What’s In Your Seafood?

Lessons Developed By:
Jill Stephens

In conjunction with UF CPET
NIH
• Introduction
• Teacher Suggestions
• Safety First
  o General Safety Practices
  o Personal Safety Equipment
    ▪ Glasses / Goggles, Gloves, Lab Coats / Aprons, Eye wash station,
      Emergency Shower, First Aid Kit.
  o Laboratory Safety: Handling Microorganisms
    ▪ Personal Protective Equipment
    ▪ Sterile Technique
    ▪ Culture Guidelines
    ▪ Disposals

• Lesson I: Dive In
  o Focus, Major Concepts, Objective, Prerequisite Knowledge, Overall time
    estimate, Vocabulary, National Science Edu Stds, Next Gen. FL Sci Stds, Basic Sci-
    Health Connection
  o Introduction, Materials and Preparation, Procedure, Assessment, Potential
    Extensions, Resources
  o Potentially Harmful Vibrio Bacteria / Health Concerns (Worksheet??)

• Oysters Anyone?
  o Problem, Hypothesis (Missing), Safety, Materials, Procedure, Disposal, Teacher
    Preparation, MEDIA Preparation,

• Detecting Water Toxicity Using Bioluminescent Bacteria – pg 12
  o Background, Problem, Hypothesis, Materials, Procedure

• Lesson II: Pick It Up, Harvest and Handling
  o Focus, Major Concepts, Objectives, Prerequisite Knowledge, Overall time frame,
    Vocabulary, National Science Stds, FL Next Gen. Stds, Basic Sci-Health
    Connection, Introduction, Materials and Preparation, Procedure, Assessment,
    Potential Extensions, Resources

• Bacterial Growth on TCBS agar
  o Data

• Identifying Vibrio Bacteria by Culture on CHROMagar plates
  o Identify the possible bacteria found on the plates below (CHROMagar):

• Lesson III: Explore
  o Focus, Major Concepts, Objectives, Prerequisite Knowledge, Overall Time
    Frame, Vocabulary, National Sci Stds, Next Gen Stds, Basic Sci-Health
    Connection, Introduction, Materials and Preparation, Procedure, Assessment,
    Potential Extensions, Resource

• Salinity & Bacteria
- Serial Dilution Plate Count Laboratory Activity Using Pictures
  - Introduction, Materials, Directions,
  - Serial Dilution Plate Counts (Worksheet)
- Real Time PCR and Bacterial Counts – Lab Activity
  - Introduction, Analyzing PCR graphs, Materials, Procedure, Analyze
- Temperature vs. Bacteria
  - Problem, Hypothesis, Personal Safety Equipment, Materials, Procedure, Questions,
- Serial Dilution: Alternate Picture Lab for Plate Counts
  - Pictures
- **Lesson IV: Flight over the Bay**
  - Focus, Major Concepts, Objectives, Prerequisite Knowledge, Overall Time
    Frame, Vocabulary, National Sci Stds, Next Gen. Stds, NOAA Ocean Literacy Stds
    (Blank), Basic Sci—Health Connection, Introduction, Materials and Preparation,
    Procedure, Assessment, Potential Extensions, Resources,
  - Activity Instruction Sheet
    - Task Sheet for Fishermen, Task Sheet for Wholesalers, Task sheet for
      ISSC/FDA, Task Sheet for NIH, Task Sheet for Consumers:
    - Group Member Responsibilities
    - Sources for Information for articles for Lesson 4, Flight Over the Bay
- Detecting Water Toxicity Using Bioluminescent Bacteria (** Don’t know where this goes)
  - Background, Problem, Hypothesis, Materials, Procedure(** Strange order),
- What’s in Your Seafood? Pre-test / Post-test
- Recall ordered for some Apalachicola Bay oysters (article from Daily News)
- Cholera and Other Types of Vibriosis: A story of Human Pandemics and Oysters on the
  Half Shell
- Salinity and Temperature Effects on Physiological Responses of Vibrio fischeri from
  Diverse Ecological Niches
  - Needs work on Figures and Tables
- Weather Changes May Predict Cholera Outbreaks: The Good News and the bad news of
  epidemic predictions.
- 22 people sickened from raw Wash. Oysters.
- Cholera oyster outbreak sickens 11 in US
- Food poisoning
- Websites for Articles
- Websites V. fischeri
Introduction:

The lessons contained in this module were developed with the concern for seafood safety as global temperatures rise causing the temperatures of the water where shellfish are harvested to increase. The major issue that develops from increased water temperature is the increase in bacteria found in shellfish, especially oysters. The information within this module engages the students in a variety of classroom and laboratory activities designed to understand the relationship between increased temperatures, bacterial growth, safe seafood handling, and human health.

Teacher Suggestions:

There are numerous activities included in this module designed to support each of the lessons. You may elect to implement all or part of them.

Teachers that are not equipped to conduct the microbiology lab activities can utilize the paper labs that are available.

Teachers who incorporate the microbiology labs involved with live culture of bacteria may decide to conduct several labs at one time by assigning a different lab to each group. This will reduce teacher preparation time and the quantity of supplies needed.

Incorporation of local resources into the curriculum is encouraged.
Safety First

Whatever activity you are engaged in while working in the laboratory, **protect yourself, protect others**, the equipment, and the facilities.  *Whenever entering a new location, you should always locate the emergency exits, fire extinguishers, and other safety equipment.*

**General Safety Practices:**
- No Running
- No Horseplay
- Work in Assigned Area
- Teacher MUST be present
- Work only if you have permission
- Wear safety gear as outlined
- Report accidents immediately
- NEVER use broken or chipped glassware or broken equipment
- Report breakage / malfunctions to the teacher immediately

**Personal Safety Equipment:**
- **Safety Glasses/Goggles:** Wear when working with chemicals, microorganisms, heating, cutting glassware
- **Gloves:** Wear when handling, chemicals, animals, toxic plants, microorganisms, sterile technique
- **Lab Coats/Apron:** Working with chemicals, microorganisms, whenever there is a need to protect skin or clothing
- **Shoes:** Sturdy, enclosed shoes are to be worn at all times in the laboratory.
- **Eye Wash Station:** Use when you get chemicals in your eyes. Flush for 10-15 minutes and seek medical attention.
- **Emergency Shower:** Use when you get LIQUID chemicals on your skin or clothing. Rinse for 10-15 minutes and seek medical attention.
- **First Aid Kit:** Use as directed by the lab manager
Laboratory Safety: Handling Microorganisms
   Basic laboratory safety should be followed at all times.

**Personal Protective Equipment:**
   Gloves  
   Lab coat/apron  
   Safety glasses

**Sterile Technique:**
   Pull back and secure long hair.

   Clean work surface with 10% bleach or 70% ethanol prior to activity and upon completion of work with bacteria

   Wash hands before putting on gloves and upon glove removal

   To reduce contamination of samples, work in a hood or a plastic storage bin that has been bleached and turned on its side.

   Spray all materials entering the sterile work space.

   Place disposable inoculation loops into bleach solution after use. Flame metal loops before/after each use. Spreaders should also be sterilized following approved techniques.

   Keep plates, bottles, and flasks closed. NEVER turn the cover upside down. Always minimize the amount of the media/culture that is exposed to the hair.

   Clean all materials and surfaces with 10% bleach or 70% ethanol upon activity completion.

   Return all materials to the proper locations.

**Culture Guidelines:**
   ALL CLASSROOM CULTURES MUST BE SEALED AND REMAIN SEALED THROUGHOUT THE EXPERIMENT.

   Place the cultures in the incubator at the specified temperature.

   ALL unused inoculated media must be disposed of properly.

**Disposal:**
   Autoclave all inoculated broth, containers, and loops/spreaders if an autoclave is available. Plates that were cultured must also be autoclaved.

   Immersion in a 10% bleach solution for a minimum of 1 ½ hours will also destroy bacteria on containers, plates, and in broth solutions.
Follow your district requirements for further steps.

**Lesson I: Dive In**

*Teachers, please feel free to incorporate as much of the lesson as you feel is appropriate for your situation.*

**Focus:** A pre-test may be administered as a bell activity and introduction to the lesson. Students will be presented with information concerning *Vibrio cholerae, Vibrio vulnificus*, and *Vibrio parahaemolyticus*. Instructors may choose to provide students with the written information as packets to read or may elect to utilize a PowerPoint presentation to disseminate information. Students will complete a graphic organizer that requires students to detect and record characteristics and health issues about each of these three bacteria. Cornell note taking may be used for PowerPoint presentation of information. Lab activities include a bioassay using *Vibrio fischeri*. Students will research topics including bacterial diseases, bioremediation, and bioassays producing a mass media project.

**Major Concepts:** Bacteria are present in the environment and can serve as disease pathogens and as beneficial members of the ecosystem. Not all bacteria within a given species are pathogenic. The *Vibrio* species of marine bacteria influencing human health are *cholerae, vulnificus, and parahaemolyticus*.

**Objectives:** After completing this activity, students will
- differentiate between pathogenic and non-pathogenic.
- understand that numerous species of bacteria are either beneficial or harmless.
- be able to explain at least one symbiotic relationship with bacteria and another organism.
- identify causes and symptoms of human diseases/illness caused by *Vibrio cholerae, Vibrio vulnificus, and Vibrio parahaemolyticus*.
- analyze the human factors that cause a person to have a greater susceptibility for affliction.

**Prerequisite Knowledge:** No previous knowledge is required; however, students with microbiology experience may have an advantage over their classmates that have not worked with micro-organisms and sterile technique. Basic skills required for microscope use are needed.

**Overall time estimate:** (2-5 days) Most of the work can be accomplished in 2 days with homework activities given. Classes conducting the internet research and creation of media presentations will add another 3 days to the lesson.

**Vocabulary:** *pathogenic, non-pathogenic, bioassay, bioluminescence, gram negative, gram positive*

**National Science Education Standards:**
A: Science as Inquiry
   - Abilities necessary to do scientific inquiry
   - Understanding about scientific inquiry
Basic Science-Health Connection: The three species of bacteria investigated in this lesson have the potential to cause intestinal discomfort and lead to possible human fatalities.

Introduction:
This activity introduces the module by focusing on the three major *Vibrio* species of bacteria that inflict humans. Although not all members within the focus species will present themselves as pathogens, it is important to recognize the potential for illness and the portion of the population that is most at risk. Subsequent activities will introduce a non-pathogenic species that will be discussed and utilized as a classroom model.

The majority of people in the United States and around the world are not cognizant of the roles that bacteria play nor do they recognize the abundance of species. One goal of this project is to raise awareness of the significant pathogenic bacteria; including signs of infection in humans, treatment, and prevention. An additional goal encourages students to investigate the countless beneficial aspects of bacteria. By educating high school students, we can develop more informed consumers and environmentally aware citizens.

Materials and Preparation:
You will need to prepare the following materials before conducting this activity:
- *Master 1.1, pre-test/post-test* (make one copy per student)
- Information Packet or PowerPoint
- Master 1.3, *3 Column Graphic Organizer* (Make one copy per student)
- Highlighters (3 colors per student)
- Microscope stations
- Prepared slides of the bacteria of interest
- Student lab notebook or accepted substitute
- Colored pencils
- Index cards
- Markers for alternate activity in part 1
Handouts: (Master 1.2, Cholera article by Dr. Morris; Master 1.4 Determining Water Toxicity Using Bioluminescent Bacteria; Master 1.5 Safety Rules; Master 1.6 Laboratory Safety: Micro-organisms) one copy per student

Procedure:
Part 1: Day 1 (& Day 2, if needed)
1. Administer the pre-test. (Master 1.1)

2. Pass out information packets to students (Master 1.2, article by Dr. Morris) or utilize a PowerPoint presentation to provide important information to the students. Students will complete the graphic organizer (master 1.3, 3 column graphic organizer) as they read the material. Cornell note taking can accompany the power point and the graphic organizer could be used as a homework activity. Vocabulary words can be placed on individual index cards.

3. Students are to use different colored highlighters to indicate the traits the bacteria have in common. (Highlight the common traits with the color assigned to each species by the instructor. One color can be used to highlight the entire trait. Additional colors indicating shared traits can be used to outline the characteristic and a third color can make diagonal lines.) possible homework assignment
   Alternate compare/contrast activity: Divide students into groups of three. Using chart paper, students will create a three circle Venn diagram. Post completed papers on the wall and have each group discuss at one item on their diagram. If some characteristics have not been explored, you may repeat discussion with a second member from each group.

4. Microscope activity: Lab partners will move to their lab stations to view three bacteria slides. (Ideally, we would like to see the three focus species of Vibrio.) Draw and label the slides in your lab notebook. Please use commercially prepared slides.
   Questions: 1. How were the organisms alike?
   2. How were they organisms different?
   Lab extension: Slides of non-pathogenic species may be viewed. Students are to analyze the slides and determine if there is a repeated visual difference between pathogenic and non-pathogenic bacteria.

Part 2: Day 2 or 3:

5. Bioassay using Vibrio fischeri (non-pathogenic)
   a. Invite a representative from an environmental water testing company to serve as resource speaker and demonstrate the implementation of the assay. The company may be willing to bring enough material for students to conduct the assay with their lab partner.

   OR

   b. Do It Yourself..... See Determining Water Toxicity Using Bioluminescent Bacteria
      Questions: 1. Record observations prior to introduction to water samples and every five minutes afterward.
      2. Did the luminescence decrease, increase, or remain the same?
      3. Why do Vibrio fischeri exhibit decreased luminescence in polluted waters?
      4. What advantages, if any do biological indicators have over the use of chemical reagents when testing water samples?
6. Internet based research: day 3-5

Students can access the internet to research diseases caused by marine bacteria, bioremediation using bacteria, bioassays using bacteria, or related topics. Students can be assigned to work individually or as a group of 3-5. Student produced materials could include a PowerPoint presentation, brochure, or public service announcement.

Assessment: (Suggestions)

Credit may be earned through a variety of means:
- Completion of the pre-test
- Completion of the graphic organizer and/or vocabulary cards
- Drawings of bacteria seen through the microscope and the comparison of the drawings
- Highlighting activity
- Informal assessment can be made throughout the activity
- Laboratory observations
- Student produced media materials (PSA, brochure, PowerPoint, etc)

Potential Extensions:
- This activity introduces students to the concept that various species of marine bacteria exist and not all of them are harmful. There are many beneficial and symbiotic relationships involving marine bacteria. Students may research other species of marine bacteria and create a presentation outlining their contribution to the marine ecosystem in which they reside.
- Finally, students interested in field research may consider developing a project that focuses upon the impact of a particular species upon the health of the ecosystem. This can be further extended to include how the health of the ecosystem impacts human health.

Resources:


http://web.uconn.edu/mcbstaff/gra/VfEs/VfEssym.htm
http://web.mst.edu/~microbio/BIO221_2004/V_fischeri.htm
http://www.nottingham.ac.uk/quorum/fischeri.htm
http://www.clubdeccm.com/PDF/20101014/20101014_3-4-5temp.pdf
http://www.youtube.com/watch?v=cH3ANoV06P0
http://departments.kings.edu/biology/lux/bacterial.html
http://www.sdix.com/Technologies/Luminescent-Bacteria.aspx
http://www.sciencedirect.com/science/article/pii/S0045653507000057  toxicity assay article
<table>
<thead>
<tr>
<th></th>
<th>Health</th>
<th>Concerns</th>
</tr>
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<tbody>
<tr>
<td>Potentially Harmful Vibrio Bacteria</td>
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The table is designed to organize information related to potentially harmful Vibrio bacteria, with columns for health concerns and potentially harmful Vibrio bacteria.
Oysters Anyone?
(Student Time: 1 day prep with overnight treatment. Spread plates on the second day and culture overnight. Count colonies on the third day. Entire class periods should not typically be needed for day 2 and day 3.)

Problem: How does handling temperature affect the bacteria levels found in oysters?

Hypothesis:

SAFETY: Follow general laboratory procedures and micro-organism safety protocols
Personal Safety Equipment:
Gloves, safety glasses/goggles, lab coats or aprons

Materials:
- 18-24 oysters
- blender
- Artificial Seawater 15% salinity (aliquot 50 ml tubes and have 1 liter bottle on hand)
- Large syringe and sterile syringe filters or filter sterilize unit (.2 micron filters)
- LB agar plates (6 per group)
- P10 or P20 micropipettes and tips or 1ml serological pipet and pump
- 25 ml serological pipets and pump/bulb
- 5ml or 10ml serological pipets and pump/bulb
- Test tube racks
- 15 ml conical tubes
- Spreaders (for inoculation)
- Incubator
- Cold storage (refrigerator)
- Ice
- Ice buckets or substitute container such as small Styrofoam cooler
- Permanent markers
- Para-film
- Scissors
- Hot plate (1 per group)
- Tongs or oven mitt
- 250ml beaker (1 per group)
- Shucking tools/knife
- Balance
- Weigh boats
- Prepared tubes of 1ml Vibrio fischeri overnight LB culture (2 per group)
- 15ml conical tube with 5 ml Vibrio fischeri (1 per group)
- Water
Procedure:

Day 1
1. Shuck the oysters.
2. Measure and record the mass of the oyster (shucked).
3. Place the shucked oysters into the blender.
4. Total the mass of the oysters. Add the milliliter equivalent of sterile ASW to the blender.
5. Cover the blender and turn on the machine. Blend until the homogenate is a liquid with few chunks. KEEP THE HOMOGENATE ON ICE.
6. Label 5 test tubes using a permanent marker. (ASW, C (cold), W(warm), CC (cooked/cold), CW (cooked/warm) (ASW, artificial seawater, is the negative control. Vf, *Vibrio fischeri*, is the positive control and is provided by your instructor.)
7. Place 5ml of sterile ASW into the tube marked ASW. Use a 10ml or 25ml serological pipet.
8. Follow the procedure in step 7. Place 5ml of the homogenate into each of the oyster tubes. (Keep tubes on ice)
9. Place .5ml *Vibrio fischeri* into each of the oyster tubes. DO NOT put it in the ASW tube.
10. Fill the beaker with approximately 150ml of water. Place the beaker on a hot plate and bring to a boil.
11. Place the 15ml tubes of homogenate marked CC & CW into a 250ml beaker of boiling water for three minutes. Place CC tube on ice.
12. Place the 15ml tubes of C, Vf (from your instructor), and CC into the refrigerator. DO NOT UNSEAL THE Vf tube.
13. The tubes marked ASW, CW and W will remain at room temperature.

