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Teaching Issues and Experiments in Ecology - Volume 10, August 2014

ISSUES : DATA SET

Effects of multiple invasive species in experimental aquatic communities

Miranda D. Redmond[1,4], Daniel L. Preston[1], Rowan M. Gaffney[2,3]

[1] Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309

[2] Department of Geological Sciences and Engineering, University of Nevada, Reno, NV 89557

[3] Itasca Denver, Lakewood, CO 80228

[4] Corresponding Author: Miranda D. Redmond (Miranda.Redmond@colorado.edu)

Two common wetland invasive species, American bullfrog (*Lithobates catesbeianus*) and mosquitofish (*Gambusia affinis* and *G. holbrooki*), that were caught with a seine from a California pond. Photo credit: Jeremy Monroe/Freshwaters Illustrated.

THE ECOLOGICAL QUESTION:
What are the effects of two invasive species, western mosquitofish (*Gambusia affinis*) and the American bullfrog (*Lithobates catesbeianus*), on a native aquatic community?

ECOLOGICAL CONTENT:
Invasion ecology, wetland communities, amphibian decline, direct and indirect effects, trophic cascades

WHAT STUDENTS DO:
This exercise is designed for upper division biology and environmental science students and could be especially useful for students in a data management, data analysis, or wetland ecology course. In this exercise, students first work in small groups to collect background information on what mosquitofish and bullfrogs consume and then modify a food web based on that knowledge. Students then develop hypotheses of how these two invasive species may affect native amphibian species, snails, and zooplankton. Following, students work individually to analyze experimental mesocosm data to determine the effects of the two invasive species on native aquatic taxa (amphibians, snails, zooplankton, and phytoplankton). Afterwards, students discuss their findings and modify their food web based on the results.

STUDENT-ACTIVE APPROACHES:

Computer-based projects, calculation, problem-based learning, cooperative learning, critical thinking

TIEE

Teaching Issues and Experiments in Ecology - Volume 10, August 2014

SKILLS:

- *Hypotheses formation:* Develop testable hypotheses and create a conceptual diagram
- *Data management and analysis:* Summarize data in excel or a relational database to create tables and graphs
- *Data visualization*: Create figures to visually represent the data
- *Data interpretation*: Interpret results and draw a hypothesized food web based on the data
- *Collaboration:* Effectively collaborate with classmates to develop hypotheses and interpret findings

ASSESSABLE OUTCOMES:

Food web diagram and hypotheses, database or summarized excel files, figures and answers to questions

SOURCE:
Preston, D.L., J.S. Henderson, and P.T.J. Johnson 2012. Community ecology of invasions: direct and indirect effects of multiple invasive species on aquatic communities. Ecology 93: 1254–1261.

OVERVIEW OF THE ECOLOGICAL BACKGROUND

Invasive species can alter the ecology and evolution of native species and are important drivers of extinction (Vitousek et al. 1997, Clavero and Garcia-Berthou 2005). Yet, when species introductions occur alongside other types of environmental change, it can be challenging to disentangle the ecological impacts of individual nonnative species relative to other stressors (Didham et al. 2005, Light and Marchetti 2007). This challenge becomes amplified when multiple nonnative species co-occur. Understanding the individual and combined effects of multiple invasive species is especially important to natural resource managers, who are often pressed to prioritize which invasive species to manage (Simberloff et al. 2005).

The consequences of biological invasions are particularly evident in freshwaters. The movement of aquatic species around the globe has occurred for multiple reasons, including the improvement of recreational and commercial fisheries (e.g., rainbow trout [*Oncorhynchus mykiss*]), the aquaculture of food items (e.g., Tilapia [*Oreochromis spp*.]), the aquarium trade (e.g., red-eared sliders [*Trachemys scripta elegans*], Eurasian milfoil [*Myriophyllum spicatum*]) and inadvertent introductions during recreational boating, fishing and travel (e.g., zebra mussels [*Dreissena polymorpha*]) (Johnson et al. 2001, Padilla and Williams 2004). In addition to their conservation implications, the cost of controlling aquatic invasive species can be high; the estimated yearly expenditure on invasive species management in the Great Lakes Basin alone is $5 billion (Great Lakes Regional Collaboration 2005).

