Chapter 2

Incorporating an Environmental Research Project Across Three STEM Courses: A Collaboration between Ecology, Organic Chemistry, and Analytical Chemistry Students

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Our collaborative research and integrated teaching programs focus on understanding chemical dynamics, ecological impacts, and human health risks posed by pyrethroid pesticides in aquatic ecosystems. We accomplish this in our courses by designing laboratory projects in which our undergraduates participate in field sampling, chemical analyses, and laboratory experiments that advance our research goals. This approach involves the research groups of three faculty and scores of students enrolled in Ecology, Organic Chemistry Lab III, and Instrumental Analysis annually. We have designed the project, in part, to demonstrate to our students the benefits of collaborating across disciplines. In this contribution, we aim to describe how this project can serve as a model for faculty who are interested in developing a similar approach.

Background

Science that addresses complex issues is often inherently interdisciplinary. Such research frequently requires effective collaborations to accomplish work that combines diverse methods. Furthermore, teams of collaborators are often needed to synthesize the results, which leads to an improved understanding of the complex and dynamic systems under investigation. We work with Seattle University undergraduates in three courses across chemistry and

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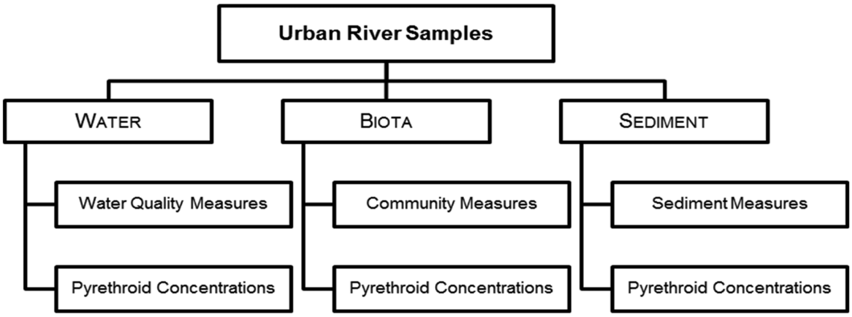
biology to examine relationships between contaminant concentrations, aquatic environmental conditions, benthic invertebrate populations, and human health concerns in the Duwamish industrial waterway in Seattle, WA. As part of this project, we also coordinate with community organizations to determine priority sites for sampling and identify stakeholders for communicating our findings. Our research objective of elucidating how pesticides in aquatic habitats impact ecosystem and human health merges with our teaching objective of modeling how scientific collaborations can be used to generate data and to better understand these complex systems.

Research on urban waterways demonstrates that greater runoff volume, greater temperature extremes, and more contamination results from increased urban and industrial development (*1*). Studies examining restoration efforts have highlighted the challenges faced in urban estuaries, since significantly altered watersheds feed these systems. As a result, projects in such settings typically focus on decreasing stressors (*2*). Within industrial waterways, smaller restoration areas are often targeted with aims to provide small-scale improvements in aquatic habitat to facilitate improvements in water quality (*3*).

A prime example of an urban waterway that has been substantially altered and degraded is the lower Duwamish River. Channelized and industrialized as Seattle developed over the twentieth century, this estuary was designated as an EPA Superfund site in 2001 (*4*). Through industrialization, the river lost its natural meanders, 92% of the estuary was filled, and 90% of the floodplain no longer floods on a regular basis (*5*). Besides physical changes, industrial and municipal activities produced high levels of legacy contaminants in the sediment, such as heavy metals, PCBs, and DDT (*6*). Despite the extent of alteration, the Duwamish still provides important aquatic habitat and a migration corridor for fish, invertebrates, and birds. Hence, restoration efforts have focused on specific shoreline sections of the river to dredge contaminated soil, to re-grade shoreline and streambed, and to seed plants (*7*). These relatively small-scale efforts have largely been driven by concern for public health and decline in salmon stocks (*2*). Recent work investigating these restored areas has suggested some success with salmon and invertebrate use, with aims to clarify future strategies (*8*).

