Using Stable Carbon and Nitrogen Isotopes of Hair to Teach About Sustainable Agriculture Through Active Learning

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ABSTRACT

The call for reform of science education is nearly three decades old (National Commission on Excellence in Education, 1983), but the implementation of such education improvements in the form of active learning techniques in large enrollment classes remains difficult. Here we present a class project designed to increase student involvement and quantitative analysis skills in a large enrollment lecture geared towards nonscience majors. We use the stable carbon and nitrogen isotopic analyses of hair to teach students about the impacts of industrial agriculture through quantitative assessment of diet in an environmental science course. Assessment of student learning, which was determined through exam questions and also through a feedback questionnaire, was overwhelmingly positive, demonstrating the usefulness of this technique in bringing active learning to the large enrollment classroom. © *2013 National Association of Geoscience Teachers*. [DOI: 10.5408/12-309.1]

Key words: active learning, large enrollment, stable isotopes, sustainable agriculture

INTRODUCTION

Improving science literacy among nonscience majors has been outlined as a major teaching goal by many national scientific organizations (American Geophysical Union, 1994; National Science Foundation, 1996; National Research Council, 1997), but most introductory sciences classes for nonscience majors are large enrollment lectures, which sometimes lack the opportunity for hands-on active learning. Many studies demonstrate that laboratory components of courses are important for the development of critical thinking skills by involving students in the process of inquiry and investigation (i.e., Hofstein and Lunetta, 1982, 2003). Traditionally, laboratory portions of classes have contributed to the active learning component of science teaching, but high enrollment classes for nonscience majors sometimes do not have accompanying laboratories (Springer et al., 1999). Thus, a major difficulty with increasing science literacy among nonscience majors is determining how to engage students in large enrollment classes.

Many studies have sought to increase material retention in large enrollment classes through active learning. Some examples of implementation of active learning in these types of classes include introduction of online course assignments (Riffell and Sibley, 2005), in-class clicker questions and small group active learning activities (Elbert-May et al., 1997; Deslauriers et al., 2011), and data analysis in workshop format (Kitchen et al., 2003). Few studies have tried to incorporate laboratory-based research projects as active learning components of the course. Here we discuss a technique to teach about sustainable agriculture to an introductory environmental geology class using stable isotopes of hair. Following a brief introductory lecture in class, students donated hair during their weekly discussion sessions as a sample population to include them in the research and teaching processes. Our goals were twofold: (1) to introduce students to the process of isotope analysis to increase participation by involving students in the research process and introduce students to some ongoing research topics at the University of Michigan, and (2) to quantitatively show the students their impact on the environment and introduce them to sustainable issues. With this class project we aimed to involve the students directly in the study through the donation of hair samples and an introduction to the laboratory instruments and capabilities during guided tours of research facilities. This type of experiment is typically done at the graduate level, and is rarely done in undergraduate classes because of large class size and access to analytical equipment.

The use of real data in the classroom for the geosciences is now common for all levels and class types, with the development of the NOAA Ocean Data Education project (NOAA, 2010, for K–12), as well as the development of modules to use real data to teach college-level online geology classes (SERC, 2012). The American Meteorological Society has shown this technique to be an effective way to increase science literacy and interest in the geosciences among students in introductory classes (Brey et al., 2010). With students participating in the data collection, these exercises become a combination of both active learning and incorporation of real data into the classroom.

The Introduction to Environmental Geology (EARTH 284 herein) class taught at the University of Michigan (UM herein) is a four-credit, high enrollment (up to 80 students) class geared toward nonscience majors and toward students whose primary concentration is in the Program in the Environment (an Environmental Studies major that requires only introductory Biology, Chemistry, and Earth Science coursework), rather than specifically concentrating in Earth Sciences. The course also serves as one of four core courses in a minor in the Environmental Science program in the Department of Earth and Environmental Sciences at UM. There is no laboratory component of the class, but there is a one-hour discussion section used to reinforce topics discussed in lecture and to introduce additional concepts. Many students take this class as a distribution requirement

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for natural sciences, and because the University only requires seven credits of natural science classes to graduate, this class is one of the few chances we may have to try to increase scientific literacy among nonscience major students. The large lecture format of the class leaves little opportunity for hands-on experimentation and development of laboratory skills. The issue that then arises with this class is how to incorporate active learning and class participation in a high enrollment class with no dedicated laboratory. After the project was successfully completed for the large enrollment class, we decided to carry out the same study in an upper level seminar style course in ecology to determine if the active learning method is also useful for smaller enrollment classes. These two case studies were used to revise the course materials for ongoing and future classroom implementation.

