

A close-up photograph of a person's hand holding a small, dark, rectangular container over a stream of water. The water is clear and flowing, with some ripples and reflections. The background is a blurred view of the stream and surrounding rocks.

The Microscopic World of Diatoms

Molly Sultany and Rebecca Bixby

Students collect stream water to study a biological indicator that can help detect pollution.

For students in biology, chemistry, or environmental science, diatoms offer excellent insight into watershed health and human impact on the environment. These microscopic algae have high species diversity, intricate geometry, and, at 20–500 micrometers (1 micrometer = 10^{-6}m), relatively large size, making them easily seen with a compound light microscope (LM). Diatoms are found globally in virtually every habitat that has sunlight and moisture, including polar seas, tropical streams, and on moist soils and mosses. Studying diatoms as biological indicators immerses students in a compelling exploration of aquatic ecosystems.

In this article, we present a novel and challenging set of activities that foster a greater understanding of scientific phenomena aligned with the *Next Generation Science Standards* (NGSS Lead States 2013; see box, p. 64). This investigation represents a two-year collaboration between a high school science teacher (MS) and a diatom researcher (RB), who mentored projects developed by 10th-grade chemistry students in Portland, Oregon. Students designed an experiment to study water quality using diatoms. Figure 1 (p. 57) outlines differentiation strategies, including group sizes, timing, and guiding questions for each activity. We conclude by discussing how to extend this lesson using a scanning electron microscope (SEM) in the classroom.

Why study diatoms?

Diatoms are one of the most ecologically important groups of marine and freshwater organisms (Stevenson and Bahls 1999). While students may be familiar with diatoms, teachers can introduce how, as *photoautotrophs*, diatoms fix carbon and produce oxygen that all animals, from zooplankton to whales, depend on for survival (Yool and Tyrrell 2003).

Diatoms are sensitive to natural disturbances and human activities (Walker and Pan 2006), including nutrient pollution from fertilizers (Potapova and Charles 2007), high silt levels from erosion (Bahls 1993), and deforestation (Bixby et al. 2009). Ubiquitous in distribution, diatoms colonize a variety of surfaces, including hard substrates (cobbles, logs, boulders), plant matter (macrophytes), and sediment (sand, silt). Given their abundance in aquatic ecosystems, rapid reproduction of one week, and ability to respond quickly to changes in their environment, diatoms and other algae are ideal biological indicators for water quality studies (Stevenson, Pan, and Van Dam 2010).

Pre-assessment: What makes a stream healthy?

In a 30-minute introductory activity, we introduce the essential question: How does living in the Anthropocene—the proposed period during which human activity has been the dominant influence on climate and the environment—affect the integrity of aquatic ecosystems? Students in teams of four brainstorm factors affecting water quality. They use a website called Scorecard (see “On the web”) to investigate sources of pollution in their neighborhoods, searching for

industrial polluters by zip code. We discuss criteria that classify a stream as impaired, emphasizing how anthropogenic stressors affect water condition, biodiversity, and ecosystem services. Students review these concepts:

- ◆ Stream health involves biological, physical, and chemical metrics.
- ◆ Water quality may be highly variable due to complex factors, including seasonality, nutrient levels, and type/extent of human activities.
- ◆ Bioindicators respond predictably to alterations in water quality with changes in species richness, abundance, and community composition.
- ◆ Sampling diatoms is noninvasive, inexpensive, and time efficient.

Next, students participate in a 60-minute pre-lab activity that uses a free online resource, SimRiver (see “On the web”), which helps students better understand how to collect and analyze diatom data, including identifying diatoms from river samples and calculating water quality. In the simulation, students select a time of year and sampling location. They specify such parameters as human population size, wastewater treatment plant locations, and land use. Working in pairs, students record their findings on a SimRiver datasheet (see “On the web”).

Overall, SimRiver helped students successfully identify diatoms by matching their appearance with images from a



FIGURE 1

Learning objectives, lesson timeline, and grouping strategies.

Type of activity or assessment	Group Size	Timing	Learning goal: Students will be able to...
Pre-assessment	4	Day 1	Explain how living in the Anthropocene affects the integrity of aquatic ecosystems
Pre-lab: SimRiver	2	Day 2	Calculate stream health using simulated diatom data
Part 1: research question	2	Day 3–5	Construct a testable, focused research question with one independent variable
Part 2: field work	Whole class	Day 6	Describe how to collect diatoms and water samples in the field, and identify experimental variables, constants, and control groups
Part 3: processing lab	2	Day 7–8	Develop analytic technique to process diatom samples and describe the scientific objectives for each step
Part 4: visualizing diatoms	4	Day 9–10	Evaluate patterns in diatom morphology using a LM and/or SEM
Part 5: data interpretation	4	Day 11–12	Compute metrics of water quality using diatoms and determine the relative health of a field site from a diatom bioassessment
Post-assessment	Individual	Day 12–14	Debate how human activity impacts local water quality using evidence from multiple sources: SimRiver, field data, and digital maps

light microscope key (Mayama et al. 2011). Students practiced the same data-collection technique used to analyze actual diatom samples later in this lesson. SimRiver reinforces how to incorporate math into community ecology, as students use mathematical equations to calculate a stream's Index of Biotic Integrity (IBI) and evaluate statistical significance in diatom populations across field sites.

