Hands-on Hypoxia: Engaging High School Educators in the science behind Marine Microbial Dynamics in Hypoxic Coastal Areas Through Field and Classroom Experiences

current THE JOURNAL OF MARINE EDUCATION

ACTIVITIES AND PROGRAM MODEL

KYLA J. KELLY
CARA A. SCHIKSNIS
DIEUWERTJE J. KAST
J. CAMERON THRASH

*Author affiliations can be found in the back matter of this article

ubiquity press

ABSTRACT

The University of Southern California's (USC) Joint Educational Project's STEM Education Programs hosted a three-day summer workshop focused on marine microbiology and coastal deoxygenation for high school educators. To increase ocean literacy in high school students from Title I schools, topical marine science research was translated into four lesson plans appropriate for classrooms that teach biology and environmental science. The lesson plans focus on how marine microbes affect and are affected by the dissolved oxygen content of seawater but covered diverse oceanography topics including microbial ecology, nutrient cycling, physical ocean dynamics, and climate change. This education framework was designed to promote and facilitate hands on discovery-based learning and making observations about the natural world. The workshop and lesson plan development were executed in partnership with faculty and graduate students researching marine microbes and oceanography from USC's Marine and Environmental Biology department to provide scientific expertise on the subject matter. At the workshop, educators were guided through each lesson plan and given classroom sets of materials to complete each of the experiments in their own classrooms. Educators also had the opportunity to experience the academic research process at both USC and the Wrigley Institute of Environmental Studies on Catalina Island, California. Teachers valued this interactive experience to learn from professional scientists and STEM educators. They left the workshop equipped with the knowledge and confidence to teach these marine microbiology and biogeochemistry concepts in their classrooms.

CORRESPONDING AUTHOR:
Kyla J. Kelly
University of Southern California, US
kylakell@usc.edu

KEYWORDS:
Marine microbes; deoxygenation; marine science education; high school; oceanography; broader impacts

TO CITE THIS ARTICLE:
Kelly, K. J., Kast, D. J., Schiksnis, C. A., & Thrash, J. C. (2023). Hands-on Hypoxia: Engaging High School Educators in the science behind Marine Microbial Dynamics in Hypoxic Coastal Areas Through Field and Classroom Experiences. *Current: The Journal of Marine Education*, 38(1), pp. 28–41. DOI: https://doi.org/10.5334/cjme.86

INTRODUCTION

Marine science education is an important component of K-12 education to promote principles of ocean stewardship in the next generation. While hypoxia is commonly taught in marine science education programs, the role of marine microbes in this process is covered less frequently. Despite this, Ocean Literacy Principles scope and sequence include marine microbes and how they shape the marine ecosystem (NMEA a). Therefore, there is a critical need for additional incorporation of the importance of marine microbes in marine science education, especially while discussing coastal deoxygenation. To this end, we have designed an educational framework that includes four lesson plans covering marine microbial diversity, ecology, and physiology in relation to physical, chemical, and biological oceanography. An emphasis was placed on discussion of how these topics interact with or relate to coastal hypoxia in coastal California.

WHY STUDY MARINE MICROBES?

What do viruses, bacteria, archaea, phytoplankton, and protozoans all have in common? They are all single-celled and microscopic, belonging to a group of organisms known as marine microbes. Referred to as the "unseen majority" (Whitman et al., 1998) because of their microscopic size, marine microbes make up the largest contingency of living material or biomass in the ocean (Hatton et al., 2021). It is estimated that in the entire ocean, there are 10^{29} bacteria and archaea – that's 100,000x more than the number of stars in the visible universe (Bar-On et al., 2018; Harvey & Howell, 2022; Whitman et al., 1998).

Marine microbes are incredibly important to food webs and biogeochemical cycling. For instance, primary producers like phytoplankton are the base of the food web, converting sunlight energy into sugars (glucose) that is transferred to higher trophic levels via predation. Furthermore, bacteria and archaea are critical to biogeochemical cycling in the ocean by functioning as decomposers to recycle organic matter like nitrogen and phosphorus back into bioavailable nutrients (NOAA a).

