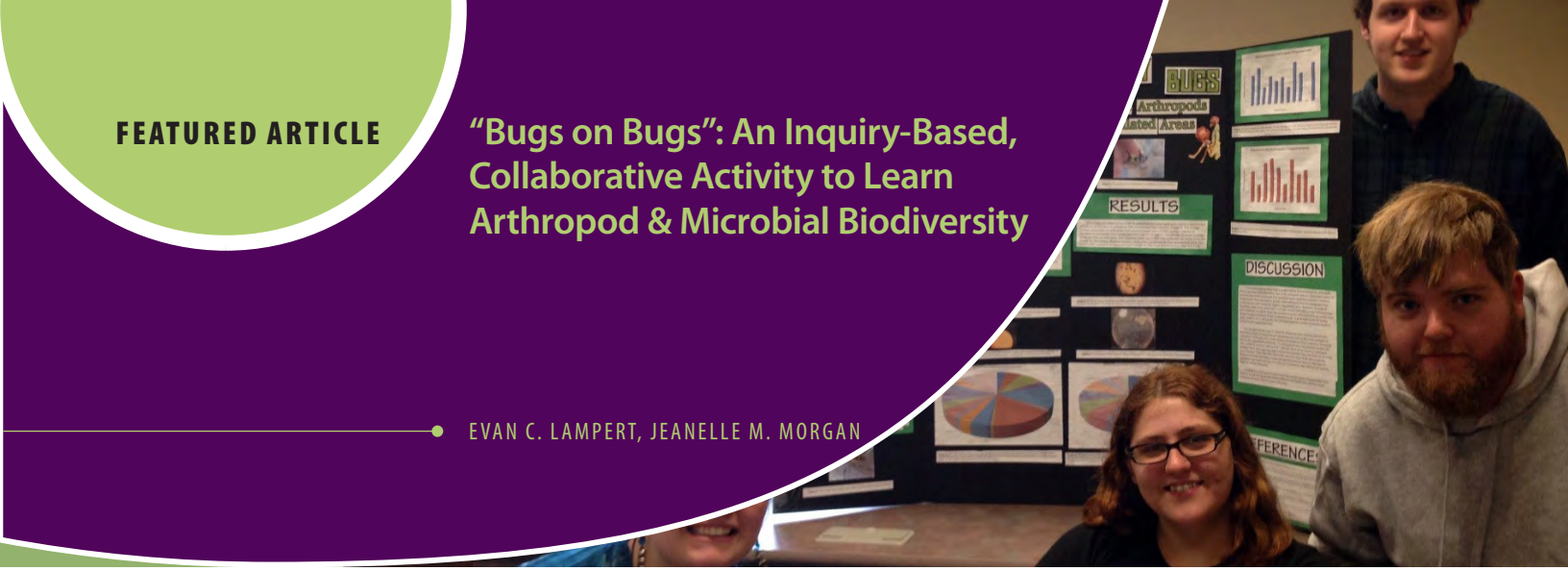
FEATURED ARTICLE

"Bugs on Bugs": An Inquiry-Based, Collaborative Activity to Learn Arthropod & Microbial Biodiversity



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Abstract

Diverse communities of arthropods and microbes provide humans with essential ecosystem goods and services. Arthropods are the most diverse and abundant macroscopic animals on the planet, and many remain to be discovered. Much less is known about microbial diversity, despite their importance as free-living species and as symbionts. We created "Bugs on Bugs" as an inquiry-based research project in which students investigate both arthropod and microbe diversity by collecting arthropods and culturing their symbionts. "Bugs on Bugs" was developed as a multiple-course project in which students from different disciplines specialize in parts of the project and collaborate in project design and data analysis. We provide instructions for use of "Bugs on Bugs" in active-learning courses, share experiences in which a biodiversity course and a microbiology course completed "Bugs on Bugs" together at our institution, and share suggestions for implementation based on our experiences.

Key Words: *Arthropods; insects; arachnids; bacteria; symbionts; culturable microbes.*

Background

The maintenance of biological diversity provides a wide variety of ecosystem services, the measurable value of which, globally, far exceeds the world's gross domestic product (Costanza et al., 1997). For example, diverse ecosystems provide soil formation and retention, water retention, pollination, and biological control services to increase agricultural productivity. Anthropogenic activities such as urban development and agriculture threaten biodiversity at global and local scales. Science students who recognize biodiversity and the extent to which it is threatened, and who can advocate recognition of its importance, will be well positioned to lead societal efforts to protect and conserve biodiversity in the coming decades.

We now know that microbes constitute most of the life on Earth.

