INQUIRY & INVESTIGATION

Inquiry-Based Laboratory Experiences Using Ecosystem Microcosms

NABT BioClub RECOMMENDATION

ROGER SAUTERER

Abstract

Self-sustaining ecosystem microcosms, called ecosystem jars, can easily be collected from local ponds, streams, or lakes. Sealed and exposed to sunlight, these miniature ecosystems can sustain themselves for a decade or more. Unlike Winogradsky columns, ecosystem jars are optimized for protist, animal, and plant observations and experiments, and are not altered by addition of sulfur, carbon, or cellulose sources, more accurately representing natural ecosystems. Ecosystem jars can support a variety of inquiry-based experiments, including student-designed projects, from middle school to college levels. Students, with instructor assistance, formulate hypotheses, design experiments, observe and catalog organisms by microscopy, then record their data in tables and graphs and draw conclusions. Students present their data in a paper or poster format or as oral presentations to the class. Potential experiments include examining biotic changes over time, the effects of added pollutants or nutrients, biotic differences between watershed types, or seasonal changes in biota. These investigations not only provide students with cooperative learning, inquiry-based lab experiences, but also help them gain appreciation for the effects of pollution or nutrient runoff on ecosystems.

Key Words: *ecosystem jars; Winogradsky columns; biotic changes; inquiry-based laboratories.*

○ Introduction

Self-sustaining ecosystem microcosms, called ecosystem jars, are easily obtained from local ponds and streams. These miniature ecosystems can sustain themselves for a decade or more, powered only by exposure to sunlight. Ecosystem jars can be used to support a wide range of inquiry-based lab exercises from middle school to college levels.

Self-sustaining ecosystem microcosms, called ecosystem jars, are easily obtained from local ponds and streams.

Using microscopes, students catalog and count different types of organisms, depending on the instructor's interest and expertise, test water quality parameters, and draw correlations between environmental conditions and proportions of different organisms. Students can examine biotic changes over time in ecosystem jars of various ages, between ecosystem jars collected at different types of watersheds, or experimentally perturb ecosystem jars collected at the same site by addition of nutrients, environmental toxins, or altered light exposure, and investigate effects of these perturbations on the biota present, promoting student hypothesis formation, experimental design, data collection, and analysis.

Ecosystem jars differ from commonly used Winogradsky columns by having a high water-to-sediment ratio and are optimized to support a wide range of protist and small invertebrate life, whereas Winogradsky columns are designed to support bacterial ecosystems and are mostly sediment with a small water column (Bodony et al., 2001; Anderson & Hairston, 1999; Rogan et al., 2005). Protists and meiofauna, unlike bacteria, are easily visible and identifiable under a microscope, and give students an appreciation of the diversity of life in local watersheds. Furthermore, Winogradsky columns are often "spiked" by the addition of cellulose, calcium carbonate, and sulfates to enhance microbial growth and may take weeks to achieve equilibrium. By contrast, ecosystem jars use unperturbed "native" ecosystem samples that better represent an actual watershed. Additionally, H_2S produced by sulfate-reducing bacteria in Winogradsky columns may enter (Rogan et al., 2005) and poison the water column.

Biology education is transitioning from traditional "cookbook" labs to inquiry-based labs that more accurately represent the scientific process (NRC, 2000; AAAS, 2011). Studies comparing traditional "cookbook" biology labs to more inquiry-based labs where students form hypotheses, design experiments, collect and interpret data, show that inquiry-based labs increase student interest in both classroom settings and in performing research later during their education (Brownell et al., 2012). Inquiry-based labs additionally improve student

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understanding and retention of core concepts, processing, and critical analysis skills (Rissing & Cogan, 2009; Lord & Orkwiszewski, 2006; Luckie et al., 2004). A meta-analysis of nearly 140 published studies (Minner et al., 2009) comparing inquiry-based K–12 science education to traditional methods of instruction showed a significant increase in knowledge and concept retention in half of the analyzed studies, especially in curricula that emphasized student active thinking, generation of hypotheses, designing investigations, and collecting and analyzing data to draw conclusions.

Like Winogradsky columns, which support inquiry-based experiments in microbiology (Anderson & Hairston, 1999), ecosystem jars, designed to support protists and small invertebrates, are versatile vehicles that support a range of inquiry-based, cooperative lab experiences from middle school to college levels. Students engage in hypothesis testing, experimental design, data collection and presentation, and in drawing correlations between environmental parameters or perturbations and biotic changes.

