CHAPTER 1 INTRODUCTION

Marine biology is the study of marine organisms, their habitats and environmental interactions. To understand the marine organisms, marine biologists study biological oceanography and related fields of chemical, physical and geological oceanography. The studies are divided into two broad spectrums in marine biology, such as functional biology and ecology. Functional biology is a study of how an organism performs roles in the surrounding environment such as reproduction, locomotion, feeding, digestion, etc. On the other hand, ecology deals with species interaction along with their physical and biological climate. The distribution and productivity of the organisms in the ecosystem are determined by these interactions.

A marine ecosystem resides in or near saltwater, meaning that marine environments can be found throughout the world, from a sandy or rocky beach of the deepest parts of the ocean. There are different types of marine ecosystems, including salt marshes, estuaries, the broad ocean the ocean floor, coral reefs, the inter-tidal zones, lagoons and mangroves. Marine environment can be divided into pelagic and benthic divisions. Both the marine environment divisions support different types of habitats and species. Maximum diversity of the organisms is observed in the littoral, sub littoral, epipelagic and mesopelagic zone. Principally due to the presence of sunlight which drives the physiological activities of various organisms. Studies of local fauna distribution and diversity are of great importance because these studies contribute to a better understanding of the structure, function and problems of local animal community (Fransozo *et al.*, 1992 and Hebling *et al.*, 1994).

1.1 INTERTIDAL ZONE

The intertidal zone or “littoral zone” is the area covered during high tide and exposed by sea water during low tide, revealing a unique ecosystem which persists under fluctuating conditions. Depending on the time of day and the tide level, the temperature of the intertidal zone is extremely variable.

However, during low tide air temperatures in the intertidal zone fluctuate considerably over a short period of time (Stillman and Somero, 1996) and reach peaks when low tides coincide with hot days (Helmuth, 2002 and Finke *et al*., 2007; Helmuth, 2002). These temperature fluctuations place a major physiological stressor on the different species within the intertidal zone. In the external medium and in surface films, salinity stress can occur. The wave action around the shore will wash away or dislodge species that are poorly suited or adapted. Species are threatened by desiccation during emersion at low tides or when they are situated in the high intertidal zones. The most common process is dehydration due to the loss of evaporative water. By migrating to a more suitable area, highly mobile organisms may escape desiccation. Less mobile species limit different activities (reduced metabolism) and contract the substrate more tightly. As a result, organism's inhabitant in the intertidal zone has developed several anatomical, physiological and behavioural adaptations that allow them to survive in this challenging environment.

1.1.1 INTERTIDAL ZONATION PATTERN

In coastal areas, the intertidal zone is known to be most diverse and productive as different types of flora and fauna are found in a few meters. The zonation of species along altitudinal, latitudinal or intertidal gradients represents their physical and biological response (Mettam, 1994). Early intertidal studies supported a significant role of physiological changes to temperature and desiccation stress in deciding patterns of vertical distribution (zonation) commonly found on rocky shores for intertidal species. Vertical zonation is the most important phenomenon or process observed in the rocky intertidal area where different bands or zones with different biodiversity are observed from the upper to the lower intertidal area (Stephenson and Stephenson, 1949; Ellis, 2003). Such regions are the biological processes that result from the rise and fall of the tidal water of the sea. Different scientists working on intertidal organism ecology have named this tide stratification according to local conditions. Such tide zones are comprising from highest to lower tide marks can be divided into Spray zone (Upper littoral zone), High tide zone (Upper mid littoral zone), Mid tide zone (Lower mid littoral zone) and Low tide zone (Lower littoral zone) (Fig. 1.1).

The Spray zone or Upper littoral zone is the zone above the upper mid-littoral zone, which during high tide can experience splash from waves but is never completely submerged at high tide. Few species in this region can tolerate the intense variations in humidity, temperature, and salinity. It is the most nutrient-poor and marginal component of the marshland ecosystem.

High tide zone or Upper mid littoral zone is flooded during the high tide only. It is a highly saline area. For a long period, parts of this area are exposed to the air as the tide recedes. Water abundance is not sufficiently high to support large amounts of vegetation, although some survive in the high tide zone. Barnacles, crabs, green algae, limpets, snails and some marine vegetation are the predominant species in this sub-region. There may also be rock pools in the high tide section. There, life is far more plentiful than in the spray environment.

Mid tide zone or Lower mid littoral zone is submerged and flooded by the tide cycle for about equivalent periods of time. The water comes twice a day, but also withdraws twice a day. Consequently, due to shorter direct exposure to the sun, temperatures are less extreme and therefore salinity is only marginally higher than ocean levels. For general, though, wave action is more intense than high tide and spray areas. The climate of mid tide zone is more complex than either the splash zone or the high intertidal zone. Organisms are often more dynamic and sometimes bigger in size than in the high tide and splash zone. There is also a much higher marine vegetation population in the middle tide zone, particularly seaweeds. In this area, species include anemones, barnacles, crabs, green algae, mussels, snails and sponges. Life in the middle tide zone, apart from being more populated, is more varied than the high tide and splash zones.

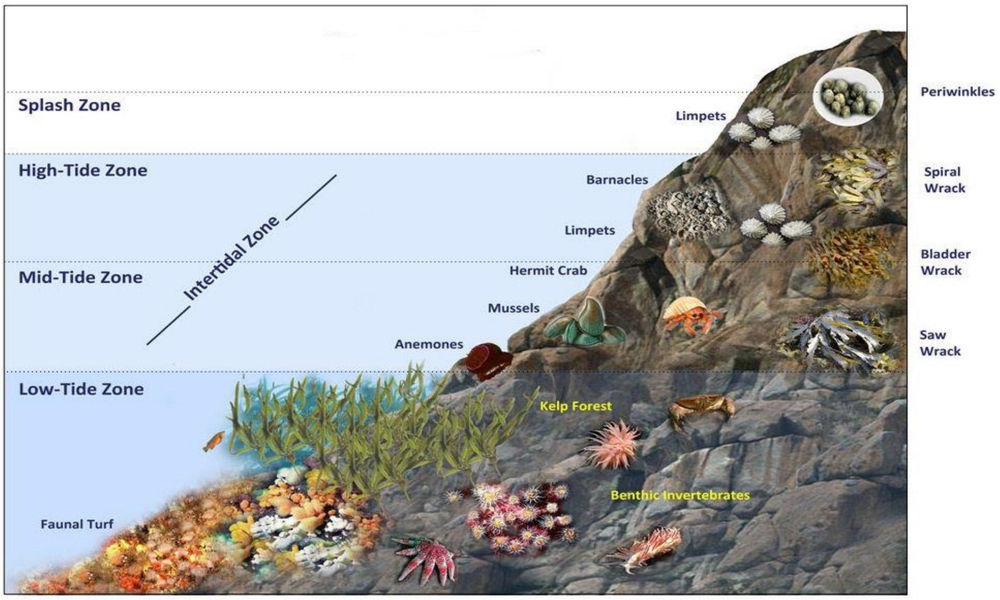

Figure 1.1 : Intertidal zonations diagram of the rocky shore **(Source:**[https://encryptedtbn0.gstatic.com/images?q=tbn:ANd9GcS0llwOWrqXK7TkH1ZYFy12w85uGtYR4YloqGj6_v_ZanzzT2fE)](https://encryptedtbn0.gstatic.com/images?q=tbn:ANd9GcS0llwOWrqXK7TkH1ZYFy12w85uGtYR4YloqGj6_v_ZanzzT2fE)