While "charismatic" animals such as large mammals and songbirds are most easily recognized by the general public, most biodiversity exists at scales that are not easily observed (Chapman, 2009). Arthropods are recognized to be the most abundant and diverse macroscopic animals by far, with an estimate of 1.2 million extant species in 15 classes known to science, and conservative estimates of 5–10 million or more species that remain to be discovered (Ødegaard, 2000; Hamilton et al., 2010, 2013). Unfortunately, many people fail to recognize and appreciate the ecosystem services these small animals provide – such as biological control of animal and plant pests, pollination, and forming the base of food webs – and instead fear arthropods, are disgusted by them, or are apathetic (Kellert, 1993).

Microbes receive even less recognition than arthropods; despite their immense ecological and medical importance in nutrient cycles and as symbionts, they are often seen only in the context of a few disease-causing "bugs." Before the invention of the microscope in the 1600s and Antoni van Leeuwenhoek's descriptions, scientists did not even know that microbes existed (Gest, 2004). We now know that microbes constitute most of the life on Earth, but we also acknowledge that we have discovered only a small percentage of the microbial diversity that is present on Earth (Saikia et al., 2011). Microbes are found in each domain of life, and *microbe* is in fact an umbrella term for organisms that are too small to be seen without a microscope: bacteria, molds, yeast, protozoa, some algae, and viruses are all included. As we explore more diverse habitats and habitats that are difficult to access, we learn more about the microbes that populate these places. It was once thought that nothing lived in the Dead Sea, for example. We now know that several Archaea and a few halophiles (salt-loving organisms), including fungi and bacteria, live there (Ionescu et al., 2012). Hydrothermal vents have been found to contain diverse bacterial communities of thermophiles and hyperthermophiles, organisms that grow at elevated temperatures (Orcutt et al., 2011).

Research in symbionts, the microbes that live on and inside other species, has exploded since molecular biology has become more accessible.

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Many of us are aware of the incredible roles they play in human health, from digestion to potentially regulating mood (Human Microbiome Jumpstart Reference Strains Consortium, 2010). It makes sense that symbionts are just as vital for other animals, including arthropods. Researchers have recently given much attention to symbionts of arthropods. In April 2014, a Web of Science search using the keywords "arthropod" and "symbiont" returned 7501 results, 2265 (~31%) of which were published since the year 2000. Most of this research has focused on gut symbionts, which play immense roles in nutrition and, thus, ecology. Other symbionts, such as those on the exoskeleton, are much less studied despite their important influence on factors such as resistance to pathogens that could kill the arthropod or potentially be mechanically vectored to humans.

"Bugs on Bugs" is an inquiry-based activity that combines two high-impact educational practices, collaborative learning and undergraduate research (Kuh, 2008), to educate students about the scope of the unseen biodiversity of arthropods and microbes, particularly symbionts. Specifically, small student teams develop questions related to arthropod and microbial diversity, sample arthropods, and sample the culturable symbionts of those arthropods. Moreover, we designed "Bugs on Bugs" to connect students in two or more courses in distinct subdisciplines of biological sciences.

○ Learning Outcomes

We developed two sets of learning outcomes for "Bugs on Bugs" partially based on the language described in Fink's (2003) taxonomy – in particular, foundational knowledge, application, and integration. Application outcomes were mostly based on acquiring new skills.

Foundational Knowledge Learning Outcomes

Upon completion, students will be able recognize that

- arthropods are the most diverse and abundant macroscopic animals;
- microbes are ubiquitious in all environments and play important ecological roles; and
- symbionts are important in life.

Application Learning Outcomes

Upon completion, students will have developed skills to

- use field marks, field guides, and keys to identify arthropods;
- calculate diversity indices to quantitatively assess biodiversity;
- use correct tools to sample arthropods from the environment;
- sample small animals for microbial symbionts using aseptic technique;
- differentiate microbes on the basis of colony characteristics and differential stains; and
- analyze data and disseminate results by written and oral presentations.

○ Course Contexts

To best connect students and integrate disciplines, we recommend that "Bugs on Bugs" be used in a learning community (for description, see Kuh, 2008) or another type of collaboration between at least two courses, including some form of organismal biology/ecology and some form of molecular-cell/microbiology course. Students in each partnered course can "specialize" in different parts of this project, and share materials and results. Our version of "Bugs on Bugs" connected two courses with different groups of students, which we describe to provide context and share our implementation strategy.

One course was the second in a two-semester introductory biology sequence for science majors. The course description includes evolution, biodiversity, and ecology; and at our institution, class and lab meetings are taught by the same faculty member (E.L.). In fall 2013, E.L. used a "flipped" course model (Fulton, 2012) in which contact time usually focused on activities, research projects (most of the lab meetings), and worksheets. We will hereafter refer to this course as the "organismal biology course."

