

# Deep - Sea Fungi: Occurrence and Adaptations

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by

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## **Statement**

As required under the University Ordinance 0.19.8 (vi), I state that the present thesis entitled “**Deep - sea fungi: occurrence and adaptations**” is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge, the present study is the first comprehensive work of its kind from the area mentioned.

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

**Samir R. Damare**

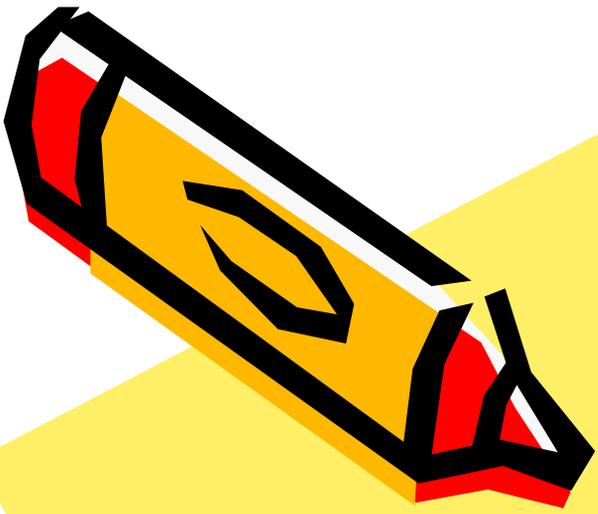
## **Certificate**

This is to certify that the thesis entitled “ **Deep-sea fungi: occurrence and adaptations**” submitted by **Samir R. Damare** for the award of the degree of Doctor of Philosophy in Department of Marine Sciences is based on his original studies carried out by him under my supervision. The thesis or any part thereof has not been previously submitted for any degree or diploma in any University or Institution.

Place:

Date:

**Dr. Chandralata Raghukumar**  
Research Guide,  
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**DEDICATED  
TO  
ALL THOSE  
WHO MADE  
THIS POSSIBLE**



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# Chapter 1

## Deep - sea Environment

The earth is uniquely favoured among the planets – it has rain, rivers and seas. Ocean basins are a primary feature of the earth's surface. Until recently, the ocean floor was hidden from human observation by miles of ocean water. Now it is possible to map the ocean bottom in detail using satellites and acoustic techniques and to observe ocean-bottom processes from the submersible. With these new techniques, major new discoveries come virtually every year. Our improved knowledge of the ocean basins is changing our view of the earth as much as the exploration of the New World changed human perspectives in the 15<sup>th</sup> and 16<sup>th</sup> centuries.

The earth is home to many different forms of life, living in many different environments. Until the 20th century, most life was found in the "normal" environment, where humans live. In the past century, scientists have found organisms that survive in conditions that are very different from the normal. Such organisms are said to live in "extreme environments." High or low temperatures, pressures, salinities, acidities, etc characterize extreme environments.

The deep sea, the largest single ecosystem on earth is an example of such an extreme environment. The sea surface occupies ~70 % of the surface of the earth, and 50 % of the surface of the earth is covered by more than 3000 m<sup>2</sup> of ocean, with a mean depth of ~ 3800 m. It is the very remoteness of the deep sea and the difficulties encountered in its exploration that have resulted in it being one of the least understood environments on earth. At present, detailed information about specific areas of the deep sea are available, but these are mere 'pinpricks' in the vastness of this environment. The understanding of the deep-sea ecosystem is entwined with some of the most exciting aspects of scientific exploration and with the development of

technologies for sampling and penetrating this environment, the information is increasing [Tyler, 2003].

Explorers and commercial interests have used the sea as a means of transport for millennia. However, it was only in the latter part of the 19<sup>th</sup> century that scientists went to the sea with the specific aim of looking downwards into impenetrable depths. One of the first was Forbes [1844], who sampled down to a depth of 600 m in the Aegean. Today one would consider this choice of sampling station as unfortunate, since now it is known that this region of the Mediterranean deep sea is faunistically poor, and the lack of animals in Forbes's samples led to the 'azoic theory' that little or no life existed below 600 m. The establishment of such a paradigm was in direct opposition to the observations of the ophiuroid *Astrophyton* being brought up on a sounding line from a depth of 1800 m in Baffin Bay [Tyler, 1980], and the pioneering work of Sars in Norwegian fjords [1864 & 1868]. Establishing the presence of a fauna in the deep sea presented irresistible challenges to a small group of scientists led by Charles Thomson. Thomson used HMS *Porcupine* to sample the ocean to the northwest of Scotland and to the west of Ireland in the late 1860s, and found fauna at depths exceeding 4000 m [Thomson, 1873]. The results of the *Porcupine* sampling programme led directly to the HMS Challenger expedition of 1872 to 1876. This expedition traversed the oceans of the globe and demonstrated a widespread and varied fauna in the deep sea, as well as taking numerous physical and chemical measurements. The results of this cruise are now considered the forerunner of modern oceanography. The Challenger expedition led directly to the 'heroic' age of deep-sea exploration, with expeditions sampling many areas of the world's oceans [Menzies et

[al., 1973; Mills, 1983](#)]. The heroic age culminated in the Danish *Galathea* expedition of 1950 to 1952, which demonstrated that life could be found in the deepest of all the oceans, in the ocean trenches. Taking stock of deep-sea ecology at this point in time would have led to the establishment of the following paradigms:

1. The deep sea was species poor.
2. It was a tranquil environment.
3. There was a slow rain of material from surface to the deep sea [[Moseley, 1880](#)].
4. No primary production occurred within deep sea.

