



From DNA Extraction to Sequence Analysis: A Semester-Long Undergraduate Research Project on Fish Mislabeling

Authors: Rumfelt, Kalee E., Wonderlin, Nicole E., Hulbert, Daniel, and White, Peter J. T.

Source: The American Biology Teacher, 82(3) : 170-174

Published By: National Association of Biology Teachers

URL: <https://doi.org/10.1525/abt.2020.82.3.170>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

From DNA Extraction to Sequence Analysis: A Semester-Long Undergraduate Research Project on Fish Mislabeling

RECOMMENDED
FOR AP BiologySALE
\$5.99/lb

KALEE E. RUMFELT, NICOLE E. WONDERLIN,
DANIEL HULBERT, PETER J. T. WHITE

ABSTRACT

In recent years, providing authentic and unique research experiences for undergraduates has become increasingly important, yet many educational institutions struggle to provide their students with such experiences. Engaging students in hands-on research is meant to increase their problem-solving skills and help them learn how to work in a collaborative environment. Unfortunately, many students never receive a genuine research experience in their undergraduate biology courses. We developed a semester-long, laboratory-based research project in which students worked in groups to investigate the prevalence of fish mislabeling in local restaurants and grocery stores using DNA barcoding. During the experimental process, students learned fundamental molecular techniques like DNA extraction, polymerase chain reaction, gel electrophoresis, and DNA sequence analysis. Students also developed soft skills linked to working in teams and science communication. Over the course of the project, students collected their own fish samples and were responsible for their team's lab workflow throughout the semester. Some groups (12/25) identified instances of mislabeling on the basis of DNA evidence. Students synthesized their results in a full scientific manuscript and ended the semester by disseminating their results in a class-wide poster symposium. Collectively, the students documented that ~21% (26/123) of the fish samples they had collected from local restaurants and at grocery stores in the Greater Lansing area were mislabeled. This project gave students the time and space needed to master molecular techniques (often through trial and error), and it engaged them in a place-based learning setting as they investigated the incidence of fish mislabeling in their local community.

Key Words: fish fraud; mislabeling; place-based learning; DNA; PCR; gel electrophoresis; Sanger sequencing.

○ Introduction

It is increasingly important for undergraduate biology students to become proficient in laboratory techniques and to have an opportunity to engage in inquiry-based lab projects. Developing a strong background in lab techniques can help students in upper-division biology courses, and, beyond a higher-ed context, these skills are often in demand in the marketplace (Barley & Bechky, 1994). In response to this, many lab curricula now incorporate a wider variety of modern lab technical skills (Shah et al., 2013). However, it can be a challenge to provide students an authentic, inquiry-based lab experience (Mennella, 2015). Many students find themselves in “cookbook” labs where they are instructed to follow directions in a step-by-step fashion, with little room to engage in an authentic scientific process. Creating an environment in which students can “do their own research,” with appropriate guidance and support structures in place, can be a beneficial experience and may even be associated with higher exam scores and lower rates of failure (Freeman et al., 2014).

There has been some work done on short-term, student-inquiry-based projects in science classrooms. For example, Arnold et al. (2017) developed a molecular biology and bioinformatics laboratory project in which students investigated the prevalence of fish mislabeling over the course of four three-hour class periods. Their stated goals were for students to (1) appreciate the uses of DNA-based testing in order to understand/perform key molecular biology techniques, (2) understand how to use bioinformatics tools to analyze data, (3) be able to present results, and (4) coherently disseminate conclusions. Fish samples were provided by the laboratory teaching team, taken from a variety of restaurants and

“What makes this approach unique is that it is a semester-long, inquiry-style lab project in which students have broad responsibility to organize and regulate the workflow of their own project.”

stores, which students then used to practice DNA extraction, polymerase chain reaction (PCR) amplification of the COI gene, gel electrophoresis, sequencing sample preparation, and DNA sequence analysis. If time permitted, the authors suggested that students collect their own experimental samples. Arnold et al. (2017) concluded that an inquiry-based lab experience was an engaging and thought-provoking experience for students, and that it was good exposure to molecular laboratory techniques.

