Adding CURE to Traditional Labs: Hands-On Microplastics Research in Freshwater Systems Conducted by First-Year Biology Students

ERIKA C. MARTIN

ABSTRACT
This project involves students in a course-based undergraduate research experience (CURE) as part of the traditional introductory biology laboratory course. Recently, research has shown that student engagement in authentic research has significant positive impacts on students, such as development of science literacy and reasoning skills. Being recently featured in the news, microplastics are a timely, interesting, and relevant topic for students. The authentic research conducted by students was the first attempt at quantification of microplastics in the Great Plains, which garnered further student excitement and engagement. Surface water and substrate samples were collected at 23 locations from small streams, rivers, ponds, and reservoirs in fall 2018. Authentic research, as broadly defined in the pedagogical context, is research conducted primarily by students. In the context of this project, authentic research is specifically defined as research done primarily by students in which the students are asking questions, designing experiments, collecting and analyzing data, and writing a final manuscript that was submitted, and accepted, as a peer-reviewed publication. This project could be incorporated at the high school or university level, for biology major or nonmajor courses. The purpose of this paper is to serve as a how-to, sharing the lesson design with specific detail on student responsibilities.

Key Words: Great Plains; Kansas; pedagogy; course-based undergraduate research experience.

Undergraduate Biology Research Experiences & Course Overview
The course-based undergraduate research experience (CURE; see Auchincloss et al., 2014) initiative has been part of the science classroom for decades, though the acronym is much more recent. It is well established that hands-on active learning benefits students, particularly women and underrepresented groups (Gregerman et al., 1998; Barlow & Villarejo, 2004; Eagan et al., 2011). We modeled our project on the procedure outlined by Auchincloss et al. (2014) for introducing an authentic research experience to undergraduate biology students. CUREs that have been successfully implemented show significant benefits to students and faculty (see Shortlidge et al., 2017). This article is designed for other biology educators as an example of how to adapt CURE methods to provide unique and flexible research experiences in their classrooms.

Getting undergraduate science students excited about science and able to conduct rigorous and high-quality scientific research on their own are difficult goals that many academic institutions strive to meet (Jordan et al., 2014). Typically, it is challenging for undergraduates to design their own research projects or transfer their mathematical skills from math class to biology class due to lack of experience or training. This fear of failure and/or of the unknown is a difficult gap for students to cross, and educators have been implementing new ways to create independent, thoughtful scientists. Involving students in authentic research experiences early in their academic career is one way to help bridge this gap. The purposes of this project were, generally, to bring an exciting and authentic scientific research project into the classroom, while allowing biology students to actively engage in the entire process of science discovery. Furthermore, the project was intended to motivate students to experience the multifaceted world of science and allow them to make discoveries on their own. The specific goals of the project described in this manuscript were to (1) get introductory biology students actively participating in the entire scientific process – formulating a question after being given guiding information, creating a testable hypothesis, designing an experiment, collecting and analyzing data, and reading and writing a peer-review-quality scientific paper; and (2) get students comfortable and confident with their ability to apply their knowledge from science and math, with the understanding that failure and “the unknown” is an integral part of science to be embraced, not feared.

Twenty-one students were enrolled in one section of Principles of Biology (GB141) at Emporia State University, a midsized four-year
Weeks 1, 2 & 3: Introducing the Topic of Microplastics

Background

Microplastics have come to the forefront of aquatic science investigation over the last decade (Law et al., 2010; Waller et al., 2017), and more recently have been reported by popular media sources like National Public Radio (Boddy, 2017), Science Daily (Forschungsverbund Berlin, 2018), BBC News (Gill, 2018), CNN (Tutton, 2018), ABC News (Jacob, 2016), and Fox News (Puhak, 2018). Popular media is considerably more accessible to freshmen-level students than peer-reviewed literature. Having students first read news coverage of a scientific article shows students that the topic they are about to study is currently relevant and gives them social and societal context before they dive into reading peer-reviewed literature.

The general definition by the National Oceanic and Atmospheric Administration (NOAA) describes microplastics as small pieces of plastic <5 mm long. Sources for microplastics are diverse and substantial. Primary microplastics are small particles introduced directly into the environment, often from cosmetic or toiletry products or as waste from the creation of large plastic products (Boucher & Friot, 2017). Secondary microplastics are small particles created from the degradation of large plastics (Boucher & Friot, 2017).

Goals

The goals were to (1) do an informal assessment of student knowledge on microplastics, (2) have a discussion where students could ask any questions, and then (3) introduce students to their research topic using layman-friendly news reports.

