Using algal microcosms in introductory biology lab. I: The influence of nutrient levels on the biodiversity of an ecological community

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Abstract

The protection of earth's biodiversity requires a sophisticated understanding of how human activities can affect the relative abundances of species in natural ecological communities. Here, we report on an introductory biology laboratory activity in which students quantified biodiversity while investigating one of the most important controls on the biodiversity of an ecosystem: nutrient availability. Students established microcosms of six species of phytoplankton ("algae") in 50-mL beakers and exposed them to five different levels of inorganic nutrients. After two weeks, students used hemocytometers to count cells and compare the relative abundances of the algal species (i.e., their community composition) at different nutrient levels. The effect of nutrient level on biodiversity (measured by Simpson's reciprocal index) was significantly curvilinear, and best described as "U-shaped." Specifically, the algal community was most diverse at the lowest nutrient level, least diverse with a small amount of added nutrients, and intermediate in diversity at the highest nutrient levels. This convenient, quantitative investigation provided students an opportunity to consider how anthropogenic influxes of nutrients into ecosystems can lead to eutrophication, and how this phenomenon can have negative effects by decreasing the biodiversity of ecological communities.

Keywords: biodiversity, eutrophication, microcosm, nutrient availability, phytoplankton, species richness, structured inquiry

Introduction

The central goals of conservation biology include identifying threats to biodiversity and evaluating methods for protecting biodiversity within ecosystems (Perrings et al., 2011; Pereira et al., 2012). In order to accomplish these goals, biologists need to be able to quantify biodiversity in a consistent and easily communicated fashion (Jacobs et al., 2014). The biodiversity of a biological community encompasses two main components: 1) the number of different taxa (e.g., species or genera) and 2) the relative abundances of the different taxa. These components are respectively called "richness" and "evenness." Statisticians have devised numerous methods to take into account both richness and evenness in a single index of biodiversity. Among the most common is the Simpson's reciprocal index, which was employed in the laboratory activity described in this paper.

One of the fundamental drivers of biodiversity in an ecosystem is the level of resources, such as inorganic nutrients, available to organisms (Huston, 1980; Wilson & Tilman, 1991; Stevens & Carson, 2002; Worm et al., 2002; Passy, 2008; Cardinale et al., 2009a; Cardinale et al., 2009b). A relatively nutrient-rich environment is likely to support more individuals, and is thus likely be more productive, than a nutrient-poor environment. In turn, it is natural to expect a positive relationship between nutrient levels and biodiversity in an ecosystem (Srivastava & Lawton, 1998; Dodson et al., 2000). However, the reverse can also be true. For instance, consider what happens when large quantities of nitrogen or phosphorus are released into lakes or estuaries. Populations of a few species of algae may take advantage of the nutrient abundance and irrupt in a phenomenon called eutrophication (Smith & Schindler, 2009; Chislock et al., 2013; Ansari & Gill, 2014). The total number of organisms in the ecosystem may increase, but only for a few species, and at the expense of individuals of other species. Thus, the biodiversity of a community can shrink with increased levels of nutrients. The expected shape of the relationship between resources and biodiversity is still an active area of research, with numerous examples of positive, negative, and hump-shaped relationships in different ecosystems (Guo & Berry, 1998; Waide et al., 1999; Dodson et al., 2000; Mittelbach et al., 2001; Fraser et al., 2015; Grace et al., 2016; Wang, 2017).

In this paper, we report on an experiment that

we have used successfully at an introductory biology level on the biodiversity of a community of phytoplankton (i.e., "algae"). After an introduction to the relevant concepts related to ecosystem productivity, biodiversity, and the algae, students were asked to brainstorm research questions, hypotheses, and potential experimental methods in their lab groups (3-4 students). Then through a whole-class discussion, the instructors guided students to an agreed-upon set of questions and experimental protocols to address the questions. Specifically, students constructed microcosms of six species of algae at five different nutrient levels in 50-mL glass beakers, and they sampled the community using hemocytometers after two weeks of growth. Students addressed three main questions with their data: 1) How did the community composition (i.e., relative abundances of the six species) vary across environmental conditions? 2) Did nutrient level affect biodiversity (as quantified by richness, evenness, and the Simpson's reciprocal index)? and 3) What is the shape of the relationship between nutrient levels and biodiversity?

