Supplemental Material

CBE—Life Sciences Education

Shim *et al*.

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SM1. **Semi-structured Interview Protocol** approved by Purdue IRB #1811021367; Participant identity and responses were made anonymous by the interviewer with names kept strictly confidential.

SM2. **Lab Manual Appendices** (examples) provide guidelines for effective teamwork, lab decorum and safety procedures, research design, data analysis, and communication guidelines for students.

SM3. **Recruitment Email** for undergraduate student participant approved by Purdue IRB #1811021367

SM4. **Zebrafish Lab Protocols** with References to Known Scientific Challenges

SM5. **Codebook Examples and Glossary**, including a Glossary of emotion codes and codebook examples to illustrate steps in Table 3: Step 2 axial and focus coding, Step 3 Process coding example, and the Step 4 final hierarchy chart for codes including those defined in Table 4.

SEMI-STRUCTURED INTERVIEW PROTOCOL

Instrument approved by Purdue IRB #1811021367 for A Qualitative Study Exploring Undergraduate Students' Perspectives on Failed Research Experience in a Biology Lab Course

Date & Time of Interview:
Type of Interview (In-Person or Phone):
Interviewer:
Interviewee (Alias/Code/ID):

Introduction:
The purpose of this research study is to explore undergraduate students' failed research experiences in the first-year lab biology course at our university. I will ask you a series of open-ended questions focusing on your experiences. Please answer these questions honestly, so that I can accurately capture and understand your experiences. As mentioned in the informed consent I provided you with earlier, your identity and responses will remain anonymous in this research study and will be kept strictly confidential. You can skip any question that you do not want to answer. Do you have any questions before we begin this interview?

Interview Questions:

- What are your interests in biology? Do you enjoy learning biology in the laboratory?
- What initial expectations did you have about your ability to complete research as a PI?
- Have you ever felt uncomfortable when you were asked to conduct open-ended research?
- Have you ever felt unsuccessful in the biology lab course? When? Why?
- How do you define failure when you conduct research?
- How did you feel when the result did not turn out the way you expected?
- What did you do when things did not go according to your plan?
- What was the problem in your research? (What challenges have you experienced while conducting your research in the lab?) How have other peers in your group approached the problem? How have you approached it?
- How did you respond to the unexpected research data or research results that were not preferable or successful?
    - Probe: What kind of help did you receive?
    - Probe: What things did you do to prepare yourself to complete the research?
- What kinds of messages did you receive from your teaching staff or peers about research data? How are these related to your perspectives about your research experience?
- How did you use or express unexpected research data while you were conducting research and in your research paper?
- What motivates you to keep pursuing research with unexpected research data?
- What are your beliefs about your ability to conduct better research in the future?
- What did you learn from this failed research experience?
- How confident do you feel about your ability to conduct successful research in the future?
    - Probe: What makes you feel confident? /What makes you NOT feel confident?
- How did this experience affect your perspective about yourself as an investigator or scientist?

- Is there anything that you would like to share with us that I did not ask you about?

Demographic Questions:

- Tell me about yourself?
    - Nationality
    - Native language
    - Race
    - Year in School
    - Major(s)
    - Minor(s)
- Do you have any other experience conducting research in the lab?
- Are you currently looking for a job/internship in biology fields in the future?

Closing:

Thank you for taking time out of your day to share your experiences with me in this interview. I have learned so much from you in this interview.

Appendix A Guidelines for Effective Teamwork and Lab Decorum

To work effectively, organize your team with strategies for efficiency and success on tasks that are done by the group. Decide on a division of labor for the tasks and assign specific roles. Write down the team member names and roles in your lab notes. Modify the duties described below as needed for a team of two or a team of four investigators. *Decide who will be the Principal Investigator for your team and decide how to take turns rotating the Principal Investigator role for each of the three Lab Module.* Rotate through the roles so you have experience with each role for one of the Lab Modules.

• *Principal Investigator (PI)*:
a. makes sure the entire team is focused on the same research question or hypothesis to test, keeps the group on task, and within the time budget.
b. makes sure each step of the task is completed in sequence.
c. looks up additional information, diagrams, and assigns readings to help the group.
d. leads the discussion in planning the task and in wrapping up the work.
e. checks that everyone has learned the techniques and understands what was done or studied.
f. takes the lead in preparing the written and oral reports to the class when the team shares their results.

• *Materials Manager (MM)*:
a. gathers all equipment and materials for the group.
b. sets up the materials or directs the setup.
c. operates the equipment and teaches others to operate equipment needed for the research.
d. consults with the teaching staff or visits other groups to get ideas or class data as needed.
e. makes sure that each group member understands how to operate the equipment.
f. organizes the clean-up by discarding lab waste appropriately as instructed.

• *Reader-Reporter (RR):*
a. locates the protocol directions and summarizes steps for the group as the work proceeds.
b. keeps everyone on track to record data, finds and records data from other lab sections when needed, records observations and notes, answers to questions, etc. as each task proceeds.
c. when new information is needed, looks for appropriate channels, develops partnerships.
d. makes sure that each group member gets the information that is collected.
e. communicates with the teaching staff about questions or problems the group encounters.
f. coordinates preparing a report on the team's work.

Suggestion: The PI may want to distribute the work by having one team member learn about the equipment, one learn about methods for communicating findings, and one to review theoretical perspectives that should be considered in planning the research so the group can use that information to make sense of any observations made during the research. All investigators are expected to learn how to operate all equipment for each module, to record and analyze the data, and to use data as evidence to test a hypothesis or answer the research question, but it can be useful to have a different investigator take the lead to teach the others.

Appendix B Lab Routines and Safe Lab Procedures

How to label containers. Label a container before something is pit in it. Write the name of the substance, its concentration, the date, along with your initials by writing on the container with a Sharpie pen (or use the team's lab tape color to write on and stick on the container).

General guidelines for discarding lab waste.
Every paper, cell, tissue, chemical solution, slide, pipet, beaker or tube has its own protocol for being discarded (or it has a place for being prepared for reuse). A good investigator does not casually throw things away, leave dirty supplies on lab benches and counters, or discard trash in the sink. Always be careful about discarding items, not only for safety reasons, but because lab waste that is not appropriately disposed of creates a hazard.

Before discarding any lab waste, know the following:

- It's chemical composition.
- If it is hazardous or nonhazardous. When in doubt, look up a substance's MSDS.
- If it is a biohazard. A biohazard is anything liquid or dry derived from a living thing or that comes in contact with living things.
- If it can be cleaned for reuse.

How to discard specific lab wastes.
Acids. Small amounts (<100 mL) or dilute acids get neutralized (check with pH paper) before slow-ly pouring down the drain followed with large amounts of water. Larger volumes of strong acids must be handled as hazardous chemical waste.

Bases. Small amounts (<100 mL) or dilute bases get neutralized (check with pH paper) before slow-ly pouring down the drain followed with large amounts of water. Larger volumes of strong bases must be handled as hazardous chemical waste.

Biohazard liquid waste (>1 mL). Cell extracts or animal blood can be autoclaved, or can be treated with a 10% Clorox solution to the liquid waste (-equal volume of 10% Clorox as volume of waste) and let stand for 30 min before pouring it down the sink with running water.

Biohazard dry waste. Disposable lab containers with cells or tissue are disposed of in the solid biohazard waste container lined with a biohazard bag.

Buffers. Most buffers can be poured down the sink. When in doubt, look up the buf-fer's MSDS.

Chemical waste, hazardous. All hazardous chemical wastes get their bottle/container labeled and/or tagged (REM provides labels to use). When in doubt, check the chemi-cal's MSDS. On the label put your name, the chemical name and percent of solution, the pH of the waste (use pH paper), the volume of chemical hazard waste, and its building location. Keep hazardous chemical waste bottles/containers in the appropriate holding area until picked up by REM.

Chemical waste, nonhazardous. Dispose of nonhazardous chemical waste as trash if solid. Nonhazardous chemical waste liquids or solutions should be poured down the drain with copious volumes of running water.

Glass, broken. Broken pieces of glassware that have not been in contact with a biohazard are swept up and put in the broken glass box in the lab; do not put broken glass in the trash can. Broken glass that has touched a biohazard is put in the sharps container (see be-low).

Gloves. If used with a biohazard then they go into the solid (dry) biohazard bag in a biohazard container. Otherwise, used gloves go into the recycling box for used gloves.

Microscope slides and coverslips. Used glass microscope slides and coverslips go in the broken glass box if they haven't come into contact with a biohazard. If they have come into contact with a biohazard then they go into the sharps container.

Needle and syringe. A used needle and its attached syringe always go into the sharps box.

Paper. Items such as paper towels, Kim wipes, or glassware wrappers go into the trash can, unless they touched a biohazard. Contaminated disposable paper and labware go into the biohazard waste con-tainer lined with a biohazard bag.

Pipetman tips. Used Pipetman tips go into the pipet tip disposal container.

Solvents. Solvents such as those used for cytological stains always go into a chemical waste container. Never pour solvents down the sink.

Transfer pipets (plastic disposable). Transfer pipets go into the trash if not used with a biohazard. If used with a biohazard, they go into the biohazard bag in a biohazard container.

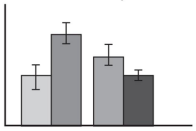
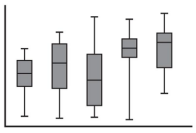
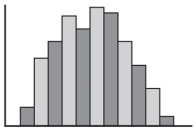
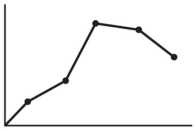
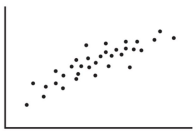
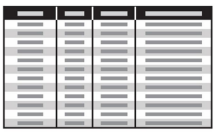
Proper attire for working in a biology lab

For safety, do not wear open-toe shoes, sandals, or flip flops. Do not wear a shirt or sweater with baggy sleeves, or tie the sleeves with a rubber band or roll them up securely. Shoulder length or longer hair is tied back so it will not fall into something or get caught in the equipment. When wearing shorts or a skirt, take care not to spill anything on bare skin, because spilling something on legs could be a health hazard.

