

Differential gene expression during *Xenopus laevis* development

Emily T. Shifley^{1*}

¹Biological Sciences, Northern Kentucky University

Abstract

In Developmental Biology classes, students are challenged with understanding how differential gene expression guides embryonic development. It can be difficult for students to realize that genes need to be turned on or off at the right time and place in order for development to proceed normally. In this lab, students working in groups perform experiments with live embryos and visualize differential gene expression allowing them to become invested in their experiment and curious about the results. This lab also addresses the benefits of *Xenopus laevis* as a model organism and allows students to observe the changes *Xenopus* embryos undergo during early embryonic stages. After the students have chosen and fixed two stages of *Xenopus* embryos, they perform an *in situ* hybridization on the embryos to visualize gene expression at two different developmental stages. They then compare their results with those from other lab groups who analyzed their embryos for different genes. The students self-reported that they better understood the concept of differential gene expression during vertebrate development and enjoyed doing this series of lab experiments working with live materials.

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Supporting Materials: S1. *Xenopus* gene expression - Fertilization instructions; S2. *Xenopus* gene expression - *In situ* hybridization instructions; S3. *Xenopus* gene expression - Lab notebook instructions; S4. *Xenopus* gene expression - Readiness Assessment Quiz 1; S5. *Xenopus* gene expression - Readiness Assessment Quiz 2; S6. *Xenopus* gene expression - Lab exam questions; S7. *Xenopus* gene expression - *In situ* hybridization probe instructions; S8. *Xenopus* gene expression - Introduction to *Xenopus* PowerPoint; and S9. *Xenopus* gene expression - Introduction to *in situ* hybridization PowerPoint.

*Correspondence to: SC Nunn Drive Highland Heights, KY 41099. Email: shifleye1@nku.edu

Learning Goal(s)

From the Developmental Biology Learning Framework

- “How do different organisms help us understand development? And what are their strengths and limitations?”
- “How does differential gene expression mediate progressive acquisition of cell fate?”

Learning Objective(s)

Students will be able to:

- Identify different stages of *Xenopus* development.
- Contrast the strengths and limitations of the *Xenopus* model organism.
- Explain the process and purpose of *in situ* hybridization.
- Compare gene expression patterns from different germ layers or organ domains.
- Compare gene expression patterns from different developmental stages.

INTRODUCTION

An important concept for undergraduate science majors learning about Developmental Biology is how differential gene expression guides embryonic development. The idea that genes are only turned on at the correct time and location in developing embryos during the differentiation of various cell types, tissues, and organs is often a difficult idea for undergraduates to grasp. A number of active learning techniques help undergraduates become motivated and successful in learning difficult concepts (1,2) and have been identified as a priority for science education (3). This series of lab exercises uses structured inquiry experiments to help students visualize differential gene expression.

Jeff Hardin has discussed how students in Developmental Biology often have trouble thinking in four-dimensions: both

the three dimensional embryo as well as developmental timing (4). In these exercises, the students observe *Xenopus* embryos as they undergo early development, which helps them understand how these embryos have biologically significant three dimensional structures that change over time. Additionally, students from different lab groups are given *in situ* hybridization probes for different genes expressed in each of the three germ layers so that while they are visualizing differential gene expression, they are also comparing how the germ layers are distinct groups of cells within the three-dimensional embryo.

Other laboratory exercises have used techniques like RT-PCR, qRT-PCR, and flow cytometry in situ hybridization to analyze gene expression differences between cancerous cell lines to help undergraduates learn through inquiry about differences in gene expression (5). Post-graduate students have also used

in situ hybridization in *Drosophila* embryos, manipulating cis-regulatory elements and transcription factors to examine the regulation of gene expression (6). Additionally, there are good examples of other labs using *Drosophila* to illustrate embryo morphology, mutant phenotypes, loss of function, gain of function, and genetic interaction studies (7). In each of these examples, students are introduced to a number of important techniques while working with different model organisms and participating in on-going research (5-7). In this series of lab exercises after the students have fertilized and observed early *Xenopus* development, they use their collected embryos to gain experience with whole mount in situ hybridization, a common and useful technique for developmental biologists. The structured inquiry nature of this lab not only allows the students to learn a new technique, it also provides an excellent visualization of differential gene expression in developing *Xenopus* embryos both spatially and temporally (Figure 1A).