The 1960s heralded a new approach to deep-sea ecology, driven by technology. Quantification became the name of the game. With the information gathered, the concept of high biodiversity in the deep sea was established, although the absolute biodiversity is still very much subject to debate, but it is now believed that the deep oceans are as diverse as tropical rain forests. Although known to be diverse, it was assumed that the deep-sea system was heterotrophic, relying on the slow sinking of material from surface waters to provide an energy source for the inhabitants. The 1970s and 1980s provided evidence that this environment was more dynamic than originally thought. The first example was the discovery of hydrothermal vents along the Galapagos Ridge in 1977.

The technology has allowed humans to penetrate the ‘remote’ environment. SCUBA diving is limited to the top water column; but the development of submersibles has allowed scientists to dive to the deep-sea bed. Current knowledge of hydrothermal vents and cold seeps would be insignificant if it were not for the

submersible. Submersibles are still used today; but the Remote Operated Vehicle (ROV) allows similar access from the comfort of the surface tender without the potential damage of manned submersibles.

Today one may summarize the paradigms for the deep-sea environment as:

1. High species diversity.
2. Periods of benthic storms perturbing an apparently gentle environment
3. Seasonal input of surface derived energy for heterotrophic organisms
4. Primary production at vents and cold seeps.

The change in understanding of the deep sea has been a function of increase in the ability of scientists to gain knowledge from this environment. Despite recent recognition of the above paradigms, all are natural phenomenon. As yet deep sea is exploited only to a very limited extent, but this may change in the future. The deep sea has also been suggested as a repository for the excess CO<sub>2</sub>, causing the so-called 'greenhouse effect'. The vastness of the deep-ocean aids its stability.

The deep sea is usually defined as beginning at the shelf break, because this physiographic feature coincides with the transition from the basically shallow water fauna of the shelf to the deep-sea fauna [Sanders *et al.*, 1965, Hessler, 1974, Merrett, 1989]. The shelf break is at about 200 m depth in many parts of the ocean, so the deep sea is said to begin at 200 m. The deep-sea floor is therefore a vast habitat, covering more than 65 % of the Earth's surface [Svendrup *et al.*, 1942]. Much of it is covered by sediment.

The deep-sea floor is an extreme environment; pressure is high, temperature is low, and food input is small. It has been characterized as a physically stable environment [[Sanders, 1968](#)].

**1. Pressure:** The pressure increases by one atmosphere for every 10 m increase in water depth. Water pressure at the surface of the ocean is 1 bar. The ocean, with an average depth of 3800 m and therefore a pressure of 380 bar, comprises approximately 70% of biosphere. Water pressure in the ocean is as high as 1100 bar the Mariana Trench (in the West Pacific, 400 km SW of Guam). It has been suggested that life originated in the deep sea some 3.5 to 4 billion years ago. Therefore hydrostatic pressure would have been very important stimulus for the early stages of life. Recently, it has been suggested that life might have originated in deep-sea hydrothermal vents, and thus it seems possible that high pressure-adapted mechanisms of gene expression, protein synthesis, or metabolism could represent features present in early forms of life [[Thistle, 2003](#)].

**2. Temperature:** The temperature generally decreases with increasing depth, reaching  $\sim 2^{\circ}\text{C}$  on the abyssal plain, but the pattern varies with latitude and region [[Mantyla & Reid, 1983](#)]. Above 500 m in mid-latitude, temperature varies seasonally, but with diminishing amplitude with increasing depth. At high latitudes, the vertical gradient in bottom-water temperature is small [[Svendrup \*et al.\*, 1942](#)]. A small vertical temperature gradient also occurs in regions where the bottom water is warm (e.g. the Mediterranean Sea and the Red Sea). Most of the water overlying the deep-sea floor is cold compared to that over most shallow-water habitats, the typical temperatures at the

ocean floor being just a little above the freezing point. At depths below ~ 800 m, temperature is remarkably constant at around 3-4°C.

**3. Salinity:** The salinity at most locations in the deep sea varies little with time. In most of the deep sea, the salinity of the bottom water is fully marine (35 ppt). Exceptions include the Mediterranean and Red Sea (> 39 ppt) and hypersaline basins such as the Orca Basin in the Gulf of Mexico (300 ppt) [Shokes *et al.*, 1976].