Other routine lab procedures

- It is always a good idea to wash hands before and after work in a lab.
- Thoroughly rinse re-useable glassware and containers like flasks, beakers, graduated cylinders, cuvettes and test tubes with tap water, then rinse with RO water before putting in the appropriate "dirty glassware" basket.
- Put "rinsed" bottles and flasks in a dirty glassware basket, and put all rinsed test tubes and cuvette tubes in a wire rack with the tube's open end pointing down.
- All paper wrappings go in the trash.
- It is always a good idea to wipe down your work area (lab bench) with paper towels and Chlorox wipes or 70% ethanol before you start working in lab, and when you finish.
- Use every piece of equipment as instructed, and treat every piece of equipment used as if it were your own. If a piece of equipment gets broken, let the person in charge know so it can be fixed or replaced.

Appendix C Guidelines for Graphs and Data Displays

Data Display Type	Usage	Advantages	Disadvantages
Bar Graph 	To compare categorical data, percentages, or summary statistics from multiple groups. Each bar represents a category; shape can be changed by moving the categories around.	Useful for understanding distributions from large datasets. Stacked bars or shading of bars can be used to distinguish the different levels within the data.	Obscures the distribution of data, number of data points, and their values.
Box and Whisper Plot 	To show distribution of data from one or multiple groups.	Shows and compares distributions of large datasets.	Should not be used for small datasets. Does not show individual data (except for outliers).
Histogram 	To show a distribution of data with the independent variable as continuous. Uses numerical data instead of categorical data.	Shows the shape of the distribution of data with a continuous variable.	Must choose the bin size wisely to avoid influencing the shape being too compressed or too dispersed.
Line Graph 	To show how a single variable or multiple variables changes over time or to show how a variable deviates from a set baseline X axis portrays categories while the Y axis portrays quantitative values.	Shows direct relationships and may be used to predict relationships between continuous variables.	Not appropriate for representing ranked, part-to-whole, or correlational data.
Dot Plot 	To show distribution of small data sets from multiple groups. The independent variable is categorical and the dependent variable is continuous.	Shows all data from multiple categories and the distribution within each category.	Not appropriate for representing a large data set because the plot will become cluttered and it will be difficult to see the individual points.
Scatter Plot 	To show individual data points from bivariate data.	Shows the relationship between variables. Shows trends in the data and any noticeable outliers.	It may be difficult to extract individual data points if they fall on the same or nearby coordinates.
Tables 	To display simple relationships between numerical values and categorical groups, so that individual values can be easily extracted from the rows and columns. Often used for small data sets.	Since values in a table are encoded as text, it is easy to extract individual values. Numbers in a table can be displayed with decimal precision. A table can also communicate multiple sets of data with different units.	Tables may make it difficult to interpret the take home message if not organized properly.

Adapted from Angra & Gardner, Adv. Physiol. Ed 40: 123-128, 2016.

Appendix D Experimentation Guidelines

Five Typically Difficult Parts to an Experiment and the Rubric for Experimental Design (RED)
Adapted from Dasgupta, Anderson, & Pelaez, CBE-LSE 13: 265-284, 2014.

Areas of Difficulty	Correct Ideas	Guidelines to Avoid Typical Difficulties
(1) Variable Property of an Experimental Subject	**Experimental subject or units:** The individuals to which the specific variable treatment or experimental condition is applied. An experimental subject has a variable property. A variable is a certain property of an experimental subject that can be measured and that has more than one condition.	**a.** Know that your experimental subject is not a variable.
		b. What groups of experimental subjects would have a variable property *in line* with the target for the stated investigation or claim to be tested?
		c. Be consistent in your use of appropriate experimental subjects with relevant variable properties throughout your proposed experiments.
(2) Manipulation of Variables	**Testable hypothesis:** A hypothesis is a testable statement that carries a predicted association between a treatment and outcome variable.	**a.** Mention BOTH the treatment and/or outcome variables in the research question or hypothesis statement.
		b. Do your hypotheses and predictions clearly indicate the expected outcome to be measured from a proposed experiment?
	Treatment group: A treatment group of experimental subjects or units is exposed to experimental conditions that vary in a specific way.	**c.** Assign treatments to experimental units in a manner appropriate for the goal of the experiment.
		d. Use only treatment conditions that are suitable physiologically for the experimental subject according to the goal of the investigation.
	Combinatorial reasoning: In experimental scenarios when two or more treatment (independent) variables are present simultaneously, all combined manipulations of both together are examined to observe combinatorial effects on an outcome.	**e.** Did you systematically consider all combinations of treatments in scenarios where the effect of two or more different treatments are to be determined?
	Controlling outside variables: The control and treatment groups are required to be matched as closely as possible to equally reduce the effect of lurking variables on both groups	**g.** Did you match appropriate variables to the research question across treatment and control groups (avoiding a prior knowledge bias)?
	Control group: A control group of experimental subjects or units, for comparison purposes, measures natural behavior under a normal condition instead of exposing them to experimental treatment conditions. Parameters other than the treatment variables are identical for both the treatment and control conditions.	**h.** Did the control group provide an opportunity to observe natural behavior conditions in the absence of the variable being manipulated in the treatment group, because conditions for both groups were suitable for all the research subjects?
		i. Were the control group treatment conditions appropriate for the stated hypothesis or experiment goal?
		j. Did you avoid any obvious differences when assigning experimental subjects to the treatment

Five Typically Difficult Parts to an Experiment and the Rubric for Experimental Design (RED)
Adapted from Dasgupta, Anderson, & Pelaez, CBE-LSE 13: 265-284, 2014.

Areas of Difficulty	Correct Ideas	Guidelines to Avoid Typical Difficulties
		vs. control group?
(3) Measurement of Outcome	Treatment and outcome variables should match up with proposed measurements for outcomes. These can be categorical and/or quantitative variable treatments. A categorical variable sorts values into distinct categories. A quantitative or continuous variable answers a "how many?" type question and usually would yield quantitative responses.	**a.** Did you mention and consider the relationship between a treatment and outcome variable? **b.** Do not reverse the treatment and outcome variables. c. Do not treat an outcome variable that is quantitative as a categorical variable.
	Outcome group: The experimental subject carries a specific outcome (dependent variable) that can be observed/measured in response to the experimental conditions applied as part of the treatment.	**c.** Did you propose outcome variables that are relevant for the proposed experimental context provided or appropriate for the hypothesis? **d.** Is your stated outcome measurable or not? **g.** Is there a good match between what the investigation claims to test and the outcome variable?
(4) Accounting for Variability	Experimental design needs to account for the variability occurring in the natural biological world. Reducing variability is essential to reduce effect of non-relevant factors in order to carefully observe effects of relevant ones.	**a.** Since a sample of experimental subjects cannot eliminate natural variability with those subjects, did you propose to collect data on variability by including a method to measure variability?
	Selection of a random (representative) sample: A representative sample is one where all experimental subjects from a target demographic have an equal chance of being selected in the control or treatment group. An appropriate representative sample size is one that averages out any variations not controlled for in the experimental design.	**b.** Did you propose uniform criteria for selecting experimental subjects for treatment vs. control groups? **c.** Did you propose criteria for selecting experimental subjects for investigating in a way that is representative of the target population?
	Randomized design of an experiment: Randomizing the order in which experimental subjects or units experience treatment conditions as a way to reduce the chance of bias in the experiment. Randomization can be complete or restricted. One can restrict randomization by using block design, which accounts for	**d.** Did you randomly assign experimental subjects to treatment vs. control group without any bias for assigning members to each group? **e.** Did you randomly assign treatments to the groups and not just random assignment of the experimental subjects?

	known variability in the experiment that can't be controlled.	
	Replication of treatments to experimental units or subjects: Replication is performed to assess natural variability, by repeating the same manipulations to several experimental subjects (or units carrying multiple subjects), as appropriate under the same treatment conditions	**g.** Did you do replications by repeating the entire experiment *at some other time* with another group of experimental subjects?
		h. Do you show evidence of replication or suggest a need to replicate as a method to access variability or to increase validity/power of an investigation?

(5) Scope of Inference of Findings	**Scope of inference:** Recognizing the limit of inferences that can be made from a small characteristic sample of experimental subjects or units, to a wider target population and knowing to what extent findings at the experimental subject level can be generalized.	**a.** Did you propose an inference from a sample that is appropriate to the intended target population? Did you refrain from under- or overestimating your findings beyond the scope of the target population? **b.** Did you carry out steps to randomly select experimental subjects' representative of the target population about which claims are made?
	Cause and effect conclusions: A cause-and-effect relationship can be established as separate from a mere association between variables only when the effect of lurking variables are reduced by random assignment of treatments and matching treatment and control group conditions as closely as possible. Appropriate control groups also in comparison to the treatment group also need to be considered.	**c.** Did you appropriately decide if a causal relationship is warranted or if the data shows only association between variables? Correlation does not establish causation.

Appendix E Lab Notes, Measurements, Numbers, and Team Problem Solving Strategies

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Lab notes. A good investigator records what they do in a lab, the data collected, and any results or the observations. Lab notes must be clear, com-plete, and thorough. If something goes wrong, anyone should be able to read the lab notes to find out what went wrong. Investigators in a research lab that has government funding are required to keep lab notes in a format that another person will be able to understand.

Entries for each lab contain the investigator's name, date, and section number on each odd-numbered page. Use both sides of the notes pages. Printed on some lab notes pages are that lab's number and lab title, but also, each part of the investigation may be given a unique title.

Procedures and flow charts. Before entering the lab, a good investigator will write on that lab's notes pages the essential "steps" for doing the procedures and flow charts for that lab. Identify each procedure and flow chart with a number or letter to keep the procedures, flow charts, and eventually the results and conclusions organized. Write the essential "steps" of what the investigators will do, and with what equipment and supplies, but refer to a page that has the protocols and do not transcribe a whole procedure from a published source like the lab manual. The goal is to be able to work from abbreviated procedures and flow charts without having to read a protocol word-for word, which would take time away from the research tasks. Investigators who come to lab with a clear outline of written procedures or flow charts will finish a lab on time, and with fewer mistakes.

