Tracking Carbon*: Photosynthesis and Respiration*

Overview

In this session participants explore the role of photosynthesis and respiration in the movement carbon through different carbon reservoirs, and identify some of the environmental factors - such as light and temperature - that control this movement. They use dissolved oxygen as a "proxy" for carbon dioxide in natural waters and several different approaches (e.g., investigations, simulations, data analyses, graphs), to gather evidence of the movement of carbon between different reservoirs as they are used by organisms. With this collection of evidence, they construct explanations for changes in oxygen, photosynthesis, and respiration throughout the day and discuss the implications for this in terms of changing atmospheric CO2 and ocean pH.

Session at a Glance

Task	Description	Time (minutes)
A. Activity: *Tracking Carbon through Respiration* (OSS 2.2)	Participants do a Quick Write to access their prior knowledge about respiration and photosynthesis. They then engage in an investigation with yeast samples and an acid indicator to answer the question, "what does eating have to do with producing carbon dioxide?"	35
B. Optional Activity: *Tracking Carbon through Photosynthesis Part 1* (OSS 2.3)	An optional activity is available that offers an in-depth classroom exploration of the use of carbon by plants and the opportunity for students to practice "argumentation from evidence" to support their ideas.	45
C. Activity: *Investigating Respiration and Photosynthesis using*	Participants conduct an experiment using "light:dark bottle" incubations and local aquatic plants to explore the relative contribution of photosynthesis and respiration to oxygen and carbon cycling in local waters.	45

a Light:Dark Bottle Experiment		
D. Activity: *Investigating Respiration and Photosynthesis using Local Water Quality Data*	Participants use the SWMP data portal to explore patterns in sunlight (PAR), oxygen, and pH observed with local NERR SWMP data. They offer explanations for the patterns they observed using evidence from the *Tracking Carbon through Respiration* and *Light:Dark Bottle Experiment* activities.	

Session Details

A. Activity: *Tracking Carbon through Respiration*

(Note to Instructor: This session is taken from the Ocean Sciences Sequence curriculum for Grades 6-8, session 2.2)

1. **Quick Write.** Have participants think about and then spend a few minutes writing to the following prompts:
    a. How do organisms use carbon?
    b. What do you already know about photosynthesis and respiration?

Do not collect the Quick Writes; participants will add to the prompts at the end of the session.

2. **(Optional) Distribute carbon cards.** Pass out a set of Carbon Cards to each small group. Explain that these cards provide examples of a diverse array of carbon reservoirs. Encourage participants to explore the back of the cards and talk to the person next to them about what kind of information is provided. Give participants about 5 minutes to explore the cards.

3. **(Optional) Watch video, It's All about Carbon**, **and Turn & Talk.** Ask participants to jot down questions or ideas as they watch the video – It's All About Carbon (3 minutes, 20 seconds) and then after the video, have them do a Turn & Talk to share their questions and ideas.

Conducting Yeast Investigations (OSS 2.2)

1. **Introduce yeast activity.** Let participants know that they will investigate what happens to carbon when living things (organisms) eat, or consume food. They will collect evidence over multiple investigations to help answer the overall guiding question: How do organisms use carbon?

2. **Introduce yeast.** Explain that in this first investigation they will be using yeast, a living, single-celled fungus, in an investigation. Yeast is used in making bread and other products, but today they will use yeast to investigate what an organism does with the food it eats. The yeast they will use was dry and dormant (not active), but when water and sugar (a food source) are added, the dry yeast will become active again.

3. **Project slides, Yeast Investigation, Part 1 and Part 2; demonstrate steps.** Each pair will do an investigation and collect observations and evidence to answer the question, "What does eating have to do with producing carbon dioxide?" Describe each of the steps on the slides and answer any questions. Distribute *Yeast Investigation Worksheets* and point out that the handouts have identical information to what is shown on the slides. They will work with a partner to do the investigation, but each of them should complete their own worksheet pages. Remind them to keep the yeast solution in the test tubes warm, either in a warm water bath, or held tightly in warm hands.

4. **Explain doing multiple tests.** Point out that each pair will test both of their yeast samples three times. Doing investigations multiple times helps to get a better sense about the reliability of the results. Knowing that the data are reliable increases your confidence in the data and thus the conclusions you can make from the data. Additionally, the more data that are collected the stronger the evidence can be to support or not support a claim.

5. **Describe BTB's reaction with CO_2.** Explain that BTB stands for bromothymol blue, a chemical that turns green or yellow when it's mixed with an acid. In water, carbon dioxide makes an acid called carbonic acid, so if the BTB turns green or yellow, that is evidence there may be carbon dioxide present.

6. **Introduce proxies.** It can be difficult to accurately measure the amount of CO_2 in a liquid, but BTB can easily be used as a proxy for indicating the presence or absence of CO_2 in the water. A proxy is: "a measured parameter used to estimate or predict another parameter that cannot be measured or quantified directly." This is helpful in understanding if it is there, but it is important to remember that using proxies does not provide quantitative data about the item we are actually interested in (e.g., we do not know how much CO_2 is present if the BTB turns green). Proxies are an important part of science when it is impossible (e.g., samples from 10,000 years ago), impractical (e.g., doing so would threaten what you are trying to observe), or extremely difficult (e.g., expensive machinery) to get data on the actual variable you are interested in investigating.

