***(Lesson Focus)***

***Learning Objectives***

***Lessons***

***Activities***

***Demos***

**SUMMARY.**

The activities in each day correspond to two scales of organisms in the aquatic microbiome: macroscopic and microscopic. **Day I** will focus on microscopic organisms in the marine environment, but will also contextualize the both scales of ecological inquiry. Students will visualize, culture, and create with aquatic microbes, and see results the next day.

**Day II** will focus on macroscopic organisms in the marine and freshwater microbiome, such as algae and copepods. Students will view large particles filtered from their own water samples under the microscope, and work with the biosensor organism Daphnia to understand environmental effects on small organisms.

***DAY I. Introduction to the Ecology of the Aquatic Microbiome.***

***Terms, Topics, and Guiding Questions -***

* Relevant terms in ecology + evolution *-* biotic, abiotic; communities and ecosystems; interactions (mutualism, parasitism); niche; competition and interdependence; diversity; photic zone/photosynthesis.
* Previous efforts to characterize or cultivate microbes of the sea - *e.g.,* TARA Oceans project - their results and implications
* Community-guided science
  + *Why it matters.*
* Guiding questions -
* *What is a microbiome?*
* *Where do these persist (soil, gut, and - most importantly - ocean!)?*
* *What are the functions of the ocean microbiome specifically?*
* *How do microbiomes differ - in terms of both function and composition - across aqueous bodies?*
* *How are global changes (climate change; rapid urbanization; pollution) affecting these systems?*
* *And what are the broader environmental impacts stemming from environmental microbiome alterations?*

***Interactive Lesson. Introduction to aqueous ecology.***

We will begin with a brief interactive introduction to relevant ecology terms (as above), with examples from the ocean and relevant freshwater systems.

We will then explore the ocean **across scales** in an interactive lesson. In this exercise, a pair of students will be given a card with a picture (or microscope image) of an organism, containing the name and a brief description. The whole group will work together to order these organisms by size from smallest (bacteriophage) to largest (blue whale) in a line they can generally agree on, placing cards together if they are unsure of the order in certain parts of the timeline. We will poll the class to see if anyone disagrees with any incorrect or ambiguous placements and ask students who have the right answer to explain their reasoning and move the card. Together, we will move around cards until we get the right order and have discussed any organisms students are unfamiliar with.

We will introduce more organisms after students have completed the size exercise, to give students a chance to further cement some intuition about the relative sizes of organisms in the ocean. This activity will include a brief discussion of mutualistic relationships between macroscopic and microscopic organisms, such as the Hawaiian bobtail squid (*Euprymna scolopes*) and *Vibrio fischeri*, a bacterium that colonizes in the light organs of the squid and contribute to its sophisticated camouflage abilities.

We will also build on the introduction to refine concepts such as relative lifespans of different organisms, eukaryotic vs. prokaryotic organisms, and whether viruses are truly “living.” We will briefly discuss visual and graphical representations of data, with an emphasis on orders of magnitude and the usefulness of log scales for data representation.

*Learning objectives*: students will get a broad introduction to foundational ecology concepts, particularly those related to the ocean. They will also begin to develop an intuition for different size scales and understand how organisms and communities at the smallest scales in an ecosystem contribute to the vast complexity and diversity at every level.

We will then break for 5-10 minutes before students reassemble in small groups (at least 2 per group) for the hands-on activities.

***Activity 1. Plating Hong Kong Ocean Water.***

Students will filter ocean water samples (their own or provided by instructors) and plate a selection of the water on two types of plates: marine broth plates, on which most marine bacteria should be able to grow; and Vibrio-selective plates, which will only allow bacteria from the genus Vibrio (or those closely related) to grow. Plates will grow overnight at room temperature and cultures will be visible within 24 hours.

***Activity 2. Making bio-art with Vibrio fischeri.***

Students will make their own designs with the luminescent bacteria *V. fischeri*. Students will use bacteria to draw on agar plates, and their designs will blossom overnight and be visible the next day.