The other course participating in "Bugs on Bugs" was a microbiology lab course for allied health majors. We selected this course because it introduced students to some basic methods and materials used in a modern microbiological laboratory. "Bugs on Bugs" gave students the opportunity to experience the demonstration and application of many of the concepts learned in the lab course's accompanying lecture. J.M. taught both the lab and the lecture; however, because they were separate courses, some students had different instructors for the lab and lecture. We will hereafter refer to this course as the "microbiology course."

Since we did not participate in a learning community, we instead connected our two courses by selecting one "liaison" to represent each team from the organismal biology course. The role of each liaison was to visit at least one lab, planned in advance, from the microbiology course, and work with a partner team in that course. The liaison provided his or her partners with the background of the research objectives and the arthropods collected and sampled. The liaison then supplied his or her partner teams with samples of microbial communities (see below), from which they could isolate and identify the specific microbes in the communities. After identifying microbes, the partners reported their results back to the liaison, and teams from both classes analyzed the data separately. Liaisons were free to visit all labs in which the microbiology teams worked on the project (various portions of four lab days).

○ How to Implement "Bugs on Bugs"

Collect Arthropods

We structured our activities in a manner similar to that described in Luckie et al. (2004), in which students complete a guided field collecting exercise before developing their own research objectives. During this first exercise, students learn to sample arthropods using a variety of methods, such as Malaise traps, aerial nets, sweep nets, and aquatic nets (we purchased all traps and nets from BioQuip Products; http://www.bioquip.com), and get a feel for the types of arthropod that each method selectively samples. Next, student teams (we recommend three or four students per team) develop research questions and hypotheses that answer their questions, design arthropod sampling protocols, and predict the outcomes of their project. Each of these should be approved in advance by the instructor. Some examples of predicted outcomes:

- Flying insects will have more diverse microbes on their exoskeletons than terrestrial arthropods.
- One type of arthropod (harvestman) will host the same microbes regardless of collection site.

- Arthropods associated with carrion will be covered with the same microbes as carrion.
- Different arthropods in decaying logs will host different microbes.
- Gut and exoskeleton microbes will be different.

Once the sampling techniques are agreed upon, students can sample arthropods during structured lab time or outside of class. In our version, E.L. required each team to collect and sample 20 arthropods to ensure a consistent sampling and identification effort. We dedicated one 2-hour lab period to sampling a deciduous forest on our campus, and we also allowed students to sample arthropods outside of class. Students had to wear gloves while sampling and place the arthropods in sanitized containers to prevent cross-contamination of normal human flora with arthropod symbionts. Arthropods should be identified to the most exclusive taxon possible using a variety of field guides or the website BugGuide.net. We found that students in our course could most accurately identify to the level of order, although upper-level ecology or entomology courses may potentially identify most arthropods to family or selected arthropods to species.

Following collections, there is a chance to demonstrate and discuss ecological aspects of arthropods, such as parasitism (e.g., we collected several venomous saddleback caterpillars [*Acharia stimulea*] covered by cocoons of *Cotesia* sp. parasitic wasps) and invasive pests (e.g., we collected red imported fire ants [*Solenopsis invicta*]). Also, fortunate students may collect amazing and fascinating arthropods (e.g., we collected bioluminescent glowworms [*Phrixothrix* sp.]).

Sample Arthropod Symbionts

Methods of sampling the microbial symbionts of arthropods vary, although it is essential that students maintain aseptic technique and use sterile plates and sampling tools. Small arthropods (<3 mm) such as ants can be vortexed in 0.5-mL sterile physiological saline to dislodge external microbes, with an aliquot of the saline streaked on agar plates using a sterile cotton swab. Larger arthropods can be sampled by directly rubbing their exoskeleton with a sterile cotton swab moistened with sterile saline. Gut symbionts can be sampled by collecting feces and vortexing them in sterile physiological saline.

The saline containing the microbes was then plated onto nutrient agar using aseptic technique and standard microbial culturing methods. Microbes were isolated by streaking for single colonies using either the quadrant or the 190° technique (Munro, 2008). We used a general-purpose nutrient agar to culture the broadest variety of microbes (Lapage et al., 1970), although other types of media can be used, depending on course-specific needs. For example, selective media allow students to inhibit the growth of "undesired" microbes and allow for the growth of specific microbes they seek. Differential media allow students to distinguish a particular species from members of the same genus. Incubators may not be needed to grow microbes; since arthropods are ectotherms, many of their symbionts may be adapted to grow at ambient temperature and may not grow at higher temperatures. Students who observe plates of microbe colonies can observe several morphological features, such as color, size, elevation, colony shape, and margin shape (Munro, 2008).

Before continuing, it is important that students record the number of different colonies (richness) growing on each plate, and how many total types of microbes each team cultures. This will inform them or their partners how many types of microbes will be isolated.