○ Materials Needed

- Sample collection jars or cleaned Mason jars of 1–2 liter capacity. (*Note*: Rinse out any soap thoroughly!)
- Microscopes and slides
- Water quality test kits (Lamotte 5870-01 test kit or equivalent)
- pH meter and, if possible, a dissolved oxygen meter
- Cameras, if microphotography is desired. (Cell phone cameras can take good photos if properly aligned with the eyepiece.)
- Identification keys for organisms of interest
- Data tables and graphs for student use

○ Sample Collection

Instructors and/or students can collect samples for ecosystem jars from any local freshwater environment. Wetlands and shores of ponds have high biodiversity, while swiftly flowing streams and large lakes generally have lower diversity and organismal density. Although the author has no experience in marine ecosystem jars, marine environments such as estuaries may also be suitable for collection.

Fill 10 percent of the jar volume with sediment, then fill it to the top with sample water (Figure 1). Include large amounts of photosynthetic organisms such as cyanobacterial mats, algae, and aquatic plants as they are essential for maintaining the ecosystem jar and are associated with numerous small animals. Sediments contain much of the bacteria involved in nitrate, phosphate, and organic matter recycling that sustains these ecosystems, and may have additional small animals. After collection, seal the jars and expose them to sunlight or bright artificial light, such as classroom windowsills. Do not unseal the jars until students perform the experiments.

○ Instructor Preparation

Instructors should explain the basic carbon/oxygen cycle, in which photosynthesizing organisms use light to convert carbon dioxide into biomass, releasing oxygen, whereas oxidative organisms consume or decay the biomass produced, using up oxygen and releasing carbon dioxide. Nitrogen, sulfur, and phosphorous cycling can also be explained if desired. Miller and Spoolman (2015) and similar environmental science texts explain these cycles and have useful illustrations. Instructors should also emphasize that alteration of an ecosystem can affect the proportions of different organisms and that ecosystems evolve over time.