Note to instructor: *If you want to explore proxies in science more deeply with your students, consider sharing with them the Tools of Science "Practice 3: Proxies in Scientific Investigations" video from http://toolsofscience.org/lessons.html.*

7. **Introduce controls.** Show participants the two demonstration test tubes, one containing tested water and the other containing sugar water, noting that this is the same as what they'll use in their investigations. Add 8 drops of BTB to each test tube, and tell them that this is how BTB reacts with the water without yeast. Explain that these are controls to help us understand what we observe. In this case, the controls will help us observe the effect of increasing the amount of carbonic acid in the water, due to CO_2 being released by the yeast. By comparing the difference in BTB reactions (color) between the treatment and controls we will be able to observe what happens to the presence of carbonic acid (and thus CO_2) in the water when we add yeast.

Note to instructor: *You may want to introduce and discuss the independent/dependent variables in the investigation. The two variables that are varying in this investigation are the yeast and the amount of CO_2 in the water. Within this investigation the independent variable is the yeast and the dependent variable is the amount of CO_2 in the water. This is because the amount of CO_2 in the water is changing among the treatments (or test tubes) and that difference is being driven by the yeast, aka it is dependent on the yeast. The changes in the yeast are not impacted by, or dependent on, the amount of CO_2 in the test tube. Understanding what the independent and dependent variables are within an investigation as well as why is helpful for having a greater conception of what is being investigated and what conclusions and inferences you can or cannot draw from the results.*

8. **Organize groups and tasks.** Have participants divide into two pairs within their table groups and tell them that one person from the table group will get the tray of materials, which contains materials for two pairs of participants. Project the first Yeast Investigation slide again. When most pairs have completed part 1, project the part 2 slide.

9. **Regain attention.** Have participants finish up their Y*east Investigation Worksheets* to prepare for a class discussion about their observations and the evidence they gathered to answer the question, what does eating have to do with producing CO_2?

Making Sense of Yeast Investigations

1. **Share observations.** Ask a few participants to share what they observed. [*Bubbles. Change in color when BTB was added to the yeast.*] Ask, **"What is your evidence that the tube with sugar was producing CO_2?"** [*Bubbles, color change.*] "**Did you find similar results each time you tested the yeast?**" [*most likely yes, unless something was done incorrectly.*]

2. **Question where CO_2 came from.** Confirm that the gas in the test tubes with sugar is carbon dioxide. Ask, **"Where do you think the carbon dioxide came from?"** Listen to a few answers, and for each idea, ask, **"Why do you think that?"** Explain that yeast and most animals, including ourselves, take in solid carbon compounds in the form of sugar when they eat, and give off (release or breathe out) carbon as carbon dioxide gas. Tell them the carbon they ate for dinner last night may be the same carbon they are now breathing out as carbon dioxide.

3. **Volunteer blows into BTB.** Have a volunteer come forward and gently blow (not suck) through the straw into the cup of blue BTB and water you prepared. (Make sure the end of the straw with the small safety hole is the end the volunteer blows into.) As the BTB changes to green and then to yellow, have participants explain to each other what is causing this change. [*Volunteer is exhaling carbon as CO_2 from the solid food they ate, which reacts with the water to form carbonic acid, which reacts with the BTB to change the color.*]

4. **Define respiration.** Tell participants that most people think of respiration as simply breathing in and out. Explain that respiration refers to the whole process of organisms breaking down carbon

containing molecules, such as sugar, for energy and for building bodies, and releasing some of the carbon as CO_2 by breathing it out into the atmosphere.

5. **Project and explain the slide, Carbon In/ Carbon Out for Respiration.** Mention that some people find it confusing that solid carbon can be made into a gas, but that's what happens during respiration. Explain that in our bodies, oxygen is absolutely necessary for the process of respiration to occur. During respiration, the atoms in sugar and oxygen get rearranged into atoms of carbon dioxide and water. During this process we get energy from the food.

*[**Note to instructor:** if your participants are struggling with understanding the process of respiration (or photosynthesis) or just want to know more, you might suggest that they do some outside reading for review.]*

6. **(Optional): Point out equal number of atoms on both sides of equation.** Point out the chemical formulas on the slide and explain that the same number of each kind of atom is on either side of the arrow. To illustrate, ask if participants can see how the six carbon atoms in sugar combined with six O_2 atoms to make six CO_2 molecules. Emphasize that atoms aren't destroyed during respiration, but are rearranged to make different chemicals.

7. **Project and explain slide, Releasing Carbon Dioxide and Methane Gas.** In addition to breathing out carbon dioxide, most animals also release carbon in methane gas (CH_4) as flatulence (farts). Ruminant animals, such as cows and sheep, also have methane burps.

8. **Project key concept.** Project the key concept and invite participants to contribute ideas about what they learned that helped to answer the guiding question, how do organisms use carbon?
    - Most organisms get energy and materials to build their bodies from molecules that contain carbon. They break down these molecules and release carbon dioxide gas. This is respiration.
    - In water, carbon dioxide makes an acid called carbonic acid, so if the BTB (an acid indicator) turns green or yellow, that is evidence that the water is becoming more acidic and that there may be carbon dioxide present.

