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THE CASE OF THE STRESSED-OUT LARVA:

A STUDY OF CLIMATE CHANGE EFFECTS ON OYSTER CELLULAR PHYSIOLOGY

Annie Schatz
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Grade Level
Advanced High School (AP/IB)

Subject area
Biology, Chemistry, Oceanography

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Title: Case of the Stressed-Out Larva: A Study of Climate Change Effects on Oyster Cellular Physiology

Focus: Using a jigsaw puzzle approach, students will piece together the results of three molecular measures to understand how warming and acidified waters affect the health of oyster larvae.

Grade Level: AP, IB, and Honors HS Biology. Also applicable to HS Chemistry and Oceanography

VA Science Standards of Learning:

LS.1/BIO.1 The student will demonstrate an understanding of scientific and engineering practices

a) asking questions and defining problems

- ask questions and develop hypotheses to determine relationships between independent and dependent variables

b) planning and carrying out investigations

- independently and collaboratively plan and conduct observational and experimental investigations; identify variables, constants, and controls where appropriate and include the safe use of chemicals and equipment

c) interpreting, analyzing, and evaluating data

- identify, interpret, and evaluate patterns in data
- construct, analyze, and interpret graphical displays of data
- compare and contrast data collected by different groups and discuss similarities and differences in their findings
- consider limitations of data analysis and/or seek to improve precision and accuracy of data

d) constructing and critiquing conclusions and explanations

- construct explanations that include qualitative or quantitative relationships between variables
- construct scientific explanations based on valid and reliable evidence obtained from sources (including the students' own investigations)

LS.2 The student will investigate and understand that all living things are composed of one or more cells that support life processes, as described by the cell theory. Key ideas include

e) cellular transport (osmosis and diffusion) is important for life processes.

LS.7 The student will investigate and understand that adaptations support an organism's survival in an ecosystem. Key ideas include

a) biotic and abiotic factors define land, marine, and freshwater ecosystems; and

b) physical and behavioral characteristics enable organisms to survive within a specific ecosystem

LS.8 The student will investigate and understand that ecosystems, communities, populations, and organisms are dynamic and change over time. Key ideas include

a) organisms respond to daily, seasonal, and long-term changes;

c) large-scale changes such as eutrophication, climate changes, and catastrophic disturbances affect ecosystems.

BIO.2 The student will investigate and understand that chemical and biochemical processes are essential for life. Key ideas include

b) macromolecules have roles in maintaining life processes;
c) enzymes have a role in biochemical processes;
e) the processes of photosynthesis and respiration include the capture, storage, transformation, and flow of energy.

CH.3 The student will investigate and understand that atoms are conserved in chemical reactions. Knowledge of chemical properties of the elements can be used to describe and predict chemical interactions. Key ideas include

a) chemical formulas are models used to represent the number of each type of atom in a substance;
c) balanced chemical equations model rearrangement of atoms in chemical reactions;
d) atoms bond based on electron interactions;

CH.4 The student will investigate and understand that molar relationships compare and predict chemical quantities. Key ideas include

b) stoichiometry mathematically describes quantities in chemical composition and in chemical reactions.

Learning Objectives:

- Students will evaluate and discuss how warmer and more acidic waters affect oyster larvae shell size, biomass (i.e. total protein), cellular stress levels (i.e. total antioxidants), and energy storage (i.e. total lipid) in a jigsaw puzzle activity
- (Advanced) Students will create a standard curve equation for their assigned molecule
- Students will use a standard curve equation to calculate the total amount of their assigned molecule
- Students will graph effects of warmer temperatures and acidic waters on their assigned molecule concentrations

Total length of time required for the lesson:

2 to 3 hours, including time at the end for class discussion and reflection. This lesson can be split into 2 – 3 class periods (depending on level, size, and schedule of the class).

- Suggestion for splitting across several class periods:
    1. First Class – Parts 1, 2, and 3
    2. Second Class – Parts 4 and 5a
    3. Third Class (optional) – 5b

Key words, vocabulary:

Climate Change Terminology

- Greenhouse Gas Effect – Humans burn (i.e. combust) fossil fuels to power cars and factories. Burning fossil fuels releases greenhouse gases (carbon dioxide (CO_2), methane (CH_4), etc.). Greenhouse gases trap heat from the Sun, in the form of radiation, in our atmosphere. The trapped heat causes the average temperature on Earth to increase over several decades.

- Ocean warming – The average temperature in our oceans has risen because of the Greenhouse Gas Effect.

- Ocean acidification – Human-produced CO_2 dissolves into the ocean. CO_2 then reacts with water and releases hydrogen ions (H^+). Tons of H^+ cause a decline in the pH, which makes the ocean more acidic.

Ecological Terminology

- Ectothermic – An organism whose body temperature is controlled by heat from the environment.

- Larvae – A stage early in an organism's life cycle that looks and behaves differently from the adult stage. For oysters, the larval stage swims unlike the non-moving adult stage.

Cellular Physiology and Molecular Terminology

- Bioenergetics – How organisms use energy in their bodies.

- Reactive oxygen species (ROS) – A harmful molecule that has a reactive oxygen atom. These molecules are naturally created during respiration since oxygen is the last electron acceptor in the electron-transport chain.

- Oxidative stress – Stress at the cellular level caused by damage to cell membranes, DNA, and enzymes from numerous ROS. Large amounts of ROS are caused by an increased demand for ATP, which increases respiration and use of the electron-transport chain.

- Antioxidants – Molecules that help fight oxidative stress by reacting with ROS to make them unreactive.

- Total Lipid – A measure of all lipid molecules in an organism (ex. fatty-acids and membrane lipids). Used as a representation for long-term energy storage levels.

- Total Protein – A measure of all proteins in an organism (ex. enzymes and structural proteins). Used a representation for biomass of an organism.

- Assay – General term for any method used to measure the amount of a cellular or chemical molecule.

- Homogenate – A solution of cells that have been broken open and made uniform throughout.

- Standard curve – An equation that is used to calculate the amount of a specific molecule from an unknown sample. The equation is created from known quantities of the molecule of interest.

Background information:

The life cycle of an oyster has two phases that have different body forms and biological functions. The first phase contains the larval stage, which swims for the purpose of finding food and a place to settle for the rest of their life. The second phase includes the adult stage that is permanently cemented on a reef-like structure with other oysters. Both phases have their own vulnerabilities to climate change, but oysters overall do not have control over their body temperature or cellular processes (i.e. they are ectothermic).

Oyster larvae need a lot of energy for development and the transition into their adult body form (i.e. metamorphosis). Along with being ectotherms, these traits make oyster larvae vulnerable to warmer and more acidic waters caused by climate change. The intersection between these two vulnerabilities as ectotherms and larvae and the impact of climate change is what I study.

The data used for this project are my actual data with the exception of the total lipid values (I am still working out which methods to use). The total lipid values that I created are based on previous research for Eastern oyster larvae. Also, more background information for this lesson can be found in the PowerPoint and answer key to the *Student Worksheet*.

For this lesson, students will explore how warmer and more acidic water within the Chesapeake Bay may affect the Eastern oyster (*Crassostrea virginica*) larvae. The students will specifically observe changes to groups of molecules important to the success of larval development. Studying the cellular level can uncover issues that may not be clear at bigger scales, like oyster shell growth. Changes at the cellular level can have an impact on the overall health and success of the organism.

The molecular groups used in this lesson are from three broad categories that are part of a holistic, bioenergetic framework created by Sokolova et al. (2012). The framework describes the level of stress that an organism may be facing under several scenarios, and the resulting consequences (Fig. 1). The main idea is that organisms prioritize giving energy to processes that are necessary for survival. All the other categories then suffer as a result (i.e. less long-term energy storage, growth, and activity like swimming).

The *Maintenance* category in the bioenergetic framework includes any cellular process needed to survive. In my research and this lesson, we will use Total Antioxidant to represent this category. Total Antioxidant is a measure of all antioxidant molecules within an organism at the time of the sample collection. Antioxidants are a group of molecules that protect organisms against oxidative stress. Measuring Total Antioxidants, then, will give us an idea of how much oxidative stress an organism might be experiencing due to warmer or more acidic waters.

Oxidative stress occurs when the number of reactive oxygen species (ROS) is greater than the pool of antioxidants within cells. Reactive oxygen species are naturally created during metabolism

by the electron-transport chain (ETC) because oxygen is used as the final electron acceptor. Antioxidants, however, are standing by to immediately remove those damaging molecules. Reactive oxygen species can be dangerous because they cause damage to lipids, membranes, DNA, RNA, proteins, and enzymes.

