Microscopic Technique as a STEM Initiative Promotes Environmental Stewardship at a Community Sailing Program

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Abstract

As part of its STEM programming initiatives, Community Boating, Inc., the nation's oldest public sailing organization introduced an "Introduction to Microscopy" course merging principles of scientific investigation with analytical techniques using compound light microscopes. Boston's Charles River provided the environmental setting for five two-day sessions. In all, 49 students between the ages of 10 and 15 took part. Students developed an ability to analyze river water samples they had collected during field expeditions in kayaks. Once in the laboratory, following instruction in the use of the microscope, wet mount slides were prepared for microscopic investigation for cyanobacteria and photosynthetic phytoplankton, diatoms, and dinoflagellates crucial for a healthy aquatic ecosystem. With these new skills students were able to better appreciate the biodiversity of the microbial life within the river, and better understand the importance of environmental stewardship in a world threatened by global climate change and the effects of urban pollution. This program demonstrated the feasibility of developing sophisticated STEM programming founded upon principles of the scientific method for important environmental initiatives in the unique setting of a community sailing program.

Key words: cyanobacteria, microscope, phytoplankton, environmental studies, scientific method

Figure 1. For over 75 years the recreational area for Community Boating, Inc. occupies the lower basin of the Charles River between the cities of Boston and Cambridge, Massachusetts. Sailboats may be seen with the CBI boathouse, boat mooring area, and docks located to the right of the photo.

Introduction

Since 1946, the mission of Community Boating, Inc. (CBI), the nation's oldest community sailing organization, has been the advancement of the sport of sailing by minimizing economic and physical obstacles. CBI enhances the community by offering access to sailing as a vehicle to empower its members to develop independence and self-confidence, improve communication and, foster teamwork. Members also acquire a deeper understanding of community spirit and the power of volunteerism. (Figure 1)

In recent years, due to the impacts of pollution and global climate change and the increasing interest of cyanobacterial blooms in recreational waters CBI and its leadership proposed broadening its educational programming within our summer junior sailing program to include environmental science topics focused on cyanobacteria and its potential public health consequences. Cyanobacteria, previously referred to as blue-green algae, are photosynthetic bacteria that occur naturally in waters used for recreation, such as swimming, sailing and waterskiing. Under certain conditions, cyanobacterial blooms may be formed due to rapid growth into dense accumulations. When toxin-producing cyanobacteria are involved constituting a public health concern, the term, harmful algal bloom (HAB) is applied. The production of toxins can pose health risks to humans and animals. Visual signs of a bloom include: surface water discoloration, reduced transparency, a disagreeable odor or thick accumulations of scum on the shoreline or water surface. Visual signs of cyanobacterial blooms have been periodically observed along the surface of the Charles River with the most common cyanobacteria identified as species of *Anabaena, Aphanizomenon, Microcystis, and Planktothrix*. (1)

Numerous environmental factors may influence cyanobacterial blooms. Environmental changes brought about by global warming due to an increase in ozone depleting substances, and other biotic and abiotic factors such as pesticides, fertilizers, sewage effluent, and industrial waste are seen as responsible for the increasing incidence of worldwide harmful algal blooms. Cyanobacterial blooms may be present without producing cyanotoxins, or evident both before and after blooms are visible. The presence of these compounds in aquatic environments, particularly in stagnant lakes, presents challenges for recreational water quality management and drinking water production. A systematic cyanotoxin monitoring system has yet to be established for the monitoring of cyanobacteria in the Charles River. In the absence of such a monitoring system, little is known about spatial and temporal patterns of cyanobacteria in the Charles River. Triggers for toxin production and toxin release are poorly understood. These provide challenges for characterizing, monitoring, and predicting toxin formation in the environment.

Community Boating serves the greater Boston community by providing extensive recreational programming on the Charles River from April through October. Its programs include a Junior Program for children aged 10-17, a Universal Access program for sailors with physical and intellectual disabilities, and an adult program. Sailing, kayaking, windsurfing, and paddle boarding are activities offered in CBI programs. To ensure a safe environment in which these activities can take place, a comprehensive review was undertaken of cyanobacterial algal bloom history, with guidance from regulatory authorities such as the U.S. Environmental Protection Agency (EPA) and the Massachusetts Department of Public Health (MDPH). (2-7) Community Boating's operations were reviewed in order to assess risk and to create a sensible but safe environment which both insures the health of the sailors who sail the Charles River while allowing maximum use of the river during times in which algal blooms are likely to occur.

