Session 4: The mystery of the disappearing sea star

Introduction: Sea stars are important predators in the rocky intertidal. They control populations of many organisms but especially the California mussel. Without sea stars, mussel populations become much larger, crowding out other organisms and decreasing the biodiversity of the rocky intertidal ecosystem. Between 2012 and 2014, there was an 81% decline in the *Pisaster ochraceus* sea star population [(Schiebelhut et al. 2018)](). Scientists thought that the dying sea stars looked like they were melting or wasting away, so the cause of the sea star decline became known as sea star wasting syndrome. Finding the cause of the syndrome posed a great science mystery that needed to be solved. In this session, learners with the help of the learning guide will solve the mystery of the decline of the sea star population, learn about the structure of DNA, and how DNA sequencing is an important tool that scientists can use to identify disease-causing agents or pathogens.

Goals:
- Have fun learning about sea stars, rocky intertidal ecology, what DNA is, DNA structure, and how to use DNA to identify the cause of sea star wasting syndrome.

Learning Objectives:
- Learners will recognize that sea stars are important predators and ecosystem engineers in the rocky intertidal.
- Learners will be able to explain that DNA is made of four types of molecules represented by the letters A, T, C, and G.
- Learners will be able to explain that a DNA molecule forms a complementary pair with another specific DNA molecule: A always pairs T and C always pairs with G.
- Learners will predict complementary pairs of an unknown DNA fragment.
- Learners will perform an activity to recognize that complementary pairing means that one DNA strand always has a complementary.
- Learners will describe how we can use DNA as a tool to identify pathogens.
- Learners will identify the organism to which a sequence of DNA belongs by comparing it with other complementary sequences and selecting the sequence with the fewest mismatches.
- Awareness that environmental stressors can make an organism more susceptible to infection by viruses or bacteria.
- Introduce citizen science tools to monitor sea star wasting syndrome.

Session Plan Outline:
- Power Point presentation with discussion questions: the learning guide's handout has the questions and the answers, the learner's handout just has the questions
- Introduce sea star ecology
- Introduce the mystery of sea star wasting syndrome and tie into Session 3 on Biodiversity.
- Introduce DNA as genetic code "recipe" for making new cells/organisms
- Introduce DNA structure

- Activity 1: make DNA model
- Introduce the fact that organisms leave DNA in their environment
- Reinforce experimental design concepts by having learners discuss design for our experiment. How many samples are needed? Do we need to sample healthy sea stars too? Which part of the sea star should we sample?
- Activity 2: Compare sequences to find the virus causing sea star wasting syndrome
- Discuss sea star ecology and what is happening with sea star wasting syndrome today.
- Tell learners how to get involved in citizen science sea star monitoring

Session Plan Details and Learning Guide Information: Learners will make observations of dying sea stars using the images in the handout. Learners will observe sea stars with missing arms and lesions (these look like white open wounds). In a discussion, learners will discuss possible causes for the melting appearance of the sea stars. During this discussion, learners will find that scientists hypothesize that a disease is causing the appearance of the sea stars and their mortality.

However, scientists need help discovering the cause of the disease. Learners probably know that bacteria or viruses cause disease, but we need a way to find which one. Viruses are too small to identify using a normal light microscope like we used in session two and most bacteria look very similar even though they are different species. When an organism is too small to identify with a microscope, scientists can identify an organism that is in an environment by using the deoxyribonucleic acid or **DNA** in the environment to look for the organism's presence or absence. Every cell of an organism contains a complete copy of an organism's unique DNA code. DNA is unique to each species and even unique among individuals in a species, so it can be used to identify if an organism is present in a given environment.

The vast majority of organisms have DNA. Some types of viruses do not have DNA. To simplify this activity, we will not address this today, because the virus that causes sea star wasting disease has DNA. DNA provides the instructions for building the organism to which it belongs. It determines everything about an organism's features from how many arms it has to how large or small it is. The uniqueness of the DNA code of an organism is one of the reasons we can use it to identify organisms in an environment, but the other reason is DNA structure.

