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INQUIRY & INVESTIGATION

Investigating Issues in the Laboratory: The Behavior of Red Swamp Crayfish as an Invasive Species

KRISSI M. HEWITT, LORI J. KAYES, DAVID HUBERT, ADAM CHOUINARD

Abstract

Recent reform initiatives in undergraduate biology call for curricula that prepare students for dealing with real-world issues and making important links between science and society. In response to this call, we have developed an issues-based laboratory module that uses guided inquiry to integrate the concepts of animal behavior and population biology into an issue of both local and global relevance. The issue associated with this module is "What should be done about invasive crayfish?" Students investigate plausible reasons why crayfish are often successful invasive species through hypothesis testing, collection of behavioral data on live crayfish, and quantitative reasoning. Students also consider economic and environmental impacts of invasive species on local and global ecosystems. We implemented this module in a large introductory biology course and conducted survey research to evaluate the module's potential to serve as an interesting and valuable learning experience for undergraduate biology students.

Key Words: *Invasive species; behavioral ecology; animal behavior; crayfish; societal issues.*

Research has shown that learners' interest in an activity can influence their relative engagement in the activity, the types of goals they set, and the learning that takes place during the activity (Hidi & Renninger, 2006). Issues-oriented science is a pedagogical approach that has been shown to increase student interest in science via their participation in authentic, inquiry-based activities while addressing important socioscientific issues that are personally and globally relevant (e.g., Lenz & Willcox, 2012). Socioscientific issues are complex social problems with technological or conceptual ties to science of personal or global relevance. Approaches to solving these issues require the application of scientific concepts and the negotiation of political, economic, and ethical factors (Sadler, 2011). Many socioscientific issues have a significant biological component and therefore require a certain level of biological literacy for public understanding. This makes the issues-oriented instructional model outlined by Lenz and Willcox (2012) an ideal approach for introductory biology lab activities. This model provides the context for risk evaluations, collective decision making, and the interpretation of scientific data through class discussions. Moreover, the model follows current recommendations for undergraduate biology education reform outlined in the AAAS's (2011) *Vision and Change in Undergraduate Biology Education: A Call to Action* by creating student-centered classroom environments based on active learning and student discourse (AAAS, 2011).

The socioscientific issue addressed is the ecological and socioeconomic impact of invasive species.

We developed the following laboratory module for undergraduate introductory biology classes. The socioscientific issue addressed is the ecological and socioeconomic impact of invasive species. We linked traditional introductory biology content (animal behavior and population biology) with a current, locally relevant issue (What should be done about invasive crayfish?). This module was designed to provide context and relevance to students learning animal behavior and to align with the core concepts and competencies outlined in *Vision and Change* toward the goal of biological literacy (see Table 1). Student learning outcomes for the module, based on course content, are as follows:

1. Define and explain important terms and concepts in invasion biology, animal behavior, and behavioral ecology.
2. Develop and test a hypothesis in order to predict winners of agonistic interactions.
3. Demonstrate the ability to collect data from live crayfish and track behavioral interactions.
4. Conduct a chi-square test to determine the statistical significance of experimental results.
5. Apply knowledge of animal behavior to the issue of invasive species.
6. Discuss possible solutions to prevent the introduction of invasive species and minimize their effect on native species.

We designed this module for use in a large, university-level, majors introductory biology course in a single 3-hour class period with ~30 lab sections per week, with 40 students per section (~1200 students). In this authentic research project, students investigate factors predicting

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Table 1. Alignment of laboratory module activities with *Vision and Change* (AAAS, 2011) core competencies.

Vision & Change Competencies	Module Activities
Ability to apply the process of science	Students generate and test hypotheses, gather data, observe nature, interpret data, and apply their findings.
Ability to use quantitative reasoning	Students use statistical tests to analyze and interpret their data.
Ability to tap into the interdisciplinary nature of science	Students are exposed to research from fisheries and wildlife, population biology, animal behavior, and environmental advocacy.
Ability to communicate and collaborate with others	Students collaborate in groups to develop their hypotheses and perform data collection and analyses. They discuss invasive-species issues in small and large groups.
Ability to understand the relationship between science and society	Through linking animal behavior to the socioscientific issue of invasive species, students explore scientific procedures and the impact of scientific data on environmental policy.

the winners of agonistic interactions between invasive *Procambarus clarkii* (red swamp crayfish) and the implications for invasive-species management. Students learn concepts and principles of animal behavior while applying this information to a real-world issue.