Low tide zone or Lower littoral zone is mostly remained submerged; it is exposed only at low tide point and during extremely low tides for a longer period of time. This area is full of life; the most significant difference to the other three with this sub-region is that there is much more marine vegetation, particularly seaweed. Organisms in this zone are usually not well suited to prolonged dryness and temperature cycles. In the lower intertidal there is higher species richness due to more interaction take place as most of the time the organisms are submerged in water. Some of the species inhabits in this zone are brown seaweed, crabs, green algae, nudibranchs, sea cucumber, sea stars, snails, sea urchins, shrimps and sponges. This area is usually occupied by Zoanthid coelenterate. Organisms can grow up to bigger sizes because more energy is available in the localized in this ecosystem and marine vegetation can also grow to larger sizes than in the other three intertidal sub-regions because of better water coverage: water is shallow enough to enable plenty of light to enter the vegetation to allow sufficient photosynthetic activity and salinity is almost natural.

1.1.2 INTERTIDAL BENTHIC COMMUNITY

Community structure is the term used by scientist for indicating what organisms are present in a given environment, in what numbers, and how they relate to each other. Unlike geographical space, differences in community structure have long been a major concern for the ecologists. Variations in species diversity are perhaps the most striking differences and have undoubtedly gained the most attention, certain aspects of population structure, including patterns of space utilization distribution of organisms, trophic structure and body size. The benthos is organisms that normally live in or near the seabed at any time during their life history. The benthic community consists of a wide variety of flora and fauna from all levels of the food web. The three methods for benthic life are (1) sessile: attachment to firm surfaces (2) mobile: free bottom movement or (3) burrowing: burrowing in sediments. Such habits are compatible with the primary ways in which benthic organisms receive food: absorption from seawater, predation, and ingestion of sediments. For living space and food, benthic organisms must be completed. The benthic ecosystem of coral reefs or intertidal beach serves as a good feeding, breeding, spawning and nursery ground to many marine organisms of economic importance. A striking character of the rocky shore population is that all the resident plants and animals are clustered or grouped in different zones, large congregations of gastropods, hermit crabs and sea anemones are found in the water-filled tide pools in the mid-intertidal zone. Crustaceans and Zoanthids form a dominant animal group in the lower intertidal zone.

1.2 PHYLUM CNIDARIA

Cnidarians have great ecological significance as they associate with a wide variety of faunal and floral species and are often characterized by a high diversity of species in reefs.

Cnidarians have two distinct morphological body plans known as polyps, which are sessile as adults, and medusa, which are mobile; throughout their lifecycle, some animals show both body plans (Fig. 1.2). Cnidarians are diploblastic, which means that they grow from two essential layers of the germ (cell): ectoderm or outer layer, and endoderm or inner layer. The outer ectoderm, or epidermis, includes the cnidocysts or the stinging cells which act as offensive or defensive order (Hessinger and Lenhoff, 1988), are the characteristic of the phylum.

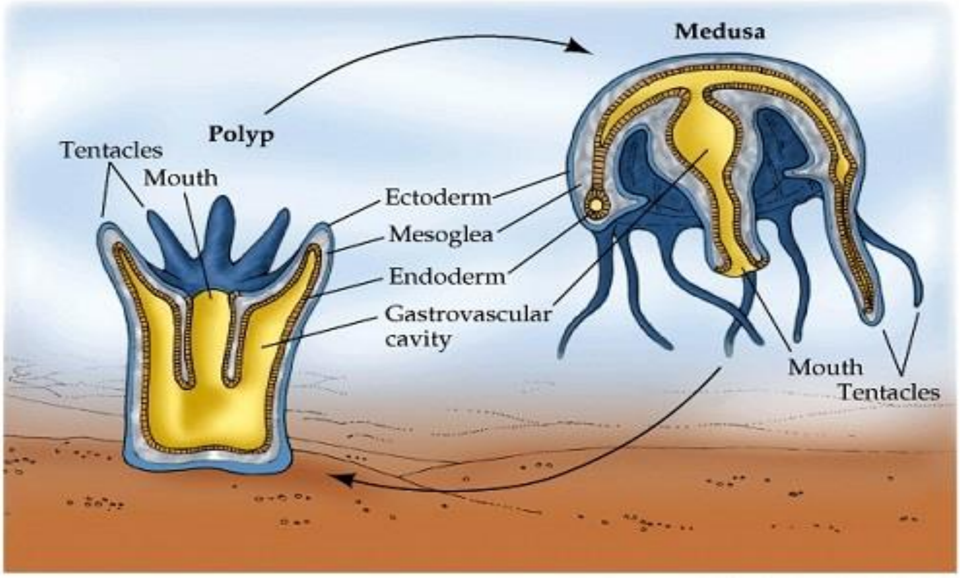

Figure 1.2: Cnidarians body forms (Polyp and Medusa)

(Source:http://cdn.differencebetween.net/wpcontent/uploads/2018/01/Difference-between-Polyp-and-Medusa.jpg)

The inner endoderm or gastrodermis, line up the gut, which in some cnidarians may be divided up by the septa (as in the Anthozoa) or elaborated into the branching canals (as in many Scyphozoa). Among two layers is a non-cellular substance called mesoglea. Cnidarians conduct extracellular digestion where enzymes break down the particles of food and nutrients are absorbed by cells lining the gastrovascular cavity.

Cnidarians have only one opening with an incomplete digestive system; the gastrovascular cavity acts as both a mouth and anus. Cnidarians have a decentralized nervous system, muscle tissue, reproductive tissue, and a hydrostatic skeleton in addition to a primitive digestive cavity. The internal pressure of fluids within the body holds a hydrostatic skeleton. Cnidarian's nervous system, responsible for tentacle movement, captured prey drawing to the mouth, digestion of food, and waste expulsion is composed of nerve cells distributed throughout the body. The basic plan of the cnidarians' nervous system is that of a nerve net that has fragmented into nerve plexuses at some sites or circular or longitudinal nerve tracts that may be syncytia. Most cnidarians neurons have the combined characteristics of sensory, motor, inter- and neurosecretory neurons at the ultra-structural stage and therefore tend to be multifunctional. Their primitive nervous system is strongly peptidergic (neurons that secrete peptide hormones as their neurotransmitters). Neuropeptide biosynthesis in cnidarians is highly effective and equivalent to that of higher invertebrates, such as molluscs and insects and vertebrates (Grimmelikhuijzen and Westfall, 1995). The currently accepted taxonomic scheme subdivides Cnidaria into two main assemblages: Anthozoa and Medusozoa, based on the structure of polyps and the existence of a medusa stage in the life cycles. The Anthozoa contain only sessile polyp forms, while the Medusozoa include species with both polyp and medusa forms in their life cycle. The distinction between Anthozoa and Medusozoa is well-supported by anatomy and life history (Salvini-Plawen 1978; Bridge et al. 1995), genome stmcture (Bridge et al. 1992) and DNA sequences (e.g., CavalierSmith et al. 1996; Odorico & Miller 1997; Kim et al. 1999; Medina et al. 2001; Won et al. 2001; Collins 2002). Cnidaria's traditional taxonomic structure reflects its phylogenetic structure, although the ranks of certain groups are incompatible with their phylogenetic hierarchical status. For instance, the class Anthozoa comprises all members of the clade Anthozoa; its sister taxon, Medusozoa, comprises the remaining classes. Thus, class and other ranks have different phylogenetic implications across the phylum (Daly *et al.*, 2007). Anthozoa is the most speciose of these classes and is further subdivided into two diverse subclasses Hexacorallia (hard corals and sea anemones) and Octocorallia (soft corals, sea pens, and gorgonians). The remaining four classes – Cubozoa (box jellies or sea wasps), Hydrozoa (hydras, hydroids, hydromedusae, and siphonophores), Scyphozoa (true jellyfish), and Staurozoa (stalked jellyfish) – are united in the subphylum Medusozoa.