SCIENTIFIC BACKGROUND

The food that we eat has distinct carbon and nitrogen isotopic signatures based upon the plants used in its production as well as its place on the food chain. These signatures are preserved when the food is incorporated into our body tissue (DeNiro and Epstein, 1978; 1981). Thus, we can use the isotopic composition of hair to determine quantitative information about diet (O'Connell and Hedges, 1999). The stable isotopic composition of an element within a compound is calculated by the following equation:

$$\delta^{heavy}X=\left(\frac{R_{sample}}{R_{std}}-1\right)\times 1000, \quad (1)$$

where R is the ratio of the heavy to light isotope of element X ($^{heavy}X/^{light}X$) and $\delta^{heavy}X$ represents the isotopic composition of element X compared to that of a standard. Isotopic compositions are reported in units of per mil (‰), which is similar to percent, but represents units of per thousand. For carbon, $R = {}^{13}C/{}^{12}C$ and $\delta^{13}C$ represents the isotopic composition of carbon in a sample compared to the international standard Vienna Pee Dee Belemnite. Differences in photosynthetic pathways between C_3 and C_4 plants cause a difference in the $\delta^{13}C$ of plant tissues. C_3 photosynthesis uses RuBisCo to capture CO_2, and strongly discriminates against ^{13}C during that process. This discrimination leads to an average offset of −19.5‰ between the $\delta^{13}C$ of C_3 plant tissue and the $\delta^{13}C$ of CO_2 in the atmosphere (Koch, 1998). The average isotopic composition of C_3 plants under current atmospheric conditions is −27‰. In the C_4 photosynthetic pathway, CO_2 is captured and concentrated within the cells by the PEP-carboxylase enzyme, which discriminates less against ^{13}C than RuBisCo. The offset between the carbon isotopic composition C_4 plants and the atmosphere is −5.5‰, leading to an average $\delta^{13}C$ of −13‰ for C_4 plant tissues under current atmospheric conditions (Tipple and Pagani, 2007). Because the main C_4 plants consumed in the United States are corn and sugar cane, with sorghum as a minor component (Schoeller et al., 1986; USDA, 2011), we can use the isotopic composition of body tissue, hair in this case, to quantify how much of carbon in our diets comes from these crops. Similarly, the isotopic composition of the diets of livestock is preserved in their tissues, so the carbon contributed to our diets through the consumption of meat and dairy also reflects the isotopic composition of the consumed animal's diet.

Equation 1 may also be used to calculate the isotopic composition of nitrogen in a sample, where $R = {}^{15}N/{}^{14}N$ and $\delta^{15}N$ represents the isotopic composition of nitrogen compared to the standard composition of air. The isotopic composition of nitrogen becomes enriched in ^{15}N moving up the food chain, increasing roughly +3‰ per trophic level (Schoeninger and DeNiro, 1984). This enrichment in body tissue is caused by proteins preferentially incorporating ^{15}N into their structures. For example, if chickens were to feed strictly on plants with a $\delta^{15}N$ of 1‰, the isotopic composition of those chickens' body tissue would be 4‰, and if a student were to eat only those chickens, the $\delta^{15}N$ of their hair would be 7‰. Animal products such as dairy and eggs are isotopically indistinguishable from meat. The feed of the livestock also influences the nitrogen isotopic composition of the animal tissue, with animals that are fed meat by-products being isotopically heavier than those fed a vegetarian diet.

Thus, the student subpopulations should be isotopically distinct based on their diets. Students consuming large amounts of corn, cane sugar, high fructose corn syrup, and industrially farmed meat products will have the highest $\delta^{13}C$ value. Students consuming low amounts of high fructose corn syrup and meat products will have a lower $\delta^{13}C$ value. Similarly, those following a vegan diet will have the lowest $\delta^{15}N$ value. Vegetarians will have a higher $\delta^{15}N$ than vegans, because of their consumption of dairy and eggs. Students eating a meat-rich diet will have the highest $\delta^{15}N$ value (O'Connell and Hedges, 1999). Fig. 1 shows the expected location of the different diets in carbon-nitrogen isotope space.

