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Developing Collaborative Lab Experiments across Disciplines through the Identification of Bacteria

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Objectives outlined in Science for All Americans include that students will be exposed to science as a collaborative effort, encompassing different scientific fields. We developed a framework for collaboration between non-science major biology and environmental science lab classes with the central theme of identifying bacteria. In this exercise, the students are tasked with identifying any bacteria that is present in the water using their complimentary skillsets. Limited directions were given in the Student Outline, so group members had to rely on one another's knowledge in order to complete the assignment. We suggest that such an activity allows students to be exposed to the concept of collaboration in the scientific community, with the goal of addressing a single problem. In this workshop, participants will go through the lab activity to identify bacteria present in a water sample and will learn how to develop their own collaborative lab activities between classes.

Keywords: non-science majors, collaboration, bacteria, water filtration

Introduction

Today's educators realize the importance of collaboration. No one person has all the answers, but together scientists can make great discoveries. These discoveries can be made by sharing knowledge with each other. One question teachers face: how do we bring non-science majors together to work and make discoveries? The ideal location is the lab or learning environment that encourages inquiry, research, observations and drawing conclusions. Laboratories and other engagements of discovery can help students to retain knowledge from lecture (Handelsman *et al*. 2004). Scientific collaboration is reinforced by frequent group activities involving members from different content disciplines (Rutherford and Ahlgren 1991).

In order to develop collaborative lessons, instructors need to find courses in which there is similar content being taught. For a true collaborative session, an experimental question should be posed to the students that requires prior knowledge from complimentary areas. We suggest that students should be assigned into groups in which each is knowledgeable in a different aspect of the pre-requisite material. This strategy ensures that the students must rely upon one another's expertise in order to fully answer the question. It is important that no student in the group knows all of the pre-requisite material, otherwise that student could potentially take over the experimental process, which will not promote collaboration with other group members. Ramirez *et al*. (2015) recently showed that this collaborative approach can be used to help both science majors and non-science majors. In that study, science majors who were familiar with sequencing the 16S rRNA gene teamed up with non-science majors who were familiar with preparing and interpreting chlorophyll spectra graphs to fully identify bacteria that were enriched in Winogradsky columns.

One of the most widely used experiments in a microbiology laboratory class is the identification of the unknown bacterium (Martinez-Vaz *et al*. 2015). This lab exercise uses a wide variety of lab techniques and has been shown to be effective in teaching important concepts in microbiology. We have taken this traditional laboratory exercise and have made it appropriate for classes of non-science majors. In contrast to a traditional laboratory in which collaboration occurs between students within a single discipline group, our exercise promotes collaboration between different students in different classes. Students with backgrounds in biology are teamed up with students with backgrounds in environmental science. Together, they are presented with a sample of

water contaminated with bacteria. Students then share their "expertise" of various laboratory techniques and the knowledge they have learned in their classes with one another to isolate and identify the bacteria present in the water. Based on previous course work, the environmental science students are experts in isolating and plating bacteria from water samples. Biology students have learned how to identify bacteria and about microscopy prior to the exercise. Together, the students learn new concepts and lab techniques from their peers. These lab techniques include an aseptic technique for culturing microorganisms from environmental water samples, gram-staining, and microscopy. By completing this activity, students are able to practice collecting, analyzing, and interpreting data. They also can practice presenting their results to their peers.

Student Outline

Brief Description of Activity

In this collaborative experiment, students in non-majors biology and non-majors environmental science will work together using the various laboratory techniques previously learned this semester to identify bacterial species present in a sample of water.

Learning Goals

By the end of this lab, students from both disciplines will:

- learn about the collaborative nature of science.
- practice setting up an experiment in order to answer a question based on an initial observation.
- learn and practice different techniques for identifying bacteria in water (microscopy, staining, differential biochemical tests based on a bacterium's ability to ferment sugar).
- practice analyzing and presenting data to their peers.

Collaboration

Begin by forming groups of four: two students from each class.

1. Write down the names of each group member in the space below.

Answer the following questions.

