The zooplankton, *Pleuromamma xiphias*. Scientists will take measurements and samples of this copepod to learn more about the circadian rhythms of this species.

Photo by: Kaitlin Noyes

**FOCUS**
Biological oceanography

**GRADE LEVEL**
Year 9-13 (UK) 8-12 (US)

**LEARNING TIME**
Two sessions of 45 minutes

**LEARNING OBJECTIVE**
The purpose of this lesson is to allow students to become familiar with the role of zooplankton in the biological pump. Students will look at the process of data analysis and learn about how carbon moves in the ocean.

**KEY CONCEPTS**

- Students will learn how to visualize data recorded from a CTD (conductivity, temperature and depth device);
- Students will learn how to measure fecal pellets;
- Students will recognize why measuring fecal pellets is important to understanding carbon flux in the ocean;
FOCUS QUESTIONS

- Identify the structure of the water column at Hydrostation “S” in the Sargasso Sea around Bermuda in May of 2019?
- At what time and average depth is *P. xiphias* generating the majority of fecal pellets?
- Utilize photos of fecal pellets and measure the length and width using the computer software Image J
- Demonstrate the average volume of fecal pellets and identify the depth and time when the maximum of fecal pellets are produced
- Brainstorm about the reasons why zooplankton migrate daily
- Apply these data the the larger concept of the movement of carbon from the upper ocean to below the mixed layer.

BACKGROUND

At the base of our ocean food web, microscopic organisms, known as plankton, dominate the sunlit layer of the ocean, or the **euphotic zone**. These plankton are plant-like (**phytoplankton**) and animal-like (**zooplankton**) organisms that play a vital role in the movement of carbon, by way of a critically important pathway known as the **biological carbon pump**. In the euphotic zone, ocean **primary producers** (phytoplankton) use the energy from the sun alongside inorganic nutrients to transform dissolved **carbon dioxide** (CO₂), into organic carbon, through the process of **photosynthesis**. A tightly knit community of zooplankton that live in the dark depths of the ocean to avoid predators during the day, make a daily migration upward to feed on phytoplankton in the surface waters at night, a process known as **Diel Vertical Migration (DVM)**. As these phytoplankton are consumed, a majority of the organic material is converted back into CO₂ through a process known as **respiration**. Concurrently, zooplankton release carbon and nitrogen-based waste products into the water column in the form of **ammonia** and **fecal pellets**. This entire chemical process from feeding to excretion to sustain life is known as **metabolism**. Although a majority of the organic carbon will be taken up in the upper ocean, there is a small percentage of carbon that will be transported to depth when the zooplankton migrate back to their daytime habitat, where it can be stored in deep, slow-moving, ocean currents for time scales of months to millennia. Understanding these cyclical processes (**rhythmicity**) and the role that individual species of zooplankton play in the movement of carbon and nitrogen in the water column, allow scientists to make better predictions of the future climate.

The DVM is a highly synchronized rhythmic event influenced by surrounding cues in the environment, such as light and temperature (**environmental variables**). Predictable, daily cycles of fluctuation, known as **circadian rhythms (CRs)**, can often prepare an organism for environmental changes by regulating their behavior and function (**physiology**). These behaviors can continue to persist under constant environmental conditions, demonstrating an underlying **circadian clock** in organisms. This clock may aid an animal in maximizing its
survival and ability to reproduce, feed and avoid predators, and balance the cost in energy (metabolic cost) needed to make such large migration.

Drs. Amy and Leo are researchers at the Bermuda Institute of Ocean Sciences (BIOS). For this expedition, they collected information on a particular member of the zooplankton community at the Bermuda Atlantic Time Series (BATS). This plankton, *Pleuromamma xiphias*, is a common migrating zooplankton, known as a copepod. Their research is focused on the following questions: do environmental variables effect when these organisms move, indicating circadian rhythms?, does this movement continue in the absence of these variables indicating a circadian clock? and how does the timing of their migration influence carbon and nitrogen production and its movement from the surface to depth (biological pump, carbon and nitrogen cycling, biogeochemistry)?

To examine this copepod, data have been collected on a 3-day research cruise on BIOS’s R/V Atlantic Explorer. Amy and Leo examined how time of day and depth influence location and production of fecal pellets that are a component of marine snow. All of these parameters are critical to understanding the role that *P. xiphias* plays in the oceanic food web at BATS and to improve calculations of carbon and nitrogen cycling that help inform and refine climate predictions.