***Demonstration. Phage plaque assay with Hong Kong Ocean Water.***

Instructors will demonstrate a plaque assay for students, as a way of visualizing the most abundant microorganisms in the ocean, bacteriophages. This exercise will reinforce phages’ important role in ocean microbiomes. Instructors will use ocean water samples collected by themselves and students to perform simple plaque assays on specific bacterial strains, which will be visualizable as “plaques” (empty spots) on a bacterial culture the next day.

**DAY II. *Environmental Impacts on Aquatic Microbes.***

Terms, Topics, and Guiding Questions -

* Building experiments: making/testing hypotheses, sample size, dilutions, data visualization

***Recap of day 1:*** *Students will examine the bacteria they cultured agar plates the previous day. They will take pictures and record observations, and be encouraged to discuss any interesting or unexpected results with the group.*

***Interactive lesson. The role of macroscopic particles in aquatic environments.***

Instructors will discuss the role of macroscopic particles and organisms in ocean and freshwater ecosystems, such as the the role of algae in forming communities of bacteria, and the role of copepods in nutrient cycling. This lesson will also include specific examples of human effects on aquatic ecosystems, such as pollutants and types of industrial run-off that have affected streams and oceans (e.g. plastic microbeads).

***Activity 1. Visualizing particles from water samples***

Students will filter their water samples with a larger filter than used the previous day, in order to collect particulate matter and any multicellular organisms. They will then rinse the filters and view what was collected under a microscope. They will record results and be encouraged to share any interesting or surprising results with the group.

*Learning objectives*: students will learn about the small scale factors and organisms present in the water in their own local environments, and gain an appreciation for their importance in ecosystems.

We will then break for 5-10 minutes before students reassemble in different small groups (at least 2 per group) for the next activity.

***Lesson 2. Biosensors.***

We will introduce students to the idea of “biosensor” species: organisms which respond to changes in the environment in a measurable way, which can be used to evaluate the health of the environment. For example, the microcrustaceans *Daphnia pulex* respond to many abiotic environmental factors, such as metals, or titanium dioxide, a common ingredient in sunscreen, with decreased growth rates, which can be measured over time.

***Activity 2.***

We will have students measure a response that can be seen in real time: heart rate, which can be measured by looking at single animals under a light microscope. We will then add one or more of the following abiotic factors to the Daphnia’s liquid: caffeine, ethanol, or temperature. We will take caffeine as our example: students will work in small groups to 1) formulate a hypothesis about the effect of caffeine on Daphnia 2) apply some knowledge from the scales exercise to calculate the relative size difference between humans and Daphnia in order to 3) decide how much caffeine to add and in what increments. Students will then measure and record the change in heart rate of the Daphnia samples upon adding different amounts of caffeine. We will graph these data points individually and all together for students to see and discuss whether the results confirm or refute their hypotheses. We will use the graphs of individual vs. combined data points as starting points to briefly discuss the effects that sample size can have on the quality of data collected and reproducibility of experiments.

Students will be invited to use their own water (post-filtration) to try to culture Daphnia. We can provide ideas for students to test the quality of the water they collected for pH and other factors, and ways to measure population growth rate.

*Learning objectives*: students will get an introduction to key processes and organisms occurring one step above the scale of bacteria in the ocean biome, and how humans have affected organisms and ecosystems at this scale.

**Potential Scratch integration:** Used USB webcams can be made into “hacked” microscopes for observation of Daphnia conditions in real time. We propose integrating these “microscopes” for use in Scratch, in which students can work in groups to develop programs which respond to the live webcam feed, including games or stories.

Timeline:

**Day I**

Intro : 20 mins

Scales of organisms Activity: 20 mins

Wrap up Activity and More Info: 15 mins

Break and splitting into groups: 10-15 mins

Activity 1: 25 mins

Activity 2: 25 mins

Wrap up: 5 mins

**Day II**

Recap of Day I: 15 mins

Macroscopic Particles Lesson: 15 minutes

Activity 1: 25 minutes

Break: 5-10 minutes

Lesson 2: 15 minutes

Activity 2: 25 minutes