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Appendix 1. Media Recipes (Modified from Bakshi et al. 2019)

Each artificial seawater medium is comprised of multiple components that are made separately and combined in the final recipe. Below we detail how to make each mix/stock, and how these are combined in the final medium. For all solutions we use acid-washed Pyrex screw-capped bottles. Note, besides the basic salts, which are made fresh for each batch of medium, the stocks and mixes can be maintained at 4°C for additional uses. We recommend remaking the stocks and mixes every 2-3 months to avoid contamination.

**Mg/Ca stock (20x)**
In 100 mL deionized, MilliQ-filtered water, dissolve MgCl$_2$·6 H$_2$O (21.2 g) and CaCl$_2$·2 H$_2$O (3.04 g). Autoclave.

**Iron stock (1,000x)**
In 100 mL deionized, MilliQ-filtered water, dissolve FeSO$_4$·7 H$_2$O (0.0028 g) and Nitrilotriacetic acid (NTA) disodium salt (0.0081 g). Filter sterilize (0.2 µm).

**AA mix (50,000x)**
This can be purchased from Sigma Aldrich (Cat #M5550). Filter sterilize (0.2 µm).

**FA mix (2,000,000x)**
Combine the following: EtOH (54.86 mL), octanoic acid (15.84 mL), decanoic acid (17.26 g), isobutyric acid (9.27 mL), butyric acid (9.14 mL), and valeric acid (10.88 mL). Filter sterilize (0.2 µm).

**Inorganic N mix (2,000x)**
In 100 mL deionized, MilliQ-filtered water, dissolve sodium nitrate (0.646 g), sodium nitrite (0.028 g), and ammonium chloride (0.053 g). Filter sterilize (0.2 µm).

**Trace metals (100,000x)**
In 100 mL deionized, MilliQ-filtered water, dissolve MnCl$_2$·4 H$_2$O (0.018 g), ZnSO$_4$·H$_2$O (0.002 g), CoCl$_2$(0.001 g), Na$_2$MoO$_4$(0.001 g), Na$_2$SeO$_3$(0.002 g), NiCl$_2$(0.001 g). Filter sterilize (0.2 µm).

**Vitamins (100,000x)**
In 100 mL deionized, MilliQ-filtered water, dissolve thiamine (1.69 g), riboflavin (0.003 g), niacin (0.985 g), pantothenate (1.013 g), pyridoxine (1.028 g), biotin (0.010 g), folic acid (0.018 g), B12 (0.010 g), myo-inositol (0.901 g), and 4-aminobenzoic acid (0.823 g). Filter sterilize (0.2 µm). Wrap container in foil to avoid photodegradation of vitamins.

**P mix for MWH-type media (1,000x)**
In 100 mL deionized, MilliQ-filtered water, dissolve orthophosphate (0.0022 mL) and KH$_2$PO$_4$(0.068 g). Filter sterilize (0.2 µm).

**Misc mix JW-type media (2,000x)**
In 100 mL deionized, MilliQ-filtered water, dissolve L-glutamine (0.146 g), dextrose (0.180 g), D-ribose (0.150 g), sodium pyruvate (0.110 g), sodium citrate (0.294 g), oxaloacetic acid (0.132 g), Sodium acetate (0.082 g), Sodium succinate (0.162 g), alpha-ketoglutaric acid (0.168 g), urea (0.606 g), glycerol (0.074 mL), glycine betaine (0.154 g), choline (0.140 g), sodium thiosulfate (0.158 g), cyanate (0.003 g), DMSO (0.056 mL), and DMSP (0.011 g). Filter sterilize (0.2 µm).
Basic salts:

|                | JW1 | JW2 | JW3 | JW4 | MWH1 | MWH2 | MWH3 | MWH4 |
|----------------|-----|-----|-----|-----|------|------|------|------|
| H2O (mL)      | 950 | 967 | 987.250 | 991.5 | 950 | 967 | 987.250 | 991.5 |
| NaCl (g)      | 23.840 | 15.901 | 7.939 | 3.969 | 23.840 | 15.901 | 7.939 | 3.969 |
| KCl (g)       | 0.746 | 0.498 | 0.248 | 0.124 | 0.746 | 0.498 | 0.248 | 0.124 |
| NaHCO3 (g)    | 0.840 | 0.840 | 0.840 | 0.840 | 0.840 | 0.840 | 0.840 | 0.840 |
| Na2SO4 (g)    | 4.270 | 2.848 | 1.422 | 0.711 | 4.270 | 2.848 | 1.422 | 0.711 |
| NaBr (g)      | 0.082 | 0.055 | 0.027 | 0.014 | 0.082 | 0.055 | 0.027 | 0.014 |
| H3BO3 (g)     | 0.026 | 0.017 | 0.009 | 0.004 | 0.026 | 0.017 | 0.009 | 0.004 |
| SrCl2 (g)     | 0.014 | 0.009 | 0.005 | 0.002 | 0.014 | 0.009 | 0.004 | 0.002 |
| NaF (g)       | 0.002 | 0.002 | 0.001 | 0.001 | 0.002 | 0.002 | 0.001 | 0.001 |
| KH2PO4 (g)    | 0.007 | 0.007 | 0.007 | 0.004 | X     | X     | X     | X     |

Final recipe- In a biosafety cabinet, add the following to the basic salts mix while stirring and filter sterilize (0.1 µm) the entire medium into a sterile container. Check medium pH, which should be ~8.2-8.3. Wrap with foil. Store at room temperature prior to dispensing.

| Mix            | JW1 | JW2 | JW3 | JW4 | MWH1 | MWH2 | MWH3 | MWH4 |
|----------------|-----|-----|-----|-----|------|------|------|------|
| Mg/Ca (mL)     | 50  | 33  | 17  | 8.5 | 50   | 33   | 17   | 8.5  |
| Iron (mL)      | 1   | 1   | 1   | 1   | 1    | 1    | 1    | 1    |
| AA Mix (µL)    | 20  | 20  | 20  | 20  | 20   | 20   | 20   | 20   |
| FA Mix (µL)    | 0.5 | 0.5 | 0.5 | 0.5 | 0.5   | 0.5   | 0.5   | 0.5   |
| Inorganic N (mL) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5   | 0.5   | 0.5   | 0.5   |
| Trace Metals (µL) | 10 | 10 | 10 | 10 | 10   | 10   | 10   | 10   |
| Vitamins (µL)  | 10  | 10  | 10  | 10  | 10   | 10   | 10   | 10   |
| Misc Mix JW (mL) | 0.5 | 0.5 | 0.5 | 0.5 | X    | X    | X    | X    |
| Misc Mix MWH (µL) | X  | X   | X   | X   | 50   | 50   | 50   | 50   |
| P Mix (mL)     | X   | X   | X   | X   | 1    | 1    | 1    | 1    |

For additional media recipes that use modifications of the basic salts solution, see:

Henson, Michael W., V. Celeste Lanclos, Brant C. Faircloth, and J. Cameron Thrash. 2018. Cultivation and genomics of the first freshwater SAR11 (LD12) isolate. The ISME Journal 12:1846-1860

Appendix 2. Order List
### Appendix 2: Order List

| Consumables (most will last >1 semester) | Supplier | Cat number | Quantity | Use |
|------------------------------------------|----------|------------|----------|-----|
| ICF instrument cleaning fluid (100ml)   | Millipore sigma | 4200-0140 | 3 | Guava flow cytometer cleaning fluid |
| Serocluster,u-btm,n/s cs100              | VWR      | 29442-396 | 1 | Cell count plate |
| SYBR green I                             | Millipore sigma | S9430-.5ML | 1 | Cell count DNA stain |
| Flow cell replacement                    | Millipore sigma | 0500-2260 | 1 | Guava flow cytometer part that is routinely exchanged |
| Easy check kit                           | Millipore sigma | 4500-0025 | 1 | Guava flow cytometer calibration check |
| 96-well, microwell microplates, round wells, pack of 5 | VWR | 10036-122 | 2 | Carbon experiment vessel |
| VWR pipette sero 50ml pr cs100           | VWR      | 89130-902 | 1 | Media dispensing |
| VWR pipette sero 10ml pr cs200           | VWR      | 89130-898 | 1 | Media dispensing |
| 50ml centrifuge tubes / bulk / cs500     | VWR      | 10025-698 | 1 | Stocks |
| 2.0ml micro tubes / pk400                | VWR      | 10025-738 | 1 | Portioning of stocks, culture, etc |
| Flasks polycarbonate 125ml               | VWR      | 89095-260 | 1 | Culturing vessels |
| Nalgene™ rapid-flow™ sterile disposable filter units with pes membrane | Thermofisher | 567-0020 | 1 | Media filtering and storage |
| VWR syringe filter .2um cs100            | VWR      | 28145-499 | 1 | Stock filtering |
| Syringe strl luerlok 60ml pk40. Cs160    | VWR      | BD309653  | 1 | Stock filtering |
| AA mix (5000x)                           | Sigma    | M5550-100ML | 1 | Media component |
| L-Glutamine                              | Sigma    | 56-85-9 | 1 | Media component |
| Dextrose                                 | Sigma    | 50-99-7 | 1 | Media component |
| D-Ribose                                 | Sigma    | 50-69-1 | 1 | Media component |
| Sodium pyruvate                          | Sigma    | 113-24-6 | 1 | Media component |
| Sodium citrate                           | Sigma    | 6132-04-3 | 1 | Media component |
| Oxaloacetic acid                         | Sigma    | 328-42-7 | 1 | Media component |
| Sodium acetate                           | Sigma    | 127-09-3 | 1 | Media component |
| Sodium succinate                         | Sigma    | 6106-21-4 | 1 | Media component |
| a-ketoglutaric acid                      | Sigma    | 22202-68-2 | 1 | Media component |
| Urea                                     | Sigma    | 57-13-6 | 1 | Media component |
| Octanoic Acid                            | Sigma    | O3907-500ML | 1 | Media component |
| Decanoic Acid                            | Sigma    | C1875-500G | 1 | Media component |
| Isobutyric Acid                          | Sigma    | K1875-5G | 1 | Media component |
| Butyric Acid                             | Sigma    | O3907-500ML | 1 | Media component |
| Valeric Acid                             | Sigma    | C1875-500G | 1 | Media component |
| EtOH                                     | Sigma    | I1754-500ML | 1 | Media component |
| Sodium nitrate                           | Sigma    | B103500-500ML | 1 | Media component |
| Sodium nitrite                           | Sigma    | 240370-100ML | 1 | Media component |
| Ammonium chloride                        | Sigma    | 12125-02-9 | 1 | Media component |
| NaCl                                     | Sigma    | 7647-14-5 | 1 | Media component |
| KCl                                      | Sigma    | 7447-40-7 | 1 | Media component |
| NaHCO3                                   | Sigma    | 144-55-8 | 1 | Media component |
| Na2SO4                                   | Sigma    | 7757-82-6 | 1 | Media component |
| Chemical          | Supplier | Catalog Number | Package Size | Notes        |
|-------------------|----------|----------------|--------------|--------------|
| NaBr              | Sigma    | 7647-15-6      | 1            | Media component |
| H3BO3             | Sigma    | 10043-35-3     | 1            | Media component |
| SrCl2             | Sigma    | 10025-70-4     | 1            | Media component |
| NaF               | Sigma    | 7681-49-4      | 1            | Media component |
| KH2PO4            | Sigma    | 7778-77-0      | 1            | Media component |
| MgCl2 x 6H2O      | Sigma    | 7791-18-6      | 1            | Media component |
| CaCl2 x 2H2O      | Sigma    | 10035-04-8     | 1            | Media component |
| FeSO4 x 7H2O      | Sigma    | 15422-250G     | 1            | Media component |
| NTA NA2 salt      | Sigma    | N0128-100G     | 1            | Media component |
| MnCl2 x 4H2O      | Sigma    | M3634-100G     | 1            | Media component |
| ZnSO4 X H2O       | Sigma    | 307491-100G    | 1            | Media component |
| CoCl2             | Sigma    | 232696-5G      | 1            | Media component |
| Na2MoO4           | Sigma    | 243655-5G      | 1            | Media component |
| Na2SeO3           | Sigma    | 214485-5G      | 1            | Media component |
| NiCl2             | Sigma    | 339350-50G     | 1            | Media component |
| B1/Thiamine       | Sigma    | T4625-25G      | 1            | Media component |
| B2/Riboflavin     | Sigma    | R4500-25G      | 1            | Media component |
| B3/Niacin         | Sigma    | N4126-100G     | 1            | Media component |
| B5/Pantothenate   | Sigma    | C8731-100G     | 1            | Media component |
| B6/Pyridoxine     | Sigma    | P9755-25       | 1            | Media component |
| B7/Biotin         | Sigma    | B4501-100MG    | 1            | Media component |
| B9/Folic Acid     | Sigma    | F7876-1G       | 1            | Media component |
| B12               | Sigma    | V2876-250MG    | 1            | Media component |
| Myo-inositol      | Sigma    | IS125-50G      | 1            | Media component |
| 4-Aminobenzoic Acid | Sigma | 127671-25G | 1 | Media component |

**Flow cytometer**
- BD, Luminex
  - Cell counts

**Bisafety cabinet or laminar flow hood**
- VWR/Fisher
  - Sterility

**Balances with 0.001gr sensitivity**
- VWR/Fisher
  - Media preparation

**Weigh paper**
- VWR/Fisher
  - Media preparation

**Filtered tips (1mL, 200uL, 10uL)**
- VWR/Fisher
  - Sterility

**Pipettes (1mL, 200uL, 10uL)**
- VWR/Fisher
  - Culture handling

**10% HCL bath**
- VWR/Fisher
  - Dishwashing

**VWR markers**
- VWR
  - Labeling

**Tape for labels**
- VWR/Fisher
  - Labeling

**10% bleach**
- VWR/Fisher
  - Sterility

**70% ethanol**
- VWR/Fisher
  - Sterility

**Latex free gloves**
- VWR/Fisher
  - Sterility
Appendix 3. Student Lab Manual (USC)-Contains student instructions

Chapter 1: Stocks and Carbon Plate Calculations

**Learning Objectives:**

- Define the Great Plate Count Anomaly and the role that medium composition might play in it.
- Be able to compare and contrast natural seawater medium and artificial seawater medium.
- Understand how Henson et al. 2016 is related to the GPCA and the overall importance of the paper to environmental microbiology.
- Calculate volumes and weights needed to make a nutrient stock at a given concentration.
- Use a balance and sterile technique to create carbon stocks that will be used in the next lab.

**Pre-lab reading**

Bacterial cells rely on macromolecules (carbohydrates, proteins, lipids, and nucleic acids) to live. Many bacterial cells are highly specialized in the types of macromolecules that can be used for life. In addition to the types of macromolecules, many bacterial cells are sensitive to the concentration of nutrients in their surroundings. **Oligotrophic** organisms need a relatively low-nutrient environment to thrive while **copiotrophic** organisms thrive on high-nutrient **eutrophic** systems.

Historically, progress in microbiology has been hindered by the difficulty of obtaining pure bacterial cultures. Most of the traditional knowledge about bacterial biochemistry, interactions, antibiotics, etc come from easily cultivated **model organisms** like *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas fluorescens*, or *Azotobacter vinelandii*. These organisms have been tremendous in the amount of knowledge that we have gathered from them, but they are not representative of environmental microbial diversity. The difficulty of cultivation of many prokaryotes has been coined the **Great Plate Count Anomaly** (Staley and Konopka, 1985) in which there is a discrepancy between number of cell types known to exist in a sample and the number of cell types that have been cultured from a sample. This anomaly is due to many reasons, but some of the most important are competition for nutrients, symbiotic interactions, and poor medium design.

**Medium** must contain all of the classes of molecules discussed earlier since they are vital for the bacteria to survive. Often, a bacterial species that is successful or a key player in biogeochemical cycles is the result of extensive evolution and natural selection acting upon populations. To successfully cultivate specialized types of cells, researchers need to have a medium that mimics the environment closely enough so that the cells are capable of making the habitat transition. Imagine humans needing to inhabit another planet. It is impossible for us to breathe an atmosphere that is too different than ours. If we try to colonize Mars as is, we will not survive the transition.

One way that researchers have been able to help solve the GPCA in aquatic systems is by using sterilized **natural seawater medium**. To use natural seawater as a cultivation medium, researchers collect seawater from the same location that they are trying to isolate bacteria from, filter out large organisms, and autoclave the seawater to ensure it is sterile. Then, they use the water as-is or add in whatever nutrients they would like in the media. This type of medium allows for certainty that factors such as nutrient content and salinity remain suitable for whatever microbes are targeted for cultivation since they were already in the system. This method has been successful and is still used, but natural seawater medium has a few key limitations. The first limitation of natural seawater medium is the **batch effect** that happens with the seawater. Depending on the time of year and characteristics of the physical movement of water, the nutrient content of water at a location will not be consistent over time. The second limitation of natural seawater medium is the **storage and transportation difficulty**. If the researcher is not close to the water body studied, they must determine how much seawater they will need to run all experiments and find a place to keep it all without contaminating it.

Natural seawater is **complex and undefined**. This means that there is a complex suite of nutrients available to the organism in culture, but researchers cannot always exactly quantify the ratio or sometimes the identity of each type. The medium type that we are using in this lab is **artificial seawater medium** designed as part of Henson et al. 2016. This specific mixture of nutrients was created to mimic the coastal Gulf of Mexico in which our isolated bacterial cells are from. This medium type is complex and **defined**, meaning there is still a complex suite of nutrients
available to the organism in culture, but we know exactly what type of molecule is there and in what concentration. Additionally, we are able to modify the medium’s salinity to account for fluctuations in the estuarine environments that coastal bacteria might encounter there.