As part of CBI's interest in the environmental stewardship of the Charles River, educating our junior members in environmental citizenship, and fostering STEM education, CBI's Junior Program members may participate in a structured program of environmental sample collection and analysis following U.S. Environmental Protection Agency Citizen Scientist guidelines and methods. (2) An important element in the EPA's Citizen Science initiative has been the fluorometric analysis of phycocyanin, a pigment exclusive to cyanobacteria and chlorophyll a, a pigment present in the majority of photosynthetic organisms. Fluorometric analysis was undertaken by high school students trained in the use of the fluorometer and supervised by a college undergraduate majoring in a scientific discipline. However, lacking from this programming has been the availability of microscopes and the training that would provide our students with the ability to recover and identify species of cyanobacteria from the Charles River. Within the context of a STEM initiative this program would incorporate elements of scientific investigation, develop student thinking and problem solving skills while integrating a real world issue present within our local environmental setting.

The microscope, a timeless and iconic piece of scientific equipment, remains the centerpiece for many fields of scientific endeavor. This instrument continues to be an invaluable asset to the fields of medicine, biology, forensics, electronics, and the environmental and physical sciences. Most children may be first exposed to the microscope as an educational toy and many might be introduced to the use of the microscope in early elementary or secondary school education. However, most students are not presented with opportunities to merge the techniques of the microscope with the principles of scientific investigation and analysis until well into their formal education. Given our unique environmental setting and guiding mission, introduction of a STEM initiative targeted to the microscope, seemed appropriate and achievable as part of a summer junior program at a community sailing organization.

The "Introduction to Microscopy" program was first introduced in 2021. Through trial and error the program evolved into the comprehensive STEM program on microscopy that was presented during the summer of 2023. The primary objective was for the student to learn how to prepare environmental samples for bright field microscopic examination and perform their own microscopic analysis to identify species of cyanobacteria and other planktonic residents of the aquatic environment.

Forty-nine students enrolled in this intensive STEM programming. This report summarizes the planning, materials, methodology, and execution of a STEM program for microscopy and scientific investigation designed to provide students an experience with methods commonly used by scientists to formulate hypotheses, conduct experiments, and ultimately to obtain knowledge.

With a generous grant provided by the Cabot Family Charitable Trust a number of pre-used compound light microscopes were acquired along with equipment and materials needed for hands-on experimentation and data collection to support the cyanobacteria monitoring research program. Funding was directed to support the Junior Program's environmental science class and cyanobacteria research while providing an enriching experience for our junior members that offer educational opportunities promoting a deeper understanding and appreciation of the natural world.

Materials and Methods

Materials required for sample collection, slide preparation and microscope analysis are shown in Table 1. Materials required for this program were obtained at modest cost. Most microscopes were binocular and came equipped with built in illumination. Acquisition of used microscopes was obtained through an online purchasing system (eBay) and funded by a generous grant from a Greater Boston philanthropic foundation. To provide students with an optimal experience for microscopic observation it was important that students have access to their own microscope. Sharing of microscopes is possible albeit less efficient when there is a limitation in the number of microscopes available. User requirement specifications for microscope purchases were identified by a professional microbiologist.

Course Administration

Recruitment of students

The course was entitled, "Intro to Microscopy" and was promoted in Community Boating's online sign up screen. Criteria for attendance included an age prerequisite of 10-15 with a class size of 12. The course would be presented on a biweekly basis on two consecutive days for a 2 hour session each day (12:30-2:30 pm). The brochure described the course as follows: *"The Charles River makes up a vibrant ecosystem, filled with microbial organisms. In this two-day course, students will undertake field studies and collect water samples in kayak based expeditions of the river. They will learn the basics of microscopy, including how to prepare samples on a slide and view them through*

Sample collection materials

Single and double Kayaks

Collection Bottles: 400 ml wide mouth transparent polyethylene terephthalate bottles

Turkey Basters

Cotton tipped applicators with wood shaft (6 Inch) individually wrapped to collect biofilm samples

