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Understanding the invasion history and dispersal patterns of the faucet snail, Bithynia tentaculata

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ABSTRACT

UNDERSTANDING THE INVASION HISTORY AND DISPERSAL PATTERNS OF THE FAUCET SNAIL, *BITHYNIA TENTACULATA*

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Invasive species pose a major threat to biodiversity and are becoming increasingly common across the globe. The success of these invasive species often comes down to an ability to disperse effectively into novel environments over time and at multiple scales. Aquatic invasive species can be particularly challenging as they are not readily visible and are often well established before being formally observed. In the Upper Mississippi River region, an invasive European snail species, the faucet snail (*Bithynia tentaculata*) has invaded from Europe via the Great Lakes. The snail serves as an intermediate host for several species of trematode parasites that have been implicated in causing large-scale waterfowl die-offs in the region. It is unclear currently whether the trematodes co-invaded with the faucet snails or if they are a product of spillback (i.e., invasive species serving as alternative hosts for native parasites). Here, we analyzed faucet snail movement both a small-scale and large-scale range. For small-scale dispersal analysis, we created a framework for undergraduate-level, student-driven experiments addressing abiotic and biotic factors' affect on faucet snail movement within their immediate

surroundings. This framework offers opportunity for continued research on small-scale dispersal as well as promoting students' applied skills and continued engagement in the field of science. For large-scale dispersal analysis, we used genomic sequencing and population analyses to estimate the overall population structure and likely invasion pathway for the faucet snail. Adult snails were collected from Europe (native range), the Erie Canal (assumed early point of introduction) and several points along the Mississippi River (current invasion forefront). Population genomic analyses indicate a large degree of differentiation between snails in the native and invasive ranges. There was a lesser degree of differentiation between Erie canal and Mississippi river populations, and almost no genetic differentiation between populations within the Mississippi river. These analyses show that gene flow is the highest among populations at the current invasion forefront indicating that populations are not moving or becoming separated after movement due to individual external factors rather that they are able to consistently move amongst themselves as they spread down the Mississippi River. This study lays the groundwork in assessing the invasion history of the faucet snail so that a similar genetic analysis of the movement of the trematodes can be used to create a parallel invasion history for the two groups to assess the likelihood of co-invasion.

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CHAPTER 1

AT A SNAIL'S PACE: LABORATORY EXPERIMENTS USING FAUCET SNAILS TO INVESTIGATE BIOTIC AND ABIOTIC VARIABLES AFFECTING SMALL-SCALE DISPERSAL

Abstract

Course-based Undergraduate Research Experiences (CUREs) allow learning that is hands-on and student-driven fostering engagement and retention of knowledge in any discipline. Here, we use this learning framework to engage students in exploring dispersal patterns as a topic in ecology. Mollusks, specifically faucet snails, are used in a laboratory setting to explore variables affecting dispersal of an invasive species. In this lesson, instructors provide basic context for experiments while students direct their own learning through brainstorming, hypothesis development, and problem solving in pairs or small groups. Students create and run their own experiments and analyze their own data to answer questions about what affects snail movement. This student-driven learning allows students to engage with the scientific process and explore core concepts of invasion biology and ecology in a more meaningful way. This lesson can be modified based on class size, skill level, and class time, meaning it is designed to take one lab period to complete at a minimum, but also functions as a unit- or semester-length project. This lesson highlights the value of learning collaboratively and fosters confidence in students' applied skills in science.

Lesson Context

Learning Goal(s):

- Develop students' understanding of the scientific method
- Understand the importance of experimental design in testing a hypothesis
- Understand how animal behavior/movement is affected by biotic and abiotic variables
- Create connection and engagement with ecology concepts through student-led hands-on experiments

Learning Objective(s):

- Collaboratively design and conduct dispersal experiments
- Problem-solve through peer consult and trial run(s) of experiment
- Analyze data to support or refute dispersal-related hypotheses
- Summarize and communicate scientific findings to a general audience

Introduction

Course-based Undergraduate Research Experiences (CUREs) have become a mainstay of scientific teaching pedagogy. CUREs as a teaching method involve student-led inquiry into a subject allowing for students to ask questions and explore a subject in a hands-on format in the classroom. CUREs allow for all students regardless of their background to have the same opportunity to experience applied lab work creating inclusivity in science teaching at the undergraduate level (Bangera & Brownell, 2014). The CURE format has been shown to increase knowledge retention as well as promote self-efficacy and confidence in students (Brownell et al. 2015, Cooper et al. 2020). Further, CUREs facilitate perseverance through failure and an understanding of the importance of failure in scientific research (Heller et al., 2020; Lopatto et

al., 2020). Overall, CUREs are a methodology that promotes critical thinking and problem-solving skills in an applied format to engage students in their own learning. Here, we use the CURE format to develop a lesson plan exploring dispersal pattern of invasive species.

Invasive species are defined as non-native species that are introduced and establish a population in a novel range (Iannone et al., 2020). Invasive species cause harm to native ecosystems through multiple means including predation, competition, disease, and parasitism (Beltrán-Beck et al., 2012; Chalkowski et al., 2018; Doody et al., 2009; Potts et al., 2010). These negative interactions often lead to decreased biodiversity and decreased ecosystem stability which is the basis for many conservation and restoration efforts (Bellard et al., 2016; Powell et al., 2011). Often, invasive species are considered generalists, meaning they can survive in a wide range of habitats disproportionately affecting native species and native ecosystems, which include many specialists – confined to a relatively narrow set of abiotic and biotic variables for survival (Clavel et al., 2011; Taillie et al., 2021). Aquatic invasive species are particularly challenging in part because they relatively inconspicuous and often able to rapidly reproduce (Hansen et al., 2013; Semenchenko et al., 2018). Perhaps the most widely known example is the zebra mussel (*Dreissena polymorpha*) which was introduced to North America through ballast water from ships and was first highlighted as a concern in the late 1900s (McMahon, 1996). Zebra mussels quickly became a major biofouling concern as well as affecting biomass of phytoplankton thereby altering food web structures (Claudi, R., Makie, 1994). These aquatic invasive species' ability to quickly reproduce is compounded by the aquatic habitat, which provides an ecological advantage by concealing harmful activity from human observation, increasing the time until intervention (Moorhouse & Macdonald, 2015).

Here, we address what specific variables play a role in the dispersal of an invasive species at a small scale to create a foundation for answering questions about large-scale dispersal events. Understanding the variables affecting the movement and dispersal of an invasive species is crucial for applied management practices. Abiotic and biotic variables, such as temperature or soil moisture and vegetation cover or pathogen infection play a major role in limiting or furthering a species' range (Neuschulz et al., 2018; Stelinski, 2019). At a continental scale, long-distance jumps of species often occur due to anthropogenic forces, such as boats and planes, and have become increasingly common as globalization increases (Bertelsmeier, 2021). Human-mediated dispersal has vastly increased the ability of invasive species to move over very long distances (i.e., from one continent to another). For example, the Argentine ant (*Linepithema humile*) has invasive populations across several continents including North America. Within the United States, they are also widespread across the states (Wetterer et al., 2009). Suarez et al. (2000) quantified the dispersal of the Argentine ant at a global and regional scale and determined that anthropogenic forces were a primary factor in the number of long-distance dispersal events (Suarez et al., 2000). Long-distance dispersal events do not only include cross-continental movements but can also occur at the level of across distances such as states or towns. For example, the gypsy moth is a Eurasian species that first invaded North America in Massachusetts in the late 1800s (Elkinton & Liebhold, 1990), but has since dispersed across states as far as North Carolina and Wisconsin (Tobin & Blackburn, 2008). The primary dispersal method employed by gypsy moths is passive transportation at a larval stage that uses silk threads and wind. Tobin and Blackburn (2008) investigated how long-distance dispersal events facilitated population movement in the past and assessed current dispersal patterns across Wisconsin. They

found that gypsy moths were able to disperse in low numbers and maintain a presence in a new location such that further dispersal events to the same area were more likely to create a stable population in those new areas. They also found that weather patterns (specifically wind currents) helped facilitate these dispersal events as the primary method of dispersal relies on passive transportation with wind. Now that gypsy moths have more dense populations in Wisconsin the frequency of these long distance dispersal events may not be as apparent as populations are already established (Tobin & Blackburn, 2008).

