

Aquatic Environments

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6.1 Introduction	6.3 Microbial Lifestyles in Aquatic Environments	6.5.2 Lakes
6.2 Microbial Habitats in the Aquatic Environment	6.3.1 Primary Production	6.6 Other Notable Aquatic Environments
6.2.1 Physical and Chemical Characteristics	6.3.2 Secondary Production	6.6.1 Brackish Waters
6.2.2 Overview of Planktonic Microbes	6.4 Marine Environments	6.6.2 Hypersaline Waters
6.2.3 Overview of Benthic Microbes	6.4.1 Marine Planktonic Communities	6.6.3 Subterranean Waters
6.2.4 At the Interface: Biofilms and Microbial Mats	6.4.2 Marine Benthic Communities	6.6.4 Wetlands
	6.5 Freshwater Environments	Questions and Problems
	6.5.1 Springs, Streams and Rivers	References and Recommended Reading

6.1 INTRODUCTION

The majority of the planet's habitat is aquatic: more than 80% of Earth's **surface** is aquatic, and the **volume** of habitat in aquatic systems is vast, spanning a range of environments (Table 6.1; Figure 6.1). These habitats are teeming with microbial life. Microorganisms are key drivers of the planet's biogeochemical cycles (Chapter 16), and this includes large roles for aquatic microbes. While the Amazon Forest has been called the lungs of the planet, roughly 50% of the oxygen that you breathe was actually produced by the photosynthesis of **aquatic** microbial primary producers. In addition, microbes are the base of aquatic food chains, which supply roughly 15% of the world's protein, and are projected to become an even larger share in the future. The water itself in aquatic environments is a vital resource, supplying water for drinking, agriculture, mining, power generation, semiconductor manufacturing and virtually every other industry. For some of these uses, aquatic microbes may be considered contaminants; as in the case of computer chip manufacturing. For potable water, contamination with pathogens results in approximately 11% of the world's

population still lacking access to safe drinking water. In this chapter, we first define the main aquatic habitat types (planktonic, benthic and their interface), then examine how microbial lifestyles (primary and secondary production) are employed in them. Finally, we describe and provide general microbial characteristics of: (1) marine systems; (2) freshwater systems; and (3) select other aquatic environments.

6.2 MICROBIAL HABITATS IN THE AQUATIC ENVIRONMENT

6.2.1 Physical and Chemical Characteristics

There are a number of typical misconceptions about aquatic habitats, due to how we think about water, for example that it tends to be well mixed. First, aquatic habitats are not homogeneous. **Stratification** is an important physical structuring of aquatic environments, established due to temperature and salinity differences (see lake example in Figure 6.2). Surface waters are warmed by sunlight, and since warm water is less dense than cold

water (water is most dense at 4°C), this temperature-driven stratification tends to persist in the absence of mixing (which *does* occur, see below). The **thermocline** is the layer in aquatic systems where a rapid change in temperature occurs. Salinity differences can also establish stratification, when precipitation or other inputs bring fresher waters over saltier ones, which are denser. (The salinity of aquatic systems can range from freshwater at 0.5‰, to

marine water between 33 and 37‰, to hypersaline systems such as the Dead Sea at 290‰; see [Information Box 6.1](#).) This layering of aquatic environments can act as a barrier

TABLE 6.1 Distribution of Water on Earth

Habitat	Volume km ³	Percent of Total Water
Oceans, seas and bays	1,338,000,000	96.5
Ice caps, glaciers and permanent snow	24,064,000	1.74
Saline groundwater	12,870,000	0.94
Fresh groundwater	10,530	0.76
Ground ice and permafrost	300,000	0.22
Fresh lakes	91,000	0.007
Saline lakes	85,400	0.006
Atmosphere	12,900	0.001
Soil moisture	16,500	0.001
Swamp water	11,470	0.0008
Rivers	2120	0.0002
Biological water	1120	0.0001

From http://en.wikipedia.org/wiki/File:Earth_water_distribution.svg.

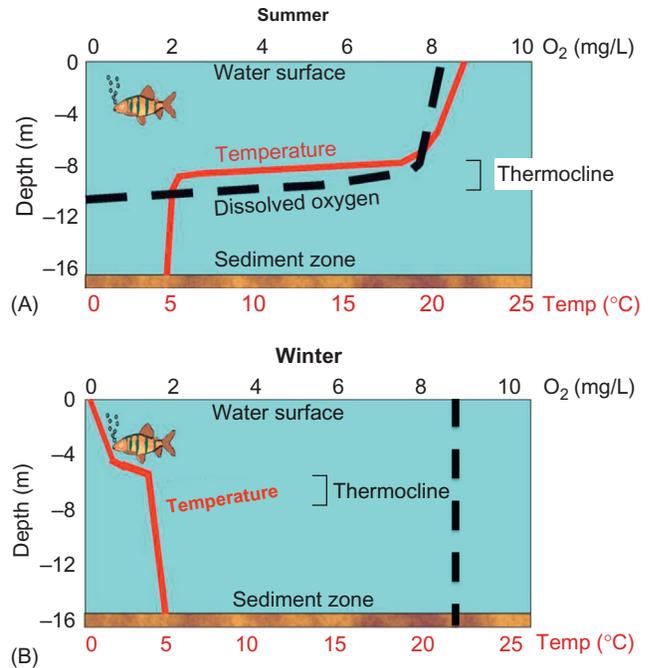


FIGURE 6.2 Stratification in a lake. This idealized view of a temperate-region, eutrophic (high-nutrient) lake shows temperature-driven stratification in the summer (A) due to warming of the surface waters. The thermocline, where the temperature drops sharply, acts as a barrier to mixing of deeper waters, thus preventing their oxygenation. In the fall and winter as the surface cools, the thermocline breaks down and mixing occurs, reoxygenating deeper waters (B).

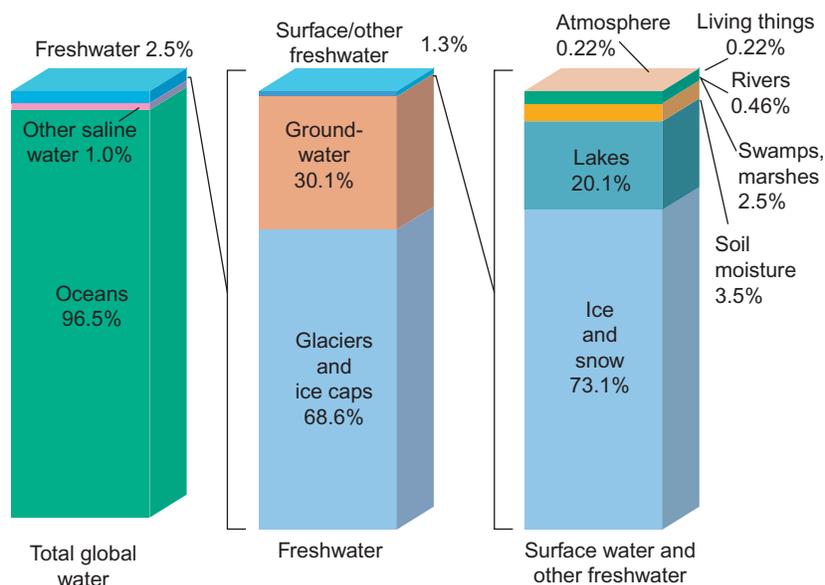


FIGURE 6.1 What are aquatic habitats? A view of the distribution of Earth's water. From <http://ga.water.usgs.gov/edu/earthwherewater.html>.

to vertical movement of organisms that move passively and/or are very small, including microbes. In addition, the layering can create markedly different chemical conditions in adjacent layers. Surface layers have higher oxygen concentrations due to diffusion and mixing from air, and they experience sunlight, but their typically high primary production can result in nutrient-limiting conditions. Microbes can be adapted for the conditions found in specific layers, and some have developed strategies for staying in certain layers. A hot topic in aquatic systems is **thin layers** (Sullivan *et al.*, 2010), which consist of layers of distinct chemistry and biology just centimeters to meters thick that can extend horizontally for kilometers. Interest in thin layers is due to the fascinating and very active biology within them, which until recently was simply missed by typically bulk-scale observations. The thermocline itself is often a transition layer of higher cell numbers and heterotrophic activity, because organic matter accumulates there. Likewise, the layer at the very bottom of the water column, directly above the sediment, often supports higher cell numbers, due to the resuspension of nutrients and carbon, as well as cells from the sediment.

A second misconception about aquatic environments is that they are static; that water's high thermal inertia and the vast size of many water bodies create extremely stable environments. In fact, aquatic systems are spatially

and temporally highly dynamic. **Mixing** counteracts stratification, and is caused by the action of winds, currents, tides, upwelling, and temperature and salinity changes. Mixing is critical to bringing oxygen and nutrients to depleted waters. High evaporation rates such as occur in the Tropics can make surface waters saltier, which can help drive mixing as the denser saltier water then sinks. Seasonal temperature changes in surface waters are a major driver of mixing in aquatic systems. Surface waters are warmed by the summer sun and are cooled in the fall and winter. This results in a decrease in the thermocline strength, permitting deeper mixing (Figure 6.2). In addition, fall and winter often bring more storms, which further mix the water column. In some systems with extreme air temperature changes such as the Polar Regions or limited water volumes such as lakes, the thermocline breaks down altogether, allowing mixing throughout the water column. Where air temperatures drop below freezing, ice forms at the surface; in shallow lakes the ice may propagate all the way to the bottom. As an interesting aside, in marine systems, the formation of ice crystals pushes out the “impurities” of salts, creating extremely salty brine channels in sea ice, an extreme habitat where unique microbiology occurs. When ice thaws in springtime in temperate, alpine and polar aquatic systems, mixing through the water column occurs once more, before summer stratification is re-established.

Light is a critical driver of habitat differences in aquatic systems. Light is able to penetrate to a depth of 200 m or more, depending on the turbidity of the water (Figure 6.3). This depth defines the **photic zone**. In lakes and coastal areas, where the amount of suspended particulate matter in the water is high, light may penetrate less than 1 m. The **aphotic zone** is the dark water where light does not reach. The presence or absence of light results in very different microbial lifestyles, diversity and activity. It is essential to consider stratification and mixing in tandem with photic zone depth, when thinking about microbes that specialize in the sunlit surface waters. If an aquatic system is highly mixed (for example, in the fall in

Information Box 6.1 What Is Salinity?

The average salt concentration in the ocean is approximately 3.5%. This is more precisely expressed in terms of salinity. Salinity (‰) is defined as the mass in grams of dissolved inorganic matter in 1 kg of seawater after all Br^- and I^- have been replaced by the equivalent quantity of Cl^- , and all HCO_3^- and CO_3^{2-} have been converted to oxide. In terms of salinity, marine waters range from 33 to 37‰, with an average of 35‰.

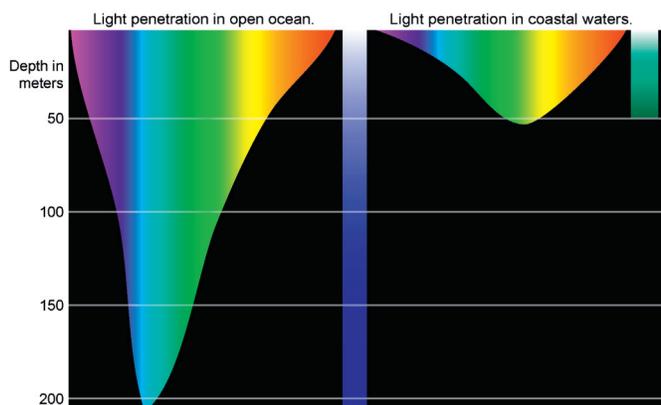


FIGURE 6.3 Light penetration through water, here shown for two ocean habitats, coastal vs. open ocean. Penetration is markedly different due to differences in turbidity resulting from dissolved and particulate matter and microbial cells. In the coastal waters, turbidity is higher, and light penetrates less deeply. Long wavelength light (red) is absorbed by water, while short wavelength light (purple) is scattered, such that the deepest penetrance is for mid-range green and blue wavelengths. Maximum light penetration may be several hundred meters in the open ocean but just tens of meters in coastal waters. From NOAA: Image courtesy Kyle Carothers, NOAA-OE.

many systems), photoautotrophic microbes may not stay in the photic zone long enough to accomplish much primary production. In contrast, stratification allows photoautotrophs to remain in the surface sunlit waters, but may result in nutrient depletion of those waters, which can also limit their primary production and growth.

Another way to consider the structuring of aquatic environments is based on habitats defined with respect to depth below surface. In particular, at the sea surface the air–water interface is referred to as the **neuston** (Figure 6.4), a habitat with high levels of harsh ultraviolet radiation, biochemicals and nutrients. Due to biomolecule interactions with the air–water interface that cause them to concentrate there, the neuston is comprised of a thin gel-like matrix of biomolecules (mainly lipids, proteins and polysaccharides) where microbes can attach or become trapped. The **pelagic zone** is a broad term used to describe the water column or planktonic habitat (see Section 6.2.2), and is subdivided on the basis of depth. In marine systems, depth from surface defines the **epipelagic**, **mesopelagic**, **bathypelagic** and **abyssopelagic** zones. Oceans range in depth up to 11,000 m in the deepest of ocean trenches. In lakes, which can be a few meters to more than 1000 m deep, depth is combined with light penetration to define the surface **limnetic** zone (where light intensity is at least 1% of sunlight), and deeper **profundal** zone. Within the pelagic zones, water can be comprised of many microhabitats and be highly structured. Floating or sinking particles create miniature islands of carbon, nutrients and substrates. **Particle-associated** microbes specialize in living on these islands, in contrast

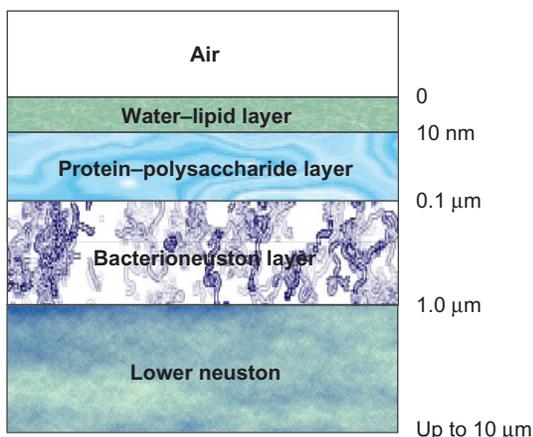


FIGURE 6.4 Schematic representation of the neuston, the upper layer of aquatic environments that can range from 1 to 10 μm in depth. The upper layer that interacts with the atmosphere consists of a water–lipid mixture that has increased surface tension. Below this is a layer of organic matter that accumulates from organic matter rising up the water column. Most scientists consider the neuston an extreme environment (see Chapter 7) because of many factors, including intense solar radiation, large temperature fluctuations, and the natural accumulation of toxic substances including chemicals, organic matter and heavy metals.

to the more intuitive **free-living** lifestyle we imagine in the pelagic habitat. Also, microbes and many macroorganisms produce exopolysaccharides, which cumulatively create an actual mesh structure to broad areas of water. Then, moving below the pelagic zone, the **benthos** is the sediment habitat underlying the water column (see Section 6.2.3).