Many microbes are also critical to controlling the Earth's climate and greenhouse gasses. Both photosynthesis and respiration by microorganisms impacts the concentration of oceanic and atmospheric oxygen and carbon dioxide concentrations (NOAA Ocean Exploration). For instance, primary producers like phytoplankton photosynthesize, absorbing carbon dioxide (CO_2) and converting it to breathable oxygen (O_2). Meanwhile, heterotrophic marine bacteria respire, taking in dissolved oxygen (DO) from seawater and converting it back to CO_2 via respiration. These two processes work synchronously: the photosynthetic organisms produce O_2 for organisms that respire, and the organisms that respire release CO_2 that can be used again by the photosynthetic organisms (Figure 1). However, these two processes are not always balanced.

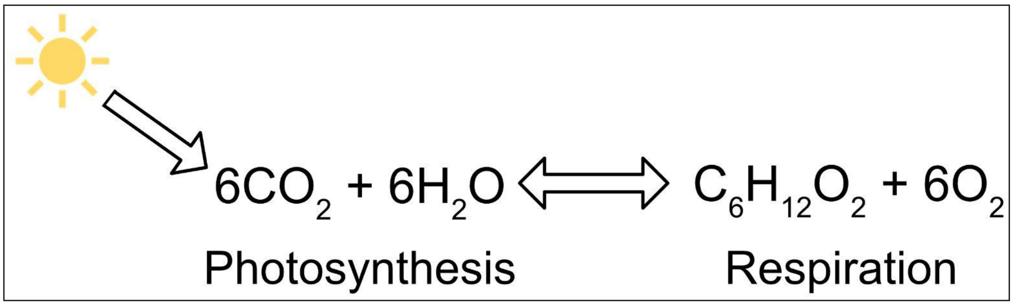

Figure 1 Photosynthesis and respiration equation.

Some regions of the ocean have low levels of oxygen and are considered oxygen deficient or hypoxic (<2 mg O_2/L). This lack of oxygen is problematic for animals and other heterotrophic, aerobic organisms that require adequate DO to respire (NOAA National Ocean Service). In some coastal regions, oxygen concentrations are low at depth due to nutrient pollution (USGS, 2017). This overabundance of nutrients fuels phytoplankton growth. When these algal blooms terminate and sink, they are decomposed by marine bacteria. This decomposition process consumes oxygen at a high rate, leaving waters hypoxic if they cannot be adequately ventilated with the atmosphere or other DO-rich waters. When DO concentrations are so low such that they can no longer sustain animal life, they are considered "dead zones" (NOAA National Ocean Service). In these severely hypoxic regions, massive die-offs of fish can occur, consequently harming fisheries (NOAA b). Furthermore, coastal hypoxia is expanding with climate change, especially in the Southern California Bight and the Gulf of Mexico (Booth et al., 2014; Turner & Rabalais, 2022). Research on hypoxia in coastal California is ongoing, and new information about what environmental and biological factors are contributing to expanding hypoxia is becoming available. Therefore, education of the general public on up-to-date hypoxia-related research is important as it impacts the communities in which they live.

To increase ocean literacy with regard to hypoxia and marine microbes, academic researchers, with expertise in marine microbes and coastal hypoxia, partnered with the USC Joint Educational Project's (JEP) STEM Education Programs to develop curricula and host a professional development workshop for both formal and informal educators. Relevant research was translated into four lesson plans that are suitable for use in high school classrooms teaching environmental and marine science, biology, and chemistry. Lesson plans were aligned to Next Generation Science Standard (NGSS), California Common Core Standards, A Framework for K-12 Science Education, and Ocean Literacy Principles Scope and Sequence (NGSS, 2017; California Department of Education, 2013; National Academies, 2012; NMEA a). The three-day workshop included an in-depth laboratory tour, a run-through of each of the four lessons, and a one-day excursion to the USC Wrigley Institute of Environmental Studies (WIES) on Catalina Island in the California Bight.

The objective of this workshop was to increase educator knowledge and confidence in teaching ocean sciences to high school students at Title I schools. Title I schools were our target audience to help increase access of disadvantaged students to hands-on science education that meets state academic content and performance standards. We aimed to increase ocean literacy in a larger population of students by equipping educators with the knowledge and resources to continue teaching our lessons year after year. Increasing ocean literacy helps students make educated decisions about the marine environment and its resources (NOAA c). The topics covered in these lessons are important and relevant, as climate change effects like deoxygenation can impact people from the communities in which our students and their families live. For instance, expanding hypoxic zones can be harmful to fisheries that people depend on for food or employment (Howard et al., 2020). Knowing how microbes influence these biological, chemical, and physical oceanographic processes is a key component of ocean literacy (NMEA b). Therefore, our lesson plans aim to increase ocean literacy and student awareness of pertinent issues facing their communities.

