Part I: Pre-activities for the classroom

Science background
Our oceans are filled with sounds of different ranges and intensities. The ambient, or background noise at any given place in the ocean is a highly variable mixture of sounds from any number of different sources\(^1\). Additionally, how a sound is perceived by a listener in the ocean depends on the local environmental conditions such as water temperature, salinity, seafloor terrain and underwater features (bathymetry), and depth\(^1\). A significant source of sound in the ocean includes those that originate from natural activity such as wind, rain, swell and current patterns, lightning strikes, seismic events, grinding sea ice (ice floes), as well as marine life such as fish, marine mammals, and snapping shrimp\(^1,2,3\). The other major contribution to background ocean noise are both purposeful and unintentional anthropogenic (man-made) sounds, including large commercial ships, sonar, polar ice-breakers, underwater explosions, and offshore drilling operations\(^1,2,3\). Unfortunately, this anthropogenic underwater noise has been increasing in both background and peak intensity levels, and it is estimated that this marine noise pollution has increased to a level ten times higher than it was only 20 years ago\(^1\). However, underwater sound has also been utilized by marine organisms such as whales and dolphins for a variety of purposes such as communication, navigation, and hunting for millions of years. Consequentially, whale and dolphin communication for navigation and hunting may be affected by man-made noise, and whale strandings linked to anthropogenic noise pollution highlights the need to address this issue\(^3,4\).

Scientists refer to the study of the behavior and propagation of sound in the ocean as “underwater acoustics”. Several properties of water allow sound to travel across vast distances of the ocean with great speed and efficiency\(^3\). To understand why sound travels well through water, an understanding of what sound is must first be gained. Sound is produced when an object vibrates, producing a **pressure wave** that radiates out in all directions away from the object similar to the ripples which travel away from a disturbance on the surface of a pond\(^3\). This wave of energy alternately compresses and decompresses (water) molecules in its path as it radiates outward from the source. These alternating patterns of compression and decompression of (water) molecules are what our ears detect as sound. Similarly, man-made sound-detecting devices such as **hydrophones** (or underwater microphones) detect these sound waves as well\(^3\).
Figure 1. Sinusoidal curve illustrating the basic components of a sound wave: frequency, wavelength and amplitude. In this example of a sound wave, the period of one cycle of this wave is 0.5 seconds, and the frequency of this wave is 2 cycles per second or 2 Hertz (Hz).

Source: http://oceanexplorer.noaa.gov/explorations/sound01/background/acoustics/acoustics.html

The basic parts of a sound wave are its amplitude, wavelength, and frequency. The amplitude of a sound pressure wave is related to its “loudness”, where the “taller” the sinusoidal curve, the louder the sound produced. The amplitude of a wave can further be analyzed using the decibel scale. The wavelength of a sound wave is related to the ‘pitch’ of the sound, and can simply be quantified by the distance between either the peaks or the troughs of the wave. Frequency is the measure of the number of wavelengths (or cycles) that pass by a given point over a given amount of time. Frequency is measured in the unit Hertz (Hz), which is an estimation of the number of cycles per second; higher frequencies are perceived as higher pitched sounds to the human ear.

Underwater acoustics provide scientists with a variety of information about the ocean environment including the movement of polar ice sheets (which relates to the processes of global warming), underwater seismic events like volcanoes and earthquakes, as well information about marine organisms such as whales, fish and coral reefs. Researchers at NOAA’s National Marine Fisheries Service, along with collaborators at the Hawai‘i Institute of Marine Biology (HIMB), have recently developed an instrument that can acoustically measure and record long-term biological processes and human activity in remote locations such as in the Northwestern Hawaiian Islands. This instrument, called an Ecological Acoustic Recorder (EAR) allows marine biologists to passively record the underwater acoustic activity of a variety of marine mammals, fish, crustaceans, etc. in both near-shore coral reef environments, and deeper open-ocean, or pelagic environments. Passive acoustic tools such as the EAR allow researchers to both monitor and characterize the activity of marine life (including the occurrence of specific species in a given area, their distribution, and perhaps trends in levels of their activity), and to monitor levels of human activity.
Data from NOAA EARS are being collected continuously, and there are now numerous recordings of our marine environment which are still being analyzed. Some interesting preliminary data of EAR recordings off O‘ahu may hold some very important and profound insight into marine behavioral acoustic ecology. NOAA scientists have observed that snapping shrimp produce pulses of sound in a crepuscular fashion (that is, peaks of snapping shrimp sounds occur at dawn and dusk). Furthermore, researchers have found that snapping shrimps are the most significant source of biological sounds on coral reefs, and these crustaceans likely play an important role in the acoustic symphony of the coral reef ecosystem.

