

Trails to Research: an Inquiry-Based Course Using Zebrafish To Provide Research Experience to Tribal College Students

Jennifer Forecki,^a Chelsea Morales,^b and Christa Merzdorf^a

^aDepartment of Microbiology and Cell Biology, Montana State University, Bozeman, Montana, USA

^bAllied Health Department, Aaniiih Nakoda College, Harlem, Montana, USA

Embryonic development is fascinating to follow and highly engaging and, therefore, lends itself for undergraduate students' first steps in experimental science. We developed the "Trails to Research" inquiry-based course, which exposes students to life science research using zebrafish as model organism. Zebrafish are ideal in the classroom: they are easy to maintain, their embryos develop rapidly, and they are easily manipulated. Further, they lend themselves to teach about embryo development and experimental design. We developed the course for undergraduates at 2-year colleges and, therefore, for students with little or no research experience. In this 5-day intensive course (which is taught during summers as a stand-alone course), students design treatment experiments for zebrafish embryos with known teratogens and with substances they select. The course comprises three modules that overlap over the 5 days: (i) introduction to developmental biology, model organisms, toxicology, and experimental design, (ii) zebrafish embryo experimental setup, and (iii) collecting, analyzing, and presenting data. Student learning was significant in the areas of experimental design, working with model systems, working with zebrafish embryos, using laboratory equipment, and presenting the results of their experiments using effective methods.

KEYWORDS inquiry, inquiry-based course, zebrafish, model organism, tribal college, 2-year college, intensive one week, undergraduate research

INTRODUCTION

Students usually self-select to participate in undergraduate research, and those with personal or familial experience in research encounter the lowest barriers (1). Studies have shown that most students are unaware of the research activities on campus during the first portion of their undergraduate experiences (2, 3). Students with the least awareness of science, technology, engineering, and math (STEM) research most likely end up excluded from learning about or participating in research on campus. Even with a desire to participate in research, students who try to obtain an undergraduate research position encounter a hidden pathway without clear directions or rules, since most positions are obtained through informal interactions with faculty (4–6). Students with less confidence or experience may be uncomfortable directly interacting with faculty (7).

One way to overcome these hurdles is to provide an introduction to research for a diverse group of students, regardless of their research background or prior coursework (1). Inquiry-based laboratory courses provide such introductory research experiences in a teaching laboratory environment (8–10). Students participate in experiences similar to real-world lab situations and increase their research-based skills while gaining an increased interest in biology and positive attitude toward learning (11, 12). Students with undergraduate research experience are some of the most successful STEM students at 4-year universities and are more likely to pursue graduate degrees (13–15). Students who participate in undergraduate research have higher GPAs, retention, and rates of degree achievement, while also building social belonging in STEM communities on campuses, which is important for all students, particularly students of minority groups (16–18).

"Trails to Research" is a week-long introduction to research for students at 2-year associate degree-granting institutions. While this inquiry-based course was initially designed for students attending tribal colleges (institutions governed by Tribal Nations), we have also taught the course at other 2-year institutions (community colleges), and it can easily be used at the freshman or sophomore level at 4-year institutions. This course helps students gain a research mindset and allows them to learn laboratory skills, such as using and caring for model organisms

Editor Nancy Maroushek Boury, Iowa State University
Address correspondence to Department of Microbiology and Cell Biology, Montana State University, Bozeman, Montana, USA.
E-mail: merzdorf@montana.edu.

The authors declare no conflict of interest.

Received: 21 December 2022, Accepted: 14 April 2023

Copyright © 2023 Forecki et al. <https://creativecommons.org/licenses/by-nc-nd/4.0/>. This is an open-access article distributed under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

and learning to use laboratory equipment. These skills are directly applicable to future undergraduate research. The structure of this course aligns with the goals of inquiry-based laboratory courses (10, 19).

This course focuses on student-designed research projects built around zebrafish (*Danio rerio*) embryonic development. Zebrafish are a model for human development, due to the similarity of early stages of development across vertebrates (20, 21). Thus, zebrafish are widely used for studying the effects of environmental pollutants (22–24). Our program joins the BioEYES program (<http://bioeyes.org/>) to enrich STEM education. BioEYES uses zebrafish in K–12 classrooms to teach lessons about the scientific method, biology, and genetics (25, 26). Thus, zebrafish lend themselves for studying early embryonic development in the classroom laboratory. They are easily maintained and their transparent embryos develop externally from their mothers, allowing microscopy and hands-on experiments. Students follow the embryos' rapid development from a few cells to free-swimming larvae that have pigment, eyes, and a heartbeat and respond to touch stimuli within 4 days. Fish and their embryos are easily tested for their response to toxins and teratogens. The latter are substances that may be harmless to an adult organism but cause birth defects. In this course, students choose substances with which to treat their zebrafish embryos and document how these substances affect embryonic development. Often, students choose substances relevant to their own lives or environment. Here, we outline this inquiry-based introduction to research using zebrafish embryos.

Intended audience

We designed this inquiry-based course for tribal college or 2-year college students who have a general interest in science, research, or are STEM majors. It can also be implemented with freshman or sophomore students at 4-year colleges. The course provides initial exposure to STEM research and intends to strengthen or inspire students' interest to participate in undergraduate research.

Learning time

Class time is an intensive 5-day week with 8 h days. Students complete most work during class time. While it is possible to finish final presentation preparations during class time, some students choose to work on their own to complete their presentations. The course is a stand-alone course and does not require other context.

Prerequisite student knowledge

While it can be helpful for students to have some basic biological knowledge before attending, this course requires no prior knowledge. Our goal is to provide the students with an introduction to life science research. By not requiring

prerequisite knowledge, the course is more inclusive and accessible to a wide range of students.

