International Journal of Education and Science Research Review ISSN 2348-6457
www.ijesrr.org Volume-4, Issue-2 April- 2017 Email- editor@ijesrr.org

ALTERED DEVELOPMENT IN APTEROUS DROSOPHILA: A PROJECT-BASED LEARNING MODULE

[1*]Ronald H. Blackmon, [2]Stephania T. Perry, [1]Moses A. McDaniel, [1]Gary L. Harmon
[1]H.G. Cooke Department of Natural Sciences, Pharmacy and Health Professions
Elizabeth City State University, Elizabeth City, NC 27909
[2]Bertie High School, Windsor, NC 27983

ABSTRACT:A major challenge in teaching North Carolina Essential Standards for Biology to high school students is that many of the processes or concepts cannot be seen, which makes mastery of the material difficult. However, students can acquire and apply new knowledge when teachers design and implement laboratory activities that emulate the processes or concepts students need to learn while capturing students' interest. The purpose of this investigation is to explore the effects of simulated acid rain on a strain of *Drosophila melanogaster*(the common fruit fly) as a collaborative study with student-scientists. There is strong evidence that acid rain damages forests, vegetable crops, buildings, aquatic organisms and even human health. This study reports an intervention aimed at helping students learn the processes of science by investigating a real life problem of environmental impacts on development in a living organism. A comparison of the pre-test and post-test data suggests that this intervention can enhance student learning and achievement.
.

KEYWORDS: Project-Based Learning, Acid Rain, Drosophila, Student-Scientists, Development

INTRODUCTION:

Acid rain, an environmental degradation factor, is precipitation produced by burning fuels such as coal and oil that emit sulfur dioxide (SO_2) and nitrogen oxides (NO_X) into the atmosphere. The SO_2 and NO_X react with water, oxygen and other chemicals to form sulfuric and nitric acids (United States Environmental Protection Agency, n.d.) As acidic rain flows it leaches aluminum and other minerals from soil depositing it in rivers, lakes, and streams. The leaching also deprives vegetation of key minerals. SO_2 and NO_X can also react in the atmosphere to form fine sulfate and nitrate particles that people can inhale into their lungs creating respiratory problems. To combat the problem, the U.S. Congress imposed strict emission regulations on industry in 1970 through the Clean Air Act, which was strengthened in 1990. By 2003, sulfur dioxide raining down on the northeastern United States had decreased by as much as 40%, but there are subtle improvements. Studies of soils in New Hampshire and Maine suggest acid rain is still impacting regions of the U.S. northeast (Driscoll *et al*., 2001). The effect of acid rain on the development of certain organisms represents an area of continued inquiry to elucidate potential deleterious effects.

RATIONALE FOR THE STUDY

This study used a developmental model system based on the life cycle of the "apterous" strain of *Drosophila melanogaster* (the common fruit fly). This organism develops from egg to adult fly in less than two weeks and thereby represents a practical model system. Science educators have ongoing interests in enhancing students' grasp of the process of science. Using a project-based learning approach, this investigation was designed to involve student-scientists. Following an explanation of the problem and discussion of the experimental design with the science teacher, students were involved in the generation of data and the execution of experimental procedures. Evaluation of the assessment results (pre- and post-test comparison) should provide insight into the value of this approach as an effective science teaching intervention.

This laboratory investigation explores the impact of simulated acid rain on the development of an apterousstrainof*Drosophila melanogaster* in collaboration with student-scientists. The experimental organism chosen for this project was a mutant of the common fruit fly. *Drosophila melanogaster* were used as subjects for this laboratory project because of their quick development, their entire genome has been sequenced, and the similarities they show to human genetics. Analysis of the *Drosophila* genome shows it shares about 60% of its DNA with humans. The life cycle from egg to adult takes about 10 days.

The apterous (ap) mutant of *Drosophila melanogaster* was first found by Miss Edith M. Wallace in 1913 while working in the laboratory of Dr. T. H. Morgan (Metz, 1914). This mutant, 'which occurred spontaneously, was shown by C. W. Metz to be recessive and located on the second chromosome near the black (*b*) locus (Butterworth and King, 1965).

The mutant apterous*Drosophila* were selected for use in this laboratory investigation since their wingless phenotype made it easy for the student-scientists to handle (Fig. 1).

Fig.1. Wild type *D. melanogaster* [left] vs. apterousmutant of *D. melanogaster* [right].

Of the several apterous mutants found in *Drosophila*, flies of the genotype ap^{56f}/ap^{56f} that were chosen for this study have the greatest pre-adult viability. In addition, they are relatively long-lived (half-life of population >15 days) and have normal fertility (Butterworth and King, 1965; Greenspan, 1997).