The intended learning outcomes for this project were that students should be able to do the following: 1) use a diversity index to quantify the biodiversity of an ecological community; 2) communicate the rationale for why nutrient levels might have a range of effects on the biodiversity of an ecosystem; 3) employ aspects of the scientific method in a structured-inquiry experiment to address an important ecological question; 4) perform statistical analyses and construct professional-quality graphs using Excel; and 5) interpret and communicate the results and their broader implications. Their achievement of these learning outcomes was assessed through the presentation of a research poster to communicate their findings.

Materials and Methods

Course overview

The microcosm experiments described here (and in a companion paper: Wise & Collins, this issue) are the principal laboratory activities of the introductory biology course BIOL 180 (Exploring Biological Diversity) at Roanoke College, a selective liberal arts institution of ~2,000 students in Salem, VA, USA. BIOL 180 is one of a sequence of three introductory courses for Biology majors, but it is also taken by some non-majors for whom this is their only biology course. Versions of these microcosm experiments have been used in 16 sections of BIOL 180 since 2015. This course meets for three two-hour periods per week, and the class is capped at 24 students. The design and data reported in this paper are from a version of the experiment used in the fall semester of 2018 in a section with 14 students.

Phytoplankton Species

Six freshwater phytoplankton species across six different genera were chosen for inclusion in the microcosm experiment (Table 1) and were obtained from a commercial supplier (Carolina Biological Supply Company, Burlington, NC, USA). This set of species included four green algae, two of which are charophytes of the family Desmidaceae, and two of which are chlorophytes of two different families. The set also included one euglenozoan and one cyanobacterium. Each species was maintained in stock culture containing an equal mix of tap water and deionized water, to which one 20-mL tube of AlgaGro® Concentrated Medium (Carolina Biological Supply Company, Burlington, NC, USA) was added per 980 mL of water.

Setting up the Microcosms

Five different nutrient-level treatments were initiated by adding the following numbers of 20-mL tubes of AlgaGro® Concentrated Medium per liter of

Table 1.

Taxonomic information for the phytoplankton species included in this study

Genus	Superkindom[1]	Phylum/Division	Family
Ankistrodesmus	Archaeplastida	Chlorophyta	Selenastraceae
Cosmarium	Archaeplastida	Charophyta	Desmidaceae
Euglena	Excavata	Euglenozoa	Euglenaceae
Gloeocapsa	Bacteria	Cyanobacteria	Chroococcaceae
Scenedesmus	Archaeplastida	Chlorophyta	Scenedesmaceae
Staurastrum	Archaeplastida	Charophyta	Desmidaceae

[1] Eukaryotic superkingdoms are as designated in Morris et al. (2016). Bacteria are at the taxonomic level of kingdom and/or domain.

aqueous medium: 3, 2.25, 1.5, 0.75, or 0 tubes.

As with the stock cultures, these growth media contained equal parts tap water and distilled water. Each microcosm consisted of a 50-mL glass beaker containing 30 mL of growth medium plus 1 mL of stock cultures of each of the six algal species. Students transferred the samples from the stock cultures using 1-mL pipettes. To prevent contamination of samples, a separate pipette was used for each stock solution. Each pipette was conspicuously labeled by genus name, and students were instructed to double-check that the name on a pipette matched the name on the stock-culture beaker before making a transfer to their microcosms.

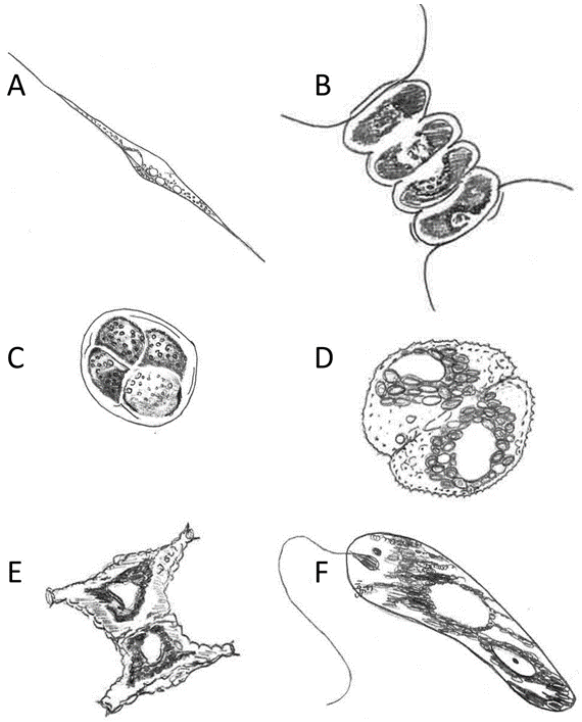
The class was split into five groups of students, and each group prepared two replicates of microcosms at each nutrient level (for a total of 10 microcosms per student group). Students covered each beaker with cellophane wrap to prevent evaporation, secured the wrap with a rubber band, and punched three small ventilation holes in the wrap using dissecting needles. The beakers were placed on a tray on a rack of shelves under constant fluorescent light for 14 days. The light was provided by four wide-spectrum tubes (F40 PL/AQ-ECO bulbs, General Electric), mounted ~40 cm above the shelf. The beakers were gently shaken daily to prevent permanent settling.

