Supplemental Material

CBE—Life Sciences Education

Indorf *et al.*

Supplemental online material:
Adding authenticity to inquiry in a first-year, research-based, biology laboratory course

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Table S1: Example research themes implemented in the University of Miami Authentic Research Laboratories (UMARL) and suggestions for their implementation

TITLE:	**Coral Bleaching Experiment**
FACULTY LEADER:	Daniel DiResta, Ph.D.
INTRODUCTION:	There are several environmental disturbances that affect the symbiosis between coral and zooxanthellae. Ecosystems comprise interactions between species and abiotic factors, and the complexity of these interactions often complicates attempts to accurately describe how any one factor may be affecting the status of a particular organism. Thus, the health of corals is often taken as a measure of overall condition of the coral reefs. Understanding how a particular ecological disturbance affects a coral requires a controlled experimental setting. In this lab exercise, students select and study the effect of a single ecological factor on coral bleaching in two different species, *Aiptasia pallida*, a solitary anemone, and *Xenia* sp., a soft coral.
OBJECTIVES:	1) Learn about environmental stress and bioindicator organisms 2) Learn about experimental design 3) Learn how to do a literature search for scientific topics 4) Learn proper formats for referencing scientific literature and internet sources
ACTIVITIES:	• Separation of algal symbionts from the tissue of the anemone host • Subsampling fractions to determine: 1) Protein content of the homogenate (Biuret Protein Assay) 2) Number of algae in the host (hemocytometer counts) 3) Chlorophyll analysis (via spectrophotometry)
STUDENT TEAMS' QUESTIONS AND/OR HYPOTHESES:	1. Light intensity There is great concern that eutrophication and sedimentation are reducing the depth to which photosynthetically active radiation (PAR) penetrates coastal waters. Hermatypic corals are sensitive to light intensity. What happens to the coral-algal symbiosis as light intensity changes? 2. Salinity

	Salinity fluctuation (both hypo- and hyper-salinity) can affect coral growth rates and trigger bleaching. What is a relevant range of salt concentrations to study? How tolerant is the coral/algal symbiosis to salinity fluctuations? 3. Temperature Bleaching episodes in coral reefs around the world are highly correlated to periods of elevated sea surface temperatures. What is a relevant range of temperatures to study? Does an increase or decrease in temperature affect any other characteristic of water quality (i.e. oxygen levels)? How and why? 4. Ultraviolet radiation A decline in stratospheric ozone (the "ozone layer") is responsible for increased intensity of UV reaching the sea surface. What affect does UV have on the coral/algal symbiosis?
EQUIPMENT/TECHNIQUES:	*Processing Corals for Algal Counts and Protein Analysis* 1. Remove 4-5 *Xenia* polyps (or 1 *Aiptasia* polyp), blot dry to remove excess water, and weigh the tissue on a balance (wet weight). 2. Homogenize the tissue in 2 to 5 ml seawater (SW). 3. Measure and record total homogenate volume. 4. Vortex homogenate and subsample it for protein analysis. The subsample should be 100-800 µl depending on size and condition of animal. Record the subsample volume. 5. Withdraw a 1.0 ml sample of the suspension for chlorophyll analysis. 6. Determine the zooxanthellae density by cell counts. *Hemocytometer Counts* Cell suspensions should be dilute enough so that the cells do not overlap each other on the grid, and should be uniformly distributed. To perform the count, determine the magnification needed to recognize the desired cell type. Now systematically count the cells in selected squares so that the total count is 100 cells or so (number of cells needed for a statistically significant count). For large cells, this may mean counting the four large corner squares and the middle one. For a dense suspension of small cells, you may wish to count the cells in the four 1/25 mm^2 corners plus the middle square in the central square. Always decide on a specific counting patter to avoid bias. For cells

	that overlap a ruling, count a cell as "in" if it overlaps the top or right ruling, and "out" if it overlaps the bottom or left ruling. To get the final count in cells/ml, first divide the total count by 0.1 mm (chamber depth) then divide the result by the total surface area counted. For example, if you counted 125 cells in each of the four large corner squares plus the middle, divide 125 cells by 0.1 mm, then divide the result by 5 mm^2, which is the total area counted (each large square is 1 mm^2): 125/0.1 = 1250. 1250/5 = 250 cells/mm^3. There are 1,000 mm^3 per ml, so you calculate 250,000 cells/ml. Sometimes you will need to dilute a cell suspension to get the cell density low enough for counting. In that case, you will need to multiply your final count by the dilution factor. For example, suppose that for counting we had to dilute a suspension of zooxanthellae 10-fold. Suppose we obtained a final count of 250,000 cells/ml as above. Then the count in the original (undiluted) suspension is 10 x 250,000 which is 2,500,000 cells/ml. *Chlorophyll Extraction and Measurement* Dilute 1ml of the homogenate in 9 ml of acetone (final acetone concentration: 90%). Vortex and extract for 10 min. Centrifuge the samples for 5 min at high speed in a clinical centrifuge. Decant the supernatant and measure absorbance at 665 nm, 647 nm, and 630 nm wavelengths. Calculate the amount of chlorophyll *a* using equation 1: (1) $Ca = (11.85A_{665}) – (1.54A_{647}) – (0.08A_{630})$, where A is the absorbance measured at each determined wavelength and C*a* is the amount of chlorophyll in µg/ml (if a 1 cm path length is used). Calculate total concentration of chlorophyll *a* in the sample using equation 2: (2) Chlorophyll *a* (µg/ml) = 5(C*a*)/L C*a* = result from (1) L = path length of cuvette (1.6 cm)
PROBLEMS/CRITIQUE:	The experiments worked well for the most part. Having the full semester to continue the work would be better than having students switch to a new project mid-semester. Groups could get a larger data set, conduct statistical analysis, work on side projects, and get more into the literature.