Day Two
14. Observe the tubes and record any visual difference detected.
15. Label 6 LB agar plates to match your 6 test tubes using a permanent marker.
16. Using a serological pipet, place .1ml from each test tube onto the center of the plate that is marked the same. Use a different pipet for each tube. Cover the plate. (Disposable, plastic pipets may be used as an economic alternative.)
17. Using a sterile spreader, start in center and spread the homogenate out toward the sides while turning the plate. Cover and let the plate rest. (This gives the liquid time to be absorbed into the agar.)
19. Incubate overnight at room temperature.

Day Three
20. Count colonies formed on the plates. You may be able to see them better with a UV light.
21. Record all data.
22. Prepare a graph illustrating your data.
   Compare/contrast the bacteria present on the plates.
   How would you handle your seafood?

Disposal:
Be sure to follow approved disposal protocol for your school district.
Teacher Preparation:

Purchase oysters from the grocery or wholesaler the day before you begin the lab if at all possible. You may have them shucked prior to the lab. If the grocery store will do it for you, I would have it done. If you plan to use the anatomy extension, the students will need to shuck the oysters.

MEDIA Preparation:

- **ASW 15% salinity** (2 liters should be enough) You may sterilize all, part, or none of the ASW ahead of time. The time spent in class will be less if you sterilize the water and make up enough 50ml tubes for one tube per group from one of the liters.
- **10% Bleach solution &/or 70% ethanol for sterile technique and disposal**
- **Luria broth culture of *Vibrio fischeri***: Inoculate enough Luria broth so that you will have 6ml per group. Inoculate on day 1 and incubate overnight at room temperature.
- **LB Agar Plates**: Pour enough plates for 6/group... If you have a biotechnology program at your school, the biotech students might pour the plates for you. (These will be needed the third day.)
Detecting Water Toxicity Using Bioluminescent Bacteria

**Background:** Numerous pollutants from both natural and anthropogenic sources find their way into the oceans on a daily basis. The increase in marine pollution is having a serious impact on the ecosystem causing fish kills, coral bleaching, mutations, and various diseases in marine organisms as well as humans. Detecting the individual pollutants and locating the source of pollution is one of many functions of marine scientists.

Detection of specific pollutants can be achieved using various techniques from water test kits to specialized equipment. Monitoring the organisms that reside in the ocean can provide scientists with a natural means of detecting toxicity. Bioluminescent bacteria such as *Vibrio fischeri* can be used to determine toxicity of the water. Scientists have observed that the luminescence of the bacteria decreases as the level of toxicity increases.

**Problem:** How can water toxicity be determined?

**Hypothesis:**

**Materials:**
- Broth culture of *Vibrio fischeri*
- 3 water samples from various sources
- 4 test tubes per group
- test tube rack (1 per group)
- 5ml or 10ml serological pipets and pump
- 4ml *Vibrio fischeri* broth culture (1 tube per group)
- Water (distilled or tapwater)
- UV light(s)
- Para-film
- Permanent markers
- Scissors
- 10% Bleach solution or 70% Ethanol

**Procedure:**
1. Collect all materials.
2. Label the test tubes. (1 for sample 1; 2 for sample 2; 3 for sample three, and Vf for the positive control)
3. Place 1ml of each sample into the corresponding test tube.
4. Place 1ml of water into the Vf tube.
5. Place 1ml of the bacteria, *V. fischeri* into each test tube.
7. Turn off the lights so that the room is as dark as possible. Use an ultraviolet light for viewing. Record observations for each sample.
8. Record observations every 5 minutes for the remainder of the class period.
9. Draw conclusions and answer questions provided by your instructor. Determine the hypothesis.
10. Clean up and dispose of materials following appropriate protocol. (refer to laboratory micro-organism safety handout)

Lesson II: Pick It Up - Harvest and Handling

Focus: Students will review seafood harvesting and handling guidelines, in addition to experiencing ISSC / FDA techniques for detecting bacteria in seafood. Particular emphasis will be placed upon detecting Vibrio species of bacteria found in oysters and other shellfish.

Major Concepts: Students will review regulations for handling, harvesting, and testing shellfish to reduce the potential for human illness resulting from consumption of shellfish.

Objectives: After completing this activity, students will
- Explain the requirements for storage temperatures of shellfish from the time it leaves the water until it reaches the table.
- Be able to identify the potential presence of V. cholera, V. parahaemolyticus, and V. vulnificus using FDA BAM information and material from ChromAgar.
- Process samples and quantify the amount of bacteria present

Prerequisite Knowledge: Students should have completed the activities in lesson one and have a basic understanding of the three major pathogenic Vibrio bacteria found in shellfish that can cause illness in humans. Familiarity with laboratory behavior and procedures along with the scientific method will be an asset.

Overall Time frame: 3 days

Vocabulary: ISSC, FDA, PCR, shellfish, aquaculture, filter feeders

National Science Standards:
A: Science as Inquiry
   Abilities necessary to do scientific inquiry
   Understanding about scientific inquiry
B: Science in Personal and Social Perspectives
   Personal and Community Health
   Natural resources
   Environmental Quality
   Natural and human induced hazards
   Science and technology in local, national, and global challenges

Florida Next Generation Standards:
SC.912.L.14.6
SC.912.L.17.2
SC.912.L.17.4
SC.912.L.17.13
SC.912.L.17.14
SC.912.L.17.20
SC.912.N.1.5
SC.912.N.1.7
Basic Science Health Connection:
Consumption of uncooked shellfish containing pathogenic bacteria can cause illness and could lead to death in individuals that are susceptible.

Introduction: We are fortunate in the United States to have established governmental agencies charged with the mission to oversee the quality of the food that is available to the consumer. In recent years, we have learned of micro-organisms that have found their way into produce, beef, chicken, eggs, and shellfish. When nationwide recalls threaten to shake our faith in the regulatory agencies, we must realize that these same agencies are in part responsibility for demanding that potentially tainted products be removed from the shelves and coolers of retail supermarkets. The system may not always be perfect, but stop and think about the rampant spread of disease caused by food borne pathogens which would occur without regulations. Government jobs initiatives may want to consider creating and funding positions for trained inspectors and microbiologists to ensure that processing plants, warehouses, and harvesting sites are adequately tested to protect the quality of US agricultural commodities.

The Food and Drug Administration, (FDA), is the federal government agency that is tasked with the responsibility of ensuring that our food supply is safe for human consumption. The United States Department of Agriculture, (USDA), is the agency that hires inspectors to regulate farms, fisheries, and processing plants. The USDA inspectors follow the parameters and testing methods accepted and approved by the FDA. Although there are many methods utilized for quality assurance, The FDA BAM, (Bacterial Analytical Manual), is what we will focus upon for the purpose of this lesson.

The Interstate Shellfish Sanitation Commission, (ISSC), is responsible for overseeing, harvesting, handling, and bacterial testing of shellfish. Shellfish are considered to be and oysters, clams, and mussels. Shellfish are of particular concern because they are filter feeders. Filter feeders reside on the seafloor in shallow water and take in bacteria as well as heavy metals from pollution. Since bacteria thrive at increased temperatures and can cause intestinal disorders, (sometimes leading to death), close monitoring of harvesting areas is necessary as our global climate continues to change and the demand for seafood continues to rise. One method of testing for *Vibrio cholerae, Vibrio parahaemolyticus, and Vibrio vulnificus* that is currently under review involves the use of real time PCR, (polymerase chain reaction).

Monitoring the water quality of the marine environment where shellfish are harvested is a critical step in maintaining a consumable supply of shellfish. Laws and regulations adopted by the ISSC provide for the sanitary control of the seafood industry. These regulations prohibit the harvest of shellfish from polluted waters and establish storage and handling temperatures and procedures.

NOAA predicts that by 2030 the United States will need 40 million tons of farm raised seafood products to meet the consumer demands. These demands are fueled in part by consumers turning to seafood for the health benefits provided. In 2005, US fish and aquatic farms recorded sales of $1.1 billion. Benefits of seafood raised through aquaculture, (farming products that are
naturally found in water), include uniform size, selective breeding for disease resistance, and supply that is available at desired prices.

Materials and Preparation:
- Brochures/handouts (print from ISSC or the Florida seafood.com website included in the reference section)
- FDA BAM website
- Handout: FDA Bacterial growth on TCBS chart
- Handouts: one copy per student (Safety Rules, Laboratory Safety-Micro-organisms, Oysters Anyone?)
- ASW 15% salinity, 7.4 pH made from Instant Ocean
- Computers
- Test tubes
- Pipet pumps
- Serological pipets (5ml or less)
- Blender or mortar and pestle
- P100 or 200 micropipette and tips
- Permanent markers 1/group
- Petri dishes with LB agar pH 7.4 5/group
- Oysters 18/group
- Vibrio fischeri culture in Luria broth 20ppt pH 7.4 20ml
- spreaders

Procedure:
1. Introduction activity: (This can take place as a bell ringer activity.) View videos from the Florida seafood website listed below or other appropriate web resource. Give students an index card upon which to write one thing they learned from the short video and one thing about which they would like additional information. Collect and review the cards.

2. Using brochures or web-based resources, students will review safe harvest and handling guidelines. Instruct students to respond to one of the following scenarios:
   a. The dockworkers that move oysters from the dock to cold storage in the warehouse go to lunch and leave Captain Bob’s oysters outside at 98°F for two hours. Identify the specific handling regulations that have been violated and the potential outcome if the oysters are eaten without cooking them.

   b. Shady Seas Fresh Oysters and Clams harvested an overabundance of product this week. The storage facility is over capacity and the temperature has been recorded at 50°F for six hours and ¼ of the seafood has died. What regulations apply to this situation? What should Shady Seas do with the shellfish in the cooler? What will happen if 100% of this product is delivered to restaurants and retailers for sale?

   c. The seafood manager at the local grocery store purchased oysters and mussels from the wholesaler on Monday. Saturday shoppers are complaining about the appearance of the shellfish. Have any handling regulations been violated? If so which one(s)? How should the manager handle the customer complaints?
3. As science and technology advance, new methods and testing protocols may be adopted. The ISSC is currently reviewing real time PCR information. Included in the resources are sample data and graphs. Using the information provided, students are to

4. Laboratory activity: Oysters Anyone?
   Students will work in lab groups to complete the experiment.

Assessment:
- Informal assessment during laboratory activities
- Completion of classroom and laboratory activities

Potential Extensions:
1. Students may design their own seafood testing experiments.
2. What does color have to with bacteria testing? Bacteria can be identified by the color exhibited when grown on specific media. In this lab, students will view bacteria cultures on TCBS agar plates, Chromagar plates, and MCPC plates. Using charts provided, students will identify the bacteria present in the pictures provided. (Teachers with access to a variety of bacteria and plate media can choose to streak enough plates for students to view and compare. Allow the plates to incubate at 37°C overnight or for 24 hours.)
3. Water quality, chemistry, and temperature influence the diversity and population of organisms within a given ecosystem. Scientists monitor the condition of the water through the use of technology. Assign students to monitor an area that provides live data feeds and determine if the potential for elevated Vibrio counts exists. Select a time frame of one day up to two weeks or more. One beneficial site is:
   http://sondes.floridaaquaculture.com/sondes/sonde_cedarkeygulfjackson.htm
4. Oyster Anatomy: Students shuck fresh oysters and view them under the dissecting microscope. Students will identify, draw and label the parts of the oyster in their log books. (Use the directions found at the following website....)
   http://www.mdsg.umd.edu/issues/chesapeake/oysters/education/anatlab/lab_i.htm

Resources:
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070830.htm
http://www.fl-seafood.com/consumers/storage.htm
http://www.fl-seafood.com/aquaculture_programs.htm
http://www.floridaaquaculture.com/SEAS/SEAS_intro.htm
http://sondes.floridaaquaculture.com/sondes/sonde_cedarkeygulfjackson.htm live water monitoring
http://www.mdsg.umd.edu/issues/chesapeake/oysters/education/anatlab/lab_i.htm (oyster anatomy lab)
Bacterial Growth on TCBS agar
Excerpt from the FDA BAM

<table>
<thead>
<tr>
<th></th>
<th><em>V. algino-lyticus</em></th>
<th><em>V. cholerae</em></th>
<th><em>V. fluvialis</em></th>
<th><em>V. furnissii</em></th>
<th><em>V. hollisae</em></th>
<th><em>V. metschnikovi</em></th>
<th><em>V. mimicus</em></th>
<th><em>V. parahaemolyticus</em></th>
<th><em>V. vulnificus</em></th>
<th><em>A. hydrophila</em>*</th>
<th><em>P. shigelloides</em>*</th>
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<tr>
<td><strong>TCBS agar</strong></td>
<td>Y</td>
<td>Y</td>
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<tr>
<td><strong>mCPC agar</strong></td>
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<td>NG</td>
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<td>Y</td>
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</tr>
</tbody>
</table>

http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070830.htm

*If you were to plan an experiment to isolate species of Vibrio bacteria, discuss how could you use the different types of color reactive plates to identify the species of bacteria contained in your sample.*
Identifying Vibrio Bacteria by Culture on CHROMagar plates

Several specialized media plates are often used to identify the presence of various species of bacteria.

When cultured on CHROMagar plates, the species listed below will exhibit certain colors:

*Vibrio parahaemolyticus*; mauve

*Vibrio cholerae*; green blue to turquoise

*Vibrio vulnificus*; green-blue to turquoise

*Vibrio alginolyticus* is colorless

**Directions:**

Use the color pictures of cultured CHROMagar plates to answer the following questions.

1. What types of bacteria are definitely present in plate 1 and plate 2?
2. What bacteria might be present based upon the color of the plate?
3. Which sample contains the greatest amount of *Vibrio parahaemolyticus*?
4. Compare/contrast the two CHROMagar plates of bacteria.

**Identify the possible bacteria found on the plates below:** (CHROMagar)

Photo from CHROMagar™
Lesson III: Explore

Focus: In Lesson III students will explore in vitro culture of bacteria to discover the relationship between bacteria growth and temperature through laboratory experience.

Major Concepts: Populations of bacteria may fluctuate according to temperature or salinity. Concentrations of bacteria in the estuaries increase during the summer months when marine water temperatures experience their annual peaks. Shellfish harvested during these months exhibit a higher concentration of bacteria.

Objectives: After completing this activity, students will
- explain the relationship between temperature and bacteria populations.
- infer the effect that temperature has upon the bacteria present in shellfish harvested at various temperatures.

Prerequisite Knowledge: Students should be able to use laboratory equipment required for bacteria culture. Students should be familiar with shellfish culture, harvest, and handling.

Overall Time frame: 2 days

Vocabulary: Serial dilution, in vitro, salinity

National Science Standards:
A: Science as Inquiry
   Abilities necessary to do scientific inquiry
   Understanding about scientific inquiry
B Science in Personal and Social Perspectives
   Personal and Community Health
   Natural resources
   Environmental Quality
   Natural and human induced hazards
   Science and technology in local, national, and global challenges

Next Generation Standards:
SC.912.L.14.6
SC.912.L.17.2
SC.912.L.17.4
Basic Science-Health Connection: Pathogenic bacteria that may be found oysters can cause illness in some humans.

Introduction:
“What will happen to bacteria levels in the oceans as sea temperatures continue to rise?” Is there a way that aquaculturalists can overcome rising temperatures and provide shellfish that are safe for human consumption?

The current practices approved by the ISSC include various techniques involving overnight cultures to determine if target organisms are present and at what concentration they occur. Scientists involved in bacterial testing must be proficient in microbiology techniques in order to attain reliable, reproducible data.

Serial dilutions of bacteria are necessary to be able to quantify the bacteria present in a given sample. The serial dilutions are plated and colonies are counted following overnight incubation. (It is crucial to practice sterile technique and utilize accurate pipetting skills.) Once the colonies have been counted and identified, data is used to determine the safety of the shellfish and the bacteria concentration in the harvest area.

Testing methods evolve over time with the development of new technology and discovery of biological assays and processes. Approval of new methodology is not achieved overnight. Rigorous testing and review is required before recommendations for acceptance can be presented to the ISSC. A method that is currently under by the ISSC involves the use of real time PCR. The PCR machine is equipped with the ability to recognize and quantify specific species of Vibrio bacteria.

Materials and Preparation:
Handouts: Temperature and Bacteria (one per student)
   PCR for Identifying and Quantifying Vibrio (one copy per student)
   PCR data
   Data Table: bacteria and plate counts (one per student)
   Serial Dilutions (one per student)
   More Salt? Lab handout

Overnight stock culture of Vibrio fischeri or your choice of classroom bacteria model

Test racks
Test tubes with caps
Inoculation spreaders
Micropipettes and tips or disposable pipettes
LB agar plates (4 per group)
Permanent markers
10% bleach or 70% ethanol
3 Incubators
Para-film
Scissors
UV lights
Sterile ASW 2liter

Procedure:
1. Temperature and Bacteria: Students will follow the steps for serial dilutions of bacteria and
then spread plate 4 consecutive dilutions determined by the instructor on eight plates, two
plates per dilution. Each group will be assignment an incubation temperature. Colonies will be
counted after 24 hours at the assigned incubation temperature.
   Questions:
   1. What influence, if any, did temperature have upon the growth of the bacteria?
   2. What impact could global climate change have upon the concentration of bacteria in
      shellfish?