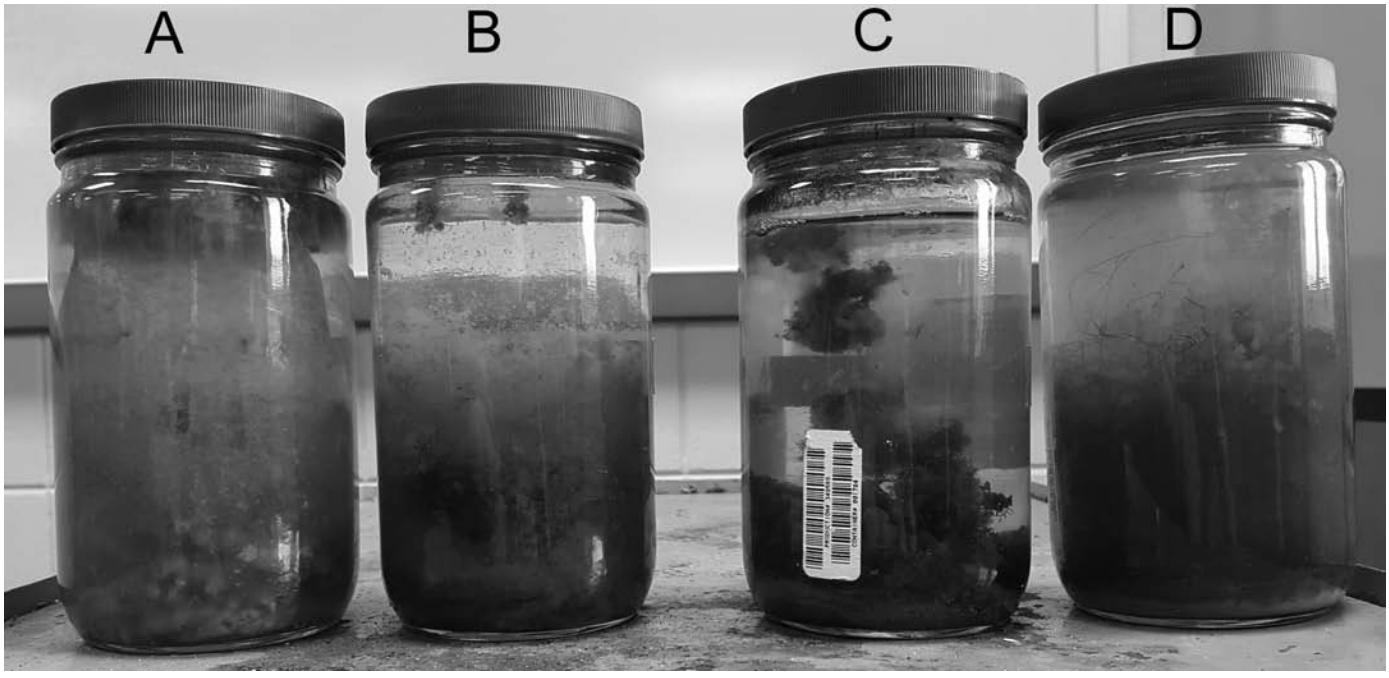

Figure 1. Photographs of ecosystem jars of different ages from the same collecting site. Samples were exposed to sunlight and sealed except for student experiments twice yearly. Sample collection dates are 2005 (**A**), 2009 (**B**), 2013 (**C**), and 2015 (**D**). Photo courtesy of student Vanessa Chapell.

Instructors should demonstrate water quality analysis and provide students practice using pH or dissolved oxygen meters and water quality test kits prior to the experiments. The Lamotte 5870-01 test kit costs about $400 and includes tests for pH, dissolved oxygen, alkalinity, hardness, nitrogen, and phosphates, and is very useful for these and other classroom experiments. Because many of the reagents in water quality test kits, especially the dissolved oxygen test, are toxic and/or corrosive, students should be warned of the hazards and closely supervised. Use of a dissolved oxygen meter reduces the hazards of performing the dissolved oxygen test.

Instructors provide for student use identification keys and appropriate data sheets (Figure 2) for the organisms of interest. Figure 3 shows an example of identification keys, using images from the Internet and assembled with a photo editing program.

○ Student Experiment and Data Collection

Warn students to keep jars closed unless they are specifically collecting samples. Student teams of 2–4 first collect water samples and perform the appropriate water quality analysis. For labs with pH or dissolved oxygen meters, have students insert the probes into the jar and record the data. Water quality data is posted for the entire class to use.

Students teams collect samples using transfer pipettes and then make multiple wet mounts on microscope slides for observation. Samples should be collected from the water column, including plant fragments or algae (often richer in organisms), and from the surface of the sediment.

Using the keys provided, students look for and count the total number of organisms and the number of each specific category of organisms in each slide. Students should observe multiple slides from each ecosystem jar to get good data. The instructor can help students identify organisms, point out interesting organisms, and explain some of their characteristics. Students calculate the total number of each organism type, the total number of organisms observed, the proportion of each type of organism relative to the total organisms counted, and the total organism per slide. Data collection and observations typically can be done in a 2- to 4-hour lab period.

Names
Class Data
BY 373

Ecosystem Data	2005	2009	2013	2015
Number of Slides	16	15	15	14
pH	9	8.8	8.8	8.1
O2 (mg/L)	7.8	7.8	7.2	6.4
PHOTOSYNTHESIS				
Filamentous Green Algae	+	+	+	+
Other Green Algae	+	+	+	+
Cyanobacteria	+	+	+	+
Fungi	-	-	+	+
ANIMALS/PROTISTS				
Ciliates	0	20	8	46
Percent	0.00%	44.44%	12.12%	46.94%
Other Protists	0	0	0	2
Percent	0.00%	0.00%	0.00%	2.04%
Nematodes	0	6	2	3
Percent	0.00%	13.33%	3.03%	3.06%
Rotifers	0	13	38	38
Percent	0.00%	28.89%	57.58%	38.78%
Tardigrades	0	4	9	0
Percent	0.00%	8.89%	13.64%	0.00%
Mites	0	0	2	6
Percent	0.00%	0.00%	3.03%	6.12%
Copepods	0	1	2	3
Percent	0.00%	2.22%	3.03%	3.06%
Daphnia	0	0	1	0
Percent	0.00%	0.00%	1.52%	0.00%
Other/Unknown	5	1	4	0
Percent	100.00%	2.22%	6.06%	0.00%
Total Organisms	5	45	66	98
Total Organisms/Slide	0.31	3.00	4.40	7.00

Figure 2. Example of a data sheet for the ecosystem jars experiment using pooled class data. This data sheet is used for ecosystem jars of various ages (line 4). Students count the number of slides observed (line 5), pH and dissolved oxygen of each jar (lines 6 and 7), and enter both the number of specific types of animals and proportion of these animals to the total animals scored. Data courtesy of student Trent Ford and the June 2016 BY 373 Cell Biology class.