TIEE

Teaching Issues and Experiments in Ecology - Volume 10, August 2014

Two common invaders in freshwater habitats in western North America are the mosquitofish (*Gambusia affinis* and *G. holbrooki*) and the American bullfrog (*Lithobates catesbeianus* [= *Rana catesbeiana*]). Both species are native to the eastern United States. Mosquitofish are the most widespread freshwater fish and have been introduced on every continent besides Antarctica, while bullfrogs are invasive throughout western North America and regions of South America, Europe, and Asia (Lever 2003, Pyke 2008). Both species have deleterious effects on native aquatic communities; mosquitofish prey on a wide diversity of aquatic invertebrates, amphibians, and other fish (Goodsell and Kats 1999, Leyse et al. 2004), and bullfrogs have negative effects on other aquatic amphibians through competition, predation, and disease transmission (Kiesecker et al. 2001, Pearl et al. 2004).

In this activity, students use data from an outdoor mesocosm experiment to explore how these two common invasive species, mosquitofish and the American bullfrog, influence the native amphibian community and other aquatic taxa (snails, zooplankton, and phytoplankton). As such, this dataset allows students to examine both the direct and indirect effects of two invasive species on native amphibians. This dataset contains a substantial amount of data (7 spreadsheets) and can therefore be especially useful for a course working with a relational database, such as Microsoft Access or PostgreSQL. The data presented here have been published in Ecology (see Preston et al. 2012) and we encourage educators to read this manuscript to gain an even better understanding of the ecological background and to read the author's interpretation of the results.

REFERENCES

Clavero, M., and E. Garcia-Berthou. 2005. Invasive species are a leading cause of animal extinctions. Trends in Ecology and Evolution 20: 110.

Didham, R. K., J. M. Tylianakis, M. A. Hutchinson, R. M. Ewers, and N. J. Gemmell. 2005. Are invasive species the drivers of ecological change? Trends in Ecology and Evolution 20: 470-474.

Goodsell, J. A. and L. B. Kats. 1999. Effects of introduced mosquitofish on Pacific treefrogs and the role of alternative prey. Conservation Biology 13: 921-924.

Johnson, L. E., A. Ricciardi, and J. T. Carlton. 2001. Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boating. Ecological Applications 11: 1789-1799.

Kiesecker, J. M., A.R. Blaustein, and C. L. Miller. 2001. Potential mechanisms underlying the displacement of native red-legged frogs by introduced bullfrogs. Ecology 82: 1964-1970.

Lever, C. 2003. Naturalized Amphibians and Reptiles of the World. Oxford University Press, New York.

Great Lakes Regional Collaboration. 2005. Great lakes regional collaboration strategy to restore and protect the Great Lakes. <http://www.glrc.us/strategy.html>

Leyse, K. E., S. P. Lawler, and T. Strange. 2004. Effects of an alien fish, Gambusia affinis, on an endemic California fairy shrimp, Linderiella occidentalis: implications for conservation of diversity in fishless waters. Biological Conservation 118: 57-65.

Light, T. and M. P. Marchetti. 2007. Distinguishing between invasions and habitat changes as drivers of diversity loss among California's freshwater fishes. Conservation Biology 21: 434-446.

Padilla, D. K. and S. L. Williams. 2004. Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. Frontiers in Ecology and the Environment 2:131-138.

Pearl, C. J., M. J. Adams, R. B. Bury and B. McCreary. 2004. Asymmetrical effects of introduced bullfrogs (Rana catesbeiana) on native ranid frogs in Oregon. Copeia 2004: 11-20.

Preston, D.L., J.S. Henderson, and P.T.J. Johnson 2012. Community ecology of invasions: direct and indirect effects of multiple invasive species on aquatic communities. Ecology 93: 1254–1261.

Pyke, G. H. 2008. Plague minnow or mosquito fish? A review of the biology and impacts of introduced Gambusia species. Annual Review of Ecology and Systematics 39: 171-191.

Simberloff, D., I. M. Parker, and P. N. Windle. 2005. Introduced species policy, management, and future research needs. Frontiers in Ecology and the Environment 3: 12-20.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of earth's ecosystems. Science 277: 494-499.

DATA SETS

- Student_Excel – this excel file contains all of the data needed to complete the core activity
- Instructor_Excel – this excel file contains all of the data as well as the accompanying graphs to answer the questions posed in the core activity
- Student_Excel_All_Data – this excel file contains additional data from the mesocosm experiment to answer the optional extension tasks
- Instructor_MS_Access_Database – this Microsoft Access database contains all of the data and the queries necessary to answer the questions in the core activity. This database can therefore serve as a useful answer key for classes in which students are expected to build a MS Access database.
- Instructor_PostgreSQL_code - this code provides all of the structured query language (SQL) code necessary to answer the questions in the core activity

using a PostgreSQL database. This code is annotated so that the purpose of each line of code is clear. This code can therefore serve as a useful answer key for classes in which students are expected to summarize their data in a postgreSQL database.

