Using Zebrafish To Bring Hands-On Laboratory Experiences To Urban Classrooms

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Abstract

Zebrafish are widely used as a model organism for research. Zebrafish embryos are also a useful resource for teaching students about vertebrate development. Here we describe a collaboration between two high school teachers and two university professors that used zebrafish to bring hands-on laboratory experiences to inner-city students, with the aim of increasing tangibility, and improving student understanding and retention, of several fundamental scientific concepts, such as the scientific method, cell division, mitosis and Mendelian genetics. We describe and provide supporting material for each of the four lab modules that we developed. We also discuss the obstacles that we encountered and include suggestions of ways to overcome these. This collaboration provides an example of how high school teachers with very little zebrafish experience can gain the knowledge and confidence to develop and implement modules such as these in a relatively short period of time. Due to the wide availability of zebrafish resources, these labs should provide a useful resource for other teachers who are interested in integrating more hands-on, inquiry-based investigations using live animals into their classes. We also hope to encourage other zebrafish researchers to collaborate with local teachers in similar projects.

Introduction

Students in urban settings often struggle with comprehension and retention of abstract ideas [1]. In addition, the limited resources available in some inner-city schools can result in a disadvantaged learning environment. Zebrafish are increasingly being used as a model organism for many different areas of biological research including genetics, developmental biology, neuroscience, toxicology and disease modeling (e.g. [2-15]). They are also a powerful teaching tool, as has been demonstrated by many other studies (e.g. [16-24]). Enabling students to utilize tangible materials, such as live zebrafish embryos, in hands-on investigations can help students to better understand otherwise seemingly abstract concepts and to ultimately forge more personal connections to the material that they are studying [25, 26]. Therefore, we decided to develop hands-on lab modules using zebrafish, that we could implement in a Title 1[27] inner-city high school. Our hope was that these experiences would increase tangibility, and hence improve student understanding and retention, of several fundamental scientific concepts, such as the scientific method, cell division, mitosis and Mendelian genetics. We also hoped that these modules would encourage student interest in Biology and inspire more students to consider further scientific studies and/or careers.

Zebrafish are valuable model organisms for biological and biomedical research due to their ease of care, widespread availability, readily accessible embryos, significant degree of genetic and developmental similarity to humans and ability to generate insights into human disease e.g. [2-15]. Most zebrafish genes are highly conserved with mammalian genes, and these genes have similar functions in zebrafish and humans. Zebrafish are easier to maintain than rodents and it is easier to obtain a steady supply of embryos from them. Individual fish can be bred every 7-10 days and they produce large numbers of progeny. For example, one female zebrafish will often release a few hundred eggs (oocytes) in a single breeding compared to the 5-10 offspring produced by a female mouse. The transparency of zebrafish embryos, combined with the fact that they develop outside the mother, allows researchers to easily investigate cell and developmental biology questions using intact embryos. In addition, because zebrafish live in water, it is much easier to expose zebrafish embryos to chemical treatments and water-soluble toxins than it would be to expose mammals. Finally, the fast development of zebrafish means that it is possible to observe behavioral and developmental phenotypes in this species as early as the first day of development [28, 29].

Many of these advantages also make zebrafish a powerful teaching animal, especially for studying vertebrate development. It is less effort for a zebrafish laboratory to generate sufficient embryos for a

teaching experiment than it would be for a mammalian lab. The ethical considerations are also fewer, given the external development of zebrafish. Zebrafish eggs can be obtained without harming any adult animals and the embryos are easily euthanized before they reach an age where they might be capable of experiencing distress [30]. It is relatively easy to obtain mutant strains that can be bred to illustrate concepts of Mendelian Genetics. It is also much easier for schools and other groups to maintain their own zebrafish colony for embryo production than it would be to locally obtain embryos from other vertebrates (e.g. see [21, 22, 24]). In addition, zebrafish embryos are easy animals for students to work with, as they are easy to handle, develop quickly, and are transparent and easy to observe with simple microscopes available in most schools. The fact that students of all ages can look down a microscope and easily see zebrafish embryos move as well as observe their hearts beating and blood cells flowing around their bodies also makes these embryos a powerful tool for capturing student attention and inspiring both a sense of wonder and interest in science [23, 31].

Two of the most successful examples to date of using zebrafish in K-12 education are BioEYES and InSciEd Out [19, 20, 32]. However, both of these projects are large-scale efforts, involving either a whole school or school district, that require considerable financial resources. In this paper, we describe a much smaller project, funded by a single National Science Foundation (NSF) Research Experience for Teachers (RET) supplement to a single investigator. This project required a relatively small investment of time (4 weeks part-time during the summer and a few meetings during the academic year) and funds ($2,000 in supplies and $6,336 in hourly pay for the teachers). This is a small enough sum of money that it could potentially be raised from donations or other sources.

This project was a collaboration between two university professors at Syracuse University and two high school teachers at Nottingham High School. Nottingham High School is a Title 1[27] public high school in the Syracuse City School District (SCSD) and it has been identified by the New York State Education Department as “persistently low achieving”. In 2016, Nottingham High School’s graduation rate was only 66%, and in previous years the graduation rate was even lower. Like all of the other SCSD high schools, most students at Nottingham High School are traditionally-underserved low-income and minority students. Approximately 74% of Nottingham High School students are considered low-income, approximately 57% are Black or African American, 12% are Asian or Pacific Islander, 8% are Hispanic and 2% are multiracial. Approximately 18% have been diagnosed with a disability and 17% have limited English proficiency (2015-2016 data) [33].

In New York State, high school students take New York State Regents Exams, administered by the Board of Regents, in order to obtain a NYS Regents Diploma. In recent years less than 60% of Nottingham High School students have graduated with a Regents Diploma[34]. To graduate with a Regents diploma, students need to pass at least one science Regents examination [35]. Many students take the Biology (called "Living Environment") exam. The success rate of Nottingham High School students in this exam is even lower than the success rate for achieving a Regents Diploma. For example, in 2015, only 43% of Nottingham High School students passed this exam, in comparison to the New York state average of 77% [36]. This suggests that failing the Living Environment exam may be one of the factors that prevents many students from graduating with a Regents Diploma. These statistics show there is a clear need to develop new methods and resources to help these students understand and retain key biological concepts. Given that, as discussed earlier, hands-on experiences can help to improve student understanding and retention [25, 26], we decided to develop lab modules using zebrafish that address key concepts in the Living Environment curriculum that many Nottingham High School students find hard to grasp. We also hoped that these modules would encourage student interest in Biology and inspire more students, and in particular low-income and under-represented minority students, to consider further scientific studies and/or careers.

The two high school teachers involved in this project (R.W. and N.A.) spent four weeks half-time in Dr. Lewis's zebrafish lab at Syracuse University as part of a Research Experiences for Teachers (RET) supplement to an NSF grant awarded to Dr Lewis. During this time, they learnt the research techniques and zebrafish husbandry skills required to implement and maintain a self-sustainable zebrafish colony and perform classroom experiments with zebrafish adults and embryos. The aim was to develop hands-on lab experiences that could be used in their classrooms, and more generally in other high schools. In collaboration, we developed lab activities appropriate for use in a "Living Environment" or Biology class with urban high school students, some with English as a second language (ESL) and/or special education needs. R.W. and N.A. then piloted these modules for three years with approximately 250 students possessing a wide range of abilities. Following the initial RET experience, the four educators met for 1-2 hours, twice a year to discuss progress and any issues that had arisen and plan further implementation steps. Each year the modules were modified to improve their usability and the quality of the student experiences. In the third year, we also piloted a student survey to assess the degree to which students' understanding of concepts included in the modules and attitudes to science and science careers had or had not changed.

In this paper, we describe the RET experience that the two high school teachers participated in and the four guided inquiry lab activities that we developed. These represent the most successful versions implemented with R.W. and N.A.'s science classes. Our aims in doing this are two-fold. First, we hope to inspire other zebrafish researchers to work with local teachers to develop their own hands-on lab activities that meet local needs, by demonstrating what can be achieved with a relatively small investment of time and money and by describing the process that we undertook. Second, we hope that the modules presented here will be useful for other teachers, either as presented, or in an adapted form, whether or not they have access to a supporting zebrafish research laboratory.

The modules that we developed begin with an introduction to model organisms in general and advance more specifically to zebrafish. We expose students to zebrafish characteristics and behaviors prior to using the fish to study more specific biological concepts. Hands-on activities include observing cell division and development in embryos, inducing morphological defects through administration of varying ethanol treatments, and breeding zebrafish with different genotypes to discover patterns of heredity. These modules provide an inquiry-based discovery of key biological concepts, accessible to a student body with a wide range of abilities. They can also be easily expanded to provide further in-depth examination for more advanced classes or for higher-level differentiation within a heterogeneous mixture of student ability within the same class, and we provide some suggestions for how this could be achieved.

Results

RET Experience

We found that a relatively short period of time (four weeks 50% time in the summer) was sufficient for two high school teachers (R.W. and N.A.) to gain sufficient knowledge of, and confidence with, zebrafish to be able to develop teaching modules that would work in their classrooms and deliver them. The two teachers were paid for this time and for additional follow-up meetings from an NSF RET supplement. The total direct costs of this supplement were $8,336, which included funds to pay the teachers at the established school district hourly rate and $2,000 for supplies to set up the zebrafish colony at Nottingham High School and equip the teachers with several years worth of consumables. Two Biology teachers were chosen with the help of the school principal at Nottingham High School, which is the closest high school to Syracuse University. As discussed in the introduction, most students at this school are low-income students and/or minority students that are under-represented in science fields and professions. We chose to recruit two teachers from the same school so that they could support each other and work together, both during the summer RET experience, and during the school year when they implemented the teaching modules.

During the four part-time weeks in the summer, the teachers were trained by K.L. and S.E. to sex zebrafish, identify different pigment phenotypes in adult zebrafish, set up breeding crosses, collect and raise embryos, identify viable embryos, recognize and remove unfertilized eggs and identify different developmental ages (called “stages”) of embryos. They also learnt how to perform toxicology experiments where embryos are exposed to specific chemicals and practiced identifying abnormal morphological phenotypes of embryos, due either to chemical exposure or genetic mutation. To help them learn how to recognize different morphological phenotypes, the teachers examined embryos mutant for a number of different developmental genes that were available in the Lewis Lab. The teachers also practiced making several different solutions, tried a variety of different microscopes that were available locally and spent time brainstorming with K.L. and S.E. about possible experiments that could be conducted in their classrooms. These experiences helped the teachers to build their expertise and confidence in working with zebrafish. R.W. and N.A. also attended weekly Lewis lab meetings where different members of the lab discussed their current research, a departmental undergraduate research poster session and talked individually to members of the Lewis Lab about their research. The teachers found these experiences very valuable for gaining more in-depth understanding of the “bigger ideas” and

aims of the research conducted by the Lewis Lab, the different areas of expertise in the lab and for the opportunity to experience research first-hand.

