RESEARCH ON LEARNING

Teaching Nutrient Cycling and Climate Change Concepts Using Excretion Experiments with Common Fish

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Abstract

Many high school students learn about nutrient cycling during biology, environmental science, and agriculture classes. These lessons often focus on soil and plants, and nutrient cycling is usually taught independently from climate change. Scientists know that animals, including fish, can have strong effects on nutrient cycling (i.e., nitrogen and phosphorus) in ecosystems. Additionally, research has shown that nitrogen and phosphorus excretion rates of animals increase with water temperatures. We worked with high school students to design and conduct nutrient excretion experiments using common fish (zebrafish) to explore the impact of climate change on nutrient cycling. This allowed students to have hands-on laboratory experience. In 2021, we worked with students participating in a residential summer program in Georgia. Meanwhile, in 2022, students enrolled in the local high school visited the university campus on two occasions to participate in the experiments, and we once again worked with students in Georgia. Students from all three groups showed an increased understanding of the role of animals in nutrient cycling and ways climate change may impact these processes, despite variable results from the excretion experiments. Students also showed increased understanding of science processes and were more likely to feel like part of the science community. We believe that these experiments can be done in high school classrooms to expand students' understanding of the scientific process, nutrient cycling, and climate change.

Key Words: *nutrient cycling; inquiry; climate change.*

○ Introduction

Nutrient cycling is included within the Next Generation Science Standards (NGSS) Life Science and Earth & Space Science disciplinary core ideas. NGSS expects middle and high school teachers to cover carbon cycling due to NGSS's emphasis on global climate change, and nitrogen cycling is included within high school classrooms in HS-LS2-4 (Achieve Inc., 2013). NGSS provides teachers considerable flexibility when teaching disciplinary ideas to make the content relatable to their location; thus, teachers may also incorporate the phosphorus cycle, but this is not explicitly required. Additionally, inconsistencies in teaching occur due to the lack of aligned resources and low teaching confidence in integrating the science practices (Achieve Inc., 2018; Fulmer et al., 2018; Pruitt, 2014). Advanced placement (AP) biology and environmental science courses also include science practices similar to those found in the NGSS standards, and these courses also teach nitrogen and phosphorus cycling (College Board, 2020a, 2020b). Despite being part of the expected curriculum, research shows that there's still a lack of understanding in high school classrooms about nutrient cycles (Faujiyati et al., 2021; O'Connell, 2010). In particular, studies have found that high school textbooks emphasize the role of soil in nutrient cycling, leading to student misconceptions about the role of the atmosphere and living organisms (O'Connell, 2010). Of course, textbooks such as Miller and Levine (2014), Miller and Spoolman (2017), and Friedland and Relyea (2015), which are frequently used in high school classrooms, emphasize nutrient cycling in terrestrial environments within the included figures and diagrams although the AP textbooks (e.g., Friedland & Relyea, 2015) do a better job explaining these cycles in aquatic environments.

Nutrients, including nitrogen and phosphorus, are important for living things as they are key components to amino acids, DNA, and ATP (Sterner & Elser, 2003). These nutrients are also important within an environmental context, especially for primary producers; human activities add large quantities of nitrogen and phosphorus to the biosphere through the addition of fertilizers to farmland and the burning of fossil fuels. Scientists recognize that nutrient cycling is an important ecosystem service as the nutrients undergo natural processes (e.g., nitrogen fixation) allowing them to be available for other biological purposes. Animals use nutrients for the development of body tissues and growth, and they release excess nutrients via excretion and egestion (i.e., urine and feces). Research shows that the nutrients released from animals via excretion and decomposition are important source of nutrients in ecosystems as they excrete nutrients, and as their bodies decompose (Frauendorf et al., 2021). While many factors of individuals and ecosystems influence the role of nutrients from animals, as with other biological rates, there is a positive relationship between temperature and excretion rates. Thus, scientists expect that nutrients released by individual animals will continue to increase under climate change scenarios (Atkinson et al., 2017).