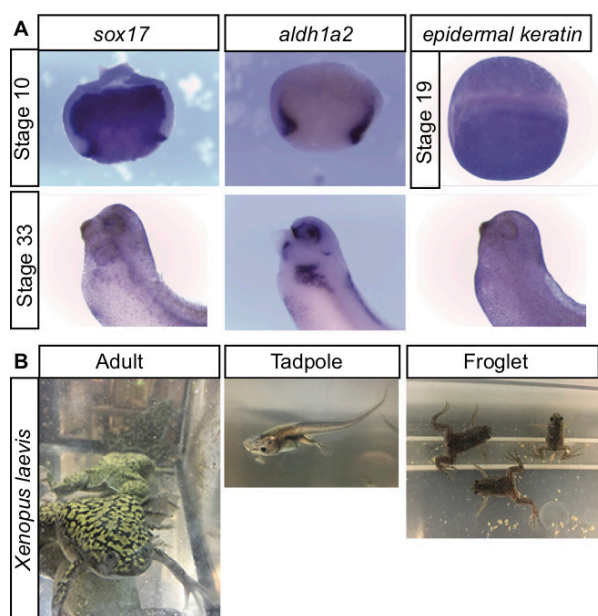


Figure 1. (A) *Xenopus laevis* differential gene expression. Students fixed embryos at early (Stage 10 or 19) and later (Stage 33) developmental time points. *In situ* hybridization shows differential gene expression in the embryos with endodermal (*sox17*), mesodermal (*aldh1a2*), and ectodermal (*epidermal keratin*) genes. The Stage 10 embryos were bisected during the *in situ* hybridization which allows students to see non-overlapping domains of endoderm and mesoderm in early gastrula embryos. Later at Stage 33, *sox17* becomes restricted to the foregut endoderm and gall bladder while *aldh1a2* is expressed in the eye, kidney, forebrain, and lateral plate mesoderm. The whole Stage 19 embryo is shown in the dorsal view so that students can notice how *epidermal keratin* is expressed throughout the ectoderm, but is excluded from the neural tube. (B) *Xenopus laevis* is an excellent model organism for studies in Developmental Biology. Students enjoyed observing not only the early embryonic stages with the *in situ* hybridization, but also the later stages as tadpoles underwent metamorphosis.

Another important concept for undergraduate science majors to explore are the benefits and limitations of various model organisms used by researchers. *Xenopus laevis* is an accessible model organism for undergraduates that provides hundreds of embryos in a single day that are large and easily manipulated (8). In the first part of this set of lab exercises, students fertilize *Xenopus* eggs, de-jelly and sort viable embryos, and observe the first cleavage. Others have successfully used undergraduates to fertilize *Xenopus* embryos

as well as perform animal cap assays and examine oocyte maturation with experiments carried out over two full days of experimentation (9). In contrast, the experiments in this lesson are spread out over three once-a-week three hour lab periods, which might be more typical of an upper level biology class at a university.

Intended Audience

This series of lab exercises was designed for a Developmental Biology class at a primarily undergraduate 4-year university. This was an upper-level elective class for Biology majors and most of the students enrolled were either Juniors or Seniors.

Required Learning Time

The first week experiment (fertilizing *Xenopus laevis* eggs) was completed in a 3 hour lab period. The students were required to occasionally check on their embryos outside of the lab period over the following two to three days and fix two different stages of embryos, which took ~15 minutes/day. The second week experiment (first day of *in situ* hybridization) was completed in a 3 hour lab period. The instructor continued the next two days of *in situ* washes outside of the lab period. The students were required to place their embryos in staining solution the day before the third lab period, which took ~30 minutes. The third week experiment (photographing and comparing their *in situ* hybridizations) was completed in ~2 hours of the lab period. The approximate times for each of the lab activities are listed in Table 1, which includes a significant time investment before the labs for the instructor to prepare all reagents and solutions for the experiments.