As an organism's metabolism rises, maybe due to outside stress, the number of reactive oxygen species goes up. If the number of reactive oxygen species exceeds the pool of readily available antioxidants, the organism must use energy to make more antioxidants. As an organism experiences prolonged stress, they will need more and more energy to try to fight oxidative stress by making more antioxidants. This situation is related to the increase in energy distributed to the *Maintenance* category in Figure 1b and 1c. If the organism cannot make more antioxidants fast enough, the reactive oxygen species can badly damage components of cells. That damage can ultimately lead to death of the organism.

The students will also be measuring Total Lipid to represent the *Energy Storage* category and Total Protein for biomass in the *Growth* category in Figure 1. Putting all three molecular groups together will give us with a better understanding of the stress experienced by oyster larvae when they are exposed to warmer and more acidic water conditions in Chesapeake Bay.

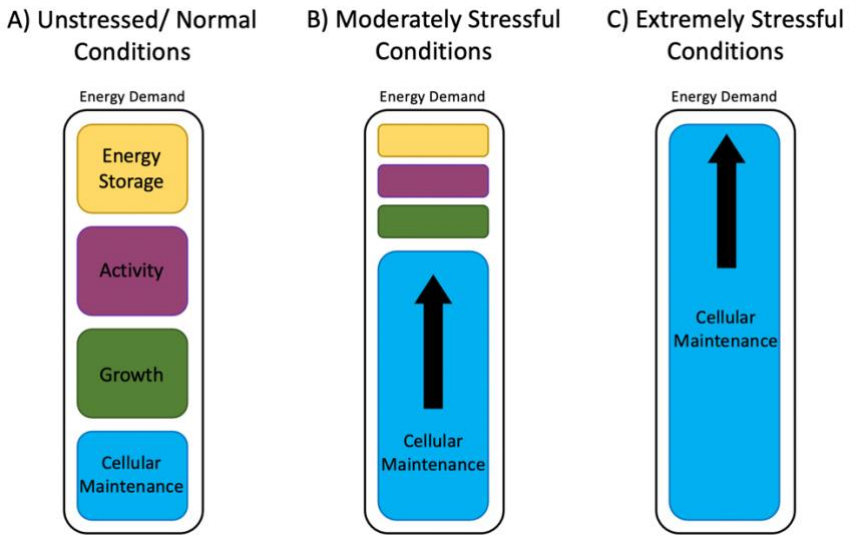

Figure 1. Adapted from Sokolova et al. (2012). The bioenergetic framework for responses to environmental stress. As stress increases, an organism will shift away from A) meeting the energy demands of all aspects of their biology to B) where an organism is spending most of their energy on processes necessary for cellular maintenance (i.e. those needed to survive) and less on all other processes. When the stress becomes extreme, C) energy is only given to the *Maintenance* category to survive. At this point, the energy supply might also begin switching from aerobic to anaerobic. Image credit: Annie Schatz.

Student handouts:

- Appendix A – Student Worksheet
- Appendix B – Total Protein Microplate Diagram and Image
- Appendix C – Total Antioxidant Microplate Diagram and Image
- Appendix D – Total Lipid Microplate Diagram and Image
- Appendix E – Student Worksheet Answer Key

Materials & Supplies:

1. Computer and projector for PowerPoint slides
2. Handouts listed above
3. Black, cannellini, red, and pinto beans (or black, yellow, red, and white beads)
4. 9 cups or jars (3 cups per group) to put the beans/beads in
5. Calculators
6. (Optional) Computers with graphing software (e.g. Excel)

Classroom Set up:

To-do in advance of the lesson:

- Make 3 sample cups per group (or jars for easier re-use) with beans that the students will count in Part 2. The three cups represent the replicates for only one of the three treatments due to time restrictions. Each group of cups will be labeled with the initials of the molecule that each group is measuring (ex. TP for Total Protein) followed by A, B, and C to represent the samples from the three replicate tanks. For example, each cup within the Total Protein group will be labeled TP-A, TP-B, TP-C. The students will use their counts to guess which of the three treatments they were given in a later section. Because the students will be identifying which treatment their samples correspond with, do not put the treatment name on the cup.
    - The black beans will represent all the other molecules that are not being "tested" and you can add however many you would like into the cups, this number does not matter.
    - Total Protein **(TP)** – Red beans – This group will be counting the Temp Treatment
        - Replicate A = 30
        - Replicate B = 25
        - Replicate C = 16
    - Total Antioxidant **(TA)** – Pinto beans – This group will be counting the Control Treatment
        - Replicate A = 18
        - Replicate B = 20
        - Replicate C = 30
    - Total Lipid **(TL)** – Cannellini beans – This group will be counting the pH Treatment
        - Replicate A = 31
        - Replicate B = 25
        - Replicate C = 28
- Print out the *Student Worksheet* in Appendix A for each student.
- Print out the *Microplate Diagrams and Images* in Appendix B – D (hopefully you can re-use these as well between class periods and year to year). This will give each group one copy.

Day-of the lesson:

- Download the PowerPoint and display on projector.
- Arrange the desks and tables so that there are three separate areas for the groups to work in.

Procedure:

Part 1 – Introduction to Global Climate Change, Physiology, and the Effects of High Temperature and Low pH on Larval Shell Size

Introduce the students to the interconnected concepts of global climate change, ocean warming, and ocean acidification using the PowerPoint provided. There are notes in the PowerPoint for each slide. The PowerPoint will also cover how we know these two environmental stressors may impact oysters, and why it is important to consider what might be going on at the cellular level (e.g. sustaining the aquaculture industry). Lastly, the slides will go over how scientists actually measure these cellular responses in the lab, as the students will mimic in this activity.

After the introduction to climate change and cellular physiology, you will show the entire class the graph of how the three treatments in this experiment affect the shell size of larval oysters. Before displaying the graph, have students answer the first two questions below. The students will use their understanding of the bioenergetic framework to decide what category shell size falls under and then hypothesize how the three treatments will affect it. This can be done either individually, in their molecule groups, or as a class.

- Q: Based on the bioenergetic framework, what category are we measuring when we observe changes to shell size? – Growth

- Q: Hypothesize how ocean warming and ocean acidification might affect larval shell size.
  There will be a range, but some possibilities are:
  - They will both decrease size
  - Temperature will increase size, while pH will decrease shell size

- Q: Was your hypothesis supported by the data, why or why not?
  Answers will differ and depend on their original hypothesis, but make sure they are using the data from the graph

- Q: How did ocean warming affect shell size? How did ocean acidification effect shell size? Why might this be happening (hint: think back to the image of the bioenergetic framework)?
  Warming – Greatly increased shell size when compared to the control.
  Why? While it is not directly tied to the bioenergetic framework, warmer temperatures increase an ectothermic organism's metabolism, which ultimately leads to more available energy (as long as the organism has enough food to support the increasing metabolic activities, which in this experiment was true).

  Acidification – Did not seem to impact shell size when compared to the control
  Why? It seems as though the acidic conditions that the larvae are experiencing might not be very stressed meaning that they are able to provide the energy needed to maintain crucial physiological processes in the *Maintenance* category and still have energy left over for continuous shell growth.

Part 2 – Hypothesis Generation and Sample Collection

Split the class into three groups (or multiples of three if you have a large class). This will be a jigsaw puzzle activity, so randomly assign one of the three molecules (Lipid, Antioxidants, or Protein) that we are measuring to each group. Based on the introductory information and class discussion on the effects of temperature and pH on larval shell size, have each student write down their hypothesis under *Section 1* on the *Student Worksheet* in Appendix A for how temperature and pH will affect their assigned molecular group after the first week (6 days old) of an oyster's larval development (this could also be done at home along with Part 1 as a pre-lab assignment). Have the group discuss their individual hypotheses and come to a consensus that they will share aloud to the class later in Part 5.

To help students understand what the biochemical assays are actually measuring within our tissue samples in the laboratory (i.e. the total amount of the specified molecule in a sample), each group will get three samples (i.e. three cups). The three samples are replicates from one of the three treatments. The students will identify which treatment their samples were collected from later in *Section 2* of the *Student Worksheet* (the answer is identified on the answer sheet as well as in the *Classroom Set-up* section). Each cup will contain beans (or beads) of two different types/colors. The colored bean will represent the assigned molecule (i.e. lipids, antioxidants, or proteins). All other molecules that the students are not measuring within our samples will be represented with the black beans.

Students will count their assigned molecule (i.e. the colored bean) and record the data in **Table A** in *Section 1* on the *Student Worksheet* in Appendix A. Because we want this part of the activity to be short and sweet, each bean will represent a specified number (i.e. multiplier) of molecules, which are listed on the *Student Worksheet*.