Learning objectives

Upon completion of the Trails to Research course, students will be able to:

1. Understand experimental design and execution
2. Understand the use of model systems
3. Use equipment to document embryos and measure dosages of teratogens, including:
 - a. Identify and use micropipettes
 - b. Use a microscope with camera to capture quality images of embryos
4. Properly stage zebrafish embryos
5. Design effective slides for their presentations

PROCEDURE

Materials

This course is an introduction to research and basic biological principles. It has three overlapping modules: (i) introduction to developmental biology, model organisms, toxicology, and experimental design, (ii) zebrafish embryo experimental setup, and (iii) collecting, analyzing, and presenting data (Fig. 1). An established 10-gal aquarium houses a group of 14 to 18 adult zebrafish (approximately equal sexes) to provide embryos. Students collect embryos from the tank (typically 100 to 500 embryos), which they keep in Petri dishes inside an incubator. Students select substances to treat the embryos, which they add to the embryo media in order to experimentally treat the embryos. Students then use stereomicroscopes to observe, measure, and document the effects of these treatments. The supplemental material contains detailed lists of equipment and materials for the 3 course modules.

Student instructions

The course consists of five 8 h days, which are devoted to developing, executing, documenting, and presenting the zebrafish embryo experiments.

(i) Module 1: introduction to developmental biology, model organisms, toxicology, and experimental design. Initial instruction includes the basics of embryonic development, the use of laboratory organisms as a model, the basics of toxicology and teratogenicity, the basics of experimental design, and the logic behind the planned experiments. This module is essential to prepare all students for participation in modules 2 and 3 since no prior knowledge is required.

(ii) Module 2: zebrafish embryo experimental setup and execution. (a) *Part 1: experiments with ethanol*

Prep Day	Day 1	Day 2	Day 3	Day 4	Day 5
Final setup of the teaching laboratory and all materials needed for the course.	Module 1 : Introduction to developmental biology, model organisms, toxicology, and experimental design. Lectures and Group Brainstorming				
	Module 2: Zebrafish embryo experimental set-up and execution		Module 3: Collecting, analyzing, and presenting data		
<u>Experiment 1</u> Evening: breeding dish containing marbles into tank for collecting embryos	<u>Experiment 1</u> Collect embryos (0 dpf) Photography Treat embryos with ethanol, retinoic acid	<u>Experiment 1</u> Clean embryos (1 dpf) Assess survival Photography <u>Experiment 2</u> Collect Embryos (0 dpf) Assess survival Calculate treatment concentrations Treat embryos	<u>Experiment 1</u> Collect Data (2 dpf) : Assess survival Developmental stage Measure length Heart rate Pigmentation Swimming behavior <u>Experiment 2</u> Clean embryos (1 dpf) Assess survival Photography	<u>Prepare Presentations</u> Develop background Information Data analysis and visual representations Effective slide design Practice presentations <u>Experiment 2</u> Collect Data (2 dpf): Assess survival Developmental stage Measure length Heart rate Pigmentation Swimming behavior	Final Presentations

FIG 1. Course structure containing 3 modules that overlap during the 5 days of instruction. The three modules of this inquiry-based course are designed to first prepare the students to conduct experiments and then to execute, analyze, and share those experiments. Module 1 includes an introduction to developmental biology, model organisms, toxicology, and experimental design and covers the preparatory materials the students need to fully participate in the course. Module 2 contains the setup and execution of two zebrafish experiments: (i) treatment of the embryos with ethanol and retinoic acid (RA), and (ii) treatment of the embryos with substances of the students' choosing. Module 3 comprises collecting data, such as survival rate, heart rate, length, developmental stage, pigmentation, and swimming behavior. Module 3 also includes the creation of a final presentation and the public delivery of the presentation. Days post fertilization (dpf) is a measure of embryonic age. For example, 0 dpf is the time from fertilization to 24 h of development, 1 dpf is from 24 to 48 h of development, and 2 dpf is from 48 to 72 h of development.

and retinoic acid. Students set up experiments in which embryos are exposed to ethanol and retinoic acid (used to treat acne). Students are familiar with these common teratogens that cause obvious birth defects in vertebrates

(27–30). Students learn how to collect and care for their zebrafish embryos. We harvest embryos from the zebrafish tank by retrieving a glass dish filled with marbles that was placed there the previous night (Fig. 2). The marbles mimic



FIG 2. Zebrafish tank with marble-filled breeding dish. Zebrafish housed in a 10-gal aquarium produce embryos for the week-long course. The glass dish full of marbles is placed into the tank late afternoon before collection of embryos the following morning (spawning begins when the automatic tank lights turn on). The marbles protect the embryos from being eaten by the zebrafish.

the natural substrate over which zebrafish like to mate, and the marbles protect the embryos from being eaten by the fish. After removing the marbles and filtering out small debris with a fine plastic mesh, the embryos are cleaned and counted by the students, using microscopes and bulb pipettes. Students learn to determine the developmental stage of the embryos. Most embryos are within a half hour developmentally, since most laying occurs right after the aquarium lights turn on in the morning. Students learn to use dissecting microscopes and the mounted camera for obtaining photos. These photos allow students to document zebrafish embryo development. Students make entries into their lab notebooks by drawing the embryos using microscopes and recording their stage and appearance.

Depending on how fecund the zebrafish are (which is largely unaffected by travel [see Appendix S1 in the supplemental material]), students work in groups of 2 to 4. Each group treats their embryos with different concentrations of ethanol (overnight treatment with 1%, 2%, or 3% ethanol) or retinoic acid (10-min treatment with 10^{-6} M, 10^{-7} M, or 10^{-8} M retinoic acid). Each substance is diluted in embryo media (Appendix S2), and 10 to 25 embryos are required for each condition. Each group also maintains a control group of untreated embryos. Students observe and photograph their embryos throughout the week.

