Definitions

Plankton: microbial communities suspended in the water column.

Phytoplankton: photoautotrophic plankton, made up of eukaryotic algae & prokaryotic cyanobacteria

Bacterioplankton: heterotrophic planktonic bacteria

Zooplankton: heterotrophic protozoans

Primary Producers: autotrophic organisms that use photosynthesis to fix atmospheric CO_2 into organic matter. Primary production is the major source of carbon & energy for the planktonic microbial community. The organic matter produced serves as food for heterotrophic plankton, and is eventually transferred down through the trophic levels of the marine food web.

Particulate Organic Matter **(POM)**: organic compounds produced by the phytoplankton, which are classified as **large** macromolecules (such as polymers), which make up the structural components of the cells (including the cellular membrane and cell walls).

Dissolved Organic Matter **(DOM)**: **smaller** compounds (such as amino acids, carbohydrates, organic acids, & nucleic acids) that are rapidly taken up by microbes & recycled.

Oligotrophic: nutrient-**poor** environment, such as a freshwater lake or the open ocean, both of which are large, deep, low-productivity environments

Eutrophic: nutrient-**rich** environment, which are usually smaller and shallower freshwater bodies

Grazing food chain: the portion of the food web which organisms are consumed by progressively larger organisms; for example: phytoplankton are eaten by zooplankton which are eaten by fish and other filter feeders. Overall the loop serves as an efficient means of DOM being utilized and released into the water column

Secondary Production: The production of bacterial biomass (as done in the microbial loop found within aquatic environments). Essentially secondary production utilizes the energy and organic matter produced by the photosynthetic primary producers, and transfers this energy & matter to progressively higher trophic levels.

What is the microbial loop, & what is its significance?

The term "microbial loop" refers to the closed-loop cycling of energy and material among phytoplankton, zooplankton, protozoans, and bacteria in aquatic (specifically pelagic) environments; and because this is a closed system energy and matter are not being lost to larger organisms. The pathway in which the energy and matter are being cycled between the microbes is depicted in Maier et al.'s (2000) Figure 6.1 shown here.

The significance of this loop lies in the ability of these microbes to work together to ultimately mineralize and breakdown organic material. It is important to note that it was not until recently that role bacteria played in this loop was found to include their predation by zooplankton, previously they had just been seen as decomposers of the dissolved organic matter (DOM). The discovery of these Bactivorous zooplankton were what enabled this cycling process to be seen as a closed loop system. While the significance of this loop may lie in the utilization of the DOM which occurs within this loop, the microbes present in this loop are often involved in larger marine processes as they are ultimately members of the much larger marine food web, as shown by Barber's (2007) depiction of the microbial loop's role in the larger marine food web.

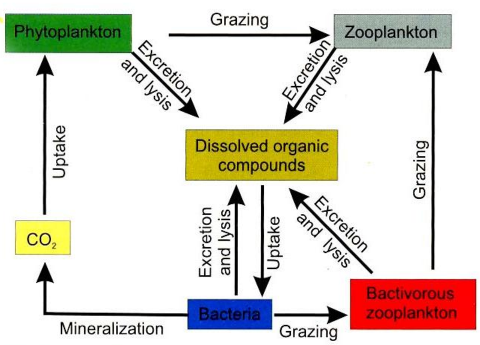
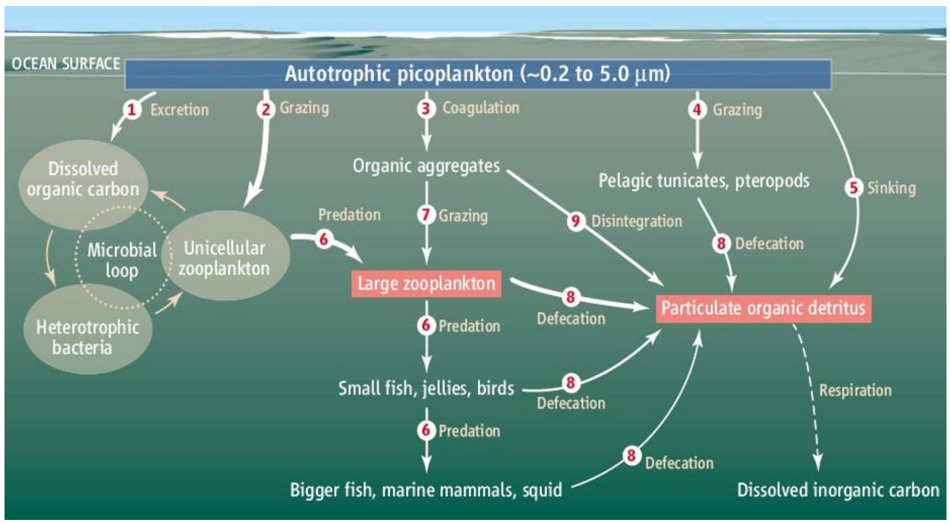


FIGURE 6.1 The microbial loop in the planktonic food web. The microbial loop represents a pathway in which the dissolved organic products are efficiently utilized. The role of bacterioplankton is to mineralize important nutrients contained within organic compounds and to convert a portion of the dissolved carbon into biomass. Grazing by bactivorous protozoans provides a link to higher trophic levels. (Modifed from Fuhrman, 1992.)



What are some of the characteristics of the benthic zone or habitat?

- Only 3% of what is produced ever reaches the benthic zone
- The benthos is a transition zone between the water column and the mineral subsurface.
- The interface collects the organic material that settles from the water and is a diffuse and non-compacted mixture of organic matter, mineral particulate matter and water.
    - This zone is characterized by a dramatic increase in the concentration of microorganisms (as much as five orders of magnitude) compared with the planktonic environment.
    - As this implies all interface environments are regions of greatly enhanced microbial activity whether they are boundaries between oxic and anoxic waters or differences between warm and cold frontal boundaries.
- Below the interface zone, microbial numbers generally decline for a variety of reasons – lack of substrate, no electron acceptor
    - Benthic microbial concentrations depend on the availability of oxygen & organic materials
- Benthic environment can support and often favors the formation of conjoined aerobic & anaerobic microenvironments.
    - Benthic habitat is an important feature of the aquatic environment.
    - The cycling of essential nutrients, such as such as C, N, & S, in this area is a major factor in geochemical recycling

What is the significance of interface environments? Give specific examples

- Interfaces, the boundary between two different environmental conditions, promote enhanced & varied microbial niches
  - For example the sulfur oxidizing bacteria, whether photosynthetic or chemoautotrophic need a supply of reduced sulfur which is only found under anoxic conditions.
  - Chemoautotrophic also need oxygen as a terminal electron acceptor and as such must exist at the interface between these two compounds that do not co-exist other than at the oxic-anoxic interface, as seen in the figure below.
- In a similar fashion, methane oxidation occurs at the sediment water interface as methane is only formed anoxically but must be metabolized using oxygen as the terminal electron acceptor.

What are microbial mats? Ecologically what is the significance of their vertical stratification?

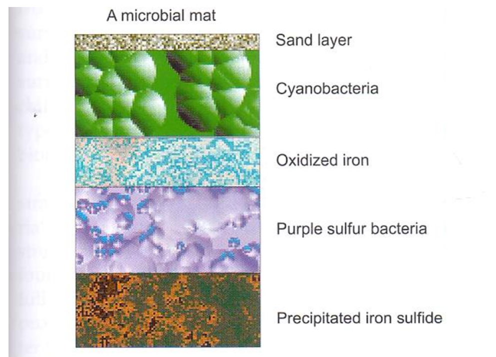
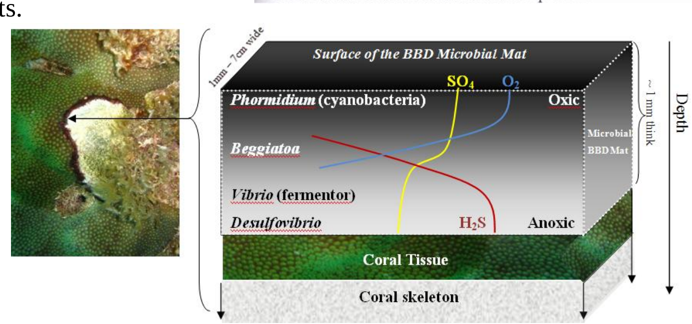


FIGURE 6.4 Schematic drawing of a microbial mat. Cyanobacteria form the surface layer of the microbial mat but may be covered by a layer of sediment, organic debris, or even cyanobacterial sheaths containing a pigment that acts as a sunscreen, blocking excessive ultraviolet radiation. Often a layer of oxidized iron appears below the cyanobacteria, followed by a layer of purple sulfur bacteria that thrive under anaerobic conditions. An extensive zone of sediment enriched in iron sulfide is often present.

- Microbial mats are an extreme example of an interfacial aquatic habitat in which many microbial groups are laterally compressed into a thin mat of biological activity.
- These microbial groups interact with each other in close spatial & temporal physiological couplings.
  - The thickness of mats ranges from several mm to a cm in depth.
  - They are also vertically stratified into distinct layers.
- Microbial mats have been found in the surface-planktonic interface of hotsprings, deep-sea vents, hypersaline lakes, & marine estuaries
- By supporting most of the major biogeochemical cycles, the mats are considered largely self-sufficient.
- Their vertical stratification is important because the mat can operate in both oxic and anoxic, as well as photic and non-photic environments.
- The communities within each zone of the mat's stratified layers has its own unique role & function



What is a stromatolite?

- Fossilized mats known as **stromatolites**, date back to 3.5 (??) billion years and are largely believed to be the primary producers on the primitive Earth
  - believed to be some of the first organisms on earth, probably formed with anoxygenic phototrophic bacteria (purple & green sulfur bacteria) due to the lack of oxygen in the atmosphere at that time
  - Stromatolites grow like corals (only the top layer is alive & growing)
  - Mats are made of mixed microbial populations
  - Mats are covered by a slime layer for protection (like corals)

What is a biofilm?

- Biofilms are not microbial mats, but rather a single layer of organic matter and microorganisms formed by the attachment and proliferation of bacteria on the surface of an object.
  - Bacteria like to be attached, particularly in Oligotrophic environments because food comes to them, and when bacteria are in a starved state, motion is the first ability to go as it requires too much energy
- The properties of a solid surface may be modified by the presence of an adherent conditioning film of organic compounds which it turn fosters the attachment of bacteria as they serve as a source of nutrients especially in oligotrophic environments.
- The initial reversible attachment of organisms to surfaces can, with time, become permanent.
  - Irreversible attachment is initiated by the excretion of extracellular polymers by the reversibly attached bacteria.
  - The extracellular polymers create a matrix that chemical bridge to the solid surface.
- Biofilms also protect the adherent community from such toxicants, which is a real problem in drinking water distribution sys.
- In some instances, biofilm development can be detrimental and there is a need to control the growth of biofilm cells using antibacterial substances. (ex. cooling towers, pipelines, heat exchangers)
- Compared with planktonic cells, biofilm formations are more resistant to antibacterial substances.
- Biofilms can harbor opportunistic pathogens and require high doses of disinfectant for their control.

Bacteria are usually found attached to solid surfaces. What benefit results from attachment?

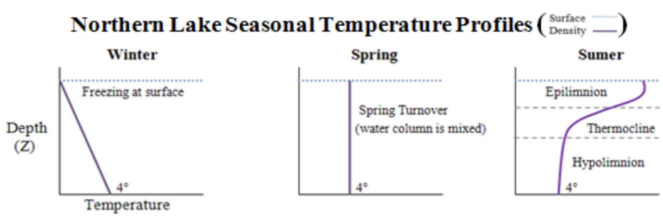
- The positive effect of attachment reported for some bacteria may be the result of indirect factors such as an increase in the local concentration of nutrients or the protective effects of the biofilm exopolymeric matrix.
  - Attachment and biofilm formation as a survival strategy are well illustrated by the distribution of bacteria in the nutrient-poor environment of high alpine streams (why?).
  - Similar situation in trickling filters.

What is the difference between lotic and lentic environments?

- The study of freshwater habitats is called **limnology** and there are two types of freshwater environments.
  - **Lotic**: **running water** habitats, including springs, streams, and rivers.
  - **Lentic** : **standing water** habitats, such as lakes, ponds and bogs.

Describe the temperature profile in lakes, what chemical properties are associated with these changes?

The only difference between the temperature profile in the ocean and in lakes is that the ocean doesn't change dramatically with season but rather always looks like the lake summer profile. The **thermocline** is the layer of water in a stratified water column in which the temperature changes substantially with depth. As shown in this figure, this layer is found between the warm surface (**epilimnion**) layer and the cooler subsurface (**hypolimnion**) layer. The epilimnion has high O2 levels, but the high primary productivity found here can deplete the nutrients, whereas the hypolimnion has low O2 & high mineral content. Significant microbial activity is found at the interfaces between the temporal layers.



Define the various zones within a lake

- **Neuston Layer:**the air-water interface which includes the uppermost mm of the water column. Nutrients are known to accumulate here. This zone has 4 zones within it.
- **Littoral Zone:** the edge of the lake where sunlight can penetrate all the way to the substrate. The **planktonic** community is composed predominantly of **algae** and secondarily of **cyanobacteria** making **primary productivity very high** in this zone.
- **Limnetic Zone:** the surface layer of open water, away from the littoral zone, where light easily penetrates. It is also dominated by phytoplankton, which force distinct community gradients based upon the wavelength and the amount of light that penetrates to a given depth.
- **Profundal Zone:** found below the littoral zone, here light penetration is less than 1% (called the "light compensation zone")
- **Benthic Zone:** lake bottom & its associated sediments

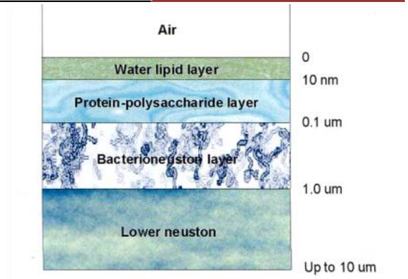
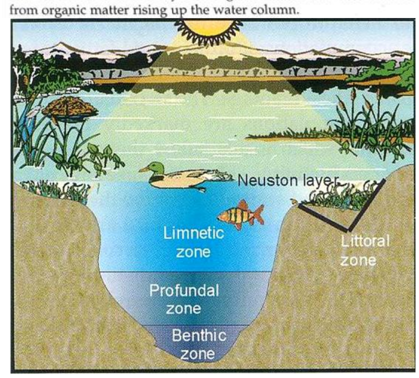


FIGURE 6.11 Schematic representation of the neuston. This is the upper layer of aquatic environments and can range from 1 to 10 μm in depth. Most scientists consider the neuston an extreme environment (see Section 6.4.1) because of many factors, including intense solar radiation, large temperature fluctuations, and the natural accumulation of toxic substances including chemicals, organic matter, and heavy metals. 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.



Where would you expect to find most algae & bacteria in a lake?

- The most algae & bacteria (cyanobacteria) would be found in the littoral zone where primary productivity is the highest

What are the differences between Oligotrophic & Eutrophic lakes?