B. Optional Activity: *Tracking Carbon through Photosynthesis* (OSS 2.3)

An optional activity is available that offers an in-depth classroom exploration of the use of carbon by plants and the opportunity for students to practice "argumentation from evidence" to support their ideas.

C. Activity: *Investigating Respiration and Photosynthesis using a Light:Dark Bottle Experiment*

Overview

In the following session, we use a "light/dark bottle" experimental design that is frequently used in oceanographic research to identify the relative contribution of photosynthesis and respiration to oxygen and carbon cycling in aquatic ecosystems. The basic approach is to "incubate" submerged aquatic plants (or plankton) in illuminated vs. dark conditions over a 24 hour period, measuring the subsequent changes in water chemistry (e.g., dissolved oxygen, pH). Students interpret these data to reveal the effects of photosynthesis and respiration on daily patterns in oxygen and pH, and then connect these to local patterns in sunlight (PAR), oxygen, and pH observed with local NERR SWMP data. The activities and learning experiences in this session set the stage for subsequent sessions where students track seasonal and long-term patterns in global carbon dioxide that are influenced by respiration and photosynthesis.

Concepts and Rationale

1) **Plants respire!** Many learners (students and teachers alike) tend to exclusively associate photosynthesis with plants (e.g. kelp, phytoplankton, eelgrass) and respiration with animals (or heterotrophs), forgetting that plants also respire. Plant respiration can make a substantive contribution to local and global patterns in oxygen, pH and CO_2 of marine and coastal waters, and this activity will help reveal this phenomenon.
2) **Student collect their own data.** Student-collected data is an important instructional gateway and first step in a learning progression to make online data (e.g. SWMP) less abstract. When students collect DO, pH or salinity data with a handheld instrument and interpret these data, their understanding and ability to conduct more advanced interpretations of SWMP data is improved. It associates a personal, hands-on learning experience with the more abstract world of online data portals. Data collected in this activity will be paired with explorations of SWMP data looking at the changes in dissolved oxygen, pH and PAR over the course of a 24 hour (or diel) period.
3) **Carbon is naturally variable**. Understanding carbon cycling is a critical piece of understanding

climate change, patterns in global carbon dioxide, seasonality, and ocean acidification. Students and teachers alike need to be able to see and articulate the natural processes that contribute to global patterns in CO_2 to provide a context for evaluating human impacts.

Introduce photosynthetic organisms in the ocean. Tell participants that just as plants on land photosynthesize, there are many organisms in the ocean that photosynthesize — in fact, most of the photosynthesis (and respiration) that happens on Earth occurs in the ocean and is done by tiny ocean organisms called phytoplankton and other microbes. These organisms also build their bodies using carbon from the carbon dioxide they take in.

1. **Project slide, Opposite Processes.** Ask, "What do you notice when you compare what happens in respiration versus photosynthesis?" [*Participants might say that the chemical formulas on either side of the arrow are flip-flopped.*] Matter is conserved because atoms are conserved in physical and chemical processes. Encourage participants to think about their prior knowledge and what they have been doing so far in the session as well.

2. **Have participants reflect on the relationship between light energy, photosynthesis, and respiration.** Referring to the "Opposite Processes" slide, have participants discuss the following questions:
    1. What do you think will happen to oxygen concentrations when plants or phytoplankton are exposed to light energy? [*oxygen will be produced by the plant through photosynthesis, and thus the oxygen concentration will increase*]
    2. Therefore, what do you think will happen to carbon dioxide concentrations when plants or phytoplankton are exposed to light energy? [*carbon dioxide concentrations will decrease because more photosynthesis is happening which requires carbon dioxide*]
    3. At nighttime and in the absence of light, which of the following is the most dominant process – respiration or photosynthesis? [*respiration*]
    4. What happens to oxygen and carbon dioxide concentrations if the amount of respiration in a system is greater than the amount of photosynthesis? [*the concentrations of oxygen will decrease because less is being made as a byproduct of photosynthesis, carbon dioxide will increase over time because more is being made as a byproduct of respiration than is being used in photosynthesis*]

3. **Using oxygen as a proxy.** Instruct participants that they will be making a prediction about how much photosynthesis and respiration is taking place during this period. Explain that for this activity they will be using oxygen as a **proxy** for photosynthesis and respiration. Remind participants that a proxy is: "A measured parameter used to estimate or predict another parameter that cannot be measured or quantified directly." Describe that because it is difficult to measure the cellular processes of respiration and photosynthesis directly – especially in the field – we can more easily measure changes in the concentration of oxygen as a proxy for respiration (i.e. consumption of oxygen and thus a decrease in oxygen concentrations) or photosynthesis (i.e. production of oxygen and thus an increase in oxygen concentrations).