This lab will result in the creation of various nutrient stocks that will aid to the cell’s production of macromolecules essential to life. To do this, we will use the dilution equation. The dilution equation is as follows:

\[(\text{initial concentration})(\text{volume transferred}) = (\text{final concentration})(\text{final volume})\]

**Henson et al. discussion:**

1. What was the motivation behind the paper?

2. What was the goal of the paper?

3. What did this paper contribute to the general scientific field?

4. What is the LSUCC?

5. Why is it important to be able to culture environmentally-relevant microbes?

Additional Notes/Questions:

**Exercise #1: Calculations for stocks**

**Materials needed:**
- Calculator

In this activity, we will perform the calculations needed to create the carbon sources that will be tested for our bacterial isolate.

Gather some of these values from your instructor:

- Assigned nutrient source(s):

- Final concentration of nutrient source needed in each well:

- Media volume in each well of plate:
Stock volume: __________________

Calculations:

1. Use the following equation to determine the mass of reagent needed to make a nutrient stock at ____________:

   Mass = formula weight * Volume MilliQ Water (L) * concentration (M)

2. Use the following equation to determine the volume of nutrient stock needed for each well of the minimal media plate:

   Volume of stock to plate = (Final concentration desired in well * Final volume in well) / Concentration of the stock

3. Use the following equation to determine the volume of culture needed to inoculate the plate:

   Volume of culture = (Concentration of culture desired in well * Final volume in well) / Concentration of culture

Do your calculations here and on the back of this page if needed for each stock:
Exercise #2: Creation of nutrient stocks

**Materials needed:**
- Biosafety cabinet
- Lab coat
- 10% bleach
- 70% ethanol
- Paper towels
- Gloves
- Pipettes and tips
- Milli-Q or other ultrafiltered water. DI acceptable.
- 1-2 balance(s) per benchtop, weigh paper, clean scoops
- Stock of carbon, nitrogen, and sulfur sources to be tested
- 1 50mL falcon tube/student
- Labeling equipment (markers and tape)
- 1 0.2µm filter/student
- 1 60mL syringe/student

Use the balance to measure your chemical out and mix it in a falcon tube containing ___________ Milli-Q H2O.

Each student will be responsible for their own stocks, but should work in groups of two.

**Usage of balance:**

- Ensure the balance is clean and nothing is on top of it.
- Press “0” or “Zero” to calibrate the balance to no weight on it.
- Put your weigh boat or weigh paper on the scale.
- Press “tare” to reset the weight back to zero.
- Measure out the needed mass onto the boat or paper with a scoopula. If you spill, you must restart.
- Record your data in the chart below.
- Put your chemical into the falcon tube of water and ensure it dissolves completely
- Fully label everything.

Fill out the table below:

| Chemical | Goal Measurement | Actual Measurement | Notes |
|----------|------------------|--------------------|-------|
|          |                  |                    |       |
|          |                  |                    |       |
|          |                  |                    |       |

**To filter your stock:**

Before entering the biosafety cabinet, fully label a new falcon tube with the following information:

- Name of chemical, stock concentration, 0.2µm filtered, initials, date

In the biosafety cabinet, filter your stock through a 0.2µm filter using sterile technique. Follow these steps:

- Tuck your lab coat into your gloves. There should be no skin showing ever.
- Use the squirt bottle and paper towels to wipe down your workspace with 10% bleach first, then 70% ethanol.
- Squirt ethanol onto your gloves and rub your hands together until dry.
- Unwrap a 60mL syringe and pull out the plunger. Make sure to stand the plunger upright with the black rubber side up.
- Attach your filter to the end of the syringe.
- Pour your stock into the syringe over a container.
- Hold the syringe over a falcon tube and pop the plunger back into the syringe. Push the entire stock through the filter and into the falcon tube. Be careful not to spill on the sides.
- Close the falcon tube tightly and store your stock with the instructor.

Exercise #3: Creation of other media types

The following tables show the composition of our medium types. What trends do you notice?

Basic salts:

|                | JW1  | JW2  | JW3   | JW4   | MWH1 | MWH2 | MWH3  | MWH4 |
|----------------|------|------|-------|-------|------|------|-------|------|
| H2O (mL)       | 950  | 967  | 987.250 | 991.5 | 950  | 967  | 987.250 | 991.5|
| NaCl (g)       | 23.840 | 15.901 | 7.939  | 3.969  | 23.840 | 15.901 | 7.939  | 3.969 |
| KCl (g)        | 0.746  | 0.498  | 0.248  | 0.124  | 0.746  | 0.498  | 0.248  | 0.124 |
| NaHCO3 (g)     | 0.840  | 0.840  | 0.840  | 0.840  | 0.840  | 0.840  | 0.840  | 0.840 |
| Na2SO4 (g)     | 4.270  | 2.848  | 1.422  | 0.711  | 4.270  | 2.848  | 1.422  | 0.711 |
| NaBr (g)       | 0.082  | 0.055  | 0.027  | 0.014  | 0.082  | 0.055  | 0.027  | 0.014 |
| H3BO3 (g)      | 0.026  | 0.017  | 0.009  | 0.004  | 0.026  | 0.017  | 0.009  | 0.004 |
| SrCl2 (g)      | 0.014  | 0.009  | 0.005  | 0.002  | 0.014  | 0.009  | 0.004  | 0.002 |
| NaF (g)        | 0.002  | 0.002  | 0.001  | 0.001  | 0.002  | 0.001  | 0.001  | 0.001 |
| KH2PO4 (g)     | 0.007  | 0.007  | 0.007  | 0.004  | X      | X      | X      | X    |

Final recipe—In a biosafety cabinet, add the following to the basic salts mix while stirring and filter sterilize (0.1 µm) the entire medium into a sterile container.

|        | JW1 | JW2 | JW3 | JW4 | MWH1 | MWH2 | MWH3 | MWH4 |
|--------|-----|-----|-----|-----|------|------|------|------|
| Mg/Ca (mL) | 50  | 33  | 17  | 8.5 | 50   | 33   | 17   | 8.5  |
| Iron (mL)  | 1   | 1   | 1   | 1   | 1    | 1    | 1    | 1    |
| AA Mix (µL) | 20  | 20  | 20  | 20  | 20   | 20   | 20   | 20   |
| FA Mix (µL) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5  | 0.5  | 0.5  | 0.5  |
| Inorganic N (mL) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5  | 0.5  | 0.5  | 0.5  |
Your instructor will give you a list of media types to make and divide up the responsibilities across groups and sections.

Use the balance to measure your chemical out and mix in __________ mL Mili-Q H2O.

Fill out the table below:

| Chemical          | Goal Measurement | Actual Measurement | Notes |
|-------------------|------------------|---------------------|-------|
| Trace Metals (µL) | 10 10 10 10 10 10 | 10 10 10 10 10 10 |       |
| Vitamins (µL)     | 10 10 10 10 10 10 | 10 10 10 10 10 10 |       |
| Misc Mix JW (mL)  | 0.5 0.5 0.5 0.5 X X X X | X X X X X X |       |
| Misc Mix MWH (µL) | X X X X 50 50 50 50 | X X X X X X |       |
| P Mix (mL)        | X X X X 1 1 1 1 | X X X X X X |       |
Chapter 2: Minimal Media Experiment

Learning Objectives:

- Define biogeochemistry.
- Be able to compare and contrast autotrophy and heterotrophy in bacteria.
- Understand where carbon is used in bacterial cells.
- Use Buchan et al. 2014 to understand where bacteria fit into a marine ecosystem.
- Use sterile technique to inoculate the minimal media experiment for our organism.

Pre-lab reading:

Biogeochemistry is defined as “the study of microbially mediated chemical transformations of geochemical interest”. Some important cycles that are microbially-mediated are the carbon, nitrogen, and sulfur cycles. Every living thing needs carbon (as you will learn in-depth in organic chemistry). Autotrophic organisms can use inorganic carbon for their life cycles while heterotrophic organisms require organic carbon. The carbon that a cell intakes is used for either biomass production (incorporation into amino acids, lipids, DNA, etc) or respiration as CO2. Nitrogen that is used for biomass production and is shunted into amino acids, proteins, and nucleic acids. Sulfur is used by cells for energy generation and the production of vitamins and amino acids.

Table 2-1: Table of terminology to describe organisms based on their energy and carbon sources. Table source: https://openstax.org/books/microbiology/pages/8-1-energy-matter-and-enzymes#17019/.

| Classification | Energy Source | Carbon Source | Examples |
|----------------|---------------|---------------|----------|
| Chemotrophs    | Chemical      | Inorganic     | Hydrogen-, sulfur-, iron-, nitrogen-, and carbon monoxide-oxidizing bacteria |
| Chemoheterotrophs | Chemical      | Organic compounds | All animals, most fungi, protozoa, and bacteria |
| Phototrophs    | Light         | Inorganic     | All plants, algae, cyanobacteria, and green and purple sulfur bacteria |
| Photoheterotrophs | Light         | Organic compounds | Green and purple nonsulfur bacteria, heliobacteria |

In environmental microbiology, it is oftentimes a slow and difficult process to determine which organisms are doing a specific piece of the biogeochemical cycle that you care about. Furthermore, it is important to remember that no piece of any cycle is ever actually independent of others. Nothing exists in a vacuum and instead has many factors that will enhance or decrease any portion of these reactions at any given time. That being said, it is still vital to understand how environmentally-relevant organisms are possibly interacting with their environment in these cycles. Culture-dependent work is important to ground-truth suggested genomic features and patterns seen in the environment. Working with bacterial cultures allows researchers to quantify the capabilities of an organism over a range of factors to try to link the microorganisms to their environmental role.
Exercise #1: Literature reading

To gather an ecosystem-level overview of aquatic biogeochemical cycles and the way that microbes are playing their part in it, use Figure 1 from the below reference to define the following terms:

Buchan, Alison, Gary R. LeCleir, Christopher A. Gulvik, and José M. González. 2014. “Master Recyclers: Features and Functions of Bacteria Associated with Phytoplankton Blooms.” *Nature Reviews. Microbiology* 12 (10): 686–98

Plankton:

Phytoplankton:

Bacterioplankton:

DOM:

POM:

Microbial Loop:

Biological pump:

Microbial carbon pump:

Viral Shunt:

Remineralization:

Record at least two questions that you’d like to discuss in class:

1.

2.
Exercise #2: Minimal Media Plate #1 Inoculation

Materials needed:
- Biosafety cabinet
- Lab coat
- 10% bleach
- 70% ethanol
- Paper towels
- Gloves
- Pipettes and tips
- Mili-Q or other ultrafiltered water. DI acceptable.
- Healthy bacterial culture
- Nutrient stocks created last class
- Base media at each benchtop
- 1 2mL microcentrifuge tube/student

Each student will test a minimal media combination to see whether it is sufficient to allow our isolate to grow.

Record the following:
  My assigned source(s):
  Well(s):

The chart below is representative of the 96-well plate that we will use for this experiment. Note the shaded gray square will be A1. Label the rows A-H and the columns 1-12. Use this to keep track with where your experimental condition will be in the plate and be sure not to accidently pipette into someone else’s well.

|      |      |      |      |      |      |      |      |      |
|------|------|------|------|------|------|------|------|------|
|      |      |      |      |      |      |      |      |      |
|      |      |      |      |      |      |      |      |      |
|      |      |      |      |      |      |      |      |      |
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|      |      |      |      |      |      |      |      |      |
|      |      |      |      |      |      |      |      |      |
|      |      |      |      |      |      |      |      |      |

In the biosafety cabinet, set up the carbon experiment. Follow these steps:
- Tuck your lab coat into your gloves. There should be no skin showing ever.
- Use the squirt bottle and paper towels to wipe down your workspace with 10% bleach first, then 70% ethanol
- Squirt ethanol onto your gloves and rub your hands together until dry.
Using a new tip each time, use the following chart to guide you and pipette the following into a labeled microcentrifuge tube:

|                               | Tube 1 | Tube 2 (if applicable) |
|-------------------------------|--------|-------------------------|
| **Base Media**                | 1.5mL  | 1.5mL                   |
| **Carbon**                    |        |                         |
|                               | _____µL of | _____µL of |
|                               | ________ | ______________ |
|                               | _____µL of | _____µL of |
|                               | ________ | ______________ |
| **Nitrogen**                  |        |                         |
|                               | _____µL of | _____µL of |
|                               | ________ | ______________ |
| **Sulfur**                    |        |                         |
|                               | _____µL of | _____µL of |
|                               | ________ | ______________ |
| **Culture Added?**            |        |                         |
| **Placed in well?**           |        |                         |

-When you have all of your media combinations ready and the chart below filled, bring your tube into the biosafety cabinet and pipette all of each tube into its corresponding assigned well.

Potential errors:
Exercise #3: Minimal Media Plate #1 Transfer into Plate #2

In the biosafety cabinet, set up the minimal media experiment plate #2. Follow these steps:
- Tuck your lab coat into your gloves. There should be no skin showing ever.
- Use the squirt bottle and paper towels to wipe down your workspace with 10% bleach first, then 70% ethanol
- Squirt ethanol onto your gloves and rub your hands together until dry.
- Using a new tip each time, use the following chart to guide you and pipette the following into a labeled microcentrifuge tube:

|                  | Tube 1 | Tube 2 (if applicable) |
|------------------|--------|------------------------|
| Base Media       | 1.5mL  | 1.5mL                  |
| Carbon           | _____µL of | _____µL of |
| Nitrogen         | _____µL of | _____µL of |
| Sulfur           | _____µL of | _____µL of |
| Placed in well?  |         |                        |
| Culture transferred? |       |                        |

- Then, **transfer** _____________ µL of the well from plate #1 into the corresponding well of plate #2.

Potential error(s):
Exercise #4: Minimal Media Plate #2 Transfer into Plate #3

In the biosafety cabinet, set up the minimal media experiment plate #3. Follow these steps:
- Tuck your lab coat into your gloves. There should be no skin showing ever.
- Use the squirt bottle and paper towels to wipe down your workspace with 10% bleach first, then 70% ethanol.
- Squirt ethanol onto your gloves and rub your hands together until dry.
- Using a new tip each time, use the following chart to guide you and pipette the following into a labeled microcentrifuge tube:

|                | Tube 1 | Tube 2 (if applicable) |
|----------------|--------|------------------------|
| Base Media     | 1.5mL  | 1.5mL                  |
| Carbon         | ______µL of | ______µL of           |
| Nitrogen       | ______µL of | ______µL of           |
| Sulfur         | ______µL of | ______µL of           |
| Placed in well?|        |                        |
| Culture transferred? |    |                        |

- Then, transfer ______ of the well from plate #2 into the corresponding well of plate #3.

Potential error(s):

For each hypothetical media result below, record an interpretation of the data:

| Plate 1 | Plate 2 | Plate 3 | Interpretation |
|---------|---------|---------|----------------|
| 1       | +       | +       |                |
| 2       | -       | -       |                |
| 3       | +       | -       |                |
| 4       | +       | -       | +              |
| 5       | -       | -       | +              |
Chapter 3: Temperature

**Learning Objectives:**
- Compare and contrast the effect of high or low temperatures on a cell.
- Define classifications of microbes based on temperature.
- Use external resources to quickly connect our lab to the external pool of data online and design an experiment.
- Use sterile technique to inoculate the salinity experiment for the semester.

**Prelab reading**

The **cardinal temperatures** refer to the minimum, maximum, and optimum temperature at which an organism can grow. Plots of this data are generally displayed with growth rate on the y axis and temperature on the x axis. At minimum temperature, the growth rate of the culture slows due to **membrane gelling** in which the cellular membrane is not as fluid as it should be and is somewhat “frozen”. At the maximum temperature, proteins **denature** and the membrane deteriorates causing cell **lysis**. At the optimum, the cell’s enzymes are functioning at peak ability and the membrane is **fluid and semi-permeable**.

We know that bacteria are found in oceans, sediments, and our bodies, but they are also documented in the Antarctic ice, hot springs, and in fracking shales. To be able to survive in extreme environments, bacterial cells often have specialized enzymes that function optimally at really cold or really warm environments. Organisms that live in the extremely hot or extremely cold environments can be classified as **extremophiles**. Bacteria can be classified by the following temperature categories: psychrophiles, mesophiles, thermophiles, hyperthermophiles as seen below.

![Temperature classifications of bacteria](https://openstax.org/books/microbiology/pages/9-4-temperature-and-microbial-growth)

*Figure 3-1. Temperature classifications of bacteria. Image source: [https://openstax.org/books/microbiology/pages/9-4-temperature-and-microbial-growth](https://openstax.org/books/microbiology/pages/9-4-temperature-and-microbial-growth)*
Exercise 1: Experimental Design
We will inoculate the temperature experiment for our isolate. With your class, decide on an experimental design. Remember that this organism was isolated from the coast of Louisiana. Spend 10-15 minutes researching temperature conditions in this ecosystem if needed. Include the following as part of your experimental design and discuss with your instructor as a class.

Media needed:

Temperatures tested:

Number of replicates per condition:

Controls:

Cell count frequency:

Predict patterns of growth with these temperatures and generate a hypothesis.

Exercise 2: Temperature inoculation and t0:
Materials needed:
-Biosafety cabinet
-Lab coat
-Safety goggles
-10% bleach
-70% ethanol
-Paper towels
-Gloves
-Pipettes and tips
-Mili-Q or other ultrafiltered water. DI acceptable.
-Healthy bacterial culture
-1-2 flasks/student
-Isolation media/benchtop

Before starting, review what you have learned about sterile technique when handling bacterial cultures in previous chapters. To determine the volume of culture needed, use the cell count provided by your instructor and the dilution equation to get a starting concentration of $10^4$ cells/mL. Show your work below.

$$C_1V_1=C_2V_2$$
Volume of culture to inoculate each flask: __

Instructions:

- Use sterile technique to put 50mL of the appropriate media into the flask.
- Pipette the volume of culture needed from above into your flask.
- Prepare the cells to be counted by portioning 200µL into a labeled microcentrifuge tube. Your instructor will fix them in 3% glutaraldehyde to perform cell counts later. Your instructor will review how glutaraldehyde preserves cells in the cell count laboratory.