Part 1: Designing a research question

While learning how pollution affects aquatic ecosystems from SimRiver, students work collaboratively to develop a testable research question. To refine their ideas, students participate in an interactive video chat with scientists who study diatoms. Students read relevant literature, brainstorm questions, and review vocabulary to prepare for this experience. Teachers can find diatom researchers by e-mailing local universities or museums.

We reached out to diatom scientists across the country, including coauthor Dr. Bixby and the authors of the website *Diatoms of the United States*, an identification guide (see “On the web”). During the video chats, each team shared their research question, receiving personalized feedback on scope, design, and feasibility. The researchers asked students questions starting with “Why?” “How?” and “What might happen if?” Incorporating insights from the video conference, students finalized the following research questions:

- ◆ How does reduced light availability caused by manufactured canopy cover (e.g., bridges) affect diatom communities that live on rocks?
- ◆ How do diatom communities in highly urbanized areas differ in the proportion of pollution-tolerant or low-pH-tolerant taxa compared to reference streams?
- ◆ How does logging along Oregon Coast streams influence water clarity, conductivity, and temperature, affecting diatom diversity and community composition?

Part 2: Collecting diatoms in the field

Ideally, students collect diatoms in sunny weather from February to June during the spring diatom bloom. They avoid sampling during or after heavy downpours, which scour rocks, washing diatom communities downstream (Stevenson and Bahls 1999). Before collections, review measures for staying safe while working outdoors near water. Needed safety gear includes gloves, eye protection, first-aid kit, personal flotation devices (PFDs), and appropriate footwear. All students complete a field trip release form with signed parental permission to be kept on file at school.

In the past, we have structured this field research in various ways. We made a class trip to an urban stream and a forested creek. Students have done independent data collection supervised by their families, collecting diatoms from diverse

microhabitats including backyard streams and ponds, and even at alpine rivers. To support the field trip, we borrow supplies such as hip waders and digital sensors from local nonprofit organizations and private companies. We encourage teachers to seek out local sources of support and explore funding from educator grants; we received financial assistance from the Diack Ecology Education Program, Northwest Aquatic and Marine Educators, and the American Chemical Society.

Before the field outing, teachers can structure a mini-lesson to help students practice using digital sensors and to assemble a simple field kit. Each kit includes

- ◆ 2 clean toothbrushes
- ◆ 1 water bottle
- ◆ 1 plastic container (e.g., school lunch tray)
- ◆ clipboard/pencil, and
- ◆ field collection datasheet (see “On the web”).

Students watch a video on diatom collection learning to use a toothbrush to scrape diatoms from stream cobbles (see “On the web” for the video and a description of the procedure). To correlate diatom data with water chemistry, students collect 100 ml of water, testing pH, conductivity, heavy metals, phosphate, and turbidity back in the lab (Figure 2).

Part 3: Processing diatoms

Before visualizing diatoms with a light microscope, students working in pairs participate in two labs. (Students wear gloves, goggles, and safety aprons for these activities.) First, they process diatom samples to remove organic matter that often obscures diatoms seen through a LM. Then, they make permanent diatom slides (see “On the web” for protocols for processing diatom samples).

When students return from the field, an easy alternative to preserving diatoms with formalin or Lugol’s iodine is to refrigerate the samples in the short-term, reducing bacterial and fungal growth. On the day of the lab, teachers can prepare a 20%

FIGURE 2

Example of student procedure for how to analyze stream water samples in the lab.

The five tests depicted below include (1) pH, (2) conductivity, (3) heavy metals, (4) phosphate, and (5) turbidity.