2. More Salt? Students will explore the impact a change in salinity has upon the growth of Vibrio
   fischeri in the laboratory.
   Questions:
   1. How was the growth of the bacteria influenced by a shift in salinity?
   2. How can this information be used by oyster farmers to reduce the quantity of
      bacteria present in oysters?

* Alternate activity:
Included with this module is a paper lab of three strains of Vibrio bacteria colonies incubated on
plates of LB Agar. The pictures and data tables can be modified for various temperatures or
salinity. Students can count the colonies and compare results. (The pictures are currently
identified by species and dilution, but could be modified to use for comparisons of temperature
or salinity by editing the master word documents.)

Assessment:
Informal evaluation throughout the exercises
Completion of experiment logs
Questions

Potential Extensions:
1. Students may design their own research projects.
2. Students may utilize the internet to research developing aquaculture practices designed to
   reduce the amount of potentially pathogenic bacteria found in shellfish.

Resources:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2703662/
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070830.htm
Salinity & Bacteria

*Follow laboratory safety guidelines and sterile technique for working with microorganisms*

**Problem:** Does salinity affect the growth of bacteria?

**Hypothesis:**

**Personal Safety Equipment:**
Gloves, safety glasses, lab coat/apron

**Materials:**
- Bacteria stock solution (Vibrio fischeri is halophytic)
- Micropipettes and tips
- LB agar plates 6/group
- 5 or 10 ml Serological pipets and pumps
- Spreaders
- 10% bleach solution or 70% ethanol
- Para-film
- Scissors
- Permanent markers
- Sterile ASW 20ppt salinity 60ml/group (7.4 pH)
- Sterile ASW 25 ppt salinity 60ml/group (7.4 pH)
- Sterile ASW 30 ppt salinity 60ml/group (8.4 pH)
- Sterile ASW 35 ppt salinity 60 ml/group (8.4 pH)
- 6 test tubes with caps/group (screw caps are preferred)
- Test tube racks
- 1.5 ml tubes
- micro-centrifuge(s) or other centrifuge able to use 1.5ml tubes

*You will begin this lab with a bacterial solution of approximately 10⁸ logs from your overnight culture.*

**Procedure:**
* Sterilize the work area by cleaning with bleach solution or ethanol.
* Always clean up using proper sterile technique.
1. Label 6 test tubes 1 through 6 using a permanent marker.
2. Label 6 LB Agar plates. Two plates will be identified as #4, 2 plates as #5, and two plates as number 6 (Write on the bottom of the Petri dish with your permanent marker.)
3. Place 4.5ml Sterile ASW into each test tube. (Keep tubes covered)
4. Place 1ml of the bacterial stock solution into a micro-centrifuge tube. Place the tube in the micro-centrifuge and spin for 3-5 minutes.
5. Pour off the supernatant and re-suspend the pellet in 1ml of ASW 20 ppt.
6. Vortex the bacterial stock solution previously prepared for 10 seconds.
7. Remove 0.5ml of the ASW 20 ppt bacterial solution and place it in Tube 1.
8. Cover with the screw cap and vortex the solution for 10 seconds. Be sure the stock solution has been covered.
9. Remove 0.5ml of the bacterial solution in tube 1 and place it into the water in tube #2.
10. Cover securely and vortex for 10 seconds.
11. Continue removing 0.5ml of the “new” bacterial solution and place it into the next tube followed by covering and vortexing for 10 seconds until all 6 solutions are completed.

**Plate the bacteria...**
13. Vortex tube four for 10 seconds. Remove 100ul of the solution. Dispense it into one of the plates labeled #4. *Do NOT leave the plates or the test tubes uncovered!!! Dispose of the pipets or tips according to approved procedure....* Repeat the procedure for the second plate labeled #4.
14. Use a spreader to distribute the bacteria on the plates. Start at the center and work the spreader to the side and back to the center while turning the plate. Be sure you have covered the entire plate.
15. Seal the plates with Para film.

17. Incubate the plates at room temperature.
15. Day two: collect your plates and count the colonies formed overnight. Record your colony counts on the data table.
18. Repeat step 15 for one week.

**Salinity shift to 25 ppt Salinity**
Follow steps 1-18, but substitute 25 ppt ASW for 20 ppt ASW.

**Salinity Shift to 30 ppt salinity**
Follow steps 1-18, but substitute 30 ppt ASW for the 20 ppt ASW.

**Salinity Shift to 35 ppt salinity**
Follow steps 1-18, substituting 35 ppt ASW for 20 ppt ASW.

**Questions:**
1. Average the number of colonies formed on your plates at the various dilutions. (Find an average number for both #4 plates, the #5 plates, and the #6 plates.
2. Compare your findings with the results from the other groups. What salinity produced the most colonies and at which dilution? Which salinity produced the least amount of growth?
3. Create a graph to depict the average growth of the bacteria.
Serial Dilution Plate Count Laboratory Activity Using Pictures

Introduction: Serial dilutions can be created using an established ratio. The dilutions that were made in this exercise were each $10^{-1}$. The dilutions in the pictures you will be using for this exercise were made by placing .9ml of buffer solution into each tube. The lab technician removed 0.1ml of the bacteria overnight culture of approximately $10^9$ logs. The receiving tube was vortexed and 0.1ml of this solution was extracted and placed in tube #2 (dilution #2). This process was repeated until all of the tubes had been inoculated from the previous tube. Dilutions 4, 5, and 6 were plated, (100 ul of each dilution was placed in the center of the plate and then spread.) The plates were placed in an incubator at 37°C for overnight culture. The plates were then removed and photographed.

Materials:
- Pictures of bacteria cultures
- Table for recording data
- Calculator (optional)

Directions:
1. Read the instructions completely prior to beginning the activity.
2. Count the number of colonies formed on each plate in the pictures provided.
3. Record the plate counts on the data sheet for each species.
4. Average the colony counts for each species at each dilution.
5. Determine the total cells present per ml.
6. Answer the questions at the end of the laboratory activity.
Serial Dilution Plate Counts

Name: ______________________

Data Collection: Plate Counts Partner 1

<table>
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<tr>
<th>Dilution</th>
<th>Plate 1</th>
<th>Plate 2</th>
<th>Average</th>
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</tbody>
</table>

Data Collection: Plate counts Partner 2

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Plate 1</th>
<th>Plate 2</th>
<th>Average</th>
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Analysis:

1. Using the information in the tables above, create a graph to compare bacterial presence.
2. Which dilution has the greatest numbers of colonies present in?
3. Which Vibrio species is most prolific?
4. How many cells were present in one milliliter of each of the dilutions per species?

Real Time PCR and Bacteria Counts - Lab Activity

Introduction:
The ISSC has established certain quantities of Vibrio species and coliform bacteria as acceptable levels that may be present in the water and in the shellfish harvested. In addition to these acceptable levels, the ISSC has specific methods which are to be employed to analyze the presence of the bacteria of concern. Many of the methods currently approved by the ISSC require large amounts of time to conduct and collect the data. The organization is currently reviewing a protocol that revolves around the use of real time PCR.

Scientists have developed a procedure that explores the point at which the species of interest begin to duplicate and the point at which the replication process maxes out and starts to decline. (These points can be represented in graphs.) The species of bacteria that shows the initial growth on the graph is the species with the highest concentration of cells.

Analyzing PCR graphs:
When using real time PCR to determine concentrations of bacteria, scientists look at the initial rise in the slope. The species of bacteria that exhibits this rise first is usually the species that is in the highest concentration.

Materials:
Graph Samples
Rulers

Procedure:
1. Review graph # 1.
2. Identify the species that has the greatest concentration of cells.
3. Which species exhibits the lowest cell concentration?

Analyze:
1. Were any of the graphs inclusive? If so, why?
2. Which of the tests showed the greatest concentration of Vibrio cholerae?
3. Which sample contained the least amount of Vibrio vulnificus?
4. What is the relationship between Vibrio parahaemolyticus and Vibrio cholerae in sample three?
5. From which water, (sample number), would you prefer to harvest oysters?
**Temperature vs. Bacteria**

*Follow laboratory safety guidelines and sterile technique for working with microorganisms.*

**Problem:** Does temperature affect the growth of bacteria?

**Hypothesis:**

**Personal Safety Equipment:**
Gloves, safety glasses, lab coat/apron

**Materials:**
- Bacteria stock solution
- Micropipettes and tips
- LB agar plates 6/group
- 2 incubators
- 5 or 10 ml Serological pipets and pumps
- Spreaders
- 10% bleach solution or 70% ethanol
- Para-film
- Scissors
- Permanent markers
- Sterile ASW 30ml/group
- 6 test tubes with caps/group (screw caps are preferred)
- Test tube racks

*You will begin this lab with a bacterial solution of approximately 10^8 logs.*

**Procedure:**

**Sterilize the work area by cleaning with bleach solution or ethanol.**

1. Label 6 test tubes 1 through 6 using a permanent marker.
2. Label 6 LB Agar plates. Two plates will be identified as #4, 2 plates as #5, and two plates as number 6 (Write on the bottom of the Petri dish with your permanent marker.)
3. Place 4.5ml Sterile ASW into each test tube. (Keep tubes covered)
4. Vortex the bacterial stock solution previously prepared for 10 seconds.
5. Remove 0.5ml of the stock bacterial solution and place it in Tube 1.
6. Cover with the screw cap and vortex the solution for 10 seconds. Be sure the stock solution has been covered.
7. Remove 0.5ml of the bacterial solution in tube 1 and place it into the water in tube #2.
8. Cover securely and vortex for 10 seconds.
9. Continue removing 0.5ml of the “new” bacterial solution and place it into the next tube followed by covering and vortexing for 10 seconds until all 6 solutions are completed.

Plate the bacteria
10. Vortex tube four for 10 seconds. Remove 100ul of the solution. Dispense it into one of the plates labeled #4. Do NOT leave the plates or the test tubes uncovered!!! Dispose of the pipets or tips according to approved procedure.... Repeat the procedure for the second plate labeled #4.
11. Use a spreader to distribute the bacteria on the plates. Start at the center and work the spreader to the side and back to the center while turning the plate. Be sure you have covered the entire plate.)
12. Seal the plates with Para film.
13. Repeat steps 10-12 for tubes and plates 5 and 6.

14. Place the plates into the incubator or room temperature area designated to your group by your instructor. (Temperatures suggested are room temperature, 30°C, and 35°C.)

15. Day two: collect your plates and count the colonies formed overnight. Record your colony counts on the data table.
16. Answer questions and draw conclusions.

Questions:
1. Average the number of colonies formed on your plates at the various dilutions. (Find an average number for both #4 plates, the #5 plates, and the #6 plates.
2. Compare your findings with the results from the other groups. What temperature produced the most colonies and at which dilution? Which temperature produced the least amount of growth?
3. Create a graph to depict the average growth of the bacteria.
Serial Dilution: Alternate Picture Lab for Plate Counts

Vibrio vulnificus - dilution 4a

Vibrio vulnificus - dilution 4b

Vibrio vulnificus - dilution 5b

Vibrio vulnificus - dilution 5a

Vibrio vulnificus - dilution 6b

Vibrio vulnificus - dilution 6a
Vibrio parahaemolyticus - dilution 5a

Vibrio parahaemolyticus - dilution 5b

Vibrio parahaemolyticus - dilution 6

Vibrio parahaemolyticus - dilution 6b
Lesson IV: Fight Over the Bay

Focus: This lesson is designed to provide the opportunity for students to engage in a debate incorporating the interests of environmentalists, health professionals, fishermen, and the government.

Major Concepts: Students will use the knowledge gained throughout the learning module to persuade others through a classroom debate.

Objectives: After completing this activity, students will
- understand the economic importance of bays.
- relate environmental issues and human health concerns.
- demonstrate the ability to work as a team to solve problems.
- use information gained through research to solve real world problems.

Prerequisite Knowledge: Students completing the first three lessons should have an understanding of how Vibrio species of bacteria affect at risk humans. Students should also be aware of safe handling and processing procedures that reduce the risk of infection from shellfish. An understanding of the environmental factors influencing Vibrio populations will assist in this activity. Additional economic concepts, ability to work with a team to solve problems, and public speaking skills will be beneficial in this lesson.

Overall Time frame:
One class period

Vocabulary: review previous vocabulary

National Science Standards:
A: Science as Inquiry
   Abilities necessary to do scientific inquiry
   Understanding about scientific inquiry
B Science in Personal and Social Perspectives
   Personal and Community Health
   Natural resources
   Environmental Quality
Next Generation Standards:
SC.912.L.14.6
SC.912.L.17.2
SC.912.L.17.4
SC.912.L.17.13
SC.912.L.17.14
SC.912.L.17.20
SC.912.N.1.5
SC.912.N.1.7

NOAA Ocean Literacy Standards:

Basic Science-Health Connection: Shellfish can contain pathogenic bacteria that can cause human illness and lead to death in at risk individuals.

Introduction:
“Over 100 confirmed cases of cholera have been detected in the United States resulting from eating oysters on the half shell harvested in Apalachicola Bay. What if anything should be done?”

Materials and Preparation:
Handouts: activity instructions
Chart paper
Markers
Timers

Procedure:
1. Students will team up to prepare a debate platform regarding actions that should be taken in response to the cholera outbreak created by eating raw oysters.
   - Divide the students into groups. Groups are: Fishermen, Wholesalers, ISSC and FDA, NIH, and consumers... (You can reduce the number of groups depending upon number of students in class.)
   - Each group should elect a leader, recorder, researcher, spokesperson, and artist. A copy of the team member responsibilities should be given to each group.
   - Provide each group with articles about cholera outbreak. Each member should have an article to read.
   - Once the students have been given adequate reading time, hand out the instructions for each of the groups. (eg: Fisherman have different instructions than the consumers....)
   - Allow the students time (10-15 minutes) to prepare five main points to present to the entire class.
   - Each group will have two minutes to present their points and propose a course of action. (Encourage students to write down points addressed that they want to refute/debate.
   - Each group will be given one minute to debate.
   - The teacher will summarize and conduct a vote of the action to take in response to the situation with the Bay oysters.
Assessment:
Team member internal evaluation
Informal observation
Written assignment

Potential Extensions:
1. Students could review minutes from meetings conducted to address similar issues.
2. Invite a guest speaker from a health organization or seafood interest to address the class.
3. Students may write to the ISSC, seafood wholesalers, or fisherman to gather information and suggestions from the people actually engaged in and affected by proposed actions.

Resources:
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070830.htm
http://www.fl-seafood.com/consumers/storage.htm
http://www.fl-seafood.com/aquaculture_programs.htm
http://www.floridaaquaculture.com/SEAS/SEAS_intro.htm
http://www.google.com/hostednews/afp/article/ALeqM5gNbMYDzfbT7tbN118PgbtC2kRHYA?docid=CNG.60d5b39646e58529e4788d62cae3ee2e.431
http://www.umm.edu/altmed/articles/food-poisoning-000064.htm

Activity Instruction Sheet

1. Divide into groups of 5 – 6 members. (1minute)
2. Elect the following positions: (2 minutes)
   - **Leader/Moderator**: This person is responsible for keeping the group on task and monitoring discussion.
   - **Recorder**: Responsibilities include writing down all ideas and preparing the final draft of position points decided upon by the group.
   - **Researcher**: This member is able to leave the group to gather outside information and directs the collection of information within the group.
   - **Artist**: Prepares graphics to enhance the presentation....
   - **Spokesperson**: Speaks for the group...This person will present the points agreed upon by the group and challenge the other groups to think the same way as the spokesperson’s group. (This may or may not be the leader)
*Allow 15-20 minutes for step 3 & 4..................
3. Read the articles that are contained in your group file. If there are several different articles in the file, be sure to distribute them amongst the group members. It is advisable to have more
than one person read the same article in order to get multiple points of view to assist in the decision making process if time allows.

4. Each group will discuss the information presented in the articles and prepare a position on the issue of closing Apalachicola Bay due to the cholera outbreak. The current schools of thought are to close the bay permanently, only during June through September, or only when Vibrio counts in the water exceed the levels established by the ISSC. The other issue on the table is to fine restaurants and consumers for eating raw oysters. Select up to five points that support your group’s position.

5. Presentation (2 minutes per group): The Spokesperson from each group will present the group’s position to the assembly. No questions or rebuttal will be allowed at this point.

6. Rebuttal/Debate (1 minute per group): Each group will have one minute to strengthen their group’s position, refute claims by other groups, or offer additional comments to persuade the assembly to vote with the spokesperson’s group.

7. Wrap Up and voting (3-5 minutes): The teacher will review the proposed actions and conduct a vote to determine what will happen to the bay.

**Task Sheet for Fishermen:**
Your livelihood depends upon the shellfish that you harvest from the bay. You must develop a persuasive speech to convince other groups and the government to continue to allow you to harvest shellfish and avoid welfare or homelessness.

**Task Sheet for Wholesalers:**
Governmental officials need to place the blame for human illness from eating shellfish on someone and are looking to the wholesalers as scapegoats. Improper storage, handling, and distribution are issues that are being scrutinized. Develop a proactive approach to reduce the potential for illness resulting from eating shellfish while maintaining your business and the people that depend upon your employment. You may consider integrating FDA food safety for the consumer into your marketing strategy.