Data and observations collected. "Dry labbing" (copying data or observations from another student or fabricating data) is not acceptable and doing so is an unethical act of academic dishonesty. A good investigator will record their own data and observations in their own lab notes. For all procedures where data or observations are collected, follow these instructions:

- Before recording raw data, put a title or description above the recorded data as lab notes.
- Determine the number of variables to be measured for a data set, as well as their relationship with each other before "laying out" the columns and rows for recording data. This means:
    a. The first column has the values of the independent variables, with subsequent columns for the values of individual dependent variables.
    b. If measuring several variables for the same sample, give each variable its own row.
    c. For a time-course study, put data in a single column, with each row labeled with the different time points.
- Arrange all rows and columns to reflect the order when data is collected.
- Take into consideration whether additional columns for subsequent calculations will be needed (totals or average counts for example); create a separate column for each mathematical manipulation so step-by-step calculations are clear.
- Write notes legibly and accurately, especially numbers:
    a. Record numerical data (number and its unit) with the correct number of significant figures; do not round off recorded "raw" numerical data values because doing so could

affect any subsequent analysis of these values.
b. Denote discrete data collected; often it's useful to record discrete data as a tally count.
c. Explain any unusual data values or observations encountered in a footnote with the collected data; a good investigator never relies just on their memory.

• Along with recorded raw data and observations, include any charts or graphs built from this raw data. Be sure to always label axes of plotted data with the units (where appropriate), include a figure legend to identify the source of the data depicted, and write a caption according to Appendix H to briefly describing what is shown in the figure, chart or graph.

Team Problem-solving Strategies: When possible, investigators work in groups to come up with and agree upon the solution to a problem. All members of the group can agree on what strategy to use for solving the problem. The problem-solving method most often used in research is known by many names: "factor-label," "unit factor," "unit analysis," "dimensional analysis," etc. All of these methods involve breaking down a problem into parts (also called "unit factors" or "equivalents") and then simply arranging all the parts or variables in the problem so all units will cancel except those required for the final answer.

First figure out a problem-identification and problem-solving procedure that is appropriate (such as outlined below). Then, generate possible solutions by solving the problem for yourself. Evaluate and test the solutions with others in the team to identify any mistakes and decide on the best solution. Finally, implement the solution, and evaluate by interpreting what the solution means and whether the answer seems logical. A good investigator always prepares to describe the solution and the steps that the team took. These six steps might be helpful:

1. What do you know? Draw a diagram. As a team, look at each other's diagrams to determine if you share the same understanding of the problem.
2. List the values and units of measure for what you are given. Determine and write down the units of measure for the final answer.
3. List all the values and units of measure for other relevant information. Determine what is needed to solve the problem.
    - Considering what information was given to start with, how each piece of information can be converted into a unit factor.
    - Write down any conversion factors that are needed to solve the problem.
    - Recognize extraneous information that is given but not needed to solve the problem.
4. Set up numbers with units in an equation. Arrange the unit factors so all units cancel except those units needed in the final answer.
5. Estimate an answer using easy number approximations. Then do the calculations. Check that your final answer has the correct units and the correct number of significant figures.
6. Check your work and write a statement about why that answer seems logical.

Example: What is the stomata density D calculated by Franks and Beerling (2009) as the number of guard cell pairs in each mm^2 of a leaf? See page I21 for a worked example.

The other terms and concepts you should recognize from the previous problem are as follows:

- **metric units and conversions**—i.e., liters, milliliters, grams, and kilograms, and conversions between them—to help in recognizing metric units from nano to giga, their symbols and corresponding factors are listed in tables below.
- **density,** defined as counts per unit area (/mm^2) or as counts per unit volume reported in counts per milliliter (/mL) or counts per cubic centimeter (/cm^3) or for solutions as grams per milliliter (g/mL) for solids and liquids and grams per liter (g/L) for gases
- **exact** versus **approximate** conversions or unit factors
- **significant figures** ("sig figs") or **significant digits**

If you are unfamiliar with any of these terms, review them and record notes as lab notes.

The conversion factors to be used in the lab come from the International System of Units (Système international d'unités or SI), which was an international standard metric system. In November 2018, the world's measurement experts revised the SI, approving a measurement system that is entirely based on physical constants of nature that took effect on World Metrology Day, May 20, 2019. The SI base units redefinitions included two of relevance to biology: the kilogram and the mole. Other SI base units of relevance to biology were unchanged: the meter and the second (http://www.worldmetrologyday.org/press_release.html) See https://www.nist.gov/pml/weights-and-measures/metric-si/si-units for a US government website on unit conversions and metric policy.

SI Metric Prefixes

	Prefix	Abbreviation	Value	
Larger quantities or whole units	tera-	T	10^{12}	Trillion
	giga-	G	10^{9}	Billion
	mega-	M	10^{6}	Million
	kilo-	k	10^{3}	Thousand
	hecto-	h	10^{2}	Hundred
	deka-	da	10^{1}	Ten
	Basic unit			
	meter	m	10^{0}	One
	gram	g		
	liter	L		
	mole	mol		
	second	s		
Smaller quantities or subunits	deci-	d	10	Tenth
	centi-	c	10^{-2}	Hundredth
	milli-	m	10^{-3}	Thousandth
	micro-	u	10^{-6}	Millionth
	nano-	n	10^{-9}	Billionth

The new definition of the mole connects Avogadro's Number (6.02 x 10^{23}) for a mol as the fundamental base unit of measurement in the SI to fundamental constants of the universe

including the speed of light in vacuum and the amount of charge in an electron. When the mole is used, the elementary entities must be specified and may be atoms, molecules, ions, electrons, other particles, or specified groups of such particles.

The SI unit of concentration is defined as the mole per cubic meter (mol/m^3), but this unit for concentration is not appropriate for life science research. In the metric system of measurement, designations of multiples and subdivision of any unit may be arrived at by combining with the name of the unit the prefixes deka, hecto, and kilo meaning, respectively, 10, 100, and 1000, and deci, centi, and milli, meaning, respectively, one-tenth, one-hundredth, and one-thousandth. In science, multiples larger than 1000 and for subdivisions smaller than one-thousandth are sometimes needed, so use the prefixes in the table above.

The following conversion equivalents are provided for your convenience and to help avoid needless human error in performing calculations:

Units of Length

10 millimeters (mm)	1 centimeter (cm)
100 centimeters	1 meter (m) = 1000 millimeters
1000 meters	1 kilometer

Units of Area

100 square millimeter (mm^2)	1 square centimeter (cm^2)
100 square centimeter	1 square decimeter (dm^2)
100 square decimeters	1 square meter (m^2)
1 square hectometer (hm^2)	1 hectare (ha)
100 square hectometers	1 square kilometer (km^2)

Units of Liquid Volume

10 milliliters (mL)	1 centiliter (cL)
10 centiliters	1 deciliter (dL) = 100 milliliters (mL)
1000 milliliters (mL)	1 liter (L)

Units of Volume

1000 cubic millimeters (mm^3)	1 cubic centimeter (cm^3)
1000 cubic centimeters	1 cubic decimeter (dm^3) 1 000 000 cubic millimeters (mm^3)
1000 cubic decimeters	1 000 000 cubic centimeters (cm^3) 1 000 000 000 cubic millimeters (mm^3)
1 cubic meter (m^3)	1 000 000 cubic centimeters (cm^3) 1 000 000 000 cubic millimeters (mm^3)

Everyday Estimation

Mass		Length	
150 g	Cell Phone	15.3 cm	Currency ($5 Bill)
5 g	Nickel Coin ($0.05)	19 cm	Pencil
2.5 g	Penny Coin ($0.01)	1 mm	Thickness of a Dime
1 g water	¬1 mL of water	7 um	Average Human Hair
600 mg	Cotton Ball		

Temperature		Volume	
100 ¬°C	Boiling Water	5 L	Blood in a Human
37 ¬°C	Human Body Temperature	3.79 L	Gallon of Milk
20 ¬°C	Room Temperature	355 mL	Can of Soda
0 ¬°C	Water Freezes	15 mL	Eye Drop Bottle
		1 mL	¬ 1 sugar cube
		1 mL	1 cm^3

Measuring Volumes: Use these Steps to Dispense Liquid Accurately with a Pipetman®

1. Select the model Pipetman® that delivers a volume in the range needed (see Table below). The maximum volume of a Pipetman® is on the end of the plunger at the top. A Pipetman® is accurate only within a certain recommended range, so do not use a Pipetman® to deliver volumes below or above these recommended volumes for that model.

Different models of Pipetman® in our lab are useful for different volume ranges, therefore it is important to choose the appropriate Pipetman® for the volume to be measured. Refer to these values for each Pipetman®

Model	Adjustable	Recommended	Smallest Increment
P-2	0 to uL	0.1 to 2 uL	0.002
P-20	0 to 20 uL	2 to 20 uL	0.02
P-100	0 to 100 uL	10 to 100 uL	0.2
P-1000	0 to 1,000 uL	100 to 1,000 uL	2.0
P-5000	0 to 5,000 uL	500 to 5,000 uL	2.0

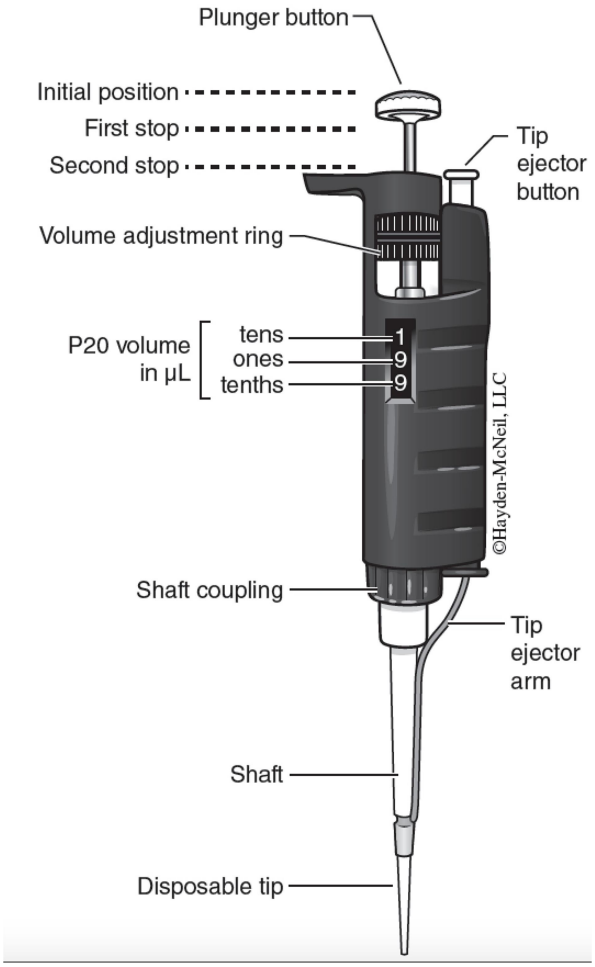
2. Set the volume you want to deliver. There are two ways to set the volume a Pipetman® delivers: using the volume adjustment ring, or turning the plunger button. Both are accurate ways to set the volume of the Pipetman®.