Part 3a – Generating a Standard Curve (Advanced)

To determine the concentration of a molecule in a tissue sample, colorimetric assays (or analyses) are used. Colorimetric assays use a reactant that will change the color of the final solution when they combine with the molecule of interest. The color of the final solution is measured with a spectrophotometer, a machine that records the wavelength of light that is transmitted (i.e. passes through) the solution, to obtain an absorbance value. The absorbance value can then be used to calculate the final concentration of the molecule in the tissue sample.

To calculate the molecule concentration from the measured absorbance of the collected sample, a relationship between known concentrations of the molecule of interest and the range of absorbances produced from the colorimetric reaction are needed. That relationship is referred to as the standard curve, which is an equation that is created by regressing the known concentrations and the measured absorbances against each other. The standard curve, misleadingly named, produces a linear equation (i.e. $Y = mX + b$) that is then used to determine the concentration (X) of the molecules within each tissue sample from the measured absorbance (Y).

Each group will work together to create a standard curve for their assigned molecule (i.e. Total Protein, Total Lipid, Total Antioxidant). Based on how much time you want to dedicate to this section, there are options for where to start your class:

Part 1 – Reading the microplate spectrophotometer output:

Students will get a picture of a fully pipetted microplate titled ***Absorbances and Attributes*** (The second page of Appendices B – D; the image will also include all of the experimental tissue samples too) and the corresponding sample ***Microplate Layout*** (the first page of Appendices B – D). The sample ***Microplate Layout*** is a diagram of the microplate that I create before running my assay so that I know which tissue sample gets pipetted into each well (i.e. the circles) on the microplate. Labeled on top of each microplate well on the ***Absorbances and Attributes Document*** will be the measured absorbance value output from the spectrophotometer.

Students will use the ***Microplate Layout*** to find the wells and known concentrations (labeled under the standard ID) that correspond to the standards used in the standard curve. Each standard will have technical duplicates that students will need to average across prior to graphing, which can be done in Excel too. The duplicates allow us to check for any technical errors due to pipetting, mixing of the reagents, etc.

Part 2 – Graphing and generating the standard curve equation (excel):

You will need a graphing program that can generate a regression line and equation given the data points from the standard curve. The instructions provided here will be for Excel:

1. In column A, the students will input the known molecule concentrations (the concentrations will be listed under the sample name on the ***Microplate Layout*** and will be different based on which molecule they were assigned to) in column A with a header (ex. "Total Antioxidant (µmol per larva)").
2. In column B under the header "Absorbance (nm)", type in the corresponding average absorbance values calculated from the values listed on the microplate image on the ***Absorbances and Attributes Document***.
3. Then go to the *Insert* tab and click on the *Scatter* plot graph
4. Right-click on the graph and navigate to *Select Data*
    a. In the *Chart data range* select all of column A and B, then make sure that the correct columns were inserted in the *X* and *Y-values*
    b. The X values should refer to column A with the molecule concentrations
    c. The Y values should refer to column B with the absorbances
5. Then go to the *Chart Design* tab -> *Add Chart Element -> Trendline -> Linear*
    a. Double or right-click on the regression line to bring up the "Format Trendline" menu -> select "Display Equation on chart" to get the regression equation needed to calculate the molecule concentrations in your collected samples

<u>Part 3b - Using a Standard Curve and Sample Absorbencies</u>

If you did not have the students generate the standard curve above, the equations will be provided to each group in the *Attributes Table* on their ***Absorbances and Attributes Document***. Feel free to take some time to explain how that equation was generated before they get started analyzing the tissue samples.

Each group will use the ***Microplate Layout*** (Appendices B – D) to find the well location for each sample. Then they will use their microplate image on the ***Absorbances and Attributes Document*** to find the measured absorbance for each sample. Each treatment replicate (e.g. Control.a) has triplicate wells (e.g Control.a – 1) to account for any technical errors from humans. Students will need to average across the triplicate wells to get the absorbance value for each treatment replicate (more detail below).

Using the *Student Worksheet* (Appendix A), students will fill in **Table B** in *Section 2* to calculate the concentration of their assigned molecule. Then they will fill in **Table C**, also in *Section 2*, to calculate their group's average molecule abundance that will be used for graphing in Part 4. These calculations will be based on the standard curve equation that the students were given or found above.

Steps for filling in **Table B**:

1. Each group will use the ***Microplate Layout*** alongside the ***Absorbances and Attributes Document*** to fill in *Absorbances 1 – 3* for each treatment replicate listed in **Table B**.
2. The *Average Absorbance* will be calculated from *Absorbances 1 – 3* for each treatment replicate.
3. Each group needs to then identify the *standard curve equation* for their assigned molecule in the *Attributes Table* on the ***Absorbances and Attributes Document***.
4. The students will use the *standard curve equation* and the *Average Absorbance* to solve for X, which is the *Molecule Concentration*.

Steps for filling in **Table C**:

1. Students will use the *Molecule Concentration* column in **Table B** to calculate the *Average Molecule Concentration* in **Table C** for each treatment.
    a. They will take the average of **replicates a-c** (i.e. individual tanks in the experiment) for the three treatments (Control, Temp, and pH).
2. In the *Attributes Table* on the ***Absorbances and Attributes Document***, students will locate the *Total Homogenate Volume* for each treatment and record it in the aptly-named column.
3. To calculate the *Total Molecule Abundance*, students will multiply the *Average Molecule Concentration* by the *Total Homogenate Volume*.
4. Going back to the *Attributes Table* on the ***Absorbances and Attributes Document***, students will record the *Standard Value* for each treatment in the identically-named column.
5. Lastly, students will divide the *Total Molecule Abundance* by the *Standard Value* to the *Final Molecule Abundance* for each treatment, which will be used to graph in the next part.

<u>Part 4 – Graphing the Data</u>

Students will use the calculated average concentrations of their assigned molecule in **Table C** to graph these amounts per treatment. The graphing can be done in Excel (advanced) or by hand using the graphing grid in "Part 3" of the *Student Worksheet* (Appendix A). Students in each group may

work together, or individually, on both the graphing and question portion. If done individually, make sure to leave time for students to think-pair-share to come to a final consensus about the treatment effects to their molecule before the Gallery Walk.

Part 5a – Gallery Walk, Reflection, and Wrap-up

Each group will present their findings to the rest of the class in either a standard presentation format in the front of the classroom or a gallery walk where each group visits the other two molecule group's final graph. Along with the final graph, each group should share their original hypothesis and state whether or not it was supported based on their findings

At this time, have all groups return to their seats if they were up doing the gallery walk, and display the three final graphs from each group to have a big-picture group discussion.

- Q: How is a high temperature affecting larval oysters? Why do you think that is?

  Warmer temperatures cause increased growth in both larval shell size, as well as their biomass (a.k.a. total protein). Increased temperature does not seem to cause a lot of oxidative stress (a.k.a. normal level of antioxidants present), but there are some signs of stress because lipid storage levels are low.

  This might be occurring because the increased temperatures cause the ectothermic larvae to increase their metabolism, which requires sufficient food/fuel. While they clearly have access to enough food energy to fuel the increased energy demands for growing, there is not enough of a surplus to put into storage.

  Also, it seems as though the antioxidants that larvae already have produced (we call these constitutive molecules) are able to withstand a natural increase in reactive oxygen species that come with the increase in metabolism, when comparing the control and temperature groups.

- Q: How is a low pH affecting larval oysters? Why do you think that is?

  Larval oyster growth is not impacted by acidic waters, but we do find that they are experiencing a larger amount of oxidative stress from the high level of antioxidants. Similarly, we see that their lipid stores are also low, which is indicative of some amount of stress.

  Likely, the reason that growth (both shell and biomass via protein) is unaffected, but we see high levels of oxidative stress, is due to an increase in metabolic activity to sustain growing (which is a priority for larvae) that also causes an increase in reactive oxygen species production. Because larvae are expending energy to continue growing and combat high levels of stress, they do not have energy left over to put away into lipid storage.

- Q: What do you think would happen to larval oysters if they experienced both environmental stressors simultaneously?

  This is also part of my research and we do not exactly know the answer to this yet, so anything that has reasonable logic behind the answer will be good.

Generally (refer to Crain et al. 2008 for more explanation), there are thought to be four main ways that multiple stressors can impact an organism: independently, additively, synergistically, or antagonistically. Additive interaction is the sum of the individual responses to the single stressors. Synergistic is when the multiple stressor response is greater than the additive in a more negative way, while antagonistic is that the response is less harmful than the additive.

- Q: Do you think the physiological effects will change throughout the larval developmental period given that this is almost halfway through?

  Again, this will generate a range of responses, which we do not exactly know the answer to.