Studies of the biotic and abiotic variables affecting fine-scale movement patterns gives insight into the specific mechanisms underlying patterns of larger scale dispersal. The lesson outlined here investigates the variables affecting fine-scale movements of an invasive freshwater snail to determine the proximate mechanisms underlying large scale dispersal throughout North America. Faucet snails (*Bithynia tentaculata*) are small freshwater snails that reach a maximum of approximately 12-15mm shell length. They graze on algae by crawling along substrates and filter feed using their gills (Jokinen, 1992). Their lifespan is anywhere from 17-39 months, both in the field and in the lab (Jokinen, 1992). The faucet snail is native to Europe but was introduced to North America in the late 1800s likely through ship ballast water (Mills et al., 1993). The faucet snail is highly reproductively prolific, getting its common name from its biofouling effects, clogging faucets and drainages, not through the small size of individuals but the large size of its populations (Nakano & Strayer, 2014). According to natural history observations, the faucet snail was first introduced to the eastern Great Lakes region in the late 1800s and has since made its way westward across the Great Lakes into the Upper Mississippi River where it is currently moving southward (Mills et al., 1993). The faucet snail is known to

outcompete native species of snails in North America (Roy et al., 2016). Beyond its competitive effects, the faucet snail is also host to several parasitic trematode species that have been implicated in major waterfowl die-offs on the Mississippi River (Poulin & Cribb, 2002; Sauer et al., 2007). Due to its ability to outcompete native snail species and its role as host to parasites also affecting native species, the faucet snail has the potential to significantly alter ecosystems in the Mississippi River. Studies of the large-scale dispersal patterns of the snail are ongoing, but studies investigating variables affecting movement at a local scale are still needed. Here, we propose a series of experiments that can be done in a classroom setting that will help to address this important gap in knowledge. Our goal is to both inform researchers looking at mechanisms of local dispersal in invasive species but also to engage students through hands-on learning experiential learning.

Intended Audience

This lesson plan is intended for undergraduate biology students. Students learn about small-scale dispersal of invasive species through classroom-based hypothesis driven experiments. The experiment is formatted as a Course-based Undergraduate Research Experience (CURE). CUREs are known to have positive outcomes in terms of self-efficacy and student engagement as well as knowledge retention (Babkoor et al., 2015; Cooper et al., 2020b; Newton et al., 2017). The experiment is designed to further student understanding of broader ecological concepts including community and population biology, species-environment interactions, and mechanisms of invasion. Students will work collaboratively to foster science communication skills and create engagement with the learning community. The lesson plan includes structured guidance for carrying out their experiments while allowing for student-

directed choice in specific biotic or abiotic variables to test. During the lesson, students will create predictions using variables that they will manipulate to assess for their effects on small-scale dispersal. Measurements of snail movement will follow a standardized protocol, but students will be free to test variables of their choice within that context. Given that each student or student group writes their own hypothesis and set of predictions, they then also collect and analyze their own data to test the hypotheses. Depending on the variable tested and the number of replicates included in the study, many students may fail to find statistically significant support for their hypotheses. This reality allows students to better understand Type I and Type II errors, gain an appreciation for the work that goes into rigorous scientific experimentation, and, perhaps most importantly, understand how "failure" is a useful result that can inform future studies (Heller et al., 2020).

Pre-requisite knowledge

Students are expected to have a basic introductory biology background. This lesson can be completed with first-year undergraduate students, but a general understanding of the scientific method prior to the implementation of the lesson is recommended. Students will be presented with an explanation of invasive species and dispersal mechanisms prior to the experiment. CUREs are an experience with working through a problem largely independently to foster student ownership of their own learning and are highly adaptable to students at all levels.

Required learning time for lesson

This lesson was designed for, at a minimum, one 2hr 50min lab period.

Scientific Teaching Themes

Active Learning

Student-directed learning promotes ownership of the learning experience and fosters student engagement. LaDue et al. (2021) presented active learning as involving engagement in four categories: behavioral, agentic, emotional, and cognitive. To briefly define these terms, behavior engagement refers to directly visible effort made by students (e.g., participation in discussions, completion of worksheets, etc.). Agentic engagement refers to student-driven questioning and leading of their own learning process. Emotional engagement refers to students' sense of belonging, interest, or value in a class. Cognitive engagement refers to self-regulation and motivation in terms of engaging with the material through thinking patterns and strategies. This overlaps with behavioral engagement in some sense as a student can engage in the behavior or completing a worksheet, but to engage with it in a meaningful cognitive way relates more towards the actual thought processes and ability to communicate their ideas (LaDue et al., 2022). This lesson is intended to be a fully hands-on experience from start to finish requiring behavioral engagement meaning students are required to complete tasks and fill out worksheets. Students will engage in setting up and running their own experiments, followed by analyzing their own data. Think-pair-share activities in which students are asked to brainstorm and discuss ideas in pairs and groups are used to generate ideas and discuss broader implications of their experiment. These student-driven questioning and analyzing processes are forms of agentic engagement. Students will work in pairs or small groups for the duration of the lesson allowing for practice in communication skills and teamwork and to encourage emotional engagement through a sense of community. Finally, students will be asked to complete an assessment which will support

cognitive engagement. Cognitive engagement is interwoven with other types of engagement and the format presented in the lesson overall as processes of thinking about concept and ideas is necessary when doing the activities listed above. Assessment specifically provides the most direct method for instructors to assess student cognitive engagement with a subject.

Assessment

The basics of assessment for this lesson include student participation in all lab activities (may be measured by completion of all worksheets given to students). The students may also be assessed on their participation in peer discussion and data collection. Additional assessments are described and encouraged but require student work outside of the lab or additional lab periods to complete. These assessments include a written summary of the study following the general format of a scientific article or an in-class presentation of their results and interpretations. Both assessments require students to find supporting scientific literature to justify their study and to place their results within a larger biological context. In addition, students are asked to critically evaluate their own hypotheses and experimental design. A rubric has been provided for the written summary and presentation that draw from key learning objectives (APPENDIX F). These rubrics will also contain sections to assess student participation both in their own groups and in the context of providing peer consultation to other groups.

Inclusive Teaching

CUREs as a general format are found to promote diversity, equity, and inclusion (Bangera & Brownell, 2014; Malotky et al., 2020). CUREs allow all students in a course to have a hands-on research experience which may have previously only been available to select students with additional resources. From the instructor perspective, CUREs promote the mindset that all

students in a class can succeed given the right tools (White et al., 2020). This lesson is largely student-driven making it widely adjustable to varying levels of knowledge and experience. The amount of instructor oversight is also highly adaptable. We encourage instructors improve inclusion by facilitating role delegation within the group (e.g., leader, data manager, etc.) to push students outside of their normal comfort zone.

Lesson Plan

Pre-Class Preparation

Snails must be collected and kept for student use. Depending on time allotted and location of collection point, students can be involved with the collection process. For identifying possible locations to find faucet snails, instructors may use the natural history observations map provided by USGS which shows and lists dates and locations where faucet snails have been reported (https://nas.er.usgs.gov/viewer/omap.aspx?SpeciesID=987). The USGS site can also be used to help familiarize instructors on how to identify the snails. Faucet snails are generally not larger than 15mm in length and have a shiny brown shell with a rounded spire and an operculum with concentric rings (Jokinen, 1992). Faucet snails can be found along shorelines that are rocky or provide vegetation such as celery beds; they are not as often found in sandy areas. The snails can be collected by hand by picking up rocks to examine the surface and by brushing along plant stalks to feel for the snails. Faucet snails may be transported using water-filled buckets with sealed lids. The buckets should be kept in a cooler during transit to keep the snails out of direct sunlight or intense heat. In the lab, snails should be kept in dechlorinated tap water at room temperature and out of direct sunlight with air stones to oxygenate the water and a food source (we used a mix of BD Bacto™ agar and dried lettuce). The snails can be kept in tanks or buckets

or cups depending on what is available to the instructor. Water should be changed once per week and food made constantly available.