We have initiated a program to determine how a class of emerging contaminants, namely pyrethroid pesticides, affects the Duwamish estuary ecosystem. Pyrethroids comprise a class of pesticides that are currently used in hundreds of agricultural, construction, commercial and household products (*9*). They have emerged as the insecticide of choice following the U.S. ban on organochlorine products in the 1970s-1980s, and the subsequent ban on their replacement, organophosphates. Recently, several papers have reported pyrethroid concentrations in natural waterways at levels that are toxic to the indigenous organisms (*10–16*). The sources of pyrethroids in waterways include spray drift, combined sewer overflow (CSO), and runoff from agriculture, golf courses, road medians, lawns, and gardens.

Pesticide monitoring in urban-dominated creeks often focuses on the water column because many high-use pesticides such as organophosphates are relatively water-soluble. For this reason, and because of the more difficult analyses associated with extracting solid samples, we have initially targeted our work on water samples that can be analyzed easily by students. Because pyrethroids are far more hydrophobic than organophosphates (log $K_{OC} \approx$ 5-6) (*17*), this presents a challenge we aim to address through a combination of measuring pyrethroids in these water samples and future sediment and tissue analyses. The expectation that these compounds should partition into sediment and biological tissues has been demonstrated (*18*, *19*). Consequently, it is important to monitor pyrethroid concentrations in multiple environmental compartments (e.g., in the water column, sediments, and biological tissues) in an urban estuary, though few recent studies have addressed this issue (*16*, *19*, *20*). Knowledge of pyrethroid concentrations in the ecosystem compartments of food sources and primary habitat is especially important to understand how pyrethroids affect food webs (Scheme 1). Our project aims to quantify pyrethroid concentrations in the different environmental compartments depicted in Scheme 1 and to assess the impacts that they have on indigenous organisms in the Duwamish waterway.



Scheme 1. Schematic depiction of our approach to studying the effects of pyrethroid pesticides on the Duwamish industrial waterway.

The success of this project *depends* on the active participation of undergraduate students— all Seattle University students enrolled in ecology, third quarter organic chemistry laboratory, and instrumental analysis are involved through their respective courses, as are many undergraduate research students working under direct mentorship of the faculty. In this chapter, we describe our study of contaminants in aquatic ecosystems as a model for faculty who are interested in developing similar collaborative research-in-teaching curricula.

In the following sections, we describe the following: (*i*) an overview of the scientific methods used, (*ii*) the specific courses involved and their respective learning outcomes, (*iii*) the design and logistics of our collaboration, (*iv*) some representative scientific results to demonstrate the quality of the data, and (*v*) some reflections on how this project enhanced the student experience vis-à-vis our learning outcomes.

Scientific Approach

We have developed a method to quantify pyrethroid concentrations in the water compartment of the ecosystem through field collections and laboratory work. Our method was adapted from the recent CALFED final report on the analysis of pyrethroid pesticides in environmental samples (*21*). This CALFED report will also serve as a guide as we extend our work to quantify pyrethroids in other environmental compartments such as sediment and tissue. The resulting analysis method was developed by students in our research groups and in the courses described later in this chapter. A summary of our procedure follows. The specific tasks assigned to students from the different courses are described later. Field sites are located across the lower Duwamish River where we have sampled water and measured ecological variables over the past five years. Water is collected in amber glass bottles, due to the photosensitivity and hydrophobicity of pyrethroids, and sterilized by filtration through 0.20 μm filters. We employ a solid phase extraction (SPE) protocol to concentrate the hydrophobic compounds contained in the water samples in preparation for trace level chemical analysis. We elute the pyrethroids from the SPE cartridges, use a stream of nitrogen gas and gentle heating to remove the elution solvent, and redissolve the eluted mixture of compounds in 1 mL ethyl acetate, thereby concentrating the pyrethroids 1000-fold. The effectiveness of our method is monitored through the use of two isotopically-enriched standards and external calibration standards. After the water samples have been filtered, 50 ng of *trans*-permethrin-$^{13}C_6$ is added to our 1.00 L sample aliquots. After the samples have been concentrated through SPE and redissolved in EtOAc, a 50 ng spike of *cis*-permethrin-$^{13}C_6$ is added. In this way, the *trans*-permethrin-$^{13}C_6$ reports the effectiveness of all of the method steps that occur following filtration while the *cis*-permethrin-$^{13}C_6$ serves as an injection standard.