Activity

The first week, all students were assigned to have read by the third week the same layman-friendly article (Joyce, 2018) about aquatic microplastics. After the assignment was announced, an informal assessment was given in which students were asked if they had heard about microplastics. About 50% of the students had heard of microplastics (mostly microbeads) before reading the article, and about 50% were unaware. The second week was dedicated only to the weekly laboratory material.

During the first week, the class had a short (30 minute) discussion about the topic and students were encouraged to ask questions. The discussion began with questions from the instructor, such as “What are microplastics?”, “Where are they found?”, and “Why are some people concerned about microplastics?” Students discussed these in small groups of two to four people and came up with answers. Instructors adapting this project to their classroom should anticipate student questions like “Where do microplastics come from?”, “What do microplastics look like?”, or “Is plastic worse than using trees for paper?” After the discussion, students were given 10 minutes to make hypotheses with their shoulder partner or small group about whether or not they expected microplastics to occur in Kansas freshwater. These hypotheses were not submitted as an assignment, but to help students use knowledge gained from a different assignment and apply it to a new topic. Students were to compose null and alternative hypotheses based on their discussions, using appropriate scientific language. Students learned about null and alternative hypotheses during the traditional laboratory exercise in week 1. The instructor went to each group individually to discuss their hypotheses and make suggestions or ask further questions. For example, when a group hypothesized that there would be microplastics in Kansas, the instructor would ask them to justify their hypothesis. Frequently the answer was “Because plastic is everywhere.” The instructor would ask follow-up questions meant to get students to be specific with their answers, such as how they knew plastic was everywhere, and continue to facilitate small discussions to solidify what the students’ thoughts were so that they could translate them from abstract ideas into specific written concepts. Solidifying students’ thoughts means that a student (or student group) must be able to create null and alternative hypotheses and justify their hypotheses on the basis of specific, demonstrable knowledge (e.g., growing their vague response of “Plastic is everywhere” to a specific response of “Based on the article I read, plastics have been found in every sample collected by scientists so far”).

Time

Week 1 discussion took ~30 minutes. The entire activity during week 3 took ~60 minutes of class time.
O Week 4: Creating the Study

Goal
The goal of this class period was to get students thinking about collection techniques and lead them to discover on their own how to collect microplastics. At the end of class, a sampling protocol was finalized.

Activity
Peer-reviewed literature was used to guide the students to create a sampling protocol (for detailed descriptions of various protocols, see Munno, 2017). Collection technique was first discussed in class, where the instructor posed a series of questions followed by discussion. Questions included items like “How do we get microplastics from the water if we can’t see them?” or “Do you think the bottom of the river, the clay and sand, would have similar amounts of microplastics as the water column?” or “What types of variables might cause increases or decreases in the amount of microplastics in a body of water?” After discussing these questions, a basic sampling protocol was written on the board using words and ideas generated by the discussion. For example, written on the board were abbreviated versions of student thoughts like “filter small things” or “differ habitats are different.” After class, the instructor wrote up the protocol and posted it to CANVAS (the university’s learning management system). Although the sampling protocol was predetermined and the students were led to it, the students themselves chose to sample the sediment and water column of streams, rivers, ponds, and lakes at each location to make comparisons of microplastic density. Sample size and replication was determined by the number of students in class and was addressed in week 5.

Suggestions
Students could have chosen a variety of options: sampling different levels in the water column (e.g., bottom, middle, surface water), or comparing similar water bodies with sand substrate to clay (e.g., ponds with sand vs. ponds with clay). Another option could have been to test different collection techniques (see Munno, 2017). Instead of the instructor researching the sampling protocol, students could have been assigned to design a protocol themselves, either by researching online or being given information from the instructor (or a combination). Having students write the protocol would be more intensively engaging. This research could be done in class or outside of class depending on class time available.

Time
The discussion lasted ~45 minutes.

O Weeks 5–11: Training in Sample Collection

Background
Given the sampling protocol created and posted on CANVAS, four sampling kits were put together by the instructor and given to students (125 μm sieve, 1 L graduated cylinder, squirt bottle, two sample jars). Sample jars were cleaned glass jars (pickle jars, mason jars, etc., of at least 12 ounces). Distilled water was obtained from the university laboratory.

Goal
Students were to design the study timeline by signing up for sample dates, and choose their sample location.