Another important and not immediately obvious set of aquatic habitats is defined by microbial associations with macroorganisms. These relationships define two additional habitats: **epibiotic**, which means attached to the surface of another organism, and **endobiotic**, which means living within another organism’s tissues. Such microbe–macrobe relationships and communication increasingly appear to be the exception rather than the rule in nature (for example, in our own bodies, see Chapter 20), and can result in some particularly innovative and exciting biology. For example, many fish and squid employ bioluminescence generated through diverse microbial relationships. *Vibrio harveyii* is one microbe that uses luminescence in its fascinating endobiotic lifestyle (see Information Box 20.3).

6.2.2 Overview of Planktonic Microbes

Plankton, from Greek word meaning “wanderer” or “drifter,” are organisms that live suspended in the water column and drift with the currents, with little or no ability to control their horizontal location. There are three functional groups of plankton, each with microbial members: phytoplankton, bacterioplankton and zooplankton. Pelagic microbial populations can be referred to as **bacterioplankton** (though notably, despite the name, these include archaeans as well as bacteria), and include photoautotrophs, chemoautotrophs (see Section 6.3.1.1) and heterotrophs. The **phytoplankton** (Figure 6.5) are the photoautotrophic plankton, which include microbes (cyanobacteria) and eukaryotes (algae, especially the single-celled dinoflagellates and diatoms). The **zooplankton** are larger heterotrophic plankton, including protozoans such as the intricate foraminiferans and radiolarians and larger organisms such as copepods. Figure 6.6 shows the relationship and interdependence of the various microbial components within a general **planktonic food web**.

Phytoplankton are the **primary producers**, which use photosynthesis to fix CO_2 into organic matter. This is a major source of organic carbon and energy, which is transferred to other trophic levels within the web (Figure 6.6). The organic compounds produced by phytoplankton can be divided into two classes, particulate or dissolved, depending on their size. **Particulate organic matter (POM)** compounds are large macromolecules such as polymers, which make up the structural components of the cells, including cell walls and membranes. **Dissolved organic**



FIGURE 6.5 Examples of phytoplankton.

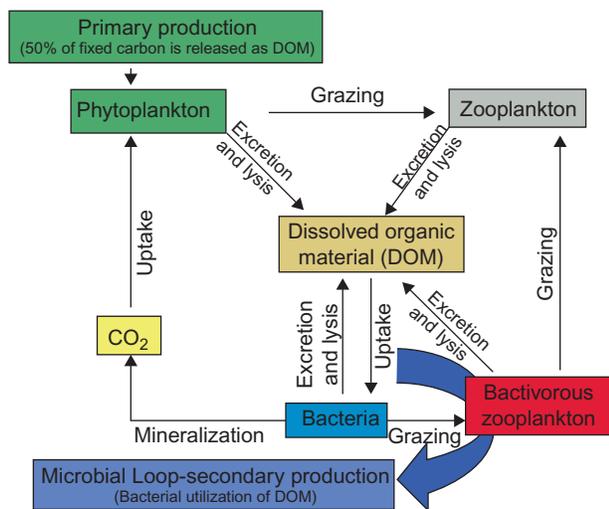


FIGURE 6.6 The planktonic food web. The microbial loop represents a pathway in which the dissolved organic products are efficiently utilized. The role of bacterioplankton is to mineralize important nutrients contained within organic compounds and to convert a portion of the dissolved carbon into biomass. Grazing by bacterivorous protozoans provides a link to higher trophic levels. Modified from Fuhrman (1992).

matter (DOM) is composed of smaller soluble material that passes through a filter (pore size 0.7 μm) including amino acids, carbohydrates, organic acids and nucleic acids, which are rapidly taken up by microbes and metabolized (Kirchman, 2004). DOM is an extremely large carbon pool, equal in size to atmospheric CO_2 (Chapter 16).

6.2.3 Overview of Benthic Microbes

The benthos is the transition between the water column and the mineral subsurface. It collects organic material that settles from above, or that is deposited from nearby terrestrial environments (through river inputs, bulk overland flow or groundwater flow). At the surface of the benthos, nutrient and carbon levels are higher than in the directly overlying waters, which often causes dramatically higher microbial numbers (as much as five orders of magnitude) and activity than in the plankton. Since activity is high, oxygen is quickly used up, leading to a steep redox gradient in the sediment; that is, oxygen is replaced by

other terminal electron acceptors such as sulfate, nitrate or iron (see Chapter 3).

The benthos supports a physiologically diverse aquatic microbial community. This is because the redox and nutrient gradients described above create numerous microenvironmental niches, of which specific physiological groups of microorganisms are strategically positioned to take advantage. In shallow-water benthic communities (mud flats, river bottoms, etc.), the surface may be dominated by photoautotrophs. Below that, and in aphotic benthic habitats, heterotrophs and/or chemoautotrophs dominate, the latter fixing dissolved CO_2 into biomass using the energy of chemical bonds (see Chapter 3). The cycling of essential nutrients, such as carbon, nitrogen and sulfur, is dependent on a combination of aerobic and anaerobic microbial transformations (Figure 6.7), and can

be viewed as biogeochemical cycling at small habitat scales (see Chapter 16).

In terms of carbon, the incoming organic matter (as POM or DOM) can be degraded aerobically to produce smaller compounds or CO_2 , or anaerobically through fermentation into organic acids and CO_2 . Organic acids can then act as electron donors for a group of strictly anaerobic bacteria that utilize CO_2 as the final electron acceptor in anaerobic respiration, thus generating methane (CH_4). The methanogenic activity in turn supports the activity of the methane-oxidizing bacteria (methanotrophs), which can use methane and other one-carbon compounds as an energy source, regenerating CO_2 . Methanotrophic activity until recently was assumed restricted to the sediment–water interface zone, because it was believed to require oxygen. We now know that **anaerobic methane oxidation**

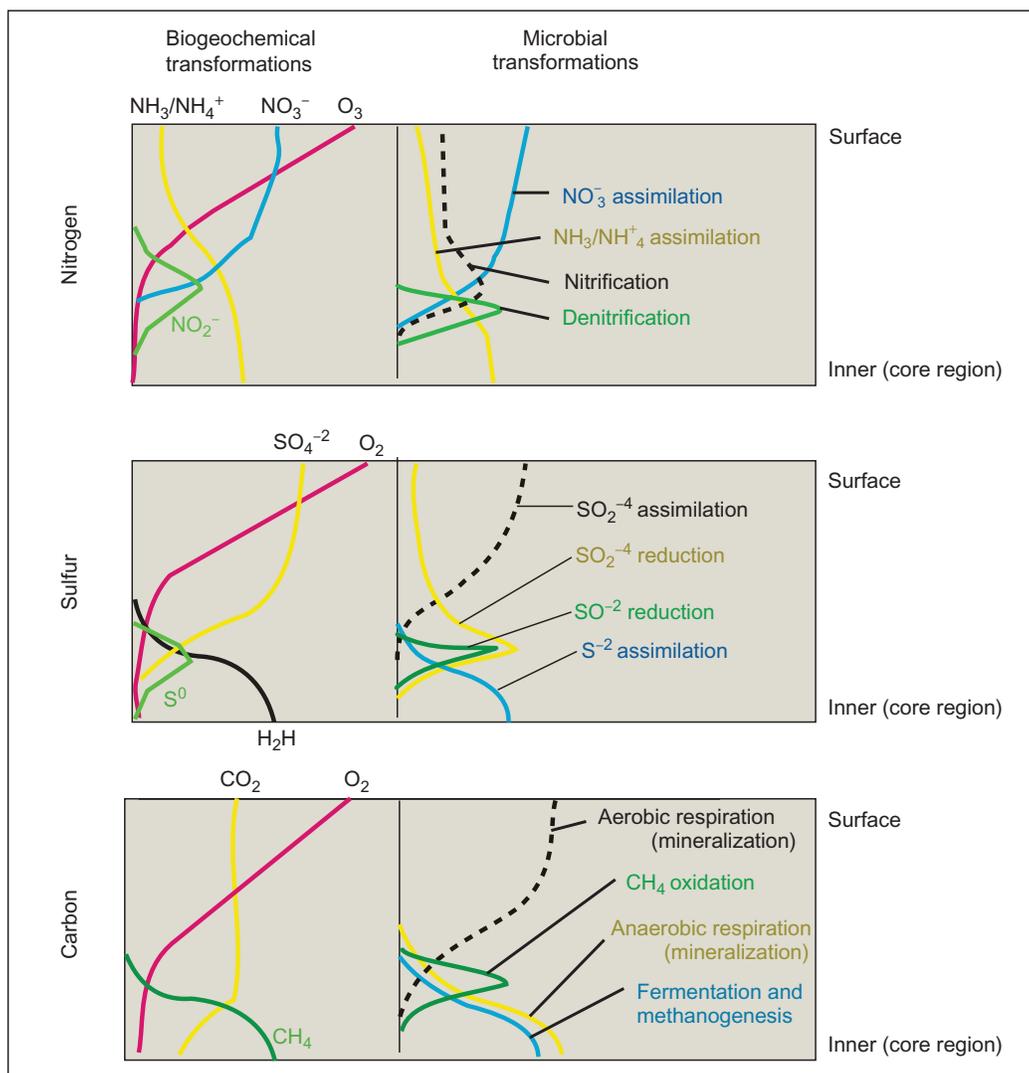


FIGURE 6.7 Biogeochemical profiles and major carbon, nitrogen and sulfur transformations that can be predicted for benthic environments in which oxygen levels are highest at the “surface” layer and are depleted by microbial activity to create anoxic conditions in the “inner” region. Adapted with permission from Pearl and Pinckney (1996).

occurs, performed by archaea and often in close syntrophic (cross-feeding) relationships with bacteria (Orphan *et al.*, 2009), although the details of this lifestyle and relationships continue to be unraveled.

In terms of nitrogen, the decomposition of organic material in the sediment layer generates ammonia from organic debris. Ammonia in the benthos may be used for two purposes: (1) biomass; that is, its assimilation as a source of essential nitrogen by planktonic and sediment microorganisms; and (2) energy; that is, its oxidation as an energy source by chemoautotrophic microorganisms. Ammonia oxidation is often localized at the sediment–water interface, where organisms utilize the release of ammonia by the decomposers and either oxygen (aerobic ammonia oxidation, by bacteria or archaea), or nitrite (anaerobic ammonia oxidation) as terminal electron acceptor (see also Section 16.3.4.2 and Case Study 19.3). The latter reaction, known as anammox, is thus far known only in the ubiquitous bacterial phylum *Planctomycetes* as its terminal electron acceptor (Kuenen, 2008). Nitrification is a two-step process of sequential oxidation of ammonia to nitrite, and then nitrite to nitrate, for example, performed in sequence by the bacterial genera *Nitrosomonas* and *Nitrobacter* (see also Section 16.3). The control of ammonia compounds can be important, especially in alkaline environments, where the undissociated NH_4OH form can be toxic to aquatic animals. The activity of the ammonia-oxidizing or nitrifying microbes can be highly sensitive to the presence of certain DOM, including naturally occurring and industrial chemicals. Therefore, the inhibition of nitrification (ammonia oxidation), which can be detected by an accumulation of ammonia or NO_2 , provides a sensitive indicator of the environmental impact of certain toxic pollutants.

6.2.4 At the Interface: Biofilms and Microbial Mats

Interfacial habitats are special, as may have become clear above. They are defined by sharp environmental gradients (for example, of UV and oxygen in the neuston, and of redox conditions in the benthos), and these gradients can create distinct niches, which microbes take advantage of through diverse life history strategies. One strategy is to team up and specialize. While we think of microbial cells in isolation, in fact they are constantly interacting with one another (see Section 19.3.1). A common way is through quorum sensing, a process by which a single cell can “sense” whether a threshold number, or “quorum,” of cells is nearby (see also Chapter 20). The coordination of microbial cells through physical, chemical and biological processes can result in the formation of complex,

specialized and diversified structures. Here we discuss the two such structural types, common on surfaces in aquatic habitats.

6.2.4.1 Biofilms

A **biofilm** is a surface association of microorganisms that are strongly attached through the production of an extracellular polymer matrix. Biofilm-harboring surfaces are usually aquatic or at least moist, and include inert surfaces, such as rocks and the hulls of ships, and living ones, such as a copepod’s exoskeleton, or an aquatic plant’s submerged leaf. As elaborated below, biofilms have been extensively studied for their role in nutrient cycling and pollution control within the aquatic environment, as well as for their beneficial or detrimental effects on human health.

Biofilm development occurs through microbial attachment to a solid surface (Figure 6.8) in two stages: (1) reversible attachment, which is a transitory physicochemical attraction (including via hydrophobic, electrostatic and van der Waals forces; Marshall, 1985); and (2) irreversible attachment, which is a biologically mediated stabilization reaction (see Section 19.3.2.1). The attached bacteria excrete extracellular polymers, which create a matrix that surrounds the cells and forms a strong chemical bridge to the solid surface. The polymers then provide a matrix for the attachment of additional cells, form internal architecture in the biofilm structure and can create a visibly “slimy” layer on a solid surface (Marshall, 1992). The exopolymer matrix is also an integral component influencing the functioning and survival of biofilms in hostile environments.

Biofilms can be so highly organized that their architecture can rival that of simple macroorganisms. Examination of mature biofilms in their native states (using microscopic techniques, such as confocal laser scanning microscopy) has revealed a complex organization (Costerton *et al.*, 1995). Biofilms can be composed of cone-, mushroom- and column-shaped clusters of cells embedded within the extracellular polymer matrix and surrounded by large void spaces (Wolfaardt *et al.*, 1994; Korber *et al.*, 1995) (Figure 6.9A). The void spaces form channels (Figure 6.9B), which function as a primitive circulatory system by carrying limiting nutrients, such as oxygen, into the exopolymeric matrix. The presence of void spaces increases the biofilm surface area and the efficiency with which nutrients and gases are transferred between the biofilm and the surrounding water. The exact nature of the biofilm architecture depends on numerous factors, including the type of solid surface, the microbial composition of the biofilm and environmental conditions.

Microorganisms benefit from membership in a biofilm community. The extracellular matrix can have several

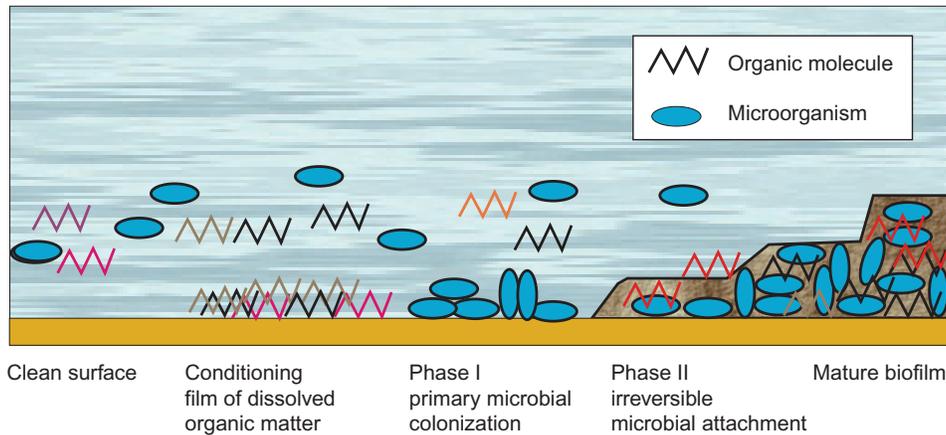


FIGURE 6.8 Representation of biofilm formation. Dissolved organic molecules of a hydrophobic nature accumulate at the solid surface–water interface and form a conditioning film. Bacteria approach the solid surface because of water flow and/or active motility. The initial adhesion (phase I) is controlled by various attractive or repulsive physicochemical forces leading to passive, reversible attachment to the surface. An irreversible attachment is a biological, time-dependent process related to the proliferation of bacterial exopolymers forming a chemical bridge to the solid surface (phase II). By a combination of colonization and bacterial growth, the mature biofilm is formed. It is characterized by cell clusters surrounded by water-filled voids. Adapted from Marshall (1992).