- When compared to Oligotrophic Lakes, Eutrophic lakes have…
  - a much thinner Epilimnion layer,
  - A much thicker layer of sulfur bacteria
  - & their anaerobic bacteria (chlorobacteria) are found above the thermocline
- In terms of primary productivity, oligotrophic lakes have higher rates (20 to 120 mg carbon/m3/day) than eutrophic lakes (1 to 30 mg carbon/m3/day) because eutrophic lakes have much higher levels of organic matter which causes turbidity and interferes with light penetration.
  - *Note:* the open ocean won't get the high carbon rates found in Oligotrophic lakes in a year
- As might be expected, in terms of secondary productivity, eutrophic lakes have much higher rates (190 to 220 mg carbon/m3/day) than oligotrophic lakes (1 to 80 mg carbon/m3/day).

What are red tides?

- An immense algal bloom that is compoed of red-pigmented dinoflagellates which produce toxins that can affect marine birds, marine mammals, & humans who consume fish which have bioaccumulated the algal toxin.

Explain the events depicted in Figure 6.14

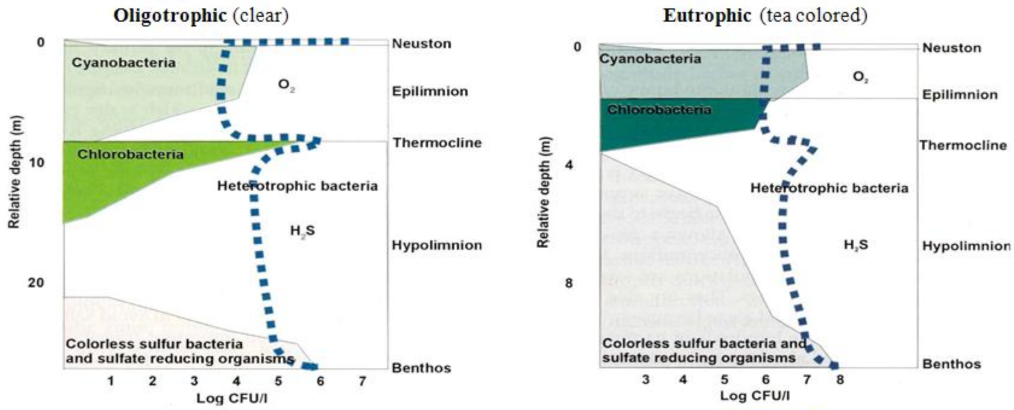


FIGURE 6.14 (A) Schematic representation of bacterial distribution in a typical oligotrophic lake. Notice especially the distribution and concentrations of the photosynthetic populations. Also note the lower concentration of heterotrophs in the upper zone, where cyanobacteria predominate. The large increase 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. The 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.)

- Both of the lakes (A. Oligotrophic & B. Eutrophic) are stratified (O_2 vs H_2S)
- Chlorobacteria: photosynthetic bacteria whose photopigments operate at a longer wavelength (IR) then the cyanobacteria
  - They don't need O_2 for photosynthesis
- Sulfur oxidizing bacteria aren't nearly as common in Freshwater because sulfur isn't as common here as it is in the Oceans
- Eutrophic lakes have much more organic material → if enough material accumulates it will become a bog
- Oligotrophic bottom sediments have 1000x more organisms than found in the overlying waters, and Eutrophic bottom sediments have 100-1000x more organisms than Oligotrophic sediments

What is an estuary?

- An estuary, which is the part of a river (fresh H_2O) that meets the sea (salt H_2O), is the best known example of brackish water

What are some characteristics of estuaries?

- Estuaries are highly variable environments because the salinity can change drastically over a relatively short distance.
- Typically the salinity (define salinity) can range from 10%o to 32%o and organisms must adapt to survive these fluctuations.
- Estuaries are extremely productive and serve as nurseries for many aquatic animals and birds, eg; Mangroves.
- In general, estuarine primary production (10 to 45 mg carbon/m3/day) is not always enough to support the secondary populations because turbidity limits planktonic productivity.
  - However, the extensive plant communities provide huge quantities of organic matter resulting in a secondary production rate ranging from 150 to 230 mg carbon/m3/day.
- The supply of nutrients can be so great that estuaries can actually become anoxic for whole seasons during the year.
- Estuaries are governed by 3 factors (Wind, Tide, & Rivers), which will determine how much freshwater there will be
- Estuarine organisms must be specially evolved to cope with changes in the osmotic pressure (due to changes in salinity)

In the thermal vent zones, what is the difference between chemoautotrophic carbon production & organic thermogenesis?

There is currently a vigorous debate about the actual source of primary organic carbon, & the process for its introduction into thermal vent ecosystems. There are 3 main explanations, each of which is based/driven more by theory than scientific evidence.

1) **Chemoautotrophic Carbon Production** theory (currently considered to be the most credible).

- Chemoautotrophs utilize CO_2 as a major and usually exclusive source of carbon.
- Primary organic carbon production in hydrothermal vent communities is based on bacterial use of H_2S, H_2 and CH_4 as electron donors, and CO_2 as a carbon source.
- Thus, according to this hypothesis, the entire food web in a hydrothermal vent community is based on chemoautotrophy, not photoautotrophy as in surface environments
  - This isn't quite correct because they need O_2 to fix methane (CH_4) & the O_2 comes from photosynthesis

2) **Organic Thermogenesis** theory

- an **abiotic** process, which is based on the observation that organic-free-free rocks initially collected near hydrothermal vents, were able to synthesize sugars & amino acids from paraformaldehyde & urea at high temperatures in the presence of carbonates.
- This idea led to the theory that the production of carbon in hydrothermal vent communities is the result of the combination of physicochemical factors found in the vent environment. These factors include: concentrated energy in the form of heat, lots of ionizing radiation present, a constant supply of all the required precursors (CO_2, N_2, NH_3, H_2, CH_4), and the advective flow around vents that provides rapid cooling & the subsequent quenching of thermogenic reactions necessary for organic synthesis reactions.
- Once the thermogenic process has converted the CO_2 to simple sugars, chemoheterotrophs can utilize the carbon to build biomass.
- Thus thermogenesis results in the primary production by heterotrophic bacteria.

3) **Advective (mixed) Plume Hypothesis**

- Primary production in hydrothermal vents is based on photoautotrophy
- The theory is based on the assumption that settling of organic carbon from near the ocean surface occurs.
- Thus the Carbon that settles from near-surface phototrophic layers, is concentrated around hydrothermal vents by advection (uprising in the wafer column due to heat), which essentially draws water & DOM in from relatively great distances

Ch. 6: Aquatic & Extreme Environments (Questions & Problems)

1) **Define what is meant by an extreme environment.** (Text book question # 7)

An "extreme environment" is an environment that fits either one or both of the following two definitions: 1) the environmental conditions of the environment (such as temperature, salinity, pH, nutrients, pressure, depth, light, etc) are either excessively high or low relative to the scale in which the conditions occur; or 2) the conditions within the environment are such that they substantially limit the diversity level of the surrounding microbes.

Extreme environments which fall under one or both of these definitions include:

- the neuston (i.g. the air-water interface): in which there is a high degree of solar radiation, accumulated toxins, diverse and intense levels of predation (coming from both the air above and water below), and constant fluctuations in the temperature, salinity, and pH
- Thermal extremes: either very hot or very cold environments
- High Salinity
- Acidic (low pH)
- High Pressure (usually accompanied by low temperatures)
- Nutrient poor or nutrient absent

2) **Describe microbial adaptations to life at high temperature.** (Text book question # 8)

Microbial adaptations to high temperature (>70°C) environments include:

- thermotolerant DNA polymerase
- large number of cations that bridge the charges between the residues of the amino acids present (aka. "salt bridges") which allow the protein to remain folded despite the high temperatures
- large concentration of saturated fatty acids in their membranes which promote stability
- unique DNA binding proteins which enable the DNA to better resist melting by arranging the DNA into special globular particles
- a unique DNA gyrase which causes positive supercoils in the DNA which enhance the stability of the DNA to the increased heat
- as well as other adaptations, which are generally the inverse of the microbial adaptations to low temperature & high pressure environments

3) **Describe microbial adaptations to life at high pressure.** (Text book question # 10)

Microbial adaptations to high pressure (> 1000 bars) environments include:

- psychrophilic (grow better in colder environments)
- can grow in the absence of light (such as the high pressure, cold, sea floor)
- large concentrations of long-chain polyunsaturated fatty acids present in their membranes, which enable the membrane to remain in a stable fluid state despite the surrounding high pressure and cold environment (ordinarily these environmental conditions would cause the membrane to gel or crystallize)
- internal control of salt concentrations
- as well as other adaptations, which are generally the inverse of the microbial adaptations to high temperatures

What are the 2 reasons why introduced bacterial species do not colonize soils?

There are several reasons microbes die shortly after being introduced into a new location.

1) the microbes that have been introduced to a site are often outcompeted for the local resources by the endemic microbes which are better suited for that particular environment.

2) the microbes may die or enter a weakened state due to environmental stressors encountered during their transport.

3) the act of inoculating the microbes into the new environment may either directly damage the microbes, or the inoculum may be damaged by the surrounding environment.

One method which has been shown to improve the transport potential of the introduced microbes is to immobilize the microbial cells in a carrier material. This carrier material would protect the microbes during the inoculation process, and can offer a slower, more gradual release into the soil reducing the shock to the introduced microbes. Biodegradable carrier materials have been found to be especially successful in preventing the cellular introduction mortality, as the medium serves as a nutrient source for the alien microbes; which in turn, decreases their immediate need to compete with the endemic microbes for the local resources and allows the introduced microbes more time to adapt to their new surroundings.

List several factors that affect microbial transport through soils

microbial transport through composite systems (i.e. soils, vadose zone materials, etc.) are governed by a variety of biotic & abiotic factors, which can be grouped simplistically into 2 categories:

1) **Hydrogeological** factors: pertains to abiotic factors such as soil characteristics & water flow
   - **Filtration** effects
   - **adhesion** processes
   - **Porous medium** characteristics
   - **Water flow** rate

2) **Microbial** factors: focuses primarily on the characteristics influencing microbial survival &/or potential for activity
   - **Physiological state** of the cell
   - **Intrinsic mobility** of the cell
   - **Predation**

ultimately it is the extensive interaction between the factors within these 2 groups that determines the extent of the microbial transport

What factors affect microbial adhesion? [cell surface charge & hydrophobicicty]

- The key cell surface factors influencing adhesion are the cell surface charge and hydrophobicity, as well as composition of the lipopolysaccharide layer and the presence of specific proteins in cell surfaces, appendages, or extracellular polymers can also play a role.

How does pH affect transport of bacteria? Viruses?

- pH doesn't seem to affect bacteria, however viruses are strongly influenced by the pH because of their capsid coating
- The pH of the matrix solution within a porous medium does not seem to have a large effect on bacterial transport.
  - However, viral transport can vary greatly depending on the pH of the porous medium
- Remember that the primary interaction limiting bacterial transport is filtration, not adsorption as it is for viruses.
- Further, overall charges on the surfaces of bacteria and viruses differ and will be affected differently by pH changes.
- This can be expressed in terms of the isoelectric point (pI).
  - The pI is the pH at which the net charge on a particle of interest = 0
  - For bacteria, the pI usually ranges from 2.5 to 3.5, so the majority of cells are negatively charged at neutral pH.
- However, viruses display a wider range of isoelectric points (see Table 7.1) making their net surface charge much more dependent on changes in pH.

How does ionic strength affect transport?

- The repulsive forces between bacteria and viruses and solid surfaces are reduced by the presence of cations in the soil solution, the concentration of **anions** (**negatively** charged) and **cations** (**positively** charged) in solution is referred to as the ionic strength of the medium.

How might cellular appendages affect movement through soil?

- Bacteria may have a variety of appendages such as **pill**, **flagella**, or **fimbriae**.
- Flagella are responsible for bacterial motility while fimbriae and pill are involved in attachment.
- The influence of bacterial motility on overall transport is generally minimal because extensive continuous water films would be needed to support microbial movement and distances covered would be minimal relative to advection.
- The primary mechanism of transport is water flow, commonly referred to as **advective transport**, which is many orders of magnitude greater than transport due to motility.
- Movement caused by flagella is usually a result of **chemotaxis**.
- Chemotaxtis is the movement of microbes toward beneficial substances or away from inhibitory substances but only **over short distances** and in response to the **presence of a chemical gradient**.
- Chemotaxis is thought to play a role in the movement toward and subsequent infection of legume roots by Rhizobium, a nitrogen-fixing bacterium.
- Rhizobia derive a variety of benefits from this association, such as protection from desiccation and predation and a generous nutrient supply, while the plant utilizes nitrogen fixed by the bacteria.
- In contrast to flagella, the presence of cellular appendages involved in attachment (pili and fimbriae) can reduce microbial transport potential.
- It is thought that cellular appendages can penetrate the electrostatic barrier thereby facilitating attachment at greater distances from the surface.

What hydrological factors affect movement through soil?

- **Soil texture** and structure, **porosity**, **water content** and potential, and wa**ter movement** through the profile are key hydrogeological factors influencing; microbial transport.
- Specific soil and vadose zone layers within a site serve as protective or attenuating zones with regard to contamination of groundwater by microbes (or chemical pollutants) via a variety of mechanisms, including filtration and adhesion. The greater the distance between the surface and the saturated groundwater, the less likely it is that groundwater contamination will occur.

What factors affect persistence and activity of introduced microbes?

- **Temperature**, **pH**, **soil texture**, **moisture content**, & the **presence of indigenous organisms** including plants, earthworms & microbes affect survival.
- Molecules slowly released from organic matter associated with matrix particles can serve as microbial nutrients.
- Access to & utilization of these solid phase **nutrients** may account, in part, for the increased survival of sorbed microorganisms
- Microbial activity can be altered by varying the **moisture content** of the terrestrial system.
- **Water content** above a certain optimal level has actually been shown to lead to a decrease in microbial numbers due to oxygen depletion as pores become flooded.
- In addition, **microbial predators** such as protozoa tend to be more active at higher soil moisture contents, perhaps because increased water tends to provide a mechanism for movement between pores.

What are ultramicrobacteria?

Ultramicobacteria, often abbreviated as UMB, are bacteria in their smallest form (≤ 0.3 μm in diameter). Given the prolific nature of bacteria and the wide expanse of nutrient poor conditions in both marine and terrestrial environments, many bacteria have developed the ability to exist in conditions of starvation by shrinking to almost one third their size to become UMBs. This dramatic decrease in size causes their cells to become rounder, and their glycocalyx layer is shed which in turn reduces the stickyness of the cell. The UMB form facilitates the transport of the bacteria through various media, as their small size allows them to pass freely through typical biological filters (both natural and anthropogenic in origin), and the absence of their sticky coating limits the cell's ability to adhere to surfaces thus allowing them to be transported further. This ease of transportation enables the UMBS to penetrate deeper into contaminated substances than they could have in their average size, thus facilitating bioremediation of the polluted site. One way in which UMBs are used to facilitate bioremediation, is when local bacteria are forced into their UMB form and then injected into porous, polluted sites. Once the bacteria have penetrated deep enough, nutrients are injected into the contaminated substrate, providing enough nourishment to the UMBs to force them out of their state of starvation and revert back to their standard size. The goal is for the bacterial cells to become large enough to plug the pores of the polluted substrate. The redevelopment of their sticky glycocalyx layer increases the likelihood of the cells adhering to the substrate while they grow, and thus be trapped within the medium instead of being expelled from it. This is often done in oil contaminated sites, because as the bacteria plug the pores of the substrate the oil is forced up to the surface, where it can then either be extracted or channeled to an extraction area to clean the area.