CONTEXT WITHIN CLASS CURRICULUM

Industrial agriculture is responsible for many negative effects on the environment, including much of the soil erosion occurring today (Montgomery, 2007). Industrial practices also release of large amounts of greenhouse gases and contribute to anthropogenic climate change, with 18% of global greenhouse emissions attributed the production of livestock alone (Steinfeld et al., 2006). Currently, corn is the most highly subsidized crop grown in the United States (Environmental Working Group, 2011) and has become the major component of livestock feed because it is less expensive than other grains. The low cost of corn has also driven its increased use as a sweetener, and has become ubiquitous in American food. The increase in the use of high fructose corn syrup also tracks the rise in obesity in America over the last 30 years (Anderson, 2007). The impact of increasing corn utilization has both public health and environmental implications, however, people often do not know about the "hidden" ingredients in their food or the provenance of their diets. The carbon and nitrogen isotopic compositions of our body tissues track the influence of industrial agriculture in our diets. Providing quantitative assessments of diet can increase people's

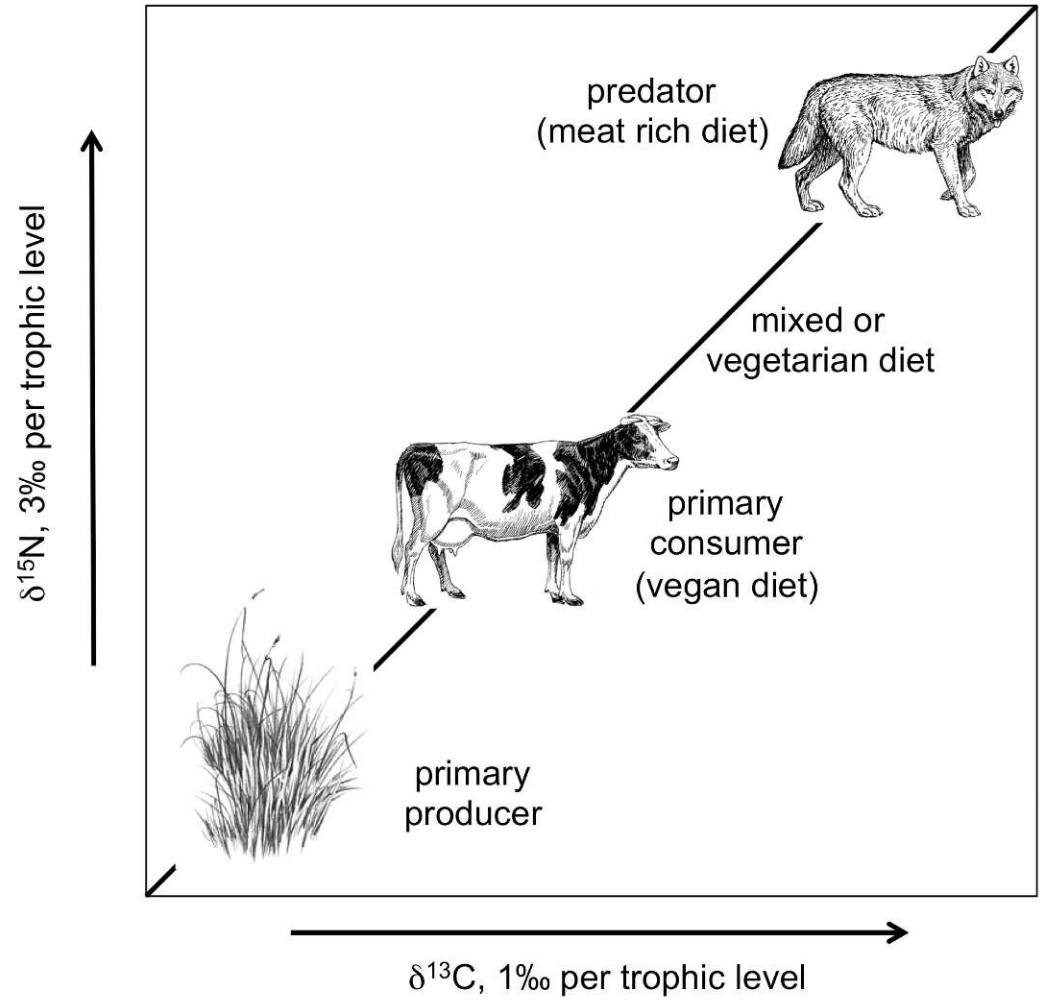

FIGURE 1: Schematic showing the enrichment of both $\delta^{13}C$ and $\delta^{15}N$ up the food chain. The enrichment in $\delta^{13}C$ is 1‰ per trophic level and the enrichment in $\delta^{15}N$ is 3‰ per trophic level.

awareness of food sources and hopefully influence their dietary choices.