Using either graph sheets provided by the instructor or graphs generated in Excel (Figure 4), students plot the proportion and/or number of organisms against environmental parameters such as pH, dissolved oxygen, age of ecosystem jar, concentration of added chemicals, etc. Either bar or line graphs can be used to present data. Most line graphs will have multiple lines, one for each type of organism (Figure 4A).

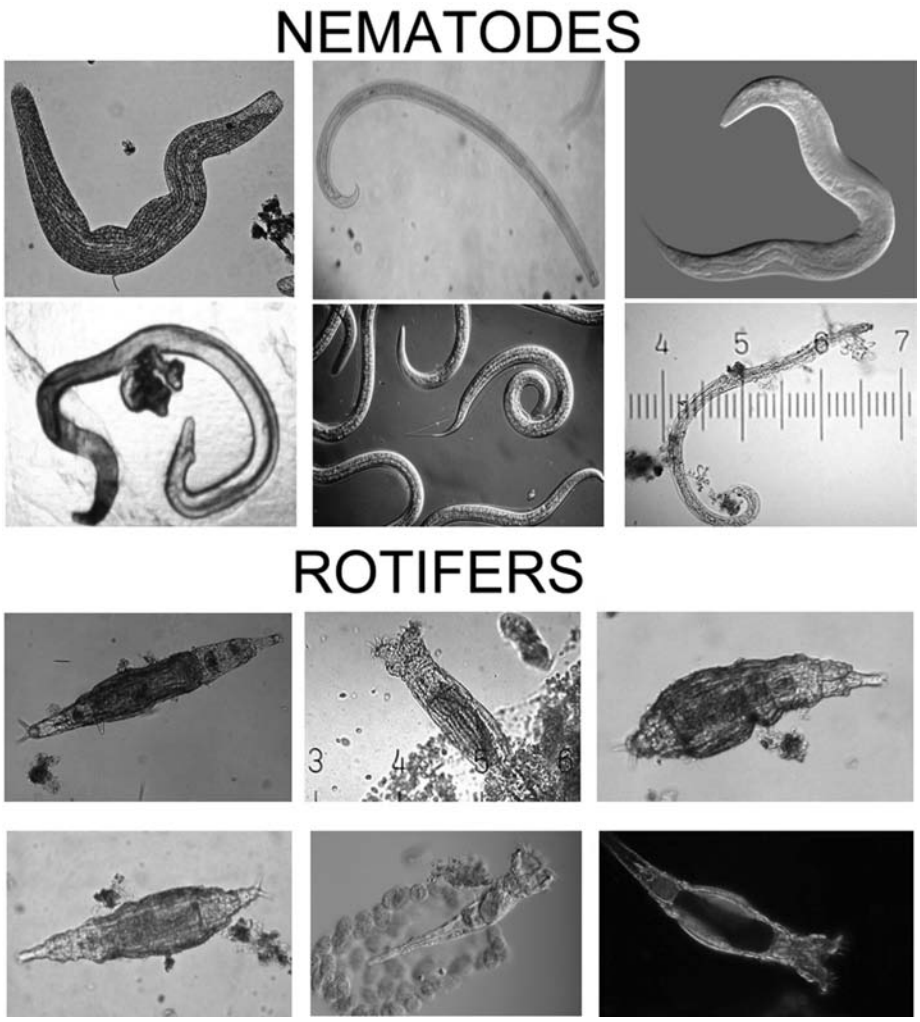

Figure 3. Sample identification keys for student use. All images under of Wikimedia creative commons license (https://creativecommons.org/licenses/by/2.0/legalcode) and were cropped, resized and positioned by author. **Nematodes**: (*top left*) Richey Schley, 2010 (https://commons.wikimedia.org/wiki/Category:Nematoda#/media/File:Nematoda_Fadenwurm.jpg); (*top center*) Christina Menta, 2012 (https://commons.wikimedia.org/wiki/Category:Mononchidae#/media/File:Nematode,_Mononchidae_-_Soil_Fauna_Diversity.jpeg); (*top right*) Bob Goldstein, 2006 (https://en.wikipedia.org/wiki/Caenorhabditis_elegans#/media/File:CelegansGoldsteinLabUNC.jpg); (*bottom left*) Mattheiu Godbout, 2007 (https://commons.wikimedia.org/wiki/Category:Nematoda#/media/File:Nematode-estomac-hemitriptere.jpg); (*bottom center*) CISRO, 2000 (https://commons.wikimedia.org/wiki/Category:Nematoda#/media/File:CSIRO_ScienceImage_2818_Group_of_Nematodes.jpg); (*bottom right*) Bob Baylock, 2010 (https://commons.wikimedia.org/wiki/Category:Nematoda#/media/File:20100814_175958_FungusOnDeadnematode_HarposporiumAnguillulae.jpg). **Rotifers**: (*top left*) Gabrial Belfort, 2009 (https://commons.wikimedia.org/wiki/Category:Bdelloidea#/media/File:Rotifera_Micrograph_(brightfield).JPG); (*top center*) Bob Baylock, 2009 (https://en.wikipedia.org/wiki/Rotifer#/media/File:Bdelloid_Rotifer.jpg); (*top right*) David Hobern , 2015 (https://commons.wikimedia.org/wiki/Category:Bdelloidea#/media/File:Bdelloidea_sp._(16068859820).jpg); (*bottom left*) David Hobern, 2015 (https://commons.wikimedia.org/wiki/Category:Bdelloidea#/media/File:Bdelloidea_sp._(16068861010).jpg); (*bottom center*) Frank Fox, 2010 (https://en.wikipedia.org/wiki/Rotifer#/media/File:Mikrofoto.de-Raedertier_Ptygura_pilula_2.jpg); (*bottom right*) Frank Fox, 2011 (https://commons.wikimedia.org/wiki/Category:Unidentified_Rotifera#/media/File:Mikrofoto.de-Raedertier-14.jpg).

