Teaching Outside the Can: A New Approach to Introductory Biology

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Abstract: We describe a new approach to teaching introductory biology. Our introductory experience for undergraduates is a laboratory course that is entirely inquiry and discovery based. We introduce our students to fundamental concepts in biology in the framework of three multi-week laboratory modules, each of which is an open-ended investigation of a current area of biological study. Students read the primary literature about the research question, learn techniques and statistical approaches, and conduct student-designed experiments. We focus on the process of doing biology, rather than on acquiring a particular body of facts. Students are actively engaged in integrative thinking about biology, and they emerge from the laboratory experience with a strong grasp of quantitative and experimental approaches and skills. Our assessments indicate that this process-based approach is an effective way to approach introductory biology.

Keywords: introductory biology, process, inquiry-based learning, laboratory

Introduction

The pace of progress in biological research is accelerating at an unprecedented rate. Greatly improved information technology, along with the rapid development of public databases housing biological data, have dramatically enhanced access by faculty and students to current advances in all biological fields. College-level textbooks for introductory biology are veritable encyclopedias of information that rapidly become dated. College faculty, as well as teachers at all other levels of education, are faced with the dilemma of how to handle all this information, while at the same time emphasizing the processes of biological investigation, quantitative reasoning and critical thinking.

We recognized the need a number of years ago to fundamentally restructure our introductory curriculum to address these issues. In addition, we noted that, as more introductory students enter our curriculum having taken an Advanced Placement (AP) Biology course in high school, there was an increased need to focus on the process of doing biology, as well as a need to emphasize more explicitly how concepts are integrated across areas of biology. Our traditional introductory sequence did not allow a fully integrated approach to biology, and seemed to give students the impression that there were clear distinctions between the sub-disciplines of biology, an impression that did not represent current practice in biology. In response to these varied challenges, we dramatically restructured our introductory sequence.

Our new introductory curriculum seeks to accomplish a number of objectives:

- Coverage of the most fundamental concepts in biology using an integrative, cross-disciplinary, topic-centered approach, focusing on the relationship between genes and function and the evolution and inheritance of traits;

- Construction of a tool kit for biology, consisting of skills and concepts essential for every biologist, including experimental design, hypothesis testing, data collection, statistical analysis, scientific communication and writing;

- Infusion of an enthusiasm for biology by introducing students to current questions in biology from the start of the first course; and

- An emphasis on process rather than content, in order to allow for and respond to the rapid pace of progress across biological disciplines.

To accomplish these goals, we designed two courses, *Biology 105: Introduction to Biological Processes,* and *Biology 106: Introduction to Biological*

Investigation. Biology 105, *Processes*, while not our focus here, is a course without a laboratory component that introduces students to major concepts in biology in the context of a current topic of biological interest and emphasizes critical thinking and writing skills. *Processes*, usually taken by first-year students whose background prior to college might not have been strong or who might not have had biology since the ninth or tenth grade in high school, provides some background content and introduction to fundamental concepts in biology. Key concepts are reviewed and reinforced. Emphasis is placed on integration across concepts and helps to provide students with adequate preparation for the *Investigations* course. Our focus here is Biology 106, the *Investigations* course. *Investigations* is an introduction to the process of biological inquiry, and is designed to provide students with many investigative skills, including observation in the lab and in the field, experimental design and hypothesis testing, data collection, statistical analysis, visual representation of biological data, and scientific communication.

Active, inquiry-driven approaches to biology have been found by us and others to be very effective at encouraging integrative learning and better retention of fundamental concepts (Wilke and Straits, 2001; Flannery, 2007). Other recent curricular changes have demonstrated increased learning with more interactive approaches in introductory biology (Wilke and Straits, 2001; McDaniel et al, 2007). Further, the Bio2010 Report underscores the need for training in experimental design, communication skills and quantitative approaches. *Investigations* is a course that addresses these issues.

The course has three laboratory modules, each lasting 4-5 weeks, that focus on a current area of investigation. Each module pursues a research question from multiple levels of analysis and is based on primary literature and current approaches to open-ended questions in biology. The modules are designed to be modified easily to build upon the data and results discovered by previous semesters' students and to incorporate individual faculty members' expertise and interest. All of the modules have the flexibility to incorporate new directions and lines of inquiry to keep pace with the progress of scientific discovery. In the next sections, we describe the overall structure and format of *Investigations*, along with a description of each module and the particular goals addressed by each. We provide more complete resources in the supplemental materials.