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NGSS not only emphasize the traditional knowledge through "disciplinary core ideas" but they also include "Science and Engineering Practices." The Science and Engineering Practices aim to address the historic lack of lab sciences in K–12 schools; therefore, this part of the framework focuses on inquiry process and the associated skills that students develop allowing them to better understand how science actually work (National Research Council, 2012; Rutherford & Ahlgren, 1989). Despite the emphasis on science practices in both NGSS and AP standards, research suggests that K–12 students get few actual inquiry experiences (Pruitt, 2014). In particular, students get few opportunities to plan and carry out investigations and make sense of empirical data, and they often struggle with experimental design (Manz et al., 2020; Wyeth & Wonham, 2018).

While students generally do not acquire a strong understanding of biogeochemical cycles and lack true science inquiry experiences, we know that it will be important for them to understand natural processes when solving environmental problems (Trevors & Saier, 2010). Our goal was to assess the impact of conducting excretion experiments, using fish, on high schoolers' understanding of the role of animals in nutrient cycling, as well as the impact of climate change on nutrient cycling. Thus, we worked with three groups of high school students during 2021 and 2022 who completed pre-surveys, conducted excretion experiments with common aquarium fish over one week, and completed post-surveys. We predicted that students would have a stronger understanding and be able to better explain the role of animals in nutrient cycling after participation in the study. We also predicted that students would display increased self-efficacy in implementing scientific investigations, conducting research, and communicating results due to their involvement in the lab investigation. Alternatively, we realized that students may overestimate their knowledge about nutrient cycles on the pre-survey due to the simplistic way in which this information is often presented. If this occurred, no increased understanding of this information would be observed in the quantitative data. Furthermore, students may opt not to respond to written questions, which would provide limited information on their understanding.

○ Methods

Group Descriptions and Demographics

Georgia

In Georgia, the participants were students enrolled in a selective summer program for rising 11th and 12th graders. To be admitted in the program, students are nominated by a teacher for a core area, and they go through a competitive application and interview processes at both the district and state levels. Ultimately, approximately 20% of the applicants are invited to attend the program each summer. There is no mandated curriculum at the summer program, and instructors are charged with creating academic experiences that are significantly different than the typical high school classroom. One of us (C.A.S.) was one of the biology instructors at this program in both summers (2021 and 2022).

During the summer of 2021, there were 24 students selected for the summer program's biology class. These students came from 23 public schools and 1 private school, and they represented 14 different school districts. Of these students, 63% of the class identified as female while the rest identified as male, and 54% of the students in this class identified as Asian while 33% identified as White. Additionally, 88% of these students were rising seniors, and 18 students had taken either AP or International Baccalaureate biology. In the summer of 2022, there were 30 students selected for the program's biology class, and 25 students participated in the surveys. The students came from 25 different public schools in 19 school districts. Of these students, 56% identified as female, and 52% identified as White while 24% identified as Asian. In this group, 55% were rising seniors, and 14 students had taken either AP or IB Biology. Two students also noted that they had taken a dual enrollment biology course.

Ohio

In Ohio, we worked with a comprehensive high school that serves students in our college town and the mostly rural surrounding areas. Students in the environmental science class were selected for participation, after discussions with teachers of that class. This was done as the project objectives aligned with this course curriculum and scheduling around other school events was possible. Thus, students from this course visited our research lab on two dates, one week apart. Each time, students came in two groups, one in the morning and one in the afternoon, so there were not too many people in the lab at once. There were 26 students in the environmental science class who completed one of the surveys, and half of these students were seniors. Of these students, half identified as female, and 81% identified as White. Only one student in this course had taken either AP or IB biology. Only 12 of the students in this class completed both the pre- and post-surveys due to absences on the dates that the surveys were completed; only the students who completed both the pre- and post-surveys were included in the statistical analyses/*t*-test.

