response to environmental conditioning elements. Tributylene (TBT), a compound widely used in anti-fouling paints for hulls, severely upsets the gender definition of molluscs, bringing about the emergence of penes in females. These false males suffer from a mortality rate much higher than that of unaffected organisms. However, the most widespread phenomenon is the feminisation of the ocean due to pollutants: increase in the proportion of females in many species of animals due to oestrogen discharge from rivers or from urban waste water, deriving from the use of contraception pills and oestrogens given to livestock. Unfortunately, apart from what we have learned from a number of local studies, we still know very little about the effects on the ocean of pollutant intake.

▶ Ultraviolet radiation

The global depletion of the ozone layer in the stratosphere, due initially to emission of CFC gases, has led to an increase in ultraviolet radiation, which though greater in the polar regions, particularly above Antarctica, extends to the tropical zones. The impact of ultraviolet radiation on the ocean remains insufficiently quantified, despite the fact that almost all marine organisms are vulnerable to it. This kind of radiation is especially harmful to eggs and larvae, which tend to accumulate in the surface waters of the ocean.

Except for the impact of habitat transformation, the Malaspina Expedition 2010 addresses all these vectors of global change in the ocean and does so globally by means of a circumnavigation project, in which special attention is paid to the tropical and subtropical oceans, which constitute most of the marine surface area.

Notes

1. The initial aim of the Haber-Bosch reaction was to produce explosives. Indeed, ammonium-chloride based fertilisers are still used today as explosives by terrorists.

The Deep Ocean: Its Exploration and Importance in Biogeochemical Cycles

Josep M. Gasol and Javier Arístegui

Most of the Earth's surface is covered by water. Since we humans are curious by nature, we can assume that exploration of the sea began at the dawn of mankind. But the sea's depths have always resisted observation. The sea being an environment hostile to man, its exploration beyond a few metres below the surface has had to wait for the development of sophisticated technology, which has been possible only in recent years. With the help of autonomous submarines, remote-controlled vehicles and oceanographic sounders, study of the deep ocean began in the mid-nineteenth century. Even so, it is reckoned that only 5% of the ocean has been observed or sampled. For the fact is that we have been studying the deep ocean for only some 150 years, approximately the life expectancy of the blue whale.

The first humans invariably feared what might be concealed in the deep ocean: a medium believed to be inhabited by mythological monsters, like the giant squid or Kraken, and which has given rise to innumerable legends since time immemorial, which prevailed into the advent of scientific rationalism. Even today, when major efforts have been made to draw up inventories of deep-ocean organisms, we are so unaccustomed to seeing them that they seem to us to come from another world[1,2]. Indeed, novelists may still attain success by describing a sea inhabited by a civilisation different from our own and determined to annihilate us[3]. Nonetheless, the sensation that monstrous creatures may live in the depths of the sea is a universal though relatively recent one, since around 1840 Edward Forbes, a renowned British oceanographer, had put forward the theory of an azoic (deprived of life) deep ocean:

> *...As we descend deeper and deeper in this region, its inhabitants become more and more modified, and fewer and fewer, indicating our approach towards an abyss where life is either extinguished, or exhibits but a few sparks to mark its lingering presence.*[4]

Forbes based his hypothesis on the fact that the abundance of organisms collected by dredging the seabed diminished as depth increased. According to him, nothing could live below 600 metres. However, subsequent dredging studies conducted by two other British oceanographers, Charles W. Thomson and William B. Carpenter, in the North Atlantic and the Mediterranean Sea, proved that life existed at great depths (globigerine mud). The HMS *Challenger* expedition (1872-1876), led by Thomson and regarded as the beginning of modern oceanography, eventually refuted both Forbes's theory and that of the British biologist Thomas H. Huxley, who claimed to have discovered the 'primordial slime' that constituted the source of all living beings: the famous *Bathybius haeckelii* (which turned out in fact to be a gelatinous precipitate of calcium sulphate).

Despite the small size of the bacteria and the difficulties involved in studying them, by the end of the nineteenth century they had been described from samples taken from a depth of 5,000 metres. The hypothesis was advanced that many bacteria lived in a 'state of suspended animation' in the deep ocean, although they were able to withstand great hydrostatic pressure (observations by A. Certes in 1882 and by B. Fischer en 1886). Nevertheless, it was not until the mid-twentieth century that Claude Zobell established the foundations of marine microbiology, on the basis of his microbiological studies in deep waters during the expedition on the Danish ship *Galathea* (1950-1952).

