Microbes dominate our planet, especially the Earth’s oceans. The distinguishing feature of microorganisms is their small size, usually defined as less than 200 micrometers (µm); they are all invisible to the naked eye. As a group, sea microbes are extremely diverse, and extremely versatile with respect to their abilities to make and eat food. All marine microbes are too small to swim against the current and are therefore classified as plankton. First we will discuss several ways to classify marine microbes.

1. Size
Planktonic marine organisms can be divided into the following size categories:

<table>
<thead>
<tr>
<th>Category</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>femtoplankton</td>
<td>&lt;0.2 µm</td>
</tr>
<tr>
<td>picoplankton</td>
<td>0.2-2 µm</td>
</tr>
<tr>
<td>nanoplankton</td>
<td>2-20 µm</td>
</tr>
<tr>
<td>microplankton</td>
<td>20-200 µm</td>
</tr>
<tr>
<td>mesoplankton</td>
<td>200-2000 µm</td>
</tr>
</tbody>
</table>

In this laboratory we are concerned with the microscopic portion of the plankton, less than 200 µm. These organisms are not visible to the naked eye (Figure 1).

Figure 1. Size classes of marine plankton
2. Type

A. Viruses
Viruses are the smallest and simplest microplankton. They range from 0.01 to 0.3 um in diameter. Externally, viruses have a capsid, or protein coat. Viruses can also have simple or complex external morphologies with tail fibers and structures that are used to inject DNA or RNA into their host. Viruses have little internal morphology. They do not have a nucleus or organelles. They do not have chlorophyll. Inside a virus there is only nucleic acid, either DNA or RNA. Viruses do not grow and have no metabolism. Marine viruses are highly abundant. There are up to 10 billion in one liter of seawater!

B. Prokaryotes
This group is comprised of the Eubacteria (commonly called bacteria) and the Archeabacteria (commonly called archea) (Figure 2). They range in size from 0.3 to 10.0 um. Prokaryotes have four major cell types: spherical, rod-shaped, spiral and comma-shaped. Prokaryotes have an outer membrane and can have flagella, which are tail-like projections used for motility. Internally, prokaryotes do not have a nucleus or membrane-bound organelles. These organisms are also highly abundant with up to 1 billion cells in one liter of seawater!

C. Eukaryotes
The group Eukaryotes, which means “true nucleus” describes all organisms that have a nucleus. This includes single-celled organisms, plants, fungi, animals and even humans. In the field of marine microplankton ecology, some of the most important microscopic eukaryotes are the single-celled organisms. Single-celled Eukaryotes have a wide diversity of external morphology. They range in size from 2 to 200 um and can have complex shapes, modified for motility. They may also have bodies made out of calcium carbonate or silica plates or shells. Internally, eukaryotes have an organized cell structure with a nucleus and membrane-bound organelles.
3. Function
A. Autotroph
Autotrophs are organisms that are capable of making their own food. Photoautotrophs make food using energy from the sun. Chemoautotrophs use chemical energy to make food. They can be both prokaryotes and eukaryotes. The most abundant photosynthetic organism on earth is the marine prokaryote, *Prochlorococcus marinus*. Significant autotrophic eukaryotic microplankton include diatoms, dinoflagellae and coccolithophores. These organisms are capable of forming massive blooms, which can be seen in the ocean from space! Microscopic organisms form the base of the food web in the ocean, and are analogous to plants on land.

B. Heterotroph
Heterotrophs are organisms that cannot make their own food and therefore must consume food made by autotrophs. In the marine environment heterotrophic eukaryotes are responsible for recycling nutrients in the euphotic zone of the ocean. The nutrients are used by the autotrophs in food production. Because marine heterotrophic prokaryotes are so abundant in seawater, they have the potential to move a significant amount of carbon up the food chain. This part of the marine food web is called the “microbial loop” (Figure 3).

![Microbial loop](image)

**Figure 3.** Schematic drawing of the microbial loop. On the left you see the traditional marine food chain. On the right you see carbon moving up the food chain mediated by marine microbes.
Now that we know some of the major types of marine microplankton, let's discuss how scientists count and identify marine microbes. The smallest marine plankton, (microplankton, nanoplankton, picoplankton, and femtoplankton) are the most abundant organisms in the ocean, but because they are so small they can be challenging to study. There are many ways to learn about the microscopic plankton in seawater. Three that we will focus on in this lab are: 1. cultivation, 2. microscopy, and 3. DNA sequencing.

1. Cultivation
Cultivation is when an organism is grown in the laboratory, usually after isolating it from other organisms. To do this one must find just the right conditions and provide all the nutrients that the organism needs to replicate itself. The mixture of nutrients and water used to grow an organism is called a medium. Photoautotrophic plankton (or phytoplankton) need nutrients, water, and, most important, LIGHT. Heterotrophs must be provided with some form of organic carbon to grow. Some organisms are highly adaptable and can be grown very easily in the lab. Others are much more challenging. Marine microbiologists have been growing bacteria from seawater for over one hundred years, so it is remarkable that the most abundant species of bacteria in the ocean were completely unknown until just recently, because marine microbiologists were using the wrong type of medium! The traditional method of plating seawater on organic-rich solid medium produces many colonies (Figure 4), but these are not the most common ones in seawater. Although we are now getting better at growing bacteria and single-celled eukaryotes, there are still many other abundant species in the ocean that have never been grown in the laboratory. Different species have different needs and we do not yet know exactly what many of these organisms need to grow. Although we cannot grow many marine microbes, we keep trying because we can learn a lot about an organism by studying it alive in the lab.