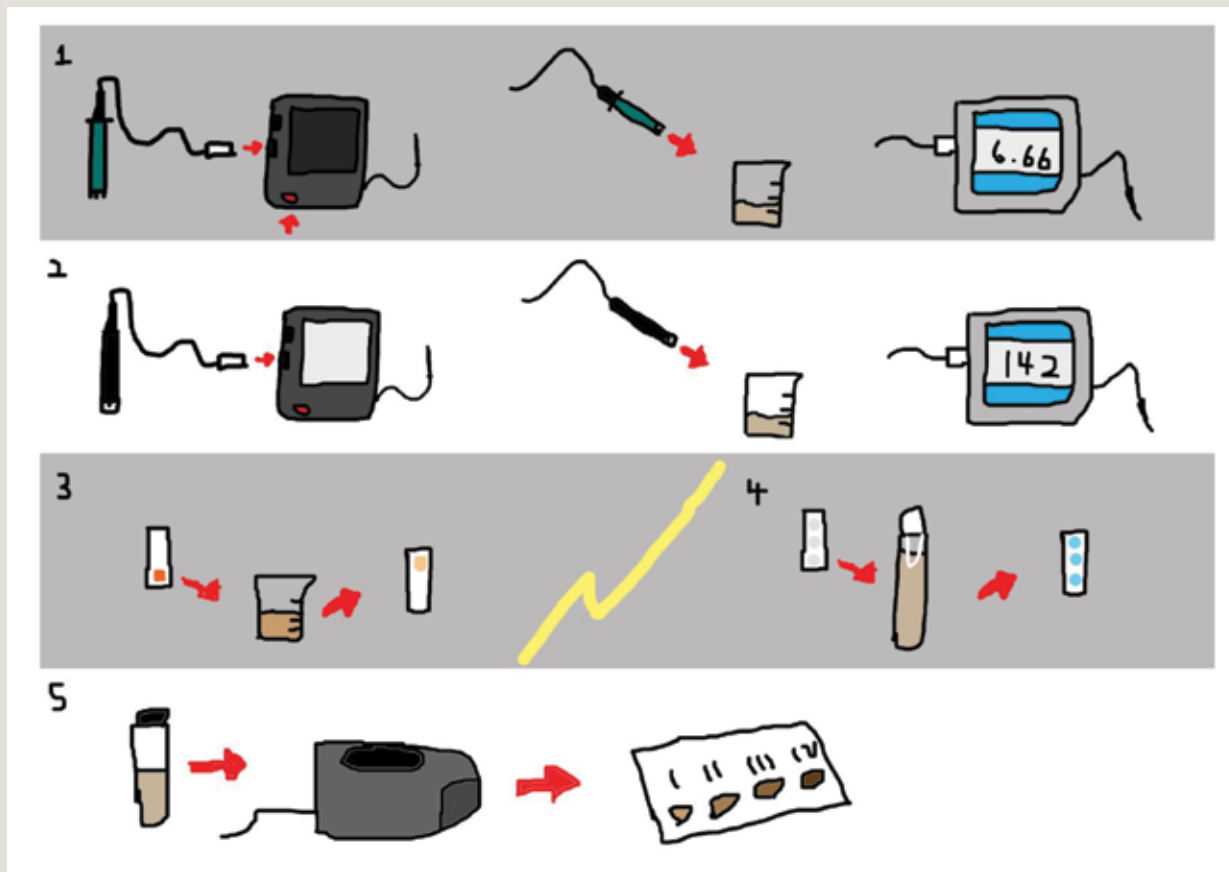


ILLUSTRATION BY STUDENT KELE STORHIM

bleach solution, which acts as an effective, inexpensive, and less caustic oxidizing agent compared to concentrated acids (Carr, Hergenrader, and Troelstrup Jr. 1986). Next, students make permanent diatom slides following a SimRiver video (see “On the web”). Instead of using a toluene-based mounting medium (e.g., Naphrax), educators should purchase light corn syrup. Corn syrup has a high refractive index (1.47) similar to Naphrax (1.65), making it an effective mounting medium to visualize diatoms with a LM (Brown 1997). We explain to students how, since the early 1900s, botanists have used corn syrup to mount diatoms on microscope slides. One of our students created a procedural sketch depicting this process (Figure 3).

Part 4: Visualizing diatoms

Students take pride in making their own slides, and identifying diatoms with a compound light, or phase contrast, micro-