Figure 1. Students streaking plates (top left) and Gram-staining microbes (bottom left). Microbe colony growth (right).

Isolating & Identifying Symbionts

Once colonies are cultured, tools such as differential stains and polymerase chain reaction can be used to further isolate, observe, and identify microbes. We used Gram staining, one of the most important and universally used staining techniques in microbiological labs (Munro, 2008), to distinguish bacteria on the basis of cell wall chemistry. In our version, students in the microbiology course used inoculating loops to remove small areas of each isolated colony (overall, individual plates had a range of 0–7 different colonies, while the eight teams had a range of 5–22 different colonies combined among their plates; see Figure 1). These were used for Gram stains. The majority of microbes stained in fall 2013 were Gram-negative, sphere-shaped bacteria. Gram stain kits and trays can be obtained from Carolina Biological Supply Company (catalog nos. 821051 and 742001, respectively).

Data Analysis

This project can generate several sets of data, which can be analyzed in a number of ways. After arthropods are collected, we recommend combining data from all students to obtain a "big picture" of arthropod diversity and of how collecting methods can influence biodiversity data. For example, in fall 2013, our students (eight teams) collected 5 classes, 17 orders, 40 families, and 50 morphospecies (conservatively estimated) of arthropods, and 136 individual arthropods were sampled (for data, see Figure 2). By comparison, a haphazard sweep-net and Malaise-trap sampling of the sample area (the forest on our campus) prior to fall 2013 yielded 230 arthropod species from 14 orders (E. Lampert et al., unpublished data). We found much lower diversity in the "Bugs on Bugs" collection compared to our previous survey because the "Bugs on Bugs" teams were free to selectively sample specific arthropods as part of their research objectives (especially grasshoppers, crickets, and harvestmen). Student teams or the whole class can calculate diversity indices; for instance, the Shannon index and the Simpson index both provide a value of a community's biodiversity, and formulas are very easy to find online. If a class samples multiple communities or uses multiple collection methods, Jaccard's similarity coefficient (Southwood & Henderson, 2000, p. 486) can be used to compare them.

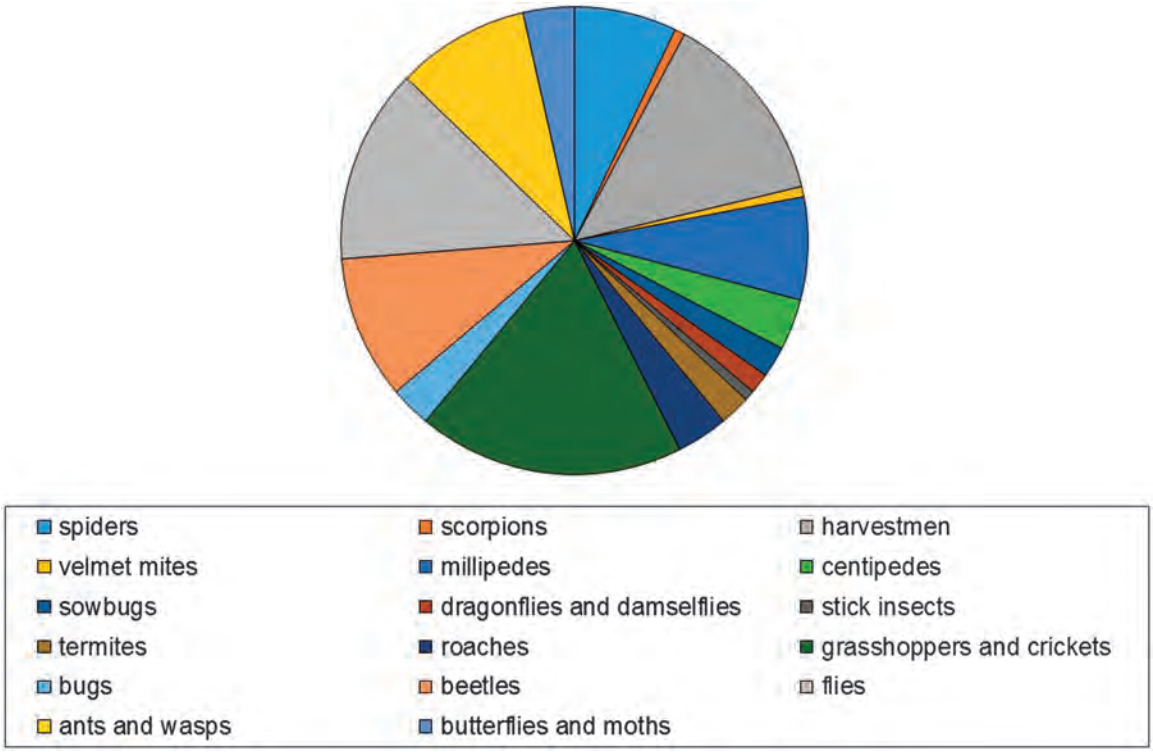

Figure 2. Sample arthropod diversity data from our version of "Bugs on Bugs," completed during the fall 2013 semester. For ease of interpretation, we combined two orders each of centipedes and millipedes into one taxon each. Harvestmen, grasshoppers, and crickets are overrepresented because two teams were selectively collecting and sampling these two groups for their own inquiry-based research objectives. Flies are also somewhat overrepresented because one team was collecting arthropods exclusively from squirrel carrion.