  With my work, I have seen the antioxidant response in the treatments that experience acidification (this includes the Temp x pH treatment) become stronger. We also found that biomass (i.e. Total Protein) starts to decline in those experiencing pH conditions around day 8.

Part 5b – Claim, Evidence, and Reasoning with Peer Review (Optional)

Using the question below, students will go through a claim, evidence and reasoning (CER) exercise; however, the question can be used in the section above if there is not time for this activity. This activity can be done during a third class period or as homework outside of class.

In this section, students will mimic the publication process that is integral to communication within science. The CER portion mimics writing the 'discussion/conclusion' section, which communicates the bigger meaning of the results that you found through your experiment. The discussion section also ties in previous scientific knowledge to help contextualize your results. The students will use the findings from the lab for the 'evidence', and the background information provided at the beginning of the lab for the 'reasoning' to support their 'claim'.

The students will then participate in the peer review process, which is what all scientific papers go through before being published. Your scientific paper is submitted to several reviewers who are scientists in your same field. The reviewers then provide feedback on your overall evidence and reasoning supporting the overall claim of your paper. You must respond to the comments provided by the reviewers before your paper can be finally published. The students will present their CERs to their peers to get feedback on their evidence and reasoning for their claims. The review process can be online as a homework assignment or in-person via verbal feedback. This is a great chance to teach students how to give and receive constructive criticism. Students should either edit their CERs or write about the feedback received and how that made them re-think the claims they made.

- Q: Given what we have learned today, how do you think oyster populations might perform in the future, given that climate change will continue to make the waters warmer and more acidic?

  Again, you will get a range of answers, which again we do not yet know the answer to.

Based on my current findings, while they do experience some amount of stress, as long as there is adequate food available, oyster larvae will be able to survive and contribute to the reproducing/adult populations.

Virtual Adaptation:

Parts 1, 3 and 4 are easily adapted for virtual classroom use, especially when using breakout rooms for the groups in parts 3 and 4.

For the bean activity in part 2, slides can be created for each cup with cut and pasted images of the beans in a pile in the middle of the slide. The kids can then interact with the slide and count the beans by moving the images around.

In part 5, the students are meant to do a gallery walk to explore how climate change has affected the other measurements in the experiment. For this portion, each group will have an assigned breakout room and can create a slide with their finalized graph and any other conclusions that they might have. Then, the students can either present their slide in the main room to everyone, or you can rotate groups between all the other breakout rooms to simulate the gallery walk.

Lastly, in the optional CER activity in part 5, one way to make it virtual outside of class is to have students record their CER and share it via Flipgrid. Then students can peer review each Flipgrid by commenting on the video.

Assessment

There is a worksheet included for the students to follow along with and complete. Within the worksheet are questions that will test the students understanding of what they learned in the introductory PowerPoint and have them apply it but will also have them analyzing their data and evaluating their findings. There will also be a larger gallery walk and group discussion where students will try to conjecture how warmer temperatures and lower pH will impact oyster larvae using all physiological measures from the lesson. There is also an option for students to mimic the science communication and peer review process with a claim, evidence, and reasoning exercise.

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Date: _____

Name: _____ Molecule Group: _____

Case of the Stressed-Out Larva:
A Study of Global Climate Change Effects on Oyster Cellular Physiology

Section 1 – Molecule Hypotheses and Sample Analysis of 6 days-old Larvae

1. As a group, hypothesize how the high temperature and low pH treatments will affect the level of your assigned molecule.

2. The biochemical assay that is used to measure your group's molecule computes the total number of molecules in a sample. You will be mimicking the assay to count how many of your assigned molecules are in the three samples in front of you (i.e. cups).

   Your samples are labeled with the replicate letter, but they are not labeled with the treatment name. In *Section 2*, your group will identify which treatment your samples came from.

   To mimic the scientific process, randomly take a sample, count, and record the total number of your assigned molecules (i.e. beans that are not black beans). Record your counts in **Table A** below.

   Because we do not want you counting all day long, each molecule you count actually represents several molecules in the real sample. See the list below for the multiplier that your group will use to calculate the *Total Molecule Abundance* and write the value down in the *Multiplier* column in **Table A** below.

   - Total Protein: 1 bean = 6 molecules
   - Total Antioxidant: 1 bean = 0.001 molecules
   - Total Lipid: 1 bean = 703 molecules

Table A

Replicate	Molecule Count	Multiplier	Total Molecule Abundance (Molecule Count x Multiplier)
1			
2			
3			

3. Now take the average of the three treatment replicates in the *Total Molecule Abundance* column in **Table A** above. This will be the average for the entire treatment.

   Average Total Molecule Abundance in Unknown Treatment: _____

4. Lastly, you need to standardize the *Average Molecule Abundance* to compare across the three treatments. The standardization value that your group will use to for your molecule is listed below. To standardize, take the *Average Total Molecule Abundance* in Question 3 and divide it by your *Standard Value* below.

   This standardization practice will come back later, so think about why it is necessary. What might be different among treatments that could bias our comparison if we did not standardize?

   - Standardize Total Protein by: 5000 Larvae
   - Standardize Total Antioxidant by: 9.074 x 10^{-5} g of protein
   - Standardize Total Lipid by: 73.913 ug of protein

   Average Standardized Molecule Abundance in Unknown Treatment: _____

Section 2 – Using the Standard Curve to Evaluate Specific Molecule Concentrations

1. Grab the microplate image on the ***Absorbance and Attribute Document*** and the ***Microplate Layout*** for your group's assigned molecule. As scientists, you have finished the protocol for your assay by placing your microplate into the microplate reader, which is a specialized spectrophotometer, and have now received the results for your measured absorbencies.

   Using the ***Microplate Layout***, identify which well on the microplate image corresponds to which sample. Record the measured absorbance (the number on the top of each well) in **Table B** below.

   After your group has filled in all the absorbances, calculate the *Average Absorbance* in the labeled column below. Locate the *Standard Curve* equation in the *Attribute Table* on the ***Absorbance and Attribute Document***. Use the *Average Absorbance* and the *Standard Curve* equation to calculate the *Molecule Concentration* in **Table B** below.

Table B

Sample	Replicate	Absorbance 1	Absorbance 2	Absorbance 3	Average Absorbance	Molecule Concentration
Example (Used made-up numbers – not from lesson)	a	0.987	0.974	0.998	$\frac{(0.987 + 0.974 + 0.998)}{3} =$ 0.986	$Y = 0.0014x + 0.1642$ Y = 0.986 X = 822 ug/mL
Control	a					
Control	b					
Control	c					
Temp	a					
Temp	b					
Temp	c					
pH	a					
pH	b					
pH	c					

2. The absorbances in **Table B** were technical replicates. Technical replicates are used to address sources of human error during the assay like pipetting or mixture of reagents.

   Now, take the average of the sample replicates (ex. Control a,b, and c) from **Table B** to get one molecule concentration per treatment. Record the averages in the *Average Molecule Concentration* column in **Table C** below.

Next, you will calculate the *Total Molecule Abundance*. Multiply the *Average Molecule Concentration* by the volume of the homogenate. The homogenate is a solution with cells that have been broken open, or lysed. The homogenate volume can be found in the *Attributes Table* on the ***Absorbances and Attributes Document***. Write down the volume in the *Total Homogenate Volume* column in **Table C** below for ease of calculation.

Lastly, you will standardize the *Total Molecule Abundance* to get the *Final Molecule Abundance* value. In the *Attributes Table* on the ***Absorbances and Attributes Document***, you can find the *Standard Value*, record it in the identified column below. You will then divide the *Total Molecule Abundance* by the *Standard Value* to obtain the standardized *Final Molecule Abundance*. You will use the *Final Molecule Abundance* value to graph the results of our experiment in the next section.

Table C

Treatment	Average Molecule Concentration	Total Homogenate Volume	Total Molecule Abundance	Standard Value	Final Molecule Abundance

3. Which treatment were your samples from in Part 2? Most likely, your average *Final Molecule Abundance* values are close, but not exactly the same between Part 2 and 3, why might this be?

Section 3 – Graphing Your Data

To this point, you have hypothesized how warmer and acidified waters might affect your assigned molecule, collected your experimental samples, processed them using a biochemical assay, and calculated the final abundance for your molecule. Now, we get to analyze how the three treatments actually affect oyster larvae and then decide whether or not the results support your hypotheses.

1. Use the graphing plane below to draw and label a bar graph showing your *Final Molecule Abundance* from **Table C** above. Because each group's final units are different, the scale on the y-axis needs to be filled in by your group. Be sure you properly label your axes with the correct variable and units.