Both R.W. and N.A. completed an anonymous (in as much as is possible with just two participants) pre-survey administered before the RET summer experience and post-survey after the end of the summer experience. The surveys and the results obtained are provided in Appendix 1. These results suggest that this summer RET experience increased both of the teachers' understanding of genetics, vertebrate development and lab research. The teachers also reported a significant increase in their confidence in several key skills for conducting research with zebrafish after the RET experience. These included: confidence in sexing and setting up breeding crosses, which on a scale of 1-10, increased from an average of 3 (pre-survey) to 9 (post-survey); identifying phenotypes in zebrafish adults (increased from an average of 3 to 8.5); determining the age (stage) of zebrafish embryos (increased from an average of 1.5 to 8) and identifying phenotypes in zebrafish embryos (increased from an average of 1.5 to 9). Their answers to the question "I would be confident conducting a zebrafish lab with my students" increased from an average of 1 to an average of 8.5. Similarly, their answers to questions about their confidence in specific general lab skills, such as pipetting and making solutions, increased considerably. While the conclusions that we can draw from a survey of just two individuals are obviously limited, these results, combined with the successful implementation of the teaching modules in subsequent years, suggest that this relatively short summer experience was successful in its aims.

Teaching Modules

Here we provide a brief description of each of the modules that we developed. These modules can be used as a complete set, or individually. All of these labs can be easily modified to fit the level and needs of the teacher and the students. Some of the lab exercises may also be adapted to other related science courses, such as Environmental Science or college level Advanced Placement Biology courses, or as enrichment opportunities for faster paced students. More detailed teacher notes, learning objectives, lesson plans and protocols for each lab module are provided in Appendix 2. More detailed information on setting up a zebrafish colony, zebrafish husbandry and generation of lab materials is provided in the Materials and Methods.

S.E. and K.L. were available for advice during the initial stages of implementing these teaching modules, but R.W. and N.A. now operate them independently. While the in-house zebrafish colony means that

Nottingham High School is now mostly self-sustaining in its delivery of these modules, the Lewis lab still provides occasional support in the form of reagents (embryo medium stock solution, anesthetizing agent Tricaine), specialized fish (like homozygous *albino* fish) and back-up embryos.

We piloted these labs in Nottingham High School, which is a Title 1 urban school [27], with the hope of making several key abstract concepts more attainable to a student body that is academically considerably behind their suburban peers. These included concepts such as the scientific method, experimental design, hypothesis formation, cell division, mitosis and Mendelian genetics (see Table 1 for more information). We also hoped to increase student interest in Biology and science, through providing more hands-on activities and opportunities for students to perform authentic experiments. Once the in-house zebrafish colony was established at Nottingham High School, we kept tanks containing adult zebrafish in the classroom that is usually used to teach Living Environment. This meant that students were exposed to the zebrafish on a regular basis before they were introduced to any of these modules. What we had not anticipated was that this instilled an interest in the fish and sense of ownership in the students, and as the year progressed, many of them began inquiring about the purpose of the zebrafish. We were able to use this opportunity to mention some of the questions that the students would ultimately explore, so that they could begin hypothesizing prior to the actual lab activities. This increased the level of anticipation for working with the zebrafish, and when the labs were conducted, students were more engaged and motivated to actively participate. As discussed below in more detail, our anecdotal and qualitative experiences suggest that students who participated in these labs developed a more thorough and robust understanding of key concepts than was our experience with students in previous years that did not have this opportunity.

Lab Module One: Introduction to Zebrafish

This lab module introduces students to *Danio rerio* (zebrafish), and explains that it is a model organism commonly used as a tool to increase our understanding of human physiology and disease. For more information about the curriculum areas and concepts that the lab addresses please see Table 1. Because of time-tabling constraints, we usually delivered the lab part of this module during one 48-minute school period, although this tended to be a bit rushed. A full hour would be preferable (Table 1). We also often used a pre-lab guided discovery and exploration of information on the web (webquest) on model organisms in the class before the lab. Webquests can provide an interactive and effective way to introduce students to particular topics as students are often more engaged if they are finding things out for themselves, rather than being taught directly by the teacher. Webquests can be used as small group

activities, fostering cooperative learning and collaborative activities. As mentioned above, for this module, we had students complete a webquest in class. However, webquests can also be completed individually as home-work by students who miss a specific class (see discussion of absenteeism later in this paper).

For this lab module, we developed a relatively simple webquest on model organisms that could be completed in one school period (see Table 1). We asked students to consult an NIH website that discusses model organisms [37] and answer various questions (see model organism webquest in Appendix 2, which we adapted from the information available on this NIH site). This activity was designed to help students understand the concept of a "model organism" and introduce them to the idea that different animals are used as model organisms and that they each have their own distinct advantages for particular types of research. At the end of this class, we usually played short British Heart Foundation videos (e.g. [38-40]), as they offer interesting examples of how zebrafish have been used to study the human heart and heart failure.

In the lab, students conducted a general investigation of zebrafish by rotating through different stations set up around the classroom. They investigated key characteristics such as sex, breeding behavior, the location of different organs using a labeled diagram and a stereomicroscope (dissecting scope) and the transparency of live embryos using a stereomicroscope. The teacher then concluded with a class discussion in order to compile and consolidate the various observations that the students had made.

Lab Module Two: Cell Division

This lab module introduces the concept of cell division as a method of growth and differentiation. For more information about the curriculum areas and concepts that the lab addresses please see Table 1. The authors feel that this lab is most effective when presented as a guided inquiry activity prior to teaching the mechanisms of cell division in vertebrates. However, it could instead be conducted in tandem or as reinforcement after teaching the concepts of this subject. Whenever possible, we taught this lab in a double school period of 96 minutes. However, occasionally, if timetabling constraints demanded, we taught it over two single periods. We only did this when we were teaching the lab to more than one class on the same day, as otherwise we would need to generate some embryos twice for the same class (see materials and methods for ways that embryo development can be slowed down so that some embryos can be re-used on different days). In this lab, students began by viewing pictures of young embryos and adult fish and forming hypotheses about how the changes between these two ages may have occurred. They

then viewed real embryos at different ages and attempted to identify the developmental age of each embryo by viewing a laminated chart of different developmental stages (ages). As a result, students were able to conclude that the embryos are "making new cells" and some cells are differentiating to become different types of cells with different jobs. The teacher then discussed the mechanisms of mitosis with students to explain how additional cells are created.

A possible extension activity that can be used to explore these concepts further involves regeneration in zebrafish. Students watch as the teacher cuts off or "fin clips" part of the tail fin of an adult zebrafish under anesthetic (see Materials and Methods). The teacher removes a small portion of the fin and the students make hypotheses regarding what will happen. Students then either view a previously clipped fish to observe the regeneration of the tail cells and try to explain how this re-growth occurred or view the same fish a week or two later.

Lab Module Three: Vertebrate Embryonic Development and Exposure to Environmental Toxins

This lab module examines the effects of environmental toxins on zebrafish embryonic development. For more information about the curriculum areas and concepts that the lab addresses please see Table 1. This module takes 5-6 school periods, spread over at least two, and ideally more, days (Table 1, Appendix 2). In this lab, students exposed zebrafish embryos to different concentrations of ethanol to observe its effects on embryonic development. Not only did this help students learn more about vertebrate development and some ways in which it can be perturbed, it also provided them with a hands-on opportunity to design and conduct all of the different stages of a scientific experiment. They developed hypotheses, designed the experiments and controls, gathered the data and formulated conclusions. In most cases, the students decided how many replicates to do for each concentration tested, how many embryos to test in each replicate, how to control for other environmental conditions and variables, and which concentrations each group would use. Occasionally, students also decided to test different lengths of exposure, for example, removing ethanol after a day in some dishes or not adding it until the embryos are a day old in other dishes. Depending on the amount of time available and also when in the day the class occurred, some students also set up breeding crosses and/or collected embryos to treat from breeding crosses set up the night before. When this was not possible, students were provided with embryos that were laid earlier in the day. Students designed their experiments, made hypotheses regarding what they thought they would see and set up the embryo treatments. On a later day (or days) they then viewed their embryos and made observations. When these observations were complete, students compiled their observations into a class data table for comparison and discussion. We did not cover statistical analyses in any detail, but the

teacher explained to the students that there are statistical tests that can be performed to see if data is statistically significantly different, as differences between treatments can also be due to noise/variation in the experiment and/or chance. The teacher performed a student's t test on the data and reported the results. Based on these results, students formulated conclusions about the effects of ethanol exposure during vertebrate embryonic development. They then hypothesized about possible effects of ethanol *in utero* during human development.

We have delivered this module in a couple of different ways, depending on the times available for particular class groups. For some groups, we used a double school period (96 minutes) to plan and set up the experiments, a single school period the next day to examine embryos and remove and record the number of dead embryos and another double period a couple of days later to analyze the embryos in more detail and collect and analyze results. However, each of the double-class sessions can also be divided into two single-period classes if necessary. If students collected and sorted the zebrafish eggs themselves, then a double class session was required for this and setting up the experiments, and the planning of the experiments was done in a previous class.

A potential extension activity here is for students to repeat their experiments using other environmental toxins such as caffeine, nicotine, calcium chloride (used on roads in winter), vinegar (to mimic acid rain), motor oil (to mimic oil spills or road runoff), nearby water sources (containing unknown toxins), fertilizer, pesticide, BPA, or any other pollutant available and approved by the teacher, to observe their relative deleterious effects. We chose to use ethanol as the "toxin" that we were testing, partly because it is inexpensive and readily available and partly because it captures students' interest and enables them to discover for themselves the potential toxicity of embryonic exposure to alcohol. Our hope was that this might also give them a better appreciation of the risks associated with alcohol consumption, especially during pregnancy, given that Onondaga county has a relatively high teenage pregnancy rate [41, 42].

For this lab, a webquest on human development can be offered as a pre- or post-lab activity so that students can think about the similarities and differences between zebrafish and human development. We usually used a webquest about human development which we obtained from [43] which uses the Mayo Clinic site [44,45]. A possible enrichment activity after the experiment, that we sometimes used to help students to make real world connections, is a webquest on fetal alcohol syndrome such as [46] or we sometimes provided students with questions to answer using an alternative fetal development and fetal alcohol syndrome website [47].