After microbes have been sampled and isolated, student teams should all have clearly identified the community of microbes associated with each of the collected arthropods. There are several ways to analyze the data, depending on specific goals and testable hypotheses of the student teams. Below, we discuss each that we used and other potential analyses, with recommendations for best practices. Data analyses are summarized in Table 1.

1. Students record richness, or number of different colony types, according to colony and cell morphology, of all 20 microbe communities. Mean richness can be analyzed in multiple ways. Individual teams can use a one-sample t-test to compare their sample means to a population mean if that is calculated (in fall 2013, our mean richness ± SE was 2.66 ± 0.10 different colonies per plate). Pairs of teams can use two-sample t-tests to compare their sample means. Lastly, class data can all be combined to compare richness among either teams or arthropod orders using analysis of variance (ANOVA). Although comparing richness among arthropod orders is highly informative (see Figure 3), for ease of implementation we recommend that ANOVA be performed only in upper-level courses or by instructors.
2. Microbial colony abundance (number of colonies) can be compared in the same ways as richness. However, abundance can be somewhat difficult to calculate, because colonies may grow in different patterns or grow together on plates. We recommend that students dilute their samples and quadrant streak for colonies if they plan to measure microbial abundance.
3. Students calculate Shannon or Simpson diversity for all 20 microbe communities. According to the Shannon index, diversity was fairly low ($H' < 1.0$ using $\log_{10}$ in all accurately calculated communities) in fall 2013, most likely because plates were dominated by specific lawn-forming or competitive microbe genotypes. It is important to share with students that this index may not accurately represent the structure of the microbe communities, because of differences in how certain microbes grow and interact on media versus on the arthropod. Shannon or Simpson diversity can be statistically tested in the same manner as richness, using t-tests and ANOVA.
4. Students use Jaccard indices to compare similarity among the most diverse, median, and least diverse of the 20 cultured communities. In this case, each team would calculate the index three times.
5. Students use t-tests to make paired comparisons of the number of each type of microbe cultured (Gram-positive vs. Gram-negative, rod-shaped vs. sphere-shaped, etc.).
6. Students can use Fisher's exact tests to determine whether a specific microbe is more likely found on one type of arthropod than on another. This might be most appropriate in a statistics course.
7. Students can use multivariate statistics (e.g., multivariate analysis of variance, cluster analysis, or ordination) to determine how similar the microbial communities are among groups of arthropods. We recommend this only in an upper-level ecological statistics course.

Table 1. Summary of statistics described in the text.

Type of Analysis	Description/Application	Example	Considerations
Total abundance	Number of arthropods in a collected sample	75 arthropods in a trap	Are students limited in how many arthropods they collect?
	Number of colonies cultured from an arthropod	200 colonies on a plate	Colonies can grow together, or form lawns; students might estimate rather than count
Richness	Number of morphologically distinguishable arthropods	6 species of crickets	Adults and immatures look different; similar-looking species
	Number of morphologically distinguishable colony types	4 different types of colonies on a plate	Colonies might look similar; falsely interpreted Gram stains or cell shapes
Shannon index (H')	Index of diversity of a sample; actual index can vary depending on the log base used	H' = 1.5 (colonies on a plate)	Easily calculated with Excel formulas; higher H' means more diversity
Simpson index (D)	Index of diversity of a sample	D = 0.7 (colonies on a plate)	Easily calculated with Excel formulas; higher D means less diversity
Jaccard index (C_j)	Index of similarity among a set of samples	C_j = 0.7 (two plates have most colony types in common)	Some equations for this can be intimidating!
Fisher's Exact test (P value)	Compares two outcomes between two groups (e.g., presence/absence of a microbe cultured from crickets vs. spiders)	P = 0.002 (microbe is significantly less likely to be present in one type of arthropod)	Only returns a P value; uses factorials; might be intimidating