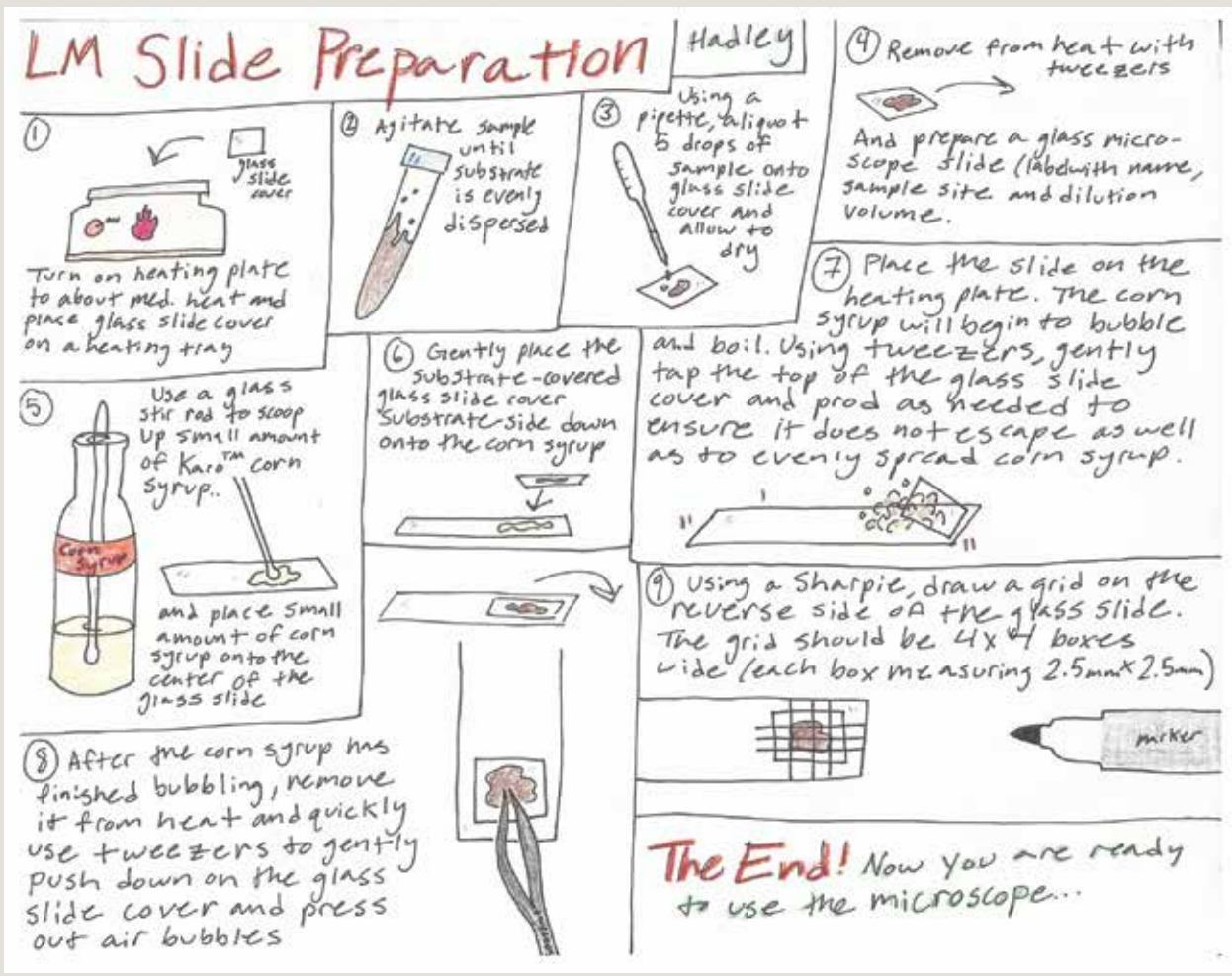
scope at 400x. Locating intricately patterned, geometric diatoms with LMs is like a biological game of Where’s Waldo? A few drops of processed diatom slurry may contain thousands of diatoms. If there are too many diatoms per slide, students adjust the dilution volume to make additional slides.

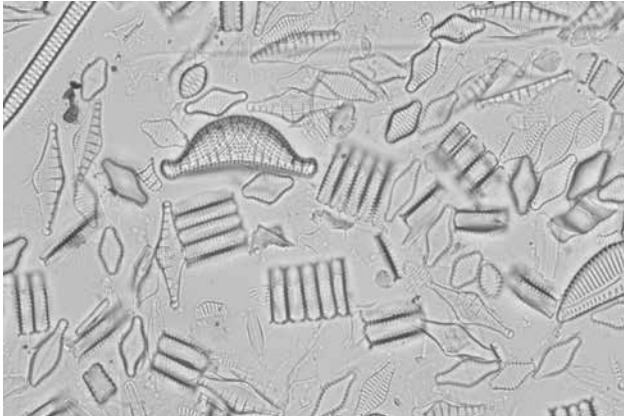
In their lab notebook, students record diatom observations, creating an informal descriptor for each genus before learning its scientific name: “guitar” for *Tabellaria*, “kiwi” for *Surirella*, “mustache” for *Epithemia*, and “birthday cake” for *Stephanodiscus* (Figure 4, p. 60). Optional extensions include viewing diatom slides under oil immersion, using digital media (tablets, computers, cell phones) to photograph diatoms, sketching each genus, or counting a larger number of diatoms for greater reliability (Stevenson and Bahls 1999).

