Investigating Global Gene Expression Changes in the Pipe Fish in Response to Exposure to Estrogen Disruptors

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Article Context:

Course

- Biochemistry
- Cell Biology
- Developmental Biology
- Genetics
- Microbiology
- Molecular Biology
- Introductory Biology
- Bioinformatics
- Evolution
- Ecology
- Anatomy-Physiology
- Neurobiology
- Plant Biology
- Science Process Skills

Course Level

- Introductory
- Upper Level
- Graduate
- High School
- Other

Class Type

- Lecture
- Lab
- Seminar
- Discussion Section
- On-line
- Other

Audience

- Life Sciences Major
- Non-Life Science Major
- Non-Traditional Student
- 2-year College
- 4-year College
- University
- Other

Class Size

- 1 – 50
- 51 – 100
- 101+

Assessment Type

- Assessment of individual student performance
- Assessment of student groups/teams
- Assignment
- Exam/quiz, in class
- Exam/quiz, take home
- Homework
- Answer clicker-type question(s)
- Answer essay question(s)
- Answer fill in the blank question(s)
- Answer multiple choice question(s)
- Answer short answer questions(s)
- Answer true/false question(s)
- Create a concept map
- Create a diagram, drawing, figure, etc.
- Create a website
- Create graph, table etc. to present data
- Design an experiment or research study
- Design/present a poster
- Give an oral presentation
- Informal in-class report
- Interpret data
- Order items (e.g. strip sequence)
- Participate in discussion
- Peer evaluation
- Post-test
- Pre-test
- Produce a video or video response
- Respond to metacognition/reflection prompt
- Self evaluation
- Solve problem(s)
- Written assignment: One minute paper
- Written assignment: Brochure
- Written assignment: Essay
- Written assignment: Figure and or figure legend
- Written assignment: Lab report
- Written assignment: Literature review
- Other

Lesson Length

- Portion of one class period
- One class period
- Multiple class periods
- One term (semester or quarter)
- One year
- Other

Key Scientific Process Skills

- Reading research papers
- Reviewing prior research
- Asking a question

- Formulating hypotheses
- Designing/conducting experiments
- Predicting outcomes
- Gathering data/making observations
- Analyzing data
- Interpreting results/data
- Displaying/modeling results/data
- Communicating results

Pedagogical Approaches

- Think-Pair-Share
- Brainstorming
- Case Study
- Clicker Question
- Collaborative Work
- One Minute Paper
- Reflective Writing
- Concept Maps
- Strip Sequence
- Computer Model
- Physical Model
- Interactive Lecture
- Pre/Post Questions
- Other

Bloom's Cognitive Level (based on learning objectives & assessments)

- Foundational: factual knowledge & comprehension
- Application & Analysis
- Synthesis/Evaluation/Creation
- Principles of how people learn
- Motivates student to learn material
- Focuses student on the material to be learned
- Develops supportive community of learners
- Leverages differences among learners
- Reveals prior knowledge
- Requires student to do the bulk of the work

Vision and Change Core Concepts

- Evolution
- Structure and Function
- Information flow, exchange and storage
- Pathways and transformations of energy and matter
- Systems

Vision and Change Core Competencies

- Ability to apply the process of science
- Ability to use quantitative reasoning
- Ability to use modeling and simulation
- Ability to tap into the interdisciplinary nature of science
- Ability to communicate and collaborate with other disciplines
- Ability to understand the relationship between science and society

Key Words: List 3 to 10 key words that are relevant for the Lesson (e.g. mitosis; meiosis; reproduction; egg; etc.)

1. RNA Seq
2. transcriptome
3. gene expression
4. estrogen disruptors
5. aquatic vertebrates
6. animal behavior

Scientific Teaching Context

Learning Goal(s)

1. Understand how modern sequencing techniques can be used to measure the expression all genes in a tissue/organism (RNA seq).
2. Identify conceptual links between genetic and behavioral/developmental data
3. Develop a hypothesis and experimental design to test whether estrogen disruptors affect fish behavior.
4. Understand and apply the proper statistical and quantitative tools used to analyze RNA Seq datasets
5. Communicate the results/conclusions of the experiment in oral form
6. Understand the function of one gene whose expression is altered upon treatment

Learning Objective(s)

Students should be able to:

1. Describe the experimental flow of RNA seq and contrast it to RT-PCR
2. Describe what portion of the Central Dogma is being measured by RNA Seq and what changes in the Central Dogma we cannot observe with this technique
3. Design a hypothesis about the effect of endocrine disruptors on fish behavior, based on prior literature.
4. Write a protocol to test the effects of endocrine disruptors on fish behavior
5. Observe and record differences in fish behavior with and without treatment
6. Describe the parameters of each quantitative tool used to work with RNA Seq data
7. Apply these tools to a new dataset
8. Construct a heat map showing which genes are significantly upregulated or downregulated with treatment
9. Choose one gene whose expression is altered upon treatment and find literature appropriate to describing the function of that gene.
10. Hypothesize about how the change in that gene's expression could lead to changes in fish behavior
11. Describe (specifically for hormone receptors and generally, in theory) how exposure to a compound in the environment can alter signal transduction, gene expression, and behavior change in an organism.
12. Present the results of the experiment and your hypothesis for how your adopted gene is connected in an oral presentation.

Vision and Change Core Competencies

1. Ability to apply the process of science: hypothesis generation, experimental design, analyze results
2. Ability to use mathematical reasoning: using computational tools to organize data and evaluate quality and significance of data.
3. Ability to tap into the interdisciplinary nature of science: connections between genetics, animal behavior, neurobiology, and computer science and math.
4. Ability to understand the relationship between science and society: studying the potential effects of estrogen mimics in our waterways.

Introduction

Intended Audience

Biology (BIO) and Biochemistry and Molecular Biology (BMB) majors, as well as students follow a pre-health curriculum at the University of Richmond typically pursue a two-semester core biology curricular sequence before advancing to upper-level courses. This sequence, Integrated Biological Principles I and II, is modeled on the AAS/NSF Vision and Change in Undergraduate Biology Education recommendations and was developed around central themes and core competencies in biology rather than material and content. Both courses in this sequence have dedicated lab sections where the same instructor teaches both lecture and lab modules; as a result, the lines between lecture and lab are blurred and students are exposed to active learning and inquiry-driven activities throughout these courses. The lab module we propose here is intended to serve as the final lab activity in the second semester of this two-course introductory sequence.

Required Learning Time

This integrated lab module is intended to be implemented over the course of four three-hour laboratory sessions (timeline presented below)

Pre-requisite student knowledge

In the previous course (Integrated Biological Principles I), students will have been exposed to fundamental genetic concepts regarding DNA transcription, translation, and gene regulation. In addition, students will have learned about key concepts relevant to this module such as homeostatic regulation, chemical communication and cell signaling, fitness and evolution, and emergent properties of complex systems. They have learned about microarrays and have executed RT-PCR to study gene expression changes at the single gene level, which will help them make the leap to RNA Seq. They have had experience in data analysis, experimental design, and interpreting data.

Furthermore, students will have been trained in searching for, digesting, and evaluating scientific literature and will have practiced oral and written communication of scientific information. We will continue to build upon these skills and content areas in the current course (Integrated Biological Principles II).

However, this module will work for students that are not part of this particular course sequence. This module is appropriate for introductory level students that are comfortable with basic laboratory practices and techniques, data collection, management and interpretation, graphing and quantitative reasoning, and careful observation. Before undertaking this model, students should be familiar with transcription, translation, and regulation of gene expression. The students should be familiar with basic cellular signalling (signal transduction) and how it can impact changes in gene expression. Through this module, students will develop an increased understanding of how cell signaling and gene expression connect to homeostatic regulation, chemical communication between an organism and its environment, fitness and evolution, and emergent properties of complex systems. Furthermore, students will gain experience in searching for, digesting, and evaluating scientific literature and will have practiced oral and written communication of scientific information. All of these skills will be employed and developed in this module.

Scientific Teaching Themes

Active Learning

This lesson plan is for a investigative lab based learning module, so it will be all active learning with the exception of a short pre-lab lecture. The students will plan and execute an experiment testing whether estrogen disruptors alter fish behavior and they will observe the animals and record results. They will be given partially processed RNA seq data obtained from animals treated with the same estrogen disruptors. They will work in groups through a case study that will guide them through the analysis of the data and production of a heat map. Each pair of students will adopt a gene whose expression is altered with treatment. They will research the function of this gene and propose how that gene might be correlated to the behavioral change in the fish. They will present their results in an oral presentation.

Assessment

In preparing for the lab, students will read material to give them a background on the topic. The reading will include questions to make them work with the data. These questions will be discussed with the entire group in lab.