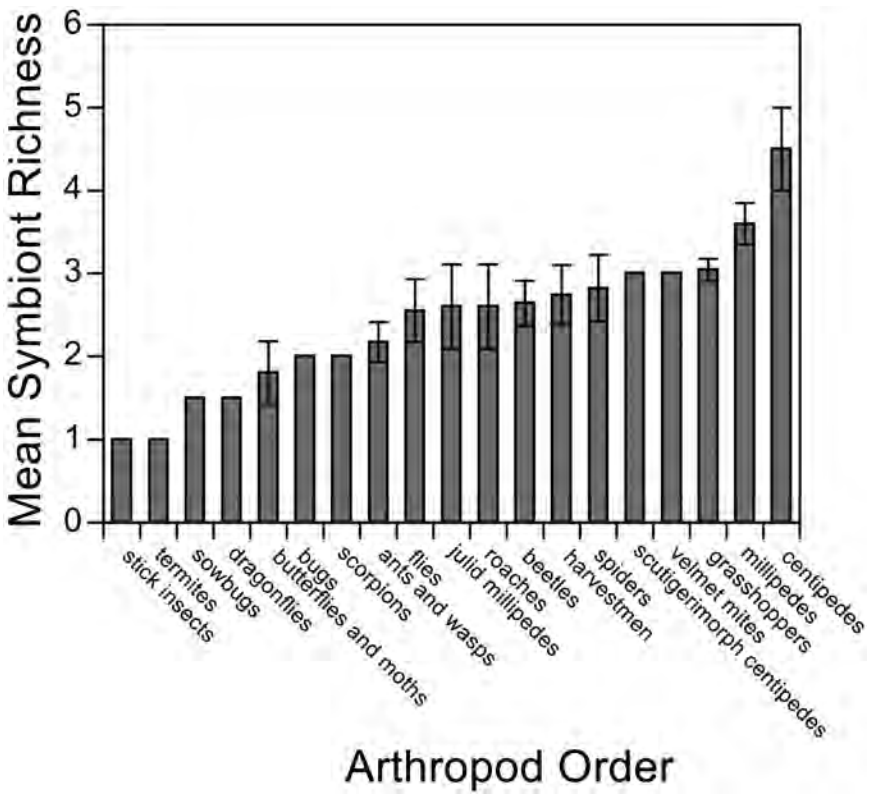

Figure 3. Sample data on microbial community richness compared among 19 arthropod orders collected. Data shown are mean numbers of microbe species collected from each order, with error bars representing standard error if at least five individual arthropods were sampled from that order. Microbial community richness was greater in centipedes and millipedes than in other arthropods, according to ANOVA ($F_{11, 106} = 2.40$, P = 0.01) and Tukey's HSD post hoc tests comparing orders with at least five individuals sampled.

Presentation/Dissemination

We recommend that each student team present a poster of their "Bugs on Bugs" project. The process of constructing and presenting posters develops skills in teamwork, formal writing, organization, public speaking, and creativity (Figure 4). Newbrey and Baltezore (2006) provide a

useful poster-construction resource for biology students of all levels.

If "Bugs on Bugs" is completed in a semester by separate groups of students in different courses, rather than in a learning community, we recommend separate poster sessions. Ideally, posters of two partnered teams with good liaisons will be similar; however, separate posters are suggested for two reasons. First, scheduling poster work times or poster presentations is logistically difficult if students from different courses have different schedules. Second, instructors from different courses may have different learning goals for the presentations and may have students working with different sets of data.

The methods for "Bugs on Bugs" are summarized in Figure 5.