Additional hydrophone research at the Hawai‘i Institute of Marine Biology
There are several research laboratories working on the subject of ocean acoustics and utilizing instruments like hydrophones in their work. Dr. Tim Tricas and his research group study the acoustic activity of fish on coral reefs. The Tricas Lab deploys hydrophones and EARS on various reefs around the Hawaiian Islands in an ongoing effort to record and characterize sounds made by fish associated with corals reefs. Dr. Tricas hopes to some day create a comprehensive acoustic library for Hawai‘i reef fish, in order to assist in monitoring the underwater environment to improve management and conservation efforts on these reefs. To date, the Tricas Lab has recorded and analyzed the frequency of more than 30 distinct fish noises (consisting of clicks, grunts, hums, and other sounds).
Drs. Paul Nachtigall, Whitlow Au, and Marc Lammers, who are part of the Marine Mammal Research Program (MMRP) at HIMB, study the bioacoustics of a variety of marine mammals and also work to develop technologies used to better understand sound production and perception by these animals. Among numerous other things, researchers at the MMRP study hearing and echolocation of dolphins and small whales, patterns of humpback whale chorusing, social acoustics of Hawaiian spinner dolphins, and the detection and characterization of the bioacoustics of several other marine organisms. The MMRP is also investigating the effects of underwater anthropogenic noise such as Navy sonar on marine mammal physiology, in an effort to better understand and possibly prevent the stranding of marine mammals.

Classroom Laboratory
As a prelude to coming to HIMB, students should carry out their own online research into the subject of underwater acoustics. We suggest the following websites for students to refer to:

- http://www.dosits.org/science/sciencesummary/

Students should provide a written definition, in their own words, of the following terms:

- Sound
- Amplitude
- Wavelength
- Frequency
- Hertz (Hz)
- Decibel scale
- SOFAR

Students should also listen to the brief (2 minutes each) example sounds provided online at http://www.pmel.noaa.gov/vents/acoustics/sounds.html by the NOAA Acoustic Monitoring...
Program\textsuperscript{5} to get an idea of the enormous variation in underwater sounds (i.e., seismic, whale, man-made, and mystery sounds). As extra credit, students can list several different types of organisms that produce sounds (e.g. known sound-producing fish, invertebrates, mammals, etc.), and a description of how that organism is able to make those sounds (i.e., snapping shrimp rapidly close a modified claw which creates a cavitation bubble, a pocket of air which then implodes, resulting in a very loud “snap”). Be sure to include any references used in your write up.

What to expect during the field trip day
During your field trip day to HIMB you will collect a variety of sea creatures in various microhabitats surrounding HIMB. Next, you will isolate snapping shrimp and other invertebrates typically found in these microhabitats, then you will record and analyze the sounds produced by them under various experimental conditions using a hydrophone.

Wearing reefwalkers, tabi or booties while collecting organisms in the water is MANDATORY.
Part II: Field trip day at HIMB

Introduction
A) Hydrophone data (or sounds recorded underwater) are typically analyzed by researchers in the form of spectrograms. A spectrogram is a visual representation (an image) of the sound and its characteristics (i.e., frequency, amplitude, intensity, etc.) over a given time period. In this lab, you will generate your own spectrograms and learn how to analyze the data you record. Below are a couple of examples of data recorded by a hydrophone at HIMB:

![Image of spectrograms]