**COMMON MISCONCEPTIONS**
These data are sampled from one time point and are a very small subset of biological, chemical and physical oceanographic data. We can however utilize these data to learn more about the migration and metabolism of *P. xiphias* and display these results graphically.

**TECHNICAL REQUIREMENTS**
Projector, access to computer with freeware “Image J”, Microsoft Excel (optional), internet access

**TEACHER PREPARATION**
- Teachers will familiarize themselves with the freeware Image J
- Teachers will be able to effectively demonstrate how to use the ruler tool on Image J to measure an object. They will also be able to “set the scale” and demonstrate this effectively
- Teachers will be prepared to move from the graph to discussions about how scientists use these data
HELPFUL LINKS

Image J freeware
https://imagej.nih.gov/ij/download.html

Using Image J
https://www.youtube.com/watch?v=xXE7MBiWJlc

What is a Thermocline?
https://oceanservice.noaa.gov/facts/thermocline.html

How to graph temperature vs. depth data?
https://www.youtube.com/watch?v=tW_welB2J8

What is a CTD?
https://oceanexplorer.noaa.gov/facts/ctd.html

PBS Changing Seas l The Fate of Carbon

The Ocean Massive Open Online Course (MOOC) l The Biological Pump
https://www.youtube.com/watch?v=hJAWIrJVvkM

Ocean Layering Explained
https://www.youtube.com/watch?v=TxdiU3LJiZ8

Concentration Gradients

The Secret Life of Plankton
https://ocean.si.edu/ocean-life/fish/secret-life-plankton

Sea Sketch l The Ocean Carbon Cycle
https://www.youtube.com/watch?time_continue=12&v=jBFcpwgp-Xk&feature=emb_logo
KEYWORDS

Biochemical respiration: a metabolic process by which an organism obtains energy (in the form of ATP and NADPH) by oxidizing (combine with oxygen) nutrients and releasing waste products.

Biological carbon pump - all those processes in the ocean that cause organic carbon formed photosynthetically by phytoplankton (primary production) in the sunlit surface layer (the euphotic zone) to be removed from contact with the atmosphere. It is a mechanism that sequesters carbon dioxide (CO₂) for weeks to hundreds or even millions of years (geological time-scales).

Carbon: A chemical element of atomic number 6 and the chemical backbone of life on Earth. Carbon compounds regulate the Earth’s temperature, make up the food that sustains us, and provide energy that fuels our global economy.

Carbon Dioxide: a colorless, odorless gas produced by burning carbon and organic compounds and by respiration. It is naturally present in air (about 0.03 percent) and is absorbed by plants in photosynthesis. Carbon dioxide is a greenhouse gas: a gas that absorbs heat.

Carbon storage: Carbon is stored on our planet in the following major sinks:

(1) as organic molecules in living and dead organisms found in the biosphere;
(2) as the gas carbon dioxide in the atmosphere;
(3) as organic matter in soils;
(4) in the lithosphere as fossil fuels and sedimentary rock deposits such as limestone, dolomite and chalk;
(5) in the oceans as dissolved atmospheric carbon dioxide and as calcium carbonate shells in marine organisms.

Circadian clock: an organism’s innate timing device over a 24-hour period. The clock is composed of specific molecules (proteins) that interact in cells throughout an organism’s body. Biological clocks are found in nearly every tissue and organ.

Circadian rhythm: are physical, mental, and behavioral changes that follow a daily cycle.

Copepod: any of numerous tiny marine or freshwater crustaceans of the order (or subclass) Copepoda, lacking compound eyes or a carapace and usually having five pairs of limbs on the thorax, some abundant in plankton and others parasitic on fish (or even in moss or banana trees if there is water!).

Copepod fecal pellets: biogenic particles that are densely packed pellets of waste products in which the composition is dependent on the feeding habits of the producer.
Diel Vertical Migration (DVM): the daily movement of zooplankton to feed in the phytoplankton-rich surface layers while visual predation is inhibited. Light is thought to be an important external control with other physical and chemical environmental factors such as food availability and predator cues.

Environmental variables: factors (variables) that exist in an organism's physical environment that influence behavior.

Euphotic Zone: The upper 200 meters (656 feet) of the ocean is called the euphotic, or "sunlight," zone. This zone contains the vast majority of commercial fisheries and is home to many protected marine mammals and sea turtles.

Halocline: a layer of water in which a high rate of change in salinity in the vertical dimension is present.