For instructors outside the range of faucet snails, substitution of another snail species will be necessary. Snails are useful for this lesson due to their generally consistent prevalence in a wide range of locations. Many species of snails are also easily kept and manipulated in a laboratory setting giving students the opportunity to get hands-on experience with animals without significant prior training or knowledge being necessary. Care should be taken to check for any necessary permits required to collect native snail species.

Kiddie pools (~1 meter diameter) should be set up ahead of time to ensure that there are no leaking or faulty pools. Pools should be filled to a few inches in depth using de-chlorinated water. Instructors can use regular tap water but a de-chlorination treatment such as Tetra AquaSafe Water Conditioner is recommended. This product can be found at most local pet or aquarium stores. Any items provided for possible student use (e.g., aquarium heaters, lights, etc.) should be tested to ensure they are in working order prior to the lesson.

Instructors should familiarize themselves with all the materials (both written handouts and equipment) before running the experiment. It is recommended that instructors run at least one trial experiment prior to implementation to familiarize themselves with the process. For faucet snails, 30 minutes per trial is sufficient to allow for snails to move from the pools center to the perimeter. If using a different snail species, more or less time may need to be allotted for trials to optimize data collection (i.e., provide snail sufficient time to move a measurable distance but avoid excessive movement such that tracking becomes difficult) (see APPENDIX C for more information about trial runs).

Several handouts should be prepared prior to class including the datasheet (APPENDIX B) and general instruction sheet for instructors (APPENDIX C). There are other optional documents including a reflection worksheet (APPENDIX E), and rubrics for assessments (APPENDIX F). During the experiment, students will need a writing implement, hard surface to write on (e.g., clipboard, hardcover notebook, etc.), a measuring tape, and a device with time keeping abilities (e.g., smartphone, watch, stopwatch).

Introductory Lecture (20 minutes)

To begin the lesson, a short lecture is used to present the overall structure of the experiment to be run and necessary background on the study species. First, the presentation introduces the CURE format for the overall lesson. Emphasis is put on the purpose of running student-driven CUREs as being a method of optimizing student engagement and knowledge retention. Indicating to the students why the lab is done in this format promotes engagement in the lesson. Second, the topics of invasive species and dispersal mechanisms are introduced, moving from broad explanations to the specific study system used for this lesson – mirroring the general information presented in the Introduction section above. The instructor will highlight how the study system fits into the local ecosystem. The last section of the lecture focuses on the scientific method, including a general overview of the steps to complete the experiment and the variables they can manipulate.

Brainstorm ideas and generate hypotheses (10 minutes)

Students will work in pairs or small groups to determine which biotic or abiotic variables affecting small-scale dispersal will be tested. Students will draw on information shared in the introductory lecture to generate hypotheses related to their chosen biotic or abiotic variable.

Table 1 shows several examples of abiotic and biotic variables students could choose to test. The resources made available by the instructor will limit the types of hypotheses that can be generated by students, so it is crucial to make students aware of what materials are available to them prior to this step. Students should then prepare hypotheses that include biological reasoning procured from exploration of scientific literature. Instructors can require that student hypotheses be stated in a standardized format such as "[expected observation] BECAUSE [biological reasoning.]" Using this format can help students associated their prediction with the factor being tested. For example: (1) Snails disperse faster at warmer temperatures because physiological processes required for movement are often optimized at higher temperatures, (2) Snails disperse faster if food is available in the pool because food is a top biological motivator of movement. Having well-reasoned hypotheses and predictions will be especially important in helping students reflect upon their results following the experiment, particularly in the case of outcomes not supporting their hypotheses. In instances where full-class synchrony or materials are limited, it may be useful for the instructor to choose the hypotheses for the class to test. In this case, students are still expected to articulate biological reasoning for the hypothesis given. Classes with statistical components in the curriculum are asked to draw a figure of their expected result to further clarify how their reasoning relates directly to the results they expect to see.

Table 1. Examples of possible biotic and abiotic variables affecting small-scale dispersal that students may choose to test.

Biotic	Abiotic
Density of snails	Temperature
Food availability	pH
Substrate present	Light
Infection status	Water composition

Trial run(s) (30 minutes)

The instructor will give a brief overview of how to run a trial either interacting with the actual materials and snails present or through verbal explanation. Using faucet snails, each official trial will run for ~30 minutes. However, in the interest of time, for the practice trials, instructors may choose a shorter run time (e.g., 10 minutes). Students should be clear on the general expectations and the roles that they will need to play during the trials before they do any hands-on work themselves. Students should run at least one full trial (including all the steps even if time allotted is minimized) to get an understanding of the entire process.

Peer consult (10 minutes)

After the initial trial is complete, students will review their work with peers. Peer review involves pairing 2-3 groups of students to evaluate the work of others and troubleshoot potential problems. In the case of an instructor-led hypothesis (i.e., where the whole class is assigned the

same hypothesis), this discussion should not only focus on potential problems, but should also be used to ensure a standardized methodology is being used by all groups. At the instructor's discretion, a full class discussion may also take place to facilitate additional problem solving. A Peer Consultation Worksheet is provided and can be used to guide students through the process of peer review.

Conduct trials (~60 minutes)

The number of trials students complete will largely depend on the amount of time allotted for the lab. Students should be asked to discuss why repeating an experiment is important and how many trials are necessary to create meaningful results (i.e., avoid Type I or Type II errors). Through each trial, group members should be assigned specific roles (in the case of pairs, the simplest division may be one observer and one recorder).

Input and Analyze Data (~40 minutes)

After completion of student trials, students are asked to input their data into an electronic format (such as Microsoft Excel). Students should keep a hard copy of their recordings to work from for input and emphasis can be put on working together in their groups to ensure accurate data is being entered. Generally pairs are optimal for this stage to keep all students engaged in the activity, but this can be modified depending on class size. Larger groups can split into smaller groups and input the same data separately from one another if necessary. Instructors will provide guidance in completing simple statistical analyses. Depending on student skill level, the mean distance traveled by the experimental and control groups, as well as the standard deviation, and standard error for each group can be calculated. The study has been designed to allow for various levels of analysis. Students can compare the means of each group graphically using bar

or box plots. If statistical significance is to be tested, the study design dictates that a t-test (or Mann-Whitney U test) will be sufficient for comparing groups. Instructors may need to provide guidance to complete these tests, though simple internet searches are usually sufficient to guide students. All tests can be done in programs such as Microsoft Excel (though the Data Analysis tool pack add-on may need to be uploaded). Instructors are encouraged to prompt students with questions about the biological significance of their results (e.g., even if you obtained statistical significance, do you think your results have biological meaning?).

Reflection (5 minutes)

A recap in the form of a brief discussion or written worksheet provides time for students to reflect on the overall concepts covered in the lesson. The reflection offers the opportunity for students to work through the biological significance of their results, something that is often missing from typical undergraduate lab activities. Some examples of questions to pose during this time include (1) Based on your experiment, what questions would you ask for future studies? (2) Was there anything about the methodology that undermines the validity of our results? (3) What are some limitations of the data? (4) What were the benefits/drawbacks of peer consultation during this process? (5) How does the experiment(s) and its results further our knowledge of broader ecological concepts? (i.e., How would their study further an understanding in the field of ecology?)