Activity
The only requirement for scheduling given to students was that all sampling had to begin in September and be completed the first week of November (weeks 5–11). Students chose aquatic locations to sample. Aquatic location was given a very broad definition of “holding water most of the year.” This definition was chosen because it increased the likelihood that a location selected by students would have water in it when they visited the site, since fall has lower precipitation than spring, which can affect stream persistence. Students could sample drainage ditches, creeks, streams, lakes, ponds, reservoirs, or lagoons but could not sample things like sidewalk puddles. Students chose sampling locations based on convenience (e.g., close to home, fall-break vacation destination). When students signed up for a date, they based their sampling location on where they would be that day.

Because students sampled over the course of approximately three months, the instructor went over the collection procedure one-on-one with the students before their collection weekend at the end of class. Since class was on Friday, the students who were sampling would meet the instructor right after class to go over the protocol. The instructions were also written and available online through CANVAS. The collection protocol is provided in the Appendix.

Suggestions
Instead of one-on-one, hands-on instruction prior to sampling, another option could be to schedule a class field trip to a nearby water body and have students practice with instructor observation. If all collections need to be done around the same time, students could sample water bodies near the school and take turns with sampling equipment throughout one or more days.

Time
Each training session took ~ 10 minutes.

O Weeks 5–11: Collecting & Preparation Methodology (Outside of Class) & Sample Processing (in Class)

Background
Sample collection (students). Students used their kits to collect their samples from their chosen locations. Samples were collected from water bodies across the state of Kansas (and one in Missouri) from September to November of 2018. Collections had to be finished on or before the first weekend of November.

Sample preparation (instructor). Each sample was fixed with 95% ethanol and stored in a safe place until all students completed their
sampling. Student samples were filtered through a 125 μm mesh sieve and thoroughly rinsed. Next, samples were placed in 30 mL polypropylene vials (Sigma-Aldrich product Z679070-500EA) labeled to retain student identification, and filled with 15 mL of 1N KOH (Sigma-Aldrich product 221473-500G) and left to process for 14 days to remove organic material. After 14 days, samples were transferred to a 125 μm mesh sieve and thoroughly rinsed, then placed in glass Petri dishes labeled with student names and dried in an oven (Thermo Scientific HERAtherm) at 60°C for 36 hours. The instructor prepared the samples for processing.

Goals
Students used scientific equipment to quantify microplastic density in the samples they collected, and recorded their data in a spreadsheet.

Activity
Sample processing (students). Once the preparation was complete, students processed their own samples. The microplastic particles in each Petri dish were counted using a compound microscope (Motic BA210) at 100× magnification. Dried contents of the Petri dish were placed on a glass slide over a 1 mm² grid, and students counted the number of microplastics in each grid section until the entire sample was processed. A “guide to microplastic identification” document available online (Barrows et al., 2017) was very useful, and photos were projected on a whiteboard for students to reference. Students were not trained prior to processing samples to know the difference between microplastics and other objects, which allowed for a hands-on learning approach. They were to use the skills they had practiced in lab to use the microscopes (learned during traditional lab exercises). Once students had their microscopes focused on their samples, the instructor went to each student and talked about the differences between plastic (irregular shape, unusual color, etc.) and inorganic debris (mostly sand). After 10–15 minutes, most students could readily differentiate microplastics from sand particles.

Suggestions
Grid stickers were used, but a Bogosh slide could be used instead. Waiting for all samples to be submitted was easier, but doing a staggered preparation for processing where each sample would be placed in the KOH solution as it arrives could be possible if the instructor wanted to get students processing hands-on earlier in the course. The traditional laboratory exercises had students using microscopes frequently. If students had not had a lot of time working with microscopes, an introductory training session would be required, likely lasting 20–40 minutes.

Time
120 minutes for discussion, learning, and processing a sample.

Week 8: Peer-Reviewed Literature

Background
When asked how they know whether a paper has been peer-reviewed, many students gave responses like “It was published in a good source” or “The author is qualified.” However, if given a paper and then asked how they could determine whether it was peer-reviewed, students were unsure. This is a very tough issue for students to grapple with, and by far the best way I have discovered to get them to learn what is or is not peer-reviewed is to have them go find an article.

Goals
Students were assigned to find and submit to the instructor a peer-reviewed scientific article.