METHODS

This project was carried out in the Introduction to Environmental Geology class (EARTH 284) at the University of Michigan. The class project was introduced initially in lecture (~15 minutes), which was followed up by a dedicated discussion section and presentation of the final results during a subsequent lecture about a week later. The initial introduction was a broad overview of the concepts of using stable isotopes to understand natural processes, with a brief introduction to issues of agricultural sustainability. The discussion section and second lecture where the final results were presented went into more detail about U.S. agriculture, and were designed to stimulate discussion about dietary choices and their potential health and environmental consequences. The project was carried in two separate classes in the Winter 2011 and Winter 2012 terms.

Students were offered the chance to donate their hair to the study. Participation in this project was not mandatory. Roughly half of each class chose to allow us to sample their hair and to participate in the analysis. More female than male students chose to donate hair because longer strands made it is easier to donate enough hair for isotopic analysis. Hair samples were collected from students and washed in a 2:1 solution of methanol/chloroform to remove lipids as well as any residue left behind by cosmetics and shampoos (Bowen et al., 2005). Depending on the length, one to multiple strands of hair were analyzed by a University of Michigan laboratory technician. Samples were loaded into a Costech elemental analyzer attached to a Finnigan Delta V+ mass spectrometer at the University of Michigan Stable Isotope Laboratory. Isotopic ratios are reported in units of per mil (‰) relative to the international standard Vienna Pee Dee Belemnite for carbon and air for nitrogen, and analytical uncertainty was better than 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. Students who chose to donate their hair were then provided with a diet survey (Fig. 2A) to determine their consumption of animal products as well as soda.

A

Food Survey:

1 a. Are you a vegetarian?

2 a. If you eat meat, how often? (every meal, once a day, once a week…)

b. Do you eat a specific kind of meat more than others? (i.e. prefer chicken over beef…)

c. Do you make an effort to eat free range and grass fed meats?

d. How often to you eat fish? (every meal, once a day, once a week…)

3. How often do you eat eggs, cheese, milk, ice cream (i.e. every meal, once a day, once a week)

4. Do you eat in the dining halls on campus?

5. How often to you drink soda? (every meal, once a day, once a week…)

6. How often do you eat fast food? (every meal, once a day, once a week…)

B

Test Questions:

In our class experiment we used the stable isotopic compositions of both carbon and nitrogen derived from hair samples provided by the class to look at the impacts of dietary choices.

a) In class, we talked about why we can use isotopes to determine the amount of corn and sugar in a person's diet. Explain how and why corn and sugar are different from other plants.

b) For both carbon and nitrogen, explain how an enrichment in the stable isotopic composition of each element is interpreted in terms of food intake. How will vegans differ from vegetarians? How will both differ from omnivores? If you prefer, you may draw a graph to answer this question.

FIGURE 2: (A) Questions used in the dietary survey given to students who donated their hair to the hair isotopic analysis project; (B) Exam questions pertaining to the hair isotopic analysis project.

During the aforementioned one-hour discussion section, students were then taken on tours of the Earth System Science and Stable Isotope Laboratories to demonstrate both the sample preparation and analysis methods to obtain the isotope data. During these tours, students were introduced to not only the procedures for the analysis of the hair, but also to other types of climate and environmental research topics being investigated by the researchers in those lab groups. For many students, this was their first chance to see and hear about ongoing research at UM. For nonscience majors, this may have been their only chance to "see behind the curtain" into an active lab. Tours involved groups of ~10 students at a time and lasted 30–45 minutes to see the ~2,000 $ft.^2$ Stable Isotope Lab. The small group size was used both to make sure that everyone could see instruments or sample processes, and also to provide a comfortable group size for asking questions.

During a lecture about a week later, the dietary and lifestyle questionnaire information (Fig. 2A) was used in conjunction with the isotopic analyses to see if the students' expectations of their diets matched their isotopic results. The isotopic results from the class were then compared to hair analyzed from a population of students in England to see the influence of corn in their diets relative to a country with no governmental corn subsidies.

Student learning was assessed using questions on an exam (Fig. 2B), and by feedback questionnaires. Test questions focused on the basic scientific principles of why tissues differ isotopically and how diet information is gathered from the data. The goal of these test questions was to determine if the students had learned how to interpret results from a dataset given the appropriate background information. Because the sustainable agriculture topic was not taught in this class prior to the addition of the isotope project, we cannot compare student learning between conventional lecture and the active learning isotope class project method of teaching. For the 2011 class, ad hoc feedback from both the associated lectures and from the discussion session were universally positive. For the 2012 class, a follow-up questionnaire (Table I) was given to assess student learning and as well as gain feedback on if the project would influence students' dietary choices.