How do surfactants affect bacterial movement in soils?

- Surfactant is a type of chemical additive used to increase the transport potential of microbes
- Reduced bacterial sorption may be due to one of several factors including an **increase in surface charge** density caused by rhamnolipid adsorption, **solubilization of extracellular polymeric** glue; or **reduced availability of sorption sites** on porous surfaces.
- **anionic** surfactant, sodium dodecyl bertzenesulfonate (DDBS), facilitated transport by preventing adsorption & reduce straining. The reduction in adsorbtion may be due to binding of the anionic (**negatively-charged**) surfactant to the cell wall, causing the net negative charge of the cell surface to increase. This would increase the electrostatic repulsion between the cell and the soil surface.
- The surfactant may also be able to **prevent flocculation** of the bacterial cells by dissolving natural polymers.

1) Compare and Contrast the major factors influencing bacterial versus viral transport through the terrestrial profile. Which type of microbe would you expect to find deeper in the profile following surface application? (Text book Q # 1)

Terrestrial transport of bacteria and viruses are **<u>similar</u>** in that…

- Their cellular surfaces and protein coats, respectively, both tend to be negatively charged, which when combined with the negative charge of the porous medium results in the transportation of the microbes through the substrate by means of electrostatic repulsion.
- Hydrophobic interactions (i.e. nonpolar groups associating in aqueous environments) appear to influence the sorption of both bacteria and viruses to the terrestrial substrate.
- The presence of cations (positively charged ions) in the soil reduces the repulsive forces between the substrate and both the bacterial and viral cells.

Terrestrial transport of bacteria and viruses **<u>differ</u>** in that…

- Filtration has little effect on the ability of viruses to travel through medium, whereas, bacteria can experience micropore exclusion, in which the bacterial cells are larger than either the pore or the opening (throat) into the pore, and are therefore prevented from entering.
- Bacteria do not appear to be affected by pH, whereas the capsid coating of viruses causes them to be strongly influenced by pH. This is because viruses have a very homogeneous surface which is easily affected by changing pHs, whereas, bacteria have such chemically diverse cellular surfaces that a change in the surrounding pH would likely have a negligible impact on the overall net surface charge of bacterium.
- In essence the fundamental difference between the bacterial and viral transport through the terrestrial profile is that bacterial movement is most limited by filtration, whereas, viral movement is the most limited by pH induced adsorption (having to do with molecular and ionic bonding of the virus to the substrate) to the soil.

I would expect bacteria, probably viable but non-culturable, to be the deepest, as saturated conditions restrict viral movement & promote adsorption to soil particles which decreases the total viral penetration depth

What are gene probes and how are they used?

- Gene probes are an application of nucleic acid hybridization.
  - Typically, probes are small pieces of DNA known as **oligonucleotides**, which are complementary to the target sequence of interest, that are marked or labled in some way in order to make them detectable.
  - They are used to identify a particular phenotype, and to test for virulence genes of suspected pathogens.
- Gene probe methodology takes advantage of the fact that DNA can be denatured and reannealed.

How is probing done (Figure 13.3)?

- To make a gene probe the gene's DNA sequence of interest must be known
  - This gene may be unique to a particular microbial species, in which case the sequence may be useful for the specific detection of that organism.
  - Alternatively, the gene may code for the production of an enzyme unique to some metabolic pathway.
- This kind of probe can be defined as a **functional gene probe**.
  - For example gene probes can be made from sequences that code for enzymes involved in nitrogen fixation.
- The gene could even be universal to all bacteria, thus allowing detection of all known bacteria.
  - Many gene sequences are now known and are readily accessible via the gene databases
  - There are also computer software packages designed to search for sequences that are unique to a particular bacterium.
- The size of the probe can range from 18 base pairs to as many as several hundred base pairs.
- The probe is then synthesized and labeled in such a way that it can be detected after it hybridizes to the target sequence.
- Early on the only labeling option was radioactivity, but nonradioactive alternatives include probes labeled with **digoxigenin** (**DIG**), **biotin**, or **fluorescein**, which can be incorporated into the sequence by chemical synthesis.
- The different labels are detected by binding the respective antibody or streptavidin-alkaline phosphate conjugate, which, when reacted with the appropriate substrate, will give a signal (Figure 13.2)

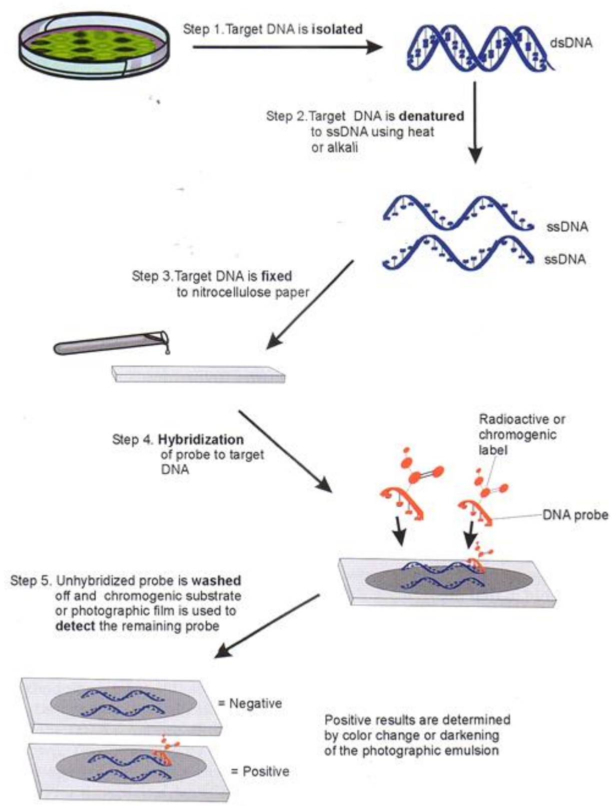


FIGURE 13.3 Alternative probe labeling and detection systems. Probes are labeled with either radioactivity, digoxigenin, biotin, or fluorescein. These labels are then detected by autoradiography or by binding the appropriate antibody or streptavidin-alkaline phosphate conjugate, which gives a signal when combined with the respective substrate. Detection of these signals can be by fluorescence, chemiluminescence, or a colorimetric assay.

- The probing process is explained using *Salmonella*. (Fig 13.3)
  - A water sample is collected and then filtered onto a membrane.
  - The cells captured on the membrane are lysed, and the released bacterial DNA is denatured into two single strands
  - The gene probe is similarly denatured into single strands & is subsequently added to the membrane with the lysed cells
  - The DNA is allowed to reanneal, and in some cases the single strand of the gene probe will anneal with the complementary target DNA.
  - After washing the membrane free of unhybridized probe, the filter is analyzed.

What are colony lifts?

- Gene probes can also be used to detect a specific gene sequence within bacterial colonies on a petri plate containing a mixed population of bacteria through use of a process termed **colony hybridization** or **lifts**.
- To perform a colony hybridization, a piece of filter paper is lightly pressed onto the petri plate so that some bacterial cells from each colony adhere to the paper.
  - The cells are lysed directly on the filter paper and the DNA is fixed to the filter.
  - The filter is then probed and detected as described above.
- After this process, only the colonies that contain the specific DNA sequence give a radioactive signal.
- Because the original petri plate contains all the intact colonies, the viable colony of interest can now be identified & retained for further study (Figure 13.4)

What is PCR and how does it work?

- The **PCR**, a technique used to amplify the amount of target DNA up to 10^6-fo1d or more, has revolutionized molecular biology methodologies.
- A typical cycle of PCR has three steps (Fig. 13.8a and b).

Step 1) The first step involves the denaturation of dsDNA into two single strands of **target** or **template DNA** (**ssDNA**).

- Added to the reaction mixture are two different short pieces of single-stranded DNA called **primers** that have been carefully chosen and commercially synthesized.
- Primers are oligonucleotides that have a sequence complementary to the target ssDNA template, so they can hybridize or anneal to this DNA, defining the region of amplification.
- One primer is described as the upstream primer and the other is the downstream primer.

Step 2) the 2nd step in the PCR cycle is primer anealing which consists of the primers hybridizing to the appropriate target sequence

Step 3) The 3rd and final step is extension

- Here, a DNA polymerase synthesizes a strand complementary to the original ssDNA by the addition of appropriate bases to the primers that have already hybridized to the target.
- The net result at the end of a cycle is two double-stranded molecules of DNA identical to the original double-stranded molecule of DNA
- Repeating the process results in PCR amplification of the DNA and an exponential increase in the number of copies.

- Temperature is a critical part of the PCR process.
  - Denaturation of the target sequence or template occurs at a temperature greater than the melting temperature of the DNA
  - For most PCR reactions this is standardized at 94^0C for 1.5 minutes, because it guarantees complete denaturation of all DNA molecules.
  - Primer annealing occurs at a lower temperature, typically between 50 and 70°C for 1 minute, depending on the base composition of the primer.
  - Is possible for a primer to anneal to a DNA sequence that is similar to the correct target sequence but which contains a few incorrect bases.
- The final step of PCR is extension.
  - The essential component of this reaction is a polymerase enzyme, such as Taq polymerase, which sequentially adds bases to the primers.
  - This enzyme was obtained from the thermophilic bacterium *Thermus aquaticus* and is uniquely suited for PCR because it is heat stable, withstanding temperatures up to 98°C, and can therefore be reused for many cycles.
  - The extension step is normally 1 minute in length and is performed at 72°C.

Design of Primers

- Detection of a target DNA that is specific to a given species or genus or a bacterium is required, then only sequences unique to that bacterium are appropriate for the design of the primers.
- **Conserved sequences** are those that are similar and are found in many bacterial species.
- Some conserved sequences are **universal** and found in all known species of a genus.
- Sequences are universal, such as some regions of 165 rDNA, and primers derived from these sequences anneal to all known bacterial species.

What is RT-PCR & what is it used for?

- A PCR method for the detection of RNA involves the use of the enzyme reverse transcriptase and is known as reverse **transcriptase-PCR (RT-PCR).**
- In RT-PCR, the first step is to make a DNA copy of the RNA sequence of interest (Fig. 13.11).
    - This copy is known as **complementary DNA** or **cDNA**.
    - The key enzyme in the reaction is re- verse transcriptase, which is an RNA-dependent DNA polymerase used to synthesize DNA from an RNA template.
- The reverse transcriptase-PCR (RT-PCR) can be used to detect RNA. Which is important in environmental samples as it can be used to detect RNA viruses, mRNA transcripts, and rRNA sequences.
- RT-PCR can also be used to detect enteric (intestinal) viruses present in environmental (particularly aquatic) samples.

What is cloning & a cloning vector?

- Cloning has enabled scientists to find new or closely related genes, as well as characterize and identify unculturable or unknown isolates.
- A **clone** is a foreign DNA fragment that is replicated in a host organism after being shuttled in by a cloning vector such as a plasmid.
- Cloning basically involves three steps:
  (1) choosing the source of DNA for cloning
  (2) producing a collection of DNA Fragments that can be inserted into a vector and in many cases ultimately creating a cDNA library
  (3) screening for the desired sequence of interest.
- Cloning results in a population of organisms that contain recombinant DNA molecules.
- Following screening for the target sequence of interest, a particular clone can be propagated to amplify the recombinant molecules, resulting in a large mass of the DNA sequence of interest.
- A **cloning vector** or vehicle is a self-replicating DNA molecules such as a plasmid or phage, that transfers a DNA fragment between host cells (Fig. 13.16).
- A useful cloning vector has three properties:
  (1) it must be able to replicate
  (2) it must be able to introduce vector DNA into a cell
  (3) there must be a means of detecting its presence.

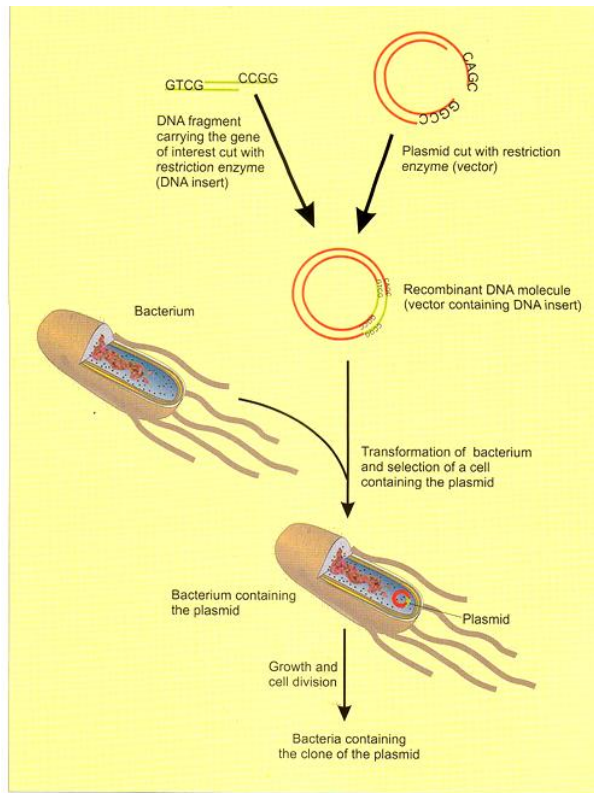


FIGURE 13.16 A foreign piece of DNA is inserted into a plasmid cloning vector. The vector can then transform a bacterial host, which will grow and divide. Subsequently, all progeny bacteria will carry the foreign DNA by plasmid replication.

What is restriction fragment length polymorphism – RLFP?

Restriction fragment length polymorphism (RFLP) is a type of analysis frequently used on bacterial isolates. First the total DNA is extracted from a pure culture of a bacterial isolate, by cell lysis and then preparing the genomic DNA using standard methods. This genomic DNA is then cut into smaller fragments by using **restriction enzymes** (endonucleases that recognize specific DNA sequences between ~4-6bp in length). Next the DNA fragments are separated using gel electrophoresis, and then visualized with ethidium bromide staining & UV illuminations. When the restriction enzymes are cut at multiple sites within the genomic DNA (producing hundreds of DNA fragments), which go through electrophoresis & probing in order to eventually **produce a fingerprint that is characteristic of the original bacterial isolate**.