PROJECT-BASED LEARNING

Learning science is something that students do, not something that is done to them. More jobs demand advanced skills, requiring that people be able to learn, reason, think creatively, make decisions, and solve problems. An understanding of science and the processes of science contributes in an essential way to these skills (National Science Education Standards, 1996). A major challenge in teaching North Carolina Essential Standards for Biology to high school students is that many of the processes or concepts cannot be seen, which makes mastery of the material difficult for students. However, the approach of teaching using collaboration enables teachers to design and implement laboratory activities that emulate the processes or concepts students have to learn while capturing students' interest and provoking serious thinking as the students acquire and apply new knowledge. Collaborative learning is an effective instructional strategy that teaches these skills. Lev Vygotsky (1962), a Russian psychologist, developed the concept of zone of proximal development. Vygotsky identified the importance of learning through communication and interactions with others to build critical thinking skills. The learner due to the knowledge, experience, and different thought processes brought about by the diversity of the group is able to perform at a higher intellectual level while retaining information longer those who work independently on tasks. Research has shown that when students work collaboratively, they are more willing to take on difficult tasks, listen to other students' ideas, and celebrate a peer's success and growth. Collaborative learning can help boost students' learning in all content areas, including social studies, science, reading, and mathematics. Problem-based-learning (PBL) is a collaborative technique that leads to deeper learning and understanding and allow students to build upon prior knowledge. The core idea of project-based learning is that real-world problems capture students' interest and provoke serious thinking as the students acquire and apply new knowledge in a problem-solving context. The teacher plays the role of facilitator, working with students to frame worthwhile questions, structuring meaningful tasks, coaching both knowledge development and social skills, and carefully assessing what students have learned from the experience. Advocates assert that project-based learning helps prepare students for the thinking and collaboration skills required in the workplace (David, 2008).

Furthermore, students in classrooms that incorporate PBL perform at least as well on standardized tests as their peers in traditional classrooms (Thomas, 2000). Walker and Leary (2009) arrive at a similar conclusion, noting that "even when the scope is limited to standardized tests of concepts, PBL is able to hold its own in comparison to lecture-based approaches." Additionally, PBL seems to facilitate success for students who have trouble learning in the traditional classroom (Barron and Darling-Hammond, 2008). PBL is also an effective tool for imparting essential non-academic 21st-century skills, including collaboration, critical thinking, and communication. Traditional educational methods that rely on rote memorization do not develop these vital skills (Barron and Darling-Hammond, 2008). In contrast, PBL has been demonstrated to improve students' ability to reason and argue clearly (Stepien *et al*., 1993), to answer conceptual problems (Boaler, 1997), and to hypothesize accurately (Purkeyand Schmidt 1996). More specifically, Cornell and Clarke (1999) as well as Wurdinger *et al*. (2007) found that project-based learning increased higher and lower performing pupils' engagement in learning activities. The first writers also reported that project-based learning not only gave all of the students an opportunity to work with each other while doing hands-on activities and discover unique skills necessary to complete projects, but also allowed the lower performing pupils to progress at their own pace. In addition, other researchers (Barron *et al*., 1998; Liu and Hsiao, 2002) discovered that academic performance; cognitive strategy use and motivation towards learning are improved when using project-based learning with low, average and high ability middle school students.

Project-based learning activities are a great way to build critical thinking and collaboration skills among students, but teachers also have to ensure that students are learning. All teachers want to see their students succeed. Atthe start of a PBL assignment, teachers should provide students with clear and challenging criteria or guidelines for success, using rubrics and examples that demonstrate intended learning outcomes from local professionals or former students (Ertmer and Simons, 2005; Barron and Darling-Hammond, 2008). This structure will provide students with clear criteria for success. The teacher also needs to track the students' progress throughout the PBL. Tracking student progress will allow teachers develop action plans that will aid in student success. Student data is a way to investigate the many questions about students, teaching practices, and learning that arise for any committed teacher. Furthermore, the data connects the teacher to the students and their learning, pushes the teacher to higher levels of reflection of their practice, and gives rise to dialogue with colleagues, students, and parents (Morrison, 2009).

STATEMENT OF THE PROBLEM

The effects of simulated acid rain on the development of apterous*Drosophila melanogaster* will be investigated with the assistance of high school students from Bertie High School in northeastern North Carolina. It is hypothesized that active participation in a real world research problem will positively impact their scientific knowledge base and their understanding of the scientific process.

MATERIALS AND METHODS

Chemical Reagents

All chemical reagents were purchased from Sigma Chemical Co., St. Louis, MO unless otherwise noted.