Almost twenty years were to go by before interest was rekindled in the study of deep-water bacteria, thanks to a fortuitous occurrence. In 1968 the submersible *Alvin*, belonging to the Woods Hole Oceanographic Institute, became detached from its mother ship and sank to a depth of 1,500 metres in the Atlantic Ocean. Almost one year later, when it was recovered by another submersible, scientists were astonished to discover that the crew's sandwiches, though thoroughly soaked, were in a good state of preservation[5]. The low temperatures, the high pressure and the 'absence of bacteria' were regarded as sufficient reason to justify the fact that the sandwiches had not decomposed. This discovery posed a number of questions regarding the role of microorganisms in the deep ocean: to what extent are microorganisms that inhabit the dark ocean active? In terms of the total biomass, are they more or less important than marine metazoans? What role do they play in the ocean's biogeochemistry? These and other questions have been posed by a host of oceanographers, microbial ecologists and biogeochemists, and thanks to research conducted in recent years we are now in a position to hazard replies to them.

The dark ocean is the greatest habitat in the biosphere, with its $1.3\ 10^{18}$ cubic metres (Figs 1 and 2). It is characterised basically by the absence of light, which makes photosynthesis impossible, although the small amount of solar irradiance that reaches down to between depths of 200 and 1,000 metres – known as the twilight zone – is enough to guide and orient many organisms. The dark ocean, unlike the sunlit waters of the epipelagic zone (0-200 metres), is characterised by high pressure, low temperature and high concentrations of inorganic nutrients. If we regard the terrestrial biosphere as a layer 38 metres in height above the continents, 99% of the terrestrial biosphere is in the sea, has an average temperature of 2°C and is subjected to an atmospheric pressure of over 100 atm. Our perception of what is important in the biosphere is clearly biased by our immediate surroundings: 'What we see constantly is not necessarily the most important'.

The dark ocean is normally subdivided into the mesopelagic zone (200-1,000 metres), with a water residence time of the order of decades and confined between the seasonal and permanent thermoclimates, the bathypelagic zone (from 1,000 to 4,000 metres) and the abyssal zone (over 4,000 metres) with water residence times of hundreds of years. Some researchers make a distinction between the abyssal zone and the hadal (or ultra-abyssal) zone, which includes the trenches, which reach depths of between 6,000 and 11,000 metres. These trenches form when two lithospheric plates converge, collide and one of them (of greater density) sinks beneath the other. They normally run parallel to the continents or to island arcs and may reach gradients of up to 45%. Despite their importance to marine life, they cover only 1% of the ocean floor.

Studying the deep ocean requires the use of specialised oceanographic instruments. The most common equipment for collecting water samples is the oceanographic rosette, which is lowered vertically by means of a cable from the surface to the ocean floor. It is provided with oceanographic bottles and sounders that measure depth, temperature, salinity, oxygen, pH, turbidity and so on, which provides us with a profile in real time of the physical and biogeochemical characteristics of the water column (Fig. 3). To study concentrations of organic matter from the surface to the ocean floor, sediment traps (Figs 3ª and 3b) are usually placed at different depths and collect sinking particles (known as marine snow). More sophisticated are remotely operated vehicles (ROVs) or submersibles either remote-controlled or with people on board. Fig. 4 shows some of these vehicles, which may reach down to even the deepest trenches.

In any case, experimental work with deep-water samples is complicated by the difficulties involved in reproducing hydrostatic pressure conditions *in situ*. Most estimates of microbial metabolism are made using decompressed samples, and it is highly probable that such metabolism differs from that which takes place in natural conditions, as the few results obtained using sampling systems that maintain hydrostatic pressure seem to indicate. Thanks to the use of video cameras attached to the rosettes, we have been able to verify that the dark ocean bears absolutely no resemblance to pure water; on the contrary, it contains an infinite number of particles, with sizes ranging between a few micras and several centimetres and which may accumulate in density interfaces between water masses, where oxygen consumption on the part of microbes is greater.