Overall structure and implementation of "Investigations"

Investigations serves as the introductory lab course for 90-120 students each term. Students have a range of backgrounds upon entering the course, from not having had a biology course for a number of years, to having just taken the *Processes* course the previous term, to having just had a high school AP Biology course and received a 4 or 5 on the AP Exam. The department typically offers four or five separate sections of the course each semester. Each section is taught by a faculty member, with one 75 minute period of classroom-based work and one 4 hour laboratory period each week. Instructors meet weekly to discuss the implementation of the course. In addition, the outgoing and incoming instructors meet at the end of each semester to discuss avenues for improvement for the upcoming semester. Finally, the department maintains a course website (Blackboard Academic Suite v. 7.1, Blackboard, Inc), where electronic copies of syllabi, assignments, class data, images and lecture notes, as well as copies of the departmentally-written laboratory manual are maintained. The textbook for the course is *Biological Science,* 2nd Edition, by Scott Freeman. The selection of the textbook is reviewed by the faculty to ensure that it is current and appropriate for the course. The text is used by both the *Processes* and the *Investigations* courses. Students are assigned readings for which they are held responsible with homework and other graded assignments. In addition, students purchase "Soil Biology Primer," a publication produced by the Soil and Water Conservation Society (Tugel et al, 2000). Other readings, particularly primary literature articles, are placed on electronic reserve or on restricted access websites.
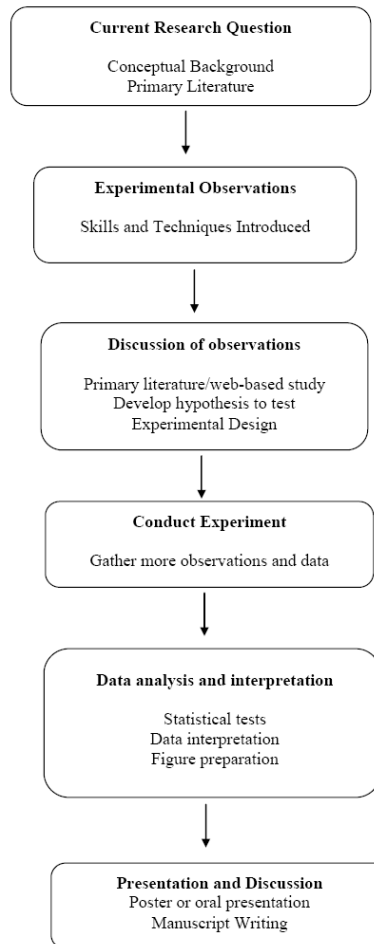
In each module, students learn the major concepts associated with a current question in biology in the classroom period with a mixture of lecture and discussion and then conduct initial observations in the laboratory in order to become familiar with the system being studied (Figure 1). Initial observations introduce both the research question and the particular skills and techniques used to address the question. Students learn the fundamental aspects of each technique, or experimental approach, not just how to operate the equipment. With specific guidance from the faculty instructors, students then read and discuss the primary literature to gain some background into the research question and, combined with the initial observations, develop specific hypotheses and conduct experiments to test them. The results are analyzed and interpreted within the framework of the initial primary literature that motivated the study. Finally, students present their findings in different written and oral formats.

The overall design of this course allows instructors to modify the particular research question being addressed from semester to semester. Each module need only address a biological question from

an integrated perspective. Further, the questions chosen for study are current, and still unresolved, areas of exploration and, as such, do not lead students through a "canned" experience. Rather, students generate testable hypotheses that yield data that could contribute to a resolution. The results can be (and are being) used as a springboard by students who wish to pursue independent research as a result of their experiences in *Investigations*.