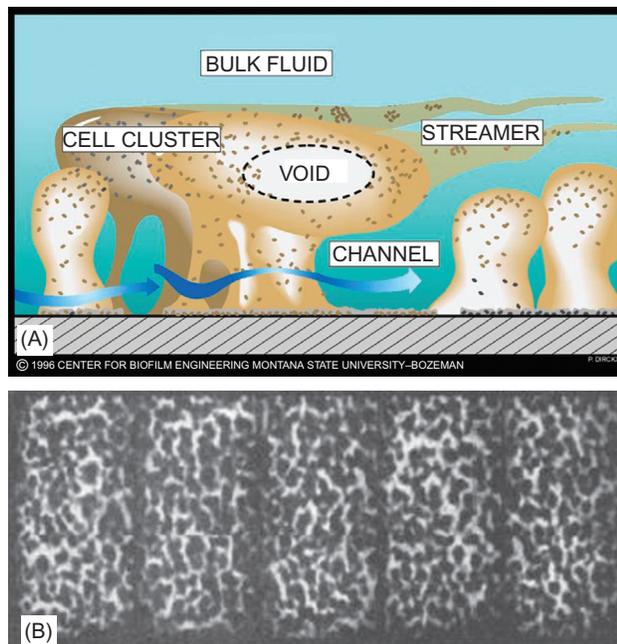


FIGURE 6.9 Biofilm communities are composed of cone-, column- and mushroom-shaped clusters of cells embedded in extracellular polysaccharide and other materials (A). Clusters of cells are surrounded by large void spaces (white networks of channels shown in biofilm in panel (B)) that allow convective flow of fluids through biofilm communities.

functions for the member cells: as an ion-exchange resin to filter and collect essential nutrients; as physicochemical protection against abiotic environmental stressors, such as desiccation or changes in pH and temperature; and against actual grazing by predators. Compared with planktonic cells, biofilm cells are far more resistant to

antibacterial substances, such as antibiotics and disinfectants; this may be due to the barrier of the extracellular matrix, or to an altered physiological state of the attached bacterial cells.

Humans contain biofilms (the most obvious example being dental plaques) and have been using them intentionally for hundreds to thousands of years. Biofilms can act as water purification systems in natural environments, and remove DOM and other contaminants from flowing waters. This property has long been exploited for use in purifying water from municipal (sewage) or industrial sources (Case Study 6.1), with crude management in ancient times giving way to sophisticated “collaboration” with the biofilms in current water purification system designs, including trickling filters in wastewater treatment (Chapter 25) and point-of-use devices (Chapter 19). On the other hand, biofilms are responsible for tooth decay and compromised medical implants. Disease caused by biofilms on medical implants is estimated to account for nearly 60% of all hospital-borne infections, lengthen hospital visits by 2–3 days and increase healthcare costs by \$1 billion per year (Davey and O’Toole, 2000). In industry, biofilm control is required for any type of pipeline (Chapter 28), in which biofilms lower the flow capacity, decrease heat-exchange efficiency and catalyze corrosion in the case of metal pipes.

6.2.4.2 Microbial Mats

Microbial mats can be considered a specialized type of biofilm. They are an extreme example of an interfacial aquatic habitat in which many microbial groups are

Case Study 6.1 Beneficial Biofilms Remove Cyanide from Gold Mine Effluent and Keep Mines in Business

The Homestake Mine in Lead, South Dakota, opened in 1877 during the Black Hills Gold Rush and operated profitably in the gold mining business for decades. As was the case with the operation of many other gold mines, cyanide was used to increase recovery of gold from ore obtained from the mine. As a result, an estimated 4 million gallons of cyanide-laden wastewater were released daily into nearby Whitewood Creek. These extraordinary levels of this toxic waste rendered aquatic life nearly nonexistent in the creek. By 1981, Whitewood Creek was listed as an Environmental Protection Agency (EPA) Superfund site. In 1977, the EPA required Homestake Mine to reduce its discharge of this toxic effluent. Traditional approaches to minimizing discharge of such toxic substances were expensive, and implementation of these approaches would result in closure of the mine.

The Homestake Mine needed an innovative, cost-effective strategy to deal with levels of cyanide in its wastestream. Jim Whitlock, a biochemist and South Dakota native, and Terry Mudder, an environmental engineer, were charged with addressing the problem (Whitlock, 1990). The solution the cross-disciplinary duo devised relied upon a bacterial biofilm, composed primarily of *Pseudomonas*, to remove cyanide and a host of other toxic substances, including ammonia and the metals nickel chromium, from the wastestream. Sets of large discs, called **rotating biological contactors (RBCs)**, served as substrates upon which the pollutant-removing biofilm grew. Each RBC consisted of disks that harbored billions of bacteria across large surface areas (100,000 to 150,000 ft²). Wastewater

passed through a train of five of these RBCs. Each disk rotated at a rate of 1.5 revolutions per minute. Approximately 40% of each disk was submerged in the wastestream at all times. The rotation allowed the biofilm community to contact the wastestream and remove pollutants such as cyanide while meeting some microbial community members' requirements for oxygen. The first two RBCs contained primarily *Pseudomonas* for the removal of cyanide and the metal contaminants, while the remaining RBCs harbored nitrifying bacteria that allowed conversion of ammonia into a less toxic form, nitrate. End products resulting from this treatment were relatively innocuous and included sulfate, carbonate, nitrate and some solids, which were subsequently removed using a clarifier. The treatment facility began operation in 1984 and became more efficient and economical over time. Cyanide removal rates of 99% (from influent levels of 4.1 mg/L to effluent levels of 0.06 mg/L) were obtained. Copper and iron were removed quite efficiently—removal rates of 95–98% were common. Removal of other metals, particularly nickel, chromium and zinc, was less remarkable. Nonetheless, the effluent was free enough of pollutants to allow rainbow trout to reinhabit Whitewood Creek. Thus, this innovative use of biofilms dramatically reduced pollution introduced into the environment by the Homestake Mine, and allowed the mine to continue operations until its closing in 2002. To date, thousands of similar RBCs have been employed worldwide to reduce cyanide levels from industrial wastestreams.

laterally tightly compressed into a thin mat of biological activity. While biofilms are typically one to several cell layers thick, microbial mats range from several millimeters to a centimeter thick, and are vertically stratified into distinct layers (**Information Box 6.2**). Another distinguishing characteristic of microbial mats is that they are based on autotrophy, the fixation of inorganic carbon into biomass, which occurs either photosynthetically or chemosynthetically. Similarly to biofilms, mat microbial groups interact with each other in close spatial and temporal physiological couplings. Microbial mats have been found associated with environments such as the benthic–planktonic interface of hot springs, deep-sea vents, hypersaline lakes and marine estuaries. By supporting most of the major biogeochemical cycles, these mats are largely self-sufficient.

In a photosynthetic microbial mat, the photosynthetic activity of the cyanobacteria creates an oxygenic environment in the upper layer of the mat. Oxygen can become supersaturated during the day, but at night, in the absence of sunlight, microbial respiration rapidly depletes all the available oxygen. Respiration by sulfate-reducing bacteria, considered a strictly anaerobic process, helps decompose the DOM from the cyanobacteria in the upper

generally aerobic layers. This apparent contradiction may be resolved temporally, with oxygenic photosynthesis occurring during the day and anaerobic sulfate reduction occurring at night, or spatially, due to the formation of anaerobic microenvironments even in the upper layers, due to the high demand for oxygen by heterotrophic activity.

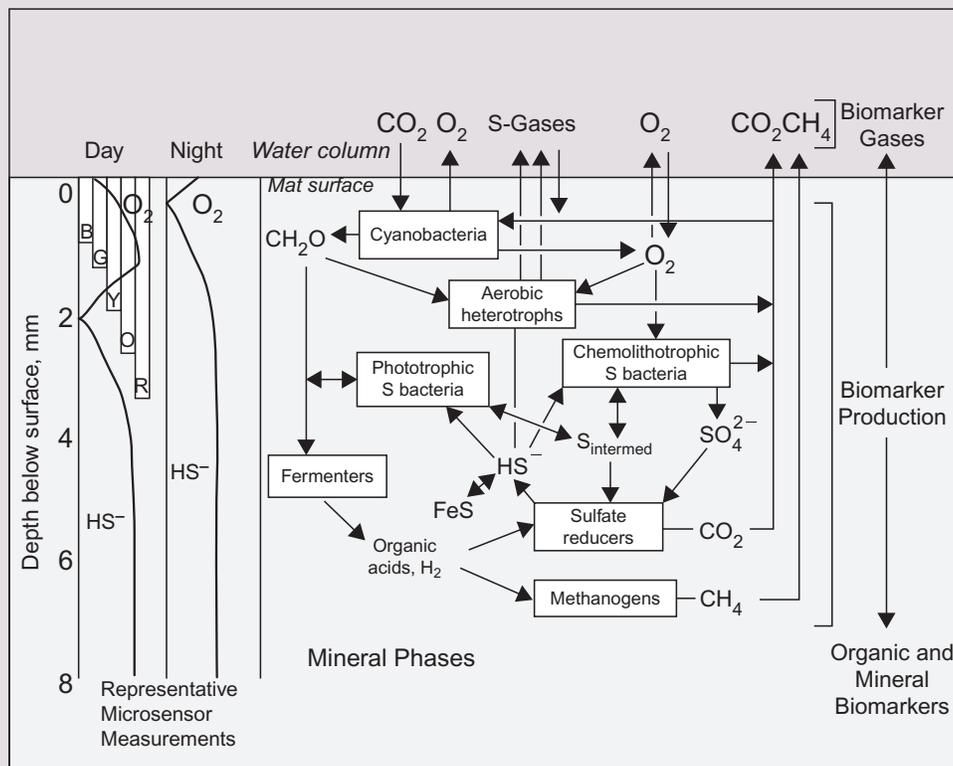
Microbial mats are unique communities because the interdependent microbial components form clearly stratified and often distinctively colored zones. Mats are often found in extreme environments or in environments where conditions fluctuate rapidly. The cyanobacteria are known to be tolerant of extreme conditions, such as high temperatures or highly saline waters, and thrive in locations where competition from other microbial groups and predation by grazing organisms are limited by the inhospitable environment. Fossilized microbial mats, known as **stromatolites**, dating back 3.5 billion years were among the first indications of life on Earth (see **Information Box 6.2**, and Section 16.1.2). At that time, Earth's atmosphere lacked oxygen, and the stromatolites from that era were probably formed with anoxygenic phototrophic bacteria (purple and green sulfur bacteria—see also Section 16.4.3.2).

Information Box 6.2 The Importance of Microbial Mats on Early Earth

The figure (top) shows a cross section of a microbial mat collected by NASA scientists from a hypersaline pond at one of the world's largest salt production facilities in Guerrero Negro, Baja California Sur, Mexico. The smallest gradations in the ruler are in millimeters.



It has been suggested that such mats forced the close proximity of the first aerobic photosynthetic microbes (cyanobacteria) with anaerobic heterotrophs. This proximity was in turn responsible for the adaptation of anaerobic heterotrophic microbes on early Earth to the presence of oxygen, which was extremely toxic to these first heterotrophic forms of life (Hoehler *et al.*, 2001). Today, as shown in the figure below, these mats provide another example of the complexity of biogeochemical cycling in aquatic environments (Des Marais, 2003). In these mats, cyanobacteria photosynthetically generate organic matter (required by heterotrophs) and oxygen (toxic for strict anaerobes). On the other hand, anaerobic heterotrophic activity recycles required nutrients back to the phototrophic community while generating toxic sulfide. Mat microbes have developed strategies to cope with the conundrum posed by these different populations. Note that in this case, the community is driven directly by photosynthesis while activity in the benthic environment is driven indirectly by photosynthesis in the form of DOM.



From (top) NASA, 2005 and (bottom) from Fig. 1 from Des Marais, D. 2003. *Biol. Bull.* **204**, 160–167. Reprinted with permission from the Marine Biological Laboratory, Woods Hole, MA.

6.3 MICROBIAL LIFESTYLES IN AQUATIC ENVIRONMENTS

6.3.1 Primary Production

Primary production in the ocean is estimated to be 50–60 petagrams ($\text{Pg} = 10^{15} \text{ g}$) of carbon per year (De la Rocha, 2006), with freshwater likely accounting for an additional one-to-several Pg (Tranvik *et al.*, 2009). This represents 50% of the total primary production globally. The amount of primary production within a given water column depends on a variety of environmental factors. These factors include: the availability of essential inorganic nutrients, particularly nitrogen and phosphorus; water temperature; the turbidity of the water, which affects the amount of light transmitted through the water column; and the degree of vertical mixing, as described above. The concentration of primary producers in aquatic environments ranges from 10^0 organisms/ml in some benthic habitats, to 10^8 organisms/ml in surface zones.

Open oceans have relatively low primary productivity because of low levels of the essential nutrients nitrogen and phosphorus. The exceptions are areas where currents cause upwelling of deeper waters bringing nutrients from the deep sea. Coastal areas are productive because of the

introduction of dissolved and particulate organic material from river outflows and surface runoff from the terrestrial environment. Upwelling can also increase productivity due to wind driven nutrient rich waters, such as off the coast of California, where upwelling-driven productivity supported the large sardine fishery made famous in John Steinbeck's *Cannery Row*. For freshwater environments, smaller and shallower freshwater bodies tend to be nutrient rich or **eutrophic**, supporting high productivity. Large, deep lakes can be nutrient poor or **oligotrophic** like the open ocean, with low productivity. However, human activities can significantly increase nutrient loading. Sources of natural nutrient loading include terrestrial runoff, rivers that feed into the lake and plant debris such as leaves. Nutrient loading from human activities includes runoff from animal manures and agricultural runoff, both of which contain high levels of nitrogen and phosphorus, the nutrients most often limiting in aquatic environments.