2. Discuss with your lab partners what it means that "science is a collaborative process." Write your thoughts in the space below.

   _____
   _____
   _____

3. Give an example of science being collaborative besides working together in groups in the teaching laboratory.

   _____
   _____
   _____

Water Quality Analysis

Each group will receive 100 mL of water. This water has up to two (2) different bacterial species. For the next four weeks (~30 minutes each week) you will work together to identify what type of bacteria are present in this sample. The potential bacterial species that may be present in your samples include the following.

- *Escherichia coli*
- *Enterobacter aerogenes*
- *Staphylococcus aureus*
- *Bacillus cereus*
- *Bacillus subtilis*

The following tests/techniques are available to you.

- Membrane filtration test
- Microscopy & Gram-staining
- Nutrient agar plates
- Citrate utilization(*)
- Lipid hydrolysis(*)

With the exception of two of these tests (*), some members of the group may notice that they are familiar with several of these techniques. Therefore, you will need to work together with your knowledge of these techniques in order to identify the bacteria present in the water.

The two tests that have not been taught to members of the group previously include citrate utilization and lipid hydrolysis.

Citrate Utilization

This test determines whether bacteria contain citrate permease, a protein that allows the bacterium to uptake citrate and use it as a carbon source. If bacteria can uptake citrate, the citrate in the medium will be metabolized and sodium carbonate (an alkaline product) will be produced. Bromothymol blue is incorporated into the medium as a pH indicator. Under alkaline conditions this indicator turns from green to blue (Figure 1). The utilization of citrate in the medium releases alkaline bicarbonate ions that cause the media pH to increase above 7.4 (MacWilliams 2009).

Day 1:

- Streak inoculate citrate agar slant with the test organisms and incubate at 37°C.

Day 2:

- Observe the tubes and record your results

Figure 1. Citrate utilization results.

Lipid Hydrolysis

This test determines whether the bacterium is able to break down lipids (a macromolecule) found in egg yolk (Yeung *et al*. 2005).

Day 1:

- Spot inoculate the test organisms on egg-yolk agar and incubate plate at 37°C.

Day 2:

- Observe the plates for a zone of opacity, indicating lipase activity (Figure 2).

Figure 2. Egg yolk results.

Your basic experimental design should cover the following.

- Week 1: Separate the bacteria from the water.
- Week 2: Identify how many different types of bacteria you have and separate them onto different plates.
- Week 3: Order tests.
- Week 4: Order additional tests and finish identifying bacteria.

Each week, groups will "order" tests that they wish to perform. Each group will have a budget of $6.00 (not real money). Each test will cost $1.00. The purpose of this is to ensure that you think critically about which tests you need, rather than just testing everything.

The properties of each potential bacterial species are in Table 1.

Table 1. Properties of potential bacterial species.

Bacterium	Gram-Reaction	Shape	Citrate utilization	Lipid Hydrolysis
Escherichia coli	Negative	Rod	Negative	Negative
Enterobacter aerogenes	Negative	Rod	Positive	Negative
Staphylococcus aureus	Positive	Coccus	Negative	Negative
Bacillus cereus	Positive	Rod	Negative	Positive
Bacillus subtilis	Positive	Rod	Negative	Negative

Worksheet

Week 1	**Starting Balance: $6.00**		
Tests Ordered	Reason	Approved by	Balance Remaining

Week 2			
Tests Ordered	Reason	Approved by	Balance Remaining

Week 3			
Tests Ordered	Reason	Approved by	Balance Remaining

Week 4			
Tests Ordered	Reason	Approved by	Balance Remaining

4. For each test ordered, state the results and how those tests informed you as to what bacteria was present in your water sample.

Week #	**Test**	**Results**	**What did this test tell you?**

5. Based on the tests performed, what bacteria (if any) are present in your water sample?

_____

_____

6. Describe, in detail, how your group came to this conclusion.

Materials

Listed below are the materials needed for each week of the activity. Recipes for each of the growth media can be found in Appendix A.

Bacteria & Water Samples

- Prior to the start of the activity, lab instructors should prepare 100 L of sterile water for each lab group.