Identifying and Counting the Phytoplankton

Our main experimental goal was to quantify the relative abundances of the six species in each microcosm. (A secondary goal was to quantify the effect of nutrient level on the densities of individuals.) To practice identifications, students made wet mounts and sketches of each species from the stock cultures. The six species we used are relatively easy to tell apart, but the colonial nature of some of the species presented a small challenge for counting (Fig. 1). For instance, when individuals of *Gloeocapsa* divide, they temporarily remain clustered within a gelatinous sheath. Most often, we found *Gloeocapsa* in groups of four, but the groups can be much larger. Similarly, individuals of *Scenedesmus* are often found in chains of four (or more) individuals. For consistency, students should count individual cells that make up the colonies. Single individuals of two other species (*Cosmina* and *Staurastrum*) are composed of two symmetrical "semicells." These two desmids pose the most difficulty for students to distinguish, but a cell of *Cosmina* appears as a pair of semicircles, while the semicells of *Staurastrum* are more angular, and depending on its orientation, an individual may look like a triangle or star, or—more whimsically—a butterfly or a pair of samosas. For consistency, students should count a pair of semicells as a single individual.

Figure 1

Key for identifying and counting the six phytoplankton genera in the microcosm experiment



A) single individual of *Ankistrodesmus*. B) single colony of four individuals of *Scenedesmus*. C) four individuals of *Gloeocapsa*, together in a gelatinous sheath. D) single individual *Cosmina*, composed of two semicells. E) single individual of *Staurastrum*, composed of two semicells F) individual of *Euglena*, the only organisms likely to be moving in the sample. Illustration by Frances E. Bosch.

To count cells, we employed disposable plastic hemocytometers (C-Chip DHC-N01, INCYTO, Korea). Each hemocytometer slide has two wells for samples, and the center of each well contains a nested set of grids to allow for flexibility in counting schemes. Because of the complexity of the grids in hemocytometers, we have found it useful to project an image of the hemocytometer grids to the class to go over guidelines for counting as a group before turning students loose to collect data.

In other versions of this microcosm experiment, we have restricted the counting to the 1-by-1 mm

central grid. This consistency in area counted would have allowed not only for differences in relative abundances of species within microcosms (our primary goal), but also for differences in densities of individuals among microcosms with different nutrient levels (a secondary goal). However, there were extreme differences in absolute densities among microcosms, such that the central grid covered only a few cells in lowest-nutrient treatment, while each row of the central grid tended to be packed with hundreds of cells in the highest-nutrient treatment. To save time, for the low-nutrient samples, students were allowed to count cells that appeared anywhere in the well, rather than just within the central grid. For the higher-nutrient samples, students were allowed to limit their counts to just two rows of the central grid. The target was to end up with at least 100 individuals per nutrient treatment in order to obtain reliable estimates of diversity. Because the total volumes of the samples differed among microcosms, we had to abandon our secondary goal of quantitatively comparing densities within species across nutrient treatments. However, the differences in abundance among nutrient levels were qualitatively obvious, both from the dispersion of cells in the hemocytometers, and from the obvious variation in the greenness of the microcosms. Thus, it was qualitatively obvious that overall productivity of the microcosms increased with increasing nutrient levels.

Reaching the target of 100 sampled cells per microcosm for the lower-nutrient treatments generally required more samples than the two wells of each slide permitted. Between samples, students cleaned and dried the wells of the hemocytometers using a plastic squeeze bottle of water and a can of compressed air. Not all student groups were able to count 100 individuals in the lowest-nutrient treatment during the two-hour class period, and only two of the five student groups were able to make counts for both replicates of each nutrient treatment. However, each group obtained counts of at least one replicate per treatment by the end of the class period. Students analyzed only the data collected by their group, but the instructors analyzed all of the class data together, and the analyses reported in this paper are from all five groups combined.