- Instructor_Powerpoint – this PowerPoint is designed for instructors to present to the students and is divided into two sections (Part I and Part II). Part I provides background on the experiment and dataset. This part should be presented at the beginning of the lesson. Part II of the PowerPoint shows figures and results that should be shown after the students have completed the activity. The PowerPoint file includes associated notes for each slide, such as background information on the methods, explanations of the results, and discussion prompts.

STUDENT INSTRUCTIONS

Overview: Invasive species have become major drivers of ecosystem change, a problem that is particularly prominent in freshwater ecosystems. Two common wetland invaders throughout the western United States are the western mosquitofish (*Gambusia affinis*) and the American bullfrog (*Lithobates catesbeianus;* previously referred to as *Rana catesbeiana*). As such, scientists and land managers are interested in quantifying the effects of these two common invasive species on native amphibian communities. As a scientist, you have established an outdoor experiment at the Hopland Research and Extension Center in Mendocino County, California, to isolate the effects of these two invasive species on native amphibian communities and other aquatic taxa (snails, zooplankton, and phytoplankton; see Photo 1). You have already collected and entered all of the data and now need to analyze the data to determine the effects of invasive species on native amphibian communities and other aquatic taxa.

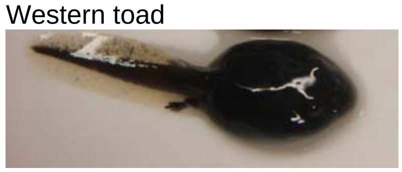
Invasive Species:	**Native Amphibian larvae:**	**Other Aquatic Taxa:**
Western mosquitofish  American bullfrog larvae 	Pacific treefrog  California newt  Western toad 	Zooplankton: *Daphnia sp.* and Copepod   Phytoplankton  Rams horn Snail 

Photo 1. The invasive species (*left*), native amphibian species (*middle*), and other aquatic taxa (*right*) examined in the mesocosm experiment. Photo Credits: *Daphnia – available from* Gewin V. 2005. PLoS Biol 3(6): e219, doi:10.1371/journal.pbio.0030219; *Copepod – available from* "Copepod" - photo by Uwe Kils is licensed under CC BY 3.0; *Phytoplankton – available from* "Phytoplankton – the foundation of the oceanic food chain" – photo by NOAA MESA Project is licensed under CC by 3.0; rams horn snail by Jeremy Monroe/Freshwaters Illustrated; all other photos were taken by D.L. Preston

Experimental Design:

The data set presents the results from your outdoor mesocosm experiment that aimed to disentangle the effects of invasive mosquitofish and bullfrogs within an experimental aquatic community. Mesocosms are small-scale representations of larger systems, which make it possible to do controlled experiments in a semi-realistic setting. The experiment involved a 2 x 2 factorial design (4 total treatments) that manipulated the presence of mosquitofish and bullfrogs within outdoor mesocosms (see Fig. 1).

A total of 20 outdoor pond mesocosms were established (Photo 2) by placing 370 L of well water, 45 ml of pond mud, 15 g of rabbit chow, 25 g of dry leaf litter, and 1.25 L of pond water containing concentrated zooplankton into each plastic tank (1.3 m Length × 0.79 m Width × 0.64 m Height). The pond mud was added to introduce algae cells, the rabbit chow provided a source of nutrients to fuel growth of primary producers, and the dry leaf litter served as a source of cover for the amphibians. In each mesocosm, 10 native snails (*Helisoma sp.*), 15 native Pacific tree frog (*Pseudacris regilla)* tadpoles, 15 native western toad *(Bufo boreas*) tadpoles and 10 native California newts (*Taricha torosa)* were added. Five of the mesocosms only had the three native amphibian species (Native treatment). For the other 15 mesocosms, five of the mesocosms had 3 invasive American bullfrog tadpoles added in addition to the native species (Bullfrog treatment), five of the mesocosms had 5 invasive mosquitofish added in addition to native species (Mosquitofish treatment), and 5 of the mesocosms had 3 invasive American bullfrog tadpoles and 5 mosquitofish added in addition to the native species (Bullfrog + Mosquitofish treatment).

