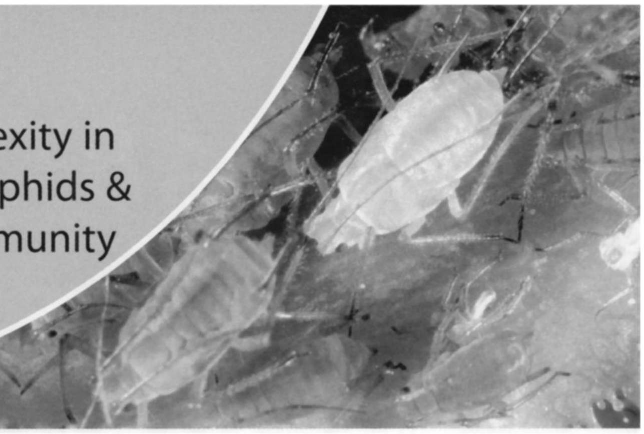
INQUIRY & INVESTIGATION

Building Ecological Complexity in the Classroom Using Pea Aphids & Components of Their Community



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Teachers face the challenge of introducing ecological concepts in an authentic way that students can apply to their lives and surroundings. Fieldwork helps achieve this goal and can have a range of beneficial impacts on participants, but opportunities to conduct fieldwork can be limited, especially in urban environments (Dillon et al., 2006). Urban environments are human-centered habitats and often lack green spaces; additionally, urban schools may have large class sizes and limited funds, which prohibit fieldwork. However, many engaging and authentic biology experiments using live flora and fauna can be done in the classroom, providing some of the same benefits as fieldwork.

Insects make an excellent model system for any life science class (e.g., Majerus et al., 1989; The University of Arizona Center for Insect Science Education Outreach, 2008) because of their prevalence and importance even in anthropogenic habitats, such as urban and agricultural environments (e.g., Axelrod, 1960; Ledieu & Helyer, 1985; Losey & Vaughan, 2006; Cooper & Rieske, 2007). We present an inquiry-based research project on insects that we used at the end of an ecology unit with 82 students in eighth grade (ages 13-14) at an urban school. We used this project to help students learn how to do science by applying concepts learned during earlier lessons and integrating those concepts with new concepts and techniques. In order to make students aware of complex ecological interactions, the lesson sequence addressed three major units: (1) designing research and collecting and interpreting data (2) how adaptations in predators and prey shape their interactions, and (3) the effects of abiotic and biotic factors on aphid populations.

Our project sequence started with direct contact and investigation of a single species, the pea aphid (*Acyrthosiphon pisum*), before including the other species involved in its complex ecological community. Aphids are ideal for use in the classroom because they are distributed worldwide and are important components of ecosystems (e.g., pests of crop and ornamental plants and food for many arthropods and some vertebrates, such as birds). Many abiotic factors (such as temperature, nutrients, and chemicals) and biotic factors (such as the host plant and predators) can cause fluctuations in aphid populations (Minks & Harrewijn, 1989; Richardson & Lagos, 2007). Ecologists long debated the relative importance of abiotic and biotic factors on the growth of populations, so students will learn about an important historical debate in ecology while learning in-depth information about the pea aphid, its host plant, and two predators of aphids (lacewing larvae [Neuroptera: Chrysopidae] and adult lady beetles [Coleoptera: Coccinellidae]).

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○ Materials & Methods

The experiment was performed in four classes at Urbana Middle School, Urbana, Illinois, during 2007. The class sizes ranged from 16-24 pupils, and they worked in groups of two or three for some parts of the project.