Figure 6. Illustration of the two ways researchers can visualize underwater acoustic data in the form of a spectrogram using software developed at Cornell University: Raven Lite 2.0. Both images show the acoustic data recorded by a hydrophone of a single snap from an Alpheus heeia snapping shrimp in a glass aquarium. The top image visualizes the snap in a waveform display format. This format allows you to analyze the sound in terms of its ‘loudness’ (Y-axis in decibels) verses time (X-axis in seconds). According to the data, this snap had a peak intensity of about 6 kU (kU is measurement analogous to decibels), and the snap had duration of about 0.2 seconds. The bottom image is the same snap visualized in a spectral frequency display format. A spectral frequency display allows you to visualize the frequency (Y-axis in kilohertz) of the snap sound wave over time (X-axis in seconds).
In this activity, you will break into groups of four students and collect the invertebrate species living in two microhabitats on the reef flats surrounding HIMB (see Appendix I for images of the two microhabitats). Collections should ideally be timed to occur during a low tide. Each group will gather tufts of *Gracilaria salicornia* (also called Gorilla ogo), an invasive algae native to the Indian Ocean and South Pacific Islands. The snapping shrimp *Alpheus heeia* lives within *G. salicornia*. Also, if you can spot the red invasive sponge *Mycale armata*, this should be collected as well because *M. armata* is home to another species of snapping shrimp called *Synalpheus paraneomeris*. Occasionally, this second species can be found in *G. salicornia* as well. Photographs of the invasive algae and sponge, and the two snapping shrimp species can be found in Appendix I. During the collection, you will use a scoop net and wear gloves to prevent any marine life from stinging your skin (be especially careful of the iridescent fireworm which is capable of stinging exposed skin, and lives in the *G. salicornia*). You will be surprised to see how many different kinds of invertebrates are living in a small clump of *G. salicornia*; try your best to identify them using the guide in Appendix II. Among the many invertebrates you may find, keep your eye out for the two species of snapping shrimp, which are needed during your experiment. While sorting through your collected *G. salicornia*, you only need to find ONE individual snapping shrimp to successfully attempt your experiment, but if you cannot find any, it may be possible to borrow shrimp and/or other invertebrates from your classmates.

Figure 7. Illustration of an example of one of the “quieter” or lower frequency and decibel level sounds made by the same species as Fig 6, *A. heeia*, in a glass aquarium. Three snapping shrimp were housed individually in separate plastic chambers which were placed into a single glass aquarium. Frozen squid was placed at the entrance to the chambers, and after about 5 minutes, the shrimp began to make the sounds which are visualized by the spectrogram above. These “feeding” sounds have not been previously reported in scientific literature, and are examples of the kind of new information you may discover during your time at HIMB.

B) In this activity, you will break into groups of four students and collect the invertebrate species living in two microhabitats on the reef flats surrounding HIMB (see Appendix I for images of the two microhabitats). Collections should ideally be timed to occur during a low tide. Each group will gather tufts of *Gracilaria salicornia* (also called Gorilla ogo), an invasive algae native to the Indian Ocean and South Pacific Islands. The snapping shrimp *Alpheus heeia* lives within *G. salicornia*. Also, if you can spot the red invasive sponge *Mycale armata*, this should be collected as well because *M. armata* is home to another species of snapping shrimp called *Synalpheus paraneomeris*. Occasionally, this second species can be found in *G. salicornia* as well. Photographs of the invasive algae and sponge, and the two snapping shrimp species can be found in Appendix I. During the collection, you will use a scoop net and wear gloves to prevent any marine life from stinging your skin (be especially careful of the iridescent fireworm which is capable of stinging exposed skin, and lives in the *G. salicornia*). You will be surprised to see how many different kinds of invertebrates are living in a small clump of *G. salicornia*; try your best to identify them using the guide in Appendix II. Among the many invertebrates you may find, keep your eye out for the two species of snapping shrimp, which are needed during your experiment. While sorting through your collected *G. salicornia*, you only need to find ONE individual snapping shrimp to successfully attempt your experiment, but if you cannot find any, it may be possible to borrow shrimp and/or other invertebrates from your classmates.
Once you are ready, your instructor will record your experiment using a hydrophone, and demonstrate how to use the computer software, RavenLite, to investigate the various types and intensities of sounds produced by the snapping shrimps under different experimental conditions housed in glass aquaria.

Below is a series of questions which will help to give you ideas about different hypotheses and tests you can perform during your time at HIMB. Read them carefully, and start to think about what question you’d like to address (hypothesis) and the experiment you’d like to conduct (method). A couple of EXAMPLE hypotheses and methods have been provided for you (see Appendix III). Please read them carefully and use them as a guide when thinking about the hypothesis you want to test and in writing your own report.