**Task Sheet for ISSC/FDA:**
This group must protect the consumer, develop and enforce regulations, as well as oversee the condition of shellfish in the bay. Use the information available and the knowledge that you have gained thus far in this learning module to prepare a position regarding the oyster situation.

**Task Sheet for NIH:**
Your interest lies in preventing disease outbreaks in humans. You are to approach this cholera situation head on to prevent people from becoming sick. Your interest is health not economics.

**Task sheet for Consumers:**
You love to eat shellfish and especially enjoy oysters on the half shell, (raw oysters.) You are tired of the government trying to tell you what to do. Prepare a persuasive speech to convince everyone to provide you with oysters and other shellfish whenever you want it!

**Group Member Responsibilities**
*Each person within the group is expected to fully participate in the activity.*
Group leader: Responsibilities include serving as group moderator during discussion and keeping group members focused on the activity at hand.

Recorder: This group member is responsible for writing down ideas and information suggested for inclusion into the group position presentation.

Researcher: Additional information may be available from other sources within the classroom. The researcher can review any available information or interview one person from another group or the teacher. Interviews are to last only one minute and the researcher can interview only one person outside their own group.

Spokesperson: This position is reserved for the person that will be presenting the persuasive speech outlining the group position. This should be someone with public speaking skills and can think quickly on their feet. If less than 5 people are in a group, the leader or recorder can also serve as the spokesperson.

Artist: Any graphics that will be used to enhance the presentation will be created by the artist. Graphics include pictures and words.....

Sources for Information for articles for Lesson 4 Fight Over The Bay

http://www.google.com/hostednews/afp/article/ALeqM5gNbMYDzfbT7tbN118PgbtC2kRHYA?docid=CNG.60d5b39646e58529e4788d62cae3ee2e.431
http://www.umm.edu/altmed/articles/food-poisoning-000064.htm
Detecting Water Toxicity Using Bioluminescent Bacteria

**Background:** Numerous pollutants from both natural and anthropogenic sources find their way into the oceans on a daily basis. The increase in marine pollution is having a serious impact on the ecosystem causing fish kills, coral bleaching, mutations, and various diseases in marine organisms as well as humans. Detecting the individual pollutants and locating the source of pollution is one of many functions of marine scientists.

Detection of specific pollutants can be achieved using various techniques from water test kits to specialized equipment. Monitoring the organisms that reside in the ocean can provide scientists with a natural means of detecting toxicity. Bioluminescent bacteria such as *Vibrio fischeri* can be used to determine toxicity of the water. Scientist have observed that the luminescence of the bacteria decreases as the level of toxicity increases.

**Problem:** How can water toxicity be determined?

**Hypothesis:**

**Materials:**

- Broth culture of *Vibrio fischeri*
- 3 water samples from various sources
- 4 test tubes per group
- test tube rack (1 per group)
- 5ml or 10ml serological pipets and pump
- 4ml *Vibrio fischeri* broth culture (1 tube per group)
- Water (distilled or tapwater)
- UV light(s)
- Para-film
- Permanent markers
- Scissors
- 10% Bleach solution or 70% Ethanol

**Procedure:**

1. Collect all materials.
2. Label the test tubes. (1 for sample 1; 2 for sample 2; 3 for sample three, and Vf for the positive control)
3. Place 1ml of each sample into the corresponding test tube.
4. Place 1ml of water into the Vf tube.
5. Place 1ml of the bacteria, *V. fischeri* into each test tube.
7. Turn off the lights so that the room is as dark as possible. Use an ultraviolet light for viewing. Record observations for each sample.
8. Record observations every 5 minutes for the remainder of the class period.
9. Draw conclusions and answer questions provided by your instructor. Determine the hypothesis.
10. Clean up and dispose of materials following appropriate protocol. (refer to laboratory micro-organism safety handout)

**What’s in Your Seafood?**

**Pre-test/post-test**

1. Organisms considered to be shellfish are:
   a. Crabs and lobsters
   b. Lobsters and oysters
   c. Shrimp and clams
   d. Clams and oysters

2. Shellfish are monitored by: (Circle all that apply)
   a. Centers for Disease Control
   b. Environmental Protection Agency
   c. Interstate Shellfish Commission
   d. United States Fish and Wildlife Service

3. Filter feeders include all of the following EXCEPT:
   a. Clams
   b. Conchs
   c. Oysters
   d. Scallops

4. Many species of bacteria are found in the oceans.
   a. True
   b. False

5. Bacteria can be (Circle all that apply)
   a. harmful to humans
   b. used to provide light
   c. used to produce oil
   d. used to test water toxicity

6. The *Vibrio* bacteria
   a. are all pathogenic (cause diseases)
   b. include the species that causes cholera
   c. include the species that causes malaria
   d. live only in the Indian Ocean

7. The primary environmental factors affecting bacteria reproduction are:
a. Light and temperature
b. Light and pH
c. Salinity and light
d. Salinity and temperature

8. Shellfish should be stored at
   a. 55°F or lower
   b. 45°F or lower
   c. 35°F or lower
   d. 25°F or lower

9. Shellfish are best for the consumer when they are
   a. Raw
   b. salted
   c. steamed
   d. non-living when purchased

10. Harvesting areas are shut down during
     a. thunderstorms
     b. high temperatures
     c. elevated bacteria levels
     d. species reproductive seasons
Recall ordered for some Apalachicola Bay oysters

Comments 19
May 10, 2011 9:55 PM
Lauren Sage Reinlie
Daily News

A rare but mild strain of cholera has led to the recall of Apalachicola Bay oysters from at least one local seafood market.

At least 11 people were sickened from eating raw or lightly steamed oysters harvested from a section of Apalachicola Bay between March 21 and April 6. None of them required hospitalization.

The U.S. Food and Drug Administration announced Tuesday that it has been asking harvesters and suppliers to recall any oysters from that area of the bay.

A few weeks ago suppliers came to Sexton’s Seafood in Destin to collect oysters that were harvested in that area, said an employee who declined to give her name.

She said no one at the business could remember any other oyster recalls in the past 20 years.

The recall didn’t affect business at the store because suppliers were able to replace the oysters with others harvested from different areas, the employee said.

No other outbreaks of illness from that particular strain of cholera have ever been reported in Florida, and officials have yet to find another case in the United States, said Sterling Ivey, a spokesman for the Florida Department of Agriculture and Consumer Services.

On April 29 the Department of Agriculture and the Division of Aquaculture closed Area 1642, a two-mile-wide stretch of Apalachicola Bay that runs along the east side of the bridge from Eastpointe to St. George Island.

The FDA is sampling oysters from the area to determine when it can be reopened.

“We hope that our work will be completed soon and the entire area can be reopened before the end of the winter season,” Ivey said.
Symptoms of the illness are nausea, vomiting and diarrhea that can begin from a few hours to five days after eating raw or lightly-cooked oysters or ingestion of surface water.

The FDA urges anyone who has recently purchased oysters to check with the retailer whether they were harvested from the affected area. If symptoms are experienced, consumers should see a doctor, according to the news release.

Florida Freedom Newspapers Staff Writer David Adlerstein contributed to this report
Read more: http://www.nwfdailynews.com/articles/oysters-40009-bay-recall.html#ixzz1Uh6liobV

INVITED ARTICLE
David Acheson, Section Editor
Cholera and Other Types of Vibriosis: A Story of Human Pandemics and Oysters on the Half Shell
J. Glenn Morris, Jr.
Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, and Baltimore Veterans Affairs Medical Center, Baltimore, Maryland

Abstract
Vibrios are ubiquitous in the aquatic environment and are commonly present in or on shellfish and other seafood. A small subset of strains/species are able to cause human disease, including the cholera toxin–producing strains of Vibrio cholera that are responsible for epidemic/pandemic cholera; thermostable direct hemolysin–producing strains of Vibrio parahaemolyticus; and Vibrio vulnificus, which can cause fulminant sepsis. Cholera outbreaks can be initiated by transmission of “epidemic” V. cholerae strains from their environmental reservoir to humans through seafood or other environmentally related food or water sources. “Nonepidemic” strains of V. cholerae and strains of other Vibrio species, including V. arahaemolyticus and V. vulnificus, are generally acquired by eating seafood (particularly raw oysters/oysters on the half shell). Although the primary clinical manifestation of infection with these strains is gastroenteritis, they can also cause wound infections and (particularly for V. vulnificus) septicemia in persons who have liver disease or are immunocompromised.

Vibrio species are free-living bacteria found in aquatic environments throughout the world. They tend to be more common in warmer waters (temperatures >17°C–20°C) [1, 2]; depending on the species, they tolerate a range of salinities. In one study in the Chesapeake Bay, Vibrio vulnificus was present in water at counts of ~10^4 organisms/mL, Vibrio cholerae–Vibrio mimicus at counts of 10^3 organisms/mL, and Vibrio cincinnatiensis at counts of 10^2 organisms/mL [2]. As would be anticipated, given their abundance in water, Vibrio species are also commonly isolated in samples from fish and shellfish, and, after concentration by filter-feeding shellfish such as oysters, may be present at concentrations that are 100-fold higher than those in the surrounding water [1]. During warm summer months, virtually 100% of oysters will carry V. vulnificus and/or Vibrio parahaemolyticus [1, 3, 4]; densities in US Gulf Coast oysters often exceed 104 organisms/g of oyster meat. Although vibrios do not appear to affect oysters, some species may be pathogenic to marine life: for example, Vibrio damsela causes lesions in damsel and other fish, and Vibrio shiloi is an important cause of coral bleaching.
Vibrio species that are associated with human illness are listed in table 1, together with Centers for Disease Control and Prevention (CDC) data on the number of reported cases and deaths in the United States in 1999. “Epidemic” strains of V. cholerae carrying a suite of specific virulence genes cause the disease cholera, and other, “non-epidemic” strains are associated with gastroenteritis, wound infections, and septicemia in susceptible hosts [5–8]. V. arahaemolyticus is a common cause of gastroenteritis and the leading cause of foodborne illness in Japan [9]; it, too, can cause wound infections and septicemia in susceptible hosts. V. vulnificus is the leading cause of death in the United States associated with seafood consumption, which is the result of its ability to cause severe wound infections and sepsis in patients who are immunocompromised or have underlying liver disease [10]. Although other Vibrio species have been linked with gastroenteritis or wound infections, the number of cases is relatively small, and, in some instances, it is unclear whether isolation of the organism represented asymptomatic colonization or infection. Given the ubiquitousness of the genus in the environment, asymptomatic colonization does occur. Perhaps one of the earliest known indicators of this was the identification of Vibrio metschnikovii DNA in the colonic contents of the 15000-year-old “Tyrolean Iceman” found frozen in an alpine glacier [11].

CHOLERA AND EPIDEMIC V. CHOLERAE
Cholera is characterized by the rapid onset of profuse, watery diarrhea, which, if untreated, can lead to dehydration, circulatory collapse, and death. Seven cholera pandemics have been recorded since 1817. The seventh pandemic (caused by V. cholerae strains in O group 1, biotype El Tor) originated in the Celebes in Indonesia in 1961 and spread through trade, tourism, and pilgrimage routes to Asia, Europe, Africa, and the South Pacific. In January 1991, explosive outbreaks of the seventh pandemic strain occurred in cities along the Peruvian coast, with further epidemic spread through South and Central America. In 2001, 184,311 cholera cases and 2728 cholera-related deaths were reported to the World Health Organization from 58 countries [12]; the actual number of cases and deaths likely is much greater than this total, because there is significant underreporting of cholera in many countries, and there are countries known to have had cholera outbreaks in 2001 that reported no cases. In contrast to the early 1990s (figure 1), when cases were concentrated in the Americas, the majority of reported cases in 2001 were from Africa and occurred in association with recent epidemics in southern and western Africa [12].

Microbiology and pathogenesis. V. cholerae is traditionally classified by O group (with 1150 O types currently recognized in the widely used Sakazaki grouping system) and by biotype (classical and El Tor) and serotype (Ogawa, Inaba, and, rarely, Hikojima) [13]. Until recently, V. cholerae strains belonging to serogroup O1 were believed to be the sole etiologic agents of cholera. However, since late 1992, V. cholerae serogroup O139 has emerged as a second etiologic agent of cholera in the Indian subcontinent and neighboring countries [14, 15], and there are suggestions that strains in other V. cholerae serogroups can cause cholera-like disease [16–18]. The signs and symptoms of cholera are caused by cholera toxin (CT), a protein enterotoxin that elicits profuse diarrhea. CT activates cyclic adenosine monophosphate, leading to increased Cl− secretion and decreased NaCl-coupled absorption. Glucose, potassium, and bicarbonate absorption, however, remain intact, as does glucose-linked enhancement of sodium and water absorption. Thus, although plain salt water is nonabsorbable during cholera and aggravates the diarrhea, the addition of glucose renders the solution absorbable, providing a physiologic basis for oral rehydration [13, 19]. Genes for cholera toxin are carried by the CTX phage and can be transferred between V. cholerae strains [20]. Virulence also has been linked
with the presence of the *Vibrio* pathogenicity island (VPI) [21], which carries key genes involved in intestinal colonization. However, both the CTX phage and VPI can be found in strains that lack epidemic potential [17], which suggests that there are other, as yet unidentified factors underlying the ability of a *V. cholerae* strain to spread in epidemic fashion. In this context, there has been some very interesting recent work suggesting that, in the human intestine, epidemic *V. cholerae* strains shift to a hyperinfectious form that is passed in the “rice water” stools characteristic of cholera, with at least short-term persistence of this form in the environment [22].

**Epidemiology.** After passage of a pandemic wave through a geographic region, cholera generally settles into an endemic pattern of seasonal outbreaks separated by periods of quiescence. In South America, for example, cases are concentrated in the summer months of January and February. Studies that we have conducted in Peru show that the seasonal cholera epidemics are often heralded 2 months before they occur by increases of *V. cholerae* in the environment (triggered, in turn, by seasonal increases in water temperature) [23], with apparent subsequent “spillover” of the bacterium into human populations. As humans become infected, *V. cholerae* present in their feces contaminate food and water sources and further increase environmental *V. cholerae* levels, resulting in amplification of the organism and initiation of the epidemic cycle. The occurrence and intensity of epidemics also has been linked with global climatic events; epidemic intensity has peaked along the west coast of South America and in Bangladesh in association with the occurrence of the El Niño southern oscillation [24, 25]. In the United States, we have a small environmental focus of potentially epidemic *V. cholerae* along the Gulf Coast, inhabited by what appears to be a single clone that has persisted for 130 years. Cholera cases caused by this strain have generally been linked to consumption of undercooked crab or raw oysters harvested from the Gulf Coast [26, 27]. Between 1995 and 2000, there were 6 cholera cases in the United States that were linked with this focus and an additional 8 cases that were associated with imported seafood [28]. However, with rare exceptions, the sporadic seafood-associated cholera cases that have occurred in the US population have not spread beyond the seafood associated index case, presumably because of the higher levels of sanitation present in this country. Other foods implicated in US cases in recent years include commercial frozen fresh coconut milk (imported from Thailand) [29] and cut cantaloupe (probably contaminated by an asymptomatic, infected food handler) [28]. Cooked rice appears to be a particularly effective vehicle for transmission of cholera, and rice has been implicated in a number of outbreaks, in settings as diverse as African funerals [30], a US oil rig platform [31], and a luxury cruise ship in Thailand (the latter outbreak was caused by an O139 strain) [32].

For unknown reasons, persons with blood group O are significantly more likely to have severe cholera [33]. Factors that predispose to hypochlorhydria (e.g., malnutrition, gastrectomy, and acid-reducing medications), by decreasing the gastric acid barrier to infection, also increase susceptibility to illness. The atrophic gastritis and hypochlorhydria associated with chronic *Helicobacter pylori* infection has also been associated with an increased risk of severe cholera [34].

**Clinical presentations, diagnosis, and management.** Despite the dread inspired by the term “cholera,” the majority of persons infected with epidemic *V. cholerae* strains do not have severe illness: 75% of persons infected with strains of the classical biotype have inapparent or mild disease, and 93% of persons infected with biotype El Tor strains (which are responsible
for the most recent pandemic) have illnesses that are inapparent or mild [35]. The incubation period for cholera ranges from 12 h to 5 days. In the most severe cases (cholera gravis), rates of diarrhea rapidly increase during the first 24 h of illness, peaking at rates of up to 1 L/h. Diarrheal stools have a pale gray “rice water” appearance. In the absence of appropriate rehydration, this degree of purging can lead to circulatory collapse and death within a matter of hours. Dehydration is reflected in a higher plasma protein concentration, hematocrit, serum creatinine level, urea nitrogen level, and plasma specific gravity. Stool bicarbonate losses and lactic acidosis associated with dehydration can result in severe acidosis, manifested by decreases in blood pH and plasma bicarbonate and an increase in the serum anion gap [36]. Despite profound potassium loss, uncorrected acidosis may be associated with a normal or high serum potassium level. Plasma sodium and chloride concentrations remain in the normal range. Among properly treated patients, deaths are rare (in 2001, the case-fatality rate for all of South America was 0.25% [12]). The clinical diagnosis of cholera is based on rapid onset of diarrhea and vomiting with dehydration and the presence of profuse “rice water” stool in an appropriate epidemiologic setting. Laboratory diagnosis is based on isolation of the organism from stool. This generally requires use of a selective medium, such as thiosulfate–citrate–bile salt–sucrose (TCBS) agar; for specimens sent to clinical microbiology laboratories in the United States, it is generally necessary to specifically request use of this medium (which is also required for isolation of other Vibrio species) for samples from suspected cholera cases. The diagnosis also can be make by serologic testing. Treatment of cholera is based on replacement of fluids lost through diarrhea by oral or, in severe cases, intravenous rehydration [19, 37]. Antibiotics, such as tetracycline (table 2), can shorten the duration of diarrhea and reduce the period of carriage. However, antibiotic therapy should always be regarded as ancillary to vigorous rehydration. It also should be recognized that many epidemic strains are resistant to recommended antibiotics. In general, resistance has been most common among isolates from Asia [28], but tetracycline resistance has been seen in the recent cholera outbreaks in Mozambique, South Africa, and Madagascar [38, 39]. These observations underscore the need for antimicrobial susceptibility testing of all V. cholerae isolates, particularly those that may have been acquired outside of the United States.