TITLE:	**Anti-colon Cancer Activity in Plant Extracts**
FACULTY LEADER:	Zhiyong Han, Ph.D.
INTRODUCTION:	Plants are rich sources of medicinal chemicals. This freshman laboratory focused on testing the anti-colon cancer activity in methanol extracts prepared from various plants growing on campus.
OBJECTIVES:	The laboratory had two principal objectives: 1) to prepare plant extracts 2) to assay the growth inhibitory effect of the extracts on cultured human colon cancer cells
ACTIVITIES:	Students were first given a lecture on medicinal plants and the general process of how medicinal chemicals, especially anticancer agents, are identified and characterized from plants. Students collected plant samples and used them to prepare methanol extracts, which were then dried and re-dissolved in dimethyl sulfoxide (DMSO). The students were taken to the Molecular Cancer Biology Laboratory in the biology department, where they learned: (1) how to culture human colon cancer cells, (2) how to count cells in a suspension using a hemocytometer under a microscope, and (3) how to prepare cell cultures to be used for the clonogenicity assay. After the students prepared their samples in DMSO, they used the samples, at various concentrations, to treat cultured human colon cancer cells. The clonogenicity assay was used to determine the effect of the samples on the proliferation of colon cancer cells. At the end of the treatment, students fixed the cells in methanol and stained the cells in crystal violet solution to visualize cell colonies. They counted the total number of cell colonies per dish in triplicates. They then used the numbers to determine the dose-dependent inhibitory effect of their extracts on the clonogenicity of the colon cancer cells.
STUDENT TEAMS' QUESTIONS AND/OR HYPOTHESES:	(1) Do the plant extracts contain potential anti-cancer activity? (2) Is production of anti-cancer chemicals tissue specific?
EQUIPMENT/TECHNIQUES:	Students learned to prepare plant extracts and investigated whether the extracts contained potential anti-cancer activity. They learned how to culture and sub-culture cells, and how to count cells using a hemocytometer under a microscope. They also learned how to record cell morphology using a microscope linked to a computer.

PROBLEMS/CRITIQUE:	• Students loved this lab and felt that it was something they could really appreciate. They were willing to spend time and effort on their research. Students were extremely excited when they found out that several extracts contained activity that inhibited the clonogenicity of colon cancer cells. • Many students asked me about research opportunities in my laboratory. • The students wished they could have continued the lab to investigate further the extracts that exhibited an inhibitory effect on the clonogenicity of the colon cancer cells. They wished that they could use molecular techniques to investigate the effects of these extracts on the expression of genes and proteins in the colon cancer cells. • Because the cell culture work was done in an active research lab, where cell culture space was rather limited, the students did not do as much cell culture work as they wished. • The students also wished that they could test the anti-cancer activity in their extracts in other cell types.

TITLE:	**Spectrophotometry of Glomalin**
FACULTY LEADER:	David P. Janos, Ph.D.
INTRODUCTION:	Glomalin, a recently (mid-90's) discovered glycoprotein that accumulates in large amounts (several mg/g) in soil is produced by mutualistic, root-inhabiting arbuscular mycorrhizal fungi. This freshman laboratory focused on glomalin and its measurement by spectrophotometry.
OBJECTIVES:	The laboratory had two principal objectives: 1) to utilize the analytic approach of quantifying an unknown against a standard curve and to emphasize the generality of this approach, and 2) to measure the biochemical "signature" of a group of ubiquitous, but highly inconspicuous soil microorganisms.
ACTIVITIES:	Student teams initially were given prepared "unknown" water samples containing phosphate, solutions for a colorimetric reaction, a phosphate standard, and a spectrophotometer. With no more instruction than "mixing the color developer solutions with water containing phosphate will cause color to develop in proportion to the amount of phosphate present", teams were asked to devise a way to measure the phosphate concentrations in their "unknown". In the next lab, by following a published protocol, they conducted an analogus spectrophotometric Bradford protein analysis of "unknown" glomalin samples provided by the instructor. In final preparation for their own projects, students were given a published scientific article about glomalin and an illustrated lecture about arbuscular mycorrhizal fungi and glomalin. They were told that they could generate research questions of any of three types: 1) a methodological question concerning glomalin extraction from soil and/or its measurement, 2) a phenomenological, descriptive question regarding "how much glomalin is where?", or 3) a correlative question regarding "how does factor 'x' affect the amount of glomalin present?" Because glomalin is both produced and degraded relatively slowly, questions about glomalin dynamics could not be addressed during the six-week laboratory.

STUDENT TEAMS' QUESTIONS AND/OR HYPOTHESES:	Most teams conducted correlational and phenomenological, descriptive projects. Some examples of questions addressed were: • "How does glomalin concentration in soil change with distance from a tree trunk?" • "Does trampling of grass diminish glomalin in the soil beneath the grass?" • "Do native versus exotic trees differ with respect to the quantity of glomalin in their vicinity?"
EQUIPMENT/TECHNIQUES:	Students learned to use micropipettors, an autoclave, a centrifuge, and a computer-controlled microplate reader. One team quantified root length using a scanner and computer software system.
PROBLEMS/CRITIQUE:	• Students responded best to using micropipettors (seen on the popular CSI television series) and the computerized microplate reader. Manually operated student spectrophotometers were not popular. • Although phosphate in soil might have been measured as a potential correlate of glomalin, the phosphate measurement exercise frustrated most students who thought it an irrelevant, waste of time. The deliberate lack of provided direction was beyond the capacity of first-semester freshmen. • Students complained about having to spend too much time outside of the designated lab period to collect soil samples and to autoclave and centrifuge them (the rate-limiting steps of the research). • Students' preparation/performance was weakest with respect to: a) consideration of autocorrelated or uncontrolled variables when sampling b) the need for replication (and understanding of pseudoreplication) c) statistical analysis (especially the idea that apparent differences might not be significant) d) graphical presentation of data (e.g., the need for "scatterplots" when two variables are measured, and the importance of error bars). • Between the "false-start" phosphate measurement exercise and losing one lab to a hurricane-related closure, there wasn't enough time for any follow-up of results.

TITLE:	**Kinematic Analysis of Limbed Locomotion in Arthropods**
FACULTY LEADER:	James C. O'Reilly, Ph.D.
INTRODUCTION:	The mechanism and neural control of locomotion in arthropods is the focus of intensive research in the United States and Europe. The principles derived from these studies are being directly applied to the most recent generation of autonomous robots and will have a wide variety of applications in science, medicine, national defense and industry.
OBJECTIVES:	There were three major goals of this laboratory: 1) To introduce the students to the basic technique of kinematic analysis – the quantification of movement using video cameras and NIH Image software. 2) To encourage the students to use their own creativity to formulate testable questions based on their own observations. 3) To help the students apply their critical thinking skills to test those questions.
ACTIVITIES:	The initial laboratory involved a basic introduction to the principles of motion analysis and an exercise designed to introduce the students to NIH Image software. Students were required to import two previously recorded high-speed video sequences of feeding behavior (toad and salamander) into NIH Image and to perform a basic analysis of jaw and tongue movements. The raw kinematic data were then used to calculate velocity and acceleration of the jaw and tongue in Microsoft Excel. This portion of the exercise included a discussion of the importance of keeping track of units during analyses and familiarized the students with the use of formulas and graphing functions in Excel. After the students were comfortable with making calculations and graphs in Excel, we explored the consequences of changing sampling rate and different "smoothing" functions on their estimates of peak velocity and acceleration. In the following laboratory, the groups were introduced to several potential study animals including cockroaches, tarantulas, scorpions and crabs. The students were encouraged to get the animals out of their cages and observe their locomotion. Each group chose a different species and started developing ideas with the laboratory instructors.