1) What are the major <u>advantages</u> of PCR when it is applied to environmental samples? (Text book question # 1)

Polymerase chain reaction (PCR) methods are an especially useful method for detecting and identifying specific microbes in the environment, because:

- PCR is not restricted to culturable microbes, and the vast majority of environmental microbes are viable but non-culturable. PCR can also detect all microbes present in the sample, regardless of their physiological.
- The primers in the PCR can be designed to detect a specific microbe, which greatly facilitates finding certain pathogens or toxins in the environment.
- PCR has very sensitive microbial detection, which means it can be used to amplify very small segments of DNA found in the environment (which is much more difficult to do with traditional culturing techniques). ICC-PCR also has increased sensitivity levels when compared to the RT-PCR or traditional PCR techniques.
- PCR can be used to detect the universal 16S ribosomal gene sequence, which is important for environmental samples, as the internal sequence can then be isolated allowing the identification of the host microbe (note: while the external 16S sequence may be universal to all eubacteria, the internal sequence is species specific).
- PCR can be used to "shotgun clone" the community DNA taken from a bacterial population within an envir. sample.
- The reverse transcriptase-PCR (RT-PCR) can be used to detect RNA. Which is important in environmental samples as it can be used to detect RNA viruses, mRNA transcripts, and rRNA sequences.
- RT-PCR can also be used to detect enteric (intestinal) viruses present in environmental (particularly aquatic) samples.
- PCR can be used to detect the normal genetic potential of a microbe, and RT-PCR can take this one step further by then detecting the actual genetic activity of the organism.
- Integrated cell culture-PCR (ICC-PCR) can be used to shorten the amount of time required to identify a virus from the typical 10-15 days of cell culturing techniques, to as few as 3 days with the ICC-PCR.
- The ICC-PCR is also important for environmental samples, as it can detect the infectious viruses.
- And last, the overall costs of using PCR is 50% less than the cost of using traditional cell culturing techniques, & takes much less time to run (PCR can run in as little as 24 hrs, as compared to the days to weeks necessary to do cell cultures)

2) What are the major <u>disadvantages</u> of PCR when it is applied to environmental samples? (Text book question # 2)

However, there are also disadvantages to using PCR methods on environmental samples; such as:

- The ability of PCR to detect all microbes present is not always advantageous, as the PCR will be detecting both the infectious and non-infectious viruses, which makes determination of the level of "harmfulness" of the environmental sample more subjective. Whereas, the much more time consuming cell culturing techniques give more information on the level of infectivity of the sample.
- PCR can also be inhibited by certain pollutants (metals, humic substances, etc.) which are commonly present in environmental samples

3) You want to identify, at the genus level, several bacterial strains isolated from the soil. Explain how you would do this using molecular techniques. (Text book question # 6a)

In order to identify all the bacterial strains present in an environmental soil sample, PCR methods should be used in which a special primer is designed. The design of the primer will depend on objective of the study; in this case we are looking for all the bacterial species of a specific genus, which are present in the sample. The primer will be designed such that it will target a specific conserved gene sequence that is known to be universal for that particular genus (in other words all species within that genus will have this specific gene sequence).

4) How would you identify a soil bacterium without prior cultivation using molecular techniques? (Text book Q # 6b)

By analyzing the 16S rDNA sequences, specific soil bacterium can be identified to the species level. This is done through the following processes:

- first the community DNA of the bacterial population is extracted from the soil sample
- next the 16S region of the extracted bacterial DNA's genome is amplified using PCR techniques
- then shotgun clone the product of the PCR amplification
- last use phylogenic analyses to analyze the sequences of the resulting clones to identify the bacterial microbes to the species level.

What is the Gaia hypothesis?

- The Gaia hypothesis is based on a 1970's theory by James Lovelock that the earth behaves like a super-organism.
- Lovelock said that "living organisms and their material environment are tightly coupled. The coupled system is a superorganism, and as it evolves there emerges a new property, the ability to self-regulate climate and chemistry."
- The basic concept is that the earth's physiochemical properties are self-regulated so that they are maintained in a favorable range for life.

- Support for the Gaia Hypothesis:
  - Over the last 4-5 billion years the sun has heated up by 30%, if the earth had not been self regulating and maintained its initial high CO_2 atmospheric levels then the earth would be roughly 290°C today; however, the earth has maintained an average surface temperature of 13°C (which is favorable to supporting life)
  - Earth's atmosphere has developed dramatically differently from that of the surrounding planets, indicating that something has drastically affected its development.
  - The Gaia hypothesis says it is the development & continued presence of living organisms which controlled our atmosphere's development. Microbial activity & plants has changed the atmosphere from the initial heat-trapping carbon dioxide-rich to the present oxidizing, CO_2 poor atmosphere.
    - The initial atmosphere was a reducing, anaerobic atmosphere, in which key abiotic reactions driven by UV light mediated the formation of organic carbon.
    - The resultant reservoir of organic matter was utilized by early anaerobic heterotrophs.
    - Microbes then developed the ability to fix CO_2 photosynthetically (e.g. stromatolites 3.5 BYA), these photosynthetic organisms were able to use the sun as an unlimited energy source which provided a mechanism for carbon recycling (ie. creating the 1st carbon cycle, which lasted for ~1.5 by).
    - Around 2 BYA, photosynthetic microbes developed the ability to produce oxygen, which, once allowed to accumulate in the atmosphere, facilitated the shift from reducing to oxidizing conditions.
    - Eventually enough O_2 accumulated to form the Ozone layer, which in turn reduced the influx of harmful UV radiation & allowed the development of higher organisms
  - When considered on a geological time scale, the biogeochemical changes that have occurred have been unidirectional, and driven by the continued evolution of organisms…so while yes the earth does act as a superorganism by responding to environmental changes, its response is SLOW (thousands to millions of years) and therefore the Gaia hypothesis should not be used to justify anthropogenic overpopulation, pollution, etc, as the Earth will not be able to adjust fast enough to maintain an environment favorable to higher life forms.

How might microbes corrode metals & concrete?

Metal Corrosion

- Metal structures that are submerged in water are vulnerable to microbially mediated corrosive damage (especially iron).
- Microbially influenced metal corrosion accounts for 15 to 30% of the corrosion failures in the gas & nuclear industries.
  - It is a major cause of pipeline failures in water treatment and chemical industries and is also associated with corrosion failures and souring in gas and oil production and storage.
- **Sulfur-Reducing bacteria (SRB)** mediated corrosion requires anaerobic conditions as they use sulfur instead of oxygen during the respiration of the organic compounds. However, O_2 is often present in the environment surrounding a metal surface. When biofilms form on the metal's surface, bacteria in the outer layers of the biofilm rapidly utilize the oxygen thus creating an anaerobic microsites which will support the growth and activity of SRB & thus lead to metal corrosion.
- Metal corrosion is initiated by 2 spontaneous electrochemical reactions.
  1) In the first reaction, a differential aeration cell is set up in which the metal surface acts as the anode to produce metal ions,& in the corresponding cathodic reaction O_2 accepts the elections produced from the oxidation of elemental iron:
     - **Anodic** reaction: $\mathbf{Fe^o \rightarrow Fe^{2+} + 2e^-}$
     - **Cathodic** reaction: $\mathbf{½\ O_2 + H_2O + 2e^- \rightarrow 2(OH^-)}$
  2) A second reaction creates a concentration cell under anaerobic or aerobic conditions in which the anodic reaction remains the same but the cathodic reaction produces H_2:
     - **Anodic** reaction: $\mathbf{Fe^o \rightarrow Fe^{2+} + 2e^-}$
     - **Cathodic** reaction: $\mathbf{2H^+ + 2e^- \rightarrow 2H \rightarrow H_2}$
- **Sulfate-reducing bacteria (SRB)** utilize H_2 as an electron donor, thereby removing it from the environment and providing a driving force for the anodic reaction.

Step 1	$Fe^{2+} + 2H_2O \Rightarrow Fe(OH)_2 + H_2$	(Spontaneous)
Step 2	$4H_2 + SO_4^{2-} \Rightarrow H_2S + 2OH^- + 2H_2O$	(SRB, i.e. *Desulfovibrio desulfuricans*)
Step 3	$H_2S + Fe^{2+} \Rightarrow FeS\downarrow + H_2$	(Spontaneous)
Overall	$2Fe^{2+} + SO_4^{2-} + 2H_2 \Rightarrow FeS\downarrow + Fe(OH)_2\downarrow + 2OH^-$	

  - Aerobic respiration using SO_4^{2-} or S^0 as the TEA
  - Completely inhibited by O_2
  - Produces H_2S which can cause metal corrosion
  - Finally, the end product of sulfate reduction is sulfide (S^{2-}), which reacts with Fe^{2+} to form metal sulfide precipitate
- How can microbially influenced **corrosion be controlled**? There are basically two strategies.
  1) coat the metal surface with bactericidal chemicals such as phenolic compounds, surface-active substances, & metals (copper)
  2) disrupt the organization of surface biofilms, thus removing the microenvironment that supports the activity of the SRB.
  3) Still another method is to pass a weak electric current along the metal pipe thus disrupting the formation of hydrogen.

Concrete Corrosion

- Microorganisms participate in concrete corrosion by producing of acidic metabolites.
- Coal is dirty because it has lots of sulfur in it, burning it can create acid rain, **Carbonic (Sulfuric) Acid**, which has a pH =4
  - Since the 1950s Germany's Black Forest has been dying due to acid rain produced in the US & carried by the jet stream
  - The Adirondack Mountains in upstate NY have also been hit hard by acid rain
- A well-documented consequence of biogenic acid production is the corrosion of concrete sanitary sewer pipes (Fig. 15.3)
  - This occurs during a two-step process involving both sulfate reducing and sulfur-oxidizing bacterial populations that cycle sulfur from sulfide back to sulfuric acid.
  - Sulfides produced at the interface between the concrete pipe & the liquid sewage (in anoxic conditions) then travel up past the sewage to form $\mathbf{H_2SO_4}$

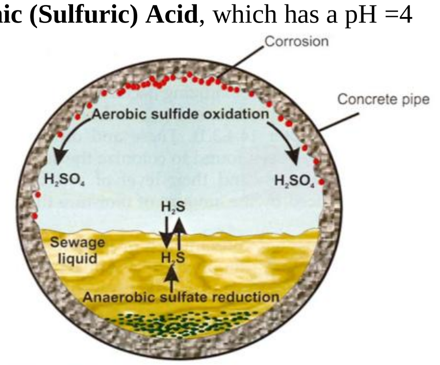


FIGURE 15.3 Cross section showing microbial involvement in the corrosion of a concrete sewer pipe. (Adapted from Sydney *et al.*, 1996.)

What is acid mine drainage?

- **Acid Mine Drainage** (**AMD**) refers to the outflow of **acidic water** from abandoned metal or coal mines. However, other areas where the earth has been disturbed (e.g. construction sites, subdivisions, transportation corridors, etc.) may also contribute acid rock drainage to the environment. In many localities the liquid that drains from coal mines is highly acidic, and in such cases it is treated as **acid rock drainage** (**ARD**). Acid rock drainage occurs naturally within some environments as part of the rock weathering process but is exacerbated by large-scale earth disturbances characteristic of mining and other large construction activities, usually within rocks containing an abundance of sulfide minerals.
- The formation of acid from pyrite ore is a complex mechanism that involves the oxidation of both iron and sulfur.
- The initial reaction leading to the formation of acid mine drainage is the spontaneous chemical oxidation of pyrite:
- This process is initiated spontaneously, but as the local pH drops, a sulfur- and iron-oxidizing bacterium, ***Thiobacillus ferrooxidans***, also begins to participate. This organism is unusual in that its pH optimum is around 2. *T. Ferrooxidans* has been intensely studied because of its role in acid mine drainage formation and for its use in metal recovery.
- Because oxidation of pyrite is an exothermic reaction, temperatures within tailings impoundments can build until they exceed 60^0C and at higher temperatures create a selective advantage for the thermophilic archaebacteria which then begin to predominate in the formation of acid mine drainage.
- The ferrous iron (Fe^{2+}) formed will, in a neutral aerobic environment, spontaneously oxidize to the ferric iron form (Fe^{3+})
- However, the autoxidation is slow, but ***T. ferrooxidans*** can oxidize Fe^{2+} quickly once the pH reaches 2.
- Oxidized iron formed in can have 2 fates.
  1) It can be precipitated as iron oxide, a reaction that generates more acid: $\mathbf{Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3\downarrow + 3H^+}$
  2) ferric iron can aid in the further chemical oxidation of pyrite(FeS_2): $\mathbf{FeS_2 + 14\ Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+}$

Note: this reaction produces acid & regenerates reduced or ferrous iron, which can then be reoxidized by *T. Ferroxidans*

- Thus, the combination of a microbially mediated reaction & a chemical oxidation reaction creates a reaction loop that speeds the oxidation of pyrite.
- The leachate from the mines is carried by surface water and groundwater flow into receiving stream & river waters

- Overall these reactions can be summarized as: $\mathbf{FeS_2 + 15\ O_2 + 14\ H_2O \rightarrow 4\ Fe(OH)_3^- \downarrow + 8\ H_2SO_4}$
- A consequence of this is that these reactions include the **dissolution of carbonate minerals**, such as calcite and dolomite; the **formation of gypsum**; and the **bacterial oxidation of certain metal sulfides**, such as copper and zinc suffixes.

How might bacteria be used to leach the sulfide ores of copper?

- It's less economical to smelt low-grade ores, so microbially mediated metal recovery is an attractive alternative to smelting
- Recently metal recovery using the organism *T. ferrooxidans* has become a well-understood, efficient, & cost-effective process
- As of 1989, more than 30% of U.S. copper and uranium production was micrcbially mediated. *T. Ferrooxidans* can participate in both the **direct** and **indirect leaching** of metals such as copper from a variety of ores.

- The reactions for removal of copper from ores such as chalcopyrite ($CuFeS_2$), chalcocite (CuS_2), & covellite (CuS) are…
  - **Direct Leaching**: $\mathbf{MS + 2O_2 \rightarrow MSO_4}$ where both metal and S are altered.
- or the metal alone may only be utilized where the oxidized Cu is soluble: $\mathbf{\underset{chalcocite}{2Cu_2S} + O_2 + 4H^+ \rightarrow \underset{covellite}{2CuS} + \underset{leached\ Cu}{2Cu^{2+}} + 2H_2O}$

- More important overall are the **indirect metal leaching reactions**: $\mathbf{Cu_2S + 2\ Fe_2(SO4)_3 \rightarrow 2\ CuSO_4 + 4\ FeSO_4 + S^o}$
  $\mathbf{2\ FeSO_4 + ½\ O_2 + H_{2S}O_4 \rightarrow Fe_{2(}SO4)_3 + H_{2O}}$

  where copper is spontaneously oxidized by the presence of the ferric ion (Fe^{3+}) & acid
  and then the resulting ferrous iron (Fe^{2+}) is reoxidized biologically by *T. Ferrooxidans*

Finally, the copper ions (Cu^{2+}) can be recovered from solution by spontaneous precipitation in the presence of scrap iron

Why might bacteria methylate metals & metalloids?

- **Bioalkylation** is the biologically mediated linking of an alkyl group (-CH_3) to a metal or metalloid element, thus forming an organometal compound.
- Bioalkylation greatly alters the physical and chemical properties of a metal and has a major effect on the fate and biological impact of metals in the environment.
- Methylation also alters the toxicity of the element because the methylated form is more volatile and more soluble in lipids.
- A consequence of increased lipid solubility is that methylated metaloids are less easily excreted and thus accumulate in living organisms causing toxicity.
- Selenium in the water is mostly in the water-soluble selenate form (SeO_4^{2-}), and the microbially-mediated methylation of selenium produces a highly volatile methylated product
    - Therefore, promotion of methylation is seen as a means of removing selenium from the water and dispersing it into the atmosphere, thus greatly reducing the level of exposure to wildlife.
- The most important intracellular agent of **mercury** methylation is believed to be **methylcobalamine** (CH_3CoB_{12}), a derivative of vitamin B12.
    - Since Hg^{2+} is highly toxic to microbes & CH_3Hg^+ is not but is fat soluble, essentially the microbes have detoxified their environment while toxifying ours
- The reason for the methylation of mercury by bacteria is not fully understood, although the methylation of certain metals may be a mechanism of detoxification.
    - Ex., the methylation of **arsenic** to methylarsenicals results in a compound that is less toxic, less reactive, and more readily excreted from the cell.
- Methylation of mercury may provide some degree of resistance to the highly toxic H^{2+}

What metals and metalloids can be methylated?