Figure. 1. Overall organization of laboratory modules

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│   Current Research Question      │
│                                  │
│     Conceptual Background        │
│       Primary Literature         │
└─────────────────────────────────┘
                 │
                 ▼
┌─────────────────────────────────┐
│   Experimental Observations      │
│                                  │
│   Skills and Techniques Introduced│
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                 │
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┌─────────────────────────────────┐
│    Discussion of observations    │
│                                  │
│ Primary literature/web-based study│
│     Develop hypothesis to test   │
│       Experimental Design        │
└─────────────────────────────────┘
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┌─────────────────────────────────┐
│      Conduct Experiment          │
│                                  │
│  Gather more observations and data│
└─────────────────────────────────┘
                 │
                 ▼
┌─────────────────────────────────┐
│  Data analysis and interpretation│
│                                  │
│        Statistical tests         │
│        Data interpretation       │
│        Figure preparation        │
└─────────────────────────────────┘
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┌─────────────────────────────────┐
│   Presentation and Discussion    │
│    Poster or oral presentation   │
│        Manuscript Writing        │
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Module 1: *C. elegans: From Genes to Behavior*

While immersed in this laboratory module, students explore the connection between genes and behavior and become familiar with the concept of model organisms in biological research, focusing on *Caenorhabditis elegans* anatomy, behavior and use in the laboratory. The overall organization of this laboratory module is described in Figure 2. We explore fundamental concepts about nerve and muscle physiology while studying two different locomotion mutants, *unc-54* or *unc-119*. The students are given one of the two mutants but are not told which mutant they have been given. The goals for this module are to characterize the phenotypes of wildtype and mutant worms, observe where the wildtype gene is expressed in the worms, determine which gene is mutated, and then use that knowledge of the gene's functions to develop a hypothesis about why a mutation in this gene would confer the particular phenotype observed.

Figure 2. Organization of *C. elegans* behavior module
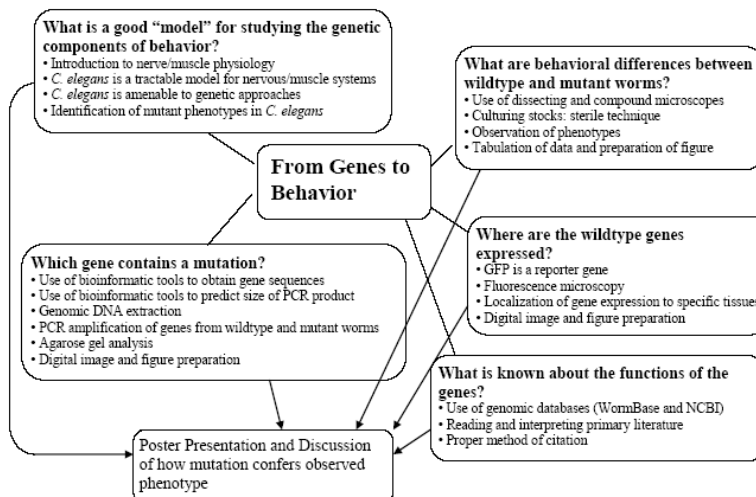
In the laboratory, students, working in pairs, learn how to use the dissecting and compound microscopes, as well as how to observe worm behavior and manipulate worms grown on nutrient medium in Petri dishes. They are introduced to sterile technique so that they can transfer worms to a new culture plate to propagate worms that they will use later for DNA extraction and PCR (polymerase chain reaction).

We next review the central dogma of molecular biology. We consider types of mutations, how mutations arise in DNA and the effects of mutations on protein function. Students learn about PCR and compare this technique with cellular replication of DNA. In the laboratory, students extract DNA from wildtype and their mutant worms and perform PCR using primers to amplify the *unc-54* and *unc-119* genes. The students read primary literature articles (Manning et al, 2004) about both gene mutations and discuss what is known and what is not yet known about the genes and their protein products.

To gain additional access to what is known about the genes, students explore two very powerful websites, WormBase (http://www.wormbase.org) and NCBI (http://www.ncbi.nlm.nih.gov). They learn how to use tools like "Sequence Extractor" (http://www.bioinformatics.org/seqext/) to predict the size of their PCR products based on the sequence of the primers. In the laboratory, students perform gel electrophoresis to compare the sizes of the PCR products from the wildtype and the mutant worm DNA. It is their task to determine which genes have which mutations and the size of the insertion or deletion in each. Students also consider the use of GFP (green fluorescent protein) as a cell biological tool. Using fluorescence microscopy, they identify cells and tissues in which the wildtype versions of the mutant genes are

expressed by observing transcriptional fusion constructs of the promoters of the genes linked to GFP. Students learn how to capture a digital image and create publishable figures that capture accurately what they have observed with the microscope. The student pairs present their data in the form of a poster and an accompanying abstract. In so doing, they learn how to prepare figures and figure legends, and to organize written information into a standard scientific format.