Students image diatoms not just with LMs but also with the Phenom Pro, a desktop scanning electron microscope

FIGURE 3

A student’s procedural sketch detailing how to make light microscope slides from processed diatom samples.





These diatoms are imaged at 1,000 \times magnification.

(SEM), to examine diatoms at a higher magnification. With an electron optical magnification up to 130,000 \times , the SEM intrigues students. Advantages include a higher resolution

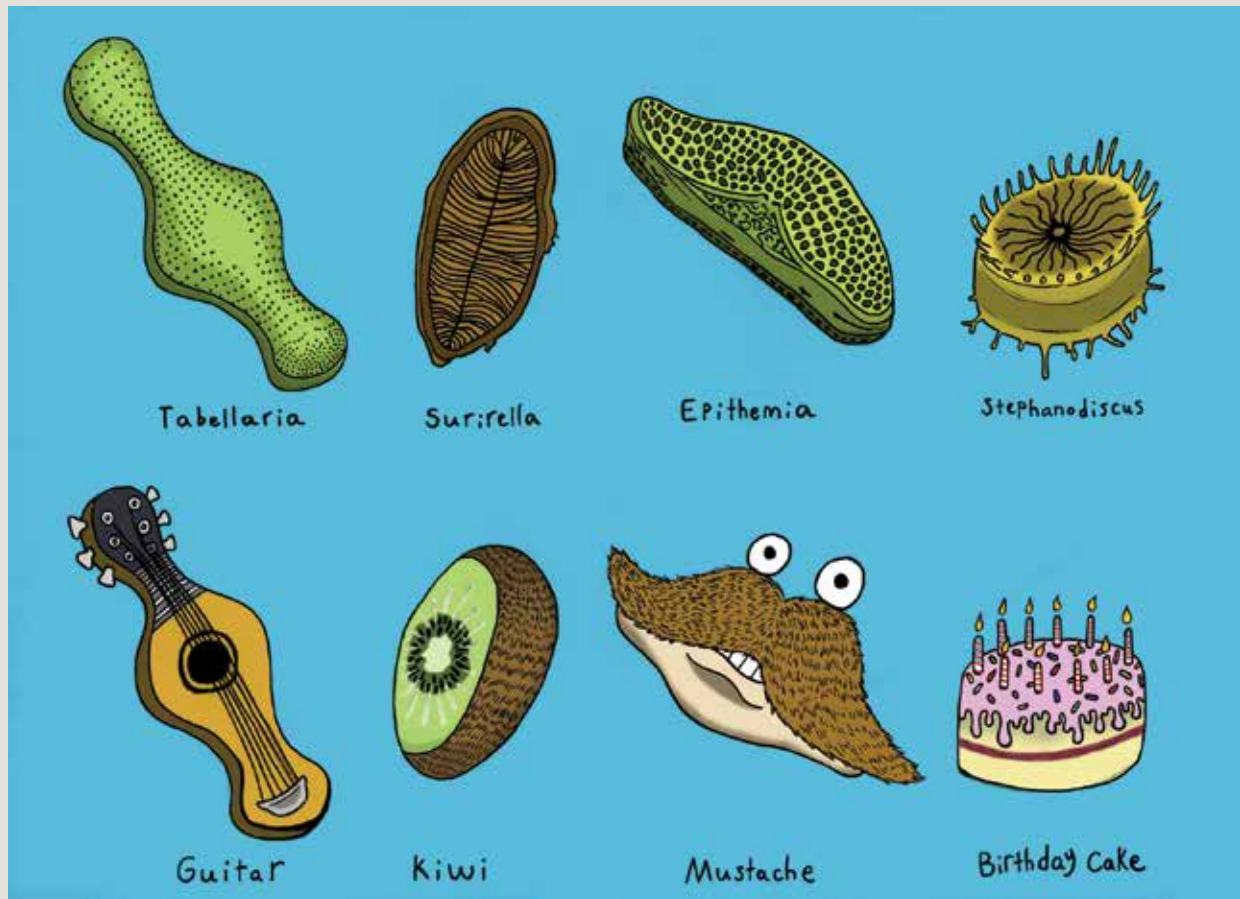
and larger depth of field, enabling a better characterization of rarer or smaller diatoms in students' samples. To access a SEM, we recommend contacting university outreach programs or science museums. Some universities loan SEM equipment, encourage students to submit samples for analysis, or offer field trips to campus. In Oregon, Project Nano, a teacher professional development program run by the Portland STEM Center and Portland State University, provides instructors with training to design a lesson using the SEM and to borrow the Phenom for two to three weeks each year.