	The remaining weeks were used for data collection and analysis.
STUDENT TEAMS' QUESTIONS AND/OR HYPOTHESES:	All of the groups developed basic testable questions in the general context of looking for principles that might be useful in developing robotic software or hardware. • Effect of surface irregularities on stride length in scorpions • Effect of surface irregularities on duty factor in terrestrial tarantulas • Effect of substrate differences on maximum sprinting speed in fiddler crabs • Effect of surface incline angle on posture in terrestrial tarantulas • Effect of surface incline on sprint speed in arboreal tarantulas
EQUIPMENT/TECHNIQUES:	Digital Video, High-Speed Video, NIH Image, Excel
PROBLEMS/CRITIQUE:	The primary problem encountered by the students was a combination of experimental error and small sample sizes that resulted in inadequate statistical power to test their hypotheses in the allotted time. The experimental error was expected as none of the students were familiar with either the animals or the analysis techniques. There is little that could be done to eliminate this problem. Sample sizes improved in the second half of the semester as the laboratory instructors made adjustments to move the students through data collection and analysis more rapidly. The rate of data collection would be greatly improved with the addition of an additional video camera to the laboratory. Also, data analysis would speed up significantly if Macintosh computers were used in the laboratory instead of Windows based computers. The process of importing color digital video sequences into NIH Image is a simple one-step process in the Macintosh version of the software, but takes several, time-consuming steps in the Windows version. This literally added many hours to the analysis for students using the regular (rather than high-speed) video camera.

TITLE:	**Population Genetics of South Florida Slash Pine (*Pinus elliottii V. densa*)**
FACULTY LEADER:	Yunqiu (Daniel) Wang, Ph.D.
INTRODUCTION:	South Florida slash pine (*Pinus elliottii* v. *densa*) is the keystone species of the pine rockland community in South Florida. Because of deforestation and severe damage by hurricane Andrew in 1992, less than 1% of the original pine forest remains. Restoration efforts are currently underway in Dade County. Determining appropriate slash pine seed sources for replanting damaged pine rocklands is an important component of restoration efforts. The research introduced in this lab course is designed to study the spatial genetic structure of South Florida slash pine using molecular markers. Our data will maximize the probability that reforestation efforts utilize genetically diverse seed stocks from appropriate seed transfer zones.
OBJECTIVES:	The laboratory had one principal objective: To teach students to describe the neutral genetic variation at microsatellite loci among selected South Florida slash pine populations, so that they can test hypotheses about how and why genetic diversity varies among populations.
ACTIVITIES:	Students initially were given an introductory talk on the history and significance of the pine rocklands to the South Florida ecosystem. The devastating facts of the current South Florida slash pine population were shown to students. The restoration plan of the pine rocklands of Miami-Dade County and the significance of obtaining genetic appropriate seed sources from existing populations for such restoration efforts were introduced to students. The concepts of how genetic diversity may function as an indicator of evolutionary fitness, and how scientists study genetic diversity using various genetic markers also were introduced to students. Based on this background information, 1) Students were asked to pick one factor to study which they believed would generate valuable information for the pine rockland restoration plan. 2) A methodological question concerning what type of genetic markers to use for our project was discussed first; then, students isolated the genetic marker, microsatellite DNA, with PCR from samples collected at various slash pine populations. Microsatellite loci were genotyped via

	an ABI 310 DNA sequencer, and loci were analyzed with the computer program GenAlEx.
STUDENT TEAMS' QUESTIONS AND/OR HYPOTHESES:	All teams conducted experiments to explore one of the following questions, which were proposed by themselves: • "Is a larger pine population more genetically diverse than a smaller population?" • "Is an old growth pine population more genetically diverse than a young growth pine population?" • "Is a pine population grown in rocky soil more genetically diverse than a pine population grown in sandy soil?" • "Does the distance between two existing pine populations affect the level of genetic relationship between them?"
EQUIPMENT/TECHNIQUES:	Students extracted genomic DNA from pine needle tissue and performed Polymerase Chain Reaction (PCR) with a thermal cycler to isolate DNA sequences of interest. Students performed DNA gel electrophoresis using both agrose and polyacrylamide as media. They set up and used Gene Scan with an ABI310 DNA sequencer to genotype microsatellite loci, and then analyzed these loci with GenAlEx (Genetic Analysis with Excel).
PROBLEMS/CRITIQUE:	• Students were exposed to a lot of techniques and lab equipment, but they struggled to relate the lab activities with the goals of their research. • Students' preparation/performance was weakest with respect to conducting statistical analyses and interpreting the results. • They need more practice before they become fully competent in most lab techniques learned as part of their research. • More samples need to be analyzed in order to generate reliable data.

TITLE:	**Using Molecular Markers to Trace the Invasive Brazilian Peppertree (*Schinus terebinthifolius*)**
FACULTY LEADER:	Dean Williams, Ph.D.
INTRODUCTION:	Brazilian peppertree was introduced to Florida around 1900 as an ornamental. The species escaped cultivation and has since become one of the most serious invasive species in the state (as well as in Hawaii and Texas). Understanding the geographic origin of invasive species and tracing their spread has both practical and theoretical implications such as facilitating the identification of potential biological control agents and quarantine efforts, as well as providing insights into colonization processes.
OBJECTIVES:	1. To use molecular markers such as nuclear microsatellite loci and a chloroplast locus to learn some basic forensic and population genetic techniques. 2. To study the spread of the exotic Brazilian peppertree to gain insight into invasive species biology and think about how this could be used to give practical recommendations for Brazilian peppertree control.
ACTIVITIES:	I introduced students in my lab section to the use of molecular markers using my study of the invasion history of Brazilian peppertree in Florida. First, I presented the students with background information on molecular markers, the importance of knowing the origins and tracking the spread of invasive species, and how molecular markers can be used to accomplish this. I then gave them background information on the invasion history of Brazilian peppertree in Florida and told them I was interested in obtaining preliminary data on several broad topics including mating and dispersal patterns, ecological associations, and screening new invasions. The student groups then had to come up with questions within these areas and use molecular methods (previously developed for Brazilian peppertree by me) to test them.