METHODS

Biological Oceanography Ph.D. candidates and STEM education experts developed four California State Science Standard and Ocean Literacy Principle-aligned lesson plans focused on marine microbes, ocean circulation, and deoxygenation. This content was created from both original ideas and adaptations of pre-existing lesson plans. The educational framework we created fills critical knowledge gaps in ocean literacy in California high school education. Furthermore, our lessons and workshop discussions place an emphasis on the marine environment off Southern California to help students connect the science to real world issues. These lesson plans follow the 5E format (Idsardi et al., 2019) and are publicly available online (*see Reproducibility*).

Eight high school educators attended a workshop on the University of Southern California's (USC) University Park Campus and the Wrigley Institute for Environmental Studies (WIES) on Catalina Island, California from July 12th to 14th, 2022 (Table 1). Most attendees came from Southern California institutions, with one teacher traveling from out of state (Washington). Informal educators comprised 25% of the attendees. Educators had a wide range of teaching experience: some with over 33 years and others just beginning their teaching careers.

Before the workshop began, educators were asked to take a survey to gather demographic information and attitudes towards teaching oceanography in their classrooms (Supplemental Document 1). Teachers also completed a pre-workshop assessment to quantify their background knowledge on the topics covered during the workshop. The knowledge assessment survey questions were graded for accuracy and averages for each question were calculated. The pre-workshop survey also had questions to quantify how interested and knowledgeable educators thought their students were with regard to oceanography topics. It should be noted that one survey was missing this information ($n = 7$), as one educator did not realize that there were two parts to the survey. To analyze this quantitative data, simple percentages were calculated.

	LOCATION	AGENDA	EDUCATIONAL STANDARDS
Day one	USC's University Park Campus	Introduction to marine microbial ecology lecture (2 h), Thrash Lab tour (2 h), Lesson 1: Exploring the Diversity of Marine Microbes (2 h)	NGSS HS-LS2-6 & HS-LS2-2; OLP 2, 3, 5; CCCS-R 3 & 4; CCCS-W 2 & 7; Framework PS3.D, LS4.C, LS2.B, ESS2.D, ESS3.D
Day two	Wrigley Institute for Environmental Studies (WIES) on Catalina Island, California	Lesson 1: Exploring the Diversity of Marine Microbes (3 h), research lab tours (3 h)	NGSS HS-LS2-6 & HS-LS2-2; OLP 2, 3, 5; CCCS-R 3 & 4; CCCS-W 2 & 7; Framework PS3.D, LS4.C, LS2.B, ESS2.D, ESS3.D
Day three	USC's University Park Campus	Lesson 2: Bacterial Ecology and Physiology (2 h)	NGSS HS-LS2-2 & HS-LS2-6; OLP 2, 3, 5; CCCS-R 3 & 4; CCCS-W 2 & 7; Framework LS2.B, ESS2.D, ESS3.D
		Lesson 3: Circulation of Nutrients (2 h)	NGSS HS-ESS3-5 & HS-ESS2-2; OLP 1, 2, 3; CCCS-R 3 & 4; CCCS-W 2 & 7; Framework LS2.B, ESS2.D, ESS3.D
		Lesson 4: Deoxygenation and Measuring Dissolved Oxygen (2 h)	NGSS HS-ESS3-5 & HS-ESS2-2; OLP 1, 3, 4, 5; CCCS-R 3 & 4; CCCS-W 2 & 7; Framework LS2.B, ESS2.D, ESS3.D

Table 1 Agenda for the workshop, time spent on each activity, and educational standards for Next Generation Science Standards (NGSS), Ocean Literacy Principles (OLP), California Common Core Standards: Reading Standards for Literacy in Science and Technical Subjects 6–12 (CCCS-R), California Common Core Standards: Writing Standards for Literacy in History/Social Studies, Science, and Technical Subjects 6–12 (CCCS-W), and A Framework for K-12 Science Education (Framework).