This module can be modified to explore different genes and phenotypes. In addition, the module can be designed to allow students to pursue an independent experiment, testing the effects of an experimental condition on mutant and wildtype worm behavior. Alternatively, students could compare two different mutations in the same gene to explore more fully the effects of various mutations on protein function. For example, students could look at mutations that affect the ability to express the protein (null mutations) and compare the effects with mutations that affect the folding of the protein. The combination of behavioral, microscopic, molecular and bioinformatics approaches provides a rich array of possible ways to explore fundamental biological concepts using the *C. elegans* model system.

### Module 2: Cyanogenic clover: Genetic variation and natural selection

In this module, students use cyanogenesis in white clover (*Trifolium repens*) to examine natural selection as an evolutionary force affecting variation within and among populations. Many plants, including many white clover strains, are cyanogenic, and there is substantial evidence that defense against herbivores is

an adaptive function of this trait. When the plant cells are damaged, the enzyme linamarase, sequestered in cell walls, comes in contact with a non-toxic cyanogenic glycoside, linamarin. Linamarase, a β-galactosidase, catalyses the removal of glucose from linamarin. The resulting acetone cyanohydrin can spontaneously break down to acetone and cyanide. Cyanogenesis is largely determined by two Mendelian loci, one affecting the production of linamarase, the other affecting the production of linamarin. Plants with at least one dominant allele at each locus are cyanogenic.

The overall organization of this module is shown in Figure 3. Students learn about the inheritance of cyanogenesis and become familiar with the enzymatic system of cyanogenesis and of the variation of cyanogenesis within and among populations of clover. In the laboratory, students examine variation among cultivars from an international seed bank using a colorimetric assay (Kakes, 1991) that allows them to determine whether and to what extent a given plant is cyanogenic. For each cultivar, they determine the frequencies of the two phenotypes (cyanogenic or not) and the frequencies of the four distinguishable genotypes. Goodness-of-fit tests are used to determine whether differences among cultivars in phenotype and genotype frequencies are statistically significant.

Figure 3. Organization of cyanogenesis module
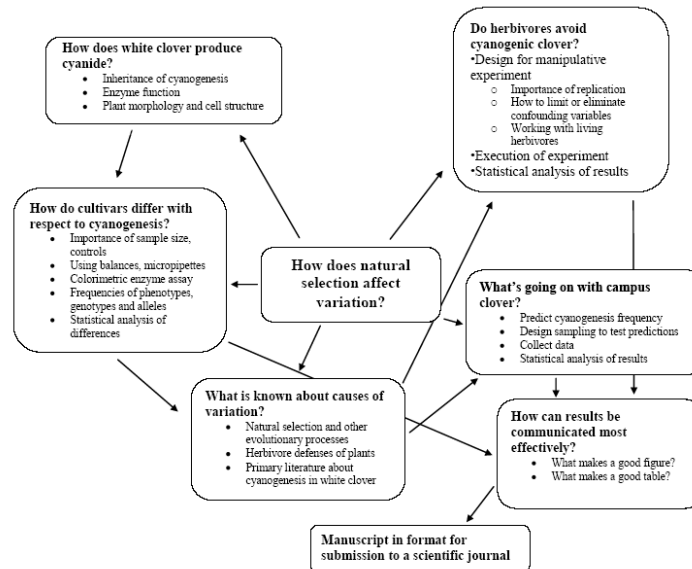
Next, we focus on microevolutionary processes (selection, mutation, gene flow, and drift) that affect variation within and among populations. Students read and discuss research reports describing broad-scale latitudinal and altitudinal variation in cyanogenesis among populations of white clover in Europe and North America (Daday, 1958; Ganders, 1990). Based on the findings reported in those papers, students generate testable hypotheses and design experiments to be carried out over the subsequent weeks. The student-designed experiments focus on the importance of proper experimental controls, adequate sample size and appropriate statistical analyses.

