Learning the Scientific Method Using GloFish

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Abstract

Here we describe projects that used GloFish, brightly colored, fluorescent, transgenic zebrafish, in experiments that enabled students to carry out all steps in the scientific method. In the first project, students in an undergraduate genetics laboratory course successfully tested hypotheses about the relationships between GloFish phenotypes and genotypes using PCR, fluorescence microscopy, and test crosses. In the second and third projects, students doing independent research carried out hypothesis-driven experiments that also developed new GloFish projects for future genetics laboratory students. Brianna Vick, an undergraduate student, identified causes of the different shades of color found in orange GloFish. Adrianna Pollak, as part of a high school science fair project, characterized the fluorescence emission patterns of all of the commercially available colors of GloFish (red, orange, yellow, green, blue, and purple). The genetics laboratory students carrying out the first project found that learning new techniques and applying their knowledge of genetics were valuable. However, assessments of their learning suggest that this project was not challenging to many of the students. Thus, the independent projects will be valuable as bases to widen the scope and range of difficulty of experiments available to future genetics laboratory students.

Introduction

LABORATORY COURSES and independent research projects offer great opportunities for students to experience the complexities of the scientific method. Learning through doing experiments also fits very well with recent ideas for reforming and improving science education through inquiry. Inquiry-based learning emphasizes student driven curricula that includes opportunities for investigation, critical analysis, reflection, and revision of ideas.¹

GloFish are commercially available, genetically engineered strains of zebrafish that offer great potential for use in inquiry-based laboratory experiments. These strains carry transgenes that cause them to express high levels of different fluorescent proteins. These fluorescent proteins cause the fish to be brightly colored under normal room light, and to fluoresce, or glow, when they absorb specific wavelengths of light (<http://www.glofish.com>). Since the transgenes in these fish are integrated into the genome, GloFish can be used in a wide array of experiments to explore fundamental concepts in genetics (<http://www.glofish.com>).² For instance, we have previously developed a laboratory protocol that enabled students in a sophomore level undergraduate course to carry out analysis of Mendelian inheritance patterns using adult GloFish.² These protocols challenged students to analyze up to three genes

and four inheritance patterns in the same cross. Importantly, they also introduced students to how chi-square statistical analysis can be used to test hypotheses, thus incorporating a math-based approach early in their undergraduate training.

Although our previous published protocol was effective in giving students experience in gathering and analyzing raw genetic data, it offered only a brief introduction to the scientific method. We have now developed three follow-up projects that enable students to successfully carry out experiments of their own design. The first project, which builds directly on the chi-square analysis protocol, gave students the opportunity to test their hypotheses about the genotype and phenotype of GloFish using PCR, fluorescence microscopy, or test crosses. Although many of the students were carrying out these techniques for the first time, they all successfully produced data that could be used to evaluate their hypotheses. This makes the project a good choice for laboratories courses, where time to problem solve experiments that are not working is limited. The second project, done by Brianna Vick as an independent undergraduate research project, explored more complex genetic concepts to uncover why there are several shades of orange GloFish. The final project, done by Adrianna Pollak for a high school science fair, used GloFish and fluorescence microscopy as tools to learn about fluorescent proteins, light and color, and transgenes.

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These projects offered students the opportunity to experience many aspects of biological research. Students doing GloFish experiments in their undergraduate laboratory course gained experience in working together to achieve their goals. The opportunity to choose their own approach for testing their hypotheses helped them gain experience in experimental design. Both Brianna Vick and Adrianna Pollak found that their initial hypotheses did not fully explain their data, and thus had the opportunity to generate a series of hypotheses that fit their growing pool of data and also reflected their increasing understanding of the science behind their projects. Thus, these ideas can serve as the bases for inquiry-driven, hypothesis-based projects appropriate for many undergraduate laboratory courses.

Materials and Methods

Fish stocks

Parental fish stocks included: the wild-type (WT) strains Tubingen (Zebrafish International Resource Center, Eugene, OR) and Zebrafish *Danio rerio* (ZDR)(Aquatic Tropicals, Plant City, FL) and the GloFish strains Glo^{YFP} (carrying the transgene mylz2:Yellow Fluorescent Protein), Starfire RedTM (Glo^{RFP}, carrying the transgene mylz2:Red Fluorescent Protein), Electric GreenTM (Glo^{GFP}, carrying the transgene mylz2:Green Fluorescent Protein), Galactic PurpleTM (Glo^{PPF}, transgene unknown), and Cosmic BlueTM (Glo^{BFP}, transgene unknown)(World of Fish, Hermantown, MN).³ Fish were raised and maintained using standard protocols.⁴

Natural breeding

Adult fish were set up to breed in the evening as single pairs in 1 L spawning tanks. The tanks were monitored for the presence of eggs until late afternoon the following day. Healthy embryos produced from each pair were maintained in a Petri dish containing aquatic system water for 7 days at 28.5°C, and then placed in a 3 or 10 L tank within a recirculating aquatic system and raised to adulthood.

Caudal fin removal

Fish were anesthetized by incubation for approximately 1 min in 0.017% tricaine methanesulfonate (MS-222) dissolved in aquatic system water. The fish were then placed on the top of a Petri dish and the fin was excised with a razor blade. Fish were returned to a recovery tank containing aquatic system water, monitored until they started swimming, and then returned to their home tank on the recirculating system.

PCR genotyping

Genomic DNA was made from fins removed as described above. The fins were placed into 1.5 mL Eppendorf tubes, extra water was removed, and 30 μ L of 50 mM NaOH was added. The mixture was heated to 95°C until the fin tissue was completely dissolved, typically 20–40 min. The tube was cooled on ice to 4°C, and then 3 μ L 1 M Tris, pH 8.0, was added. After mixing, the debris was pelleted by centrifugation for 5 min at 14,000 g in a microcentrifuge. The PCR reactions contained 2 μ L of undiluted genomic DNA, 0.2 μ M forward RFP primer (5'-GTA ATG CAG AAG AAG ACT ATG GGC TGG GAG-3'), 2 μ M reverse RFP primer (5'-GCG GAT CTT GAA GTT CAC CTT GAT GCC-3'), 12.5 2X PCR Master Mix

(Invitrogen), 1 μ L taq polymerase, and milliQ water to bring the total volume to 25 μ L. The fragment of the RFP gene was amplified using the following program: step 1: 95°C for 2 min; step 2: 94°C for 30 sec; step 3: 65°C for 30 sec; step 4: 72°C for 45 sec; step 5: go to step 2 29 times; step 6: 72°C for 5 min; step 7: hold at 4°C. The resulting products were resolved on a 2% agarose gel and the DNA visualized using SYBRSafe (Invitrogen) according to the manufacturer's directions.