3. Fit a disposable tip on the end of the shaft the Pipetman®. Be sure it is the right size tip - most are color-coded to the plunger button color. Insert the tip enough to make a tight seal.

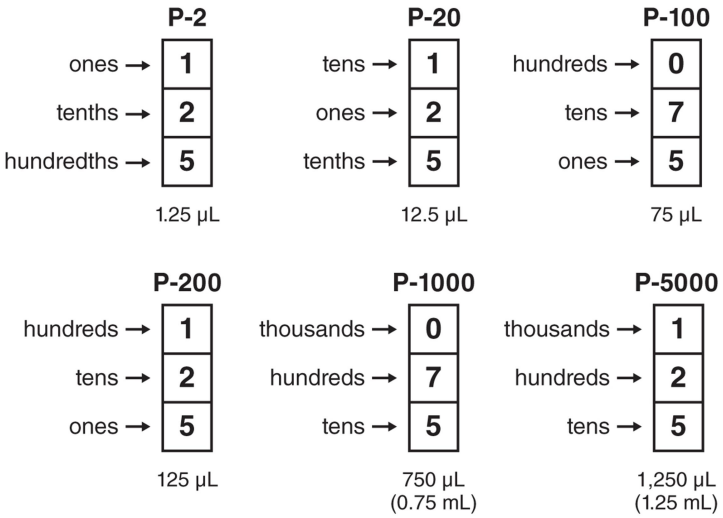
4. Immerse the tip between 1-2 mm for micro-volume pipettes and up to 6-10 mm for large-volume pipettes.

4. Press the plunger of the Pipetman® to its "first stop."

5. Keep the Pipetman® vertical, then immerse and hold the tip in the liquid sample. The angle of your pipette tip in the sample surface should be close to 90° degrees and should not deviate more than 20° degrees from vertical.
6. Pre-rinse the tip two or three times to form a liquid film in the tip to increase the accuracy. This will neutralize capillary effects in micro-volume pipettes and, for large-volume tips, the air temperature inside the tip will equalize with the temperature of the sample. To confirm that the Pipetman® is delivering the desired volume according to the adjusted Pipetman® volume scale, compare the amount of liquid pulled into the tip with the maximum capacity for that tip. The digital volume scale is read from top to bottom (top being the plunger end, and bottom the shaft end). Depending on the Pipetman® model (volume range), different digits represent different volume increments. The Figure in the corner below shows how to read some sample volume settings for the different models in our lab.



7. Allow the plunger button to return slowly to its up position. For larger volumes - typically 1 ml or greater - pause about 1 second or more after the sample pick up, with the tip still in the liquid. This will allow the sample to fully aspirate. Withdraw the tip from the liquid sample. Check the volume again. Confirm there are no air bubbles in the sample in the tip.
8. Dispense into the liquid or onto the surface of the receiving solution. Be careful to avoid picking up sample after dispensing. Also maintain consistent speed and smoothness when pressing the plunger to dispense the sample. Uncontrolled action can cause bubbles, splashing, aerosols and contamination of the pipette shaft and piston and can also lead to loss of sample.
9. Press the plunger to the second stop to expel any remaining liquid. While keeping the plunger fully depressed, remove the Pipetman®



from the receiving container. Once out, slowly let the plunger return to its up position.
10. Eject the disposable tip by pressing the tip ejector button. Discard it into a tip disposal container. Use a fresh tip for each different sample to prevent sample "contamination."
11. When finished, return the Pipetman® to its storage rack.

Checking the Accuracy of a Pipetman®. To confirm that a Pipetman® is delivering the volume it is set to deliver, periodically check its accuracy by dispensing volumes of RO water and weighing the water delivered with an electronic balance. This accuracy check uses the fact (actually a close approximation listed in a table above) that at room temperature, 1 mL with a volume of 1 cm^3 has a mass of 1 g for water.

Measuring Dry Chemicals: Use these Steps to Measure with an Electronic Balance
1. Check the bubble level of the balance by opening its lid; use the adjustable feet to level the balance. With the lid open, clean off (with a brush) the balance's weigh pan if needed and then close the lid. Press the "Zero" button on the balance to zero it; the display shows 0.000 g. Gently lift the 200 g calibration weight with a forceps. Lift the balance lid and place the calibration mass onto the center of the weigh pan. The display should show 200.000 g (the calibration weight's mass) and give you some information about the number of significant digits. It is normal for the readout to fluctuate ± 0.003 g due to surrounding drafts. When near an electronic balance, try to be as still as possible. Be patient, it may take a moment before a balance's readout to stabilize. Remove the calibration weight and return it to its storage container. NOTE: When you record any measurement, the last few digits are approximations.

2. If calibrated, when you press the "Zero" button again, the display shows 0.000 g. Lift the lid and place an empty weigh boat or weigh paper onto the center of the weigh pan. Then press the "Zero" button again to tare the balance (display shows 0.000 g). Always use a weigh boat, or a weigh paper when measuring the mass of substances or liquids with an electronic balance; Once you tare the balance, the mass of the weigh boat or weigh paper placed in the center of the weigh pan of the balance will not be included in the mass of the sample to be measured.

3. Gently place the object to be weighed in the weigh boat or on the weigh paper, then read the mass of the sample. Remove the weigh boat, paper, or beaker with the sample from the tray/pan. If liquid or a chemical gets spilled on the weighing pan or anywhere on the balance, wipe it up or clean off any particles from the weigh pan with a brush immediately.

Calibrating an Electronic Balance
With the bubble level in balance, the lid open, and the balance's weigh pan clean, close the balance's lid. Press the "Zero" button on the balance to zero it; the display shows 0.000 g. Once zeroed, press the "Cal" button to start calibrating the balance; the display will show 200.000 g (the calibration weight's mass). Press the "Enter" button to confirm (CAL will show on the display when the “Enter" button is pressed), and then the display will flash 200.000 g (the calibration weight's mass). Lift the lid and gently place the 200 g calibration weight onto the center of the weigh pan; the display will stop flashing, and just show 200.000 g. Wait until the 200.000 g display “settles” to insure calibration of the balance. Then remove the calibration weight, return it to its storage container, and close the lid.

Appendix H Guidelines for Creating Figures and Figure Captions for Research Communication

Before creating a panel of figures to report findings, make a plan to analyze the data. For example, sketch graphs to show how the sample is expected to vary; predict two different outcomes: one graph prediction might portray expected findings if there IS an interesting pattern and a second graph prediction might display expected findings if there is NO pattern; include measures of variation from replication (p. A4); write captions for figures with units of measure. In brief, include the following:

- Give a clear description of what is displayed.
- State briefly how it was done - the method - but without detail.
- Identify the research subject.
- Include units of measure, but know that variables can be continuous or discrete and may have no units.
- For photos:
  - Define the calibration scale.
  - Explain symbols in a legend.
  - Include a control or alternative condition panel for comparison.

An important factor in scientific research is reproducibility, meaning the ability for a study to be read and replicated. Reproducibility requires that a publication be transparent about methods and data. A key section in a scientific paper is in a figure and its legend, which should give a reader enough background about the methods and data to independently analyze the figure. Since there is limited pace in which to adequately describe the figure, a competent scientist must be succinct and clear. When undergraduate students begin conducting research, they typically have a scientist as a mentor. One of the things these mentors teach is data presentation. The following guidelines for making figures are meant to stimulate a conversation between mentors (the teaching staff) and trainees to develop more competent future scientists.

Information that appears in an effective figure caption can be divided into four competencies: experimental subject, experimental design, research tools, and interpretation.

Item	Description	Questions to ask yourself
Experimental Subject Information about the specimens or model species for this research		
Cell or tissue type	The kind of cells or tissue that the image is showing. This gives a reader information about your model system and the approximate scale of the image.	What was my model system? Did I use tissue samples or cultured samples? If I am using a tissue, am I interested in specific cells or the whole tissue?
Source of cells (tissue and species)	This goes along with cell type, and they can be written together (ie. CHO cells = Chinese hamster ovary cells), but if you don't use a known acronym, remember to include the source of your cells as well as type.	What was my model system? What kind of cells or specimen am I imaging?
Research Tools		

Information about the tools and materials used to complete the research		
Type of microscopy	The type of microscopy used to obtain the images. It is not necessary to name the specific model of microscope in the legend. Be sure to include bright-field/dark-field where applicable.	What kind of microscope did I use? Stereomicroscopy illuminates the sample from above; light microscopy passes a light beam through the specimen.
What is being stained and/or expressed in the cells	A reader cannot interpret an image without knowing what is being stained or expressed in the cells. This can be written within the figure itself or in the legend. For immunostaining, specific antibodies do not need to be named, just the target.	What am I trying to show with my picture? Did I stain anything, and if so, what? Were my animals/cells transgenic, and if so, what for?
Scale bar	This allows a reader to interpret the sizes of cells that appear in the images and to understand the magnification for the image capture. Include a size conversion either on the figure or in the legend.	How will I use the scale bar and team problem-solving to calculate density or sizes of cells in a typical specimen?
Experimental Design Information about the experiment and its relationship to a research question or hypothesis		
Sample size	Sample size can include how many subjects were studied, how many samples were prepared, how many cells were analyzed. Exactly which sample size is informative will vary based on the experiment.	How many subjects did I use? How many samples did I prepare? How many cells were analyzed per sample? Did I include graphed data from my images and/or statistics referring to them?
Experimental treatment	A description of the experimental procedure. What did you do to your experimental subject in this experiment? It should relate back to your research question and the title of the figure set it's found in.	What did I do to the cells/tissue/ subject before capturing the data? How did the treatment vary between different experimental groups? What was my hypothesis and how did I test it?
Experimental groups	These group names can be written in the figure itself with its corresponding image(s). This allows the reader to easily interpret your images without having to refer back and forth between the figure and legend to identify each image. Elaborate upon these groups in the legend.	How many different conditions are displayed with images? How did these conditions vary? What is a succinct name that I can give to each group that describes what is different in each?
Experimental variables	Describe the differences between the experimental groups. This can be a specific treatment, a length of time, a region in a tissue sample, or something else that aligns with your hypothesis and the title of the figure set.	What is my independent variable? What is my dependent variable?
Experimental controls	Controls are important so that reader can see how a specific variable changes the subjects. You do not always need to include negative controls in the figure set since it is assumed that non-specific binding was tested and either	How many controls are needed for my experiment? What are my controls for comparison purposes? How are my controls informative for a reader?

	not present or was accounted for.	