**Exercise 2: Temperature diversity**
Spend 20 minutes to create the following presentation about your assigned temperature category.

Category:

Bacteria that fits into this category:

Temperature range:

Culture dependent or independent work?

Has this organism ever been isolated? If so, from where?

Include image of environment that fits this criteria.

Sources:
Chapter 4: Microbial Growth

Learning Objectives:
- Label the parts of a bacterial growth curve
- Compare and contrast multiple methods of counting cells in a bacterial culture
- Discuss why oligotrophy vs. copiotrophy could affect the mode of cell counting that is used

Pre lab reading:
When bacterial cells grow, they divide by binary fission and this results in a single cell becoming two clonal cells. Depending on the generation time, cells can be classified as oligotrophic or copiotrophic. Oligotrophic organisms are generally slow growing and only need small amount of nutrients to grow. Copiotrophic organisms, however, are fast growers and have historically been the most easily studied type of organism.

![Figure 4-1](https://openstax.org/books/microbiology/pages/9-1-how-microbes-grow)

The number of bacterial cells over time is plotted as a growth curve. There are several regions of a typical bacterial culture’s growth curve. Lag phase occurs when a transfer into new medium happens and the population of cells does not grow. This is potentially due to the cells needing an “adjustment period” to up or down regulate their transcription of genes in response to the new environment. Exponential growth is the phase of growth in which cell counts are growing the fastest. This stage is when cultures are the healthiest and rapidly using up the provided nutrients. Stationary phase is when a limiting essential nutrient is completely used or when too many metabolic byproducts accumulate in the culture. Cells can no longer continue with exponential growth and the growth will slow to a halt. At this stage, cell growth and death are balanced. Death occurs after stationary phase when the number of dividing cells is lower than the number of dead cells.
When a culture exists in medium with multiple substrates available to use, sometimes growth curves will show **diauxic growth** patterns. When the organism uses one substrate fully, there is an additional lag period in which the cells shift their transcription to be able to swap to another substrate. When grown in the presence of two substrates, *E. coli* uses the preferred substrate (in this case glucose) until it is depleted. Then, enzymes needed for the metabolism of the second substrate are expressed and growth resumes, although at a slower rate.

Bacteria are too small to count with our eyes, so there are many different ways that we can count cells and track their growth. Some of the common methods are:

1. **Direct counts with light microscope**: Fluorescence microscopy is one way that cells can be counted. Some cell types can be seen with only a microscope, but oftentimes cells need to be stained to increase contrast and more easily see the cells under a microscope. Some stain types that are frequently used are DAPI and SYBR Green.

2. **Serial dilutions** allow a mathematical way to determine cell count when organisms can be cultured on agar plates. The dilution continues until there is a countable number of colonies on a plate. This is an estimate that can be done with the naked eye.
3. **Optical density:** Spectrophotometers are used to indirectly count cell cultures by measuring the amount of light that passes through the culture. Higher cell counts result in a higher turbidity of the culture and allow for less light to pass through a cuvette.

![Figure 4-4 (left): Serial dilution involves diluting a fixed volume of cells mixed with dilution solution using the previous dilution as an inoculum. The result is dilution of the original culture by an exponentially growing factor. Image and caption source: https://openstax.org/books/microbiology/pages/9-1-how-microbes-grow](https://openstax.org/books/microbiology/pages/9-1-how-microbes-grow)

![Figure 4-5: (a) A spectrophotometer. (b) A spectrophotometer works by splitting white light from a source into a spectrum. The spectrophotometer allows choice of the wavelength of light to use for the measurement. The optical density (turbidity) of the sample will depend on the wavelength, so once one wavelength is chosen, it must be used consistently. The filtered light passes through the sample (or a control with only medium) and the light intensity is measured by a detector. The light passing into a suspension of bacteria is scattered by the cells in such a way that some fraction of it never reaches the detector. This scattering happens to a far lesser degree in the control tube with only the medium. Image and caption source: https://openstax.org/books/microbiology/pages/9-1-how-microbes-grow](https://openstax.org/books/microbiology/pages/9-1-how-microbes-grow)

4. **Flow cytometry:** This is an automated counting method and is the most sensitive method of counting cells. Cells must be stained with a dye that incorporates into the DNA of the organism. Cells are sucked through a capillary in single file and hit with a laser that causes the DNA stain to fluoresce. The fluorescence and scattering of light around the cell is detected and used to quantify cell counts. The flow cytometry method allows for multiple stain types in a single sample.
Exercise 1: Plots

- Work with your instructor and classmates to plot a growth curve in R Studio.

Figure 4-6: In flow cytometry, a mixture of fluorescently labeled and unlabeled cells passes through a narrow capillary. A laser excites the fluorogen, and the fluorescence intensity of each cell is measured by a detector. Image and caption source: https://openstax.org/books/microbiology/pages/20-5-fluorescent-antibody-techniques
Chapter 5: Growth Rates

Pre-lab reading:
When comparing bacterial growth across species or experimental conditions, we express the data as growth rates. It is important to note that growth rates are never about a single cell, rather it is the rate at which an entire population grows.

Copiotrophs generally have fast growth, and many copiotrophic organisms are able to grow faster when nutrients are high and lower their rates when nutrients are low. Oligotrophic organisms, however, cannot do this and will not usually flux their growth rate to accommodate a higher nutrient content. Oftentimes when culturing from environmental samples, the copiotrophic organisms with faster growth rates will quickly outcompete the oligotrophic organisms that grow more slowly. In the majority of ecosystems on Earth, however, the abundant and environmentally relevant organisms are predicted to have slow growth rates since many of the natural environments on Earth are oligotrophic. This is one of the fundamental reasons for the existence of the GPCA and why many lab-strains and model organisms are fast-growing copiotrophs. To see a table comparing oligotrophs and copiotrophs, see the following review article: Kirchman, David L. 2016. “Growth Rates of Microbes in the Oceans.” Annual Review of Marine Science 8: 285–309.

Calculating Growth Rates of a culture

To find the growth rate of an organism, use the following:

Number of generations:
\[
n = \frac{\log(N_t) - \log(N_0)}{\log(2)}
\]

Generation time:
\[
g = \frac{n}{t}
\]

Growth rate (k):
\[
k = \frac{1}{g}
\]

To find the growth rate of an organism, use the following:

Number of generations:
\[
n = \frac{\log(N_t) - \log(N_0)}{\log(2)}
\]

Generation time:
\[
g = \frac{n}{t}
\]

Growth rate (k):
\[
k = \frac{1}{g}
\]
If: \( N_0 = 1,000 \text{ cells} \cdot \text{mL}^{-1} \) \( N_t = 5,000 \text{ cells} \cdot \text{mL}^{-1} \) \( t = 120 \text{ hours} \)

Then:

\[
\begin{align*}
n &= \frac{\log(5,000) - \log(1,000)}{0.301} = 2.322 \text{ generations} \\
g &= \frac{2.322}{120} = 0.01935 \text{ generations per hour} \\
&= 0.01935 \text{ generations per hour} \times 24 \text{ hours/day} = 0.4644 \text{ generations/day} \\
k &= \frac{1}{0.4644} = 2.153 \text{ days per generation}
\end{align*}
\]

Interpretation: This organism doubles every 2.153 days under the given conditions.

**Exercise 1: Calculate the growth rates of the temperature experiment in excel**

Use the growth plots that you made last week to calculate the growth rate of each replicate in each condition of temperature growth. Optional- this exercise could be checked/done differently using sparse-growth-curve

(https://github.com/thrash-lab/sparse-growth-curve)

---

**Chapter 6: Salinity**

**Learning Objectives:**

- Define the ways that we classify bacteria based on salinity preferences
- Understand osmosis movements and what happens to a cell when placed in solutions outside of its optimum.
- Compare and contrast how limnic vs coastal vs ocean organisms might need to control their osmolarity.
- Connect lab information to the broader pool of knowledge to design an experiment to test salinity preferences in our organisms.

**Pre-lab reading:**

Some of the ways that we classify bacteria cell’s relationship to salinity is nonhalotolerant, halotolerant, halophile, extreme halophile. Water’s passive movement into and out of a cell is called osmosis and is affected by the tonicity of the environment that the cell exists in. Water will flow from the low solute concentration to the high solute.
concentration in an attempt to balance out the net concentration of solutes in and out of the cell. When water leaves the cell and moves into a hypertonic solution, the cell will crenate. When water goes into the cell and moves from a hypotonic solution, the cell will swell and lyse. If the solution is isotonic, the water moves in and out of a cell at equal rates so that the cell remains healthy and intact.

Figure 3-1: Net movement of water. Image source: https://openstax.org/books/concepts-biology/pages/3-5-passive-transport.

One of the ways that bacterial cells respond to osmotic stress is through incorporation of compatible solutes. Compatible solutes are molecules in which the cell takes in to balance the ionic strength inside and outside of the cell. Generally, these molecules are not used for biomass production or energy generation since they are not always metabolized by the cell.

Marine bacteria don’t really have to worry about rapid salinity fluctuations since the salinity of the oceans stays relatively constant. Bacterial cells that exist in coastal systems, however, must be able to respond to sudden changes in ionic charge on the outside of the cells. Estuarine systems are areas in the land/water interface and are the point that freshwater from rivers and lakes mix with the salt water from the oceans. For example, search usgs.gov for estuary salinity data at a fixed location such as Barataria Bay in Louisiana to understand the types of salinity changes organisms that live in estuaries face on short timescales. Organisms that live in places such as these must be able to adjust their cellular machinery to account for the rapid ionic flux. Today we will set up an experiment that will test the range of salinities that our cultures can grow in.

**Exercise 1: Experimental design of a salinity experiment**

Today we will inoculate our salinity experiment for our isolate. With your group, decide on an experimental design. Remember that this organism was isolated from the coast of Louisiana. Spend 10-15 minutes to research salinity fluctuations in the isolation environment and design your experiment. As a class, discuss your design with your instructor.

Include the following:

- Media needed:
- Salinities tested:
- Number of replicates per condition:
- Controls:
- Count frequency:

Predict patterns of growth with these temperatures and generate a hypothesis.
Exercise 2: Salinity experiment inoculation and t0

**Materials needed:**
- Biosafety cabinet
- Lab coat
- 10% bleach
- 70% ethanol
- Paper towels
- Gloves
- Pipettes and tips
- Mili-Q or other ultrafiltered water. DI acceptable.
- Healthy bacterial culture
- 1-2 flasks/student
- Media that has a range of salinities/benchtop

Before starting, review what you have learned about sterile technique when handling bacterial cultures in previous chapters.

To determine the volume of culture needed, use the cell count provided by your instructor and the dilution equation to get a starting concentration of \(10^4 \text{ cells/mL}\). Show your work below.

\[
C_1V_1 = C_2V_2
\]

Volume of culture to inoculate each flask:

Use sterile technique to put 50mL of the appropriate media into the flask.

Pipette the volume of culture needed from above into your flask.

Prepare the cells to be counted by fixing them in 3% glutaraldehyde. Your instructor will review how glutaraldehyde preserves cells in the cell count laboratory.
Appendix 4. Informal Essay 1

In paragraph format, write an informal essay that highlights your initial impression about what it means to take part in a Course-based Undergraduate Research Experience (CURE) lab. There is no strict word count on this assignment, but your goal should be about half a page.

Here are some topics to cover:

- Background about yourself such as your major, goals you hope to achieve with your degree, interest (or lack of) in research, previous exposure (or lack of) to research, and conceptions about who does research
- What you've heard about CURE labs (if anything), your worries about the course, excitement towards the course, and what you hope to get out of this course
- Do you think that research experience applies to you and your goals? Why or why not?
Appendix 5: Social Media Assignment

Purpose: The Social Media Assignment is designed to get you engaged with the scientific community on Twitter — which many biologists have chosen as their preferred form of rapid scientific communication. By following scientists on Twitter, you will be exposed to up-to-date research articles, current interests of the scientific community, and ongoing scientific events such as conferences. Many biologists tweet articles they’ve recently read or talks they’re currently attending and find interesting. Finally, you will need to perform some outreach of your own.

Week 1: Sign up for a Twitter account at https://twitter.com and follow your TA. Each TAs Twitter handle can be found in the syllabus. Complete the assignment on Moodle for Week 5 by telling your TA your Twitter handle so they know who to actually accept and/or follow back.

- Feel free to make your Twitter account private, but you will have to follow a few people throughout the semester.
- Your TA will not necessarily follow you back, you following them is a confirmation step. Class-relevant communication will take place over Moodle, Email, or potentially Slack, but not Twitter.
- One exception: if your Twitter account is marked as "protected", your TA will have to follow you to add you to their list. You must add them back to receive credit.

Week 2: Follow a scientist at your university and one from a different university on Twitter. Retweet a science-related tweet from each that you like, with a comment about why you liked the tweet (you cannot simply retweet, it must be a "Retweet with comment"). Submit the following for each scientist:

1. The name of each scientist you are following and the university or institution where they work.
2. A screenshot of your "Following" list showing that you are, in fact, following said scientists.
3. A link to, or screenshot of, your retweet (link preferred, links may not work if your account is protected).

Week 3: Follow a scientific organization on Twitter. Visit their website and tweet a sentence or two about the organization and include their web page. Submit the following:

1. The name of the organization you’re following.
2. A screenshot of your "Following" list showing that you are, in fact, following said organization.
3. The link to the web page of the organization
4. A link to, or screenshot of, your retweet (link preferred).

Week 4: Follow a scientific government department, agency, etc. on Twitter. Retweet a science-related tweet from them and comment on the tweet. Suggestions include NASA, the USGS, DoE, etc. Submit the following:

1. The name of the agency you’re following.
2. A screenshot of your "Following" list showing that you are, in fact, following said agency.
3. A link to, or screenshot of, your retweet (link preferred).

Week 5: Tweet about something you learned in any of your science classes this week that you found interesting or surprising. Be sure to include the course name in your tweet. Submit the following:

1. The name of the course.
2. A link to, or screenshot of, your tweet (link preferred).

Week 6: Tweet a sentence or two about a scientific news story (not a research article). Please avoid clickbait stories and stick to articles with primary sources as references. Submit the following:

1. The title of the story.
2. A link to the story.
3. A link to, or screenshot of, your tweet (link preferred).
Week 7: Tweet a sentence or two about a scientific video you watched and include the link to the video. It can be from any source such as YouTube, a news site, a university website, etc. As with last week, avoid clickbait and stick to videos about real science. This video needs to have taught you something new or easily described a concept you’ve previously found difficult. Submit the following:

1. The title of the video.
2. A link to the video.
3. A link to, or screenshot of, your tweet (link preferred).

Week 8: Tweet a sentence or two about your lab work thus far. Focus on skills you’ve learned or anything interesting that is happened. Submit the following:

1. A link to, or screenshot of, your tweet (link preferred).

Week 9: Choose a scientific paper tweeted by any scientist you’re following and read the abstract (you do not have to read the whole article). Retweet the article and write one sentence about the abstract (you don’t need much detail). This assignment can be turned in whenever you read the article. Submit the following:

1. The title of the scientific paper.
2. A link to the scientific paper.
3. A link to, or screenshot of, your retweet (link preferred).

Week 10: Tweet a sentence or two describing any scientific event—seminar, workshop, etc.—you have attended this semester and not previously tweeted about. Include the name of the event, who hosted it, and a link to their site (if applicable). This assignment can be turned in whenever you attend the event (you do not have to wait until Week 13). Examples include department seminars, etc. Feel free to use any scientific event, this is merely a suggestion. Submit the following:

1. The name of the event.
2. Who hosted the event.
3. The date of the event.
4. The website of the event (if applicable).
5. A link to, or screenshot of, your tweet (link preferred).

Week 11: Tweet a sentence or two about your semester (not necessarily this class). You will be graded equally for a positive or negative tweet as we are not trying to force good publicity for this lab. A tweet on your poster presentation from Week 13 is also acceptable. Submit the following:

1. A link to, or screenshot of, your tweet (link preferred).

At the end of the semester, all of the above should be combined into a single PDF and submitted for grading. All links/screenshot dates will be checked to ensure interaction throughout the semester rather than all posts occurring at once.
Appendix 6. Presentation: Minimal Media Assignment

Each person has been assigned a carbon, nitrogen, and sulfur source that we will test whether our organism can use as the sole C/N/S substrates for growth. This presentation will give you practice obtaining relevant primary literature online to answer the questions below and help you familiarize yourself with your substrates. Your instructor has assigned you to groups, and each person per group will prepare one slide about their carbon source with the following information:

1. The name of the C/N/S compounds
2. The chemical formula of the compounds
3. A picture of the compounds’ structure
4. A fact about where these compounds are naturally found
5. A fact about how these compounds are relevant to bacteria in the environment
6. Cite your source(s) in the footnote of your slide

You will each be given a maximum of two minutes to present your substrates. Be sure to practice this presentation to ensure appropriate timing.
Appendix 7. Elevator Pitch Assignment
Modified from Becky Carmichael, Kyle Sirovy, Scott Kosiba, Mindy Brooks, and Courtnie DiCapo.

Part 1: In class assignment

Scientists communicate their findings through a number of mediums to a wide variety of audiences that range from fellow scientists within the same field of study to the general public. The composition of your audience should always dictate how you discuss your science. If you were to present the findings of your research to a room of non-scientists but your presentation was more appropriate for fellow scientists, the presentation would fall flat. What’s the point of communicating if those receiving that communication don’t understand?