Data Display and Analysis

Students focused on three response variables: species richness, biodiversity, and evenness. The species richness (S) of a microcosm is the number of different species found in the samples of the microcosm. For quantifying biodiversity, we used the Simpson's reciprocal index (1/D). An attractive feature of this index is that it does not involve logarithms, which tend to be non-intuitive to students. The Simpson's reciprocal index is calculated from the relative abundances (p_i) of all the species using the following formula:

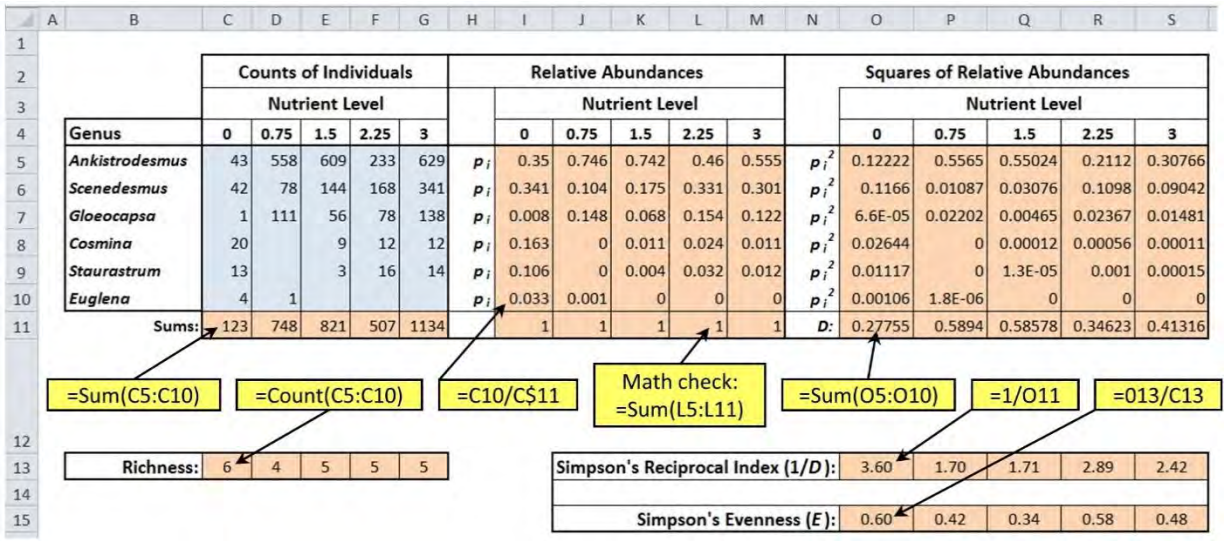
$$1/D = 1 \div \sum_{i=1}^{S} (p_i^2)$$

where Σ indicates the sum across all species, and i = 1 through S. The larger the value of 1/D, the greater the diversity of the community, with a maximum value of S occurring if p_i is equal for all species. Evenness has to do with the equitability of the abundances among species. Evenness can be seen as a component of diversity that remains after factoring out species richness. To quantify the Simpson's evenness (E) of a community, one simply divides the Simpson's reciprocal index by S. If p_i is equal for all species, the evenness is maximal, and E = 1. (Figure 2 displays an annotated snapshot of an Excel spreadsheet with data from one of the student groups and formulas for the calculation of relative abundance, richness, evenness, and Simpson's reciprocal index.)

Students generated graphs using Excel to display how the biodiversity index, richness, and evenness varied across the five nutrient treatments. They used simple linear regressions to assess whether nutrient level affected diversity index, richness, or evenness in a linear fashion. To assess whether there was a significant curvilinear relationship (i.e., either hump-shaped or U-shaped), students were instructed to perform a multiple regression that included both a linear and a squared term as independent variables (i.e., nutrient level and the square of the nutrient level). A significant positive coefficient for the squared term would indicate a concave upward (U-shaped) relationship, while a significant negative coefficient would indicate a concave downward (hump-shaped) relationship. (To create a quadratic regression line in an Excel scatterplot, check the "Polynomial" Trendline Option in the Format Trendline menu, and choose Order "2.") Students also made graphs ("charts") using Excel to compare the community compositions of the algae among the different nutrient treatments. Some students made pie charts, while most used bar ("column") charts. Some students made five separate bar charts, while others were able to display results for all five nutrient treatments on one bar chart. The diversity of displays

Figure 2

Sample spreadsheet used for calculating diversity-related metrics in the microcosm experiment



