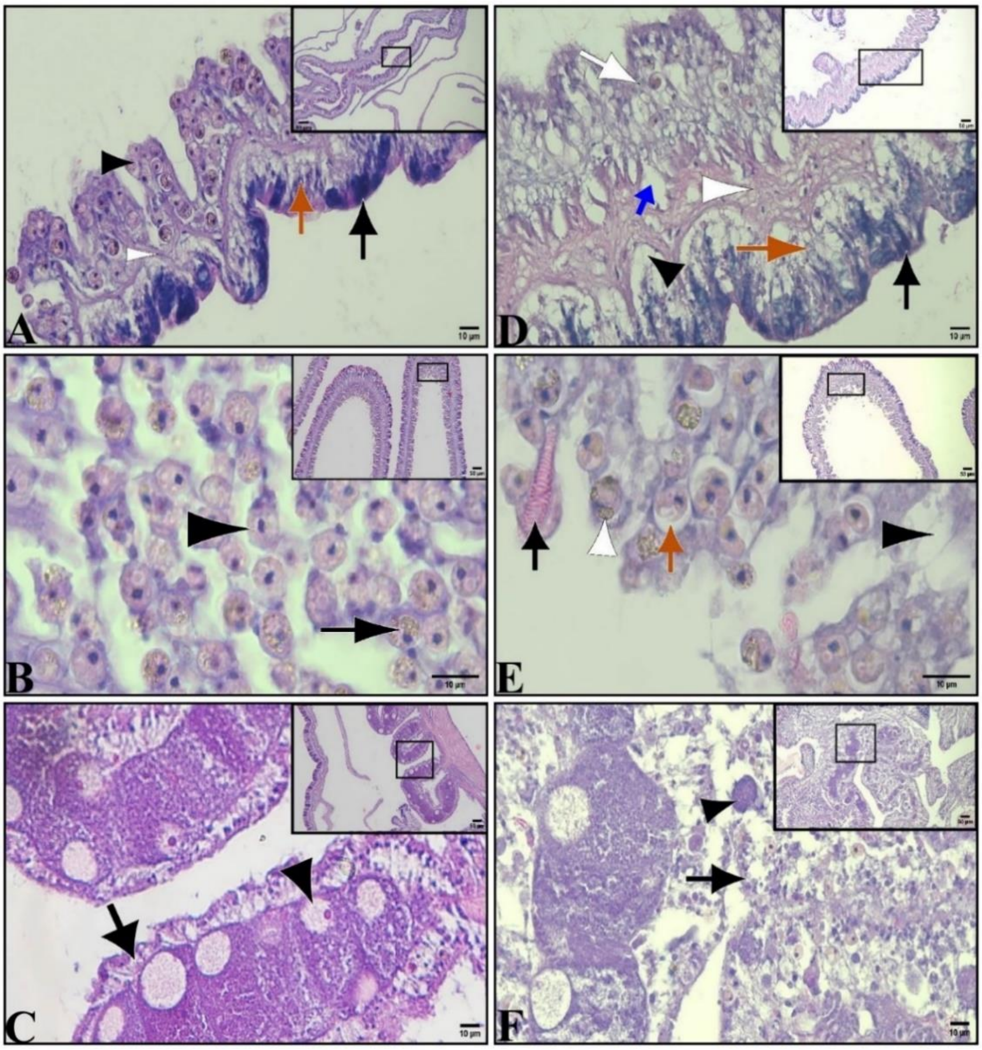


**Fig. 7.2*: H&E images of the surface body wall of control and heat-treated A*iptasia* tissue of captive animals**. The images represent higher magnification shots of the region marked within rectangles in the inset shots. **A) The epidermis of the control animal at 40X. Note homogenous columnar epithelium (orange arrow) with mucocytes (black arrow), distinct mesoglea (white arrowhead) and gastrodermis (black arrowhead) replete with zooxanthellae. B) Zooxanthellae of control animal's tentacle at 100X. Note the regular, even margin of zooxanthellae along with the visible nucleus (black arrowhead) and greenish-brown accumulation body (black arrow). C) Well developed oocytes of control animal at 40X. Note the oocyte with visible nucleoli (black arrowhead) and the uniformly arranged cuboidal supporting cells (black arrow). D) Epidermis of the heat-treated animal at 40X. Note pyknosis of epidermal cells (orange arrow), enlarged mucocytes (black arrow), increased space between mesoglea and gastrodermis (blue arrow) and cleft formation between epidermis and mesoglea (black arrowhead) with hypertrophied mesoglea (white arrowhead) and atrophied**

**gastrodermis (white arrow) at 40X. E) Zooxanthellae of the heat-treated animal's tentacle at 100X. Note the loss in shape of paler zooxanthellae with a prominent space between the symbiont and the symbiosome (orange arrow), irregular shaped accumulation body (white arrowhead.), empty symbiosome (black arrowhead), and the expelled spirocysts (black arrow). F) Necrotic oocytes (black arrowhead) of the heat-treated animals at 40X. Note diffuse liquefaction necrosis of supporting cells of oocytes (black arrow).**

Hypertrophied mesenterial filaments and cnidoglandular caps were observed in the heat-stressed animals. Supporting cells of the gonads manifested diffuse liquefaction necrosis with loss of tissue architecture (Fig. 7.2F, 7.3F). Epidermal cells of the basal body in heat-treated animals were diffusely necrotic exemplified by hypereosinophilia and pyknosis (Fig. 7.4A, 7.4B). The basal body was also marked with the presence of expelled zooxanthellae and spirocysts (both broken and intact) in the heat-treated animals (Fig. 7.4C, 7.4D). Overall, tissue staining was weaker in the heat-stressed animals as compared to the control set and the reaction was more dramatic in the wild set as compared to the captive setting.