4. **Explain to participants that they will be setting up an experiment to measure changes in oxygen as a result of light energy, photosynthesis, and respiration.** Lead a discussion with the participants to develop an experimental investigation of these processes and make predictions. The depth and nature of this discussion and level of participant involvement will depend on time constraints, aquatic plants being used in the incubations, experimental setup, participant knowledge, and ability to foster participant involvement. This can range from a scenario where the experimental incubations are completely set up by the instructor ahead of time, to one where participants are given a list of available materials and asked to design an experiment to test the effect of light, photosynthesis and respiration on oxygen concentrations. Regardless of the extent of participant involvement in this process, it is critical to ask them to make and explain their predictions of the incubations and then test those with results when the incubations are complete.

Setting up the light:dark bottle experiment

Experimental setup will depend on what types of submerged aquatic vegetation (SAV) or other photosynthetic organisms you available in local habitats or NERR. Macrophytes such as eelgrass, seagrasses, kelp, and sea lettuce have substantial biomass and can rapidly influence oxygen concentrations in a small (500 – 1000ml) container or flask. Phytoplankton can also be used, however there are some adjustments to make given their relatively small size and proportional influence on dissolved oxygen concentrations. Using phytoplankton may be valuable if planktonic communities play an important role in oxygen cycling in local waters, or SAV are not available.

A. Collection of Samples

Seagrass and eelgrass (including their roots and rhizomes) can be gently harvested from the sediment and transferred into trays or plastic bags with seawater to keep them wet. Ideally, plants are harvested the day of the experiments, but can be kept in the refrigerator for a couple of days until they can be transferred to flasks for the incubations. *Macroalgae* (e.g. *Macrocystis, Nereocystis, Ulva*) should be collected the day of the experiment and stored in saltwater until transfer to incubation flasks. When collecting SAV for the investigation, also collect enough seawater to fill the total number of flasks that will be used. (NOTE: This can also be conducted with fresh water using *Elodea* or other FW plant).

Water samples with phytoplankton should be collected the day of the experiment (otherwise larger grazers such as copepods may eat all the photosynthesizers before your experiment begins!). If you have very productive or eutrophic waters, you may be able to collect surface waters and transfer them directly into bottles for the experiment and still get measureable changes in oxygen concentrations as a result of photosynthesis. Another option is to supplement the phytoplankton in your sample water by using a small mesh size (<100um) plankton net to collect additional plankton. This concentrated plankton sample can then be added to the larger water sample before use in the experiment, essentially "boosting" the photosynthesis in your sample by adding a concentrated dose of phytoplankton.

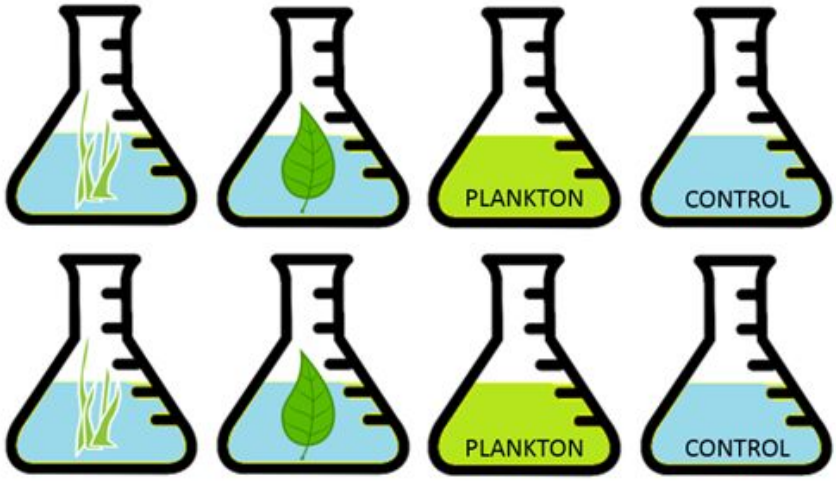
B. Incubation Setup

Water Sample Collection: Use a single, large (10-20L) container to hold the seawater that will be used in your experimental setup. This will allow you to record one value for the initial oxygen concentration (i.e. t_0 or "tee zero") that can be compared as a starting point to all subsequent measurements of the incubations. A 10L cubitainer with a spigot is ideal, but any large container or bucket will work. Seawater being used for the experiment can be collected ahead of time. If possible, store the water at a similar temperature to that of the experimental incubations. Water collected from aquaria in your interpretive center or a flow-through seawater system can also work.

Light/Dark Bottles: Below are steps for setting up the incubations. Engage the students (or workshop participants) in as many of these steps as possible (e.g. labelling, filling bottles), but if you have time constraints a lot can be done beforehand.

1) Assemble the flasks that will be used for each plant type and treatment. Use a minimum of two flasks per treatment (i.e. plant type, plus controls (seawater but no plants)). Narrow mouth glass

flasks (e.g. 1L Erlenmeyers) work well because when they are filled to the neck they reduce the amount of gas exchange with the atmosphere during the incubations. Below is an example of a flask set up that includes eelgrass, seaweed, plankton and a control. A second set of these eight flasks would be duplicated and kept in the dark (e.g. inside a cooler) during the incubations.