**4. Oxygen:** Oxygen enters the ocean by exchange with the atmosphere and as by-product of photosynthesis by marine plants in the euphotic zone. The dissolved gas is carried to the deep-sea floor by the descent of surface waters. The water overlying most of the deep-sea floor is saturated with oxygen or nearly so (5-6 ml L<sup>-1</sup>). Oxygen concentration also varies with depth in the sediment. Oxygen enters the pore water of deep-sea sediments by diffusion and by the activities of organisms that pump or mix water into the sediment. The depth of oxygen penetration into the sediment limits the vertical distribution of organisms. Oxygen is consumed by animal and microbial respiration and by chemical reactions in the sediment. Where the deposition rate of labile organic matter is relatively high and the oxygen concentration in the bottom water is low, as in the basins of the California Continental Borderland, free oxygen disappears within the first centimeter [Reimers, 1987]. Where organic deposition rates are low and bottom water is well oxygenated, as beneath the oligotrophic waters of the North Pacific, abundant free oxygen is present several centimeters into the seabed [Reimers, 1987].

**5. Light:** Light intensity decreases exponentially with the depth in the water column because incident photons are absorbed or scattered. Particles suspended in the water increase both the absorption and scattering, but even in the clearest ocean water no photosynthetically useful light reaches the sea floor below about 250 m. Therefore, the deep-sea floor differs from more familiar ecosystems as the plant primary production does not occur. Except for hydrothermal vent and cold-seep communities, the food of deep-sea floor organisms must be imported.

### **Benthic storms**

In much of the deep sea, the near bottom water moves slowly as compared to that in shallow-water environments. The flow does move some material, in particular phytodetritus, which accumulates down. The water is never still, because tidal forces move water at all ocean depths. On the continental slope intense currents may be generated by internal tides and water column instability causing breaking at internal waves. Theoretical considerations reveal that prevailing synoptic weather systems can create strong ( $20 \text{ cm s}^{-1}$ ) near-bottom currents in the deep ocean. The maximum speed of these oscillating bottom currents depends on both atmospheric parameters (magnitude of the wind-stress curl anomaly, spatial scale of weather system and speed of large-scale background wind) and oceanic parameters (total water depth and combined effects of horizontal diffusion and bottom friction). Benthic storms are characterized by periods of daily averaged flow of more than  $15 \text{ cm s}^{-1}$  maintained for two or more days. During intermittently strong peaks in flow of more than  $40 \text{ cm s}^{-1}$ ,

the top few millimeters of sediment may be completely stripped [Hollister *et al.*, 1984; Hollister & McCave, 1984]. Such events occur due to vorticity propagated from upper water column in areas of strong surface flow, such as Gulf Stream [Weatherly & Kelley, 1985]. However, similar peaks in eddy energy may arise from atmospheric storm-driven motions in areas far from the continental margin [Gardner & Sullivan, 1981], from disturbances associated with intermittent flow over sills [Dickinson *et al.*, 1982] and from bottom-trapped topographic waves [Grant *et al.*, 1985].

### **Deep-sea sediments**

Deep-sea sediment deposits are accumulations of minerals and rock fragments from the land mixed with insoluble shells and bones of marine organisms and some particles formed through chemical processes occurring in sea water. Much of the information about Earth history comes from study of such deposits. Particles in sediment deposits come from different sources like:

1. Terrigenous (derived from land)
2. Biogenic (derived from plants and animals)
3. Authigenic (formed on or in the sediments on the sea floor)
4. Volcanogenic (particles from volcanic eruptions)
5. Cosmogenous (particles from outer space)

**Table 1.1 Major sediment input to the oceans**

<b>Source</b>	<b>Estimated amount (10<sup>9</sup> tons / year)</b>
Rivers	18.3
Glaciers and ice sheets	2.0
Wind blown dust	0.6
Coastal erosion	0.25
Volcanic debris	0.15
Groundwater	< 0.48

**1. Terrigenous sediments:** These are derived from the erosion of the continents and are transported into the ocean as particles of gravel, sand or mud. Their mineral composition varies and reflects the source rock and weathering process (climate). Most of the world's largest rivers are located in the wet tropic regions where there is also high relief and intense chemical weathering so these are areas of high mud input into the oceans. The Ganges River discharges the most sediment per year – about 1500 million tonnes. Sediment is also blown off the continents into the ocean, particularly on the west coast of continents adjacent to the major deserts. Usually only very small particles (less than 20  $\mu\text{m}$ ) are carried long distances. The Abyssal clay or "red clay" that covers much of the deep ocean floor is largely of aeolian origin. While aeolian dust is deposited everywhere, it only dominates on the abyssal regions where low biological productivity and the dissolution of calcium carbonate prevent dilution. Melting of ice sheets and icebergs has been, and continues to be, a major provider of sediment to the sea floor in high latitudes. Ice is indiscriminate in what it carries: giant boulders to finely ground clay.

**2. Biogenic sediment:** This contains organically produced particles and is defined as any deposit which has more than 30% biogenous constituents by weight. There are three main groups of organic sediments – calcareous, siliceous and phosphatic.