Prerequisite Student Knowledge

The students enrolled in this course were required to have completed Introduction to Biology courses and therefore had basic knowledge of genetics and general lab skills such as pipetting and proper use of stereomicroscopes. In lecture, we had covered chapters on identity and patterning, fertilization, differential gene expression, and cell to cell communication before performing the lab, although these exercises could also easily accompany later chapters on amphibian development.

Prerequisite Teacher Knowledge

The instructor should be familiar with *in situ* hybridization procedures and have some background knowledge in Developmental Biology. For this course we used the 11(th) Edition of Developmental Biology by Gilbert and Barresi as our textbook resource. Other great resources on the *Xenopus* model organism include Xenbase (8) <http://www.xenbase.org/entry/> and Early Development of *Xenopus laevis*: A Laboratory Manual (10).

SCIENTIFIC TEACHING THEMES

Active Learning

With structured inquiry, students are tasked with fertilizing their own plates of embryos, observing and keeping their embryos alive over several days, and then performing an *in situ* hybridization on these same embryos (Supporting File S1: *Xenopus* gene expression - Fertilization instructions and Supporting File S2: *Xenopus* gene expression - *In situ* hybridization instructions). This creates a sense of ownership and investment for each lab group in the experiment. The students work in groups of 3 or 4 performing all aspects

of the exercises gaining hands-on experience caring for, manipulating, washing, and photographing developing embryos. The groups are assessed together with a Readiness Assessment Quiz (RAQ) every week on the previous week's lab.

Assessment

The students are required to document their work in an individual lab notebook where they record their procedures, results such as sketches or photographs of the developing embryos, and answers to thought questions at the end of each lab (Supporting File S3: *Xenopus* gene expression - Lab notebook instructions). The lab notebooks are graded twice during the semester for completion. Additionally, each week the lab groups are given a Readiness Assessment Quiz (RAQ) which tests their basic understanding of the previous week's lab (Supporting File S4: *Xenopus* gene expression - Readiness Assessment Quiz 1 and Supporting File S5: *Xenopus* gene expression - Readiness Assessment Quiz 2). Lastly, the students take individual lab exams at three points during the semester testing their understanding of the learning objectives of each of the lab exercises performed (Supporting File S6: *Xenopus* gene expression - Lab exam questions). At the end of the semester, students responded to a survey questioning their impressions of the lab (approved by the Institutional Review Board of Northern Kentucky University).

Inclusive Teaching

The syllabus for the course includes a diversity statement as well as a statement listing the instructor's pronouns and welcoming students to share their own pronouns with the instructor. The syllabus also has a detailed outline of all of the assignments and due dates for the semester to provide a highly structured course including many low stakes assessments throughout the semester, which helps increase student success and decrease the achievement gap (11-13). Students are first introduced to the topic of differential gene expression in an active learning lecture which includes the use of clicker questions and think-pair-share activities, both of which engage a diverse population of students (14,15). During the lab period, the material is accessible in several different formats to facilitate different learning styles including a textbook, a short power-point lecture with diagrams, and videos of early amphibian development. All students are given the opportunity to perform Developmental Biology lab techniques in this series of exercises. They are in permanent lab groups of 3-4 people to foster collaboration and every student is encouraged to take turns at the microscope and with the embryos. Others have found that using guided inquiry work with living organisms to introduce laboratory skills improved their underserved STEM student's attitudes and success (16). The groups were self-selected on the first day of lab, but could certainly be assigned to encourage a mixture of students.

LESSON PLAN

Xenopus laevis and Other Supplies

If your institution has a colony of *Xenopus laevis* frogs available, you would need approximately 1 male and 4 female frogs for each lab section. Using four female frogs means that even if some of the females do not lay viable eggs, you will have plenty as long as one of the other females lays viable eggs.