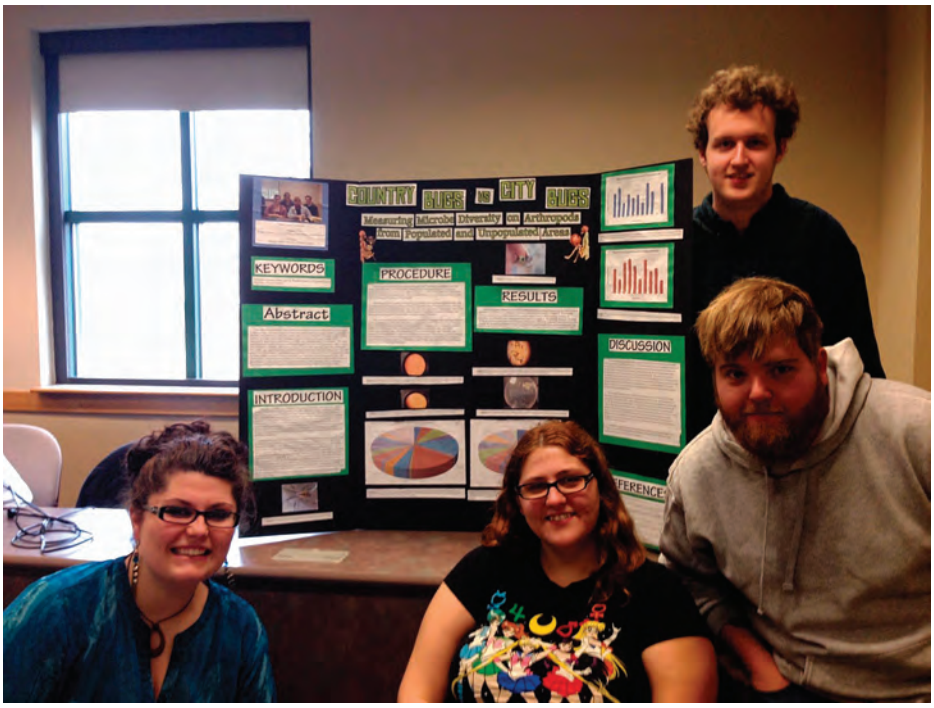

Figure 4. Example student poster presentation, fall 2013 semester.

Student Responses to "Bugs on Bugs"

We gave our students two separate surveys in fall 2013. One survey, used to evaluate students in the two courses that communicated and collaborated with each other, was given to the students in the microbiology course. Items on this survey were related to clear and accurate communication. We found that the eight liaisons visited an average of 2.1 times, with two liaisons attending only the one required meeting and two visiting all four possible days. Overall, liaisons were rated as average (see Figure 6).

The second survey, given to students in the organismal biology course, was used to assess how the project's learning goals were met. Items on this survey included questions about what students learned, as well as items about advice to future students and best practices. This survey showed that students overwhelmingly preferred the "hands-on" parts of the project, especially going outdoors to collect arthropods (see Figure 7). Not surprisingly, the most common best practices they mentioned were to communicate better with partners in the microbiology course, not procrastinate, follow directions, and better understand the goals of their projects.

Discussion

Best Practices

"Bugs on Bugs" succeeds in engaging both students and instructors when it is taught using an inquiry-based model, in which students are free to develop their own objectives and hypotheses as well as ways to meet these objectives by collecting and

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5
Course 1 Activities	- Learn field sampling techniques - Form teams - Develop questions, hypotheses - Design research protocol	- Collect arthropods - Record, analyze arthropod diversity data - Sample arthropods for microbes - Begin microbe cultures	- Collect arthropods - Record, analyze arthropod diversity data		- Students present research with poster or oral presentations
Shared Activities			- "Liaison" shares protocol, questions, hypotheses - "Liaison" provides plates	- Colony and microbe data are shared with "liaisons"	
Course 2 Activities			- Form teams - Colonies are isolated from plates	- Gram stain bacteria - Record all colony and microbe data	- Students present research with poster or oral presentations

Figure 5. Synopsis of the collaborative version of "Bugs on Bugs." Although students work on the project over several labs, each part of the project may take only a portion of a lab or multiple lab meetings.

analyzing data. This model can be more difficult on the instructor, who must ensure that students are looking at the "big picture," understand their own objectives, and are developing methods to meet them. Students inexperienced in inquiry-based teaching have a slight learning curve, which requires the instructor to provide moderate levels of support. Moreover, instructors must ensure that student teams have the research infrastructure (time, place to collect, equipment) needed to complete their project. Despite these difficulties, the engagement and stimulation from an inquiry-based activity makes it worthwhile.

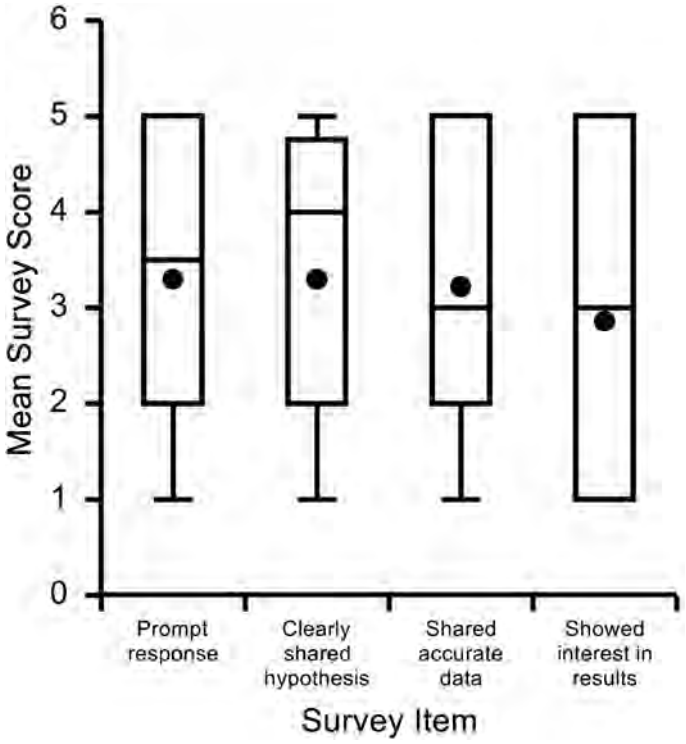

Figure 6. Responses to surveys asking students in the microbiology course to rate their liaisons from the organismal biology course. Ratings are on a 1–5 scale, with 1 being the lowest rating. Surveys were collected in fall 2013. Means are represented by symbols (•), while other points represent the 10th, 25th, median, 75th, and 90th percentiles, respectively.