Prevention. Outside of the context of endemic or seasonal epidemic cholera, the risk of acquiring the disease is low and can be further reduced by maintenance of good sanitation and provision of safe potable water. However, as long as there are environmental foci of epidemic strains (such as the one seen along the US Gulf Coast), it is not possible to totally eliminate the risk of V. cholerae infection. Cooking of seafood and shellfish reduces but does not eliminate the risk: in studies conducted by the CDC, epidemic V. cholerae could still be isolated from infected crabs that had been boiled for 8 or steamed for 25 min, cooking times that resulted in crabs that were red in appearance, with meat that was firm and appeared to be well cooked [26]. Although a great deal of recent work has been done on development of cholera vaccines, including development of oral attenuated vaccines, no cholera vaccine is currently commercially available in the United States [40].

NONEPIDEMIC V. CHOLERAE

Strains of V. cholerae that do not carry the virulence factors necessary to cause epidemic cholera have been implicated as causes of diarrheal disease, wound infections, and, in susceptible hosts, septicemia [5–8, 41, 42]. Epidemiologic studies and studies involving volunteers have linked the occurrence of diarrheal illness to production of a heat-stable enterotoxin (NAG-ST) similar to that produced by enterotoxigenic Escherichia coli [43, 44];
diarrheal illness has also been linked to production of CT or a CT-like toxin and to the ability of a strain to colonize the intestine [41, 42]. In contrast to V. cholerae O1, which is not encapsulated (and which, with 1 or 2 possible exceptions, does not cause sepsis), 190% of nonepidemic V. cholerae produce a polysaccharide capsule; heavily encapsulated strains are significantly more likely to be isolated in samples from patients with septicemia than are strains with minimal or no encapsulation. As is true for other vibrios, nonepidemic strains of V. cholerae are part of the normal, free-living bacterial flora in estuarine areas throughout the world. In areas such as the US Gulf Coast, these strains are several orders of magnitude more common than are epidemic V. cholerae strains in the environment. Isolation is not associated with the presence of fecal coliforms, which is the marker currently used by state and national regulatory agencies in the United States to regulate shellfish harvesting waters. Although illness due to nonepidemic V. cholerae has been linked at a global level to contaminated water and a variety of foods, particularly seafood [41, 42], oysters appear to be the primary vehicle of infection in the United States [45]. The most common manifestation of nonepidemic V. cholerae infection is diarrhea. The incubation period, judging by outbreak reports and volunteer studies, is short (<24 h). Abdominal cramps may be prominent; bloody diarrhea is occasionally reported [45]. Illness is usually mild and self-limited, although a diarrheal stool volume of 5.3 L was seen in a volunteer who received 106 cfu of one nonepidemic strain [43]. Nonepidemic V. cholerae strains have also been isolated from persons with septicemia. The case-fatality rate in a recent case series from Taiwan was 47% [7]; in older US literature, the rate exceeds 60% [46]. Illness appears to be confined to persons with cirrhosis or other liver disease or who are immunocompromised [7, 46]. The route of entry of the organism in these cases is not well defined, although foods containing the organism would be a likely source. As with epidemic V. cholerae, treatment of diarrhea is dependent on adequate rehydration. Septicemia requires aggressive antibiotic therapy and supportive care. Although no controlled studies are available, a combination of minocycline (100 mg q12h po) and cefotaxime (2.0 g q8h iv) [47] or use of a “newer” fluoroquinolone [48] has been recommended for treatment of sepsis caused by V. vulnificus and would appear to be reasonable in management of sepsis caused by V. cholerae.

V. PARAHAEOMOLYTICUS

In the fall of 1950, there was an outbreak of food poisoning in Osaka, Japan. Of 272 patients with acute gastroenteritis, 20 died. These deaths led to an intensive investigation of the outbreak and, ultimately, to the identification of an etiological agent that was named “V. parahaemolyticus.” V. parahaemolyticus is halophilic, or salt loving, and requires NaCl for growth. Isolates from ill persons have traditionally been differentiated from (presumed nonpathogenic) isolates from environmental sources on the basis of hemolytic activity seen when these isolates are grown on special medium (Wagatsuma agar); this is termed the “Kanagawa reaction,” named for the Japanese prefecture where the original study was done. Hemolytic activity in Kanagawa-positive strains of V. parahaemolyticus has been linked with production of thermostable direct hemolysin (Vp-TDH) [49]. TDH-related hemolysins have also been identified that appear to have phenotypic activity similar to that of Vp-TDH and share sequence homology with Vp-TDH [50]. Although V. parahaemolyticus has always been recognized as an important enteropathogen, there has been a striking increase in the incidence of V. parahaemolyticus infections since the mid-1990s. This increase has been noted in multiple countries, including Japan [51] and the United States [52], and appears to be associated with the appearance of a new clonal group with pandemic potential, which includes isolates in serotypes O3:K6, O4:K68, and O1:K untypeable [53]. It has been suggested that rising water temperatures in shellfish-growing areas have served as a cofactor in the increasing incidence of cases of V.
V. parahaemolyticus infection in the United States [52, 54]. Further work is needed to assess the relative impact of water temperature and of the appearance of O3:K6 and related strains. Before 1980, V. parahaemolyticus outbreaks in the United States were associated with seafood but not specifically with consumption of raw oysters; in contrast, in the 1990s, raw oysters were the vehicle of transmission in 11 (69%) of the 16 V. parahaemolyticus outbreaks reported to the CDC [52]. V. parahaemolyticus infection most commonly causes gastroenteritis. In a summary of clinical data from 202 patients with V. parahaemolyticus gastroenteritis that were reported to the CDC between 1973 and 1998 [52], manifestations included diarrhea (98%), abdominal cramps (89%), nausea (76%), vomiting (55%), and fever (52%); 29% of patients reported bloody diarrhea. In foodborne outbreaks, the median incubation period was 17 h (range, 4–90 h), and the median reported duration of illness was 2.4 days (range, 8 h to 12 days). A frank dysentery-like syndrome has been reported in association with V. parahaemolyticus in India and Bangladesh [55]. Although this syndrome is not as common in the United States, there is 1 report of a US patient with blood and leukocytes in stool specimens and superficial colonic ulcerations noted on sigmoidoscopy [56]. V. parahaemolyticus also is a cause of infection in wounds with contact with seawater [5, 8] and has been linked with occasional cases of primary septicemia (i.e., septicemia without an obvious focus of infection). As is true for other Vibrio species, serious wound infections and sepsis occur most commonly in persons with underlying liver disease, alcoholism, or (particularly for wound infections) diabetes [52]. In the 1973–1998 CDC case series [52], the case-fatality rate among persons with V. parahaemolyticus septicemia was 29%. Blood agar and other nonselective media support the growth of V. parahaemolyticus, but isolation from feces generally requires the use of a selective medium, such as TCBS. As is true for other diarrheal diseases, the key to management of V. parahaemolyticus gastroenteritis is provision of adequate rehydration. Although there are no data on antimicrobial efficacy, patients with persistent diarrhea (duration 15 days) may benefit from treatment with tetracycline or a quinolone. In the absence of data, it would appear reasonable to use the treatment protocols recommended for V. vulnificus infection (outlined below) for severe V. parahaemolyticus wound infections or septicemia.

V. vulnificus

V. vulnificus, which was first identified in 1979 simply as a halophilic, lactose-positive marine vibrio [10], causes severe wound infections, septicemia, and gastroenteritis [10, 57–60]. The majority of clinical and environmental V. vulnificus isolates reported to date are of biotype 1. Strains of biotype 2 (now known as “serovar E”) cause sepsis in eels but do not affect humans; biotype 3 strains have been described in association with wound infections related to handling of live fish (tilapia) from fish farms in Israel [61]. As has been reported for non-O1 V. cholerae, V. vulnificus strains produce a polysaccharide capsule that has been strongly linked to virulence [62]. Typing systems based on the capsule have not been developed, in part because of the great diversity seen in capsular types: in one study of 120 strains, 96 different capsular types (“carbotypes”) were identified [63]. V. vulnificus is very sensitive to the degree of binding of iron by transferrin in the host and to serum ferritin concentration [64, 65]. The organism grows rapidly in serum with transferrin that is 170% saturated with iron, with growth restricted at lower saturation percentages (in healthy adults, transferrin is ~30% saturated). This may explain the increase in the risk of disease in persons with hemochromatosis or who are alcoholic and malnourished and therefore have low concentrations of transferrin and correspondingly high saturation of transferrin [64]. Estrogen has been found to play a protective role in mouse models of V. vulnificus sepsis [66], which is interesting in light of the fact that the majority of
human cases occur in men. Like other vibrios, *V. vulnificus* occurs naturally in estuarine or marine environments. The highest numbers (in water and oysters) are found in areas with intermediate salinities (5–25 parts per thousand) and warmer temperatures (optimally, 120_C) [1, 3, 4]. *V. vulnificus* is the most common cause of serious vibrio infections in the United States (table 1); the incidence reported in community-based studies in coastal regions is ∼ 0.5 cases/100,000 population/year [10, 57, 60]. Bacteremia without an obvious focus of infection (primary septicemia) occurs in persons who are alcoholic or who have chronic underlying illnesses, such as liver disease, cirrhosis, or hemochromatosis (table 3) [10, 57–60]. In one study, an increased risk of infection was associated with consumption of as little as 30 mL (1 oz) of alcohol per day [60]. Infection is generally acquired through consumption of oysters containing the organism. Wound infections occur after exposure to estuarine water. Typical exposures include wounds acquired while opening an oyster or in a boating accident. Wounds may become infected in healthy hosts. However, the most severe manifestations are seen in persons with underlying defects in host defense mechanisms. Based on the number of cases reported to the Florida Health Department between 1981 and 1992, the annual rate of illness from *V. vulnificus* infection for adults with self-reported liver disease in Florida who ate raw oysters was 7.2 cases/100,000 adults, 80 times the rate among adults without known liver disease who ate raw oysters (0.09 cases/100,000 adults) [67]. Given the frequency of isolation of *V. vulnificus* from oysters, the incidence of disease is still much smaller than might be expected, even when the need for an appropriate host is taken into account [67]. This may reflect the need for a high infectious dose or possible differences among strains of *V. vulnificus* in their ability to cause illness. In support of the latter hypothesis, there are suggestions that certain ribotypes, carbotypes, or 16S rRNA sequence polymorphisms [68] are more common among clinical strains than among strains from the environment. Patients with primary septicemia present with fever and hypotension (table 3); one-third have shock at initial presentation or become hypotensive within 12 h of hospitalization [57]. Distinctive bullous skin lesions have been reported in 50%–90% of patients [57, 58]. Thrombocytopenia is common, and there is often evidence of disseminated intravascular coagulation. More than 50% of patients with primary septicemia die; the mortality rate exceeds 90% for those who are hypotensive within 12 h of initial presentation [57]. Wound infections range from mild, self-limited lesions to rapidly progressive cellulitis and myositis. Patients who survive severe *V. vulnificus* infections often have some degree of residual disability. This does not appear to be related to the actual infection, which clears readily with antibiotic therapy, but rather to the consequences of multiple organ system failure and the prolonged hospitalization associated with occurrence of a shock syndrome [42]. A presumptive clinical diagnosis of *V. vulnificus* sepsis can be made on the basis of (1) the occurrence of shock or hypotension, or other signs suggesting sepsis (for wound infections, evidence of rapidly progressive cellulitis or myositis); (2) a history of cirrhosis, chronic alcoholism, immunosuppression, or hemochromatosis; (3) a history of recent consumption of raw oysters or exposure of wounds to estuarine water; and (4) the presence of characteristic bullous skin lesions [42]. A definitive diagnosis requires isolation of *V. vulnificus* from samples of blood, from wounds or skin lesions (if present), or of stool. Blood agar and other nonselective media, including media used in commercial blood culture systems, are adequate for isolation from blood and wound samples; TCBS agar is necessary for isolation from stool.

The early administration of antimicrobial agents is critical to successful treatment. Case-fatality rates increase significantly with increasing time between onset of symptoms and initiation of therapy [57]. There is a suggestion, from a large series of cases in Florida, that antimicrobial therapy combinations that included tetracycline were more efficacious [57]. Recent in vitro
and animal studies from Taiwan have led to recommendations that patients be treated with minocycline (100 mg q12h po) and cefotaxime (2.0 g q8h iv) [47] or a “newer” fluoroquinolone [48].

**SUMMARY**

As long as oysters and other shellfish are harvested from warm waters and eaten raw or with minimal cooking, there is risk of infection with *Vibrio* species. In the developing world, sporadic seafood-associated infections with epidemic *V. cholerae* may serve as a trigger for cholera epidemics. In the United States, despite the presence of an environmental focus of epidemic *V. cholerae*, there is less risk of epidemic disease because, presumably, of the extant level of sanitation. However, the risk of sporadic vibrio infection remains. The risk is greatest for persons who are immunocompromised, have cirrhosis (or a history of heavy ingestion of alcohol), or have conditions predisposing them to increased saturation of transferrin with iron. Persons in these risk groups should avoid eating raw oysters (including oysters on the half shell), particularly during the summer and early fall, when water temperatures may exceed 20°C, and should try to minimize exposure of wounds to warmer estuarine or marine waters.

<table>
<thead>
<tr>
<th><em>Vibrio</em> species</th>
<th>Clinical presentation</th>
<th>Gastroenteritis</th>
<th>Wound or ear infection</th>
<th>Septicemia</th>
<th>No. of cases (no. of death)*</th>
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<tbody>
<tr>
<td><em>V. cholerae</em></td>
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<tr>
<td>Epidemic (O1, O139)</td>
<td>++</td>
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<td>--</td>
<td>6 (0)</td>
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<tr>
<td>Nonepidemic</td>
<td>++</td>
<td>--</td>
<td>--</td>
<td>45 (10)</td>
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<tr>
<td><em>V. mimicus</em></td>
<td>++</td>
<td>+</td>
<td>--</td>
<td>10 (0)</td>
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<tr>
<td><em>V. parahaemolyticus</em></td>
<td>++</td>
<td>+</td>
<td>(+)</td>
<td>116 (1)</td>
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<tr>
<td><em>V. fluvialis</em></td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>19 (10)</td>
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<tr>
<td><em>V. furnissii</em></td>
<td>++</td>
<td>--</td>
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<td>1 (0)</td>
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<tr>
<td><em>V. alginolyticus</em></td>
<td>++</td>
<td>++</td>
<td>--</td>
<td>60 (11)*</td>
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<tr>
<td><em>V. alginolyticus</em></td>
<td>--</td>
<td>++</td>
<td>--</td>
<td>28 (0)</td>
<td></td>
</tr>
<tr>
<td><em>V. denezelia</em></td>
<td>--</td>
<td>++</td>
<td>--</td>
<td>2 (0)</td>
<td></td>
</tr>
<tr>
<td><em>V. cincinnatiensis</em></td>
<td>--</td>
<td>--</td>
<td>(+)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><em>V. carlivi</em></td>
<td>--</td>
<td>(+)</td>
<td>--</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><em>V. marinae</em></td>
<td>(+)</td>
<td>--</td>
<td>(+)</td>
<td>1 (0)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** ++, Most common clinical presentation; +, neither rare nor most common clinical presentation; (+), rare clinical presentation.

* Data reflect *Vibrio* infections reported to the Centers for Disease Control and Prevention during 1999. Data are from the 36 states that reported cases, for many of these states, reporting of *Vibrio* infections is not routine, and consequently nos. may not reflect the true no. of cases. Data were kindly provided by P. Tracey, US Centers for Disease Control and Prevention, Atlanta.

* Data include 4 cases associated with foreign travel.

* The 21 reported deaths are from a group of 76 cases for which data on death were available.

**Table 1.** *Vibrio* species implicated as causes of human disease and number of deaths associated with infection with these species.
### World Health Organization recommendations for antibiotic therapy for cholera.

<table>
<thead>
<tr>
<th>Antibiotic regimen</th>
<th>Dose recommended for patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children</td>
</tr>
<tr>
<td>Doxycycline, single dose</td>
<td></td>
</tr>
<tr>
<td>Tetraacycline, q.i.d. for 3 days</td>
<td>12.5 mg/kg</td>
</tr>
<tr>
<td>TMP-SMX, b.i.d. for 3 days</td>
<td>5 mg/kg TMP and 25 mg/kg SMX</td>
</tr>
<tr>
<td>Furazolidone, q.i.d. for 3 days</td>
<td>1.25 mg/kg</td>
</tr>
</tbody>
</table>

**NOTE.** Erythromycin or chloramphenicol may be used when the antibiotics recommended above are not available or when *Vibrio cholerae* 01 is resistant to them. Doxycycline is the antibiotic of choice for adults (except pregnant women) because only 1 dose is required. TMP-SMX is the antibiotic of choice for children; and furazolidone is the antibiotic of choice for pregnant women. TMP-SMX, trimethoprim-sulfamethoxazole. Adapted from [57], with permission.