Explanation of Assignment or Presentation (5 minutes)

This time is used to explain any assessment options given for the work. The assignment proposed here is a scientific summary including an Introduction, Methods, Results, and Discussion in a written format. Another option is for students to prepare an oral presentation that

follows a similar format and can be presented to the instructor or the class. The lesson in its current form does not include time for student presentations. Including this option would require additional class time.

Discussion

The standardized protocol for measuring the movement of the snails was tested with the help of two undergraduate research assistant students. The undergraduate students were tasked with trialing the experimental portion of the lesson plan using temperature as the factor being tested for effects on dispersal. The students were introduced to the study system at the start of their time working in the lab. Students were provided scientific literature to read to further familiarize themselves with the system, and they were asked to communicate the knowledge gained in their own words at the end of the lesson to assess their retention of broad concepts underlying the experiment. The students were also asked throughout the experiment for their input on clarity of methods, hypothesis development, and any teaching strategies they felt would increase self-efficacy. At the end of the lesson, students wrote personal reflections. Both students reported that the lesson was more engaging than traditional lessons they have experienced in other undergraduate lab courses and indicated their desire to perform this type of lab as part of an official course. They were excited by the process of working directly with live snails and conducting their own experiments. Both students also indicated increased feelings of confidence in their ability to think scientifically and carry out experiments independently. One student reported an increased desire to pursue a scientific career path that involved research. These responses are similar to those reported by students conducting other CUREs (Cooper et al., 2020b; Lopatto et al., 2020).

Modifications and extensions

This lesson can be modified to fit the context of specific class size and layout. The overall format can be adjusted to fit a range of time availability as well as class sizes. Instructors should anticipate taking time to adjust this lesson plan to fit the goals of their specific class. In general, this lesson plan has a framework for students to gain practical skills such as proficiency at searching for and comprehending current scientific research, hypothesis generation, critical review of experimental methodology, written and verbal scientific communication, and teamwork. Instructors' goals may focus on all the skills to varying degrees based on the background knowledge of their class and intended learning outcomes for their course.

Pieces of the lesson can be expanded or condensed into a single lab period or unit- and semester-length projects. Changes would require more adherence to a set plan as far as options for student hypotheses as well as any specific methodology. For labs that have the time and resources to run this lesson for an entire unit (several weeks), there will be more time to explore in the most direct student-driven manner. For example, instructors may ask students to find and present relevant current scientific research related to measuring dispersal. In this case, a full lab period may be dedicated to instructing students in how to find relevant peer-reviewed literature on a topic and how to read scientific papers for comprehension. Students may then have multiple lab periods to run trials, critically review their methodology, and then complete official experimental runs of data collection. After the experiment, another separate lab period may be used to instruct students on analyzing their data using basic statistical tests. Assessments can also be scaled up or down to include all parts suggested here or only a single part of it. For example, single lab period lessons may only require completion of all worksheets given or a very brief

written or verbal summary whereas unit-length projects may involve multiple drafts of a longer written summary that includes an editing process as well as an in-class presentation from each group on their work.

The lesson is adaptable to an introductory-level or an upper-level course. In an introductory-level setting, the instructor might pare down the options in terms of what the students investigate and the amount of time they spend generating and troubleshooting ideas. In an upper-level course, students will be given more autonomy to lead their own experiments as independently as possible. For background on the system, the information should be tailored based on the level of the class as well. Introductory level classes should focus on broad concepts of invasive species and dispersal and the faucet snail as a species-specific example. The goal at the beginner level is to use this species-specific example to connect broad concepts of invasive species and dispersal to a real-world example to promote foundational comprehension and knowledge retention. The focus in advanced classes should delve deeper into specific mechanisms and link faucet snails to a larger context of invasive species and patterns of dispersal. At this more advanced level, students already have a foundation of understanding, so the goal is to allow them to build on their knowledge. Here, students are guided in learning how to critically analyze new information to fit into or change their current understanding of a topic which is a highly relevant skill for students as they advance in any field of study. Any modifications implemented by the instructor will change the level of specificity in student generated ideas and reasoning for those ideas (i.e., introductory students receive more guidance and are required to produce less robust reasoning for their conclusions whereas advanced students work more independently and are asked to explain their reasoning in more detail).

Regardless of constraints of time or level of the course, this lesson is intended to foster a group effort in furthering understanding of ecological concepts by connecting them to real world examples.

CHAPTER 2

A GENOMIC APPROACH TO MAPPING THE INVASION HISTORY OF THE FAUCET SNAIL IN ITS INVADED RANGE

Introduction

Invasive species have become increasingly common worldwide. The introduction of invasive species to novel ecosystems poses a major threat to native populations and biodiversity as a whole (Doherty et al., 2016; Dueñas et al., 2021; Molnar et al., 2008). Invasive species disrupt native ecosystems through predation, competition, parasitism, and disruption of ecosystem functions (Damas-Moreira et al., 2020; Doherty et al., 2016; Dukes & Mooney, 2004; Laverty et al., 2017). Because of invasive species' potential to drastically alter native ecosystems, management practices of these populations are crucial to maintaining biodiversity in the system. Understanding how an invasive species was able to establish populations in a novel range can be crucial to create effective management strategies.

One of the first questions that arises in looking at an invasive species is where they came from and how they successfully moved from beyond their native range. Though invasive species are a cause for concern, they often remain elusive until the point they are causing major observable consequences to a native system (Darling & Blum, 2007; Lodge et al., 2006). This delay between invasive species' original introduction to a novel system and their consequences becoming apparent makes identifying how they were introduced difficult. Natural history observational data is often the first record made of a species. Although natural history data does provide information regarding where a species is, it does not necessarily provide a full

understanding of when introductions took place or in what order multiple introductions of a species occurred. As such, it is difficult to retrace invasion pathways through observation alone. Invasion pathways can be recreated through use of genetic tools. Genetic tools can reveal an invasion history that either corroborates natural history observations or that provides further insight into cryptic pathways of invasion.

Faucet snails (*Bithynia tentaculata*) are an invasive species originating from Europe. Faucet snails were first introduced to the Great Lakes in the late 1800s, likely through ballast water of ships (Mills et al., 1993). Since then, they have made their way into the Mississippi River likely through recreational boating and have slowly moved southward. Faucet snails have most recently been recorded in two lakes in Montana, also likely via transfer on recreational boats (Roy Véronique St-Louis Jared House et al., 2016). The invasion of the faucet snail has been highly successful. Faucet snails are generalists, as many invasive species often are, meaning they can maintain strong populations across a range of conditions. This is very similar to the case of zebra mussels which is perhaps the most well-known example of an aquatic invasive (Strayer, 2009). The commonality between these two invasive species lies in their ability to be highly reproductively prolific. Through their high proliferation and their generalist capabilities, faucet snails have a competitive advantage compared to native snails in North America (Schock et al., 2019). Beyond disturbing native species and ecosystems, faucet snails are an intermediate host of several species of trematodes including *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus* (Hoeve & Scott, 1988; Sauer et al., 2007). These species of trematodes have been linked to large-scale waterfowl death (particularly lesser scaup [*Aythya affinis*] and American coot [*Fulica*

americana]) as waterfowl are the definitive host in this trematode life cycle system (Sauer et al., 2007).

Here, we use population genomics tools to assess differences between populations and to compare these results with natural history observations. The goal of this study is to recreate the pathway of invasion of the faucet snail in North America. Though natural history observations of the faucet snail suggest they were introduced to the Great Lakes and invaded in a generally east to west route, a comprehensive invasion history is still lacking. Using genomic tools allows for a current estimate of shared alleles between populations to be made which can then be compared to our understanding through natural observations to assess whether the genomic population structure lines up with the natural observations or if there is an area of disconnect between the two. The two possible overarching outcomes for this assessment are either: (1) the pathway of invasion follows natural history observations or (2) the pathway of invasion does not follow the pattern described by natural history observations. Within these two scenarios, there is room for further elucidation of patterns of multiple introductions or multi-dimensional spread from an initial introduction point(s). Genomic analyses allow for clarification of a more accurate pathway of invasion than through natural history observations alone. With natural history observations, time at which an invasive species is first reported may not line up with when that invasive species actually first arrived in that location. Often invasive species go through a lag period in which their populations grow before spreading, so the invasive species effects on the ecosystem may not become readily apparent until the expansion stage (Havel et al., 2015).