Lab Module Four: Mendelian Genetics

In this lab module, students learn about Mendelian ratios of inheritance during sexual reproduction, and mutations. For more information about the curriculum areas and concepts that the lab addresses please see Table 1 and Appendix 2. We found that this module is most useful after the basic concepts of Mendelian genetics have been covered in class, to make those concepts more tangible and memorable. This module took either 2 or 3 school periods, depending on whether students collected zebrafish eggs from the test breedings themselves. Students were first introduced to adult *albino* fish, a common zebrafish mutant phenotype. (Note: *albino* fish are often available at pet stores. If *albino* fish are not available, any other mutation with a visible adult phenotype can be used). Students viewed live wild type (WT) and *albino* adult zebrafish and made hypotheses about their genotypes. Students also made hypotheses about what the phenotypes of any offspring would be, if different combinations of adult fish were used in breeding crosses. Students then either set up actual crosses to view two or three days later or the teacher provided pre-prepared embryos to examine and count. Students counted the numbers of WT and *albino* embryos and the teacher compiled a class data table for a larger sample size comparison. Students observed ratios of normal to mutant phenotypes and discussed the concepts of dominant and recessive alleles, and homozygous and heterozygous genotypes. Lastly, students made hypotheses regarding the genotypes of hypothetical F2 generations if the F1 generations were to be grown up to adulthood and used in breeding crosses again.

If other mutant strains are available, for example in a neighboring zebrafish research lab, students may also examine these and generate hypotheses regarding how the specific effects of these mutations are caused. On different occasions, we have used mutations in *evx1*, which have a fin phenotype as adults [48], as well as recessive mutations where homozygous adults are not viable and heterozygous adults have no obvious phenotypes but mutant embryos have clear morphological phenotypes visible by two-days such as *mindbomb*, [49-52], *smoothened* [53, 54], and *scl* [55]. Each of these offers different possibilities for wider discussion. For example, *scl* mutants have heart edema and no blood flow. This can lead to a discussion about the value of zebrafish as a model system for studying heart development. *mindbomb* mutants also have heart edema. In addition, they lack trunk pigment as a result of loss of neural crest, which, in higher-level classes could be used to introduce neural crest and the idea that this is a vertebrate innovation (it evolved in the vertebrate lineage) [56-58]. *smoothened* mutants can be used to talk about Hedgehog signaling and the fact that a gene originally found in fruit flies (its name is based on its initial mutant phenotype in flies) is essential for many different aspects of vertebrate development and is involved in many cancers [59-

[61]. The similarity of the *smoothened* mutant phenotype to that caused by the chemical cyclopamine and the fact that cyclopamine was identified because sheep eating the corn lilly, *Veratrum californicum*, had cyclopic lambs is another interesting topic that we have covered in some classroom discussions and that could also be used as an extension activity after the lab.

Assessment of Modules

In comparison with previous years of teaching cell division and Mendelian genetics, R.W. and N.A. observed a greater level of student engagement and motivation to explore these topics when using these zebrafish modules. An unexpected advantage of establishing the classroom colony of zebrafish was that this instilled in many students an interest in, and sense of ownership of, the fish, even before they had conducted any experiments with them. Our qualitative experiences with students suggests that the students that completed these lab activities better understood and were better able to recall concepts that students in previous years had struggled with, presumably because they were able to view actual organisms rather than images or models and were engaged in more active learning. They also had a better grasp of what was occurring on a cellular level and the steps involved in the scientific method. Our experiences with the students also suggest that many of them improved their critical and analytical thinking skills as a result of their participation in these modules. In addition, the use of one model organism allowed students to become intimately familiar with this animal, which increased depth of understanding when applying new concepts. The consistent use of the same model also decreased time spent on introducing a new subject, allowing students to begin scientific investigations sooner. Students were eager to help maintain the fish tanks and frequently asked when they would be working with the zebrafish again. They seemed to take pride in conducting actual science experiments and expressed appreciation of the opportunity.

In the final year of piloting these modules, when we were confident that we were implementing activities that worked well in the Nottingham High School Living Environment classes, we piloted a survey that we asked students to take before working with zebrafish and then again, after they had completed some of these lab modules. The aim of these surveys was to try and assess quantitatively if these hands-on experiences increased student's understanding of key concepts and/or changed their attitudes to science and/or science careers. We asked students several questions about areas of science that the modules addressed and to assess attitudes we used questions from the STEM Semantics Survey and the Career Interest Survey, as described in [62], as these attitudinal surveys have been validated in other studies [62]. The surveys were piloted with four classes, but due to extremely high levels of absenteeism only 70 students

completed the pre-survey, and only 37 completed the post-survey. Only 30 students took both. It is hard to draw any conclusions from this initial pilot as the sample size is so small and it is from just one cohort of students. In addition, due to unusual timetable constraints that year and the very high levels of absenteeism, most of the students who completed both surveys had only experienced the first two zebrafish modules. However, we gained some valuable insights from this pilot effort, which we will use to improve the survey in future years (see discussion).

Materials and Methods

Ethics Approval

All of the experiments conducted as part of this collaboration were approved by the Syracuse University Institutional Animal Care and Use Committee (IACUC), as part of a teaching and outreach IACUC protocol written by KEL. All four authors underwent standard training at Syracuse University in lab safety and working with zebrafish and are named personnel on this protocol. Adult zebrafish that were transferred to Nottingham High School for their zebrafish colony were transferred off this protocol with IACUC approval.

IACUC approval was required for all experiments conducted at Syracuse University or with Syracuse University zebrafish because Syracuse University receives federal Public Health Service (PHS) funding [63, 64]. Institutions that do not receive PHS funding, which would include most K-12 institutions, do not require IACUC approval to use zebrafish adults or embryos in experiments. However, some science fairs may require prior ethical approval for projects using zebrafish [65].

Setting up a Zebrafish Colony

In this case, adult zebrafish were obtained from Syracuse University. Wild type (WT), *leopard* (a mutation that produces a spotty pigment pattern) and *albino* strains of zebrafish can usually be obtained from a local or online pet store, or through an educational science supply company such as Ward's Science or Carolina Biological Supply. Educational supply companies are also increasingly carrying zebrafish breeding kits and even eggs. For Lab Module 4, any adult fish with a visible mutant phenotype can be used (it does not need to be *albino*).

To maintain the adult zebrafish in a classroom we established two 10-gallon aquariums, which were kept in the back of the room on a table (Figs 1A & B). The tanks were each set up with a Marineland Biowheel Penguin 75 power filter (10 gallon size), a Tetra 26447 submersible aquarium heater set to 27°C, and a HDE LCD Fish tank submersible thermometer to monitor temperature (Figs 1A & B; these items can be obtained from pet stores and/or online merchants). To make cleaning easier, we did not add any aquarium stones. Each tank can easily hold 20 zebrafish. These should ideally be an equal mix of males and females to prevent the females from becoming "egg-bound". (Females that are kept separate to males do not lay eggs and this can lead to a distended abdominal area, poor quality eggs and eventually a blockage so that the female won't lay even when placed with a male). Fish were fed once daily, in the morning, 2 flakes

each of Tetramin tropical fish flakes (available from pet stores and online merchants). During school vacations, an automatic feeder (Fig. 1C; Fishmate F14 Aquarium Feeder, PM207 [66]) was used to deliver the same daily feed. Once each month we used an aquarium syphon (available from pet stores and online merchants) to remove 20% of the tank water and replaced it with water that had been dechlorinated by exposing it to the air for 24 hours. At the same time, any algal growth was removed using an algae sponge (available from pet stores and online merchants), and the filter cartridge was changed. When we first established the tanks, we ran them without fish for a month to check that they worked reliably, and to condition the water. The fish were then added 10 at a time to minimize ammonia blooms.

As zebrafish usually lay their eggs when the light comes on in the morning, we acclimatized them to a light/dark cycle by turning on the lights every morning at about the time that we wanted to obtain eggs (usually before the first class in the morning). We accomplished this by closing the window blinds when we left each day and having electric lights on a timer. We used inexpensive desk lamps with flexible necks so that the light could be directed at the tanks (e.g. Fig. 1E).

To try and collect embryos or observe breeding behaviors later in the day, we covered breeding tanks containing fish with a box before turning on the classroom lights in the morning and then removed the box when we wanted the fish to start breeding. We have found that this often works, particularly if we only want to delay breeding by an hour or so. Alternatively, the breeding boxes or fish tanks can be kept in a room or cupboard with electronic lights programed to a different light cycle.

We euthanized all embryos before 8 days post fertilization (dpf) based on ARAC guidelines for the use of zebrafish in the NIH Intramural Research Programme [67], which suggest that this is the earliest developmental time point at which zebrafish larvae might be considered capable of experiencing pain, distress or suffering. Embryos were euthanized by placing them on ice for at least 20 minutes and then treating with 16.6% household bleach (1 part bleach to 5 parts water) before disposing of them down the drain. If necessary, adult fish were euthanized by prolonged immersion in 200-300mg/L of Tricaine (also known as MS-222 or ethyl 3-aminobenzoate methanesulfonate salt, Sigma, A5040) until there was no longer a heart beat. These protocols are also described in the ARAC guidelines for the use of zebrafish in the NIH Intramural Research Programme [67].

Breeding Zebrafish and Embryo Collection

Two male fish and two female fish were placed in a specialized breeding or crossing tank (Fig. 1D) on different sides of a divider using a fish net (Fig. 1F). Depending on the class, we often had students do this themselves under supervision. The breeding tank consisted of an inner tank with holes in the floor that fits inside a slightly bigger tank. This means that when eggs were laid they fell through the holes and the fish could not eat them. We used breeding tanks kindly donated by Aquaneering (Fig. 1D) [68].

We maintained the fish at a temperature between approximately 27°C and 28.5°C while they were set up in breeding crosses. We have found that this improves embryo production and helps to make sure that the embryos develop normally. To achieve this, we use inexpensive room heaters with a temperature dial that we calibrated in the constant temperature fish rooms at Syracuse University before using them at Nottingham High School. We used Delonghi Safeheat Ceramic Heaters (model number DCH5090EL) for this purpose, but other similar heaters should also work. Lamps (bendable desk lamps work well) were set up on an electronic timer so that the fish had 10 hours of dark and the lights came on about 30 minutes before we wanted eggs to first be laid (Fig. 1E). Whenever possible, we placed the fish on this light-dark cycle for at least a few days before we wanted to breed them. We also fed them extra protein (Golden Pearl 500-800 from Artemia International) for a few days before breeding.

When the lights came on, on the breeding day, the inner tank was moved to a clean outer tank with clean fish water of a shallower depth (so that the fish will only be in about an inch of water) and the divider was removed. When the fish had laid eggs, they were returned to their normal larger tank and the eggs were collected using a tea strainer / tea net (Fig. 1F). Eggs were washed from the tea strainer into plastic 100mm diameter petri dishes with embryo medium (EM) and unfertilized eggs were removed. (Unfertilized eggs can be recognized by the fact that they do not progress beyond the 1-cell stage).