The vertebrates and invertebrates that inhabit the deep ocean are very striking in appearance (Fig. 5), although the greatest biomass and diversity correspond to prokaryotic organisms (bacteria and archaea). These latter are responsible for most of the metabolic activity and, by extension, of the global biogeochemistry of the dark ocean. Compared to surface waters, deep-water bacteria account for 75% of the biomass and approximately half the ocean's total activity (Fig. 6).

Most of the organic carbon resulting from photosynthesis in the epipelagic zone is breathed in the mesopelagic zone by prokaryotes and, consequently, may return to the atmosphere in the form of carbon dioxide after months or even years. However, part of the organic carbon manages to avoid degradation and reaches the bathypelagic zone, where sooner or later it will be breathed, remaining sequestered for centuries in the form of CO_2 until the slow thermohaline circulation of deep waters takes it back to the surface. Even so, it is believed that about one third of the ocean's global respiration takes place in the dark ocean.

Until recently it was assumed that most of the dark ocean's prokaryotes lived freely in the water. However, recent studies based on the genomics of isolated individuals and on the metagenomics of natural samples question this assumption and suggest that microorganisms are associated with particles, probably small ones, with insignificant sedimentation rates: what is known as particulate organic matter in suspension. A distinction is usually made between organic matter available to organisms, which is fresh and composed of easily assimilable monomers, and older matter, resistant to decomposition and normally of high molecular weight.

In general, polymers tend to be more numerous (around 90%) than monomers, and deep-ocean bacteria and archaea must synthesise specialised enzymes in the breakage of these molecules before they can incorporate and use them. The activities of these enzymes increase with depth, at least in a relative way, while bacterial growth efficiency also increases, so that those bacteria that inhabit the deep ocean tend to 'use up' more energy in the maintenance of their cell structures and in synthesising enzymes than those that inhabit the surface waters. This observation coincides with the fact that the genomes of isolated individuals from the ocean depths tend to be characteristic of bacteria that have not reduced their genome; on the contrary, they have a wide repertoire of biochemical mechanisms that allow them to use multiple types of organic matter.

The presence of these generalist mechanisms suggests that organic matter is produced not only by particles formed on the surface, which either sink to the bottom by their own weight or else are transported by intense vertical mixture processes. It may also be that areas of primary production exist in the dark ocean itself and areas of accumulation of particulate matter in suspension, between water mass interfaces, coming from the continental fringes. The chemoautotrophic fixing of inorganic carbon occurs in the dark ocean by the action of bacteria that oxidise ammonium, hydrogen and N_2O, possibly representing up to 50% of phytoplankton production that is exported to the dark ocean.

The diagram in Fig.7 provides a synthesis of current knowledge of carbon biogeochemistry in deep waters: labile and semi-labile dissolved organic carbon – the primary substratum of prokaryote communities – is made available mainly through transformation and dissolution of particles, instead of through direct export of organic carbon dissolved in surface waters. Both microorganisms like zooplankton and small migratory fish take part in the destruction and transformation of particles, thereby determining the magnitude of organic carbon export in this form. Tiny suspended particles may originate from the transformation of others of greater dimensions, although they may also join together to form larger particles that settle as sediment. In general, suspended matter seems to be more labile than particulate matter, and probab
constitutes a good substratum for bacterial growt
although decomposition capacity of particles w
depend on the presence of inorganic forms of nitr
gen and phosphorus, which increase the efficien
of bacterial remineralisation.

Recent discoveries that reveal a heterogeneou
dynamic deep ocean, with a relatively high degree
microbial metabolism and with complex process
of transformation and remineralisation of organ
matter, contrast with the prevailing idea of this env
ronment at the end of the nineteenth century:

> (...) *life must be very monotonous in the deep se*
> *There must be an entire absence of seasons, no d*
> *and night, no change in temperature. Possibly the*
> *is in some places a periodical variation in the supply*
> *food falling from above, which may give rise to a litt*
> *annual excitement amongst the inhabitants.*[6]

One century later, study of the deep ocean a
quired great importance with the discovery in 197
of dense, thriving populations of invertebrates
hydrothermal vents at a depth of some 2,600 me
tres (Fig. 8). Soon it was deduced that several type
of bacteria – that had symbiotic relationships wit
other organisms – played the role of primary pro
ducers through chemosynthetic processes, usin
reduced inorganic compounds which accompa
nied the flow of materials that emerged from th
hydrothermal vents. This source of organic matte
fed an entire community of metazoans, includin
molluscs, crustaceans and vertebrates.