1.2.1 CLASS ANTHOZOA

Anthozoans are recorded from the intertidal zone to the deep oceans. The exact number of anthozoans is still not clear (Burnett et al., 1997 and Reimer et al., 2007), but the record is updated with an estimate of 7500 extant species of Anthozoa by Crowther, 2011.Anthozoans, the representative of Phylum Cnidaria, are the largest group of organisms that play an important role in the development of coastal forms. In tropical seas, the symbiotic association between Anthozoans and Symbiodinium has received extensive attention and is considered an adaptation to life in oligotrophic environments which may have played a crucial role in the development of early (Triassic) coral reefs. The relationship of Anthozoans-Symbiodinium has essential ecological significance in the marine environment because it enables the holobiont to survive in nutrient-poor waters and helps to grow reefs and create some of the largest bio constructions on Earth. Anthozoans have a cylindrical body crowned by a ring of tentacles surrounding the mouth. The mouth leads into a tubular pharynx which decreases for some distance into the body before opening into the gastrovascular cavity. Unlike other cnidarians, however, the cavity is subdivided by many radiating partitions, or separations. The gonads are also located within the cavity walls. Anthozoans lack a medusa stage. Anthozoan's reproduction is observed using both sexual and asexual methods. Many species of Anthozoa are gonochoric, some species are hermaphroditic, and during their lifespan, others may change sex. Gametes are generally broadcasted, but some species are known to breed their embryos in the coelenteron (e.g. *Bunodactis hermaphroditica*, *Anthopleura hermaphroditica*) and others are attached to the polyp surface. The predominantly polypoid nature of the Anthozoan life cycle is sometimes viewed as a group synapomorphy (Hyman 1940; Brusca and Brusca 1990), but this trait is shared with at least a few medusozoans and may be pleisiomorphic (Collins et al., 2006a). The class Anthozoa has two subclasses classified on the basis of their body fold symmetry, the Octacorallia and the Hexacorallia. Octocorals almost always have eight pinnate tentacles, while Hexacorals typically have six or rather a multiple of six tentacles.

1.2.1.1 Subclass Hexacorallia

There are about 4,300 extant species documented in the Hexacorallia subclass (Doumenc and Praet, 1987). Subclass Hexacorallia comprises Ceriantharia (tube anemones), Scleractinia (true or stony corals), Corallimorpharia (jewel anemones), Actiniaria (sea anemones), Zoantharia (encrusting anemones) and Antipatharia (black corals). Since the morphology of Hexacoral polyps is more complex than that of Octacoral polyps, it became difficult to interpret the group's monophyly and relationships within it. Most of the traditional diagnostic features of each order are not relevant to members of other Hexacorallian subclasses, making it difficult for Hexacorallians to determine shared history (Lang, 1984). Budding is a general term used for Scleractinian asexual reproduction (Kramarsky and Loya, 1996; Chadwick-Furman and Spiegel, 2000; Ryland, 1997a). Although the longitudinal fission cycle in Actiniarians and Corallimorpharian is closely parallel to the process of extra tentacular budding in Scleractinians (Cairns, 1988). Hexacorallian phylogeny is not resolved purely because of the incompatibility of character or terminological inaccuracy, but because specific anatomical features do not restrict the same exclusive classes. For example, the histology of mesenterial filaments links zoanthids and Actiniarians; the arrangement of mesentery groups of Actiniarians with Scleractinians and Corallimorpharians. Hexacorallians have a complex life cycle with a mobile planktonic phase and a subsequent sessile phase among all Cnidarians.

1.2.1.1.1 Order Actiniaria

Actiniarians (sea anemones) are the most diverse and successful members of the Phylum: Cnidaria, Class: Anthozoa, Subclass: Hexacorallia, occupying marine benthic habitats across all depths and latitudes. Worldwide, approximately 1,200 known species in 46 families of sea anemones have been recorded which inhabit all oceans and habitats from the deep sea to coral reefs and estuaries. Many species of sea anemones inhabit rocky shores, especially where tide pools remain submerged at low tide. Even though their many species are adapted to relatively cold waters, their greatest diversity is in the tropics. Most species cling to rocks, shells or submerged timber, often hiding in cracks or under algae, but some burrow in sand and mud, and very few are pelagic.

1.3 MORPHOLOGY OF SEA ANEMONES

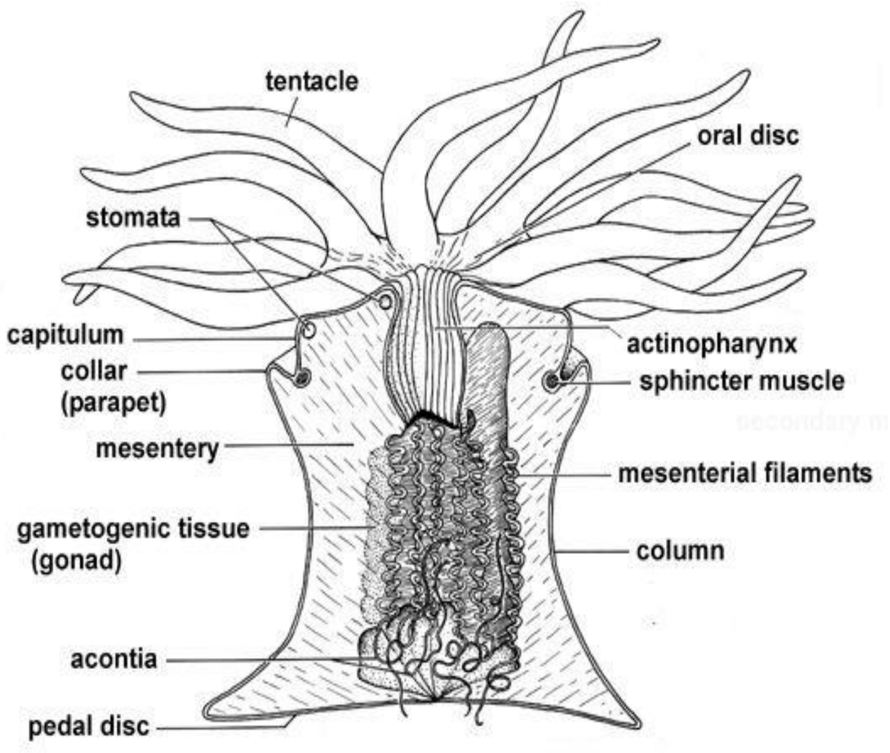

Figure 1. 3 : Schematic diagram of sea anemone anatomy

(Source:https://www.google.co.in/url?sa=i&source=images&cd=&ved=2ahUKEwjx19CfwOTmAhXNyDgGHdPcD34QjRx6BAgBEAQ&url=https%3A%2F%2Fslideplayer.com%2Fslide%2F13486582%2F&psig=AOvVaw3rhtiw2vja173dyRycJYH1&ust=1578040242376613)