Mosquitofish

		–	+
Bullfrogs	I	Native Treatment (Control) Native Amphibians Only	Mosquitofish Treatment Native Amphibians + Mosquitofish
	+	Bullfrog Treatment Native Amphibians + Bullfrog	Mosquitofish + Bullfrog Treatment Native Amphibians + Bullfrog + Mosquitofish

Figure 1. A diagram depicting the 2 X 2 factorial design of the experiment. The positive sign (+) indicates that the particular invasive species was added to that treatment while the negative sign (-) indicates that the particular invasive species was not added to that treatment.

Photo 2. This photo is of the outdoor mesocosms used to experimentally evaluate the effects of mosquitofish and bullfrog tadpoles on native aquatic communities. Photo Credit: D.L. Preston

Part 1: Background Research and Hypotheses

A. A key way to understand how an invasive species may affect other organisms in the community is to build a food web to show connections between organisms and the food they consume. You have been provided with a food web depicting all of the important organisms in the mesocosm experiment except for the two invasive species, American bullfrog and western mosquitofish (see Fig. 2).
In small groups, collect background information on what the two invasive species consume and then add these two species to the food web in Figure 2. *Note: In this experiment, the American bullfrogs, treefrogs, toads, and newts are all in the larval stage. Please provide at least two citations that you used to complete the food web.

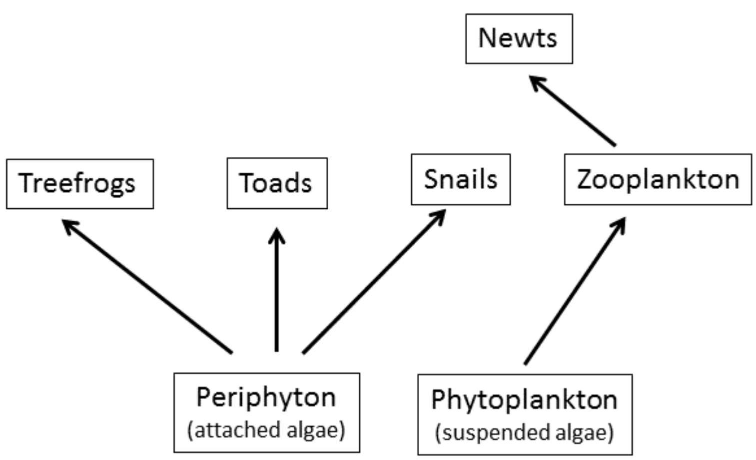


Figure 2. An aquatic food web illustrating the relationship between the native amphibian larvae (treefrogs, toads, and newts), snails, zooplankton (i.e., small

planktonic crustaceans, including *Daphnia* and copepods), phytoplankton (i.e., suspended algae), and periphyton (i.e., attached algae). The arrows points in the direction of the energy flow (i.e., the arrow points to the consumer).
B. Based on your new knowledge of mosquitofish and American bullfrogs, please write a hypothesis detailing how these two invasive species might affect the native amphibian species, snails, and zooplankton (*Daphnia* and copepods).

Part 2: Data Analysis and Interpretation

Invasive species effects on native amphibian species

Your goal is to determine the effect of each treatment on the three native amphibian species (Pacific treefrog, California newt and Western toad). Please answer the following questions addressing the effects of invasive species on native amphibian communities using the data in the excel spreadsheet. For this section, you will need to use the 'Treatment_Assignments', 'Amphib_Survival', and 'Amphib_Mass' worksheets in the excel data. Be sure to read the *Important Data Notes* part at the bottom of this section for details about the data and calculations.

1. How did the presence of the two invasive species (bullfrogs and mosquitofish) influence the survival of native amphibian species? Did the two invasive species affect native amphibian survival in the same way for all species? In addition to providing a written response (3-5 sentences) to these questions, please also include a figure that shows the mean percent survival of each native amphibian species in each treatment. Be sure to include standard error bars in your figure (see Important Data Notes).

2. How did the presence of these two invasive species influence the mass (an indicator of amphibian growth) of the native amphibians that survived? In your answer to this question, please also include a figure that shows the effects of the treatments on native amphibian mass. Be sure to include standard error bars in your figure. **Hint – To calculate the mean mass in each treatment and standard error, you first need to calculate the mean mass of each species in each tank since each individual amphibian is not a true replicate. Following, you then need to use the mean mass value in each tank to calculate the mean and standard error in each treatment.*

3. What mechanisms may be responsible for the observed effects of mosquitofish and bullfrogs on native amphibians? In your answer, please describe whether each mechanism was a direct effect or an indirect effect. *Note: a direct effect is an interaction between two species, such as predation, whereas an indirect effect is when the effect is mediated by a third (or more) species.