During one lab section, the students will work through a case study (developed by the instructors) that will guide them through the analysis of the data and production of a heat map. In addition, the case study will help connect the biological problem they are studying to other data in the field and the societal issue of accumulation of estrogen in waterways. The case study will test learning objectives #1-8. The instructors will make sure that the students remain on track as they proceed through the activity, since it will build throughout the assignment. The case study will be graded for assessment.

Finally, the students will explore the literature and learn about the function of one gene whose expression is altered with treatment. The students will work in pairs and peer review their selection of papers and their summary of the gene function. They will work together to prepare an oral presentation for the lab, using a rubric as a guide. The instructors will grade their presentation using the same rubric.

Inclusive Teaching

The students will be able to influence the trajectory of their assignment by choosing the gene they will explore. This element of choice will allow students to express their own interests within the assignment. By combining lab work, group work, problem based learning, and oral presentation preparation, we hope to reach students with different learning styles and preferences.

Lesson Plan

Overview and context

Endocrine disrupters (ED) represent a wide variety of chemical compounds that can mimic hormones and can interfere with a variety of physiological processes. EDs are common in industrial and household waste and many are known pollutants that can cause serious health problems in humans and wildlife. For example, exposure to EDs may be linked to increased risk of cancer and other diseases and may also affect behavior and development. In this module, student will expose fish (pipefish and/or zebrafish) to a suite of known or suspected EDs (e.g. Bisphenol A, Bisphenol S, Octylphenol, Nonylphenol, Tributyltin, Endosulfan, Methoprene, and/or Fipronil) at a range of concentrations, and monitor impacts on fish behavior, development, and gene regulation. The students will receive partially processed RNA Seq data (derived under similar experimental conditions) to study the gene expression changes that correlate with their treatment. We anticipate 4-6 laboratory sections of 16-18 students and will thus encourage our students to employ a fully factorial design with adequate replication.

The goals of this activity are to allow students to develop and test hypotheses, design experimental approaches, collect, analyze, and interpret a variety of data, and synthesize information to draw conclusions about the environmental impact of ED pollution. Students will reconcile and evaluate different lines of data (e.g. behavioral and gene regulatory) and will search and interpret scientific literature to develop data-driven conclusions about impacts of ED pollution.

Analyzing RNA Seq Data

As this is an introductory level course with freshman and sophomores, we will not be able to devote the time to process all of the data. Instead, much of the typical data analysis will be done in advance by the instructors, but we will walk the students through the ideas behind that data analysis through their case study, using fictional data sets or small subsets of the genuine data set.

Specifically, the instructors will generate the sequences, de-multiplex, align and annotate, and then perform statistical tests to quantify the depth and coverage of the sequencing. The students will analyze some of these parameters as part of their case study. They will use a GUI based program to generate a Heatmap from the data, such as HeatmapGenerator (Khomtchouk et al., 2014). By sticking with a GUI based program, the students will be able to generate a representation of the data without having to learn a programming language, which is out of the scope of this course. However, by analyzing the methods behind some of the quantitation, they will gain an appreciation for the role of computer science, statistics, and quantitative assessment in analyzing these data sets.

Timeline of Experiments and Assignments

Week	Activity	Pre-lab Assignment	Follow-up Assignment
1	Discuss impacts of EDs on humans and other organisms; discuss and develop experimental design for ED experiment	Read pre-lab case study and prepare answers to discussion questions	Finalize formal hypotheses and experimental design

2	Set up replicate fish tanks and establish dosage regime; finalize behavioral methodology and plan for data collection; search literature for genetic and behavioral impacts of EDs		Continue literature search for research on specific endocrine disruptors and their impacts on behavior, development, and gene regulation
3	Discuss literature search results; collect behavioral data; start processing RNAseq data	Complete literature search	Complete graphical and statistical data analysis on behavior data
4	Finish analyzing RNAseq and analyze behavioral data; choose one affected gene to study		Generate final figure(s) and figure legends; research adopted gene; prepare oral presentation
5	Present outcomes of experiment including discussion of relevant literature; interpret results and discuss implications of the group's findings	See previous follow up assignment	

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References

1. Khomtchouk et al.: "HeatmapGenerator: High performance RNAseq and microarray visualization software suite to examine differential gene expression levels using an R and C++ hybrid computational pipeline." Source Code for Biology and Medicine, 2014 9:30]