In 2018, we conducted an expanded version of this approach, using fish-mislabeled detection techniques to create a semester-long, inquiry-based lab project for undergraduate students. This project was intended to (1) engage students in a broader place-based lab project, (2) give students responsibility for the pacing of their own projects, and (3) provide students the opportunity to troubleshoot and problem-solve along the way when their lab work did not yield successful results. While our teaching team acted as facilitators in the lab, students were in control of their own workflow and made decisions about how best to organize themselves and collaborate within their groups in any given lab period during the semester. The most significant difference between Arnold et al.'s (2017) project and ours was the scope and duration of the student investigations. Ours was a semester-long curriculum, whereas Arnold et al.'s (2017) version lasted only two to three weeks. This time increase was implemented to allow for a more realistic research experience, with more in-depth troubleshooting, brainstorming, and investigating.

Below, we describe our semester-long fish-mislabeled lab project. This lab project was implemented in a 15-week, five-credit introductory cell and molecular biology course in which students spent 160 minutes per week in class (2 × 80 minute sessions) and 220 minutes per week in lab (2 × 110 minute sessions) working on their project. However, the fish-mislabeled curriculum was carried out mostly during the lab portion of the course, whereas the lecture portion was used to develop a deeper understanding of the laboratory techniques used. Laboratory techniques were always

covered in lecture prior to the start of experimentation in lab. This was done to give students a good basis of understanding before performing these techniques on their own. Each lab session was staffed with a teaching team consisting of one graduate teaching assistant and two undergraduate learning assistants (typically third- or fourth-year undergraduate students who had previously taken introductory cell and molecular biology).

○ Lab Project Overview

The semester-long lab project was divided into four components: (1) background investigations, (2) fish sample collection, (3) lab work, and (4) dissemination of student results. We detail each of these steps below (see also Table 1). These components largely occurred sequentially (from 1 to 4), with the exception that student background investigations were weaved into both the lecture and lab components of the course. This was done at strategic points throughout the semester to ensure that students learned about the molecular process of experimental techniques in their lecture prior to using that specific technique in the lab.

1. Background Investigations

Background investigations were composed of a number of different activities, including (a) constructing an annotated bibliography, (b) lab and lecture homework, and (c) lab and lecture quizzes.

a Annotated Bibliography

At the beginning of the semester, students were required to write an annotated bibliography on the topic of fish fraud and fish mislabeling as a lab assignment. This required students to complete a review of scientific literature, describing three primary sources that they found (three per student, 12 per team). Each annotation was required to include the main result of the work and the relevance of the work to the intended fish-mislabeled

Table 1. Timeline of project assignments.

Week	Working on . . .	Deadline to turn in . . .
1	Sample collection	
2	Research question & introduction	
3	Annotated bibliography	Research proposal
4	Annotated bibliography & project timeline	
5	Project timeline	Revised proposal no. 1 & annotated bibliography
6	Peer review	Revised proposal no. 2
7	Peer review	Revised proposal no. 3
8	Title & methods	
9	Discussion & conclusion	
10	Discussion & conclusion	
11	Poster	Final manuscript
12	Peer review & poster	
13	Poster	Revised manuscript
16		Poster

project. In particular, students were encouraged to find sources that documented fish fraud and mislabeling in markets and restaurants, seminal barcoding research, and the financial/consumer implications of fraud and mislabeling.

b Lab and Lecture Homework

Weekly lab and lecture homework was assigned to ensure that students did background reading and engaged in video resources that described lab techniques. Homework assignments consisted of completing short-answer questions, drawing diagrams, and matching. Students were also required to make summary notes of their readings and various video assignments. Lecture homework was aligned with the lab techniques that were being completed in a given lab that week.

c Lab and Lecture Quizzes

Lab and lecture quizzes were given on a weekly basis, corresponding to the dates on which homework was due. The purpose of these quizzes was to test students on the theoretical and practical aspects of the lab techniques being used in a given week. Lecture quizzes tended to focus on the theoretical aspects of a test (e.g., Explain how a pair of primers can be used to amplify a gene), whereas lab quizzes tended to focus on the main points of a particular test (e.g., List four of the components that need to be included in a PCR mix, prior to running PCR).