After collecting survey responses, the workshop began with a USC associate professor and principal investigator (PI) giving an introductory lecture on marine microbes, coastal deoxygenation, and the research their lab group at USC is conducting (*see Reproducibility*). Next, the PI gave a tour of their research lab in USC's Marine and Environmental Biology Department. Educators were introduced to several instruments used to conduct research, including environmental chambers for growing microbes, biological safety cabinets for conducting sterile culture work, and flow cytometers for counting cells (Figure 2). A graduate student in the lab demonstrated flow cytometry analyses of a sample they were working on, allowing teachers to see research in real time.

Figure 2 Educators look at a culture flask full of microbes (left) and learn about flow cytometry and cell sorting (right). Photos by Dr. Dieuwertje Kast.

After the lab tour, we returned to the teaching laboratory to run through Lesson 1: Exploring the Diversity of Marine Microbes (*see Reproducibility*). We provided some additional background information to prepare the educators for the lesson's content. The broad objectives of this lesson are to (a) gain a better understanding of marine microbes and why they are important, and (b) to learn about quantifying and characterizing microbial diversity in the ocean. One of the activities in this lesson instructs students to use a plankton tow to capture phytoplankton from the ocean, then identify species present under the microscope. While the real plankton tow was done on day two of the workshop at WIES (see below), this activity was simulated in the classroom using a makeshift plankton tow made from a 2-liter soda bottle, pantyhose, and string (Kelly & Kast, 2020; NJ Sea Grant Consortium, 2014). Glitter was poured in a large tote bin full of water to represent phytoplankton in the ocean. Teachers took turns towing the plankton net to collect the glitter "phytoplankton".

In addition to analyzing microbial community diversity, this lesson also asks students to examine how phytoplankton morphology (physical structures and shapes of organisms) aids them in staying near the surface of the ocean. This surface water retention of cells is important for phytoplankton access to adequate light for photosynthesis. To better understand this concept, we incorporated the Great Plankton Race (Deep-C Consortium, 2013; SIMS, 2020) into our lesson plan (Figure 3). The objective of this lesson was to craft the slowest sinking phytoplankton. Educators were introduced to phytoplankton morphology and structures that increase drag, helping cells stay buoyant. Next, teachers were given foam, paperclips, pipe cleaners, sponges, and straws to build their own slow-sinking phytoplankton. After ~15 minutes, the sinking speed of each plankton was recorded. We then discussed why some phytoplankton did better than others, focusing on surface area and its relation to drag. Science and math standards may be incorporated here, as students could calculate the surface area of their crafted phytoplankton and relate these values to sinking speed.

Figure 3 Teachers participate in the great plankton race activity from Lesson 1. A few examples of the phytoplankton teachers created are displayed. Photos by Dr. Dieuwertje Kast.

On day two of the professional development workshop, educators were brought to USC's field site on Catalina Island: the USC WIES. Teachers gained hands-on experience practicing experimental techniques and performing measurements used by oceanographers doing research in the field. To measure microbial respiration rates in the water off Catalina Island, educators deployed a Niskin bottle to collect a water sample (Figure 4). This water sampling method collects water at a specified depth by deploying a "messenger" to close the bottle, sealing the sample inside. Once the Niskin bottle was brought back onto the dock, the attached hose was used to collect water in opaque glass containers. These containers were then sealed to prevent any oxygen exchange with the air, so that oxygen consumption could be measured. A negative control was created by adding carbonyl cyanide m-chlorophenyl hydrazone (CCCP), which disrupts respiration. These special bottles were equipped with optode sensors, an oxygen detection technique that uses light and fiber optic cables to read the DO content of seawater. We showed educators how to take these measurements and they recorded initial values for time point zero. The samples were then left to incubate in a temperature-controlled water bath for a few hours. More optode measurements were collected each hour to demonstrate the change in DO levels over time. We further discussed the data with them on day three of the workshop, connecting this research tool and data to microbial respiration.

Next, an educator on staff at WIES led teachers in doing two plankton tows off the dock (Lesson 1). They used a 20-micron net to capture phytoplankton and an 80-micron net to capture zooplankton. These samples were taken up to the lab for microscopic analysis. Additionally, teachers practiced making standard water quality and environmental measurements: salinity using a refractometer, temperature, wind speed and direction using an anemometer, pH using pH strips, and turbidity using a Secchi disk (Figure 4). This data is important to record when collecting the plankton tow, as water's chemical and physical properties can impact plankton community composition. This is the standard procedure done by researchers who perform weekly net tows along many of the southern California piers to assess phytoplankton community composition (SCCOOS, 2022).