2. Do these results support your group's hypotheses, why or why not?

3. How did warmer water temperatures and acidic water conditions impact your group's molecule abundance?

    a. What do you think may be causing these results?

    b. How might these results impact the outcome of the two other molecule abundances? Recall what you learned earlier about the bioenergetic framework under environmental stress.

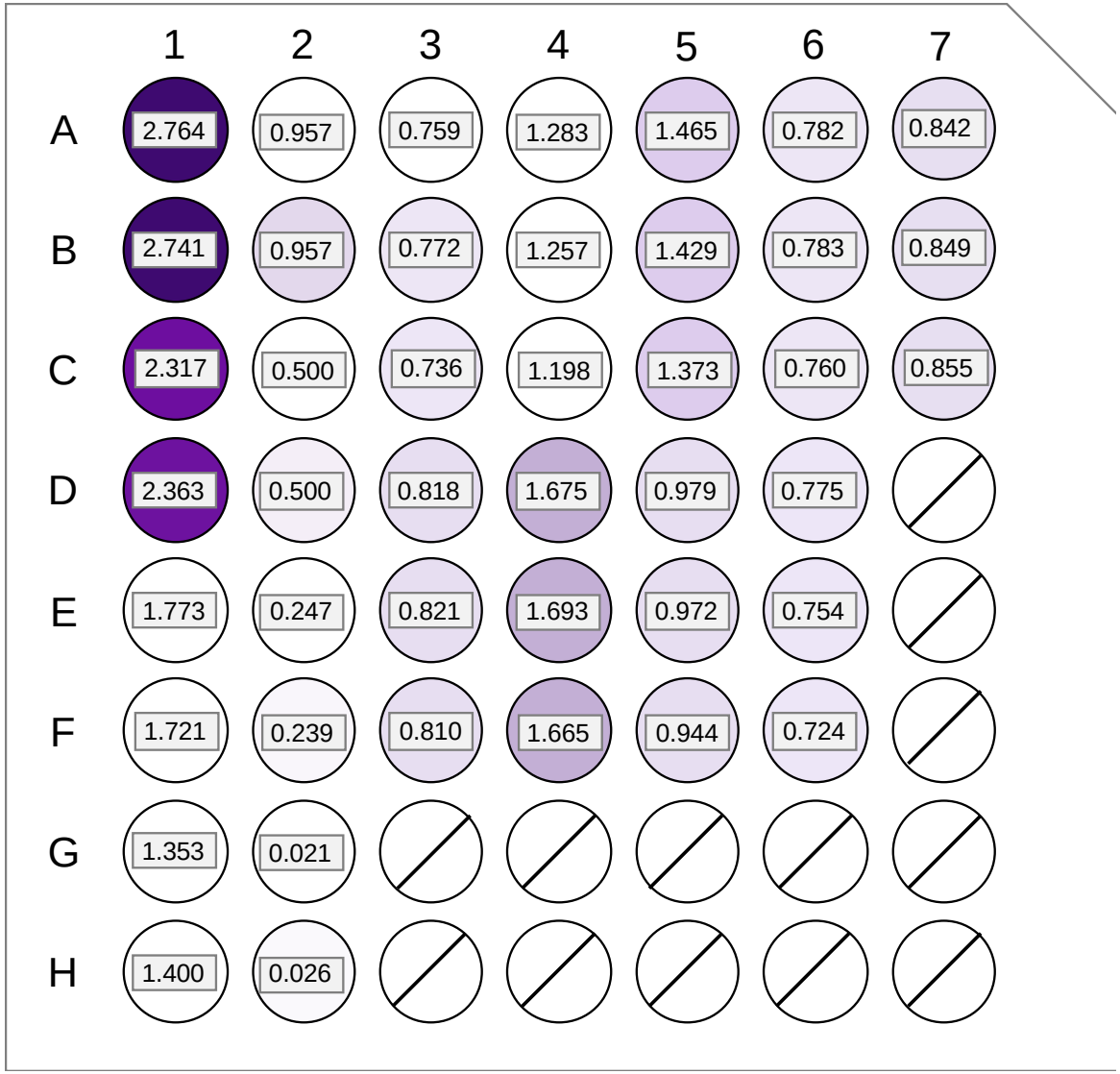
Total Protein Microplate Layout

	1	2	3	4	5	6	7
A	Std 1.a 2000 ug/mL	Std 5.a 500 ug/mL	Control.a – 1	Control.c – 1	Temp.b – 1	pH.a – 1	pH.c – 1
B	Std 1.b 2000 ug/mL	Std 5.b 500 ug/mL	Control.a – 2	Control.c – 2	Temp.b – 2	pH.a – 2	pH.c – 2
C	Std 2.a 1500 ug/mL	Std 6.a 250 ug/mL	Control.a – 3	Control.c – 3	Temp.b – 3	pH.a – 3	pH.c – 3
D	Std 2.b 1500 ug/mL	Std 6.b 250 ug/mL	Control.b – 1	Temp.a – 1	Temp.c – 1	pH.b – 1	
E	Std 3.a 1000 ug/mL	Std 7.a 125 ug/mL	Control.b – 2	Temp.a – 2	Temp.c – 2	pH.b – 2	
F	Std 3.b 1000 ug/mL	Std 7.b 125 ug/mL	Control.b – 3	Temp.a – 3	Temp.c – 3	pH.b – 3	
G	Std 4.a 750 ug/mL	Std 8.a 25 ug/mL					
H	Std 4.b 750 ug/mL	Std 8.b 25 ug/mL					

The standard labels, like **Std 1.a**, can be read as **Standard** = 1 and **Technical Replicate** = a

The sample labels, like **Control.a-1**, can be read as **Treatment** = Control, **Sample Replicate** = a, and **Technical Replicate** = 1

Total Protein Absorbances and Attributes



	1	2	3	4	5	6	7
A	2.764	0.957	0.759	1.283	1.465	0.782	0.842
B	2.741	0.957	0.772	1.257	1.429	0.783	0.849
C	2.317	0.500	0.736	1.198	1.373	0.760	0.855
D	2.363	0.500	0.818	1.675	0.979	0.775	
E	1.773	0.247	0.821	1.693	0.972	0.754	
F	1.721	0.239	0.810	1.665	0.944	0.724	
G	1.353	0.021					
H	1.400	0.026					

Total Protein Attributes

- Standard curve: Y = 0.0014x + 0.1642
  - Y = Absorbance
  - X = Protein Concentration
- Total Homogenate Volume:
  - Control = 0.166 mL
  - Temp = 0.167 mL
  - pH = 0.165 mL
- Standardizing Value = 5000 larvae