- Instructors should prepare overnight cultures of each of the following bacteria (grown for 12 to 14 hours at 37 °C, 200 rpm).
    - *Escherichia coli*
    - *Enterobacter aerogenes*
    - *Staphylococcus aureus*
    - *Bacillus cereus*
    - *Bacillus subtilis*

- The day of the activity, instructors should prepare a 1:100 (v/v) dilution of each bacteria culture in sterile water. Five microliters of one or two of the diluted bacteria types should be added to each group's 100 mL sterile water.

Week 1: Water Filtration

- Vacuum pump (at least one per class)
- Sterile Membrane Filtration Chamber (one per group)
- 47 mm, 0.45 µm gray nitrocellulose membrane (one per group)
- Forceps (one per group)
- Ethanol (95%) in a glass beaker for sterilization of forceps (one per group)
- Luria-Bertani agar plates (in 60 x 15 mm dishes) (one per group)
- Lab marker/sharpie to label plates (one per group)
- Incubator set to 37 °C (one per class)

Week 2: Isolation of Bacteria

- Luria-Bertani agar plates (in 60 x 100 mm dishes) (number depends on how many different types of bacteria colonies they have isolated)
- Inoculating loops (at least one per group)
- Bunsen burner to sterilize inoculating loop (one per group)
- Lab marker/sharpie to label plates (one per group)
- Incubator set to 37 °C (one per class)

Weeks 3 & 4: Identifying Isolated Colonies of Bacteria

- Microscope slides (one box per class)
- Cover slips (one box per class)
- Inoculating loops to prepare slides of each bacteria colony for Gram-staining (at least one per group)
- Bunsen burner to heat fix cells prior to staining (one per group)
- Gram-stain kits (one per group)
    - Crystal violet
    - Gram's Iodine
    - Ethanol (95%)
    - Safranin O
- Bibulous paper (one booklet per group)
- Compound light microscope (one per group)
- Oil Immersion for microscopy (one bottle per group)
- Simmons Citrate Utilization Tubes (approximately two per group)
- Egg Yolk Lipid Agar Plates (approximately two per group)

Notes for the Instructor

Pre-Requisite Knowledge

Prior to beginning the laboratory activity, students should be familiar with the listed concepts/laboratory techniques. If this experiment is being done collaboratively between two classes, then the pre-requisite knowledge can be divided up between the two classes.

- Experimental design
- Metabolism of sugars and fermentation
- General properties of prokaryotes, most notably from Domain Bacteria
- How to properly use the microscope
- Proper staining of slides
- Proper aseptic technique and plating bacteria
- Water quality analysis and membrane filtration

Instruction

This lab works best if instructors for both of the participating classes are present for the activity. Students will have access to an instructor with whom they are familiar, and another who likely has a different expertise than their usual teacher. In addition, having both instructors present improves the student to teacher ratio, and keeps the lab running smoothly.

Principles Behind Each Component of the Activity

Part 1: Water Filtration

Prior to the start of the experiment, the instructor should take the water samples and inoculate them with known bacterial strains (*Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, Bacillus cereus* or *Bacillus subtilis)*. Inoculate each water sample with up to two different strains. If you are going to use two bacteria, it is recommended that you choose one Gram-positive and one Gram-negative bacterium. Due to the low concentration of bacteria typically found in water, bacteria are detected using a membrane filtration system (Forster and Pinedo 2015). This system utilizes a nitrocellulose membrane that has a pore size of 0.45 µm. As water passes through the membrane, most bacteria will be caught on the membrane. The membrane can then be placed on nutrient agar plates and incubated overnight. A video demonstrating this technique can be found at: https://www.youtube.com/watch?v=Ev5B6PxITh0 .

Normally the membrane filtration test is used by scientists to look for an indicator microorganism (typically *E. coli*) (Forster and Pinedo 2015). To select for the growth of *E. coli*, special nutrient agar (mFC agar or mENDO agar) is used with an incubation temperature of 44.5 °C. However, since we do not want to select for just one type of bacterium, we have revised this protocol to use non-selective growth media (Luria-Bertani agar) at 37 °C to allow for the growth of different bacteria. Further information on the membrane filtration test and pictures showing each of the equipment needed can be found at: http://www.asmscience.org/content/education/protocol/protocol.3982 .