	Counts of Individuals						Relative Abundances						Squares of Relative Abundances					
	Nutrient Level						Nutrient Level						Nutrient Level					
Genus	0	0.75	1.5	2.25	3		0	0.75	1.5	2.25	3		0	0.75	1.5	2.25	3	
Ankistrodesmus	43	558	609	233	629	p_i	0.35	0.746	0.742	0.46	0.555	p_i^2	0.12222	0.5565	0.55024	0.2112	0.30766	
Scenedesmus	42	78	144	168	341	p_i	0.341	0.104	0.175	0.331	0.301	p_i^2	0.1166	0.01087	0.03076	0.1098	0.09042	
Gloeocapsa	1	111	56	78	138	p_i	0.008	0.148	0.068	0.154	0.122	p_i^2	6.6E-05	0.02202	0.00465	0.02367	0.01481	
Cosmina	20		9	12	12	p_i	0.163	0	0.011	0.024	0.011	p_i^2	0.02644	0	0.00012	0.00056	0.00011	
Staurastrum	13		3	16	14	p_i	0.106	0	0.004	0.032	0.012	p_i^2	0.01117	0	1.3E-05	0.001	0.00015	
Euglena	4	1				p_i	0.033	0.001	0	0	0	p_i^2	0.00106	1.8E-06	0	0	0	
Sums:	123	748	821	507	1134		1	1	1	1	1	*D*:	0.27755	0.5894	0.58578	0.34623	0.41316	

Richness:	6	4	5	5	5

Simpson's Reciprocal Index (1/*D*):	3.60	1.70	1.71	2.89	2.42
Simpson's Evenness (*E*):	0.60	0.42	0.34	0.58	0.48

The individual count data are entered into the blue-shaded cells, and the values shown in pink-shaded cells are calculated from formulas once the count data are entered. The yellow callouts indicate formulas typed into the orange cells. (Analogous formulas are found in adjacent cells.) Note that if the "Math check" cells do not equal 1, then there were errors in data or the formulas typed into the cells. This table shows data collected by one of the five student groups participating in the experiment.

enabled class-wide discussion of the relative merits of different types of graphs during the poster session that served as the main assessment metric for this project.

Students used only the data collected by their group for their analyses, rather than the combined class data. In addition to simplicity, this strategy had the benefit of motivating each group to collect a complete, high-quality set of data. In addition, having different sets of data for each poster made the presentation session more interesting and interactive. For the results presented in this paper, we combined the data for all five student groups. In the analyses of the effect of nutrient level, we include a "block" term to represent the variation that can be attributed to differences in algal communities (or student observational skills) among the student groups. These analyses are thus not strictly regressions, as they include student group as categorical variable, which was treated as a random-effects factor. The analyses reported here were performed using JMP-in 4.0.4 (SAS Institute, Cary, NC, USA). The effect of student group was not always significant, but leaving this blocking factor in each of the models serves to illustrate how much the data differed among student groups. A finding of little or no differences among student groups would serve as evidence of the robustness of the results.

Results

The regression models indicated that the level of nutrients did not have a simple linear effect on any of the three diversity-related metrics (Table 2: $P > 0.05$ for the nutrient factor). However, the quadratic models indicated that the relationship between nutrient level and Simpson's reciprocal index was significantly curvilinear (Table 2A: $P = 0.02$ for the nutrient^2 factor). The greatest biodiversity occurred at the lowest nutrient level (Fig. 3A). As nutrient level increased, there was first a sharp decrease in biodiversity, then a gradual increase, resulting in a U-shaped pattern. This U- shaped pattern was also largely reflected in the values for species richness (Fig. 3B) and evenness (Fig. 3C), but the quadratic coefficients were not statistically significant for either of these metrics (Table 2B and 2C). The mean richness did not vary much (4.0-5.0) across nutrient treatments. The mean evenness was more variable (0.42-0.66), and evenness appeared to drive the U-shaped pattern in the Simpson's reciprocal index more strongly than did species richness.

Table 2

Summary of results of statistical models testing for linear and quadratic effects of nutrient level on: A) biodiversity index, B) species richness, and C) evenness. A significant nutrients^2 factor (i.e., the square of nutrient level) indicates curvature in the relationship. Student group was treated as a random-effects factor.