○ Experimental Protocol

There were three main phases to this project: pre-surveys, experiments, and post-surveys. The surveys included three sections: demographic information, science content knowledge, and science skills/practices. In the section on demographics, students provided information about their grade, gender, ethnicity, and high school; they also provided a list of the science classes they had completed. In the section on science content, students were asked "yes"/"no" questions about if science concepts have been covered in their science courses. Students were asked to rank their familiarity with different content topics on a 1–5 scale with 1 being "not familiar" and 5 being "very familiar." If the student selected "no," the survey advanced to the next science concept, and their familiarity with the topic was recorded as a 0. If students selected "yes," further questions (including free response questions) were asked to evaluate their understanding of these concepts. The last section of the survey included over 30 questions that asked students about their comfortableness with various science skills and practices. These questions were on a 1–5 Likert scale, and many of these questions were sourced from the Student Assessment of Learning Goals instrument, which has been widely used across science courses to assess student learning (Seymour et al., 2000).

The nutrient cycling experiments conducted by students involved measuring how much nitrogen and phosphorus fish excrete at different temperatures. To prepare students for the excretion experiment, the teacher started by facilitating a brief overview of both the nitrogen and phosphorus cycles. This overview included reviews of images of the cycles, discussion of animal contributions to nutrient cycling, and introduction to several experimental approaches (i.e., full lake studies, mesocosms, lab studies, etc.) that are used for aquatic studies. Students were then presented with the question of "How does temperature impact nutrient cycling from fish?" and they worked in groups to design an experiment that would address this question. Using large chart paper, students created a visual of their experimental design including sample numbers, a list of needed supplies, and a description of their variables (independent, dependent, and controls). All of the groups presented their experimental design to the class.

At this point, the teacher facilitated a class discussion exploring the various experimental designs. Students considered the strengths and weaknesses of the submitted designs as well as their ability to answer the guiding question. This resulted in student questions on ethical use of some animals, collection and measuring techniques, and concerns about animal viability in certain temperatures, which were addressed. Students then selected the best-constructed experimental design that could also be altered to fit the time constraints of our interaction and available supplies. Doing this, brought students to a consensus on a warming experiment conducted within a lab setting over a weeklong period with pet-store fish.

Students then helped set up tanks at three different temperatures (approximately 21, 23, and 25°C) and placed 12 zebrafish (*Danio rerio*) in each tank, so they could acclimate for one week. In Ohio, students used traditional fish aquaria, while in Georgia, the students used large plastic tubs as these were readily available in the classroom setting. Daily, during the acclimation period, the tanks were cleaned, and the fish were fed.

The following week, students conducted the experiment, using a method routinely used by scientists who study animal-mediated nutrient cycling (Downs et al., 2016). To measure excretion rates, the fish were transferred to individual, snack-sized (16.5 cm × 8.2 cm) Ziploc bags with 100 mL of filtered water. The Ziploc bags were placed upright in coolers and plastic containers within the classroom, so the water would not spill. The fish were left in the bags for ~45 minutes, removed and weighed using a classroom scale, and then returned to their initial tanks. The nutrients excreted by the fish in the Ziploc bags were analyzed using Hach kits (TNT 829 for Ammonia and TNT 843 for Phosphorus). The phosphorus Hach kits were spiked using potassium phosphate monobasic to ensure that the phosphorus levels would be above the detection limit; this amount was subtracted from the final amounts. Students followed the directions from the Hach kits for analysis, and the samples were assessed with a spectrophotometer. In 2021, students used Ocean Optics USB-650 spectrophotometers, while Hach DR2800 spectrophotometers were used during 2022. We also measured ammonia and phosphorus on water samples before fish were added, and excretion rate was calculated as the difference in N or P mass from before fish were added and after they excreted for 45 minutes. Students recorded their data in a spreadsheet template, and data were pooled across the class. Once excretion experiments had been conducted on all fish, the students were able to discuss trends in the data.

After students had completed the post-survey, we used the unique identifiers to pair pre- and post-data from the students. Paired *t*-tests were conducted on students' content knowledge and science skills to assess the changes in responses between the pre- and post-surveys.

○ Results and Discussion

Excretion Experiments

In the experiment conducted by Georgia students in 2021, a warming effect can be seen as both phosphorus (P) and nitrogen (N) excretion rates increase with temperature. However, the P excretion rates collected by this group were much higher than expected (Figure 1). The fish excretion data collected by the students in Georgia during 2022 was highest at the medium temperatures; yet the N excretion rates were lower than what would be expected (Figure 1). In Ohio, the N excretion rates collected by the students in the morning were higher with increased temperatures, and the highest nitrogen excretion was at the medium temperature. P excretion rates were highest at the high temperature, but the medium temperature had lower excretion than the low temperature (Figure 1). In the afternoon group, trends in N excretion were similar to those in the morning, while P excretion increased steadily with temperature. Due to the variability in data collected by the two groups, the pooled data from Ohio students did not show clear trends with warming (Figure 1).