4. If the US Fish and Wildlife Service can only eradicate one of the invasive species from California wetlands, which species do you recommend they eradicate to protect native amphibian species?

Important Data Notes:

1.) Species codes (used on many of the spreadsheets):
    - PSRE = Pacific Tree Frog (*Pseudacris regilla)*
    - BUBO = western Toad (*Bufo boreas)*
    - TATO = California newt (*Taricha torosa*)
2.) No_of_Survivors (a field in the Amphib Survival datasheet) = Number of survivors of the specified species in the specified tank
3.) How to Calculate Standard Error: *Standard Error = Standard Deviation / sqrt (sample size)*
    - The standard error of survival (or mass) in each treatment is the standard deviation of the survival (or mean mass) values from all of the tanks within a treatment divided by the square root of 5, because there were 5 tanks in each treatment.

<u>Invasive species effects on other aquatic taxa</u>

You are also interested in the effects of each treatment on other taxa in the experimental aquatic community, particularly snails, zooplankton (i.e., small planktonic crustaceans, including *Daphnia* and copepods), and phytoplankton (i.e., suspended algae). Please answer the following questions addressing the effects of invasive species on snails, zooplankton, and phytoplankton. In answering each question, include a written response as well as a figure with standard error bars. For this section, you will need to use the 'Treatment_Assignments', 'Snail_and_Zooplankton, and 'Phytoplankton' worksheets in the excel data. Don't forget to read the *Important Data Notes* part at the bottom of this section for details about the data.

1. How did the presence of invasive species influence the density of snails?
    a. Did the two invasive species (bullfrogs and mosquitofish) affect snails differently?
    b. If there was any effect of the invasive species on snail density, what mechanism may be responsible for the effect?

2. How did the presence of invasive species influence the density of zooplankton (*Daphnia* and copepods) and the density of phytoplankton (i.e., relative phytoplankton fluorescence)?

a. Did the two invasive species (bullfrogs and mosquitofish) affect zooplankton and/or phytoplankton differently?
b. If there was any effect of the invasive species on the abundance of zooplankton or phytoplankton, was the effect direct or indirect?

Important Data Notes:

1.) Number of Zooplankton = Number of *Daphnia* + Number of Copepods. To calculate density, please note that a total of 6.9 L of water were sampled in each mesocosm for zooplankton.
2.) Run Number = Each sample was run five times. Use the mean of the five runs as the value for that mesocosm.
3.) Phytoplankton Fluorescence = A relative value that measures the amount of light absorbed by chlorophyll in the sample. These numbers do not have units because they are relative to a sample blank, rather than absolute measurements.

Part 3: Data Synthesis

In small groups, discuss whether the data supports your initial hypothesis and how your food web compares to the results. What may be the mechanisms that explain the relationships between the invasive species, native amphibians, and other aquatic taxa? Please modify your food web based on your new knowledge and add in dashed arrows to indicate the indirect effects of the two invasive species on the aquatic community.

NOTES TO FACULTY

This dataset and accompanying activity could be used in a variety of courses and altered appropriately. This material was taught in a Biological Data Management course for upper division undergraduate students at the University of Colorado – Boulder. As such, we focused more on the data management and visualization aspect and had students create databases (Microsoft Access and PostgreSQL) with queries that summarized the data for each question. However, this dataset is also well suited for a statistical analysis course where students are able to practice performing two-way Analysis of Variance tests and checking for normality and homoscedasticity.

The data presented here provide important insights on the direct and indirect effects of invasive species on the aquatic communities, and as such, are appropriate for helping students better understand wetland ecology, food web dynamics, and invasion ecology. We have provided figures that summarize the data (see Instructor_Excel_file). These figures can be useful for courses that want to focus more on data interpretation rather than data management and analysis.

Classroom Management

Introduction: We recommend using part 1 of the PowerPoint to introduce the students to the important background information of the activity. We have provided detailed notes on each slide and recommend that the instructor actively engages students during the PowerPoint with questions (see suggested questions in PowerPoint notes).