(b) *Part 2: experiments with substances chosen by the students.* At the end of day 1, students brainstorm and discuss with their peers ideas for substances to test. Students use their personal interests and the Internet for ideas of everyday substances, substances that might have effects on human health, or substances present in local ecosystems. Day 1 ends with students deciding which substances to test and setting up the marble dish in the zebrafish tank (Fig. 2). Early on day 2, students collect and clean the second batch of embryos. Subsequently, students search for and read published materials on their chosen substances for two purposes. (1) They develop hypotheses with their predictions regarding the effects that their substances might have on the development of their embryos. (2) They determine the maximal recommended daily dose or Environmental Protection Agency (EPA) limit for their substances. These values are used to calculate concentrations of the chosen substances for zebrafish embryo treatment (Appendix S3). The students test each substance at three different concentrations over a wide range to help ensure interesting results. Generally, the students use the maximal recommended dose or EPA limit as their lowest dose, since neither would be expected to result in abnormalities, plus two higher concentrations that might produce visible abnormalities. The students learn concepts of preparing stock solutions and making dilutions. They set up their day 2 experiments by treating the day 2 batch of embryos and establishing control groups. Students are taught the essential skill of using micropipettes before and while setting up their experiments.

Each morning, students perform the basic animal husbandry practices of transferring the embryos from their day 1 and day 2 experiments to fresh embryo media. Observations of 1 day post fertilization (dpf) embryos include assessment of

developmental stage, gross morphology (by microscopy), and survival rate (Fig. 1; see also Appendix S3). These observations and photos are part of the students' data and are used for their final slide presentations.

(iii) Module 3: collecting, analyzing, and presenting data. Over the course of the next 2 to 3 days, students continue to monitor developmental stages, gross morphology, and survival rates of their embryos. On the third day, when embryos from the first experiment are 2 dpf, students assess swimming behavior and pigment development. They also measure heart rate and length of their embryos (Fig. 1, Appendix S3). Students use the camera-equipped stereomicroscope to capture images and videos that document morphology and behavior. Students collect similar data for their second experiments (Fig. 1).

The students' research projects culminate in short slide presentations. They receive guidance on the creation of effective slides (Appendix S3) and different ways to visually represent their data (as graphs or tables). For their introductory slides, students consolidate the background information they previously collected, along with newly researched information, to put their projects into context, for example, information about biomedical or environmental relevance and health impacts of their tested substances. In addition, these talks contain an introduction to zebrafish, experimental methods, data, results, interpretation of their data, and conclusions, including future directions. An example presentation and presentation rubric are in the supplemental materials (Appendices S4 and S5).

The students present to an audience of their peers, family, and tribal college staff and faculty. Community members always show interest in the effects that the tested substances might have on them, their children, environment, and animals.

Faculty instructions

This course is designed for small classes of 10 to 20 students with 2 instructors. Limiting the student number fosters a community where students have ample and direct access to instructors in an intimate setting. The instructors work extensively with small groups of students, which ensures that the experience is accessible and nonintimidating, allowing students and instructors to collaborate and share during learning.

Because the course is targeted to all students, especially those without prior research experience, instructors provide instructions for all tasks, laboratory skills and use of equipment. For example, working with zebrafish embryos is universally a new experience for students. Students need assistance handling and caring for zebrafish embryos and setting up their experiments. Other aspects of experimental design, research skills, and terminology are typically new to the students. The close contact between students and instructors allows the instructors to engage in many conversations and guide students when they need help. These interactions strongly impact

the ability of students to learn, share, interpret their findings, etc.

(i) Preparation instructions. *(a) Three months prior to the course.* The course uses vertebrate animals and therefore the activities during the course have to be approved by the Institutional Animal Care and Use Committee (IACUC). The IACUC is responsible for the humane treatment of all animals used in classrooms and laboratories, as well as ensuring proper veterinary care, and alignment with federal regulations.

(b) Three weeks prior to the course. At 3 weeks prior to the course, instructors establish a fish tank for housing adult zebrafish to provide embryos for the students' experiments. For materials and brief fish maintenance instructions see Appendix S1. To ensure that the zebrafish produce a large number of embryos, the zebrafish should be accustomed to their environment (light cycle, temperature, tank environment) for at least 2 to 3 weeks prior to the course. The care of zebrafish is described at <https://zfin.org/> or in the Zebrafish Book (31).

(c) One day before the course. The evening prior to the course, instructors place a glass dish filled with marbles into the fish tank. Fish spawn over the marble substrate when the tank light turns on the next morning. Instructors also prepare the ethanol and retinoic acid solutions for treating embryos on day 1.

Suggestions for determining student learning

Several measures assess student learning: a pre- and posttest and final presentations. The pre- and posttests determine if students achieved the learning objectives (LOs) of understanding experimental design and execution, understanding the use of model systems, and working with pipettes (learning objectives 1, 2, and 3a) (Appendix S6). The final presentations are used to assess if the students properly stage zebrafish embryos, use a microscope with camera to capture quality images of embryos, and design effective slides for their presentations (learning objectives 3b, 4, and 5). Instructors use a rubric to assess the final presentations (Appendix S5).

Sample data

Students collect data about the effects of alcohol and retinoic acid in the first round of experiments. These known teratogens result in significant birth defects (Fig. 3B, panels 2, 3, and 8). In the second set of experiments, students explore how different substances affect the development of zebrafish embryos (Fig. 3B, panels 4 to 7). Student often choose substances that have particular relevance for them or their community. For example, students have chosen to study the effects of tobacco and nicotine, caffeine, and pain killers on embryonic development. Students have also been interested in testing components found in agricultural runoff waters, acid mine drainage, contaminated well

water, and brine from oil fields, which are all substances that affect rural reservation communities in Montana. Students collect data in the form of images taken with a dissecting microscope (Fig. 3) to document the morphology of zebrafish embryos. Students also measure heart rate, size, and survival, and observe pigmentation and swimming behavior. Students compile these data into tables or graphs (Fig. 4) for their final presentations (Appendix S4).