If your institution does not have *Xenopus*, you can purchase breeding pairs of frogs from Carolina Biological, Ward's Scientific, Nasco, or *Xenopus* Express for approximately \$60. However, the husbandry and upkeep for the adult frogs may not be worth the time and expense if they are only going to be used for this series of lab exercises once a year. Another convenient option is to purchase fertilized embryos, which are available from Nasco, *Xenopus* Express, and Carolina Biological for approximately \$40. Although the students would not be able to observe the cortical rotation or early cleavage, they would still be able to practice staging the embryos, to observe the embryos develop and change three-dimensionally over time, and to fix them for *in situ* hybridization. This would also not require the submission of an IACUC protocol if you are not using adult *Xenopus* or keeping older tadpoles.

There are a several solutions required for the fertilization and *in situ* hybridization lab exercises. These will need to be prepared over the course of several days before the lab, and are listed at the end of the student lab instructions (Supporting File S1: *Xenopus* gene expression - Fertilization instructions and Supporting File S2: *Xenopus* gene expression - *In situ* hybridization instructions).

Additionally, before running this lab, the instructor will need to acquire *in situ* hybridization probes. The *Xenopus* research community is incredibly generous in sharing the plasmids for *in situ* probes if you contact them. The ones used in this lab (like *sox17*, *aldh1a2*, and *epidermal keratin*) are available by contacting the author (shifleye1@nku.edu). There are also many other excellent options of probes that give interesting expression patterns, especially if your students want to look at a specific region or organ domain of the developing embryo. For instance, some additional good options of genes with distinct expression patterns include mesodermal markers *nkx2.5*, *foxf1*, or *tbxt* and ectodermal markers *sox9*, *foxi1e*, or *snail2* and endodermal markers *hhex*, *endodermin*, or *mixer*. If you search on <http://www.xenbase.org/entry/> for any gene of interest, it will provide a page with lots of information on your gene and under the expression tab, you can view *in situ* hybridization images that have been generated from different labs and publications including the corresponding author (8). After acquiring the plasmids with your genes of interest, you will need to digest them to use as a template to make the *in situ* probes using the instructions included in Supporting File 7 (Supporting File S7: *Xenopus* gene expression -*In situ* hybridization probe instructions).

Xenopus laevis Fertilization and Early Development - Lab Week 1

The afternoon before the lab period, the instructor primes the female frogs by injecting near their nuptial pads 1ml of Human Chorionic Gonadotropin (HCG) to stimulate the frog to begin laying eggs. Females will start to lay eggs ~9 hours after injection if they are kept at RT or ~14 hours after injection if they are kept at 15°C, and will continue to lay eggs for ~8 hours (10). The morning of the lab period, the female frogs are placed in a high salt solution to keep them happy and healthy (10).

At the beginning of lab, the instructor gives a short ~10 minute power-point presentation introducing the benefits of *Xenopus laevis* as a model organism as well as the three-

dimensional structure of the early cleavage and gastrula stage embryos using images and time-lapse videos (Supporting File S8: *Xenopus* gene expression -Introduction to *Xenopus* PowerPoint). The instructor also demonstrates how to remove the testes from an anesthetized male frog. The groups of students then each collect a petri dish full of embryos from the high salt tanks holding the egg-laying female frogs and fertilize them with a piece of the testes following the lab procedure (Supporting File S1: *Xenopus* gene expression - Fertilization instructions). Once the embryos have undergone cortical rotation, the students de-jelly the embryos and examine them under a dissecting microscope to begin to sort viable from inviable embryos. Students are instructed to remove white, marbled, or unfertilized eggs from their plates. It can be challenging initially for students to find the dead or dying embryos with their transfer pipet while looking through the dissecting microscope, but encourage them to take turns within their group. While the students are waiting for their remaining viable embryos to develop, they can sketch and label prepared sections of the various stages of frog embryos (available from Carolina Biological or Anatomy Warehouse).