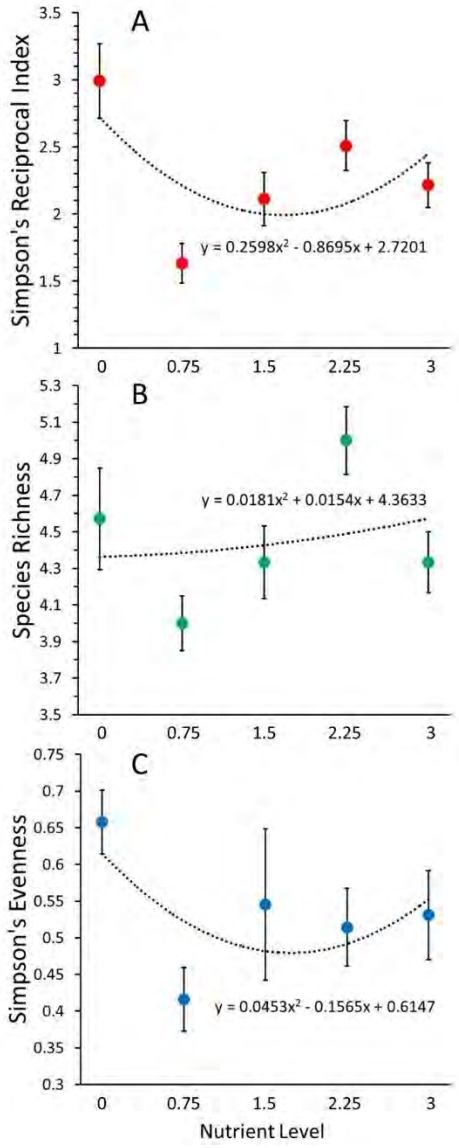
Source of variation	df	Mean square	F-ratio	P-value
A. Simpson's Reciprocal Index (Linear Model)				
Student group	4	0.36970	0.7767	0.55
Nutrients	1	0.28698	0.6029	0.44
Error	26	0.47602		
Simpson's Reciprocal Index (Quadratic Model)				
Student group	4	0.36001	0.8848	0.49
Nutrients	1	2.48329	6.1032	0.02
Nutrients^2	1	2.20429	5.4174	0.03
Error	25	0.40689		
B. Species Richness (Linear Model)				
Student group	4	2.29946	4.1210	0.01
Nutrients	1	0.39238	0.7032	0.41
Error	26	0.55799		
Species Richness (Quadratic Model)				
Student group	4	2.29529	3.9598	0.01
Nutrients	1	0.00321	0.0055	0.94
Nutrients^2	1	0.01627	0.0281	0.87
Error	25	0.57965		
C. Simpson's Evenness (Linear Model)				
Student group	4	0.06691	2.9260	0.04
Nutrients	1	0.02250	0.9840	0.33
Error	26	0.02287		
Simpson's Evenness (Quadratic Model)				
Student group	4	0.06754	3.2225	0.03
Nutrients	1	0.08842	4.2186	0.05
Nutrients^2	1	0.07053	3.3651	0.08
Error	25	0.02092		

The effect of student group was statistically significant for richness and evenness, but not for the Simpson's reciprocal index. Even when they were statistically significant, differences in values among student groups accounted for a relatively small percentage of the total variation (e.g., 32% and 26% for richness and evenness, respectively, in the quadratic models). Importantly, none of the inferences would have been different had the student-group factors been omitted from the models.

The community composition of the microcosms varied substantially across nutrient levels (Fig. 4). Students tended to be more comfortable first interpreting the values in the arithmetic scale (Fig. 4A). However, the fact that one can see the values for the less-abundant species much better on the logarithmic axis provides a good opportunity to persuade students of the value of using logarithms for some types of data (Fig. 4B).

Figure 3.

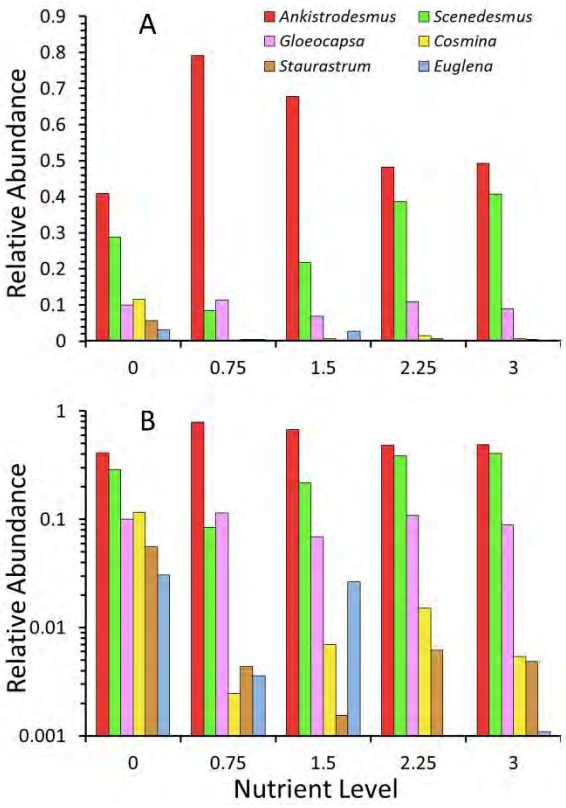
Relationship between nutrient level and biodiversity in the microcosm experiment