MAINTENANCE OF *DROSOPHILA MELANOGASTER (APTEROUS STRAIN)*

Flies used in this study (apterous strain of *Drosophila melanogaster*; genotype ap^{56f}/ap^{56f}) were purchased from Carolina Biological Supply Co., Burlington, NC. Flies were cultured on Formula 4-24® Instant Drosophila Medium (Carolina Biological Supply Co) which was mixed in a 1:1 ratio of Formula 4-24® to dH_2O (15 ml medium to 15 ml dH_2O per vial). Standard culture required new vials were set up every three weeks. The course instructor maintained the cultures and increased the number of vials (flies) to accommodate the number needed for the groups of participating students.

Student-scientists were divided into groups of four students for a total of twelve groups. Guidelines (see student instructions and data sheets in Appendix A) were given and explained to the student-scientists to help them begin their investigation. For each group, a "control" vial of Formula 4-24® with dH_2O was prepared. Each group also prepared an "experimental" vial of Formula 4-24® with 2% acetic acid (pH 4.0--simulated acid rain) instead of dH_2O. A concentrated stock solution of yeast (*Saccharomyces cerevisiae*) was prepared by the course instructor and dispensed five drops per vial. Vials were labeled by the students with the following: date, group number and either control or experimental as appropriate. After two minutes, student-scientists added ten female flies and one male to each vial.

ASSESSMENT

Student achievement was evaluated using the following pre- and post-test (see Appendix B). The tests were designed to cover topics and subject areas used to prepare students for the end-of-course test.

DATA ANALYSIS

Statistical analysis was performed using GraphPad QuickCalcs® software. The statistical significance of any differences in mean larval, pupal, adult counts and adult weights under control versus experimental conditions was determined usingStudent's *t-test.* P values less than 0.05 were considered statistically significant.

RESULTS

Under the guidance of the course instructor, students completed data collection with the following results. Data has been summarized and includes results from all twelve groups of student-scientists.

Ten of the 12 student groups reported more larvae in the control vials than in the experimental vials in which simulated acid rain exposure occurred. In two groups, there were more flies in the experimental vials than in the control vials. In one group, there were no larvae in the control or the experimental vials on day 10.

The mean number of larvae from the 12 student groups was lower ($p<0.05$) in the experimental group than the number in the control vials (Fig. 1).

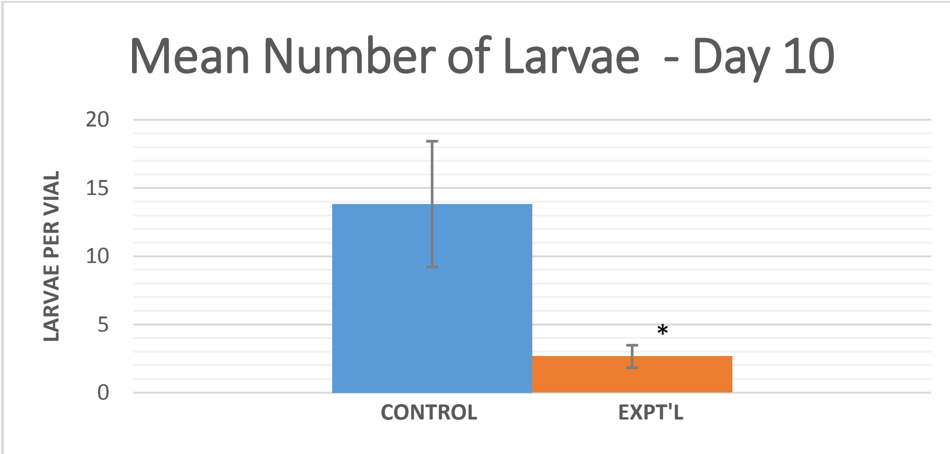


Figure 1: The mean number of larvae across all groups for control and experimental vials on day 10. [* = $p<0.05$]

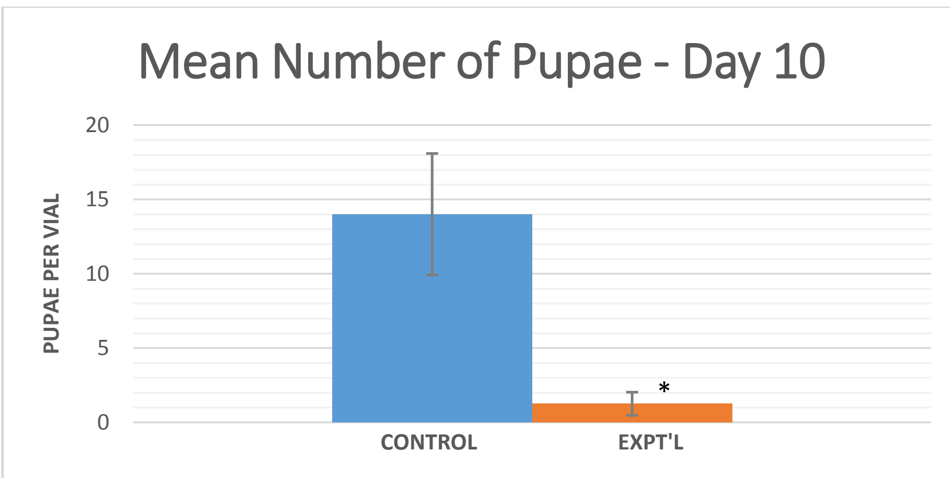


Figure 2: The mean number of pupae across all groups for control and experimental vials on day 10. [* = $p<0.05$]

The mean number of pupae across all groups was also significantly higher in the experimental vials than in the control vials. However, one group had no visible pupae on day 10 (Fig. 2).