An anemone is essentially a polyp consisting of a coelenteron (internal body cavity), which is surrounded by the column wall (Fig: 1.3). The basic structure of the Actiniarian tissues consists of ectoderm, or outer layer of cells; and endoderm, the inner layer of cells; and mesoglea, consisting of connective tissue, located between the two layers.

A few tentacles and oral disc ectoderm cells have muscle processes; besides these, the epidermis contains independent muscle fibres. Mesoglea is a gelatinous matrix comprising protein collagen-formed fibres. The volume of mesoglea in Anthozoa is very high and dense, approaching its maximum degree of differentiation. Cells are usually loosely distributed throughout the mesoglea, but not in a layer that is defined. The innermost layer of gastrodermis or endodermis consists of columnar epithelio-muscular cells where the cell bases are drawn into muscle fibres. Such muscle fibres are circular in arms, oral disks, columns, and basal disks, but they form strong longitudinally running retractor muscles on the mesenteries.

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A proximal end is either rounded, in which case the species is buried in the soft substratum, or it forms a well-developed flat pedal disc, which it uses to attach to the hard substratum. A particularly important feature of taxonomy and biology of sea anemone is the morphology of the column and column margin. The column may smooth or has different structures such as verrucae (Hollow, a wart-like and adhesive excrescence of the column), tenacula (More or less solid papillae situated on the column, tall and sometimes lobed), tubercles (Solid mesogleal excrescence on the column), vesicles (Ampullaceous, nonadhesive evagination of the column), marginal spherules (Alternate term for acrorhagi), marginal pseudospherules (Vesicles situated at the margin, resembling acrorhagi, often with an aperture, containing same cnidae as a column) or marginal projections. Running down the column from the distal end to the proximal end of the anemone, the often brightly colour oral disk is surrounded by the tentacles; the actino-pharynx joins the mouth to the coelenteron, and the pedal or basal disk. The oral disc (at the distal end) is usually circular; it can be drawn into lobes in some species. The mouth can be surrounded by lips and is usually raised on a hypostome. The tentacles are simple, hollow and usually arranged in alternating cycles in the hexamer. They come from the margin and/or the oral disc, and almost never have astrospheres (Spherical tentacle tip which clearly demarcated from the stalk). Several species have special fighting tentacles that can be removed or discarded; they carry large amounts of special cnidae used for protection.

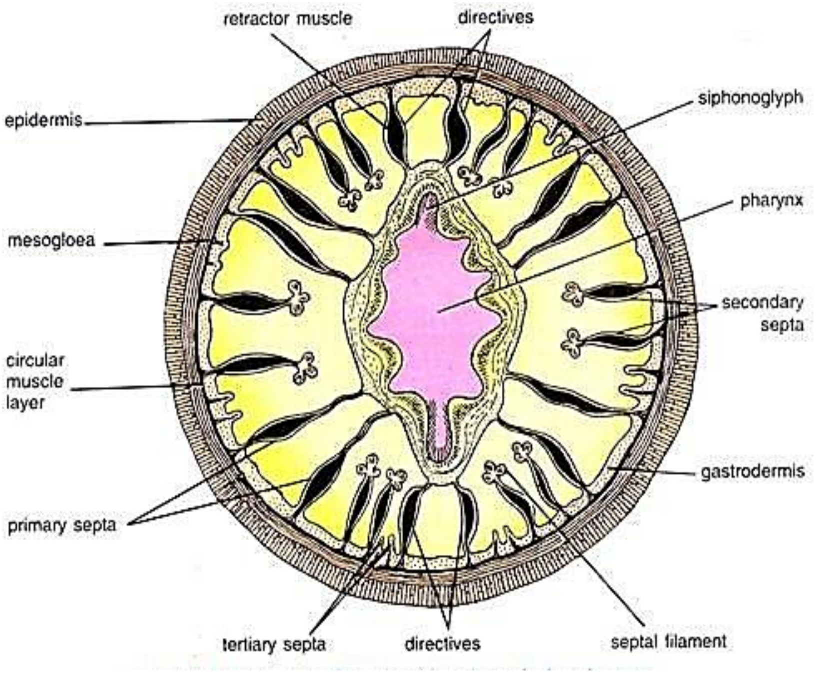

Figure 1. 4 : Cross section through the pharynx of sea anemone

(Source:http://cdn.biologydiscussion.com/wpcontent/uploads/2016/04/clip_image007-12.jpg)

The coelenteron is split up into many partitions by means of various septa, which are called mesenteries. These are shaped from endoderm and mesoglea sheets that grow out of the wall of the column. Such mesenteries are usually referred to in pairs, as there are usually two mesenteries of the same length next to each other. They can either reach all the way from the column to the actino-pharynx, in which case they are named perfect mesenteries, or they can reach only part of it and then they are named imperfect mesenteries (Fig. 1.4).

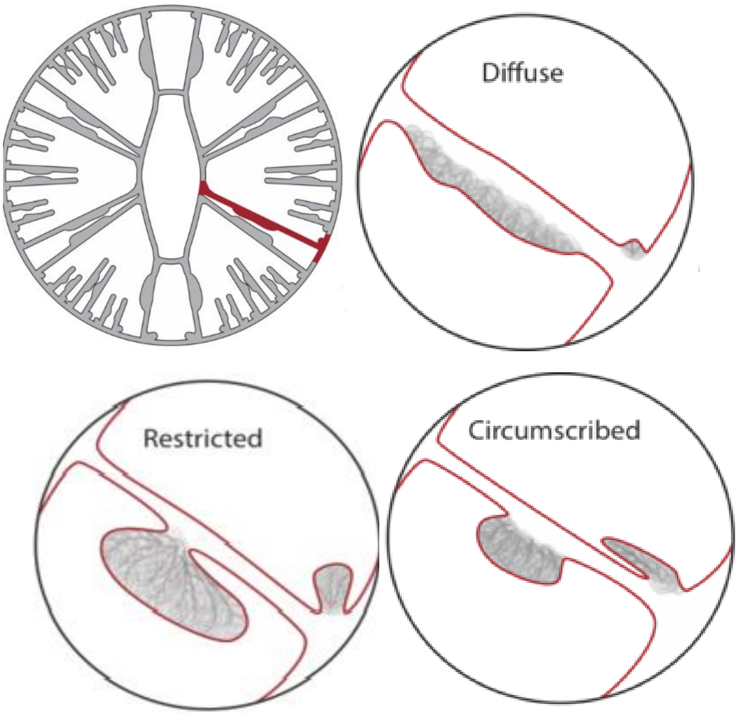

Figure 1. 5 : Sea anemone mesentrial muscle.