Students test the hypothesis that the frequency of cyanogenic clover varies with local winter soil temperature. For this experiment, we take advantage of a system of underground steam heat tunnels that produces strips of warmer ground where snow thaws more rapidly. We have buried retrievable temperature probes in clover patches for several months to show that the soil is warmer over the steam lines. Using the colorimetric assay, students test the specific hypothesis that clover growing over the steam lines is more likely to be cyanogenic than that growing away from the steam lines. They analyze their data statistically and relate their findings back to the primary literature.

Because generalist herbivore density tends to be lower at higher elevations (Horrill and Richards, 1986), students also test the hypothesis that herbivores exhibit a preference for acyanogenic clover. Students determine the cyanogenesis profile for particular clover leaves of a variably cyanogenic cultivar of clover from

the international seed bank which they assessed at the beginning of the module. Students then introduce herbivores such as locally harvested land snails (*Cepaea nemoralis*) or commercially available beetle larvae (*Zophobas morio*) to clover leaflets of known genotype. Students determine the amount of clover eaten by measuring leaflet surface areas before and after the feeding trial, then they conduct paired, one-tailed *t*-tests to assess whether the herbivores have eaten significantly more acyanogenic clover. The final assignment for this module is a manuscript prepared in the format for submission to a peer-reviewed journal.

This laboratory module is also amenable to modification in a number of ways. Students can test other hypotheses that might explain the results of the primary literature they read, including effects of soil water content or other factors that might exhibit a similar latitudinal or altitudinal cline. Alternatively, they can examine developmental aspects of cyanogenesis, where young plants exhibit less

cyanogenesis than do adult plants (Hayden and Parker, 2002).

Module 3: Biodiversity and Soil microbial ecology

In this module we investigate how variation in the local environment affects the biodiversity of soil communities. The overall organization of this module is shown in Figure 4. We introduce this module with a discussion of biomes, ecological communities and global patterns of biodiversity. We consider several current hypotheses explaining those patterns. This allows us to discuss how biologists develop and test hypotheses, as well as to introduce important concepts in ecology and conservation biology. We then narrow our focus to local soil communities and the effect of soil properties on different types of organisms. The students read and discuss a recent research article (Fierer and Jackson, 2006) that provides background material and motivation for the study of soil properties and microorganismal diversity.

Figure. 4. Organization of soil microbial diversity module
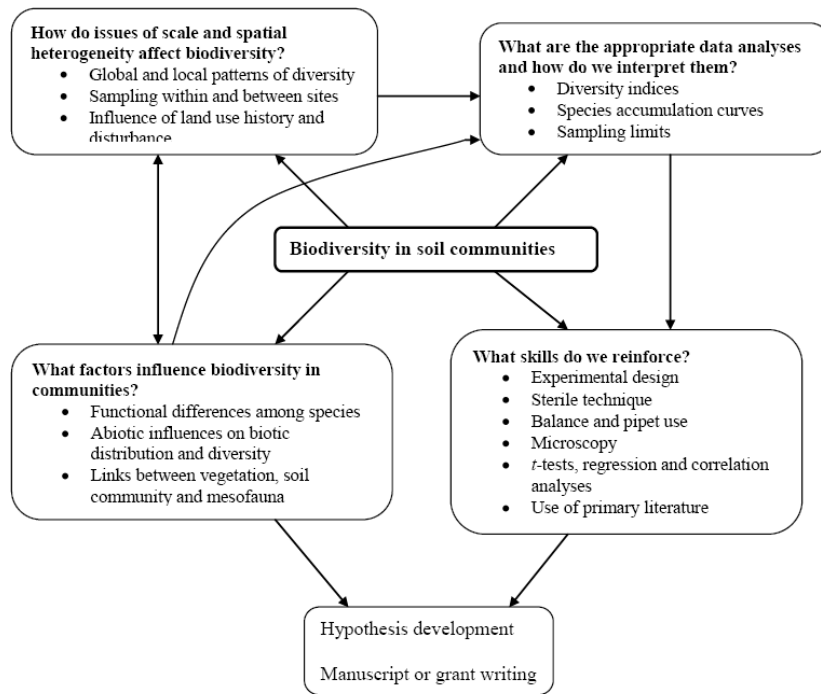
After gaining insight into the relationships between diversity and soil properties, the students move to an experimental field-based approach to test specific hypotheses about patterns of biodiversity in soil communities. They learn how sampling techniques are integrated into experimental design and discuss the limitations of sampling. Student pairs use a