We have also developed an effective method for collecting reliable data on water quality across multiple sites in the lower Duwamish River. Through a consistent sampling regimen, including regular intervals (i.e., monthly) and responses to storm events, we can quantify relevant water quality parameters that potentially affect pyrethroid concentrations. With each water sample, the battery of measurements includes: temperature, pH, dissolved oxygen (HACH HQ40d probes); nitrate, nitrite, ammonia, total nitrogen, phosphate, total phosphorus, sulphur, copper, COD, turbidity (HACH SR2800 field spectrophotometer); and salinity (refractometer). Using this sampling protocol, we build on our preliminary results that suggest CSOs are potential sources for higher turbidity, higher nitrate, and lower oxygen levels. By detecting pyrethroid concentrations in ecosystem compartments and measuring water quality, we can identify parameters corresponding to pyrethroid sources, fates, and impacts. By designing our project to include temporal, spatial, and chemical variations among samples, we are able to make inferences about how pyrethroid pesticides impact the environmental health of the Duwamish.

Institutional Context

Our collaboration is between Ecology, Organic Chemistry Lab III, and Instrumental Analysis, which are standard courses that are common to most American undergraduate chemistry or biology curricula. As such, the topics covered in each course are fairly standard relative to corresponding courses at other universities. Because Seattle University operates on the quarter system, these courses each meet for 12 weeks; however, conducting a similar collaboration on the semester schedule would likely alleviate some of the time constraints that we sometimes confront. We conduct our collaboration during the spring quarter because all three courses are offered each spring; this enables meaningful and timely interaction between students in the different courses. A brief description of each course follows, some of which is summarized in Table 1.

Table 1. Summary of information about each course in our collaboration

	Ecology	*Organic Chemistry Lab III*	*Instrumental Analysis*
Course enrollment	20 - 25	60 - 80	12 - 18
Number of sections	1	5 - 7	1
Lab hours / week	4h	3h	7h (2 x 3.5h)
Student body [a]	Jr. & Sr.	So. & Jr.	Jr. & Sr.
Weeks involved [b]	1 - 4	1	5 - 6

[a] So. = sophomores; Jr. = juniors; Sr. = seniors. [b] Number of weeks that students in each course work on this collaborative project.

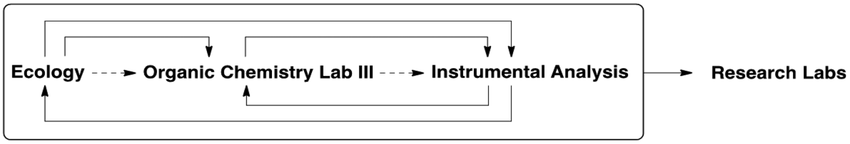
Our ecology course (BIOL 470) is a one-quarter course taken primarily by junior and senior biology majors. This course introduces undergraduates to interactions between organisms in biological communities and the relationship of biological communities to the environment. Ecology meets for three hours of lecture and four hours of laboratory per week. Lecture topics include population growth and regulation, competition and predation, community energetics and nutrient cycling, comparative ecosystem analysis, and ecosystem evolution. Laboratory exercises include field sampling techniques, experimental population manipulations, and ecosystem modeling. The initial field labs introduce students to methods and relevant issues at local study sites through meetings with community partners, sample collection, and data analysis. Based on these introductions, students design independent and team projects to further investigate more specific hypotheses.

Organic Chemistry III Lab (CHEM 347) is the third lab course in a standard year-long organic chemistry series and each section meets once weekly for three hours. As the terminal course in a year-long series, the third quarter is dedicated to research projects that change periodically according to the interests of the faculty who are teaching the course. Applications of organic chemistry to other fields are emphasized, and recent research projects have links to fields such as medicinal chemistry, biochemistry, cell biology, materials science, environmental chemistry, and food science. This is enabled by the design of the three quarter series wherein the first quarter lab course focuses on organic chemistry lab techniques, and the second quarter lab is dedicated to performing organic chemistry experiments taken from the primary literature.