In this study, snails were collected from locations in their native range (Netherlands) and their invasive range (including New York, and several locations along the upper Mississippi

River) to examine possible scenarios of invasion based on genomic analysis. The results from this study may also allow for some commentary on the future potential spread of the faucet snail, although there are many other factors to consider in the case of future movement of an invasive species. This study is a foundational step toward understanding the invasion dynamics of this system and the evolutionary processes that may have facilitated the invasion.

Methods

Between April 29 - June 2, 2016, hundreds of adult faucet snails were collected from seven locations including the Netherlands, Oneida Lake in New York, and 5 Navigation Pools (hereafter, Pools) in the upper Mississippi River (Figure 1). The river is divided by a series of Lock and Dams that divide it into separate but not isolated Pools. Snails were collected from two locations in Pool 7, the Fred Funk Boat Landing (n=13) and the spillway (n = 5). The two locations in Pool 7 are approximately 6 km apart, on opposite ends of Lake Onalaska, a large back water pool of the Mississippi River. We found almost no genetic differentiation between individuals from these two locations and so for simplicity, they have been combined into a single Pool 7 sample. The Fred Funk boat landing collection location was used for estimates of geographic distance (e.g., isolation by distance). At all sites, snails were collected by hand from rocks and vegetation at the shoreline and transported live back to University of Massachusetts - Dartmouth. Snails were subsequently euthanized in the lab by freezing and stored at -20°C until further processing. DNA extractions of at least 30 whole snails (shells removed) per location were done using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA). The amount of DNA in each extraction was quantified using PicoGreen (Ahn et al., 1996). Samples that met submission criteria (e.g., >10 ng/µL) were submitted for Restriction-site Associated

DNA sequencing (RADseq) to Floragenex, Inc. (Eugene, Oregon, USA). Floragenex, Inc. completed all library preparation steps, sequencing, and initial data quality checks.

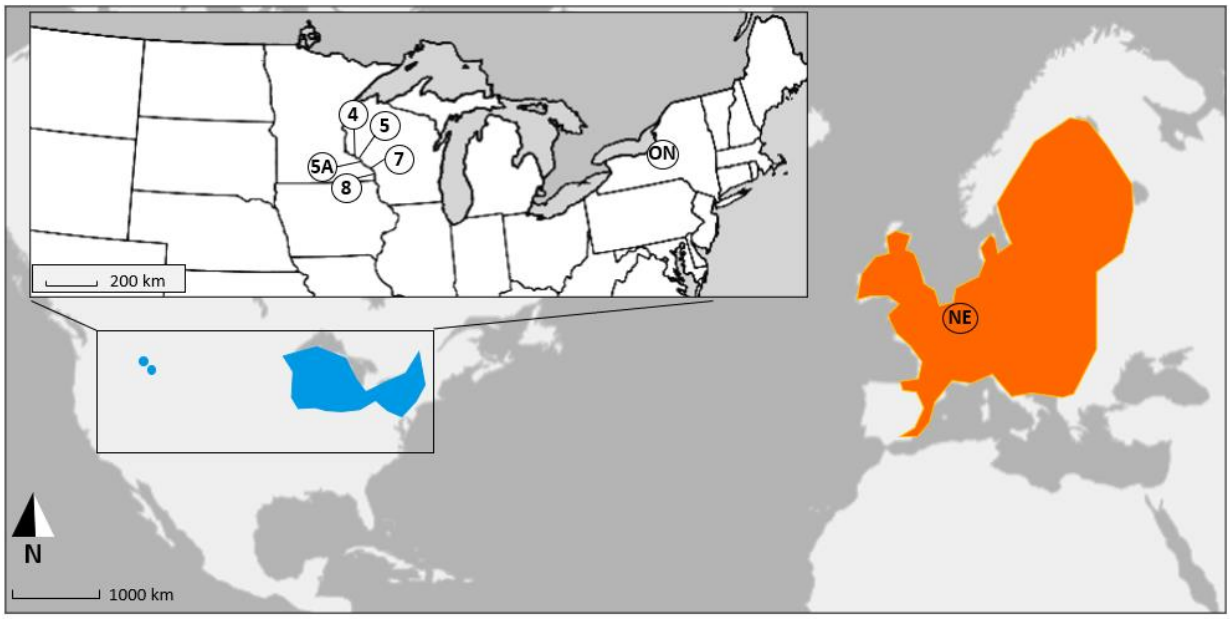

Figure 1. Map of faucet snail range and sample locations. Large map shows native (orange) and invasive (blue) ranges of faucet snail. Inset map of upper Unites States shows sampling locations. In the native range, sampling locations with coordinates are as follows: (NE) Netherlands (51.794139, 5.8043167); (ON) Oneida Lake, NY (43.22378, -76.10426); (4) Mississippi River Navigation Pool 4 near Alma, WI (44.33526, -91.95453); (5) Navigation Pool 5 near Cochrane, WI (44.21484, -91.84962), (5A) Navigation Pool 5A near Winona, MN (44.12089, -91.70734); (7) Pool 7 near La Crosse, WI, the Fred Funk Boat Landing (n=13) (43.93134, -91.30851) and the spillway (n = 5) (43.870114, -91.276394); (8) Pool 8 near Genoa, WI (43.67771, -91.22389).

In total, 95 samples were submitted for sequencing. These included samples from the Netherlands (n = 14), Oneida (n = 14), Pool 4 (n = 7), Pool 5 (n = 14), Pool 5A (n = 14), Pool 7 (n = 18), Pool 8 (n = 15). Briefly, the RAD-seq process involves digesting genomic DNA using a restriction enzyme (SbfI) followed by a ligation at the P1 adapter. A multiplexing barcode is then attached to each DNA fragment before the samples are pooled for shearing, size selection, P2 adapter ligation, and PCR enrichment. This creates a library that can then be sequenced using single end 100 bp sequencing.

Sequence data was initially processed using Stacks v 2.61 (Catchen et al., 2013). The command *process_radtags* demultiplexed and removed barcodes from samples. It also filtered out low-quality reads. Since no reference genome is published for faucet snails, the denovo_map.p1 pipeline was used to build loci de novo (ustacks), assemble a catalog (cstacks), and match samples against the catalog (sstacks) (Rochette & Catchen, 2017). Sequencing read depth coverage was checked to ensure all samples has > 10x coverage (all 95 samples met this criteria) which reduced the possibility of overestimating heterozygote diversity.

An Analysis of Molecular Variance (AMOVA), calculated using GenoDive (version 3.0) (Meirmans, 2020) was used to calculate measures of variance within and between populations. F_{ST} and G_{IS} values were also estimated using GenoDive. A Mantel test was used to assess for a correlation (i.e., isolation by distance) using pairwise F_{ST} and geographic distances. Distances between locations were calculated in kilometers (km) using Google Earth. Since the distances did not have a normal distribution, they were log transformed. The log transformed distances were then tested for normality using the D'Agostino-Pearson omnibus normality test and the Shapiro-Wilk normality test. The tests showed that the log transformed distances did not pass for

normal distribution, so Spearman's r was used for the Mantel test as it is a non-parametric measure of rank correlation.

We also ran a set of analyses using STRUCTURE v. 2.3.4 (Evanno et al., 2005). STRUCTURE uses a Bayesian algorithm to group individuals with similar SNPs in the absence of a priori population information. K-means methods were then applied for K=2-7 to find the most appropriate value. Cluster Markov Packager Across K (CLUMPAK) was used to create a visual representation of the data from the STRUCTURE analysis.