Materials

- pea plants (*Pisum sativum*–enough for two per group and stock plants)
  a. bush pea seeds (Seeds of Change, San Jose, NM; http://www.seedsofchange.com/)
  b. pots
  c. all-purpose potting soil
  d. watering can
  e. natural sunlight or fluorescent, full spectrum, or "grow" light bulbs
- pea aphids (Berkshire Biological, Westhampton, MA; http://www.berkshirebio.com)
- two fine-mesh bags per group
- metal twist-ties
- two wooden skewers per group
- lacewing larvae (Rincon Vitova Insectaries, Ventura, CA; http://www.rinconvitova.com) and lady beetle adults (Carolina Biological Supply Co., Burlington, NC; http://www.carolina.com)
- Petri dishes–two per group
- microscopes and magnifying glasses (optional)
- small eyelash brushes (can be made by cutting off all but a few bristles on inexpensive, plastic paint brushes)
- pictures of a pea plant, aphid, and each predator for each student
- students should bring a picture of their favorite animal to class
- Miracle-Gro® Liquid All-Purpose Plant Food

Pea aphids are considered plant pests and should not be released outdoors. Predators reared in artificial environments or not captured in the local environment also should not be released outdoors. All pea plants and insects should be killed by freezing for at least 48 hours before disposal.

Prior Knowledge Needed

Students need to be able to differentiate between abiotic and biotic factors and understand which of these affect arthropods, such as temperature, nutrients, predators, host plant, etc. Students also should have a general understanding of predator-prey interactions, an understanding of evolutionary adaptation, and the ability to read graphs. Evolutionary adaptation can be a difficult concept to understand and we explained it to students as the change of an anatomical structure or behavioral trait of an organism over a period of time that makes the organism better-suited to its local environment (Ricklefs, 2001). Our students had prior experience working with insects (Richardson & Hari, 2008). However, if students have not had experience working with plants and insects, teachers may want to prepare them for this activity by giving them magnifying glasses and letting them explore the plants and animals prior to starting this lesson sequence.

Timeline

This experiment is best conducted from spring through autumn as predators and prey are readily available and active (The University of Arizona Center for Insect Science Education Outreach, 2008). We concluded our project the first week of December and had satisfactory results.

Six to Eight Weeks in Advance

Plant 10 pots with 3-4 bush pea seeds for stock plants and two pots per group with 3-4 bush pea seeds for experiments. Keep plants in a sunlit area or under fluorescent, full spectrum, or "grow" light bulbs.

Four to Six Weeks in Advance

Order aphids two weeks after the initial planting date and transfer them to the 10 stock plants. Plant 10 additional pots of peas every two weeks after the initial planting date and transfer aphids to each potted plant to create a continuous supply of aphids. Transfer aphids by placing the new two-week-old plants next to old plants; aphids will migrate from the old plants onto the healthy foliage.

One Week in Advance

Order adult lady beetles and lacewing larvae.

One to Two Days in Advance

Deprive predators of food so they will be hungry during the project. Predators may need to be housed individually; otherwise they may consume each other.

○ The Activity

Class One: How To Design a Research Project

We led the students in a discussion of the major steps required to initiate a research project: select a research system, select the most important components of a research system, propose hypotheses to test, and design the project (including making a list of needed materials). We focused the discussion on aphids and had each student list all abiotic and biotic factors that may influence the population size of aphids. We discussed the factors and the students predicted whether they would have a positive or negative effect on aphids. Students then developed hypotheses about which abiotic and biotic factor would impact aphids the most. Our students decided to test two hypotheses: (1) supplementing the aphids with nutrients (an abiotic factor) will increase their population, and (2) introducing predatory lady beetles (a biotic factor) will decrease the population of aphids. Students designed their project and developed methods for measuring the effects of the factors on aphids. Nutrients were supplemented to half the plants with Miracle-Gro® Liquid All-Purpose Plant Food with each watering. These nutrients affect the plant directly and the aphids indirectly through the plant (another biotic factor). Using other abiotic factors, such as temperature or wind, would allow students to test the direct influence of an abiotic factor on populations of aphids.

Class Two: Adaptations of Plants, Herbivores & Predators

We divided the class into groups of two or three students and assigned each group a pea plant stocked with aphids, one lady beetle and lacewing (in separate Petri dishes), and pictures of pea plants, pea aphids, and predators. Students labeled morphological features on these pictures and proposed an adaptive function for each feature. We directed students to observe the live organisms and answer the following questions: What adaptations do plants have for survival? What adaptations might protect them against herbivores? How do aphids feed? What adaptations allow them to feed? Where are aphids found on the plant? What adaptations do predators have that may allow them to hunt or catch prey?