Towards the end of the lab period (~90 minutes after fertilization at room temperature), the embryos should undergo their first cleavage and the students can again sort their plates, removing embryos with irregular cleavage planes. Students then place their plates of embryos at 16-18°C at the end of the lab period for overnight incubation. Each group is tasked with sketching or photographing their developing embryos, clearing their plates of dead embryos, and replacing the culture medium for the next 2-3 days. They can move their plates of embryos out to room temperature the next day or keep them at cooler temperatures to slow their development (a chart outlining the speed of development at different temperatures is included in their instructions). Each group is instructed to fix a set of embryos at two different developmental stages (ideally at least 6 embryos per stage). They pipet the embryos into a collection jar or vial, remove most of the culture media, and then cover the embryos with MEMFA fixative and place at 4°C.

Working with live organisms always carries a risk that things may not go as planned. By having 4 females lay eggs, there are likely to be hundreds of viable eggs, but it is possible that none of the females lay good eggs on the day of the lab. If they do not, students can at least sketch the prepared slides of *Xenopus* embryos and watch the videos showing the early development. If possible, it would be worthwhile for the instructor to have some reserve embryos (~50 young stages, ~50 tailbud stages) fixed and stored in ethanol at -20°C so that if the frogs do not lay eggs, the groups can still use these extra embryos for the *in situ* hybridization part of the lab.

In situ Hybridization - Lab Week 2

The fixed embryos are dehydrated with ethanol and can be stored at -20°C for any amount of time until the instructor is ready to start the *in situ* hybridization lab. For this week of lab, the instructor again gives a short ~10 minute presentation explaining how *in situ* hybridization works and why the results of *in situ* hybridization are useful (Supporting File S9: *Xenopus* gene expression -Introduction to *in situ* hybridization PowerPoint). The students follow the procedure for Day 1 of *in situ* hybridization on their *Xenopus* embryos (Supporting File S2: *Xenopus* gene expression - *In situ* hybridization instructions). As part of day 1, the instructor demonstrates how

early stage *Xenopus* embryos can be bisected for better staining during the *in situ* hybridization. This is the most technically challenging part of the lab because the student must position a round embryo on an agarose coated plate and cut it in half with a razor blade. The instructor can rotate through to help each group. Letting each student try to bisect an embryo is a good experience. Usually there are so many embryos it's not a problem if most of them get crushed rather than cut. Each group really only needs one or two successfully bisected embryos to get a good picture. I would also suggest each group leaves several young embryos whole in case none of the bisected embryos make it through the *in situ*. The whole embryos will still stain with the protocol (for instance *epidermal keratin* in Figure 1A), but if the students are successful in bisecting an embryo, they are neat to photograph because cavities like the archenteron are apparent and it is easier to compare mesodermal and endodermal genes (for instance *sox17* and *aldh1a2* in Figure 1A).

Each group is assigned a different gene and given a prepared Digoxigenin (Dig) labeled probe. During some of the long washing steps of this lab, students can also work through an additional excellent exercise by Dr. Cebra-Thomas making Play-doh models of the different stages of gastrulation (17) https://www.swarthmore.edu/NatSci/sgilber1/DB_lab/Frog/frog_gast_model.html. The students should at least get their embryos into the pre-hybridization wash before the end of the lab period depending on how long their bisection takes. After this lab period, the instructor or a teaching assistant can take over the *in situ* washes, performing Day 2 and Day 3 washes on subsequent days. If you have labs that meet more than once a week, your students could be involved with some of these washes as well. The embryos are left in the last 1X MAB wash at 4°C over the weekend, and each group is tasked with completing the Alkaline Phosphatase washes on the day before their next lab period. This takes ~30 minutes for a student to complete. The embryos remain in the staining solution overnight at room temperature.

In situ Hybridization - Lab Week 3

After a brief review of the steps of *in situ* hybridization that have been completed, students view their embryos under a microscope to determine if a specific staining pattern has appeared. The probes provided to each group typically give strong staining. They complete several washes to stop the staining and then each group takes turns photographing their embryos with a microscope imaging system. We used a Leica dissecting scope with a camera. If this is not available the students could hold their phone cameras carefully above a microscope eyepiece to take a picture of their embryos. All the photographs from each of the lab groups are shared online so that the students can compare their gene's expression pattern with others and students also post their photographic results in their individual lab notebooks. This last part of the lab does not take the entire 3 hour period and could accompany another lab exercise or activity within the same lab period. The students can also use the extra time to research their gene expression pattern, make comparisons between groups, and write conclusions in their lab notebooks.