- **Nickel (Ni)**
- **Tin (Sn)**
- **Antimony (Sb)**
- **Lead (Pb)**
- **Germanium (Ge)**
- **Arsenic** (**As**) salts are green and used to be used as dyes & pigments in wallpaper
    - In England molds grew on the wallpaper and gasified the As & killed 2 children
- **Selenium** (**Se**) levels increased in California resevoirs & was somehow mobilized
  → birds ate it & their egg shells became weak (much like DDT poisoning)
- **Mercury** (**Hg**): Metallic Mercury is used as an electrode → UV lights; it is found in thermometers; it bioaccumulates in fish; and it is used as a preservative in hats ("mad as a hatter"), vaccines (→ autism), and in paper (→ Minamota)

1) Think about the activities involved in the carbon, nitrogen, and sulfur cycles and develop your own idea for applying one of these activities in the commercial sector. (Text book question # 1)

Aspects of the sulfur cycle could be used to corrode sculptures and statues (made of metal, concrete or stone) in order to give the piece a more antiqued, weathered appearance which may be desirable to the consumer.

2) Methylation of metals increases their volatility. Is it a good idea to use microbial methylation as a basis for removal of metals such as mercury and selenium from contaminated soils? (Text book question # 4)

Methylation is both a naturally and anthropogenically induced process, in which a methyl group is linked to a metal(loid). Among the metal(oids) known to be susceptible to biomethylation (i.e. microbial methylation) are: Nickel (Ni); Tin (Sn); Antinomy (Sb); Lead (Pb); Geranium (Ge); Arsenic (As); Selenium (Se); and Mercury (Hg). In addition to greatly altering both their physical and chemical properties, biomethylation is also known to change the toxicity of the metalloids by making them more volatile (evaporate faster) and soluble in lipids.

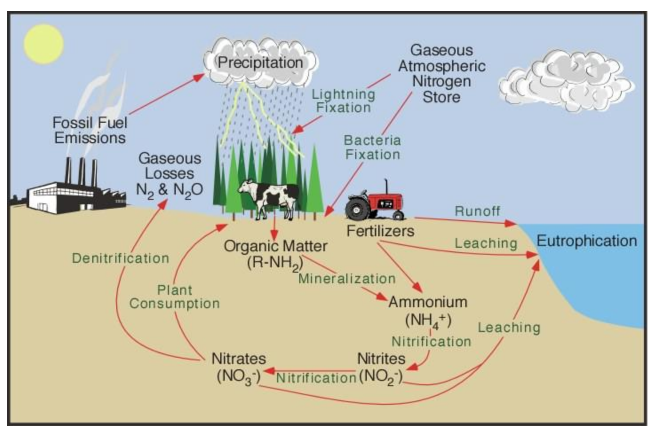
Generally speaking microbial methylation of metals is not a good method of soil decontamination, as their methylated forms are often more prone to bioaccumulation; as is the case with mercury, tin, and possible iron. Bioaccumulation of these metals is of health concern, as high organisms on the food chain are easily poisoned by the high accumulation of toxins in their prey (ex. consumption of fish and shell fish that have high levels of mercury built up in their systems can be fatal to humans). Microbially methylated selenium, however, is thought to be an environmentally friendly method of soil decontamination; as the process removes the selenium from the seawater and disperses it into the atmosphere, which, in turn, decreases the selenium concentration in the water that had previously been poisoning the wildlife (ex. birds began to produce eggs that were to thin and fragile to produce viable offspring). Therefore, before microbial methylation is considered as a means of soil decontamination, the effect of the mathylated metal on the surrounding environment should be considered for the individual metal in question.

3) Groundwater under cattle feedlot operations is often found to have nitrate contamination. Explain the microbial basis for nitrate contamination of groundwater in these areas. (Text book question # 6)

Nitrogen generally does not accumulate in natural, undisturbed environments. This is because these ecosystems do not have very much excess ammonia (NH_4^+). The presence of ammonium is significant because it is easily converted to nitrate (NO_3^-) by aerobic, chemoautotrophic nitrifying bacteria, and nitrate is an anion (negatively charged ion) which makes it very mobile in soil and thus easily carried to the water table when produced in excess.

Through the anthropogenic conversion of natural systems to agricultural systems, humans greatly increased local ammonia concentrations, through their direct application of ammonium to the substrate as crop fertilizers, and indirectly by the ammonium present in the fecal waste produced by their livestock (shown in the figure below). The excess ammonia is then converted to nitrate which is then carried into the surrounding watersheds.

High nitrite concentrations in the groundwater are of concern for many reasons. The nutrient loaded fresh water can be carried downstream and emptied in to oceans, where the local physical and biological processes react to these high nitrate concentrations in such a way that anoxic layers within the water column are created (e.x. hypoxia, a.k.a "dead zone"). High nitrate concentrations in drinking water can affect human babies, as their stomachs have not yet become acidic enough to prevent the growth of denitrifying bacteria. Denitrifying bacteria in the gut will convert the nitrate to nitrite (NO_2^-)) the nitrite then binds to the hemoglobin & prevents it from transporting oxygen throughout the body (ex. methemoglobinemia, a.k.a. "blue baby syndrome"). This process in human adults can cause the formation of nitrosamines which tend to be cancerous.



- The dead zone, located off the Louisiana coast, is an area of low oxygen (< 2.0 mg/L)driven by anthropogenic land change
- Dead zone receives 41% of the US's water drainage from the Mississippi River Basin (MRB)
- 58% of the MRB is predominantly agricultural land
- 21% of the MRB is predominantly open range or barren land
- 1/3 of the Mississippi comes down & Joins the red River to form the Atchafalya Basin
- Oxygen in the water column is usually ~9 or 10 mg/L, in June it can drop to) mg/L
- Ocean oxygen is mixed by storms, water temperature, winds, etc.
- Oxygen levels drop due to respiration

How Hypoxic Zones are Formed

1) Mississippi River brings in nutrient rich waters
2) Algae feed & grow on these nutrient rich (fertilized) waters
3) zooplankton eat the algae
4) bottom water bacteria eat the dead algae that have fallen from the surface → anoxia
5) Organisms can't survive in anoxic conditions → organisms flee the area

- Upper water pycnocline (density stratification prevents oxygen mixing & aeration)
- Hypoxic zone can be a thin bottom layer or a very thick (20m) bottom layer

- Offshore water movement → hypoxic zone pushed closer to the shore
  - Fish get trapped between the hypoxic zone & the shore
    - Brief period of good fishing conditions
      - If hypoxic conditions last too long there are massive fish kills

- The Dead (Hypoxic) zone is not completely "dead", but rather…
  - Direct mortality
  - Altered migration
  - Decrease in suitable habitats
  - Increase in susceptibility to predation
  - Changes in food resources
  - Susceptibility of early life stages

- **How dead zones affect fisheries**
  - Louisiana fisheries depend on high densities of bottom-feeding fish (croaker) & shrimp
  - Fisheries bring billions of dollars into the Louisiana economy
  - Avoiding the dead zones, bottom-feeding fish are found at the periphery & are increasingly leaving the area altogether

- Every time oxygen levels decrease below 1 mg/L the food chain is re-set because the benthic organisms have died
- in the Mississippi River there is a strong relationship between primary & net productivity

Hypoxic History

- the amount of agricultural lands in the US haven't really changed since the 1900s; however, the intensity in which they are cultivated has dramatically increased by 1920 most forests in the US had been plowed into flat lands
    - no vegetation left to hold the nutrients in the soils
        - nutrients are washed away → 1930s Dust Bowl
- 2/3 of America's productive soil has been lost

- **Nitrogen Loses under different land uses** (kg/km^2yr)

Tillage & Cropping Treatment	
Continuous Blue Grass (native cover)	11
Corn, wheat, clover rotation	485
Continuous corn	1,213
Plowed 4 inches (fallowed)	2,172

- after all the forested areas had been plowed, the wetlands were drained so that they could be plowed
- the majority of today's agriculture is restricted to the Midwest
    - there are counties in which 95% are either corn or soybeans
    - sharp loss of diversity, very homogenous landscape

- Today more nitrogen is fixed through anthropogenic chemical processes than is produced naturally
- Land use drives water quality worldwide and also in this watershed
- The amount of nitrogen carried by the water didn't really change until after the 1950s
- There is a positive correlation between increasing nitrogen concentrations and algal (Diatom) abundance
- Can track oxygen changes in the soil through micropaleontology (change in the abundance of forams requiring oxygen vs forams which don't require oxygen)

- Today we can predict with 95% accuracy where the hypoxic zones will be 2 months from now
- As time goes on the size of the hypoxic zone is increasing for the same amount of nitrogen (because the sediment is storing carbon)
- Increased nitrogen concentrations → increased phytoplankton production → decrease oxygen concentrations
- Diatoms require a large difference between silica and nitrogen. However today these levels are almost equal
    - Diatoms are being replaced by flagellated algae → HABs
- Fish feed on zooplankton which feed on diatoms
- Current trends could cause the fisheries to collapse & an increase in HABs
- Essentially hypoxia is causing the marine food web to collapse

Explain Figure 5

Turner and Rabalais' (2003) Figure 5 (shown on the right), depicts the resultant nitrogen (N) and phosphorous (P) losses due to the conversion of a Missouri prairie to agricultural fields. The native prairie vegetation was a type of bluegrass which had relatively deep root systems (>1m long) and provided the area with continuous ground cover. The extensive roots of this native plant facilitated the binding of both the N and P in the substrate, thus preventing both soil erosion and depletion of the nutrient concentrations. When this native cover was plowed and replaced with a continuous cover of wheat crops, nutrient losses increased dramatically: Nitrogen losses increased from an annual average loss of ~11 kg/km^2 under bluegrass conditions to an astonishing 596 kg/km^2; while less dramatic, phosphorous losses also increased from 4 to 173 kg/km^2. This increase in the annual loss (or release) of the nutrient concentrations within the substrate were caused by a combination of the disruption of the soil during the removal of the bluegrass and planting of the wheat, and the inability of the wheat's root system to provide adequate soil stability. As shown in figure 5, the wheat roots were half the length of those of the bluegrass, and much less expansive horizontally. Combinations of soil erosion, and losses due to the growth requirements and harvesting and of wheat crops soon caused the substrate to be so depleted of nutrients that it could no longer support wheat crops. At this point the wheat was replaced with corn crops, which further depleted the nutrient concentrations of the substrate, with average annual losses of 1213 kg/km^2 and 350 kg/km^2 for nitrogen and phosphorous respectively. When plowed and left unseeded (fallowed) the substrate lost an astounding 2172 kg/km^2 of N and 699 kg/km^2 of P, this resultant release of essential nutrients is almost 200 times the annual loss under the region's native bluegrass ground cover.

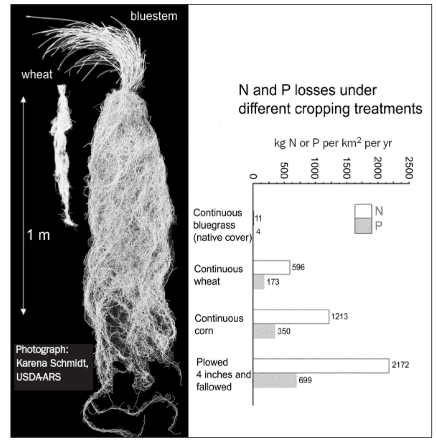


Figure 5. Average nitrogen (N) and phosphorus (P) losses (kilograms per square kilometer per year), 1 May 1926 to 1 May 1928, in runoff from experimental plots at the Missouri Agriculture Experiment Station (Miller and Krusekopf 1932). A belowground vertical profile of the roots in wheat (Triticum aestivum) and native big bluestem (Andropogon gerardi) prairie vegetation is shown.

What effect does marsh cover have on nitrogen release?

Studies have found that the percentage of marsh cover appears to be inversely correlated to the amount of nitrogen in the watershed, as shown by the rapid decline in the nitrogen yields of watersheds that follows increases in percentage of marsh cover in the area. Turner and Rabalais' (2003) Figure 6 (shown on the right) depicts this inverse relationship between nitrogen concentrations and percent marsh cover in watersheds. For this reason marsh, and overall wetland, restoration can be used to decrease abnormally high nitrogen concentrations and attempt to restore the overall water quality of the watershed.

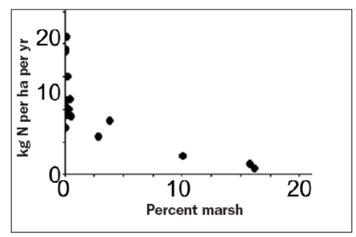


Figure 6. Relationship between the percentage of a watershed in Iowa that is marsh and nitrogen from nitrate and ammonium (kilograms per hectare per year) for 17 watersheds over 100 hectares, 1971 to 1973. Adapted from data in Jones and colleagues (1976).

Describe the major events that lead up to hypoxia in the "dead zone"

Hypoxic is caused by the simultaneous occurrence of two main factors: 1) stratification of the water column; and 2) increased nutrient concentrations within the watershed. As is typical of most estuaries, the water column of the Mississippi river delta is stratified, with the river fresh water lying above the more dense oceanic salt water. As will be discussed in much greater depth in the following questions, poor agricultural practices have allowed massive nutrient depletion of the Mississippi River Basin (encompassing almost 41% of America). Cultivation of the land has caused soil erosion, which allows the nutrients to be released from the substrate and transported down the Mississippi River to the Gulf coast. The stratified water column combined with the anthropogenic nutrient loading causes massive increases in the abundance of the filamentous algae which feed on these nutrients, resulting in algal blooms. When the algae eventually die they sink to the bottom of water column, where benthic bacteria consume their remains. During consumption of the algae the bacteria use up the surrounding dissolved oxygen, which combined with the stratified water column, causes the formation of an anoxic layer. This region of anoxia is often referred to as hypoxia. Given that very few marine organisms can survive in anoxic conditions, organisms flee the hypoxic area, thus altering their typical migration patterns and making them more vulnerable to predation. Off shore water movement can intensify the effect of hypoxia by forcing the hypoxic region closer to the shore, thus trapping fish and other organisms between the shoreline and the hypoxia. If the organisms are trapped here long enough the forced alteration in their natural food web combined with the increasingly anoxic conditions will causes massive fish kills (which is why hypoxic regions are also referred to as "dead zones").

1) Describe in detail the events as they occur in the Gulf of Mexico.