Students next predicted what would happen when aphids were placed in the Petri dish: Will predators move directly toward them in a straight line? Will aphids defend themselves? Will a predator keep eating until it is full or will it ration its food?

Students clipped off two leaves from the pea plant containing aphids and placed one leaf in each Petri dish. An alternative to our method is to release the predator onto a plant that has a population of aphids. Aphids often drop from leaves when approached by a predator, a defensive behavior that cannot be observed in Petri dishes. Students observed the predator-prey interaction and noted the following: What are the predator's behaviors (i.e., searching behavior, antennal movements, extension of mouthparts, feeding, etc.)? What does the aphid do when the predator approaches? How does the predator capture and hold its prey? Do the lady beetle and lacewing larva use their mouths similarly? How do the mouthparts of the insects differ? Do predators of different species have any anatomical features or behaviors in common?

Students brought a picture of their favorite animal to class or drew their favorite animal if they forgot a picture. On the picture, students labeled morphological features and proposed their adaptive functions. We were primarily interested in having the students identify adaptations that helped an animal acquire food, defend itself, attract a mate, and adapt to environmental conditions.

For homework, students drew their own fictional animal and gave it adaptations that made it perfectly adapted to its environment. Students were asked to explain the habitat of the animal and how its adaptations helped the animal cope with environmental conditions, defend itself, and acquire food or mates.

Class Three: Effects of Abiotic & Biotic Factors on Aphids

We divided the class into groups of two-three students. Each group was given two plants, one of which was supplemented with nutrients during each watering. Half of the groups were given two lady beetles, while the other half were not given a predator (testing the effects of nutrients only). Each group carefully transferred four apterous (wingless) adult aphids from stock plants to each of their plants with an eyelash brush. We used adult aphids because they can reproduce daily via cloning, and apterous aphids because they produce more offspring than winged aphids. We recommend transferring 10 aphids to each plant to allow for larger populations to develop faster.

The groups with lady beetles added one lady beetle to each of their plants. Mesh bags were used to enclose the plants and arthropods. They were closed with metal twist-ties and held upright with wooden skewers. Plants were placed in adequate lighting for one week.

Class Four: Collection & Interpretation of Data

The students counted aphids on each of their plants after one week and entered the data into Microsoft Excel. The teacher can enter the data into Excel if there is a lack of computers, time, or student familiarity with this software. Alternatively, students can create the graph by hand on a piece of paper or the blackboard because this method may help them understand how to design and interpret a graph. We combined data from the entire class and made a graph so the students could determine how the abiotic and biotic factors influenced aphid populations. Differences between treatments in abundance of aphids were tested by ANOVA (PROC GLM, SAS Institute, 2002), blocked by nutrient level and presence/absence of a predator, including the interaction term. We tested differences between means initially with full ANOVA models, but eliminated insignificant interactions and random effects step-wise from models, starting with highest order interactions, and leaving only significant terms (see Milliken & Johnson, 1984). Data were $\log_{10}$-transformed to meet assumptions of ANOVA. Differences between individual means were tested with Tukey-Kramer means separation test (Sokal & Rohlf, 1995). We present statistical results to rigorously analyze our activity. However, teachers can discuss the importance of the treatments they use in their classrooms by making general conclusions based on visual interpretations of the graphs. Each student completed a written explanation of the graphs (this may need to be done at the start of a fifth class in order to have time to compile the data and make graphs).

Assessment of Students

We used mixed methods to assess students before, during, and after completion of the activity. Students were given a pre-activity quiz to assess their knowledge of ecological terms associated with this lesson (Table 1). Students then retook a quiz nearly identical to the pre-activity quiz after completion of the activity and answered additional questions that allowed them to provide feedback on the activity, including how much they felt they learned (Table 1; Table 2).