TABLE I: Results of the feedback questionnaire for the EEB 410 and 2012 EARTH 284 classes. Responses were scored on a 1 through 5 scale, where 1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, and 5 = Strongly Agree. Students responded more favorably in the lower level large enrollment EARTH 284 class than the upper level small enrollment EEB 410 class, though generally feedback from all students was positive.

Question	Class EEB 410	2012 EARTH 284
I learned about both the unsustainable and sustainable agricultural practices in the United States.	3.46 ± 0.78	4.13 ± 0.60
I learned about the sources of food in the United States.	4.14 ± 0.54	4.25 ± 0.52
I was surprised by the percentage of carbon coming from corn present in my diet.	4.15 ± 0.99	4.20 ± 0.92
This project was useful to demonstrate the effect of my dietary choices on the environment.	3.79 ± 0.89	4.21 ± 0.69
Knowing my isotopic composition will influence my choices about food in the future.	3.60 ± 0.83	3.85 ± 0.98
I have learned enough about isotopes to explain issues about food sources with friends and family.	3.60 ± 0.86	3.86 ± 0.61

1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Strongly Agree

Although this study focused mainly on using isotopes to teach sustainability in diet choices to a large format class comprised of predominately nonscience concentrators, it is clear that this method could also be useful for small enrollment classes of science concentrators. The same isotopic study was carried out in a senior-level Ecology and Evolutionary Biology capstone seminar (EEB 410) in the Fall of 2011 to test the effectiveness of this teaching style on upper-level science concentrators, and also to gauge interest in expanding the project to include other student demographics. Eighteen students and two instructors contributed hair for isotopic analysis. Feedback from this upper-level class was collected from the students through the same feedback questionnaire as the 2012 EARTH 284 (Table I) class administered after the discussion of the isotopic results.

RESULTS

Isotopic Results

Thirty students from the 2011 EARTH 284 class, 27 from the 2012 EARTH 284 class, and 18 students from the EEB 410 class donated hair for analysis. Seven graduate students and two instructors also donated hair to the study. There were a total of 71 omnivores, 12 vegetarians, and one vegan. The average carbon and nitrogen isotopic composition from each class was statistically indistinguishable. The mean $\delta^{13}C$ for the students was -18.21 ± 0.99‰, with a range of 5.0‰ (-21.51 to -16.51‰). This range in $\delta^{13}C$ corresponds to C_4 plants making up 20–57% of the students' carbon intake through direct consumption of C_4 plants and corn fed animal products. The mean $\delta^{15}N$ for the students was 8.40 ± 0.60‰, with a range of 2.89‰ (+6.94 to +9.82‰). This nitrogen isotope range equates to the difference between one trophic level. Figure 3A shows the isotopic distribution of hair analyses for the class, graduate students and instructors, while Fig. 3B shows the average isotopic compositions of omnivores and vegetarians compared to the one isotopic analysis from the vegan student.

As predicted, the average isotopic composition of omnivores is more enriched in both ^{13}C and ^{15}N than that of vegetarians. The average isotopic composition of