Activity
Students were shown how to use university library and internet resources during class. We discussed what peer-reviewed means. Students were shown Google Scholar and the university library’s website, which has database searches (e.g., JSTOR, Web of Science) specifically for peer-reviewed literature. Students were asked to use the knowledge they learned in class discussions about peer review, plus the knowledge of the study design and data they collected, to find an appropriate article that was peer-reviewed and related to their research topic. Students e-mailed their choice to the instructor with a few sentences about why they thought the article was peer-reviewed. The instructor reviewed the article, and if it met the criteria it was posted on CANVAS to share with the rest of the class. If the article was not peer-reviewed, it was explained to the student how that was determined, and they tried again. Most students took one or two tries, but a couple students took more than five attempts. Each student had to choose a unique article that had not been selected by another student. Students reviewed their article and wrote a two- to three-page summary that was submitted electronically. The quality of the summaries was surprisingly good. I expected casual writing, grammatical mistakes, skipping over complex terminology, and other shortcuts, but overall the students submitted good (or great) work. Good and great work include accurate use of scientific terminology and ability to define and/or explain scientific terms, concepts, and techniques while using proper grammar.

The summary was to include (1) how they knew the article was peer-reviewed, (2) why the study was conducted, (3) how the study was conducted (sample size, number of treatments or observational studies, location), and (4) the conclusions of the study. Students were given over a month to choose their article and write their summary. Students e-mailed their choice to the instructor with a few sentences about why they thought the article was peer-reviewed. The instructor reviewed the article, and if it met the criteria it was posted on CANVAS to share with the rest of the class. If the article was not peer-reviewed, it was explained to the student how that was determined, and they tried again. Most students took one or two tries, but a couple students took more than five attempts. Each student had to choose a unique article that had not been selected by another student. Students reviewed their article and wrote a two- to three-page summary that was submitted electronically. The quality of the summaries was surprisingly good. I expected casual writing, grammatical mistakes, skipping over complex terminology, and other shortcuts, but overall the students submitted good (or great) work. Good and great work include accurate use of scientific terminology and ability to define and/or explain scientific terms, concepts, and techniques while using proper grammar.

The summary was to include (1) how they knew the article was peer-reviewed, (2) why the study was conducted, (3) how the study was conducted (sample size, number of treatments or observational studies, location), and (4) the conclusions of the study. Students were given over a month to choose their article and write their summary. The instructor reviewed the article, and if it met the criteria it was posted on CANVAS to share with the rest of the class. If the article was not peer-reviewed, it was explained to the student how that was determined, and they tried again. Most students took one or two tries, but a couple students took more than five attempts. Each student had to choose a unique article that had not been selected by another student. Students reviewed their article and wrote a two- to three-page summary that was submitted electronically. The quality of the summaries was surprisingly good. I expected casual writing, grammatical mistakes, skipping over complex terminology, and other shortcuts, but overall the students submitted good (or great) work. Good and great work include accurate use of scientific terminology and ability to define and/or explain scientific terms, concepts, and techniques while using proper grammar.

Suggestions
Grid stickers were used, but a Bogosh slide could be used instead. Waiting for all samples to be submitted was easier, but doing a staggered preparation for processing where each sample would be placed in the KOH solution as it arrives could be possible if the instructor wanted to get students processing hands-on earlier in the course. The traditional laboratory exercises had students using microscopes frequently. If students had not had a lot of time working with microscopes, an introductory training session would be required, likely lasting 20–40 minutes.

Time
120 minutes for discussion, learning, and processing a sample.

Suggestions
Grid stickers were used, but a Bogosh slide could be used instead. Waiting for all samples to be submitted was easier, but doing a staggered preparation for processing where each sample would be placed in the KOH solution as it arrives could be possible if the instructor wanted to get students processing hands-on earlier in the course. The traditional laboratory exercises had students using microscopes frequently. If students had not had a lot of time working with microscopes, an introductory training session would be required, likely lasting 20–40 minutes.

Time
120 minutes for discussion, learning, and processing a sample.
constructive comments, and students could revise their work before the final submission. Nearly every student took advantage of this option.

**Time**
The presentation by the librarian lasted ~20 minutes. Discussion about peer review led by the instructor took ~20 minutes. Seventy-two hours were needed to complete the draft manuscript.

**Week 12: Understanding & Analyzing Data**

**Background**
Having students process and analyze data (i.e., hands-on experience) gives students ownership and a deeper appreciation for how analyses work. Working with their own data also allows students to connect statistical results to real-world ecological locations.