○ Crayfish as Invasive-Species Models

Red swamp crayfish was an advantageous study species for these topics because of its value as a model for both ecological invasion and behavior. Specifically, we chose crayfish because they provide an excellent example of a locally relevant ecological issue. Given the cosmopolitan nature of their role as invaders, this module will have local relevance in a great many other places and can be adapted to many geographic areas. It is appropriate for both secondary and post-secondary biology courses.

Additionally, while several hypotheses exist regarding the mechanisms that facilitate the exclusion of native species by invasive crayfish, there is agreement that agonistic (aggressive and submissive) interactions between and within species play a large role in the exclusion of native species (Gherardi & Daniels, 2004; Gherardi, 2007). Agonistic behaviors are well documented, reliable, and easy to observe in a laboratory setting (Tierney et al., 2000), making them ideal for students who lack experience with behavioral analyses. Finally, crayfish are readily available from distributors and easy to maintain in a laboratory setting.

○ Materials

Table 2 outlines the materials needed for instructors to successfully run the laboratory module in a large or small course.

○ Methods

Part 1: Agonistic Behavior

Crayfish Care and Rotation: When not in use by students, crayfish were stored individually in disposable Tupperware containers with holes in the lid. If crayfish are stored together, they will establish a dominance hierarchy and will not interact during class (Bergman & Moore, 2005). The housing containers were filled with 400 mL of spring water or treated tap water, and a small square cup was kept in the container to provide shelter and was used to catch and release crayfish. For the best results, each crayfish pair should interact only once. To ensure that students and instructors could track which crayfish had interacted, the containers were labeled (A–J) and the crayfish carapace and containers were painted with corresponding colors of nail polish. Students could select any two crayfish that had not been previously paired in a contest. They recorded the pairings on a tracking sheet that was used for subsequent class sections to ensure that crayfish were matched in novel pairs for each contest. To prevent mortality or experimental bias, instructors should not allow pairing between recently molted crayfish or disparately sized crayfish. After the completion of the laboratory module, crayfish should be disposed of, first by immersion in clove oil or Eugenol (0.125 mL/L) and then by freezing for 3 weeks (to ensure death). This is a humane euthanasia method (AVMA, 2013). After freezing, you can dispose of the crayfish by double bagging them for trash collection.

Setting the Stage. Students completed a premodule exercise in which they watched a CBS video (http://youtu.be/mUssO68D2eM) and read an article about invasive species and behavior as a proximate cause of invasion success (Holway & Suarez, 1999). They were required to answer questions, including brainstorming about which physical attributes of crayfish might be useful to measure when comparing invasive and noninvasive crayfish and ways to standardize behavioral observations. In the laboratory class introduction, students watched a video about invasive crayfish in Oregon (http://www.opb.org/news/article/invasive-crayfish-may-be-class-pets-first/) that were initially released from classrooms; read a local pamphlet about the crayfish invaders; and then discussed, in groups, why people are concerned about invasive species, the types of problems they cause, their major sources of introduction, and ways to deal with populations of invasive crayfish. As a class, they discussed the cost and social ramifications of invasive-species control or the lack thereof. We structured this discussion as a think–pair–share of the following two questions: "Should we stop spending as much money on trying to control invasive species? Why/Why not?" and "What would be the societal ramification of stopping?" Students were briefly introduced to the link between behavior and invasive species, building on the article they read prior to class (Holway & Suarez, 1999), and how to measure animal behavior. For more information on methods for data collection and analysis in animal behavior research, Martin and Bateson (2007) provide a thorough yet accessible introduction. Students watched a video of a local researcher talking about his research and how the current module would contribute to his research on the behavior of red swamp crayfish in Oregon. Finally, students were instructed in respectful handling of live crayfish.

Table 2. Materials list per group of 4–8 students. Crayfish supplies are enough for 15 lab sections of 40–45 students each.