2) Using a handheld DO meter (e.g. YSI, PASCO), measure and record the initial dissolved oxygen concentration in the large container of seawater. Stir the container with the sensor to make sure you are getting an accurate measurement that is representative of the entire container. *This initial reading is important because it will be your "baseline" to which all subsequent measurements are compared*. Record both percent saturation and mg/L. (NOTE: Percent saturation is a better parameter to use with students/teachers because it clearly reveals effects of photosynthesis vs. respiration (i.e. anything over 100% is driven by photosynthesis, anything less is respiration). Percent saturation also corrects for changes in temperature that might occur. Milligrams per liter is also a relevant unit because this is typically used for setting water quality standards and the units most commonly reported in media or scientific literature).
3) Label the flasks, including plant type, replicate and light vs dark (e.g. eelgrass A - dark).
4) Distribute the eelgrass and/or macroalgae into the appropriate flasks. If you have lots of flasks and plant material, you can also set up flasks with differing amounts of each plant as an additional experimental component. If you really want to get quantitative and do a more in-depth interpretation of data, the participants can weigh the plant material placed in the flasks (to make sure it is equal among all replicates) or quantify the biomass in each flask in some other way.
5) Transfer seawater into each flask. Leave enough headspace at the top so the flask doesn't

overflow when you place the handheld sensor inside to measure dissolved oxygen. The seawater can be poured into each flask using a funnel, but it is preferable to transfer using tubing attached a spigot on the cubitainer or siphoned from the bucket and filling each flask from the bottom. This will limit the introduction of oxygen by splashing and bubbles created during transfer. (NOTE: A 500ml Erlenmeyer flask will accommodate a YSI Pro2030 sensor to measure oxygen, although the conductivity port will not be submerged and not allow salinity measurements).

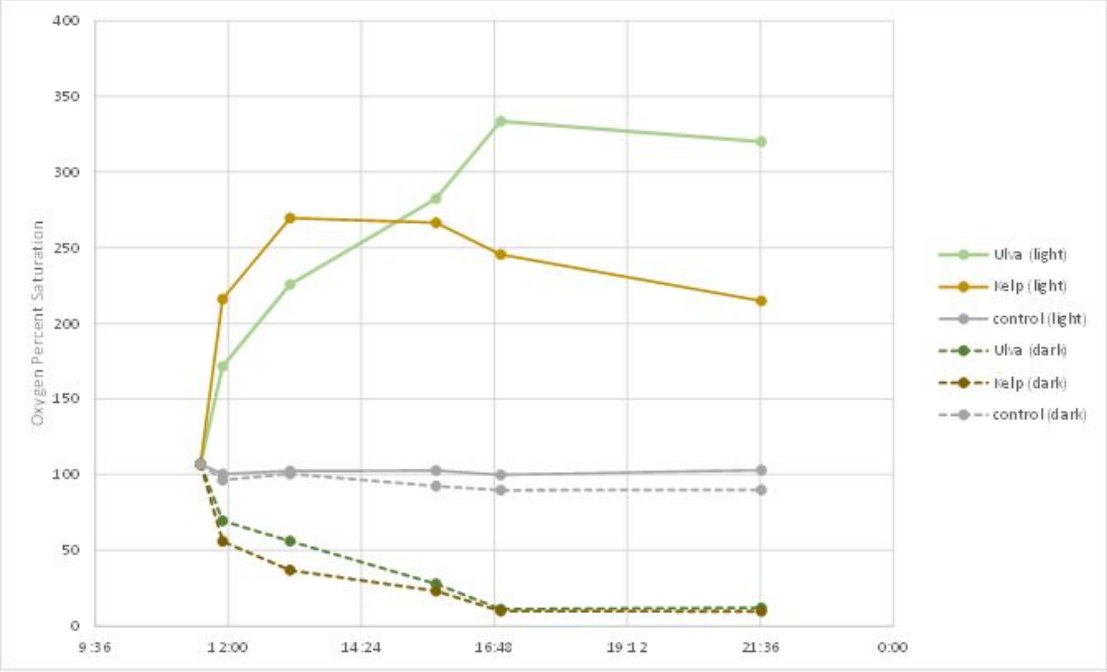
6) Place one set of flasks in full sunlight and in a location where they will continue to get full sun during the entire day. Place the flasks for the dark incubation in a larger cooler or other light-tight container.
7) Every 1-3 hours (or as time allows), record the time of day and measure oxygen concentrations (or pH) in each of the flasks or bottles. Make note of any observations (e.g. bubble formation, sunlight conditions). PAR data can also be obtained recorded using the local NERR or other meteorological station.

Examples of incubation setups for light:dark bottle experiments (clockwise from top left). 1) Incubation setup using using clear, recycled drink bottles in a free-standing water bath, 2) Setup at Kachemak Bay

NERR, including two types of SAV (kelp and sea lettuce) and flasks with control water. An identical set of flasks was replicated in the dark by placing them in a large cooler. 3) Three 1L beakers filled with eelgrass from Padilla Bay and incubated in direct sunlight for 4 hours. A duplicate set of three were incubated in the dark.