6.3.1.1 Photoautotrophy vs. Chemoautotrophy

Aquatic primary production is considered, and quantified, almost exclusively as photoautotrophy occurring in sunlit waters. Because photosynthesis is mediated by photopigments with characteristic absorption spectra such as

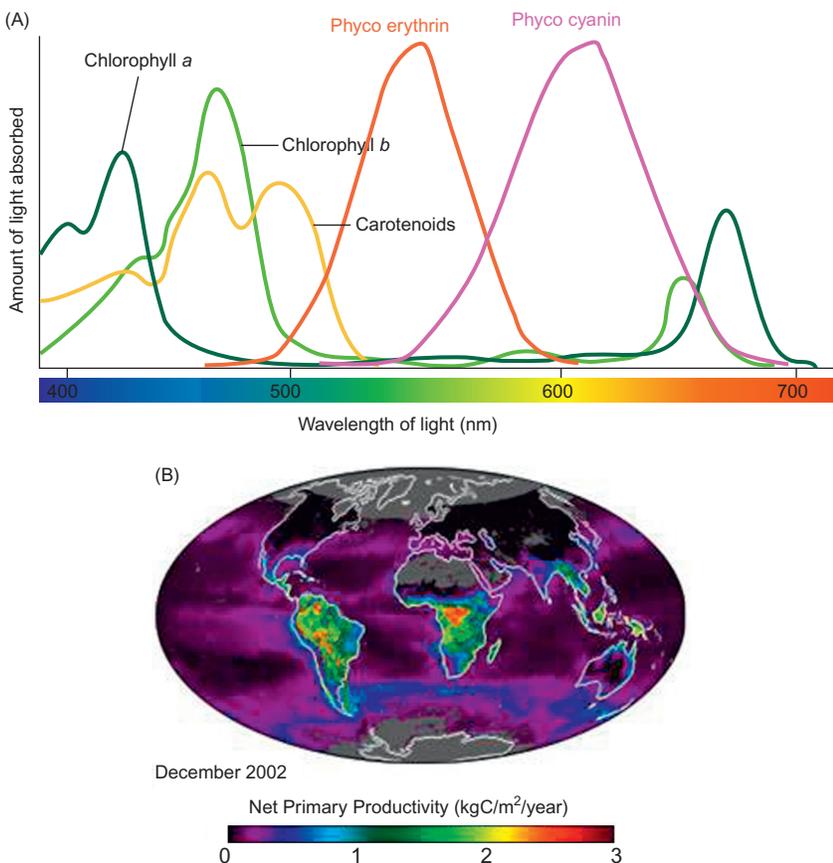


FIGURE 6.10 The absorption spectra of photosynthetic pigments (A). Modeled global primary production (B), from satellite data on chlorophyll abundance, measured by its characteristic spectral signature. *Source:* NASA.

chlorophyll (Figure 6.10A), it can be measured from space through satellite imagery (Figure 6.10B). Chemoautotrophy, the ability to fix carbon using chemical bond energy (i.e., the oxidation of reduced compounds) (see Chapter 3), does not have a similar such marker that is visible from space! Initially, chemoautotrophy was believed to occur in extreme aquatic environments such as geothermal hot springs and oceanic hydrothermal vents, but gradually it has been increasingly recognized to occur in many more aquatic habitats. The question has now evolved from “where does chemoautotrophy occur?” to “where does chemoautotrophy not occur?”

Chemoautotrophs are seen throughout the water column, especially in oxygen minimum areas, which occur when high microbial decomposition (usually linked to high overlying primary productivity) strips oxygen from the water. The Gulf of Mexico’s “Dead Zone” (Figure 6.11) is an example of a particularly large oxygen minimum area, and received its name because the extreme lack of oxygen in mid-depth waters causes fish die-offs. These areas are expected to increase with global change, due to increased water stratification from warmer temperatures, combined with more extreme precipitation events flushing agricultural fertilizers into aquatic systems. Chemoautotrophs have been discovered to be abundant in oxygen minimum waters, as well as in benthic

systems (see Section 6.4.2.2), and are present at varying levels throughout aquatic habitats (Reinthal *et al.*, 2010; Swan *et al.*, 2011). When inorganic carbon fixation is measured throughout the water column, consistent though low amounts can be seen through the dark subphotic waters (Figure 6.12), cumulatively (due to the large volume involved) representing a large and important though still poorly defined contribution to marine primary production.

Ammonia oxidizing archaeans are one form of ubiquitous pelagic chemoautotrophs, virtually unknown until the mid-1990s (Schleper and Nicol, 2010). These marine pelagic Crenarchaota are a minor component of photic communities, but reach 35–40% of open-ocean microbes below 1000 m (Figure 6.13) (Karner *et al.*, 2001). A variety of molecular methods, including gene and transcript surveys, genomics and metagenomics (see Chapter 21), show that chemoautotrophy via aerobic ammonia oxidation is likely the dominant lifestyle for these microbes (Church *et al.*, 2010).

6.3.2 Secondary Production

Although we may think of the general planktonic food web in the aquatic systems as simply involving

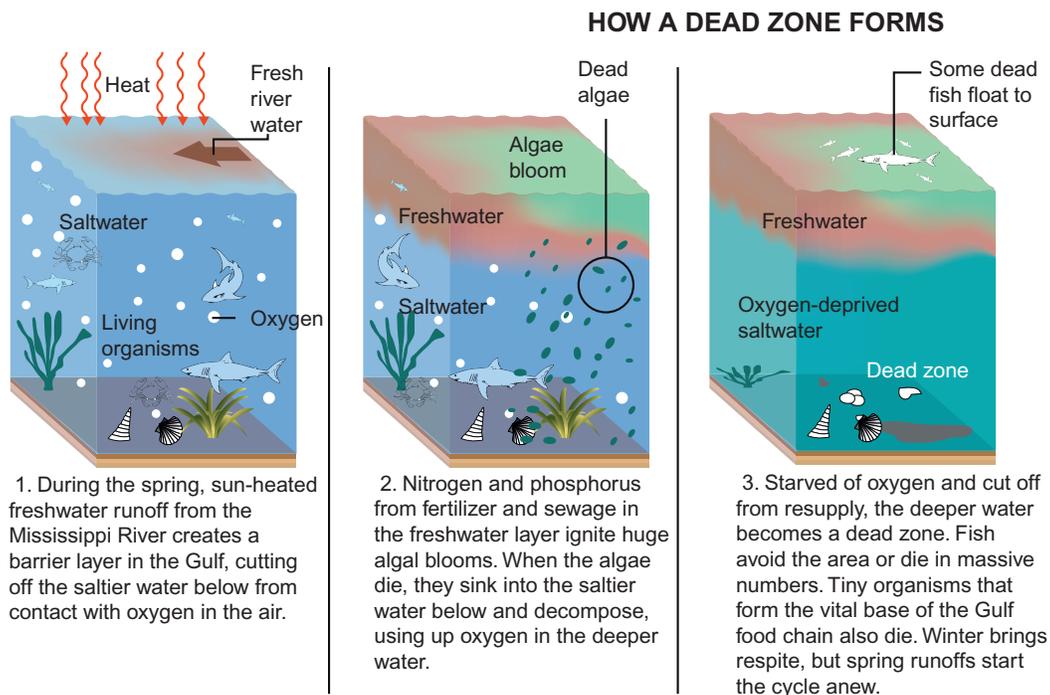


FIGURE 6.11 A so-called Dead Zone, where heterotrophic decomposition has stripped the water of oxygen, leading to large regions of anoxia and sometimes resulting in massive fish die-offs.

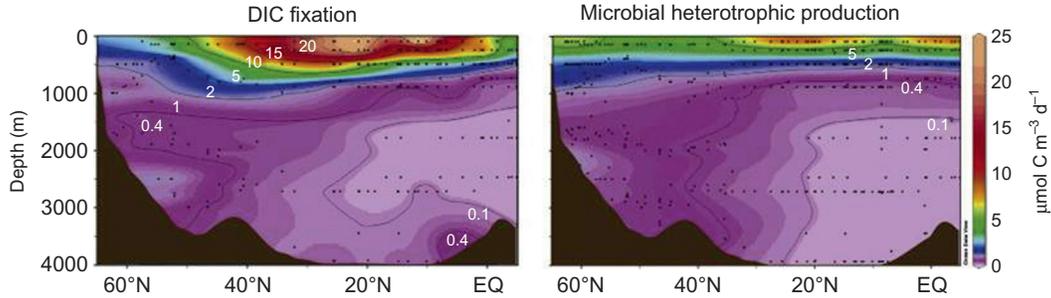


FIGURE 6.12 Chemoautotrophy in the eastern North Atlantic. On the left, dissolved inorganic carbon (DIC) fixation through the water column, measured by ^{14}C -bicarbonate fixation. The strong signal of photosynthesis in surface waters is clear, but low amounts of fixation continue throughout the vast dark waters below. On the right, microbial heterotrophic production as measured by ^3H -leucine incorporation showing that the subphotic pelagic chemoautotrophy is on the same order of magnitude as the heterotrophic production at those depths. Image from Reinthaler *et al.* (2010).

bacterioplankton and zooplankton consuming phytoplankton, the primary producers, which in turn are consumed by progressively larger organisms, the actual transfer of carbon and energy between trophic levels is much more complex (Figure 6.6; see also Figure 2.19 for an alternate image of a pelagic food chain). DOM represents a very large pool of carbon, roughly equivalent to the CO_2 in the atmosphere. This is because $>50\%$ of the carbon fixed by photosynthesis is released into the water column as DOM, which is rapidly utilized by heterotrophic microbes in a pathway in the aquatic food web referred to as the **microbial loop**. In this loop, bacterioplankton remineralize a portion of the DOM into CO_2 and nutrients, which in turn fuel new primary production—in fact, microbially recycled nutrients in the ocean’s surface waters fuel roughly 80% of marine primary production (Duce *et al.*, 2008). Bacterioplankton also assimilate DOM to produce new biomass of their own, which is referred to as **microbial secondary production**. Thus, the microbial loop serves to efficiently utilize the DOM released into the water column. Because there is so much DOM, and the microbial loop allows its nutrients, carbon and energy to be retained in the sunlit surface waters to support more growth, **the microbial loop is a key concept in aquatic systems.**

Why is there so much DOM, and where does it come from? The DOM pool comes primarily from phytoplankton, with contributions from zooplankton and bacterioplankton, as well as from larger organisms through excretion and the lysis of dead cells. Among the phytoplankton, it is known that both “healthy” cells and “stressed” cells (those under some form of environmental stress) release DOM into the water column. In addition, “sloppy” feeding habits of zooplankton and larger organisms that eat phytoplankton may release a portion of their biomass as DOM into the water column. Finally, evidence indicates that as much as 6 to 26% of DOM is released during the lysis of phytoplankton and bacterioplankton by viruses (Ashelford *et al.*, 2003) (see Figure 6.6).

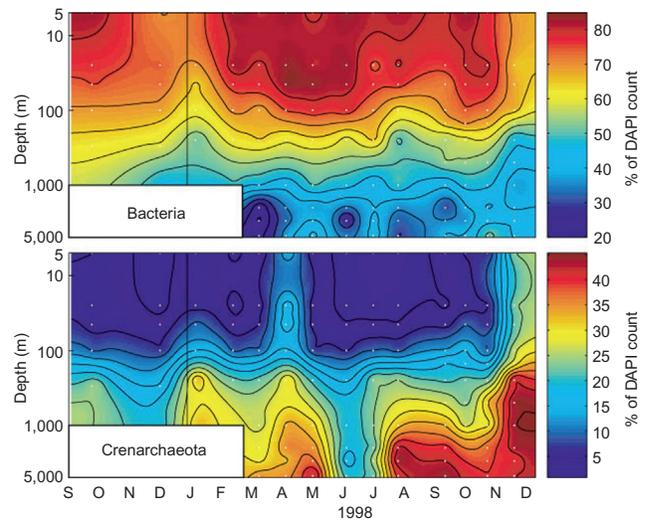


FIGURE 6.13 Marine Crenarchaea are abundant over space and time. This example shows the relative abundance of Bacteria (top) and Crenarchaeota (bottom) at Station ALOHA in the north Pacific off Hawai'i, through the water column over 15 months. Total cell counts were made by epifluorescence using the DAPI nucleic acid stain (see Chapter 13), and the relative proportion of each group was obtained using targeted probes with fluorescence *in situ* hybridization (FISH; see Chapter 13).

Thus, in a real aquatic food web, the heterotrophs (the bacterioplankton and zooplankton) consume each other, DOM, POM and autotrophs (phytoplankton, the main primary producers). The zooplankton in turn are consumed by larger organisms such as fish and other filter feeders. In the open ocean it takes approximately five steps or trophic levels to produce exploitable fish. In coastal zones it takes 1.5 to 3.5 steps to produce fish because primary production levels are higher. There is often a temporal lag between primary and secondary production; Figure 6.14 shows that an increase in phototrophs (as measured via chlorophyll *a*, a photosynthetic pigment) is followed by an increase in heterotrophs in a marine system.

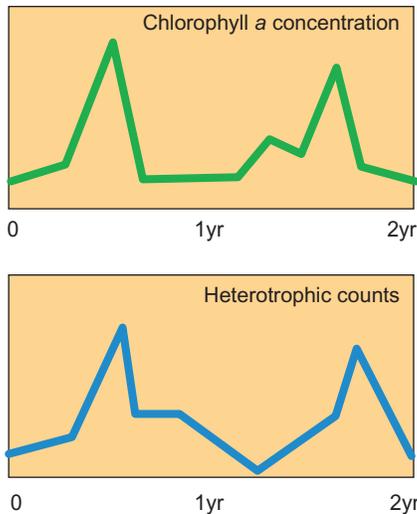


FIGURE 6.14 Diagram of the interrelationship between the concentration of chlorophyll *a*, a photosynthetic pigment and heterotroph density. The concentration of chlorophyll in water is related to the amount of primary production. This in turn influences the amount of secondary production by heterotrophic populations. In this figure, it can be seen that as the chlorophyll *a* concentration increases, it is closely followed by an increase in heterotrophic populations. Thus, secondary production is intimately tied to primary production. Adapted from Rheinheimer (1985).

6.3.2.1 Photoheterotrophy: A Newly Appreciated Microbial Lifestyle

Until recently, microbial life in aquatic systems was imagined to occupy the two main categories of (1) photosynthesizers, living in the surface water and forming the base of the food chain, and (2) the heterotrophs, living solely off that sunlight-fueled primary production. Increasingly, we know this was too simplistic a view. As described above, chemoautotrophy is likely an appreciable additional though poorly understood source of primary production in many aquatic systems. In addition, heterotrophs may not be as exclusively reliant on primary production as we have understood. They may be able to supplement their energy, but not carbon, requirements through the use of sunlight. This **photoheterotrophy** is not photosynthesis because it does not involve carbon fixation, and, since CO₂ is not being fixed, these organisms are still fundamentally “heterotrophs.” There are several different ways that heterotrophs can directly, or indirectly, use sunlight for energy (reviewed in Moran and Miller, 2007), and two of these ways are highlighted briefly here.

First, **aerobic anoxygenic phototrophy (AAnP)**, which uses bacteriochlorophyll to capture sunlight for energy without producing oxygen, appears to be widespread among diverse marine microbial lineages (Moran and Miller, 2007). In the freshwater and marine sites examined so far, the percentage of surface microbial cells carrying the genes for AAnP ranges from <1% to 10% of the

community (Figure 6.15A) (Yutin *et al.*, 2007). Second, a simple type of light-harvesting molecule related to the rhodopsins in our own retinas was discovered in marine microbes (reviewed in DeLong and Béjà, 2010). Since it was initially described in a Proteobacteria, it was termed **proteorhodopsin**, and was demonstrated in the lab to be a light-activated proton pump that generates proton gradients available for ATP production (Figure 6.15B). In addition, it is expressed in the environment, tuned to different wavelengths of light at different depths, and confers growth benefits in some cultured microbes. The gene encoding proteorhodopsin is present in a remarkable 13–80% of marine surface bacteria and archaeans (DeLong and Béjà, 2010). The current understanding for how both of these strategies works is as a way for heterotrophs to survive lean times, and be faster to respond when new food is again present. This has significant impacts on marine carbon cycling, energy budgets and ideas about the ecology and evolution of these communities. Yet, the prevalence of photoheterotrophy was unknown until very recently, and there is much about it that remains to be understood.