STUDENT TEAMS' QUESTIONS AND/OR HYPOTHESES:	The research topics that some of the student groups pursued included: 1. Testing whether there was an association between the two genetic types of Brazilian peppertree that were introduced into Florida and different habitat types (pineland and urban areas) in South Florida. Students found that the two genetic types of peppertree do not segregate by these habitats. 2. Screening of Brazilian peppertree samples from a nursery in northern Florida to see if they may have come from trees already present in Florida or if new stock was being imported illegally from South America. This group discovered that the nursery samples appear to have been derived from stock taken from the wild in Florida. The implication of this finding is that the nursery plants may not represent a new threat by adding novel genetic variation to the established invasive population when they inevitably escape cultivation. 3. One group tested peppertree samples from Texas to see if they may have come from Florida or possibly from new stock in South America. This group found that the Texas samples appear to have been derived from Florida stock. This study highlights some similar findings for other species, that Florida has often been a staging ground for further invasions of tropical regions. 4. One group compared peppertree samples from Hawaii and Florida to see if there was evidence for a genetic bottleneck and founder event in Hawaii since those islands are more isolated than Florida. This group found very clear evidence that there was a genetic bottleneck in Hawaii and that the original small numbers of founding individuals were probably from Florida stock. 5. One group was interested in testing for multiple paternity using seedlings collected from a single female plant. This group found evidence that female peppertrees are indeed pollinated by multiple male plants.
EQUIPMENT/TECHNIQUES:	The students learned some basic molecular techniques including micropipetting, DNA extraction, DNA amplification using PCR, and the interpretation and analyses of DNA fragment profiles. The students utilized basic lab equipment such as pipettors, microcentrifuges, and a thermocycler (PCR) machine. They also used an

	ABI 310 Genetic Analyzer and the software program GeneScan to genotype their samples. I also introduced the students to a genetic analysis program (GenAlEx v 6.0; Peakall and Smouse 2005) that runs as an add-in for Excel. This program was specifically developed for teaching and was sufficient to allow the students to perform some basic analyses of their data.
PROBLEMS/CRITIQUE:	1. In general, the students were very excited to extract and analyze DNA. Many of them were familiar with Brazilian pepper from high school courses, and because the species is a local problem they seemed to be interested in the overall goals of the project. 2. Students had difficulty (especially initially) merging what they learned in last semester's class (BIL 150) with some of the concepts used in forensics and population genetics such as Mendelian inheritance, a locus versus an allele, diploid versus haploid etc. As expected, many of the students had difficulty understanding the differences between the basic evolutionary forces (e.g. drift, selection, non-random mating, migration, and mutation) and how these were related to the interpretation of their data. 3. I was pleased with the students' facility with computers, teaching them to use the genotyping software and the Excel program was easier than teaching grad students to use them! The interpretation of statistical results is still difficult for them though (what exactly does a P value mean?), and I suppose it will take them awhile to build up that particular skill. On the other hand, the students needed lots of "encouragement" to simply look for patterns in their data in addition to using the statistics. 4. Constructing figures and tables is still a problem for many of the students. They did fairly well at picking the correct type of figure, but do poorly at labeling them correctly. I think there needs to be a more concerted/organized set of assignments where they are given data that they have to construct figures and tables for during the semester (as take-home assignments).

TITLE:	**Retinoic Acid Effect on the Transcription of Anterior-posterior Identity Genes *hox* in Zebrafish**
FACULTY LEADER:	Isaac Skromne, Ph.D.
INTRODUCTION:	Retinoic Acid is a derivative of Vitamin A that serves as a signaling molecule during vertebrate development to instruct cells where along the anterior-posterior axis of the embryo they are located. The information conveyed by Retinoic Acid to the cells causes the specific activation of the transcription factor genes *hox*, which instructs the cells whether they are positioned in the cervical, thoracic, lumbar or caudal portion of the embryo. Because of Retinoic Acid's important role in regulating the identity genes *hox*, defects in Retinoic Acid signaling can result in congenital malformations.
OBJECTIVES:	The laboratory has 4 principal objectives: 1) to understand the power that signaling molecules have over gene regulation, 2) to understand that the same signaling molecule can have different effects over cells depending on the time, place and concentration, 3) to understand the importance of internal and external controls for quantifying changes in gene expression, and 4) to learn data analysis and presentation.
ACTIVITIES:	Student teams tested the function of Retinoic Acid during zebrafish development by testing a single parameter in Retinoic Acid signaling. This parameter was carefully selected under the advice of the faculty leader to ensure that tests across teams complemented each other, opening the possibility for the comparison of results across teams. Students treated embryos with exogenous Retinoic Acid or a Retinoic Acid inhibitor at different times, concentrations, etc. several hours prior to the laboratory. During the laboratory, students took pictures of the embryos, extracted RNA, and diluted the samples to appropriate concentrations. Outside the laboratory, the teaching assistant ran a Reverse Transcription/quantitative PCR on samples using several primers for *hox* genes and internal controls. Students in class analyzed percentage change in *hox* expression relative to an internal, housekeeping gene (*actin*) and compared them to control, untreated embryos.
STUDENT TEAMS' QUESTIONS AND/OR HYPOTHESES:	Teams conducted correlational and phenomenological projects. Some of the questions addressed were:

	1) What happens to the embryos and the expression of *hox* genes when they are exposed to Retinoic Acid synthesis inhibitor at different times in development? 2) What happens to the embryos and the expression of *hox* genes when they are exposed to Retinoic Acid synthesis inhibitor at different concentrations? 3) What happens to embryos and the expression of *hox* genes when they are exposed to Retinoic Acid at different times in development? 4) What happens to embryos and the expression of *hox* genes when they are exposed to Retinoic Acid at different concentrations? 5) Can the effect of inhibiting Retinoic Acid synthesis be rescued by treating embryos with exogenous Retinoic Acid at different times in development? 6) Can the effect of inhibiting Retinoic Acid synthesis be rescued by treating embryos with exogenous Retinoic Acid at different concentrations?
EQUIPMENT/TECHNIQUES:	Zebrafish embryos are obtained from the College's Zebrafish Core Center. qPCR machines are available for use in the Molecular Core Facility. Micropipettors and microscopes are available in the teaching and in the leader's labs.
PROBLEMS/CRITIQUE:	1) Students responded best to the use of live specimens and their phenotypic analysis using semi-quantitative scales of their own design (e.g., eyes are bigger, equal or smaller than control embryos). 2) Students responded positively to the use of micropipettors and the extraction of RNA using kits. 3) Students responded the least positive to the quantitative analysis of gene expression and its changes relative to internal standards and external controls. Analysis is not very intuitive and can be complex. 4) Student's weakest part is the quantitative data analysis and its presentation in graphs and tables. 5) Some students are disappointed that the experiments are not fool-proof, despite that they are constantly reminded that the focus is on the research process and the way science works, not on the end product.