Safety issues

All laboratory activities occur in a biosafety level I (BSL-I)-certified teaching laboratory. Prior to the course, instructors develop standard operating procedures (SOPs; following the university's chemical safety guidelines for handling chemicals) and review these SOPs with the students on the first day before the start of activities. Students receive proper personal protective equipment, including lab coats and protective eye wear. Students hear the safety guidelines and practices in a BSL-I lab, and all procedures executed by instructors and students adhere to ASM guidelines for biosafety in teaching laboratories.

Instructors working with this curriculum have proper training in the care and use of zebrafish by online and/or in-person training specific to the institution.

The procedures for this course were approved by the institution's IACUC committee. Instructors should refer to the "Guide for the Care and Use of Laboratory Animals" for additional questions about the care and use of animals in a laboratory setting (32).

DISCUSSION

Field testing

Due to the intensive all-day nature of this experience, the courses are held in the summer. The initial test of this course occurred in the summer of 2015 at Montana State University (MSU). During the summers of 2016 to 2019, we conducted one course at MSU plus two additional courses that rotated between Aaniiih Nakoda College (Harlem, MT), Chief Dull Knife College (Lame Deer, MT), Little Big Horn College (Crow Agency, MT), and Fort Peck Community College (Poplar, MT). Subsequently, the COVID-19 epidemic forced a hiatus.

For the courses held at MSU, students travel from Montana's seven tribal colleges, stay in the dormitories, and attend the course in a cell and molecular biology teaching laboratory. For courses held at tribal colleges, we travel with most equipment and the fish on the day prior to the course. Upon arrival, we set up the fish and all equipment in a teaching laboratory at the respective college. Bringing equipment and supplies alleviates the need for the tribal colleges to provide any specialized equipment, ensuring the courses are accessible to all campuses.

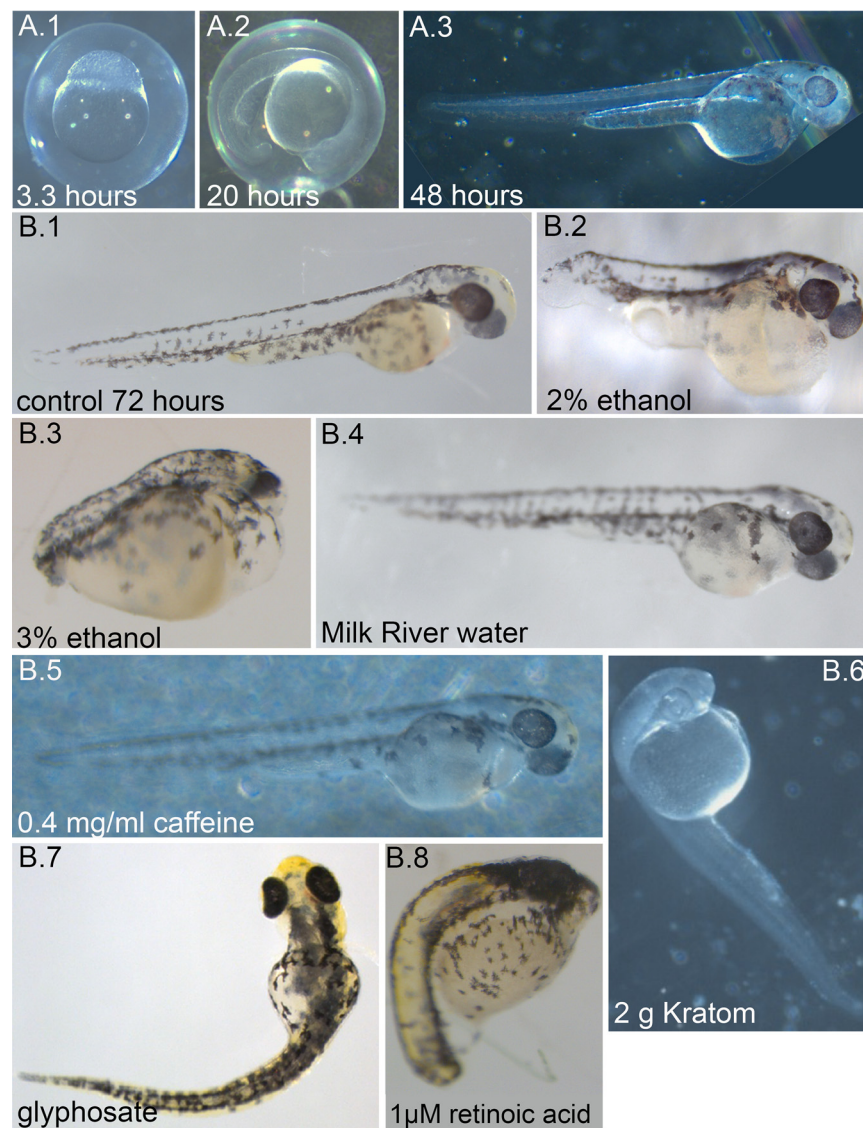


FIG 3. Sample images of embryos taken by course students. (A) Images of control embryos at different developmental stages. (A1) The 3.3 h embryo is an accumulation of cells on top of the yolk, protected by the chorion, which is the transparent membrane surrounding the embryo; (A2) at 20 h the embryo is still inside the chorion, the head-to-tail axis is clearly visible and curled around the yolk, and eyes have begun to form; (A3) at 48 h the embryo has hatched from the chorion. (B) Control and treated 72-h embryos. (B1) A control embryo at 72 h has developed pigment, a heartbeat, and responds to touch stimuli. (B2) Abnormal development in 2% ethanol-treated embryo, particularly in the head and tail regions. (B3) A 3% ethanol-treated embryo lacks head structures and shows stunted tail development. (B4) An embryo treated with water from the Milk River in northern Montana, an area with heavy agriculture, shows normal development. (B5) An embryo treated with 0.4 mg/mL caffeine has slight edema in its heart region. (B6) An embryo treated with an extract prepared from 2 g kratom is developmentally delayed, but otherwise developing normally. (B7) An embryo treated with the herbicide glyphosate shows a curved body and abnormal swimming behavior. (B8) An embryo treated with 1 μ M retinoic acid has a disrupted body plan and severe reduction of head and tail structures. All images were taken by students participating in the courses.