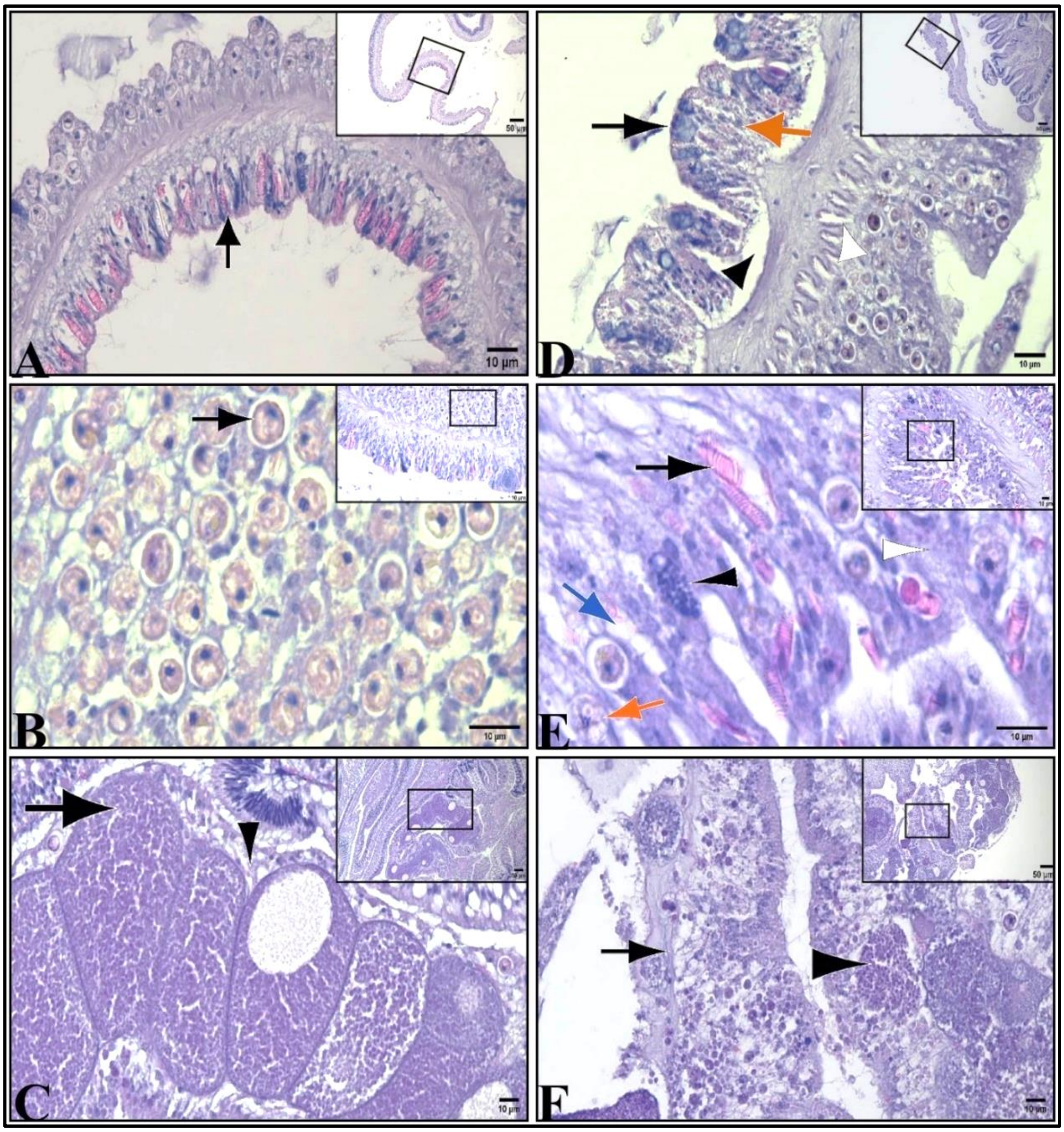


**Fig. 7.3: H&E images of the surface body wall of control and heat-treated *Aiptasia* tissue of wild animals.** The images represent higher magnification shots of the region marked within rectangles in the inset shots. **A) Normal epithelium at 40X. Note homogenous columnar epithelium of epidermis with evenly distributed spirocysts (black arrow). B) Zooxanthellae of control animal's tentacle at 100X. Note the even margin of zooxanthellae along with the visible nucleus (black arrow). C) Well developed oocytes of control animal at 40X (black arrow). Note the uniformly arranged cuboidal supporting cells (black arrow). D) Epidermis of the heat-treated animal. Note the enlarged mucocytes (black arrow), necrotic columnar epithelial cells (orange arrow), increased space between epidermis and mesoglea (black arrowhead), and the cleft between hypertrophied mesoglea and the gastrodermis (white arrowhead). E) Reduced number of zooxanthellae of the heat-treated animal's tentacle**

**at 100X. Note clumping and dissociation of the gastrodermal tissue (white arrowhead), pale irregular zooxanthella (orange arrow), expelled mucocytes (black arrowhead) and spirocysts (black arrow) and the empty symbiosome (blue arrow). F) Necrotic oocytes (black arrow) of the heat-treated animals at 40X. Note diffuse liquefaction necrosis of supporting cells of oocytes (black arrowhead).**