Part 5: Guiding data interpretation

Once students tally numbers and types of diatoms present in their samples, they enter the data into a spreadsheet (for an example, see "On the web"). To make counting easier, students create a color-coded key of diatoms using LM or SEM images (Figure 5). Using values from primary literature, students add additional data into their spreadsheets

FIGURE 4

An example of how students used diatom features to invent morphological descriptors.



for diatom pollution tolerance and saprobity. Many online resources exist with coded numeric scales describing environmental preferences of each diatom genus. Our students accessed a regional Pacific Northwest diatom database courtesy of a local diatom researcher for this information; however, Table 1 in Hill et al.'s (2001) paper represents a helpful resource to understand the fundamental niche of diatom genera.

To answer the essential, guiding question of how living in the Anthropocene affects the integrity of aquatic ecosystems, students analyze diatom data using diverse metrics. For a comprehensive list of diatom indices, we refer students to the U.S. Environmental Protection Agency's rapid bioassessment protocol for periphyton (see "On the web"). Ideas include:

- ◆ **Water quality class:** What is the numeric value for stream health based on diatom sensitivities to nutrient pollution? After accessing diatom *saprobity values* (the level of decomposition of organic matter in water) from primary literature (Van Dam, Mertens, and Sinkeldam 1994), students use the same calculation introduced in SimRiver. Diatom saprobity reveals information about a stream's biological oxygen demand, oxygen saturation level, and available nutrients. *Polysaprobic* diatoms can be found in heavily polluted, oxygen-poor waters, while

oligosaprobic diatoms are located in minimally polluted waters with greater oxygenation (Figure 6).

- ◆ **Percent community similarity index:** How similar are diatom communities between two field sites? Students hypothesize that stream sites located in close geographic proximity may have more similar diatom assemblages compared with localities in different watersheds. This metric provides an interesting comparison of genus-level biodiversity across two sites.

FIGURE 5

Example of a student's color-coding for a diatom SEM image with two visible genera.

The blue diatoms are *Navicula* and the purple ones are *Nitzschia*. Students do not count broken diatoms.

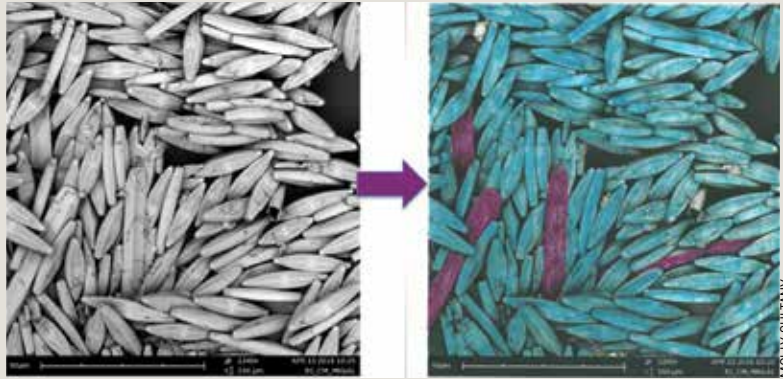
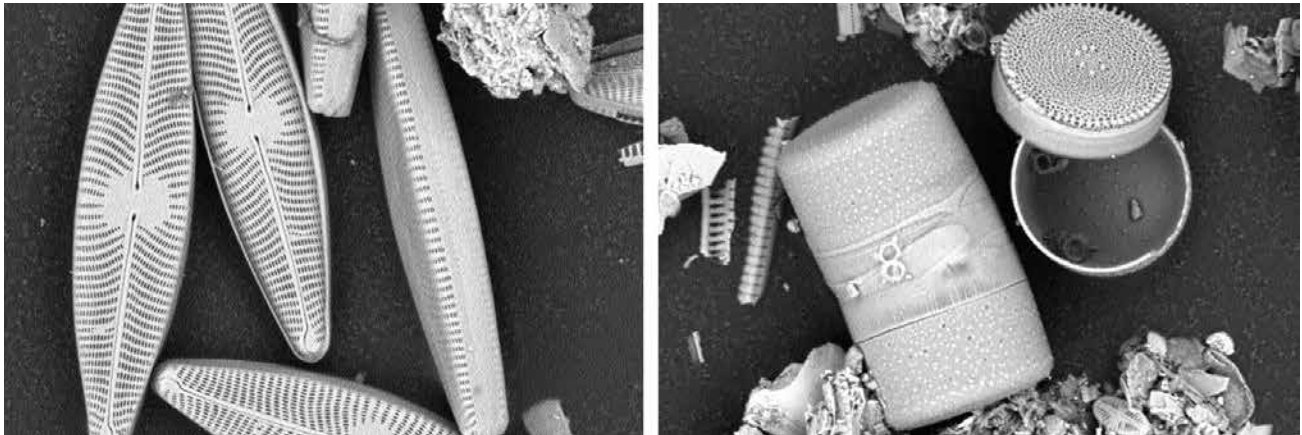


FIGURE 6

Linking diatom nutrient preferences to a stream's pollution profile.