2. Fish Sample Collection

Students worked in teams of four throughout the semester-long project. A comprehensive list of sushi-selling restaurants and grocery stores was provided to the students, and each group was randomly assigned two locations from the set. Ultimately, each student team collected fish samples from one local restaurant and one local grocery store. Students were encouraged to collect ≤ 10 different fish samples from each location (“different” was defined as being from a different species). The fish was brought into the lab and stored at -15°C for use later in the semester.

3. Lab Work

The teaching team served as project facilitators and in-class consultants for the students. The teaching team (in consultation with the course instructor) made sure that the lab was stocked with the necessary reagents and that they were available to help student teams as they learned how to use the various pieces of equipment needed for the project (e.g., bench-top centrifuges, thermocyclers, gel electrophoresis boxes). The teaching team did not lecture or perform weekly demonstrations for the class. Students had full reign to decide how their experimentation was conducted, which included things like troubleshooting, pace of experimentation, and choice of technique. When students got stuck, the teaching team was there to help guide students in the right direction but generally refrained from making decisions for them.

Students extracted the DNA from each of their fish samples using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Next, they amplified the 16S gene from their DNA extractions using the primers 16Sar and 16Sbr (Ivanova et al., 2007). Each reaction mix was made using a GoTaq Flexi DNA Polymerase Kit (Promega) and consisted of the following reagents (and concentrations) in 50 μL

reactions: reaction buffer (1x), MgCl_2 (2.5 mM), dNTPs (0.2 mM each), forward and reverse primer (0.2 μM each), DNA polymerase (1.25 μL), and a DNA template (quantification was not performed). A positive and a negative control were used. The positive control was a salmon DNA sample prepared and tested by the teaching team. The PCR conditions were as follows: an initial denaturation for two minutes at 94°C , 35 cycles of 94°C for 30 seconds, 52°C for 40 seconds, and 72°C for one minute, and a final step of extension at 72°C for 10 minutes. Students visualized their PCR results via gel electrophoresis using the MiniOne gel electrophoresis system (Embitec, San Diego, CA) and a 1% agarose gel. The MiniOne system allows students to view their gel results as electrophoresis is occurring and has a hole at the top of the hood so that students can take pictures of their gels with their cell phones. There is no need for an external ultraviolet light rig, making this a very efficient system for students running electrophoresis. Students determined that PCR products were successfully amplified if a band (500 bp) was visualized during gel electrophoresis. In instances where electrophoresis indicated that a successful PCR reaction occurred, excess product that was not used during electrophoresis was cleaned up using the Wizard SV Gel and PCR Clean-Up System (Promega). Clean samples were combined with the reverse primer (16Sbr) and sent to the Michigan State University Sequencing Facility. When sequences returned, students analyzed their results using a chromatogram viewer known as FinchTV. After sequences were cleaned on FinchTV, NCBI BLAST was used to compare the obtained sequences to known fish sequences.

Students were responsible for troubleshooting their own lab work when failure occurred, in consultation with the teaching team. A failed PCR reaction was defined as seeing no band during electrophoresis. If all samples in a given run yielded no band (including the positive control), students reattempted PCR. If the positive control exhibited a band but other samples did not, students typically identified the DNA extraction step as where an error occurred, and students often chose to repeat their DNA extractions. In cases where the negative control exhibited a band, contamination was often suspected, and students typically chose to repeat their PCR step with fresh reagents. Failed sequencing reactions were noted when multiple nucleotide signatures (i.e., peaks in the Sanger readout) were detected at the majority of nucleotide positions. Students remedied this issue by repeating any number of the prior steps (i.e., DNA extraction, PCR).