Imaging

Live adults and larvae were anesthetized as described above. Live adult fish were photographed with a Panasonic DMZ-TZ3 digital camera or a Sony Cybershot Model # DSC-H20. Larvae were photographed using an Olympus SZZ12 stereomicroscope fitted with a Cannon PowerShot A520. Fluorescent and bright field images of the excised fins and trunks of live fish were gathered using a Nikon Eclipse 801 Epifluorescent Microscope connected to a Spot digital camera.

Use of vertebrate animals

All procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC) as part of a Teaching Animal Care and Use Protocol and, because transgenic organisms were used, by the U of MN Institutional Biosafety Committee (IBC). Brianna and Adrianna completed all of the chemical and animal training needed to do research in a U of MN laboratory. Students in the course were required to complete three animal training modules: Ethics of Animal Use in Research, Use of Animals in Research & Teaching at the U of M, and Biomedical Research Animal Use (<http://cflegacy.research.umn.edu/iacuc/training/>) (Supplementary Material 1; Supplementary data are available online at www.liebertpub.com/zeb). Students were trained in all techniques before the onset of their experiments, and a laboratory member or instructor was present at all times to ensure correct techniques were used.

Statistical analyses

Assessment surveys were taken by students in the undergraduate genetics laboratory course three times during the semester (see Results section). Questions 1–8 were multiple choice questions that focused on facts relating to designing and analyzing their experiments. These were scored as “correct” or “incorrect.” Two questions about the value of the GloFish project for learning (V1–V2) were scored as strongly agree (5) to strongly disagree (1). All answers from a single assessment by a particular student were entered into the same line in a JMP table. Questions that were not answered were scored as missing. A Wilcoxon rank sum test, a paired Wilcoxon signed rank test, and chi-square tests were carried out using JMP 10 software on subsets of the data as described in the Results.

Results

Experiencing the scientific method in the undergraduate classroom

This laboratory exercise was carried out in a sophomore level genetics laboratory course. It gave the students the opportunity to go through each step of the scientific method and successfully test their hypothesis about Mendelian inheritance

patterns of GloFish transgenes. The students in each of the three laboratory sections of the course came to a consensus about which hypothesis they wanted to test, and then divided into smaller groups based on interest so as to take different approaches to test this hypothesis. The use of GloFish gave students a great deal of choice and control in the design of their experiments and a high chance of success in producing data that could be used to make a conclusion.

Step 1: Make Observations

During the first meeting of the laboratory during the semester, students carried out our previously published GloFish experiment.² Students characterized several groups of adult progeny from parents carrying GloFish transgenes and mutations that cause changes in pigment pattern and fin length. They counted the progeny with each phenotype, generated a hypothesis about the inheritance patterns of each trait, and then tested their hypotheses using chi-square statistical analysis. In the simplest clutches of progeny, students analyzed the inheritance of only a single gene and trait. In the most complex, they analyzed the inheritance of three genes and three different inheritance patterns. This first laboratory gave them experience in working together and analyzing and interpreting raw data.

Step 2: Generate a testable hypothesis

The data the students gathered in the first laboratory were used to make a testable hypothesis about the inheritance pattern of one of the observed traits. This was first done through a homework assignment that guided them through the basics of designing a test cross (Supplementary Material 2). In the second step, all of the students in the class shared the ideas they had generated in this homework, and together decided on one hypothesis to test as a group. The class then divided into smaller groups depending upon the approach the students decided to use, with the choices of setting up fish in single pair matings and assaying the phenotypes of the progeny, using fluorescence microscopy to characterize which fluorescent proteins were present, and PCR genotyping (Supplementary Materials 3–5). The students in each smaller group worked together to do their experiment and gather and interpret their data. Here, we summarize the approaches taken and data gathered by two sections of students.

Step 3: Test the hypothesis

Hypothesis 1. Glo^{RFP} is dominant over Glo^- . In the first example, students chose to test the hypothesis that the presence of the Glo^{RFP} transgene and the associated trait of red body color was dominant over absence of the transgene (Glo^-) and gray body color (Fig. 1). The students taking a PCR genotyping approach made genomic DNA from the tail fins of progeny from a cross between a Glo^{RFP}/Glo^- and a homozygous WT fish. A portion of the RFP coding sequence was then amplified and the products analyzed by gel electrophoresis (Fig. 1C and Supplementary Material 4). If their hypothesis was correct, they expected that the red progeny would all carry the transgene and the gray fish would all lack the transgene. As part of their experimental design, the students assayed more gray fish than red fish, reasoning that if the gray

fish lacked the transgene, then this would help rule out the alternative hypothesis that the red body color was recessive to gray. The PCR reactions containing DNA from red fish served as positive controls to ensure the PCR reactions could successfully produce a product. PCR reactions that lacked primers or genomic DNA were used as controls to rule out contamination of reagents. The students PCR reactions closely matched their expectations, with PCR producing a product from almost all of the red fish DNA ($n=3/4$ fish) and no product from the gray fish DNA ($n=5/5$ fish), reactions containing no DNA ($n=7/7$ reactions), and reactions containing no primers ($n=7/7$ reactions). Class discussions and a follow-up homework (Supplementary Material 6) enabled students to learn about the importance of controls in PCR experiments: why PCR can fail to produce bands even when a template containing the target sequence is present and how contamination of reagents can cause bands to be present in reactions missing essential components.

The students taking the crossing approach bred a Glo^{RFP} homozygous female with a WT male (Fig. 1B). If Glo^{RFP} was dominant over Glo^- , they expected all of the progeny to be red. However, they found that under white light, a pink color was apparent in the majority of the progeny, while a small subset appeared colorless (Fig. 1B). In a complementary approach, the students assayed the pink and colorless fish by fluorescence microscopy. In contrast to the red body color, the fluorescence was present in all of the progeny of the experimental cross, and the strength of the fluorescent signal appeared to correlate with the amount of pink body color (Fig. 1B). Thus, the students concluded that the fluorescence produced by the Glo^{RFP} transgene was dominant over lack of fluorescence/transgene presence.