Slide preparation materials

Microscope slides (1 x 3 in)

Rectangular coverslips (24mm x 50 mm)

Transfer Pipettes (LDPE 7.0 ml capacity, 3.0 graduated 155mm length, large bulb, 3.2 ml bulb draw

Disposable 9 in diameter paper plates to use as clean work spaces

Disposable paper cups

Stainless steel forceps with curved serrated tip

Kim-wipes

Pencil/drawing paper

Cyanobacteria Atlas: Nienaber MA and Steinitz-Kannan M. *A Guide to Cyanobacteria: Identification and Impact.* University Press of Kentucky, Lexington, Kentucky, 2018. (8)

Microscopes

Microscopes: Pre-used microscopes obtained through eBay included a number of microscope manufacturers (Leitz, Bausch and Lomb, AO Spencer, AmScope). The cost of pre-used microscopes averaged $250. The majority were binocular microscopes fitted with three objectives: high power (X40), low power for scanning (X4), and an intermediate power (X10). A maximum magnification of X400 could be achieved using X10 ocular lenses.

Illuminators: built-in for most microscopes, one microscope without a built-in illuminator required external lantern light to provide illumination

Prepared microscope slides: (Labvida – LVQ091) of various stained mammal, bird, insect, amphibian and plant specimens.

Table 1. Materials Required to Conduct the Introduction to Microscopy Course

a microscope. In so doing, students will be able to observe cyanobacteria, diatoms, dinoflagellates, and green algae species, many observing these microscopic species for the first time. Communities of microorganisms in biofilms will also be recovered by scraping boat hulls and observed microscopically." This course was one of a number of offerings and was coordinated with a number of other activities that involved sailing instruction and other environmental science events. Table 2 summarizes the organization of the two day course.

Day 1 (Two Hours)
Kayak expedition of fresh water river ecosystems
Grab sample collection of water and vegetation
Labeling of sample containers and refrigeration for overnight storage
Day 2 (Two Hours)
Orientation of microscope parts and operation
Prepared slide familiarization and practice of microscopic analysis
Instruction in the preparation of wet mount slides
Wet mount slide preparation
Microscopic analysis of specimens
Discussion of findings with students.

Table 2. Elements of STEM Microscope Instruction

Instructional staff

Five individuals were responsible for providing support and instruction for the program. Staffing included a professional environmental microbiologist (gcd), a class coordinator and expedition leader majoring in chemical engineering (eg), a graduate student in marine biology (dn), and two high school students as laboratory assistants (cq, al). The instructional staff also leveraged many of the assets including trained staff and safety equipment already in place at CBI.

Organization of classroom

Conference tables were configured in a "U" shape with a projector, and screen positioned at the open end of the "U" for the orientation presentation. Seating was modified so smaller students would have easy access to viewing through the microscope's ocular lenses. Extension cords with surge protection were used to connect microscope illuminators.

Collection methodology

Sample collection

Students were led in kayaks by the expedition leader to locations within lagoon recreational areas where a broad range of planktonic animal and plant species would be most prevalent. Sample collection could have easily been performed without watercraft from accessible shoreline areas, particularly where surface vegetation, scum or algal blooms were visible. Each student was equipped with a clear plastic 400 ml collection vessel and a turkey baster. Turkey basters were used to aspirate the river water and then release the sample into the collection vessel. (Figure 2) Alternatively, students could submerge the jars directly into the river. Before setting out on the kayak expedition into the lagoon, students were advised on how much water to collect to ensure a volume that would provide optimal representation of the river's resident biota. Visual observation of the collected samples revealed the presence of organic debris containing microscopic plant and animal species. Some students introduced flowers from lily pads or small floating sticks into their jars to augment their microscopic observation of the biofilms that would form on these surfaces. During each expedition students could collect samples from a number of locations within the shallow parts of the lagoon. Students were asked to label their samples with the date, location, surface water temperature, and time of collection. Samples were refrigerated at 2-8° C for overnight storage. On the following morning collection bottles were removed for warming.