stratified random sampling method to collect soil and leaf litter samples from two local sites. They make observations about vegetation types, topography, and current disturbance patterns at each location. In the laboratory students examine soil and topographic maps of the sites, giving them a good sense of the underlying geology of the area. They measure several soil

properties for each of their soil samples, including pH, soil water content, and soil organic matter content.

To investigate the mesofaunal diversity in their leaf litter and soil samples, students set up Berlese funnels and then identify the mesofauna at the level of Order using dichotomous keys. Students learn serial dilution plating techniques to assess bacterial and fungal diversity for each of their soil samples. They examine their plates and describe all the morphospecies they see, constructing a class "morphospecies library." The students determine both the total morphospecies richness as well as which morphospecies are present for each sample. The data on the morphospecies found per sample are used to create a species accumulation curve, allowing us to revisit issues of experimental design and sampling.

In the final section of this module, students reinforce and extend their understanding of statistics by using Student's *t*-tests to analyze their data for differences in soil properties, bacterial and fungal morphospecies richness and abundance, and overall mesofauna diversity between sites. We also contrast the use of regression and correlation analyses when they test their hypotheses about how the various soil properties might affect patterns of diversity in the soil. They use the number of colonies of each morphospecies to calculate a Shannon-Wiener index as an overall measure of diversity for different types of organisms at each site. Finally, they calculate Jaccard indices for the bacteria, fungi and mesofauna to examine community similarity between the sites. The culminating assignment for this module is an individually written manuscript. This assignment builds on the scientific writing skills of the previous module and allows students to focus more on aspects of interpretation and presentation of results.

This module, like the others, can be modified to accommodate different interests and expertise. Different soil properties can be measured. For example, in one semester, the study sites differed in levels of soil arsenic (because one site was an old orchard). Alternatively, the module could include an experimental angle, by culturing the soil microbes under different environmental conditions to test hypotheses about what factors control biodiversity. Another intriguing direction, described in a number of research articles (eg. Pace, 1997), would be to incorporate a molecular assessment of bacterial diversity, using PCR and either sequencing or RFLP analysis to identify 16S rRNA variants. This last approach would build on skills developed in the first module.

Assessment and Discussion

Prior to 2004, we offered a two-semester introductory sequence. Each course was formatted as a lecture period three days each week bundled with a four-hour laboratory, one focusing on cellular biology and the other concentrating on ecology, transmission genetics and evolution. During this period, we found that 40-60% of our faculty teaching effort was devoted to the 100-level curriculum, constraining the development of new intermediate and advanced courses. Another consequence of our former introductory curriculum was that students identified themselves as either "cell biologists" or "ecological biologists," and most were resistant to taking intermediate and advanced courses that ran counter to that identity. We also found that the content and memorization-focused lecture/laboratory format was dampening many students' enthusiasm for biology.

The new introductory curriculum has reduced the teaching effort at this level to 25-30%, which has increased our flexibility and ability to offer new courses at other levels of the curriculum. Perhaps the most striking improvement is that our students approach biology with a much more integrative perspective while still addressing substantial content and concepts, no longer distinguishing based on field or level of approach. We have also seen a recent impressive increase in the number of biology majors, which we attribute, at least in part, to our changes at the introductory level.

Graded assessment of student learning

In order to assess how well students mastered the skills and concepts of *Investigations*, each module has a number of short, graded assignments that take the form of homework, problem sets or other short written assignments. These assignments focus on content and concepts covered in class and in the textbook-assigned reading. In addition, each module has a culminating assignment that stresses a major form of scientific communication. The cumulative final exam consists of a conceptual essay-style section and a skills-based practicum. The instructor of each course section poses essay style questions designed to examine how well students mastered particular integrative concepts covered in each individual course section. The skills practicum portion of the final exam is a 45 minute-long exercise in which those being examined move among approximately 20 stations. The stations contain questions that examine particular skills emphasized throughout the term (see Table 1 for examples). Some stations have microscopes, others have balances, and still others have other small equipment used throughout the semester. Quantitative and statistical skills requiring computer-based software or a calculator are also included in the skills practicum. Several questions

examine critical thinking skills pertaining to experimental design, data interpretation and hypothesis testing.