Student engagement

To measure student attitudes and engagement, each institution's IRB approved the use of the Undergraduate Research

Student Self-Assessment (URSSA) (33). The assessment measures students' self-reported ability to think and work like a scientist, their overall research skills, personal gains related to research, and their overall attitude toward research. High self-

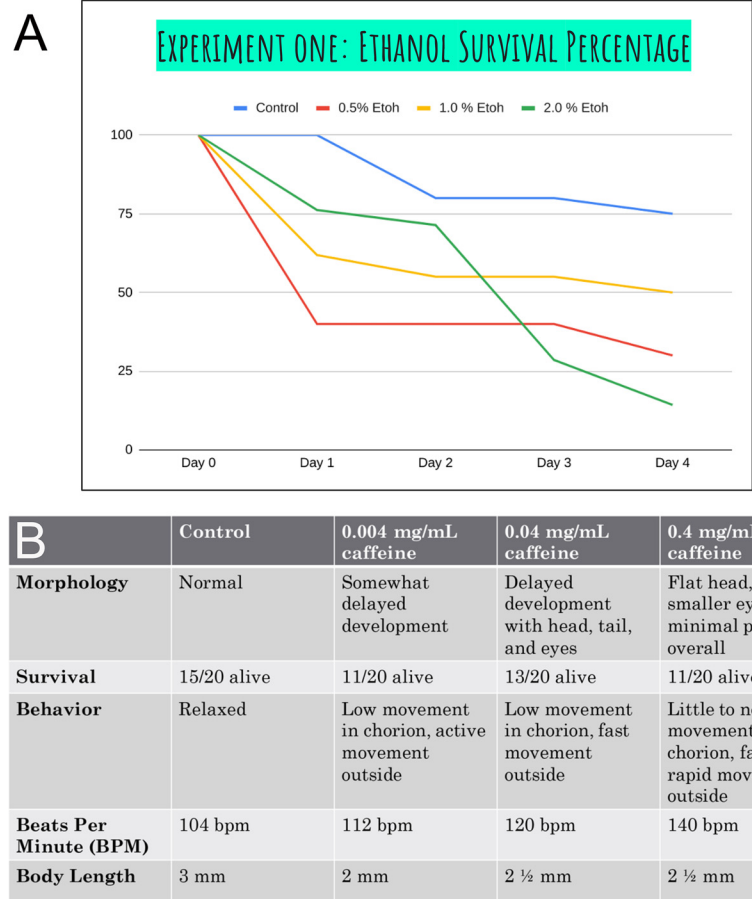


FIG 4. Data samples prepared by students for their final presentations. (A) One group of students created a line graph showing embryo survival for 0 to 4 dpf control embryos and embryos treated with 0.5%, 1.0%, and 2.0% ethanol. (B) A different group of students presented their observations of caffeine-treated embryos in a table. The assessed parameters included morphological observations, overall survival, swimming behavior, heart rate, and body length. The caffeine concentrations are listed along the top of the table. In order to determine the amount of caffeine to use, the students found that a pregnant woman should not consume more than 200 mg caffeine per day (approximately 2 cups of coffee) (<https://www.nhs.uk/common-health-questions/pregnancy/should-i-limit-caffeine-during-pregnancy/>). A 200-mg dose of caffeine in the 5 liters of blood of an average female results in 0.04 mg caffeine/mL blood (middle concentration used by the students). The 200 mg of caffeine distributed in the total wet body weight of an average female results in ca. 0.004 mg/mL (lowest concentration used). Weak drip coffee has ca. 0.4 mg/mL caffeine (ca. 96 mg caffeine per 8-ounce cup of coffee); this was the highest concentration chosen by the students. The fish embryos were removed from the caffeine solutions on the next day, when human embryos would have had the equivalent age of ca. 1 month post fertilization. Similar research and calculations are applied to all substances that the students choose.

efficacy is positively correlated with engagement and is key to motivation in the classroom (34, 35). Here, we discuss the results for the first two measures listed above from two courses in 2018 at MSU and Aaniiih Nakoda College on the Fort Belknap Reservation, the two courses with the most complete data (total of 22 students) (Table I). Results for the other two measures from the URSSA, along with demographic information on the students in these two courses, is reported in Appendix S7.

At the end of each course, we asked students to rate their overall ability to think and work like a scientist before and after their participation in the Trails to Research course on a Likert scale from no ability to great ability (Table I). We found that

18.2% of the students indicated good or great ability before the course and 86.4% reported good or great ability after the course. Thus, the majority of the students indicated an increase in their ability to think and work like a scientist.