The distribution of **calcareous biogenous sediments** is largely determined by what is known as the calcite compensation depth (CCD; sometimes erroneously referred to as the carbonate compensation depth). This is the depth, usually several km, below which calcite does not accumulate because it is a level on the sea floor where the rate of carbonate supply is equal to the rate of carbonate dissolution. It is analogous to the snow line on land. The depth, at which dissolution starts, is called the lysocline, (generally located 500-1000 m above the CCD). The CCD exists because the carbon dioxide rich deep water of the oceans is undersaturated with respect to calcite. Calcite also becomes more soluble with increasing pressure and lower temperatures. The warm tropical surface waters are supersaturated with calcium carbonate. Thus, the level of the CCD rises in high latitudes where the cold CO<sub>2</sub> rich water is at the surface and is depressed in low latitudes where the supply of calcite raining to the sea floor is higher. The dissolution of calcite recycles Ca as organisms precipitate more CaCO<sub>3</sub> than can be supported by the flux of calcium to the oceans from rivers. It is estimated that approximately 90% of the calcium carbonate precipitated by organisms in the upper layers of the oceans is dissolved in the deep ocean. Examples of carbonate sediments are – foraminefera, coccoliths and pteropods.

**Foraminefera** are single-celled protozoans that are both benthic, which live in sediments on the sea floor, and planktonic, which live in the upper 100 m or so of the ocean. Of the estimated 4000 species living today, 40 are planktonic but because of their great abundance they secrete more calcite than all other foraminifers. Foraminiferal shells (called 'tests') of both groups occur in a variety of shapes, and typically range from 0.1 mm to 1 mm in size (but have been found up to 18cm!). The shells of all planktonic and most benthic species are composed of calcite. The fossil record of benthic foraminifera dates back to more than 550 million years. Planktonic species have been around for the last 200 million years but really got going about 100 million years ago. Because of the large number of species (it is estimated that there are over 40,000 in the rock record), their wide distribution and environmental sensitivity, they can be used to determine past climate conditions. In addition, because of their rapidly changing form (species generally exists for about ~5-15 million years), they can be used to determine the age of sediments in which they occur.

**Coccolithophores** are a common group of phytoplankton - single cell algae (plants). They are unique in that the single cell is surrounded by armour of at least 30 calcite plates (called coccoliths) to form a sphere only 30  $\mu\text{m}$  in diameter. Scientists estimate that the organisms deposit more than 1.5 million tons (1.4 billion kilograms) of calcite a year, making them the leading calcite producers in the ocean and together with the forams they deposit more calcite on the floor of the deep ocean than all the shells and corals on the continental shelves. Consequently they are responsible for many thick chalk and limestone beds. They first appear in the fossil record in the Jurassic and were particularly common in the Cretaceous, when they produced many

chalk deposits, like the White Cliffs of Dover. They were almost wiped out at the Cretaceous -Tertiary boundary but have persisted to the modern day. A related, but now extinct, group is the Discoasters, which secreted microscopic star-shaped calcite crystals. Together these microfossils are important in micropaleontology for evolutionary and environmental studies. Because of their small size they are often called nannofossils. Today Coccolithophores live mostly in subpolar regions. They are often found in nutrient poor water that cannot support other types of plankton. They form blooms, which because of the structure of their plates are visible from space. They appear as milky white or turquoise patches. Blooms are a regular occurrence off the north coast of Australia.

**Pteropods** are small gastropod mollusks, basically floating snails whose foot is modified for swimming. They produce large mucus feeding webs for trapping phytoplankton. They have a coiled shell composed of aragonite into which they can retreat if threatened (some species do not have the shell and are just a gelatinous blob). They favour tropical and warm-temperate seas. In some equatorial areas of the Indian Ocean, pteropod shells dominate the sedimentation, resulting in a subset of the carbonate ooze - the pteropod ooze.

**Siliceous sediment** is composed of siliceous shells or skeletons of opaline silica, a form of hydrated silicon dioxide. The principal silica producers are the radiolarians (animals) and diatoms (plants). On the whole the ocean is undersaturated with silica, therefore you might expect biogenic silica to dissolve and not be present in sediments. The solubility of silica decreases with increasing pressure and decreasing

temperature – that means that there is more of it in the deep ocean (the opposite to carbonate). Siliceous organisms are generally found in nutrient rich waters (areas of upwelling) that have high silica content. The shells do dissolve (pretty slowly), but because of high productivity there is a lot of them and they get buried before they get destroyed. The siliceous content of sediment is highest in deep water where calcareous sediment is absent (because of the CCD). Radiolarians are protozoans that construct beautifully complex silica exoskeletons that often have many spines extending outwards. They form oozes on the sea floor that over time can evolve into hard sedimentary rocks called radiolarian cherts or, if mixed in with calcareous ooze they form individual flint nodules in chalk. They have been around for the last 540 million years and are useful for dating rocks, because their skeletons are very well preserved in the sediment. During the Cretaceous-Tertiary mass extinctions, radiolarians did well in comparison to other planktonic life forms. Radiolarians absorb dissolved silica from seawater to construct their beautiful skeletons. Some sponges produce calcareous spicules that are common in some shelf and slope sediments. Diatoms are single cell algae (plants) that incorporate silica into their cell wall to form ‘frustules’. Diatoms occur in both benthic and planktonic forms. The benthic ones are restricted to water depths of less than 100 meters. Diatom blooms are common in rivers and upwelling zones (due to the high nutrient content) and can be toxic to other organisms because of oxygen depletion or the biotoxins produced by some species.