6.4 MARINE ENVIRONMENTS

Marine environments are one of the dominant groups of habitats on the planet. Oceans cover $\approx 70\%$ of Earth’s surface, such that a more accurate name for the planet might be Ocean. Beyond surface area, the ocean’s vast **volume** makes its importance as a habitat even greater. The ocean’s **average** depth is ≈ 4000 m, and its deepest spots are $\approx 11,000$ m; this ocean habitat is mostly dark (except for bioluminescence) and under high pressure (Figure 6.16 and Table 6.2). It encompasses a remarkable diversity of conditions and life forms, and we are only at the early stages of mapping these.

Marine microbiology has had a major role in propelling environmental microbiology forward (see Information Box 6.3), through the discovery of new physiologies (e.g., photoheterotrophy, Section 6.3.2.1), and the use of cutting-edge methods (e.g., metagenomics; see Chapter 21). These advances were led over the last several decades by a small number of individuals, and have resulted in a now large, robust community of marine microbiologists and microbial oceanographers. The number of global exploration efforts centered on marine microbiology are one indication of the success of this field; these include the International Census of Marine Microorganisms (ICoMM), which has profiled microbial communities from more than 500 global sites using the 16S rRNA gene (see Chapter 21 for how such profiling works); the Global Ocean Survey, which profiled roughly 150 communities using shotgun sequencing (thus capturing a random sampling of microbial genes rather than just the 16S rRNA marker gene; see Chapter 21 for details);

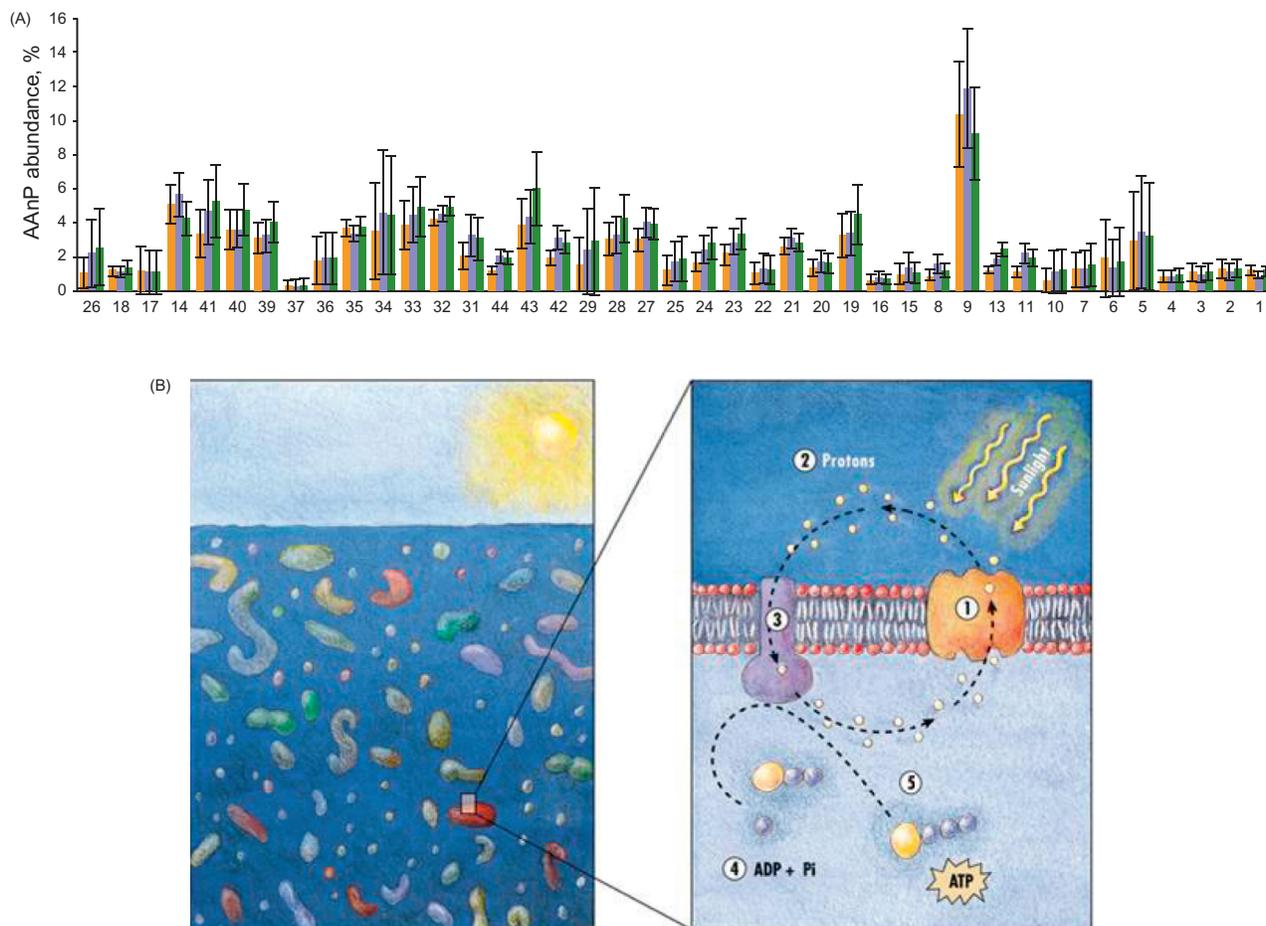


FIGURE 6.15 Phototrophy. (A) The percentage of bacterial cells calculated to contain genes involved in anaerobic anoxygenic phototrophy, in Atlantic and Pacific surface waters, using the metagenomic data from the Global Ocean Survey (numbers on the X-axis refer to sampling station numbers). From Yutin *et al.* (2007). (B) An artist’s rendition of microbial cells in the surface ocean, and how proteorhodopsin works in the cell membrane to capture light energy to pump protons, producing the proton gradient that ATPase can then harness to make ATP. From DeLong and Béjà (2010).

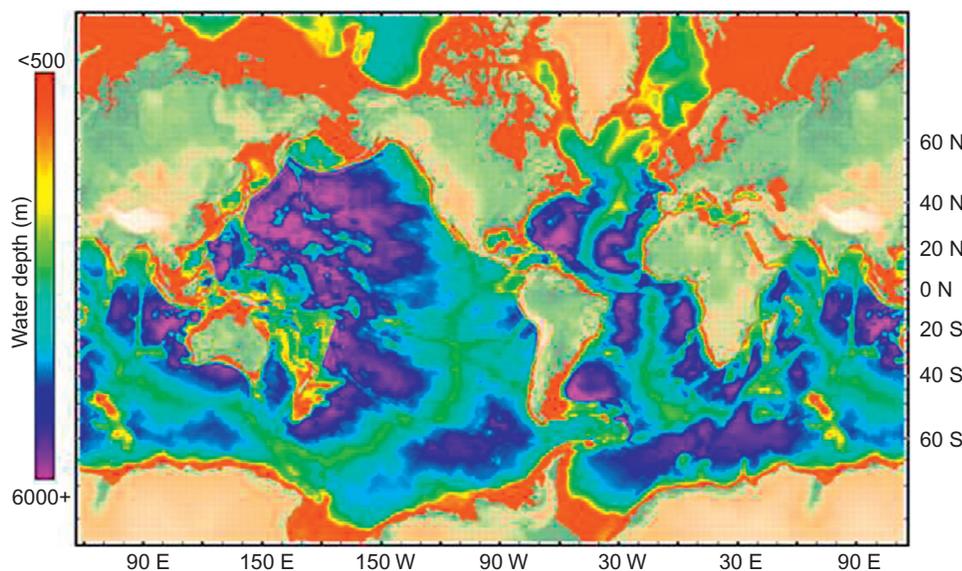


FIGURE 6.16 Global map of ocean water depth. The water depth scale is from less than 500 m (red) to 6,000+ m (purple). From Orcutt *et al.* (2011).

and the Tara Oceans survey, which is undertaking a trophically integrated virus-through-zooplankton survey across more than 150 global sites, using a variety of molecular and microscopic techniques. The extremely large volumes of data coming from such efforts continue to fuel discoveries and novel analyses of marine microorganisms' ecology and evolution that are not yet possible for microbes in most other habitats.

TABLE 6.2 Estimated Volumes of Ocean Habitats

Habitat	Vol. (m ³)
Water column (<200 m below sea level)	3.0×10^{16}
Water column (200+ m below sea level)	1.3×10^{18}
Hydrothermal plumes ^a	7.2×10^{13} (yr)
Subsurface ocean	10^{16}
Sediment, all	4.5×10^{17}
Shelf sediment	7.5×10^{16}
Slope sediment	2×10^{17}
Rise sediment	1.5×10^{17}
Abyssal sediment	2.5×10^{16}
0- to 10-cm layer	3.6×10^{13}
Ocean crust ^b	2.3×10^{18}

Adapted from *Orcutt et al. (2011)*.

^aThe volume of hydrothermal plumes is given as the volume of plume fluid produced per year.

^bThe volume of oceanic crust was assumed by multiplying the average thickness of the oceanic crust (7 km by the assumed area of seafloor underlain by crust (65% of Earth's surface, or 3.3×10^{14} m²).

6.4.1 Marine Planktonic Communities

The ocean contains diverse microbial habitats, both vertically (neuston to abyssopelagic depths) and horizontally (coastal upwelling regions versus open ocean gyres, the Mediterranean versus the Antarctic Ocean). As a general rule, microbial concentrations are highest within the neuston and drop markedly below this region (*Figure 6.17*); surface waters contain up to $\approx 10^8$ microorganisms/ml and decrease by more than 10-fold at a depth of 100 m. Coastal oceans support on average 10-fold higher microbial numbers than open oceans, due to terrestrial nutrient and carbon inputs; this is especially true in populated coastal areas (*Rheinheimer, 1985*). Oceans have profiles similar to those

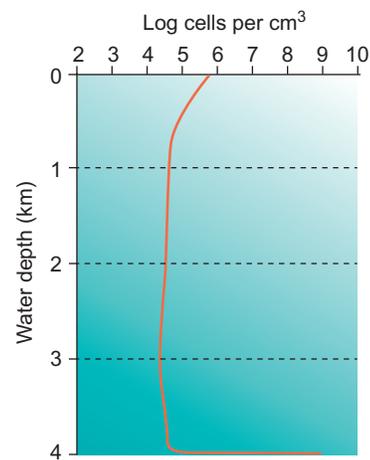


FIGURE 6.17 Cell density through the water column and surface sediments at station BIOTRANS in the north-east Atlantic. Note log scale. From *Jørgensen and Boetius (2007)*.

Information box 6.3 Marine Microbes as Drivers of Discovery: Doubling the World's Known Proteins by Sequencing the Sargasso Sea

The oceans harbor a staggering number of microorganisms that mediate biogeochemical cycles which affect the entire planet. In 2003, a team of scientists led by J. Craig Venter (a leader of sequencing the human genome) embarked on round-the-world yacht trip to sample surface marine microorganisms and sequence their genomes. This monumental effort, termed the Global Ocean Survey, undertook the use of metagenomic analysis (sequence analysis of all the DNA in a sample, see Chapter 21) to increase knowledge of ocean microorganisms (*Falkowski and de Vargas, 2004*; *Venter et al., 1994*). The nutrient-limited Sargasso Sea, a two-million square mile portion of the North Atlantic Ocean, was chosen for the journey's starting place on the assumption that its oligotrophic status would support a simpler community, more amenable to sequencing.

Sargasso Sea water samples were passed through filters (from 0.8 μ m down to 0.1 μ m) to capture microbial members of this

marine environment. Once microorganisms were harvested, the scientists extracted DNA from the filters. Shotgun sequencing, a high-throughput technique commonly used in the field of genomics, was then used to characterize the community DNA. Using this approach, the team generated truly staggering amounts of data over the next years of their journey. *Yooseph et al. (2007)* organized these data into protein clusters of similar sequences, as a means of analyzing it without relying on reference databases for matching and identification (an approach which remains more common as it is easier, but is highly biased by, and limited to, the composition of the reference database(s) used and typically only permits analysis of a subset of the data). Using this approach, *Yooseph et al.* discovered that the marine microbial protein diversity discovered by their team to date—which was just a portion of the final total—doubled the world's known diversity of protein types.

of lakes presented later in the chapter, depending on whether the marine environment is oligotrophic like the open ocean, or eutrophic like coastal waters, especially coastal waters where sewage outpours may be present. Due to numerous efforts such as the International Census of Marine Microorganisms (ICoMM), we now know fairly definitively that pelagic bacterial communities are dominated by surface Cyanobacteria (particularly *Prochlorococcus*, described below), Alphaproteobacteria (driven by SAR11, described below) and Gammaproteobacteria (Zinger *et al.*, 2011) (Figure 6.18A and B).

6.4.1.1 Making Half the Oxygen You Breathe: Marine Phytoplankton

Overall, marine phytoplankton (Figure 6.5) make half of the oxygen you breathe, and this contribution is in turn divided about 50:50 between the cyanobacteria (Figure 6.19) and the eukaryotic algae. Cyanobacteria, dominated by the genera *Prochlorococcus* and *Synechococcus*, account for a quarter of global primary production. As the “coccus” of their names implies, they are small, round cells that are morphologically relatively

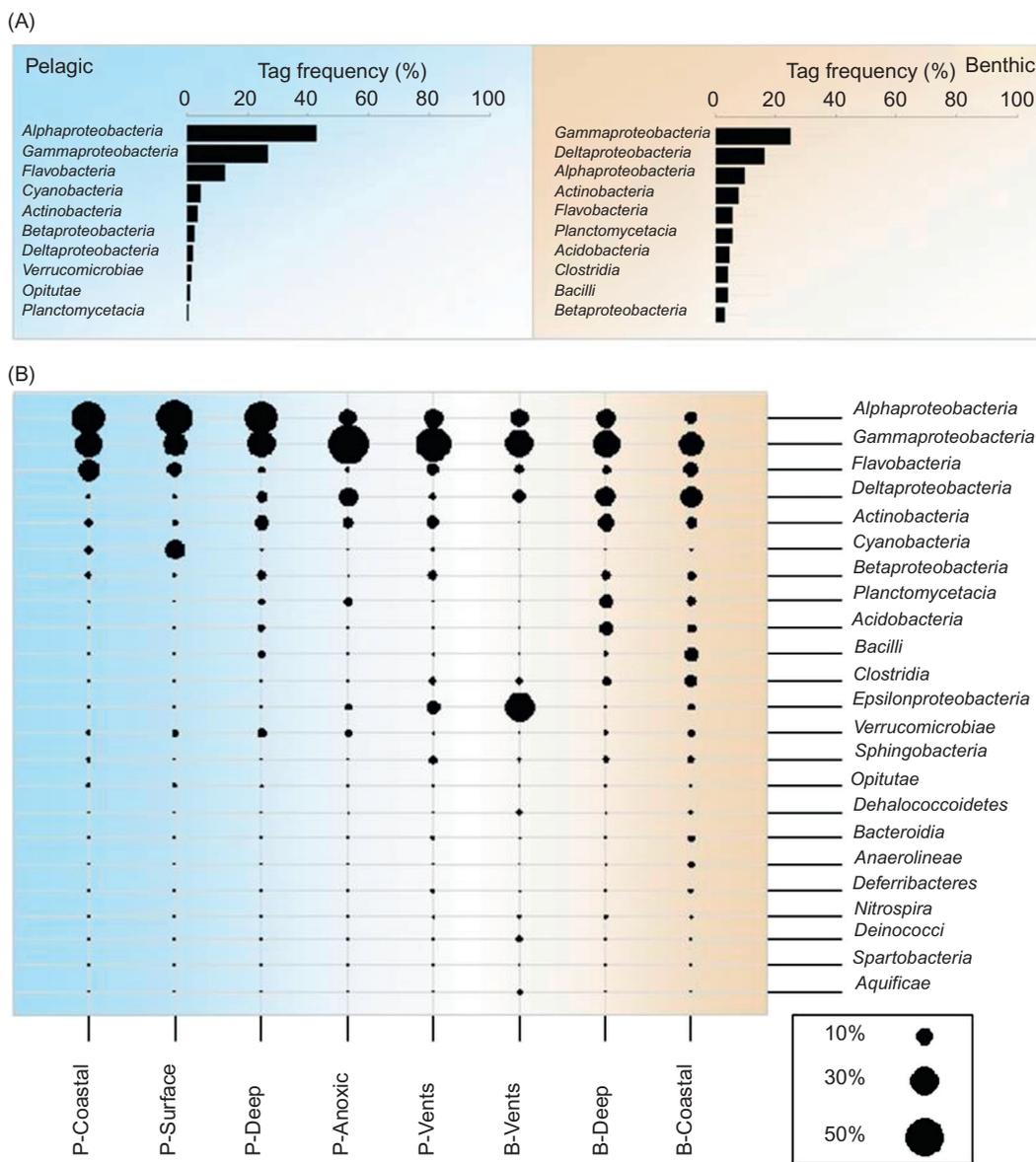


FIGURE 6.18 Bacterial community composition across 509 samples spanning the ocean’s pelagic and benthic habitats, from the International Census of Marine Microorganisms. This represents 9.6 million sequences of amplicons of the 16S rRNA gene V6 hypervariable region, sequenced by 454 pyrosequencing (see Chapter 21). (A) The top 10 bacterial classes in each habitat (not including hydrothermal vents and anoxic habitats in the benthic averages). (B) Average abundances of bacterial taxa in various pelagic (P) and benthic (B) ecosystem types. From Zinger *et al.* (2011).