Assessment

Lab groups are assessed with a Readiness Assessment Quiz (RAQ) each week in lab on the previous week's material (Supporting File S4: *Xenopus* gene expression -Readiness

Assessment Quiz 1 and Supporting File S5: *Xenopus* gene expression - Readiness Assessment Quiz 2). Every student is responsible for keeping an individual laboratory notebook documenting the procedures, results, and answers to discussion questions for the lab (Supporting File S3: *Xenopus* gene expression - Lab notebook instructions). Additionally, students have individual lab exams assessing material from the labs (Supporting File S6: *Xenopus* gene expression - Lab exam questions). In the final grade calculations, the group RAQs were worth 8%, the individual lab notebook was worth 5%, and the lab exams were worth 15%. For the RAQ and lab exams, the questions are mostly at remember or understand levels of Bloom's taxonomy, although some questions ask them to apply what they had learned to a new situation. The questions at the end of each lab exercise that the students answer in their lab notebooks also require analyzing.

TEACHING DISCUSSION

This series of lab exercises worked well in giving the students experiences with living organisms, specifically *Xenopus laevis*. The ownership and responsibility the students felt towards their embryos during the lab exercises was encouraging to see. The students were also able to learn about and participate in a common experiment used by Developmental Biologists, *in situ* hybridization, to examine differential gene expression. Visualizing the different layers or groups of cells staining dark purple with endodermal, mesodermal, or ectodermal genes was a great way to help students see that as different germ layers form and cells differentiate in the embryo, they express different genes (Figure 1A). Additionally, depending on the gene assigned and the stages they fixed, students were able to see temporal changes in gene expression over developmental time as well (Figure 1A). For instance, SRY-box 17 alpha (Sox17a) is expressed throughout the endoderm in early stage embryos where it helps specify the endoderm, but at later tailbud stages its expression becomes restricted to the foregut endoderm and gall bladder (18-20). Aldehyde dehydrogenase 1 family member A2 (alhd1a2) is a retinoic acid generating enzyme that is expressed in the gastrulating *Xenopus* mesoderm (21) and plays diverse roles during vertebrate development (for instance (22)) while epidermal keratin is expressed in the non-neural ectoderm (23-25). When the students compared their gene expression pattern with other groups, they were able to contrast these different gene expression patterns and see how they changed over developmental time.

Student Impressions

The students who were enrolled in this class came in with a mixture of experience or no experience in undergraduate research as well as a mixture in their level of interest in Developmental Biology (Figure 2A). Nearly all students reported being more interested and more informed about the types of experiments Developmental Biologists perform at the end of the semester (Figure 2A). The students clearly enjoyed the *Xenopus* series of lab exercises. Many of them wrote that their favorite part was "working with live organisms" and "getting to see all of the stages of development starting from fertilization." They also reported learning more from this lab than a typical lab and agreed or strongly agreed that the lab helped them visualize embryonic development and differential gene expression (Figure 2B). One aspect of the lab that was a concern was the requirement that the students come to the lab

outside of the regularly scheduled lab period to document, fix, or wash embryos. The student responses show that this was a problem for 20% of the class (Figure 2B). Indeed, at our institution ~40% of the undergraduates are first-generation college students and many are working outside jobs at the same time as attending school or commuting. We talked about trying to coordinate within their group of 3 or 4 students to share this responsibility and the instructor also offered to cover a certain day if they needed help. However, the goal was still to encourage them to observe the developing embryos themselves, if possible, on the 2-3 weekdays following the fertilization. This aspect could also be addressed at the beginning of the semester when the groups are first formed to make sure each group has someone who can come into lab outside of class time. I also included in the IACUC protocol for this lab exercise to house extra tadpoles from our fertilization over the course of the semester and the students loved seeing the tadpoles start metamorphosis by the end of the semester, which was something they could observe from week to week in lab and did not require outside class time.