Biofilm collection from boat hulls

CBI's fleet of sailboats provided surfaces that supported a visible biofilm containing microscopic communities that thrive at the air-water interface. Using 6 inch sterile cotton tipped applicator swabs biofilm material could be easily collected and transferred to the laboratory for microscopic analysis. Slides would be prepared with swabs rolled onto a clean glass slide expressing the biofilm material and followed by application of a coverslip.

Figure 2. STEM students collecting river water samples using turkey basters and 400 ml plastic collection bottles

Power point introductory presentation – Introduction to Microscopy

A Power Point presentation provided the students an orientation organized into three parts:

Part 1: The use, mechanics, and operation of the compound light microscope was explained. During this part of the orientation each student would be sitting in front of a compound light microscope fitted with three or more objective lenses. Parts of the microscope were described including the rotation of the turret to select the high, and low power objectives, ocular eyepieces, and knobs for coarse and fine adjustment. The function of the mechanical stage, diaphragm, and light source was demonstrated. The field of view, calculation of magnification, adjustment of the light source, manipulation of coarse and fine adjustment knobs and operation of the mechanical stage were described. Using their microscopes students learned to correctly seat the objective lens and adjust the focus using the coarse adjustment knob by bringing the stage up towards the objective and stopping when the image of the specimen comes into view. Once the image could be seen the fine focus adjustment knob is adjusted to sharpen the details of the specimen. Using bright field microscopy specimens could be magnified from 40X to 400X by alternating between three objectives. At these magnifications animal cells, plant cells, bacteria, and protozoa could be easily observed.

Part 2: Preparation of wet-mount slides for microscopic analysis was described. With the use of the transfer pipette, one to two drops of sample containing visible organic debris would be deposited onto the center of the slide. Using forceps, the coverslip would be placed onto one side of the water drop and gently lowered onto the slide. Placing the slide on the microscope stage student would first use the lowest power scanning objective to locate examples of phytoplankton or zooplankton. Once in focus the specimen could be viewed at higher magnifications to identify species.

Part 3: Overview of phytoplankton and zooplankton species found in the Charles River and biofilms of surfaces of boats was provided. This orientation included diagrams of the morphology and microphotographs of common cyanobacteria, diatoms, dinoflagellates, and green algae that students might encounter under the microscope. These projected images would be available during the session to assist students in identifying major genera or species of their microscopic findings.

As part of the orientation process each student's microscope had been previously set up with a prepared slide already in focus at an appropriate magnification. This allowed the student to familiarize themselves with the focusing, control of slide movement around the microscope stage, and description of visual landmarks in prepared slides of stained plant and animal tissues. Examples of prepared slides included; Root tip of allium plant, frog blood smear, smooth muscle, cardiac muscle, spinal cord cross section and insect leg. Students were given sufficient time to practice with their microscopes prior to the preparation of their own slides.

Figure 3. Students conducting microscopic examination of the biodiversity in river water samples with instructors providing assistance. Sample collection bottles are shown in the foreground. Note: microscope without a built-in illuminator required a battery powered lantern to provide illumination of the specimen.

Results and Discussion

Class make-up: Demographics to assess diversity and inclusivity

The demographics of the students attending the course was evaluated from signup sheets to assess diversity and inclusivity. In all, 871 children attended one or more of the classes offered in CBI's summer Junior Program. Of these, we were surprised that 108 students expressed interest for the "Introduction to Microscopy" course with 16 to 28 students signing up for each of the five sessions. Since class size was limited to 12 students each session became immediately oversubscribed. While we did have prior experience with students sharing a single microscope, this situation was not optimal as the time limits of the course did not allow adequate opportunity for each student to conduct their own microscopic analysis. Those actually selected for enrollment was determined by those first applying. Those not selected were placed on a waitlist or were offered another activity. Ultimately, between 9 and 11 students were enrolled for each session for a total of 49 students. This number allowed most students to have their own microscope although in some cases two students were assigned to a single microscope. A photograph of the classroom set up is shown in Figure 3.

Forty-nine students between the ages of 10 and 15 enrolled in the course. There were 28 male and 19 female students with 2 non-reporting. Students originated from

24 different communities in and around the Greater Boston area and included one student from Texas and one student visiting from Spain. Ethnic breakdown of students enrolled was reported by 36 students: Caucasian (12), African-American (5), Asian-Pacific Islander (14), Hispanic (2), and Caucasian/Asian/Pacific Islander (2).