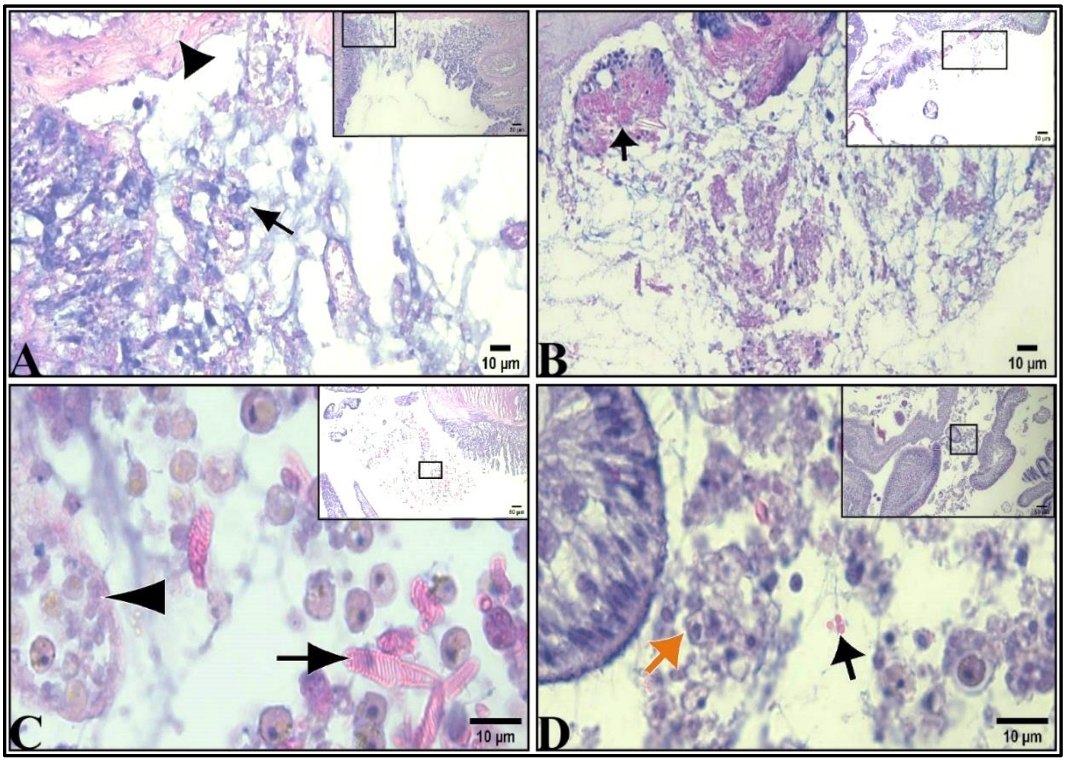


**Fig. 7.4: H&E Images of the basal body wall of heat-treated *Aiptasia* tissue of captive and wild animals.** The images represent higher magnification shots of the region marked within rectangles in the inset shots**. A) Basal body epidermis of the heat-treated captive animal showing loss of tissue architecture at 40X. Note liquefaction necrosis of epithelial cells and mucocytes (black arrow) and the thickened mesoglea (black arrowhead). B) Basal body epidermis of the heat-treated wild animal hypertrophied at 40X. Note hypereosinophilia and liquefaction necrosis of the epidermis (black arrow). C) Region of the basal body of the heat-treated captive animals at 100X. Note the expelled zooxanthellae (black arrowhead) and the cluster of expelled spirocysts (black arrow). D) Region of the basal body of the heat-treated wild animal at 100X. Note the liquefied necrotic gastrodermis tissue with expelled zooxanthellae (orange arrow), and broken expelled spirocysts (black arrow).**

### 7.3.3. Immunohistochemistry observation

Site of expression and the differential expression of HSPs in our study corroborated with the regions of histopathological changes after heat stress experiment. The control animals showed much weaker staining of HSP60 (SPA 807) as compared to the heat-stressed animals with only diffuse staining in the apical epidermis, tentacles, and oocytes (Fig. 7.5a-c). Mesoglea, mucocytes, and the spirocysts remained unstained (Fig. 7.5b, d; 7.6b, d) in both control and heat-treated animals. Positive staining of HSP60 (SPA 807) was mostly localized in the apical epidermis (Fig. 7.5d), gastrodermis (Fig.7.5d, e), and the oocytes of the heat-stressed captive animals (Fig. 7.5f). Wild animals showed a more drastic response than the captive ones with stronger staining for HSP60 in the swollen epidermis of the tentacles (Fig. 7.6d), gastrodermis containing degraded zooxanthellae (Fig. 7.6d, e), and in the oocytes of the treated animals (Fig. 7.6f).

The control animals (both captive and wild set) had a weaker constitutive expression of the HSP90ß, specifically in the apical cells of epidermis (Fig. 7a, 8a), tentacles (Fig. 7.7b, 7.8b), and in the oocytes (Fig. 7.7c, 7.8c) as compared to the heat-treated animals (Fig. 7.7d, 7.8d). HSP90ß (SPA 842) was over expressed in the pyknotic surface body epidermis (Fig. 7d), epidermis and gastrodermis of tentacles (Fig. 7.7e) as well as in the cnidoglandular caps (Fig. 7.7f) of the heat-treated captive animals. The wild animals showed sloughing of the epidermis with specific staining in the epidermis (Fig. 7.8d), in dissociated gastrodermis and symbionts in the tentacles (Fig. 7.8e), and the cnidoglandular caps (Fig. 8f) of the heat-treated animals. Spirocysts and mucocytes remained unstained in both captive and wild set of animals.

Experimental positive control for SPA 807 and SPA 842 (human colon cancer tissue and human breast cancer tissue, respectively) stained positive. Experimental negative controls (*Aiptasia* tissue without primary antibody incubation) showed no antibody signal.