**3. Authigenic (sometimes called hydrogenous) deposits:** These are deposits precipitated from seawater as a result of chemical reactions, (some of which may be assisted by bacteria). They include ferromanganese nodules (often called manganese

nodules) phosphorites, glauconites and evaporites. **Manganese nodules** form in the deep ocean and are particularly common in the Pacific where they are estimated to cover 30-50% of the sea floor. They are made up predominantly of manganese oxide ( $\text{MnO}_2$ ) and iron oxide ( $\text{Fe}_2\text{O}_3$ ) - average contents of 30% manganese and 20% iron. They are dark brown in colour, slightly flattened rough spheres, 5 to 10 cm in diameter and are generally found in water depths of 4000 to 6000 meters. In cross section, the nodules show concentric layers, or growth rings around a core - like tree rings. The core can be a fragment of anything, a bit of basalt, skeletal material etc. The growth rate of the nodules is very slow - nodules in the Pacific Ocean are estimated to be 2 to 3 millions years old. Nobody is quite certain about how manganese nodules form, but it seems likely that bacteria are involved. It is thought that the major sources of manganese in seawater are leaching of sea floor basalts and of hydrothermal activity along mid-ocean ridges.

**4. Volcanogenic sediments:** Three things come out of volcanoes: lava, tephra and gas. Tephra is all ejecta blown through the air or water by explosive volcanic eruptions. Tephra comes in different sizes classified as - blocks, bombs, lapilli, cinders and ash. Large-sized tephra generally falls close to the volcano. Smaller fragments are carried away by the wind. Volcanic ash can travel hundreds to thousands of kilometers downwind from a volcano. As the cloud of ash and gas moves away from the volcano, it loses altitude and ash falls to the ground forming a layer of sediment. Volcanic ash in deep-sea sediments may be in discrete layers or dispersed through other sediments. Size sorting by the wind may occur with distance from the source.

5. **Cosmogenous sediments:** These are extraterrestrial in origin. These are the least abundant sediment type and are generally found diluted by other sediments. There are two main sources: **Cosmic dust** - silt and sand-sized particles and **Comets & asteroids**. Research by the Ocean Drilling Program and others has revealed a thin and distinctive band of clay present in sediments around the world. This band is highly enriched in Iridium (Ir) and corresponds to the Cretaceous-Tertiary (KT) boundary. Iridium is a rare-earth element that is found at very low concentrations in the earth's crust but is common in meteorites. The source of the iridium in this clay band is thought to be a comet that hit the earth 65 million years ago. The impact produced a layer of sediment that can contain up to 20% cosmogenous material. Comets and asteroids are also capable of producing particles called tektites. They are dark-coloured, rounded silicate glass particles that can be less than a millimetre (microtektites) to several cm in size. They are found concentrated in areas around the world that are referred to as strewn fields. The tektites are formed by impact melting of surficial sediments. Microtektites are found in deep-sea sediments within the Australasian strew field (a large area which is thought to have resulted from an impact on the Indochina Peninsula). Ocean Drilling Program researchers examining cores from the Ninety East Ridge (Eastern Indian Ocean) and the Sulu Sea (both located in the Australasian strewn field) have found increased levels of Ir. The Ir concentrations and microtektites distribution have lead them to propose that the Australasian impact could have excavated a crater between 15 and 19 km in diameter.

Much of the deep-ocean floor is covered by such deposits, which accumulate slowly, particle by particle. Typical accumulation rates are between 0.1 and 1 cm per

thousand years. Since deep-ocean sediments accumulate slowly, particles may spend years suspended in seawater or exposed to the overlying waters while being slowly buried in the bottom.

### **Special habitats in deep sea**

Apart from the above mentioned parameters of the deep-sea environment, there are a few deep-sea habitats which need a special mention. Ex.: Cold-seeps, hydrothermal events.

**Cold seeps:** A cold seep, sometimes called a cold vent, is an area of the ocean floor where hydrogen sulphide, methane and other hydrocarbon-rich fluid seepage occurs. Cold seeps are distinct from hydrothermal vents that the former's emissions are of the same temperature as the surrounding seawater, whereas the latter's emissions are super-heated. Cold seeps constitute a biome supporting several endemic species.

Cold seeps were first discovered in 1984 by Dr. Charles Paull in the Monterey Canyon, just off Monterey Bay, California, at a depth of 3,200 m. Since then, seeps have been discovered in the other parts of the world's oceans, including the Gulf of Mexico, the Sea of Japan, and in the waters off the coast of Alaska. The deepest seep community known is distributed between the Kuril and Japan trenches and the Kashima seamount in the Sea of Japan, at a depth of 5,000 to 6,500 m.

Unlike hydrothermal vents, which are volatile and ephemeral environments, cold seeps emit at a slow and dependable rate. Owing to the differing temperatures and stability, cold seep organisms are much longer-lived than those

inhabiting hydrothermal vents. Recent research has revealed seep tubeworms to be the longest living noncolonial invertebrates known, with a minimum lifespan of between 170 and 250 years.