For ease, the Lewis Lab provided a 60X EM stock, which we stored at +4°C. We diluted this stock solution with reverse osmosis (RO) water (EM 1x final concentration: 5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2.2H_2O$, 0.33 mM $MgSO_4.7H_2O$, buffered to pH 7.8 with HEPES) and then added a small amount of methylene blue (0.1% w/v solution in sterile water, final concentration: 0.00002% methylene blue) in order to prevent fungal growth.

Embyros were kept at a density of no more than 100 embryos per 100mm diameter petri dish in embryo medium + methylene blue. Smaller petri dishes were used for experiments and observations. Embryos were normally incubated at 28.5°C although see discussion below about other permissible temperatures.

The developmental ages (stages) of embryos were identified according to [69], which provides a detailed description of zebrafish development. We usually talk about "developmental stage" rather than "age" as embryos develop at different speeds depending on temperature and environmental conditions (see discussion below). Consequently clear morphological criteria have been described for identifying different developmental stages [69]. These criteria are usually called a "staging series".

Obtaining Embryos at Specific Stages

The easiest way to obtain embryos at specific stages is to grow them at 28.5°C from the time that they are laid. In this case, embryos should reach particular developmental stages at the times indicated in the zebrafish staging series [69]. This paper is freely available online [70] and key stages are also listed on the online zebrafish database ZFIN [71]. For most of the lab modules included in this paper precise stages are not required. The hardest stages to obtain as live embryos are the early stages for lab 2. Embryo development can be sped up by placing embryos at a higher temperature and slowed down by placing them at a lower temperature. This can be exploited to obtain embryos at particular stages of embryonic development at specific times. The degree to which the rate of development changes is described in [69] or at [72]. We do not advise using temperatures higher than 32°C or lower than 25C, as the overall rate of development can become asynchronous. In addition, in our experience, if embryos are placed at 25C in the first few hours after they are laid, some of them will not develop normally, although others will. Zebrafish embryos are particularly sensitive to lower temperatures for the first 10-12 hours (until they finish gastrulation and reach "bud" stage). However, once embryos are a day old, if they are at placed at approximately 18°C their rate of development slows down to almost a complete stop. This is a useful technique for using the same embryos or larvae on successive days. We have even successfully done this with mid-somitogenesis stage embryos.

Fixing Embryos

Embryos were fixed by the Lewis Lab as backup material (in case fish did not lay and/or to more easily provide early developmental stages) by placing them in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) overnight at +4°C. They were then stored at 4°C in 4% PFA and rinsed several times with PBS before using for classes. We then returned them to 4% PFA for long-term storage at 4°C and used them again in subsequent classes. The PFA solution can be reused several times. We have embryos that have now been stored for over 4 years and they are still as good as when we first fixed them. Fixed embryos are a resource that it would be easiest to obtain from a willing zebrafish laboratory. They can easily be shipped so the laboratory does not have to be local. However, they can also be generated "in

house". For example, PBS can be made using sterile water and PBS tablets from Sigma (Cat # P4417-100TABS) or other suppliers. The Lewis Lab obtains PFA as a powder from Fisher Scientific (ACROS Organics AC416785000) and re-suspends it at 4% in PBS by heating the solution to 68°C for approximately 1 hour and then adjusting the pH to 7.4 to ensure that the PFA fully goes in to solution. We then filter-sterilize the solution and store it in 10mL aliquots at -20°C until needed. However, 4% PFA in PBS can also be bought as a premade solution from several suppliers including Fisher Scientific and Santa Cruz Biotechnology. Embryos are placed in either small (1-2ml) plastic tubes or small glass vials, as much embryo medium as possible is removed, and 4% PFA is added. The tubes/vials should then be placed on their sides in a fridge, so that all of the embryos are exposed to the PFA. The next day they can be stored vertically in a fridge.

Microscopy

We used LW Scientific S Series stereomicroscopes although any standard school stereomicroscopes with transmitted light (light source below the sample) should work.

Anaesthesia

For anaesthetizing embryos so that they could be examined more easily at later ages when they are moving, we used Tricaine (also known as MS-222 or ethyl 3-aminobenzoate methanesulfonate salt, Sigma, A5040). We made a 0.672% (40x) solution dissolved in sterile water and adjusted the pH to 7.0 using 1M Tris-HCl, pH 9.0, before filter-sterilizing and aliquotting into 10-15 mL tubes. We then stored this stock solution in a freezer compartment of a standard classroom fridge/freezer. We diluted this stock solution in EM (final concentration: 0.0168%) to anaesthetize moving embryos for observation. The same concentration of Tricaine was also used to anaesthetize adult fish for "fin clipping" experiments (see lab module 2 description in the appendix). Once embryos or fish have been observed they are transferred back into EM to wash off the Tricaine.

Discussion and Conclusion

This article describes a collaboration between two high school teachers and two university professors that, in a relatively short period of time (four weeks half-time) and with very little capital investment, produced four different hands-on lab modules for use in an inner-city high school and provided the high school teachers with the knowledge and confidence to deliver these modules to their students. We describe these lab modules and provide teacher notes, lesson plans and information and protocols for the materials and preparation work that they require. We initially started to pilot these labs with adult zebrafish and embryos brought in from the Lewis Lab at Syracuse University on a class-by-class basis. However, we now have established a small adult zebrafish colony at Nottingham High School. This has had additional positive consequences as it has stimulated student interest in zebrafish in general, given them a sense of "ownership" of the lab zebrafish and increased engagement with the labs. It also means that the activities are now more self-sustainable as they require very little input from the Lewis Lab, allowing that Lab to now move on to supporting additional teachers in other schools to deliver similar experiences.

Some of the strengths of these modules are that they are relatively easy and inexpensive to implement and that teachers can easily and quickly be trained to deliver them. Most schools would not need to purchase additional equipment, especially if zebrafish embryos and adults can be obtained from a nearby research lab. In addition, and most importantly, these experiments are very effective at capturing student attention and increasing student interest in the subjects being covered, across a wide range of student abilities. Some of these modules are similar to experiments/activities that have been used in other countries and other types of schools (e.g. [21-24]) and they have been equally effective in engaging students in these other environments, suggesting that these modules should have wide applicability and appeal to a variety of settings and student groups.

One difficulty that we encountered when implementing these modules was adapting them to short lesson times. Periods at Nottingham High School are currently 48 minutes long, with double periods once every four days. This resulted in some lessons being a bit rushed. Longer lesson times would definitely be an advantage for many of these labs. To try and compensate for this, we taught modules 1 and 2 straight after each other, so that we had more flexibility with time. As a consequence, students spent almost a full week of their Biology classroom time working with zebrafish. During this time, a mobile laptop cart was checked out of the library for webquests and other research. Module 3 also took a full week, although it was taught slightly later in the year.

Another difficulty arose due to the timing of classes and zebrafish embryonic development. We could not easily provide students with live embryos at all of the different developmental times (stages) that we wanted them to see, due to the different times at which classes occurred during the day. For example, early morning classes were easier to obtain newly laid embryos for, as zebrafish normally lay their eggs when the lights come on in the morning. Eggs laid later in the day can sometimes be obtained by using a different dark-light cycle (if the breeding boxes are in a cupboard or room that is not being used in the day) or by covering the breeding boxes with a cardboard box to keep them in the dark until embryos are required (for more details see Materials and Methods). However, even with these methods it is often not possible to obtain all of the different stages of live embryos that are needed for this module. Therefore, we developed two different types of back-ups, both of which have worked successfully. First, we have photographs of embryos at different ages (stages) that students can study and second, we have fixed embryos at different ages (stages), including early cleavage stages (just a few hours old), that we can store long term and wash out of fixative and into PBS or an equivalent medium for students to observe. We found that as long as students were able to view at least one developmental time point in live embryos, they were able to envision the other time points sufficiently, even if they were represented with a digital image.

In addition, the zebrafish did not always lay enough eggs to work with. This is a common problem when working with live animals and it has been encountered by other projects that use zebrafish (e.g. see [24]). For the labs that involved observation of zebrafish embryos, we tried to have live embryos for the students to observe as we feel that this is much more engaging. However, whether embryos are being obtained from a neighboring zebrafish lab or from an in-house colony of adult fish, there is always the very real possibility that either no embryos or too few embryos will be obtained. This may often be the case when using an in-house small-scale zebrafish colony. Some things that can help encourage the fish to lay are setting them up in breeding crosses 4 weeks and 2 weeks before the required date, to get them used to breeding and separating males from females a few days before breeding. In addition, feeding extra protein and using electric room heaters to maintain the breeding tanks at a constant temperature between 27°C and 28°C can be helpful. (For more information see Materials and Methods). However, none of these strategies ensure breeding success. This is another reason why the back-ups mentioned above (fixed embryos and laminated images) are useful.

Another common problem that we encountered when delivering these modules and in particular, when teaching labs that span more than one school period, was the high rate of absenteeism at the school where

we piloted these modules. This was particularly problematic when students were working as a pair or larger group. A large percentage of students were absent at least once a week, and often they were absent for two or more days. For module 3, wherever possible, we placed students who had missed days into a group with students that were present on that day or those days so that they could be brought up to speed with what was happening in the experiment. For the other modules, students were asked to complete the activities in order. For example, if a student did not attend class on the first day of a multi-day zebrafish experiment but did attend on the second day, in most cases they were required to complete the first day's activities prior to advancing in the module, even if this meant using the laminated images rather than live material. They were then given the opportunity to stay after school to get caught up on the lessons while materials were still available, and many students did this because they were excited to conduct the activities. In this way, more students were able to complete the module, and even those that didn't were able to experience part of the module.

As mentioned in the results, our qualitative experiences with students suggest that they were more engaged with the concepts being taught in the zebrafish lab modules than students had been when these same topics were taught without these interactive experiences in previous years. In addition, our experiences suggest that the students that completed the zebrafish lab modules better understood and were better able to recall concepts that students in previous years had struggled with. They also developed a better grasp of the scientific method and a higher level of critical and analytical thinking skills than similar students did in previous years. Unfortunately, as discussed earlier, our pilot survey to try and test whether our qualitative assessments about the effects of implementing these modules were supported by quantitative data did not provide useful results as extremely high levels of absenteeism meant that very few students completed both the pre-survey and the post-survey, and most of those that did had only experienced the first two zebrafish modules. In future years, we plan to amend this survey and the way that we implement it, based on the insights gained from this pilot effort. For example, we realized that the high degree of absenteeism at this particular school means that we need to track individual students so that we can compare survey results with attendance during each module. In future, each student will be provided with a unique code that they will use when filling in the surveys and the teachers will also provide attendance data for each student, associated with the code rather than a name. As the teachers will know the code for each student, they will not examine any individual survey results. Instead, an independent assessor will be provided with the anonymous (but linked) survey answers and attendance data. In the future, we will also ask students to re-take the survey one year after taking the modules to assess if there are long-lasting effects on knowledge retention or attitudes to science. Ideally, we would

compare students who take part in these modules to a control group of students that do not, but this is problematic, both in terms of providing all students with the same educational opportunities and because of the difficulties of identifying classes of students that are equivalent with respect to other potential confounding factors, especially in a school with a large range of student abilities and a high number of English as a second language students. However, as we start to work with additional teachers and schools, we can collect before and after data, using the year before any zebrafish modules are implemented as a base-line to compare later data to.