Results

In total, DNA from 95 faucet snail from 7 locations was sequenced. Following filtering steps, 2404 variant loci were included. An analysis of molecular variance (AMOVA) was used to determine F_{IT}, F_{IS}, and F_{ST} values all of which can range from 0 to 1. F_{IT} is an overall inbreeding coefficient explaining the level of inbreeding within an individual relative to the total populational meaning the closer the value is to 1, the higher the level of inbreeding. The AMOVA showed that the level of variance found to be related to variation within individuals was 66.2% (F_{IT} = 0.338, standard deviation (SD) = 0.005). F_{IS} explains the level of mating among relatives within a subpopulation meaning the closer the value is to 1, the higher the level of inbreeding. The variance related to variation among individuals was 19.9% (F_{IS} = 0.232, SD = 0.004, p = 0.010). F_{ST} explains the level of mating that occurs within rather than between subpopulations meaning the closer the value to 1 the more mating that occurs within subpopulations not between subpopulations. The variance related to variation among populations was 13.9% (F_{ST} = 0.139, SD = 0.004, p = 0.010) (Hahn, 2019; Wright, 1922). Pairwise F_{ST} values between collection locations ranged from 0.016 to 0.370. Pairwise F_{ST} values were highest in

comparisons between native and invasive locations (Table 2). G_{IS} values, a measure of inbreeding, ranged from 0.131 to 0.383, and the highest value belonged to the Netherlands population (Table 2).

Table 2. Pairwise F_{ST} values and G_{IS} values for each faucet snail population (based on 95 individuals; 2024 loci). All F_{ST} values were shown to be significant ($p<0.001$).

F_{ST}	Invasive Range						Native Range	G_{IS}
	Pool 4	Pool 5	Pool 5A	Pool 7	Pool 8	Oneida	Netherlands	
Invasive Range								
Pool 4	-	0.113	0.079	0.061	0.046	0.168	0.370	0.237
Pool 5		-	0.064	0.050	0.054	0.135	0.342	0.131
Pool 5A			-	0.032	0.047	0.105	0.322	0.209
Pool 7				-	0.016	0.099	0.309	0.227
Pool 8					-	0.124	0.326	0.231
Oneida						-	0.288	0.232
Native Range								
Netherlands							-	0.383

Both STRUCTURE and GenoDive found the optimal value of K = 2 for K-means method analysis. This determination has been documented to be found more often than would be expected by chance (Janes et al., 2017), thus, biological interpretations of higher K values are sometimes needed. We also found reasonable support for K = 3. Figure 2 shows the results of the K-means method for K = 2 - 4. The optimal K values (K = 2 or K = 3) indicate that there has been sufficient time for some genetic differentiation to occur between the native and invasive populations. Within the invasive range, there is also some genetic differentiation between locations. In the case of K = 2, the Mississippi River showed no genetic differentiation. The Oneida location showed some similarities between the native and other invasive populations indicating a large proportion of shared alleles with both native and invasive populations. For K = 3, the Oneida population became more differentiated from either the native population or all other invasive populations studies. Oneida locations had largest shared alleles at Pool 5 with lessening overlap as distance from Pool 5 increased.

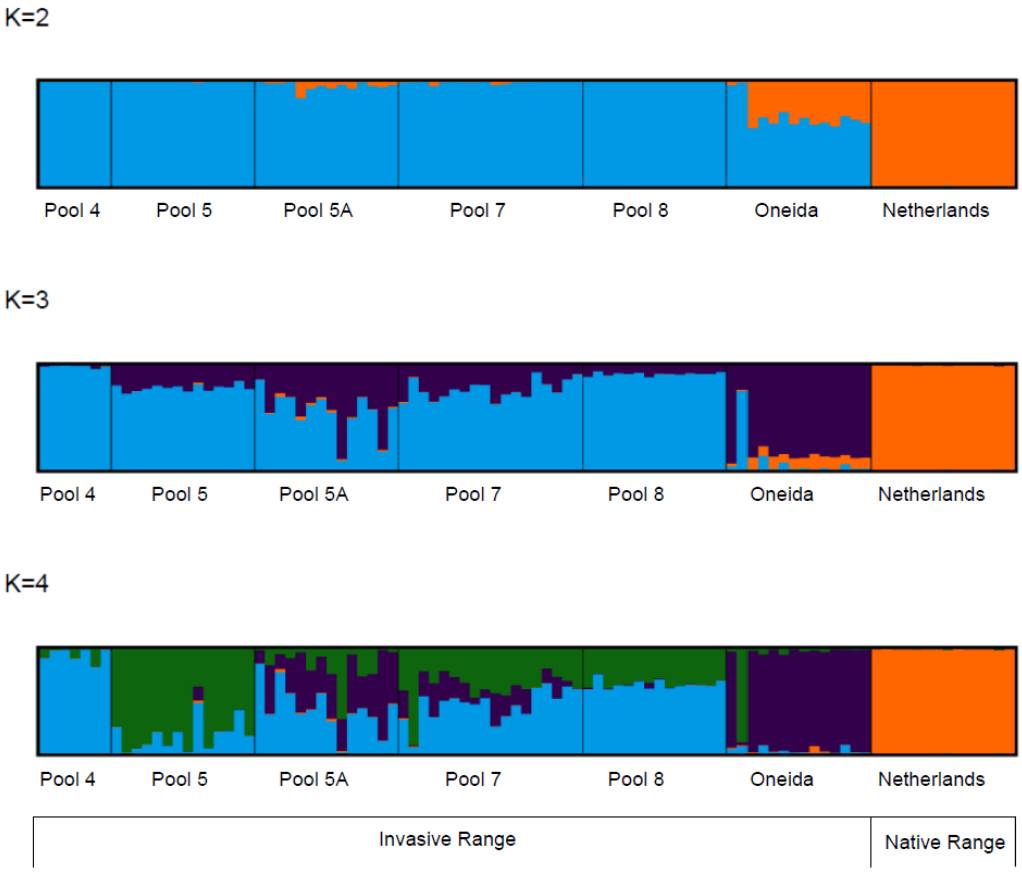

Figure 2. Plot of genetic differentiation of faucet snail. Visual representation of STRUCTURE analysis using CLUMAK. Depicts STRUCTURE iterative runs for K = 2 through K = 4 with each individual represented by a vertical line and each location indicated by black borders and labeled underneath. Colors in each plot infer a genetically distinct cluster. Optimal value of K = 2 and K = 3 was identified.

Isolation by distance analyses showed a significant positive correlation between pairwise F_{ST} values and geographic distances (log-transformed) (Mantel. r = 0.817, p = 0.014).

Discussion

Here, we used genomic analyses to evaluate the population structure of the faucet snail to map the most likely invasion pathway into and across North America. Natural history observation suggests the original introduction of the faucet snail to North America occurred at least as far back as 1869 (USGS, 2019). Our analysis supports natural history observations in that the native population showed clear genetic distinction from any of the populations within the invasive range. Given that the snail has a 1-year generation time, and the original introduction occurred in the late 1800s, sufficient time has likely passed to lead to the observed degree of genetic differentiation between native and invasive populations. However, given that we only sampled one population within the native range, we cannot exclude very likely confounding variable that we did not sample from the true source population. Therefore, measures of genetic distance between native and invasive populations should be interpreted with caution. Isolation by distance analysis also supported this differentiation showing a positive correlation between genetic and geographic distance meaning that the more distance between the locations geographically, the more genetically distinct they are from each other.