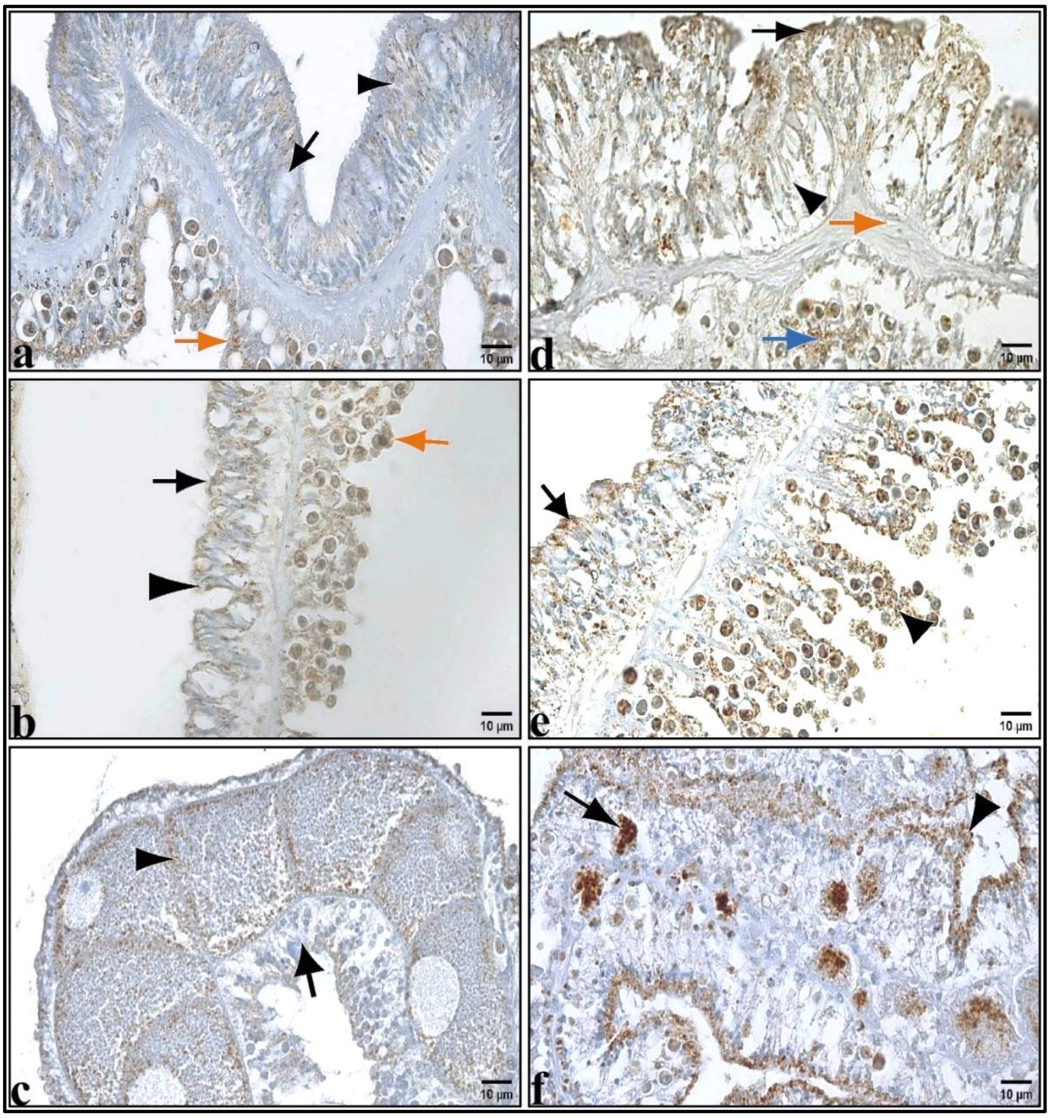


**Fig. 7.5: Expression of HSP60 (SPA 807) in captive animals under control and heat stress conditions at 40X. a) Control, note diffuse staining in the apical surface of the epidermis (black arrowhead), and the gastrodermis (orange arrow). Mucocytes remained unstained (black arrow). b) Tentacle of the heat-treated animal. Note diffuse staining of HSP60 in the gastrodermis and symbionts (orange arrow) and in the apical epidermis (black arrow). Spirocysts remained unstained (black arrow head). c) Oocytes of the control animal. Note diffuse staining (constitutive expression) in the oocytes (black arrowhead). Supporting cells remained unstained (black arrow). d) Epidermis of the heat-treated animal. Note overexpression of HSP60 in the apical surface of the epidermis (black arrow) and in the gastrodermis (blue arrow). Spirocysts (black arrowhead.) and mesoglea (orange arrow.) remained unstained. e)**

**Tentacle of the treated animal. Note overexpression of HSP60 in the apical epidermis (black arrow) and in the dissociated gastrodermis (black arrowhead). f) Oocytes of the heat-treated animal. Note overexpression of HSP60 in the oocytes (black arrow), and in the and apical surface of supporting cells (black arrowhead). Scale bar = 10 µm.**