The mean number of adult flies that eclosed from the 12 student groups was significantly lower ($p<0.05$) in the experimental group than the number in the control vials (Fig. 3).

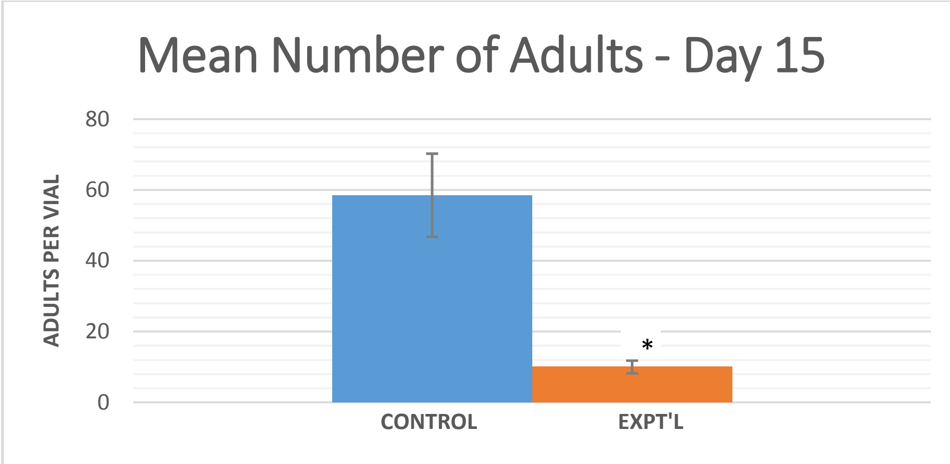


Figure 3: The mean number of adult flies across all groups for control and experimental vials on day 15.
[* = p<0.05]

While one group had no detectable larvae or pupae on day 10, there were 6 or fewer adults in the vials of that group on day 15. It is likely that the small number of larvae and pupae developed in the Drosophila medium and were not visible on the sides of the vials above the medium and thus not counted by the students. However, once eclosion occurred, the resulting adult flies were detected and counted.

Student-scientists also collected data on the average weight per fly in milligrams. As shown in Figure 4, the average weight of adult flies in the experimental group was significantly lower than the weight of the flies in the control group.

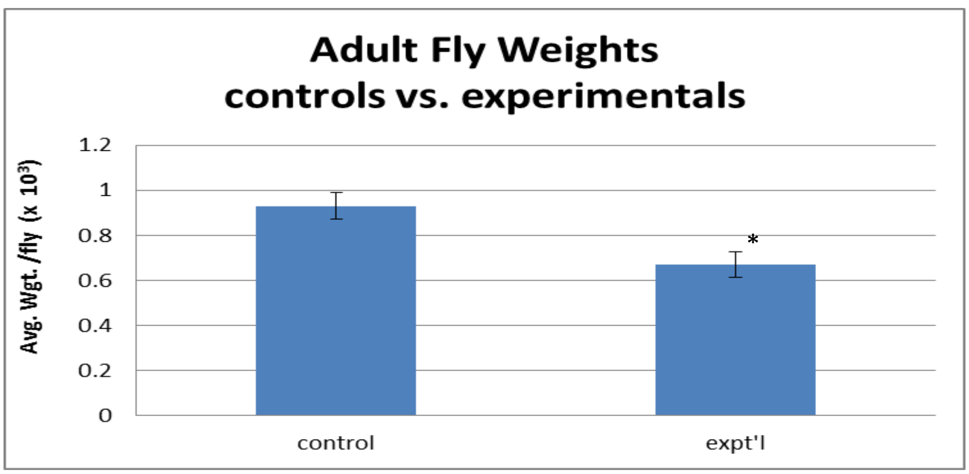


Figure 4: Comparison of the weights of the adult flies. The first bar is the mean weight (in milligrams) of the control stock (±SE) and the second is the mean weight (in milligrams) of the experimental stock (±SE).
[* = p<0.05]

The assessment of student scores on the pre-test versus the post-test indicates that improvement was achieved in the students' ability to answer test questions correctly (Fig. 5). As a consequence, the grade distribution changed such that fewer students scored lower grades after the project-based intervention. In addition, more students scored higher grades after the intervention and, in some cases even higher than the highest grades earned by students who took the pre-test (Fig. 6).