Genetic differences within the invasive range are less apparent, but intriguing. Using the structure K-means analysis, K=2 and K=3 were both found to be optimal. Because statistically K=2 shows up more often by chance, it is necessary to interpret higher values of K for sound biological reasoning as well (Janes et al., 2017). In the case of K=2, there are two clear

genetically distinct populations between the Netherlands and all the Mississippi River populations with the Oneida population sharing alleles of both as a middle ground. Oneida sharing alleles with both populations indicates that Oneida is an older established – but not entirely isolated – population which is supported by biological interpretation. The natural history observations show the snails were first observed in the Erie Canal (and moved into areas of New York and the Great Lakes) before moving into and down the Mississippi River, so the Oneida location may represent a potential initial location of invasion (USGS, 2019). In the case of K=3, there are 3 genetically distinct clusters of populations – Netherlands, Oneida, and the Mississippi. Again, this population structure falls in line with natural observations as well, both K values indicate a general East to West movement of the snails. In contrast to K=2, Oneida in K=3 shows distinct alleles rather than showing only shared alleles with both the Netherlands and the Mississippi River. The distinct alleles in Oneida indicate that genetic differentiation has occurred between older and newer established populations in the invasive range. Looking at Oneida and the Mississippi River, we see the most shared alleles between Oneida and Pool 5A indicating that Pool 5A may represent a point of introduction of snails from Oneida. Looking at the Pools surrounding Pool 5A, the number of shared alleles with Oneida goes down moving both north and south away from Pool 5A. This pattern indicates that snails are moving both up and down the Mississippi River rather than displaying unidirectional movement. Overall, the populations in the Mississippi River show relatively little genetic variation (particularly in the case of K=2) which would indicate that they either haven’t had enough time to differentiate, or they are not subject to strong isolation or selective pressures. Given the first sightings of the

faucet snail in the Mississippi River were in the early 2000s and the snails' year-length generation time (USGS, 2019), the latter appears more likely.

Future work in this system would benefit from more sampling locations across the native range and in the older invasive range. Since only one very small portion of the native range was sampled in this study, it is difficult to draw many conclusions about the level of genetic variation in the snails' native range. Studies including multiple locations in both native and invasive ranges can allow for extrapolation of likely regions from which introduced populations originated (Stepien et al., 2002). Looking at genetic variation in the native range also allow for insight into how the snails may or may not genetically differentiate based on isolation or other pressures which would improve our understanding of the possibilities for the snails to adapt in their invasive range (Stepien et al., 2002). Native sampling would also be crucial to understanding more specific possible source populations for the original invasion. More sampling in the older established invasive range would further expand on whether there is isolation between older and newer populations and whether that could explain any of the dynamics at play at the forefront of the invasion – namely the higher virulence and die-offs of waterfowl we see due to the trematode species the faucet snail hosts. Since our study does not include multiple locations in the older invasive range, conclusions about genetic differentiation causing higher virulence at the forefront of the invasion cannot be made. However, another study analyzing invasive populations of faucet snails based on microsatellite markers presented support for a single North American population suggesting genetic differentiation may not be a factor in virulence dynamics (Perez et al., 2016).

Overall, this study is foundational for future work looking at the population structure of the trematode parasites that use the snails as hosts. Creating a parallel invasion history would clarify how these two organisms function together in terms of their invasion. There are two likely possibilities for how the invasion could have happened: (1) co-invasion or (2) spillback. In the case of co-invasion, the trematodes moved with the snails from their native range to their new invasive range together either through a single introduction or multiple introduction events. Studies of another parasite-host system have shown certain species of parasitic worms have been shown to co-invade with invasive fish species into novel aquatic systems. The worms are then able to parasitize other native species of fish in addition to the initial relationship with invasive fish (Sayyadzadeh et al., 2016). Since the faucet snail is often outcompetes native snails in its invasive range (Harman, 2000), the trematodes using native snails as intermediate hosts is less of a concern. For this system, co-invasion would be of higher concern in the context exposing native species of waterfowl – which act as a definitive host and are facing major die offs – to these trematodes. Spillback, on the other hand, would have involved the snails invading while the trematodes were already present in North America meaning the trematodes were able to find a suitable host that allowed them to then spread and exist in higher numbers than before the invasion. Other aquatic parasite-host systems have shown a spillback effect in which a high population of invasive mollusks allows for an increased abundance of the intermediate stages of a trematode parasite (Mastitsky & Veres, 2010). This increased abundance of trematode parasites increases the likelihood that the definitive host will ingest the parasite which again causes concern for waterfowl species acting as the definitive host in this system. It is likely to be some combination of spillback and co-invasion taking place. The faucet snail is known to host several

species of trematodes in its native European range, including several but not all trematodes, that have been implicated in waterfowl deaths in North America (McLaughlin et al. 1993). The combination of the two invasion methods adds complexity to understanding the mechanisms behind the success of this invasion.

Managing invasive species is crucial for maintaining biodiversity which is a major indicator of ecosystem stability and resilience (Loreau & de Mazancourt, 2013). With increased globalization and continued anthropogenic forces of transport, understanding the pathways successful invasive species have followed is a key step in mitigating damages. Establishing patterns across many specific invasive species individually allows for analysis of possible larger-scale patterns of invasive species as a whole. Here, we analyzed a singular species of aquatic mollusk, but future work should aim to interpret any patterns that hold true for multiple species of aquatic mollusks to identify possible overarching management practices for aquatic systems. Identifying broad-scale mechanisms behind successful introduction and establishment of invasive species provides a lens through which to create management practices to minimize the damages caused by these invasion events and preserve native ecosystems.

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APPENDIX A

TIMELINE FOR SNAIL DISPERSAL LESSON

Table 3. Timeline for snail dispersal lesson

Activity	Description	Time	Notes
Pre-class preparation			
Find and collect snails	1. Determine mollusk species available near you 2. Find a location with mollusks 3. Collect + bring snails back to lab	Variable; Should plan for several days and possibly multiple outings searching for snails if instructor is not aware of a location that consistently has snails	Collection note: Do not work alone. For safety always have at least one other person present.
Print Handouts	1. Print and review all handouts/datasheets 2. Decide on assessment for lesson	5-10 minutes	Find datasheet/worksheets under APPENDIX B, D, E
Prepare supplies	1. Gather supplies: - Kiddie Pools - Clipboards and writing implements - Measuring tapes - Optional equipment for altering pool conditions (e.g., heaters, lights, substrate) 2. Fill pools with water (check for leaks) 3. Test all optional equipment (e.g., heaters, lights)	10-15 minutes	Pools can be set up during the lab period or prior depending on available space

(continued on following page)

Table 3 (continued)

Activity	**Description**	**Time**	**Notes**
Practice trial	1. Review datasheet 2. Run through at least one trial to become familiar with general process	30 minutes	If a different mollusk species was chosen, plan on extra time to determine optimal time period to allow the mollusks to disperse before measurement – instructions on how trials will run under APPENDIX C
In-class activities			
Introductory lecture + brainstorming	1. Introduce topics: CURE format, invasive species, dispersal, etc. 2. Review materials available for experiment 3. Brainstorm: students generate hypotheses (in pairs/small groups)	25-35 minutes	
Trial run(s) + discussion	1. Review instructions and materials 2. Optional demonstration by instructor 3. Students run 1 trial (minimum) 4. Discussion: Students work in small groups to troubleshoot/improve methodology	40 minutes	For more structured lessons, instructor can inform students to perform their trial runs for a shorter period of time than a full trial if trial time is not a factor instructors want students to direct themselves.
Data collection	1. Students run trials (number of iterations based on the allotted time)	1 hour (minimum)	Ensure students decide on roles (one observer and one recorder)

(continued on following page)

Table 3 (continued)

Activity	**Description**	**Time**	**Notes**
Data input + analysis	1. Instructor explains data analysis 2. Students enter data to Excel (or other available data analysis program) 3. Students run basic statistical tests with instructor oversight	~40 minutes	Data entry can be done outside of class time if necessary (with instructor providing directions either in class or as a recording/handout)
Wrap-up discussions + assessment	1. Full class discussion of the experiment 2. Reflection on results and application to broader concepts (verbal discussion and/or worksheet) 3. Instructor explains assessment	10 minutes	Class discussion is highly recommended for reflection with or without the use of a worksheet Assessment options/rubrics – APPENDIX F

APPENDIX B

SNAIL DISPERSAL DATA SHEET

Snail Dispersal Data Sheet

Name of observer: _____
Name of recorder: _____

Hypothesis: _____

_____

_____

Data collection					
Pool 1			Pool 2		
Conditions			Conditions		
Trial	Snail	Distance	Trial	Snail	Distance

APPENDIX C

DIRECTIONS FOR TRIALS, DATA COLLECTION, AND ANALYSIS

Directions for Trials and Data Collection and Analysis (for instructors)

Materials:

- Kiddie pools (2/group)
- Snails (20/pool max. recommendation)
- Measuring Tape
- Datasheet
- Clipboard (or other writing surface)
- Time keeping device (smartphone, watch, stopwatch)
- Optional materials:
    - Aquarium heaters
    - Various Substrates/Food sources
    - Lights
    - Etc.