Instrumental Analysis (CHEM 426) is the second of two analytical chemistry courses offered at Seattle University and it meets for two lecture hours and approximately seven laboratory hours per week. Scientific concepts covered include the theory, methods, and techniques of spectrophotometric, chromatographic, and micro-analytical procedures in instrumental analysis as well as introductory statistics and quality assurance. Examples from environmental chemistry are often used in the lecture as a means to introduce students to these scientific concepts. Most of the laboratory exercises focus on open-ended environmental research questions. The students work together in small groups and perform their experiments on a rotating basis. Each group has two lab periods (one week) to complete a given study before advancing to the next experiment. Experiments at the beginning of the quarter often involve method development and validation, whereas those later in the quarter focus on quantitative analysis using the methods developed by earlier groups.

Design and Logistics of Our Collaboration

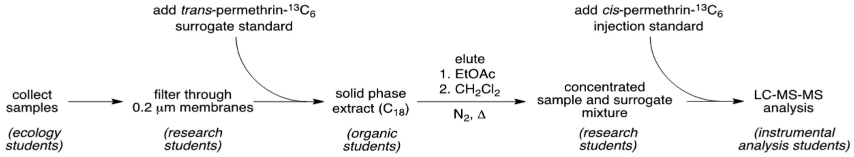
Our initial aim was to design a project that provided an example of *bona fide* scientific collaboration, addressed a local environmental issue, and supported understanding of fundamental course concepts, within the constraints of available laboratory time. Other than serving as a useful example of course concepts, by design, this project had no direct impact on the lecture portion of these courses. Overall, this collaborative project requires the entire academic quarter to conduct; however, the amount of time dedicated to the project in any one course is less than 20% of the total time in lab. Thus students in each course conduct many additional unrelated experiments over the 12-week quarter. Before the quarter starts, the faculty members plan the logistics of the collaboration, starting with the dates of sample collection by the Ecology students, a decision that is partially dependent on tidal conditions. Because we have multiple sections of Organic Chemistry Lab III, it takes an entire week to conduct the reverse-phased solid phase extraction (RP SPE), elution and evaporation. Ideally the sample collection and SPE can be completed in the first two to three weeks of the quarter, leaving ample time (3-5 weeks) for students in Instrumental Analysis to optimize LC and MS parameters, and then conduct their measurements. This leaves us only a few weeks to gather students across courses, permitting them to share and discuss their data. Scheme 2 illustrates the sharing of samples and dissemination of data between students; the faculty involved and their research groups provide oversight on all aspects of the project.



Scheme 2. Diagram illustrating the sharing of samples (dashed arrows) and data (solid arrows) between student groups participating in this research project.

Students' experience of the collaboration begins when the students from all three courses (*ca.* 100) assemble for an evening viewing of *Poisoned Waters*, a PBS Frontline documentary (available on DVD) about anthropogenic pollution in the Puget Sound and Chesapeake Bay. This serves to introduce students to the need for scientific collaboration in general, and also to instill interest, curiosity, and a common baseline of background knowledge about this collaborative project. The Frontline documentary is also noteworthy for our students because the Duwamish drains into the Puget Sound; their study site directly impacts one of the locations under scrutiny in the documentary. Another way we generate student interest and a sense of connection to the research project is by organizing a field trip to the Duwamish River. About halfway through the term, we arrange for all the students to participate in an ongoing community service project aimed at restoring the river. To simplify our workload, we work with Duwamish Alive, a non-profit citizen's group that aims to protect and restore habitat in the Puget Sound. Students meet with the leaders of these citizen's groups to ask questions about local environmental issues, and they participate in restoration activities (e.g., invasive plant removal and water quality monitoring).