Item	Numbered Required	Notes
5½ gallon aquarium	1	
Cardboard box	1	Ideally only slightly larger than aquarium, cut to a height ~3 inches *below* the top of the aquarium (prevents crayfish from focusing on students peeking in)
Digital scale	1	Must be accurate at least to 0.1 g
Tray for scale	1	Sides must be high enough to keep crayfish from escaping (we used 1890-mL Tupperware containers)
Crayfish housing containers	12	Must have lid and be able to hold water (we used 1890-mL Tupperware containers)
Fast-drying nail polish of variable colors	10[a]	Used to mark crayfish (make sure it is easy to distinguish between colors)
Small square cups (with holes in bottom)	12	Need to be able to fit in container when closed; used as shelter for crayfish and to transport them between containers (we used 3-inch planting pots)
Timer	1	
Small plastic ruler	1	
Medium aquarium fish net	1	
Local invasive species pamphlet	2	
Rabbit food pellets, small bag	1[a]	Each crayfish will need 1 pellet every few days
Crayfish (*Procambarus clarkii*)	12	Best to order all one size, as the extreme size differences do not work very well (we ordered from Carolina Biological Supply)

[a] Quantity will be sufficient for entire class.

Figure 1. Students are given a demonstration on proper handling of crayfish. Crayfish should be picked up directly behind the thorax to prevent pinching.

Physical Characteristic Variables. Students were given a demonstration of how to handle the crayfish (Figure 1) and how to collect the data for three variables (sex, weight, and length of the outer claw; Figure 2).

Group Hypothesis Development. Although students were previously informed that the aggressive behavior of the red swamp crayfish is important for its invasion success, they were also informed that crayfish behavioral patterns are still unclear and that there are still many open scientific research questions about crayfish social interactions. For example, local researchers are studying what factors may predict the winners of agonistic interactions between conspecifics. Students were told that they should write a hypothesis about which physical characteristic they thought would be the best predictor of a winner of an agonistic interaction (contest) between two crayfish. Students were asked to discuss how their hypothesis would relate to contests between invasive and native crayfish as a link to the topic of invasive species. The variables described above were selected for their ease of measurement and because the latter two are likely to differ between the native and invasive crayfish species (i.e., the natives weigh less and have shorter claws).

Animal Behavior Data Collection. Prior to each of the three contests, students recorded data for each of the three variables mentioned above for the two crayfish that were selected to participate in the contest. They also recorded the nail-polish color on the crayfish, and the letter on the container, to track the crayfish. To begin observing the agonistic interaction, crayfish were simultaneously released into a 5-gallon tank (inside a box, to prevent crayfish responding to students; it is important to ensure that the students are quiet and remain unseen by the crayfish). In multiple experimental trials done during the development of the lab module, it took

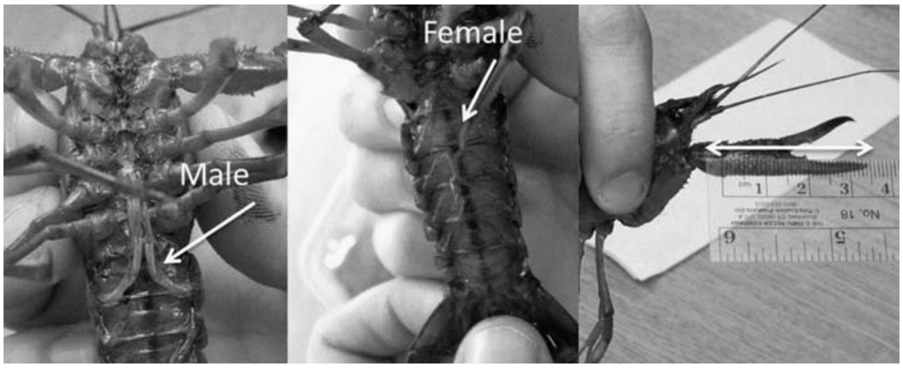

Figure 2. Measurements of sex and claw length were taken in addition to weight. Males have four calcified swimmerets just below the lowest legs, whereas females lack the swimmerets. Claw length was measured on the outer side of the claw.

Table 3. Ethogram of crayfish behaviors used to determine winners. Numbers in parentheses indicate whether the behavior was an attack or retreat (+/–) and the weight of the behavior (1/2). Each tally mark in the behavior table is multiplied by the number in parentheses corresponding to that behavior and then totaled.