The lack of color in some of the fish carrying the Glo^{RFP} gene was surprising, as other crosses suggest that the red body color is a dominant trait (Fig. 2). One likely explanation was that the RFP protein levels were too low at this stage of development (~ 1 month post fertilization) to produce the red body color. Consistent with this, positive control progeny from a cross of parents both homozygous for Glo^{RFP} were much brighter red when they were assayed at 2 months post fertilization (Fig. 1A). This raises a potential difficulty of doing these experiments in the classroom. In our experience, the red body color is the easiest to detect in early larva. Thus, this experiment would be even more difficult with any of the other colors of GloFish.

Hypothesis 2. Orange fish carry both the Glo^{YFP} and Glo^{RFP} transgenes. In a second example, students tested the hypothesis that fish that appear orange under white light are carrying both the Glo^{RFP} and Glo^{YFP} transgenes. Students used two crosses to test this hypothesis (Fig. 2). In the first, a fish homozygous for the Glo^{RFP} transgene was crossed with a fish homozygous for the Glo^{YFP} transgene. If the hypothesis was correct, then all of the progeny would carry one copy of Glo^{RFP} and Glo^{YFP} and would appear orange under white light, which exactly matched the results of the experiment (Fig. 2A). In the second cross, an orange GloFish of unknown genotype was crossed with a WT, gray fish. As expected from their hypothesis, this cross produced orange, yellow, red, and gray progeny (Fig. 2B).

In a second approach, students used fluorescence microscopy to gain insight into the genotypes of the orange GloFish.

FIG. 1. (A) Punnett square and images outlining the expected and observed phenotypes and genotypes of the parents and progeny of the control cross. All fish in this cross were homozygous for Glo^{RFP} , and thus red whether the transgene was dominant or recessive. Thus, it served as a control to ensure that red body color could be effectively detected. (B) Punnett square and images of the expected and observed phenotypes of the cross testing the hypothesis that the red color and red fluorescence (arrows) are dominant over the gray/no fluorescence. If the hypothesis was correct, the students expected all of the progeny to have a red body color and to be red fluorescent. (C) Example gel showing products from PCR genotyping. The expected 200 bp band for the RFP gene is present only in the lane with red fish DNA and all of the components of a PCR reaction. Several of the lanes have a very small "primer dimer" product that was likely produced by the forward and reverse primers annealing to one other. In addition, some of the lanes show products that are not expected (*), suggesting that the annealing temperature was not high enough, and the primers were annealing to additional sites outside of the RFP gene. All images of fish are lateral views with anterior to the left.

Hypothesis

Orange fish are carrying both the Glo^{RFP} and Glo^{YFP} transgenes

A Cross 1: Can red and yellow parents produce orange progeny?

B Cross 2: Can orange fish produce red and yellow progeny?

FIG. 2. Crosses to determine the origin of the body color of orange GloFish. **(A)** Punnett square describing the first test cross and images of parental and progeny phenotypes. If the hypothesis was correct, the students expected all of the progeny to have an orange body color, which exactly matched the results of this test cross. **(B)** Punnett square describing complementary test cross and images of parents and selected progeny. If the hypothesis is correct, the cross should produce red and yellow progeny. Consistent with the hypotheses and the predictions from the Punnett square, yellow ($n=5$), red ($n=2$), orange ($n=5$), and gray ($n=1$) progeny were produced. All images are lateral views with anterior to the left.

FIG. 3. Patterns of fluorescence are consistent with orange GloFish carrying both the Glo^{RFP} and Glo^{YFP} transgenes. Progeny from Cross 2 in Figure 2 were assayed for emission of fluorescence after excitation by different wavelengths of light. Each row contains images of the same fish. The first column has images of the whole fish, and the remaining columns have images of the surgically removed caudal fins. The prominent structures are the fin rays (open white arrowheads). The red fish, consistent with the properties of RFP, fluoresces weakly under blue light and strongly under green light. In contrast to the yellow fish, the fluorescence is present in both the fin rays and the mesenchymal tissues in between. The yellow fish, consistent with the known properties of YFP, fluoresces strongly under blue light (480 nm) and only weakly under green light (545 nm). Fluorescence under both wavelengths is largely localized to the fin rays. The orange fish fluoresces strongly when excited by blue and green light, and fluorescence is present in the fin rays and in between. None of the GloFish fluoresce under UV light (350 nm). Analysis of all of the progeny demonstrated that fish with the same color under white, room light also have matching fluorescence patterns (Supplementary Material 7). All images are lateral views with anterior to the left.

The students reasoned that if their hypothesis was correct, the fluorescence of the orange GloFish should be a sum of the fluorescence patterns in yellow and red GloFish. Our previous studies indicated that the GloFish transgenes are expressed in the bony rays of the fins.² Thus, the students used caudal fin tissue for their fluorescent assays. This tissue has many advantages for use in the classroom. It is flat, and so easy to mount on a slide. Removing small pieces of the tail fin of anaesthetized adult fish is an established technique to gather tissue for PCR genotyping and for studies of regeneration. Fish recover normal swimming patterns minutes after fin removal, and the fin completely regenerates in approximately 2 weeks.⁵ Consistent with the absorption spectra of RFP and YFP, red fish did not fluoresce when excited by blue light and fluoresced strongly when excited by green light, and yellow fish fluoresced strongly under blue light and weakly under green light (Fig. 3 and Supplementary Material 7). Interestingly, the pattern of fluorescence under green light was not only different in strength, it also had a slightly different pattern. The fluorescence in the red fish was diffuse throughout the fin, while the fluorescence in the yellow fish was almost exclusively in the fin rays (Fig. 3). The pattern of fluorescence of the orange fish was consistent with both RFP and YFP being present, with strong fluorescence under both blue and green light and expression in the fin rays and in the tissue in between the rays (Fig. 3 and Supplementary Material 7).

Step 4: Analyze results and draw conclusions

The predicted results for each of the students' experiments closely matched their data (Figs. 1–3). Thus, both of the hypotheses were strongly supported. In addition, their data also help rule out alternative hypotheses. For instance, another possibility was that the color of the orange fish came from expression of an orange fluorescent protein. If this was correct, the cross between an orange and a gray fish would have produced only orange and gray progeny, and the fluorescence pattern would have been shifted from that of YFP and RFP.