Evaluation of the STEM programming

Five two day sessions were made available every two weeks throughout the summer of 2023. Planning for the program took place in the spring led by the Executive Director of Community Boating. The program was interposed within a wide array of other activities centered on sailing instruction and environmental science subjects. The "Citizen Scientist" initiative based upon U.S. Environmental Protection Agency guidance engaging the public to participate in monitoring recreational waterbodies for the presence of harmful algal blooms was already in place. Daily monitoring of pre-determined river locations using fluorometric analysis of samples representative of the aquatic environment had been previously established and managed by students. Because of the limited opportunity for microscopic analysis it was determined that for the summer of 2023, introducing the microscopic element to this existing program would afford more students with greater exposure to the basics of the scientific method using the environmental stewardship of the Charles River as a backdrop for these activities.

Due to the comprehensive programming offered to our junior sailors the time allocated for the "Introduction to Microscopy" program was limited to a total of four hours, two hours for field studies in areas likely to contain species of cyanobacteria as well as other phytoplankton and zooplankton. This two hour period was also used for sample collection. The second two hour period was devoted to the actual instruction and use of microscopes in which students were afforded the opportunity to prepare samples for microscopic observation and to use the microscopes to analyze the samples they had previously collected. We believe this limited time was sufficient to accomplish the basic elements of scientific investigation but limited the student's ability to probe more deeply into the other aspects of the scientific methods such as experimentation, data analysis and drawing conclusions. While we were able to touch on these aspects in a superficial way the limitation of time prevented a deeper involvement. Cyanobacteria atlases and drawing paper were provided for each student intended for additional efforts to speciate and record more precisely their observations. (8) However, this activity was sporadic in practice. We did provide a Power Point slide deck depicting the morphology of the phytoplankton and zooplankton which assisted in classification. However, the use of "I phones" to photograph microscopic fields was entirely student driven and a technology that will be explored in the 2024 programming.

Preparing wet mount slides for microscopic analysis

Using a 9 inch disposable paper plate as a clean work surface students prepared wet mounts of the samples they had collected the day before. Using the transfer pipette they extracted approximately 3 ml from their sample collection vessels including visible material. As instructed they deposited one to two drops of water including the visible portion onto the slide surface. The coverslip was gently dropped onto the fluid trapping the visible portion of the specimen. Extra fluid was absorbed with Kim wipes. To maximize the observation experience the procedure was improved by allowing collection vessels to stand undisturbed facilitating the concentration of material. Using the turkey baster, samples were removed from the bottom of the sample vessel and transferred to paper drinking cups. This modification improved recovery of microscopic flora and fauna. We also learned that collection in the field could be improved by transferring small branches, leaves, and surface scum directly into the collection vessels.

Student Experience gained through the use of the microscope

In order to maximize the student's interaction with microscopes a subjective assessment was solicited at the conclusion of each class by asking each student his or her opinion as to the value of this experience. In general, students expressed excitement over their new found skills and abilities in viewing into the microscopic world of the river ecosystems. One student packaged her wet mount slide to bring to her science teacher. Another student's enthusiasm and curiosity lead to his claim that a new species of phytoplankton had been identified. When asked if students would be interested in a scientific career most students answered in the affirmative. Individual responses were not recorded. Student's attitudes toward the value of this programming will be assessed more objectively through a questionnaire to be developed for the 2024 season.

Through the use of the microscope this course promoted a better understanding of fresh water river ecology and encouraged curiosity and enhanced levels of inquiry among the students. Students gained a greater appreciation of the biodiversity within the microscopic populations that form biofilms. In any given microscopic field of vision students could readily observe the unicellular and multicellular morphology of species of phytoplankton and zooplankton including the intricate beauty of the diatoms, the unique motion of the dinoflagellates such as *Vorticella sps* and the copepods that feed on the cyanobacteria and green algae. Colonies of *Pediastrum sps* and *Spirogyra sps* representing green algae could be readily identified.