The goal of this assignment is to expose you to one of the many ways scientists communicate their research to specific, well-defined audiences and highlight the broader impacts of their research in a way that is both relevant and interesting to that audience. This exercise will help you identify your audience, cater your communications with that audience, and aid in the development of an elevator pitch for your final posters.

Please read the following questions before listening to the science communication. Feel free to make notes throughout and then provide detailed responses to these questions:

Title of science communication:_____________________________________________________

Presenter’s name, title, and affiliation:_________________________________________________

Type of science communication (podcast, seminar talk, blog post, etc.):____________________

1. Do you feel that the presenter has a strong grasp of the research they conduct? What gave you this impression? What aspects could you model for your own pitch/what do you want to incorporate?

2. Who is the target audience for this communication? How can you tell?

3. Were they effective at communicating their research to this audience? What did you find specifically effective or not? How did the presenter “hook” the listener and sustain attention?

4. List the main takeaways (broader impacts/results) of the research.

5. Was the importance of their research clear? What was it?
6. What did you like best about the presentation of the material?

7. What clarifying or engaging question would you want to ask the presenter? What was left unanswered?

8. After listening to the science communication, what aspects of the presenter’s delivery style will you model for your own presentation?

9. List specific aspects from your CURE research that you will highlight including the big picture take-away, and relatable example.
Part 2: Homework

An elevator pitch is a short talk used to introduce yourself and your research to others and includes the question being addressed, the importance of the work, and major findings. You’ve already evaluated a science communication piece (podcast episode, seminar talk, published paper, blog post, etc.) and analyzed that researcher’s pitch. Now, you will use your previous evaluation to craft an elevator pitch on existing research. Use the instructor suggestions to find a relevant piece of scientific work and create an elevator pitch on that work. Fill out the prompts below:

Title of scientific research: ____________________________________________

Presenter name(s), title(s), and affiliation: ______________________________

Type of science communication (podcast, seminar talk, blog post, etc.): ____________

When composing any sort of science communication, be it a poster or paper to be submitted to a peer-reviewed journal, two components are essential: A well-defined target audience and a clear story.

**Step 1. Identify your target audience**

The reason many communications of science fail to resonate with those listening or reading is because the authors did not adequately identify who they are communicating to. By zeroing in on a specific audience you can cater components of your speech to their level of interest, understanding of complex relationships and methodologies, and formulate broad impacts that will resonate.

1. What are some aspects of your target audience that you can define? Think: education level, location/affiliations(s), occupation(s), age, familiarity with broad topics you’ll discuss. List as many specifics as possible. Example: *College educated, mostly PhD and MS degree seeking.*

   Your target audience:

2. Using your response above, create a single person that fits into this target audience. In a few sentences, write a general description of this person—this is who you’ll be crafting your pitch for!

**Step 2. Find your story**

When communicating science in any form, it is crucial to have a clear idea of the story you want to tell. A clear story makes communicating your results more interesting to your audience and helps to convey your desired message effectively.

3. Write a two-sentence summary of the research question and the answer (main result/conclusion) to that question. This two-sentence summary will form the backbone of your elevator pitch.
4. From the two-sentence summary you’ve created above, you should include a brief summary of the approach used to obtain the main result. This does not need to be overly detailed but should be clear and specific enough that your audience understands the general approach taken. Now, rewrite your two-sentence summary to include a sentence (or two) about the general approach taken to obtain the main result--this should be sandwiched between your research question and the main result/conclusion.

5. Consider why the main result is important from the perspective of your audience. It’s not enough to state a result if nobody understands the point of it all. Rewrite your two-sentence summary on the back of this sheet to include a sentence (or two) discussing why the results are important from an ecological or biological perspective and also why this should be interesting/relevant to your audience.

6. Practice saying your elevator pitch and come to class ready to present it to your classmates in small groups.

**Elevator Pitch Rubric**

3 min or less – 3 pts
Over 3 min – 0-2 pts

**Introduction**
Relevant/concise – 4 pts
Short/choppy or Too long/Rambling – 2 pts

**Objectives of investigation**
States objectives – 3 pts
Unclear objectives – 2 pts

**Methods**
Concise and relevant to results shared – 4 pts
Not relevant to results shared – 3 pts

**Results**
State main point that matches the objective – 3 pts
Main point doesn’t tie back to objective/unclear – 2 pt

**Discussion**
Communicate the importance of the result – 3pts
Weakly communicate importance of result – 2 pts
Appendix 8. Writing Assignment 1: Nutrients

Use what you have learned about bacterial interactions with nutrients for writing assignment #1. Format your writing as follows:

Introduction:
- Present information in a “funnel” that begins with the most general information and ends with the goal of the project
- General statement about the importance of bacteria
- Relate bacteria to nutrients
- Why do we care about bacteria and nutrients together?
- Why are we doing this experiment?
- Hypotheses about the experiment and controls

Methods:
- What did we do? List volumes and concentrations - be specific.
- Avoid including information about labeling or type of pipette used.

Results:
- What should the result table look like for the entire class? Create the table but leave it empty until all results are completed.

Discussion:
- What controls did we place?
- What kind of results could we see with the controls?
- What would each of these mean?
| Section       | Category                                                                 | Points         |
|---------------|---------------------------------------------------------------------------|----------------|
| Style         | Name, Section Number, Section Headings, Double-spaced                      | 4              |
|               | Title                                                                     | 1              |
| Introduction  | General funnel of information                                            | 2              |
|               | Define bacteria and their importance                                      | 2              |
|               | Importance of carbon to life                                              | 3              |
|               | Diversity of bacterial carbon usage and rationale for experiment          | 3              |
|               | Hypothesis                                                                | 3              |
| Methods       | Sterile technique                                                         | 2              |
|               | Volume of media, carbon source, culture                                   | 5              |
|               | Carbon substrate                                                          | 3              |
| Results       | Table of carbon substrates with appropriate caption                        | 6              |
|               | Written explanation of results                                            | 4              |
| Discussion    | Discussion of possible controls and results                               | 3              |
|               | Support or reject hypothesis and why                                      | 4              |
| Citations     | Copy-and-paste information in text                                        | -6 per instance|
|               | Invalid sources                                                           | -1 each instance|
|               | No in-line citations                                                      | -3 each instance|
|               | No citations                                                              | -5             |
| **Total**     |                                                                          |                |
Appendix 9. Writing Assignment 2: Temperature

Use what you have learned about the effect of temperature on bacterial growth for writing assignment #2. Format your writing as follows:

Introduction
- Funnel of information:
  - What is temperature, and why do we care about it in a bacterial sense?
  - Scope/diversity of temperatures that bacteria are found to thrive in
  - Why should we test temperature in our bacteria?
  - Hypothesis. Expected temperature range of the bacteria and why?

Methods
- What did we do?
  - Be sure to include things such as sterile technique, media, media modifications if needed, temperatures, volumes, etc.

Results
- What is the temperature min, max, and optimum for your organism
  - Graph – what we plotted in class.
  - Figure caption

Discussion
- How does the data support or reject your hypothesis?
- Is this temperature range surprising given the isolation locations?
- What kind of data are our controls here?
| Section       | Category                                                                 | Points          |
|--------------|--------------------------------------------------------------------------|-----------------|
| **Style**    |                                                                           |                 |
|              | Name, Section Number, Section Headings, Double-spaced, Title appropriate  | 5 possible      |
| **Introduction** | General funnel of information                                      | 2              |
|              | Define temperature and the relationship between bacteria and temperature | 5              |
|              | Diversity of temperature for bacteria and rationale for experiment       | 5              |
|              | Hypothesis                                                               | 3              |
| **Methods**  |                                                                           | 10 possible     |
|              | Sterile technique                                                        | 1              |
|              | Volume of media and culture                                              | 3              |
|              | Temperature range and replicates                                         | 4              |
|              | Description of how graphs were made                                      | 2              |
| **Results**  |                                                                           | 10 possible     |
|              | Cardinal temperatures                                                    | 2              |
|              | Temperature growth curve and figure caption                               | 6              |
|              | Paragraph explaining results of graph                                     | 2              |
| **Discussion** |                                                                           | 10 possible     |
|              | Controls                                                                 | 3              |
|              | Support or reject hypothesis and why                                      | 4              |
|              | Temperature range in connection to the environment                        | 3              |
| **Citations** | Copy-and-paste information in text or no citations                      | 0 on paper      |
|              | Invalid sources or no in-line citations                                 | -3 each instance|
| **Total**    |                                                                           | 50              |
Appendix 10. Homework 1: Downloading R and RStudio

Download R:
1. Go to the following address: https://cran.cnr.berkeley.edu
2. Select the download that is most appropriate for you (Linux, Mac, or Windows)
3. Follow any download instructions that appear

Download RStudio:
1. Go to the following address: https://www.rstudio.com/products/rstudio/download/
2. Choose the free RStudio Desktop Open Source License option
3. Follow any download instructions that appear

Show your instructor the downloaded programs at the start of class.
Appendix 11. Homework 2: Growth Curves

Now that you are all professionals at plotting temperature data in RStudio, your homework is to do the following:
1. Download the two .csv files containing temperature data from the other sections’ organisms
2. Input the data into RStudio
3. Change the R code to match headings or regroup data if needed
4. Change the title to the strain number and your name
5. Plot the data and save a PDF to submit

Write a short paragraph of what you think about the data that you see. This should include the differences and similarities of each organism’s min, max, and optimum. Your final product will be two growth curve plots and one paragraph interpreting the data.
Appendix 12. Homework 3: Poster critique

Find a scientific poster displayed somewhere on campus or use one provided by your instructor to answer the following questions:

Based on the TITLE ONLY, what do you think the poster will be about?

What was the main research question and was the title an accurate description of it?

Was the main conclusion clearly stated? What was it?

Were the major ideas of each table/figure clearly stated in the table/figure legend? Were the tables/figures themselves clear and easy to understand? Were they all necessary? Please comment.

Was the order of sections in the poster formatted in a logical/easy to read way?

What did you like best about this poster?

What did you think was the worst thing about this poster?

How long did it take you to understand this poster?

Please feel free to add pages in order to fully explain your responses
Appendix 13: Homework 4: Growth Rates

After our in-class review of the new R code for plotting growth rates, plot growth rates for the temperature experiment. Submit a brief interpretation of results and a PDF of the curve with appropriate title, labels, and figure caption. In your results interpretation, include an outside primary source that contains some kind of temperature data from any waters in coastal Louisiana and decide whether our organism might do well in that environment.

Your final product will be one plot of growth rates with a written interpretation and brief discussion of the data. Most of this assignment will be complete before leaving class.
Appendix 14. Final Writing

For this writing assignment, you should stop thinking of carbon substrate usage, temperature, and salinity as unrelated experiments. Rather, remember that this entire course is actually a single project to characterize our organisms. This writing assignment is meant to be a formal, comprehensive scientific report about the organism that you have grown to know and love. This paper should be written so that an audience completely unrelated to our class could understand it. This assignment will be broken into multiple parts:

Title: Should be an original descriptive title that includes key words of the project

Introduction:
- This section MUST include at least three outside references that you find on your own to support your ideas
- Why are bacteria important to study?
- Explain why carbon usage, temperature, and salinity are important factors relating to bacteria and link that information back to our isolation site.
- Introduce our organism. Include where it was isolated, how it was isolated, anything important about the media, why this media is good/bad, where the isolate currently exists, anything that had been published prior to this project, etc.
- Introduce our research questions, hypotheses, etc.

Methods:
- Be comprehensive, nonrepetitive, and concise.
- Use separate subheadings for:
  - Sterile Technique
  - Cell Counts
  - Minimal media
  - Temperature
  - Salinity
  - Growth rate calculations
  - Graphing

Results: At this point, results should be comprehensive!
You should have the following figures with captions:
- Carbon Table
- Temperature Growth Curves
- Temperature Growth Rates
- Salinity Growth Curves
- Salinity Growth Rates
- Data in figures must be also typed out and reported in the text. Reference this in the text as relevant.
  - EX: LSUCC0135 is a curved rod (Figure X).
- Break this category into relevant subsections as needed.
- There should be no interpretation of these results.

Discussion:
- Use this section to tie in our results to what we know from existing literature and things that we have learned in class. This section is for the bigger picture connections from what we see in our data to existing data and the world around us.
- There should be citations here

Future Directions:
- What would you like to see happen next in this series of experiments with our organism?
References:
- Follow APA style and list in order of appearance
- Use numerical in-text citations
Rubric:

| Section   | Category                                                                 | Points       |
|-----------|---------------------------------------------------------------------------|--------------|
| Peer Review | Thoughtful comments and relevant feedback on peer’s paper               | 40 possible  |
| Style     | Name, section number, appropriate title, section headers, appropriate grammar, easy to read and concise | 10 possible  |
|           | Unit symbols where necessary                                             | 7            |
| Introduction | Importance of bacteria                                             | 5            |
|           | Synthesis of bacterial relationship with carbon, temperature, and salinity | 15           |
|           | Introduce organism and any known information about it                   | 7            |
|           | Describe the rationale of this experiment and brief description of it    | 8            |
| Hypothesis |                                                                              | 5            |
| Methods   | Sterile Technique                                                        | 3            |
|           | Counting: Instrument, stain, limit of detection                          | 4            |
|           | Carbon                                                                    | 4            |
|           | Temperature                                                               | 4            |
|           | Salinity                                                                  | 7            |
|           | Computation: R or RStudio                                                | 4            |
|           | Computation: ggplot2                                                      | 4            |
| Results   | Carbon Table and caption                                                  | 5            |
|           | Temperature growth curves and caption                                      | 5            |
|           | Temperature growth rates and caption                                       | 5            |
| Section                  | Description                                                                 | Score |
|--------------------------|-----------------------------------------------------------------------------|-------|
| Salinity growth curves   | and caption                                                                  | 5     |
| Salinity growth rates    | and caption                                                                  | 5     |
| Written results          | in addition to the figures                                                  | 5     |
| Discussion               |                                                                             | 5     |
| Connection to ecology/  | bigger idea                                                                  | 20    |
| Well thought-out         | acceptance/rejection of hypothesis                                          | 10    |
| Future Directions        |                                                                             | 5     |
| Thoughtful idea of a     | follow-up experiment                                                        |       |
| References               |                                                                             | 5     |
| In-text citations and    | properly formatted citations                                                 | 5     |
| Total                    |                                                                             | 200   |
Appendix 15: Poster Assignment

Now that you’ve written your final paper, let’s practice another form of science communication. A poster is a great way to discuss your research and present your data, while personally connecting with your audience. The general construct of a scientific poster is similar to the scientific papers you have been writing; however, the poster is concise and highlights the key points and findings. Essentially, condense your paper into poster format.

Like we discussed in class, your poster should be aesthetically pleasing and not cluttered.

Your poster needs to include:
1) Title
2) Your name and your lab partner’s name, and the department where the research was conducted
3) Introduction
   a. Briefly highlight the relevant background, what makes this research important/what gap in the knowledge we are filling with our work, and your research question/hypothesis.
4) Methods
   a. Briefly discuss how we conducted our research and make sure to explain each experiment distinctly. Feel free to be creative with figures, charts, diagrams, etc.
5) Results
   a. Provide the data produced from each experiment
   b. Make sure that each figure/table has an informative caption
6) Discussion
   a. Briefly discuss the main findings of our investigation and any conclusions we can draw from them
   b. Briefly discuss the relevance of these conclusions to the broader scientific community
   c. Bullet points are encouraged here
7) Acknowledgements
   a. The purpose of this section is to thank anyone who made this research opportunity possible
   b. Be sure to thank the department, the university CURE program and any staff that coordinate/direct the program, the instructor of record, the Principle Investigator, and your Laboratory Teaching Assistant.
8) References
   a. APA format and numerical in-text citations

Make sure your poster is sized for:
   Width: 48 inches
   Height: 36 inches

For the presentation, aim for 3-5 minutes.

Things to keep in mind:
- Your poster should be easy for your reader/audience to read and follow along
- No clutter, no distracting background
- Make sure the font is large enough/figures are readable
- Keep your poster spatially organized
| Section                  | Category                          | Points     |
|--------------------------|-----------------------------------|------------|
| Design and Layout        |                                   |            |
|                          | Organization                      | 10 possible|
|                          | Readable figures and text         | 10 possible|
| Heading                  |                                   |            |
|                          | Names and Affiliation              | 5 possible |
|                          | Clear and accurate title           | 5 possible |
| Content                  |                                   |            |
|                          | Introduction                       | 20 possible|
|                          | Methods                            | 10 possible|
|                          | Results                            | 10 possible|
|                          | Figures, Tables, and Captions      | 20 possible|
|                          | Discussion                         | 20 possible|
|                          | Acknowledgements and References    | 10 possible|
| Presentation             |                                   |            |
|                          | Time                               | 10 possible|
|                          | Professionalism and body language  | 20 possible|
| Total possible           |                                   | 150 possible|
Appendix 16. Informal Essay 2

In paragraph format, write an informal essay that highlights your experience now that you have taken part in a Course-based Undergraduate Research Experience (CURE) lab. There is no strict word count on this assignment, but your goal should be about half a page.

Here are some topics to cover:

- After revisiting your Blog Post #1, address your overall thoughts about your experience in the class. Was your initial impression with the course consistent throughout the semester? Did your views on research and what it means to be a researcher shift throughout the semester? Would you now consider yourself a researcher or at least capable of being a researcher?