Step 5: Science writing

One of the main goals of this course was to give students experience and training in science writing. Accordingly, their largest assignment in this course was writing a paper in the form of a scientific manuscript (Supplementary Material 8). Students in previous years had expressed the desire to choose which experiment they used for their paper, so this year's students were given the choice of writing their paper on their GloFish experiment or on an experiment in *Drosophila* genetics. Over the past few years, several positive changes have been made in the science writing assignments, largely in response to student feedback. First, the paper was prepared in sections, with due dates for each section spread throughout the semester (Supplementary Material 8). This helped students manage their time and enabled the instructors to get comments and critiques back to the students more quickly. In addition, the students had the opportunity to revise their paper in small steps over the course of the semester, learning from and incorporating the instructors' feedback. This approach also moved the greatest demand on students' time to earlier in the semester. This greatly reduced late papers and removed the challenge of preparing the paper while studying

for final exams. Finally, the first drafts were weighted less than the final draft, offering an incentive for effort put into the revision (Supplementary Materials 8 and 9).

Assessment

To evaluate the usefulness of this GloFish project, we assessed undergraduate students' knowledge of the scientific method and statistical analysis pre- and post-laboratory and again at the end of the semester. We formed an eight-question (Q1–8) assessment with all questions in multiple-choice, closed question format to simplify analysis of the results (Fig. 4 and Supplementary Material 10). The student's overall score was the percent correct for Q1–8. The pre-laboratory assessment was given during the second meeting of the class, just after finishing the first step (initial observations) of the laboratory and prior to generating a testable hypothesis. The post-laboratory assessment was given just after completion of the laboratory, and the post-semester assessment was given on the last day of class (Supplementary Material 10). Comparing the three assessments (pre-lab, post-lab, and post-semester) with a Wilcoxon rank sum test did not find a significant change in the mean scores for Q1–8 ($p=0.80$) (Fig. 4A). However, the power of this analysis was limited. First, the assessment surveys were not coded so that we could track the performance of individual students across the semester and carry out repeated measures analyses. Second, the number of assessments returned to us varied ($n=51$ –52 pre-laboratory assessments, $n=36$ –39 post laboratory assessments, and $n=46$ –47 post-semester assessments; there is a range in the number of responses within a assessment because some students did not answer all of the questions). These factors could have limited the ability of the statistical analysis to find significant changes.

Despite this, several trends could be identified. The proportion of correct answers in the questions related to experimental design (Q1–3, 5) were high, even in the pre-assessment. This suggests that the majority of students already had a strong understanding, and our assessment could have been focused instead on more advanced concepts (Fig. 4B). For instance, in the pre-laboratory assessment, 100% of the students were able to place the steps of the scientific method in order (Q1) and correctly answer the question "A hypothesis can be accepted or rejected but never proven to be true or untrue" (Q5). Further, the percentage of students who correctly answered questions about positive (Q2) and negative controls increased in the following assessments, suggesting that the GloFish laboratory had a positive impact on students understanding of controls (Q3) (Fig. 4B). In contrast, there was no change or even a negative change in the percentage of students with correct responses to the questions related to statistics and the meaning of a p value (Q6, 7, 8) (Fig. 4B).

In the post-laboratory assessments, we asked the students to provide feedback on the value of the GloFish experiment for their learning as well as answer the eight questions (Fig. 4C and Supplementary Material 10). This enabled us to determine whether there were any correlations between the responses of individual students to different parts of the survey. 81% of students reported this project improved their understanding of experimental design (V1), while only 57% reported a positive effect on their ability to interpret p values

FIG. 4. Evaluation of undergraduate student knowledge on experimental design. **(A)** Comparison of student performance on Questions 1–8 (Q1–8) in the pre-laboratory, post-laboratory, and post-semester assessments. **(B)** Comparison of the percentage of students that answered Q1–8 correctly in the pre-laboratory and post-laboratory and post-semester assessments. **(C)** Student post-laboratory self-evaluations of how carrying out the GloFish laboratory influenced their learning. All assessments were done voluntarily and anonymously.

(V2) (Fig. 4C). A Matched Pair Wilcoxon Signed Rank test indicated a significantly higher positive response to V1 compared to V2 ($p=0.017$). Chi-square analyses that paired the each of the questions Q1–8 with the responses to V1 and V2 found no meaningful correlations. For instance, there was

no evidence that correct answers to any of the questions related to experimental design (Q1–3, 5) correlated with agreeing that the GloFish project increased knowledge of experimental design (V1) ($p>0.32$). Further, there was no association between the overall scores on Q1–8 and responses

for V1 and V2, with the exception of two students who had low overall scores. One of these students had low ratings for both V1 and V2 and the other a high rating for V1 and a low rating for V2.

At the end of the semester, the students were asked to reflect upon the value of the GloFish laboratory (Supplementary Material 10). Their responses suggested largely positive experiences. The majority of the students in this course were sophomores, and their previous experiences with research were mostly in other laboratory courses ($n=36/40$ responses). When asked "What was the most valuable part of doing the GloFish experiment?", the most common response by far was the opportunity to learn and do a new technique ($n=22/40$ responses), with PCR as the most common technique listed. The other most common answers related to improving their understanding of genetics ($n=10/40$) and appreciating the opportunity to do their own experiment ($n=5/40$). For the opposite question "What was the least valuable part of doing the GloFish laboratory", the most common response was to leave this section blank ($n=14/40$) or indicate that they had nothing to list as a response ($n=4/40$). Because the majority of students who left this section blank answered all of the other questions, the most likely explanation is that they had no immediate critiques of the laboratory. The other most common responses were very logical, and included doing a technique that did not work ($n=7/40$) and doing the same technique repetitively ($n=4/40$).

Independent undergraduate research

During our efforts in developing GloFish laboratories and projects for undergraduate courses, we noticed variations in the shade of orange between different fish. For example, some orange GloFish were much redder than others, although still clearly distinct from red GloFish (Fig. 5). Our previous results demonstrated that orange GloFish carry both the Glo^{YFP} and Glo^{RFP} transgenes (Fig. 2).²

This observation suggested a way that we could bring more complexity into the projects of future students in the genetics laboratory course. Building this initial observation into a project for the classroom was well matched with the expertise of an undergraduate student, Brianna Vick, who had just joined the laboratory. Brianna was well prepared for research through her participation in the Pathways to Advanced Degrees in the Life Sciences Program, which gave her in-depth training in critical problem solving, professional speaking, poster presentations, debate groups, as well as in laboratory skills such as pipetting, PCR, gel electrophoresis, and ELISA (<http://www.d.umn.edu/brpa/>).