Students kept a journal throughout the activity, which included a record of the class discussions, their investigations, and the results for each class. We gave students a sheet with the questions listed throughout this project, which they also answered in their journal. Their journal allowed us to evaluate the following:

1. Did they list multiple abiotic and biotic factors that may affect aphids?
2. Did they develop a hypothesis stating how abiotic and biotic factors impact the size of aphid populations?
3. Can they identify adaptations and state how they help pea plants, aphids, and predators survive and reproduce?
4. Did they use their knowledge of adaptations of predators and prey to predict the outcome of predator-prey interactions? How well do their observations of predator-prey interactions match their predictions?
5. Can they apply their knowledge of adaptations by creating a fictional animal, labeling

Table 1. Sample quiz administered before the activity to assess student knowledge and following the activity to assess student learning.

1. What does biotic mean?
2. Please provide an example of one biotic factor that affects insects.
3. What does abiotic mean?
4. Please provide an example of one abiotic factor that affects insects.
5. What is an adaptation? a. Incorporation of any material into the cells, tissues, or fluids of an organism. b. A reversible change in the structure or behavior of an organism in response to environmental change. c. The levels of a food chain. d. An anatomical structure or behavioral trait of an organism that has evolved over a period of time in order to increase an organism's chances for survival.
6. Please provide an example of an adaptation that you have and what it does.
7. Do predators ration their food or eat prey until they are full?
8. If you were designing an ecological experiment, which would you do first? a. Collect data b. Pick what to work with c. Observe your organisms d. Gather research materials
*9. Please tell us at least one thing you liked the most about this project.
*10. Please tell us at least one thing you did not like about this project.
*11. Is there anything about the project that you found confusing?
*12. Please name a predator and its prey.

*Questions 9-12 were only on the post-activity quiz.

Table 2. Survey questions posed to the students in which they assessed how much the activity helped them learn key ecological ideas. Students answered the questions on a scale of 1-5, with one meaning "not at all" and 5 meaning "greatly".

QUESTION	MEAN SCORE (± SE)
Do you think this activity helped you understand the difference between abiotic and biotic?	3.41 (0.136)
Do you think this activity helped you understand what an adaptation is?	3.43 (0.144)
Do you think this activity gave you a better understanding of how to design a research project?	3.64 (0.126)
Do you think this activity gave you a better understanding of how to do a research project?	3.69 (0.125)

the adaptations and explaining them? In particular, do their adaptations address how it acquires food, attracts a mate, defends itself, and copes with its environment?

6. Did they successfully measure the initial size of aphid populations and the size of aphid populations after one week?
7. Can they successfully identify which factor affected aphids? Graph interpretation was assessed with the following questions: Which treatment(s) increased the aphid populations? Which treatment(s) kept the aphid populations from getting large? If you wanted to control aphid populations, which treatment would you use?