We also used the URSSA to assess changes in the students' perception of their research skills on a Likert scale from no skill to great skill (Table I). The majority of students in our sample (63.7%) said that they had a little to moderate research skills before participating in the course. After participating in the course, the majority of students (90.9%) felt they had good skill or great skills. While all the students indicated that they had some skill (0% reported no skill) before participating in the course, almost all students

TABLE 1
Table of student responses to evaluation questions^a

Survey statement	% of students who responded that their ability or skills were:				
	None	A little	Moderate	Good	Great
A. Rate your overall ability to think and work like a scientist					
Before participation	0%	45.4%	36.4%	18.2%	0%
After participation	0%	0%	13.6%	63.6%	22.8%
B. Rate your overall research skill					
Before participation	0%	27.3%	36.4%	27.3%	9.0%
After participation	0%	0%	9.1%	59.1%	31.8%

^aThese questions were administered in the postcourse survey ($n = 22$). Students ($n = 22$) were asked to evaluate any gains due to their participation in the zebrafish course in the areas of “thinking and working like a scientist” (A) and research skills (B). The instrument to measure these outcomes was the Undergraduate Research Student Self-Assessment (URSSA), which was administered at the end of the course (33). Students rated their responses on a 5-point Likert scale. Increases were observed in both abilities to “think and work like a scientist” and in overall research skills. These survey results are from courses at MSU and Aaniiih Nakoda College on the Fort Belknap Reservation in 2018, two courses with the most complete data. Additional URSSA results and student demographics are in the supplemental materials (Appendix S7).

felt that after participating in the course, they had obtained good or great research skills. Thus, the course improved the students’ self-efficacy in the research environment.

Evidence of student learning

To provide evidence of student learning, the courses included a pre- and posttest that targeted the LOs. We show results from the same two courses mentioned above ($n = 22$). The pre- and posttests included questions about how to properly design an experiment and the purpose of model organisms when studying developmental biology (LOs 1 and 2). Relevant questions from the pre- and posttests were grouped according to LOs, and mean student learning gains were calculated (Table 2). We observed significant learning for LOs 1 and 2.

We used the rubric in Appendix S5 to assess the final presentations and determine if students met LOs 2, 4, and 5 (Fig. 5). Students scored 83% for both LOs 2 and 5 (the ability to understand the use of model systems and the ability to design effective slides for their presentations, respectively). They scored 69% for LO4 (the ability to properly stage

zebrafish embryos). These scores indicated that students met these learning objectives.

We assessed understanding the use of model organisms (LO2) by two methods: by the pre- and posttests (Table 2) and using the final presentation rubric (Fig. 5). Both metrics indicated that students met this learning objective.

We assessed the students’ ability to use equipment (LO3) in two parts. We report on these separately, since our data showed unequal effectiveness in meeting LO3a and LO3b. LO3a (identification and use of micropipettes) did not show significant learning gains (Table 2), while for LO3b (using a microscope with camera to capture quality images of embryos), students scored 79% (Fig. 5), indicating that LO3b was met. We concluded that students require more hands-on practice with micropipettes to meet LO3a.

Besides an opportunity to assess students’ learning, the presentations were open to all faculty, staff, families of the students, and the wider community. In this way, students shared with the community and communicated how the course affected them. These presentations gave students the opportunity to hone their communication skills and the ability to answer questions.

TABLE 2
Gains in learning objectives 1 to 3^a

LO [question no. on pre- and posttest]	Mean student learning gain (<i>P</i> value)
1. Understand experimental design and execution [10, 11, 12]	0.68 (<0.001)
2. Understand the use of model systems during embryo development [13]	0.69 (0.003)
3. Use equipment to document embryos and measure dosages of teratogens: (a) identify and use micropipettes [4, 6, 7]	0.35 (0.175)

^aThe learning gains for learning objectives 1, 2, and 3a were determined by combining the scores from relevant questions (in brackets) on the pre- and posttests (see Appendix S6). According to the scores from the two 2018 courses, significant learning gains occurred for learning objectives 1 (understanding experimental design and execution) and 2 (understanding the use of model systems). The gains for learning objective 3a (using equipment, specifically micropipettes) were not significant. The *P* values were calculated using a two-tailed *t* test comparing the scores for the pre- and the posttest scores ($n = 22$).

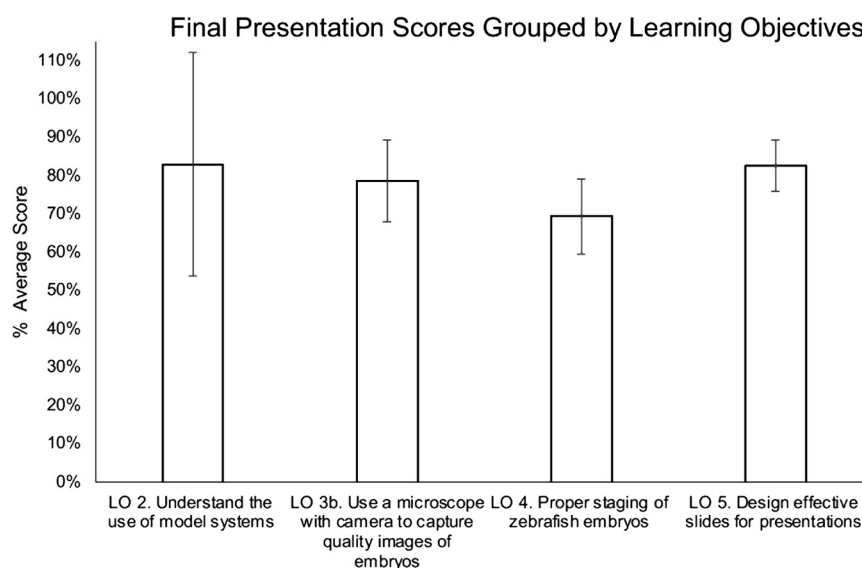


FIG 5. Final presentation evaluation for learning objectives 2, 3b, 4, and 5. The final presentations from the two courses were scored using the rubric in Appendix S5 of the supplemental material. The scores were grouped by their respective LOs: LO2, understanding model organisms; LO3b, using equipment, specifically a microscope with camera, to capture quality images of embryos; LO4, proper staging of zebrafish embryos; LO5, designing effective slides for a presentation. The average percent scores for these parameters are shown in the graph. LOs 2, 3b, 4, and 5 had average scores of 83%, 69%, 79% and 83%, respectively. The error bars are the standard deviations of the scores ($n = 22$).