- Has your confidence in your lab abilities grown this semester? Do you feel that any skills you have learned are relevant to you and your goals? Would you recommend a CURE lab to a friend?
Appendix 17. Poster Symposium Worksheet

| Criteria                          | Poster #:________ |
|----------------------------------|------------------|
| **1. Content:** short, understandable | 1 2 3 4 5 6 7 8 9 10 |
| **2. Organization/clarity:** easy to follow and understand, flows well | 1 2 3 4 5 6 7 8 9 10 |
| **3. Audience:** detail appropriate; big picture catered to audience | 1 2 3 4 5 6 7 8 9 10 |
| **4. Completeness:** detail and depth is appropriate | 1 2 3 4 5 6 7 8 9 10 |
| **5. Volume:** Projects voice, appropriate for group size | 1 2 3 4 5 6 7 8 9 10 |
| **6. Pace:** Relaxed pace, easily understood given accent/vocal style | 1 2 3 4 5 6 7 8 9 10 |
| **7. Diction:** pronunciation is clear and deliberate | 1 2 3 4 5 6 7 8 9 10 |
| **8. Enthusiasm/energy:** shows interest through tone and energy | 1 2 3 4 5 6 7 8 9 10 |
| **9. Body language/posture:** does not fidget, upright posture | 1 2 3 4 5 6 7 8 9 10 |
| **10. Eye contact:** maintains eye contact, engages audience member | 1 2 3 4 5 6 7 8 9 10 |
| **11. Audience questions:** clear and thoughtful response | 1 2 3 4 5 6 7 8 9 10 |

**OVERALL POSTER SCORE**

1. Based on your overall impression of the presentation, do you feel that they have a strong grasp of the experiment they conducted and its relevance to the field? Were they able to effectively communicate this to their audience? Be specific with your feedback.

2. What did you like best about the presentation of this poster?

3. What could have been improved or refined? Use the scores you provided to guide your response.

4. What clarifying or engaging question did you ask and what was the answer given? Did you feel that they answered it thoroughly and with the audience in mind?
Appendix 18. Dishwashing protocol

The following steps should be used to ensure clean dishware:
- Rinse with copious amounts of water
- Scrub using brushes or sponge with soap and hot water
- Rinse with hot water
- Rinse with cold water
- Rinse with DI water
- Allow to air dry on racks

If the dishware has an open top or will be used for measurement, cover with foil and put away.

Flasks and glassware should be acid washed overnight in 10% HCl. After acid washing:
- Rinse 4x with DI water
- Rinse 4x with nanopure water
- Allow to air dry on racks
- Cap flasks and bottles before autoclaving
Appendix 19. Flow cytometry parameters (Modified from Bakshi et al. 2019)

This protocol assumes that users have received the proper training in flow cytometry and understand how to use their equipment. The following parameters are used with the Guava easyCyte 5HT (Millipore) flow cytometer for enumeration:

**Gain settings**
Forward scatter 1  
Side scatter 2.83  
Green fluorescence 4.56  
Yellow fluorescence 8  
Red fluorescence 8

**Counting**
3,000 events or 90s.

**Controls**
Negative: unstained sterile medium  
Negative: stained sterile medium  
Positive: medium with a common marine bacterial heterotroph (e.g., E.coli).

**Cell fixation**
Cells can be fixed in 2-4% glutaraldehyde and stored at 4°C if the instructor would like to complete cell counts all at once rather than once per day. This would be ideal if the access to the flow cytometer is limited. Glutaraldehyde should be used in a fume hood and fixed cells should be stored in a secondary container that is labeled according to the institution’s EHS standards.

Additional details and gating examples can be found in:

Thrash, J. Cameron, Jessica Lee Weckhorst, and David M. Pitre. (2015) *Cultivating Fastidious Microbes*. In Hydrocarbon and Lipid Microbiology Protocols, vol. 4 (*Cultivation*). Edited by Terry J. McGenity, Kenneth N. Timmis and Balbina Nogales.
Appendix 20. Example Setup of Carbon/Minimal Media Plate

|         | Sulfate | Thiosulfate | L-Cysteine | DMSO | Sulfate | Thiosulfate | L-Cysteine | DMSO | Methionine | Methionine |
|---------|---------|-------------|------------|------|---------|-------------|------------|------|------------|------------|
| Citrate | A1      | A2          | A3         | A4   | A5      | A6          | A7         | A8   | A9         | A10        |
| Bicarbonate | B1   | B2          | B3         | B4   | B5      | B6          | B7         | B8   | B9         | B10        |
| Succinate | C1   | C2          | C3         | C4   | C5      | C6          | C7         | C8   | C9         | C10        |
| Dextrose | D1     | D2          | D3         | D4   | D5      | D6          | D7         | D8   | D9         | D10        |
| Acetate | E1      | E2          | E3         | E4   | E5      | E6          | E7         | E8   | E9         | E10        |
|         | Urea    | Ammonium    | Urea       | Ammonium | Urea   | Ammonium    | Urea       | Urea | Ammonium   |

Note that this implementation includes different organic and inorganic sulfur and nitrogen sources in addition to the carbon sources on the left. This kind of approach can be used to find a defined minimal medium for the strain in question, and thus, the alternative schedules in Appendix 28 refer to the “minimal medium” experiment instead of a “carbon plate” experiment.
Appendix 21. Code for Growth Curves

Student Version

# File: Growth_Curves.R
# Source: Lanclos et al., 2018
# Authors: V. Celeste Lanclos, Alex Hyer, Jordan Coelho

# Useful reminders:
# 1. Any text after "#" is ignored by R. Use this feature to take notes.
# 2. You need to change any text in ALL CAPS to match your data file.
# 3. RStudio let's you export graphs from the Plots tab. NO SCREENSHOTS!

# This script takes a CSV with the following columns as input:
# 
# Salinity (int) or Temperature (int): the salinity or temperature at which
# the culture was grown
# Day (int) or Hours (int): how long the culture has been growing
# Replicate (int): trial number at a given salinity
# Cell.Count (int): the concentration of cells counted on each day

# Purpose:

# rm(list=ls())

# install.packages("ggplot2")

# library(ggplot2)

# DATA_NAME <- read.csv("NAME_OF_CSV_FILE_HERE.csv", header = T)

# DATA_NAME$Replicate <- factor(DATA_NAME$Replicate, levels = unique(DATA_NAME$Replicate))

# ggplot(DATA_NAME, aes(x = COLUMN_FROM_CSV_FOR_X_AXIS, y =
# COLUMN_FROM_CSV_FOR_Y_AXIS, color = COLUMN_FROM_CSV_FOR_SERIES, fill =
# COLUMN_FROM_CSV_FOR_SERIES)) +
# geom_line() +
# facet_wrap(~COLUMN_FROM_CSV_FOR_INDEPENDENT_VARIABLE) +
# labs(x = "X-AXIS_TITLE", y = "Y-AXIS_TITLE",
# title = "FIGURE_TITLE") +
# scale_y_log10() +
# theme_bw()
**Instructor Version**

```r
# File: Growth_Curves_Instructor.R
# Source: Lanclos et al., 2018
# Authors: V. Celeste Lanclos, Alex Hyer, Jordan Coelho

# This script graphs the growth curve of an organism at different salinities or temperatures.
# The code below is in terms of salinity, but can easily be modified for temperature.

# This is the Instructor Edition of this code and thus has the
# "right answers" as well as useful tips. Please provide students with the
# Student Edition and take time to note what information they need to
# fill in for themselves.

# This script takes a CSV with the following columns as input:
#
# Salinity (int) or Temperature (int): the salinity or temperature at which the culture was grown
# Day (int) or Hours (int): how long the culture has been growing
# Replicate (int): trial number at a given salinity
# Cell.Count (int): the number of cells counted on each day
#
# You can, of course, edit the code and file to match your needs.

# Removes any data from R's memory.
# Note that this removes all environmental variables, please exercise caution.
# In particular, don't execute this line after opening up an R Project file.
# We strongly recommend that you execute this file in it's own R session.
# This line is included to ensure a clean environment and prevents students
# from unwittingly using previously stored data from their global environment
# and getting perplexing results.
rm(list=ls())

# Install the graphing software "ggplot2".
# Comment this out/delete it if you've already installed it.
install.packages("ggplot2")

# Load ggplot2 into R so that we can make our plot later.
# This library will be removed by the `rm(list=ls())` which means students
# can't proceed with plotting until they re-run this. It serves as a useful
# check that they actually went through the whole file.
library(ggplot2)

# Read your CSV file into R and saves the dataframe as "salinity" or "temperature".
# Put the path to your file in between the quotation marks below.
# You can use the tab key to more easily find your file in RStudio.
# We found the GUI window was useful for students new to software.
salinity <- read.csv("salinity_growth_curve_example_data.csv", header = T)

# Tells R to treat your replicates as categories instead of a number series.
# We found that student's frequently skipped this line whenever they were
# troubleshooting or trying to execute the code on their own for homework.
# If this line is skipped, R will treat the replicates as a number series
# which results in three obvious and incorrect features:
# 1. There will only be a single series on their graph.
```
# 2. The line is largely blue with black segments instead of a solid color.
# 3. Each salinity on the x-axis will have a vertical spike.
# If you notice these features on a student's graph, they have skipped this
# critical line.
salinity$Replicate <- factor(salinity$Replicate, levels = unique(salinity$Replicate))

# Render the graph using ggplot2.
# Commenting out lines below and re-making the graph will help students
# learn what each line does.
ggplot(salinity, aes(x = Day, y = Cell.Counts, color = Replicate, fill = Replicate)) + # aes = aesthetic
  geom_line() + # Make this graph a line graph
  facet_wrap(~Salinity) + # Make a new graph for each salinity
  labs(x = "Time (Days)", y = "Cell Concentration (cells ∙ mL\(^{-1}\))", # Change the graph labels
       title = "Test Organism Salinity Curve") + # Change the graph title
  scale_y_log10() + # Scale the y-axis by log10
  theme_bw() # Change the theme to a clean black and white theme

# Students can now export the graph using the Export function in RStudio.
# Don't let students submit screenshots.
Appendix 22. R Code for Rates

Student Version

# File: Growth_Curves.R
# Source: Lanclos et al., 2018
# Authors: V. Celeste Lanclos, Alex Hyer, Jordan Coelho

# Useful reminders:
# 1. Any text after "#" is ignored by R. Use this feature to take notes.
# 2. You need to change any text in ALL CAPS to match your data file.
# 3. RStudio lets you export graphs from the Plots tab. NO SCREENSHOTS!

# This script takes a CSV with the following columns as input:
# 
# Temperature (int) or Salinity (int): the temperature or salinity at which the culture was grown
# Replicate (int): trial number at a given temperature
# Rate (float): the rate at which a replicate grew

# Purpose:

# rm(list=ls())

# install.packages("ggplot2")

# library(ggplot2)

# DATA_NAME <- read.csv("NAME_OF_CSV_FILE_HERE.csv", header=T)

# DATA_NAME$Replicate <- factor(DATA_NAME$Replicate, levels=unique(DATA_NAME$Replicate))

# ggplot(DATA_NAME, aes(x = COLUMN_FROM_CSV_FOR_X_AXIS, y = COLUMN_FROM_CSV_FOR_Y_AXIS)) +
# geom_jitter(size = 5, width = 0.0, height = 0.0, alpha = I(0.5)) +
# geom_smooth(method = "auto", formula = y ~ x, span = 0.85, se = FALSE) +
# labs(x = "X-AXIS_TITLE", y = "Y-AXIS_TITLE",
# title = "FIGURE TITLE") +
# scale_x_continuous(breaks = c(CONDITION1, CONDITION2, CONDITION3, ETC),
# labels = c("CONDITION1", "CONDITION2", "CONDITION3", "ETC")) +
# theme_bw()
Instructor Version

#' File: Growth_Rates_Instructor.R
#' Source: Lanclos et al., 2018
#' Authors: V. Celeste Lanclos, Alex Hyer, Jordan Coelho

#' This script graphs the growth rate of an organism at different salinities or temperatures.
#' Note: The code below is for salinity but can be easily modified for temperature.

#' This is the Instructor Edition of this code and thus has the
#' "right answers" as well as useful tips. Please provide students with the
#' Student Edition and take time to note what information they need to fill
#' fill in for themselves.

#' This script takes a CSV with the following columns as input:
#' #
#' # Salinity (int) or Temperature (int): the salinity or temperature at which the culture was grown
#' # Replicate (int): trial number at a given salinity or temperature
#' # Rate (float): the rate at which a replicate grew
#' #
#' # You can, of course, edit the code and file to match your needs.

#' Removes any data from R's memory.
#' Note that this removes all environmental variables, please exercise caution.
#' In particular, don't execute this line after opening up an R Project file.
#' We strongly recommend that you execute this file in it's own R session.
#' This line is included to ensure a clean environment and prevents students
#' from unwittingly using previously stored data from their global environment
#' and getting perplexing results.
rm(list=ls())

#' Install the graphing software "ggplot2".
#' Comment this out/delete it if you've already installed it.
install.packages("ggplot2")

# Load ggplot2 into R so that we can make our plot later.
# This library will be removed by the `rm(list=ls())` which means students
# can't proceed with plotting until they re-run this. It serves as a useful
# check that they actually went through the whole file.
library(ggplot2)

#' Read your CSV file into R and save it as "salinity" or "temperature".
#' Put the path to your file in between the quotation marks below.
#' You can use the tab key to more easily find your file in RStudio.
#' We found the GUI window was useful for students new to software.
salinity <- read.csv("salinity_growth_rate_example_data.csv", header=T)

#' Tells R to treat your replicates as categories instead of a number series.
#' We found that student's frequently skipped this line whenever they were
#' troubleshooting or trying to execute the code on their own for homework.
#' If this line is skipped, R will treat the replicates as a number series
#' which results in three obvious and incorrect features:
#' 1. There will only be a single series on their graph.
# 2. The line is largely blue with black segments instead of a solid color.
# 3. Each salinity on the x-axis will have a vertical spike.
# If you notice these features on a student's graph, they have skipped this
# critical line.
salinity$Replicate <- factor(salinity$Replicate, levels=unique(salinity$Replicate))

# Render the graph using ggplot2.
# Commenting out lines below and re-making the graph will help students
# learn what each line does.
ggplot(salinity, aes(x = Salinity, y = Rates)) + # aes = aesthetic
  geom_jitter(size = 5, width = 0.0, height = 0.0, alpha = I(0.5)) + # Graph data points
  geom_smooth(method = "auto", formula = y ~ x, span = 0.85, se = FALSE) + # Draw average rate line
  labs(x = "Salinity", y = "Growth Rate (k)", # Change the graph labels
       title = "Test Organism Salinity Growth Rate") + # Change the graph title
  scale_x_continuous(breaks = c(6, 12, 23, 35), # Manually set axis breaks to match salinity
                     labels = c("6", "12", "23", "35")) + # Manually label the breaks
  theme_bw() # Change the theme to a clean black and white theme

# Students can now export the graph using the Export function in RStudio.
# Don't let students submit screenshots.
Appendix 23. Quizzes

Fall 2018

Quiz- Pipettes and Carbon (post-lab)

1). You have three mechanical pipettes available for use: P10, P100, P1000.
   A) Which is/are the pipette(s) that can hold 20 μL?

   B) Which is/are the pipette(s) that can hold 100 μL?

   C) Fill the boxes (right) to read as the volume readout should on the P10 pipette if you were to use it to deliver 9.2 μL volume.

2) List 2 reasons that all organisms need carbon.

3) What are the two general forms of carbon?

4) Explain the difference between autotroph and heterotroph:

5) Why do we care to test Carbon use in our isolates?

6) Are all bacteria bad for you? Explain something beneficial that bacteria can do. Be as specific as possible and use the back of the page if needed.

Pipettes and Carbon KEY

1. A. P10, P100
   B. P100, P1000
   C. 092

2. Carbon is used for structural components of a cell. Carbon is also used for energy within cells.

3. Inorganic and Organic

4. Autotrophs can use inorganic carbon while heterotrophs need organic carbon to live

5. Knowing the types of carbon molecules that an organism can use will help to predict its function in the environmental nutrient cycling and maybe interactions between organisms.

6. No, not all bacteria are bad for you. The bacteria in your gut can help break down compounds that we cannot process whole. This allows for a healthier gut.
Quiz - Temperature (post-lab)

1. The definition of temperature that we are using for this class is: “A measurement of the __________________ of the molecules within a ____________.

2. Rapid movement of the molecules equates to (hot / cold) temperature.

3. List 2/3 effects temperature has on bacteria’s ability to survive:
   
   1. 
   
   2. 

4. T/F: Bacteria can regulate their own internal temperatures.

5. Why are temperature graphs shaped like a bell curve?:

6. Draw a diagram with the 3 Cardinal Temperatures. The y axis should be Growth Rate and the x axis should be Temperature:

7. BONUS: Draw the graph from above with the bell curves and labels of the 4 temperature -phile types we saw in class.
**Temperature KEY**
1. kinetic energy/system
2. hot
3. growth rate/enzyme function/
4. F
5. Organisms will have a min, max, and optimum temperature when plotting growth rates vs. temperature
6. The diagram should look like a bell curve. The y axis has Growth Rates and the x axis has temperatures from cold to hot. The leftmost low in which the rate is non-zero is the minimum, the peak is the optimum, and the rightmost low in which the rate is non-zero is the maximum.
7. See Figure 3-1 in Appendix 11

**Quiz - Salinity (post-lab)**

1. Which is not a category to describe the salinity of water?
   a. Brackish
   b. Fresh
   c. hyperbrackish
   d. Briny
   e. saline

2. Order the classifications from Q1 from most fresh to most salty:

3. Osmosis is the active/passive movement of water through cell membranes in response to solute concentrations outside of the cell.