Student Content Knowledge

In Georgia in 2021, 83% of students indicated that they had studied the nitrogen cycle before being involved in these experiments, and 21% shared that they had previously studied the nitrogen cycle in aquatic environments; 58% of students shared that they had previously studied the phosphorus cycle at school, and 29% noted that they had studied the phosphorus cycle in aquatic environments; 54% of students indicated that they had learned about the role of consumers (animals) in nutrient cycling; 83% of students noted that they had learned about climate change, but only 21% students had learned about climate change impacts on aquatic environments. In Georgia in 2022, 76% of students had previously learned about the nitrogen cycle, and 32% had learned about nitrogen cycling in aquatic environments. Meanwhile, 44% of these students indicated that they had studied the phosphorus cycle, and 24% had learned about this within aquatic environments. Similar to the year before, 52% of students had studied the role of consumers in nutrient cycling, 84% had studied climate change, and 20% had studied climate change impacts on aquatic environments. In Ohio in 2022, 67% of students shared that they had previously studied the nitrogen cycle and 33% had student this cycle within aquatic environments. Only 33% of students had learned about the phosphorus cycle, but 25% had also learned about it in aquatic environments; 50% of students indicated that they had studied the role of consumers in nutrient cycling; 83% shared that they had studied climate change, and 33% indicated learning about how climate change will affect aquatic ecosystems (Table 1).

Student understanding of all of these concepts increased (Figure 2). At the end of the experiment, students best understood the role of consumers in nutrient cycling while improvements to their understanding of phosphorus cycling were generally largest. All learning improvements were found to be significant based on paired *t*-tests (Table 2).

Within the pre- and post-surveys, students also had the opportunity to respond to open-ended questions. In Georgia in 2021 and 2022, twelve students wrote responses to these pre-survey questions, while three students in Ohio responded to these open-ended questions. In the pre-survey responses, students were more likely to write about the importance of nutrients for the soil or share that they had been taught about nutrient cycling but didn't remember