Activity: We recommend having the students work in small groups (3-4 students) for Parts 1 and 3 to collect background information, discuss their hypotheses and the mechanisms behind their findings. For Part 2, we recommend having the students work individually. The primary objective for Part 1 of the activity is to get students familiarized with the native aquatic community and the invasive species in the experiment. Finding well documented sources describing the diet of each species can be challenging, particularly for American bullfrog tadpoles and will require some degree of interpretation. After each group finishes part 1, we recommend bringing the class together for 5-10 minutes to compare food webs and hypotheses among the groups. At this time, we also recommend discussing the importance of independent samples for statistical analyses (and calculating standard errors) and then discussing why multiple measurements within a mesocosm (e.g., amphibian mass and phytoplankton) are not independent of one another. In these instances, the students first need to calculate the mean value per mesocosm before calculating the standard error. This is also briefly explained within the activity, but we recommend reviewing it as a group as well because it can be confusing.

Discussion: After the students have completed the lesson, the instructor should use Part II of the PowerPoint presentation to lead a discussion regarding the interpretation of the results and underlying mechanisms. The PowerPoint presentation includes notes about the most plausible underlying mechanisms that generated the observed results and we also give our interpretation of these results below. Specific discussion questions (and answers) that can be asked during the PowerPoint presentation include:

1) What could explain the differential effects of mosquitofish on native amphibian survival?

Answer: The most likely explanation for these results is that toads are highly toxic and distasteful as larvae. Toads produce a potent toxin called bufotoxin. Treefrogs do not produce any toxins. California newts are highly toxic as adults, but they are not toxic as larvae and they are readily preyed upon. These results therefore reflect differences in the palatability of each amphibian and its susceptibility to predation by mosquitofish.

2) Why did bullfrogs reduce the mass of treefrogs but not newts at the end of the experiment?

Answer: Bullfrogs compete directly with treefrogs for resources (both are herbivores as larvae). In contrast, newts are carnivores as larvae so they were not affected strongly by bullfrogs.

3) Why might toads have a larger mass in the presence of bullfrogs and mosquitofish than with just bullfrogs alone?

Answer: Toads compete with bullfrogs for resources (algae), so the presence of bullfrogs reduced their growth rates. The addition of mosquitofish slightly increased toad growth rates because mosquitofish preyed on treefrogs, thereby reducing competition between treefrogs and toads (an indirect positive effects of mosquitofish on toads).

4) What mechanism could explain the differences in snail density across treatments?

Answer: The change in snail density is likely due to competition from bullfrogs. Snails had less to eat in the presence of bullfrogs and therefore produced fewer offspring. Mosquitofish neither competed with nor preyed on snails.

5) Explain the relationship between mosquitofish, zooplankton, and phytoplankton that was found in the experiment.

Answer: The zooplankton results show a direct effect of mosquitofish predation; zooplankton were virtually eliminated in the mesocosms with mosquitofish. This led to cascading indirect effects on phytoplankton abundance. The mesocosms with low levels of zooplankton had high levels of phytoplankton due to release from grazing. This is an example of a trophic cascade.

After discussing the mechanisms behind each figure, we recommend drawing the food web on the board as a class (see Figure 3 for an answer key). In addition, we would highlight the indirect effects that mosquitofish and bullfrogs had on the native amphibians (see Figure 4).

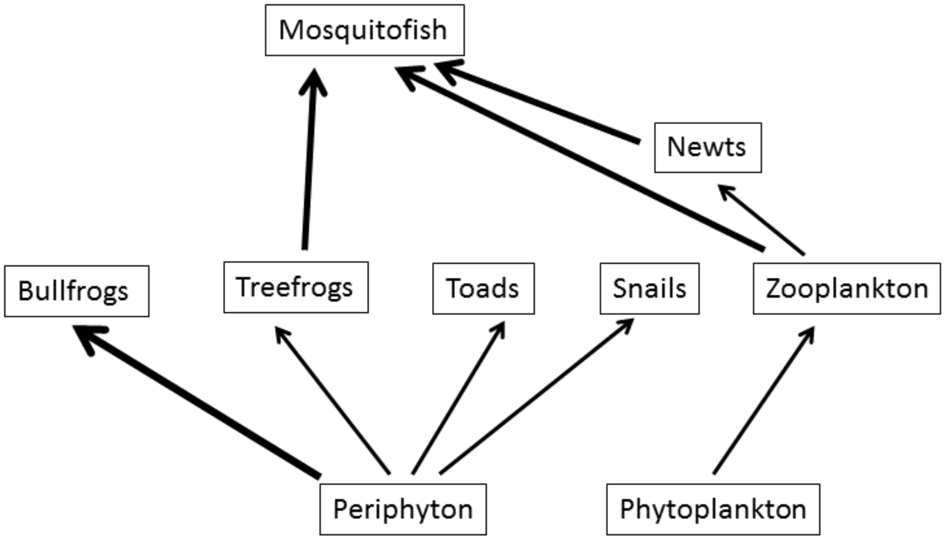


Figure 3. This food web depicts the hypothesized trophic interactions involving the invasive species (bold arrows) based on the mesocosm data.