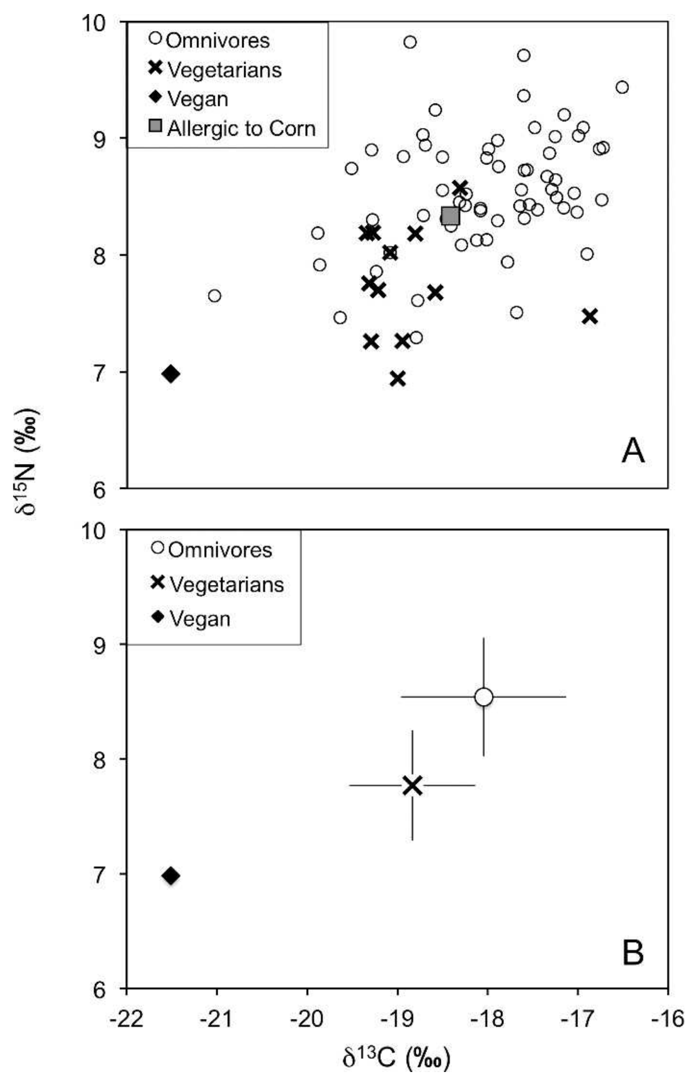

FIGURE 3: (A) Individual hair isotopic analyses for students, broken down by omnivores, vegetarians, vegans, and the one student allergic to corn; (B) Average isotopic composition of omnivores, vegetarians, and vegan students in the class. Error bars are 1σ around the mean.