Amongst this lush biota students marveled at free living flatworms such as planaria, and roundworms found in these fresh water habitats. Once a new organism was observed, other students would be directed to that student's microscope who had made the initial discovery. In fact, students modified the class plan of drawing the unique morphology of representative species by utilizing smart phone technology. By placing the camera lens of their smart phones over the ocular lens of the microscope students were able to capture superb images of the field of vision to be easily shared with all class members. As most students lacked extensive prior experience with the operation of compound microscopes the skills needed in focusing the instrument using the various objectives proved to be the most challenging. However, despite their initial inexperience most students did gain the skills to properly focus the microscope. One important concept was learning to safely position the objective over the slide. Once this was accomplished one could then look into the microscope and focus upward using the coarse and fine adjustment knobs without damaging the specimen. Reversing this process, especially with the higher powered objectives risked destroying the slide and potentially damaging the objective lens. This event occurred only once by a single student.

It was crucial to reserve an appropriate amount of time for students to initially develop two important skills with the prepared slides; ease of focusing the specimen with the different objectives and movement of the slide using the mechanical stage. After sufficient practice time each student was asked to describe the specimen in sufficient detail at a particular magnification (color of tissue, shape of what they were seeing). If there was an unusual or interesting observation in one or more of the prepared slides, then all students were asked to look through that particular instrument. This encouraged the detailed description of their observations and the verbal sharing of data. Students were encouraged to ask any questions or concern regarding this familiarization exercise. Two to three instructors were available to address each student's concern. Before moving on to their own specimens, students were asked to raise their hand if they felt comfortable with their newly acquired skills.

Conclusion

Sophisticated STEM programs were shown to be feasible in our unique environmental setting. Combining the opportunity for sample collection during field studies with laboratory analytical techniques allowed students to experience many aspects of the scientific method in the context of a real world environmental problem. The real world concern of harmful algal blooms exacerbated by climate change in their own environment provided a centerpiece activity in which students could better understand the natural world threatened by global environmental factors. With modest funding, access to scientific equipment and materials needed to conduct sophisticated investigations was feasible. Students experienced the thrill of scientific discovery while developing a greater appreciation of the complexities of biodiversity with a better understanding of the important microbial communities present in an urban fresh water ecosystem.

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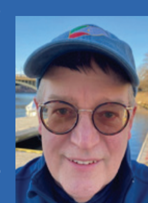
Connor Quigley, a 2024 Brookline High School, MA, graduate, is a recognized young scientist and journalist. His scientific endeavors include significant research on toxic algae at the Cyanobacteria Lab at Community Boating, Boston, leading to a presentation at the 2023 SETAC Europe Annual Meeting in Dublin. As a 2023 Whipple Writing Fellow, Connor excelled in crafting narrative nonfiction on emerging technologies and AI. As 2023-2024 Editor-in-chief of The Cypress, Brookline High's monthly newspaper, Connor effectively managed a team of sixty-three. Connor is set to commence his college education in fall 2024, continuing to integrate his passions for science and journalism.

Anna Lena Leutiger is a sophomore at the Newman School in Boston's Back Bay. Originally from Munich, Germany she moved to Boston MA when she was nine years old. Since then, she has spent every summer at Community Boating, sailing, helping to educate children, and collecting samples for the Cyanobacteria Lab as well as for microplastic research. When she is not on the Charles, she is helping middle school students learn mathematics as part of GLAM (Girls Learn Advanced Math). She is also a contributor to the school newspaper.

Dana Norton is a marine toxicologist whose interests revolve around harmful algal blooms (HABs) and microplastic pollution. She earned her Bachelor of Science in Marine Biology at the College of Charleston in 2017 and her Master of Arts in Biology from the Citadel in 2024, working with Dr. John Weinstein. Her research focuses on monitoring local algal levels through community-centric efforts and microplastic accumulation and toxicity in aquatic environments.

Charles Zechel has led Community Boating Inc. (CBI) as Executive Director since 2002. CBI is the nation's oldest and largest community sailing organization, located in the heart of Boston along the shore of the Charles River. During Mr. Zechel's tenure CBI's programs have expanded to include the nation's largest accessible sailing program serving over 250 sailors with disabilities each year. Additionally, CBI hosts 12 high school sailing teams and operates a youth program serving over 1000 children each summer. With the understanding that water quality of the Charles River has become of paramount importance for all sailors at CBI our STEM programming is empowering children to become future leaders of environmental stewardship.



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