**Guiding Questions**

In groups of four develop a single hypothesis, one that you are interested in exploring, after thinking about the following questions:

- Do you expect each creature to make distinct, recordable sounds in captivity? Why?
- Will snapping shrimp make sounds without any external stimuli (i.e., gently touching them with a plastic rod, feeding, exposing them to other invertebrates, etc.)?
- What are the biological reasons for being able to create a powerful snap underwater? Do you think the different species of snapping shrimp use their snapping abilities for the same reasons?
- Do similar sized snapping shrimp of the same species make snaps of similar intensity? Is this always true? What about similar sized snapping shrimp of different species?
- Do snapping shrimp make sounds when they feed? How do these sounds differ from their snaps?
- Will snapping shrimp make different sounds in response to different types of food? Do different species of snapping shrimp respond in the same way to food?
- Which other species of invertebrate found in the microhabitats (i.e., crab, fire worm, feeble shrimp, etc.) will elicit a snap from the snapping shrimp? Why? Do you think the snap will be a “predatory” or “defensive” snap?
- How many snaps do you expect a snapping shrimp to make in a period of time (e.g., one, three, five minutes)? Will there be a pattern to the snaps, or will they be singular in nature? Why? Will this pattern vary by species of snapping shrimp?

**Tools available**

In the lab we can conduct listening experiments using a hydrophone by manipulating the environment surrounding the snapping shrimp and other invertebrates you found. Glass vials with open tops will be provided for you to conduct your experiment in. You can isolate
individuals and listen to each animal separately in the vials, or together as you manipulate the conditions within them. You will have approximately 3 minutes to conduct whatever experiments you devise. This means that you can test a continuous 3 minute experiment, or, for example, break up your investigation into something like a series of 3 one minute recordings where you test several approaches to explore your hypotheses. We will demonstrate how to use these tools in the lab, after which you will have all the necessary information to create and test your own hypotheses (see example in the Appendix III).

**Materials to bring:**
- Shoes to get wet/muddy
- Pen/pencil and paper/notebook

**Materials available:**
- Hand “scoop” net
- Metal forceps
- Glass vials
- Petri dishes (60 x 15 mm)
- Dissecting scope (integrated with digital camera if available)
- 5 gallon glass aquaria
- Marantz handheld solid state recorder (Model PMD661)
- High Tech hydrophone with pre-amplifier (Model HTI-96-Min)
- Metric ruler
- Raven Lite 2.0 software
- Personal computer with speakers
- Squid, fish, shrimp, or pellet feed

**Exploring the data (data processing and organization)**

A) For each sound recorded, you should be able to generate 2 separate audio spectrograms; a waveform and spectral frequency image using the free, downloadable program, Raven Lite 2.0 ([http://www.birds.cornell.edu/brp/raven/RavenOverview.html](http://www.birds.cornell.edu/brp/raven/RavenOverview.html)).

B) You will generate spectrograms of the whole time interval you recorded. You will also zoom in on specific interesting sounds you observe within the longer recording, and generate other spectrograms to be used in more in-depth analyses.

**What you will do with the data back in the classroom?**

When you return to your home classroom after your visit to HIMB, you will need to begin the process of analyzing your data. Your instructor should have already downloaded a copy of Raven Lite 2.0 for you to use in class. You will compare results, discuss your hypotheses, make conclusions, and reflect on the importance of your findings.
Part III: Post-activities back in the classroom

Step by step analysis
Begin class with a general discussion of your experience at HIMB. Were you prepared for the lab exercise and able to complete the activity? Did you enjoy your time there? Now begin to discuss the subject matter:

A) Were your hypotheses supported by the data? Did all of the invertebrates you collected make sounds? Did the snapping shrimp snap in response to your predetermined external stimuli? Was there a pattern to the snap, and did the number of snaps vary? Do you think the snapping shrimp snapped in an offensive or defensive manner?

B) Was there a difference in the snapping response between the two species of snapping shrimp? Did the two species of snapping shrimp respond in the same way to the various other invertebrates found in their microhabitats? Did the number of snapping shrimp versus the number and type of other invertebrate species vary between groups? Did the peak intensities (amplitude and frequency) vary between snaps of snapping shrimp?

C) Do your findings influence the way you view the ocean? Did this activity make you more aware of sounds (natural or man-made) in the ocean?

Lab report
For your laboratory exercise at HIMB, you will be expected to eventually produce an in-depth laboratory report including the following independent sections:

- **Title**: summarize the entire experiment in several words.

- **Introduction**: in one half to three-quarters of a page describe the subject of ocean acoustics, how sounds are used by animals in the ocean as well as how humans now contribute significantly to ocean noise pollution, and why we should care.