Possible modifications

In the two courses on which we report, we did not achieve significant gains in LO3a, students' use of micropipettes. Thus, we developed a more structured and rigorous exercise. We made note cards indicating different volumes. To make this a bit like a game, teams of two draw cards and set micropipettes, which are scored for accuracy. After 10 cards per team, the winning team is determined. This new approach was successfully tested once and can now be field-tested rigorously during the coming year.

This inquiry-based course can be taught to students at any 2-year college and to freshman and sophomore students at 4-year colleges. Instructors can adapt this course model for students without prior experience to other areas of STEM research.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, DOCX file, 5.6 MB.

ACKNOWLEDGMENTS

We thank the students who took our courses and filled in the surveys represented here. Funding for development of these courses was provided from the NSF and by two internal grants from MSU (Recruitment and Retention

award from the Provost's Office and an Outreach and Engagement award from the President's Office).

We have no conflict of interest to declare.

REFERENCES

1. Bangera G, Brownell SE. 2014. Course-based undergraduate research experiences can make scientific research more inclusive. *CBE Life Sci Educ* 13:602–606. <https://doi.org/10.1187/cbe.14-06-0099>.
2. Healey M, Jordan F, Pell B, Short C. 2010. The research-teaching nexus: a case study of students' awareness, experiences and perceptions of research. *Innov Educ Teach Int* 47:235–246. <https://doi.org/10.1080/14703291003718968>.
3. Spronken-Smith RA, Brodeur JJ, Kajaks T, Luck M, Myatt P, Verburgh A, Walkington H, Wuetherick B, Helen Walkington, Brad Wuetherick. 2013. Completing the research cycle: a framework for promoting dissemination of undergraduate research. *Teach Learn Inquiry* 1:105–118. <https://doi.org/10.20343/teachlearninqu.1.2.105>.
4. Carlone HB, Johnson A. 2007. Understanding the science experiences of successful women of color: science identity as an analytic lens. *J Res Sci Teach* 44:1187–1218. <https://doi.org/10.1002/tea.20237>.
5. Eagan MK, Sharkness J, Hurtado S, Mosqueda CM, Chang MJ. 2011. Engaging undergraduates in science research: not just about faculty willingness. *Res High Educ* 52:151–177. <https://doi.org/10.1007/s11162-010-9189-9>.
6. Seymour E, Hunter A-B, Laursen SL, DeAntoni T. 2004. Establishing the benefits of research experiences for undergraduates in the