4. If solute concentration outside of the cell is higher than inside the cell, water:
   a. Moves inside the cell
   b. Moves outside the cell
   c. Moves in and out at an equal rate
   d. No movement

5. If solute concentration inside of the cell is higher than outside the cell, water:
   a. Moves inside the cell
   b. Moves outside the cell
   c. Moves in and out at an equal rate
   d. No movement

6. What is an osmolyte?

7. The following questions are in regard to our artificial seawater media that we use in class:
   a. Which paper was the one to create this media?
   b. What is the difference in medias that we are using for this salinity experiment? Your answer should go beyond they are different salinities and explore what makes them different salinities.
Salinity KEY
1. C
2. Fresh, Brackish, Saline, Briny
3. Passive
4. B
5. A
6. An osmolyte is a charged molecule that the cell will store to balance the ionic charge inside and outside of the cell
7. a. Henson et al. 2016 / b. The media types that we use have the same types of salts at varying concentrations so that the total ionic strength of each type is different. The nutrients are added to the media at equal concentrations though.

Quiz- Bacterial Growth (post-lab)

1. Bacteria reproduce sexually/sexually.

2. Culture growth refers to what?

3. What is the technique we use to measure growth of our cultures?
   a. Colony forming units
   b. Flow cell cytometry
   c. Hemacytometer
   d. Optical density

4. Draw a standard bacterial growth curve, and include the phases of growth:

5. SYBR-green stains what part of the bacteria?
   a. Cell wall
   b. Mitochondria
   c. Proteins
   d. DNA

6. How do you add a comment in R Studio?

7. This is the code that we have been using. Circle the part of the code that allows you to change the axis labels. Put a square around the part of the code that allows you to change the way the data is grouped.

```r
ggplot(Temp, aes(x = Day, y = Cell.Count, color = Replicate, fill = Replicate)) +
```
8. What was the motivation of Henson et al. 2016?

9. What was the goal of Henson et al. 2016?

10. What was the measurement of cultivation success/failure in Henson et al. 2016?

**Bacterial Growth KEY**
1. Asexually
2. When the total count of cells in the culture increases exponentially. Individual cells are living and dying, but the total cell population size is increasing.
3. B
4.
5. D
6. Pound sign (#)
7.
8. The Great Plate Count Anomaly was the major motivation of Henson et al. 2016. The GPCA is the phenomenon in which <1% of cells that we know are in a sample are able to be cultured on an agar plate and grown in a lab.
9. The goal of Henson et al. 2016 was to use high throughput dilution to extinction culturing with artificial seawater medium to isolate bacterioplankton from the Gulf of Mexico.
10. % viability was the metric used to evaluate success of the experiments. This is XXX.

**Quiz 5- Growth Rates (post-lab)**

1. Define what a “growth rate” is.
2. Where do we get data to calculate growth rates?

3. What is generation time?
   a. Time it takes for cells to double in size
   b. Time it takes for a single cell to divide
   c. Growth of cells over a period of time
   d. Time it takes for the population size to double

4. What do we NOT need to calculate generation time?
   a. No
   b. k
   c. Nt
   d. n

5. Fill in the equation
   \[ n = \frac{(\log(\text{current}) - \log(\text{initial}))}{0.301} \]

6. Which is the line that assigns data for your x-axis and y-axis?
   1. `ggplot(nameyourdata, aes(x=XXXX, y=XXXX)) +`
   2. `geom_jitter(size = 5, width = 0.0, height = 0.0, alpha = I(0.5)) +`
   3. `geom_smooth(method = "auto", formula = y ~ x, span = 0.85, se = FALSE) +`
   4. `labs(x="Enter a label", y="Enter a label", title="Enter a label") +`
   5. `scale_x_continuous(breaks=c(12,24,33,40), labels=c("12","24","33","40")) +`
   6. `theme_bw()`

7. Is the code above for growth curves or rates?

8. What is the difference in axes for growth curves vs growth rates?

9. Draw what a growth curve graph looks like on the left and growth rate graph on right.

**Growth Rates KEY**
1. The rate at which a population of cells grow
2. The data comes from cell counts over time
3. D
4. B
5. \[ n = \frac{\log(N_1) - \log(N_0)}{0.301} \]
6. Line 1
7. Growth rates
8. Growth curves have time on the x axis and cell counts on the y axis. Growth rates have a variable on the x axis and rates on the y axis.
9. See Figures 2-3 in the main text
Appendix 24. Example Final Exam Fall 2018

Experimental Theory (150 points)

General:

1. What were 2 of the ways that we used sterile technique to minimize possible contamination in this lab? (2 points each, 4 points total)
   1)  
   2)  

2. Describe a specific way that bacteria uniquely affect their environment. (4 points)

3. List 3 reasons that bacteria can be beneficial to humans. (2 points each, 6 points total)
   1.  
   2.  
   3.  

4. What are the two types of controls that we should have with experiments? (2 points each, 4 points total)

5. Define the predicted outcome of each of the controls from above and give an example of each: (2 points each, 4 points total)
   1.  
   2.  

6. Which of the following nutrient cycling do bacteria NOT help with: (2 points)
   a. carbon  
   b. sulfur  
   c. nitrogen  
   d. phosphorous  
   e. bacteria help with all of the above  
   f. bacteria don’t participate in any of the above

Scientific literature:

7. What is the difference between a motivation and an objective? (2 points each, 4 points total)
   Motivation:
Objective:

8. What are the 6 major sections of a scientific paper? (1 point each, 6 points total)

9. Fill in the section of a scientific paper that the following information would be found: (2 points each blank, 8 points total)

| Excerpt from paper                                                                                                                                                                                                 | Section of paper |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| Within the environment, chromium mainly persists in two forms: Cr(III) and Cr(VI) (Bartlett, 1991). Cr(VI) is highly toxic, soluble, and can be easily transported across cell membranes of both eukaryotic and prokaryotic organisms via sulfate and other active transporters (Ackerley et al., 2004b; Cheng, Holman & Lin, 2012). |                  |
| Though a “core” metabolic and genomic structure was seen among our isolates, our data suggests that Cr(VI) reduction discrepancies within these isolates could be related to strain-level genetic and metabolic variation. Further, chromate resistance may be intertwined with the ability of a bacterium to reduce and transport chromate as well as the type of stress response the organism might have. |                  |
| Each genome did contain genes with sequence homology to the chromate reductases, chrR and yieF, of non-model organisms. The putative chrR-like genes found in the isolates are homologous to a chrR gene (GenBank accession number AM902709) found in T. scotoductus. (Opperman, Piater & Van Heerden, 2008). |                  |
| Whole genome shotgun sequencing was performed by multiplexing the genomic DNA onto one lane using the Illumina HiSeq 2000 platform with 100 bp paired end reads using V2 chemistry at Cincinnati Children’s Hospital Medical Center’s Genetic Variation and Gene Discovery Core Facility. |                  |

Carbon:

10. Autotrophs are associated with organic / inorganic carbon. (2 points)

11. Heterotrophs are associated with organic / inorganic carbon. (2 points)

12. Rocks, respiration, and sediment are sources of organic / inorganic carbon in aquatic ecosystems. (2 points)

13. In 3 sentences or less, why did we perform transfers when characterizing the carbon usage of your organism? (3 points)
Use the following result table to answer questions 14-17:

| Well  | Carbon Source         | P1 | P2 | P3 |
|-------|-----------------------|----|----|----|
| A1    | Sodium acetate        | +  | -  | -  |
| A2    | Sodium succinate      | +  | -  | -  |
| A3    | Sucrose               | +  | -  | -  |
| A4    | Urea                  | +  | +  | +  |
| A5    | Glycine               | +  | +  | +  |
| A6    | Bacteria + media      | +  | +  | +  |
| A7    | Bacteria + carbonless media | + | + | + |
| A8    | Carbonless media only | +  | +  | +  |

14. Which wells contain the carbon sources that this organism can use? (3 points)

15. Which wells contain the controls? Label them as positive or negative controls. (6 points)

16. Can we trust this data? (2 points)

17. Why or why not? (3 points)

18. Fill in the blank to go through the protocol for the carbon experiment: (3 points each blank, 15 points total)

We started the first carbon plate with 1.5mL of__________________ media already in the wells of Plate #1. We then added our ________________ to give our organism food to grow. Then we inoculated Plate #1 with our bacteria- ________________. We waited for 2 weeks, then Plate #1 was counted using in the Lab. The source of the bacteria for Plate #2 was __________________ since this portion of the experiment was a transfer rather than an inoculation.
19. What are the fates of carbon for a respiring bacterium? (3 points)
   a. CO2
   b. Waste
   c. Biomass
   d. All of the above
   e. None of the above

20. T/F: All bacteria found in the same environment use the same types of carbon substrates. (1 point)

21. T/F: Bacteria can regulate their temperature. (1 point)

22. Rank from coldest to hottest: psychrophile, thermophile, hyperthermophile, hyper-psychrophile, mesophile (4 points)

23. Since temperature directly affects bacterial growth rate, if a bacterium is growing in a system that is outside its optimum temperature range it will be more / less active. (2 point)

Use the following graph from XX to answer questions 24-27:
24. Which organism has the lowest range of possible temperatures? (3 points)
   a. L. sansranciscensis I
   b. L. sansranciscensis II
   c. C. milleri

25. If all of these organisms were in a flask together, which do you think would dominate the culture? Hint: Which would outgrow the others? (3 points)
   a. L. sansranciscensis I
   b. L. sansranciscensis II
   c. C. milleri

26. What temperature range would you expect to see all of these organisms in the environment co-existing? (3 points)

27. Explain your answer for number 26 above. What about the graph tells us that this is the correct range? (4 points)
Salinity:

28. T/F: In the salinity experiment, we used media with different types of salts to figure out which salts the organism liked best. (2 points)

29. T/F: If we adjust an organism’s media from a salinity value of 5 to a salinity value of 10, it will definitely be okay because both of these values are in the brackish range. (2 points)

30. Osmosis is active/passive. (1 point)

31. Osmolyte transport is active/passive. (1 point)

32. T/F: Defining salinity tolerances helps in understanding bacterial distribution. (1 point)

33. If a cell is placed from a fresh habitat into a salty habitat, describe what happens to the water inside of the cell. Draw an image if you’d like. (5 points)

34. Why are coastal areas commonly brackish? (5 points)

35. The saltiest type of water is: (2 points)
   a) brackish
   b) briny
   c) fresh
   d) saline
   e) super saline

Microbial Growth and Growth Rates:

36. Fill in the blanks or circle the appropriate answer pertaining to microbial growth and growth rates. (3 points each, 15 points total)

   Bacteria reproduce through binary _____________________. This process is when one cell becomes two through (sexual / asexual reproduction). Because of this type of reproduction, cultures of bacteria experience ____________________ growth, which is indicated in the
growth curves as the part of the curve with the steepest slope. If we can find the number of
cells at the inflection points of a growth curve, we can calculate the ________________
of the culture. This value allows researchers to easily compare microbial growth. Once rates are plotted,
they form a (sigmoidal / bell curve) shape in which we can see the minimum,
maximum, and optimum condition of a bacterial culture.

37. T/F: The way an organism will grow in a lab is the exact way that it will grow in the environment (2 points)

38. T/F: Generation time is the time it takes for one cell to become 2. (1 point)

39. The equation: \( n = \frac{\log(N_t) - \log(N_0)}{0.301} \) is used to calculate what? (3 points)
a. The time at which lag phase ends  
b. The time between \( N_t \) and \( N_0 \)  
c. The cell count at the beginning of stationary  
d. Generations between \( N_t \) and \( N_0 \)  
e. Generations per time  
f. Time per generation  
g. Growth rate

40. The equation: \( g = \frac{n}{t} \) is used to calculate what? (3 points)
a. The time at which lag phase ends  
b. The time between \( N_t \) and \( N_0 \)  
c. The cell count at the beginning of stationary  
d. Generations between \( N_t \) and \( N_0 \)  
e. Generation time  
f. Growth rate

41. The equation: \( k = \frac{1}{g} \) is used to calculate what? (3 points)
a. The time at which lag phase ends  
b. The time between \( N_t \) and \( N_0 \)  
c. The cell count at the beginning of stationary  
d. Generations between \( N_t \) and \( N_0 \)  
e. Generations per time  
f. Time per generation  
g. Growth rate

Bonus: Explain in detail something that you learned this semester that was not on the exam. You can earn up to 5pts.
Final Exam Answer Key

1. Gloves/ Ethanol and Bleach our workstation/ Ethanol our gloves/ Use a biosafety cabinet
2. Answers can vary depending on in class discussion. Most answers with any nutrient cycling are acceptable and some examples we used in lecture are:
   - When bacteria degrade nutrients that flow into the Gulf of Mexico from the Mississippi River, they can use up all the oxygen in the bottom waters and cause the seasonal hypoxic zone that we see in the summer.
   - Bacteria often are the ones that can break down large complex carbon molecules into forms that are more usable for other organisms to use. An example of this is aromatic hydrocarbons. The ring structures are difficult to break, but some bacteria can sever the bonds and release the ring into chains that other organisms can use.
   - Plant roots often have symbiotic bacteria that help with nitrogen fixation and turns nitrogen into a form that is usable for the plants.
3. Nutrient cycling, food microbiology (lactobacillus in yogurt), gut microbiome, etc
4. Positive and negative controls
5. Positive control results should respond to the tested stimuli in alignment with known results. EX: The carbon experiment’s positive control was our isolate inoculated into its regular growth medium since we know that the organism can grow in this medium.
   A negative control should be designed so that there will be no response to the tested stimuli. EX: One of the carbon experiment’s negative controls was the medium without our organism inoculated into it. If there is growth in that well, it is likely that the experiment was contaminated and we should not trust the results.
6. e
7. Motivation is the reasoning for the study often presented as the problem or gap in knowledge. Objective is the specific aims of what a study hopes to accomplish.
8. Abstract, Introduction, Methods, Results, Discussion, References
9. Introduction, Discussion, Results, Methods
10. Inorganic
11. Organic
12. Inorganic
13. We transferred the carbon plates multiple times to eliminate the carryover carbon that would exist from the inoculum in the first plate. If the cells can grow after 3 transfers, we know mathematically that there wouldn’t be enough carryover carbon to sustain life.
14. A4,A5,A6,A7,A8
15. A6 is the positive control and A8 is the negative control
16. The data seems untrustworthy at first since the negative control is positive.
17. Carbonless media only should not be positive since there is no bacteria inoculated into it. It is likely that this experiment got contaminated with an autotroph.
18. carbonless media, carbon sources, LSUCC0135, Plate#1
19. d
20. F
21. F
22. hyper-psychrophile, psychrophile, mesophile, thermophile, hyperthermophile
23. less
24. c
25. b
26. around 10˚C to right under 35˚C
27. The organisms based on temperature alone in theory could overlap in the temperatures at which the growth rate is not 0
28. F
29. F
30. passive
31. active
32. T
33. The water inside of the cell would rush outside of the cell to try to balance the ionic potential of each
34. Rivers often input large amounts of fresh water when they drain into salt water near coasts.
35. b
36. fission, exponential/logarithmic, growth rate, bell curve
37. F
38. F
39. D
40. E
41. G

Bonus: Can be anything that is correct and was covered in the course.
## Appendix 25: Instructor timeline

| Week | Topic | Quiz | In-Class Activity | Assign to students | Instructor Prep for following week |
|------|-------|------|-------------------|--------------------|-------------------------------------|
| 1-2 months before class | | | | | Order all materials (A2) |
| 1 week before class | | | | | Create and distribute syllabus, instructor should get familiar with cell counts (A3,19) |
| 1 | Introduction | Syllabus | Pipette practice | Informal Essay 1 (A5), Social Media Assignment (A5) | Select nutrient sources |
| 2 | Nutrient Stocks | | Create nutrient stocks used for minimal media experiment | Presentation 1 (A6) | Make ASM-C (A1) |
| 3 | Minimal media plate #1 | Pipettes and Nutrients | Set up and inoculate minimal media plate #1, science communication example | Elevator pitch (A7), Writing 1 (A8) | Make temperature media (A1) and set incubators |
| 4 | Temperature | Temperature inoculation | | Writing 2 (A9) | Daily cell counts on temperature exp (A3, 19), Make ASM-C (A1), Count minimal media plate #1 (A3, 19) |
| 5 | Minimal media plate #2 | Temperature | Transfer minimal media plate #1 to plate #2, elevator pitch | Homework 1 (A10) | Distribute temperature cell count data to students, Upload growth curve graphing instructions and code (A21) |
| 6 | Growth curves | | Plot temperature growth curves | Homework 2 (A11) | Make ASM-C (A1), Count minimal media plate #2 (A3, 19) |
| 7 | Minimal media plate #3 | Bacterial Growth | Transfer minimal media plate #2 to plate #3, Poster evaluation | Homework 3 (A12) | Upload growth rate graphing instructions and code (A22) |
| 8 | Growth rates | | Plot temperature growth rates | Homework 4 (A13) | Make salinity media (A1), Count minimal media plate #3 (A3, 19) |
| 9 | Salinity | Growth rates | Salinity inoculation | Poster (A15), Final writing (A14) | Daily cell counts on salinity exp (A3, 19), Distribute salinity cell count data to students |
| 10 | Data round-up and poster drafts | Salinity | Review data, poster drafts, | | |
| 11 | Exam review and posters | | Poster presentation and review | Informal Writing 2 (A16) | Create Final Exam (A24) |
| 12 | Final exam | | Final Exam | | |
Appendix 26: Alternative schedule and assignments/assessments/activities from Spring 2020

