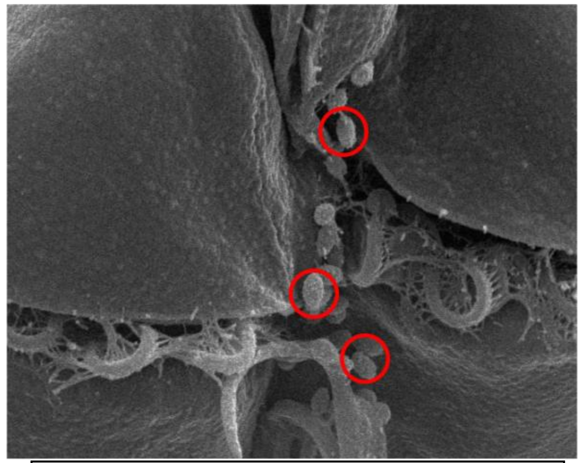
ECOHAB: *Karenia*

RECENT FINDINGS:
BACTERIA AND THE MICROBIAL LOOP

Bacteria in Marine Water

Bacteria are crucial to the health and functioning of the marine environment. In typical coastal waters, there can be as many as 5 million bacteria in one milliliter of water, or 250,000 bacteria in about one drop of water. These bacteria are responsible for a variety of crucial processes in seawater including photosynthesis, nitrogen (N) and phosphorus (P) fixation, and decomposition. Through N & P fixation, the bacteria covert these compounds into more readily usable, or bio-available, forms of the nutrients that can be utilized by other organisms. Likewise, through decomposition, bacteria release the nutrients bound in decomposing organisms back into the ecosystem to be recycled and used again.



Bacteria cells located on the sulcal groove of a *Karenia brevis* cell.

Bacteria play an essential role in the marine ecosystem in the cycling of elements in marine waters since they have a key role in the transfer of nutrients between sources and more complex organisms. One important pathway involving bacteria is called the **microbial loop**. The microbial loop consists of bacteria, which break down complex organic nutrients into simpler organic compounds, and small marine flagellates which feed on bacteria. In turn, the bacteria use the newly formed organic matter for their own growth. Ultimately, when the bacteria are consumed, those nutrients are returned to the food web. Thus, bacteria are natural recyclers, allowing otherwise inaccessible nutrients to be used again and again within the system. In some cases, the microbial loop actually allows **heterotrophic** production to exceed **autotrophic** production.

Marine phytoplankton in coastal waters (including bacteria) fix carbon and nutrients using energy from the sun. Grazers consume phytoplankton and are in turn consumed by other marine organisms in the classic food chain. All organisms excrete waste products in the form of particulate and dissolved organic matter (POM and DOM respectively) and can be utilized by bacteria for growth. When bacteria are consumed themselves, the POM and DOM utilized by the bacteria is effectively recycled and returned back into the system.

Scientists conducting experiments as a part of the ECOHAB: *Karenia* project have found that bacteria produce biomass at higher rates in large blooms of *Karenia*. This means that bacteria could be playing an important role in supplying and cycling nutrients to *Karenia* blooms. To determine what the role of bacteria is in Florida waters the following questions are being examined:

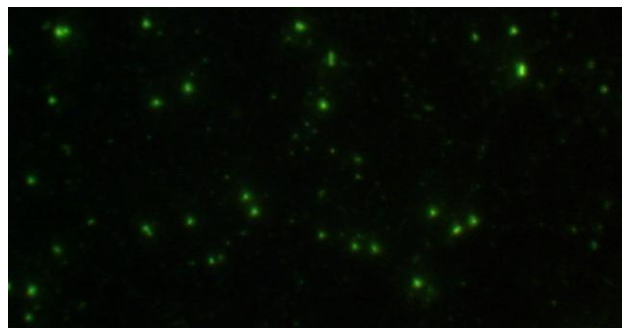
1: Are bacterial communities the same inside and outside of *Karenia* blooms? What bacteria are there and in what numbers?

2: What is the rate of bacterial production (growth or increase in cell number)? Does production increase or decrease when *Karenia* is present?

3: What nutrients are going through the microbial loop (being recycled) and at what rates do bacteria providing additional nutrients for *Karenia* blooms?

Bacteria research in ECOHAB:*Karenia* project

Bacterial enumeration: The two methods used to count bacteria are **epifluorescent microscopy** and **flow cytometry**. Under light microscopy, bacteria are very difficult to locate and enumerate due to their small size. Epifluorescent microscopy involves the use of the fluorescent DNA stain 'SYBR Green'. When this dye is added to a sample, it binds to DNA and "glows" green when illuminated by blue light. Through the use of an epifluroescent microscope, a sample is illuminated with blue light and the bacteria and viruses present in the sample glow green. Under magnification the glowing organisms can then be enumerated. Although accurate, this method is labor intensive due to the amount of time required to prepare and count a sample.



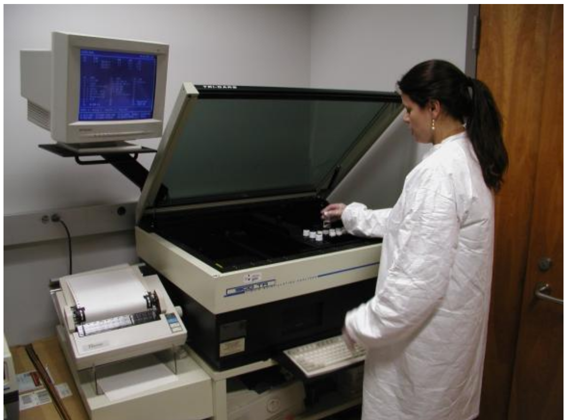
A water sample stained with SYBR Green dye. The "stars" are viruses and the "planets" are bacteria (1000x magnification).

Flow cytometry is an automated method for counting particles or cells in a stream of fluid. Flow cytometry has two benefits over the bacterial enumeration method, the time required and data provided. First, a machine called a flow cytometer passes a stream of cells (individual bacteria, particles, or plankton in single-file) by multiple laser beams. The light from the lasers can either be scattered by the cells or the light energy can be absorbed by the cells and re-emitted as a lower energy light color or **fluorescence**. At rates up to 300 cells per second, a flow cytometer can quickly and automatically provide accurate counts of bacteria in water samples as well as physical, chemical, and biological data from the light scattering and fluorescent properties of the cells.

DNA sequencing: DNA sequencing is a method which provides data in the relative percentage of different bacteria in the water samples. In this method, DNA is isolated and extracted from water sample filters and run through **Polymerase chain reaction**

(**PCR**), a process that allows scientists to replicate specific portions or strands of DNA. In this case, common bacterial species will be targets for PCR DNA replication. PCR provides more DNA to be sequenced and compared to databases of known sequences.

Bacterial production: This method quantifies the amount of carbon bacteria fix into cell tissue, (i.e. how much energy bacteria are supplying to the food chain) using the radioactive isotope Tritium (^{3}H). To measure bacterial production, tritium is attached to the amino acids **leucine** and **thymidine**. Leucine is a building block of many proteins produced by cells, and thymidine is one of the four amino acids found in DNA. Adding ^{3}H labeled leucine or thymidine to a water sample with bacteria, incubating for a period of time, and then measuring the amount of isotope found is cells gives a measure of the uptake of these amino acids. Further calculations and conversions allows for a measure of carbon fixation by bacteria within water samples. This provides data on the importance of marine bacteria to carbon cycling and how the bacteria and microbial loop are contributing to the transfer of carbon through the food chain.



Scientist working on a scintillation counter measuring sample radioactivity.