As the first step in her project, Brianna hypothesized that the presence of these different shades of orange was due to different dosages of the Glo^{RFP} and Glo^{YFP} transgenes (Fig. 5). Gene dosage refers to the number of copies of a specific gene present in a cell. As the gene dosage increases, increased transcription and translation would lead to higher levels of the protein. If the dosage of one gene is higher than another, the phenotype of the progeny would more prominently resemble the gene with higher dosage. For instance, a fish having one copy of Glo^{RFP} and two copies of Glo^{YFP} would be yellow-orange, while a fish having two copies of Glo^{RFP} and one copy of Glo^{YFP} would be red-orange (Fig. 5B). To test this hypothesis, we completed several distinct crosses to produce

progeny with different dosages of the Glo transgenes (Fig. 6 and Supplementary Materials 11–15).

Our results were consistent with the gene dosage hypothesis. Most notably, the progeny of Cross C (Fig. 6 and Supplementary Material 13), which would include fish with two chromosomes containing the Glo^{RFP} transgene and one chromosome containing Glo^{YFP} , had orange fish with a redder-orange color, while the orange fish in Cross D (Fig. 6 and Supplementary Material 14), which at most had one chromosome containing Glo^{RFP} , were less red. Although these crosses provided some evidence that fish with higher doses of the transgenes are distinct in color from fish with fewer copies of the genes, a quantitative method of measurement would be beneficial for future research. One way to quantitatively test the presence of the protein in homozygous versus heterozygous progeny would be to complete replicate real time quantitative PCR, which has already been used to determine zygosity of Glo^{RFP} transgenic zebrafish.⁶ Addition of this experiment would allow for more accurate measurements of the relative copies of Glo transgenes and determine whether the hypothesis can be supported by statistical analysis.

We also found that, in some cases, orange fish with the same genotype had different shades of orange. For instance, in cross A, one of the GloFish was clearly redder than its sibling fish with the same genotype (Fig. 6 and Supplementary Material 11). Direct gene dosage must not be the only factor causing variation of shades in orange GloFish. Transgenes often insert into the genome in large tandem arrays.^{7–9} Thus, one possible explanation is that multiple tandem copies of the Glo transgenes are interacting in ways that affect levels of expression.^{7–9} For example, uneven crossing over between the tandem repeats during meiosis could cause gametes to have different numbers of copies of the transgene in their tandem arrays, causing the progeny to have a different levels of expression from each other and from their parents.

FIG. 5. Hypothesis that different dosages of Glo transgene loci affects the shade of color in orange GloFish. **(A)** Examples of two adult GloFish with different shades of orange and a red GloFish for comparison. The squares to the right are magnifications of similar regions in the trunk of each fish. Fish are homozygous for the *golden* mutation, which causes lack of pigment in their stripes. **(B)** Matrix describing the hypothesis about how the genotypes at the Glo^{RFP} and Glo^{YFP} loci influence the body color of the GloFish.

FIG. 6. Different shades of orange in developing GloFish with the same genotype. Comparisons between selected red and orange fish resulting from the indicated test crosses. The *squares to the right* of each group of whole pictures are magnified views of the fish in a region just posterior to the pectoral fin, arranged in the same order as the whole fish pictures. *Arrows* in the images for Crosses A and B indicate the location of the pectoral fins. Note that there are different shades of orange fish in crosses A and B even though they all have the same complement of transgenes. All images are *lateral views with anterior to the left*.

The number of copies of the transgene in the tandem array could be directly proportional to the expression level, similar to our gene dosage hypothesis. The fish that was redder than its siblings could have had more copies of the Glo^{RFP} gene. However, the opposite effect is also possible. Garrick and colleagues found that the presence of “silencing repeats” in an array of transgenes in mice caused decreased transgene expression.⁹ By eliminating copies of the transgene, they were able to increase expression.⁹ Thus, the fish that was red-orange could have instead had fewer tandem repeats of Glo^{RFP}.

In addition, there are several other factors that may have been playing a role in the shades of the orange GloFish. Wild-type zebrafish, especially the males, tend to be slightly yellow in color. This could have caused some of the orange GloFish to have a more yellow cast. In addition, fish purchased at a pet store could have any combination of transgenes. We were able to produce fish that were consistently yellow-orange by breeding a Glo^{RFP} fish with a Glo^{GFP} fish (Fig. 6E and Supplementary Material 15). Thus, although we did not completely solve the mystery of why there are so many different shades of orange GloFish, we generated many hypotheses that will be the basis of future research by students.

Science Fair Project

GloFish also offer the opportunity for middle school and high school students to design their own science fair projects. Adrianna Pollak, a student at Cloquet High School, carried out a science fair project that generated data that will contribute to the design of new GloFish experiments for the undergraduate classroom and enabled her to learn about fluorescence and transgenes. While fluorescence microscopes are still not available in most high schools, the process Adrianna went through as she advanced through her project offers an excellent example of how independent research gives students the opportunity to drive their own learning.

In the first year of her science fair project, Adrianna gained experience in the zebrafish system by carrying out crossing experiments to identify the inheritance patterns of the Glo^{RFP} and Glo^{YFP} transgenes, similar to our previously published experiments.² At the time Adrianna was deciding on her project for her second year, we obtained the whole range of GloFish strains, which included the red (Glo^{RFP}), orange (Glo^{RFP}; Glo^{YFP}), yellow (Glo^{YFP}), lime green (Glo^{GFP}), blue (Glo^{BFP}), and purple (Glo^{PFP}) (<http://www.glofish.com>). This offered a great opportunity for Adrianna to pursue her interest in the fluorescence phenotype of GloFish.

Instead of generating a single testable hypothesis, Adrianna made several sets of hypotheses, revising them as she gathered preliminary data and carried out additional research. Adrianna first generated questions that she wanted to answer: (1) What effect does the color of light have on the fluorescent protein in *Danio rerio* (zebrafish)? (2) Can the color of the fluorescent protein of *Danio rerio* be used to predict genotype? Based on these questions, she generated her first, most general hypothesis: If there is a relationship between the color of light being absorbed and the color being emitted by zebrafish fluorescent protein, then each fish will emit a different color under different wavelengths of light and the genotype will be predictable.

Adrianna then generated more complex hypotheses that made predictions of how each strain/color of GloFish would fluoresce when excited by each wavelength of light available on

our fluorescent microscope. Adrianna quickly realized that her initial hypotheses were not matching her preliminary data. For instance, she predicted that green GloFish would have a combination of blue and yellow fluorescence, which is a correct prediction if yellow and blue pigments were combined, but not correct for a protein like GFP that emits green light.