Entire communities of light independent organisms develop in and around cold seeps, most relying on a symbiotic relationship with chemoautotrophic bacteria. These bacteria, both archaea and eubacteria, process sulphides and methane through chemosynthesis into chemical energy. Higher organisms, namely vesicomyid clams and siboglinid tube worms use this energy for their life processes, and in exchange provide both safely and reliable source of food for the bacteria. Other bacteria form mats, blanketing sizable areas in the process.

Cold seeps develop unique topography over time, where reactions between methane and seawater create carbonate rock formations and reefs. These reactions may also be dependent on bacterial activity.

**Hydrothermal vents:** The discovery of hydrothermal vents in 1977 was certainly the most important event for the marine biologists because it changed the perception of the deep sea as a cold, dark, high-pressure and nutrient poor environment inhabited by psychrophilic, oligotrophic to barophilic microbial communities. By contrast, deep-sea vent areas are hot to warm and inhabited by animal communities whose densities may reach  $50 \text{ kg m}^{-2}$ . Invertebrates living in these warm biotopes are in endosymbiotic relationships with autotrophic sulphur-oxidising bacteria. In the hot areas of the ecosystem, temperatures often reach  $350^\circ\text{C}$ , and precipitation produces mineral structures (black smokers) that contain thermophilic microorganisms.

Vents are known to exist in the Pacific and Atlantic oceans. Most are found at an average depth of about 2,100 meters (7,000 ft) in areas of seafloor spreading along the Mid-Ocean Ridge system — the underwater mountain chain that snakes its way around the globe. In some areas along the Mid-Ocean Ridge, the gigantic plates that form the Earth's crust are moving apart, creating cracks and crevices in the ocean floor. Seawater seeps into these openings and is heated by the molten rock, or magma, that lies beneath the Earth's crust. As the water is heated, it rises and seeks a path back out into the ocean through an opening in the seafloor. As the vent water bursts out into the ocean, its temperature may be as high as 400°C. Yet this water does not boil because it is under so much pressure from the tremendous weight of the ocean above. When the pressure on a liquid is increased, its boiling point goes up. Chimneys top some hydrothermal vents. These smokestacks are formed from dissolved metals that precipitate out (form into particles) when the super-hot vent water meets the surrounding deep ocean water, which is only a few degrees above freezing. So-called “black smokers” are the hottest of the vents. They spew mostly iron and sulfide, which combine to form iron monosulfide. This compound gives the smoker its black colour. Geologists are intrigued by how rapidly vent chimneys grow - up to 9 meters (30 ft) in 18 months. A scientist at the University of Washington has been monitoring the growth of “Godzilla,” a vent chimney in the Pacific Ocean off the coast of Oregon. It reached the height of a 15-story building before it toppled. It is now actively rebuilding. These underwater geysers are believed to play an important role in the ocean's temperature, chemistry, and circulation patterns.

## Chapter 2

# Microbial Life

in

# Deep-sea Sediments

As oceanic crust moves away from spreading centres, it becomes covered with increasingly thick layers of slowly accumulating sediments, which can accumulate to depths of several kilometers. Sediment layers can be much thicker at continental margins, where they accumulate from weathering of continental platforms and accretion of sediment scrapped off of subducting tectonic plates. The study of biodiversity of subsurface microorganisms from the sediments is only just beginning. A case can be made that the subsurface is both the largest portion of Earth's biosphere and the most poorly catalogued. In most subsurface studies to date, bacterial diversity has been evaluated through cultivation and enumeration of various metabolic functional groups. In most cases, only aerobic heterotrophs were characterized. In recent years, other functional groups have been routinely enumerated in the subsurface, including denitrifying bacteria, dissimilatory metal reducing bacteria, and methanogenic archaea. Relatively few of these organisms have been characterized mostly because of late surge in this field of study and lack of facilities for the studies.

As mentioned in the previous chapter, the deep sea was thought to be devoid of life till mid 19<sup>th</sup> century. This assumption was refuted in 1860 by the discovery of corals and sponges attached to a transatlantic cable that was hauled up from the seafloor for repair. From 1868 to 1870, Thomson led multiple cruises of HMS *Lightning* and HMS *Porcupine* to dredge the Atlantic seafloor at depths as great as about 4,600 meters. By the end of his project, he had discovered diverse life on much of the ocean floor. Since then, marine biologists have come to accept the existence of life on the seafloor and in the first few meters of deep-sea sediments. The existence of high-pressure life was first documented by the oceanographic surveys of the *HMS*

*Challenger*, during the 1870s. This pioneering expedition for the first time made extensive collections of deep-sea biota, with more than 100 dredgings at depths of more than 1 km. These collections revealed an unexpected abundance of life in the abyssal depths and thus started the fantastic journey of human beings to look into the life in deep sea.

As discussed earlier, conditions like low temperature, high pressure, and low nutrient levels combine to make the deep sea a potentially hostile environment. Most of the deep-sea bottom is stable, cold and dark; therefore it is possible that very ancient life forms may be present in a state of suspended animation in the world's largest refrigerator. In 1968, the research submarine *Alvin* accidentally filled with seawater and sank to a depth of 1540 m in the North Atlantic, where it remained for about one year. Upon recovery, it was observed that a boxed lunch consisting of bouillon, a bologna sandwich, and apples were all remarkably well preserved. This famous "bologna sandwich experiment" and its successors initiated a fascinating and controversial chapter in modern marine microbiology.