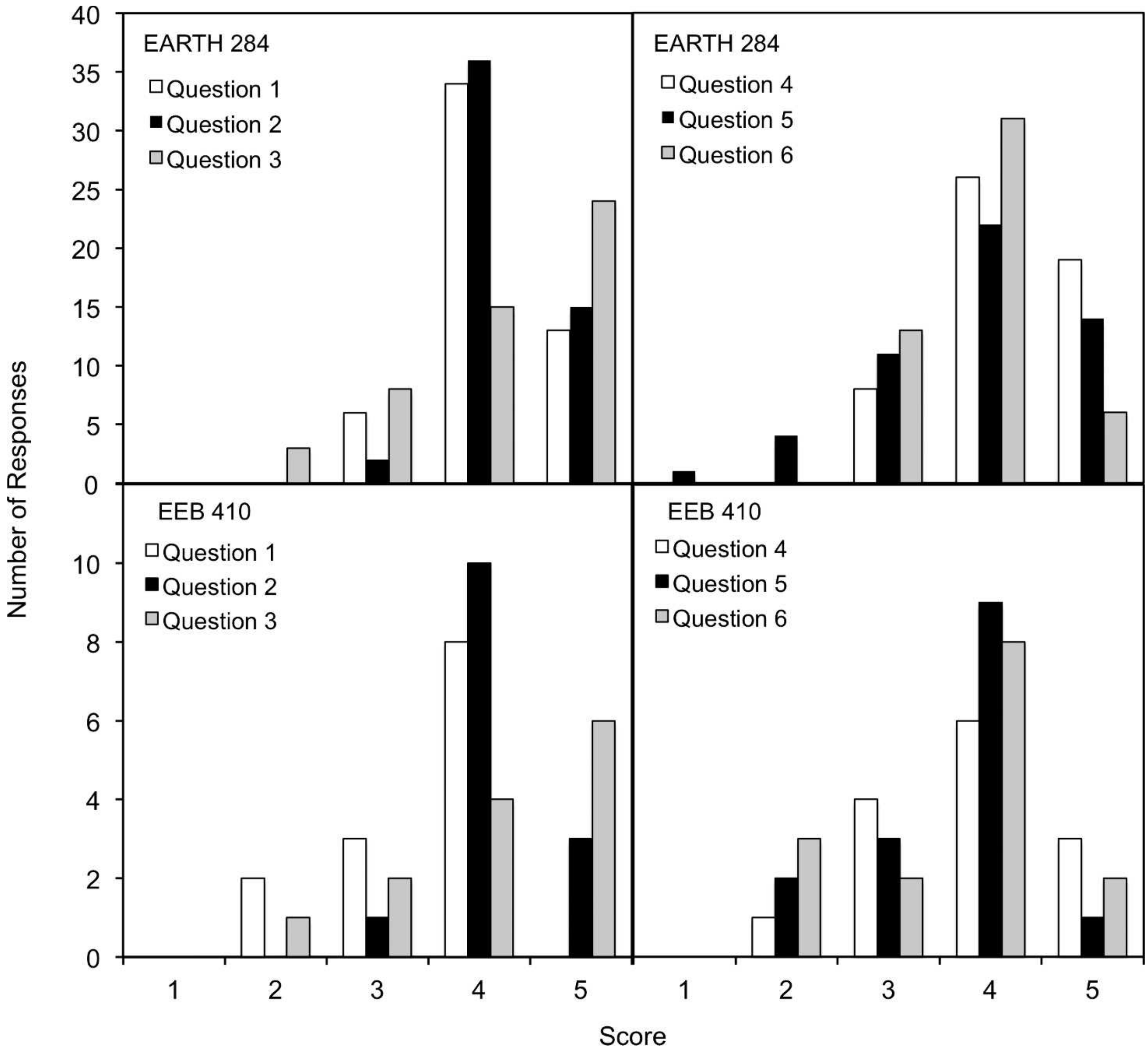

FIGURE 4: Histograms displaying the breakdown of student responses to the feedback questionnaire. Responses were scored on a 1 through 5 scale, where 1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, and 5 = Strongly Agree. Question numbers refer to the questions displayed in Table I.

vegetarians is also enriched in both ^{13}C and ^{15}N compared to the vegan. One student in the class was allergic to corn, but still had a $\delta^{13}C$ value of −18.31‰ (Fig. 3A), showing a considerable amount of C_4 plant-derived carbon in her diet. The individual isotopic measurements of each student, as well as other trends in the data including an isotopic difference between male and female students, can be found in the *Supplemental Material* and *Data files* (available online at dx.doi.org/10.5408/12-309s1 and dx.doi.org/10.5408/12-309s2).

Assessment of Teaching

The effectiveness of this class project was assessed mainly through a feedback survey given to the students after the project had been completed. Table I displays the results of these feedback questionnaires for the EEB 410 and 2012 EARTH 284 classes. Figure 4 displays histograms of the responses to individual questions for each of the classes, with the top panels representing EARTH 284 and the bottom panels representing EEB 410. According to these questionnaires, both classes responded favorably to the project. On a scale of 1 to 5, with 1 representing "strongly disagree" and 5 representing "strongly agree," most students rated the exercise with 3s, 4s, and 5s with regard to learning about sources of food and sustainability. For example, the students of EARTH 284 responded with an average of 4.21 ± 0.69 that the project was a useful way to demonstrate the effect of dietary choices on the environment. The introductory large format class generally scored the activity higher than the upper level class.

The majority of the students agreed that they had learned about sustainability in agriculture (Question 1: 4.13 ± 0.60 for EARTH 284; 3.46 ± 0.78 for EEB 410), and that they were surprised by the amount of corn in their diet (Question 3: 4.20 ± 0.92 for EARTH 284; 4.15 ± 0.99 for EEB 410). Interestingly, both classes indicated that learning about the quantitative contribution of corn to their diet through isotopes would influence their dietary decisions in the future. The feedback questionnaire also offered students the chance to write in comments for the instructors. In this written feedback, students commented that they thought the project was "fun and enlightening," "very interesting," and their "favorite part of the course so far."

While the available sample size is small, in both 2011 and 2012 students in EARTH 284 answered exam questions about sustainable agriculture correctly at higher rates, 88% and 91% respectively, than other exam questions on other topics from the same exam (84% and 82%). In 2009 and 2010, with no isotope and sustainability exercise, students had average scores of 83% on the equivalent exam. Thus, the data suggest that student performance was better on the active-learning exercises than on lecture material, and that overall test performance was better as well.

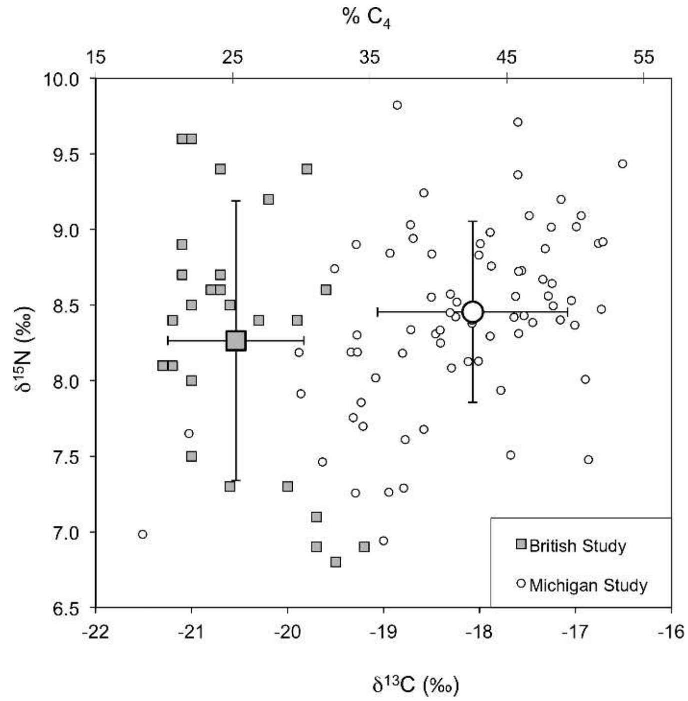

FIGURE 5: Comparison between Michigan and British studies, showing the carbon isotopic compositional difference between the diets of the two countries. Individual isotopic analyses are displayed by the smaller symbols, while the average isotopic compositions are represented by the large symbols. Error bars are 1σ around the mean.