After doing additional research about fluorescence microscopy and the basic physics of the absorption and emission of light by fluorescent molecules, Adrianna generated a brand new set of hypotheses (Fig. 7). For instance, she hypothesized that the Cosmic Blue GloFish were likely carrying Blue Fluorescent Protein, and therefore should emit blue light when excited by light at 350 nm (Fig. 7A). An important tool was the excellent “Fluorescence SpectraViewer” website developed by Invitrogen to generate maps of the absorption and emission spectrum of fluorescent molecules (Fig. 7B and C) (<http://www.invitrogen.com/site/us/en/home/support/Research-Tools/Fluorescence-SpectraViewer.html>).

To test her revised hypotheses, Adrianna helped pilot the method of assaying fluorescence using the caudal fins of adult fish (Fig. 8). Importantly, removal of the caudal fin does not harm the fish, as required by the rules of most science fairs. She found that the yellow, red, green, and orange fish all had specific patterns of fluorescence, while the fins of the Glo^{BFP} and gray (nontransgenic) fish did not fluoresce under any of the wavelengths of light tested (Figs. 7 and 8). These patterns largely matched Adrianna’s hypotheses (Table 1 and Figs. 7 and 8). For instance, the Glo^{YFP} fins were fluorescent when excited by blue light, as expected. However, there were also a few exceptions. For instance, the yellow fish were also fluorescent under green light, although the fluorescence was lower than for the orange and red fish that express RFP (Fig. 8). Adrianna’s closer examination of the absorption and emission spectra for YFP revealed that this protein can be excited to a low level by green light, thus providing the likely explanation for the unexpected results (Fig. 7B). In a more dramatic exception to her hypotheses, the fin from the blue fish did not fluoresce under any of the wavelengths of light (Table 1 and Fig. 8).

The promoter used to drive expression in the Glo^{RFP}, Glo^{GFP}, and Glo^{YFP} transgenes, and presumably the others as well, was isolated from the muscle specific *myl2* gene. Thus, we next assayed fluorescence in the trunk of the fish, as this is where the skeletal muscle is most concentrated. These results largely matched the expression patterns in the fin, although in general the fluorescence was much brighter in the trunk (Table 2 and Fig. 9). In addition, the trunk of the Glo^{BFP} fish fluoresced as expected when excited by UV/blue light (Table 2 and Fig. 9).

Adrianna was also able to evaluate her original hypothesis. Using the data from the trunks of the GloFish, as this fluorescence was much brighter, she made predictions about what combinations of Glo transgenes are expected to give completely distinct fluorescence patterns, and which combinations will not (Tables 3–5). Adrianna’s next project will be to test these predictions by making dihybrid crosses carrying two different transgenes and analyze these crosses using fluorescence and PCR genotyping (Tables 4 and 5). Her work will, importantly, also generate protocols and ideas that can be used by the next generation of genetics laboratory students.

We still do not know why the Glo^{BFP} fish showed such differences between the fin and body fluorescence, especially

FIG. 7. Summary of hypotheses about the how each color fish would fluoresce under different wavelengths of light. **(A)** Hypotheses generated by Adrianna after her research on the relationship between wavelength and color of light and how fluorescent proteins work. The labels above the columns indicate the peak wavelength and color of the light used to illuminate the fish. The predictions were based on Hypothesis graph after looking at Fluorescence Spectraviewer graphs. **(B and C)** Examples of Fluorescence Spectraviewer graphs (<http://www.invitrogen.com/site/us/en/home/support/Research-Tools/Fluorescence-SpectraViewer.html>) that Adrianna used to make her hypotheses. **(B)** This graph suggests that Glo^{GFP} fish should fluoresce when excited by blue light (~480 nm), as this wavelength falls within the absorption spectrum of GFP. **(C)** In contrast, Glo^{RFP} fish should not fluoresce or should fluoresce only at low levels when excited by blue light, as the ability of RFP to absorb this wavelength is very low. The vertical lines mark the approximate position of the 488 wavelength, which is within the range of blue light used to excite the GloFish on our epifluorescence microscope.

as the fluorescence patterns in the other strains matched between these two tissues. Adrianna's initial hypotheses are that the BFP protein is expressed at too low a level or not at all in the Glo^{BFP} fin. Adrianna's hypotheses will be a great start for the students who take on the next step of this project.

Discussion

Each of the GloFish projects was successful by several measures. On the most tangible level, the students all designed experiments that effectively tested their hypotheses. All of the students produced data and showed high proficiency in analyzing their data. Considering that the large majority of students were having their first experiences with research, this is a high achievement. In addition to opening up

the possibility for students to gain experience in a wide variety of experimental approaches, many students reported that part of their engagement in this research came from working with such a beautiful animal. As Adrianna stated, "Just looking at the detail of the fins, and the fish themselves, under the microscope was such a wonderful experience. It still amazes me how one small portion of a zebrafish's caudal fin can contain such beautiful detail."

Experiencing the scientific method in the undergraduate classroom

One of the main outcomes from the assessments will have impact on future iterations of the undergraduate genetics

FIG. 8. Different colored GloFish have distinct patterns of fluorescence in their caudal fins. GloFish with different body colors under regular room light were assayed for fluorescence emission after excitation by different wavelengths of light. Each row contains images of the same fish. The *first column* has images of the whole fish, and the *remaining columns* have images of the surgically removed caudal fin. A range of wavelengths was used to excite the fluorophores, and the peak wavelength is listed above each column. The fins are in *lateral views with anterior to the left and dorsal to the top*. The same exposure times were used for each fish for each wavelength of light (20 msec for bright field and 400 msec for each of the specific wavelengths of light). The exception to this was fin of the green GloFish, which was extremely bright under blue (480 nm) light, so image at this wavelength was taken with a 200 msec exposure.

laboratory course. Data suggest that this laboratory was too easy for many of the students. Most strikingly, most of the students answered questions about experimental design correctly even before doing the GloFish laboratory. This suggests that the difficulty of this laboratory could be increased to focus on more complex ideas. This was supported by feedback from many of the students in the end of semester assessment. Many of the students wanted more choices of GloFish strains to work with, more freedom in designing the experiment, and the addition of more complexity to the analysis of the DNA/molecular genotype of the fish.