Chemosynthesis processes in hydrotherma
vents are carried out by chemolithoautotrophi
bacteria similar to those found also in therma
springs on the planet's surface (such as geyser
and fumaroles). Nevertheless, in no other ecosys
tem is the process intense enough to maintain suc
complex trophic networks as in hydrothermal vent
Probably, the bacteria-metazoan symbiosis allow
direct, efficient transference of organic matter fror
the production source (bacteria) to the consume
(metazoa).

Hydrothermal vents are found in areas of activ
volcanism in the Atlantic, Indian and Pacific ocean
where the lithospheric plates separate. When th
lava that has been belched out from the subso
cools, it contracts and opens, allowing seawater t
penetrate. The water reacts with the basalt mantl
at high pressure and temperature and become
a highly reduced acid fluid abundant in metals
hydrogen and hydrogen sulphide (H_2S). This fluic
escapes through the openings in the solidifie
lava and enters into contact with the oxygen i
the seawater. Often calcium sulphates are precipi
tated which give rise to chimneys through which
the hot water (up to 350ºC) exits, charged with re
duced substances that endow it with its dark tone
Between the exit point of the chimney and the
seawater gradients are formed of temperature, o
reduced metals and of H_2S. In those places where
H_2S and oxygen coexist, the chemolithoautotrophic
bacteria can break down the former and use its
electrons in the process of aerobic breathing (tha
is, consuming O_2) to fix CO_2.

In the study of these kinds of ecosystems, submersibles and ROVs are indispensable, not only to approach hot springs but also to carry out precision sampling of structured though unstable environments. For example, the turbid waters of the fountains of the Galapagos Rift (00°47'N, 86°08'W) contain from 10^6 to 10^9 prokaryotic cells per millilitre. These organisms also form mantles in the sediment, on which a peculiar purple coloured fish (*Bythites hollisi*) feeds, giving rise to a trophic chain of only two links. From this and other hydrothermal vents bacteria and archaea have been isolated with the ability to reproduce at high pressure and temperatures (for example, *Methanococcus jannaschiae* with optimum growth at 86°C and a duplication time of 26 minutes).

Dominant invertebrates around the vents are mussels (*Calyptogena magnifica*) and giant tubular worms (*Riftia pachyptila*). They all have symbiont bacteria; sometimes they even host several kinds of bacteria that operate biochemically in a differentiated way (for example, methanogens and sulphate reducers). The worms acquire great dimensions (up to 2.5 metres in length) and lack both mouth and intestine. Gas exchange with the exterior takes place in the 'gills'. Given that symbionts need oxygen and sulphide simultaneously, they possess an efficient system of gas transport, based on haemoglobin. 50% of their bodies consists of a spongy tissue where the symbiont bacteria live that may account for up to 75% of the animal's weight. Molecular studies suggest that there is normally a single type of symbiont per animal species.

Many different types of hydrothermal vents exist in which both their geological structure and chemical properties vary, as a result of which their associated organism communities are also very varied, with bacteria and archaea using different kinds of metabolism and metazoans of very different types that feed on them. Hydrothermal vents are not, however, the only 'exotic' environments of great biogeochemical, evolutionary and biodiverse interest that may be found in the deep ocean. The *mud volcanoes* found in the SE Mediterranean (such as the dome of Naples or the Olympus mud volcano) have associated *cold springs* that form in areas of sediment, where a number of prokaryotes produce methane as a waste product. This methane is freed from the sediment in the form of small bubbles, which other bacteria exploit for their growth. On the other hand, large white blankets of sulphur oxidising bacteria of the *Beggiatoa* genus may be observed.

Other special environments are the *hypersaline anoxic basins*, which are particularly important in the Mediterranean and the Red Sea (for example, L'Atalante, Bannock, Discovery, Urania, Kebrit Deep and Shaban Deep). They are found in the deepest regions (below 3,000 metres) of the basin that formed during the Messinian Event (5-8 million years ago), when the Mediterranean became almost completely desiccated, and they filled with evaporites, which were subsequently redissolved in the sea. Their ecosystems are characterised by very high salinity (160-300 per thousand), high hydrostatic pressure and anoxia. The high salinity prevents their waters from mixing with the seawater beyond; consequently their environments have remained isolated from the rest of the ocean for millions of years. They contain their own particular bacterial diversity, and highly specialised new eukaryotic organisms have even been identified that live in anaerobiosis.