Student participation in the research portion of this study starts with ecology students collecting river water samples (*ca.* 40 – 60 L) from several sites along the Duwamish River. In addition to the samples that are brought back to the lab, the Ecology students also conduct field measurements of numerous abiotic measures of ecological import (e.g. pH, temperature, dissolved O_2, salinity, total suspended solids, [Cu], $[NO_3^-]$ and $[PO_4^{3-}]$) as well as biotic ones (e.g., density and size of amphipod, isopod and polychaete benthic invertebrates). The river water samples are then sterile filtered within 24 h by our research students to prevent microbial degradation during storage. Each pair of organic chemistry students conducts reverse-phased solid phase extraction (RP SPE) on one river water sample (1 L each) using commercially available SPE cartridges. They then elute the retained mixture of organic compounds using an organic solvent, and evaporate the solvent, leaving a solid residue. This residue is provided to the students in Instrumental Analysis, who dissolve it in a small volume of organic solvent and analyze the resulting solution by LC-MS-MS, quantifying the concentration of each member of a panel of 5 pyrethroids and two isotopically-enriched standards. This flow of material and data between the groups of students is depicted in Scheme 3.



Scheme 3. Diagram illustrating the collection, processing, and analysis of Duwamish water samples by the various groups of undergraduate students participating in this research project.

The data generated are communicated between the Ecology and Instrumental Analysis students so that (*i*) they can practice their presentation skills, and (*ii*) they can make use of the composite data, as appropriate. For example, in presentations and posters generated by students for a final symposium, field ecological data with laboratory chemistry data were combined to demonstrate that sites with detectable pyrethroid concentrations are higher in water turbidity, suggesting surface runoff as a likely source. Because Organic Chemistry students are not ready for the types and volume of data generated by students in the other two, more advanced, courses, we supply Organic Chemistry Lab III students with only the mass spectral data, which they use to identify the pyrethroids detected. Using mass spectral data to characterize organic molecules is an appropriate assignment for these students and solidifies the link between the Organic Chemistry and Instrumental Analysis courses.

Representative Scientific Results

Although this chapter is focused on providing a model to faculty for future course development, we wish to provide examples of the type of data our students have generated in this study. Our results thus far indicate working with students across the three courses enables us to quantify low levels of pyrethroids in water, quantify differences in water quality, and investigate relationships with invertebrate densities. We have found differences among study sites suggesting potential sources from surface runoff associated with urban development (Figure 1). Elevated levels of turbidity, nitrate, and phosphate at the Hamm Creek site suggest potential higher volumes of runoff compared with other sites. Since runoff often carries higher loads of suspended solids, turbidity functions as an effective indicator of runoff, and these solids may often carry excess nutrients (e.g., nitrate and phosphate), which can have negative consequences for aquatic systems through eutrophication and hypoxia. Due to the chemical nature of the pyrethroids, they may also be more likely to accumulate in areas with increased turbidity and runoff.

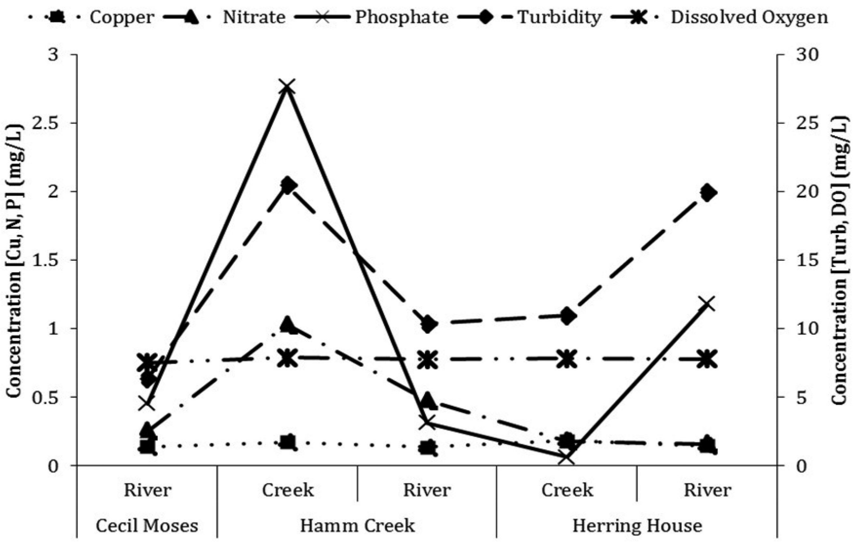


Figure 1. Data collected by students during the ecology class exemplifying the variation among sites and the similarities in concentrations patterns between turbidity, phosphate, and nitrate, suggesting potential runoff sources.