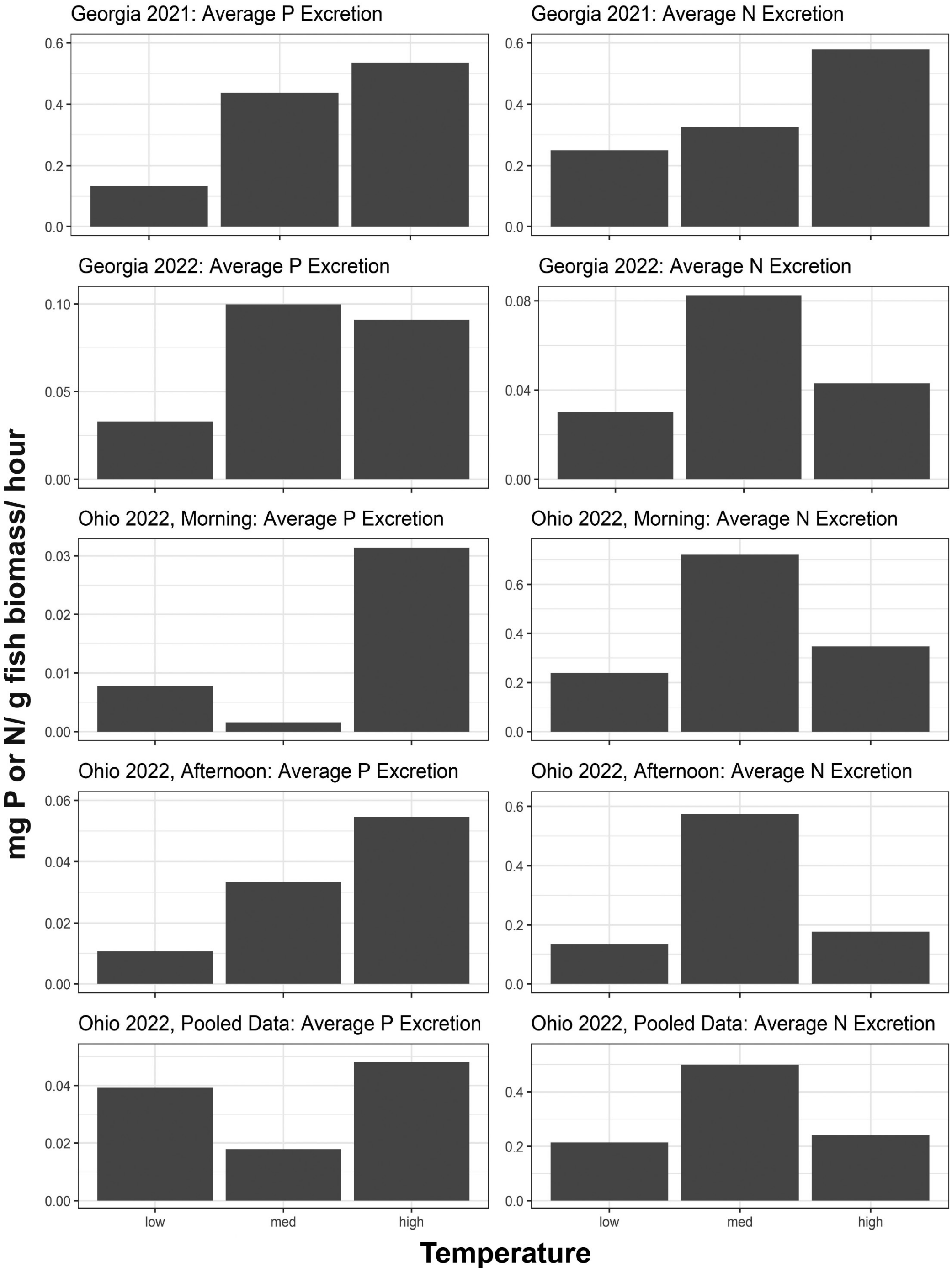

Figure 1. Average nitrogen and phosphorus excretion of the fish at each temperature from each student group.

Table 1. Percentage of students who indicated that they studied the topics in the pre-survey. Specifically, the survey asked if students had previously learned about nitrogen cycle (in general), the nitrogen cycle in aquatic environments, the phosphorus cycle (in general), the phosphorus cycle in aquatic environments, the role of consumers (animals) in nutrient cycling, the impacts of climate change, and the impacts of climate change on aquatic environments.

	N Cycle	N Cycle Aquatic	P Cycle	P Cycle Aquatic	Consumers	Climate Change	Climate Change Aquatic
Georgia 2021	83	21	58	29	54	83	21
Georgia 2022	76	32	44	24	52	84	20
Ohio 2022	67	33	33	25	50	83	33