4. Dissemination of Student Results

Students disseminated their project information in two ways: (a) a final manuscript, written by each team over the duration of the semester; and (b) a conference-style poster presentation during the final week of the semester.

a Final Manuscript

Students wrote their manuscripts in a structured iterative fashion over the duration of the semester. Students started writing their project in the form of a research proposal (as they were still learning the lab techniques and getting familiar with the fish mislabeling literature, etc.). They were then

assigned to write their manuscripts in the order of introduction, methods, results, and discussion. TA feedback was provided at various junctures (Table 1). The students were able to use any data acquired by the class in order to develop in-depth hypotheses and research questions. By the end of the semester, their papers resembled manuscripts that one might submit to a peer-reviewed scientific journal.

b Poster Presentation

Students prepared their poster presentations (based on their manuscripts) during the last two weeks of the semester. They had to include the following: title, abstract, introduction, methods, results, discussion, conclusion, and figures/graphs to communicate their main findings. During the last class period, students presented their posters in a group setting to their peers, teaching team, and other professors in the college who volunteered to attend the session.

○ Results

In all, 25 groups of four students each ($N = 100$ students) completed the fish-mislabeled lab project in the spring 2018 semester. Among these, 12 groups found instances of fish mislabeling. Collectively, students documented that ~21% (26/123) of the fish samples they had collected from local restaurants and at grocery stores in the Greater Lansing area were mislabeled.

Students made similar conclusions in their manuscripts, including that (1) restaurants had a higher incidence of mislabeling than grocery stores; (2) small and large grocery stores had similar rates of mislabeling; (3) common fish species tended to be mislabeled at a lower rate than rare fish species; and (4) fish retailers that were branded “organic” had a mislabeling rate of 0%, compared to the 19% mislabeling rate among retailers not branded “organic.”

In order to make these conclusions, students deemed that “small” stores were defined as having <500 locations in the United States and all other stores were considered “large.” Students determined that “common” and “uncommon” fish categories were based on the National Fisheries Institute’s (2012) list of the most commonly consumed fish. The top five consumed fish were defined as “common” and all other fish were defined as “uncommon.” Students deemed a retailer “organic” if the store was USDA-certified for selling only organic products (USDA, 1990); stores without that certification were deemed “non-organic.”

○ Discussion

During the final poster sessions and while writing their final manuscripts, students posed various reasons why they thought fish mislabeling was occurring (e.g., restaurants purposely making substitutions in an attempt to lower their costs, or staff not having the training needed to distinguish some types of fish from others). These reasons were largely drawn from the literature that students had reviewed when constructing their annotated bibliography. Students also postulated that the reason why grocery stores had lower instances of fish mislabeling might be that they are held to higher accuracy standards than restaurants, or that restaurants use less accredited fishing companies to supply their seafood (Everstine et al., 2013; Bosco et al., 2018).

In addition, the striking comparison of mislabeling rates in organic retailers (0%) and non-organic retailers (19%) led students to suggest that organic retailers also may use more accredited fishing companies or are held to more strict regulations pertaining to species identity (Everstine et al., 2013; Bosco et al., 2018). These ideas presented by students demonstrated that their results and literature reviews allowed them to try to provide answers to the issue of fish mislabeling using critical thinking. Unlike Arnold et al. (2017), we required our students to engage in literature review throughout their project. Based on our students’ conclusions, we believe that this difference allowed students to better understand scientific literature and draw meaningful conclusions from it.

What makes this approach unique is that it is a semester-long, inquiry-style lab project in which students have broad responsibility to organize and regulate the workflow of their own project. Working on one project for the entirety of the semester had many benefits. For example, students had the opportunity to develop and refine many different laboratory techniques (e.g., using micropipettes, DNA extraction, gel electrophoresis, and troubleshooting protocols based on results). In the lab experience that we facilitated for our students, the repetition of lab procedures was often facilitated by errors that students made in their DNA extraction or PCR preparation. Students had a vested interest in “getting it right” the second or third time through a particular lab protocol. This repetition allowed students to perfect their molecular biology techniques. While, undeniably, there is a degree of “cookbookery” involved when following DNA extraction protocols or preparing PCR cocktails, a deeper understanding of the processes is required in order to figure out where a mistake may have been made. Thus, the semester-long project gave the student groups leeway to try multiple troubleshooting ideas when they got unanticipated results (i.e., no gel electrophoresis band, or failed Sanger reactions). Similarly, this project evoked a high degree of “student ownership.” Learning experiences that feature student ownership can increase effort and accountability (Walters et al., 2017; Williams & Reddish, 2018). For this project, students took on the role of a biology researcher. Any successes or failures that teams experienced were a direct result of the care, time, and attention that they paid to their lab protocols. Some groups even engaged in friendly competition to see who could produce the best results (i.e., who can successfully identify the most fish?).