Crayfish Agonistic Behavior Name & Weight	Behavior Description
Approach (+2)	Crayfish walks toward the other crayfish, generally with claws extended (perhaps posturing). Speed is greater than average walking speed. Ends when movement ceases or direction changes.
Posturing (+1)	Crayfish raises claws and front part of body but does not approach other crayfish. Generally, claws are very open during this behavior. Ends when crayfish lowers claws below plane of body, or when crayfish moves toward the other crayfish.
Retreat (–1)	Crayfish turns and walks away from other crayfish or postures and walks backwards; if the former, walking will be faster than average walking speed. Ends when movement ceases or direction changes (generally to approach).
Tail flip (–2)	A very obvious behavior. Rapid flick of telson (tip of tail), which propels the crayfish backward with its claws extended together and down in a streamlined position. Ends when all walking legs make contact with aquarium floor again.

an average of 4 minutes for the crayfish to acclimate and begin the interaction. It is important that the students have a consistent baseline to start recording their observations; this acclimation period of 4 minutes serves as the standardized value for this baseline and is consistent with the behavioral-observation protocols used by crayfish researchers. After 4 minutes, students started recording interactions in a behavior table, using an ethogram developed in association with a local researcher (Table 3). Each student in the group observed and recorded the interactions of both crayfish on their own and tallied the different behaviors for 6 more minutes. After the total 10 minutes, crayfish were removed from the arena tank and returned to their original containers. Students determined which crayfish was the winner by totaling the positive and negative tally marks for each behavior (note that some behaviors are positive and some are negative, as indicated in Table 3). During the tallying process, students discussed any differences they may have had in their observation numbers and took the average of their tallies across the behaviors. This allowed them to assess the inter-rater reliability of their observations. The crayfish with the highest total score was the winner. Each crayfish was indicated as a winner or loser in its data table, in association with its weight, sex, and claw length. Students repeated the data collection and contest two more times per group for a total of six crayfish per group. Class data were compiled by students or instructor as the numbers of winners and losers based on different traits (heavier weight, longer claws, male vs. female). For example, if the winner of contest 1 was a male in a male–female interaction, with longer claws and greater weight than the female, you would add 1 to each of those three categories. Note: In some male–female contests, the male will mate with the female rather than displaying aggressive behavior, depending on the season.

Data Analysis. Quantitative reasoning is an important skill to develop at the collegiate biology level (AAAS, 2011). Although many students have not yet had statistics, careful scaffolding of this activity can allow them to do an analysis using a simple chi-square test. This module requires that they use quantitative reasoning to test their original hypotheses and interpret the data. Students use the class totals to do a chi-square analysis based on the hypothesis that each group came up with at the beginning of the class. Chi-square tests are used in this module because they are easy for students to understand and are used to compare observed data with data we would expect to obtain according to a specific hypothesis (for more information on using chi-square tests in biology, see http://mathbench.umd.edu/modules/statistical-tests_chisquare_intro/page15.htm or https://www.youtube.com/watch?v=WXPBoFDqNVk). The expected values are based on the total number of winners observed if the outcome is based on chance (i.e., sum of winners + losers / 2). Students are asked to relate their results to their hypothesis and think about how the advantage of the physical attribute they explored – which either did or did not result in winning – might affect native crayfish populations in the event of an invasion.

Part 2: Foraging & Reproductive Behaviors

Crayfish invasions are not mediated solely by agonistic interactions; multiple other factors are likely to be involved in the displacement of native species by invaders (Gherardi, 2007). For this reason, the

Table 4. Questions for students in order to address the issue of invasive crayfish (modified from Maben, 2004).

Five Questions for Students to Answer		
1.	**Sounding the Alarm**	How can we, as citizens, call attention to the problem of invasive crayfish?
2.	**Scientific Data Gathering**	What evidence or data did you collect about crayfish behavior during this lab that would help in our understanding of whether, and in what way, invasive crayfish can be an environmental problem?
3.	**Risk Assessment**	What do you think should be done about invasive crayfish, and how expensive is your proposed effort likely to be? Be specific.
4.	**Public Awareness**	How will you let the public know that invasive crayfish are an environmental issue? What should they do about it?
5.	**Ongoing Follow-up**	What should we do to investigate whether or not your efforts have an impact?

Table 5. Closed-response survey statements with corresponding course data percentages (n = 397).