Interpretation

Information that helps the reader understand the take-home message of the figure

Title	This is the first sentence of your legend and is usually bolded. It either summarizes the conclusions to be drawn from the figure or the experimental approach followed to make the figure.	What does this figure mean? Why did I put the items in this figure set together? How does this relate to my hypothesis or research question?
Clear labeling system	Clearly label a legend that goes with each figure set. If you have more than one component in the figure set, label them each with numbers or letters and then use that system to refer to each component in the legend. If there is a set of images that go together, lettering is not needed for each individually as long as the image clearly differentiates between them (ie. by giving stain, experimental group name, etc.)	What do I want the reader to notice about this figure and how can I call attention to meaningful patterns?
Description of regions of interest	A region of interest (ROI) is a particular area in your image that you want to draw attention to. You can use symbols to achieve this (see below). Reference the symbol in the legend, explain what the ROI is, and why it is relevant in this figure.	Do I have a region of interest in my image? Did I add any symbols to my image? Why am I pointing out this area as a region of interest?
Arrow	Arrows may be used to point to an ROI in an image. They are used to point to a specific spot in an image.	What specific spot in a photograph must the reader notice?
Asterisk	Asterisks may be used to point to an ROI in an image. They are used to point to specific spots or to mark the center of a particular cell.	What spots in a photograph must the reader notice?
Box	Boxes are used to highlight a whole area in an image that is an ROI. They are also frequently used to show an ROI at another magnification	Do I need a box to help the reader relate two panels of the same feature at different magnifications?
Lines and dotted lines	Lines and dotted lines are used to highlight a large ROI in an image with a shape. They can also be used to help orient the reader and label specific structures within a histological section. They can be used to separate different regions or structures in an image.	Must I separate different features in a set of images – such as to indicate a feature that was removed for clarity in the final presentation?
Showing images in sets	Showing multiple images in one figure set allows the reader to compare experimental conditions and controls. Organizing the figures in a clear way is important. One option is to use a grid system in which the axes are labeled with experimental controls, stains, time points, or whatever may be applicable to the image set.	What temporal or spatial or treatment patterns must the reader notice for causal interpretations or a finding that a particular treatment di not cause the expected outcome?
Showing images with graphed data derived from images	Showing images with graphed data will allow the reader to see the data from multiple images rather than just one and helps the	How can data be depicted in a graph to reveal measures of variation, such as 95% confidence intervals, that are not

	reader derive the take-home message of the figure. If there are more images than just the one(s) shown in the figure, state that the image(s) in the figure are representative images.	apparent in representative images? Have I reviewed Appendices B (p. A4) and J (p. A20) in deciding how to graph the data?

Recruitment Email Script

Approved by Purdue IRB #1811021367 for A Qualitative Study Exploring Undergraduate Students' Perspectives on Failed Research Experience in a Biology Lab Course

Subject: Research Request - to learn about failed research experiences in a biology laboratory course

Dear Student,

My name is Soo Won Shim and I am a graduate student in the Department of Biological Sciences. An interdisciplinary team of researchers from the College of Education and the College of Science is collaborating to examine what happens when students in a lower division laboratory course for biology majors conduct a course-based undergraduate research investigation that fails. Specifically, the purpose of the research study is to understand students' perspectives about and responses to an experience with negative results or research that failed.

In order to participate in this study, you must be a student 18 or older who completed a laboratory course for biology majors and who self-identifies as having experienced challenges due to a component of failure in your team's research investigation in the course. We are asking for your participation in an individual interview where you will be asked a series of open-ended questions related to your failed research experiences in a lab course. The individual interview will take approximately 45 minutes. If you participate in this study, you will receive $30 in the form of Amazon gift codes as compensation.

If you are interested in participating in this study, please contact Soo Won Shim at shim40@purdue.edu to sign the attached form and to schedule a time and date for your individual interview.

If you qualify but do not want to complete the form or if you have any questions regarding the informed consent form or this study, please contact Soo Won Shim at shim40@purdue.edu.

Thank you for your time.

Sincerely,
Soo Won Shim

Reminder Email Script
Subject: Reminder about Upcoming Interview

Dear Student,

My name is Soo Won Shim. I am contacting you to remind you about your upcoming interview on [date/time] at [location] to learn about your research experiences in a biology lab course. As mentioned in the informed consent form, I provided you with earlier, your identity and responses will remain anonymous in this research study and will be kept strictly confidential.

If you have any questions about your upcoming interview or any aspect of the research study, please contact me at shim40@purdue.edu.

Sincerely,
Soo Won Shim

SUPPLEMENTARY MATERIAL

Context for Failure in An Introductory Biology Laboratory Course: A Zebrafish Population Study Lab Protocols, Known Scientific Challenges, and Associated References to the Research Literature

Soo Won Shim and Nancy Pelaez

Purdue University Department of Biological Sciences

Research Setting. In the course, students had a laboratory preparation lecture (45 minutes) and a laboratory course (180 minutes) each week over a semester. There were three modules set up as Course-Based Undergraduate Research Experiences (CUREs) according to Auchincloss et al. (2014), which means that each lab course module involved students with use of science practices, collaboration, discovery about a topic of broad relevance, and iteration to engage students in learning the research process and how to conduct their own investigations. One of the modules was a zebrafish module. For the zebrafish module, students were expected to work as a team of three students to design their own research and collect and analyze data over the course of a semester. The participants in this study all took charge of the zebrafish research module as principal investigator, meaning that they assigned roles for their team members, checked that everyone understood what was done or studied, planned the research, coordinated an oral presentation to share results with the class, and they wrote a manuscript suitable for publication that was peer-reviewed by other members of the team before submitting a final report at the end of the semester.

Disciplinary Context. The students in this study were learning about biology lab equipment, including microscopes, Personal Protective Equipment (PPE), measurement tools, water quality, and policies such as animal care and use regulations and hazard awareness for research with live animals, in this case, zebrafish (*Danio rerio*). Hundreds of mutant traits are known for zebrafish including many that are similar to human clinical disorders. Ten striped fish that had been donated previously had produced many offspring in our lab classroom. These were distributed among six 10-gallon tanks. Most were AB/AB (wild type) descendants of fish donated by a research professor at a time when the zebrafish bred for research were not always surviving or breeding. In fact, one problem was identified when lab water from a Reverse Osmosis system in the building was used to culture microbes. Results were positive for aerobic bacterial growth of various environmental species commonly isolated from a wide array of sources including soil, groundwater, and in some cases, highly purified water. All of the species isolated were oligotrophic, meaning that they can survive in extremely low-nutrient environments. The class had also been given AB/Sandy hybrid fish by another research professor. Such hybrid fish are descendants of a transgenic fish with the mutated tyrosinase enzyme, so that they carry an allele that would make some offspring transparent, which is useful for research. A sentinel program for zebrafish disease monitoring in the building was used to screen both teaching and research zebrafish facilities on an annual basis. Throughout the academic year prior to this study, whenever a fish died, whether from natural or accidental causes, it was frozen. Students were informed that before this study frozen fish from the lab classroom as well as sentinel fish housed in the small animal facility for research all tested positive by polymerase chain reaction (PCR) for *Myxidium streisingeri*, and *Pseudoloma*

neurophilia. *Myxidium streisingeri* is a fairly common parasite of the zebrafish urinary tract, and it is thought to have a complex life cycle that requires both a vertebrate and an invertebrate host, with the infected invertebrate host shedding actinospores that infect the vertebrate host (Whipps et al., 2015). The invertebrate host of *Myxidium streisingeri* could be a small free-living worm that was observed in several of the zebrafish tanks. The other pathogen detected, *Pseudoloma neurophilia*, is also a prevalent infectious agent of laboratory zebrafish. It is an obligate intracellular spore-forming unicellular parasite that preferentially infects neural tissue. Infection may only result in clinical signs such as emaciation and spinal deformities when fish are old or stressed, although it has been reported to be associated with reduced growth and decreased reproductive fitness (Ramsey et al, 2009). *Pseudoloma* can be transmitted from mother to offspring by being encapsulated within the oocyte (Sanders et al., 2013).

Teams of students studied the zebrafish tanks from August through November, 2018. This provided adequate time for the population to mate and produce offspring that could then be introduced into the tank about ten weeks later when they reached sexual maturity (Westerfield, 2007). A large fish tank was used to house the adult fish. Attached to this tank was a small "flow-through" tank, used as a nursery in which fry were placed after they hatched and were swimming. Separate from these tanks, an incubator held the eggs and newly hatched fry in small containers kept at a constant temperature. Microscopic observations made it possible to identify phenotypes 2-3 days after fertilization (Lamason et al., 2005). To keep the population healthy, safety protocols were followed when handling and feeding the fish.