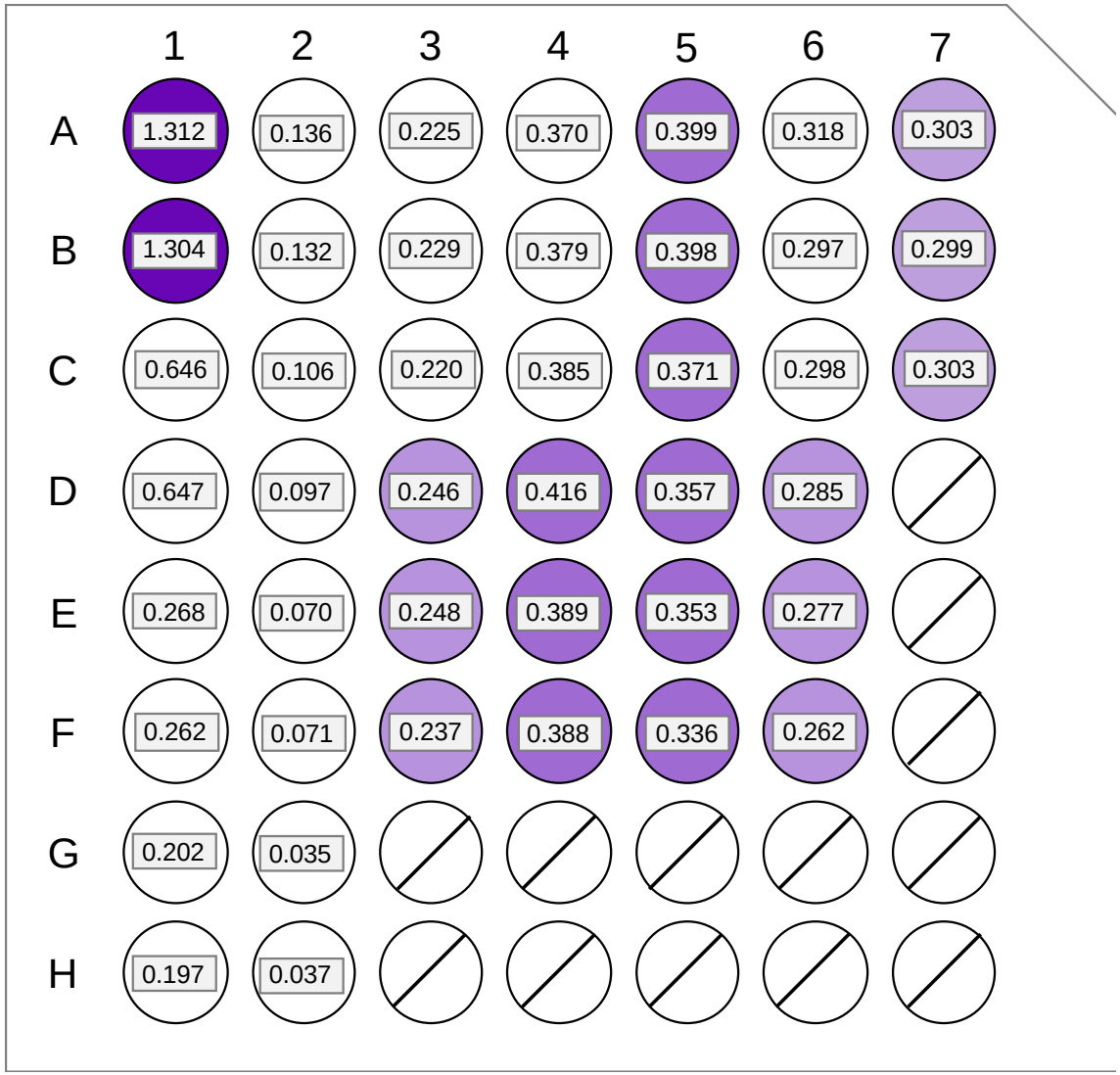
Total Antioxidant Microplate Layout

	1	2	3	4	5	6	7
A	Std 1.a 1000 umol/L	Std 5.a 100 umol/L	Control.a – 1	Control.c – 1	Temp.b – 1	pH.a – 1	pH.c – 1
B	Std 1.b 1000 umol/L	Std 5.b 100 umol/L	Control.a – 2	Control.c – 2	Temp.b – 2	pH.a – 2	pH.c – 2
C	Std 2.a 500 umol/L	Std 6.a 75 umol/L	Control.a – 3	Control.c – 3	Temp.b – 3	pH.a – 3	pH.c – 3
D	Std 2.b 500 umol/L	Std 6.b 75 umol/L	Control.b – 1	Temp.a – 1	Temp.c – 1	pH.b – 1	
E	Std 3.a 200 umol/L	Std 7.a 50 umol/L	Control.b – 2	Temp.a – 2	Temp.c – 2	pH.b – 2	
F	Std 3.b 200 umol/L	Std 7.b 50 umol/L	Control.b – 3	Temp.a – 3	Temp.c – 3	pH.b – 3	
G	Std 4.a 150 umol/L	Std 8.a 25 umol/L					
H	Std 4.b 150 umol/L	Std 8.b 25 umol/L					

The standard labels, like **Std 1.a**, can be read as **Standard** = 1 and **Technical Replicate** = a

The sample labels, like **Control.a-1**, can be read as **Treatment** = Control, **Sample Replicate** = a, and **Technical Replicate** = 1

Total Antioxidant Absorbances and Attributes



Total Antioxidant Attributes

- Standard curve: Y = 0.0013x + 0.0036
  - Y = Absorbance
  - X = Antioxidant Concentration
- Total Homogenate Volume:
  - Control = 1.06×10^{-4} L
  - Temp = 1.07×10^{-4} L
  - pH = 1.05×10^{-4} L
- Standardizing Value:
  - Control = 9.17×10^{-5} g protein
  - Temp = 1.42×10^{-4} g protein
  - pH = 7.42×10^{-5} g protein

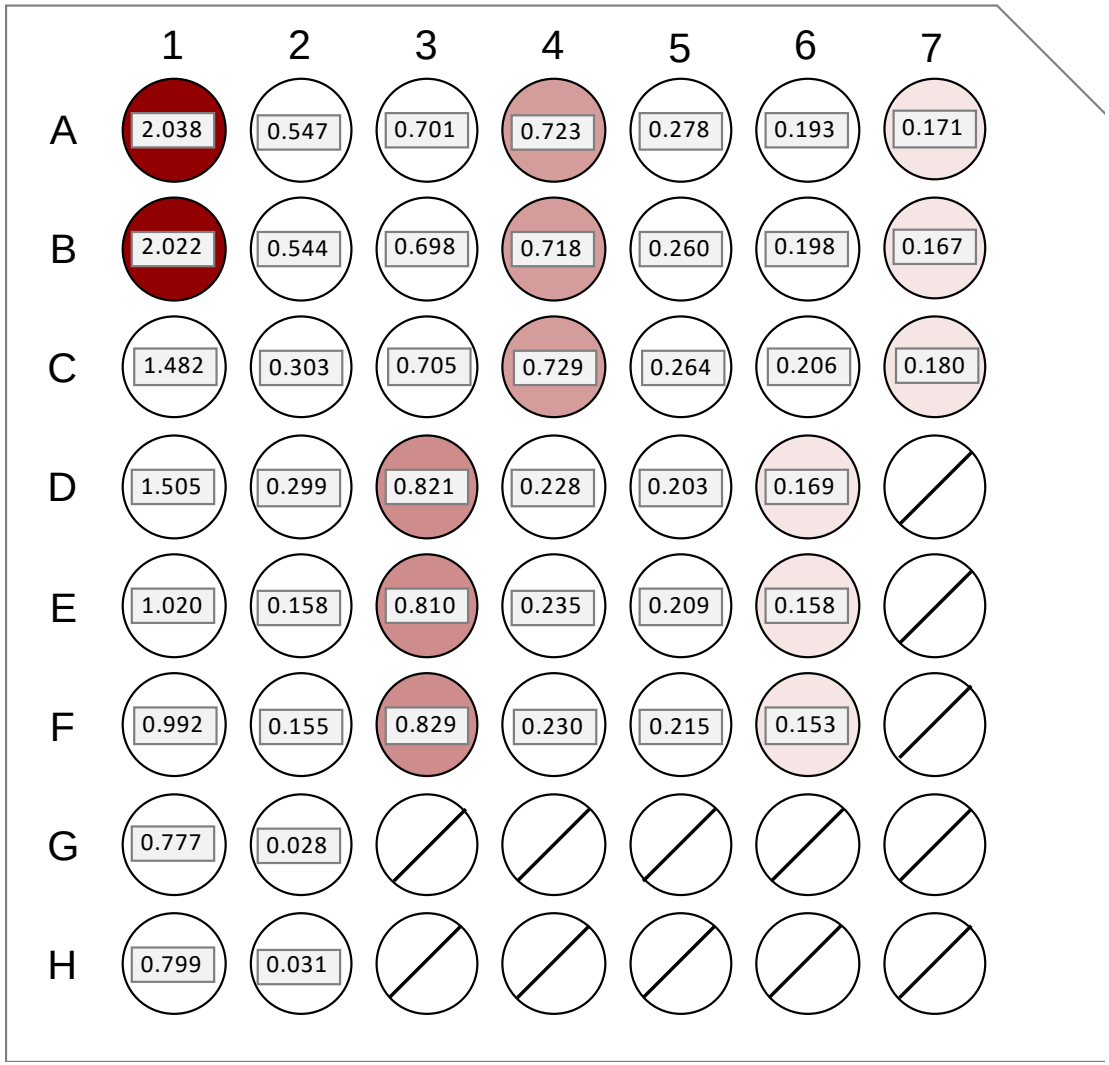
Total Lipid Microplate Layout

	1	2	3	4	5	6	7
A	Std 1.a 2000 ng/uL	Std 5.a 500 ng/uL	Control.a – 1	Control.c – 1	Temp.b – 1	pH.a – 1	pH.c – 1
B	Std 1.b 2000 ng/uL	Std 5.b 500 ng/uL	Control.a – 2	Control.c – 2	Temp.b – 2	pH.a – 2	pH.c – 2
C	Std 2.a 1500 ng/uL	Std 6.a 250 ng/uL	Control.a – 3	Control.c – 3	Temp.b – 3	pH.a – 3	pH.c – 3
D	Std 2.b 1500 ng/uL	Std 6.b 250 ng/uL	Control.b – 1	Temp.a – 1	Temp.c – 1	pH.b – 1	
E	Std 3.a 1000 ng/uL	Std 7.a 125 ng/uL	Control.b – 2	Temp.a – 2	Temp.c – 2	pH.b – 2	
F	Std 3.b 1000 ng/uL	Std 7.b 125 ng/uL	Control.b – 3	Temp.a – 3	Temp.c – 3	pH.b – 3	
G	Std 4.a 750 ng/uL	Std 8.a 25 ng/uL					
H	Std 4.b 750 ng/uL	Std 8.b 25 ng/uL					

The standard labels, like **Std 1.a**, can be read as **Standard** = 1 and **Technical Replicate** = a

The sample labels, like **Control.a-1**, can be read as **Treatment** = Control, **Sample Replicate** = a, and **Technical Replicate** = 1

Total Lipid Absorbances and Attributes



Total Lipid Attributes

- Standard curve: Y = 0.001x + 0.0317
  - Y = Absorbance
  - X = Lipid Concentration
- Total Homogenate Volume:
  - Control = 135.89 uL
  - Temp = 136.87 uL
  - pH = 135.26 uL
- Standardizing Value:
  - Control = 137.88 ug protein
  - Temp = 124.17 ug protein
  - pH = 74.23 ug protein

Case of the Stressed-Out Larva:
A Study of Global Climate Change Effects to Oyster Cellular Physiology

Section 1 – Molecule Hypotheses and Sample Analysis of 6 days-old Larvae

1. As a group, hypothesize how the high temperature and low pH treatments will affect the level of your assigned molecule.