Points and bars represent means ± SE for five student groups: n=7 microcosms each for nutrient levels 0 and 0.75, and n=6 for nutrient levels 1.5, 2.25, and 3 tubes of AlgaGro per liter of aqueous medium. The dotted lines and equations represent the results of quadratic regressions of: A) Simpson's reciprocal index, B) species richness, and C) Simpson's evenness on nutrient level.

Figure 4.

Algal community structure at five nutrient levels in the microcosm experiment



Nutrient levels indicate number of tubes of AlgaGro per liter of aqueous medium. From left to right, the bars represent the relative abundances of *Ankistrodesmus, Scenedesmus, Gloeocapsa, Cosmina, Straurastrum,* and *Euglena* in: A) arithmetic, and B) logarithmic scales.

The two chlorophyte species (*Ankistrodesmus* and *Scenedesmus*) dominated the communities at all nutrient levels; however, they were at their least dominant in the lowest-nutrient treatment (Fig. 4). The non-chlorophyte species constituted 30% of the individuals in the lowest-nutrient treatment, but only 10-13% in the other four treatments. As a result, a much more even (and thus diverse) distribution of relative abundances can be seen in the lowest-nutrient treatment. *Gloeocapsa* remained steady at approximately 10% across all nutrient levels. The other three species (*Cosmina*, *Staurastrum*, and *Euglena*) were always less common, but they all attained their highest relative abundances in the microcosm with the lowest nutrient level.

Discussion

The availability of nutrients had a substantial, but not particularly straightforward, effect on the diversity of the phytoplankton communities in this microcosm experiment. It was qualitatively obvious that increasing the nutrient level greatly increased the growth and reproduction of the algae. Therefore, higher nutrients led to greater productivity over the short term for the community as a whole. However, the effect of nutrient levels was not the same on all species: Some species benefited at the expense of others. The overall result was that diversity was greatest at the lowest nutrient level, least at intermediate nutrient levels, and intermediate at the highest nutrient level. That is, the nutrient-biodiversity relationship was significantly nonlinear, and the concave-upward curve made the relationship approximately U-shaped.

Previous studies across a diversity of ecosystems have found a variety of relationships between nutrient level and diversity. Some studies have found a linear increase in diversity with increasing nutrients, others have found a linear decrease, and many have found a hump-shaped relationship, such that diversity is maximized at intermediate levels (Wilson & Tilman, 1991; Guo & Berry, 1998; Mittelbach et al., 2001; Cardinale et al., 2009a; Fraser et al., 2015; Groendahl & Fink, 2017). Our results were a bit unusual in having the lowest diversity at the intermediate levels of nutrients (cf., Huston, 1980; Waide et al., 1999; Mittelbach et al., 2001; Wang, 2017). Nevertheless, this pattern was consistent among the five student groups, and we have obtained similar results in other years with similar experiments. The fact that this U-shaped pattern was not anticipated by the students made the experiment and the poster presentation all the more interesting.

Examination of the details of the community composition provides insight into how increases in nutrient levels led the patterns of biodiversity of the communities in this experiment. At the lowest nutrient level (without an addition of AlgaGro), it is likely that the population growth of all six species was kept in check by limiting resources. No one species was able to grow to the extent that it completely dominated the others. *Ankistrodesmus* seemed to be the most sensitive to additional nutrients. In particular, with the addition of just 0.75 tubes of AlgaGro per liter, *Ankistrodesmus* took over, making up 79% of the entire phytoplankton community. This dominance by one species in the intermediate-nutrient environments led to overall low evenness and low biodiversity. With higher and higher levels of nutrients, *Scenedesmus* became a stronger and stronger competitor, nearly drawing even with *Ankistrodesmus* at the highest nutrient level. This led to greater evenness in the highest nutrient level, but not as great as in the lowest level. It seems that competition between these two chlorophytes largely drove the pattern of diversity across the range of nutrient levels.