**Activity**
Students’ data were recorded in a single spreadsheet for analysis. Two analyses were conducted by the instructor using sophisticated statistical software appropriate for scientific publication: a t-test comparing average density of microplastics in water versus sediment and an analysis of variance (ANOVA) comparing microplastic density among water body types (stream, river, pond, lake). The spreadsheet had six columns (Student Name, Sample Date, GPS, Water Body Type, Water or Sediment, Microplastic Density). However, students learned about and calculated a t-test by hand in class. Throughout the semester, students practiced statistical tests (chi-square and t-test). By doing different types of tests with different data, students explore the impacts of sample size, variation, and null hypotheses on the setup and output of statistical tests.

**Suggestions**
Depending on what statistical background students have, students could simply make graphs by hand or with computer software. If they are more advanced, students could conduct these analyses as homework with a written assignment provided by the instructor. Very advanced students could run the t-test and ANOVA.

**Time**
Organizing the data and running a t-test by hand took 60 minutes. If students had not had prior experience with a t-test, the time to conduct one in class with the students would likely double. The sophisticated analyses for the published manuscript were completed by the instructor in 24 hours.

**Week 14: Concluding the Research**

**Goals**
Students submit a reviewed and edited scientific manuscript.

**Activity**
Finally, the students reviewed the draft manuscript. A hard copy of the paper prepared by the instructor was given to every student in week 14. Students were required to correct mistakes, highlight areas they found confusing, write questions in the margins, and make general comments throughout the paper. Students found several grammatical errors, highlighted areas that they found confusing, and asked good questions. This final step allowed students to see their own work presented in manuscript format. Science communication is critical, and this exercise is a hands-on way for students to connect the scientific process with the scientific product. Their paper was submitted to a peer-reviewed journal and accepted (see Martin et al., 2019).

**Student Response**
While there are no quantitative assessments on student learning at this time, the response students had to this project was overwhelmingly positive. Every student completed every task. Previous in-class research projects in which students were split into small (two-to four-person) groups and directed to come up with their own projects often resulted in the stereotypically negative “group work” problems; some students did the work and others did not. Whereas many students in the previous small groups chose safe topics (e.g., what factors influence bread mold, or what color of light do plants prefer), this experience brought the students to the forefront of authentic and relevant science without the concern their research might fail.

Several students sent e-mails to me personally or came to class early after their sampling event, excited to discuss their experience with the instructor and fellow classmates, this type of enthusiasm has not been present in the traditional small-group projects. After the semester ended, a few students inquired about other courses with similar experiences. Two students began work as undergraduate research assistants in the biology department.

**Conclusions**
At this time, there are no quantitative data to compare gains in student knowledge, understanding, or persistence; however, given the student feedback and the anecdotal success of the project described here, there is currently an effort to quantify the differences these authentic research experiences are having on the students. Future plans include quantifiable assessments and surveys comparing classes that continue the traditional small-group research projects to classes that adopt whole-class, innovative, and inquiry-based research projects like the one described here.

**Acknowledgments**
I thank Emporia State University’s Katherine K. White Faculty Incentive Grant Program for funding, and the students of Fall General Biology 141: Julia Avila, Sierra Behrens, Danielle Berry, Leah Cona, Ashley Feldmann, Khushi Ghanchi, Emily Hall, Jenna Hindelilter, Tanner Lane, Samantha LeMay, Mackenzie Loar, Kolin Loewen, Zachary Museousky, Scott Nelson, Austin Ohlfs, Bethany Ortega, Conner Ryan, Hannah Seidel, Anna Straub, and Katelynn Stucky. I also thank M. Sundberg, T. Burnett, and the anonymous reviewers for valuable comments on the manuscript.
References

Auchincloss, L., Laursen, S.L., Branchaw, J.L., Eagan, K., Graham, M., Hanauer, D.I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE–Life Sciences Education, 13*, 29–40.

Barlow, A.E. & Villarejo, M. (2009). Making a difference for minorities: evaluation of an educational enrichment program. *Journal of Research in Science and Teaching, 41*, 861–881.

Barrows, A.P.W., Neumann, C.A., Pieper, C., Berger, M.L. & Shaw, S.D. (2017). Guide to microplastics identification: a comprehensive methods guide for microplastics identification and quantification in the laboratory. Marine & Environmental Research Institute, Blue Hill, ME.

Boddy, J. (2017). Are we eating our fleece jackets? Microfibers are migrating into field and food. National Public Radio, *The Salt*. https://www.npr.org/sections/thesalt/2017/02/06/511843443/are-we-eating-our-fleece-jackets-microfibers-are-migrating-into-field-and-food.

Boucher, J. & Friot, D. (2017). Primary microplastics in the oceans: a global evaluation of sources. IUCN, Gland, Switzerland.