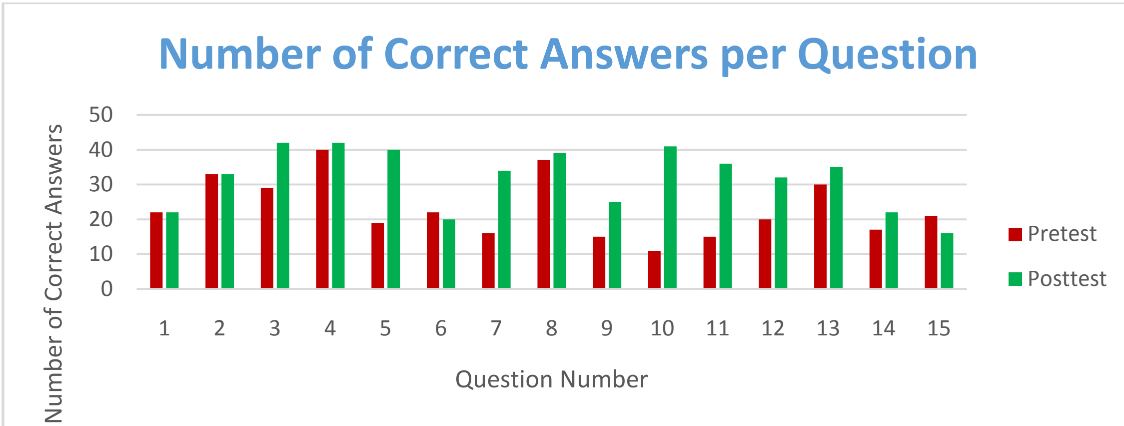


Figure 5: The chart is the results of the student assessment data. The red bars represent pre-test and the green bars are post-test results.

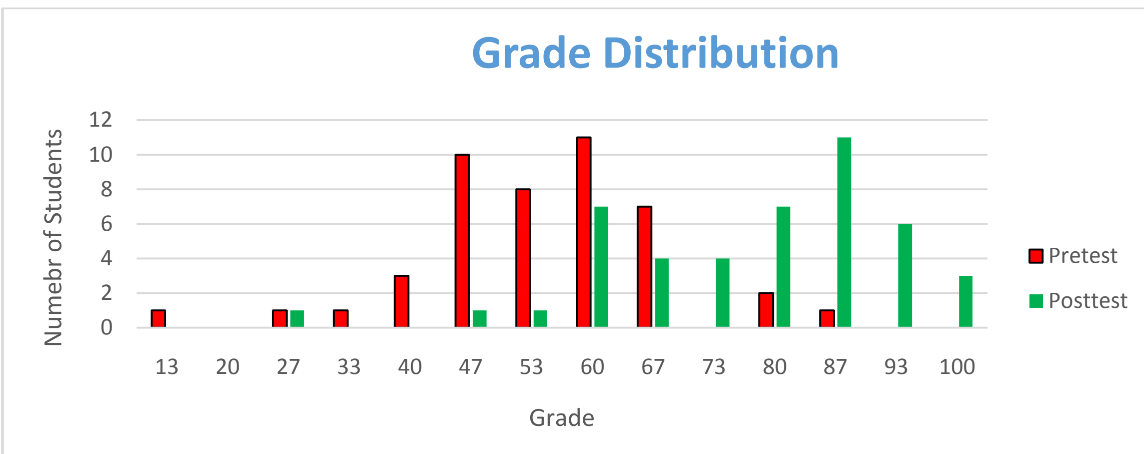


Figure 6: Numerical grade distribution for the pre-test and the post-test. The red bars show the pre-test data and the green is the post-test data.

DISCUSSION

This investigation into the effects of simulated acid rain on the development of an apterous strain of *Drosophila melanogaster* (fruit flies) was undertaken to enhance understanding of pollution in the environment and how it can disturb biological systems. High school science teachers have as one of their primary goals to enhance and enrich student's science knowledge base and more importantly, their understanding of the process of science. Using project-based learning, this study became a collaborative partnership with student-scientists from Bertie High School in northeastern North Carolina.

Acid rain is caused by reactions in the environment. It is a result of air pollution and can be carried great distances in the atmosphere. As a consequence, the source of the pollutants can be in neighboring states, across the country and even in different continents from the places where the acidic precipitate falls.

Although northeastern North Carolina is relatively isolated, the presence of acid rain may still negatively impact the ecosystem.