The current hypoxic areas present in the Gulf of Mexico are the result of more than 200 years of poor land management in the midwestern United States. When European settlers first arrived in America land seemed to be an unlimited resource, especially when compared to their homelands, and as a result for the first couple centuries of settlement land was viewed as cheap and expendable, and thus little, if any, attention was paid to sustainable cultivation of the land. By 1860 at least 15% of the Mississippi River Basin (MRB), which encompasses roughly 41% of the contiguous united states, was either under cultivation or its nutrients had already been exhausted. As the MRB drains predominantly through south eastern Louisiana, it is perhaps no surprise that records show New Orleans had the highest suspended sediment concentrations during the 1800s, when land development for agricultural purposes was at its height. While the specific hypoxic repercussions of America's poor land management may not have been anticipated two hundred years ago, the rapid and extensive rate of nutrient depletion due to agriculture was recognized, as shown in the following quote by a visitor to the US in the 1800s, "There is no portion of the globe that is being exhausted of its fertility by injudicious cultivation, so rapidly as the Mississippi Valley." Despite this early acknowledgement, land continued to be treated as expendable well into the 1900s. It wasn't until the 1930s that Americans were forced to face consequences of their poor land management, when a nationwide drought brought agriculture to a standstill and the already exhausted Midwest became a barren "dust bowl." As discussed in question 3, the dramatic change from the native diverse forms of land cover to only a handful of agricultural crops resulted in massive soil erosion throughout the MRB, with the nutrients being washed into the surrounding watersheds and eventually carried down stream by the Mississippi River and emptying into the Gulf of Mexico. Nutrient loading of the watersheds was further exasperated by the addition of loose fertilizers to the now impoverished farmland. These nutrient rich waters have been accumulating over time at the mouth of the Mississippi River delta, where the coastal Gulf waters are becoming polluted to the point where hypoxic conditions occur. The high nutrient concentrations have greatly affected the atomic ratio of silicate to nitrate. Historic silicate to nitrate ratios have fallen from 4:1 to nearly 1:1, which has severe consequences to the surrounding coastal food web, as dips below this 1:1 ratio causes the microbial loop to switch from a diatom based system to a flagellated algae dominated system. As diatoms are at the base of the food chain for the coastal fisheries, this anthropogenically induced nutrient loading of the coasts could cause the local fisheries to collapse. In addition, the eutrophic conditions cause massive increases in the populations of the flagellated algae, forming "harmful algal blooms" (HABs). As the algae die they will sink to the bottom of the water column, where benthic bacteria will consume them. As discussed in question 1, the bacteria use dissolved oxygen in the water in order to breakdown the algal corpses; however, due to the large amount of algae being consumed combined with the stratified water column (formed by the fresh river water laying on top of the more dense oceanic salt water), causes the oxygen to be almost entirely used up forming an hypoxic layer. Louisiana fisheries feed on benthic fish and shrimp, however, these organisms are not adapted to survive in anoxic condition and thus either flee the hypoxic regions or die. Since these benthic organisms are a crucial part of the food chain for local fisheries, the fish are being forced to the periphery of the hypoxic zones in search of food, or they are being trapped between the coast and the hypoxic area where they eventually starve resulting in massive fish kills (thus giving hypoxic regions their alternative name, "dead zones"). In effect hundreds of years of careless agricultural practices and overall poor land management have brought about the present hypoxic conditions along the mouth of the Mississippi River, and out into the surrounding Gulf of Mexico. In addition to dramatically altering the distribution and makeup of the coastal marine food web, hypoxia has also caused massive increases in harmful algal blooms (HABs), and greatly threatens the stability and sustainability of the coastal fisheries, which bring in billions of dollars into the Louisiana economy alone every year.

2) How does soil disturbance affect nitrogen concentrations in the watershed?

In an undisturbed system, natural ecologic processes keep nutrients bound (stored) in the sediments. The nutrients in the soils provide enough nourishment to support large plant growth (i.e. forests). These large plants, in turn have substantial root systems which stabilize and prevents erosion of the soils. The stability of the substrate keeps the nutrients from washing away, and thus allowing the cycle (nutrients → plants → soil stability→ nutrients→ plants…) to continue. When soils are disturbed this cycle is disrupted and the nutrients, specifically nitrogen, are released into the environment where they can be washed or blown away. A prominent example of this (which is discussed in much greater detail in Questions 2 above & 4 below) is when native environments are disrupted by humans in an effort to create farmland. First the forests are clear cut and the underlying sediments are tilled in order to prepare the land for cultivation. By removing the deep root systems and protective cover of the once overlying vegetation, the substrate becomes vulnerable to erosion. Meade et al (1990) found that the conversion of forests to agricultural fields caused orders of magnitude increases in soil erosion (Turner and Rabalais, 2003). The loose, eroded soil containing the nutrients is then washed into the surrounding rivers and streams, resulting in dramatic increases in the nitrogen concentrations in the surrounding watersheds. Pierce et al (1972) reported that natural forests in New Hampshire had annual nitrogen retentions around 200 kg/km^2; however, when these forests were clear cut not only was the nitrogen no longer retained in the system, far more nitrogen was lost (~3,800 – 5,700 kg/km^2) than could have been produced in that year. The end result of agriculture related soil disturbances tends to be exhausted, infertile soil unable to support the intended crops, and eutrofication of the surrounding water systems.

History of Marine Oceanography

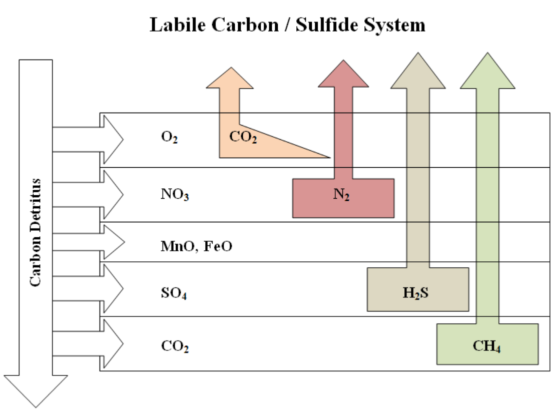
- 1880's: Challenger mission: the deep sea needed to be surveyed so that telegraph lines could be laid
- 1960's: marine microbiology revolved around culturing
  → now you just have to be able to isolate the DNA from the other genetic material in order to prove you have a microbe
- 1967: Platechtonics Theory took over
- 1977: galapagoes vent communities are discovered
- 1980: Colleen Cavanaugh suspects chemosynthesis in tubeworms, she knew tubeworms were filled with crystallized sulfur
  She got samples from Meridth & found out that they were filled with bacteria
- 1981: Felback et al. they proposed that there was chemosynthesis in mussels
  refused to acknowledge bacterial presence because chemosynthesis in Echinoderms is a much bigger discovery
  → in actuality there was bacteria present & they had a symbiotic relationship with the mussels
- 1985: Gulf of Mexico Cold Seeps are found

Note: Bob Ballard did **Not** discover chemosynthesis in hydrothermal vents

Challenger Discoveries

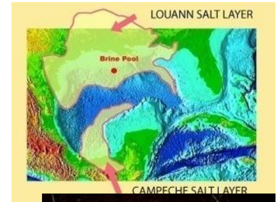
1) plenty of Metazoan Life
2) Zones of fauna based on depth
3) Amount of organisms decreases with increasing depth (i.e. total biomass decreases with depth)

- It was already known that plants weren't found below the first few hundred meters (the photic zone) which meant that the organisms found below this depth were heterotrophs
- **Refractory Carbon:** the stuff microbes can't eat
- **Labile Carbon**: what microbes can eat
- Studies found that the labile carbon was rapidly consumed with increasing depth
- The general pattern for the microbial distribution in the oceans is based on the amount of labile carbon present
- Demand >> Flux (organisms want more food then there is)
- Chemosynthesis must have an anaerobic/aerobic interference
  - The problem is that the water column is aerobic, so where are the interfaces?
- Overall there is a disconnect between the number of organisms and the amount of available food, we can't explain what's supporting the high biomass given the limited food supply.



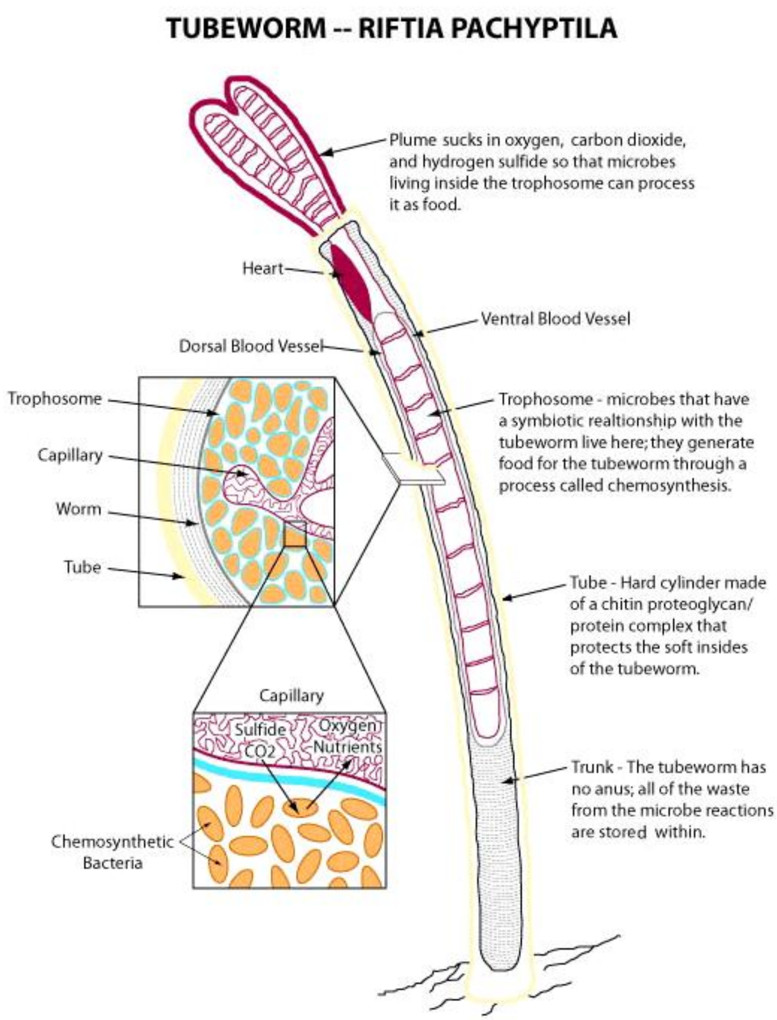
- Ripping off O_2 is very inefficient!
- Bacteria is getting the biggest bang from the sulfide because they're getting the most from the detritus and have chemosynthesis

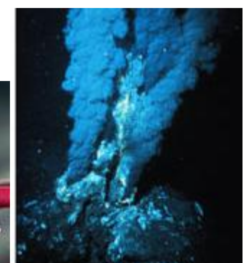
"Wormy Mud, hydrocarbon seeps, & hydrothermal vents: The exciting relevance of reduced sediments"

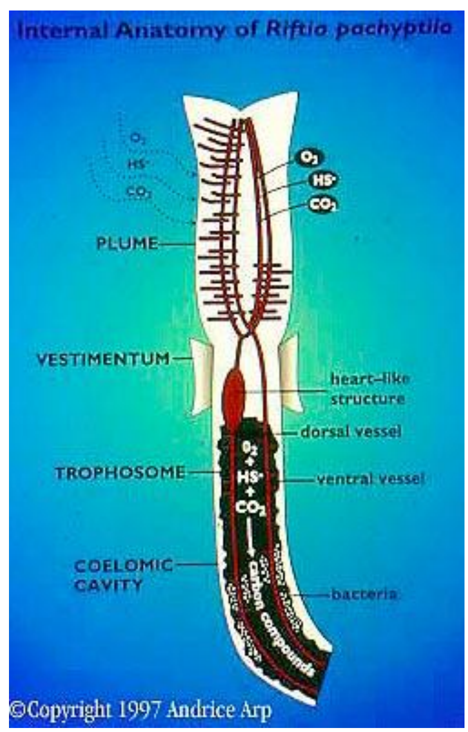


- The gulf of mexico is techtonically active
- Hydrothermal vents & cold seeps are both unique in that they're both sulfuric environments
- Salt is providing the mechanism for bringing hydrocarbons to deep sea cold seeps
- The Deep sea is the "kingdom of echinoderms"
- Deep sea cucumber: blind, gelatinous animal, that biolumeneces with threatened
- Mussels are probably relying on either methane or sulfide deep in the sediments
- Organisms can scavenge low concentrations of sulfide
  → but sulfide is highly reactive with O_2 & doesn't last long
- Bush-like tube worms provide the interface between aerobic and anaerobic environments
  - these bush-like worms have a very small plume and most of the worm extends into the rock
  - grow <1cm/yr and may be the longest living organism

- Dune "Grass" are actually tube worms that are feeding on the sulfide being emitted from the spreading sea floor







Ch. 10.3 Symbioses of chemoautotrophic prokaryotes with animals (Chapter Summary)

In recent years, technological advances have greatly facilitated scientific studies of prokaryotic associations with marine organisms, such as the importance of the symbiotic relationship between *Archaea* and sponges, and other marine animals. The importance of understanding these symbiotic relationships has increase as these relationships are found to have great biotechnical potential.

10.3.1 Chemoautotrophic endosymbionts in hydrothermal vent animals

Surprised by the discovery of dense deep sea communities surrounding hydrothermal vents, scientists have worked hard over the last few decades to better understand these unique ecosystems and justify not only their existence but also their high productivity in such an extreme environment. Hydrothermal vents are considered an extreme environment because the depth at which they occur has no light penetration and extremely high pressure. Initial studies ruled out organic matter as the primary food source for these communities, as it would take too long for the organic material to settle from the surface waters and too much of the matter would be consumer in the upper layers of the water column to provide enough food to support these communities. For this reason scientists began to suspect the presence of chemoautotrophic bacteria and their likely involvement in the nourishment of these vent communities through symbiotic relationships.

Carbon isotope ratios, taken from sample of bivalve cellular material, further supported this theory, as the ratios were dramatically different from the ratios found in photic zone organisms. Thus scientists were able to show that these deep sea organisms were not feeding on organic matter in which the carbon had been fixed in the photic zone. Anatomy studies of these deep sea organisms ruled out filter feeding as the primary mode of nourishment; as the digestive tract and filtration mechanisms of the deep sea clams was greatly reduced when compared to their photic relatives, and the giant deep sea tube worms had no gut and could not have absorbed enough organic material from the warm vent waters to support their high metabolism. Microscopic studies of the tubeworms revealed the presence of endosymbiotic, gram-negative bacteria in the large central cavity of the worm (the trophosome organ). Studies eventually showed that the gill plume extending from the vest of the tubeworm, would take in O_2, H_2S and CO_2 from the warm vent waters, and transport them to the trophosome where the bacteria would oxidize the reduced sulfur to get the energy to fixate the CO_2 into organic material, which would then feed the host tubeworm. Similar chemoautotrophic bacterial processes were found in the deep sea clam, though their endosymbiotic relationships were not nearly as sophisticated or efficient as those found in the tubeworm. Deep sea mussels were found to have even less sophisticated symbiotic bacterial systems, though they were found to have methanotrophic bacteria, in addition to sulfide-oxidizing bacteria.

10.3.2 Episymbiotic bacteria on vent animals

In 1985 large shrimp populations were found around the mid-Atlantic Ridge vent chimneys; however, these shrimp were not found to have endosymbiotic bacteria, but rather were found feeding (grazing) directly on chemoautotrophic bacterial mats found along the vent chimney walls. Episymbiotic bacteria were also found along the mouths and internal exoskeletons of these vent shrimp, which seemed to "farm" the chemoautorohic bacteria within the microbial mats by looking for specific O_2 and sulfide concentrations and ensuring optimal gas exchange between the microbial mats and the surrounding water by fanning out the warm vent waters.