In conclusion, we hope that this example of how teachers with very little zebrafish experience quickly gained the skills and confidence to implement these experimental modules, our descriptions of the modules themselves and our discussion of potential obstacles and suggestions of ways to overcome them, will provide a useful resource for other teachers who are interested in integrating more hands-on, inquiry-based investigations using live animals into their classes. We also hope that this paper will encourage other zebrafish researchers to collaborate with local teachers in similar projects. The authors all found this to be a very rewarding project. In addition to the outcomes described in this paper, these experiences also helped SE and KL improve their other K-12 outreach activities so that they are now more relevant to local students needs and the Living Environment curriculum. In addition, N.A and R.W gained a renewed confidence in several different laboratory skills, an expanded understanding of Genetics and vertebrate development and a broader understanding of, and appreciation for, university-based scientific research.

Acknowledgements

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Conflict of Interest/Competing Interests

The authors have no competing interests.

Figure 1: Equipment used for zebrafish maintenance and experiments.

(A) The two 10 gallon aquariums in which zebrafish are kept at Nottingham High School. The tanks are each set up with a Marineland Biowheel Penguin 75 filter, a Tetra 26447 submersible aquarium heater set to 27°C, and a HDE LCD fish tank submersible thermostat to monitor temperature. (B) A close up view of one of these tanks. (C) The automatic feeder used to feed the fish during the holidays. (D) An Aquaneering breeding tank with divider in place. Note the level of water shown in this picture is the appropriate level for when the divider is removed and fish are breeding. The water should be filled almost to the top overnight while the divider is in. (E) Our breeding set up with a breeding tank and a desk lamp, which is plugged into a timer. We used the same desk lamps, directed at the 10-gallon aquariums, to maintain a light-dark cycle for the fish. (F) Equipment used to collect embryos. A net for moving fish, a tea net / tea strainer for collecting embryos, a squeeze bottle filled with EM for rinsing embryos out of tea net / tea strainer and a small petri dish for examining embryos (embryos were first collected into larger petri dishes).

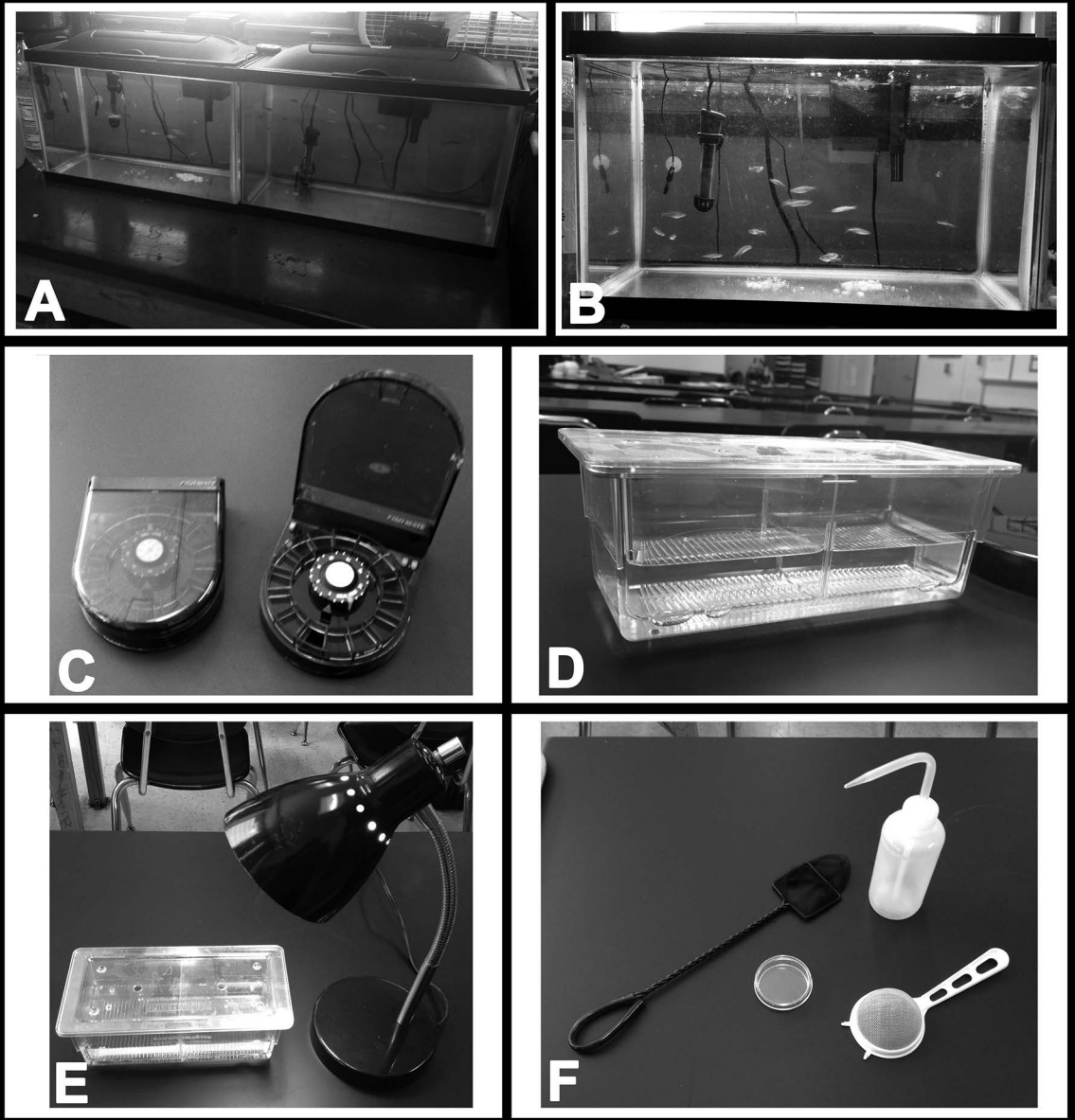
A
B
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Table 1. Approximate length of lab modules and curriculum areas/concepts that they address.

For each lab module included in this paper we provide a list of key curriculum areas and concepts that the module covers, including laboratory skills that are required to pass the New York State Regents examination in the Living Environment. In addition, all of these activities count as laboratory experience. As a prerequisite for admission to the Regents examination in the Living Environment, students must have successfully completed 1200 minutes of laboratory experience with satisfactory written reports for each laboratory investigation. We also provide the specific New York State Living Environment Curriculum (see http://newyorksciencteacher.com/sci/pages/cores.php) standards that are addressed, at least in part, by the activities contained within the module and the number of 48-minute school periods that we usually dedicate to each module. For cases where the amount of time can be varied, we have indicated this.

Lab Module	Curriculum Area(s) / Targeted Concepts	Relevance to New York State Living Environment Curriculum	Number of 48 Minute School Periods Dedicated to the Module
1. Introduction to zebrafish	Model organisms Embryogenesis and development Hypothesis formation Data analysis Formulation of conclusions	Standard 1: Key Ideas 1 & 2 Standard 4: Key Ideas 1, 4 & 5	1 school period for webquest. At least 1 school period for lab (slightly longer would work better as 48 minutes is a bit rushed).

	Use of stereomicroscope Observations of biological processes		
2. Cell Division Possibly to include fin regeneration as an extension activity.	Cell division / mitosis Cell differentiation Embryogenesis and development Vertebrate body plan Hypothesis formation Data analysis Formulation of conclusions Regeneration of vertebrate tissues Use of stereomicroscope Observations of biological processes	Standard 1: Key Ideas 1 & 2 Standard 4: Key Ideas 1 & 4	2 school periods for lab. Ideally a double period. Fin regeneration experiment is 5-10 minutes and can be done at the end of this lab (if there is sufficient time) or at the beginning of the next class. It then requires 5-10 minutes to reexamine the fin a week later.
3. Embryonic development	Scientific method	Standard 1: Key Ideas 1, 2 & 3	1 school period to plan experiment and develop hypotheses.

and exposure to Environmental toxins	Hypothesis formation Experimental design Need for control groups Data collection Data analysis Formulation of conclusions Toxic effects of chemicals on embryos Toxic effects of ethanol Embryogenesis and development Use of correct scientific instruments Use of stereomicroscope Observations of biological processes Assumptions and limitations of experiment	Standard 4: Key Ideas 1, 4, 5 & 7	1 double period class to set up the experiment and treat the embryos (if students are collecting and sorting embryos themselves). If students are provided with embryos, 1 school period may be sufficient. 2 school periods (on different days) to examine embryos and collect data. One of these can be just a few minutes to remove dead embryos. 1 school period to analyze and discuss data.

4. Mendelian genetics	Scientific method Hypothesis formation Mendelian genetics Heredity Dominant/recessive Data collection Data analysis Formulation of conclusions Use of stereomicroscope Observations of biological processes	Standard 1: Key Ideas 1, 2 & 3 Standard 4: Key Ideas 1, 2, 3 & 4	1 school period to analyze adult phenotypes, make hypotheses and set up fish mating crosses. 1 school period to collect and sort embryos if students do this themselves. (This is not needed if students are just provided with embryos) 1 school period to analyze embryonic phenotypes and draw conclusions.