**Table 2.** World Health Organization recommendations for antibiotic therapy for cholera.

---

### Epidemiologic characteristics of and clinical manifestations in patients with primary septicemia caused by *Vibrio vulnificus*.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Gastroenteritis (n = 231)</th>
<th>Primary septicemia (n = 191)</th>
<th>Wound infection (n = 180)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean risk factors*</td>
<td>14</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Liver disease</td>
<td>14</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Diabetes</td>
<td>14</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>Neutropenia disorder</td>
<td>0</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>0</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Malignancy</td>
<td>15</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Immune deficiency</td>
<td>5</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Renal disease</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Gastrointestinal surgery</td>
<td>11</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Any of the above</td>
<td>39</td>
<td>97</td>
<td>98</td>
</tr>
<tr>
<td>Patient characteristic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>55 (18-84)</td>
<td>64 (24-82)</td>
<td>59 (4-81)</td>
</tr>
<tr>
<td>Male sex</td>
<td>57</td>
<td>66</td>
<td>88</td>
</tr>
<tr>
<td>Symptom or sign</td>
<td>10</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Fever</td>
<td>100</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>44</td>
<td>53</td>
<td>50</td>
</tr>
<tr>
<td>Nausea</td>
<td>71</td>
<td>59</td>
<td>49</td>
</tr>
<tr>
<td>Vomiting</td>
<td>54</td>
<td>54</td>
<td>50</td>
</tr>
<tr>
<td>Lesion*</td>
<td>0</td>
<td>84</td>
<td>30</td>
</tr>
<tr>
<td>Localized cellulitis</td>
<td>0</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Boutsous skin lesions</td>
<td>0</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>69</td>
<td>97</td>
<td>99</td>
</tr>
<tr>
<td>Death</td>
<td>9</td>
<td>61</td>
<td>17</td>
</tr>
</tbody>
</table>

**Notes:**
- Data are percentages of patients, unless otherwise indicated. Data are from Shaw et al., 1991. Table adapted from Morris, 1992, with permission.
- Risk factors are not mutually exclusive.
- *Lesion* was defined as any ulceration, erosion, or necrosis of the skin.
- Two patients with underlying medical conditions (cirrhosis and alcoholism) died; there is a reasonable likelihood that these patients had underlying septicemia.

**Table 3.** Epidemiologic characteristics of and clinical manifestations in patients with primary septicemia caused by *Vibrio vulnificus*. 
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References
MICROBIOLOGY OF AQUATIC SYSTEMS

Salinity and Temperature Effects on Physiological Responses of Vibrio fischeri from Diverse Ecological Niches

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*NOTE: See original journal article for Figures and Tables.

Abstract

Vibrio fischeri is a bioluminescent bacterial symbiont of sepiolid squids (Cephalopoda: Sepiolidae) and monocentrid fishes (Actinopterygii: Monocentridae). V. fischeri exhibit competitive dominance within the allopatrically distributed squid genus Euprymna, which have led to the evolution of V. fischeri host specialists. In contrast, the host genus Sepiola contains sympatric species that is thought to have given rise to V. fischeri that have evolved as host generalists. Given that these ecological lifestyles may have a direct effect upon the growth spectrum and survival limits in contrasting environments, optimal growth ranges were obtained for numerous V. fischeri isolates from both free-living and host environments. Upper and lower limits of growth were observed in sodium chloride concentrations ranging from 0.0% to 9.0%. Sepiola symbiotic isolates possessed the least variation in growth throughout the entire salinity gradient, whereas isolates from Euprymna were the least uniform at <2.0% NaCl. V. fischeri fish symbionts (CG101 and MJ101) and all free-living strains were the most dissimilar at >5.0% NaCl. Growth kinetics of symbiotic V. fischeri strains were
also measured under a range of salinity and temperature combinations. Symbiotic V. fischeri ES114 and ET101 exhibited a synergistic effect for salinity and temperature, where significant differences in growth rates due to salinity existed only at low temperatures. Thus, abiotic factors such as temperature and salinity have differential effects between free-living and symbiotic strains of V. fischeri, which may alter colonization efficiency prior to infection.

**Introduction**

Years of research with various members of Vibrionaceae have shown temperature and salinity to be integral agents in governing Vibrio population dynamics [1, 2], physiological stress responses [3], and evolution [2, 4]. For example, brackish, coastal, and pelagic waters are each uniquely inhabited by distinct Vibrio populations [2]. Since temperature and salinity gradients are known to change over these environments, their variability can determine the fitness of each unique Vibrio population. Furthermore, Vibrio species found in freshwater are prominent since they possess a low Na+ requirement for growth and starvation survival (e.g., Vibrio cholerae and Vibrio mimicus [2]). In addition, members of the Vibrionaceae occur naturally in the digestive tract and on the skin surface of marine animals [1]. In general, the genus Vibrio—along with their close relatives such as Photobacterium—are thought to be especially adapted to engaging in pathogenic and benign host–microbe interactions, with these symbiosis traits probably having a deep and ancient common ancestry, arising independently numerous times during the evolution of Vibrionaceae [5]. The association between sepiolid squids (Mollusca: Cephalopoda) and Vibrio fischeri has become a model system for studying the physiological and molecular signaling between hosts and their bacterial symbionts. The association is mutualistic, since the bacterially produced bioluminescence provides camouflage for the squid hosts in a cryptic behavior termed counterillumination [6]. Interestingly, V. fischeri is also a bioluminescent symbiont of monocentrid fishes, including the genera Monocentris and Cleidopus [7]. Moreover, some strains of V. fischeri that are free-living are unable to develop a light organ association with squid or fish hosts, making them symbiotically incompetent [8]. V. fischeri isolated from monocentrid fishes are only capable of colonizing sepiolid squids in laboratory experiments at a reduced efficiency and possess a lower carrying capacity within cephalopod hosts [9]. Prior data has also demonstrated that symbiotically incompetent V. fischeri and those colonizing Euprymna, Sepiola, and monocentrid fishes are genetically distinct from each other [5, 10–13]. All extant species of Euprymna (Cephalopoda: Sepiolidae) are largely allopatric and distributed in the Indo-West Pacific [14]. Previous research has shown that V. fischeri strains native to one Euprymna species will out-compete conspecific symbionts that are non-native [9, 15]. These and other data suggest that host specialization and competitive dominance may be the result of symbionts locally adapting to Euprymna species in their environment. However, observations of the stratigraphical distributions of V. fischeri and V. logei, two symbiotic species found in Mediterranean sepiolids, determined that temperature and not host squids established Vibrio distribution in the Mediterranean Sea [16, 17]. Numerous Sepiola species exist sympatrically in the Mediterranean, and most of these host species simultaneously co-occur with both V. fischeri and V. logei [16, 18]. Thus, contrary to the competitive dominance observed in Euprymna, Vibrio symbionts in Sepiola are host generalists [16]. Other studies with bivalve and vertebrate hosts have also demonstrated that salinity and temperature influence host colonization [19–22], abundance and distribution [23–25], physiological state and survival [26–28], and the adhesive capabilities to host epithelia [29]. Hence, previous research has made it apparent that salinity and temperature influence all life cycle stages of Vibrio species, including the biogeography of free-living cells, host attachment with subsequent proliferation during symbiosis, and the alternative evolutionary trajectories
available to different host ranges. Especially important is the question as to how evolutionary lifestyle as a host generalist, host specialist, fish versus squid symbiont, and free-living cell influences growth limits of V. fischeri to abiotic factors such as salinity and temperature. Studying the microbial growth of marine symbionts constantly experiencing shifts between marine free-living and host phases is critical since the osmotic pressure can change dramatically between these two environments [30, 31]. This subject definitely needs to be addressed with the remarkable illumination in recent years that virulence and osmoregulation possess links within pathogenic Vibrio species [32]. Therefore, we studied the physiological performances of various V. fischeri strains isolated from different host and free-living environments over a gradient of NaCl concentrations ranging from 0.0–9.0% to observe any correlations between V. fischeri lifestyle and osmotic effects on microbial growth. We also measured synergistic effects of salinity and temperature on V. fischeri growth to determine if these factors had any influence on bacterial fitness during competition with one another.

Methods

**Bacterial Strains, Media, and Culture Maintenance**

Table 1 lists collection sites, squid hosts, and isolated strains of V. fischeri used in this study. Once isolated from squid light organs [9], strains were stored at −80°C in cryo-vial tubes containing a final concentration of 20% glycerol in either seawater tryptone (SWT: 70% 32 ppt Instant Ocean artificial seawater, 0.5% Bacto tryptone, 0.3% Bacto yeast extract, 0.3% glycerol, pH 7.5–8.0; [33]) or Luria–Bertani high salt liquid media (LBS: 1.0% Bacto tryptone, 0.5% Bacto yeast extract, 2% NaCl, 0.3% glycerol, 50 mM Tris–HCl, pH 7.5; [34]). The day before each experiment, strains from the −80°C freezer stock were streaked for use onto SWT agar plates (1.5%) to isolate single colonies.

**Bacterial Growth over Salinity Gradients**

To study V. fischeri’s ability to grow over a wide salinity range, isolates were acquired from diverse niches (Table 1), namely as obligately free-living (i.e., symbiotically incompetent and unable to colonize a fish or squid host), fish symbionts (procured from Monocentris and Cleidopus), squid-host generalist symbionts (isolated from Sepiola squid), and squid-host specialist symbionts (isolated from Euprymna squid). Individual colonies of each strain from SWT plates were used to inoculate 18×150 mm test tubes containing 5 mL SWT. These test tubes served as starter cultures for the experiment. Tubes were incubated at 28°C while shaking at 225 rpm for 16 h. Thereafter, 10 μL of each overnight starter culture was used to inoculate test tubes containing 5 mL of fresh SWT liquid media. The subsequent cultures were incubated at 28°C and shaken at 225 rpm for 3 h. After 3 h of growth, a Uvikon XL spectrophotometer was used to measure optical density (OD600) readings of each culture were measured at each concentration (n=5).

**Monoculture Growth Studies on V. fischeri from Euprymna Hosts**

Strains of V. fischeri isolated from various Euprymna species (Table 1) were grown at different temperatures (12°C, 28°C, and 32°C) and salinities (24, 32, and 38 ppt) to observe how these parameters affect generation times in SWT. Salinity was measured using a refractometer (ATAGO® Co., LTD, Japan). These particular temperatures and salinities were chosen since they
are representative of the environments V. fischeri encounters in nature outside the host. Three-hour cultures of each strain were grown in the same manner as the optical density–salinity gradient studies. After 3 h of growth, a Uvikon XL spectrophotometer was used to take optical density measurements (OD600) of each culture. Cultures were inoculated in triplicate into 125-mL flasks containing 50 mL of SWT to bring the initial cell density to 5×10^5 CFU/mL. Salinity and temperature were measured at the following settings: 24 ppt/12°C, 24 ppt/28°C, 24 ppt/32°C, 32 ppt/12°C, 32 ppt/28°C, 32 ppt/32°C, 38 ppt/12°C, 38 ppt/28°C, and 38 ppt/32°C. We also examined the effect of nutrient-limiting media on growth rates using minimal ribose media [35] with two symbiotic V. fischeri strains, ES114 and EM17. Flasks (125 mL) were aerated at 225 rpm and maintained at the appropriate temperatures for three hours prior to inoculation to guarantee the media was at the correct temperature. OD600 measurements were measured from each of the flask cultures every 30 min for 8 h to obtain growth curves for each strain. OD600 measurements were natural log transformed to calculate each strain’s generation time. Since the experiment was designed with a two-way factorial (or two crossed factors) in a completely randomized design, our analysis used a two-way ANOVA with interaction and when the interaction was present, means of the factor combinations were separated by pairwise t tests. When interaction was not present, the means at the three temperatures and salinities were separated by pair-wise t tests (α=0.05).

**V. fischeri ES114 and V. fischeri EM17 Competition Growth Studies**

To search for the possibility of antagonism or allelopathy between strains, competition growth experiments were completed with V. fischeri ES114 and EM17, since both had similar growth rates across the entire range of salinities and temperatures examined. Triplicate 125-mL flasks with 50 mL SWT were co-inoculated with equal numbers (50:50 ratio) of both strains. The initial cell densities of V. fischeri ES114 and EM17 were each half of the monoculture inoculations (2.5×10^5). This was to achieve the same starting total cell population as in the monoculture growth studies. Salinity of the SWT media and temperature at which they were incubated were as follows: 24 ppt/12°C, 32 ppt/28°C, and 38 ppt/32°C, which represented low, intermediate, and high conditions. Flasks were aerated at 225 rpm and maintained at the experimental temperatures for 3 h prior to inoculation to guarantee the media was at the correct temperatures. Cell enumeration of each strain was ascertained through plate counts by sampling from each of the replicate flasks once every hour. Bacterial ratios were obtained by counting the number of visibly luminous colonies in the dark (EM17), and subsequently the total number of colonies in the light (EM17+ES114). The difference between the two counts yields the total number of V. fischeri ES114 colonies. Since V. fischeri ES114 is not visibly luminous, this allows quantification of both strains then grown together [36]. The competition growth rate data was then subjected to t tests to detect any significant differences using the software package SAS. The usage of either optical density (OD600) or cell density (CFU/mL) yielded similar growth rates for identical strains; therefore, we used the cell density as an approximation of each strain.

**Results**

**Effects of Salinity Gradients on V. fischeri from Different Ecological Niches**

V. fischeri native to Euprymna species exhibited variable growth throughout the salinity gradient compared to Sepiola strains (Figs. 1 and 2). This trend was especially important at lower salinities (<2.0 NaCl, Fig. 1), even when comparing those data to non-squid strains (Fig. 3). In contrast, V. fischeri isolated from Sepiola squids exhibited the most uniform growth throughout
the entire salinity gradient (Fig. 2). V. fischeri ET401 and EB12 were the least able to grow at low salinity (<1.0% NaCl) of all the strains isolated from host animals. Conversely, non-squid V. fischeri exhibited more variability at high salinities (>5.0% NaCl, Fig. 3). No bacterial growth was observed for any of the strains at either the low or high ranges measured in this study (0.0, 7.0, 8.0, or 9.0% NaCl; Figs. 1, 2, and 3). Of all the strains, V. fischeri ES114 demonstrated the best overall growth at low salinities (<3.0% NaCl) and V. fischeri ET401 at higher salinities (>4.0% NaCl). The one exception was free-living V. fischeri CB37, which exhibited the best overall growth over most of the salinity range (Fig. 3). Of all the strains examined, V. fischeri ATCC 7744 (a free-living isolate) had the most constrained growth across the entire salinity gradient. Therefore, host generalists V. fischeri (from Sepiola species) were the least different from each other in their ability to grow from 0.0–9.0% NaCl when comparing them to V. fischeri from the host genus Euprymna (host specialists) and non-squid niches (fish symbionts and obligately freeliving bacterioplankton).

**Temperature and Salinity Growth Studies of V. fischeri from Euprymna**

Growth rates of the Australian and Japanese V. fischeri were tested to assess if any correlations existed between generation times for symbionts as result of being isolated from the same host species (Fig. 4) or the same geographical location (Fig. 5). Mean generation times for strains at each salinity–temperature combination examined (α=0.05) are shown in Table 2. Generation times with dissimilar letters (a, b, c, or d) within the column of each strain were significantly different from each other, whereas growth rates possessing the same letter are statistically equivalent. For example, all values with the letter “a” are equal to one another, and all values with the letter “b” are the same. However, all generation times with the letter “a” are statistically different from those with the letter “b”. Temperature significantly affected all five V. fischeri strains, whereas a significant salinity result was detected only in V. fischeri ES114 and ET101. A significant synergistic interaction between temperature and salinity was also observed within these same two strains (Figs. 4 and 5). At 12°C, growth rates for all strains were significantly lower than those at 28°C and 32°C, while growth rates between these two later temperatures were similar (Figs. 4 and 5). Significant salinity effects for V. fischeri ES114 and ET101 were observed only at 12°C. Increasing the salinity at this temperature led to more rapid generation times for V. fischeri ET101 (Table 2). Mean generation times for the competition growth studies of V. fischeri ES114 and EM17 in nutrient-rich SWT were not significantly different from monoculture generation times of these two strains in the same media at higher salinity and temperature conditions (Table 3). However, a significant difference was observed between the competition and monoculture generation times at 24 ppt/12°C (Fig. 6).

Monoculture generation times of V. fischeri ES114 and EM17 in minimal ribose media were significantly slower than those in SWT for the same temperature (Table 3). Generation times (as noted with different letters, Table 3) were also significantly different from each other. V. fischeri EM17 and ET401 generation times behave most similarly to each other than to any other Vibrio symbionts across different temperature and salinity conditions (Table 2), as neither displayed a significant salinity–temperature interaction. Salinity and temperature had a significant interaction on the growth rates of V. fischeri ET101 and ES114. Similar to V. fischeri EM17 and ET401, these two strains have similar generation times at 28°C and 32°C. However, salinity had a dissimilar significant effect on V. fischeri ET101 and ES114 growth rates at 12°C. Although V. fischeri ET401, ET101, and EM17 are the only strains to annually experience temperatures as low as 12°C (Table 4), they seem to lack growth rates that are uniquely adapted for those temperatures. No significant difference in generation times among Vibrio symbionts was observed at 12°C. Comparably, V. fischeri ES114 is from Hawaii, where temperature is nearly
constant: the difference between surface temperature and that of 100 m below sea level is only a few degrees centigrade (www.nodc.noaa.gov), yet V. fischeri ES114 generation time at 28°C is not significantly faster than the other symbionts (Table 3). In this regard, V. fischeri EM17 and ET401 are derived from environments extensively variable in temperature throughout the year. V. fischeri ES114 experienced a significant salinity effect and salinity—temperature interaction on its growth rate where V. fischeri EM17 and ET401 did not, implying the microbial physiology of V. fischeri ES114 is more sensitive to variable environments. However, this does not necessarily allude to the conclusion that V. fischeri EM17 and ET401 are better adapted to variable environments.