In order to connect our data on water quality and ecological measures with aquatic chemistry, we have developed a selective and sensitive SPE-LC-MS-MS method to monitor selected pyrethroids (*cis*- and *trans*-permethrin, bifenthrin, cyhalothrin, and cypermethrin isomers) in our post-SPE samples. Figure 2 shows a calibration curve for these compounds. Instrument detection limits for these analytes are approximately 5 ng/mL, meaning environmental concentrations of a few ppt (ng/L) are detectable given our SPE concentration step. These detection limits are on par with what others have reported for the more common GC-MS-MS methods used for pyrethroid analysis (*21*). Preliminary tests show that bifenthrin was present at detectable levels. Unfortunately, we were not able to quantify the detected bifenthrin because of poor recoveries of our permethrin-^{13}C standards. After our research students later optimized our analytical procedures, recoveries of spiked standards from Duwamish water were greater than 90%. As our research project progresses, we intend to adapt our LC-MS-MS method to accommodate additional high-use pyrethroids (e.g., deltamethrin, esfenvalerate, and cyfluthrin). All of these analytes can be monitored simultaneously by our analytical instrumentation. Another important area for future development is to adapt our analytical procedures to allow for analysis of pyrethroids from solid samples (i.e., sediment and tissue) given the importance of understanding pyrethroid fates in different compartments within the Duwamish. Work on this analytical challenge provides future students with opportunities to develop, adapt, and test new methods during summer research opportunities. At the end of the school year, all the data generated feed into our research programs, where our research students then build upon the preliminary findings obtained.

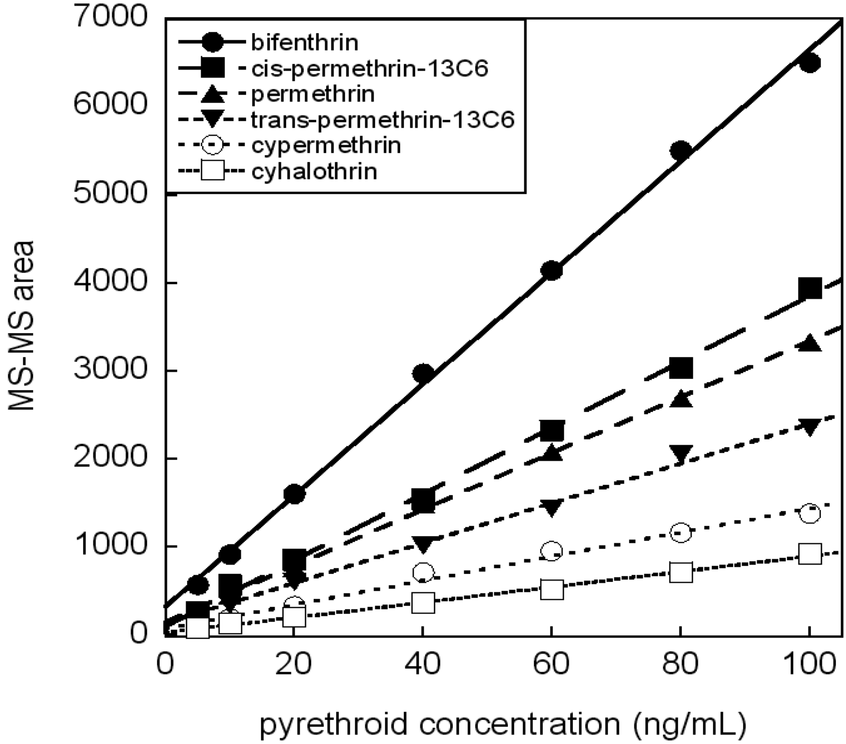


Figure 2. Student generated liquid chromatography-tandem mass spectrometry (LC-MS-MS) calibration curve for selected pyrethroid pesticides.

Reflections on Our Learning Outcomes

This project fits well with the learning objectives of each of our courses. In the Ecology course, the learning outcomes include comprehending fundamental concepts underlying environmental issues, exploring local ecosystems, experiencing essential elements of field research, and contributing to local issues through science. These are matched perfectly by this project that aims to investigate ramifications of emerging environmental contaminants to the aquatic food web of the Duwamish River. During their field work, the Ecology students achieve nearly all of these course goals.