Appendix 1 – Teacher Surveys

Pre-Survey

Please answer the first 8 questions using a scale of 1-10 (as indicated)

1. I feel confident sexing zebrafish and setting up breeding crosses (1= strongly disagree; 10 = strongly agree)

---

2. I feel confident identifying phenotypes in zebrafish adults (1= strongly disagree; 10 = strongly agree)

---

3. I feel confident staging zebrafish embryos (1= strongly disagree; 10 = strongly agree)

---

4. I feel confident identifying phenotypes in zebrafish embryos (1= strongly disagree; 10 = strongly agree)

---

5. I am confident in my understanding of the scientific research process
(1= strongly disagree; 10 = strongly agree)

---

6. I would be confident conducting a zebrafish experiment with my students (1= strongly disagree; 10 = strongly agree)

---

7. I feel confident about my general lab skills such as pipetting and measuring accurate volumes
(1= strongly disagree; 10 = strongly agree)

---

8. I feel confident about my ability to accurately calculate and make lab solutions (1= strongly disagree; 10 = strongly agree)

What are you hoping to get out of this experience (please carry on overleaf if necessary)

Post-Survey

Please answer the first 13 questions using a scale of 1-10 (as indicated)

1. How interesting did you find this summer experience? (1= very boring; 10 = extremely interesting)

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2. How useful did you find this summer experience? (1= not useful; 10 = extremely useful)

---

3. I feel confident sexing zebrafish and setting up breeding crosses (1= strongly disagree; 10 = strongly agree)

---

4. I feel confident identifying phenotypes in zebrafish adults (1= strongly disagree; 10 = strongly agree)

---

5. I feel confident staging zebrafish embryos (1= strongly disagree; 10 = strongly agree)

---

6. I feel confident identifying phenotypes in zebrafish embryos (1= strongly disagree; 10 = strongly agree)

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7. This experience has increased my understanding of Genetics (1= strongly disagree; 10 = strongly agree)

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8. This experience has increased my understanding of vertebrate development
(1= strongly disagree; 10 = strongly agree)

---

9. This experience has increased my understanding of lab research (1= strongly disagree; 10 = strongly agree)

---

10. I feel confident about my general lab skills such as pipetting and measuring accurate volumes
(1= strongly disagree; 10 = strongly agree)

---

11. I feel confident about my ability to accurately calculate and make lab solutions

(1= strongly disagree; 10 = strongly agree)

---

12. I am confident in my understanding of the scientific research process

(1= strongly disagree; 10 = strongly agree)

---

13. I would be confident conducting a zebrafish experiment with my students

(1= strongly disagree; 10 = strongly agree)

In your opinion, what was the best part of this experience / what has worked best? And why? (Please carry on, on the next sheet

[More space was provided on the sheet for each of these open-ended questions]

Is there anything that you think would improve this experience in the future?

Is there anything that you still need support with to be able to use this experience in your teaching?

Any other comments?

(or continuation of any of the sections above)

Results From the Teacher Surveys

The pre-survey was administered before the teachers started the summer RET experience and the post-survey was administered after they had finished it. Both surveys were completed anonymously, so we do not know whether teacher A in the table of results (Table 2) corresponds to teacher 1 or teacher 2. The survey was administered to obtain feedback on the RET experience with the aims of offering similar experiences in the future and hopefully improving them. At this stage, none of the authors of this paper had considered writing this paper. In addition to the scored responses detailed below, the post-surveys also had the following comments in response to the question about the best parts of the experience: “The

best part for me was the opportunity I got to observe and learn from almost everyone in the Lewis Lab"; "I experienced first hand real world research for the first time in a long time"; "The lab meetings and poster sessions were a good way to gain more in-depth understanding of the "bigger ideas" and aims of the research. Dedicating a time to speak with each student about their research was also extremely helpful because each had a different area of expertise to share." Neither teacher identified anything that could be improved about the experience and both were confident that they had the support they needed.

Table 2: Results From Teacher Surveys.

Question Number (pre-survey/post-survey)	**Teacher 1** (pre-survey)	**Teacher 2** (pre-survey)	**Average score** (pre-survey)	**Teacher A** (post-survey)	**Teacher B** (post-survey)	**Average score** (post-survey)
1/3	3	3	**3**	9	9	**9**
2/4	3	3	**3**	9	8	**8.5**
3/5	2	1	**1.5**	8	8	**8**
4/6	2	1	**1.5**	9	9	**9**
5/12	9	9	**9**	10	10	**10**
6/13	1	1	**1**	8	9	**8.5**
7/10	3	3	**3**	10	10	**10**
8/11	2	2	**2**	9	9	**9**
NA/1	N.A.	N.A.	N.A.	10	10	**10**
NA/2	N.A.	N.A.	N.A.	10	10	**10**
NA/7	N.A.	N.A.	N.A.	10	10	**10**
NA/8	N.A.	N.A.	N.A.	10	10	**10**
NA/9	N.A.	N.A.	N.A.	9	9	**9**

Appendix 2 – Lab Modules

Lab Module One: Introduction to Zebrafish

Time that lab requires: 1 period of 48 minutes. (An hour would be better).

Learning Objectives

After completing this lab module students should:

1. Be able to describe the morphological differences between male and female zebrafish
2. Be able to identify the locations of major organs in zebrafish embryos and larvae
3. Be able to describe similarities between zebrafish and human body plans
4. Appreciate the advantages of zebrafish embryos being transparent for studying embryonic development
5. Be able to identify advantages of zebrafish as a model organism for biological and biomedical research
6. Have more experience and skill at formulating hypotheses based on observational data
7. Be able to use a stereomicroscope independently to examine embryonic zebrafish

After the associated webquest students should also be able to:

1. Name five different model organisms used for biomedical research
2. Identify at least one advantage of each of these organisms for biomedical research
3. Identify at least two human diseases that are being investigated using model organisms

Teacher Preparation

Obtain zebrafish embryos from an in-house colony or elsewhere so that you will have embryos that are 25-32 hours old (the key thing is that a beating heart is clearly visible) and larvae that are 4-5 days old. Zebrafish embryos and larvae are best observed on a stereomicroscope (dissecting scope) with a light source below the petri dish that contains the embryos. Embryonic development can be sped up or slowed down if necessary by incubating embryos at different temperatures (see Materials and Methods in the main paper) but for this module precise stages are not required. However, these methods can be exploited to enable the same embryos and larvae to be used for several classes within the same day and/or classes on adjacent days (see Materials and Methods in the main paper).

Set up the stations as described below in different positions in the classroom so that students can easily move between them. For stations one and two, set up at least three fish tanks or aquariums with dechlorinated water and label them "Tank A", "Tank B", or "Tank C". Up to four students can easily view one tank at a time, so multiple tanks may be necessary for each letter depending on the size of the class. Place several male zebrafish in "Tank A", several female zebrafish in "Tank B", and equal numbers of male and female zebrafish in "Tank C". Note: male and female zebrafish should be kept in separate tanks overnight and mixed immediately before viewing to maximize breeding behaviors. Breeding behaviors are more likely to be observed if the class occurs in the morning as zebrafish usually breed when the lights first come on. However, breeding behaviors can also sometimes be observed later in the day, if fish are kept in the dark until just before the males and females are combined in the same tank(s).

Materials

- Male and female adult zebrafish
- Fish tanks and fish water (to set up the three different types of tanks)
- Fish net (for moving fish into the different tanks)
- Zebrafish embryos at 25-32 h and zebrafish larvae at 4-5 days post fertilization
- Photographs of embryos/larvae at these ages and/or fixed embryos as a back-up
- Annotated diagrams of 25-32 h embryos and 4-5 day old larvae showing major organs
- Embryo medium (see Materials and Methods) to keep the embryos and larvae in
- Petri dishes
- Tricaine solution (see Materials and Methods) for anesthetizing the larvae
- Dissecting microscopes / stereomicroscopes with transmitted light (a light source below the base)
- Plastic pipettes
- Plastic pipettes with a small plastic micropipette tip inserted in the end (or other similar tool) for rolling embryos around to look at them.

Procedures / Instructions for Students

Background

The zebrafish, *Danio rerio,* is a common freshwater aquarium fish that originally comes from India and Bangladesh. People have zebrafish in their aquariums at home as they are relatively easy to maintain. The

zebrafish is also widely used in Developmental Biology, Genetics and Biomedical Research as a model organism.

Objectives

- Identify male and female zebrafish
- Observe breeding behaviors in adult fish
- Observe fish embryos at different stages (ages) of development

Station 1: Identifying Male and Female Zebrafish

- Observe the two fish tanks for a few minutes (Tank A and Tank B).
- Hypothesize which tank you believe has female and which has male fish.
- Explain the reasons for your observations.
- Ask your teacher to reveal the correct sexes.
- Make a drawing of one male and one female zebrafish, labeling the parts visible to you and the differences between the two sexes.

Station 2: Observing Breeding Behavior

- Tank C contains both male and female zebrafish. Describe what you see happening.
- Based on your observation of Tank C, hypothesize the reason for the differences in male and female behaviors.

Station 3: Observing Zebrafish Embryos

- Using the dissecting microscope, observe the one-day-old embryos. Use the annotated diagram to locate the yolk, eyes, ears, brain, and somites in the trunk (that make the muscle). Can you see the heart? Is blood flowing? Draw and label one embryo and label its parts.

- Observe the older embryos (larvae). How are these similar and how are they different to the younger embryos? Are you able to see the same structures as in one-day-old embryos? Are there any structures that you can see in the older embryo that are not visible in the one day old embryos? Is blood flowing? Can you see fins? How many chambers does the heart have? Is the swim bladder visible? Draw and label one embryo and its organs.

- Is the body plan of the older embryo similar at all to the organization of a human body? Look down on the embryo from above and see how its eyes, ears, fins and body are arranged. Make a note in your book of any differences and similarities with a human.

Lab Module One. Model Organism Webquest. Based on information from
http://publications.nigms.nih.gov/classroom/supermodels/menu.html

Name/Group _____ Date_____

Model Organism Webquest

Directions:

Go to http://publications.nigms.nih.gov/classroom/supermodels/menu.html. Answer the following questions regarding each commonly used model organism by clicking on its picture and completing the tutorial including the multiple-choice question at the end. If you get the question wrong – try again.

Organism One: *C. elegans*

What is *C. elegans*?

Why is it considered a useful model organism?

What is GFP and why is it useful?

How has *C. elegans* allowed scientists to understand how animal brains are organized?

Organism Two: Zebrafish

Why is zebrafish used to study development?

What causes I-cell disease?

What do scientists hope to discover by studying bone development in zebrafish?

Organism Three: Fruit Fly

Name three traits (genetically determined characteristics) scientists study in fruit flies.

What can alter the time of day or night that flies walk around, eat and sleep?

Describe a disease in humans that scientists hope to cure by studying these mutations?

Organism Four: Yeast

What types of things do we use yeast for in our daily lives?

Describe Friedriech's ataxia.

How are scientists searching for ways to diagnose and treat this disease in humans?

Organism Five: Mouse

List three reasons why mice are useful model organisms.

What problems can gene changes cause in Huntington's disease?

How might scientists find a cure for this disease?

Additional Questions

Based on what you've learnt today – why do you think that scientists use different types of model organisms in their research?

Which model organism would you use to study how the gut develops in an embryo and why?

Lab Module Two: Cell Division in Zebrafish Inquiry Investigation

Time that lab requires: 2 periods of 48 minutes. Ideally this would be one double period, although alternatively the stations can be set up for two periods on different days and students complete on day 2 what they didn't finish on day 1. However, if you are using live embryos this will usually require generating the early stages twice. See Materials and Methods section in the main paper for a discussion of ways to slow down embryonic development and use embryos on successive days.