Directions:

1. Students will split into pairs/small groups and brainstorm hypotheses and how to test them based on the materials available to them. Students will write these hypotheses on the top of their datasheet.
2. Students will set up their pools (e.g., if they are assessing how food presence affects dispersal rate, they might choose to put food in one pool and no food in the other).
3. Students then decide roles. One student is the primary observer, watching the snails and doing the physical measuring with the measuring tape. The other student will be the primary recorder and will fill out the data sheet.
4. Students then will do a trial run. Instructions for a trial is as follows:
    - Students will place their snails in the center of the pool (use of a petri dish or other circular disc ensures snails are being placed at relatively the same original diameter every trial. Petri dishes used in experiment design were plastic and did not need to be weighted down).
    - Students will begin their timer once snails are in place. At this point, both partners will observe snail movement. Emphasis should be placed on watching for excessive movement of snails back to the center. If within the time frame, many snails are dispersing out and then returning, then time for each trial may need to be shortened. In our study, we found 30 minutes to be optimal for maximum snail dispersal with minimal backward movement. This may not be the same in your classroom which is why running practice trials prior to class is important. Instructors may also choose to measure dispersal in other ways such as time needed for x number of snails to reach the edge of the pool. It is up to the instructor's discretion to either allow students to set their own trial lengths or give a standardized time to the class depending on level of structure desired.
    - After the time period is complete, the observer will use the tape measure to measure the distance from the center point of the pool to each snail (in centimeters). The observer will read out these measurements for the recorder to write down in the data sheet. At this point, one full trial is complete.

5. Students will then form small groups to discuss the trial and any issues they may have had. This is a chance to articulate their ideas and provide feedback to other groups. Students may decide to alter their methods at this time.
6. Students will conduct trials for analysis. The number of trials will be constrained by time allotted for this lesson. Remind students to make sure their roles are in place (observer/recorder) before the start of each trial to minimize any confusion over tasks.
7. Students will then enter their data into Microsoft Excel where they will run basic statistical analyses. We recommend having them find the average, standard deviation, and standard error for their datasets and using T-tests to compare data between their pools. They can also create bar graphs with error bars to represent their results in a visual format. The analysis of their data may need to take place either at home or in an additional class period based on time constraints. We found analysis could easily fill an entire lab period by itself if instructors plan has students do everything listed above.
8. Students will lastly reflect on the overall process and their results. This can be a discussion in class and/or a worksheet (example worksheet provided in APPENDIX E), but it is recommended that a class discussion is facilitated whether or not a worksheet is also given. The discussion allows students to really articulate what it is that they did and how they can use what they learned at a broader scale.

APPENDIX D

PEER CONSULT WORKSHEET

Peer Consult Worksheet

Name(s) of your group: _____
Name(s) of group you are commenting on: _____

Peer review is a crucial part of the scientific process. We gain valuable insight from discussing our ideas and hearing feedback on them. You will pair up with another group and ask them about their experiment. They should discuss their hypothesis and their methodology. Ask any clarifying questions as they come up. This discussion should be a back-and-forth conversation not a one-way presentation. Questions are listed below to guide you through your discussion. Questions are related to the group to whom you are providing feedback (i.e., you list the other group's hypothesis not your own and your comments are on their methodology).

What is the group's main hypothesis?

How are they testing their hypothesis?

Provide feedback on their methods: Are their methods logical? Do their methods actually test what they are presenting as their hypothesis? Provide at least one positive and one **constructive** criticism.

Additional comments/discussion points:

APPENDIX E

SNAIL DISPERSAL REFLECTION SHEET

Snail Dispersal Reflection

Name: _____

Now that you have completed your experiment and your analysis of your data, it is time to reflect. Interpreting the significance of your results is one of the most important parts of completing an experiment. This is where you assign meaning to your data in a broader sense (i.e., You explain why your results should matter to other people in your field or even beyond your field of study). Please fill out the following questions in full sentences.

1. Based on your experiment, what questions would you ask for future studies?

2. Was there anything about the methodology that undermine the validity of your results?

3. What are some limitations of your data?

4. What were the benefits/drawbacks of peer consultation during this process?

5. How does your experiment(s) and its results further our knowledge of broader ecological concepts? (i.e., How does your data further knowledge in the field of ecology?)

APPENDIX F

RUBRICS FOR ASSESSMENT

Rubrics for Assessment

Example Rubric for Written Summary:

For a written summary, students will be asked to write a scientific summary of their experiment including an Introduction, Methods, Results, and Discussion. The rubric presented here is a foundation for assessment. Instructors implementing this lesson as a unit- or semester-long project may choose to have students hand in sections for feedback throughout the unit and then produce a revised version as a final product. Points for editing will need to be incorporated into a revised rubric.

Introduction presents a thorough and logical justification for the study. Hypotheses are clearly stated near the end of the introduction.	15 points
Methods are thorough and contain enough information for another scientist to reasonably repeat the study.	15 points
Results are reported in a factual manner absent of interpretation. They are presented with appropriate language in terms of significance.	15 points
Figures and graphs are formatted and labeled appropriately. Figure legends are present and contain appropriately detailed information.	15 points
Discussion interprets the results and whether they support or refute the hypotheses. Logical arguments are presented and supported by scientific sources. Limitations of methods, analysis, and future directions for research are discussed at the end.	15 points
References are included and citations are formatted properly.	15 points
Grammar and spelling are correct.	5 points
Student participated fully in the lesson and offered meaningful feedback to peers.	5 points
Total:	100 points

Example Rubric for Presentation:

For a presentation, students will follow the same general format of a written summary (intro, methods, results, discussion). However, students will be asked to present this information in visual and verbal format. Students will present in their groups. Instructors should ensure expectations for equal time speaking in the presentation.

Introduction: presents a logical justification for the study moving from broad themes to specific study system. Hypothesis clearly stated.	15 points
Methods are clear and contain enough information to be understood without going into minor details.	15 points
Results are reported in a factual manner absent of interpretation.	15 points
Figures and graphs are labeled and formatted appropriately. Presenter orients audience to the axis of the graph and overall takeaway is explained clearly.	15 points
Discussion interprets the results and whether they support or refute the hypotheses. Presenter offers support for their arguments and introduces them in an organized manner. Limitations of results and further research questions are explained.	15 points
Any photos or supporting text acquired from outside research is properly cited.	5 points
Presentation was aesthetically pleasing – used appropriate font colors and sizes.	5 points
Presentation was free of grammatical/spelling errors.	5 points
Presentation style was appropriate: Presenters spoke in a loud and clear tone, engaged with their audience, and contributed equally to presenting.	5 points
Student participated fully in the lesson and offered meaningful feedback to peers.	5 points
Total:	100 points