Data Analysis and Interpretation

Oxygen concentrations can be collected periodically during the incubations, or recorded using a deployable logger and downloaded at the end. Regardless of the method of data collection, the end goal is to generate a visualization of oxygen (or pH) over time for interpretation (see figure below). This data visualization is from a light:dark bottle incubation conducted at Kachemak Bay BNERR and used sea lettuce (*Ulva*) and kelp that were collected on local beaches. This particular investigation provided an excellent example of the effect of photosynthesis and respiration on oxygen concentrations, and also revealed potential differences in photosynthetic efficiency among the two plant species. An effect of planktonic respiration was also detected, although small, as evidenced by the decrease in oxygen in the dark control flasks. as an example).



Although the type and frequency of data will vary with each instructor, class or setting, there are four basic outcomes of this activity to work towards:

1) Participants have the experience of collecting data and then translating this user-collected data

into a visualization that can be interpreted by the group;

2) Participants gain expertise plotting time series data and understand that change over time can be easily detected by comparing time as an independent variable (x-axis) and oxygen concentration (or percent saturation, pH) as a dependent variable (y-axis);
3) Participants will compare and contrast differences in patterns between light and dark treatments, identify the accumulation or depletion of oxygen over time, and offer explanations of the mechanisms;
4) Participants will explain the relationship between light, photosynthesis, respiration, oxygen and carbon dioxide in the experimental manipulations, which will provide base knowledge and comfort when exploring similar parameters through the SWMP data portal.

D. Activity: *Investigating Respiration and Photosynthesis using Local Water Quality Data*

Note to instructor: *This activity provides students the opportunity to work with professionally-collected data, make predictions about oxygen production and consumption (i.e. photosynthesis and respiration) in the marine environment, and test their predictions using real-time environmental data recorded at a their local NERR reserve (and others across the country). The activity builds on the previous investigations in this session on respiration, dissolved oxygen and light (i.e. light:dark bottle experiments), and their interpretations of the results and observations. Collectively, these activities allow students to further demonstrate an understanding of the relationship between oxygen, carbon dioxide, photosynthesis, respiration, and the variability of these in natural waters - and navigate online data portals. For this activity, it is important that participants understand that photosynthesis is fueled by light energy, and that this results in the production of molecular oxygen (O_2).*

Materials and Preparation Needed

1. **Identifying local diel data to use**: Examples of SWMP data have been provided that illustrate the desired relationship between PAR and dissolved oxygen over a 2-4 day period. However, you may prefer to use more recent and locally-relevant water quality from the closest NERR and replace the data used in the handout “Predicting changes in O2 and CO2 in surface waters”.

2. **Class Handout**: If you are adding local PAR data to the handout, modify the template with a screen capture of the relevant SWMP data. Then prepare enough handouts for each participant to have a copy of the Modify the "Predicting changes in O2 and CO2 in surface waters

Activity Details

1. **Reflecting on the effect of photosynthesis and respiration on oxygen, CO2 and pH.** Have participants reflect on evidence of the role of photosynthesis and respiration on the movement of carbon through different reservoirs. Have them revisit their responses to the prompts below and make predictions of what relationships they will find when observing water quality data collected in local waters.
    a. What do you think will happen to oxygen concentrations during the day? At night?
    b. Based on observations from the light:dark bottle experiment, have participants predict what they think will happen to dissolved oxygen and pH over the course of the day. What about dissolved CO_2 concentrations? [*the concentrations of oxygen will decrease at night because less is being made as a byproduct of photosynthesis, carbon dioxide will increase over time because more is being made as a byproduct of respiration than is being used in photosynthesis*]

2. **Making predictions about respiration and photosynthesis.** Tell participants that they can use this graph to estimate the changes in dissolved oxygen and carbon dioxide they would expect to find in the water throughout the day as a proxy for photosynthesis and respiration. Have participants work in a small group to discuss the following prompts:
    - Thinking about the relationship between light energy, photosynthesis and phytoplankton in the water, what do you predict would happen to dissolved oxygen concentrations as light intensity increases? [*With more light the rate of photosynthesis would be higher because light energy is needed for photosynthesis; this would lead to higher dissolved oxygen concentrations because oxygen is released during photosynthesis*]
    - Assuming plankton respiration is relatively constant throughout at 24 hour period, what would you predict would happen to dissolved oxygen concentrations at night in the water column? [*The concentration of O_2 will decrease because less is being produced through*

photosynthesis].

- What do you predict will happen to CO_2 concentrations between high light (day) and low light (night) periods? *[The concentration of CO_2 shows an inverse relationship to O_2 with low levels of CO_2 during the day and high levels at night.]*

Optional: Remind participants to think about the following relationships to make predictions:

- when the rate of photosynthesis is greater than the rate of respiration, the dissolved oxygen concentration in the water will increase;
- in the absence of light, the rate of respiration will exceed the rate of photosynthesis and dissolved oxygen concentrations will decrease.