Learning Objectives

After completing this lab module students should:

1. Be able to describe the major stages of vertebrate embryonic development
2. Have a better understanding of mitosis and its crucial role in development and growth
3. Be able to describe similarities between zebrafish and human body plans
4. Appreciate the advantages of zebrafish embryos being transparent for studying embryonic development
5. Have more experience and skill in formulating research questions
6. Have more experience and skill in backing up conclusions with data
7. Be able to use a stereomicroscope independently

Teacher Preparation

This lab is best conducted with live embryos. However, we have found that fixed embryos at various ages also work. If either of these are not available at all ages (stages), they may be supplemented with images that have been printed and laminated or enclosed in paper protectors. We usually set up the lab as stations around the room with different ages of embryos on different microscopes or images available for stages (ages) that you don't have embryos for. Alternatively, if you are just using images, these may be attached together using a binder ring.

Embryos at each of the seven most widely recognizable stages (ages) should be represented. The images or embryos should be labeled as "mystery" stages A-G and placed out of order around the room or in the binder clip. The zygote stage cannot be produced as live embryos for each class, so we usually use fixed embryos and/or an image for that stage. We used the following stages and would be happy to provide the images that we used on request: Zygote – 1 cell; Cleavage - 4-32 cells; Blastula - 512 cells – high stage; Gastrula - shield – 75% epiboly; Segmentation - 8-12 somites; Pharyngula - 25-28 h and Larval - 3-5

days. Students are also provided with an example of an adult zebrafish (swimming around in a tank – not under the microscope) and an image of an adult zebrafish. To observe the live embryos, you will need a stereomicroscope (dissecting scope) with a light source below the base on which you place the petri dish that contains the embryos. We recommend having two dishes of larval embryos for students to look at: one dish where the embryos are just in EM and are swimming and one dish where the embryos have been anesthetized with Tricaine.

A possible extension to this lab is to perform a "fin clipping" experiment to demonstrate tissue regeneration. This procedure is often used by zebrafish labs to collect a tissue sample for DNA extraction. To perform this experiment we placed a single adult zebrafish into 300mL of fish water with 7.5 mL of 40x Tricaine (see anaesthesia section above) in a small glass beaker. Once the fish was no longer swimming – it was carefully removed from the beaker using a plastic teaspoon. Excess liquid was allowed to pour back into the beaker by tilting the spoon and the fish was placed on its side in a small petri dish. The fish was then observed on a stereomicroscope with reflected light (light source above the sample). Students examined the intact caudal fin (tail fin) and recorded its appearance and length by drawing or taking a photograph. We used a demonstration microscope with a camera so that an image of the fish was projected onto a screen for the class to see. The fish was then scooped back up with the plastic teaspoon by gently pushing the spoon under the head and towards the tail. Its position was adjusted on the spoon so that the tail protruded over the edge of the spoon. The fin was then cut with a sharp pair of clean small scissors, sterilized by dipping in 70-100% ethanol. Approximately 1/3 to 1/2 of the fin was cut off or "clipped"– i.e. to about the level of the notch. Don't clip into the flesh of the tail. The fin tissue usually stuck to the scissors. The fish was then placed back under the microscope to record what the cut fin looked like. The fish was then put into a tank of fish water and left to recover from the anesthetic. The fish usually recovered within a few minutes and started to swim around normally. Then a week or two later this procedure was repeated (without the cutting of the fin) to observe the regenerated fin. More frequent observations can also be made. A slightly different method of cutting the tail fin, which gives an indication of how much can be cut off, can be seen at the beginning of the video at [73, 74]. A protocol for a similar experiment performed by high-school students can be obtained in [21].

Materials

- Live zebrafish embryos of different stages (ages) in Petri dishes with embryo medium (or fixed embryos washed into PBS or other equivalent solution)

- Plastic pipettes with a small plastic micropipette tip inserted in the end (or other similar tool) for rolling embryos around to look at them.
- Tricaine solution (see Materials and Methods) for anesthetizing the larvae
- Laminated images of different stages and/or fixed embryos as a back-up
- Dissecting microscopes / stereomicroscopes with transmitted light (a light source below the base)
- Lab reference pages with stages (ages) of zebrafish development – assembled from pictures in [69] or a similar source

For fin-clipping experiment

- Adult fish
- Fish net
- Glass beaker
- Tricaine solution (see Materials and Methods) for anesthetizing the fish
- Fish water
- Plastic teaspoon
- Small petri dish
- Dissecting microscopes / stereomicroscope – ideally with a camera so that image can be projected
- Sharp pair of small scissors
- Small amount of 70-100% ethanol (for sterilizing scissors)

Student Instructions

Background

Most vertebrates undergo the same progression of changes and progress through the same different developmental stages (ages) as they grow from a single cell to a many-celled adult. In zebrafish these stages are called zygote, cleavage, blastula, gastrula, segmentation, pharyngula, and larval stages. The amount of time it takes to move through these stages is approximately 3-4 days at a temperature of 28.5°C. We refer to "stages" rather than "ages" as embryos grow at different speeds, and hence reach these developmental stages after different lengths of time, depending on temperature and other environmental conditions.

Your teacher will provide you with images of a newly fertilized zebrafish egg (known as a single-celled zygote) and a sexually mature adult zebrafish, 90 days later. There will also be embryos of different stages (ages) at different stations around the room.

Objectives

- Observe and describe the process by which an embryo becomes an adult
- Compare the number of cells in an embryo to that of an adult
- Observe zebrafish embryos at different stages and identify mystery stages A-G

Procedures

1. Look at the pictures of the newly fertilized zebrafish egg (known as a single-celled zygote) and the sexually mature adult zebrafish. You can also look at the live adult fish. Formulate a hypothesis about what changes have to occur for a zygote to become an adult zebrafish and write this down.
2. Visit each mystery stage station. You need to visit them all but they do not need to be in a particular order. At each station, view and draw the embryos under 100x and 400x magnification. Include the station letter next to each drawing so you can check your predictions later.
3. Using the reference pages provided, determine which stage of development your embryo is in.
4. For each station, provide an explanation of why you chose that stage.

Concluding Questions:

1. How does an embryo become an adult?
2. Provide the evidence you saw in your investigation to support your claim.
3. Create three alternate research questions you could ask relating to today's concepts.

Student Instructions for Fin-Clipping Experiment.

1. Draw what the fin of the adult fish looks like before the surgery
2. Draw what the fin looks like after the surgery
3. Hypothesize what you think will happen over the next week to the fin

A week later

4. Draw what the fin looks like now and compare to what it looked like after surgery
5. What has happened?
6. How is this different to what would happen with humans?
7. Why might it be useful for scientists to study what has happened with the fish?

Lab Three: Vertebrate Embryonic Development and Exposure to Environmental Toxins

Time that lab requires: Ideally 5-6 periods of 48 minutes. (Only 10-15 minutes are required during the 4th period – although a longer length of time can be used to analyze embryos in more depth, see discussion below).

Learning Objectives

After completing this lab module students should

1. Be more experienced and skilled at designing experiments
2. Understand the importance of control groups in experiments
3. Be more experienced and skilled at collecting and analyzing data
4. Be more experienced and skilled in drawing conclusions and formulating additional hypotheses based on experimental data
5. Have a better understanding of the scientific method
6. Be able to describe the advantages of using zebrafish embryos for toxicology experiments
7. Be able to describe some of the possible toxic effects of ethanol exposure on zebrafish embryos
8. Be able to use a stereomicroscope independently
9. Be able to use a micropipette correctly

Teacher Preparation

This lab can be conducted as an open-ended inquiry or with instructor guidance. The teacher may choose to prepare various appropriate ethanol treatments ahead of time and allow students to each choose one, or the teacher may allow students to select their own treatment concentration. We have found that students sometimes have a difficult time selecting an appropriate concentration of ethanol to test due to lack of understanding of proportions and of relative toxicity levels, so some guidance is sometimes necessary. We often ask students what the legal limit for driving is, for example, to guide them towards testing lower concentrations. Alternatively, the teacher may allow students to select a percentage rather than a concentration and challenge them to determine and/or perform the necessary calculations. Depending on the length of the classes and the number of school periods that can be devoted to this lab, the first class can be used to plan the experiments and the last class can be used to consolidate, analyze and discuss the data. This is particularly helpful when class-times are relatively short. In addition, one class is required to set up the experiment and at least one class is needed to observe and analyze the results. If sufficient time is available to use one school period to design the experiments, students can be guided to choose how

many embryos to include in each dish, how many control dishes to do, what concentrations to test and how many replicates of each concentration to do. If there is sufficient time, they could also set up breeding crosses at the end of this lesson.

In our hands, we have found that concentrations of 3% ethanol and above kill all of the embryos by the time they are 24 hours old. Therefore, we suggest that at least most concentrations tested should be lower than this. 2.5% also makes embryos very sick and most die, 2% results in severe phenotypes and 1% is not too badly affected at 24 hours old but has phenotypes at later stages.

For the class where students set up the experiments, if it is in the morning and there is sufficient time available, students can collect embryos from breeding crosses themselves and sort viable embryos from fertilized eggs into dishes. This can help students be even more engaged in the experiment, but it also takes quite a bit of time, especially as it is probably best if the teacher and/or a volunteer used to working with zebrafish checks that the students have accurately removed all of the unfertilized eggs. (The alternative is to treat fertility as another variable in the experiment).

If time available for this lab module is limited, it is possible to just remove, and record the numbers of, dead embryos the day after the ethanol treatment (which is relatively quick) and then analyze the embryos in more detail on either the next day or the day after, depending on what fits the class schedule best. Ideally, dead embryos would be removed daily so that they don't poison the water. However, for students with lower attention spans, it is preferable to perform the detailed phenotype analysis on one day (2 or 3 days after ethanol exposure), as, even though the embryos will look different at different ages, the students may get bored "repeating" the analysis on different days. By day 3 or 4 (2 or 3 days after ethanol exposure) many different phenotypes can be assayed, including movement. Just doing one day of detailed analyses also enables the lab to take up less overall time, which can be an advantage given dense curriculum requirements.

In addition to testing different concentrations of ethanol it is also possible to test the effects of applying the ethanol at different times. This would require either setting up treatments on successive days or having eggs laid both that morning and the day before available when setting up treatments. It is also possible to test what a shorter period of exposure does – by removing the ethanol – for example, after one day of exposure and comparing this to embryos that continue to be exposed. Students can be encouraged to raise these sorts of questions and design these experiments by asking them leading questions such as "do you

think it would make any difference if the embryos were not exposed to ethanol until later in development?" followed by "how could you test that?"

As an extension of this project - students can be asked to prepare research posters, either in class or as an additional out-of-class activity. We have done this with considerable success when using this lab as part of a more intensive summer research experience.

This lab is also a good opportunity to teach students how to use a micropipette. In fact, teachers may opt to conduct an introductory lab on using a micropipette prior to conducting this lab module.