○ Student Presentations and Assessment

Students present their data and conclusions in individual or group lab reports using scientific paper format, and/or as an oral group presentation. Either format instructs students on how to report scientific data and allows for instructor assessment of their projects. Additionally, examination of data sheets, observations of student activity, and exam or quiz questions can further assess student learning from this project.

Types of Ecosystem Jars Experiments

Ecosystem jars can be used to investigate a wide range of questions related to ecosystems and biota. Each set of observations can be performed in a single 2–4 hour lab, depending on level of detail desired. Examples include:

Changes in ecosystem jars over time.—This demonstrates to students that ecosystems evolve and change over time, with relative proportions of organisms varying as the ecosystem ages. Instructors need to collect multiple samples from the same location over a period of several years.

Seasonal differences.—Collect samples from the same site in summer, fall, spring, and winter over a few years to determine if organismal proportions differ on a seasonal basis.

Different types of water bodies.—Collect samples from small ponds, larger lakes, streams, or other water bodies, or from different locations in the same water body, such as pools and rapids of a stream, near shore and off-shore of ponds or lakes. Students can investigate whether types and proportions of organisms differ between different micro-environments in the same water body.

Environmental Perturbation Experiments

These types of experiments are especially valuable for inquiry-based lab experiments, requiring hypothesis generation, experimental design, periodic observations, and data collection and analysis. Each set of observations takes two to four hours and should be performed at daily to weekly intervals over a period of a few to several weeks.