TITLE:	**Aphid Biology**
FACULTY LEADER:	Alexandra C. C. Wilson, Ph.D.
INTRODUCTION:	Aphids are insects that feed on plant phloem sap. They are able to reproduce sexually and asexually, they feed on a large diversity of host plants, transmit hundreds of plant viruses and are dependent on an obligate intracellular bacterial symbiont for nutritional provisioning. In this freshman laboratory we formulate and test biological hypotheses using aphids and their host plants as an experimental system.
OBJECTIVES:	(1) To formulate and test a biological hypothesis (2) To collect and analyze data (3) To communicate science, in the form of oral presentations supported by slides, in written reports and in posters.
ACTIVITIES:	Week 1: I provide students with a lecture form introduction to aphid biology, highlighting aspects of their biology that are amenable to experimental manipulation within the four week period the students have to execute their experiments. Students then spend time in their groups formulating their questions and hypotheses and then design their experiment. Students plant the seeds for the plants they will need for their experiment. Week 2: Students place adult asexual female aphids on plants to raise the next generation aphid progeny they need to set up their experiment in Week 3. Week 3: Students set-up their experiment using the plants they planted seeds for in Week 1 and the next generation aphids born between Weeks 2 and 3. Week 4: Students collect data. Data collection can include number of adult aphids, number of juvenile aphids, aphid mass (mg), and host plant measures related to size such as height (cm), number of leaves and above ground dry weight (mg). Students also have the opportunity to use stable isotope analysis to quantify the % nitrogen content of host plants. Week 5: Data analysis. Weeks 6: Final presentations.
STUDENT TEAMS' QUESTIONS AND/OR HYPOTHESES:	Students usually formulate hypotheses around the following: (1) Host plant preference (2) Within species competition (a question facilitated by the fact that pea aphids have two color morphs and so a pink genotype can be competed against a green genotype and the pink and green aphids can easily be counted and weighed)

	(3) The effect of nitrogen fertilizer on aphid (and host plant) growth (4) Genotype x Environment (host plant) interactions (5) The effect of simple insecticides (like soap) on aphids.
EQUIPMENT/TECHNIQUES:	Students are provided with access to three different genotypes of the pea aphid (*Acyrthosiphon pisum*). Typically, two lines will be pink lines and one will be a green line. They also are provided access to seeds from five different host plants; fava, alfalfa, sweet pea, pea, and clover. Students have access to nitrogen stable isotope analysis for %N quantification. They have access to a balance, a control temperature growth room, and growth chamber. They have access to a balance (mg).
PROBLEMS/CRITIQUE:	In general students report loving this lab because they get to do the experiment from the beginning to the end, and the materials are really tangible and accessible to them. There is little that is mysterious and so this allows them to formulate questions and hypotheses and test them. The one thing that needs improvement with this lab is the data analysis part – that part tends to be a bit of a mysterious "black box" for the students.

TITLE:	**Stable Isotope Ecology**
FACULTY LEADER:	Leonel Sternberg, Ph.D.
INTRODUCTION:	Stable isotope analysis provides a powerful tool to ask questions about the environment ranging from determining diets of organism to adulteration of honey with sugar. This freshman laboratory introduces this technique to students.
OBJECTIVES:	The principal objectives of the laboratory are: 1) Learn how to formulate a hypothesis which can be tested with stable isotope techniques. 2) Learn how to sample populations in order to test the results statistically. 3) Apply quantitative methods describe the results and present them in a clear concise way.
ACTIVITIES:	In the first exercise students sample hair tissue to determine its carbon and nitrogen isotope ratios. This familiarizes students with the sampling technique for stable isotopes. As a follow up of the above exercise, the data is tabulated and students learn how to describe the data, ask questions about the data and answer it statistically. Students learn how to use Excel to analyze data as well as graphing and presentation. Having familiarized with the technique each group is asked, as a second exercise, to come up with a hypothesis to be tested using isotope techniques. The instructor and TA go over each project with each student group and discuss the design of the experiment, how to best collect samples, how to replicate and most importantly whether the proposed sampling really tests the hypothesis. Student then learn how to process samples, operate the mass spectrometer and analyze the results using techniques learned in the first exercise. Finally students learn how to present their results in a PowerPoint presentation and poster session.
STUDENT TEAMS' QUESTIONS AND/OR HYPOTHESES:	Students generally ask two types of questions: 1) adulteration of foods with corn or sugarcane products, such as determining whether maple syrup is adulterated or not; 2) ecological interactions such as determining the trophic level of different organisms or determining the effect of abiotic factors on plant isotope composition.
EQUIPMENT/TECHNIQUES:	Students learn to use an analytical balance and a computer controlled elemental analyzer coupled to an isotope ratio mass spectrometer.

PROBLEMS/CRITIQUE:	We lack sufficient analytical balances to accommodate the students need for weighing samples. These balances break easily. Currently I am using balances from my own laboratory for this student research. The time is too short to follow-up and re-run experiments.

Table S2: Example syllabus for the University of Miami Authentic Research Laboratories (UMARL) course

BIL 151 INTRODUCTORY BIOLOGY LABORATORY Fall '05

supported by the

HOWARD HUGHES MEDICAL INSTITUTE

Section RY, 1 Cr., Thursdays 2:00 pm – 4:50 pm, SA 104 & 106

Instructors: [redacted]

Teaching Assistants: [redacted]

Undergraduate Peer Facilitators: [redacted]

Date:	**Activity:**	**Submissions:**
August 25	Introduction to Topic 1	
September 1	Background & question development	Student 1 presentation
8	Data collection & analysis	Student 2 presentation
15	Data collection & analysis	Student 3 presentation
22	Data collection & analysis	Student 4 presentation
29	Data collection & analysis	Student 5 presentation **Abstract drafts due**
October 6	Final analysis & reporting	Student 6 final presentation
13	Introduction to Topic 2	(Switch labs) **Topic 1 final abstracts due & posters due at printer**
20	Background & question development	Student 1 presentation
27	Data collection & analysis	Student 2 presentation
November 3	Data collection & analysis	Student 3 presentation

10	Data collection & analysis	Student 4 presentation
17	Data collection & analysis	Student 5 presentation **Abstract drafts due**
24	NO LAB: Thanksgiving Recess	
December 1	Final analysis & reporting	Student 6 final presentation
December 2		**Topic 2 posters due at printer**
December 13; 2:00 – 4:30 pm	**POSTER SESSION**	**All posters displayed;** **Topic 2 final abstracts due**

Goal & Objectives:

The goal of these laboratories is to introduce you to the activities in which research scientists engage when conducting scientific discovery. In addition to learning about specific research topics, you will learn: 1) to formulate questions, 2) techniques of investigation, 3) quantitative and statistical analysis, 4) graphical display of data, and 5) oral and written communication of research results.