- sciences: first findings from a three-year study. *Sci Educ* 88:493–534. <https://doi.org/10.1002/sce.10131>.
7. Cotten SR, Wilson B. 2006. Student–faculty interactions: dynamics and determinants. *High Educ* 51:487–519. <https://doi.org/10.1007/s10734-004-1705-4>.
 8. Russell B, Weaver G. 2011. A comparative study of traditional, inquiry-based, and research-based laboratory curricula: impacts on understanding of the nature of science. *Chem Educ Res Pract* 12:57–67. <https://doi.org/10.1039/C1RP90008K>.
 9. Weaver GC, Russell CB, Wink DJ. 2008. Inquiry-based and research-based laboratory pedagogies in undergraduate science. *Nat Chem Biol* 4:577–580. <https://doi.org/10.1038/nchembio1008-577>.
 10. Auchincloss LC, Laursen SL, Branchaw JL, Eagan K, Graham M, Hanauer DI, Lawrie G, McLinn CM, Pelaez N, Rowland S, Towns M, Trautmann NM, Varma-Nelson P, Weston TJ, Dolan EL. 2014. Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci Educ* 13:29–40. <https://doi.org/10.1187/cbe.14-01-0004>.
 11. Howard DR, Miskowski JA. 2005. Using a module-based laboratory to incorporate inquiry into a large cell biology course. *Cell Biol Educ* 4:249–260. <https://doi.org/10.1187/cbe.04-09-0052>.
 12. Shaffer CD, Alvarez C, Bailey C, Barnard D, Bhalla S, Chandrasekaran C, Chandrasekaran V, Chung HM, Dorer DR, Du C, Eckdahl TT, Poet JL, Frohlich D, Goodman AL, Gosser Y, Hauser C, Hoopes LLM, Johnson D, Jones CJ, Kaehler M, Kokan N, Kopp OR, Kuleck GA, McNeil G, Moss R, Myka JL, Nagengast A, Morris R, Overvoorde PJ, Shoop E, Parrish S, Reed K, Regisford EG, Revie D, Rosenwald AG, Saville K, Schroeder S, Shaw M, Skuse G, Smith C, Smith M, Spana EP, Spratt M, Stamm J, Thompson JS, Wawersik M, Wilson BA, Youngblom J, Leung W, Buhler J, et al. 2010. The genomics education partnership: successful integration of research into laboratory classes at a diverse group of undergraduate institutions. *CBE Life Sci Educ* 9:55–69. <https://doi.org/10.1187/09-11-0087>.
 13. Rodenbusch SE, Hernandez PR, Simmons SL, Dolan EL. 2016. Early engagement in course-based research increases graduation rates and completion of science, engineering, and mathematics degrees. *CBE Life Sci Educ* 15:ar20. <https://doi.org/10.1187/cbe.16-03-0117>.
 14. Lopatto D. 2007. Undergraduate research experiences support science career decisions and active learning. *CBE Life Sci Educ* 6:297–306. <https://doi.org/10.1187/cbe.07-06-0039>.
 15. Russell SH, Hancock MP, McCullough J. 2007. The pipeline: benefits of undergraduate research experiences. *Science* 316:548–549. <https://doi.org/10.1126/science.1140384>.
 16. Fechheimer M, Webber K, Kleiber PB. 2011. How well do undergraduate research programs promote engagement and success of students? *CBE Life Sci Educ* 10:156–163. <https://doi.org/10.1187/cbe.10-10-0130>.
 17. Walton GM, Cohen GL. 2011. A brief social-belonging intervention improves academic and health outcomes of minority students. *Science* 331:1447–1451. <https://doi.org/10.1126/science.1198364>.
 18. Gregerman SR, Lerner JS, von Hippel W, Jonides J, Nagda BA. 1998. Undergraduate student–faculty research partnerships affect student retention. *Rev High Educ* 22:55–72. <https://doi.org/10.1353/rhe.1998.0016>.
 19. Dolan EL. 2016. Course-based undergraduate research experiences: current knowledge and future directions. National Science Foundation, Arlington, VA.
 20. LaBonty M, Pray N, Yelick PC. 2017. A zebrafish model of human fibrodysplasia ossificans progressiva. *Zebrafish* 14:293–304. <https://doi.org/10.1089/zeb.2016.1398>.
 21. Lohi O, Parikka M, Rämetsä M. 2013. The zebrafish as a model for paediatric diseases. *Acta Paediatr* 102:104–110. <https://doi.org/10.1111/j.1651-2227.2012.02835.x>.
 22. Zhang Z, Liu W, Qu Y, Quan X, Zeng P, He M, Zhou Y, Liu R. 2018. Transcriptomic profiles in zebrafish liver permit the discrimination of surface water with pollution gradient and different discharges. *Int J Environ Res Public Health* 15:1648. <https://doi.org/10.3390/ijerph15081648>.
 23. Frame JM, Lim S-E, North TE. 2017. Hematopoietic stem cell development: using the zebrafish to identify extrinsic and intrinsic mechanisms regulating hematopoiesis. *Methods Cell Biol* 138:165–192. <https://doi.org/10.1016/bs.mcb.2016.08.004>.
 24. Kalueff AV, Echevarria DJ, Homechaudhuri S, Stewart AM, Collier AD, Kaluyeva AA, Li S, Liu Y, Chen P, Wang J, Yang L, Mitra A, Pal S, Chaudhuri A, Roy A, Biswas M, Roy D, Podder A, Poudel MK, Katore DP, Mani RJ, Kyzar EJ, Gaikwad S, Nguyen M, Song C, International Zebrafish Neuroscience Research Consortium. 2016. Zebrafish neurobehavioral phenomics for aquatic neuropharmacology and toxicology research. *Aquat Toxicol* 170:297–309. <https://doi.org/10.1016/j.aquatox.2015.08.007>.
 25. Shuda J, Kearns-Sixsmith D. 2009. Outreach: empowering students and teachers to fish outside the box. *Zebrafish* 6:133–138. <https://doi.org/10.1089/zeb.2008.0568>.
 26. Shuda JR, Butler VG, Vary R, Farber SA. 2016. Project BioEYES: accessible student-driven science for K–12 students and teachers. *PLoS Biol* 14:e2000520. <https://doi.org/10.1371/journal.pbio.2000520>.
 27. Eason J, Williams AL, Chawla B, Apsey C, Bohnsack BL. 2017. Differences in neural crest sensitivity to ethanol account for the infrequency of anterior segment defects in the eye compared with craniofacial anomalies in a zebrafish model of fetal alcohol syndrome. *Birth Defects Res* 109:1212–1227. <https://doi.org/10.1002/bdr2.1069>.
 28. Lee LMY, Leung C-Y, Tang WWC, Choi H-L, Leung Y-C, McCaffery PJ, Wang C-C, Woolf AS, Shum ASW. 2012. A paradoxical teratogenic mechanism for retinoic acid. *Proc Natl Acad Sci U S A* 109:13668–13673. <https://doi.org/10.1073/pnas.1200872109>.
 29. Lee GS, Liao X, Shimizu H, Collins MD. 2010. Genetic and pathologic aspects of retinoic acid-induced limb malformations in the mouse. *Birth Defects Res A Clin Mol Teratol* 88:863–882. <https://doi.org/10.1002/bdra.20712>.
 30. Serrano M, Han M, Brinez P, Linask KK. 2010. Fetal alcohol syndrome: cardiac birth defects in mice and prevention with folate. *Am J Obstet Gynecol* 203:75.e7–75.e15. <https://doi.org/10.1016/j.ajog.2010.03.017>.
 31. Westerfield M. 2000. The zebrafish book: a guide for the

- laboratory use of zebrafish (*Danio Rerio*). University of Oregon Press, Eugene, OR.
32. National Research Council. 1996. Guide for the care and use of laboratory animals. National Academies Press, Washington, DC.
 33. Weston TJ, Laursen SL. 2015. The undergraduate research student self-assessment (URSSA): validation for use in program evaluation. *CBE Life Sci Educ* 14:ar33. <https://doi.org/10.1187/cbe.14-11-0206>.
 34. Ouwenel E, Schaufeli WB, Blanc PML. 2013. Believe, and you will achieve: changes over time in self-efficacy, engagement, and performance. *Appl Psychol Health Well Being* 5:225–247. <https://doi.org/10.1111/aphw.12008>.
 35. van Dinther M, Dochy F, Segers M. 2011. Factors affecting students' self-efficacy in higher education. *Educ Res Rev* 6:95–108. <https://doi.org/10.1016/j.edurev.2010.10.003>.