[Zobell & Johnson \[1949\]](#) first coined the term barophile and [Zobell & Morita \[1957\]](#) obtained the first evidence of piezophilic growth in mixed microbial cultures recovered from the deep sea. [Yayanos \[1979\]](#) reported the first isolate of pressure-adapted bacteria in 1979. Subsequently, many psychrophilic piezophiles with various optimal growth pressures have been isolated and characterized physiologically and genetically. Deep-sea hydrothermal vents are interesting sources of novel isolates, many of which were discovered in the course of investigation into the origin of life. Thermophilic microorganisms have also been examined physiologically under high-

pressure conditions. Thus, studies on the effects of pressure on microorganisms have been mainly performed using two types of microorganisms, psychrophilic peizophiles and thermophilic peizophiles.

As subsurface strata may be effectively isolated from each other, as well as from the subsurface, it is possible that microbial diversity may vary as a function of depth. Certainly, the abundance of broad functional groups of microorganisms has been reported to vary according to geochemical controls, such as the availability of electron acceptors.

Although most studies on diversity discuss the number of species, other aspects are at least as important, for example, there is cladistic or phylogenetic diversity, morphological diversity, ecological diversity and genetic diversity. All four are harder to study and to quantify than species diversity, and all are independent of it [[Williamson, 1997](#)]. Phylogenetic diversity gives the relationship of cellular organisms in the form of diagrammatic trees. The higher plants, animals and fungi occupy three small, closely related branches. At a molecular level, multicellular organisms may be very uniform, and this also reminds us that any one measure of biodiversity is insufficient. Morphological and ecological diversity are perhaps more relevant to the preservation of biodiversity, but there are no well-defined guidelines available about how either should be measured. Measuring ecological diversity is even harder, but probably nearer to what is needed for conservation decisions. So it is not surprising that quantitative studies of biodiversity have, apart from a nod to phyletic diversity, been based almost entirely on species count. It is well known that most species have not been described.

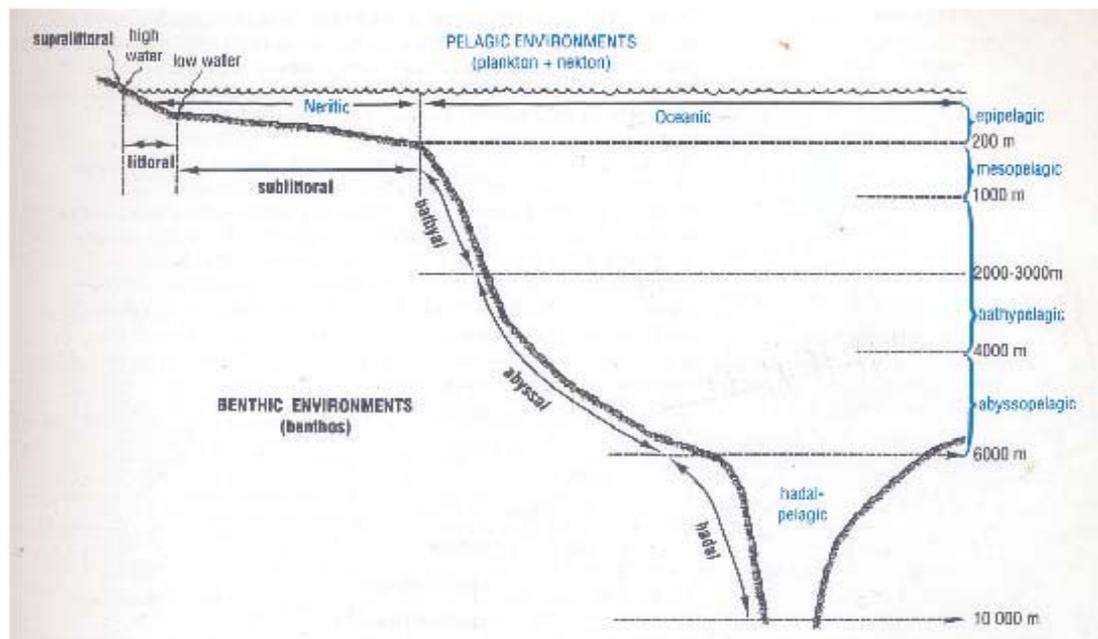
High species richness of deep-sea sediments was first given prominence by Howard Sanders at the Woods Hole Oceanographic Institution in the 1960s. With his co-workers, he found that by washing the mud from anchor-dredge samples of the deep-sea ooze off New England through fine screens, a surprisingly large number of clearly differing species were revealed [Sanders *et al.*, 1965]. He focused his attention on the macrobenthos, a size fraction of the sediment-dwelling community of mainly invertebrate animals that are retained on screens with meshes of 0.25-0.5 mm and have an upper size limit, arbitrarily determined by visibility in sea bed photographs, separating them from the megabenthos.