Figure 4 Educators take turns collecting water in a Niskin bottle to measure dissolved oxygen with an optode sensor (top left), measuring turbidity with a Secchi disk (top right), collecting phyto- and zooplankton in net tows (bottom left), and measuring salinity with a refractometer (bottom right). Photos by Dr. Dieuwertje Kast.

In the lab, teachers viewed samples from their plankton tows under the microscope (Figure 5). They were given identification guides and asked to identify and describe the organisms they saw. These observations of phytoplankton morphology (i.e., cellular shape and extracellular structures) were discussed in the context of the great plankton race activity on day one. The water was full of a diverse community that included phytoplankton (like *Chaetoceros sp.*), radiolarians, copepods, barnacle larvae (nauplii), and polychaetae worm larvae. We discussed the Latin names of these groups of organisms and how we can use language to help students understand the names of these different groups of organisms. For example, planktos is Greek for "to wander", and scientists define plankton as organisms that drift with the current and are unable to swim against it. The procedure for this plankton tow and microscopic identification activity are outlined in Lesson 1 (*see Reproducibility*).

Figure 5 Educators use microscopes at WIES to look at the phyto- and zooplankton samples they collected (left). One microscope was connected to a screen to project the view under the scope (right). Photos by Dr. Dieuwertje Kast and Kyla Kelly.

Lastly, educators toured a lab actively conducting research on foraminifera. The lab's PI briefly talked about the values of foraminifera research and the insights they can provide with regard to paleoclimate change research. Teachers had the opportunity to watch these researchers feed the organisms they were working with.

On day three of the workshop, we were back in the teaching lab on USC's main campus guiding educators through the rest of the lesson plans. We connected the concepts and activities to the educators' experiences at WIES. Starting with Lesson 2: Bacterial Ecology and Physiology (*see Reproducibility*), we engaged educators in the material by asking teachers what they already knew about ecology and physiology. We discussed how each of these terms can be applied to marine microbes. One of the objectives of this lesson was to perform a growth assay by plating bacteria and counting colony forming units (CFUs) to quantify bacteria present in a sample. This method is commonly used for water quality assessments such as determining the amount of *E. coli* present in a water sample to assess the risk of exposure of beachgoers to harmful pathogens (CA.gov, 2022). This method involves performing serial dilutions (another lesson objective), which can be related to state math standards. We first demonstrated this conceptually by serially diluting a water sample with dark blue food dye (Figure 6). This demonstration is helpful for visual learners, as one cannot directly observe the differences in concentrations when serially diluting bacteria.

Figure 6 A visual example of serial dilutions was made using food coloring and distilled water (top). In Lesson 2, teachers performed serial dilutions with bacterial cultures and plated them on agar (middle). Twenty-four hours later, the number of bacterial colonies on each plate were counted and quantified (bottom). Photos by Dr. Dieuwertje Kast and Kyla Kelly.

In pairs, educators performed their own serial dilutions with a concentrated culture of innocuous *E. coli* (strain NCM3722) and distilled water. To visualize the concentration of cells in each dilution, cells were plated on agar and left to incubate for 24 hours. Plates were checked the next day, and the data was sent to workshop attendees (Figure 6). During the workshop, we went over calculations of CFUs that are used to quantify bacteria in an environmental sample (*see equation*).

fl fi fi fifl fi fi
fl fl fi ufi fi fi

Equation 1: This equation is used to calculate colony forming units (CFUs) in Lesson 2.

They practiced these calculations with example plates previously made as a demonstration.

Next, we went over lessons focused on the ocean's physical and chemical properties. From the day one introductory lecture and the previous day's DO measurements, teachers were ready to engage with the concepts covered in Lesson 4: Deoxygenation and Measuring Dissolved Oxygen (*see Reproducibility*). Knowing that DO concentrations are dependent on certain ocean physical and chemical properties, the educators were tasked with measuring the DO in various water samples. They used dissolved oxygen test kits from Monitor Aquarium and Aquaculture Water Quality to measure DO in saline water (made using table salt) and freshwater, each at three different temperatures: cold, hot, and room temperature (Figure 7). Results were recorded and discussed in relation to the DO concentrations measured the previous day (using the optode method) on Catalina Island.