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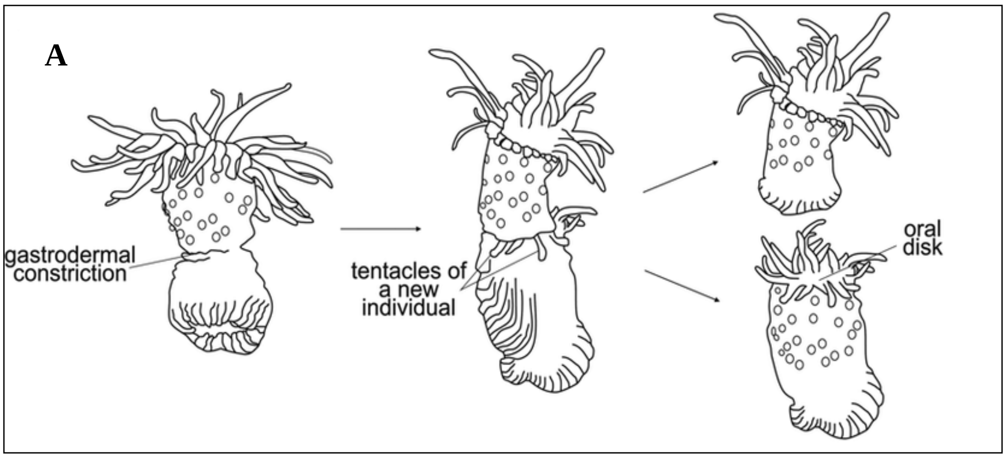
The endocoel is the space enclosed by the mesenterial pairs, while the exocoel is the space enclosed by multiple pairs of mesenteries. Directive mesenteries are pairs of perfect mesenteries usually attached to the siphonoglyphs and are characterized by the endocoel-free retractor muscles. Mesenterial inserts are the lines in which the mesenteries join the oral or pedal disks and can also be seen on the column.

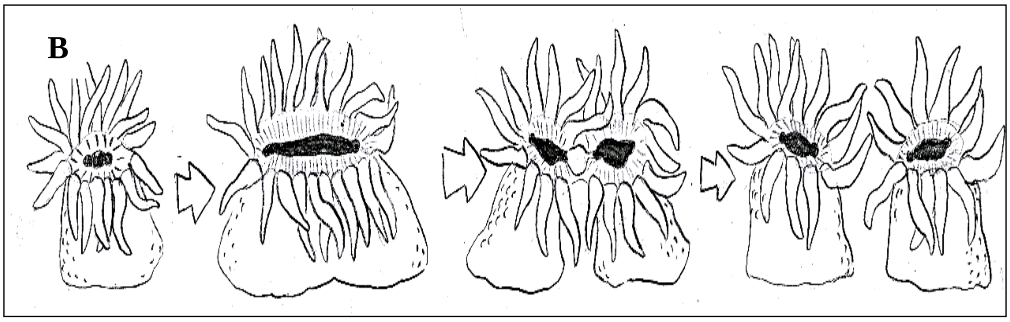
The actino-pharynx, moving from the mouth, is typically covered with rugae or longitudinal furrows that allow the structure to expand when large prey is ingested. Siphonoglyphs are deep grooves that are usually placed opposite each other on the distal end of the actino-pharynx and help them to feed. A strong sphincter muscle around the margin surface, just below the tentacles, allows some anemones to cover the retracted tentacles carrying their prey and close the upper column over the prey, preventing their escape. It is usually found along the margin of anemone and can either be lodged in the mesoglea (called a mesogleal sphincter) or in the endoderm (called an endodermic sphincter). The muscle can be further described as either diffuse or extended along the upper column length, confined to a smaller area when concentrated, or circumscribed when extremely concentrated (Fig. 1.5).

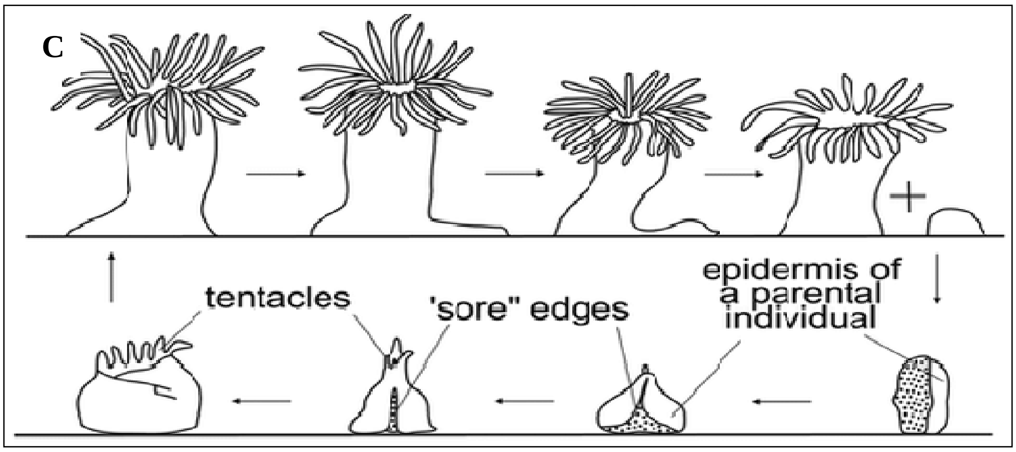
All cnidarians are distinguished by Cnidae, which are hollow shafts or tubules with pointed tips in a transparent capsule. The tightly coiled thread attached to the tubule, when triggered by a stimulus, most usually tactile, serves as a trigger, reinforcing the capsule and firing the pointed tubule into the predator or prey tissue (Anderson and Bouchard, 2009). Two forms of cnidae are present in anemones: nematocysts that are stinging capsules and spirocysts that are typically used for attachment. Typical Actiniarians cnidae generally described in the study include basitrichs, holotrichs, atrichs, micro basic p-mastigophores, microbasic amastigophores and spirocysts. The cnidom belonging to each species is the set of cnidae found in tissues from an assortment of anatomical areas of the anemone. Acontia is complex threads filled with a high nematocyst concentration. The thread bases are attached below the filaments to the mesenterial walls and the tips are free to be extruded either through the mouth or through cinclides, which helps in defense and prey capture. Definitions of typical morphological characteristics were supplemented by information from the Illustrated Glossary of Sea Anemone Anatomy (Sebens, 1998).