The discovery question posed to students was to predict how the pigmentation patterns of fish in their tank would look at the end of the semester and to gather data to test and refine

their prediction throughout the course of the semester. In addition to the wild type fish with short tails and black stripes (AB), some fish in each tank lacked black pigment, either because the fish had the golden phenotype and were homozygous for the slc45a2 insertion (Lamason et al., 2005) or because new fish with the absolute phenotype recently added to the tanks had the double ednrb1a^b140; mitfa^b692 recessive mutations (Engeszer et al., 2008). Panels B and C in Figure 1 illustrate the diversity of fish phenotypes and science practices used such as measurement tools, PPE, and data records. In the lab, students were also taught about different types of lab water, how to maintain optimal temperature and water quality for aquatic organisms, how to capture microscopic observations with zoom stereo and light microscopes, and use of precautions to reduce the potential of spread of infections among fish and from animals to humans when breeding, cleaning eggs, handling zebrafish, and raising the fry.

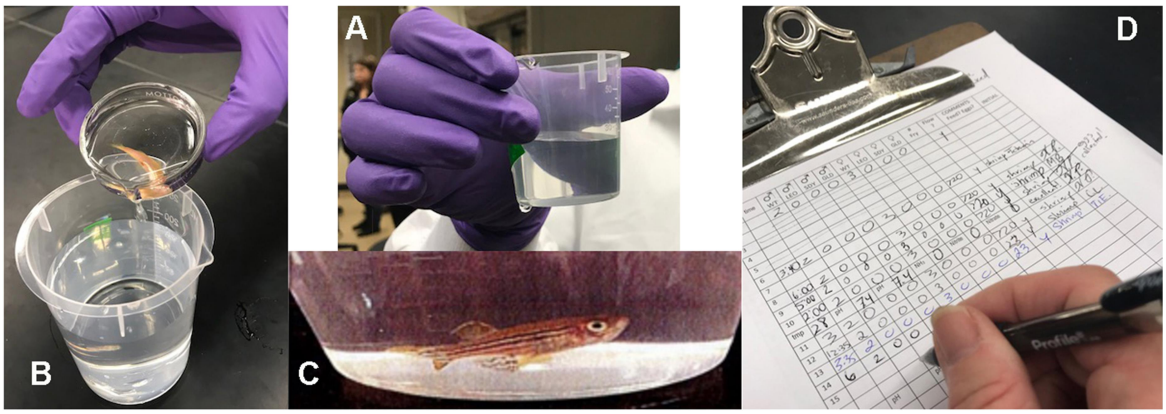


FIGURE S1. Golden and wild type striped zebrafish and some of the laboratory research tools and practices employed to study the fish. Panel A shows use of PPE and a beaker for measuring the volume of water to dilute Tricaine, a drug used as a fish sedative. Panel B shows a sedated fish of the golden phenotype being transferred to a recovery beaker for observation before

returning it to the tank. Panel C shows a wild type striped zebrafish being observed following sedation. Panel D shows daily records kept on the team's zebrafish population for pooling data across the lab sections.

In terms of their aim to discover something about a topic of broad relevance, some teams decided to find out what was affecting the breeding of zebrafish and others wanted to understand more generally the connection between gene variants such as slc24a5, a putative Cation Exchanger that affects pigmentation in zebrafish and human skin pigmentation (Lamason et al., 2005). Most teams started by examining the proportions of zebrafish phenotypes to make predictions for evolution of their population in future generations using the Hardy-Weinberg Equilibrium (HWE). Biological evolution can be defined as being the sum total of genetically inherited changes in the gene pool for all the individuals who are the members of a population. For instance, at the beginning of the semester, one tank held a population of 16 fish: 12 were striped and 4 of the absolute (transparent) phenotype. Assuming that a pigmentation trait is determined by the inheritance of a gene with two alleles, in the Hardy-Weinberg Equilibrium Equation ($p^2 + 2pq + q^2 = 1$), p is defined as the frequency of the dominant allele and q as the frequency of the recessive allele for a trait controlled by a pair of alleles. A team might assume that all of the fish were homozygous at the start of the semester (Figure 2 A). If mutation is not occurring, all members are breeding, all mating is random, every individual produces the same number of offspring, natural selection is not occurring, and there is no immigration or emigration (no gene flow into or out of the population), then with p=3/4 and q=1/4 the tank for a future generation at Hardy-Weinberg Equilibrium might have only 1/16 of the fish showing the absolute phenotype (Figure 2 B). However, if adults of the absolute

phenotype fail to survive or breed (Figure 2 C) or if more fish of the absolute phenotype were added to put gene flow into the population (Figure 2 D), then the proportion of transparent fish could be much smaller or larger than the expected 1/16. At the end of the semester, p equals all of the alleles in individuals who are homozygous dominant and half of the alleles in individuals who are heterozygous for this trait in a population. As a pre- or co-requisite, students enrolled in this lab had studied examples of the Hardy-Weinberg Equilibrium in a lecture course and details about the instructions to apply this model to real data in this lab course is published elsewhere (Liu et al., under review). To summarize, by analyzing the changing proportions of zebrafish phenotypes, the students might conclude if evolution had occurred in the zebrafish population in their tank (Pelaez, 2018). However, there were occasions when the zebrafish did not breed. All of the case study participants reported here were faced with this challenge. The data collected from their tanks throughout the semester did not allow a comparison of the patterns of phenotypes between the parent and offspring generations.

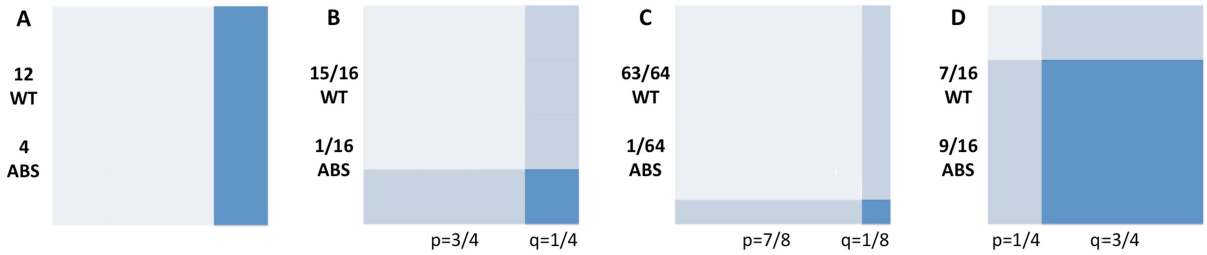


FIGURE S2. Application of the Hardy-Weinberg Equilibrium equation to zebrafish populations (large squares). Panel A illustrates the assumption that an initial population of 16 fish could all have been homozygous, with 12 wild type (WT) fish (light rectangle) and 4 fish of the absolute phenotype (dark rectangle). Panels B, C, and D illustrate the Hardy-Weinberg Equilibrium

equation ($p^2 + 2pq + q^2 = 1$), where p is defined as the frequency of the dominant allele and q as the frequency of the recessive allele for a pigmentation trait controlled by a pair of alleles. In other words, p equals all of the alleles in individuals who are homozygous dominant (lightest shaded area) and half of the alleles in individuals who are heterozygous (medium shaded areas) for this trait in each population. Likewise, q equals all of the alleles in individuals who are homozygous recessive (darkest shaded area) and the other half of the alleles in individuals who are heterozygous (medium shaded areas). Because there are only two alleles in this case, the frequency of one plus the frequency of the other must equal 100%, which is to say $p + q = 1$.

With respect to group settings in a laboratory course, students were randomly assigned into a group of three students. They were expected to collaborate with each other for all laboratory activities. In the lectures, the students from teams who studied the same tank in laboratory sections that met on different days had chances to interact and discuss the potential causes of fry death in their tanks. The failure of the fish to reproduce in a particular tank could be viewed as a natural selection pressure that changed a zebrafish population, but in this study many participants perceived dealing with this unexpected data as a failed research experience.

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SUPPLEMENTARY MATERIAL 5

Codebook Examples and Glossary

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Codes were developed by using descriptive coding and emotion coding (Saldaña, 2013). Descriptive coding is useful to identify the content of the data by representing their actions with the participant's own words and phrases. Emotion coding captures the participants' emotions in order to understand their experiences internally and socially. Although the codebook was started using participants' own words and phrases, for clarity in describing the patterns, a Glossary of definitions for emotion codes was adopted, modified from the American Psychological Association *Dictionary of Psychology* at https://dictionary.apa.org/ .
NOTE: A coping strategies Glossary with code definitions is included as Table 4 within the manuscript.

Glossary of emotion codes
Anxiety - apprehension and tension in which an individual anticipates impending danger, catastrophe, or misfortune.
Confusion - a mental disturbance characterized by bewilderment, disorientation, and inability to think clearly or act decisively.
Daunted - feeling intimidated, feeling fear, and disheartened.
Doubtful - a mental state with lack of confidence or an uncertainty about something or someone, including the self.
Frustrated - the feeling from the thwarting of impulses or actions that prevents one from obtaining something they have been led to expect based on past experience.
Nervous - a transient emotional state of anxious apprehension or an excitable, highly strung, or easily agitated disposition.
Overwhelmed - feeling submerged by thoughts and emotions about current problems, to the point where one lacks efficacy and feels frozen or paralyzed.
Stressed out - having difficulty relaxing and quieting one's mind and needing to take control.
Uncertainty - a lack of confidence or clarity about something or someone, including the self.
Worried - a state of mental distress or agitation due to concern about an impending or anticipated event, threat, or danger.