○ Results

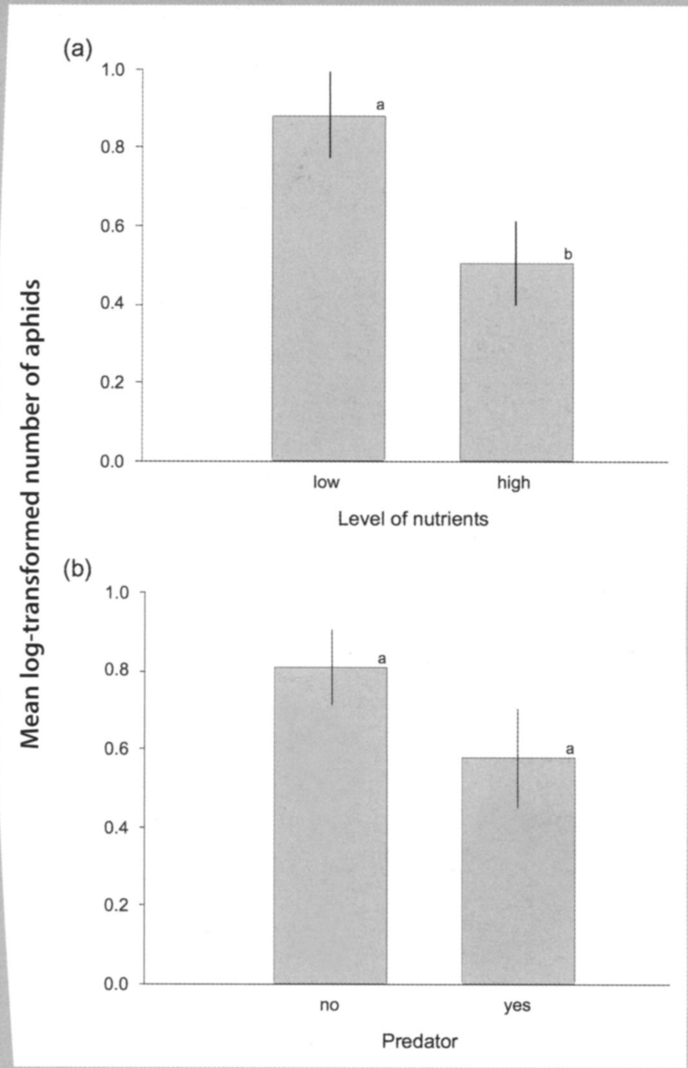
The average score on the homework, in which students created their own ultimately-adapted animal, was 8.9 ± 0.365 out of 12. Points were deducted most often because students neglected to explain the function of a morphological adaptation.

The students found that aphid populations were highest on plants that were not supplemented with nutrients, whereas predators did not influence populations of aphids (overall ANOVA, $F_{2,51}$ = 4.43, p = 0.02; nutrient level, F_1 = 6.33, p = 0.02; predator presence/absence, F_1 = 2.22, p = 0.14; Figure 1a, b). Students scored an average of 3.14 ± 0.148 out of 5 points on the graph interpretation.

We used a paired t-test to determine whether the average grade of the students was higher on the post-activity quiz compared to the pre-activity quiz. If a student did not complete both quizzes, his/her grades were not included in the statistical analyses. The average grade on the pre-activity quiz was 5.27 ± 0.211 out of 8 points, whereas the average grade for the post-activity quiz improved to 5.93 ± 0.203 (t = -4.30, n = 69, p <0.001). Closer examination of the data revealed that the normalized learning gain was 24.2%, and 70.5% of the students improved their grade, not including students who had scored 100% on the pre-activity quiz.

The students had mixed responses to the activity, but overall they thought it helped them learn key ecological terms and how to design and perform a research project (Table 2). In response to the survey question, "Please tell us at least one thing you liked the most about this project," most students (82.4%) said they liked working with plants and animals, doing a hands-on experiment, and seeing the interactions between the plants, pea aphids, and predators. In response to the survey question, "Please tell us at least one thing you did not like about this project," 25% of the answers indicated that they did not like collecting data or taking notes. Another 25% of the respondents either had a dislike for one or more of the insects, did not like seeing the predator eat the prey, or thought the aphids were too small and difficult to see and count on the plant. The last major dislike (9.72%) was that students did not like working in groups, or one or more members of their group were not productive. In response to the question, "Is there anything about the project that you found confusing," 70% of the students said no. The second most common response (8.6%) was that the graphical data or results were confusing.

Figure 1. Mean $\log_{10}$-transformed number of pea aphids (± 1 SE) on pea plants with: a) a low and high level of supplemental nutrients; and b) predatory lady beetles. Means with different letters are significantly different (Tukey-Kramer means separation test, P < 0.05).