1.4 LIFECYCLE OF SEA ANEMONES

Sea anemones are capable of reproducing both sexually and asexually (Stephenson, 1928 and Hyman, 1940) and known for their variety of reproduction combinations (Bocharova and Kozevich, 2011). The mode of reproduction may vary from population to population even in the same species (Schmidt, 1970; Rossi, 1976; Chia, 1976). The common asexual reproduction variants in anemones are transversal fission, longitudinal fission and laceration. Transversal fission was described for some sea anemone species belonging to two primitive families: Gonactiniidae and Aiptasiidae (Schmidt, 1970; Stephenson, 1935; Chia *et al.,* 1989, Crowell and Oates, 1980; Hand and Unlinger, 1992). Young species individuals start their fission by appearing in the middle of the polyp body like a tentacle rudiment band. Then the overstretching comes from under the rudiments of the tentacle and divides the body into two parts. The upper half of the body begins regeneration of the pedal disk after separation, while the lower side forms the oral disk (Fig. 1.6A).







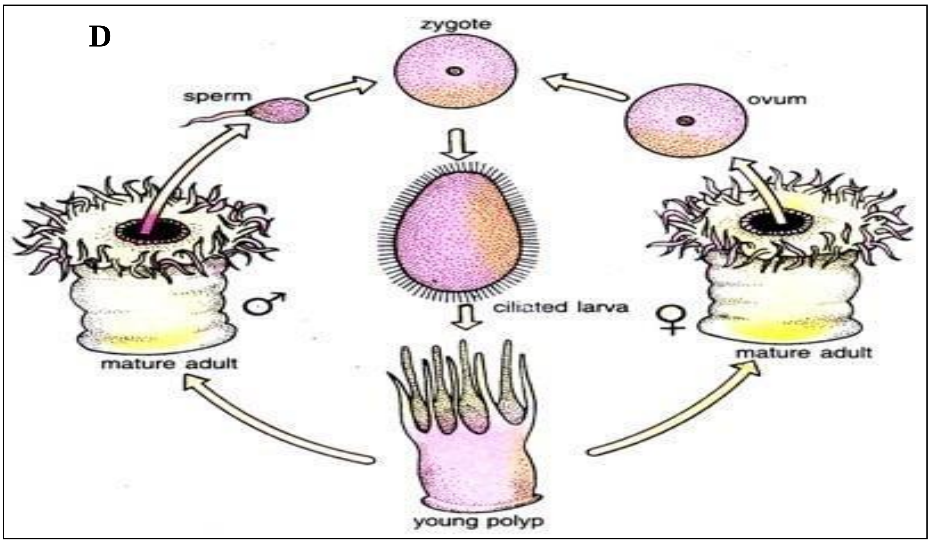


Figure 1. 6 : Reproduction modes of sea anemones.

A: scheme of transversal fission (Asexual reproduction) in *Anthopleura stellula* (According to Schmidt, 1970), B: scheme of longitudinal fission (Asexual reproduction) in *Anthopleura elegantissima* (According to Sebens, 1983), C: scheme of pedal laceration in sea anemones (Asexual reproduction) (According to Polteva, 1970) (Adapted from Bocharova, 2016) D: Ascheme of sexual reproduction in *Metridium* sp.

(Source:http://cdn.biologydiscussion.com/wpcontent/uploads/2016/04/clip_image013_thumb2-2.jpg)

Longitudinal fission is the most common type of asexual reproduction of marine anemones and occurs in *Aiptasia, Sagartia, Metridium, Anemonia, Haliplanella* and members of other genera. Both the apical and the basal ends will start the fission. In the last case, the base extends as far as the body of the sea anemone is separated (Fig. 1.6B).

During the laceration reproduction mode, small fragments of the irregular shape is attached to the substratum dissociate from the parental pedal disk, while an adult sea anemone crawls or remains stationary. Each separate fragment includes pedal disk parts, mesenteries, and lateral body wall sections. The edges of "sore" fuse after separation and new tentacles and mesenteries are created alongside the "sore." The new individual often has an abnormal number of syphonoglyphs and mesenteries as a result (Hyman, 1940) (Fig. 1.6C). In sexual reproduction, Males may release sperm to stimulate females to release eggs, and fertilization occurs either in the gastro-vascular cavity or in the water column.

The sperm and eggs, or larvae, are thrown through the mouth. The fertilized egg grows into a Planula larva that drifts into a juvenile sea anemone for a while before sinking into the seabed (Fig. 1.6D).

Many species only have sexual reproduction, others only have asexual reproduction and a significant number of species tend to combine various mechanisms of these reproductive modes, considering changing environmental conditions. Many authors have (Stephenson, 1929; Ayre, 1984; Billingham and Ayre, 1996) supported this statement.

1.5 IMPORTANCE OF SEA ANEMONES

Despite anemones being a relatively low diversity group when compared, for example, with the crustacean order Amphipoda of the polychaete order Phyllodocida they are highly successful in an ecological sense (Carlgren, 1949). Since the life-span of individual sea anemones of many species may span a century or more (Dunn, 1981a), Actiniarians comprise an important part of the marine ecosystem. The advantages of anemonefish symbiosis include the exclusion of mucus and necrotic tissue from the host (Arvedlund *et al*., 2006), the use of ammonium-rich fish excrement in the host food, and the reduction of predatory fish predation of the host (Fautin and Allen, 1997). The fish, in exchange, are not only covered by predators, but also feed on scraps leftover from anemone meals (Fautin and Allen, 1992). More than 50 fish species are possible symbionts of the following families of sea anemones: Actiniidae, Stichodactylidae, and Thalassianthidae (Randall and Fautin, 2002; Patzner, 2004). A specific symbiosis has been recorded between both hermit crabs in gastropod shells (Hazlett, 1981; Mercier and Hamel, 2008; Gusmao and Daly, 2010) and lives gastropods (Fautin and Goodwill, 2009) with sea anemones attached to their shells. Many tropical anemones have found a complex relationship with zooxanthellae. Anemone houses dinoflagellates in tentacles, oral disks and occasionally in the upper column, providing light and protection for organisms, while anemones benefit from photosynthesis -produced sugars. Sea anemones not only serve as hosts for a variety of marine fauna but also as opportunistic predators form an important link in the marine food web. The predation of anemones on mobile predators, such as isopods, may be important in the structuring of these communities; whereas the eating of sedentary species, such as bivalves, was opportunistic and occurred only when these species were accessible (Kruger and Griffiths, 1998). Despite the fact that sea anemones are major secondary consumers in rocky shore environments, a number of species often prey on them.

Sea anemones are attractive invertebrates that divers enjoy viewing underwater, especially the large tropical species that harbour anemone fish along with other interesting symbionts. As diving operations are a major source of income for local businesses in many parts of Indian water, protecting the invertebrates that form an important part of the reef is in the interests of local people.

The toxins created by sea anemones are of common use to Hawaiian tribes, who use them on their arms as a poison and as a stimulant to the heart (Barry et al., 1977 and Dunn, 1981a). Now a day, Sea anemone tissues are being extensively studied for protein and their structural behaviour. Actinoporins are used to analyse pore formation, a type of protein isolated from anemones

(Alegre-Cebollada *et al.,* 2007). Another group of proteins isolated from anemones is used in many cancer research aspects and extracts from a few Actiniarian species have been shown to have a negative effect on mice tumours (Dunn et al., 1975). In addition, various existing medicinal and pharmacological uses of benefits for human sea anemones have been recorded (Wang *et al*., 2000; Tu *et al*., 2003; Honma and Shiomi, 2006; Moran *et al.*, 2006; Monroy-Estrada *et al.,* 2007; Zaharenko *et a*l., 2008 and Morales *et al*., 2009). Studies of biological and pharmaceutical activities such as antifouling, antimicrobial, antitumor and antifungal from some marine anemones have been recorded from the Indian coast (John, 2009; Manilal *et al*., 2010; Thangaraj *et al*., 2011; Bragadeeswaran *et al.,* 2011; Thangaraj and Bragadeeswaran, 2012).