Table 3 Step 2 Codebook with axial and focus coding

Before failure			
Codes	**Description**	**Sub codes**	**Examples**
Initial career goals	Student's career goal in the beginning of the semester	Anesthesiologist Biology researcher Medical school Naturalist	"I wanted to be an anesthesiologist." "Getting a degree here if I want to continue on the graduate school for Entomology or continue on with biology." "I want to be in a medical field." "I maybe want to be a naturalist one day."
Prior knowledge	Student's answer to questions about Hardy Weinberg Equilibrium and Mendel's law	Elaborate the models well and applied	"The data table we will use will take into account the amount of fish observed, the change in allelic frequency, and the traits observed, The former two will allow for us to determine if the Hardy-Weinberg equilibrium will hold up into following generations, as in order for Hardy-Weinberg to remain true, there must be a large breeding population, random mating, no change in allelic frequency, no immigration or emigration, and no natural selection."
		Some wrong answer but applied	"Using the Hardy-Weinberg theory we can't really predict the genotypes of the current population because none of the population expresses the recessive phenotype. If a fish is born that expresses the recessive phenotype, then we can use the Hardy-Weinberg equation to start predicting things more accurately. As of now all we can do is to assume the amount of heterozygous and homozygous dominant fish there are. To record evidence, we will keep an expected population count of each type and an accurate count of all the fish that are actually there. Using the data table, we will conduct a chi square test to see how well the actual population fits the expected."
		Did not elaborate but applied	"Throughout the course of this semester, I am keeping track of the information that I collect in lab of each generation. I would use the same Ho and Ha we came up with in class for the corn. I am watching the zebrafish pigmentations and making note of them. The possible phenotypes for this are LEO (recessive) and Wildtype and golden (mutant)."
Initial expectation (self-efficacy)	Student's reported expectation about their ability to conduct open-ended research	No trouble	"I didn't think that I was going to have any trouble. I thought that it was going to go smoothly just because I mean the only like close to lab type of experience I had was in high school where things were very structured, and I was like well you're going to get results no

			matter what.”
		No stated expectation	“I guess I didn't really have any expectations for it.”
		Would be difficult and need a lot of work	“I guess, Initially I thought it was going to be difficult to take a lot of work in like designing how the experiment would function. That's how I felt about initially.”
		Would do it even if it was complicated	“It felt rather complicated because in the beginning of the year it's a lot of very like complicated and advanced terms and subjects, it just felt a little complicated in the beginning. But then like as the year went on, I sort of understood it more and more.”
		Not sure of their ability and the research process	“You know in the beginning I was very nervous, and I wasn't sure how a good of an investigator I was.”
Motivation	The reasons why a student keepd working on the research	Grade	“I'd be lying if I said that grade wise, it didn't mean anything because that definitely means something. I don't want to do bad in school.”
		Desire to learn	“I really wanted to know what was going on. And so I would spend a lot of time looking at the fish tank and looking at the data and doing outside research and just trying to figure out what could possibly be happening.”
		Responsibility	“I wouldn't say it was all on me but I definitely take some of the responsibility for it.”
		Curiosity	“Sometimes you just have ambition that you just want to know why it's not working. Why aren’t all tanks did work at all.”
Previous lab experience	Student’s previous biology lab experience	A structured lab experience	“In the high school lab courses, everything was laid out like you were given. Basically, it's like instructions on how to do the lab and what your data should look like more or less.”
		No lab experience	“I guess, since I haven't been really taking any biology courses before that, that was like my first one since high school.”
During failure			
Emotional reactions	Student’s emotional reactions to failure	Anxious	“I was going to get data and just be able to write a paper with no problem, but having this trouble definitely brought some uncertainty into what exactly I was going to do. So had a bit of anxiety over it.”
		Worried	“I mostly just felt worried about, because writing the paper thinking I have nothing to write about.”
		Overwhelmed	“I guess I just a little bit overwhelmed and stressed about it.”
		Stressed out	“Before actually doing the work on that sort of stressed me out,

			because I had no idea whether I was right or wrong really."
		Frustrated	"I was frustrated and kind of a little annoyed. I guess I'm not annoyed at anyone or anything specifically just having to then write a paper without any data. It was difficult and it was very frustrating."
		Doubtful	"I doubted about myself."
		Daunted	"I initially thought that I'd be able to do it in the end, but it was a little bit daunting in the beginning."
		Nervous	"I was very nervous for myself, and I wasn't really sure if I'd be able to do it."
Reasons for negative emotions	Students' explanations for their emotional reactions	Uncertainty	"Having this trouble definitely brought some uncertainty into what exactly I was going to do."
		Worried about grade	"I was a little overwhelmed because I had no idea what I'm going to do. I had to write a paper on this and [this paper was] a major part of my grade."
		Unfamiliar	"Before actually doing the work on it that sort of stressed me out… because I had no idea whether I was right or wrong really."
		Comparison	"I heard so many times people talking about how the PIs for this tank had the worst because we didn't have anything to go off of."
		The semester-long project	"Because my research paper was a little bit different than the other people in my group primarily because my experiment took place over the course of the entire semester. And because of that I basically I had like the entire semester to do in away."
		A concern of judgement	"I wasn't really sure if I'd be able to do it. And I thought I'd be letting my group down by not being able to research and analyze well... I never feel like I'm as quite on the same level as everyone else."
		Burden of being the PI	"I wanted to be able to answer questions from my team members if they had questions, so I wanted to know enough about this topic, zebrafish. To be able to make sure that my investigation was going well, running smoothly… I guess that's what I expect of myself as a PI."
Other factors that influence students'	Students' various characteristics	Characteristics	"I am goal-oriented, and I would say I'm optimistic for the most part and just motivated. So, once put my mind to something I want to reach that goal."
		Levels of interest	"I love being in the lab and I love collecting data and looking at

coping strategies		(Ambition)	everything...I'm always down for learning new things. If there's ever something I don't understand, I'm always asking questions."
		Maturity level	"I was the oldest student of course. My first semester where they were all freshmen and then I'm here twenty-one years old. I just think I was different maturity level about everything else that's happening where I have in life I've had enough experiences of things that didn't exactly go my way and even in college if I've already had these experience of things has been then. That didn't exactly go as I planned them. So I kind of already know how to work through them."
		Other personal issues	"I was going through some other issues at the moment too."
	Students' different perceptions of levels of difficulty	Perceived difficulty	"It felt rather complicated because in the beginning of the year it's a lot of complicated and advanced terms and subjects and stuff and it felt a little complicated in the beginning."
	Outside class experience	Another lab experience	"In my other class I had a different experiment and that went really well. So, it was about the same time. So, it's like, it didn't really turn me off of doing research."
		Previous practices in high school	"Not Like in class. They [high school teachers] are never like directed or allowed. I never really felt like I was allowed to interact with people that were outside of my group."
	The ways of processing the suggested information	Did not negate the stress	"… that was one of the things that was difficult because I was so focused on the goal of reaching conclusions that supported my hypothesis. That was something that potentially could have screwed me up. Once I didn't reach the data that I expected, I felt like I had failed."
		Negate the stress	"[After talking with teaching staff], the shift was just me coming to the realization that error, errors in science happen and that you should still acknowledge them."
	Support from peers or teaching staff	No help from peers	"They [peers] didn't have much to help with because it wasn't like they are part of our lab like they worked P.I. And honestly we were all pretty stopped. We didn't know what was going on but I didn't want to over worry that with my paper knowing that they had to write their own paper. Of course we talked about it because we're a group. But in terms of going out of my way to try to figure out what was going on they didn't have much to do with that."
		Help from peers	"At first we just got along well together anyway. But then realizing

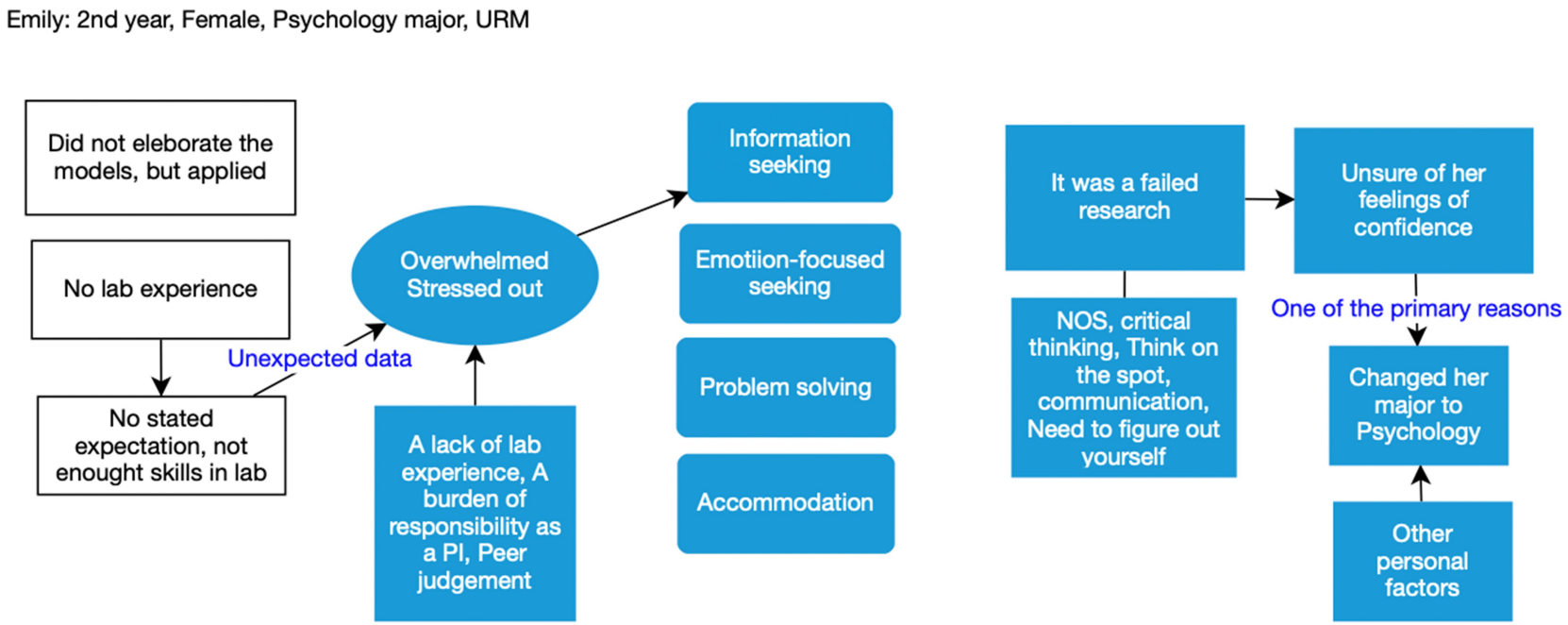
			that we would be helping each other a lot with the project and helping each other through the class and through other classes as well. So I thought it was nice to be good group members I guess and just be friends and talk more to succeed in the course." "They [peers] did also give feedback on their own thoughts about what was happening with the tank."
		Teaching staff support	"Teaching staff had talked about how you're not always going to get the results you want but that's OK. So I was encouraging in a way. They mentioned that if you get data that isn't what you want. That's OK. Just as long as you talk about maybe why it didn't work out right."
	Constraints	The restrictions that impose constraints on the course-based research	"I didn't really have a conclusion on what happened with the zebrafish because several things that I'd have to test for that, the equipment I simply didn't have, nor did I have the time to do it." "Well, the constraints were, many groups working with this one tank. And it's hard to coordinate between all of them… work to try and fix it."
After failure			
Perception of failure	Student's reported perception of the failed experiences	Not failure	"I understood that it wasn't a failure and that it actually was something that I could analyze and talk about and improve upon in the future if I'm given the opportunity. Then I felt like it wasn't a failure, that I still had reached the goal even if it wasn't the one that I set out to reach."
		Total failure	"I think failure is when you set a goal at the beginning of your research and you're trying to find something, like the phenotypes, and then you don't have any data to go off of by the end of your research. So, I wanted to figure out what the phenotypes of this offspring would be that I didn't have offspring. So I would say that's a failure. But then again, if I maybe could have found a reason why they didn't. Then I probably wouldn't say it was as much of a failure, but I didn't find any reason to why they weren't surviving. I'm just saying that was just a total failure all the way around sadly for me."
		Failure but okay	"I think failure would be, with me, just a complete inability to collect any meaningful data. Because like I said with null hypothesis