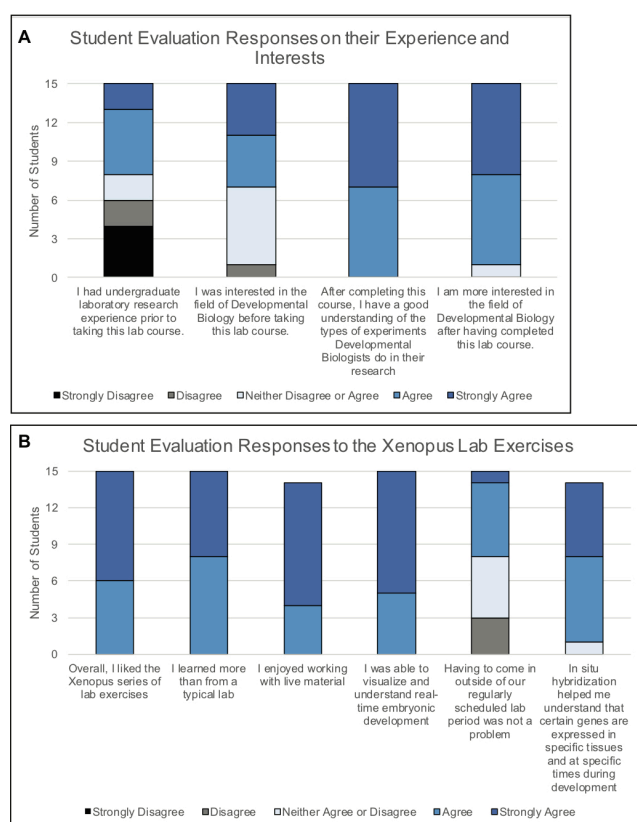


Figure 2. (A) Student evaluation responses on their experience and interests. The students reported an increased interest and understanding of Developmental Biology research after having completed the course. (B) Student evaluation responses to the *Xenopus* series of lab exercise. Overall, the students enjoyed the lab, especially working with live materials. The students felt that the learning objectives were achieved. Some students had a problem coming into lab outside of the regularly scheduled period.

Extensions

In this lab, the students only observed the normal development of *Xenopus laevis* and normal gene expression patterns. One great way to expand on this lab would be to

allow groups of students to perform different manipulations on their developing embryos. Others have outlined inquiry-based labs where students were tasked with researching the impacts of environmental toxins on development, writing and screening their research proposals, and then carrying out their manipulations on sea urchins or chick embryos and finally writing a report on their results (26). This series of labs was found to increase the students' motivation and performance (26). Students have also done genetic and environmental manipulations with developing zebrafish and *Caenorhabditis elegans* models (27). For *Xenopus*, if the female frogs are laying a good number of eggs, each group of students could perform any number of environmental manipulations on half of their embryos, keeping the other half as controls. One example is to treat some of the embryos with Lithium Chloride as outlined by Justin Spenillo (28) https://www.swarthmore.edu/NatSci/sgilber1/DB_lab/Frog/JA_Spenillo/justin2.html). There are also a large number of small molecule inhibitors that *Xenopus* embryos can be cultured in if there is a specific genetic signaling pathway the students would like to manipulate in their embryos (29) http://www.xenbase.org/common/jsp/showWiki.jsp?Small_Molecules_for_Xenopus_Research. Having students commit time before performing these manipulations to do background research into primary literature would be important and would give the instructor time to order any reagents necessary. Gehring and Eastman describe how they scaffolded information literacy assignments in a Developmental Biology class, which helped their students identify and apply valid sources to their own inquiry-based labs (30). Another great extension of this lab would be to spend more time instructing the students on the imaging of the *Xenopus* embryos. Watson and Lom describe a series of labs where their students created image portfolios and posters, rather than written lab reports, which helped their students hone their scientific communication skills throughout the semester (31).