Marine Snow: Aggregations of organic and inorganic particles in the surface waters of the ocean, primarily composed of phytoplankton, bacteria, microzooplankton, biominerals, terrestrial derived components and detritus. As these materials sink, they collect other floating debris, including fecal material (poop), dead and decaying animals, suspended sediments (e.g., silt), and other organic material that may have been transported from the land to the sea.

Metabolic cost: The amount of energy consumed as the result of performing a given work task

Metabolism: The entire chemical process from feeding to excretion that sustains life

Mixed surface layer: The surface layer of the ocean water mixed by wave and tide motions to produce relatively isothermal and isohaline conditions.

Phytoplankton: microalgae that contain chlorophyll and require sunlight in order to live and grow.

Photosynthesis: occurs in plants and some bacteria, wherever there is sufficient sunlight – on land, in shallow water, even inside and below clear ice. All photosynthetic organisms use solar energy to turn carbon dioxide and water into carbohydrates (food) and oxygen: $\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$.

Physiology: the study of normal function within living creatures. It is a sub-section of biology, covering a range of topics that include organs, anatomy, cells, biological compounds, and how they all interact to make life possible.

Physiological respiration: the movement of oxygen from the outside environment to the cells within tissues, and the transport of carbon dioxide in the opposite direction.
Plankton: floating or drifting organisms with limited locomotion powers. Oceanic plankton constitute the largest reservoir of biomass in the world's oceans and play a significant role in the transfer of energy and materials within the oceanic ecosystems. The major plankton subdivisions include bacteria, phytoplankton, and zooplankton.

Primary Production: the amount of organic matter (usually expressed as grams of carbon per square meter per day) produced by organisms that are able to manufacture food from simple inorganic substances using energy from sunlight (in the case of photosynthesis).

Pycnocline: A layer of water in which a high rate of change in density in the vertical dimension is present. This layer of water has a high gravitational stability and thus physically isolates adjacent layers of water.

Rhythmicity: describing a cyclical process.

Thermocline: A layer of water beneath the mixed layer in which a rapid change in temperature can be measured in the vertical dimension.

Zooplankton: A diverse group of animals (usually tiny, but up to several meters in length for some jelly fish), that drift in aquatic environments. Several aquatic animals live out their life as part of the zooplankton (such as copepods and amphipods), while the egg and larval stages of some animals (such as crabs, clams and some fish species) are part of the zooplankton only until they grow large enough to start their adult life on the sea floor or other habitats. Zooplankton are also an important first tier of the food chain.

PRE-ASSESSMENT STRATEGY
To garner useful information on student knowledge of the biological carbon pump, students can trace a molecule of CO₂ from the atmosphere through the ocean food web to look at the conversion of inorganic sources of carbon to organic ones and sources of return of inorganic carbon to the atmosphere.

Key driving questions

1. How is CO₂ absorbed by the ocean?
2. What is the process that phytoplankton use in the conversion of inorganic CO₂ to organic CO₂?
3. What are the mechanisms by which CO₂ is: a) returned to the atmosphere? b) sinks to the deeper layers of the ocean? and c) used or consumed by other organisms?
LEARNING PROCEDURE

Working With CTD Data

A. Open the Excel File CTD_DATA_BIOS. You will see that there are three tabs at the bottom: CTD_TEMP; CTD_SALINITY and CTD_DENSITY. In the first tab Column A is “Temperature” and Column B is “Depth”.

<table>
<thead>
<tr>
<th>Date</th>
<th>5-21-2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel</td>
<td>R/V Atlantic Explorer</td>
</tr>
<tr>
<td>Location</td>
<td>Hydrostation &quot;S&quot;</td>
</tr>
<tr>
<td>Chief Scientist</td>
<td>Dr. Amy Maas</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.2264</td>
<td>1</td>
</tr>
<tr>
<td>22.8137</td>
<td>25</td>
</tr>
<tr>
<td>21.7925</td>
<td>50</td>
</tr>
<tr>
<td>20.6222</td>
<td>75</td>
</tr>
<tr>
<td>20.0303</td>
<td>100</td>
</tr>
<tr>
<td>19.8926</td>
<td>115</td>
</tr>
<tr>
<td>19.8234</td>
<td>125</td>
</tr>
<tr>
<td>19.7841</td>
<td>137</td>
</tr>
<tr>
<td>19.7102</td>
<td>150</td>
</tr>
<tr>
<td>19.6555</td>
<td>160</td>
</tr>
</tbody>
</table>
B. Select both columns A (temperature) and B (depth) by placing the cursor over the letter at the top of column A, holding down the mouse and moving it over to the letter at the top of column B. Both columns should be highlighted.