- **Hypothesis**: based on your background knowledge of ocean acoustics and the information about what tools are available in the lab at HIMB, describe the hypothesis you tested.

- **Materials and Methods**: develop and describe, in detail, the experiment you conducted to test your hypothesis; include all of the materials you used to complete it as well.

- **Results**: compile your data and express them visually and where appropriate in graphs, tables, or figures.

- **Discussion**: analyze your data in essay form; discuss the results, emphasize what did and didn’t work, and propose a new experiment or changes to your original experiment which may help explain the results.

- **Conclusion**: in a paragraph or so, summarize your results and make concise conclusions about them; also include a sentence or two conveying your general conclusions about your
results in the context of bioacoustics in the ocean and why sounds are important to life in the ocean.

References

Science background information condensed and/or compiled from the following sources:


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Appendix A:
Relevant Next Generation Science Standards

Science and Engineering Practices
1. Scientific Knowledge is Open to Revision in Light of New Evidence
   a. Most scientific knowledge is quite durable, but is, in principle, subject to change
      based on new evidence and/or reinterpretation of existing evidence.
2. Engaging in Argument from Evidence
   a. Evaluate the claims, evidence, and reasoning behind currently accepted
      explanations or solutions to determine the merits of arguments.
3. Analyzing and Interpreting Data
   a. Apply concepts of statistics and probability (including determining function fits to
      data, slope, intercept, and correlation coefficient for linear fits) to scientific and
      engineering questions and problems, using digital tools when feasible.
4. Obtaining, Evaluating, and Communicating Information
   a. Communicate scientific information (e.g., about phenomena and/or the process of
      development and the design and performance of a proposed process or system) in
      multiple formats (including orally, graphically, textually, and mathematically).
5. Planning and Carrying Out Investigations
   a. Plan and conduct an investigation individually and collaboratively to produce data
      to serve as the basis for evidence, and in the design: decide on types, how much,
      and accuracy of data needed to produce reliable measurements and consider
      limitations on the precision of the data (e.g., number of trials, cost, risk, time), and
      refine the design accordingly.

Connections to Nature of Science

6. Scientific Investigations Use a Variety of Methods
   a. Scientific inquiry is characterized by a common set of values that include: logical
      thinking, precision, open-mindedness, objectivity, skepticism, replicability of
      results, and honest and ethical reporting of findings.

Disciplinary Core Ideas
1. LS4.D: Biodiversity and Humans
   a. Biodiversity is increased by the formation of new species (speciation) and
      decreased by the loss of species (extinction).
   b. Humans depend on the living world for the resources and other benefits provided
      by biodiversity. But human activity is also having adverse impacts on biodiversity
      through overpopulation, overexploitation, habitat destruction, pollution,
      introduction of invasive species, and climate change. Thus sustaining biodiversity
      so that ecosystem functioning and productivity are maintained is essential to
      supporting and enhancing life on Earth. Sustaining biodiversity also aids
      humanity by preserving landscapes of recreational or inspirational value.
2. LS2.D: Social Interactions and Group Behavior
   a. Group behavior has evolved because membership can increase the chances of
      survival for individuals and their genetic relatives.

Crosscutting Concepts
1. Stability and Change
   a. Much of science deals with constructing explanations of how things change and how they remain stable.

2. Cause and Effect
   a. Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects.

Common Core State Standards Connections:
ELA/Literacy -
1. SL.11-12.5
   a. Make strategic use of digital media (e.g., textual, graphical, audio, visual, and interactive elements) in presentations to enhance understanding of findings, reasoning, and evidence and to add interest. (HS-LS1-4)

2. RST.11-12.1
   a. Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account. (HS-LS2-2)

3. RST.11-12.7
   a. Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem. (HS-LS2-6)

4. WHST.9-12.7
   a. Conduct short as well as more sustained research projects to answer a question (including a self-generated question) or solve a problem; narrow or broaden the inquiry when appropriate; synthesize multiple sources on the subject, demonstrating understanding of the subject under investigation. (HS-LS2-7)

5. WHST.9-12.9
   a. Draw evidence from informational texts to support analysis, reflection, and research. (HS-LS4-4)

6. WHST.9-12.2
   a. Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes. (HS-LS2-2)

Mathematics -
1. MP.2
   a. Reason abstractly and quantitatively. (HS-LS2-2)

2. HSN.Q.A.3
   a. Choose a level of accuracy appropriate to limitations on measurement when reporting quantities. (HS-LS2-2)
Appendix B:
II. Examples of various invertebrates found in *G. salicornia*

Left column, top to bottom: Iridescent fireworm (*Eurythoe complanata*), Small crab #1 (*Liomera spp.*), Small crab #2 (*Liomera spp.*).