Important information also comes from the decrease in the ability of students to answer questions about statistical analysis. There are several potential explanations. The number of students returning the assessments decreased from the pre-laboratory assessment to the later assessments. This could have introduced bias into analysis if students who answered correctly in the first assessment were less likely to complete the later tests. Another possibility, which has important implications for student learning, is that a mismatch between the type of analysis done in class and the type of analysis on the assessments could have caused confusion. For analysis of

TABLE 1. FLUORESCENCE PATTERNS OF GLOFISH FIN TISSUE

	<i>Bright field</i>	<i>UV light (350 nm*)</i>	<i>Blue light (480 nm)</i>	<i>Green light (545 nm)</i>
Red Fish	Was visible	Did not fluoresce	Did not fluoresce	Fluoresced high
Orange Fish	Was visible	Did not fluoresce	Fluoresced	Fluoresced high
Yellow Fish	Was visible	Did not fluoresce	Fluoresced	Fluoresced low
Green Fish	Was visible	Did not fluoresce	Fluoresced	Did not fluoresce
Blue Fish	Was visible	Did not fluoresce	Did not fluoresce	Did not fluoresce
Purple Fish	Was visible	Did not fluoresce	Did not fluoresce	Fluoresced low
Gray Fish	Was visible	Did not fluoresce	Did not fluoresce	Did not fluoresce

*Fish were illuminated with a band of light, and the peak wavelength is listed.
This summary is based on the images in Figure 8.

TABLE 2. FLUORESCENCE PATTERNS OF GLOFISH IN THE CENTER OF THEIR BODY

	Bright field	UV light (350 nm*)	Blue light (480 nm)	Green light (545 nm)
Red Fish	Was visible	Did not fluoresce	Did not fluoresce	Fluoresced high
Orange Fish	Was visible	Did not fluoresce	Fluoresced	Fluoresced high
Yellow Fish	Was visible	Did not fluoresce	Fluoresced	Fluoresced low
Green Fish	Was visible	Did not fluoresce	Fluoresced	Did not fluoresce
Blue Fish	Was visible	Fluoresced	Did not fluoresce	Did not fluoresce
Purple Fish	Was visible	Did not fluoresce	Did not fluoresce	Fluoresced
Gray Fish	Was visible	Did not fluoresce	Did not fluoresce	Did not fluoresce

*Fish were illuminated with a band of light, and the peak wavelength is listed.
This summary is based on the images in Figure 9.

their GloFish experimental data, the students used chi-square analysis. For this analysis, the null hypothesis was that there was no significant difference between the observed values and the values expected from the postulated inheritance pattern. Thus, the null hypothesis and the experimental hypothesis were the same. In contrast, the assessments asked the students to interpret an experiment where a control group and an experimental group of plants were raised under different conditions (Fig. 4 and Supplementary Material 10). In this kind of experiment, the null hypothesis (that there is no difference between the two groups) would typically be opposite to the experimental hypothesis (that the two groups of

plants will grow differently). The students may not have been able to translate the different ways null hypotheses are used in these different kinds of analysis. An important goal will be to give students the opportunity to apply several different kinds of statistical analysis to their data. In addition, we plan several improvements into our assessment design that will increase our ability to evaluate how successful we are in helping students learn how to apply mathematical and statistical approaches. Importantly, in the future, students will code each of their assessment surveys so that we can track learning of individual students across the semester. This will give us the power to identify significant changes in the responses of

FIG. 9. Different colored GloFish have distinct patterns of fluorescence in their trunk. GloFish with different body colors under room light were assayed for fluorescence emission after excitation by different wavelengths of light. A range of wavelengths was used, and the peak wavelength within each range is listed above each column. Each row contains images of the same fish. The *first column* has images of the whole fish, and the *remaining columns* have images of the center dorsal region of the trunk of the fish, with the fish positioned in a *lateral view with anterior to the left and dorsal to the top*. Brightfield images were taken with a 20 msec exposure, images of fish exposed to 350, 480, 545 nm light were taken at 30 msec exposure, and images of fish exposed to 700 nm light were taken at 200 msec exposure. The exception to this was green fish under 480 nm light, which was taken at a 10 msec exposure because the fluorescence was so bright.

TABLE 3. PREDICTIONS ABOUT WHICH COMBINATIONS OF TRANSGENES CAN BE DISTINGUISHED FROM ONE ANOTHER BY THEIR FLUORESCENCE PATTERNS

	Glo^{RFP}	Glo^{YFP}	Glo^{GFP}	Glo^{BFP}	Glo^{PPF}
Glo^{RFP}	NO	YES*	YES	YES	NO
Glo^{YFP}		NO**	NO	YES	NO
Glo^{GFP}			NO	YES	YES
Glo^{BFP}				NO	YES
Glo^{PPF}					NO

*"YES" means this would be a good cross to use in the classroom for using fluorescence to determine genotype. For instance, if you cross a Yellow (Glo^{GFP}) fish with a Blue (Glo^{BFP}) fish, this would be good choice. This is true because you can easily tell if an offspring contains both GFP and BFP because each these proteins will fluoresce under a different wavelength of light.

**"NO" means this would not be a good cross to use. For instance, crossing a Yellow (Glo^{RFP}) with a Red (Glo^{YFP}) would be a bad choice. This is true because you cannot tell if an offspring contains both YFP and RFP because each of these proteins will fluoresce under 545 nm wavelength light. Therefore, cannot easily tell the difference between a fish that is only carrying the Glo^{YFP} and one carrying both Glo^{YFP} and Glo^{RFP} using the fluorescence in the body of the fish. Note that the answer would be "YES" for this cross if you were instead using fluorescence in the tail fin (see Fig. 3).

These predictions are based on the data in Figure 9, and provide a guide for which dihybrid crosses can be accurately assayed by fluorescence microscopy.

subsets of students that may not be detectable within the larger group.

The goal of the laboratory was to give students hands-on experience with the scientific method: to bring the process of scientific inquiry into the classroom. Thus, another striking outcome from the assessments was that the majority of students reported that learning techniques was the most valuable part of the laboratory. Interestingly, this differential between the goal of the teacher and the goals of the students has been noted in many other studies.¹⁰ As was the case here, it has been found that teachers largely aim at improving students' cognitive skills and problem solving abilities, whereas students are more focused on gaining practical skills.¹⁰ Thus, an important challenge will be to increase engagement by finding ways to bridge the gap between the goals of the students and the instructors.