C. Click on the “Insert” tab on your Excel toolbar

D. Select “X Y Scatter” Icon at the bottom right hand corner

E. Select “Scatter with Smooth Lines”

F. You will see a graph appear that looks like this and goes from the deepest measurement to the shallowest depth near the surface. We now need to flip the “Y” axis.
G. Click to highlight the "Y-axis" and then right click and choose "Format Axis"

H. Tick the box that notes "Values in Reverse Order"

I. Now the graph will read the temperature from the surface to 1000 meters
J. Next, click to highlight the “x-axis” and then right click and choose “Format Axis”
K. You will also want to move your axis to the bottom of the graph. This is completed by choosing the “labels” tab and positioning the label as “high”.

L. The graph will now look very similar to the one below

M. The next step is to label our “X” and “Y” axis with the units of depth and temperature and give our graph a title by using the “Chart Design Tab” and selecting “Add Chart Element” to add both the axis and chart title
N. From this graph you will be able to identify the depth at the beginning of the thermocline.

O. Repeat this process to create salinity (CTD_salinity) and density (CTD-density) graphs.

P. The density graph should look like the below. From here, identify the mixed surface layer and the beginning of the pycnocline.
Fecal Pellet Production

A. The depth of the surface mixed layer has now been established and the next data set you will analyze will be fecal pellets produced by P. xiphias collected from four different time-points. There are 31 pellets to analyze and below is a table of the time, depth and number of pellets collected.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Depth Category</th>
<th>Towing Depth</th>
<th>Time</th>
<th>Pellets produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shallow tow</td>
<td>mixed surface layer</td>
<td>Surface-200m</td>
<td>2AM</td>
<td>21</td>
</tr>
<tr>
<td>Shallow tow</td>
<td>mixed surface layer</td>
<td>Surface-200m</td>
<td>10PM</td>
<td>5</td>
</tr>
<tr>
<td>Deep tow</td>
<td>below the mixed surface layer</td>
<td>400-600m</td>
<td>9AM</td>
<td>4</td>
</tr>
<tr>
<td>Deep tow</td>
<td>below the mixed surface layer</td>
<td>400-600m</td>
<td>3PM</td>
<td>1</td>
</tr>
</tbody>
</table>

B. After opening Image J on your computer go to File → Open, and then open the file labeled 2AMstack (The file is provided alongside this lesson as a downloadable file .tiff file) This file contains 21 photos of fecal pellets from organisms at 2:00 AM.

C. Click on the line icon shown in the screenshot below.
D. Take this tool and draw a line from the start of the ruler on the board to 1 mm cm; this is going to be our “known measurement” and will allow us to Set the Scale.

![Image of ruler and measurement tool]

E. To set the scale now that you have your known measurement, select Analyze on your toolbar and Set Scale. This will give you a window like the one below. The computer has the known amount of pixels in the box to now equate with your known distance of mm. Make sure you add 1.0 mm as your known distance (this should be approximately 1 mm = 1589.47 pixels). Select Global in this window and this will save the scale for this set of photos.

![Image of window with set scale options]
F. Now that the scale is set you can measure your fish pellet by taking your tool by taking the longest length and width of each pellet. You can then go to the toolbar and select Analyze → Measure (or Control + “m” / Apple “m”). When you are complete, there should be 42 measurements in the window. “a” is the major axis diameter and “b” is the minor axis diameter.

G. Your data file will look like this when you are complete in analyzing all 21 pellet photographs.
H. Copy and paste these data into Excel and label your first tab 2AM. The column that we are interested in is the “length column” and other columns can be deleted. To calculate the volume of an ellipse you must know the radius of our two measurements. The radius is $r = \frac{d}{2}$.

I. To calculate the volume of each fecal pellet as observed we will follow the known factor that fecal pellets are predominately cylindrical, elliptical or near spherical. For this lab we will approximate fecal pellet geometry as an ellipsoid (Turner 2002; Shatova et al. 2012). “a” and “b” are the radii of the ellipses major radius and minor radius respectively.

$$V = \left(\frac{4}{3}\right)\pi ab^2,$$

J. Calculate the volume of each fecal pellet and generate the average volume in mm$^3$. Students will need to calculate the average fecal pellet volume in mm$^3$, the standard deviation within each stack and the standard error for each time point.

Excel Formulas

=average for Average

=STDEV for Standard Deviation

=STDEV/Sqrt(sample size) for calculation of the standard error

You will need your standard error for the addition of error bars for your graphs.