Eagan, M.K., Sharkness, J., Hurtado, S., Mosqueda, C.M. & Chang, M.J. (2011). Engaging undergraduates in science research: not just about faculty willingness. *Research in Higher Education, 52*, 151–177.

Forschungsverbund Berlin (2018). An underestimated threat: land-based pollution with microplastics. *ScienceDaily*, February 5. https://www.sciencedaily.com/releases/2018/02/180205125728.htm.

Gill, V. (2018). Microplastics are ‘littering’ river beds. *BBC News*, March 12. https://www.bbc.com/news/science-environment-43363545.

Gregerman, S.R., Lerner, J.S., von Hippel, W., Jonides, J. & Nagd, B.A. (1998). Undergraduate student-faculty research partnerships affect student retention. *Review of Higher Education, 22*, 55–57.

Jacobo, J. (2016). Breakdown of biodegradable plastics in ocean ‘extremely slow’, UN report says. *ABC News*, May 24. https://abcnews.go.com/US/breakdown-biodegradable-plastics-ocean-extremely-slow/story?id=39345322.

Jordan, T.C., Burnett, S.H., Carson, S., Caruso, S.M., Clase, K., Delong, R.J., et al. (2014). A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. *MBio, 5*, e01051-13.

Joyce, C. (2018). Beer, drinking water and fish: tiny plastic is everywhere. National Public Radio, *All Things Considered*, August 20. https://www.npr.org/sections/thesalt/2018/08/20/636845604/beer-drinking-water-and-fish-tiny-plastic-is-everywhere.

Law, K.L., Morét-Ferguson, S., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J. & Reddy, C.M. (2010). Plastic accumulation in the North Atlantic subtropical gyre. *Science, 329*, 1185–1188.

Martin, E.C., Sorell, C., Avila, J., Behrens, S., Berry, D., Cona, L., et al. (2019). Assessment of microplastics in the Great Plains: comparing densities in water and benthic sediment across Kansas. *Transactions of the Kansas Academy of Science, 122*(3–4).

Munno, K.E. (2017). Microplastic retention by type in several species of fish from the Great Lakes. Master’s thesis, University of Toronto.

Puhak, J. (2018). 93 percent of bottled water contains microplastics, study says. *FOX News*, March 16. https://www.foxnews.com/food-drink/93-percent-of-bottled-water-contains-microplastics-study-says.

Shortlidge, E.E., Bangera, G. & Brownell, S.E. (2017). Each to their own CURE: faculty who teach course-based undergraduate research experiences report why you too should teach a CURE. *Journal of Microbiology & Biology Education, 18*(2), 182-29.

Tutton, M. (2018). It's not just the oceans: microplastic pollution is all around us. *CNN*, April 22. https://www.cnn.com/2018/04/22/health/microplastics-land-and-air-pollution-intl/index.html.

Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., et al. (2017). Microplastics in the Antarctic marine system: an emerging area of research. *Science of the Total Environment, 598*, 220–227.

ERIKA C. MARTIN is an Assistant Professor in the Department of Biological Sciences, Emporia State University, Emporia, KS 66801; e-mail: emartin7@emporia.edu.

Appendix: Sample Collection Protocol

Equipment to get from Dr. Martin:

1. Graduated cylinder
2. Sieve
3. Two glass collection bottles

Equipment you need to have on your own:

1. GPS locator. Most smartphones have this capability. For iPhone, find the “Compass” app. This app comes free with the phone. If you have deleted it, be sure to download it before you go out. The GPS is at the bottom of the screen. As an example, the GPS location of your classroom, SH47 is 38°24’55”N and 96°10’53”W.
2. A one-cup measuring cup
3. One squirt bottle filled with filtered water.

Protocol:

Go to water body.
Take GPS location.
To collect water sample

1. Fill graduated cylinder with water, pour through sieve. Repeat filling graduated cylinder and pouring through sieve FIVE times.
2. Rinse sieve with water squirt bottle into collection jar.

To collect substrate sample

1. Take measuring cup and scoop one cup of substrate from bottom of water body.
2. Put substrate into sieve.
3. Submerge the sieve just enough to rinse the substrate but not deep enough so that water goes over the edge of the sieve!! VERY IMPORTANT!
4. Use water squirt bottle to put sieve contents into collection jar.
   Fill measuring cup and sieve ONE time.

When you have finished collecting both the water and substrate samples, bring equipment and both collection jars to the main biology office and tell the secretary it is for Dr. Martin. Be sure your name and date is on it!