Attendance policy:

Attendance of all scheduled laboratory sessions is MANDATORY. Unavoidable absences must be excused by the instructor IN ADVANCE. Unanticipated, unexcused absences may result in a lowered grade.

Requirements & Grading:

Students will investigate two topics, one with each professor. For each topic (half semester) each student is responsible for four products: two individually written exercises, one oral presentation, and a group poster (all posters are to be presented publicly at the Cox Science Building during the final exam period).

Although students will work cooperatively in teams, students individually will write about their team's results. Each student will INDEPENDENTLY write an **extended abstract** (no more than TWO double-spaced pages of text, to be accompanied by appropriate tables and figures with LEGENDS) of her/his group's project for each topic. A DRAFT of the abstract is due at the sixth lab session on a topic. It will be constructively criticized and then returned for revision. Final versions that will be graded are due after the final lab on each topic (see schedule, overleaf).

A second individually-written, **graded exercise** will be designed by each professor. This exercise may take any one of several possible forms such as a written quiz on

techniques and concepts used in lab, a closed-book problem set, a library research report, or a written critique of a poster.

Five-minute **oral presentations** (accompanied by PowerPoint slides) are to be given by one member of each research team at the beginning of each lab (with the exception of the first on a topic). A twelve-minute, final presentation that may utilize slides from prior presentations will be given at the conclusion of each topic. One presentation each week will be selected for display on the "information commons" projector in the Cox lobby.

In addition, each lab group will prepare a **poster** cooperatively (one poster for each of two topics). **Due dates for posters are indicated on the accompanying schedule and must be met in order for all posters to be printed on time.** All students in a group must contribute to preparing the poster, and all will receive the same grade for their poster.

For each of the two topics during the semester, a student will receive two grades, one for the extended abstract and one for the written exercise. The maximum points awarded for each graded written exercise will be 100 points. Therefore, the highest point total possible for the semester will be 400 points. Final letter grades will use the University of Miami standard percentage scale shown below. The oral presentations and posters will be graded on a Pass/Fail basis (i.e., Pass+, Pass, Pass-, Fail); two or more plusses or minuses among these Pass/Fail grades collectively may affect the final grade by elevating or diminishing it one step on the letter grade scale (e.g., raising a B+ to an A-).

Total score range:	**Percentage range:**	**Letter grade:**
388 to 400	97 to 100	A+
372 to 388	93 to 97	A
360 to 372	90 to 93	A-
348 to 360	87 to 90	B+
336 to 348	84 to 87	B
320 to 336	80 to 84	B-
308 to 320	77 to 80	C+
296 to 308	74 to 77	C
280 to 296	70 to 74	C-
268 to 280	67 to 70	D+
256 to 268	64 to 67	D
240 to 256	60 to 64	D-

0 to 240	0 to 60	F

FALL 2005 LAB (GLOMALIN)

WEEK:	ACTIVITIES:
1	a) Introduction to lab, requirements, and grading b) "Macro" colorimetric Bradford protein assay to assess a potential "bioterrorism" letter with "mysterious" white powder c) CN standard curve generation and powder assessment
2	a) PowerPoint presentations from four groups b) Lecture on mycorrhizas, arbuscular mycorrhizas, and glomalin c) Demonstration of accessing Web of Science d) Practice microplate protein assay standard curve Homework: Develop a research question.
3	a) PowerPoint presentations from four groups (glycoprotein & SOM) b) Impromptu instructions on micropipette use, microplate assay, & dilution c) Practice on running a microplate standard curve Homework: Bring in soil samples.
4	a) PowerPoint presentations from four groups (std. curves & mainly research questions and methods. Rem. Justification/Rationale) b) Begin research
5	a) PowerPoint presentations from four groups (refinement of questions) b) Soil collection for some groups, extractions for others c) In the open lab period and in lab, demonstration of how to convert glomalin OD values to mg/g of soil.
6	a) PowerPoint presentations from four groups (progress reports)

	b) DEG and TG extractions continuing for some groups, first effort at final protein assays for others c) Extended Abstract drafts due!
7	a) Quiz! b) Final PowerPoint presentations from all four groups c) Finish data collection d) Start posters

Table S3: Students omitted prior to propensity score matching to avoid biasing the treatment (University of Miami Authentic Research Laboratories; UMARL) and control (traditional biology labs) groups used in hierarchical logistic analyses on research credits obtained, STEM major at graduation, graduation in four years, or graduation with honors.

Excluded Student Group	N	Reason for Exclusion
Matriculated as undeclared	947	Students undeclared at matriculation may have been intending to major in STEM. Major at matriculation, either STEM or non-STEM, was included as a variable in data analyses.
Missing SAT/ACT scores and/or final GPA	93	Propensity score matching does not allow missing data.
Matriculated after fall 2010 (matriculated spring 2011 or later)	1,878	Students had not had the opportunity to graduate in four years by 2014, nor did they all have similar amounts of time to earn research course credits.
Participated in special research program	96	These programs are geared towards increasing the number of students going on to pursue STEM PhD degrees. Program students are required to have high GPAs and be STEM majors. Many are required to take the UMARL lab courses.
Graduated in less than 3 years	4	Students did not have similar amounts of time to earn subsequent research course credits.

Took introductory biology lab in their junior or senior year	95	Students did not have similar amounts of time to earn subsequent research course credits.

Table S4. Descriptive statistics of variables used in propensity scores for student populations in traditional biology lab courses or University of Miami Authentic Research Laboratories (UMARL) before and after matching. Gender, race/ethnicity, and graduation status were used in exact matching while SAT score and STEM major at matriculation were used as covariates.