K. Open the next stack file (eg. 9AM) and complete this process for the other 3 timepoints. The scale should remain the same, but you may have to re-set it.

L. Generate a summary table and graph to compare fecal pellet volume across time points. There will be slight variation from student to student.

<table>
<thead>
<tr>
<th>Fecal Pellet Volume</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2AM</td>
<td>0.0045</td>
</tr>
<tr>
<td>9AM</td>
<td>0.0119</td>
</tr>
<tr>
<td>3PM</td>
<td>0.0145</td>
</tr>
<tr>
<td>10PM</td>
<td>0.0070</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2AM</td>
</tr>
<tr>
<td>9AM</td>
</tr>
<tr>
<td>3PM</td>
</tr>
<tr>
<td>10PM</td>
</tr>
</tbody>
</table>
M. After the summary table is complete students will create two bar graphs that address these two questions?

1. Average volume of fecal pellets (mm³) produced by *P. xiphias* at four time points
2. Number of fecal pellets produced by *P. xiphias* at four time points

**ASSESSMENT AND EVALUATION**

Students will be asked to complete questions in the accompanying worksheet and attach their graphs.

**STANDARDS**

Next Generation of Science Standards | High School

Disciplinary Core Ideas

**LS1.C: Organization for Matter and Energy Flow in Organisms**
- As matter and energy flow through different organizational levels of living systems, chemical elements are recombined in different ways to form different products. (HS-LS1-6),(HS-LS1-7)
- As a result of these chemical reactions, energy is transferred from one system of interacting molecules to another. Cellular respiration is a chemical process in which the bonds of food molecules and oxygen molecules are broken and new compounds are formed.

**LS2.B: Cycles of Matter and Energy Transfer in Ecosystems**
- Plants or algae form the lowest level of the food web. At each link upward in a food web, only a small fraction of the matter consumed at the lower level is transferred upward, to produce growth and release energy in cellular respiration at the higher level. Given this inefficiency, there are generally fewer organisms at higher levels of a food web. Some matter reacts to release energy for life functions, some matter is stored in newly made structures, and much is discarded. The chemical elements that make up the molecules of organisms pass through food webs and into and out of the atmosphere and soil, and they are combined and recombined in different ways. At each link in an ecosystem, matter and energy are conserved. (HS-LS2-4)

Crosscutting Concepts

Systems and System Models
- Models (e.g., physical, mathematical, computer models) can be used to simulate systems and interactions—including energy, matter, and information flows—within and between systems at different scales. (HS-LS2-5)

Energy and Matter
- Changes of energy and matter in a system can be described in terms of energy and matter flows into, out of, and within that system. (HS-LS1-5), (HS-LS1-6)
- Energy drives the cycling of matter within and between systems. (HS-LS2-3)
Ocean Literacy Principles

**Principle 2: The ocean and life in the ocean shape the features of earth**

2D The ocean is the largest reservoir of rapidly cycling carbon on Earth. Many organisms use carbon dissolved in the ocean to form shells, other skeletal parts, and coral reefs.

**Principle 3: The ocean is a major influence on weather and climate**

3E The ocean dominates Earth’s carbon cycle. Half of the primary productivity on Earth takes place in the sunlit layers of the ocean. The ocean absorbs roughly half of all carbon dioxide and methane that are added to the atmosphere.

3F The ocean has had, and will continue to have, a significant influence on climate change by absorbing, storing, and moving heat, carbon, and water. Changes in the ocean’s circulation have produced large, abrupt changes in climate during the last 50,000 years.

3G Changes in the ocean-atmosphere system can result in changes to the climate that in turn, cause further changes to the ocean and atmosphere. These interactions have dramatic physical, chemical, biological, economic, and social consequences.

**Principle 4: The ocean made earth habitable**

4C The ocean provided and continues to provide water, oxygen, and nutrients, and moderates the climate needed for life to exist on Earth (Essential Principles 1, 3, and 5).

**Principle 6: The ocean and humans are inextricably interconnected**

6A The ocean affects every human life. It supplies freshwater (most rain comes from the ocean) and nearly all Earth’s oxygen. The ocean moderates the Earth’s climate, influences our weather, and affects human health.

**Principle 7: The ocean is largely unexplored**

7E Use of mathematical models is an essential part of understanding the ocean system. Models help us understand the complexity of the ocean and its interactions with Earth’s interior, atmosphere, climate, and land masses.

**Author**
Kaitlin Noyes