Part 2: Identifying the Number of Different Types of Bacteria Present in Water and Re-Streaking

When bacteria grow on agar plates, each bacterial cell replicates by binary fission and eventually forms into colonies. After incubation of the nitrocellulose membrane, students will examine for the presence of colonies. Bacterial colonies can be classified according to color, shape, edge, and whether there is any elevation. Differences in colony properties can be used to determine how many types of bacteria are present (the students will not be able to determine the identification of each bacterial colony at this point). Students will make observations about the colonies they see and then pick one of each and re-streak them onto new Luria-Bertani agar plates. By doing this, the students are separating the bacteria. This ensures that there is no cross-contamination of bacteria during their identification procedures.

Part 3: Gram-Staining and Other Identification Processes

The first procedure in identifying the bacteria in this experiment is to perform a Gram-stain. The Gram-stain is a differential stain used to classify bacteria according to the composition of their cell envelope. The procedure for the Gram-stain is as follows.

- Using your sterile inoculating loop, take a loop full of culture and spread onto a glass slide.
- Allow the slide to dry for several minutes.
- Heat fix your slide.
- Add a few drops of crystal violet. Sit for 1 minute.
- Rinse off crystal violet with distilled water.
- Add a few drops of iodine. Sit for 1 minute.
- Rinse off iodine with water.
- Rinse with ethanol until run-off is clear (no more than 10 to 15 seconds). Then rinse with water.
- Add a few drops of safranin. Sit for 1 minute.
- Rinse with water and dry using bibulous paper. View results using bright-field microscopy.
- A video of the Gram-stain procedure can be seen at: https://www.youtube.com/watch?v=6QCFlBryJUo .

Gram-positive bacteria have a thick cell wall of peptidoglycan and will stain purple. Gram-negative bacteria have a thin cell wall of peptidoglycan and an outer membrane. They will stain pink. This technique will also allow the students to identify the shape of the bacteria cells. From the table of bacteria properties (Table 1), we see that all of the bacteria are either rod-shaped or cocci (spherical)-shaped.

The students will have to perform two other tests (citrate utilization and lipid hydrolysis) to determine the identity of the bacteria in their sample. The instructions for both of these tests are described in the Student Outline for this activity. The test for citrate utilization determines whether bacteria contain citrate permease, a protein that allows the bacterium to uptake citrate and use it as a carbon source. If bacteria can uptake citrate, the citrate in the medium will be metabolized and sodium carbonate (an alkaline product) will be produced. Bromothymol blue is incorporated into the medium as a pH indicator. Under alkaline conditions, this indicator turns from green to blue (Figure 1). The utilization of citrate in the media releases alkaline bicarbonate ions that cause the media pH to increase above 7.4 (MacWilliams 2009). Table 1 indicates which bacteria should test positive and which should test negative for citrate utilization.

The lipid hydrolysis test determines whether the bacteria are able to break down lipids in egg yolk. After performing the test, a zone of opacity will appear around a

positive result, indicating that the bacteria can hydrolyze the lipids present. Table 1 indicates which bacteria should test positive and which should test negative for lipid hydrolysis.

Proper Handling and Disposal Instructions

This lab uses biohazardous materials. Lab coats, gloves, and goggles should be provided for the students when completing this lab activity. All instructors should be familiar with their institution's disposal guidelines and the American Society of Microbiology's Laboratory Safety Guidelines (http://www.asm.org/index.php/guidelines/safety-guidelines). All waste material contaminated with bacteria should be placed in biohazardous containers and autoclaved prior to disposal.

Sample Data to the Activity

The description below shows how students can identify a water sample that contains both *Staphylococcus aureus* and *Escherichia coli*.

Results from Week 1

After the water is filtered, the nitrocellulose membrane is placed onto a Luria-Bertani agar plate and is incubated for 24 hours at 37 °C. Figure 3 shows one plate after incubation.