Independent research

Not surprisingly, the independent projects gave students more opportunity for reflection, discussion, and revision of ideas and hypotheses. While the genetics laboratory students

chose to test hypotheses that were very likely to be supported, Brianna and Adrianna both chose projects where the answer was not known. Further, they both generated data that did not fit their initial hypothesis, and so had the opportunity to challenge their initial ideas about how the biology was functioning. As Adrianna reported in response to a query about which parts of this experience were the most valuable for her "one of the parts that was most interesting was figuring out how fluorescence works in the fish. It took a while to gain an understanding of fluorescent proteins, but the correlation between fluorescence and genetics is so fascinating, and I cannot wait to learn as much about them both as possible." Similarly, Brianna reported that for her, one of the most rewarding parts of her experience was "learning from an original hypothesis that we could not support, and developing new hypothesis based on observations from our collected data." As these types of experiences that require students to construct their knowledge have been associated with positive effects on learning,¹ we hope that Adrianna's and Brianna's experiences will serve as templates for bringing these types of more complex scientific projects into the classroom.

Brianna and Adrianna also independently identified another fundamental key to success in research, the importance of working together as a group to learn and solve problems. Adrianna reported "I think one of the most valuable things for me by doing this project was being able to work in a college lab and interacting with people of different educational backgrounds." Brianna similarly reported that regular laboratory meetings, which were used to brainstorm, evaluate data, and problem solve, were important factors in her success.

Challenges for the future

While successful, these projects also illuminate major challenges in improving science education. The first of these challenges is how to identify the key characteristics that make laboratory projects beneficial to students. Currently, there are only a handful of studies that give insight into these characteristics, and almost all of these are for K-12.^{1,10-13} The second of these challenges is bringing ideas developed locally, such as those presented here, to a larger audience. In particular, many of the ideas for using zebrafish in undergraduate courses could be easily brought into K-12 classrooms. This special issue of *Zebrafish* and other similar efforts such as BioEyes and InSciEd Out (<http://www.bioeyes.org/>, <http://www.insciouted.org/>) are important beginnings. How can we go beyond this and measure the wider range impact of these projects? Traditional methods such as measuring how many

TABLE 4. EXPECTED FLUORESCENCE PHENOTYPE PROGENY FOR A "YES" CROSS

Parental Genotypes: Glo^{GFP}/Glo^- (Lime Green) X Glo^{BFP}/Glo^- (Blue)

Progeny genotype	Excitation wavelength*		
	350 nm	480 nm	545 nm
Glo^{GFP}/Glo^-	Will not fluoresce	Will fluoresce	Will not fluoresce**
Glo^{BFP}/Glo^-	Will fluoresce	Will not fluoresce	Will not fluoresce
$Glo^{GFP}/Glo^-; Glo^{BFP}/Glo^-$	Will fluoresce	Will fluoresce	Will not fluoresce**
Neither Glo^{GFP} nor Glo^{BFP}	Will not fluoresce	Will not fluoresce	Will not fluoresce

*A band of wavelengths was used, and the peak wavelength is listed; **will have only very dim fluorescence.

Note that each genotype gives a different fluorescence pattern. See Table 3 for further explanation of a "YES" cross.

TABLE 5. EXPECTED FLUORESCENCE PHENOTYPE PROGENY FOR A “NO” CROSS

Parental genotypes: Glo ^{YFP} /Glo ⁻ (Yellow) X Glo ^{RFP} /Glo ⁻ (Red)			
Progeny genotypes	Excitation wavelength*		
	350 nm	480 nm	545 nm
Glo ^{YFP} /Glo ⁻	Will not fluoresce	Will fluoresce	Will fluoresce
Glo ^{RFP} /Glo ⁻	Will not fluoresce	Will not fluoresce	Will fluoresce
Glo ^{YFP} /Glo ⁻ ; Glo ^{RFP} /Glo ⁻	Will not fluoresce	Will fluoresce	Will fluoresce
Neither Glo ^{YFP} nor Glo ^{RFP}	Will not fluoresce	Will not fluoresce	Will not fluoresce

*A band of wavelengths was used, and the peak wavelength is listed.

Note that every different genotype does not have a distinct fluorescence pattern. See Table 3 for further explanation of a “NO” cross.

times an article is cited seem insufficient, as much of what happens in classrooms is never published.

The key to both of these challenges may be to find more ways to build bridges between teachers and scientists. Such bridges would enable us to generate assessments that could be used to identify effective laboratory experiences for students through their whole development as learners, for kindergarten through seniors in college. Ideas and knowledge fundamental to K–12 teachers, such as how to create a curriculum and measure impacts could cross over to the scientists, and ideas about how to use strangely colored zebrafish to learn about biology could cross over into more classrooms.

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Author Contributions

Brianna M. Vick recently completed her B.S. degree in Biology at the University of Minnesota Duluth. Her research was funded through the Pathways to Advanced Degrees Program, which she began in June 2011. Brianna established and piloted the caudal fin fluorescent imaging experiments for the Genetics Laboratory course and led the research project designed to uncover the causes of color variation in orange GloFish. She developed and analyzed the assessments for the students in the Genetics Laboratory course. Her re-

search was presented at the University of Minnesota Duluth 2012 Undergraduate Symposium.

Adrianna Pollak is a high school student at Cloquet Senior High School in Cloquet, MN. Adrianna carried out the experiments that characterized the fluorescence emission patterns of different GloFish strains as part of her 2011–2012 Science Fair project. She presented her work at several conferences, including the MN Academy of Science State Science Fair, National American Indian Science and Engineering Fair (NAISEF), and the Tri-State Junior Science and Humanities Symposium.

Cynthia Welsh is a science teacher at both Cloquet Senior High and Middle School, and the director of the NE Minnesota and American Indian Science and Engineering Regional Fair. She was instrumental in matching Adrianna with the Liang laboratory, guiding Adrianna through the research needed to revise her hypotheses, and helping her create the figures to present her data and ideas in her research paper and poster presentations. You can read more about her work at <http://thewomantoday.net/womantoday/august2012/index.html>.

Jennifer O. Liang is an Associate Professor in the Biology Department at the University of Minnesota Duluth. She mentored Brianna Vick and co-mentored Adrianna Pollak in their GloFish research, and provided training and technical support for some of their experiments. She combined the caudal fin fluorescence imaging method that Brianna and Adrianna developed with other techniques for the GloFish experiments done by the students in her Genetics Laboratory course.

Disclosure Statement

No competing financial interests exist.

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