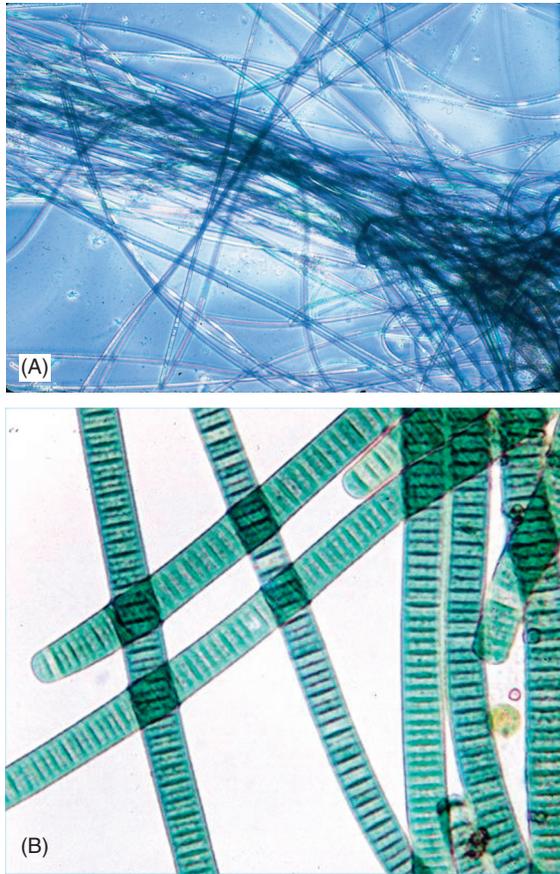


FIGURE 6.19 Example of cyanobacterial phytoplankton. (A) *Lyngbya* and (B) *Oscillatoria*.

nondescript. *Synechococcus* tend to dominate in coastal regions, while *Prochlorococcus* rule the vast oligotrophic surface waters in the center of oceans. In fact, *Prochlorococcus* are the numerically dominant photosynthetic cells on the planet—and yet were not discovered until 1988! These two genera have also been cultured in the lab, and been the focus of extensive physiological studies including the distributions and diversity. These cyanobacterial genera have played an important role in linking microbial diversity to physiology and ecology in both cultures and wild populations. Their studies lent great support to the microbial “ecotype” concept: microbial “species” are extremely difficult to define (see Section 19.2.3), but the rigorous diversity and ecological studies of *Prochlorococcus* clearly revealed related phylogenetic clusters that share defining ecological characteristics (for example, high-light adapted vs. low-light adapted) (Moore *et al.*, 1998). Other important cyanobacteria include lineages that fix nitrogen, including *Trichodesmium*, *Crocospaera* and *Anabaena*, with *Trichodesmium* believed to account for about half of global marine nitrogen fixation (Bergman *et al.*, 2013). As in all habitats, in the oceans nitrogen gas must be fixed into ammonia to be biologically available (see Chapter 16 for

details of this process and the nitrogen cycle). Although heterotrophic marine nitrogen fixers exist, cyanobacterial nitrogen fixers are currently considered to be the group performing the vast majority of marine nitrogen fixation.

Eukaryotic phytoplankton are responsible for roughly the other half of marine primary production, thus about a quarter of the primary production on the planet. They are also some of the singularly most beautiful microorganisms known (Figure 6.5). The remarkable morphologies many possess are created by hard outer coverings with intricate designs, the shapes of which can help keep them from sinking out of the photic zone. Eukaryotic phytoplankton are also responsible for much of the periodic bioluminescence seen in surface waters at night. Important groups include the diatoms, coccolithophores and dinoflagellates. Diatoms are responsible for about 20% of global photosynthetic primary production, and thus the majority of eukaryotic phytoplankton’s contribution. They are also involved in the cycling of silica in the oceans: 4–50% of the dry weight of their cells is made of silica, which is used in a two-valved extracellular skeleton called a frustule. Their cells may be unicellular or form long chains, depending on the species and conditions. Coccolithophores are a second major group of eukaryotic phytoplankton, whose name comes from the Greek word for “round stone-bearers” due to the calcium carbonate (CaCO_3 , i.e., chalk) plates that cover their surfaces. They are small but significant primary producers (on the order of $\approx 5\%$ of the global signal), and like many phytoplankton grow to high abundance known as “blooms” in places like the Sargasso Sea and the Gulf of Alaska. The settling of such blooms to old ocean floors millennia ago during the Cretaceous formed the famed White Cliffs of Dover, England. The final important group of eukaryotic phytoplankton is the dinoflagellates. This diverse group actually includes members unable to photosynthesize (and thus living heterotrophic lifestyles), as well as groups that live symbiotically with marine organisms such as corals. Much of the planktonic bioluminescence in the sea is due to dinoflagellates.

Phytoplankton abundance and community composition vary depending on the season and conditions. **Algal blooms** occur when waters are eutrophic, warm and calm, and certain algae or cyanobacteria proliferate rapidly resulting in blooms. Such blooms are a natural part of the yearly cycling of many lake and ocean ecosystems. However, extreme eutrophication and bloom events can adversely affect the water quality in several ways. As described above, high amounts of primary production settling through the water column can strip oxygen from the water during its decomposition. In addition, water experiencing algal blooms tends to be unpleasant for recreation (fishing, boating and swimming) because of odors and slime. In the worst cases, **harmful algal blooms** are composed of algae that

produce potent toxins, sometimes resulting in coastal **red tides**. These get their name from the most notorious sources, red-pigmented dinoflagellates. The red tides of Florida, known for massive fish-kills washing onto beaches and human respiratory irritation, are caused by the dinoflagellate *Karenia brevis* blooming throughout the Gulf of Mexico, and then accumulating along the Southeast U.S. coast. *K. brevis* is a highly toxic alga that causes human illness, shellfish toxicity, animal and bird mortalities, and reddish water discoloration. As global change conditions exacerbate eutrophication and bloom-supporting conditions (warmer, more stratified waters), harmful algal blooms and red tides are likely to become more frequent and intense.

6.4.1.2 Heterotrophic Marine Microbes

There are several ubiquitous, abundant marine heterotrophic microbes, analogs to the major photoautotrophs *Prochlorococcus* and *Synechococcus*, described above. By far the most important is the alphaproteobacterial **SAR11** clade typified by *Pelagibacter ubique*, whose name literally means ubiquitous pelagic bacteria. This clade was discovered in 1990 in 16S rRNA gene surveys and is seen throughout the world's oceans, from the tropics to the poles. Since it could not be grown, it bore the name SAR11, based on its first description as a sequence clade, from the 11th clone sequenced from a clone library made from Sargasso ("SAR") seawater. Finally, in 2002, this clade's "birth announcement" came after *Pelagibacter ubique* was successfully grown in the lab and its physiology began to be studied (Rappé *et al.*, 2002). Remarkably, **about a third of all bacterial cells in the ocean are from the SAR11 lineage**, which may be its own family in the Alphaproteobacteria. They are equally abundant in shallow and deep waters (Zinger *et al.*, 2011). Why is SAR11 so successful? It has a highly streamlined genome, which allows it to survive and reproduce even in very low-nutrient conditions. It also has diversified into a number of "ecotypes" specialized to different subhabitats, like *Prochlorococcus*. Lastly, its success may be partly due to its ability to supplement its energy needs (though not its carbon needs) by phototrophy, since at least some of the clade's members have the ability to perform photoheterotrophy (see Section 6.3.2.1) (Giovannoni *et al.*, 2005), which may allow them to get through lean times and channel more scarce resources into biomass rather than energy.

There are a number of other important heterotrophic groups in the oceans. They include microbes, such as the Deltaproteobacteria that often dominate deeper water, and Euryarchaeota. Euryarchaeota are less abundant overall than pelagic Crenarchaeota (which are highlighted in Section 6.3.1.1), but are ubiquitous, and appear to be

seasonally important members of surface communities. A recent population genome assembly of one (from a metagenome, see Chapter 21) suggests a photoheterotrophic lifestyle via proteorhodopsin (see Section 6.3.2.1) with their carbon substrates of choice being proteins and lipids (Iverson *et al.*, 2012). Other important marine pelagic heterotrophs include protozoa such as flagellates and viruses (see next section), as well as fungi. Heterotrophs not only consume DOM and POM, but can be active predators; it is estimated that protozoa and viruses are responsible for similar amounts of bacterial mortality (Wommack and Colwell, 2000).

Fungi in the marine habitat occur in endolithic associations with limestone, the shells of sea creatures, sponges and corals (Golubic *et al.*, 2005). They have also been isolated from carbon-rich areas of the water column and benthic habitat. The distribution of fungi in aquatic environments is not well studied; however, there is increasing interest in useful secondary metabolites that marine fungi may produce. Another surprising fact is that fungi have been isolated from sediment samples taken at depths of 5000 m in the Central Indian Basin (Damarem *et al.*, 2006).

6.4.1.3 Marine Viruses

Viruses are important to almost any ecosystem so far studied (see Section 2.4 for a general overview of viruses), and are the most abundant biological entities on the planet (10^{31} on Earth), commonly outnumbering bacteria about 10 to 1. If laid end to end, 10^{31} viruses would stretch 10^8 light years away from Earth, and comprise a biomass equivalent to ≈ 75 million blue whales. Such astronomical numbers have led to speculation that viruses represent the largest unexplored genetic reservoir on Earth.

Ocean viruses were thought nearly nonexistent until 1989, when seawater was concentrated onto electron microscopy grids, allowing their direct observation. Previous culture-based studies had used nonmarine microbial hosts, and thus had unsurprisingly failed to recover marine viruses. Now marine viruses are perhaps the best-studied environmental viruses, and are known to play diverse roles in marine ecosystems (reviewed in Breitbart, 2011). They impact microbes through mortality (cell lysis), horizontal gene transfer and the modulation of host metabolisms. Additionally, they alter global biogeochemistry. In fact, through lysis and the production of POM and DOM, they are responsible for the largest ocean carbon flux (150 Gt/yr) dwarfing all others by >five-fold. Viruses that infect phytoplankton are also important to nutrient cycling, because their lysis of phytoplankton's primary production promotes secondary production, as described in Section 6.3.2. Marine viruses also infect larger marine animals such as fish and crabs, and can

thereby cause considerable economic losses for the fishing industry, particularly in aquaculture settings with high host densities.

With new genomics-based windows (Chapter 21) into wild viral communities, the extent of viral impacts on large-scale biogeochemistry, beyond simple lysis, has been increasingly revealed. For example, viruses can contain “host” genes, which are expressed during infection to metabolically reprogram infected cells, in order to maximize viral production. The mechanisms by which this occurs can have profound biogeochemical impacts. The most surprising discovery of this is that cyanobacterial viruses carry “host” photosynthesis genes (reviewed in [Breitbart, 2011](#)). These genes are expressed during infection, allowing the infected cells to maintain photosynthesis (and thus energy production) longer, and are hypothesized to power the production of more progeny viruses. A significant portion of marine cyanobacterial photosynthesis in fact appears to be performed by phage-encoded photosynthesis proteins ([Sharon *et al.*, 2007](#)). Evolutionarily, these virally encoded photosynthesis genes are a dynamic gene pool, with the diversity generated among phage copies able to recombine back into the host gene pool ([Sullivan *et al.*, 2006](#)). This means that viruses appear to help shape global photosystem evolution! Such discoveries demonstrate the central ecological and evolutionary roles of viruses. However, in spite of advances in marine virology, environmental virology is bottlenecked by “unknowns.” Fundamental questions such as “who infects whom?” remain open; 90% of each viral genome and metagenome is typically new to science, and cultured model systems insufficiently represent wild diversity. This is an area open for many more discoveries.

6.4.2 Marine Benthic Communities

Benthic environments are habitats of steep redox gradients and abundant microniches, as described in [Section 6.2.3](#) along with the general characteristics of benthic aquatic microbes. The seafloor provides diverse habitats that can be grossly divided into soft-bottom sediments, hard-bottom rocky ocean crusts and hydrothermal vents. The latter is an extreme environment covered in detail in [Section 7.4.1](#). There are a number of other notable but less abundant benthic habitats whose microbial communities have been studied in some detail; these include cold seeps and, though the concept may be surprising, “whale-falls,” where the carcasses of whales fuel thriving successional ecosystems (see *Osedax* the “bone-eating” worm, [Figure 6.20](#)). Symbioses between microbes and macrobes are a hallmark of many of these systems.

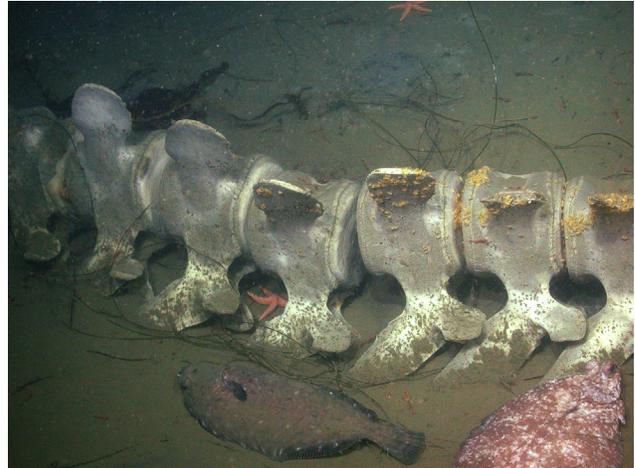


FIGURE 6.20 Two flatfish lurk near the vertebrae of a dead whale about 600 meters below the ocean surface in Monterey Canyon. Researchers at the Monterey Bay Aquarium Institute (MBARI) towed this whale carcass off a beach in Monterey Bay and placed it on the seafloor so that they could study the animals that fed on and colonized the carcass over time. The orange specks on the bones are amphipods, which consume the flesh of the recently sunk whale. MBARI researchers also discovered a new genus of worms (*Osedax*) that burrow into the whale bones after the flesh has been consumed. Instead of legs, these worms have “roots” which infiltrate the whale bone and contain unique endosymbiotic bacteria that can digest collagen within the whale bones and thus provide nutrition for the worms. © 2007 MBARI.