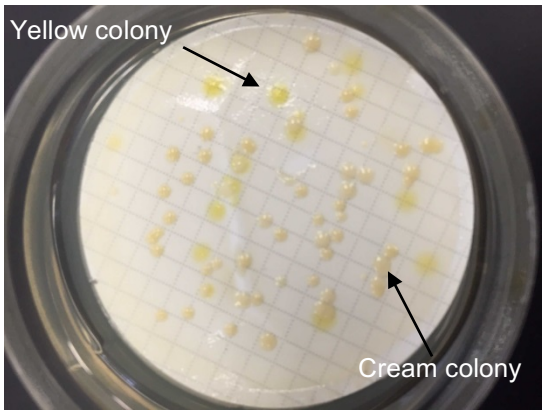

Figure 3. Bacteria recovered on nitrocellulose membrane after incubation for 24 hours on Luria-Bertani.

As seen in Figure 3, there are two distinct colonies – yellow and cream colored. This is indicative of at least two different types of bacteria present in the water. During the second week of the activity, students re-streak each colony onto separate Luria-Bertani agar plates to separate the two strains of bacteria.

Results from Week 2

Figure 4 shows the separation of the two bacteria. The separation is successful since we see only one colony type on each plate.

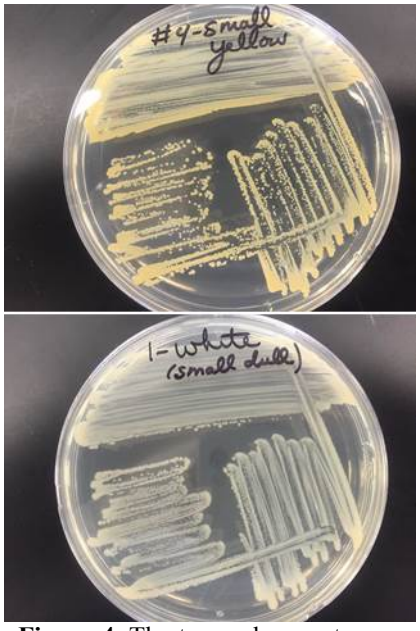

Figure 4. The two unknown types of bacteria colonies grown separately on Luria-Bertani agar.

Results from Weeks 3 & 4

Once the students see they have isolated the different bacteria that are in their water, they may begin to identifying which bacteria they have during the third week of the activity. If students were not successful in separating the bacteria, then the students should continue working on their isolations.

To begin identifying the bacteria, students should start with the Gram-stain. The Gram-stain is used to identify what type of cell envelope the bacterium has. Gram-positive bacteria have a thick cell wall comprised of peptidoglycan. Gram-positive bacteria will appear purple after staining. Gram-negative bacteria have a thin cell wall of peptidoglycan and an additional outer membrane. Gram-negative bacteria will appear pinkish red after staining.

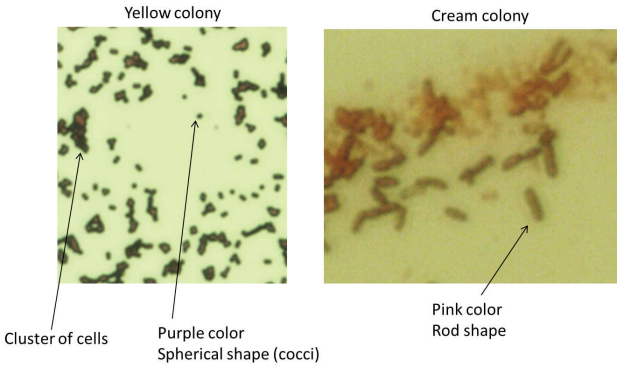

Figure 5. Results of Gram-staining.