			that oh would maintain hardy Weinberg equilibrium that was technically true. We did maintain that equilibrium, but I considered it failed because we didn't get any new fish to say that our second generation was still in that same ratio of genotypes. It was because we had no second generation…. It's OK to have a failed experiment because that happens."
What students learned (values)	Student's reported values of the failed experiences	Personal growth	"I feel like it grew as a person at least even if it wasn't in biology, I felt like I figured more out about myself."
		Evidentiary foundation of science	"Real research is messy. It's not going to be straightforward answers and straightforward ways of doing things. Definitely, it requires a lot of thought and critical thinking."
		Research process	"What I learned about is more about using theory and applying it to a real-life situation…I learned more about how to observe. Better observing a living system."
		Learning experience	"It was a learning experience for sure. It definitely made me a better thinker, a critical thinker because you have to think through those things and try to figure out how to make this work with what you have."
		Communication skills	"Communicating with other people. I didn't put a lot of value on that before and for as much value as I should. Working in groups and being able to talk about other people who are in the same situation really helped."
		Problem solving skills	"I guess I learned just how to problem solve a little bit better. Problem solving was not exactly my forte."
		The difficulty of dealing with animals	"I learned a lot about zebrafish. I learned that it's difficult to maintain tank conditions."
		The importance of asking questions	"If you don't get the results that you really expecting in this research, you should ask a different question, why did you get this? Why did you get this result than one you originally expected? and then, from there, just examine things and trying to test it again." "I learned that if I don't know what's going on, ask other people for help."
		Insight for the future research	"I think number one make sure you can quantify the amount of being fed and then number two just making sure that that temperature stays consistent the entire time and that can be a little

			tricky, of course that is water and the heating elements that are being used."
		Patient	"I learned that I was much more patient with myself than I thought I would be."
Research self-efficacy	Student's reported feelings of confidence in conducting open-ended study	Feel more confident	"That [the zebrafish module] makes me more confident now I'm doing research and I've gained those skills in analyzing things as they're happening."
		Feel accomplished but unsure of ability	"I guess I felt a little more accomplished that I actually had a full paper it wasn't exactly where I wanted it to be, it was rough draft, but it was a full paper. So I think maybe being a scientist in that biology class made me think out of the box. Instead of just, you know, having an answer for everything you have to dig for those answers. I don't know what makes me not feel confident. I don't even know, I don't really feel confident. If more as I just don't see myself doing that. You know for a good career or anything. I don't know if I feel confident in myself for that. I just don't think I have any aspiration to do that."
		Feel more confident but insignificant	"That's really what makes it.. Makes me feel more confident about it in the end… Just sort of made me feel a little bit insignificant but in the fact that like there's a lot of factors that I cannot control really negatively to the experiment potentially messing up. It just makes me feel a little bit insignificant because there's just some things that I don't know and I can't really prepare for, but like I at least know what to do now when that stuff does happen."
Investigator identity	After having a failed research experience, student's reported identity as an investigator	Science identity (investigator)	"I felt like I completed some research and I feel like that's what it takes to be an investigator you to complete research right off what you get and understand your results and just like everything that you performed. That's I see myself as an investigator." "It feels phony to call myself an investigator. But through the process of like reading the research paper at the end, actually coming up with thoughts on the subject. Definitely felt more like, I was investigated in a way." "I guess it actually solidifies that I want to do research. I know I wanted to before…Even though I had this unexpected data and the

			failed experience. It just made you want to do research more.”
		Did not have an identity as an investigator	“No, I don't have an answer for that [identity as an investigator]. I don't think so.”
Zebrafish research	Student’s research plans and final paper regarding the zebrafish research	Initial plans	“Initially the way it was designed was that we were just going to look at the phenotypes of the parents’ generation and then we would do Hardy Weinberg calculations based off of that and look at the offspring and then perform chi score test to see how accurate our predictions were.”
		Paper conclusions	“I had my original question. I don't think I added to the question itself. I think I kept my question but then qualified like in my, like in talking about my data when I was talking about my results. I kind of qualified it with why I thought the results did not go with my predictions.” “The best thing to do is trying to figure out why it didn't go according to your plan. So, that's what I did. That's what I wrote my paper over you know, trying to figure out why it didn't work. Maybe the water temperatures or the P.H. levels or something in the water wasn't right.”

Table 3 Step 3 process coding example. Participant interview transcripts were re-read several times after coding their coping strategies. Example themes were visualized in process maps to understand the sequence of adopting coping strategies using NVivo12. Findings are summarized in Figures 2 and 3 of the manuscript.



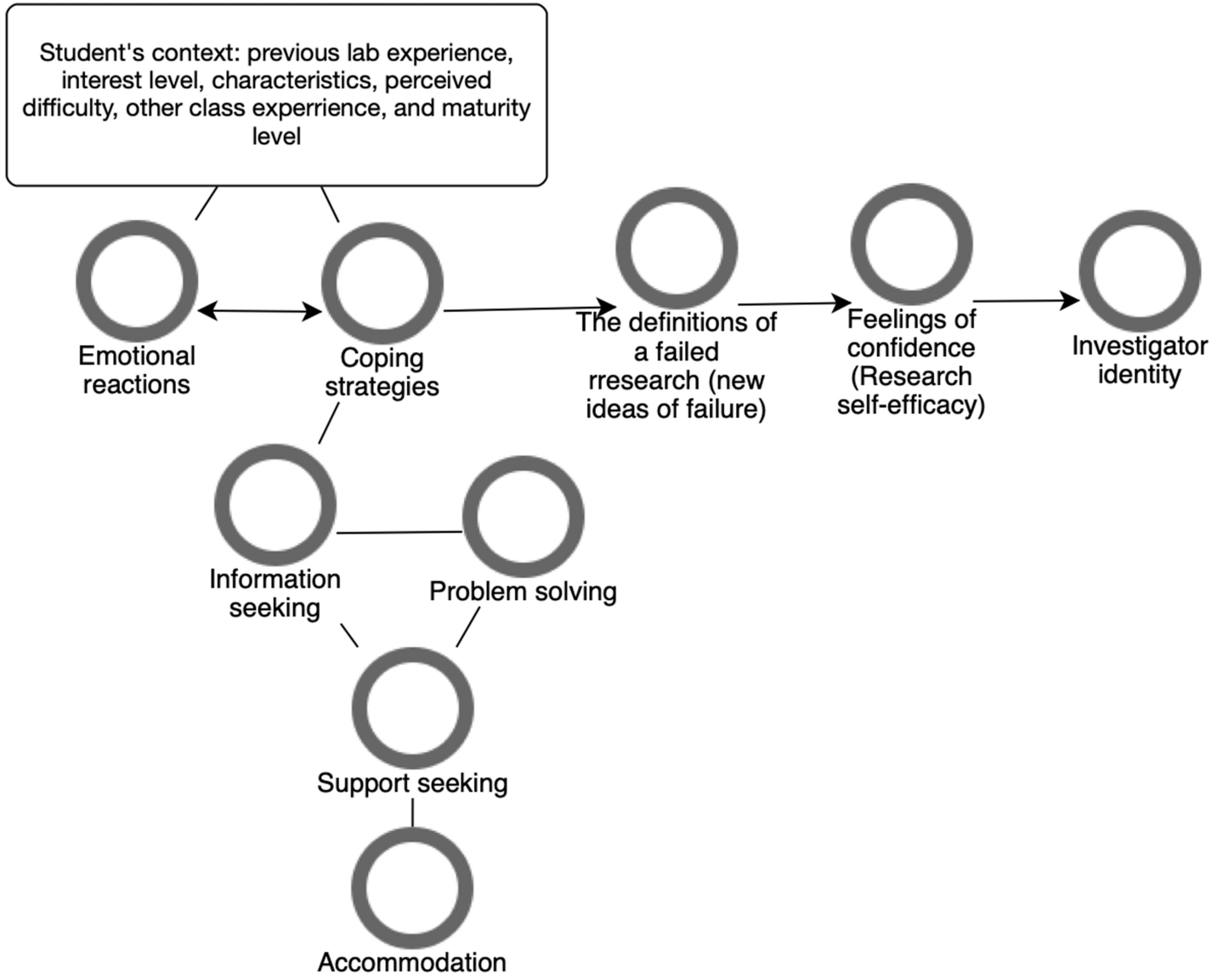

Student's context: previous lab experience, interest level, characteristics, perceived difficulty, other class experrience, and maturity level
Emotional reactions
Coping strategies
The definitions of a failed rresearch (new ideas of failure)
Feelings of confidence (Research self-efficacy)
Investigator identity
Information seeking
Problem solving
Support seeking
Accommodation

Table 3 Step 4 Final hierarchy chart for codes from N'Vivo12 used for analyzing the data based on the theories