Another upside to this project is that it capitalizes on the benefits of place-based learning, an approach that utilizes aspects of the local culture and the natural environment, integrating them into the educational experience. This type of curriculum emphasizes a hands-on, real-world learning experience (Sobel, 2004) and has been positively linked to student gains in learning (Hasni et al., 2016). In our project, students seemed to like the idea that they were investigating whether fish mislabeling was occurring in *their* local community. They also enjoyed the fact that the answer to the question of local fish mislabeling had not previously been investigated. After documenting cases of mislabeling, many groups discussed why proper fish labeling might be important for the community. Some of their ideas fell in line with the research papers they had read while conducting their literature review. These ideas included ethical standards, health concerns, and economic impacts (Jenssen et al., 2012; McGuire et al., 2016; Van Ruth et al., 2018).

○ Common Mistakes

Here, we have reported the results as they were presented by our students. The results should be interpreted with appropriate context: the students were relative novices in terms of research skills, meticulousness, and experience (e.g., as compared to an experienced molecular biologist); it was not possible to determine whether the student results reflect the true rate of mislabeling at local restaurants and grocery stores, or whether the cumulative mislabeling figure is artificially inflated or deflated as a result of student errors or mix-ups.

We noted that there were a few common mistakes that student teams tended to make. First, students often used too little fish tissue for DNA extraction compared to the dime-sized pieces that the protocol calls for. This decreased the DNA concentration and, at times, seemed to result in failed PCR or Sanger reactions. Another common mistake was in the “wash” steps of the DNA extraction protocol. The protocol calls for careful extraction of supernatant after fish flesh has been macerated and treated with cell lysis, RNase, and protein precipitation solutions. This was followed by two washes (one with isopropanol and one with ethanol), with two different supernatant amounts saved during aspiration (50 μ L saved during isopropanol aspiration and 25 μ L saved during ethanol aspiration). Students commonly saved too much of the isopropanol and kept too little of the ethanol. Finally, particularly early in the semester, we noted that some students struggled with their pipette skills, often improperly using their pipettor, resulting in incorrect volumes that undoubtedly impacted PCR or Sanger reactions.

○ Conclusion

We designed and implemented a semester-long lab project that engaged students with an investigation of fish mislabeling in their local community. Students were asked to carry out many of the steps that a researcher would complete when engaging in a similar project in the “real lab world,” including a literature review, sample collection, mastering of key molecular biology techniques (DNA extraction, PCR, gel electrophoresis, PCR cleanup, DNA sequence analysis), conducting data analysis, and disseminating results by preparing a manuscript and a poster presentation. Our project also benefited students because it gave them the time and space needed to master molecular techniques (often through trial and error) and engaged them in a place-based learning environment as they investigated the incidence of fish mislabeling in their local community.

References

- Arnold, M.L., Holman, D. & Zweifel, S.G. (2017). Using molecular biology and bioinformatics to investigate the prevalence of mislabeled fish samples. *American Biology Teacher*, 79, 763–768.
- Barley, S.R. & Bechky, B.A. (1994). In the backrooms of science: the work of technicians in science labs. *Work and Occupations*, 21, 85–126.
- Bosco, S.A., Foley, D.M. & Hellberg, R.S. (2018). Species substitution and country of origin mislabeling of catfish products on the U.S. commercial market. *Aquaculture*, 495, 715–720.
- Driver, R., Asoko, H., Leach, J., Scott, P. & Mortimer, E. (1994). Constructing scientific knowledge in the classroom. *Educational Researcher*, 23(7), 5–12.