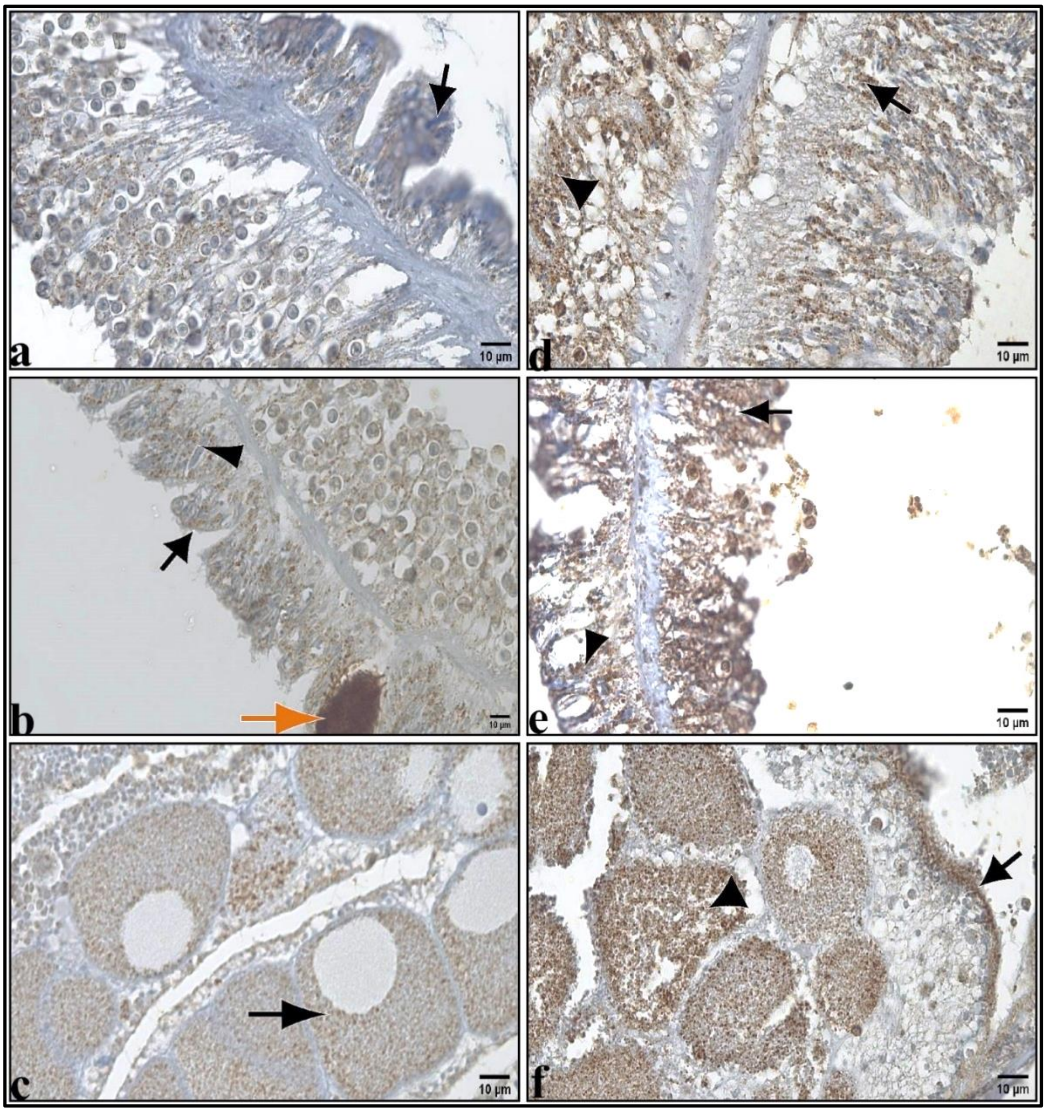


**Fig. 7.6: Expression of HSP60 (SPA 807) in the wild animals under control and heat stress conditions at 40X. a) Epidermis of control animal, note the unstained mucocytes (black arrow). b) Tentacle of the control animal. Note diffuse staining in the epidermis (black arrow and overexpression in the bacterial aggregate (?) (orange arrow). Spirocysts remained unstained (black arrowhead). c) Oocytes of the control animal. Note diffuse constitutive expression in the cuboidal oocytes (black arrow). d)Epidermis of the heat-treated animal, note over-expression in the epidermis (black arrow), and in gastrodermal layer with symbionts (black arrowhead). e) Tentacle of the heat-treated**

**animal. Note over-expression in the epidermis (black arrowhead) and in the gastrodermis with symbionts (black arrow). f) Oocytes of the heat-treated animal. Note overexpression of HSP60 in the oocytes (arrowhead) and apical surface of supporting cells (arrow). Scale bar = 10 µm.**

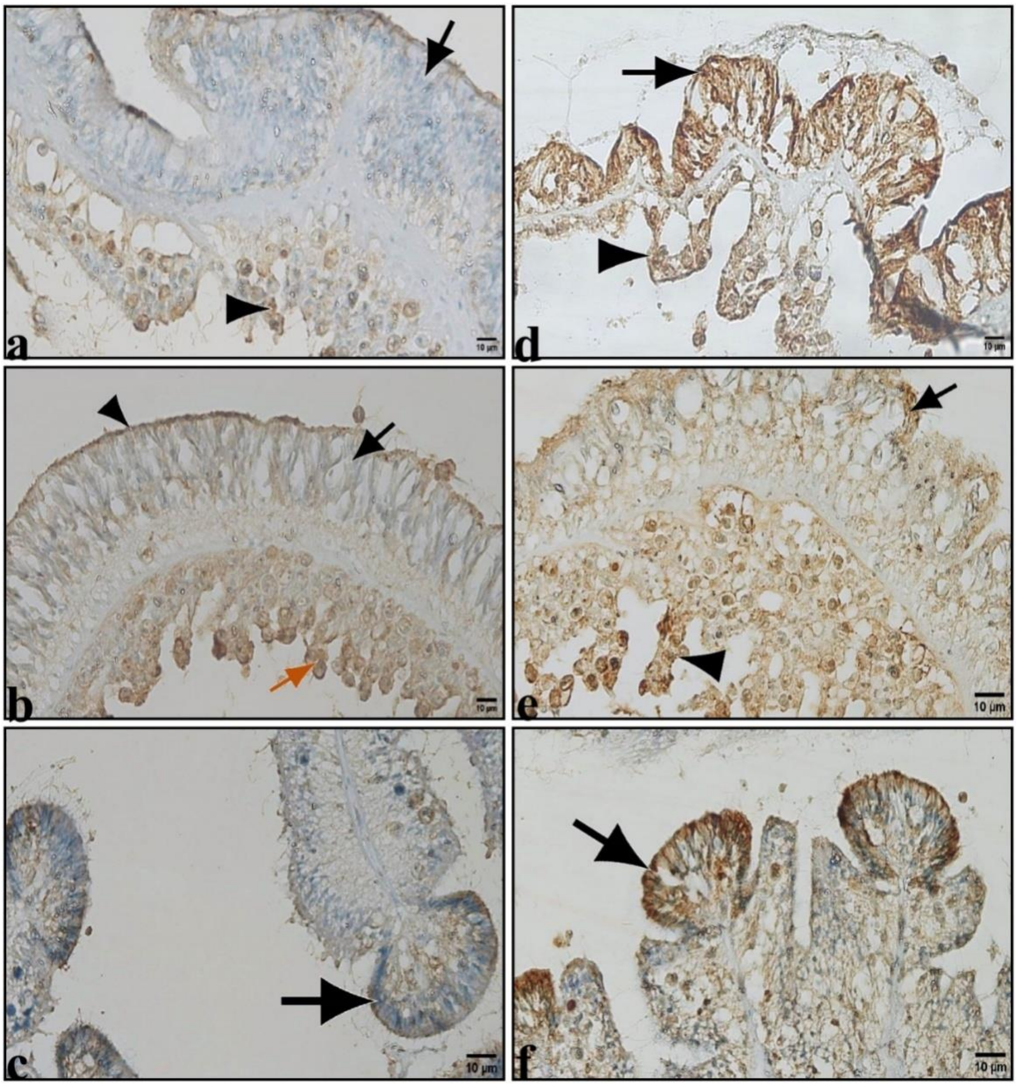


**Fig. 7.7: Expression of HSP90 ß (SPA 842) in captive animals under control and heat stress conditions at 40X. a) Epidermis of the control animals. Note, unstained mucocytes (black arrow), and diffuse staining in the gastrodermis (black arrowhead). b) Tentacle of the control animal. Note diffuse staining in the apical epidermis (black arrow) and the distal end of gastrodermis (orange arrow). Spirocysts remained unstained (black arrow. c) Normal-looking mesenterial filaments and Cnidoglandular caps of the control animal. Note no staining in the apical epidermis of Cnidoglandular caps (black arrow). d) Pyknotic epidermis of the heat-treated animal. Note overexpression of HSP90ß in the epidermis (black arrow) and gastrodermis (black**