Survey Statement	Agreed[a] (%)	Neutral[b] (%)	Disagreed[c] (%)
The crayfish lab held my interest.	91.44%	5.81%	2.44%
The crayfish lab challenged me intellectually.	46.18%	36.09%	17.74%
During the crayfish lab, discussing with my classmates helped me learn.	80.74%	13.46%	5.81%
The crayfish lab helped me better understand the importance of studying animal behavior.	86.55%	9.48%	3.97%
The crayfish lab made me want to learn more about biology topics in general.	63%	28.44%	8.56%
The crayfish lab was a valuable learning experience for me.	73.7%	18.96%	7.03%

[a] Answers include "Strongly Agree" and "Agree."
[b] Answers include "Neither Agree Nor Disagree."
[c] Answers include "Disagree" and "Strongly Disagree."

curriculum also explores the additional factors of foraging and reproductive behaviors. To examine these behaviors in crayfish, students visited computer stations set up with presentation slides about foraging and reproductive behavior that they had not yet considered, discussed it with their groups, and answered questions related to how the differences in these behaviors between native and invasive crayfish provided an advantage to the invasive crayfish. They also considered how parental care was related to invasive species survival in novel habitats. Depending on the time of year, it may also be possible for students to observe live crayfish with eggs or young.

Part 3: Scientific Ideas & Modern Society

At the end of the module, students answered questions that tied their newly constructed knowledge of invasive crayfish behavior to possible solutions or ways to prevent the spread of invasive species (Table 4). The questions had them consider issues related to environmental policy and decision making (Maben, 2004).

Assessment of Student Learning

Students were formatively assessed throughout the module via sharing their data, answering questions, and discussing their action plan with their instructor and classmates at checkpoints written into the curriculum. The students were also assessed via a postmodule quiz based on their learning outcomes. Our GTAs are extensively trained by an education professional in pedagogical theory, including on how to lead discussion, Bloom's taxonomy, how to do effective formative and summative assessment, and how to be scientific teachers.

Survey Research on Laboratory Module

The research of the module focused on the students' perceptions of their learning experience immediately after completing the module (Table 5). An anonymous survey was composed of modified survey questions from assessments of another postsecondary science laboratory module (Kalinowski, 2012) and several questions developed by the authors that reflected the potential gains of teaching an issues-oriented science module (Lenz & Willcox, 2012). Closed-response survey questions used a rating system, as follows: 1 = Strongly Agree, 2 = Agree, 3 = Neither Agree Nor Disagree, 4 = Disagree, and 5 = Strongly Disagree. Of the 483 students who were asked to participate in the survey, 397 agreed, for a response rate of 82%. Table 5 reports the percentage of students (n = 397) who agreed, disagreed, or were neutral with regard to the survey statements.

As is apparent from the survey results, this module was incredibly successful with the students in terms of holding their interest (91.44% agreed) and in giving them a better understanding of the importance of animal behavior (86.55% agreed). The students felt that opportunities for discussion with their groups helped them learn (80.74%). The majority of students (63%) also indicated that it made them want to learn more about biology in general. Many students (46.18%) agreed that the module challenged them intellectually, but many were also neutral in regard to this statement (36.09%), and a minority of the students (17.74%) disagreed.

○ Discussion & Implications

We have developed and implemented this laboratory module successfully in a large introductory biology course for majors, taught by 14 graduate teaching assistants. Both the students and the teaching assistants reported being engaged in the laboratory module.

The survey assessment shows the effectiveness of the activity on students' engagement, interest, and valuing of the learning experience. The use of an easy-to-implement paper survey in class has allowed us to think about future improvements to the module. For example, some of the students did not report being intellectually challenged by the activity, so we are currently adjusting the module to be more challenging by having students use population biology concepts and modeling to predict the spread of invasive crayfish in their local waterways. In addition, this module could be made more inquiry-based by having the students come up with a wider range of hypotheses to test and by giving them less context initially.

This module allowed us to teach traditional animal behavior content with population and invasion biology while informing our students about an important issue in their local and global communities. Although we were unable to use native crayfish in the contests because of the difficulty of keeping them alive in lab conditions, we had the students relate their hypotheses and their results to the potential interactions of invasive and native crayfish as a means to get them to think about this connection and the design of their experiment. The module was carefully designed on the basis of core concepts and competencies outlined in *Vision and Change* (AAAS, 2011), and it is highly adaptable to be made locally relevant to both postsecondary and secondary biology classes, especially in large introductory lab courses.

○ Possible Module Extensions

If you have more than one lab period for this exercise, students can develop their own ethogram as a class during the first lab period. Another possible extension of this activity for an animal behavior course would be to do a methodological comparison between counts of behaviors and duration of time spent in various behavioral states to compare the two measurement techniques and discuss which would be the best method in this species. Students could also do some independent research on native crayfish behavior and physical characteristics for comparison purposes.

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