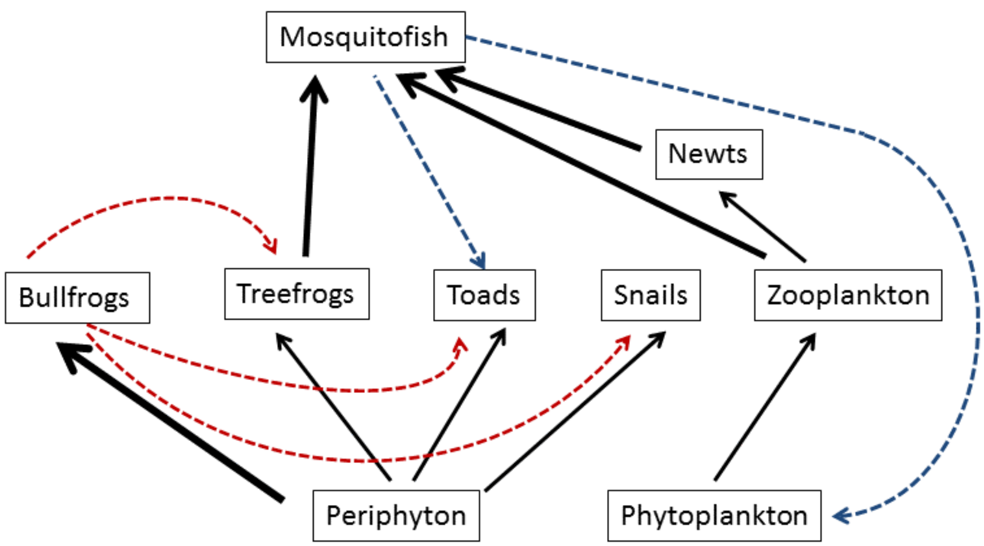


Figure 4. This food web depicts the hypothesized trophic interactions (bold arrows) based on the mesocosm data as well as the positive (dashed blue arrows) and negative (dashed red arrows) indirect effects that mosquitofish and

bullfrogs had on the aquatic community. Bullfrogs negatively indirectly affected treefrogs, toads, and snails through competition (they were all competing for the same resource, periphyton). Mosquitofish indirectly positively affected toads by reducing the abundance of tree frogs (a competitor), resulting in more periphyton for the toads to consume. Mosquitofish also indirectly positively affected phytoplankton by reducing the abundance of zooplankton.

Optional Extensions tasks

In the excel file titled 'Student_Excel_All_Data', we provide data on water nutrients and snail size. These measurements were taken at the end of the mesocosm experiment and provide additional insights on the effects of the two invasive species.

Water Chemistry:

With these data, instructors can ask a variety of questions, such as: What were the effects of mosquitofish and bullfrogs on nutrients? What mechanisms may be responsible for the observed effects?

Important Data Notes:

1.) DOC = Dissolved Organic Carbon
2.) TDN = Total Dissolved Nitrogen
3.) NH4+ = Ammonium
4.) TDP = Total Dissolved Phosphorous

Snail Size:

With these data, instructors can ask: what were the effects of mosquitofish and bullfrogs on snail size? If the focus of the course is on data organization, another potential question is: what is the proportion of snails with a shell width greater than 5 mm in each treatment? That way students will need to spend time organizing the snail_shell_width datasheet and also create a slightly more complicated query if they are using a database.

Statistical Analysis:

One simple way to analyze the data is to use a two-way analysis of variance (ANOVA) with bullfrog presence and mosquitofish presence as independent variables. Separate tests can be run with amphibian survival, amphibian mass, snail density, zooplankton density, and relative phytoplankton fluorescence as response variables. In these analyses, it should be emphasized that multiple measurements within a mesocosm (e.g., amphibian mass) are not independent of one another. In these instances, the response should be the mean value per mesocosm. ANOVA assumes normality of the data and homogeneity of variances between treatments. The students can use data visualizations (e.g., histograms) to test these assumptions.

STUDENT EVALUATIONS

The answer guide below highlights the key points that students should discuss when answering each question. In addition, we provide a rubric for each figure.