**arrowhead). e) Tentacle of the heat-treated animal. Note over-expression in the apical epidermis (black arrow) and gastrodermis (black arrowhead). f) Cnidoglandular caps after heat treatment. Note overexpression of the apical cellular layer of the Cnidoglandular caps (black arrow). Scale bar = 10 µm.**

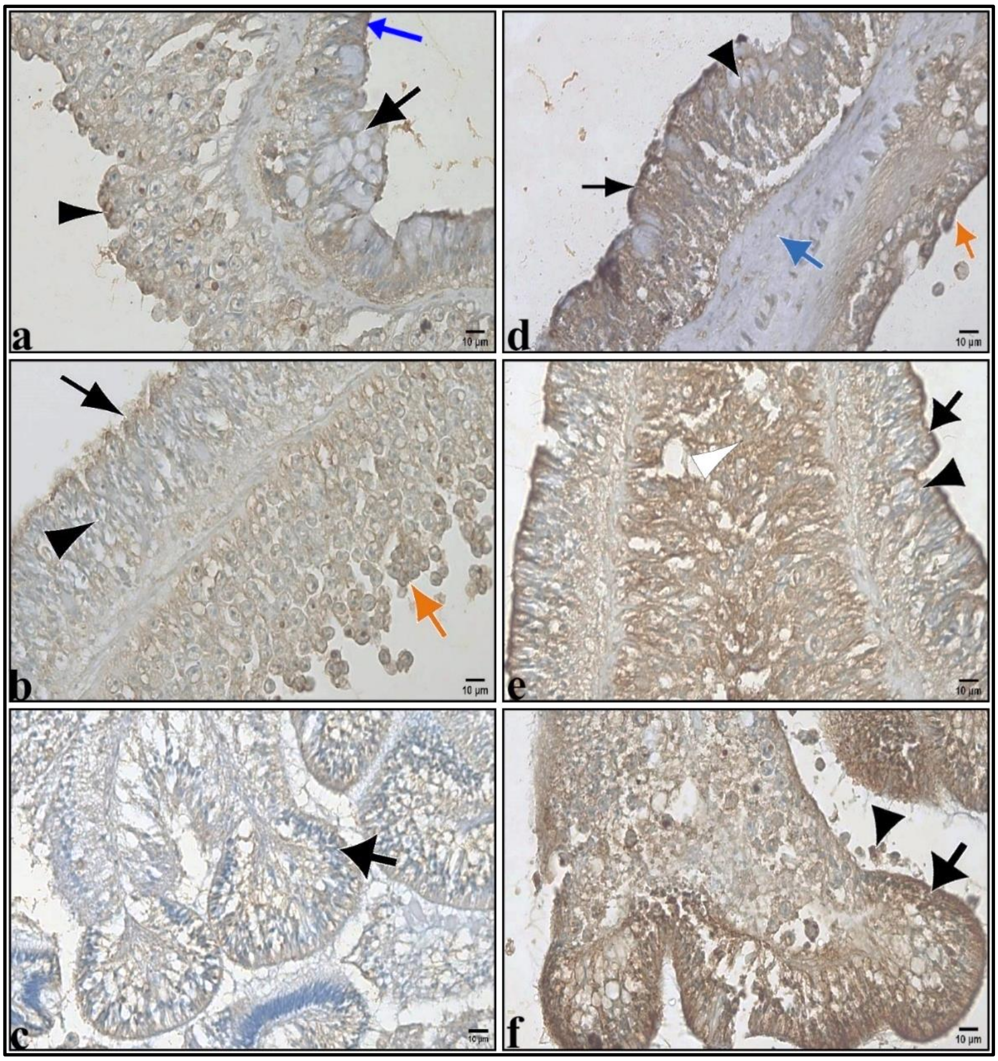


**Fig. 7.8: Expression of HSP90ß (SPA 842) in wild animals under control and heat stress conditions at 40X. a) Epidermis of the control animals, note diffuse stain mainly in the apical cells of the epidermis (blue arrow), and gastrodermis (black arrowhead). Mucocytes (black arrow.) remained unstained. b) Tentacle of the control animal. Note diffuse staining in the apical epidermis (black arrow) and gastrodermis (orange arrow) Spirocysts (black arrowhead) remained unstained. c) Mesenterial filament and cnidoglandular cap of the control animals. Note the unstained basophilic nucleated cells of the cnidoglandular caps (black arrow). d) Epidermis of the heat-treated animal. Note overexpression of HSP90ß in the epidermis (black arrow), and**

**gastrodermis (orange arrow). Mesoglea (blue arrow) and mucocytes (black arrowhead.) remained unstained. e) Tentacle of the heat-treated animal. Note overexpression in the apical epidermis (black arrow), and gastrodermis (white arrowhead). Spirocysts remained unstained (black arrowhead). f) Mesenterial filament and cnidoglandular cap of the heat-treated animal. Note overexpression of HSP90ß in the apical layer of cnidoglandular caps (black arrow), and in the expelled zooxanthellae (black arrowhead). Scale bar = 10 µm.**