   Some hypotheses might be:
   - Both will increase/decrease molecule
   - Warming will increase/decrease molecule, while acidification will increase/decrease molecule

   Might want to have them explain how they developed their hypothesis. For instance, because antioxidants are necessary to the survival and *Maintenance* of an organism, the total antioxidant group may think that both temperature and pH will increase antioxidant abundance within the organism.

   The total protein group may hypothesize that it will react similarly to the shell size data that they just saw since protein falls mostly within the *Growth* category in the bioenergetic framework.

   Lastly, the total lipid group may hypothesize that because it falls within the *Storage* category, that larvae in the temperature and acidic treatments will have lower amounts of lipid since most of their energy will be going to the *Maintenance* category.

2. The biochemical assay that is used to measure your group's molecule computes the total number of molecules in a sample. You will be mimicking the assay to count how many of your assigned molecules are in the three samples in front of you (i.e. cups).

   Your samples are labeled with the replicate letter, but they are not labeled with the treatment name. In *Section 2*, your group will identify which treatment your samples came from.

   To mimic the scientific process, randomly take a sample, count, and record the total number of your assigned molecules (i.e. beans that are not black beans). Record your counts in **Table A** below.

   Because we do not want you counting all day long, each molecule you count actually represents several molecules in the real sample. See the list below for the multiplier that your group will use to calculate the *Total Molecule Abundance* and write the value down in the *Multiplier* column in **Table A** below.
   - Total Protein: 1 bean = 6 molecules
   - Total Antioxidant: 1 bean = 0.001 molecules
   - Total Lipid: 1 bean = 703 molecules

Table A – Total Protein (ug)

Replicate	Molecule Count	Multiplier	Total Molecule Abundance (Molecule count x Multiplier)
1	30	6	180
2	25	6	150
3	16	6	96

Table A – Total Antioxidant (umol)

Replicate	Molecule Count	Multiplier	Total Molecule Abundance (Molecule count x Multiplier)
1	18	0.001	0.018
2	20	0.001	0.020
3	30	0.001	0.030

Table A – Total Lipid (ng)

Replicate	Molecule Count	Multiplier	Total Molecule Abundance (Molecule count x Multiplier)
1	31	703	21793
2	25	703	17575
3	28	703	19684

3. Now take the average of the three treatment replicates in the *Total Molecule Abundance* column in **Table A** above. This will be the average for the entire treatment.

   Average Total Molecule Abundance in Unknown Treatment:

   Total Protein = 142 ug
   Total Antioxidant = .0227 umol
   Total Lipid = 19,684 ng

5. Lastly, you need to standardize the *Average Molecule Abundance* to compare across the three treatments. The standardization value that your group will use to for your molecule is listed below. To standardize, take the *Average Total Molecule Abundance* in Question 3 and divide it by your *Standard Value* below.

   This standardization practice will come back later, so think about why it is necessary. What might be different among treatments that could bias our comparison if we did not standardize?

   - Standardize Total Protein by: 5000 Larvae
   - Standardize Total Antioxidant by: 9.074×10^{-5} g of protein
   - Standardize Total Lipid by: 73.913 ug of protein

   Average Standardized Molecule Abundance in Unknown Treatment:

   Total Protein = 0.028 ug/larva
   Total Antioxidant = 250.17 umol/g protein
   Total Lipid = 266.31 ng/ ug protein

Section 2 – Using the Standard Curve to Evaluate Specific Molecule Concentrations

1. Grab the microplate image on the ***Absorbance and Attribute Document*** and the ***Microplate Layout*** for your group's assigned molecule. As scientists, you have finished the protocol for your assay by placing your microplate into the microplate reader, which is a specialized spectrophotometer, and have now received the results for your measured absorbencies.

   Using the ***Microplate Layout***, identify which well on the microplate image corresponds to which sample. Record the measured absorbance (the number on the top of each well) in **Table B** below.

   After your group has filled in all the absorbances, calculate the *Average Absorbance* in the labeled column below. Locate the *Standard Curve* equation in the *Attribute Table* on the ***Absorbance and Attribute Document***. Use the *Average Absorbance* and the *Standard Curve* equation to calculate the *Molecule Concentration* in **Table B** below.

Table B – Total Protein

Sample	Replicate	Absorbance 1	Absorbance 2	Absorbance 3	Average Absorbance	Molecule Concentration
Control	a	0.759	0.772	0.736	0.756	422.52 ug/mL
Control	b	0.818	0.821	0.810	0.817	465.95 ug/mL
Control	c	1.283	1.257	1.198	1.246	772.52 ug/mL
Temp	a	1.675	1.693	1.665	1.678	1081.07 ug/mL
Temp	b	1.465	1.429	1.373	1.422	898.59 ug/mL
Temp	c	0.979	0.972	0.944	0.965	571.94 ug/mL
pH	a	0.782	0.783	0.760	0.775	436.29 ug/mL
pH	b	0.775	0.754	0.724	0.751	419.13 ug/mL
pH	c	0.842	0.849	0.855	0.852	491.45 ug/mL

Table B – Total Antioxidant

Sample	Replicate	Absorbance 1	Absorbance 2	Absorbance 3	Average Absorbance	Molecule Concentration
Control	a	0.225	0.229	0.220	0.225	170.10 umol/L
Control	b	0.246	0.248	0.237	0.243	184.41 umol/L
Control	c	0.370	0.379	0.385	0.378	287.82 umol/L
Temp	a	0.416	0.389	0.388	0.398	303.28 umol/L
Temp	b	0.399	0.398	0.371	0.389	296.59 umol/L
Temp	c	0.357	0.353	0.336	0.349	265.64 umol/L
pH	a	0.318	0.297	0.298	0.304	231.33 umol/L
pH	b	0.285	0.277	0.262	0.274	208.21 umol/L
pH	c	0.303	0.299	0.303	0.301	229.08 umol/L

Table B – Total Lipid

Sample	Replicate	Absorbance 1	Absorbance 2	Absorbance 3	Average Absorbance	Molecule Concentration
Control	a	0.701	0.698	0.705	0.701	669.63 ng/uL
Control	b	0.821	0.810	0.829	0.820	788.30 ng/uL
Control	c	0.723	0.718	0.729	0.723	691.63 ng/uL
Temp	a	0.228	0.235	0.230	0.231	199.30 ng/uL
Temp	b	0.278	0.260	0.264	0.267	235.63 ng/uL
Temp	c	0.203	0.209	0.215	0.209	177.30 ng/uL
pH	a	0.193	0.198	0.206	0.199	167.30 ng/uL
pH	b	0.169	0.158	0.153	0.160	128.30 ng/uL
pH	c	0.171	0.167	0.180	0.173	140.97 ng/uL

2. The absorbances in **Table B** were technical replicates. Technical replicates are used to address sources of human error during the assay like pipetting or mixture of reagents.

   Now, take the average of the sample replicates (ex. Control a,b, and c) from **Table B** to get one molecule concentration per treatment. Record the averages in the *Average Molecule Concentration* column in **Table C** below.

Next, you will calculate the *Total Molecule Abundance*. Multiply the *Average Molecule Concentration* by the volume of the homogenate. The homogenate is a solution with cells that have been broken open, or lysed. The homogenate volume can be found in the *Attributes Table* on the ***Absorbances and Attributes Document***. Write down the volume in the *Total Homogenate Volume* column in **Table C** below for ease of calculation.