Overall course assessment

In order to gauge the effectiveness of *Investigations* at achieving its goals, we administered a skills questionnaire to all students at the beginning of the semester and again at the end of the semester. This skills assessment tool gauged student learning, as perceived by them, of laboratory skills as well as quantitative skills like statistical measures, experimental design and data analysis. We queried 114 students, distributed among six separate sections of the course, taught by five different faculty members in 2007. Students were asked to indicate their level of familiarity with a given skill, from "fluent", meaning considerable exposure to the use of the skill, to "tried once or twice" to "never before encountered."

The skills questionnaire includes a variety of skills taught in *Investigations*, including some that students might well have encountered before coming to college (eg. using graduated cylinders, Fig. 5A). The responses of the students to the survey at the beginning of the semester reflect perceived skill familiarity prior to taking the course. These responses were tallied across the six sections surveyed. As is evident from Figure 5A ("Before"), at the beginning of the semester, 79% of the students felt "fluent" in the use of graduated cylinders. In contrast, only 5% of the students felt highly skilled in PCR (Fig. 5B). Only six percent of the students felt "fluent" in the use of a model organism for biological research, with 58% of the students having never encountered a model organism prior to the course. Eighty-three percent of the students had never encountered bioinformatics approaches to biological questions.

Table 1. Examples of skills practicum questions on the final exam
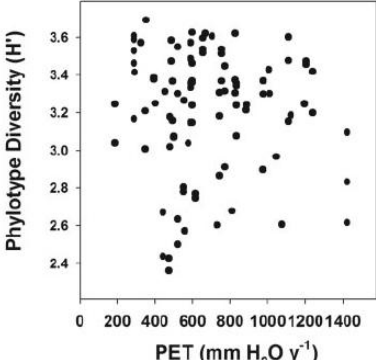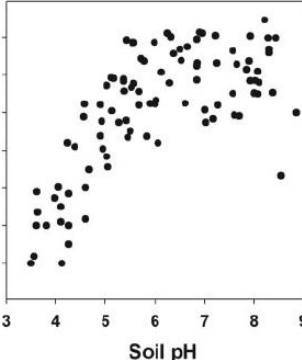
1. Pipet 170 μl of distilled water into the next preweighed, empty tube from the yellow rack. Close the tube and put it in the white rack. Write the # of your tube and the micropipette you used on your answer sheet, and write your name on the list next to the # of your tube. TUBE# _____ MICROPIPET USED:-----
2. Use the provided mesofauna data table to answer the following questions: a. Calculate Jaccard's index comparing the two sites [re: Cj= a/a+b+c) b. Which index (richness, H', Cj) or combination of indices gives you the most complete picture of biodiversity at these two sites?
3. Shown below are two scatterplots from the Fierer and Jackson 2006 paper. Correlation analysis was performed, and the correlation coefficients (*r*) are shown below each graph. The critical *r* value (to determine statistical significance) is **0.195**.   *r* = 0.14　　　　*r* = 0.83 **a)** Is **PET** correlated with biodiversity?　Yes　No **b)** Describe how you made this conclusion. **c)** Is this correlation positive, negative, or no correlation?　Circle correct answer **d)** Is **pH** correlated with level of biodiversity?　Yes　No **e)** Describe how you made this conclusion. **f)** Is this correlation positive, negative, or no correlation?　Circle correct answer
4. Please identify the *C. elegans* on the plate as either wildtype or a mutant strain.
5. You need to make serial dilutions to perform a plate dilution assay on a soil sample. You begin with 10 g of soil and add it to 95 ml water for your first dilution (10^{-1}). For tubes with a final volume of 10 ml, how much of your 10^{-1} dilution and how much water do you need to make a 10^{-2} dilution? How would you create a 10^{-4} dilution sample?