DISCUSSION

The students' carbon and nitrogen isotopic compositions were compared to the isotopic compositions of a British population published by O'Connell and Hedges (1999). This comparison is displayed in Fig. 5. The two populations are isotopically similar for nitrogen but University of Michigan students are more enriched in ^{13}C compared to the British population, showing that the two populations of people are eating roughly similar amounts of meat, but that their carbon sources are very different. Using a conservative estimate of −24‰ as an end-member for food derived from C_3 plants (e.g., Tipple and Pagani, 2007), we calculate that the average Michigan student diet is made up of 46% C_4 plants compared to 24% for the British population. The difference in $\delta^{13}C$ values and thus, the amount of dietary C_4 carbon between the University of Michigan students and the British population is likely due to the use of corn in agriculture in the United States, though it could also be interpreted as a larger contribution of cane sugar to the diets of Michigan students than to those in the United Kingdom.

The feedback collected from the two classes demonstrates that this activity is a useful way to increase student interest in geological and environmental issues at the introductory level and also to convey concepts effectively for senior level classes. Interestingly, most students from both classes generally agreed that they had learned enough about diet and isotopes to discuss food source and sustainability issues with friends and family. This result is especially important for the nonscience concentrator students in the large enrollment class because it demonstrates the ability of this exercise to increase science literacy in nonscience concentrators such that they are comfortable discussing these issues outside of the classroom.

According to the student feedback, the introductory level EARTH 284 class responded more favorably to the activity than the upper level class, showing that this exercise is useful for both levels of students but may be more useful for lower-level, large-enrollment classes. This discrepancy between classes may have arisen because some of the upper-level students were likely already introduced to the issues of agricultural sustainability in previous classes, so the concept of tracking diet using stable isotopes may have been new but the students may have already learned about the environmental issues associated with diet. Nevertheless, the student feedback indicates that using isotopes of students' hair to quantitatively determine diet is a useful way to teach aspects of sustainable agriculture as well as increase student involvement and active learning for multiple student demographics.

APPLICATIONS OF THIS EXERCISE

For instructors that do not have access to the analytical equipment necessary for isotopic analysis of students' hair, there are a few options for how to implement this exercise in their classrooms. First, if funds are available from the university to carry out this exercise, there are multiple commercial laboratories that could process samples collected from students. The cost is approximately $10 per sample, depending on the university. If funds are not available for outside analysis, we have provided the individual analyses and student responses to the food questionnaires within the supplemental material, along with an outline activity that could be modified by the individual instructor according to his or her preferences about which themes were most important to emphasize. We suggest that instructors have students plot the data themselves and encourage them to draw their own conclusions prior to discussion of the data within the lectures. Through this exercise, students are engaging in active learning techniques even if they cannot directly participate in the data collection and research. In future classes, we also expect to carry out this exercise within the discussion section of EARTH 284 to increase the development of critical thinking and analytical skills in nonscience majors.

CONCLUSIONS

The feedback questionnaires given after the project was completed indicated that the majority of students in the large enrollment EARTH 284 class had learned enough to share information about sustainability with their friends and family, which demonstrates that this class project was successful at increasing science literacy among nonscience majors. Feedback from students in both the large-enrollment introductory class and the small-enrollment upper level class were overwhelmingly positive, though feedback was higher in the introductory level class than the upper level class. This feedback shows that this activity is an effective way to incorporate active learning techniques to engage students of all levels, nonscience and science concentrators alike, but may be more useful for engaging students at the introductory level. Although there is not yet enough data to be conclusive, exam results to this point also suggest that student performance is enhanced through the active learning activity, both relative to other questions on the same exam and relative to previous years' results.

From the feedback questionnaires, students also indicated that they were now more aware of the sources of their food and sustainable and unsustainable agricultural practices in the United States. The students who actively tried to avoid corn products were isotopically different from those who did not, showing that their dietary choices can be seen in the isotopic composition of the hair. The students also indicated that project was a useful way to show the influences of their diet on the environment, and that this exercise might influence their food choices in the future, demonstrating that this class project may increase awareness of sustainability issues in the United States.

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