Since the cycle from egg to adult is approximately ten days, the timeframe for the investigation was practical for a study which utilized high school students as science partners. The data collected included the number of larvae detected ten days after the cultures were established. The mean number of larvae was significantly higher in the control vials which were set up using deionized water than the number in the experimental vials which were set up with simulated acid rain (2% acetic acid). The simulated acid rain appeared to reduce the number of larvae to less than 20% of the number that developed in the control vials. These results suggest that an environment that is more acidic than normally occurs has a negative effect on eggs developing into viable larvae. It remains to be determined if the effect of the simulated acid rain is the blockage of egg development into first in star larvae or whether the negative effect is the failure of first instar larvae to progress to second instar or second instar to third instar. Consistent with the pattern that was observed with larvae in this investigation, pupae were also negatively affected by the presence of simulated acid rain. The mean number of pupae in the control vials was more than 11 times higher than the number of pupae in the experimental vials. It was not unreasonable to expect this trend since larvae that do not progress or develop cannot become viable pupae. Again, it remains to be determined if the reduced numbers of pupae is a stage-specific phenomenon, i.e., affecting only larvae, or is the consequence of low numbers as development proceeds from the larval stage to the next stage.

Following the determination of the mean number of pupae, the next data set collected was the mean number of adults that eclosed by the fifteenth day after the cultures were established. There was a statistically significant reduction in the mean number of adults that eclosed in the experimental vials (>80% reduction) than in the control vials. In addition, those adults that did eclose in the experimental vials were smaller than those from the control vials based on average weight per fly in milligrams. The reduction in adult fly weight in the experimental vials suggests that one of the toxic effects of simulated acid rain is reduced size of the adults. It would be interesting to see if the reduced size had any impact on total lifespan or whether acid rain affects all organs in the same manner.

Long term exposure to simulated acid rain has been shown to be detrimental to development and reproduction of the carmine spider mite *Tetranychus cinnabarinus* (Wang *et al*., 2006). The simulated acid rain prolonged the immature stages of development which is different from the observations with Apterous*D. melanogaster*. In the fruit flies, the simulated acid rain resulted in the immature stages, the larval stages, not developing into pupae. This would seem to suggest that the spider mites were more resilient and adaptable to the effects of the simulated acid rain. In the spruce-living spider, *Pityohyphantes phrygianus*, simulated acid rain caused low mortality in the low pH environment but the negative long-term effects could not be ruled out (Gunnarsson and Johnsson, 1989). While mortality was not studied in the experiments with Apterous*D. melanogaster*, it likewise remains to be seen what long-term effects simulated acid rain would have on these organisms. The assessment of student learning was primarily accomplished through the administration of a pre-test and, following the experimental intervention, a post-test. The test was designed to cover subject areas that are examined via end-of-course tests. The results indicate that the vast majority of students improved following the acid rain experiment intervention. In some cases, the improvement was significant. Likewise, the numerical grade distribution was shifted from **41 grades ≤ 67**to **18 grades ≤67**. In addition, the numerical grade distribution was also shifted at the higher end from**3 grades ≥ 73**to **27 grades ≥ 73**.

LIMITATIONS AND RECOMMENDATIONS

One of the limitations of this study is that it was a “one-shot deal.” Due to the restrictions required by the school system, teachers are expected to cover an approved amount of material each semester. The intervention that we used for this study requires additional time and effort that would take away from the time allotted to cover all of the topics expected by the school system administration. In addition to time spent at Bertie High School in class, additional effort was required so that students could be transported to laboratories in the H.G. Cooke Department of Natural Sciences at Elizabeth City State University (ECSU). While most of the experimental work was executed at Bertie High School, the initial set up and the final data set (assessing adult fly weights) took place at ECSU. Both transportation associated costs (busses and bus drivers) and the cost for a substitute teacher had to be approved and provided by the school district.

The simulated acid rain intervention appeared to have a positive effect on student achievement. The data strongly support the concept that collaboration with student-scientists benefits the students and helps to create new citizen-scientists. With the many challenges that are impacting our ecosystem, a more knowledgeable populace could be of value in helping to find solutions to our problems. For those students who go on to higher education, project-based learning will strengthen their grasp of the scientific method and the process of science.

As a result of this study, we recommend that more project-based learning in the sciences be used to more efficiently develop understanding of and competency in executing scientific endeavors for high school students.

ACKNOWLEDGMENT

This work was supported by U.S. Department of Education MSEIP grant #P120A120117-14 to R.H.B. and G.L.H.

APPENDIX A

STUDENT INSTRUCTIONS:

Your name: ________________ Group members names: ____________________

Group #: ______

Purpose: The Effects of Simulated Acid Rain on the Development of *Apterous Drosophila*

Research:

- What is acid rain? ____

- What are *Apterous Drosophila*? ____

Hypothesis: Will acid rain have an effect on the development of *Apterous Drosophila*? Explain your answer.

