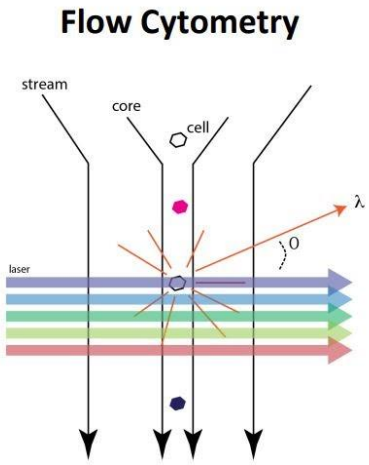
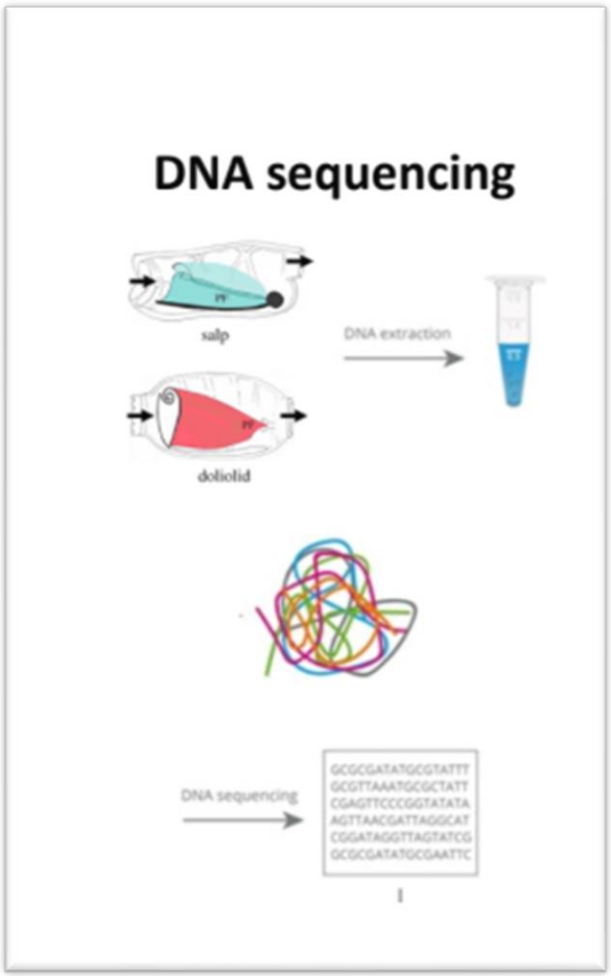


Graphic illustration of flow cytometry

Flow Cytometry is an important technique we use in our study of the predator-prey relationship between gelatinous grazers and ocean microorganisms. Flow cytometry detects and measures cell characteristics of the microbial prey consumed by gelatinous grazers. A flow cytometer instrument has three main components (fluidics, optics, and electronics) that work together to provide a complete system of cell analysis. We begin the process with a sample of phytoplankton cells taken from the gut of gelatinous grazers, suspended in a fluid and injected into a flow cytometer. It takes measurements of the cells, one by one, as they pass by its laser at rates of many thousands of cells per second. Since the cells we have extracted have a natural fluorescence, the light is absorbed and then scattered in a band of wavelengths that is characteristic to the cells and their components. In this process, the instrument's optical system generates a photocurrent which is digitized. The data is then gathered and processed by a computer for our scientific analysis.

Gelatinous Grazers and their microbial prey (phytoplankton) are the focus of our study. We seek to understand the impact gelatinous grazers (salps, appendicularians, doliolids, pyrosomes and pteropods) and their high feeding rates have on microorganism populations in oceanic food webs. Using flow cytometry, we can examine the microbe composition in the guts of gelatinous grazers and in the seawater samples to determine grazing rates, selectivity of prey, and mechanisms involved in filtration and retention. We take advantage of the autofluorescence of phytoplankton to detect, count, differentiate and sort them into groups based on their unique combinations of pigment and cell size. Flow cytometry provides an accurate, quantitative description of the microorganism community preyed upon by gelatinous grazers and helps us understand their role in food web models.

DNA Extraction and Sequencing



DNA sequencing serves as a vehicle in our discovery of marine gelatinous grazers and the diverse roles they play. DNA will be extracted from the guts of several taxa of gelatinous grazers for sequencing of the microbial community present.

These DNA technologies help us discover the microbial prey of gelatinous grazers. We are given an inside look, from the cellular level, on the food web of the ocean's microbial ecosystem. DNA sequencing allows us to see which organisms are eating which microbes and how the feeding mechanics of the different gelatinous grazers impact the prey they consume. This sequencing approach allows us to identify consumed prey with high taxonomic resolution, even if they are partially degraded or present in low abundance.

DNA extraction and sequencing helps us understand marine gelatinous grazers and the roles they play in their communities and ultimately global microbial processes.

SCUBA, the Self-Contained Underwater Breathing Apparatus, is a research tool that enables scientific diving underwater to retrieve samples or conduct field observation of gelatinous grazers and related predator prey relationships. The apparatus allows a single diver to operate autonomously and in situ without the need of support from the surface and gives mobility in underwater environments.

SCUBA diving in blue water is used to efficiently observe and collect gelatinous grazers in their underwater habitat to further determine predator prey relationships, DNA, cellular characteristics, and ecosystem viability. SCUBA based sampling allows researchers to observe and experiment with fragile gelatinous animals in their respective environments. Net based capture of gelatinous animals can mangle specimens beyond identification and introduce microbial contaminants into the study. With SCUBA, experiments can be performed with the feeding of these animals in their natural prey fields without the harmful effects of air exposure or nets.

In Situ **Sampling**

In Situ is a Latin phrase that translates to “on site” or “locally” and refers to the process of observing and sampling gelatinous grazers in their local environment. This means gelatinous grazers are not observed nor sampled under controlled conditions, but rather the variables that exist in an ocean ecosystem where they are present. With a SCUBA research tool and methodology, it is possible to observe and collect gelatinous grazers as they are in the environment.