As seen in Figure 5, the yellow colony that was isolated has cells that stain purple, and have a spherical or coccus shape. The purple color indicates that this bacterium is Gram-positive. When reviewing the properties of the potential bacteria in the Student Outline, we see that this result indicates that the yellow colony is *Staphylococcus aureus*. The cream colored colony has cells that stain pink and are rod shaped. The pink color indicates that this bacterium is Gram-negative. When reviewing the properties of the potential bacteria in the Student Outline, we see that this result indicates that the cream colony is either *Escherichia coli* or *Enterobacter aerogenes*.

According to the table listing the properties of potential bacteria, both *E. coli* and *E. aerogenes* do not perform lipid hydrolysis. Therefore, using that test will not help in differentiating the two species. Since *E. coli* does not produce citrate permease and *E. aerogenes* does, the citrate test should be used to identify this unknown. The cream color colony is streaked onto the citrate agar (Figure 6). If the citrate agar turns blue after incubation, then the bacterium does produce citrate permease. If the citrate agar remains green after incubation, then the bacterium does not produce the permease.

After 24-hour incubation, the citrate tube remained green. This indicates that the cream-colored colony was *E. coli*.

Figure 6. Results of Citrate Utilization Test.

Assigning & Adapting this Activity to Any Laboratory or Classroom

This laboratory is designed to bring two different non-science major classes together to increase their understanding and awareness of the meaning of collaboration in science. At our institution, this activity was assigned to students in biology and environmental science courses. Such an activity can be done between any two classes that cover the topics that are addressed in this lesson. Alternatively, a non-science major class can be teamed up with an upper-level major class to complete this activity. Recently, it was shown that teaming up non-science majors with science majors helps the non-majors have a better understanding of the concept of interdisciplinary collaboration (Ramirez *et al*. 2015).

As stated in the beginning of the Student Outline and in the Notes for the Instructor, the students must rely upon previous knowledge obtained in their respective lab classes in order for them to complete the assignment. Hence, limited directions are given in the Student Outline. Biology students should be familiar with the technique of identifying bacteria according to shape and Gram-stain. Environmental science students should be familiar with how to isolate bacteria from water and how to streak it onto nutrient agar plates. It is recommended that before this activity is assigned, students are familiar with basic microbiology techniques (sterile technique, streaking microorganisms onto nutrient media, Gram-staining, and microscopy) and have a basic understanding of water filtration to isolate microorganisms. If desired, instructors can modify the introduction of this assignment to include more background information on these techniques. This information can be found in the Notes for the Instructor section.

If collaboration between two different classes is not possible, then this activity can be modified for use in a single class. Instructors would have to make sure that their class has all of the pre-requisite knowledge before beginning the activity. If necessary, the lab protocol can be modified to provide more detailed directions. For advanced classes (i.e. upper level microbiology or environmental science classes), students could go and collect their own environmental water samples and try to identify the bacteria found in that sample. For that activity, students will have to use additional identification techniques and research the metabolic properties of commonly found waterborne bacteria using Bergey's Manual of Determinative Bacteriology. These techniques can include, but are not limited to:

- starch hydrolysis
- glucose utilization (oxidative-fermentation, methyl red, Voges Proskauer)
- lactose utilization

- casein hydrolysis
- motility
- indole production
- catalase test
- oxidase test
- nitrate reduction

Building Collaborations

We suggest that having students assume the role of a teacher for particular techniques in a small group is a basis for establishing collaboration with non-science majors. Students knowledgeable in one topic or technique can show this to their peers. This promotes mutual learning and engagement across all individuals. To first establish collaboration, instructors should look at their curriculums and identify concepts or lab techniques that are common to both of them (Figure 7). Perhaps one class learns the basics of a lab technique and an upper-level course learns specialized uses of that technique.

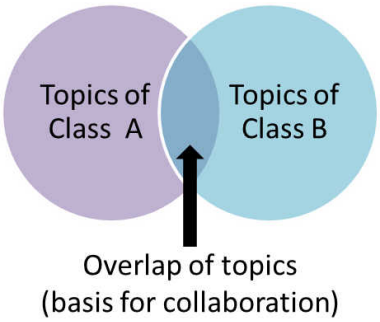

Figure 7. Identifying topics for collaboration.