Right column, top to bottom: Brittle starfish (*Ophiocoma brevipes*), Feeble shrimp (*Palaemon debilis*), Hawaiian blood-spotted crab (*Portunus sanguinolentus hawaiiensis*).

Photo credit: Kai Fox
Appendix C. 
An example of how to phrase a hypothesis and materials and methods section

The following section describes an EXAMPLE of a possible hypothesis and experiment you could conduct at HIMB. By reading through this example, you will also get a better sense of how to use the tools available in the lab. Your experimental design must be DIFFERENT from this activity, and must directly address your hypothesis. The techniques and tools (i.e., the plastic jars, collecting invertebrates from microhabitats, recording sound waves with the hydrophone and recorder, and analyzing the sound intensities with the software) described in this experiment are the same ones you will be using, but you must come up with your own question and your own way to answer it.

Hypothesis (EXAMPLE):
A) If snapping shrimp are exposed to other species of invertebrates (or heterospecific) which can be found naturally in close proximity to their living environment (shrimps, crabs, brittle stars, etc.) for 5 minutes, they will respond with a series of defensive (or offensive) snaps to maintain their “territory”.

B) If snapping shrimp are exposed to frozen squid for 5 minutes, they will begin to feed and in the process produce distinct, measurable underwater sounds which are different than their typical “snap” which is used as a defensive behavior.

Methods (EXAMPLE):
A) Exposure of snapping shrimp to heterospecific invertebrates

1. I collected a snapping shrimp (A. heeia) and a feeble shrimp (Palaemon debilis), from a tuft of G. salicornia. Then I separated the animals into individual 50 mL plastic containers.

2. I assembled my hydrophone apparatus such that the hydrophone was suspended 5 cm off the bottom in a small aquarium filled with fresh seawater.

3. I then placed a metric ruler on the bottom of the tank, with one end directly under the center of the hydrophone.

4. Next, I scooped the feeble shrimp from its container and placed it into the container housing the snapping shrimp. I quickly snapped the lid closed, and submerged the plastic container (containing both shrimps) into the small aquarium.

5. I placed the plastic container with the two shrimps at the end of the ruler opposite the hydrophone.

6. I allowed the surface of the water in the aquarium to settle, and then I began recording acoustic data by pressing the “Record” button on the recorder. I took notes during the recording to remind me what I did during the experiment and what I observed at what time.

7. Following 5 minutes of recording, I stopped the recording by pressing the “Stop” button on the recorder. I then removed the plastic container from the glass aquarium and again separated the invertebrates into their own individual containers with fresh seawater.
8. I then downloaded the file containing my data from the recorder to the computer at my lab station. I then opened the file using the Raven Lite 2.0 software and began to analyze my recording data.

9. At the end of the day, I released all invertebrates back into the ocean (near the point of capture), however all invasive algae and sponge were dried and thrown away in the trash.

B) Exposure of snapping shrimp to frozen squid

1. I used the pre-existing hydrophone apparatus described above in steps 1-3.

2. I kept a snapping shrimp in a separate plastic container, snapped the lid closed, and submerged the plastic container into the small aquarium.

3. I placed the plastic container with the snapping shrimp at the 15 cm mark on the tank’s bottom.

4. I then placed a small (1 cm$^2$) piece of defrosted squid on top of the holes drilled in the lid of the plastic container.

5. I allowed the surface of the water in the aquarium to settle, and then I began recording acoustic data by pressing the “Record” button on the recorder. I took notes during the recording to remind me what I did during the experiment and what I observed at what time.

6. Following 5 minutes of recording, I stopped the recording by pressing the “Stop” button on the recorder. I then removed the plastic container from the glass aquarium, and added some fresh seawater to the container.

7. I downloaded the file containing my data from the recorder to the computer at my lab station. I then opened the file using the Raven Lite 2.0 software and began to analyze my recording data.

8. At the end of the day, I released all invertebrates back into the ocean (near the point of capture), however all invasive algae and sponge were dried and thrown away in the trash.