Figure 4. Bacterial colonies growing on a solid medium. Each colony started out as a single cell, but multiplied on the plate to form a mound of cells big enough to seen by eye. Many different colors, sizes, and textures of colonies indicate that there are different types of bacteria present (Image: G. Steward).

2. Microscopy
A microscope is a device used to magnify and visualize objects that are too small to be seen with the unaided eye. The first light microscope powerful enough to see bacteria was made by Anton van Leeuwenhoek in the late 1600s. He observed large bacteria and single-celled eukaryotes in all sorts of samples including some in seawater. Even after
hundreds of years of refinement to the light microscope, however, most bacteria in the
ocean could still not be seen and counted with a traditional light microscope, because
they were too small and below the resolution limit. Because most bacteria could not be
easily seen, and because they would not grow on the common media, marine
microbiologists did not know how many bacteria were in seawater until the 1970s!
Around that time an important new microscopy technique called **epifluorescence microscopy** was developed and applied to marine microbiology. In epifluorescence microscopy, a sample is illuminated with specific wavelengths of light that cause the sample to fluoresce, and the light given off as fluorescence is collected and viewed. Phytoplankton, which contain chlorophyll, are naturally fluorescent and will glow red when illuminated with blue light (Figure 5). Other organisms that don’t have chlorophyll, need to be first stained with a fluorescent dye to be seen (Figure 5). Since all cells contain DNA, samples are usually stained with a fluorescent dye that binds to DNA so that all the cells can be seen. Even the smallest bacteria that are too small to be resolved by traditional light microscopy can be detected using this method, because they glow against a dark background. With this method, scientists made the first total direct counts of bacteria in seawater and were surprised to find that there were about 1 million per milliliter! The method was later refined even further so that now even viruses, most of which are much smaller than bacteria, can be easily detected.

![Figure 5. Epifluorescence microscopy images. The picture on the left shows phytoplankton fluorescing red because they contain chlorophyll (image modified from www.sinice.cz). The picture on the right shows heterotrophic bacteria glowing blue because they have been stained with a fluorescent blue DNA stain (Image G. Steward). Without the stain they would be invisible.](image)

**Electron microscopy** is another much more sophisticated form of microscopy that uses a beam of electrons rather than photons (light). The first electron microscope was built in 1931. Because the wavelength of electrons is much smaller than the wavelength of visible light, the electron microscope has a much greater resolving power and can be used to image not only cells and viruses, but even the detailed structures inside of them (Figure 6). Because most marine bacteria are so small, this is the best way to get a good close look at them.
Figure 6. Electron microscopy images. The picture at left shows a flagellated protist in the center with many bacteria of different shapes around it (Image: G Steward). The image in the middle is a section through a bacterium showing the lack of organelles inside; a characteristic of prokaryotes (Image: K. Lounatmaa/SPL). The image on the right is a section through a protist showing the internal structure and organelles characteristic of a eukaryotic cell (Image: C. Brussaard).

Microscopy is a great way to see and count marine microbes, but we cannot always tell different organisms apart based only on what they look like.

3. DNA Sequencing
DNA, which stands for deoxyribonucleic acid is the hereditary material of most living organisms. The information in DNA is stored as a “code” made up of four nucleotides: adenine (A), guanine (G), cytosine (C) and thymine (T). The nucleotides make up the “rungs” of the DNA ladder (Figure 7). An average microbial gene is made up of 1000 nucleotides, therefore an average gene would look like a DNA ladder with 1000 rungs. DNA sequencing is a method that determines the order of the nucleotides in long stretches of an organism’s DNA. This information is useful for several reasons. First, DNA is like a blueprint for the proteins that make up cells and tissues. Knowing what types of proteins an organism is capable of making can give you insight into their function and role in the environment. Second, DNA sequences are similar between closely related organisms. For example, if you had three DNA sequences of the same length: GGAATTCC, GAAATTCC, and TCGGCCAT, you can clearly tell that the first two sequences are more similar. They only differ by one nucleotide (the second G). One could predict from these sequences that the first two organisms were closer ancestors than the first to the third or the second to the third. If you have an unknown organism and you sequence a portion of its DNA, you can use that sequence to search for the identity of the organism.
In order to search for DNA sequences similar to that of an unknown organism, one must have a database of known sequences. Fortunately, several large databases of DNA sequences exist, several of which are open access. One of the most used databases is GenBank. GenBank is an open access, worldwide sequence database that is run by the United States National Institute of Health (NIH). The database contains millions of DNA sequences from everything from viruses to humans. You can search the database for sequences similar to yours using an online search tool called BLAST. Blast stands for basic local alignment search tool. This search engine will find all the DNA sequences similar to one being searched and report the results. Sequences can be displayed in a phylogenetic tree, which is a branching diagram showing the inferred evolutionary relationships among various biological species or other entities based upon similarities and differences in their genetic characteristics (Figure 2). In Figure 2 we see a phylogenetic tree that was made using the rRNA gene. This gene is particularly useful because all organisms have one, and it contains information about the evolutionary relationship of organisms.