6.4.2.1 Marine Sediments

The seafloor is the final resting place for “marine snow” (sinking organic particles) and larger dead organisms, and this rain of organic matter from above can be an important food source for heterotrophic marine microorganisms. It can also add seasonality to the seafloor, where temperatures and other physicochemical conditions may remain more constant through the year. Rates of decomposition typically decline with depth, as does the amount of organic matter reaching the ocean floor. Concomitantly, the number of cells in the surface sediments typically declines with the seafloor depth.

From the global perspective enabled by ICoMM (one of the few broad efforts to survey diverse benthic habitats), Gammaproteobacteria comprise an average of $\approx 25\%$ of global benthic bacterial communities surveyed (their analyses so far have focused on bacteria), while Deltaproteobacteria are the next most abundant phylum at $\approx 16\%$ ([Zinger *et al.*, 2011](#)) ([Figure 6.18](#)). Overall, these groups, along with the next three most abundant phyla, the Planctomycetales, Actinobacteria and Acidobacteria, contain chemoautotrophs and anaerobic or microaerophilic heterotrophs, making them suited for benthic living. Coastal sediments are distinguished by lineages typically considered terrestrial or indicative of human contamination, the Clostridia and Bacilli ([Zinger *et al.*, 2011](#)). In deep-sea sediments, it appears that Acidobacteria in the

upper sediments give way to Chloroflexi and the candidate division JS1 in lower sediments (Zinger *et al.*, 2011).

Other research reveals that Archaea and Eukarya are important components of the soft benthos. Crenarchaeota and Euryarchaeota are abundant in sediments, and benthic diatoms, forams and radiolarians contribute to the benthic food web.

6.4.2.2 Rock-eaters Under the Sea

When we consider seafloor communities, we typically think of soft-bottomed habitats. However, much of the ocean's floor is made of basaltic crust, which extends several kilometers below the seafloor. Due to the resulting massive volume, basaltic crust is actually the largest potential habitat on Earth. But is it actually a habitat—can things live on rocks at, and below, the bottom of the sea? Discoveries just within the last decade suggest that chemolithoautotrophs—“autotrophs” meaning they fix their own carbon, “chemo” meaning they use chemical energy to do it and “lithos” meaning that the chemical energy comes from rock—are abundant, diverse and active in basaltic crusts. In fact, microbial cell numbers on seafloor basalt are typically 3–4 orders of magnitude higher than in the overlying waters (Santelli *et al.*, 2008), then decrease in the subseafloor. The physiologies of these abundant cells are just beginning to be investigated, and will likely be diverse since their communities appear more diverse than those in either deep or surface ocean waters (Santelli *et al.*, 2008). However, chemolithoautotrophy appears to be a major lifestyle, through various proposed mechanisms including iron, sulfur and manganese oxidation (Santelli *et al.*, 2008), and as supported by the presence of diagnostic gene sequences, isotopic investigations and incubation experiments (Lever *et al.*, 2013). Even before the last decade's discoveries in this habitat, subseafloor microbes had been estimated to comprise 10–30% of the total living biomass of Earth (Whitman *et al.*, 1998). Understanding planetary carbon and energy cycles is simply not possible without understanding these systems, and their continued exploration is certain to yield new discoveries for years to come.

6.5 FRESHWATER ENVIRONMENTS

Freshwater environments, such as springs, rivers and streams, and lakes, are those not directly influenced by marine waters. The science that focuses on the study of freshwater habitats is called **limnology**, and the study of freshwater microorganisms is **microlimnology**. There are two types of freshwater environments: running water, including springs, streams and rivers; and standing water, including lakes, ponds and bogs. These freshwater environments have very different physical and chemical

characteristics, and correspondingly different microbial communities and activities. For instance, the microbial community in a lake in Egypt is not the same as the microbial community in one of the Great Lakes in the northeastern United States. In this section we define various freshwater environments and outline the types of microorganisms that inhabit them.

6.5.1 Springs, Streams and Rivers

Springs form wherever subterranean water reaches Earth's surface. Microorganisms, especially bacteria and algae, are often the only inhabitants of springs. In general, photosynthetic bacteria and algae dominate spring environments, with communities ranging from 10^2 to 10^8 organisms/ml. These primary producers are present in the highest concentrations (10^6 to 10^9 organisms/ml) along the shallower edges of the spring and in association with rock surfaces, where light is available and inorganic nutrients are in highest concentrations (Rheinheimer, 1985; Kaplan and Newbold, 1993). Although heterotrophs are also present, numbers are usually low (10^1 to 10^6 organisms/ml) because DOM is low. As they mature and die, photosynthetic populations provide the initial source of organic matter for downstream heterotrophic populations. However, the largest portion of DOM found in surface freshwater originates from surrounding terrestrial sources. This organic input, which originates from sources such as plant exudates, dead plants, animals and microbial biomass, is transported into standing water habitats by mechanisms such as terrestrial runoff, seepage and wind deposition. Thus, we have the image of spring water starting at its source with very low concentrations of DOM and heterotrophs. The DOM and the heterotrophic populations steadily increase as the spring moves away from the source and as inputs of terrestrial organic matter and microbial biomass continue to accumulate (Kaplan and Newbold, 1993).

Springs, as they flow away from their subsurface source, merge with other water sources to form streams and rivers that eventually flow into other bodies of water such as lakes or seas. Streams contain primary producer communities, especially when light can penetrate to the bottom of the stream. Photosynthetic populations range from 10^0 to 10^8 organisms/ml and tend to be present as attached communities associated with biofilms because of the flowing nature of the water column. Phytoplankton (free-living) communities also exist in streams, but because of the constant water movement, they are not spatially stable populations (Rheinheimer, 1985).

As a stream progresses and becomes larger, it tends to accumulate DOM from surface runoff and sediments. The increase in DOM limits the penetration of light and consequently begins to limit photoautotrophic populations.

In turn, heterotrophic populations begin to increase in response to increased DOM. In general, the concentration of heterotrophs in streams and rivers ranges from 10^4 to 10^9 organisms/ml, with microbial numbers increasing as DOM increases. Because of their flow patterns, stream and river waters are for the most part well aerated, meaning that their microbial inhabitants are predominantly aerobic or facultatively aerobic. Although isolated pools that form in rivers act as DOM and POM sinks and support fairly stable heterotrophic planktonic communities, the only truly stable populations in the flowing habitats of streams and rivers are the biofilm and sediment (benthic) communities (Rheinheimer, 1985).

6.5.2 Lakes

Lakes are among the most complex of the freshwater environments. They may range from small ponds to vast lakes that generate their own weather patterns, such as Russia's Lake Baikal, which holds roughly one-fifth of the world's unfrozen surface freshwater, and is the deepest lake on the planet, and the U.S.A.'s Great Lakes. Although often regarded as nonflowing environments, lakes have inflows and outflows. Lakes may have unique chemical composition, and can form extreme environments (see Chapter 7); examples include salt lakes (see Section 6.6.2), bitter lakes that are rich in $MgSO_4$, borax lakes that are high in $Na_2B_4O_7$ and soda lakes that are high in $NaHCO_3$.

Lake microbial communities and their interactions are complex and diverse, reflecting the complexity of the habitat. Lakes contain extensive primary and secondary productive populations that interact dynamically. The primary productivity in the shallow near-shore waters is high, driven predominantly by algae and secondarily by cyanobacteria. The attached communities here are dominated by the presence of filamentous and epiphytic algae. Central lake waters are dominated by phytoplankton, which form distinct community gradients based upon the wavelength and the amount of light that penetrates to a given depth (Figure 6.21). One example of a lake-dwelling microbial phototroph with a specialized niche is *Chlorobium*, a green sulfur bacterium. *Chlorobium* can use longer wavelengths of light than many other phototrophs, meaning they can live deeper. They are also anaerobic organisms, using H_2S rather than H_2O for photosynthesis (see Section 16.4.3.2). Thus, they have a competitive advantage in establishing a niche at depths lower in the water column or even in the surfaces of sediments, where only small amounts of light penetrate, little or no oxygen is present, but hydrogen sulfide is available.

In addition to their phototrophic populations, lakes have extensive heterotrophic communities. Heterotrophic concentrations vary with depth, but there are three areas

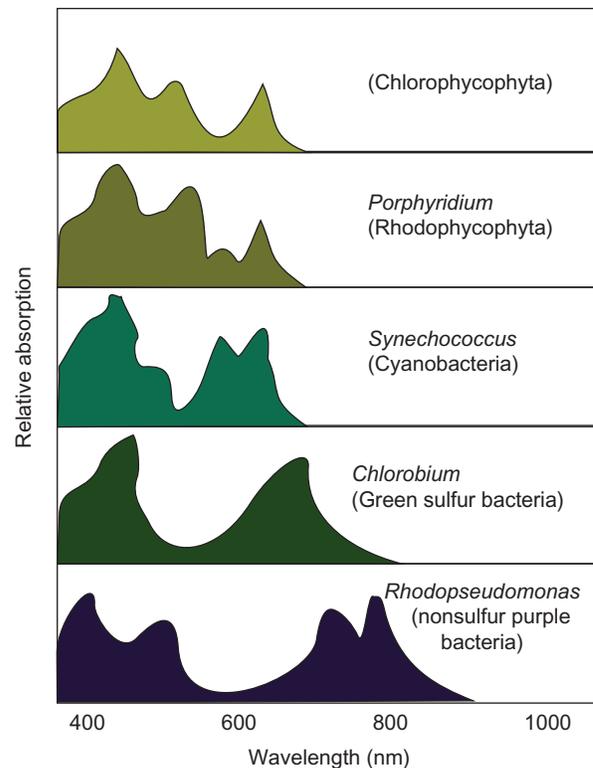


FIGURE 6.21 Graphs showing the light absorbance spectrum of common phytoplanktonic algae and photosynthetic bacteria. It can be seen that each of these groups has a different profile. This enables groups to take advantage of their niche. In general, organisms that are capable of utilizing longer wavelengths are found deeper in the water column. Thus, they do not have to compete with organisms higher in the water column that absorb the shorter wavelengths. Adapted from Atlas and Bartha (1993).

that generally have elevated numbers of heterotrophs (with some parallels to marine systems). These are areas with higher nutrients and organic matter: the neuston layer; the thermocline, where organic debris tends to settle and accumulate; and the upper layer of the benthos, where the heterotrophic populations are mainly anaerobic.

There are some striking differences between oligotrophic and eutrophic lakes; Figure 6.22A and B compare the major bacterial populations typically found in each (Konopka, 1993). Oligotrophic lakes have higher rates (four- to 20-fold) of primary production than eutrophic lakes, due to their deeper light penetration. In oligotrophic lakes, as might be expected, the amount of secondary production is directly coupled to primary production, and secondary production in the photic zone is generally 20 to 30% of primary production. Eutrophic lakes have much higher (\approx three to 80 times) rates of secondary production than oligotrophic lakes, and of a decoupling from primary production than oligotrophic lakes (Atlas and Bartha, 1993).

Apart from their microbial and algal populations, streams, rivers and lakes also contain fungi, protozoa and

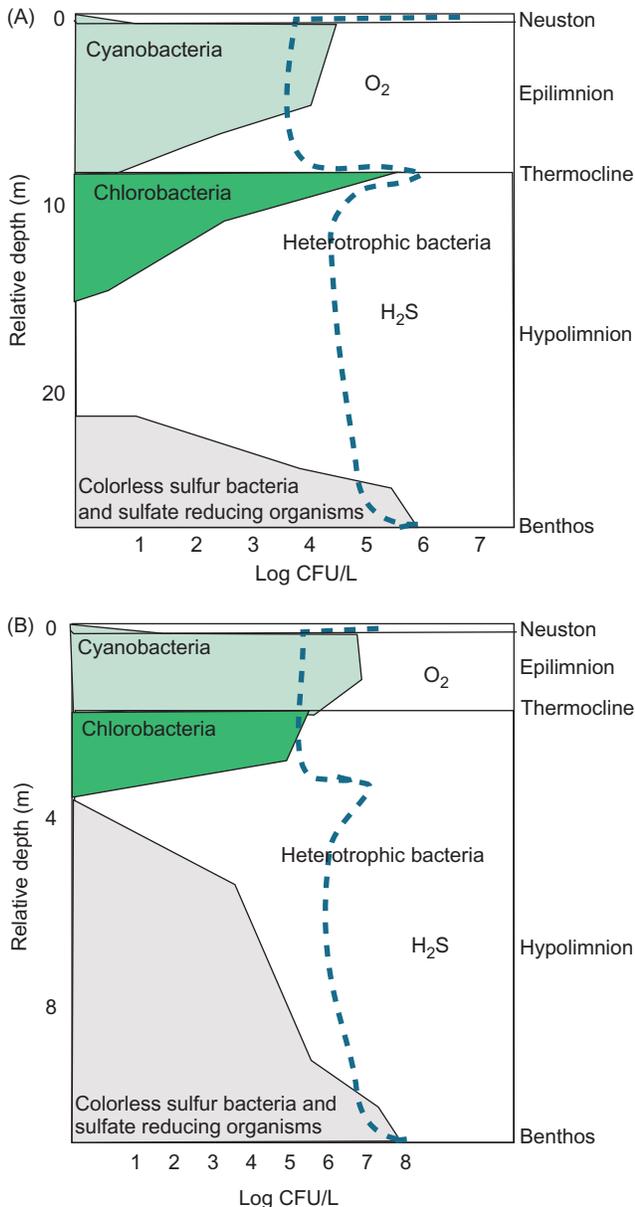


FIGURE 6.22 (A) Schematic representation of bacterial distribution in a typical oligotrophic lake. Notice especially the distribution and concentrations of the photosynthetic populations. Also note the lower concentration of heterotrophs in the upper zone, where cyanobacteria predominate. The large increase in the heterotrophic population between the epilimnion and the hypolimnion is related to the presence of a zone where organic matter accumulates. This area is known as a thermocline and is a zone where the sunlight-warmed surface water (less dense) and the deeper colder water (more dense) meet, forming a density gradient where organic matter accumulates. (B) Schematic representation of a typical eutrophic lake. This figure shows the same groups of organisms as in (A) indicating the localization and relative concentrations throughout the water column. Notice that both the photosynthetic and the heterotrophic populations are considerably higher in a eutrophic lake. Adapted from Rheinheimer (1985).

viruses, which interact and contribute to the functioning of the food web. Fungi serve as parasites of planktonic algae, preventing overpopulation and allowing light to penetrate farther into the water column. Other fungi have simple lifestyles, colonizing surfaces and often forming fungal lawns, whereas still others can have complex predatory lifestyles. A fascinating example is *Zoophagus insidians*, which live attached to filamentous green algae in rivers and lakes. The fungi have long hyphae which trail down into the water column forming fishing lines: when touched by prey such as rotifers, the hyphae rapidly secrete a sticky substance, ensnaring the microscopic animal. The hyphae then grow quickly into the mouth of the rotifer and form a fungal mycelium that absorbs the contents of the animal's body from the inside.