Variable		Before Matching						After Matching					
		Traditional			UMARL			Traditional			UMARL		
		N	%	Mean ± SD	N	%	Mean ± SD	N	%	Mean ± SD	N	%	Mean ± SD
Gender	Female	1,741	63.2%		334	61.7%		327	61.5%		334	61.7%	
	Male	1,015	36.8%		207	38.3%		205	38.5%		207	38.3%	
Race/ Ethnicity	2 or More Races	52	1.9%		11	2.0%		7	1.3%		11	2.0%	
	American Indian or Alaska Native	11	0.4%		3	0.6%		2	0.4%		3	0.6%	
	Asian or Pacific Islander	279	10.1%		82	15.2%		81	15.2%		82	15.2%	
	Black	277	10.1%		45	8.3%		44	8.3%		45	8.3%	
	Hispanic	624	22.6%		87	16.1%		87	16.4%		87	16.1%	
	Unknown/ Missing	178	6.5%		34	6.3%		32	6.0%		34	6.3%	
	White	1,335	48.4%		279	51.6%		279	52.4%		279	51.6%	
Graduation Status	Not yet graduated	558	20.2%		94	17.4%		91	17.1%		94	17.4%	

	Graduated	2,198	79.8%		447	82.6%		441	82.9%		447	82.6%	
STEM Major at Matriculation	Non-STEM	751	27.2%		42	7.8%		41	7.7%		42	7.8%	
	STEM	2,005	72.8%		499	92.2%		491	92.3%		499	92.2%	
SAT Score (or ACT equivalent)		2,756		1278 ± 122	541		1318 ± 117	532		1314 ± 114	541		1318 ±117

Table S5. All possible predictor's coefficient values, Wald test statistics, *P*-values, odds ratio, and odds ratio 95% confidence interval (CI). Each predictor was included in the first step of hierarchical logistic regressions to examine the likelihood of its effect (odds ratio) on subsequent individual research experiences, graduating with a STEM major, graduating in four years, and graduating with honors. The effect of all predictors was accounted for prior to determining the effect of participating in the University of Miami Authentic Research Laboratories (UMARL; step 2) on each of the outcomes.

Outcome	Step	Predictor	Coefficient value (β)	Wald χ^2	*P*	Odds Ratio (e^{β})	Odds Ratio 95% CI
Subsequent individual research experience	0.	Constant	-6.312	33.633	< 0.001	0.002	_
	1.	Female	0.005	0.001	0.973	1.005	0.744 – 1.359
	1.	Race/Ethnicity	_	7.660	0.264	_	_
	1.	2 or more races	-0.081	0.020	0.889	0.922	0.295 – 2.878
	1.	American Indian or Alaska Native	-20.710	0.000	0.999	0.000	0.000
	1.	Asian or Pacific Islander	-0.055	0.027	0.870	0.946	0.488 – 1.8536
	1.	Black	-0.520	1.582	0.208	0.594	0.264 – 1.337
	1.	Hispanic	-0.068	0.041	0.840	0.934	0.482 – 1.809

	1.	White	-0.457	2.208	0.137	0.633	0.347 – 1.157
	1.	SAT score	0.003	16.270	< 0.001	1.003	1.001 – 1.004
	1.	STEM major at matriculation	2.131	16.316	< 0.001	8.424	2.995 – 23.692
	2.	Participation in UMARL	0.421	8.164	0.004	1.534	1.141 – 2.034
STEM major at graduation	0.	Constant	-5.419	23.889	< 0.001	0.004	_
	1.	Female	-0.258	2.237	0.135	0.773	0.551 – 1.083
	1.	Race/Ethnicity		12.037	0.061		
	1.	2 or more races	0.115	0.034	0.853	1.122	0.332 – 3.786
	1.	American Indian or Alaska Native	-0.084	0.007	0.932	0.919	0.131 – 6.447
	1.	Asian or Pacific Islander	1.081	8.125	0.004	2.947	1.402 – 6.196
	1.	Black	0.226	0.310	0.578	1.254	0.566 – 2.778
	1.	Hispanic	0.229	0.424	0.515	1.257	0.632 – 2.502
	1.	White	0.327	1.055	0.304	1.386	0.743 – 2.585
	1.	SAT score	0.002	10.288	0.001	1.002	1.001 – 1.004
	1.	STEM major at matriculation	1.830	38.106	< 0.001	6.234	3.487 – 11.145

	1.	Number of semesters in introductory biology lab	0.672	14.858	< 0.001	1.958	1.391 – 2.756
	2.	Participation in UMARL	.445	7.493	0.006	1.560	1.135 – 2.145
Graduation in four years or less	0.	Constant	-3.700	17.301	< 0.001	0.025	_
	1.	Female	0.505	13.673	< 0.001	1.657	1.268 – 2.166
	1.	Race/Ethnicity	_	5.496	0.482	_	_
	1.	2 or more races	0.769	1.214	0.271	2.157	0.549 – 8.467
	1.	American Indian or Alaska Native	-1.202	1.545	0.214	0.301	0.045 – 2.000
	1.	Asian or Pacific Islander	-0.197	0.381	0.537	0.821	0.439 – 1.535
	1.	Black	-0.487	1.895	0.169	0.614	0.307 – 1.229
	1.	Hispanic	-0.208	0.437	0.508	0.812	0.438 – 1.506
	1.	White	-0.192	0.455	0.500	0.825	0..473 – 1.441
	1.	SAT score	0.002	11.786	0.001	1.002	1.001 – 1.003
	1.	Research course credit	0.396	27.672	< 0.001	1.487	1.282 – 1.723

	1.	Number of semesters in introductory biology lab	0.549	14.726	< 0.001	1.731	1.308 – 2.291
	2.	Participation in UMARL	0.447	10.924	0.001	1.564	1.200 – 2.040
Graduation with honors	0.	Constant	-10.980	95.426	< 0.001	0.000	_
	1.	Female	0.208	1.647	0.1999	1.231	0.896 – 1.692
	1.	Race/Ethnicity	_	16.083	0.013	_	_
	1.	2 or more races	-0.582	1.137	0.286	0.5105	0.144 – 1.772
	1.	American Indian or Alaska Native	-1.133	0.901	0.342	0.22	0.031 – 3.340
	1.	Asian or Pacific Islander	0.395	1.292	0.256	1.485	0.751 – 2.937
	1.	Black	-0.637	1.886	0.170	0.529	0.213 – 1.313
	1.	Hispanic	-0.521	2.059	0.151	0.594	0.291 – 1.210
	1.	White	0.017	0.003	0.957	1.017	0.549 – 1.884
	1.	SAT score	.008	92.704	< 0.001	1.008	1.006 – 1.009
	1.	STEM major at matriculation	0.049	0.027	0.870	1.050	0.584 – 1.888
	2.	Participation in UMARL	0.535	12.011	0.001	1.707	1.262 – 2.311