Figure. 5. Skills assessment questionnaire: The percentage of students rating each skill as "fluent" was determined the first day of the semester (Before) and again at the end of the semester (After). A) Skills students might have encountered before taking *Investigations*. B) Laboratory techniques specific to *Investigations*. C) Conceptual and integrative skills involving scientific communication, experimental design and statistical analysis.
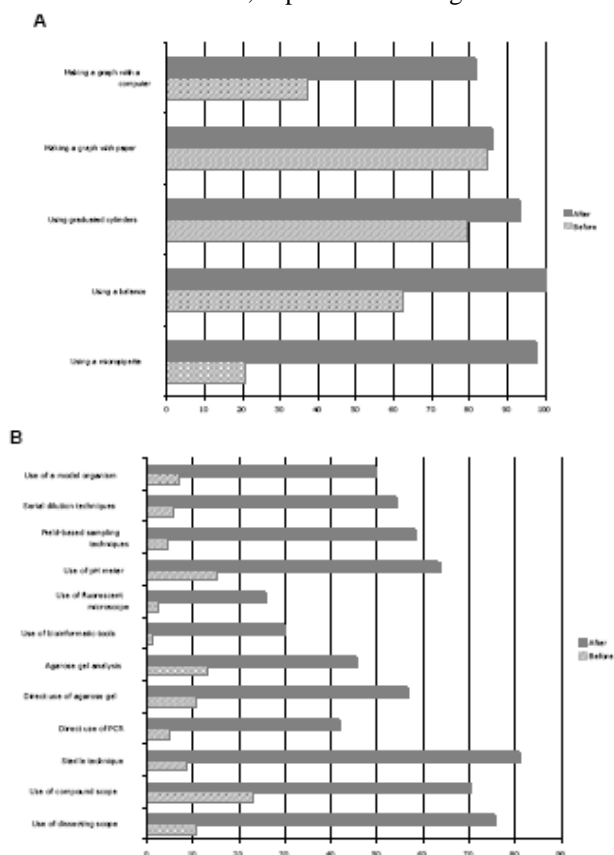
The responses were tallied in the same way at the end of the semester and the difference in percent of students feeling fluent for each skill was determined ("After", Figure 5). These responses indicate the change in the familiarity or exposure to a skill, as perceived by the students. While it is possible that some of the skills were also developed in other science courses that were being taken concurrently, many of them are covered only in *Investigations* and not in introductory chemistry, physics or math. Thus, changes in the perception of mastery of a skill most likely reflect learning (or perceived learning) on the part of the student as a result of taking this course. The change in level of perceived mastery of skills specific to introductory biology was quite dramatic. The percentage of students feeling "fluent" in the use of a dissecting microscope changed from 10% at the beginning of the semester to 75% at the end of the semester. Similarly, the percentage of students feeling skilled in the use of bioinformatics approaches increased from 1% at the beginning of the semester to 30% at the end. Thus, from the students' perspective, the course format led to an increase in skill level in virtually all of the skills addressed by the course. We are in the process of conducting follow-up questionnaires with this cohort of students to address longer-term retention of skills and concepts.

Conclusions

Our new introductory curriculum has contributed to an increased interest in the biology major. The curriculum introduces our students to fundamental concepts in biology in a topical, integrative, discovery based manner that seems to accomplish the stated goals. Faculty teaching *Investigations* are excited about

being able to teach open-ended, discovery-based laboratory projects and find the relevance of the projects to the primary literature to be an important and positive element of the course. One challenge we face with the course is our own comfort levels with a process-focused approach to introductory biology concepts as opposed to the traditional content-focused march through a list of concepts. While a few faculty feel that students seem to be less-prepared for work at the intermediate level of the biology curriculum, most faculty report a greater ability of students to engage the primary literature and no noticeable deficiencies in preparedness for intermediate level course work, as compared with the traditional two-semester lecture/lab courses that preceded *Investigations*. After four years with this new curriculum in place, we find our students engage intermediate and advanced courses with confidence and enthusiasm, with no notably apparent gaps in their basic knowledge and with substantially greater sophistication in their abilities to read the primary literature and approach topics integratively and experimentally. They also approach their intermediate and advanced laboratory work with skill and confidence. We continue to monitor and evaluate our student preparedness for advanced level work in biology. Perhaps the most notable change since we implemented the new introductory curriculum is that students no longer identify themselves as "cell people" or "ecology people."

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