○ Discussion & Implications for Teaching

We started this project with an in-depth study of each organism, but used the pea aphid as the main focus of our investigations. This type of in-depth study, which focuses on a single organism (e.g., Tomkins & Tunnicliffe, 2001), is highly motivating to students because they feel like they know and understand the organism. Ecological lessons should strive to start on this level before building to more complex concepts. Without this proper introduction, the complexity of ecological systems, combined with a lack of knowledge about whole-organisms biology, can inhibit ecological teaching (Barker & Slingsby, 1998). Our project aims to avoid these pitfalls while still meeting National Science Education Standards for grades 5-8 in Life Science (Content Standard C) and two of the three overarching goals for science students in Illinois: Students should "understand the processes of scientific inquiry and technological design to investigate questions, conduct experiments and solve problems" (State Goal 11); and "understand the fundamental concepts, principles and interconnections of the life ... sciences" (State Goal 12).

The students found that varying levels of nutrients influenced populations of aphids, and predators were not effective in limiting populations of aphids. We are unsure why the abundance of aphids was lower on plants that received supplemental nutrients because we expected the opposite pattern. The plants provided with nutrients may have been healthier and more able to induce a chemical defense that limited growth of the aphid population. We are also unsure why predators had no influence on populations of aphids. This experiment would need to be repeated multiple times to determine if our outcome is the norm. However, even these contradictory results are useful because they demonstrate the normal outcome of many scientific investigations.

One of the values of this project is that it is inquiry-based. We did a pilot project in 2006 with ~100 eighth grade students in which we used tall goldenrod (*Solidago altissima*), an aphid (*Uroleucon nigrotuberculatum*) that lives on goldenrod, and predators that we caught in the wild. Students chose predators as the most important biotic factor during the pilot study and in 2007. However, students chose temperature as the abiotic factor during the pilot study and nutrients in 2007. This flexibility to choose abiotic and biotic factors allowed the students to feel like they have more control and input in the project. Multiple students on the survey remarked that they enjoyed doing independent research, observing the system, watching the developments over time, and learning new methods to interpret results. Depending on the number of students in a classroom, their prior knowledge of scientific research, ability to work independently, and the classroom resources, each student or group may be allowed to pick its own project instead of focusing the entire class on the same abiotic and biotic factors.

Our lesson sequence, which broke the experiment into sections, kept the students on task, helped them learn the key ecological terminology, and helped them successfully manage a large-scale project without feeling overwhelmed. For example, our lesson sequence allowed students to have a better understanding of adaptations because they had guided help applying the knowledge in the classroom with live animals before they had to independently apply their knowledge on a homework assignment. However, we found that they are much better at identifying morphological adaptations than explaining their function. Some students struggled with the concept that an adaptation is only beneficial in certain habitats and situations, and could be a handicap in others. Students also struggled with interpreting graphical data. We would recommend that the ability to read graphs should be required background knowledge prior to doing this activity. We gave our students more practice interpreting graphs after the activity.

Although some students did not like working with insects, we persist in this choice for many reasons. They are an excellent model system for classrooms because they can be purchased, reared, and manipulated. They also provide students an opportunity to observe common fauna that is often overlooked. Many students remarked at the end of this project that they looked more favorably upon insects and lost some of their fear of them. While this project does not replace the need and benefit of fieldwork, it provides opportunities for students to overcome some of their ecological illiteracy.

○ Conclusion

This was the culminating project in the ecology unit for the students at Urbana Middle School, and thus a multi-faceted and long-term project. We think it is worthwhile doing all parts of the activity; however, it can be broken into smaller components. For example, the focus during the pilot project was only on developing an experiment to test the impact of one abiotic and one biotic factor on goldenrod populations (Classes Three and Four in this activity). The students enjoyed the pilot project and gained knowledge, however more prior knowledge about the plant-insect community was necessary prior to looking at factors that influenced the population of aphids, which is why we expanded the project the second time we taught it. An alternative could be to highlight predator-prey interactions and emphasize biological control of aphids (see The University of Arizona Center for Insect Science Education Outreach, 2008). Regardless of whether you choose to use the full experimental method we have described, or shorten or modify the lesson, we hope you and your students will find exploring plant-insect relationships as accessible and exciting as we do.

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