Each diatom genus has a different saprobity value, which is an index of its nutrient preferences. Using these values, students make inferences about the level of nutrient pollution and dissolved oxygen within a stream (Van Dam et al. 1994).

Saprobity value	Scientific term	Explanation
1	oligosaprobic	Minimally polluted, high levels of inorganic nutrients, high dissolved oxygen levels
2	beta mesosaprobic	Moderately polluted, nitrogen in the form of ammonia present
3	alpha mesosaprobic	Heavily polluted, nitrogen in the form of amino acids present, decreased dissolved oxygen concentrations
4	alpha meso/polysaprobic	Very heavily polluted
5	polysaprobic	Highly polluted with sewage or organic nutrients, high bacterial activity, and production of ammonia and hydrogen sulfide, oxygen absent



Diatoms differ in cell size, patterning, and ornamentation, making them easily identifiable with a SEM.

- ◆ **Sensitivity to organic pollution:** What is the proportion of pollution-sensitive, indifferent, and tolerant diatoms at each field site? If students calculate a high proportion of pollution-sensitive diatoms in a stream (e.g., *Nitzschia sinuata* var. *Tabellaria*), they may compare this bioassessment to chemical metrics of stream health.
- ◆ **Motile taxa:** What percentage of diatoms sampled (*Navicula* + *Nitzschia* + *Surirella*) are capable of movement? This metric can be used as an indirect measure of how much silt and fine sediments are suspended in the water column; these diatoms may be better adapted to an aquatic environment with high erosion (Stevenson and Bahls 1999).

As a class, students counted 2,947 diatoms, detecting evidence of anthropogenic pollution across all 10 Pacific Northwest field sites. Students calculated statistically significant differences in diatom populations in slightly polluted vs. highly impaired waters. After counting 220 diatoms from a farm in Washougal, Washington, students classified water quality as slightly polluted based on a diatom saprobity index of 1.77 (student calculation, Figure 7; saprobity index key, Figure 6, p. 61). At this site, students counted a high proportion of *Eunotia* (46% of diatoms), a diatom that thrives in low pH waters. Another student group sampled diatoms along an industrial section of the Willamette River, classifying the water quality as moderately polluted (2.8). After counting 224 diatoms within nine genera, students learned that the diatom community consisted of 9% pollution-sensitive diatoms, 89% indifferent, and 2% pollution tolerant. Students

identified a high proportion of *Melosira* (60% of diatoms), a pollution-indifferent species that thrives in nutrient-rich (i.e., eutrophic) waters. Overall, our students were amazed at the specific, nuanced information about water quality and aquatic ecosystem health that they inferred from conducting a diatom bioassessment.

Assessment strategies

Throughout this lesson, teachers can assess students with an emphasis on process (see “On the web” for differentiation strategies). After the first week and a half, student teams submit a research proposal as a formative assessment. This assignment helps students articulate clear goals and design a feasible procedure, becoming scaffolding for a formal lab

FIGURE 7

Example of a student’s spreadsheet calculations for water quality class using saprobity values.

Diatom Genus	Abundance (N)	Pollution Tolerance Class	Saprobic Value (S)	N x S
<i>Pinnularia</i>	4	2	1.5	6
<i>Navicula</i>	54	2	3	162
<i>Achnantheidium</i>	54	3	2	108
<i>Eunotia</i>	102	3	1	102
<i>Humidophila</i>	5	2	2	10
<i>Surirella</i>	1	2	1.5	1.5
Sum total of N x S column:				389.5
Sum total abundance (N):				220
Water quality class calculation (N x S)/(N):				$389.5/220 = 1.77$

report. In-class workshops provide a structural framework to introduce students to specialized vocabulary and critical-thinking skills inherent to science writing. Each week, students collaboratively wrote their proposal during class, discussing ideas with their peers. Homework involved reading papers from primary literature and revising the research proposal in Google Docs. As an additional formative assessment, our students developed an inventive identification guide to learn the names and features of common Portland-area diatoms (see “On the web”).

The final, culminating summative assessment can be a lab report or poster (see “On the web” for a rubric). In the past, our students constructed research posters and defended their findings during a classroom poster session. AP Environmental Science students can be assessed using AP lab report guidelines. Recently, our students wrote in-depth research papers, articulating what they learned about water quality in the context of environmental chemistry.