Part 1: Background Research and Hypotheses

A. The primary interactions that can be included in the food web are:
   - Mosquitofish consuming newts, zooplankton, and treefrogs.
   - American bullfrogs consuming periphyton.

   Below are a few potential sources that the students may cite:
   - Kiesecker J.M., Blaustein A.R., and C.L. Miller 2001. Potential mechanisms underlying the displacement of native red-legged frogs by introduced bullfrogs. Ecology 82:1964–1970.
   - Lawler, S. P., Dritz, D., Strange, T. and Holyoak, M. (1999), Effects of Introduced Mosquitofish and Bullfrogs on the Threatened California Red-Legged Frog. Conservation Biology, 13: 613–622.
   - Kupferberg, S.J. 1997. Bullfrog (*Rana catesbeiana*) invasion of a California river: the role of larval competition. Ecology 78:1736–1751.

B. Hypotheses about the effects of the invasive species can include:
   - Bullfrogs will have a negative effect on treefrogs, toads, and snails due to competition for resources (i.e., periphyton).
   - Mosquitofish will have a negative effect on newts, zooplankton, and treefrogs due to direct consumption. *Note* – most students will hypothesize that mosquitofish will negatively affect toads, and we recommend taking off zero (or very few points) for that hypothesis.
   - Mosquitofish will have a positive effect on phytoplankton by consuming zooplankton because zooplankton consume phytoplankton.

Part 2: Data Analysis and Interpretation

<u>Invasive species effects on native amphibian species</u>

1. The answer should include the following:
   - Neither invasive species affected toad survival.
   - The survival of both treefrogs and newts was negatively affected by mosquitofish, but not by bullfrogs.

Figure rubric:

- Correct calculations of mean and standard error
- Y-axis is percent survival
- Treatments are clearly labelled
- Logical grouping of data by native amphibian species and treatment.
- Error bars are included for each data point

2. The answer should include the following:
    - Mosquitofish decreased the mass of treefrogs and newts but did not have a strong effect on toads. Some students may say that mosquitofish appear to have a slight positive effect on toads, particularly when bullfrogs are present, which is okay.
    - Bullfrogs decreased the mass of treefrogs and toads but did not have a significant effect on newts.

    Figure rubric:

    - Correct calculations of mean and standard error
    - Y-axis is mass in grams
    - Treatments are clearly labelled
    - Logical grouping of data by native amphibian species and treatment
    - Error bars are included for each data point excluding instances where the sample size is one (i.e., the mosquitofish treatment for newts and the bullfrog/mosquitofish treatment for newts)

3. Direct effects include:
    - Mosquitofish predation of treefrogs, zooplankton, and newts.

    Indirect effects include:

    - Decrease in mass of treefrogs and toads in the presence of bullfrogs due to competition for periphyton.
    - Increase of phytoplankton in the presence of mosquitofish due to mosquitofish predation on zooplankton.
    - Slight increase of toad mass in the presence of mosquitofish due to mosquitofish predation of treefrogs, which reduced the competition for periphyton.

4. The discussion should include the following:
    - Mosquitofish have a substantially greater impact than bullfrogs on native amphibian populations.
    - To protect the native community, we should prioritize eradicating mosquitofish over bullfrogs.

Invasive species effects on other aquatic taxa

1. The answer should include the following:
    - Bullfrogs negatively affected snail density while mosquitofish had no effect on snail density.
    - Snail density decreased due to competition with bullfrogs for periphyton.

    Figure rubric:
    - Correct calculations of mean and standard error
    - Y-axis is density in units of # per mesocosm (or unit volume)
    - Treatments are clearly labelled
    - Logical grouping of data by native aquatic taxa and treatments.
    - Error bars are included for each data point.

2. The answer to part A should include the following:
    - Phytoplankton increased when mosquitofish was present.
    - Zooplankton drastically decreased when mosquitofish was present.
    - Bullfrogs had no effect on phytoplankton or zooplankton.

    Response to part B should include:
    - Mosquitofish directly negatively affected zooplankton density through predation.
    - Mosquitofish had an indirect, positive effect on the phytoplankton population by decreasing zooplankton densities.

    Figure rubric:
    - Correct calculations of mean and standard error
    - Y-axes are zooplankton density (# per liter) and phytoplankton fluorescence
    - Treatments are clearly labelled
    - Logical grouping of aquatic taxa and treatments

- Error bars are included for each data point

Part 3: Data Synthesis

Students should modify the food-web based on the results of the experiment. See Figure 3 and Figure 4 in the Notes to Instructor section for the appropriate food web diagrams

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