1.6 SEA ANEMONE DIVERSITY DOCUMENTATION AT MORPHOLOGICAL AND MOLECULAR LEVEL

1.6.1 MORPHOLOGICAL IDENTIFICATION OF SEA ANEMONES

Actiniarians have high ecological and pharmaceutical importance. Considering the roles of Actiniarians as an integral part of marine biodiversity and ecosystem services, marine anemone studies have gained worldwide. The baseline data for further ecological and pharmaceutical research can be provided by diversity studies and documentation at the species level. Several factors may be significant in the study of the external morphology of anemone identification: habitat preferences and colour pattern (Daly and Fautin, 2004), specimen size and oral disk diameter, form and size of tentacles and their arrangement on the oral disc, and verrucae pattern on the column (Fautin *et. al*, 2008). The types and size of nematocyst from different body parts of anemone is also an important criterion for identification (Ostman *et al*., 2013). Many of the anemone taxonomy features can only be investigated by histological sections. Basilar musculature, for example, has traditionally been used to distinguish large groups within Actiniaria on taxonomic keys (Stephenson, 1935 and Carlgren, 1949). Taxonomic reviews have historically been confused by high levels of morphological variation within and between supposed species (Burnett *et al*., 1997).

Moreover, since the early '70s, numerous studies have established several secondary sources of phenotypic variation at smaller scales. Some of these variations are usually considered as the inherent responses of the sea anemone to ecological factors such as space competition, food availability, or low tide water loss (e.g. Hart and Crowe, 1977). The existence of mixed morphs may also indicate that some of these phenotypic differences are passed down to the offspring (e.g. Haussermann, 2004b), but it has not been checked for how many generations they are retained and whether they could ultimately encourage the formation of new species (Spano, 2019). The emergence of molecular methods has revolutionized the ecological and evolutionary study and thus the challenge of species delimitation.

1.6.2 MOLECULAR IDENTIFICATION OF SEA ANEMONES

Identifying Anthozoans species based solely on morphological characters has contributed to controversy due to the phenotypic plasticity of these organisms and has often called for additional support of genetic studies (Reimer *et al*., 2006a, b, 2010). In the case of the order Actiniaria, the lack of consensus on morphological characteristics based on phylogeny makes the DNA data particularly important. This, in turn, makes the selection of markers and the potential of such markers to more critically reconstructing phylogeny. There is currently a strong focus on using a mitochondrial marker, in particular a fragment of the cytochrome oxidase I gene (COI) for this purpose While there is ample evidence that this marker is indeed appropriate for delineating species across a wide taxonomic spectrum, it has also become apparent that a nuclear marker system supplement could be useful (Sonnenberg *et al*., 2007). For Actiniaria fauna, Dohnaand Kochzius, 2016 evaluated three genetic markers namely the cytochrome oxidase subunit I (COI), a COI Intron (with the Homing Endonuclease Gene (HEG)) and the Internal Transcribed Spacer II (ITS II), to consider their usefulness for the identification of the species at a molecular level. Both the ability of COI and COI Intron to separate species is constrained by events with very low inter-specific sequence differences and not by high intra-specific diversity. This finding implies that this problem will not be solved by more comprehensive taxon sampling. Regarding problematic groups, where inter-specific genetic variation clearly impedes the delineation of species, efforts should concentrate on exploring other markers or supplementary ID systems (Concepcion *et al.,* 2008; Huang *et al*., 2008; Sinniger *et al*., 2008, Dohna, 2016).

The mitochondrial genome of Basal animals typically evolves more slowly than the genome of bilaterians. This discrepancy in rate complicates the analysis of relationships between members of these lineages and the identification of cryptic species within them or the testing of concepts of morphological species. Dally *et al*., 2010 studied the properties of mitochondrial and nuclear ribosomal genes in Order Actiniaria using both ordinal and family taxa samples. The fragments of 12S rDNA and 18S rDNA most efficiently recover well-supported nodes among the markers analysed here; those of 16S rDNA and 28S rDNA are less successful. The 12S and 18S are remarkably similar in terms of resolution and support groups. Nevertheless, in terms of their utility at other taxonomic levels and their amplification profile, these markers differ in terms of their practical value for studying the phylogenetic of marine anemones. However, 18S easily amplifies and aligns across the order, the subclass and the phylum, and thus has importance for studies that compare widely. The recovery of relationships within the more cantered taxon sample is also successful through the 18S marker.

1.6.3 MORPHOLOGY VS MOLECULAR TAXONOMY

After the Bar-coding system has been evolved, the question comes to mind why is a taxonomic method required to identify the species when gene-level information is available? Most of the studies published over the past few years have been analysed here, and there is no evidence that DNA bar-coding is replacing conventional taxonomy. Mitochondrial DNA barcodes can't provide enough taxa information to help make decisions about species status, so bar-coding technicians don't have all the new taxa identification expertise and the sequence. Molecular taxonomy can enable us to differentiate between very close and non-distinct similarities at species level and morphology. Molecular taxonomy or barcode identification can be useful for authenticating the-field species identification and can also act as perfect documentation of information when kept in the laboratory or museum. We can say that morphology and molecular taxonomy both work in a symbiotic relationship for Actiniarian species identification and molecular techniques provide support for taxonomic methods for species identification.

1.7 THERMAL STRESS IMPACT AND ECO-PHYSIOLOGY OF SEA ANEMONES

Eco-physiology is the branch of ecology that studies the relationships between species and their surroundings. In particular, it explores how the environment affects the physiology or the natural functions of the body or the organisms. Coral reef ecosystems are ideal model systems for biotic interaction and environmental stress studies because they harbour species that undergo broad spatial and temporal variations in environmental conditions due to steep gradients in wave effect, food availability, temperature, and desiccation (Paine, 1977; Newell, 1979; Denny, 1988; Menge and Farrell, 1989; Dahlhoff *et al.*, 2001). Scientists at Great Barrier Reef had made a series of elegant experiments and started the modern research to understand the coral symbiosis physiology. They showed that symbiotic corals consume phosphate and ammonia from the ambient seawater by day and release it at night. The increased absorption of phosphate in the light indicates a relationship with the symbiotic photosynthetic activity of algae-zooxanthellae (Goreau *et al.*, 1979).