| Week | Topic | Quiz | In-Class Activity | Assign to students | Instructor Prep for following week |
|------|-------|------|-------------------|--------------------|------------------------------------|
| 2 months before class | | | | | Order all materials  
Begin culturing strain for course |
| 2 weeks before class | | | | | Create syllabus  
Begin culturing strain for course |
| 1 week before class | | | | | Transfer cultured strain into fresh media  
Distribute syllabus |
| 1 | No Class | | | | Transfer cultured strain into fresh media  
Make SYBR-Green for course experiments |
| 2 | Introduction + Syllabus | Lab Safety | | | Transfer cultured strain into fresh media  
Calculate mass needed for each chemical for each nutrient stock  
Weigh and aliquot excess dry chemical to bring into the lab |
| 3 | Macromolecules and culture medium | Macromolecules and Minimal Media Methods | Nutrient stocks for minimal media experiment | Pre-lab reading assignment (due week 4) | Transfer cultured strain into fresh media  
Make base MWH2 media (no C, S, N sources)  
Autoclave the 96-well plate cover  
Aliquot 1.5mL Base MWH2 and control media into wells of a sterile 96-well plate  
Aliquot MWH2 into controls wells  
Count cultured strain day of the lab prior to class to calculate inoculation volume of cultured strain into plate |
| 4 | Minimal Media Experimental Design | Microbial Physiology and Minimal Media Methods | Set-up and inoculate Minimal Media Plate #1 | | Transfer cultured strain into fresh media  
Dishwash, acid wash, and autoclave necessary number of flasks for Temperature Experiment  
Make MWH2  
Count cultured strain day of the lab prior to class to calculate inoculation volume  
Aliquot 50mL of MWH2 into each cleaned/autoclaved flask  
Set desired temperature for incubators  
Daily time-points for 1 week for the temperature experiment |
| 5 | Temperature | Temperature Experiment Inoculation | Elevator Pitch (due week 7) | | Transfer cultured strain into fresh media  
Autoclave the 96-well plate cover  
Aliquot 1.5mL Base MWH2 and control media into wells of a sterile 96-well plate |
| Day | Minimal Media Experiment | Minimal Media Experiment Protocol | Journal Club & Minimal Media Plate #2 | Aliquot MWH2 into control wells
Continued daily time-points for temperature experiment |
|-----|--------------------------|----------------------------------|--------------------------------------|----------------------------------------------------------------------------------|
| 6   |                          |                                   |                                      | Transfer cultured strain into fresh media Count Minimal Media Plate #1 Upload Plate #1 count data to Blackboard Dishwash, acid wash, and autoclave necessary number of flasks for Salinity Experiment Make MWH1, MWH2, MWH3, MWH4 Count cultured strain day of the lab prior to class to calculate inoculation volume Aliquot 50mLs of each media type into separate flasks for Salinity Experiment Daily time-points for 1 week for the temperature experiment |
| 7   | Salinity                 | Salinity                          | Salinity Experiment Inoculation      | Transfer cultured strain into fresh media Autoclave the 96-well plate cover Aliquot 1.5mL Base MWH2 and control media into wells of a sterile 96-well plate Aliquot MWH2 into control wells Continued daily time-points for salinity experiment |
| 8   | Minimal Media Experiment | Scientific Literature             | Minimal Media Plate #3 & “Meet the Microbiologist” Podcast | Count Minimal Media Plate #3 & Upload Plate #2 count data to Blackboard Clear space in Thrash Lab for students to work Day of class, set out pipette sets, pipette tips, 96-well count plates, and thaw SYBR-Green for students |
| 9   | Flow Cytometry           | Minimal Media Experiment Data Interpretation | Optional activity-students observe flow cytometer cell counts | Remind about Peer Edits & Scientific Reading Assignment |
| 10  |                          |                                  |                                      | Upload Temperature and Salinity data to Blackboard |
| 11  | Bacterial Growth         | Microbial Growth                  | Data Visualization in R – Temperature and Salinity Growth Curves | Temperature and Salinity Growth Curves (due week 12) & Final Lab Report (Due week 15) Did not happen due to COVID lab shutdown: Count Minimal Media Plate #3 Upload Plate #3 count data to Blackboard |
| 12  | Bacterial Growth Rates   | Calculate Growth Rates for        | Final Exam (Virtual during Week 14)  | Create poster template and upload to Blackboard |
|   |   |   |   |
|---|---|---|---|
|   | Temperature and Salinity Experiments & Data Visualization in R – Temperature and Salinity Growth Rate Curves | Add extra hour for taking the exam in case of internet shortages/lack of quiet space in home for studying and taking exam. |
| 13 | Poster Design and Construction | R-Studio, Salinity Growth Rate Curve | Poster Drafts |
|   | Reminder about Final Exam and Final Lab Report | Poster Presentation (recorded presentation due Week 16) |
| 14 |   | Virtual Final Exam |   |
| 15 | Study Period | Time for questions and help with Posters and Final Lab Report |   |

**Spring 2020 Quizzes**

**Quiz 1 – Macromolecules and Minimal Media Methods (Pre-lab)**

1. Natural seawater is:
   a. Complex and undefined
   b. Minimal and defined
   c. Minimal and complex
   d. Complex and defined
   e. Minimal and undefined

2. ________ prompted scientists to create better growth media.
   a. Artificial Seawater Media
   b. The Great Plate Count Anomaly
   c. Natural Seawater Media

3. It is ok for the nutrient solutions to be non-sterile because we will not be using them again.
   a. True
   b. False

4. Fill in the blank. We used a ________ to maintain a sterile environment ________ filter our nutrient stocks using a __________ filter and ___________.
   (laminar flow hood, filter sterilize, 0.2 micron, syringe)

**Quiz 2 – Microbial Physiology and Minimal Media Methods (Pre-lab)**

1. The carbon that heterotrophic microorganisms consume has two general gates, _____ and/or ________.
   a. Incorporated into biomass/respired as CO2

Page 81 of 120
b. Incorporated into biomass/remains unchanged from its original form
c. Remains unchanged from its original form/respired as CO2
d. Never seen again/incorporated into biomass

2. Microorganisms are important in the carbon, sulfur, and nitrogen biogeochemical cycles.
   a. True
   b. False

3. An organism that uses light to consume and digest organic carbon is a ________.
   a. Photoheterotroph
   b. Photoautotroph
   c. Chemoautotroph
   d. Chemoherotroph
   e. None of the above

4. Short answer. How many carbon, nitrogen, and sulfur substrates will each student be testing in our minimal media experiment?
   (5 carbon, 2 nitrogen, 5 sulfur)

Quiz 3 – Enzyme Physiology (Pre-lab)

1. Enzymes are biological catalysts that increase the rate of chemical reactions by lowering the __________ of the reaction.
   a. Product concentration
   b. Substrate availability
   c. Activation energy
   d. None of these

2. Cardinal temperatures refer to:
   a. The minimum and maximum temperatures that an organism can grow
   b. The minimum, maximum, and optimum temperatures that an organism can grow
   c. The optimal temperature that an organism can grow
   d. None of these

3. Temperature does not affect the activity of enzymes.
   a. True
   b. False

4. Extreme temperature disrupts the stability and structure of cell membranes.
   a. True
   b. False

Quiz 4 – Minimal Media Experiment Protocol (Pre-lab)

1. Prior to adding anything to the wells, what is the volume of media in each well?
   a. 1.5 mL
   b. 2 mL
   c. 1.5 uL
   d. 2 uL

2. What is the source of inoculum bacteria for Plate 2?
   a. Plate 3
   b. Plate 1
   c. Culture flask

3. The volume of stock added to the well in the plate was ________ and to a final concentration of ________.
   a. 2 uL / 3.2 x 10^-5 M
   b. 2 uL / 3.2 x 10^-2 M
   c. 2 mL / 3.2 x 10^-2 M
4. The base media used in this experiment is unamended from its original form and contains all macromolecules necessary for growth.
   a. True
   b. False

Quiz 5 – Salinity Quiz (Pre-lab)

1. Hydrophilic and large polar molecules can pass freely across the plasma membrane.
   a. True
   b. False
2. Water moves from ______ water / ______ solute concentration to ______ water / ______ solute concentration.
   a. High / low …. Low / High
   b. High / high …. Low/ low
   c. Low / high …. High / low
   d. Low / low …. High/ high
3. Bacteria use compatible solutes as a way to fight osmotic stress during salinity changes in their surrounding environment.
   a. True
   b. False
4. Compatible solutes are:
   a. Can be used for biomass
   b. Can be used for energy generation
   c. Are used as a charge buffer to balance the ionic strength outside the cell
   d. All of the above

Quiz 6 – Scientific Literature (Post-lab)

1. A textbook is an example of a ________:
   a. Primary source
   b. Secondary source
   c. Tertiary source
2. A primary source is peer-reviewed from 2-3 experts in the field of research prior to the work being published.
   a. True
   b. False
3. Where do these two components fit into the structural organization of a primary research paper: what is already known about the topic, and the gaps?
   a. Introduction
   b. Methods
   c. Results
   d. Discussion
4. The results section is where you both describe the observed data and interpret it’s findings.
   a. True
   b. False

Quiz 7 – Minimal media Experiment interpretation (Post-lab)

1. Anything below ________ cells/mL we are not considering positive growth.
2. Short answer. How will we determine that LSUCC0713 is growing on the compounds supplied, and why? (Positive growth on three consecutive plate transfers, to determine that LSUCC0713 is not consuming the carryover nutrients from the initial inoculation.)

Quiz 8 – Microbial Growth (Pre-lab)

1. Compared to copiotrophic organisms, oligotrophic organisms typically grow ______ and need ______ nutrient concentrations to grow.
   a. Quickly / high
   b. Slowly / low
   c. Quickly / low
   d. Slowly / high

2. Regarding microbial growth, binary fission leads to exponential growth.
   a. True
   b. False

3. Which is NOT a phase in a microbial growth curve?
   a. Lag Phase
   b. Death Phase
   c. Exponential phase
   d. Saturation phase
   e. Stationary phase

4. Diauxic growth patterns in batch culture occur when the organism consumes and depletes a substrate during growth, then transfers to another available substrate. During this process you see a lag phase while they shift their gene expression to catabolize the new substrate.
   a. True
   b. False

Quiz 9 – RStudio, Salinity Growth Rate Curve (Post-lab)

x-axis = Salinity (PSU)
y-axis = Growth rate (divisions per day)
Title – “Growth Rate vs. Salinity, LSUCC0713”
Data points plotted along with interpolation curve

Quiz 10 – Poster draft (Post-lab)

Drawn schematic of how they envision their poster.
Spatial organizational flow of information determined, all figures to be included are specified, and flow charts/visuals for methods are determined
Spring 2020 Alternative assignments/assessments/activities

Name: ________________________________

BISC 221 Lab – CURE
Spring 2020
Pre-Lab Assignment #2

Directions: Please read Carini et al. 2014 found on Blackboard, and answer the following questions regarding the paper. Hand your completed assignment to your instructor at the beginning of class.

Due date: February 11th

1. Fill in the blank:
   “…relatively little is known about vitamin biogeochemistry or the affect of vitamins on
   the ______________________ and ______________________ of planktonic
   communities.

2. In Figure 1, what the reactions labeled in black mean? What do the reactions labeled in red mean?

3. What organism is this study working with? Is it a type of bacteria, archaea, or eukaryote? What Earth
   system does it live in/isolated from (ex: soil, lakes, ocean, subsurface, etc.)?

4. Fill in the blank:
   “Cells for counts were stained with ______________________ and counted with
   a Guava Technologies ______________________ ...

5. What evidence lead these researchers to hypothesize that Ca. P. ubique is auxotrophic for HMP?
6. Figure 2c represents nucleotide sequences, each base represented by a different color. Above the aligned sequences, is a sequence illustration with varying font sizes – what does this sequence represent.

7. The authors state that, “Thiamin and AmMP were ineffective at restoring thiamin-limited growth at pico- or nanomolar concentrations.” Why? Hint – Figure 3.

8. In Figure 4, at what time of day and at what depth did the researchers find the highest concentration of HMP? Approximately, what is the highest concentration they found?

9. True/False: Thiamin cycling in the oceans may follow complex patterns and involve multiple processes and intermediates.

10. How was their thiamin stock solution contaminated with HMP?

Pre-lab Assignment #1 Answer Key

1. Structure, composition
2. Black = encoded in the genome. Red = missing from the genome
3. SAR11, bacteria, ocean
4. True
5. SYBR Green I, flow cytometer
6. Missing genes. No transporter, and also missing genes for biosynthesis
7. Consensus sequence
8. No ThiBPQ or TenA
9. 8pm, ~110 meters depth/DCM, ~ 35 pM
10. True
11. Background HMP concentrations from manufacturing

Meet the Microbiologist: SAR11 and Other Marine Microbes

1. Who provides the carbon for the marine ecosystems? What “zone” do they live in?

2. What is the “zone” referred to where “you might barely get to read a newspaper”? What are the microorganisms that live there consuming? There did that source of food originate from?

3. Why are the microbes that live in the deep ocean biochemically different than those that live at the surface?

4. How does the distribution of cells change as you go deeper in the water column?

5. In regard to the carbon cycle, how much of global photosynthesis is occurring in the global oceans?

6. Why does the surface have so little nutrients?

7. How is the carbon cycle different in the ocean than it is on land?
8. Do the oceans act as a sink for atmospheric CO2?

9. What is the total census for SAR11 cells?

10. Where in the water column do SAR11 cells exist?

11. What percent range of photosynthetically fixed carbon are SAR11 cells consuming?

12. Are SAR11 cells vulnerable to infection? By what? Are these entities usually as abundant as SAR11?

13. What two gaseous compounds are SAR11 cells producing from DMSP? Are those compounds known to volatize into the atmosphere from the ocean?

14. What technology did the Giovannoni lab use to isolate marine microbes 30 years ago? How does this work? What medium did they use for isolating these organisms?

15. What is a characteristic of ocean gyres? What is the causing these regions to expand?

16. What analogy did Dr. Giovannoni use to describe the carbon source/“food” that SAR202 eats?
Final Lab Report Guidelines

This lab report is a synthesis of all the experiments done this semester. Augment your Introduction and Methods sections, and make sure to implement any feedback that I have given you. You can access this feedback on your turn-it-in assignments. You need to include the following sections:

Title:
The title should be descriptive of our objectives this semester

Introduction:
This section must include at least four outside references to support the message you are conveying in your introduction. You will need to discuss why bacteria, particularly marine bacteria, are important. Incorporate ideas of why nutrient usage, temperature, and salinity are important to bacteria, and marine and coastal environments. Introduce LSUCC0713, and you MUST discuss where LSUCC0713 was isolated from and describe that system.

You need to include how it was isolated, the type of media used for isolation, where this isolate is stored. That being said, you MUST cite Henson et al. 2016 as a reference – this citation is not counted in your 4-reference minimum. Follow up with introducing the experiment, and what our objectives are.

Methods:
Your methods must be organized, accurate, comprehensive, and nonrepetitive. You should organize your methods into different subheadings for each experiment, including sterile technique. Do not forget to discuss the controls we implemented in each experiment, and to add Cell Counts and RStudio into your methods.

Results:
All figures must have the appropriate figure/table captions. The captions provide your reader with the information needed to interpret the figure. Think of it like a map legend. The figures that you need to include are:
- Minimal media experiment data
- Temperature Growth Curve
- Temperature Growth Rate Curve
- Salinity Growth Curve
- Salinity Growth Rate Curve

Remember, you are stating your results in this section, and will be interpreting them in the discussion.

Discussion:
Here is where you will interpret your data and address trends that you notice. I am not looking for a specific answer, but I want you to form conclusions based on the data and to explain why you believe this to be true.

I have provided you with the other section’s minimal media experiment data. You can use this as a point of reference in interpreting your own data – under no circumstance should you include this in your results section.

Additionally, you can use this section to tie the results from our experiments into the bigger picture. How does our experiment and results connect to existing data and to the marine environment. Use citations to back up your claims if necessary.

Future Directions:
If you could continue, what experiments would you like to do? What direction would you take this research?

References:
APA format
Introduction/Methods Rubric

This assignment is loosely graded. Essentially it is meant to get them thinking about how to write about environmental microbiology, a way to provide them with feedback on the introduction), and to make sure that their methods are correct. I told them to use the “Structural Organization of Primary Research Papers”, (page 7) in Chapter 4 of their lab manual, to guide them in their writing.