One of the four academic learning outcomes for the organic chemistry students is to “apply the fundamental lab techniques of separation, purification and characterization to investigate current scientific research questions.” Participation in this project addresses this learning outcome as students apply their knowledge of thin-layer chromatography, column chromatography, gas chromatography, and mass spectrometry to SPE and LC-MS. In addition, their participation enables an increased throughput of environmental samples, thus allowing for multiple replicates of each measurement and the obtainment of higher quality data by the Ecology and Instrumental Analysis classes. Students in Organic Chemistry Lab III learn about SPE and LC-MS-MS because of their participation in the collaboration. SPE and LC-MS are significantly more specialized than what is typically taught in most sophomore organic chemistry courses; however, they are widely used in many research fields including environmental science, food science, cell biology, natural products chemistry, and medicinal chemistry. Although we would not argue that SPE and/or LC-MS-MS ought to be added to typical organic curricula, we are happy to have our students learn additional and modern techniques such as these. For these reasons, this course almost always involves a few modern techniques, depending on the nature of the research projects the faculty choose to include.

Among the academic learning outcomes for the instrumental analysis are to (*i*) operate sophisticated scientific instruments commonly found in chemistry laboratories; (*ii*) choose and successfully employ appropriate instrumental and calibration techniques depending on the particular experimental parameters; and (*iii*) collect, critique, and use spectroscopic, chromatographic, and mass spectrometric data to determine the identity of unknown analytes and to quantify their concentrations. By using primary samples from this collaborative research project, the students are faced with all the challenges and decisions of doing actual analytical chemistry research. The students gain first-hand experience developing analytical methods and operating highly sophisticated instrumentation. They also learn about MS-MS quantification methods and using isotopically-enriched standards to assess the performance of their analytical method.

Besides meeting the learning objectives of the three courses, the research-based and community-oriented characteristics of this project provide significant advantages to our students. First, our students are excited to work on a real research project. This is especially true for the organic chemistry students, who are mostly sophomores who have not yet had the opportunity to participate in research projects. In addition, our students enjoy the fact that this project focuses on a site that (*i*) is an environmentally disturbed Superfund site; (*ii*) is a real-world problem of local importance; and (*iii*) is a problem that disproportionately affects the racially and economically disadvantaged people who live along the river. These facts help our students to understand how science can be applied to problems that many of them already care about. Finally, this project helps our students to understand the role of meaningful scientific collaboration; throughout this project, students learn that none of the faculty's individual research groups have all the skills and knowledge needed to address the problem of collecting, concentrating, and quantifying emerging contaminants in the Duwamish industrial waterway. This becomes apparent when we gather everyone together and students realize that none of their individual faculty are able to answer all of their questions, a realization that becomes obvious as they see us turn toward each other for advice.

Summary

Taken together, our work with students in our courses assesses contaminant concentrations, water quality, and invertebrate populations across sites to determine ramifications for the foundation of the aquatic food web. To investigate relationships among these factors, we developed protocols in our chemistry courses to measure pyrethroid pesticides across a panel of sites over time. The students have been able to analyze pyrethroid variability within and across sites to explore relationships with invertebrate densities and water quality. Students have also been able to quantify how pyrethroids can interfere with natural responses to predation threats, with ramifications for overall food web dynamics.

Our collaboration is focused on detecting levels of emerging chemical contaminants and monitoring the potential ecological impacts. While always being mindful of our primary research aim of elucidating how anthropogenic chemicals ultimately impact the environmental health of the Duwamish River estuary, we also focus on providing a rich and meaningful learning experience to our undergraduate students. We integrate several areas of inquiry through our collaboration among chemists and ecologists, with particular skills and interests in environmental health sciences. Distinctive components of our project include: work within a Superfund site known to be impacted with legacy pollutants, measuring emerging contaminants using sophisticated instrumentation, investigating ecologically relevant changes in food webs, and carrying out all of this research with undergraduate students across multiple linked science courses. We hope this contribution will serve as a catalyst for future innovative course development.

Acknowledgments

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