____

Experiment:

1. Obtain two plastic vials containing food (white powdery substance).
2. On a piece of blue tape write today's date (XX-XX-16), group number, and control stock and place it on one of the plastic vials.
3. On a piece of red tape write today's date (XX-XX-16), group number, and experimental stock and place it on the second plastic vial.
4. To the vial labeled control stock add: 15 ml dH_2O (all of the blue labeled tubes)
5. To the vial labeled experimental stock add: 15 ml 2% HOAc (all of the red labeled tubes)
6. Allow the vials to rest for two minutes.
7. To both of the vials add five drops of concentrated yeast solution.

8. Allow the vials to rest for five minutes.
9. To each of the vials, control stock and experimental stock, add ten females by brushing them gently into the vial and one male.
10. Allow the flies to develop for ten days.
11. On day ten remove the adult flies and count the pupae and larvae and record the count in the data table
12. Allow the flies to develop for an additional five days, count the adult flies and record the count in the data table

Data:

Day 10		
	Control Stock	Experimental Stock
# of larvae		
# of pupae		

Day 15		
	Control Stock	Experimental Stock
# of adults		

Analysis and Conclusion:
Does Simulated Acid Rain have an effect on the development of *Apterous Drosophila*? Use the data gathered from your experiment to support your answer.

____

____

APPENDIX B

PRE-TEST AND POST-TEST:

Name: ______________________________ Block: _________ Date: ________________

1. When deforestation occurs in an area, what immediate effect does this have on the water cycle?
   A. More precipitation is formed.
   B. There is less runoff water.
   C. More water is returned to the atmosphere.
   D. Less water is returned to the atmosphere.

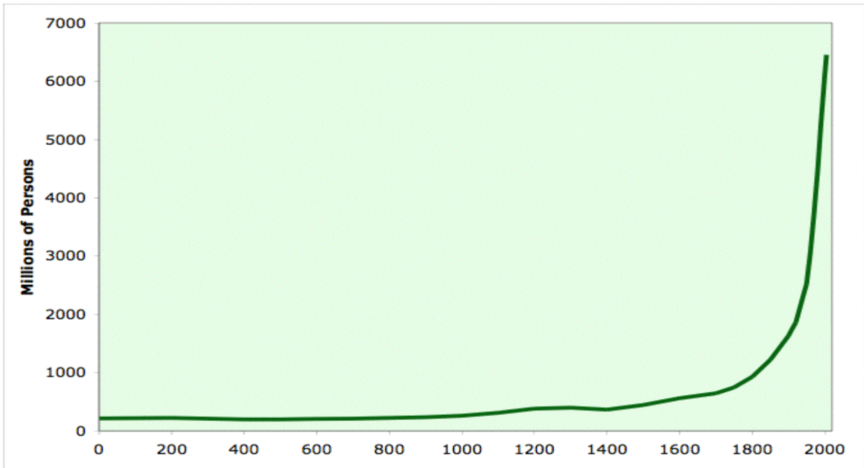
2. What will most likely happen if the human population continues to grow at current rates?
   A. There will be fewer natural resources available for future generations
   B. There will be an increase in nitrogen levels in the atmosphere
   C. There will be a decrease in water pollution
   D. There will be an increase in the number of hurricanes

3. What is the main suspected environmental problem associated with the burning of fossil fuels?
   A. depletion of fresh water   B. depletion of ozone
   C. global cooling   D. global warming

4. Which does pollution affect?
   A. the environment only
   B. living organisms only
   C. the environment and living organisms
   D. neither the environment nor living organisms

5. Which of the following global concerns is indirectly responsible for all of the others?
   A. waste disposal   B. resource depletion
   C. overpopulation   D. poor sanitation

6. This graph represents changes in human population over a period of 2,000 years.



7. What can be concluded from the graph?

   A. Human population grew at a constant rate over the last 2,000 years.
   B. Human population grew exponentially over the past 200 years.
   C. Human population reached its carrying capacity around the year 1900.
   D. Human population will begin to level off around 2010.

8. What is the main source of acid rain?
   A. sulfur dioxide   B. nitrogen oxide   C. magnesium oxide
   D. both A and B   E. both B and C   F. both A and C

9. To help reduce environmental pollution we must
   A. assess the situation
   B. educate the public
   C. predict the consequences of environmental interventions
   D. all of the above

10. Acid rain is found in which part of the United States?
 A. East Coast B. West Coast
 D. North-East D. Central (middle states)
 E. A, B, C & D

11. Which environmental factor would cause the greatest decrease in the number of species of plants and animals living in some of the lakes in the United States?
 A. increased in dissolved oxygen levels B. global warming
 C. ozone destruction D. acid rain

12. Which side effect of environmental pollution could be a danger to human offspring, even if pollution were to stop?
 A. cancer B. damage to internal organs
 C. infectious diseases D. birth defects from gene mutations