Conclusion

Overall, this series of lab exercises is an excellent way to get undergraduate students working with live organisms and observing early stages of development. Additionally, using the *in situ* hybridization protocol, students can visualize and document how differential gene expression occurs during different stages of vertebrate development.

SUPPORTING MATERIALS

- S1: *Xenopus* gene expression - Fertilization instructions
- S2: *Xenopus* gene expression - *In situ* hybridization instructions
- S3: *Xenopus* gene expression - Lab notebook instructions
- S4: *Xenopus* gene expression - Readiness Assessment Quiz 1
- S5: *Xenopus* gene expression - Readiness Assessment Quiz 2
- S6: *Xenopus* gene expression - Lab exam questions
- S7: *Xenopus* gene expression - *In situ* hybridization probe instructions
- S8: *Xenopus* gene expression - Introduction to *Xenopus* PowerPoint
- S9: *Xenopus* gene expression - Introduction to *in situ* hybridization PowerPoint

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Table 1. *Xenopus* Gene Expression - Teaching Timeline

Activity	Description	Estimated Time
Preparation for Lab		
Acquiring <i>in situ</i> probe templates	Contact corresponding author / <i>Xenopus</i> labs for <i>in situ</i> probe template plasmids.	Several weeks beforehand
Prepare <i>in situ</i> hybridization probes	Transform, mini prep, and digest template plasmid to use to transcribe labeled <i>in situ</i> probe.	Several days
Prepare reagents and supplies	Prepare all solutions and buffers listed on the lab instructions.	Several days
Induce female frogs to lay eggs	Prime female frogs with 1ml of HCG the afternoon the day before lab period and then place in High Salt solution the morning of the lab period.	~30 minutes
Fertilizing <i>Xenopus</i> Embryos: Week 1 Lab		
Mini-lecture on <i>Xenopus laevis</i>	Short lecture with images of early frog embryos and time-lapse videos.	~10 minutes
Preparation of male testes	Instructor demonstrates dissection of testes.	~10 minutes
Collection and fertilization of <i>Xenopus</i> eggs	Groups gather dishes of eggs from female frogs and fertilize with testes.	~30 minutes
Examination of embryos	While waiting for cortical rotation and cleavage, students sketch sections of early <i>Xenopus</i> embryos.	~30 minutes
Sorting of embryos	Embryos are de-jellied and sorted for viable embryos.	~30 minutes
Observation of cleavage	2-cell embryos (~1.5 hours after fertilization at room temperature) are examined and sorted again for viable embryos.	~30 minutes
After Lab Tasks		
Documentation and care for developing embryos	Groups observe developing embryos and keep dishes clean of debris.	~15 minutes/day for 2-3 days
Fixation of embryos	Groups fix embryos at an early and later stage of development.	~15 minutes
<i>In situ</i> Hybridization: Week 2 Lab		
Mini-lecture on <i>in situ</i> hybridization	Short lecture explaining the uses and steps of <i>in situ</i> hybridization.	~10 minutes
<i>In situ</i> hybridization washes	Students complete Day 1 washes, including a bisection of early stage embryos after re-hydration.	~3 hours
Group RAQ quiz	Lab groups complete an RAQ on the previous week's lab during a long <i>in situ</i> wash.	10 minutes
After Lab Tasks		
<i>In situ</i> hybridization washes	Instructor finishes Day 2 and Day 3 washes.	Several days following the lab (2 days of ~6 hours of total washing)
<i>In situ</i> hybridization washes	Students complete Day 4 washes to put embryos into staining solution the day before their lab period.	~30 minutes
<i>In situ</i> Hybridization: Week 3 Lab		
Mini-lecture on <i>in situ</i> hybridization	Short lecture reviewing the uses and steps of <i>in situ</i> hybridization.	~10 minutes
Completion of <i>in situ</i> hybridization	Students complete final Day 5 washes.	45 minutes
Group RAQ quiz	Lab groups complete an RAQ on the previous week's lab during a long <i>in situ</i> wash.	10 minutes
Analyzing results	Groups take turns photographing embryos; students answer discussion questions in their lab notebooks.	~1 hour