An oligochaete worm (the "Pompeii worm") was found in the walls of the East Pacific smoker chimney vents, which had a rather extreme temperature gradient along its body (from 85°C at the anterior end within the chimney wall to ~20°C at the posterior end that extended into the water column). The worm was found to be covered with Episymbiotic filamentous bacterial colonies that were large enough to be seen by the naked eye. Some of these bacteria were found to be chemoautotrophic, though their exact relationship with the worm is not yet known.

10.3.3 Chemoautotrophic endosymbionts in non-vent animals

Chemoautotrophic algae are also found to have symbiotic relationships with a wide range of organisms in varying habitats, though many of these habitats have similar balances between sulfide concentrations and reducing conditions. For example, methanotrophic symbiosis was found in cold-seep mussel communities, in which the bacteria use the methane from the cold seep as both the carbon and energy source. Some mussels have two types of chemoautotrophic bacteria present, one which oxidizes the sulfur and another which oxidizes the methane; the mussel will adjust the ratio of the populations needed of each type of bacteria according to the sulfide and methane concentrations present in the surrounding environment. The presence of multiple types of symbiotic bacteria within a host greatly enhances both the ecological and physiological flexibility and overall robustness of the host.

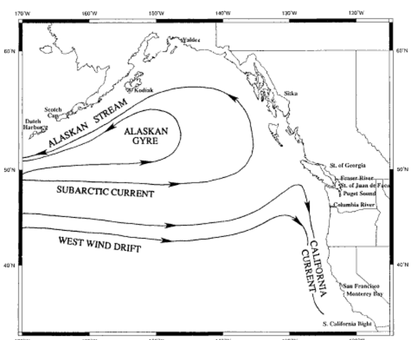
10.3.4 Phylogeny and acquisition of symbiotic bacteria

How organisms acquire their symbiotic algae is not entirely known, though it seems to have been through the co-evolution of both the host and the algae.

Dr. Sibel Bargu's Guest Lecture on Harmful Algal Blooms (HABs)

- **Algal Blooms**: favorable environmental conditions trigger periods of rapid cell reproduction called blooms
- When a bloom occurs there are different outside factors that effect the length & strength of the bloom:
  - light availability
  - nutrient availability
  - phytoplankton species
  - mixing depth
  - temperature
  - grazing pressure
- when talking about Algal Blooms (& HABs) species matters!
  - "Bloom has regional, seasonal, & species specific aspects; it is not simply a biomass issue"
  - some phytoplankton species can form cysts, which allows them to survive periods of starvation

- **Harmful Algal Blooms** (HABs) are increasing both in distribution and frequency
  - It was initially thought that this might just be because more people were looking for HABS (like with coral diseases); however, more evidence points to increased anthropogenic loading causing increases in Eutrophic waters are actually what are promoting these blooms
  - Time series of nutrient & *Pseudo-nitschia* in the Mississippi delta shelf supports this (Turner & Rabalais 1991)
- **Do natural phenomena also change the environment?**
  - Yes, California has naturally occurring algal blooms caused by seasonal & regional changes in the SSTs of the ocean currents
  - Figure 1. Schematic representation of the major currents on the US Pacific Coast. → (Adapted from Dodimead et al 1963).



- **Establishment of harmful algal species**
  - Naturally occurring
  - Introduced Specied
    - Currents
    - Ballast Water dumping (anthropogenic)
    - Aquaculture transporting (anthropogenic)
- **Ecological effects of HAB Species**
  - When they are very abundant…
    - Shading (blocking the sunlight)
    - Oxygen depletion (excessive respiration or decomposition)
    - Mechanical irritation (algal appendages can damage fish gills)
    - Toxin production (which can kill many high levels within the food web)

- *Note*: not all red tides are toxic, they're red tides because the cells have red pigments, and thus when there is a bloom in that species the water will appear red.
- *Note*: all toxin producing Algal blooms cause red tides, because only algal can be toxic without having a high enough density to change the water color

- **Cell Toxin Content** is affected by**…**
  - Light
  - Growth rate
  - Dissolved inorganic & organic nutrients
  - Temperature
  - Strain of species
  - May change (decline) when grown in culture
- **What advantage, if any, does the toxin confer on an alga**? To Avoid predation, &/or Kill their competition
- **Toxins produced by phytoplankton**:
  - Uptake of minerals (ex. Iron dumping to increase oceanic productivity)
  - Reduce the metal toxicity (ex. copper is a sensitive compound, in high concentrations it can poison cell, some algal are immune to copper, so they will accumulate it and then excrete it to poison their competition
  - Grazing deterrents (by poisoning predators you can avoid top down predation)

Harmful algal Blooms (**HABs**)

- Classified according to the symptoms produced by the toxins in humans & by the species believed to be producing the toxin
- West Coast has mainly ASP & PSP, whereas, the east coast has many many types of HABs

- **Paralytic Shellfish Poisioning** (**PSP**)
  - *Alexandrium* spp., *Pyrodinium bahamense*, *Anabaeana* spp.
  - Produces Saxitonins which are toxic at concentrations of 1000s cells/L
- **Ciguatera Fish Poisioning (CFP)**
  - *Gambierdiscus toxicus*
  - Produces Ciguatoxin/Maitoxin
- **Diarrhetic Shellfish Poisioning (DSP)**
  - *Dinophysis* spp.
  - Produces Okadaic acid, pectenotoxins, yessotoxins, which are toxic in low concentrations (100s of cells/L)
- **Neurotoxic Shellfish Posioning (NSP)**
  - *Karenia brevis* which produces Brevetoxins
  - Huge problem in Florida, especially because the toxin can be aerosolized
- **Amnesic Shellfish Posioning (ASP)**
  - *Pseudo-nitzschia* spp. (only diatom producing toxins,
  - Produces Domaic Acid which is toxic in concentrations >50,000 cells/L
  - Problem is that not all of these diatoms produce this toxin…and its hard to identify those that do
  - Animals (Domaic Acid Poisoning): anchovies & Krill pass the domaic acid to high trophic levels. Symptoms include: diarrhea, confusion, disoriented, scratching
    - 1961: birds died in California in Alfred Hitcock's home town → his movie "The Birds"
    - 1991: > 200 Sea birds died from this
    - 1998 & 2000: more than 150 sea lions strandings
- **Cyanobacterial Toxins**
  - *Microcystis, Nodularia, Anaaena, Lyngbia*…
  - Produces Microcystins and Nodularins, cylindrospermopsin, anatoxins, and saxitoxins
  - Spring bloom in march → increased chance of consuming toxic algal
- **Trophic Transfer of Algal Toxins**
  - Toxic Algal bloom → vectors (fat soluble, water soluble, etc.) → High Trophic Levels

- Coastal Toxic Phytoplankton is well studied, however, Oceanic Toxic Eukaryotic phytoplankton are not as well understood or as frequently studied. *Pseudo-nitzschia* producing Domoic Acid (causes ASP) is an oceanic toxic algal, which can also affect coastal waters

- **Scientific HAB Methodology**
  - **Cell Identification**: use epiflourescent probes which bind only to the species of interest; but it isn't available for all species
  - **Toxin Detection**: pregnancy test-like tests which are set for a certain toxin threshold
  - **Remote Sensing**: wont tell you the species, but it will show the geographic distribution & duration of the bloom

- **Monitoring Programs & Health Standards**
  - Safety standards for seafood: 20µg DA/g mussel tissue, 0.8µg STX/g in mussel tissue
  - Fluid handling system permits autonomous collection of samples & timed application of multiple reagents *in situ*, subsurface. → Problem is that this wont get the spatial distribution, it will only id it where the instrument is.
  - There is no way for civilians to know if their personal sea food catches have toxins…the toxins can only be indentified by scientific labs

The Role of Eutrophication in the Global Proliferation of Harmful Algal Blooms

(Gilbert et al. 2005 publication summary)

- **Cultural Eutrophication** is when coastal waters are polluted by nutrient loading
  - Human population growth & subsequent increases in agriculture cause major changes in the landscape
  - These changes in turn, increase anthropogenic runoff (agricultural, sewage discharge, etc.)
  - Increased use of chemical fertilizers since the 1950s has also worsened Eutrophication
  - While both Nitrogen & Phosphorous cause eutrophication, the effects of N_2 are much more serious because:
    - far more N_2 based synthetic fertilizers are used globally
    - N_2 is more prone to runoff than phosphorous which binds to the soil
    - N_2 limits primary production
    - *Note:* China consumes 25% of the global tones of nitrogen ($\frac{20}{85}$ million tonnes N_2 consumed globally per year)
  - Cultural Eutrophication is considered to be one of the largest global pollution problems

- Eutrophication promotes the geographic and temporal expansion of harmful algal bloom (HAB) species
  - Eutrophication seems to cause larger (spatial area), more toxic, longer lasting, and more widespread HAB distributions
  - China is a good example of the correlation between increased nutrient loading & HAB frequency in coastal waters , since 1970 China's nitrogen consumption went from <5 to > 20 million tones & their HAB outbreaks went up 20 fold

- ↑ human population → ↑sewage discharge → ↓ N:P molar ratio → ↑ HAB outbreaks

- HABs are ultimately caused by a complex interdependence of environmental factors, physiological factors, & trophic interactions
  - Thus they're not the result of eutrophication alone, and not all HAB species react the same way to eutrophication

  → there's no single criterion (like a N:P ratio) for HAB prediction

HAB Paradigms:
- **Old**: HABS are stimulated by nutrient loading only if there is a direct & visibly evident increase in the biomass of the algal bloom
- **New:** nutrient loading can indirectly stimulate HABs in subtle, but more significant ways over the long term,
  - ex. ↑hypoxia → kills the filter-feeding shellfish that would normally have consumed the HAB species→ unchecked HABs
- Nutrient inputs include:
  - **Inorganic** : ammonium, nitrate, nitrite, phosphate, & silicate
  - **Organic**: dissolved organic material (DOM), particulate organic matter (POM)

Note: atmospheric processes account for as much as 40% of the total inorganic & organic nitrogen input into aquatic systems

- The fraction of total fertilizer composed of organic material & manures is increasing at greater rates
  - ex. the amount of **urea** used in nitrogen fertilizers & feed additives went up more than 50% in the last decade

- quantification of DOM's composition, & the extent to which the DOM contributes to coastal eutrophication is very difficult
- some cyanobacteria grow faster on urea than on other nitrogen sources
- Florida's cyanobacteria are positively correlated with urea nitrogen uptake,& negatively correlated with inorganic nitrogen uptake
- **mixotrophs** are species that mix their methods of nutrient uptake
- organic nutrients can stimulate bacteria (or heterotrophs) which in turn can stimulate the development of heterotrophic or mixotrophic HAB species by favorably altering their microenvironment.

- Different strains within the same species can have different traits (growth, toxicity, blooms, response to nutrient, etc)
  - Nutrient pulses can be assimilated by cells of the same species at different rates depending on the strains present, their natural history, & their growth rates

- Ultimately the success of a given species depends on its ability to exploit the quantity, quality, timing, & intensity of available nutrients, as well as their interactions with competitors, consumers, and other environmental factors

Ch. 12: Human disease – toxic dinoflagellates and diatoms (Chapter Summary)

12.1 "Red tides" and "harmful algal blooms"

The spatial and temporal distribution of marine plankton growth depends on a many environmental factors. Attempting to understand these factors is important as plankton blooms not only affect the marine food webs, but blooms of toxic plankton can affect human health as well. Phytoplankton blooms are commonly referred to as "red tides" or "harmful algal blooms" (HABS). Unfortunately, both of these names have their limitations, as they cause misconceptions about the algal blooms; as not all algal blooms are red (or the bloom may not reach a high enough density for the red color to become obvious), and HABs imply that the algae must reach high densities in order to become harmful which is not always the case given the extreme toxicity of some plankton. Toxic algae and HABs have been occurring worldwide for many centuries, including bible references to red tides occurring around 1290BC; however, it was not until relatively recent changes in their distribution, increased frequency, and growing affect on the economy, made their study of such great importance. To better put toxic algae into context it is important to understand their place within the greater marine phytoplankton community; there are 4,000 known species of marine phytoplankton, of these 200 are known to cause "blooms," and within these 200 only 70 species are known to be toxic (most of these are dinoflagellates). Humans are affected by the algal blooms because we eat fish and shellfish which have accumulated high levels of the toxins by eating the phytoplankton.

12.2 Shellfish poisoning

Shellfish poisoning in humans is characterized by the rapid (minutes to hours after eating the contaminated organism) onset of the disease symptoms; these symptoms include diarrhea, vomiting, and various neurological problems. What makes shellfish poisoning especially difficult to control, is that the toxins are all nonproteinacious, which means that they will not neutralized through cooking of the contaminated meat.

12.2.1 Paralytic shellfish poisioning (PSP)

PSP has a global distribution and is perhaps the best known of the HAB resultant diseases found in humans. PSP is caused by saxitoxins produced by dinoflagellates. These saxitoxins bioaccumulate mainly in the tissues of shellfish (i.e. clams, oysters, mussels, etc) but can also be found in some crustaceans (such as crabs and lobsters). As is typical of shellfish poisoning, PSP is characterized by the rapid onset of its disease symptoms in humans (within minuets). The common treatment for PSP is stomach pumping and medically administered charcoal which is used to absorb the remaining toxins.

12.2.2 Neurotoxic shellfish poisoning (NSP)

NSP is caused by brevetoxins produced by the *Gymnodinium breve* dinoflaggelate species. *G. breve* is easily characterized by the red color & predictable seasonality (spring to summer) of its blooms. *G. breve* is also known to cause massive fish kills, which can in turn cause anoxic conditions along the coast. The wave action of the surf can also facilitate the transport of the brevetoxin aerosols by the wind onshore, these airborne toxins are known to cause eye irritation and shortness of breath in humans.

12.2.3 Diarrhetic shellfish poisoning (DSP) and azaspiracid poisioning

DSP is caused by the consumption of shellfish which have high levels of the okadaic acid toxin which is produced by two dinoflagellate genuses. DSP causes vomiting, diarrhea, abdominal pain, and is known to be a carcinogen possibly resulting in cancer along the digestive tract of humans. Bioaccumulation of this toxin in shellfish does not seem to be caused by phytoplankton blooms, but rather by long term exposure to relatively low levels of the toxins produced by the dinoflaggelates.

12.2.4 Amnesic shellfish poisoning (ASP)

The symptoms of ASP are quite different from the other types of shellfish poisoning, as ASP often results in disorientation, dizziness, and short-term memory loss, in addition to vomiting. ASP is believed to be caused by a neurotoxin (domic acid) produced by diatoms. This neurotoxin is also known to produce disease symptoms in marine birds and mammals.

12.3. Ciguatera fish poisoning (CFP)

CFP incidence is often unpredictable and has a localized distribution. The disease is caused by fat soluble toxins which bioaccumulate in fish, and can be transported through the food chain, which is why most human causes of CFP have been caused by the consumption of a contaminated large predatory fish. CFP symptoms often develop within hours of consuming the intoxicated organism, and include diarrhea, vomiting, abdominal pain, and various neurological abnormalities (such as numbness, weakness, sore teeth, and altered temperature sensations).