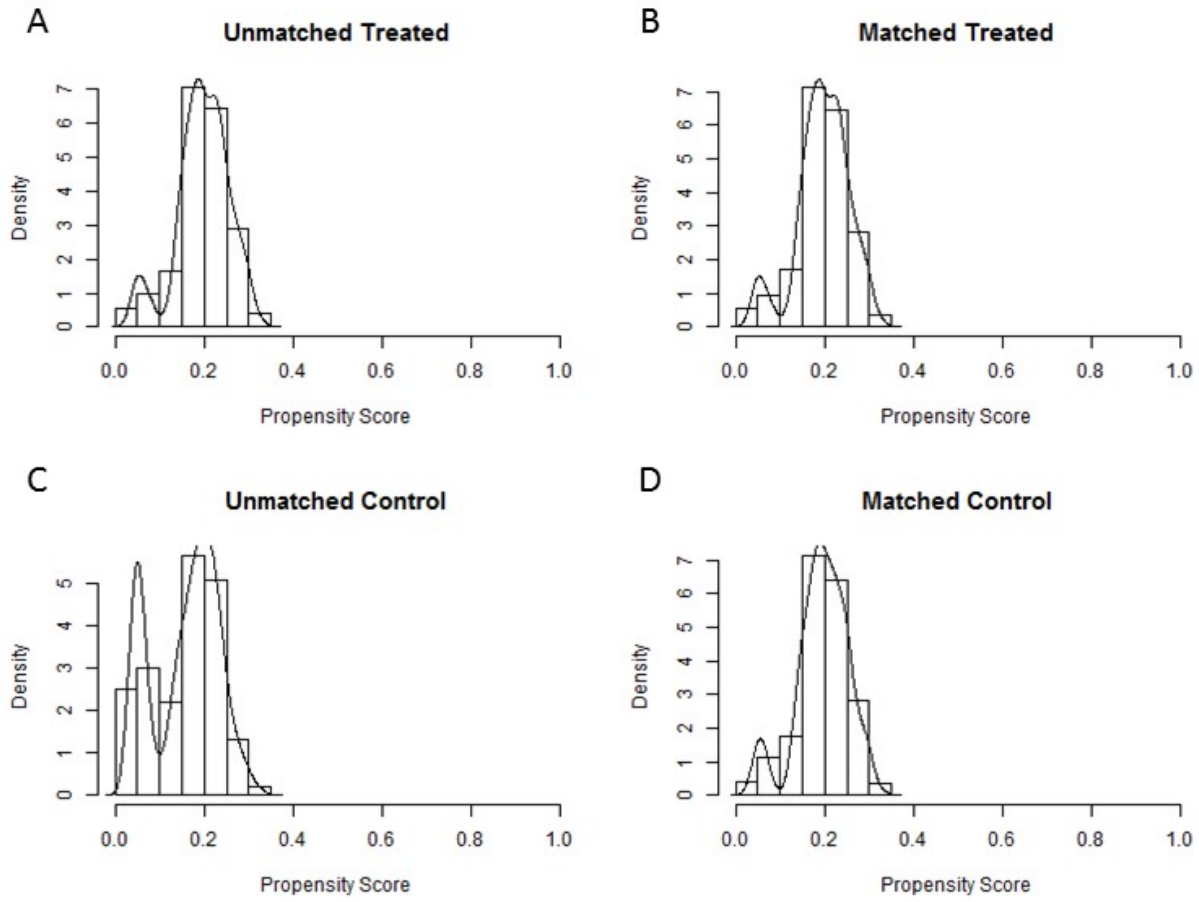

Figure S1. Propensity score distributions of University of Miami traditional (Control; C, D) and Authentic Research Labs (UMARL) (Treated; A, B) lab student groups before (A, C) and after (B, D) matching. We used gender, race/ethnicity, and graduation status (0 = not yet graduated, 1 = graduated) in exact matching and SAT score and STEM major at matriculation (0 = non-STEM, 1 = STEM) as covariates for nearest neighbor logistic regression matching.

Table S6. Weighted mean ratings (1 = No gain, 5 = Very large gain) and weighted standard deviations of self-reported benefits gained by students from participation in the University of Miami Authentic Research Labs (UMARL) and by other CURE students nationwide from the Classroom Undergraduate Research Experiences (CURE) survey implemented by Grinnell College. Items are ordered by difference between mean UMARL student response and student responses nationally from greatest to least difference. The national responses include UMARL student responses.

	UMARL N = 280		**National N = 38,160**		***t***
Question	Weighted Mean ***	Weighted SD	Weighted Mean	Weighted SD	
Skill in how to give an effective oral presentation	4.01	0.32	3.03	0.08	45.32
Skill in science writing	4.03	0.24	3.30	0.09	44.23
Tolerance for obstacles faced in the research process	4.05	0.28	3.46	0.07	29.87
Self-confidence	3.75	0.32	3.16	0.12	24.75
Readiness for more demanding research	3.95	0.33	3.39	0.08	23.51
Skill in interpretation of results	4.07	0.19	3.53	0.05	37.15
Understanding how scientists work on real problems	4.11	0.20	3.57	0.06	39.80
Understanding how knowledge is constructed	3.95	0.31	3.42	0.09	26.13
Ability to read and understand primary literature	3.82	0.35	3.29	0.14	24.69
Becoming part of a learning community	3.92	0.32	3.41	0.06	25.78
Understanding science	4.04	0.27	3.57	0.05	25.72
Understanding how scientists think	3.84	0.23	3.37	0.06	24.67
Ability to analyze data and other information	4.19	0.18	3.73	0.04	29.40
Learning laboratory techniques	4.21	0.21	3.75	0.10	23.26

Ability to integrate theory and practice	3.92	0.27	3.47	0.04	23.04
Understanding the research process	3.87	0.30	3.43	0.10	20.09
Understanding that scientific assertions require supporting evidence	4.08	0.26	3.64	0.05	25.98
Clarification of a career path	3.35	0.44	2.93	0.21	13.89
Learning ethical conduct	3.48	0.45	3.10	0.15	12.74
Confidence in my potential as a teacher	3.25	0.29	2.88	0.07	16.08
Learning to work independently	3.43	0.36	3.30	0.07	4.60

*** All UMARL means are significantly different from national means at $P < 0.0001$, $df = 279.83$