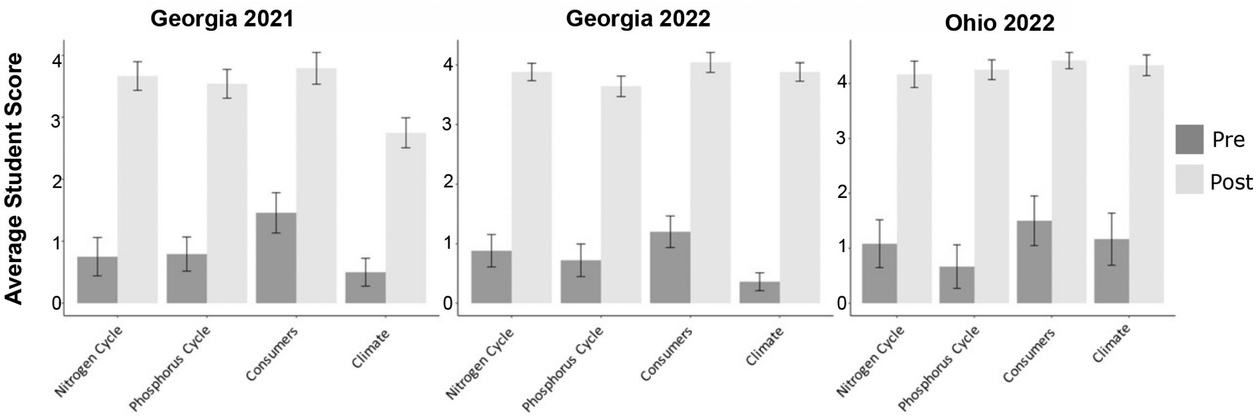

Figure 2. Bar plots showing the average student familiarity with content topics (nitrogen cycle, phosphorus cycle, impacts of consumers in nutrient cycling, and impact of climate change on nutrient cycling) in pre- and post-surveys for each group.

Table 2. Paired *t*-test results (*P* values). The paired *t*-test was conducted on a survey question in which students rated their understanding on a 0–5 scale on the following content topics: the nitrogen cycle, the phosphorus cycle, impacts of consumers on nutrient cycling, and the impact of climate change on nutrient cycling. All *P* values <0.001, and they show very strong evidence for student learning.

	Nitrogen	Phosphorus	Consumers	Climate
Georgia 2021	1.27E-08	1.22E-08	1.27E-06	8.92E-09
Georgia 2022	1.00E-10	8.08E-10	1.04E-10	1.36E-15
Ohio 2022	3.93E-05	7.06E-06	6.42E-05	0.000123

Table 3. The five science skills and practices in which students showed the most growth practices for each student group. The areas that were common between at least two student groups are noted in italics, and the values shown represent the increase in average response between the pre- and post-surveys.

Georgia 2021	Ohio 2022	Georgia 2022
Understanding science journal articles (+0.79)	*Making oral presentations about science research (+0.77)*	*Feeling like part of the scientific community (+0.76)*
Making oral presentations about science research (+0.76)	*Interacting with scientists from outside your school (+0.68)*	*Figuring out the next step in the research process (+0.64)*
Figuring out the next step in the research process (+0.55)	Doing well in future science courses (+0.63)	*Interacting with scientists outside your school (+0.52)*
Writing scientific papers and reports (+0.53)	Understanding concepts guiding research (+0.60)	Contributing to science (+0.60)
Interacting with scientists outside your school (+0.47)	*Feeling like part of the scientific community (+0.53)*	Defending an argument when asked questions (+0.52)

the content. In all three groups, over 90% of the students completed the open-ended questions in the post-survey, and these student responses demonstrated a stronger understanding of nutrient cycling, the role of consumers, and the impacts of climate change (available in the Supplemental Table S1 online).

Science Skills and Self-Efficacy

The results from the survey questions asking about students' science skills and self-efficacy included considerable variation (available in the Supplemental Table S2 online). However, students from each group exhibited growth in similar areas from participating in the excretion experiments. When considering the top five areas of growth for each group, there were three skills that showed significant growth among two student groups. The students in Georgia in 2021 and the students in Ohio both made positive gains in their confidence to make oral presentations about science research. Meanwhile, the students in Georgia in 2021 and 2022 both made positive gains in their confidence to figure out the next step in a research process and feel like part of the scientific community. Students in all three groups made significant improvements in their ability to feel confident interacting with scientists from outside their school (Table 3).

○ Limitations

There are several limitations that must be considered with this study. First, many students lacked basic science skills, perhaps due to online learning during the pandemic. Some contamination was observed within and between samples. While this may have limited the quality of the excretion data, this could easily be avoided with further practice and instruction. The lack of student experience with science practices also suggests that it is even more important to engage students in inquiry activities post-pandemic. Although the goal was to provide a learning experience for the students, and not to collect research-quality data, high variability in the data could cause students to rate their learning experience lower than if data showed clear trends.