Conclusions

After analyzing diatom data, students successfully answered the essential question: How does living in the Anthropocene affect the integrity of aquatic ecosystems? One student reflected, “This research is incredibly important because the quality of the Earth’s water, as well as its ecosystems, impacts every living thing, including humans.”

This diatom lesson marks a scientific rite of passage for our sophomores. Our students have presented diatom work at local watershed conferences and regional and state science fairs. Last fall, they shared their research with Dr. Jane Goodall at the Oregon Youth Summit. Students described the experience as “inspiring, humbling, and incredibly rewarding.” ■

Molly Sultany (msultany@nacademy.org) is a high school science teacher at Northwest Academy in Portland, Oregon, and Rebecca Bixby (bbixby@unm.edu) is a research professor in the biology department at the University of New Mexico in Albuquerque, New Mexico.

On the web

Collecting diatoms (video): <http://bit.ly/Diatom-collect>

Diatoms of the United States: <http://westerndiatoms.colorado.edu>

Field collection procedure and datasheet, protocols for processing diatom samples, sample student data spreadsheet, diatom identification key, rubric, differentiation strategies: www.nsta.org/highschool/connections.aspx

Scorecard: <http://scorecard.goodguide.com>

SimRiver: <http://bit.ly/2aLnrKw>

SimRiver datasheet: <http://bit.ly/2bdWkWN>

SimRiver video on slide preparation: <http://bit.ly/2aFeH4W>

U.S. Environmental Protection Agency’s rapid bioassessment protocol for periphyton: <http://bit.ly/2aVpNnV>

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Connecting to the *Next Generation Science Standards (NGSS Lead States 2013)*.

Standards		
<p>HS-LS2 Ecosystems: Interactions, Energy, and Dynamics HS-ESS3 Earth and Human Activity</p>		
Performance expectations		
<p>The chart below makes one set of connections between the instruction outlined in this article and the <i>NGSS</i>. Other valid connections are likely; however, space restrictions prevent us from listing all possibilities. The activities outlined in this article are just one step toward reaching the performance expectations listed below.</p> <p>HS-LS2-2. Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales.</p> <p>HS-ESS3-6. Use a computational representation to illustrate the relationships among Earth systems and how those relationships are being modified due to human activity.</p>		
Dimension	Name and <i>NGSS</i> code/citation	Specific connections to classroom activity
<p>Science and Engineering Practices</p>	<p>Asking Questions and Defining Problems</p> <ul style="list-style-type: none"> Ask questions that arise from examining models or a theory to clarify relationships. <p>Planning and Carrying Out Investigations</p> <ul style="list-style-type: none"> Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence. <p>Engaging in Argument From Evidence</p> <ul style="list-style-type: none"> Construct an oral and written argument or counterarguments based on data and evidence. <p>Obtaining, Evaluating, and Communicating Information</p> <ul style="list-style-type: none"> Communicate scientific ideas (e.g., about phenomena and/or the process of development and the design and performance of a proposed process or system) in multiple formats (including orally, graphically, textually, and mathematically). 	<p>After investigating a stream’s pollution profile, students develop testable questions on water quality.</p> <p>Students design an investigation, participate in fieldwork to collect diatoms, and use digital sensors to quantify stream health.</p> <p>Students share empirical questions with diatom scientists via Skype, and defend their findings using scientific evidence.</p> <p>Students communicate their results with lab reports, research papers, and poster presentations.</p>
<p>Disciplinary Core Ideas</p>	<p>LS2.C: Ecosystem Dynamics, Functioning, and Resilience</p> <ul style="list-style-type: none"> Anthropogenic changes (induced by human activity) in the environment...can disrupt an ecosystem and threaten the survival of some species. (HS-LS2-2) <p>ESS3.C: Human Impacts on Earth Systems</p> <ul style="list-style-type: none"> The sustainability of human societies and the biodiversity that supports them requires responsible management of natural resources. 	<p>Students investigate how anthropogenic stressors disrupt diatom communities, impact aquatic food webs, and degrade water quality.</p> <p>Comparing diatom and stream chemistry data allows students to infer human impact on stream ecology.</p>
<p>Crosscutting Concept</p>	<p>Scale, Proportion, and Quantity</p> <ul style="list-style-type: none"> In considering phenomena, it is critical to recognize what is relevant at different measures of size, time, and energy and to recognize how changes in scale, proportion, or quantity affect a system’s structure or performance. (HS-LS2-2) 	<p>Imaging diatoms with microscopes (LMs, SEMs) facilitates the study of the metric system and scientific notation.</p> <p>Diatom communities change in response to natural and anthropogenic disturbances, revealing information about ecosystem stability.</p> <p>Diatom structure relates to ecological function. Some diatoms form large colonies with many individuals, connected by silica spines, an adaptation to evade predation by snails.</p>

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