Discussion
V. fischeri is a cosmopolitan microbe with a ubiquitous distribution in oceans, estuaries, brackish waters, and marine sediments throughout the world [37], as either part of free-living bacterioplankton or as a mutualistic symbiont [7, 38]. Although most V. fischeri strains are “facultative” symbionts with cyclical free-living and mutualistic lifestyles, V. fischeri strains exist that persist strictly as members of the bacterioplankton and are symbiotically incompetent, essentially becoming obligately free-living [8]. Clearly, V. fischeri is establishing its worldwide dissemination through oceanic water currents as host animals are known to be limited in their dispersal ability [10, 11]. Ocean temperatures can range between −1.0°C and 30°C with salinities ranging between 5 to 38 ppt (www.nodc.noaa.gov) [39]. Previous studies investigating the microbial ecology of luminous bacteria suggests that species composition of a particular environment was largely determined by patterns of temperature, salinity, nutrient concentration, solar radiation, and other abiotic factors [7, 40–43]. Although this idea may continue to hold for microfloral planktonic communities, more recent research has demonstrated that selective pressures in marine bioluminescent bacteria for specificity toward their host fishes and cephalopods with light organs can preside over normal evolutionary physiological requirements. For instance, Photobacterium leiognathi typically is more abundant as a free-living microbe in warmer waters; however, this species can be found as a symbiont in both temperate-water and tropical leiognathid fishes [7]. V. fischeri itself is usually a temperate-water species but can be found in hosts inhabiting both tropical and temperate waters [38]. This provides evidence that the distribution, ecology, and evolution of luminescent bacterial species in marine environments can be partially driven by symbiosis as opposed to abiotic factors. Previous work has demonstrated luminous Vibrio species colonizing light organs of the Mediterranean genus Sepiola was determined by temperature and not squid-host specificity [16, 18]. Alternatively, V. fischeri symbionts colonizing the squid genus Euprymna from the Indo-west Pacific were primarily determined by host specificity [9, 15]. Such outcomes governed by abiotic or host specificity may be dependent on the number of hosts available, utilization of different host animals, and whether hosts are allopatric or sympatric. Additionally, the host animal can directly influence symbiont abundance and distribution via seeding the oceanic water column with bacteria through daily venting cycles [44]. Due to the complexity of host interactions and abiotic factors in directing the community structure of marine luminescent microorganisms such as V. fischeri, roles of both ecological determinants need further investigation to better understand how this microbe resides in the diverse niches it occupies [7, 17]. Our study measured the effects of salinity and temperature on growth rates (i.e., generation times) of V. fischeri from several Euprymna species in nutrient-rich media (Table 2). Nutrient-rich media have previously been used to simulate a host environment when studying effects of salinity and temperature on the microbial physiology of Vibrio species [26]. Since growth rates of microorganisms have characteristics that represent underlying
physiological processes of single cells (e.g., biosynthesis of macromolecules), understanding how abiotic factors influence Vibrio generation times will facilitate the illumination of the cellular events responsible for changes in microbial populations during symbiosis [45]. V. fischeri ET101 encounters considerable variation in temperature (Table 4), and growth appears more sensitive to changes in salinity and temperature than V. fischeri ES114. Correlations between significant effects on generation times by abiotic factors and constant/variable environments are absent. Finally, V. fischeri ET101 and ET401, two E. tasmanica symbionts isolated from squid from two distinct locations (Table 1) has demonstrated no detectable competitive dominance [9]. However, both V. fischeri isolates are genetically distinguishable [11]. V. fischeri ET101 was isolated from E. tasmanica inhabiting Melbourne, Victoria and V. fischeri ET401 was isolated from E. tasmanica living in Townsville, Queensland (Table 1). Waters near Victoria typically range in temperature from about 12–17°C, whereas Queensland is much warmer (24–26°C). V. fischeri ET101 and ET401 possess generation times that were uniquely affected by salinity and temperature (Fig. 4). For instance, V. fischeri ET401 possess the fastest logarithmic growth at low salinity and low temperature (24 ppt/12°C), yet V. fischeri ET101 has the most rapid generation time at high salinity and low temperature (38 ppt/12°C; Table 2, Fig. 4). Salinity only has this growth-altering effect at 12°C and not at the higher temperatures. This outcome may be the result of underlying differences in regional physiological adaptations. V. fischeri EB12 and EM17 are isolates from two Japanese hosts, E. morsei and E. berryi. E. morsei tends to occur in cooler temperate waters of northern Japan, while E. berryi is more frequently found in southern Japan’s temperate warm waters, including along the coast of China [46]. Distributions of E. morsei and E. berryi do overlap partially. Nevertheless, these hosts are believed to be sibling species that are either reproductively isolated or hybridization is rare [47]. Similar to V. fischeri ET101 and ET401, generation times of Japanese strains may respond differently to salinity and temperature due to physiological differences resulting from evolution within their respective thermal niches. At low salinity and low temperature (24 ppt/12°C), V. fischeri EM17 had a shorter generation time than V. fischeri EB12. As the salinity increased at low temperature (12°C), the generation times between these two strains became more similar (Table 2, Fig. 5). Although V. fischeri EB12 infects E. berryi, which is restricted to sub-temperate/warm waters (17–25°C), this strain may also experience temperatures as low as 2°C during its free-living planktonic phase in northern Japanese waters. V. fischeri ES114 and EM17 both have generation times of approximately 30 min at 32 ppt/28°C in SWT media, but V. fischeri ES114 still out-competes EM17 in E. scolopes under similar environmental conditions [15]. Interestingly, a “competition” effect was observed at 24 ppt/12°C; both strains grew significantly slower in the presence of the other. Additionally, V. fischeri EM17 generation time was more negatively affected by the presence of V. fischeri ES114 than ES114 was by EM17 at 24 ppt/12°C (Fig. 6). Hence, microbial allelopathy may at least be partially responsible for competitive dominance in Euprymna, especially at lower temperatures (e.g., 12°C). Vibrio symbionts have quite similar generation times over the temperatures and salinities examined in nutrient-rich media, yet native strains still out-compete non-native ones under laboratory conditions that approximately simulate natural habitats. If these results can be extrapolated to the nutrientrich environment of the host, competitive dominance in Euprymna would not be solely the result of native Vibrio symbionts possessing faster generation times or growth rate constants (growth parameters g and k) than non-native ones while in the host. Rather, V. fischeri strains may be more actively competing against one another via faster generation times when they are part of the free-living bacterioplankton, where the oceanic water column serves as a semi-starving environment relative to the host. Growth rates of V. fischeri ES114 and EM17 in minimal media demonstrate that symbionts can have differential growth rates when nutrients may be more limiting. The
possibility remains that competitive dominance may be a combination of faster growth rate parameters and microbial amensalism, as these two phenomena are not mutually exclusive. Despite the presence of competitive dominance of native V. fischeri during Euprymna colonization [15], population genetic surveys of host squid and V. fischeri symbionts suggest secondary colonizations occur [11], whereby previously allochthonous strains become established in a novel animal host. Particular attributes of the Vibriosepioid squid symbiosis engender native symbionts of Euprymna spp. susceptible to at least partial displacement by non-native invaders. Attachment and proliferation of V. fischeri within axenic squid hatchlings emerging from eggs can be initiated and completed with as little as ten cells [8], creating severe symbiont founder effects and genetic bottlenecks during the colonization of the hosts each generation. Symbiont populations may undergo considerable genetic drift upon acquiring new hosts, exposing residential symbionts to potential deleterious effects of Muller’s ratchet [48, 49]. An upper bound then crystallizes and confines the magnitude of adaptation that native symbionts achieve to their hosts, diminishing the likelihood of a permanent evolutionarily enduring advantage gained by native vibrios over non-native ones in host colonization. Specific morphological changes are triggered within squid hatchlings upon colonization by Vibrio cells, and these immense transformations continue to occur throughout the early stages of the symbiosis [50], which make continuous and serial re-colonization from free-living symbionts less probable after maturity of the association. These properties could permit invading non-native V. fischeri to retain occupancy of foreign Euprymna, despite the prevailing presence of competitive dominant strains in an area, providing the non-native symbionts arrive and settle into host animals first. Non-native V. fischeri initially outnumbering native strains is key to this scenario to offset competitive dominance. Low temperature environments (i.e., winters, cold temperate climates) appear to foster conditions and alternative salinities where allochthonous vibrios could accomplish this inside hosts during early stages of symbiosis by exploiting their faster generation times (Table 2), if optimal conditions were sustained. Perhaps localities combining low temperature, low salinity, and semi-starvation with free-living bacterioplankton in estuaries and deltas during cold periods provide the best opportunities to function as reservoirs for non-native Vibrio symbionts. Although marine environments normally range in salinity from 3.3–3.7% [40], investigating the effects of salinity on V. fischeri growth is important considering this Table 4 Annual mean temperatures and mean salinities for Euprymna species (www.nodc.noaa.gov, www.cephbase.utmb.edu) Host squid Distribution 0–100 m usual temperature range 0–100 m usual salinity range E. tasmanica Australia/Tasmania 12–25°C 20.0–35.8 ppt E. morsei Japan (cool temperate waters) 2–17°C 32.2–34.0 ppt E. berryi Japan (warm temperate waters) 17–25°C 34.2–34.8 ppt E. scolopes Hawaiian Islands 22–26°C 34.6–35.2 ppt E. hylebergi Thailand 21–28°C 31.4–34.4 ppt E. hoylei Philippines/North Western Australia/Marshall Islands 21–29°C 34.0–35.0 ppt Biogeographic data was obtained from the National Oceanographic and Atmospheric Administration and published literature [46, 47, 55] Salinity and Temperature Effects on Physiological Responses of V. fischeri 147 species ability to invade and thrive within novel hosts and environments is related to its capacity to manage stress [51]. The genus Vibrio includes some of the most common culturable marine bacteria, but the general ecology of Vibrio in the oceans still remains poorly characterized [52]. Furthermore, the physical, chemical, and biological variation that serves as the impetus for the adaptation and diversification of Vibrio species is yet to be well described. This study demonstrates different V. fischeri strains have various ranges of salinity that they are able to tolerate and grow (Figs. 1, 2, 3). Surprisingly, slight changes in salinity (e.g., Δ0.1% NaCl=Δ1 ppt) led to dramatic changes in
growth. As a result, the most prevalent strain within a given geographical region can be quite dynamic, depending on the level of salinity fluxes that occur as a consequence of local water currents and seasonal changes. In some instances, sudden and sharp demarcations existed where a particular strain grew significantly and where it did not grow at all, indicating gradual zones of decreasing growth are not always present with subtle changes in salinity. Hence, minute salinity changes in the marine environment could dramatically influence host squid and V. fischeri symbiotic relationships (Figs. 1, 2, 3). Some overlap existed in the physiological response among different strains isolated from Euprymna, Sepiola, and non-squid (i.e., obligately free-living and fish symbionts). These overlapping salinities may represent where different strains can coexist simultaneously in the open ocean. Obligately freeliving strains failed to grow above 6.0% NaCl. Therefore, undertaking a symbiotic lifestyle may select for V. fischeri more adapted to higher salinities, although this needs to be further investigated. V. fischeri CB37 was the only symbiotically incompetent strain able to grow above 6.0% NaCl (Fig. 3).

Understanding how temperature and salinity modulate the biogeographical distribution of Vibrio populations can allow us to predict whether these populations are greatly influenced by abiotic pressures. For example, V. fischeri ET101 and ET401 both occur in Australian waters. The former grows faster at 24 ppt/12°C and the later at 38 ppt/ 12°C. The two strains have similar generation times at all other combinations of salinity and temperature. The salinity 38 ppt is not a common salinity in their habitat (Table 4). Stabilizing selection of growth rates at high and low salinities at 12°C is not a suitable explanation of their cooccurrence within E. tasmanica. Instead, a more plausible hypothesis is V. fischeri ET401 maintains its population by growing more quickly within this host at low salinity and is more prone to expulsion from the light organ through ventilation cycles, while V. fischeri ET101 maintains its presence by colonizing the squid more efficiently throughout the host’s temperature and salinity ranges. Therefore, V. fischeri ET101 would be less likely to be expelled into the water column. This is analogous to an r-selected (ET401) versus K-selected (ET101) strategy of survival. Further studies investigating intra-strain variation throughout large environmental gradients are planned in future studies. Although microbial growth was principally sensitive at high and low NaCl concentrations, other parameters such as oxygen concentration, trace metals, radiation, hydrostatic pressure, and stress responses affect marine microbial populations and need to be considered [40]. For instance, stress is known to affect the quality of organic carbon produced by vibrios living in simple, microbial loop foodwebs. This phenomenon influences the quality of carbon available to other trophic levels [53]. Illumination in recent years that the multitude of microbial physiological responses to stress (e.g., heat shock, starvation) are coupled and cross-talk elevates the complexity and provides fertile ground for intriguing research [54]. Future work will examine the extent to which V. fischeri symbionts are capable of adapting to different environmental niches (extreme temperatures, low/high salinities, and feast/famine nutrient conditions) and whether the evolutionary potential to adapt to these environments are correlated with those Vibrio symbionts (e.g., strains, species) most abundant in the habitats, leading to a greater understanding of microbial diversity, speciation, and evolution.

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Table 3 Mean generation times (min) for Vibrio fischeri ES114 and V. fischeri EM17 from competition and minimal media growth studies (monoculture generation times in SWT are included in parentheses for comparison) Strain 24 ppt 12°C 32 ppt 28°C 38 ppt 32°C Minimal ribose 28°C EM17 80.3d (63.6c) 30.4b (26.9b) 26.4b (30.8b) 89.8d (30.0ba) ES114 82.7d (73.0a) 28.4b (30.8b) 29.9b (27.4b) 114.5e (28.9ba) Generation times with different letters and colors denote values significantly different from each other. For example, all values with the letter “a” are equal to one another, and all values with the letter “b” are also equivalent. However, all generation times with the letter “a” are statistically different from those with the letter “b”.

Mean generation times obtained from averaging different growth studies completed at 28°C

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Inside Science: Authoritative science news

Weather Changes May Predict Cholera Outbreaks:
The Good News and the bad news of epidemic predictions.
By Joel N. Shurkin, ISNS Contributor

(ISNS)—Scientists working with data from the cholera-plagued Tanzanian archipelago of Zanzibar have found that even a slight variation in temperature or rainfall could herald an epidemic.

That’s both good news and bad news.

The good news is that by monitoring the weather and changes in the climate, epidemiologists may be able to predict the arrival of a disease epidemic up to four months in advance, early enough to make maximum use of vaccines.

The bad news is that with global warming, it is likely cholera epidemics will increase in frequency, and with the climate and rain patterns changing, epidemics could be even more frequent. It’s happening already—recent epidemics in Haiti and Cameroon indicate a resurgent disease.
Cholera is a particularly ghastly disorder caused by the Vibrio cholerae bacteria and is transmitted through drinking water contaminated by human feces. Its main symptom is violent, severe diarrhea, followed by dehydration. In some recorded epidemics, the death rate for infected individuals can be as high as 50 percent. For some patients, the time between feeling healthy and death can be as little as 24 hours.

Cholera originated in the Indian subcontinent and is a disease usually found in developing areas of the world. It arrived in Europe in the early 19th century, reaching pandemic proportions several times.

The relationship between weather, seasons and cholera is long-established. Sea surface height, sea surface temperature and the concentration of chlorophyll in the ocean have already been shown to be predictive in earlier studies in India and Bangladesh.

Researchers from the International Vaccine Institute in Seoul, South Korea, went back to disease and environmental records in Zanzibar between 2003-08. Reporting in the June issue of the American Journal of Tropical Medicine and Hygiene they studied rainfall totals in Zanzibar, high and low temperatures, humidity, and sea surface temperatures. Similar techniques have been successfully used to plot malaria and dengue fever.

They found that a one degree Celsius increase in the average monthly minimum temperature was a sign that the number of cholera cases would double within four months. Further, a 7.8 inch increase in monthly rainfall totals was predictive of a substantial increase in cases within two months.

Giving vaccines to a population that may already have been infected is less effective than vaccinating them before infection, so being able to get ready for an outbreak would save lives. An epidemic now underway in Yaounde, the capital city of Cameroon, has been blamed on unusual heavy rains coming well before their normal time. The researchers think that is a good example of environmentally driven disease.

The 2010 earthquake in Haiti also triggered cholera. More than 300,000 people have been sickened and 5,000 died. The rainy season is about to start, and the researchers fear an explosion of the disease.

While the study from Zanzibar is useful it would be more useful if it could be extrapolated to other areas, and so far it cannot, said David Sack, professor of international health at the Johns Hopkins Bloomberg School of Public Health in Baltimore.

Sack studies cholera in Bangladesh. He said that the disease comes in March in the south of that country, October and November in the north, and year round in Dacca, the capital city—all this in a country the size of Florida.

So what is true in Zanzibar may not be true in Nigeria.

But the seasonality of the disease is well-established and more extreme summers may cause more grief.
"Climatologists predict a 1.4-5.8 C (2.5-10.435-42 F) degree rise in mean temperature over the next 100 years," the vaccine researchers wrote. "Increased sea temperatures and levels associated with global warming intuitively suggest the possibility of increased cholera incidence in many resource-poor regions of the world."