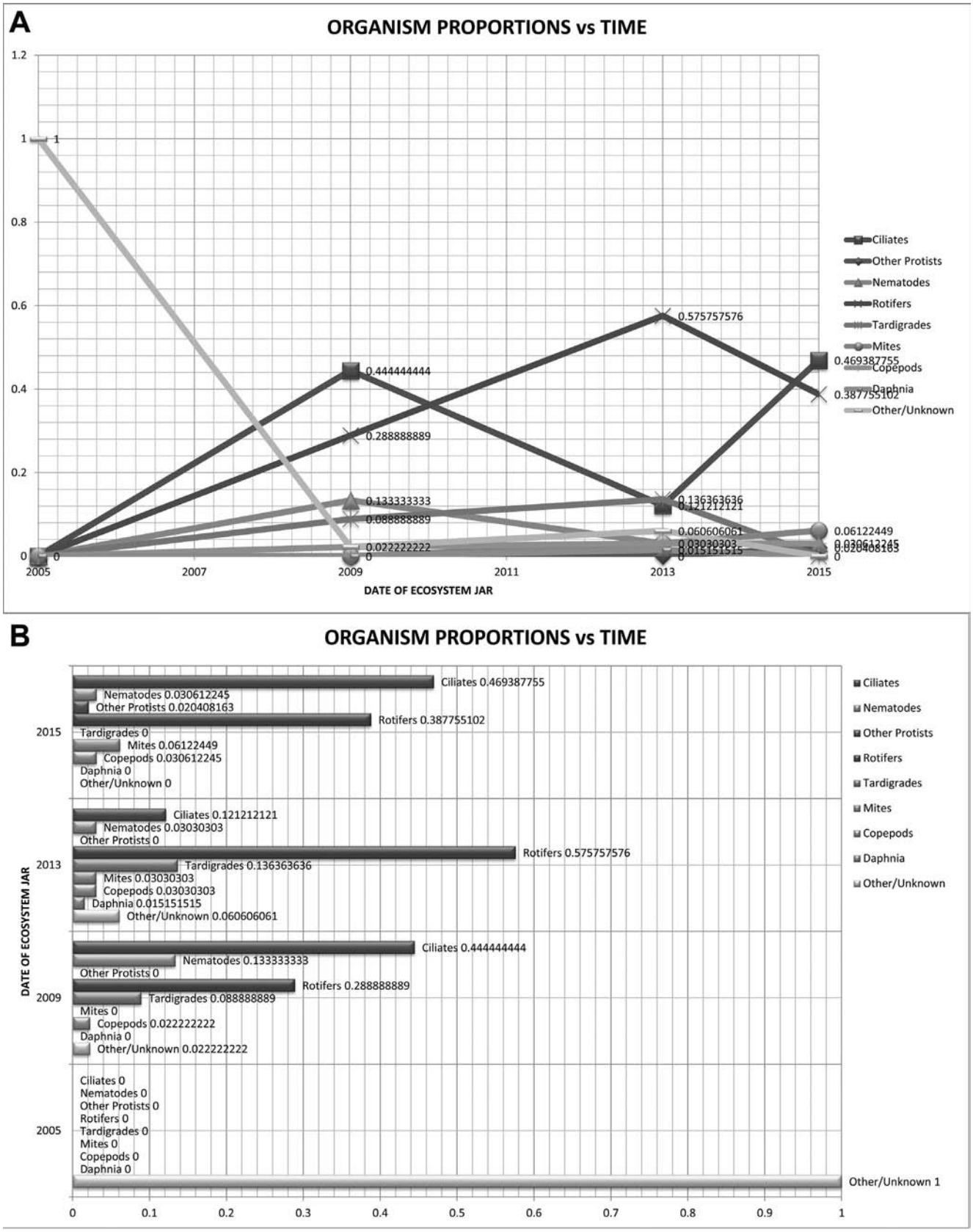

Figure 4. Sample graphs of organism proportions vs. collection date of ecosystem jar. Data can be expressed as a line graph (**A**) with multiple lines, each one representing a specific group of organisms, or as a bar graph (**B**). Data courtesy of student Trent Ford and the June 2016 BY 373 Cell Biology Class.

Effects of pH.—Several samples are collected from the same location, their pH measured, and then small a amount (2–5 mM) of phosphate buffer is added to each jar, and the pH is adjusted to the desired value. The control jar should be adjusted to the same pH as was initially measured. pH ranges should be between 5 and 9 or few organisms will survive. Students analyze the organismal proportions periodically (daily to weekly) over a period of weeks to determine changes in organismal numbers and proportions. This exercise gives students an appreciation of the effects of acidic rain or runoff on aquatic ecosystems and that organisms vary in sensitivity to pH.

Effects of nutrients.—High nutrient loads in watersheds are a serious environmental issue (Meuller & Helsel, 1996). EPA recommended limit for phosphates is 50 ug/l (expressed as P), and the limit for nitrates in drinking water is 10 mg/l (Meuller & Helsel, 1996). Students initially determine numbers and proportions of organismal categories in selected sample jars, then add varying amounts of nitrates (as potassium, sodium, or ammonium nitrate) or phosphates (sodium or potassium phosphate), combinations of nitrates and phosphates, or commercial fertilizers (up to 100 mg/liter) to the jars. Concentrations should range from half the EPA limit up to 100 times the EPA limit. When adding phosphates, make sure the pH is kept to that of the control. Students should measure pH and dissolved oxygen and analyze biota in each sample jar on a weekly or biweekly basis to determine the effects of nutrient load on the biota.