Note: we suggest that dishes are labeled on the lid (easy to see) but also on the side, so that if lids are put back on the wrong dish by mistake this can be discovered and corrected. It is not a good idea to put labels on the base of the dishes as that interferes with observing the embryos on the microscope.

Materials

- Live zebrafish embryos laid that day (exact stage is not crucial – but should be recorded) or, if class time allows breeding crosses of zebrafish set up the day before and tea nets / tea strainers, petri dishes and squirt bottles of embryo medium to collect eggs.
- If testing different times of exposure – potentially embryos laid the previous day
- Approximately 4 dishes of zebrafish embryos per group
- Dissecting microscopes / stereomicroscopes with transmitted light (a light source below the base)
- Ethanol solution
- Disposable plastic pipettes
- Plastic pipettes with a small plastic micropipette tip inserted in the end (or other similar tool) for rolling embryos around to look at them.
- Gilson P200 micropipettes or equivalent with 0.1-10µl tips
- Incubator kept ideally at 28.5^{o}C – but the exact temperature and the precision of the incubator is not crucial
- Tricaine solution (see Materials and Methods) for anesthetizing older-stage embryos (so that they can be examined in depth even when they are old enough to swim).
- Possible back-up resources incase embryos don't lay. These could be embryos that were treated with different concentrations of Ethanol ahead of time and then fixed, combined with data about how many

embryos died in the respective dishes and photographs of the dead embryos. Alternatively students could examine a similar published study [75] that includes a video of the experiment and analyze the data included in this publication.

If students are setting up their own breeding crosses and collecting embryos

- Small fish breeding tanks with dividers for setting up breeding crosses
- Room heaters
- Timer
- Light source
- Fish water
- Petri dishes
- Tea nets / Tea strainers and squirt bottles of embryo medium for collecting eggs
- Embryo Medium

Student Instructions

How to Calculate Volumes by Percent

Step One: Calculate 1% volume by multiplying the total volume in the Petri dish (in this case 40mL of embryo medium) by 0.01

Example: 1% = 40mL x 0.01 = 0.4mL

Step Two: Multiply your answer from step one by whatever percentage you wish to use

Example: 2.5% = 0.4mL x 2.5 = 1.0mL

5% = 0.4mL x 5 = 2.0mL

Step Three: Convert to micrometers (μL) if using a micropipette by multiplying by 1000.

(1000 μL = 1mL)

Example: 1% ethanol = 0.4mL = 400 μL

2.5% ethanol = 1.0mL = 1000 μL

Background

The environment plays a role in 85% of all diseases. New science is showing that the effects of exposure to chemicals at low doses, and in combination, can have an impact on human growth and development. Some chemicals, pollutants, foods, and other behavioral changes that may have minimal adverse effects in adults, may impact a developing fetus and have long-lasting (chronic) effects even into adulthood.

Various toxins persist in the environment, but today you will observe the effects of alcohol on embryonic development.

Objectives

- Design and conduct an experiment to test the effects of alcohol on embryonic development

Procedures

1. Obtain zebrafish embryos from your teacher.
2. Determine which concentrations your lab group will use in your treatment dishes.
3. Obtain an ethanol solution from your teacher.
4. Label each of your 3 treatment dishes with the chemical name and concentration, your group number, the age of the embryos, and the date and time. Place your labels on the dish lid and side – not the base as this makes it hard to see the embryos when you use the microscope.
5. Label your control dish as the control, your group number, the age of the embryos, and the date and time. Place your labels on the dish lid and side – not the base.
6. Administer the appropriate concentration of ethanol into the three treatment dishes using the micropipette.
7. Place your dishes on your class tray in the incubator for 24 hours.
8. On day 2, view your embryos under the microscope. Count and remove dead embryos. Make a note of how many embryos you remove in your notebook and on the lid of the dish.
9. On day 3 (or 4), view your embryos and make observations of the dead and live embryos in your notebook, including drawings, data apes and descriptions of behavior. Start by looking at your control embryos so that you know what "normal" looks like and then carefully compare this control dish to the treatment dishes to note any differences and/or abnormalities in the treated embryos. You will probably need to anesthetize the embryos to look at them carefully so that they are not swimming around the dish. To do this, pipette them carefully into a dish that contains Tricaine. Once you have observed the embryos, pipette them into a dish of embryo medium to wash off the Tricaine and then back into their original experimental dish.
10. Enter your data into the class spreadsheet so that we can compare all of the data together.

Concluding Questions:

1. What effect does alcohol have on embryonic development?

2. Based on your observations during this investigation, predict the effects of alcohol on human embryonic development. How is this information important for pregnant mothers?
3. Do you think consuming alcohol is more detrimental during early or late pregnancy? Why?

Lab Four: Mendelian Genetics in Zebrafish

Time that lab requires: 2-3 periods of 48 minutes. (3 periods if students collect eggs from breeding crosses themselves, 2 if they do not).

Learning Objectives

After completing this lab module students should

1. Have a better understanding of Mendelian genetics and inheritance during sexual reproduction
2. Have a better understanding of the concepts of dominant and recessive genes
3. Be able to predict the result of breeding crosses of adult zebrafish with different combinations of phenotype and/or genotype
4. Be able to identify the possible genotypes of live adult zebrafish with different phenotypes
5. Be able to predict whether a particular mutant phenotype in zebrafish is controlled by a dominant or recessive gene and how it is inherited.

Teacher Notes

Images of various zebrafish mutant phenotypes may be substituted for live fish, if necessary. As with the cell division lab, fixed embryos may also be used. The length of time between collecting embryos and being able to observe the phenotype may differ for different mutations. We used *albino* zebrafish as the mutants. This works well as they are recessive viable, so it is possible to see the homozygous mutant phenotype in the adults, and the phenotype is very obvious in both adults and embryos. The embryonic phenotype is visible by two days and becomes more pronounced over the next couple of days. Fixed embryos can be saved and used year after year as back-ups in case the different breeding crosses don't lay eggs. You just need some embryos with WT pigment for crosses of mutants with WT fish and *albino* embryos for the crosses of homozygous mutants with each other. You can also use a Mendelian ratio of *albino* and WT embryos to illustrate what would happen if the embryos from the cross of the mutant fish with WT fish were grown up and then crossed to each other. However, any other mutation with a visible adult phenotype could be substituted for the *albino* zebrafish. If time is limited, then the setting up of breeding crosses and collection of embryos can be removed from the module and fixed or live embryos can be supplied to students instead. In this case, the module could be completed in just two class sessions. If students set up breeding crosses and collect embryos at least three class sessions are required and the second one needs to be relatively early in the morning (unless the teacher removes the dividers in the

breeding crosses – in which case the fish will lay eggs in the morning and viable embryos can be collected later in the day).

Materials

- Images or live adult zebrafish - wild type (WT) and mutants. One of the easiest examples is to have *albino* fish and WT fish.
- Fixed embryos for different crosses as a back-up incase fish don't lay
- Plastic pipettes with a small plastic micropipette tip inserted in the end (or other similar tool) for rolling embryos around to look at them and counting embryos.
- Dissecting microscopes / stereomicroscopes with transmitted light (a light source below the base)

If students are setting up their own breeding crosses and collecting embryos

- Small fish breeding tanks with dividers for setting up breeding crosses
- Room heaters
- Timer
- Light source
- Fish water
- Petri dishes
- Tea nets / Tea strainers and squirt bottles of embryo medium for collecting eggs
- Embryo Medium

Instructions for Students

Background

Inside the cells of every living organism are the instructions that control everything that happens genetically to the organism. The instructions are located on chromosomes inside the cell's nucleus and are known individually as genes. Each gene dictates the kind of protein that will be manufactured. These proteins, alone or in groups, result in the formation of characteristics or traits in all living things. However, not all genes function directly to control a particular trait. Some may instead function as the switch to turn other genes on or off.

In most sexually reproducing plants and animals, offspring inherit two copies of each gene for a particular trait. One copy of the gene for a trait comes from the male parent and the other comes from the female

parent. In zebrafish, the trait for pigmentation (color) is inherited in this manner. Zebrafish offspring exhibit particular appearances, or phenotypes, based on the traits inherited from both parents.

Objectives

- Examine different zebrafish phenotypes and consider the possible sources of the differences
- Predict the results of breeding crosses of adult zebrafish with different combinations of phenotypes and/or genotypes
- Set up zebrafish breeding crosses to test particular hypotheses about the inheritance of particular traits

Procedures

Exercise I: Making Observations and Predictions

1. Examine the Wild Type (WT) zebrafish and then record in your lab book the phenotypes you observed.
2. Examine the mutant zebrafish. Compare their features to those of the WT fish. Write down all the distinguishing features you are able to see in both groups.
3. What may have caused the phenotypes you observed in the mutant fish?
4. Make a prediction of the phenotypes you might observe in embryos as a result of crossing the mutant fish with each other or the WT fish with each other. Explain your reasoning.

Exercise II: Determining Whether a Phenotype is Dominant or Recessive

For this part of the lab you will design and perform experiments to answer the experimental question: Is a particular phenotype a dominant or recessive trait in zebrafish?

1. Formulate a hypothesis – do you think that this phenotype is a dominant or a recessive trait?
2. Based on your hypothesis – what phenotype or phenotypes do you think the embryos would have if you crossed a WT fish with a mutant fish?
3. Perform your experiment with the zebrafish provided by your instructor

a) Set up at least one breeding cross

b) Next day – collect embryos from your fish and make a note of the number of eggs laid. Clearly label your dish, remove any unfertilized eggs and put your dish in the incubator. If you have more than 100 embryos divide them between different dishes.

c) 2-3 days later – look at your embryos. What do you see? Do all of your embryos have the same phenotype? Describe and count all of the different phenotypes that you see.

d) Does your data support your hypothesis? Or does it suggest a different hypothesis?

4. We will combine your data with the whole class data and discuss what we find. In your discussions, consider this question: What are the probable genotypes of the parents of the zebrafish used (provided by your instructor) in this experiment? Explain your reasoning.

5. Based on your data in this experiment, is the phenotype a dominant or recessive trait in zebrafish? How do you know? (Does your data support your hypothesis? Why or why not? Explain.)

6. Based on your hypothesis – if you grew up embryos from a cross between a WT and a mutant zebrafish and then, once those embryos were adults, mated them to each other – what would you expect their embryos to look like?

Further Exploration: Solving Problems When the Genetics Are Unknown

1. You have been provided with a wild type (WT) looking zebrafish (a fish with a WT phenotype). What test would you perform to find out if this fish is heterozygous or homozygous for the WT pigment phenotype? Explain your answer.

2. If you know the parents' genotype for autosomal genes (alleles A and a), how can you determine what type of offspring they will produce? Explain your answer by means of Punnett squares.

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