- Everstine, K., Spink, J. & Kennedy, S. (2013). Economically motivated adulteration (EMA) of food: common characteristics of EMA incidents. *Journal of Food Protection*, 76, 723–735.
- Freeman, S., Eddy, S.L., McDonough, M., Smith, M.K., Okoroafor, N., Jordt, H. & Wenderoth, M.P. (2014). Active learning increases student performance in science, engineering, and mathematics. *Proceedings of the National Academy of Sciences USA*, 111, 8410–8415.
- Hasni, A., Bousadra, F., Belletête, V., Benabdallah, A., Nicole, M. & Dumais, N. (2016). Trends in research on project-based science and technology teaching and learning at K–12 levels: a systematic review. *Studies in Science Education*, 52, 199–231.
- Ivanova, N.V., Zemlak, T.S., Hanner, R.H. & Hebert, P.D. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7, 544–548.
- Jenssen, M.T.S., Brantaeter, A.L., Haugen, M., Meltzer, H.M., Larssen, T., Kvale, H.E., et al. (2012). Dietary mercury exposure in a population with a wide range of fish consumption—self-capture of fish and regional differences are important determinants of mercury in blood. *Science of the Total Environment*, 439, 220–229.
- Juyoung, L. & Jihyeong, S. (2015). Importance of exploratory writing in critical thinking and learning. *ITAA*, 1, 1–2.
- Kågesten, O. & Engelbrecht, J. (2007). Student group presentations: a learning instrument in undergraduate mathematics for engineering students. *European Journal of Engineering Education*, 32, 303–314.
- McGuire, J., Kaplan, J., Lapolla, J. & Kleiner, R. (2016). The 2014 FDA assessment of commercial fish: practical considerations for improved dietary guidance. *Nutrition Journal*, 15(1).
- Mennella, T.A. (2015). Designing authentic undergraduate research experiences in a single-semester lab course. *American Biology Teacher*, 77, 526–531.
- National Fisheries Institute (2012). NFI top ten list, a familiar school of fish. *About Seafood*, September 24. https://www.aboutseafood.com/press_release/nfi-top-ten-list-a-familiar-school-of-fish/.
- Shah, K., Thomas, S. & Stein, A. (2013). Affordable hands-on DNA sequencing and genotyping: an exercise for teaching DNA analysis to undergraduates. *Biochemistry and Molecular Biology Education*, 41, 388–395.
- Sobel, D. (2004). *Place-Based Education: Connecting Classrooms & Communities*. Great Barrington, MA: Orion Society.
- Thompson, K.J., Switky, B. & Gilinsky, A. (2012). Impromptu presentations: boosting student learning and engagement through spontaneous collaboration. *Journal of Education for Business*, 87, 14–21.
- USDA (1990). National Organic Program. Electronic Code of Federal Regulations, accessed October 11, 2018. https://www.ecfr.gov/cgi-bin/text-idx?tpl=/ecfrbrowse/Title07/7cfr205_main_02.tpl.
- Van Ruth, S.M., Luning, P.A., Silvis, I.C.J., Yang, Y. & Huisman, W. (2018). Differences in fraud vulnerability in various food supply chains and their tiers. *Food Control*, 84, 375–381.
- Walters, S.R., Silva, P. & Nikolai, J. (2017). Teaching, learning, and assessment: insights into students’ motivation to learn. *Qualitative Report*, 22, 1151.
- Williams, L.C. & Reddish, M.J. (2018). Integrating primary research into the teaching lab: benefits and impacts of a one-semester CURE for physical chemistry. *Journal of Chemical Education*, 95, 928–938.

KALEE E. RUMFELT was an undergraduate learning assistant involved in facilitating the fish fraud labs. She is now a graduate student at the School of Public Health, University of Michigan, Ann Arbor, MI 48109. NICOLE E. WONDERLIN (wonderl1@msu.edu) is a graduate student and DANIEL HULBERT (hulberd@msu.edu) is a postdoctoral researcher in the Department of Entomology, Michigan State University, East Lansing, MI 48824. PETER J.T. WHITE (pwhite@msu.edu) is an Assistant Professor in Lyman Briggs College and the Department of Entomology, Michigan State University.