12.4 *Pfiesteria piscicida*

Pfiesteria is known to cause mass mortalities in estuarine fish; however, it has a very complex life cycle which makes it very difficult for scientists to study.

12.5 Why do dinoflagellates and diatoms produce toxins?

Toxins produced by dinoflagellates and diatoms are complex secondary metabolites, the diversion of energy required for the creation of these toxins is believed to have some ecological benefit to the organism.

12.6 Why are HABs and toxin-associated diseases increasing?

Phytoplankton blooms are generally the result of several environmental factors combining in specific combinations; these factors often include: temperature, sunlight, water stratification, nutrient levels, etc.

12.7 Monitoring and control of HABs

Remote sensing and satellite technology can be used to detect large scale and remote occurrences of HABs.

- **Background**

  On September 11, 2001, the world was shocked by the unprecedented attacks on New York City and Washington, D.C. As we came to grips with the tremendous scale of the tragedy, a new fear loomed that terrorists would strike again with biological weapons. The show follows *New York Times* reporters and authors of the best-selling book ***Germs: Biological Weapons and America's Secret War***, Judith Miller, Stephen Engelberg, and William Broad, as they delve into the past and present of biological weapons development, and grapple with the current threat of anthrax & other attacks

- **Summary of the documentary**
  - Anthrax, smallpox, ebola and more-- The frightening past and lethal future of biological warfare.
  - In the chilling aftermath of the terrorist attacks on the World Trade Center and Pentagon, America finds itself threatened by powerful biological weapons potentially as lethal-- but more portable-- than a nuclear arsenal.
  - NOVA follows three reporters from The New York Times as they travel the world researching biological weapons for their headline-grabbing bestseller, Germs, and for this in-depth documentary.
  - NOVA, along with authors **Judith Miller**, Stephen Engelberg and Pulitzer Prize-winner William Broad, trace the veiled history of "black biology" and discover the frightening truth about the United States' involvement in the development of biological weapons, devastating Soviet biological stockpiles, classified government research projects and a world totally unprepared for germ warfare.
  - visits abandoned Soviet germ factories in central Asia and goes to Kazakhstan, where dozens of Soviet scientists worked in the world's largest bioweapons facility.
    - NOVA viewers will accompany Miller as she visits abandoned Soviet germ factories in central Asia that produced enough biological poisons during the Cold War to kill everyone in the world many times over.
    - In Kazakhstan, 800 Soviet scientists toiled in perhaps the world's largest bioweapons facility, built expressly for creating a new, more lethal variant of anthrax.
  - profiles 2 Soviet germ scientists who defected to the US in the early '90s & shared details of the Soviet bioweapons program
    - With the implosion of the former Soviet economy, many of its bioweapons experts have taken their skills elsewhere
    - We'll meet two Soviet germ warriors who defected to the U.S. in the early '90s.
    - They'll detail how far the Soviet program progressed & what we have to fear from their discoveries
  - Dr. William C. Patrick III , the chief of the U.S. Army's **Fort Detrick** facility
    - Dr. William C. Patrick III spent over three decades at Fort Detrick
    - From 1951 to 1969, he developed germ agents for warfare.
    - When the US officially ended its offensive program in 1969, Patrick's work turned to germ defenses
    - *Note:* 7 ½ grams (see figure) of anthrax could infect everyone in a 14-story building.
    - Among the secrets revealed on the show is a U.S. plan to spray so-called "incapacitating" agents on the population of Cuba in order to make them too sick to resist invading American forces


Dr. Patrick showing how a weaponized biological agent (like athrax) can be used.

  - explores evidence of Saddam Hussein's secret bioweapons program and speculates on its possible links to terrorist group
  - recombinant DNA technology allows scientists to genetically manipulate germs, in order to make them more deadly
  - bioweapons experts, former Russian military leaders, biologists, unprecedented access to biological factories, and insights from high-level military personnel answer the toughest questions:
    - Where do germ weapons come from?
    - Who has access to them?
    - Can the air we breathe become a weapon?
    - And can America defend itself against germ warfare?
  - profiles ways the United States is working to fight bioterrorism and defend ourselves from a biological attack
    - To combat that threat, the Defense Advanced Research Projects Agency, or DARPA, has set up a futuristic research program encouraging "extreme innovation."
    - If it succeeds, the research may not only neutralize a BW attack, it could revolutionize medicine, too.
    - But until such advances are realized, the United States is far from prepared.
    - As recent events reveal, there is no single magic bullet that will defend us against a biological assault on American soil. NOVA and the *New York Times* present a disturbing and groundbreaking search for answers.

1) Why do you want to decode an organism's genome?

- By decoding the genome of a virus or infectious bacteria, you can better understand its virulence and hopefully design a vaccine (ex. this is currently being done with the H1N1 influenza virus)

2) Why would one want an agent that would attack plants and animals?

- Modern terrorists think in terms of wide-scale acts that will be the most **disruptive** but not necessarily the most destructive
- For this reason agriculture is a popular target of bioterrorists, as a diseased crop has to potential to kill the livestock feeding on it, humans feeding on it or the livestock, and cause massive economic damage (i.e. destroy the infrastructure)

3) What agents did Iraq develop as bioterror agents?

- By the time of the Gulf War cease-fire in 1991, Iraq had **weaponized anthrax**, **botulinum toxin**, and **aflatoxin** and had several other lethal agents in development (such as: gas gangrene, trichothecene, mycotoxins, wheat cover smut, ricin, hemorrhagic conjunctivitis virus, rotavirus, camel pox)
- Iraq is known to have unleashed chemical weapons in the 1980s, both during the Iran-Iraq war and against rebellious Kurds in northern Iraq. But there is no evidence that the Iraqi state has ever used its biological arsenal
- What is almost certain, though, is that this arsenal still exists in 2001. In fact, with the aid of former Soviet experts and UNSCOM inspectors kept at bay, the Iraqi arsenal is likely growing in power.

4) What is the ATCC and what agents were sought?

- **American Type Culture Collection** (ATCC)
- Established in 1914 and originally incorporated by scientists in 1925 to serve as a worldwide repository and distribution center for cultures of microorganisms
- Iraqis legally bought *Bacillus Antracis* which is a military strength form of anthrax developed by the US military

5) What is the significance of Fort Dietrich?

- As the Cold War intensified, American research into biological warfare accelerated.
- Fort Dietich was started in 1943 in Maryland, in order to develop biological weapons
- In 1948, the US built a huge sealed spherical test chamber at Fort Dietrich, to test the aerosol dispersal of pathogens
- The US found biological weapons were far more effective for large scale aerial attacks than typical weapons, as biological weapons had the ability to wipe out entire populations.
- Among the biological weapons developed here is the military strength form of anthrax

6) What are the 3 forms of Anthrax?

	Reparatory (Inhalation) Anthrax	**Cutaneous (Skin)** Anthrax	**Intestinal** Anthrax
Spores	Spores are airborne	Spores enter skin	Spores are eaten
Background	The **most severe** type of anthrax. As of 2001, there were 10 confirmed cases 4 of which were fatal	Most common. As of 2001, there were 7 confirmed cases.	Brought on by consuming contaminated meat. As of 2001, no cases had been reported in the past 50 yrs
Symptoms	May resemble a cold or flu virus, or a cough. Rapidly develops into severe breathing problems & total body function collapse.	Begins as raised, itchy bump resembling an insect bite. Develops into a blister & then painless ulcer. Fever, swelling, & headache may follow. Has characteristic black lesion.	Starts with nausea, appetite loss, vomiting, & fever, followed by abdominal pain, vomiting of blood, & severe diarrhea.
Prevention	Most inhalation anthrax exposures have occurred through mailed letters	Wash hands, bandage wounds, & treat blisters	Cook meat well.
Treatment	antibiotics, both for adults and children	antibiotics, both for adults and children	antibiotics, both for adults and children
Fatality	**~ 90%**	20%	25 – 60%

Note: Prior to the fall 2001 cases, the most recent case of inhalation anthrax in the United States was reported in 1976. This was one of only 18 cases of inhalation anthrax reported in the last 100 years. The risk of contracting any of the 3 types of anthrax remains very unlikely.

7) Why does one favor Anthrax (as opposed to an agent you can't control)?

- Anthrax is a disease caused by the bacterium known as *Bacillus anthracis,* which resides inactive in soils.
- Warm-blooded animals such as cows, sheep, & goats can contract the disease by eating food contaminated with spores. It is rare to find infected animals in the United States, its infectious to humans, it cannot be transmitted from person to person.
- Anthrax is preferred over other biological agents because:
    - Non-contagious → You can control how you spread it (Use plane, mail, etc. to release the invisible respiratory anthrax)
    - There's a delay between the delivery and the infectious effect of the microbe

8) What is the importance of Kazahstan?

- In Kazakhstan, 800 Soviet scientists toiled in perhaps the world's largest bioweapons facility, built expressly for creating a new, more lethal variant of anthrax. (currently its being maintained for scientific study)
- They made enough Anthrax to kill billions of people & enough biological weapons to kill the world 18 times over
- The soviets were much more efficient at producing these weapons than the US.
    - Ex. if the US could produce 1 metric ton of dried anthrax per year, the soviets could produce 4,500 metric tons

9) What was the thought behind Soviet bioweapons and what diseases did they pursue?

- They tested germs for maximum killing power → essentially tried to make wars as horrible as possible
- They concentrated on hemorrhagic diseases (which cause massive blood loss)
- They weaponized: Anthrax, Smallpox, Plague (*Yersinia pestis*), Marburg virus (lethal, no cure), Tuberculosis, and tried to weaponized Ebola

10) What is the significance of biological weapons versus explosives and nerve agents?

- Biological weapons have the ability to kill many more people than either explosives or nerve agents
- Biological weapons can also be more easily manipulated in order to control their: containability, method of spread, level of contagiousness, and overall pathicity
- Fort Dietrich's Dr. Patrick: "I can make a very good case for biological warfare as a more humane way of fighting war than with the atom bomb and chemical warfare. We can incapacitate a population with less than 1 percent of the people becoming ill and dying. And then we take over facilities that are intact. When you bomb a country, you not only kill people but you destroy the very facilities that are needed to treat them -- the electricity, water, all the infrastructure is gone when you bomb. So, to my way of thinking, if you must have warfare, if you use incapacitating agents, it is more humane then what we refer to now as "conventional warfare" with bombs and conventional weapons."
- Overall Biological weapons are **more persistent** & you get **more bang for your buck**

11) What is the significance of smallpox as a bioterror agent?

- Smallox is highly contagious & has a hemorrhagic form
- It is caused by the *Variola* virus which needs human cells to reproduce, but kills the cells in the process
- 30% of adults and 80% of children infected will die
- 1/3 of the survivors were blind, while other side-effects included skin scars and infertility in males
- Smallpox killed ~60 million Europeans in the 18th century alone
- Fort Dietrich's Dr. Patrick: "Smallpox represents one of the **ultimate weapons of biological warfare**, because …
    - You can grow it to a high concentration
    - You can dry it **(**it is very stable as a dried agent and lasts for years)
    - It requires only 2 or 3 virons, or virus particles, to produce a respiratory infection
    - You also start an **epidemic**, because if you infect 1 person, this person will probably infect 20 to 30 people, And these people, in turn, will infect a subsequent number of people
    - so smallpox, as well as plague, is something that keeps on giving and giving and giving."
- However, smallpox has very obvious disease signs, which would make it difficult for infected people to enter into closed systems when they are the most contagious → perhaps smallpox isn't the ideal bioterrorism weapon afterall

12) Where do we find smallpox today?

- By 1979, Worldwide vaccinationsenabled Smallpox to become the only disease to ever become completely eradicated from nature
- Today smallpox stocks can be sound at the US Center of Disease Control (CDC), and the Russian State Research Center of Virology & Biotechnology (VECTOR) in Koltsovo
- There are also evidence of genetically altered smallpox strains that are able to overcome immune responses (even in vaccinated individuals) created by the Soviets at the VECTOR institute in the 1990s

13) What was project Bacchus?

- **Project Bacchus** was a covert investigation by the Defense Threat Reduction Agency US Defense Department to determine whether it is possible to construct a bioweapons production facility with off-the-shelf equipment.
- Essentially used to see if a bioterrorism lab could be built without detection
- In the 2yr simulation, the facility was constructed, & production of anthrax-like bacterium was successfully completed
- They found that while growing germs in large quantities is easy, weaponizing them is much more difficult
    - The ionic charge on the germ particle makes airborne pathogenic dust difficult to manufacture
- Bacchus is the Greek god of wine, & wines are produced though fermentation, like the bacteria.

14) What is the importance of 2 to 5 micron size?

- Biological aerosols work best when they are between 2-5 microns, because they are small enough to get between the lungs & stick there

15) What is the significance of dry versus liquid bioterror agents?

- Dry agents are much harder to make then liquid, but they are **MUCH easier to disseminate**
- Electrostatic charge is an inherent property which makes microbes hard to disseminate
- The 2001 anthrax scare involved aerosol anthrax which was traced to a Texas strain isolated by vets

16) Why would one use non-contagious germs such as *Salmonella*?

- *Salmonella* was chosen because it was non-contagious & debilitating
- Thus you can incapacitate an enemy without killing them or harming their infrastructure

17) What were the US plans against Cuba?

- *Salmonella* was considered as a bioweapon against Cuba because…
  - Incapacitate the entire Cuban population in as little as 3 days to 3 weeks
  - Wouldn't kill the population
  - Wouldn't harm their infrastructure
  - The US army would be able to go in & take over the entire country with relative ease

18) What are the "oldie moldies"?

- Oldie moldies are standard disease agents such as: anthrax, plague, botchilism, etc.
- Modern technology enables scientists to take the genetic, recombinant DNA of the original "oldie moldies" and make them worse than ever before by improving their stability, reduce the number of cells needed to infect the respiratory system, etc.
- Thus instead of killing 1 million people with a traditional oldie moldie disease, the scientifically engineered form may now hill 10 million people

19) What was the mortality of plague epidemics?

- There are may types of Plague
- Bubonic plague (Black Death) is perhaps the most well known
  - Caused by the enterobacterium *Yersinia pestis*
  - Carried by fleas and rodents → bite humans and infect them
  - Disease has an incubation period of 1-6 days
  - Has the largest death toll of any non-viral epidemic: Killed 1/3 of Europe's population (~75 million people)
- Plague as a Biological Weapon
  - During WWII Japan developed a weaponized form of plague which they deliberately released on the Chinese, Koreans, & Manchurian civilians
  - After WWII both the US and Soviet Union began to develop different forms of weaponized plague
  - Inhaled Plague becomes Weaponized pneumonia → untreated pneumonic plague has a 50-90% fatality rate

20) What means are there to defend against bioterror?

- Bioterrorism is especially hard to fight because it tends to cause mass confusion, the severity & dire consequences of making the wrong choice tends to make individuals of authoritative positions refuse to take responsibility or action
- There are so many checks and balances to prevent the wrong choice from being made, that the system becomes so complicated & disjointed that decisions are very slow (if they can even be agreed upon)
- High tech equipment can be used to check for airborne pathogens
- Mass & frequent training of medical officials in order to promote awareness & symptom recognition