Additionally, there were numerous absences among the local Ohio students, which caused our student sample size to be much smaller than in the Georgia groups. However, we acknowledge that variable attendance is a typical factor that teachers regularly face and cannot control. Furthermore, content knowledge was not assessed by the researcher beyond student perception; thus, we do not know if student's ability to apply this knowledge to scenarios improved. Also, there were some science skills and efficacy questions that students rated lower on the post-survey than the pre-survey. It is suspected that students may have overestimated some of their abilities, especially when considering open-ended tasks (Clauss & Geedey, 2010). Additionally, the students in Georgia participated in additional activities beyond the experiment with the researcher. This may have influenced some of the improvements for the science skills and attitudes beyond the realm of the experiment.

In terms of the excretion experiment, there is a balance between time and water volume, and this is well-known from excretion literature (Whiles et al., 2009). When fish are placed in smaller volumes of water, this can be stressful, but the incubation time needed for detectable nutrients is much shorter. Meanwhile, fish can be placed in a larger volume of water, but they will need to be left for a longer incubation time for the excretion to be detectable. This has no drawbacks for the fish, but this can present logistical problems when scheduling for student class times. Considering these factors led to our decision to place fish in 250 mL of water for 45 minutes.

○ Future Recommendations

Based on the successes and challenges faced during this project, we also provide recommendations for teachers working to implement these experiments in the future. First, one challenge that was encountered across all groups was the lack of basic lab skills including knowledge on how to use micropipettes and collect data in Excel. When students were provided with a bit of dedicated time to practice these essential lab skills, the excretion experiments ran much smoother. If conducting this as part of a high school course, we recognize that there are also time and budget constraints. Due to the nature of these experiments, students working in groups of up to four individuals will have ample opportunities to participate and develop lab skills. Student groups may collect data for a particular nutrient (nitrogen or phosphorus), or the teacher may decide to focus solely on nitrogen, which generally provided cleaner data in our experiment. Furthermore, the excretion data collected by students could be pooled within a class or across classes. Finally, we recognize that there are ethical considerations when using vertebrate animals, and the experiment could be conducted with another species such as snails; invertebrates also tolerate a wider range of temperatures which could help students obtain results with clearer temperature trends.

Meanwhile, establishing a partnership between a school district and a local college would provide additional opportunities in terms of both lab resources and interactions. For instance, students in our Ohio experiment who came to the university campus were more likely to feel like part of a scientific community. This collaboration also allowed for the use of more precise spectrophotometers than what would typically be available in a high school laboratory. If planning such a partnership, we recommend beginning the planning process early to allow for scheduling and paperwork, which can be considerable and delay implementation of the project. This collaboration may require some innovation in terms of transportation and space usage (especially when university research labs are smaller than typical classrooms). For instance, in our experience, we worked with high school students in the university lab in groups of 15 students to ensure safety.

○ Conclusion

We compared pre- and post-surveys data to assess student understanding of nutrient cycling concepts as well as science skills. Across all three groups of students, there were significant improvements in student understanding of the science concepts, and the lab investigation proved to be beneficial to the development of student identity and science skills, even though excretion rates were often very variable and did not conform to predictions in terms of temperature effects. Since students typically lack understanding of how consumers influence nutrient cycling, we recommend that teachers integrate experiments with consumers into their courses. The results indicate that even with imperfect data, students will gain an increased understanding of the role of consumers and will also be able to connect this ecological concept with climate change. As directed by the NGSS, it is becoming increasingly important for students to understand impacts of climate change, and students need to develop the skills necessary to confidently design and conduct experiments and disseminate results to peers (Achieve Inc., 2018; Pruitt, 2014).

With the NGSS expecting educators to shift from having students being knowers of science to doers of science, excretion experiments provide opportunities for students to build epistemic agency. Students in all three groups were able to build science knowledge while also utilizing prior knowledge, and they developed science skills in ways that were meaningful and integrated with disciplinary knowledge. Furthermore, our results support the idea that students can have epistemic agency and build science knowledge even with they do not seem to be "ready" in terms of the expected prior knowledge (Miller et al., 2018). We acknowledge that allowing students to build epistemic agency is difficult as this requires changes in classrooms that challenge many of the historic educational norms (Achieve Inc., 2018; Miller et al., 2018; Pruitt, 2014). While the excretion results did not always conform to expectations based on literature, our data on student learning suggests that these experiences are still beneficial to student learning related to both disciplinary core ideas and scientific practices.

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