Figure 7 Teachers measure dissolved oxygen content of water samples with different salinities and temperatures in Lesson 4. Photos by Dr. Dieuwertje Kast.

Lastly, teachers participated in the activities outlined in Lesson 3: Circulation of Nutrients and Oxygen (*see Reproducibility*). First, teachers simulated ocean stratification by layering waters of differing densities in a clear plastic bin. Hot fresh water, hot salt water, cold fresh water, and cold salt water were each dyed a different color using food coloring. One at a time, each type of water was slowly poured down a sloping surface into the clear bin (Figure 8). Teachers observed the resulting layers (stratification) and we discussed how different water properties affect the density of water masses in the ocean. We made connections between this lab demonstration, stratification in the natural environment, and how this impacts circulation of nutrients and DO between the surface ocean and deep waters.

In addition to demonstrating how water masses of different densities form layers in the ocean, teachers also participated in an activity that showed how water bodies of different densities are unable to mix. Two cups of water with different densities (cold and salty vs. warm and fresh) were dyed blue or red (respectively), then placed in a shallow container of water. Each cup was poked with a tack to let the water slowly seep out. Teachers observed that the two water masses did not mix and formed their own layers (Figure 8).

At the end of the workshop, teachers were asked to participate in another post-workshop knowledge assessment that asked the same questions as the pre-workshop knowledge assessment to determine the effectiveness of our workshop in increasing educator knowledge of marine microbes and ocean deoxygenation (Supplemental Document 1). Additionally, this questionnaire included a quantitative survey that asked teachers to rate how much they valued the workshop and provided space for feedback. We graded this knowledge assessment portion for accuracy of answers and compared average scores for each question to scores from the pre-lesson knowledge assessments. Simple percentages were calculated for quantitative portion. Although eight teachers attended the workshop, only seven post-workshop surveys were collected, as one educator left early due to a schedule conflict.

Figure 8 In Lesson 3, educators poured water of different densities down a gently sloping surface (top left). The different water bodies (indicated by different colors) separated into layers based on density (top right). To demonstrate the separation of bodies of water with different densities, cups with cold, saline water (blue) and hot, fresh water (red) were placed in a shallow container of water (bottom left). Holes were poked in the cups to allow water to slowly trickle out. The water masses separated in the container, based on density (bottom right). Photos by Dr. Dieuwertje Kast.

Lastly, teachers were sent home with bags containing most of the supplies needed to do these lessons in their own classrooms. These supplies included DO measurement kits, test tubes, agar plates, disposable pipettes, plastic bins, build-your-own-plankton-tow supplies, and more. The supplies not provided were easily accessible "grocery store" items. Furthermore, teachers were given the opportunity to apply for continuing education units for participating in the workshop.

ALIGNMENT TO EDUCATIONAL STANDARDS

To make our lesson plans broadly available to California high schools, we aligned our lesson plans to NGSS and California Common Core Standards (NGSS, California Department of Education). We also aligned them to A Framework for K-12 Science Education standards and Ocean Literacy Principles Scope and Sequence to make these lesson plans applicable to educational standards in other states (National Academies, 2012; NMEA a). The NGSS standards covering biodiversity and populations in ecosystems and changing environmental conditions relate to our lessons about microbial diversity and climate change, while the standards covering geoscience relate to our lessons about the effects of physical and chemical oceanography on dissolved oxygen and nutrient concentrations. California Common Core Standards about carrying out experimental procedures and understanding science-specific words relate to performing the experiments in our lesson plans and analyzing the results, as well as comprehending the scientific content and vocabulary presented. A Framework for K-12 Science Education standards covering cycles of matter, cellular energy generating processes, adaptation, and climate change related to our lessons' discussion of the role of microbes in biogeochemical cycling, energy generation via photosynthesis, phytoplankton adaptations to life in the surface ocean (e.g., the great plankton race), and consequences of global change. Lastly, Ocean Literacy Principles covering ocean circulation, biogeochemical cycling, climate change, oxygen production, primary productivity, and ecosystem and biological diversity relate to our lessons about the role of phytoplankton and bacteria in ocean biogeochemistry and DO content.