Marine sediments overlay two-thirds of the earth's surface and harbour diverse and abundant fauna. It was estimated earlier that of the  $3.8 \times 10^{30}$  prokaryotes calculated to be in the unconsolidated subsurface sediments, 97 % or  $3.7 \times 10^{30}$  occur at depths shallower than 600 m. The estimated number of prokaryotes for deeper sediments is only  $0.13 \times 10^{30}$  cells. This value was uncertain because it was based on extrapolation, but it still represents considerable microbial biomass. One would then wonder about their function, metabolism and biogeochemical role at these depths. In other words, what do they eat, how do they respire and what roles do they play?

Thus the study of microorganisms isolated from the deep sea promises to provide new information about the origin of life and its evolution, contributing to the overall marine biodiversity, which is poorly described so far. The study of these extremophiles also gives an opportunity to investigate how life processes work at some of the extreme temperatures (both high and low) and pressures of the biosphere.

Marine organisms can be classified according to the marine environments they inhabit. Thus, there are oceanic species and neritic species depending upon whether the organisms are found in offshore or coastal waters, respectively. Similarly, plants or animals that live in association with seafloor are collectively called benthos. The benthos includes attached seaweeds, sessile animals like sponges and barnacles, and those animals that crawl on or burrow into the substrate.

**Fig. 2.1 The basic ecological divisions of the ocean. The neritic (inshore) pelagic zone is separated from the oceanic (or offshore) pelagic zone by the edge of the continental shelf, which is generally at about 200 m depth. Benthic habitats are in black font whereas pelagic divisions in blue (figure not to scale)**



Source: *Biological Oceanography An Introduction, 2<sup>nd</sup> Edition* (Lalli CM, Parsons TR, Eds.) p. 3, Butterworth-Heinemann Publications

In spite of the vast majority of the seafloor permanently submerged below tidal levels, relative to the intertidal regions, comparatively less is known about life in the bathyal, abyssal and hadal zones (Fig. 2.1). This is mainly due to their relative

inaccessibility. Although it is possible to dive to several thousand metres in submersibles or to employ remote-controlled cameras, the number of hours of direct observations in the deep sea are extremely less. Most of the information on deep-sea ecology comes from indirect inferences based on animals contained in benthic samples obtained from ships. Whatever the method, expense is the limiting factor in the deep-sea research. Compounding this expense problem is the fact that animal life is just not very abundant in many deep-sea areas, so that it is desirable to have large numbers of samples. But now with new techniques for collection and observation, combined with accumulating numbers of analyzed deep-sea samples, assessment of benthic life in deeper water, is improving.

Most animal phyla are represented in this dark environment of low temperatures, high pressures and predominantly soft substrates of the deep sea. Some deep-sea residents have a cosmopolitan distribution and are found in all the major oceans; other species are restricted to relatively small areas. In general, species become more limited in geographic range as water depth increases. Only about 20 % of the species present below 2000 m in the Atlantic Ocean are also found in the Pacific or Indian Oceans. Many species found in areas deeper than 6000 m are endemic to the hadal region, and many are restricted to a particular trench.

The benthic animals are separated in to infaunal and epifaunal species, depending upon whether they live within sediments or on the surface of the seafloor respectively.

The benthic animals can also be separated in different categories based on the size (relative to the mesh size of sieves used to separate animals from sediments) as follows –

**Macrofauna** (or macrobenthos) are those animals that are retained by a 1.0 mm mesh sieve. These are the largest benthic animals, including starfish, mussels, most clams, corals, etc.

**Meiofauna** (or meiobenthos) are those animals retained by a 0.1 to 1.0 mm mesh sieve. These are small animals commonly found in sand or mud. The group includes very small molluscs, tiny worms, several small crustacean groups (including benthic copepods), as well as less familiar invertebrates.

**Microfauna** (or microbenthos) are those animals that are smaller than 0.1 mm in dimension. This smallest size category is largely made up of protozoans, especially ciliates.

Bacterial abundance in deep sea sediments exceeds 100000 cells/cm<sup>3</sup>, even at a depth of few kilometers below the seafloor [[Raghukumar \*et al.\*, 2001](#)]. Bacteria play a key role in all major biogeochemical cycling processes in deep-sea sediments, where they contribute up to 90% to the benthic biomass [[Pfannkuche, 1992](#)].

Apart from bacteria, recognition of viral influence on pelagic processes has in the past few years led to an increased focus on the role of viruses in benthic environments [[Danovaro \*et al.\*, 2001](#); [Hewson \*et al.\*, 2001](#); [Middelboe \*et al.\*, 2003](#); [Mei & Danovaro, 2004](#); [Breitbart \*et al.\*, 2004](#)]. Such studies have verified that viruses are abundant, diverse and dynamic members of benthic communities. There are 10<sup>7</sup>–10<sup>9</sup> viruses cm<sup>3</sup> of surface sediment, which is 10–100 times higher than densities

usually found in the overlying water column of the same area [[Middelboe \*et al.\*, 2003](#)], and their abundance and activity have been shown to be positively correlated with benthic microbial activity [[Middelboe \*et al.\*, 2003](#); [Glud & Middelboe, 2004](#)]. Due to such high numbers, viruses can therefore potentially affect the bacterial population and biogeochemical processes in the marine sediments.

In the next chapter, we will see the distribution and occurrence of fungi in the marine environment.