Once a topic has been identified, a lesson/lab activity needs to be prepared. A successful collaboration has learning objectives that target both sets of classes. Therefore, instructors must ask themselves when preparing such a lesson, "What benefits do each of these classes get from this collaboration"? If one class of students is not learning anything new, then the collaborative lesson loses its value.

After the lesson objectives have been identified, logistics must be considered. This can include asking questions such as: How do we get the classes together? Do these classes meet at the same time? How many times should the classes meet? Where should they meet? (i.e. is the room big enough?) Can we still cover the rest of the curriculum material? To address the concern of finding a big enough location, instructors can consider using online group meeting spaces like Wikis to promote collaboration between individuals (Augar *et al*. 2004).

Student/Teacher Reactions to the Assignment

- "I was very excited to see my environmental students having that "aha" moment as they were identifying bacteria using techniques they would not have used in class. I think they were also surprised to see that they had areas of knowledge that their biology counterparts did not, and that they were able to share those to a collaborative end." (J. Huxster, Adjunct Professor of Environmental Science)
- "This experience was great and a chance to use skills I hadn't used in some time. Being able to not only relearn techniques but teach those techniques to someone with no experience was very rewarding." (S. Moss, teaching assistant for Biology)
- "This exercise was an excellent collaborative learning experience for students. Each was able to contribute something to the project and learn new techniques from other members. This gave a great basic representation of how professional scientists work together." (E. Bilyk, teaching assistant for Environmental Science)
- "The water column experiment was unlike any other I had experienced in bio lab, it was very cool to be able to see how different science fields interact with one another. Working with environmental science students was interesting because they were able to complete a task we didn't know how to complete, and we were able to help them understand our field as well. It really proved that science is a community and all sciences can interact with one another and help each other solve a problem or mystery. It honestly felt like we we're real scientists working together to understand a phenomenon". (A. Dominici, Biology student)
- "This experiment was a great way to collaborate with our different skill sets. There were some parts of the experiment that I felt comfortable doing, but for other parts, I had to rely on my group mates to teach me a new skill." (A. Carson, Biology student)
- "Working with students from another science class broadened my understanding of how different techniques and sciences are used to put together a successful experiment. I appreciate the opportunity to collaborate with the students and to learn from our discussion of the results. I found that I had a better understanding of the concepts that I was learning when I was able to apply them and share them with other students." (A. Szymanski, Biology student)

- "The water column experiment showed how you needed to communicate your knowledge with the members from the other class so you could go through the procedures of identifying the bacteria." (K. Viola, Environmental Science student)

Assessment

We asked our students to complete surveys both before starting, and after completing the experiment to assess whether they had a better understanding of the collaborative nature of science and how to identify bacteria from water samples. The surveys can be found in Appendix B.

In regards to collaboration, the percentage of students having a proper understanding of what collaboration meant increased from 33% to 71%. In pre-surveys, many students confused collaboration with peer-review. Students thought collaboration meant that a scientist checks the work of another. In post-surveys, students thought collaboration allowed scientists to learn from one another. One survey respondent wrote "it is helpful to have people with different knowledge to help with parts I didn't know how to do."

In regards to identifying bacteria from water samples, the percentage of students understanding why filtration is used to determine whether bacteria is present in water rose from 10% to 79%. Understanding of methods to identify bacteria (Gram-staining, biochemical properties, shape, etc.) increased from 27% to 85%.

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About the Authors

Brian M. Forster received his Ph.D. from Cornell University. In 2011, he joined the faculty of Saint Joseph's University. Dr. Forster is the laboratory coordinator for the natural science laboratory-based classes designed for students who are not science majors. He teaches courses in general biology, heredity, environmental science, and a course in microbiology designed for students wishing to enter nursing or allied health programs.

Emily C. Bilyk received her B.S. in Chemical Biology from Saint Joseph's University in spring, 2016. She has spent two years as a teaching assistant for biology and

environmental science courses. Ms. Bilyk has presented research at the 2016 Biophysical Society Annual Meeting on “Determining the CQC-mediated interactions in the MUC1 transmembrane homodimer”. She is continuing her education as a medical student at the Philadelphia College of Osteopathic Medicine (PCOM).