Lastly, you will standardize the *Total Molecule Abundance* to get the *Final Molecule Abundance* value. In the *Attributes Table* on the ***Absorbances and Attributes Document***, you can find the *Standard Value*, record it in the identified column below. You will then divide the *Total Molecule Abundance* by the *Standard Value* to obtain the standardized *Final Molecule Abundance*. You will use the *Final Molecule Abundance* value to graph the results of our experiment in the next section.

Table C – Total Protein

Treatment	Average Molecule Concentration	Total Homogenate Volume	Total Molecule Abundance	Standard Value	Standardized Molecule Abundance
Control	553.66 ug/mL	0.166 mL	91.97 ug	5000 larvae	0.018 ug/larva
Temp	850.53 ug/mL	0.167 mL	142.04 ug	5000 larvae	0.028 ug/larva
pH	448.95 ug/mL	0.165 mL	74.08 ug	5000 larvae	0.015 ug/larva

Table C – Total Antioxidant

Treatment	Average Molecule Concentration	Total Homogenate Volume	Total Molecule Abundance	Standard Value	Standardized Molecule Abundance
Control	214.11 umol/L	1.06×10^{-4} L	0.023 umol	9.17×10^{-5} g protein	250.82 umol/ g protein
Temp	288.50 umol/L	1.07×10^{-4} L	0.031 umol	1.42×10^{-4} g protein	218.31 umol/ g protein
pH	222.87 umol/L	1.05×10^{-4} L	.023 umol	7.42×10^{-5} g protein	309.97 umol/g protein

Table C – Total Lipid

Treatment	Average Molecule Concentration	Total Homogenate Volume	Total Molecule Abundance	Standard Value	Standardized Molecule Abundance
Control	716.52 ng/uL	135.89 uL	97367.90 ng	137.88 ug protein	706.18 ng/ug protein
Temp	204.08 ng/uL	136.87 uL	27932.43 ng	124.17 ug protein	224.95 ng/ug protein
pH	145.52 ng/uL	135.26 uL	19683.04 ng	74.23 ug protein	265.16 ng/ug protein

1. Which treatment samples did you analyze earlier in Part 2? Most likely, your average *Standardized Molecule Abundance* values are close, but not exactly the same between Part 2 and 3, why might this be?

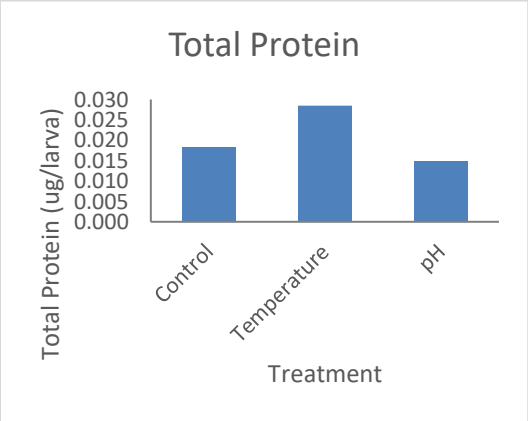
   Total Protein – Temp
   Total Antioxidant – Control
   Total Lipid – pH

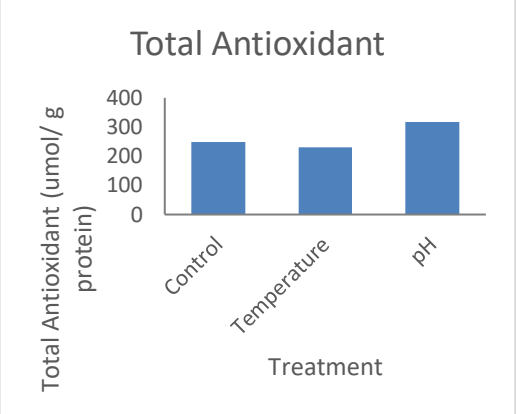
   The numbers will be different depending on how students rounded throughout their calculation to the final number.

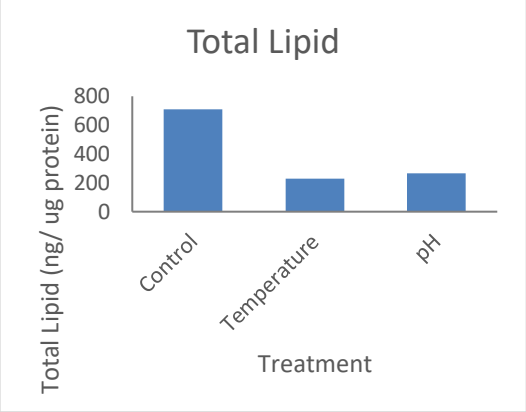
Section 3 – Graphing Your Data

To this point, you have hypothesized how warmer and acidified waters might affect various cellular physiological processes, collected your experimental samples, processed them in your specified biochemical assay, and calculated the final abundance for your assigned molecule. Now, we get to analyze how the three treatments actually impact oyster larvae and then assess whether or not the results support your hypotheses.

1. Use the graphing plane below to draw and label a bar graph showing your *Final Molecule Abundance* from **Table C** above. Because each group's final units are different, the scale on the y-axis needs to be filled in by your group. Be sure you properly label your axes with the correct variable and units.







2. Do these results support your group's hypotheses, why or why not?
   Variety of responses, but make sure they understand that the data only do or do not support their hypotheses, the data do not prove them.

3. How did warmer water temperatures and acidic water conditions impact your group's molecule abundance?
   Total Protein – high water temperatures greatly increased total protein, while acidic conditions slightly lowered protein (Feel free to discuss that when the statistics are run, the difference between the control and acidic treatments would probably not be designated as statistically different)

   Total Antioxidant – warmer water does not seem to cause an increase in total antioxidant molecules, while acidic conditions do. This means that acidic conditions seem to cause more harmful reactive oxygen species, or oxidative stress, to be produced causing the larvae to increase the production of antioxidants to fight off these damaging molecules.

   Total Lipid – Both warmer and acidic waters have low lipid storage levels

   a. What do you think may be causing these results?
      Total Protein – Similar to the effect of temperature on shell size, warmer water temperatures increase the metabolism in ectothermic organisms, so with adequate food to supply their metabolic needs, there is more than enough energy to go around.

      Total Antioxidant – (this one isn't as straight forward, so it's ok if the students do not the exact reason, as I do not know either since I am currently researching it) – While the warmer water should in theory cause an increase in the production of reactive oxygen species due to the increased metabolism, it is possible that the organism already had enough antioxidants to combat this stress since they have similar levels to the organisms in the control treatment. The increase of antioxidants in the larvae in the acidic treatment indicates the increase of the malicious reactive oxygen species, indicative of stressful conditions. This increase could potentially be due to the increase in the energetic requirement to build and maintain (remember that we did not see a size difference in the larval shell between those in the control and pH treatments) the larval shell in acidified waters. Keeping up with that energy requirement for growth means an increase in metabolism, which therefore increases the production of reactive oxygen species.

         Too Long: Didn't Read – Larva have a set amount of antioxidants laying around to be used at anytime, due to the similarity in levels between the control and temp treatments, warm water can be handled without excess energy to produce more antioxidants. Acidic conditions on the other hand have increased reactive oxygen species production (possibly due to increased energy needs for keeping up with building their shells in acidic waters), which causes them to increase antioxidant production.

Total Lipid – Larvae in the temperature treatments are prioritizing growth (as seen in the shell data from earlier, and while the students in this group will not know this yet, this is also seen in the total protein data). Because the warmer water increases metabolism, it is possible that while they are getting enough fuel from their food for growth requirements, there is not surplus energy supply left over to be put into storage. Similarly with those in the acidic conditions, because they are keeping pace with the growth demands of both their shell and protein but also combating increased reactive oxygen species, or oxidative stress, they also may have just enough energy being supplied via food to maintain those activities, but not enough to put away for storage.

b. How might these results impact the outcome of the two other molecule abundances? Recall what you learned earlier about the bioenergetic framework under environmental stress.

You will get a variety of responses, but some thoughts may be:

From the Total Protein group, the increased growth in the temperature group but no change in the pH group may result in similar levels of antioxidants and lipids because they clearly have enough energy for growing and if they were stressed, based on the bioenergetic framework from earlier, they would struggle with growth if they didn't have enough energy.

From the Total Antioxidant group, because the temperature group do not seem stressed (similar levels of antioxidants compared to the control group), they would be expected to have unaffected growth and lipid storage. The larvae in the acidic treatment, on the other hand, seems to be experiencing stress, so the students might expect their growth and lipid storage to suffer as a result.

From the Total Lipid group, because both the larvae in the temperature and pH treatments had low lipid levels, they might expect that the antioxidants are high due to increased stress experience and maintenance costs, while protein would be low due to no energy for anything but maintenance.