3. **Participants work on PAR and Dissolved Oxygen handout**: Participants work individually, then discuss their predictions in small groups as they examine four days of light energy (PAR) recorded at a NERR meteorological station. They make predictions about the pattern in dissolved oxygen, pH and carbon dioxide they expect based on these PAR conditions and concepts from the respiration activity and the light:dark bottle experiments. As they work through the handout, remind them to compare and discuss their findings when after they finish Question #3, then again for Question #5. Questions on the handout [*with answers*] include:
    a. In the figure above, indicate the approximate location of "noon" and "midnight" along the plotted data. [*Noon will be at the peak of the PAR curve each day, midnight at the trough. The x-axis can also be used to identify the time of these events.*]
    b. What do you predict would happen to dissolved oxygen concentrations as light intensity increases? What would happen at night? [*Dissolved oxygen will increase as PAR increases, and decrease at night.*]
    c. Draw a line in the figure above representing the pattern in dissolved oxygen you would expect to see over the four day period. [*Dissolved oxygen will generally reflect the pattern in PAR. Participants with more advanced understanding may include a time-lag in the increase in dissolved oxygen, with highest concentrations occurring after the highest PAR measurements. Others with knowledge of the potential effects of tidal flushing may say that it is too difficult to predict accurately the pattern in dissolved oxygen.*]
    d. Using a different color pen or pencil, draw a line that represents your prediction of

dissolved CO_2 concentrations, then of pH, over the four day period. [NOTE: *This part of the activity may involve extensive sense-making to work through the relationships between oxygen, carbon-dioxide and pH. Encourage all the participants to explain, justify and talk through their predictions with one another and help them come to more complete understanding. Correct predictions would have similar, overlapping lines for dissolved oxygen and pH, and an inverse pattern for dissolved carbon-dioxide (e.g. when oxygen and pH are elevated, carbon dioxide will be low).*]

4. **Testing predictions with NERR SWMP data:** Tell participants they will be testing their hypotheses (i.e. prediction) using water quality data collected during the time period represented on their handouts. Provide the Reserve name, MET and WQ station names, and dates for them to visualize SWMP data. Alternatively, display and discuss screen captures of the PAR and DO data downloaded from the NERR CDMO website.
    a. Once participants have downloaded, graphed and discussed the appropriate SWMP data to test their predictions, display the same SWMP data visualization on the screen. Using the Data Literacy Framework principles, have participants volunteer to walk through "Orientation" and "Interpretation"steps. Assist with this process if necessary, until all the relevant patterns are discussed. Focus on data included in the visualization, rather than discussing mechanisms or making predictions about what is not on the graph (this is the "Synthesis" stage).
    b. **Orientation**: Make sure participants note the two separate y-axes, the parameters being measured (and what the units mean), that data were collected every 15 minutes over a multi-day period. Additional information about where and how the data were collected is appropriate to discuss at this time.
    c. **Interpretation**: Have participants identify the patterns in the different variables and point out positive (or negative) correlations. Discuss all the patterns before moving to the step of synthesis, where mechanisms, causation, and other potential driving factors or variable (e.g. CO2) can be discussed. Prompts might include:
        i. What time of day do you see the highest light intensity? The lowest? [*midday. night*]
        ii. How do you think the weather might have been different on the days data were collected? What is your evidence for this? [*TLook at variabillity in PAR to*

indicate cloudy weather].

d. **Synthesis**: Encourage the participants to discuss and interpret patterns based on their knowledge of photosynthesis and respiration. Have them predict pH, then test this by looking at the SWMP data with pH as a parameter.

Appendix A: Materials Needed and Preparation for Yeast Experiment

For the class

- PowerPoint presentation
- Digital/data projector
- Whiteboard or flip chart paper and pens
- Masking tape
- Access to video:
    - Carbon Tracker online video https://www.youtube.com/watch?v=O4WMdwIwrSw

For each participant:

- Yeast Investigation Worksheets

For the A. Activity: *Tracking Carbon through Respiration (OSS 2.2)*

- 1 oz active dry yeast (4 packets)
- 16 oz distilled or other water (tested to be sure it doesn't change BTB's color)
- ½ tsp measuring spoon
- ¾ tsp baking soda
- 1 black permanent marker (fine point)
- 1 measuring pitcher, 500 mL
- 3 pipettes
- 2 test tubes
- plastic container with sealable top
- 1 bottle (18.75 g) glucose powder
- 1 plastic drinking straw

- 1 pair scissors
- 1 clear plastic cup (9 oz)
- 1 bottle bromothymol blue (BTB)
- 1 test tube cleaning brush

For each group (2 pairs) of participants:

- 1 cafeteria-style tray
- 2 clear plastic cups (9 oz)
- 4 test tubes with yeast culture
- 1 15 mL bottle of BTB (0.04% solution)
- 2 small plastic cups (1 oz) with 2 pipettes of tested water
- 2 small plastic cups (1 oz) with 2 pipettes of glucose solution
- 1 copy of Carbon Cards in an envelope
- 4 pipettes
- 2 test trays
- 4 stir sticks
- (optional) colored pencils

For B. Activity: *Tracking Carbon through Photosynthesis Part 1* (OSS 2.3)

- 1 tree branch
- (optional) jar with Elodea and BTB solution* (optional activity set up a few days before the start of this session)
- scissors or paper cutter

Preparation of Materials

Before the day of the session:

1. **Set up projection system/review multimedia.** Set up and test the projection system to be sure all participants will be able to see the videos at https://www.youtube.com/watch?v=O4WMdwIwrSw and https://www.youtube.com/watch?v=ypbb9Zi5Tao
2. **Duplicate copies.** Copy the following pages:

- Make one copy for each participant:
  - Yeast Investigation Worksheets
  - Evaluate the Evidence: The Source of Most Matter in Plant Structures handout
  - Researching Photosynthesis handout
  - Following the Water in Photosynthesis (reading). There are two copies of this reading on one sheet of paper; make the copies then cut the paper in half to provide a copy on ½ sheet of paper for each participant.
  - PAR and DO handout
- Make one copy for each small group of 3:
  - Concept Cartoon–Mass
- Optional handouts
  - Optional: Carbon in food
  - Optional: Reflection – Carbon sugar to air

3. **Label yeast test materials.** Using a permanent marker and Figure 2–1 on page 158 from OSS Unit 2 as a guide, (**a**) mark "S" on half of the test tubes; (**b**) mark "NS" on the other half of the test tubes; (**c**) mark half of the pipettes with "S" and mark "NS" on the other half; (**d**) mark half of the 1 oz cups "Sugar" and the other half "Water;" and (**e**) mark all test trays by writing "S" on the left side and "NS" on the right side.
4. **Prepare trays.** Set the following materials for 2 pairs of participants on each tray:
   - 2 clear cups, each holding 1 "S" test tube and 1 "NS" test tube
   - 4 stir sticks
   - 2 small cups marked "Water"
   - 2 small cups marked "Sugar"
   - 2 "NS" pipettes and 2 "S" pipettes
   - 1 bottle BTB
   - 2 marked test trays
   - (optional) yellow, blue, and green colored pencils
5. **Prepare demonstration straw.** Use scissors to cut a small (~1 cm long) diamond-shaped opening near one end of the straw. This opening will prevent the volunteer from accidentally sucking up liquid through the straw.
6. **Prepare Plant Investigations envelopes (OSS 2.3) for each group.** Copy Plant Investigations (one for each group of three). Cut apart the three descriptions and place them in an envelope

(one envelope for each group).

7. **Make copies of the Carbon Cards.** Make one set of 2-sided copies for each small group of 4 participants.

One to two hours before the session:

1. **Prepare yeast culture.** The following makes enough for 1 class of 32 participants:
    a. At least 1 hour before class begins, use the sealable container (in case you're doing this at home and need to transport the culture to school) to mix 1 oz of active dry yeast with 4 oz (125 mL) of body-temperature **(~99ºF)** tested water (see below in notes for success). Let it sit open to the air for at least 1 hour.
    b. After the yeast culture has been sitting for 1 hour, add 3/4 tsp of baking soda, and gently mix. This is to raise the pH slightly so the CO_2 that's produced effects a color change to BTB from blue to yellow.
2. **Make glucose (sugar) solution.** The following quantity is enough for 2 classes of 32 participants: Add 6 oz of tested water (see below in notes for success) to the measuring pitcher. Sprinkle in the contents of 1 bottle of glucose powder while stirring. Keep stirring until all the glucose is dissolved.
3. **Fill test tubes, water and sugar cups on trays.**
    a. Using one of the pipettes, transfer 2 pipettes (see below in notes for success) of yeast culture into all 32 test tubes for participants. (If you go by height, this quantity of yeast should reach ~3.5 cm up from the bottom of the test tubes.)
    b. Using a second clean pipette, place 2 pipettes of tested water into each water cup.
    c. Using a third clean pipette, place 2 pipettes of glucose (sugar) solution into each sugar cup.
4. **Prepare demonstration cup.** Add approximately 5 oz of tested water to a clean 9 oz clear plastic cup. Add enough drops of BTB to color the water a deep blue. Place the prepared straw near the cup and set this aside until needed in the session.
5. **Prepare BTB demonstration test tubes (controls).** Add 2 pipettes of tested water to one clean test tube marked "NS." Add 2 pipettes of sugar water to another clean test tube marked "S." Set both test tubes aside until needed.

Optional: Immediately before the session

Determine if you will use a warm water bath to keep the yeast warm and actively respiring during the investigation. Alternatively, you can ask participants to hold the tubes tightly in their hands to provide warmth to the yeast during the investigation. If your room is warm, these steps are likely not needed.

Notes for success for OSS 2.2:

- Test the water you intend to use—add some drops of BTB to a water sample. The water should be a strong blue. If it turns at all greenish, then use a different water source. Some bottled water and tap water sources cause the BTB to change color, so distilled water is recommended to eliminate variables that will affect the expected color change.
- The pipettes are calibrated. If you pinch the top of the pipette and release, there should be from 2.5 to 3.0 mL of liquid inside pipette. When measuring "2 pipettes," any quantity from 5 to 6 mL is fine.
- A made-up batch of yeast will be good for about 1½ days.
- If storing overnight, cover the yeast culture, and place it in a refrigerator. Allow an hour or two for the culture to come to room temperature before proceeding.
- Be sure **not** to use "rapid rise" or "highly active yeast." These have sugar and ascorbic acid added, which interferes with the BTB indicator.
- Important: Keep the yeast warm as it respires during the investigation – use a warm water bath or have participants hold the test tubes tightly in their warm hands as they are reacting.