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**22 people sickened from raw Wash. oysters**

By VANESSA HO Seattlepi.com  
Story Published: Aug 5, 2011 at 8:49 AM PDT

SEATTLE -- The state Department of Health is advising people to cook local shellfish, after 22 people became sick from eating raw oysters harvested in Puget Sound and along the Washington coast.
Officials said the oysters contained the Vibrio parahaemolyticus bacteria. The naturally occurring bacteria thrives in July and August with warm temperatures and low tides. Four of the illnesses stemmed from recreational harvesting in the Sound and along the coast. Eighteen of the cases came from commercial operations.

Vibriosis poisoning symptoms include diarrhea, nausea, cramps, vomiting, fever, headache and chills. Health officials had the following tips for safe oyster harvesting:

- Put oysters on ice or refrigerate as soon as possible after harvest.
- If a receding tide has exposed oysters for a long time, don't harvest.
- Cook oysters thoroughly. Fifteen seconds at 145 degrees destroys the Vibrio bacteria.

The advisory comes a day after health officials closed all King County shorelines to shellfish harvesting, after finding risky levels of the Paralytic Shellfish Poison toxin.

Seattlepi.com is a media partner of KOMO News.

Cholera oyster outbreak sickens 11 in US

(AFP) – May 10, 2011

MIAMI — As many as 11 people have reported getting sick from eating raw oysters contaminated with cholera bacteria in northern Florida, officials said on Tuesday.

The oysters came from Apalachicola Bay, near Panama City in northern Florida, about 300 miles (482 kilometers) from New Orleans along the Gulf of Mexico coast, and the US Food and Drug Administration issued a warning not to eat them.

"There is ongoing, collaborative discussions among all state and federal partners as we look at this new pathogen to analyze the first ever outbreak of this unique strain of Vibrio cholerae," Florida’s Department of Agriculture said in a statement.

State officials said 11 cases of illness were reported, while the FDA said eight of those have so far been confirmed as "caused by toxigenic Vibrio cholerae O75... No one was hospitalized or died."
The high number of cases is unusual, given that the Centers for Disease Control typically logs one to two cases per year, an FDA spokesman told AFP.

"From 2000-2010, a total of 17 persons with toxigenic V. cholerae O75 infection were reported to CDC, the numbers are greatest when the water is warm," spokesman Douglas Karas said in an email.

The FDA said the affected oysters were harvested from Area 1642 in Apalachicola Bay between March 21 and April 6.

The Florida Department of Agriculture said it closed the area to oyster harvesting on April 29 and was investigating the cause of the outbreak.

"To date, we have learned of two events that may be the cause of the cholera related illnesses. First, there was a dredging operation near the 1642 harvesting area that may have stirred up organisms on the floor of the ocean," it said.

"We have also learned there was a sewer break in East Point and we are investigating whether it had any impact on oysters in 1642. The harvesting area will remain closed until our investigation is complete."

Area 1642 is home to about 10 percent of the state's oyster harvest, and oysters taken from there are mainly consumed in Florida, Georgia and Alabama.

The strain is different from the one that has killed more than 4,850 people in Haiti -- identified as toxigenic Vibrio cholerae serogroup O1.

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**Food poisoning**

**Introduction:**

Food poisoning occurs when you eat food contaminated with bacteria or other toxins. Symptoms include diarrhea, vomiting, and stomach cramps, and generally start 4 - 36 hours after eating contaminated food. While food poisoning is often caused by bacteria, it can also result from eating poisonous plants (some mushrooms, for instance) and animals (pufferfish). Every year, more than 76 million people get sick from food poisoning, especially during summer when food may not be kept cold enough to prevent bacteria from growing.

**Signs and Symptoms:**

The typical signs of food poisoning are nausea, vomiting, abdominal cramping, diarrhea, head or muscle aches, and fever. Specific bacteria may cause these signs and symptoms:

- *Clostridium botulinum* (*C. botulinum*, or botulism): weakness, blurred vision, sensitivity to light, double vision, paralyzed eye nerves, difficulty speaking and swallowing, paralysis that spreads downward, respiratory failure, death
- *Salmonella* spp., *Shigella* spp., and *Campylobacter jejuni* (*C. jejuni*): fever, chills, bloody diarrhea
- **Escherichia coli** (*E. coli*): hemorrhagic colitis (diarrhea with very little stool and large amounts of blood), occurring up to 3 days after eating contaminated food
- **Mushroom poisoning**: affects the liver, the neurological system (brain), or the gastrointestinal tract, including symptoms such as stomach upset, delirium (confusion), vision difficulties, heart muscle problems, kidney failure, death of liver tissue, and death if left untreated

Fish poisoning causes nausea, vomiting, diarrhea, abdominal pain, dizziness, and headache. Specific types of fish poisoning can cause other signs and symptoms, such as:
- Ciguatera (caused by toxins in some fish, including grouper, snapper, mackerel, and barracuda): numbness or tingling around the mouth, feeling of loose teeth, impaired touch sensation of hot as cold and cold as hot, itching, muscle and joint pain, slow heart rate, low blood pressure
- Pufferfish poisoning: numbness or tingling around the mouth, trouble coordinating movement, difficulty swallowing, excess saliva, twitching, loss of ability to talk, convulsions, paralysis that spreads upward, respiratory failure, death
- Shellfish poisoning (caused by toxins in algae that are then eaten by shellfish): numbness or tingling around the mouth or in the arms and legs, trouble swallowing, difficulty speaking.

**What Causes It?**

Usually bacteria and algae cause food poisoning, but poisonous plants and animals may also be the cause.

Common bacterial toxins include:
- *E. coli* in undercooked hamburger, unpasteurized apple juice or cider, raw milk, contaminated water (or ice), vegetables fertilized by cow manure, or spread from person to person
- *Listeria monocytogenes* (*L. monocytogenes*) in cole slaw, dairy products (mostly soft cheeses from outside the United States), and cold, processed meats
- *Salmonella* spp. in poultry, beef, eggs, or dairy products
- *Shigella* spp. from raw vegetables or cool, moist foods (such as potato and egg salads) that are handled after cooking
- *Staphylococcus aureus* (*S. aureus*) in salad dressing, ham, eggs, custard filled pastries, mayonnaise, and potato salad (usually from the hands of food handlers)
- *C. jejuni* in raw milk and chicken
- *C. botulinum* in improperly home canned foods (in children under 1 year of age, mostly from honey but also from corn syrup)
- *Clostridium perfringens* (*C. perfringens*) in meat and poultry dishes and gravies, mostly foods that were cooked more than 24 hours before eating and were not reheated well enough
- *V. cholerae* in bivalve (two shelled) shellfish (such as mussels, clams, oysters, and scallops), raw shellfish, and crustaceans (such as lobsters, shrimp, and crabs)

Common types of fish poisoning include:
- Scombroid poisoning from bacteria in dark meat fish (tuna, bonito, skipjack, mahi-mahi, mackerel) that are not refrigerated well
- Ciguatera poisoning in tropical fish (grouper, surgeonfish, snapper, barracuda, moray eel, shark) that have eaten toxic plankton
- Puffer fish poisoning from the organs and flesh of puffer fish
- Poisoning from shellfish that feed on certain algae
Mushroom poisoning occurs from eating wild poisonous mushrooms, especially *Amanita phalloides*.

**Who's Most At Risk?:**
Infants and the elderly are at greater risk for food poisoning. Other risk factors include:
- Having a pre-existing medical condition, such as chronic kidney failure, liver disease, or diabetes
- Taking antibiotic, antihistamine, or steroid medicines
- Having sickle cell anemia and other problems with red blood cells
- Weakened immune system, pregnant women and people over age 65 are most at risk
- Traveling in an area where contamination is more likely

Listeriosis is most common in pregnant women, fetuses, and people with immune problems. When a fetus is infected with listeria, it may be born prematurely or die.

**What to Expect at Your Provider’s Office:**
Your health care provider will examine you for signs and symptoms of food poisoning, such as stomach problems, and dehydration. Your health care provider may also ask about foods you have eaten recently, where you may have traveled, and if you have had contact with people showing similar symptoms. Tests of your vomit, blood, and stool can identify the cause. In the case of botulism, your health care provider may request electromyography (a test to measure electric impulses in the muscles) to confirm the diagnosis. A lumbar puncture (spinal tap) may be done to check for signs and symptoms related to central nervous system disorders.

**Treatment Options:**
**Prevention**
These steps can help prevent food poisoning:
- Wash your hands and clean any dishes or utensils when you are making or serving food.
- Keep juices from meat, poultry, and seafood away from ready to eat foods.
- Cook foods to proper temperatures.
- Promptly refrigerate any food you will not be eating right away.
- If you take care of young children, wash your hands often and dispose of diapers carefully so that bacteria can't spread to other surfaces or people.
- If you make canned food at home, make sure to follow proper canning techniques to prevent botulism.
- Don't feed honey to children under 1 year of age.
- Don't eat wild mushrooms.
- When traveling where contamination is more likely, eat only hot, freshly cooked food. Boil water before drinking. Don't eat raw vegetables or unpeeled fruit.
- Always refrigerate fish.
- Don't eat tropical fish caught during blooms of poison plankton.
- Eat pufferfish only in specially licensed restaurants with chefs trained to cook it.
- Don't eat shellfish exposed to red tides.

If others may have eaten a food that made you sick, let them know. If you think the food was contaminated when you bought it from a store or restaurant, tell the staff and your local health department.

**Treatment Plan**
Treatment for most cases of food poisoning involve replacing fluids and electrolytes (such as sodium, potassium, magnesium, and chloride). While experiencing vomiting and diarrhea, the person should avoid solid food but increase clear liquids. In more severe cases, a person may need help either breathing or stopping vomiting. In most cases, health care providers do not prescribe antibiotics because they may prolong diarrhea. If you have eaten certain toxins (such as from mushrooms or shellfish), your health care provider may take steps to clean out your stomach (a process called lavage, or pumping the stomach) and administer activated charcoal, which can help absorb the remaining toxin.

**Drug Therapies**
Depending on the symptoms, cause, and severity of food poisoning, a health care provider may prescribe drugs, including:
- Oral rehydration therapy
- Antibiotics
- Antitoxin to neutralize toxins from *C. botulinum* (only given within the first 72 hours)
- Amitriptyline to control the numbness and tingling from ciguatera poisoning
- Apomorphine or ipecac syrup to cause vomiting and help rid the body of toxins
- Atropine for mushroom poisoning
- Diphenhydramine and cimetidine for fish poisoning
- Mannitol for nerve-related symptoms of ciguatera poisoning

**Complementary and Alternative Therapies**
If you are suffering from severe food poisoning, seek conventional medical treatment. Complementary and alternative therapies are best used to strengthen the body and aid in the prevention of food poisoning. For example, animal studies have shown that certain vitamins and nutrients may protect against some food toxins while others may actually worsen the effects of toxins. Milk thistle is an herb commonly used in Europe as a primary treatment for mushroom poisoning. Homeopathy may help treat diarrhea in children (which is sometimes caused by food poisoning) in developing countries.

**Nutrition**
The following general nutritional guidelines may be helpful in the case of food poisoning:
- Drink plenty of fluids (to prevent dehydration).
- Drink barley or rice water (to soothe inflamed stomach or intestine).
- Probiotics, such as *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*, can help restore the balance of good bacteria in the intestine. If you are traveling to an area where the food and water may be contaminated, in addition to taking the precautions above, taking probiotics both before and during your trip may help maintain intestinal health.
- Apple cider vinegar is a traditional remedy that, although it has not been studied scientifically, may have some antimicrobial properties. Mix 2 tsp. in one cup warm water and drink several times a day.

For specific types of food poisoning:
- Alpha-lipoic acid -- Several reports indicate that alpha-lipoic acid, an antioxidant commonly found in broccoli, spinach, and beef, may help treat *Amanita* (mushroom) poisoning, especially when combined with milk thistle (*Silybum marianum*). Seek medical treatment if you suspect mushroom poisoning. Do not self treat.
- Vitamin A -- Studies on rats show that vitamin A offers some protection against salmonella. Rats infected with *Salmonella* appeared to eliminate the bacteria from their bodies faster when pretreated with vitamin A rather than with placebo, according to one study. They also gained more weight and had a greater immune response than rats given placebo.
- Calcium phosphate -- One animal study suggests that rats receiving calcium phosphate supplements may be protected from *Salmonella* poisoning. Researchers think that calcium phosphate helps boost *Lactobacillus*, the good bacteria found in the intestine, which helps fight off *Salmonella*. 

**Supplements to avoid:**
- Fish oil -- In a study of mice infected with the bacteria *Listeria*, animals that regularly consumed diets rich in fish oil had significantly more bacteria in their spleens than animals that consumed diets rich in lard or soybean oil. Until researchers can determine what these results mean to humans, people with *Listeria* infection should avoid foods containing fish oil.

**Herbs**
Various herbs have been used traditionally to treat different types of food poisoning, though in most cases more research is needed.

Milk thistle (*Silybum marianum*) is often used for liver disorders and is widely used in Europe to treat *Amanita* mushroom poisoning. Studies show that patients with *Amanita* poisoning can be effectively treated with pharmaceutical silibinin (the primary active component of milk thistle) up to 48 hours after eating the deadly mushrooms.

Animal studies of Chinese and Japanese combination herbal remedies used for *Listeria* suggest they may be effective for food poisoning. Active ingredients include:
- Asian ginseng (*Panax ginseng*)
- Astragalus root (*Astragalus membranaceus*)
- Chinese cinnamon bark (*Cinnamomum aromaticum*)
- Ginger root (*Zingiber officinale*)
- Licorice (*Glycyrrhiza glabra*)
- Peony root (*Paeonia officinalis*)
- Skullcap (*Scutellaria lateriflora*)

Seek the advice of a trained and licensed herbalist or practitioner of Traditional Chinese Medicine who will guide your individual treatment. Do not self treat with these herbs. Some of these herbs should not be taken if you have heart disease, or high blood pressure, or take blood thinning medication. In addition, some of these herbs interact with other herbs, supplements, and prescription medications, so it is important to make sure all your health care providers know what you are taking.

Laboratory (test tube) studies suggest that the following herbs have antibacterial or antimicrobial properties, although there is no evidence they are effective for treating food poisoning in humans. Do not use these herbs without speaking to a physician or knowledgeable herbal practitioner. Some side effects can be dangerous:
- Bittervine (*Mikania micrantha*)
- Goldenseal (*Hydrastis canadensis*)
- Oregon grape (*Mahonia aquifolium*)
- Chamomile (*Matricaria recutita*)

Barberry (*Berberis vulgaris*) has also been used traditionally to treat diarrhea from infectious causes such as *E. coli* and *V. cholera*. Berberine, the active ingredient in barberry, can cause
brain damage in newborns. Speak to a physician before using berberine containing herbs with children of any age.

**Homeopathy**

No studies have examined the effectiveness of homeopathic remedies for food poisoning. Before prescribing a remedy, homeopaths take into account a person's constitutional type -- your physical, emotional, and intellectual makeup. An experienced homeopath assesses all of these factors when determining the most appropriate remedy for a particular individual. Below are some more common remedies for food poisoning or diarrhea:

- **Arsenicum album** -- for foul smelling diarrhea from food poisoning or traveler's diarrhea with burning sensation in the abdomen and around the anus. This remedy is most appropriate for individuals who feel exhausted yet restless and whose symptoms tend to worsen in the cold and improve with warmth. Vomiting may also occur. *Arsenicum* may also be used to prevent diarrhea when traveling.
- **Chamomilla** -- for greenish, frothy stool that smells like rotten eggs. Used primarily for children, especially those who are irritable, argumentative, and difficult to console.
- **Calcarea carbonica** -- for children who fear being in the dark or alone and who perspire heavily while sleeping. Stools have a sour odor.
- **Podophyllum** -- for explosive, gushing, painless diarrhea that becomes worse after eating or drinking. Exhaustion often follows bowel movements, and the individual for whom this remedy is appropriate may experience painful cramps in lower extremities.
- **Sulphur** -- for irritable and weepy children. May have a red ring around the anus and diarrhea with the odor of rotten eggs.

**Prognosis/Possible Complications:**

Most cases of food poisoning are mild and clear up on their own within 4 - 7 days. However, with mushroom poisoning, up to half of people may die. With botulism, less than 10% die, and some people may need help breathing for months afterwards. More than half of poisonings from pufferfish are fatal. Death is rare in other fish poisonings, but nerve related symptoms can continue for months.

The following are some possible after effects of food poisoning:

- After shigellosis, white blood cell problems and kidney problems
- After *E. coli* infection, kidney problems and bleeding problems
- After botulism, long hospital stays (1 - 10 months) with fatigue and difficulty breathing for 1-2 years, or muscle weakness followed by respiratory failure
- After salmonellosis, Reiter syndrome (an arthritis like disease) and inflammation of the heart lining
- After campylobacteriosis, Guillain-Barré syndrome (a nerve disease)

**Following Up:**

For a severe case of food poisoning, you may need to stay in the hospital to receive fluids and electrolytes, so health care providers can monitor your breathing. Doctors may need to insert a tube down the throat (intubate) or connect you to a machine to help with breathing. Dialysis may be required. Cathartics (substances that help the body remove waste), enemas, and lavage may help eliminate toxins.

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Supporting Research


Yonekura K, Kawakita T, Saito Y, Suzuki A, Nomoto K. Augmentation of host resistance to

**Websites for Articles**


http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19870


**Websites for V. fischeri**

http://web.uconn.edu/mcbstaff/graf/VfEs/VfEssym.htm

http://web.mst.edu/~microbio/BIO221_2004/V_fischeri.htm

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http://departments.kings.edu/biology/lux/bacterial.html

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