Metals and other toxins.—Students can investigate the biotic effects on ecosystem jars collected from the same site by adding various amounts of metal ions or other common pollutants (such as oil or gasoline) to ecosystem jars and examining each jar periodically (daily to weekly) for organismal numbers and proportions (over a period of weeks. Each jar should be analyzed for water quality and biota prior to adding pollutants. Pollutants can be added individually or in combination. Concentrations should include a range from below the EPA recommended limit to at least ten-fold EPA limit. Recommended limits in freshwater ecosystems of common metal pollutants (U.S. EPA, 2017) are: iron 1mg/l, nickel 52 ug/l, zinc 90 ug/l, chromium (III) 74 ug/l. Metals can be added as chlorides or sulfates. Calculate concentrations on the basis of the metal, not the overall compound. The pH of the jars should be measured and adjusted if necessary after addition of the metals or other pollutants, as some may alter pH. Warn students about the toxicity of chromium and nickel. These experiments give students appreciation of the effects of common industrial pollutants and show that organisms vary widely in their sensitivity to these agents.

○ Educational Outcomes and Benefits

Ecosystem jars can support a wide range of cooperative, inquiry-based investigations, including student-designed experiments. Experiments using ecosystem jars and analysis of their biota can be oriented to the particular interests and background of the instructor, including investigations of plants or algae, invertebrate animals, or protists. Additionally they are inexpensive, and sampling sites are widely available close to the school or institution. Benefits and learning outcomes are listed below. NGSS numbers are from the Next Generation Science Standards for high school (NGSS, 2013).

- The use of student teams encourages cooperative learning both in data collection and analysis and in the development of the paper or presentation. *Assessment*: Observations of student teams; attribution of sections in the lab report or presentation.
- Students generate hypotheses about effects of perturbations on the ecosystem jar biota and design experiments to test their hypothesis. A sample hypothesis is: "Some categories of organisms are more sensitive to iron than others" (NGSS HS-LS2-1, HS-LS2-2, HS-LS2-6, HS-LS4-5, HS-LS4-6, HS-ESS3-3). *Assessment*: Examination of student hypotheses and experimental design for appropriateness, controls, and data generated.
- Students collect data, prepare appropriate tables and graphs, and attempt to correlate biotic changes with environmental parameters to draw conclusions (NGSS HS-LS-2-1, HS-LS2-2, HS-LS4-5, HS-LS4-6, HS-ESS3-3). *Assessment*: Observation of student notebooks, data sheets, and reports.
- Students present their data in one or more scientific formats, including paper or poster formats, or oral presentation. *Assessment*: Evaluation of quality of the report and/or presentation.
- Students gain experience in water quality assessment (using water test kits) and instrumentation (using pH or dissolved oxygen meters), as well as in microscopy and organismal identification (NGSS HS-LS2-1, HS-LS2-2, HS-LS2-7). *Assessment*: Instructor observations of student data collection; quality of data observed in student notebooks and reports.
- Students gain appreciation for the effects of pollutants, nutrients, or acidic rain and runoff on freshwater ecosystems, and actually see and measure these effects (NGSS HS-LS2-2, HS-LS2-6, HS-LS2-7, HS-LS4-5HS-LSS3-4). *Assessment*: Student reports and presentations, test or quiz scores.
- Students gain appreciation for biotic changes as ecosystems evolve over time (NGSS HS-LS4-5). *Assessment*: Evaluation of student reports and presentations, quiz or test performance.
- Students gain appreciation for the diversity of small organisms commonly found in local waters. Instructors have an opportunity to describe the features and habitats of many of the organisms observed.

Ecosystem jars in biology laboratory exercises, therefore, are a valuable addition to the lab exercises in a wide range of curricula and levels, and are worthy of consideration and adoption.

○ Acknowledgments

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ROGER SAUTERER is an Associate Professor of Biology at Jacksonville State University, Jacksonville, AL 36265; e-mail: sauterer@jsu.edu