"Bugs on Bugs" as described here requires several class meetings to complete. E.L. used all of two 2-hour labs and brief portions (30–45 minutes) of two others, along with a few "open lab" times. J.M. used portions of four 2-hour lab meetings. We consider this investment of time well justified, given that the project can be used as an inquiry-based alternative to multiple "survey" labs that are traditionally used to teach biodiversity.

Effective collaborative learning is an important part of science education and is especially important for "Bugs on Bugs." Instructors must commit to collaborative teaching and learning, and plan and structure the collaborations among different classes. We believe that learning communities are exceptional environments for this project and for others of its nature. If the participating courses have separate groups of students, we strongly recommend structured meetings between liaisons. If whole teams can meet each other to discuss objectives and findings, that is preferred. At the very least, partner teams should meet to share samples and results from their specialized portions.

We have taught "Bugs on Bugs" both as a long-term collaborative project (as described above) and as a short-term laboratory activity completed with just one group of students. In the latter implementation, students collect and sample the arthropods during one lab period and then observe the plates during a second lab period. The microbe colonies are not Gram stained, but students are still able to collect diversity data that can be analyzed as described above. The shorter implementation was used during summer 2014, a term too short to implement the collaborative version.

It is important that students know that culture-based surveys of microbial communities offer only a partial picture of the true structure of those communities. It is estimated that globally, only 1% of microbes can be cultured (Pace, 1997); and many symbionts of insects cannot be cultured (Broderick et al., 2004). Microbes that cannot be cultured can instead be surveyed by using molecular techniques to examine genetic sequences such as 16S rRNA genes. Molecular techniques may be a useful addition to future iterations of "Bugs on Bugs," particularly if this project is used in upper-level molecular biology and genetics courses.

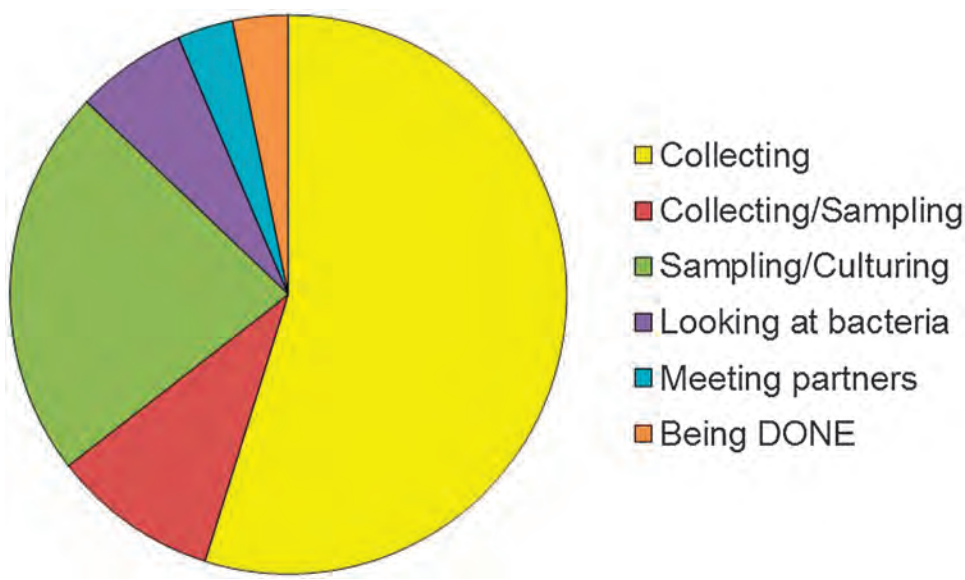

Figure 7. Responses to surveys asking 38 students in the organismal biology course to name their favorite part of "Bugs on Bugs." Surveys were collected in fall 2013.

Conclusions

"Bugs on Bugs" is an engaging, hands-on, and fun project that integrates multiple disciplines of biological sciences and teaches important collaborative and quantitative skills along with biology content. Students will be

engaged by the opportunity to develop and answer their own research questions and will enjoy the hands-on learning outside of the classroom. Discovering what microbes are growing on a plate, and realizing that they were present naturally on a tiny arthropod, is captivating. We as instructors are also very stimulated intellectually by the inquiry-based project, since we can have no idea, in advance, what results students will discover. We believe that with careful planning and design, "Bugs on Bugs" can be successful at other institutions, and we hope that others will be interested in adding it to their biology curricula.

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