## 7.4. Discussion

This study was designed to study the specific expression of heat shock proteins as well as the morphological and histological changes in *Aiptasia*, (both wild and captive) on exposure to heat stress under laboratory conditions. The study revealed that heat stress has damaging effects on the tissue and cells of model organism *Aiptasia*. Morphologically, the animals showed contraction behaviour (both tentacles body) indicating a high-stress state (Dunn et al. 2004). In the present study, the captive animals seemed to have responded better to heat stress as compared to the wild animals as evident by both morphological and histological changes. The captive animals shrunk and turned darker, possibly due to the condensation of Symbiodiniaceae, while the wild animals shrunk and turned paler (due to loss of symbionts). Similar morphological changes were observed by Weizmann & Levy (2019) in *Aiptasia* subjected to a gradual increase in temperature up to 34°C. According to their findings, contraction is evidence of adaptive plasticity in cnidarians linked to their epigenetics modification. It is a possibility that the stress experienced by captive *Aiptasia* during acclimatization in the laboratory provided them with a slightly better tolerance to heat stress as compared to the wild *Aiptasia*. There is strong evidence that environmental history can significantly impact the coral’s response to elevated temperature and their overall tolerance to extreme stress (Hawkins & Warner, 2017), by adjusting their physiological and molecular response to heat stress (Ainsworth et al. 2016; Berry & Gasch, 2008). Based on a model of

Great Barrier Reef’s Sea Surface Temperature (SST), Ainsworth et al. (2016) showed that the present events would render the reefs physiologically better prepared for the main stress event. Recent studies also indicate the role of coral holobiont (Diaz et al., 2016; Glasl et al. 2016), host genotype (Bellantuono et al. 2012; Hawkins et al. 2015), epigenetic modifications such as DNA methylation (Eirin-Lopez & Putnam, 2019; Dixon et al. 2015), and upregulation of heat shock proteins to provide adaptability and survival in corals in response to environmental stress (Leggat et al. 2011).

The histological changes observed in the present study are very similar to tissue changes in other corals and *Aipatsia* as reported in the literature (Gates et al. 1992; Mise & Hidaka, 2003; Ladriere et al. 2008; Fujise et al. 2013). The observed loss of tissue and cellular integrity in epithelium, gastrodermis, and signs of both internal and external damage in zooxanthellae. Loss of epithelium tissue architecture, resembling wound healing was observed in heat-stressed branches of *Acropora hyacinthus* by Traylor-Knowles (2019). According to the observations of Traylor-Knowles et al. (2017), heat stress in *A. hyacinthus* disrupted the epithelium, releasing spirocysts and microbasic mastigophores from lysed cells. The release of undischarged spirocysts observed in this study is an indication of host cell necrosis and detachment and was also reported by Bhagooli & Hidaka (2004) in coral *Galaxea fascicularis* after heat exposure at 32°C.

In the present study, the gastrodermal layer, housing Symbiodiniaceae was affected the worst with the marked reduced density as well as the reduction in symbiont size. O Heogh Gul (1987) and Ainsworth & Guldberg (2008) observed a shift of zooxanthella towards the distal portion of the gastrodermis in corals exposed to environmental stress. Moreover, a higher concentration of zooxanthellae towards the distal end of the host gastrodermis in heat-stressed animals was also observed. In contrast, control animals had an even distribution of zooxanthellae in the gastrodermis. Hoegh-Guldberg et al. (1987) and Ainsworth & Guldberg

(2008) described these changes as circumstantial evidence of exocytosis. The increase in the number of damaged zooxanthellae as compared to the healthy-looking zooxanthellae corroborates with the work of Bhagooli & Hidaka (2004), also reported a significant increase in degraded zooxanthellae in coral *Galaxea fascicularis* after heat exposure at 32°C. The increase in the number of mucocytes under heat stress corroborates the work of Fransolet et al. (2013). This has an adaptive significance as the increase in mucocytes and spirocysts increases the efficiency of catching prey after the bleaching episode (Fransolet et al. 2013) and may partially compensate for the lack of symbionts by relying more on the heterotrophic mode of nutrition (Tremblay et al. 2015). Heat stress causes the generation of reactive oxygen species (ROS) in Symbiodiniaceae, which disrupts the cellular integrity of gastrodermis, causing the release or degradation of zooxanthellae by many different cellular mechanisms (Nielsen et al. 2018; Bieri et al. 2016). In-situ degradation of zooxanthellae has been reported in corals during natural bleaching events (Ainsworth & Hoegh-Guldberg, 2008; Brown et al. 1995) and in corals and anemone on exposure to temperature and/or light stress in the laboratory (Downs et al. 2009; Dunn et al. 2004). The vacant vacuolar spaces in the gastrodermis indicate spaces left after the expulsion of zooxanthellae and the apparent space between the symbiosome and the host membrane signifies retraction of zooxanthellae inside its vacuole (Ladriere et al. 2008). Vacuolization observed in the supporting cells of oocytes may be a sign of nutrient deficiency under stress. Reproduction is an energy-consuming process and it is possible that under high-stress cnidarians conserve energy by reduced reproduction. It has been well documented that coral bleaching has adverse and long-lasting effects on corals and leads to reduced fecundity and survival (Ward et al. 2000). Differential expression of HSPs in *Aiptasia* provided evidence for heterogeneity of cells in cnidaria. High expression of HSP60 and HSP90ß in heat-stressed *Aiptasia* indicates 31°C is a stressful temperature for the anemones. However, a lower constitutive of expression of both HSP60 and HSP90 indicated their role in normal physiological functioning. Choresh et al. (2001) also reported mild constitutive expression of HSP60 in anemone *Anemonia viridis,* whereas, an upregulated expression in animals stressed at 31°C indicating the thermotolerant role of HSP60.The specific site of HSP generation in *Aiptasia* corroborates with the finding of histopathology sites as observed in H&E slides. Epithelium, zooxanthellae, and oocytes were the sites with the most cellular and tissue damage and were concurrently the site of over expression of HSPs.