Climate change is a well-known phenomenon in the history of Earth. But the most pressing issue in today's world is the accelerating climate change, which is the primary consequence of global warming. Global warming is a process of earth heating, and such earth heating is caused by greenhouse gasses that absorb heat and light from the sun, resulting in high temperatures. The sensitivity of corals to such climate change conditions is also reflected by coral skeletal discontinuities or other indications of stress such as coral bleaching. Coral bleaching occurs when the zooxanthellae density decreases dramatically and/or when the photosynthetic pigment concentration within the zooxanthellae decreases. Zooxanthellae are diverse taxa of photosynthetic dinoflagellates living symbiotically in reef-building coral gastro dermal tissues (Blank and Trench, 1985; Rowan and Powers, 1991a, b). Many environmental factors have been identified to cause host and symbionts dissociation, i.e. extremes of temperature (heat shock and cold shock), high irradiance, prolonged darkness, heavy metals (particularly copper and cadmium) and pathogenic microorganisms (Hoegh-Guldberg, 1999). Globally, however, the high sea surface temperature is known as a major contributor to the significant events of coral bleaching (Stone *et al*., 1999).

1.7.1 SEA SURFACE TEMPERATURE AND ANOMALY

Sea Surface Temperature (SST) is and has been one of the most evaluated variable in the ocean and has gained substantial scientific attention as a result. Sea surface temperature (SST) is an important indicator of productivity, pollution and global climate change, which can be measured using optical satellite thermal infrared (IR) bands. Since the 1970s, global mean SST has risen from decade to decade, with consequences for global weather patterns and ocean ecosystems. Elevated sea surface temperature (SST) caused by E1 Niño Southern Oscillation (ENSO) events is known to cause coral stress and mortality through a phenomenon known as bleaching. The increasing frequency of coral reef mass bleaching events (Hughes *et al.,* 2017) is most striking. The pattern usually distinguishes between two states: warmer than normal central and eastern Pacific SSTs (El Niño) and cooler than normal central and eastern Pacific SSTs (La Niña). Such thermal fluctuations have a significant impact on the prevailing biodiversity.

The term temperature anomaly indicates a deviation from a reference value or a long-term average. A positive anomaly indicates that the temperature observed was warmer than the reference value, whereas a negative anomaly shows that the temperature observed was colder than the reference value. Sea surface temperature variations influence the atmosphere by altering the flow of latent and sensible heat from the oceans, resulting in anomalous heating patterns. When the maximum water temperatures of the season coincide with the anomalies of warm water, coral bleaching occurs. The frequency of bleaching events in the last two decades of El Niño poses a "worst-case scenario" in predicting the future of coral reef ecosystems, particularly when linked to a warming sea-surface temperature history (Glynn *et al.,* 2000). SST is a critical factor in the well-being of zooxanthellae host animals such as corals, sea anemones and giant clams (Krishnan *et al*., 2011). Corals usually recover from bleaching as SST condition improves, but in extreme cases, they die (McClanahan, 2004). Severe bleaching can lead to mortality of the corals. It is therefore important to determine how SST affects the reefs of the coral. SST disturbances demonstrate possible disruption to coral reef ecosystems and sometimes reveal potential changes in species richness and abundance that could be prioritized for efforts in marine conservation (Palumbi *et al*., 2014). Monitoring of daily SST anomalies accompanied by field monitoring of bleaching responses in real-time may help develop and implement effective management strategies to reduce environmental stress on coral reef ecosystems.

1.7.2 THERMAL STRESS AND HSPs (Heat Shock Proteins) EXPRESSION

Stress can affect an individual at a variety of different levels—cellular, tissue or organismal, which can affect the life history and fitness of an organism. Stress might cause an organism to redirect its resources to basic physiological processes to maintain homeostasis, away from growth or reproduction (Charbonnel *et al.* 2008). In its natural habitat, however, it is unlikely that any organism will be subjected to only one stressor at a time—an organism is much more likely to encounter several different stressors concurrently, both biotic and abiotic. The effects of thermal stress are of importance to marine organisms. Intertidal organisms may be faced with the effects of not only fluctuating seawater temperatures, but fluctuating air temperatures, as well. This has been seen in several species of intertidal sea anemones—a change in temperature affects energy expenditures and oxygen consumption, as well as behaviour in different species of sea anemones (Shick 1991). Additionally, many marine organisms, particularly those living in the littoral zone, are already living at the threshold of their thermal tolerances (Harley *et al.* 2006).

Physiologically, the coral bleaching phenomenon is due to the breakdown of the mutualistic phototrophic symbiosis between Scleractinian corals and dinoflagellates endosymbionts (Symbiodinium spp. genus), commonly known as zooxanthellae. This breakdown of symbiosis can result from a wide range of environmental stressors (Brown; Douglas 2003, Lesser 2004), but one of the most significant for mass coral bleaching is unusually high sea surface temperatures that can act synergistically with high solar irradiance (Coles,1978; Glynn,1990; Richier, 2008). Cellular bleaching mechanisms include a number of processes including in situ degeneration of zooxanthellae, the release of zooxanthellae from mesenterial filaments and release of algae inside the host. At the molecular level, the photoinhibition process undergone by the zooxanthellae is the first stage of temperature or light-induced coral bleaching (Bouchard and Yamasaki, 2008; Smith *et al*., 2005; Warner *et al.,* 1996).

A defensive mechanism known as the Heat Shock or Stress Protein (HSP) response (Miller and McLennan, 1988; McLennan and Miller, 90; Sanders *et al*., 1991; Sanders, 1993; Sharp *et a*l., 1997) has been implicated in the adaptation of species to highly variable environmental parameters. The response of stress proteins, present in almost all organisms studied including Sea anemone, involves the rapid synthesis of a set of proteins called heat shock proteins or stress proteins. HSPs often include participants that are both constitutive and stress-inducing. Their function under normal cell physiological conditions is mostly expressed in controlling homeostasis of proteins (e.g., degradation of abnormal proteins), guiding the folding and assembly of other proteins, and carrying proteins within the cell (Lindquist, 1986; Sanders, 1993;

Parsell and Lindquist, 1993). The character of an emergency response is stress protein induction (Lindquist, 1986; Parsell and Lindquist, 1993). The increase in the synthesis and accumulation of abnormal proteins serves as a catalyst for a rapid and irreversible increase in the expression of HSP genes (Craig and Gross, 1991).

HSPs stabilize denaturing proteins as molecular chaperones and refold those already denatured, thus the HSP response could be considered as an ecologically and evolutionarily significant factor in thermal adaptation, setting thermal tolerance limits and improving the resistance of an animal to thermal stress (Hartl and Hartl-Meyer, 2002; Tomanek, 2010). Nevertheless, between different taxa, physiological responses to thermal stress are not uniform (Tomanek, 2010; Feder and Hofmann,1999; Sorensen and Kristensen, 2003) In tropics, subtidal sessile species live at or just below their upper thermal limit while undergoing relatively constant conditions compared to intertidal counterparts (Coles and Jokiel, 1978). Thermal stress and the induction of a molecular heat shock response may result in very small increases in temperature.

Most of the Studies regarding thermal stress and their impact on organisms have been investigated in corals. However, sea anemone shows a similar response to thermal stress. Some of the sea anemone species have been investigated for the expression of different genes such as HSP60, HSP70 and HSP90 that react to the thermal stress. Such a study suggests the use of HSPs gene expression as a tool for stress detection in marine invertebrates.