Protozoa and viruses are important predators of aquatic microorganisms. Protozoan populations are typically several orders of magnitude lower than bacterial numbers. They are able to affect the numbers of bacteria and algae because each protozoan is able to consume hundreds of bacteria and algae per day. Protozoa and their food species often exhibit the cyclic, temporally offset population dynamics typical of predator–prey relationships, where the prey is abundant first, then the predators' numbers rise and cull the prey, until food is limiting and predators decrease again. Viral and host populations fluctuate in a similar fashion (Wommack and Colwell, 2000). Viruses in freshwater environments can be very abundant, with viral numbers exceeding bacterial by two orders of magnitude (versus one in most marine habitats). Like marine viruses, freshwater viruses have diverse hosts, including microbes, microalgae, protozoa and larger organisms. As in marine systems, viruses can cause an appreciable amount of bacterial mortality (20 to 60%) (Suttle, 1994; Hennes and Simon, 1995), and the associated release of DOM fuels secondary production (Middledoe *et al.*, 1996), as discussed in Section 6.3.2. Together, protozoan and viral populations help to control the concentrations and biomass of the bacterial and algal communities (Wommack and Colwell, 2000).

6.6 OTHER NOTABLE AQUATIC ENVIRONMENTS

6.6.1 Brackish Waters

Brackish water is a broad term used to describe water whose salinity is between that of fresh and marine water, and these are often transitional areas where such waters mix. An estuary, which is the part of a river that meets the sea, is the best-known example of brackish water.

Estuaries are highly variable environments because the salinity can change drastically over a relatively short distance, ranging from 10‰ to 32‰ (Information Box 6.1), and over time of day due to tidal cycles (for example, high tide bringing saltier marine waters farther up into the estuary). Seasonal increases in freshwater due to rainfall or snowmelt will decrease the salinity at a given point in the estuary. In order to survive here, resident microbes must be adapted to these large fluctuations in salinity. Despite this challenge, estuaries are very productive environments. In general, estuarine primary production is low, due to poor sunlight penetration as a result of high turbidity, which occurs from suspended organic matter brought by river inflow and tidal mixing (Ducklow and Shiah, 1993). However, heterotrophic activity and secondary production are high. Primary and secondary production are decoupled in these systems, because of the large amounts of organic carbon brought by terrestrial runoff and river inflow. In fact, the supply of carbon and nutrients can be so great that in many cases estuaries can actually become anoxic for whole seasons during the year (Ducklow and Shiah, 1993).

6.6.2 Hypersaline Waters

Hypersaline environments include coastal lagoons, salt and soda lakes, salterns (human-made hypersaline ponds for producing salt; Figure 6.23A), deep-sea brine pools (formed from the dissolution of salt during seafloor tectonic activity), brine channels in sea ice, and fermented foods and pickling brines. Hypersaline environments have higher salinities than seawater ($\approx 35\%$) and may even be salt saturated. At room temperature, saturation of freshwater with sodium chloride results in $\approx 270\%$. The saltiest aquatic habitat on Earth may be the hypersaline lakes of McMurdo Dry Valleys in Antarctica, where the salinity can reach $\approx 440\%$ —see Section 7.1.1 for a detailed discussion of these lakes. Hypersaline environments are considered extreme because normal cell physiologies cannot withstand the strong salt concentrations: the salinity gradient from inside to outside the cell causes it to rapidly desiccate, losing its cellular water.

Halophiles, a type of extremophile (see Chapter 7 for other examples) adapted to salty habitats, have mechanisms for accumulating or producing nontoxic solutes inside their cells to be isosmotic with the external environment. Their proteins are also specially modified to prevent denaturation at high salt concentrations. For surface-sunlit hypersaline habitats such as salterns, high UV levels are also a challenge, and many of the microbes there have pigments to protect them, as well as efficient DNA protection and repair systems. The microbial pigments, often carotenoids and bacteriorhodopsins, typically lend shallow salt habitats a pink or orange color. While halophiles occur in

all three domains of life, there are comparatively few eukaryotic halophiles and an abundance of archaeal ones. One of the most notable halophiles is the ubiquitous genus *Haloquadratum* (Figure 6.23B), a euryarchaeon within the class Halobacteria (note the counterintuitive “-bacteria” ending despite being within the Archaea), which is shaped just like a flat, square salt crystal! Like other extreme environments, the harsh conditions of hypersaline waters result in lower microbial community diversity, since fewer lineages are able to survive in them (Figure 6.23C). Halophiles are of particular interest to astrobiologists (biologists who study life on Earth that may be similar to life on other planets, and search for that extraterrestrial life), since remnant water on Mars is likely to be highly salty, and also UV levels at the Martian surface are high.

6.6.3 Subterranean Waters

The groundwater environment is in the subsurface and includes shallow and deep aquifers. The characteristics and microbial communities of the groundwater environment have been discussed in Sections 4.2.1.3 and 4.6. Briefly, microorganisms are the sole inhabitants of these environments and bacteria and archaeans are the dominant types of microbe present. In general, levels of microbial activity are low, especially in intermediate and deep aquifers. As shown in Figure 4.18, activity is orders of magnitude lower in these aquifers than in other aquatic habitats, due to low nutrient levels. Many subsurface environments may even be considered extreme from a nutrient perspective (Chapter 7).

6.6.4 Wetlands

Wetlands are a habitat type where soils are seasonally or permanently saturated with water, which can be freshwater, brackish or marine. They contain distinct plant and microbial communities, and represent a diverse and important aquatic habitat. They were discussed in Section 4.2.1.4, and are simply highlighted here. Marine wetlands include mangrove swamps that provide “nursery” habitat for the larvae of many fish species. Brackish wetlands include estuarine marshes that can act as massive filtration systems to decrease total carbon and nutrient loading on coastal waters. Related to this, human-made wetlands are often created as part of wastewater treatment (described in Section 25.6). Freshwater wetlands are the largest natural source of the potent greenhouse gas methane (CH_4) to the atmosphere (Denman *et al.*, IPCC report, 2007). Freshwater wetlands also include peat bogs, which contain a vast amount ($>30\%$) of the planet’s stored soil carbon pool. This stored carbon may be in danger of release to the atmosphere under continued climate change (Denman *et al.*, 2007).

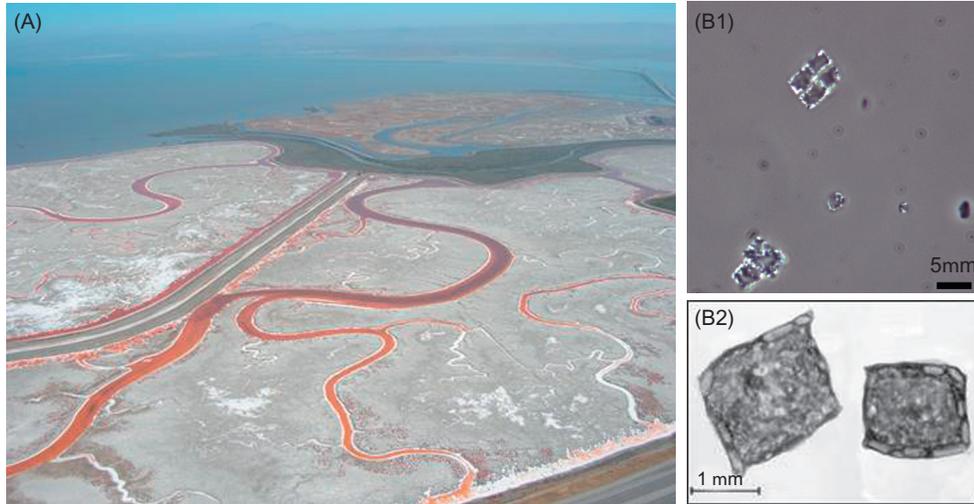
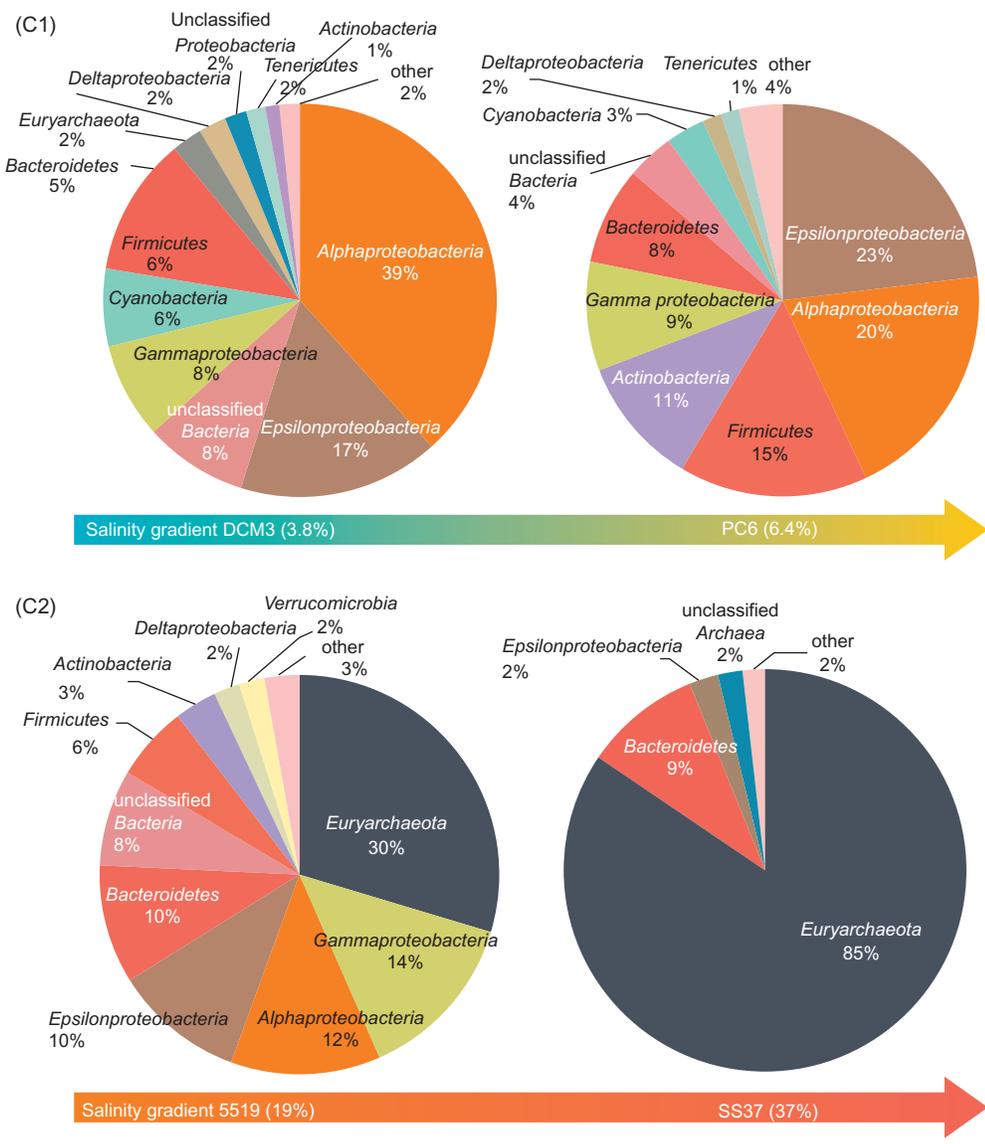


FIGURE 6.23 Hypersaline communities. Diverse hypersaline habitats exist including salt ponds, like those on the shores of California’s San Francisco Bay (A). (B) *Haloquadratum* is one of the most memorable halophiles for its box-like shape, reminiscent of a salt crystal. (C) Microbial community composition shifts and becomes less diverse as salinity increases. A study compared Mediterranean warm waters at 35‰ salinity, a hypersaline coastal lagoon in the Galapagos Islands at 6‰, and two Spanish salterns (salt ponds) at 190‰ and 370‰. The research team profiled the communities using metagenomic sequencing (Chapter 21), and these pie charts show the taxonomic breakdown of 16S rRNA gene sequences pulled from those metagenomic datasets (using sequences longer than 100 bp, identity >95% to the taxonomy indicated, total number of 16S rRNA reads per dataset ranging from 1408 to 3333). *Haloquadratum walsbyi* (shown in the inset of (B)), within the Euryarchaeota, was by far the dominant microbe in the two most saline sites, at 15% and 64% of the total reads, respectively. (B) © Mike Dyall-Smith. <http://www.nature.com/news/2004/041011/full/news041011-3.html> and http://web.aanet.com.au/~aanet/DGBHome/Research/PC_square4_7_opt_L.jpg. (C) From Ghai *et al.* (2011).



QUESTIONS AND PROBLEMS

1. What are the most “important” aquatic habitats by volume and/or by relevance to humans?
2. Describe large-scale and small-scale ways in which aquatic environments are not homogeneous.
3. What is meant by the term microbial loop? Roughly how much primary production is fueled by recycled nutrients?
4. What are biofilms and microbial mats? Where would you expect to find each? Why are they of particular interest to humans?
5. A newly trained environmental engineer is hired to investigate solutions to clogging of water distribution lines by a persistent, thick and gelatinous material. The astute engineer quickly recognizes that this recurring problem may be caused by microorganisms and rushes to isolate and characterize the microorganisms clogging the pipelines. The engineer is successful in culturing several microorganisms in broth cultures (i.e., flasks containing liquid microbiological media) from the material found in the pipelines. In these broth cultures, the engineer determines the amount of an antimicrobial compound necessary to kill these microorganisms. To be certain an adequate amount of this antimicrobial compound is delivered, the engineer adds twice as much as the broth culture-based tests suggested would be necessary. Much to the engineer’s surprise and dissatisfaction, the treatment is ineffective in killing the microorganisms found within the pipelines. Why did this treatment fail? What additional measures might the engineer need to take to solve the company’s problem with the clogged pipes? Can you devise any novel strategies based on material presented in this chapter?
6. What is photoheterotrophy? Give two specific examples of microbes that live this lifestyle.
7. What is aquatic chemoautotrophy, and why may it be important?
8. What is a thermocline?
9. Describe how marine environments differ from freshwater environments physically, chemically and microbially.
10. What roles do aquatic viruses play in ecosystems?
11. Your first job is as an environmental microbiologist is at a wastewater treatment plant, where you are in charge of the sludge bioreactors. Even before you read the later chapter on wastewater treatment, why do you care about aquatic viruses in your system?
12. You have been hired fresh out of college by a geoengineering consulting firm, Geoengineering Real Solutions. You are put on a team evaluating ocean fertilization with iron as a way to sequester carbon from the atmosphere. The idea is that iron is a

limiting micronutrient for a number of phytoplankton in the oligotrophic open oceans. Fertilizing large areas of ocean by dumping iron filings off of tanker ships should cause phytoplankton to bloom, fixing more CO₂ out of the atmosphere, resulting in more carbon getting buried in the deep sea due to sinking particles. How would you use your logic to evaluate whether this solution is likely to be successful, and what additional pieces of information might you need to help your team decide?

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