All these special habitats are of great importanc
in terms of biogeochemistry and marine diversit
despite their limited extension in comparison t
the vastness of the ocean depths. They constitut
unique ecosystems and have been called 'the la
frontier of life' by virtue of the extreme conditions i
which their associated organisms grow and thriv
Nonetheless, they might also be called 'the fir
frontier of life', because life on our planet originate
probably in conditions very similar to those whic
the microorganisms of these environments inhab

▶ Postscript

We dedicate this chapter to the memory of ou
friend and colleague Francesc Pagès, a pioneer i
the study of the life forms of the deep ocean an
one of the first Spanish oceanographers to descen
to the depths in a submarine. Few better than hi
managed to convey the beauty of the organism
that populate this environment, which not so lon
ago was regarded as being deprived of life.

Our thanks to Josep Maria Gili, Gerhard Hern
Alexander Bochdansky, Anne-Loise Reysenbac
and Álvaro Roura for allowing us to use their pho
tographs.

Notes

1. See the CeDAMar project website (http://www.cedamar.org
or this bestiary of the abyssal kingdom (http://www.pbs.or
wgbh/nova/abyss/life/bestiary.html).
2. http://www.eurocoml.org/public/publications/deeper-tha
light.
3. Frank Schätzing, *El quinto día*. Ed. Planeta, 2006.
4. Edward Forbes, The *Natural History of the European Seas*. Lo
don: John van Voorst, 1859.
5. http://www.whoi.edu/page.do?pid=10737
6. Henry N. Moseley, *Nature*, April 22 1880, p. 592.

Biodiversity and Genetic Resources of the Deep Ocean

Carlos Pedrós-Alió, Ramon Massana, Xabier Irigoien and Jesús M. Arrieta

Oceans cover 70% of the planet's surface and their average depth is of 3,800 metres. Consequently, the deep ocean is Earth's most extensive ecosystem. It was said in the past that we are less familiar with the topography of the ocean bed than with that of the surface of the Moon. Although this statement is no longer as true as it was, it highlights the little we know about the depths of the sea. The two major characteristics of this ecosystem are its constant moderate temperature (around 4°C) and darkness. For this reason, all the organisms that inhabit this ecosystem must of necessity be chemotrophic, that is, they can obtain energy only from redox reactions, not from light.

Nonetheless, ascertaining what these living beings are, how many exist and to which species they belong continues to pose a challenge. Oceanographic campaigns to sample the deep ocean require many days' sailing and instruments and apparatus sturdy enough to work at depths of thousands of metres. All this is very costly; consequently, most of our knowledge of the sea is restricted to the coastal zones and, to a somewhat lesser extent, the continental shelves.

Aware of these difficulties, in 2000 a global network of marine diversity researchers began work on drafting the Census of Marine Life (ICOM, Ausubel, 2010; McIntyre, 2010). The aim of this multidisciplinary international project was to draw up a census of all living beings present in the ocean and to estimate what proportion of its biodiversity was known and how much was yet to be discovered. The project was completed in 2010. During the first decade of the twenty-first century over five hundred expeditions took place, from the tropics t
the polar regions, involving at least 2,700 scientist
from more than eighty countries.

The results have spectacularly increased ou
knowledge of marine life. In the case of fauna, it i
estimated that some 250,000 species exist in th
sea. Each year of ICOM's existence some 1,650 ne
species of animals were described, most of the
crustaceans and molluscs; but what most aston
ished researchers was the fact that 156 species o
new fish were described each year, since fish wer
regarded as one of the best known groups of ma
rine animals. In the case of microorganisms, esti
mates proved to be more difficult.

ICOM used the 30 million observations mad
not only to study diversity but also to determine th
abundance and distribution of many species. Whe
all the data had been analysed, it was reckoned tha
microorganisms account for up to 90% of the ma
rine biomass. Zooplankton, fish and large mammal
therefore represent only 10%. This need not surpris
us, however. Each millilitre of water contains aroun
one million prokaryotes (bacteria and archaea), 1
million viruses and thousands of eukaryotic micro
organisms (protists). Therefore the entire ocea