## 7.5. Conclusion

Studies on the pattern and specific expression of HSPs in cnidarians are scarce, as most of the research in cnidarians so far has focussed on western blotting to quantify HSP expression. This is one of the first studies to use immunohistochemistry to study the pattern and location of heat shock protein expression in *Aipatsia* and can be an important tool for similar studies in scleractinian corals. This information will be very useful in future to study the effects of climate change on corals by using *Aiptasia* as a model organism.

# Chapter 8

## Summary and future scope

Coral patches on the west coast of India are relatively understudied and have ecological as well as aesthetical value for the local community. An account of the biodiversity of these coral patches in MMS and Goa is crucial to appreciate the true beauty of the reefs. The present study has updated the diversity of corals from the highly understudied reefs of the central west coast of India. The diversity of hard corals is more in Grande Island (25 spp.) as compared to Malvan (19 spp.). Mapping revealed shallow areas to be rockier and more dominated by encrusting corals like *Porites*, whereas sandy deeper patches dominated by monospecific growth of *Turbinaria* and *Goniopora*. The presence of critically endangered and vulnerable coral species (IUCN red list), as well as ecologically sensitive species of Sea slugs in the study area, indicate the significance of these reef communities and points towards better sustainable management practices to protect them. Habitat mapping revealed that the sites with dominant rocky substratum had higher abundance of encrusting corals like *Porites*, *Favites, Goniastrea*, whereas the sites with a sandy substratum had soft corals and foliose coral, *T. mesenterina.* Habitat distributing mapping also revealed the sites with a sandy bottom had a lower diversity and abundance of hard corals, therefore these sites can be used for boat anchoring.

Both the regions are highly affected by adverse climate conditions and anthropogenic influence, especially the unplanned tourism. The corals are subjected to annual bleaching events with *Porites* corals affected the worst. Underwater surveys during October 2014 revealed mild, 4-6% coral bleaching in Grande Island; 5-7 % during April 2015 which increased to a mean of 50.5 % during the global bleaching event of 2015/16. November 2015. MMS witnessed a mean of 15% bleaching during 2014, which rose to 70 %

bleaching during 2015/16. This study also analysed the change in community structure after the bleaching event in Grande Island, Goa. The reef witnessed seasonal changes in benthic communities which were exacerbated by the bleaching event. With a continuous decline in the percentage of live coral, a sharp increase in the coverage of macroalgae and rubble increased from mean of 17.88% in November 2015 to a mean of 35.83% in April 2016 in the study area. The study also revealed the spread of coral eroding sponge-*Cliona* spp., after the bleaching event. It was recorded that foliose corals, *Turbinaria* spp. were most susceptible to sponge infestation, increasing from affecting mean 25% *Turbinaria* colonies in 2015 to mean 40 % infestation in 2017.

Despite the growing research in the field of coral-symbiosis relationships, the cellular and molecular mechanisms of bleaching remain poorly understood. Organisms have the capability of tolerating stress to a certain extent by regulating their gene expression and generating molecules like heat shock proteins (HSP). HSP induction under stress conditions has been reported in various zooxanthellate cnidarians including the model coral organism, a sea anemone-*Aiptasia*. This study provides insight into the histological and cellular changes in *Aiptasia pulchella* under heat stress, and the localization of expression of HSP60 and HSP90 using the tool of Immunohistochemistry. The present study shows that the heat stress at 31°C causes drastic changes in the tissue and cellular structure changes between the heat-stressed and control animals in both captive-raised and wild set. The gastrodermis layer housing the symbionts were affected the worst and HSP expression and histological damage was more in wild animals than the captive. The results obtained from this comparative study could provide further insights into the cellular and histological basis of coral bleaching in scleractinian corals and provide the foundation for further research about mechanisms of heat tolerance conferred by heat shock proteins in cnidarians.

This study also establishes the presence of skeletal tissue growth anomalies in the disturbed marginal reefs of the west coast of India and revealed a salient shift in bacterial communities between the healthy and STA-affected corals. The disease prevalence was highest in foliose coral, *Turbinaria mesenterina*. Growth anomalies on *T. Mesenterina* appeared as distinct globular, pigmented to partially bleached masses of coral skeleton. The diseased samples exhibited higher bacterial diversity and were dominated by opportunistic phyla like Planctomycetes, Cyanobacteria, Verrucomicrobia, and Acidobacteria as revealed by 16S metagenomics analysis. Several human pathogens and copiotrophic coral pathogens were observed in the diseased samples. Functional annotation of the bacterial communities revealed major changes in sulphur and nitrogen cycling in corals and may have a role in disease. Coral tumors could have formed in reaction to environmental stress (UV, heat stress, high nutrients, and sedimentation load), however, the shift in the bacterial community may have aggravated the already immunocompromised coral health and led to changes in metabolic pathways. It would be interesting to further study the histopathology of the affected corals to shed light on the etiology and disease pathogenesis.

Widespread damage was recorded in the study area mainly due to unregulated tourism activities like SCUBA and boat anchors dropping on vulnerable corals like *Turbinaria mesenterina.* Coral Damage Index revealed a severe degree of damage in most of the sites, much higher than the critical-cut-off of 4% with sites S1 (16.7%), S2 (15.78%), S4 (17.83 %) of Grande Island, and MMS1 (19.1 %), and MMS2 (13.5 %). Site MMS1 had the highest damage, possibly due to proximity to the Sindhudurg fort which gets hundreds of visitors every day, and is also the main diving spot in MMS. Categorising sites according to the coral morphology revealed that the sites dominated by foliose corals had a greater number of broken colonies as compared to the reef site dominated by massive and sub-massive corals.

The present situation calls for urgent intervention by policy-makers, stakeholders, and management strategists to conserve the reefs along the Central West coast of India. Sites with lower diversity of foliose corals could be used for recreational SCUBA diving activities and mooring buoys should be deployed in the sites to minimise the damage. Also, the number of boats and tourists should be within the carrying capacity of the reefs. At the local level, the tourists, as well as the tour operators, should be trained about the conservation of coral reefs. Unless strict immediate actions and regulations are implemented in the Indian reefs, we might not be able to enjoy these wonderful and highly productive marine ecosystems in the coming years.

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