13. Which type of energy impacts the environment and produces over 60% of all of the electrical power in the United States?
 A. water B. wind C. solar power D. fossil fuels

14. How does human population growth differ from other species?
 A. other species control their resources
 B. humans have control of one other species
 C. humans can consciously change their environment
 D. other species can improve their infant survival rates

15. The burning of fossil fuels has greatly increased the level of CO_2 in the atmosphere. Ifthe levelof CO_2 continues to rise, which will most likely occur in an ecosystem?
 A. acid rain will have a much higher pH
 B. the atmospheric temperature will increase
 C. the levels of groundwater will increase significantly
 D. the number of producers and consumers will increase

16. Which environmental factor would have the greatest impact on the pH levels of lakes in North Carolina?
 A. putting several different species of fish into the lake
 B. beautification of the lake by planting flowers and bushes
 C. air pollution created in areas that are miles away from the lake
 D. increasing the size of the lake by cutting down trees and other vegetation

REFERENCES

1. Barron, B., and Darling-Hammond, L. (2008). Teaching for meaningful learning: A review of research on inquiry-based and cooperative learning.
2. Barron, B., Schwartz, D., Vye, N., Moore, A., Petrosino, L., and Bransford, J. (1998). Doing with understanding: lessons from research on problem and project-based learning. Journal of the Learning Sciences, 7(3), 271–311.
3. Butterworth, F.M. and King, R.C. (1965). The Developmental Genetics of Apterous Mutants of *Drosophila melanogaster*. Genetics. 52: 1153 – 1174.
4. Cornell, N. A., and Clarke, J. H. (1999). The Cost of Quality: Evaluating a Standards-Based Design Project. NASSP Bulletin. 83 (603), 91 – 99.
5. David, J. L. (2008). Teaching Students to Think. Educational Leadership 65.5 80-82.

6. Driscoll, C. T, Lawrence, G. B., Bulger, A. J., Butler, T. J., Cronan, C. S., Eagar, C., Lambert, K. F., Likens, G. E., Stoddard, J. L., and Weathers, K. C. (2001). Acidic Deposition in the Northeastern United States: Sources and Inputs, Ecosystems Effects, and Management Strategies. BioScience. 51: 180 – 198.
7. Ertmer, P. A., and Simons, K. D. (2005). Scaffolding teachers' efforts to implement problem-based learning. International Journal of Learning, 12(4), 319-328.
8. Greenspan, R. J., (1997). Fly Pushing: The Theory and Practice of Drosophila Genetics. Cold Spring Harbor Laboratory Press, NY pp. 17 – 19.
9. Gunnarsson, B. and Johnsson, J. (1989) Effect of simulated acid rain on growth rate in a spruce-living spider. Environ. Pollut. 56: 311-317.
10. Liu, M., and Hsiao, Y. (2002) Middle school students as multimedia designers: a project-based learning approach. Journal of Interactive Learning Research, 13(4), 311–37.
11. Metz, C.W. (1914) An apterous Drosophila and its genetic behavior. Am. Naturalist 48: 675 - 692
12. Morrison, Jennifer. (December 2008/January 2009).Why Teachers Must Be Data Experts. Educational Leadership 66.4.
13. National Science Education Standards (1996). National Academy Press, Wash, D.C.
14. Purkey, W. W., and Schmidt, J. J. (1996). Invitational counseling: A self-concept approach to professional practice. Pacific Grove, CA: Brooks/Cole.
15. Stepien, W. J., Gallagher, S. A., and Workman, D. (1993). Problem-based learning for traditional and interdisciplinary classrooms. Journal for the Education of the Gifted Child, 16, 338–357.
16. Thomas, J. W. (2000). A review of project-based learning. Autodesk Foundation, San Rafael, CA. Retrieved from http://www.bie.org/index.php/site/RE/pbl_research/29.
17. Vygotsky, L.S. (1962). Thought and Language. Cambridge, MA: MIT Press. (Original work published in 1934).
18. United States Environmental Protection Agency (n.d). Acid Rain. Retrieved from https://www.epa.gov/acidrain/what-acid-rain.
19. Walker, A. and Leary, H. (2009). A Problem Based Learning Meta-Analysis: Differences across Problem Types, Implementation Types, Disciplines, and Assessment Levels. Interdisciplinary Journal of Problem-based Learning, 3(1), 6-28.
20. Wang, J-J., Zhang, J-P., He, L., and Zhao, Z-M. (2006) Influence of long-term exposure to simulated acid rain on development, reproduction and acaricide susceptibility of the carmine spider mite, *Tetranychus cinnabarinus*. Journal of Insect Science, 6:19, available online: insectscience.org/6.19.
21. Wurdinger, S., Haar, J., Hugg, R., and Bezon, J. (2007). A qualitative study using project-based learning in a mainstream middle school. Improving schools, 10(2), 150-161.