It is important to point out that the vast majority of the studies available in the literature analyzed the effects of nutrient levels on species richness (i.e., strictly the number of different species), rather than a diversity index that also considered evenness (but see: Wilson & Tilman, 1991; Laird et al., 2003; Groendahl & Fink, 2017). Because our microcosm experiment was designed to be performed in a class setting, it was constrained to be smaller and shorter than most published studies on the topic. Specifically, because we had a pool of only six species growing over a period of two weeks, it is not surprising that we did not see a statistically significant effect on species richness. Nevertheless, the pattern that we found for species richness was consistent with our diversity-index pattern. Moreover, the fact that we used small communities in well-controlled environments enabled us to incorporate measures of community evenness, as well as to calculate a more comprehensive biodiversity index. Therefore, our results are probably more sensitive (and powerful) in terms of examining the subtle effects that nutrient levels can have on the diversity of biological communities.

A microcosm experiment such as this one cannot fully address the scale and complexity of what happens to natural aquatic communities when fertilizers and wastewater flow into lakes and estuaries (Smith & Schindler, 2009). However, this experiment did provide insight into one of the more subtle negative effects of eutrophication. The increase in nutrients in our experiment spurred an increase in overall productivity, which by itself might seem like a positive outcome. However, nutrient addition created winners and losers, with the overall effect of decreasing the diversity of the community. Such a depleted community is likely to be less able to provide vital ecosystem services to other organisms, including to humans (Loreau et al., 2001; Balvanera et al., 2006; Duffy, 2009; Perrings et al., 2011; Hooper et al., 2012; Pereira et al., 2012; Chislock et al., 2013; Ansari & Gill, 2014; Jacobs et al., 2014).

The overall results may vary from experiment to

experiment, and all student groups may not obtain the same outcome. While such variation can lead to frustration in canned experiments, there is no outcome to this experiment that is uninteresting or inexplicable. Such variation in outcomes can be an important lesson in and of itself. Allowing students to interpret and present their own group's results, then showing and discussing the combined results collated by the instructor, also teaches the lesson of the importance of replication in supporting scientific conclusions. As long as instructors take steps to ensure students set up the experiment correctly and collect data assiduously, this experiment should provide interpretable and satisfying results.

One source of variation that is not completely under control of the instructor is the speed at which students are able to count their algae using hemocytometers. Allowing time to practice using the microscopes and identifying the algae species prior to the experiment helps. Checking each group's interpretation of the hemocytometer grid during the counting is also important for consistency. Even with incorporating these measures, variation in students' acumen and enthusiasm tend to cause large differences in how quickly they collect their cell-count data. In the version of the experiment described in this paper, each group was instructed to collect two replicates of data at each nutrient level during the lab period. However, analyses of the data could still be performed with a single replicate, so it was fine if a group did not complete both replicates. In other iterations of this experiment, very slow-counting groups were required to come in after lab to finish their counting.

A second potential complication is that some species of algae replicate much faster than others. Therefore, it is important for the instructors to sample their stock cultures prior to the students' setting up their experiments to make sure each species is at a reasonable density. In particular, we have often found it useful to dilute the *Ankistrodesmus* stock cultures (and sometimes *Scenedesmus* as well) because they tend to reproduce much more quickly than the other species in the environmental conditions of our microcosms.

Conclusion

The experiment described in this paper provided students a hands-on opportunity to use original, authentic data to address an interesting experimental question using structured inquiry and the scientific method. Students hypothesized potential outcomes to the question of how nutrient addition might influence the biodiversity of an ecosystem. They performed an experiment to test their hypotheses, used their data to calculate biodiversity indices, and analyzed these data statistically to make inferences about the effect of nutrients on biodiversity. Because the answers to the experimental questions were not obvious prior to performing the study, students felt a greater sense of ownership of their investigations, which made the presentations of their results to the class especially engaging.

One of the greatest pedagogical assets of the phytoplankton microcosm employed in this study is its flexibility. For instance, we have had our classes look at the complementary question of how the biodiversity of algal communities affects the productivity of the ecosystems in microcosm experiments (Wise and Collins, this issue). We have added to the complexity of the system in BIOL 180 and in upper-level ecology courses by including such factors as competition, herbivory, disturbance, assembly order, and invasibility. This study system has also been employed by students for independent research projects in the senior seminar course for biology majors.

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