Sarah A. Moss received her M.S. in Biology from Saint Joseph's University in spring, 2015. Ms. Moss conducted her thesis research on "A study of nesting diamondback terrapins (*Malaclemys terrapin)* at three locations in coastal New Jersey". For the fall of 2015, she joined a research project in collaboration with The Leatherback Trust and the United States Geological Survey (USGS) and is continuously looking to expand her experiences in wildlife and natural resource conservation.

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Appendix A
Growth Media Recipes

Luria-Bertani and Simmons Citrate can be purchased from most laboratory supply companies. Difco and several companies sell these growth media as dehydrated medium. Be sure to follow all directions indicated on the bottle. Listed below are the recipes if you wish to prepare your own medium.

Luria-Bertani agar plates (500 mL) (MacWilliams and Liao 2006)

- 5 g tryptone
- 5 g sodium chloride
- 2.5 g powdered yeast extract
- 5 g of agar
- 500 mL of deionized water

- Add tryptone, salts, yeast, agar, and water to flask.
- Autoclave (121 °C, 20 minutes, 103 kPA).
- Put flask into water bath to cool (do not allow it to cool to the point where the agar hardens). When cool, pour plates.

Simmons Citrate Agar Tubes (500 mL) (MacWilliams 2009)

- 0.1 g magnesium sulfate (heptahydrate)
- 0.5 g ammonium dihydrogen
- 0.5 g dipotassium phosphate
- 1.0 g sodium citrate (dehydrate)
- 2.5 g sodium chloride
- 5 g of agar
- 0.04 g bromothymol blue
- 500 mL deionized water

- Dissolve salts in deionized water. Adjust pH to 6.9.
- Add agar and bromothymol blue. Gently heat, with mixing, to boiling until agar is dissolved. Dispense 4.0 mL into test tubes.
- Autoclave (121 °C, 20 min, 103 kPA).
- Cool in slanted position (long slant, shallow butt). The uninoculated medium should be green due to bromothymol blue and the pH of the medium.

Egg Yolk Agar Plates (500 mL) (Adapted from Yeung *et al*. 2005)

- 5 g tryptone
- 5 g sodium chloride
- 2.5 g powdered yeast extract
- 5 g of agar
- 500 mL of deionized water

- Add tryptone, salts, yeast, agar, and water to flask.
- Autoclave (121 °C, 20 min, 103 kPa).
- Put flask into water bath to cool (do not allow it to cool to the point where the agar hardens).
- Once cooled, add 6 mL of egg yolk (see directions for egg yolk).
- Pour plates once egg yolk has mixed well with the agar.
- Egg yolk plates are good for less than 1 week.

Egg Yolk – make fresh solution the DAY you are making egg yolk agar plates

- Put on a pair of gloves and spray both your gloves and the egg with 70% ethanol.
- Crack open the egg and save the egg yolk.
- Take a pipet and stab the egg yolk and recover liquid from within the egg yolk.

- Transfer this liquid to a sterile conical tube (15 mL).
- Add an equal amount of 1X phosphate buffered saline (PBS) such that you now have made a 1:1 (v/v) dilution of your egg yolk with PBS.
- **DO NOT GET ANY EGG WHITE INTO YOUR PREP**

Appendix B
Assessment Survey

Circle the course you are in: BIOLOGY ENVIRONMENTAL SCIENCE

Thank you for participating in the assessment of the Water Filtration & Bacteria Identification experiment your class is working on this semester. Your answers to the following questions will help us to evaluate these new teaching efforts. Please answer each question to the best of your ability. Your course grade will not be affected by the responses you give. DO NOT PUT YOUR NAME ON THIS PAPER.

FREE RESPONSE QUESTIONS

1. Explain in one or two sentences what the following phrase means to you: "*Science is collaborative*"

_____

_____

_____

2(a). How can you isolate bacteria from a water sample? _____

(b). Name one way you can identify an unknown bacterium.

_____

Mission, Review Process & Disclaimer

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