The first activity of this session is about learning DNA structure. DNA is made up of four building blocks we will refer to as **DNA molecules** represented by the letters **A, T, C**, and **G**. DNA is stable in the environment outside of an organism, because it is made up of two strands that form a double helix (see images in presentation and handouts). That double helix is formed because one strand of DNA molecules is paired with a complementary strand. Each **A is matched with a T** and each **C with a G**. As a result of this **complementary pairing**, no matter which strand of DNA is sampled from the environment it can be matched to the complementary strand. This structure combined with the uniqueness of the DNA sequence for each species can help us identify the pathogen that causes sea star wasting syndrome. Learners will follow the

instructions in their handout with the help of the learning guide to build their own DNA double helix with candy gumdrops and toothpicks.

The next activity requires the DNA of our pathogen. The objective of this discussion is to guide learners through the experimental design process and introduce them to the process of sampling DNA and how that DNA on a swab becomes a sequence that we can read. Learners will discuss how many swabs they need from both healthy and diseased sea stars and where they need to swab to get sample DNA that likely has the pathogen. Then they will learn how DNA on a swab becomes DNA sequences. First DNA is extracted. This is the process of opening cells so that we can get DNA out and then cleaning that DNA by removing proteins and fats, which will hinder the sequencing process. Extracted DNA is broken apart into smaller fragments so that it can be put in a sequencing machine that will output sequences in letter form so that scientists can compare them. For simplicity, we stick to these most essential and basic steps that are common to all DNA sequencing processes but there are many ways of preparing DNA to be sequenced which have more steps.

After sequencing learners will find out that an unknown sequence was more frequently found on diseased sea stars. This sequence most likely belongs to the pathogen. Learners will match the unknown fragment of DNA to the known sequences of other organisms trying to find the complementary second fragment to identify the pathogen. Learners will match up the DNA sequences and record in their table in the handout how many DNA molecules match and how many do not. The one that has the most matches is the cause of the disease in this case this is the Denso virus. Now that learners have solved the mystery of the disappearing sea stars, they will likely want to know if the Denso virus is still out there and are sea stars still dying.

The learning guide will conduct a follow up discussion after the second activity regarding the current state of sea star wasting syndrome. Though sea star wasting syndrome is not prevalent now. There is a chance that it can become so again. The Denso virus is ubiquitous on the west coast of North America. However, it is not dangerous until temperatures get too hot. The virus only caused disease in 2012-2014 because of unusually high-water temperatures on our coast. As the climate warms it is likely that heating events like the one that precipitated sea star wasting syndrome are going to become more frequent, so that is why we need citizen scientists (all the learners) to go out into tide pools to count sea stars and record your findings on the sea star wasting network website (https://marine.ucsc.edu/data-products/sea-star-wasting/). On this website you can find data work sheets and images that will help you identify what type of sea stars you are in the tidepools and whether they have sea star wasting syndrome. Then all the learners can be part of real data collection and help monitor sea stars populations in the rocky intertidal. We can all be citizen scientists and help protect our coastal home.

Tie into session 2 on Microscopy – Learners will initially want to look for the cause of sea star wasting using a microscope which is a connection to session 2 in which they learned to use microscopes to identify phytoplankton

Tie into session 3 on Biodiversity - loss of sea stars has resulted in less pressure on sea urchins and mussels. In the rocky intertidal, this means that mussel populations

expand and crowd out other organisms. In the kelp forest, unchecked populations of urchins, which eat kelp, devour kelp forests leading to loss of habitat for other organisms.

Activities: Identify cause of sea star wasting disease using DNA data. Walk learners through how we find the viral cause of the disease by studying DNA in, on, and around affected sea stars

- **Discussion/presentation of sea star ecology and set up of the problem or mystery.**
- **Activity 1**: Introduce DNA and build DNA double helix out of gumdrops and toothpicks following instructions in the learning guide handout.
    - o   This video link demonstrates step by step how to build the DNA molecule. https://www.teachengineering.org/activities/view/cub_biomed_lesson09_activity2
    - o   Each participant will receive:
        - ▪   ~25 toothpicks
        - ▪   ~30 gum drops
- **Discussion/presentation of DNA extraction of sea stars with sea star wasting disease to link between DNA structure and using DNA to identify pathogens.**
- **Activity 2**: Match the sequences following instructions in the learning guide handout.
    - o   Each learner should have a complete set of sequences from the sequence set provided in the session packet
    - o   These should be printed on cardstock paper then cut out, so they are easily aligned.
    - o   The unknown and the Denso virus sequences should fit perfectly like a puzzle
    - o   Each of the other species when aligned with the unknown will have mismatches.