**Introduction – 10 points**

*Introduce the topic of environmental microbiology/marine microbial ecology (3 points)*

- They make no connection to marine microbial ecology (1 point)

*Introduce the topic of the importance of cultivation (1 point)*

- *This is important, but at this point in their writing they might not have picked up on it yet. This will be required and emphasized for the final lab report.*
- No mention of it – 0 points

*What type of organism is LSUCC0713? – A bacterium (1 point)*

- No mention – 0 points

*Where was LSUCC0713 isolated? (1 point)*

- No mention – 0 points

*Why is it unique/what type of system? (1 point)*

- Mention it is brackish – 0.5 points
- No mention – 0 points

*What is the motivation for this investigation? (3 points)*

- They provide a generic motivation without any context – 1 point
- No mention of motivation – 0 points

**Methods – 10 points**

*Carbon stocks – 2 points*

- Why did we make these/what experiment are they for? – 0.5 point
- Concentration of stock/volume of water – 1 point
- Filtered – 0.5 points

*Minimal media – 2 points*

- Final concentration of stock in the plate – 0.5 points
- The matrix format – 0.5 points
- Repeated the plate 3 times – 0.5 points
- The volumes of culture/stock/amended medium – 0.5 points

*Temperature – 2 points*

- Type of media – 0.5 points
- Volume of media/volume of culture inoculated – 0.5 points
- Incubation length and conditions – 0.5 points
- Cell fixing for counts – 0.5 points

*Salinity – 2 points*

- Type of media – 0.5 points
- Volume of media/volume of culture inoculated – 0.5 points
- Incubation length and conditions – 0.5 points
- Cell fixing for counts – 0.5 points

*Sterile Technique – 2 points*
Final Lab Report Rubric
50 points

Title: 2 points
Includes LSUCC0713 – 1 points
Includes the characterization aspect – 1 points

Introduction: 10 points
Introduce the topic of environmental microbiology/marine microbial ecology – 3 points
They make no connection to marine microbial ecology (1 point)
Introduce the topic of the importance of cultivation – 1 point
No mention of it – 0 points
What type of organism is LSUCC0713? – 1 point
No mention – 0 points
Where was LSUCC0713 isolated? – 1 point
No mention – 0 points
How was LSUCC0713 isolated? – 1 point
No mention – 0 points
Why is it unique/what type of system? – 1 point
Mention it is brackish – 0.5 points
No mention – 0 points
What is the motivation for this investigation? – 2 points
They provide a generic motivation without any context – 1 point
No mention of motivation – 0 points

Methods: 14 points
Carbon stocks – 2 points
Why did we make these/what experiment are they for? – 0.5 point
Concentration of stock/volume of water – 1 point
Filtered – 0.5 points
Minimal media – 2 points
Final concentration of stock in the plate – 0.5 points
The matrix format – 0.5 points
Repeated the plate 3 times – 0.5 points
The volumes of culture/stock/amended medium – 0.5 points
Temperature – 2 points
Type of media – 0.5 points
Volume of media/volume of culture inoculated – 0.5 points
Incubation length and conditions – 0.5 points
Cell fixing for counts – 0.5 points
Salinity – 2 points
Type of media – 0.5 points
Volume of media/volume of culture inoculated – 0.5 points
Incubation length and conditions – 0.5 points
Cell fixing for counts – 0.5 points
Sterile Technique – 2 points
Cell counts – 2 points
Method used – 0.5 points
Stain used – 0.5 points
Dilutions made to each sample – 1 point
RStudio – 1 points
Why did we use RStudio – 0.5 points
What packages did we use – 0.5 points

Results: 7 points
Minimal media data – 1 points
Temperature Growth Curve – 1 point
Temperature Growth Rate Curve – 1 point
Salinity Growth Curve – 1 point
Salinity Growth Rate Curve – 1 point
Informative figure and appropriate captions – 2 points

If figures are blurry, -0.25
Any mention of the other class’s data will get an automatic deduction of 5 points.

Discussion: 10 points
Interpret data and draw conclusions from them – 3 points
Explanation of why they think that conclusion to be true – 2 points
Extrapolating these conclusions out to the coastal Louisiana system – 3 points
Comparing these results with similar studies – 2 points

References: 2 points
Henson et al. 2016 – 1 points
At least 4 references – 1 point

Quality of writing: 6 points
Poor sentence structure, unclear ideas, poor grammar – 3 points
### Appendix 27: Alternative schedule and assignments/assessments/activities from Fall 2021

| Dates       | Week | Topic                              | Quiz  | In-Class Activity                  | Assign to students | Instructor Prep for day of teaching |
|-------------|------|------------------------------------|-------|------------------------------------|--------------------|-------------------------------------|
| 8/23-8/29   | 1    | No lab                             | No lab| No lab                             | Order all materials |
|             |      |                                    |       |                                    | Instructor should create and distribute the syllabus, Instructor should get familiar with cell counts |
| 8/30-9/5    | 2    | Intro./safety                      | Lab safety | Read lab manual CH for next week | Instructor should familiarize themselves with safety material locations in the classroom |
| 9/6-9/12    | 3    | Macromolecules and Cultivation     | Lab Quiz #1 | Creation of Nutrient Stocks | Pre-lab #1, (Exercise #1 in CH 3 of lab manual), Checked in lab manual in week #4, turned in (on 9/22), Lab manual CH for next week |
|             |      |                                    |       |                                    | Bring chemicals, falcon tubes, filters, syringes, weigh paper, and Milli-Q |
| 9/13-9/19   | 4    | Minimal media plate #1            | Lab Quiz #2 | Minimal media plate #1 inoculation | Pre-lab assignment #2 (A26) (Due 9/22), Lab manual CH for next week |
|             |      |                                    |       |                                    | Fill 96-well plates with ~1.5 mL of base media (AMS1-C/N/S), Bring nutrient stocks to the lab, Count during week 6. |
| 09/20-09/26 | 5    | Temperature                        | Lab Quiz #3 | Scientific paper, Carini et al. 2014 discussion, Temperature inoculation | Pre-lab assignment #3 (A27) due next class (9/29) |
|             |      |                                    |       |                                    | Count inoculum cultures on Accuri, fill flasks with ~50mL of AMSW1, Bring flasks, inoculum culture, and tubes to the lab, Take daily time points (~7 days) for temperature experiment |
| 9/27-10/3   | 6    | Minimal media plate #2            | Lab Quiz #4 | Plate #1 transfer to plate #2, Gene editing journal club w/ lecture instructor (~1hr) | Podcast assignment (A26) (Due 10/6), Post-journal club review paper (A27) (Due 10/20), Lab manual CH for next week |
|             |      |                                    |       |                                    | Fill 96-well plates with ~1.5 mL of base media (~C/N/S), Bring nutrient stocks, pipettes, sterile tips, and culture to the lab, Count during week 9. |
| 10/4-10/10  | 7    | Cell membranes and salinity        | Lab Quiz #5 | Salinity lab activity (non-mCURE activity/flex week) | Intro. and methods draft (Due 10/20) |
|             |      |                                    |       |                                    | Bring red onion, make hypotonic, isotonic, and hypertonic solutions, Set up microscopes, Get sheep blood, Show students how to use microscopes, Show example images of the cell wall effects after all groups have completed the lab activity (non-mCURE activity/flex week) The instructor can substitute in their own lab activity to teach about the topic of “Cell membranes and salinity” |
| Date       | Week | Activity                                                                 |
|------------|------|---------------------------------------------------------------------------|
| 10/11-10/17 | 8    | No class, fall break                                                      |
| 10/12-10/17 | 9    | Minimal media plate #3                                                   |
|            |      | Plate #2 transfer to plate #3, Climate change journal club w/ lecture instructor (~1 hr) |
|            |      | Post-journal club review paper (A27) (Due 11/10), Lab manual CH for next week |
|            |      | Count minimal media plate #2, Fill 96-well plates with 1.5 mL of base media (AMS1-C/N/S), Bring nutrient stocks to the lab, Count during week 12. |
| 10/25-10/31 | 10   | Flow cytometry and microbial characterization lab |
|            |      | Microscopy characterization lab and virtual flow cytometry activity (A27) |
|            |      | Bring laptops for Next Classes, Lab Manual CH for Next Week |
|            |      | Bring Diatoms, Dinoflagellates, Trichodesmium, and Crocosphaera cultures/pasteur pipettes/ ice water buckets for Diatoms and Dinoflagellates, Set up microscope stations, Make video using flow cytometer, keep the Cyanobacteria in a window in the classroom when not using them during lab |
| 11/1-1/7   | 11   | Bacterial growth rates and data visualization in R |
|            |      | Lab quiz #6 and Lab quiz #7 |
|            |      | Discuss temperature data, Final paper, and Posts |
|            |      | R-Markdown of growth curve figure draft (Due 11/10) |
|            |      | Bring laptop to demonstrate plotting for students |
| 11/8-11/14 | 12   | Bacterial growth rates and data visualization in R |
|            |      | Lab quiz #8 |
|            |      | Discuss data, final paper, and posters, Discuss concepts from CH 8 pt 2 and how to do growth rate calculations |
|            |      | Growth rate calculations due next class |
|            |      | Count minimal media plate #3, Bring data to class for students to make tables with |
| 11/15-11/21 | 13   | Final data round up |
|            |      | Lab quiz #9 |
|            |      | Work on posters/lab Report |
|            |      | Posters (A27) (Due 12/1), Lab report (A27) (Due 12/8) |
|            |      | Remind students of lab report and poster due dates, but they should have been working on this for longer |
| 11/22-11/28 | 14   | No class, Holiday |
|            |      | Lab quiz #10 |
|            |      | No class, Holiday |
| 11/29-12/5 | 15   | Poster presentations |
|            |      | Poster presentations |
|            |      | Take notes during presentations for grading later |

**Alternative Schedule Notes:**
- Lab report turned in during finals week instead of a lab final exam being given
- The salinity experiment was omitted in this alternative schedule
- The social media assignment was omitted in this alternative schedule. Instead, two journal club review writing exercises were implemented.
- The carbon utilization experiment was amended to be a minimal media experiment testing nitrogen and sulfur utilization in addition to carbon.
Fall 2021 Quizzes

Quiz 1 (pre-lab)
1. Multiple Choice: Natural seawater is:
   a. Complex and undefined
   b. Minimal and undefined
   c. Complex and defined
   d. Minimal and defined
   e. Minimal and complex

2. It is okay if our nutrient stocks are not totally sterile because it will not affect our minimal media experiment in the future.
   True False
   Incorrect Feedback: Our nutrient stocks must be sterile because we need them to conduct our growth experiments with our single bacterium. They are not useful to us if they get contaminated.

3. Pick the two correct answers. This week, we will be using a ____ attached to the end of a _______ inside of a biosafety cabinet.
   60mL syringe
   0.2um filter
   250mL flask
   2um filter

4. The _____________ prompted scientists to create better growth media in modern times.
   a. The Great Plate Count Anomaly
   b. Natural Seawater Media
   c. Artificial Seawater Media
   d. Storage and Transportation Difficulty

5. Match the type of organism to the nutrient type it prefers.
   a. a. Oligotrophic organisms a. low-nutrient environments
   b. b. Copiotrophic organisms b. high-nutrient environments

6. True/False: The reaction that organisms use to catalyze the connection of monomers to form polymers is called hydration synthesis.
   True False
   Incorrect Feedback: Go back to the lab manual and look over the reactions again. The reaction that organisms use to form polymers is called 'dehydration' synthesis because a water molecule is lost from the reaction.

Quiz 2 (pre-lab)
1. An organism that uses a chemical energy source to consume and digest organic carbon is a
   a. Photoheterotroph
   b. Photoautotroph
   c. Chemautotroph
   d. Chemoheterotroph
2. Microorganisms are important in the carbon, sulfur, and nitrogen biogeochemical cycles.
   **True** False

3. How many carbon, nitrogen, and sulfur substrates will each student be testing in our minimal media experiment?

   **1 nitrogen, 1 sulfur, and 5 carbon sources.**

4. The carbon that heterotrophic microorganisms consumes has two general fates, ________ and/or ________.
   a. incorporated into biomass/ respired as CO2
   b. incorporated into biomass/remains unchanged from its original form
   c. remains unchanged from its original form/respired as CO2
   d. Never seen again/incorporated into biomass

5. Heliobacteria are an example of a photoautotroph that use organic carbon and light to survive.
   **True** False
   Incorrect Feedback
   This is actually an example of a photoheterotroph from the lab manual. These organisms use light, but they use organic carbon (hence the heterotrophic lifestyle).

6. Organisms commonly use both nitrogen and sulfur for amino acid formation in cells.
   **True** False

**Quiz 3 (pre-lab)**

1. Enzymes are biological catalysts that increase the rate of chemical reactions by lowering the __________ for that biochemical reaction.
   - activation energy
   - substrate availability
   - product concentration
   - None of these

2. Cardinal temperatures refer to:
   - the minimum, maximum, and optimum temperatures that an organism can grow.
   The minimum and maximum temperatures that an organism can grow.
   The optimal temperature that an organism can grow.
   None of these.

3. Temperature does not affect the activity of enzymes.
   **True** False

4. Extreme temperatures disrupt the stability and structure of cell membranes.
   **True** False

**Quiz 4 (pre-lab)**

1. A hypothesis needs to be _____ and _______ to be useful in the context of scientific investigation.
1. a. testable
b. provable
c. falsifiable
d. definitively correct

2. What is the trial group called in which the independent variable is kept at an established value or at zero?
   a. control
b. dependent
c. prediction
d. independent

3. When trying to figure out how to write/format my lab report in a few weeks, what should I use to do this? (one answer only)
   a. Chapter 4 of my lab manual
b. Just copy the first suggestion that comes up on Google
c. Chapter 2 of my lab manual
d. Chapter 3 of my lab manual

4. When reading scientific papers, it is important to take notes, look at the figures, look up words that I don't know, and make sure that I understand the conclusions that the paper is drawing.
   True False

5. Which parameters need to be defined before the actual experimental methodology is put on paper? (select all appropriate answers)
   a. The variables
b. levels of treatment
c. replication
d. controls

6. Depending on the environment they live in, bacteria like to grow at different temperatures, so temperature is important to test when studying an organism. This is why we did the temperature experiment for our US3C007.
   True False

Quiz 5 (pre-lab)

1. Hydrophilic and large polar molecules can pass freely across the plasma membrane.
   True False

2. Water moves from _______ water / _______ solute concentration to, _______ water / _______ solute concentration.
   high water / low solute ...
   high water / high solute ...
   low water / high solute ...
   low water / low solute ...

3. Bacteria use compatible solutes as a way to fight osmotic stress during salinity changes in their surrounding environment.
True False

4. Compatible solutes are:
   - not used for biomass
   - not used for energy generation
   - are used as a charge buffer to balance the ionic strength outside the cell
   All of the above

Quiz 6 (post-lab)

1. Which organism that we observed last week was coccoid in shape and slightly green-colored under the microscope?
   a. *Trichodesmium erythraeum*
   b. *Crocosphaera watsonii*
   c. *Pseudo-nitzschia*
   d. *Alexandrium*

2. What was the type of stain that the Thrash lab (and our experiments) use for flow cytometry?
   a. Syber Green
   b. DAPI
   c. Ethidium Bromide
   d. Acridine Orange

3. Which organisms that we observed last week had 'puff' and 'free trichome' morphologies?
   a. *Crocosphaera watsonii*
   b. *Trichodesmium*
   c. *Alexandrium*
   d. *Pseudo-nitzschia*

4. How would you describe this morphology, based on your morphology guide sheet that you received last week?
   (insert image of a Streptobacillus bacterium)
   a. Streptobacilli
   b. Streptococci
   c. Vibrio
   d. Palisades

5. Which organism did we look at last week that was motile?
   a. *Alexandrium*
   b. *Pseudo-nitzschia*
   c. *Trichodesmium erythraeum*
   d. *Trichodesmium thiebautii*

6. How would you describe this morphology, based on your morphology guide sheet that you received last week?
   (Insert image of a Spirochete bacteria)
   a. Spirochete
   b. Cocci
Quiz 7 (pre-lab)
1. What do you need to download before our lab on Wednesday this week?
   a. Rstudio & R
   b. Python
   c. Matlab
   d. JavaScript

2. What do I need to bring with me to lab this week?
   a. My lab manual chapter 8 that I have thoroughly read all the way through
   b. My laptop (not just a tablet/phone) because we are coding
   c. Just my phone/tablet
   d. Just a notebook

3. What is it called when there is a plateau in the number of living bacterial cells and the rate of cell division and death are roughly equal?
   a. Stationary phase
   b. Log phase
   c. Lag phase
   d. Death phase

4. The homework following class today is to knit your RMarkdown file and submit the .html AND your RCode files to Blackboard for grading.
   True False

Quiz 8 (post-lab)
1. We should always use the library() command to re-load packages (like ggplot2) every time that we open R so that the program knows what packages that we want to use.
   True False

2. What did we use to format and output our .html file once we made our plot?
   a. knit(knitr)
   b. ggplot2
   c. .html generator
   d. ggridges

3. Why did we use RMarkdown and submit our figure as a .html file?
   a. RMarkdown shows that your code is reproducible
   b. RMarkdown is good for reports or other resources as it is easily formatted and easy to read.
   c. We can only make an RMarkdown file in RStudio.
   d. We did not use RMarkdown to output our plots to a .html file.

4. What kind of file with our data in it did we read into RStudio so that RStudio could 'understand it' properly?
   a. .csv
5. What package did we use to make our plots?
   a. Knitr
   b. ggplot2
   c. ggridges
   d. tidyverse

6. When we made our plots, we saw that our organism grew best at 37 degrees celcius.
   True False

Quiz 9 (post-lab)
1. What are the most frequently used plot types?
   a. line graphs
   b. bar graphs
   c. dotplots
   d. density plots

2. What is Nt in the growth rate calculation?
   a. The final cell density over the time period of observation.
   b. The starting cell density over the time period of observation.
   c. The number of generations
   d. The growth rate

3. Many natural environments are oligotrophic, so many organisms in oligotrophic regions have slow growth rates.
   True False

4. Most growth curves, like the main plot that we made in class, are ____ graphs/plots because it shows how the cell density changes over time.
   a. Line
   b. Bar
   c. Boxplot
   d. Histogram

Quiz 10 (post-lab)
1. The positive control with our artificial seawater and inoculated US3C007 is not important when considering the results of our minimal media experiment.
   True False

2. Which temperature did our organism appear to grow best at in our temperature experiment?
   a. 25 deg C
   b. 12 deg C
   c. 30 deg C
   d. 4 deg C
   e. 37 deg C
3. Our temperature experiment and minimal media experiment were monitored and counted by use of the ______?
   a. Flow cytometer and cells stained using Syber Green.
   b. Hemocytometer and cells stained using Syber Green.
   c. Flow cytometer and cells stained using Syber Yellow.
   d. Hemocytometer and cells stained using Syber Blue.

4. Our temperature experiment was conducted over the course of 7 days by incubating our cultures at 5 different temperatures, and by taking daily cell counts on the flow cytometer.

   True False

5. The stain that was used on our cells whenever a cell count was taken was a ___ stain.
   a. DNA
   b. Bacterial only
   c. Mitochondrial
   d. Golgi Aparatus

6. Please type the answers to both blanks with a comma in between them:
   US3C007 is in the genus _____ and the class _______.
   Evaluation Method     Answer     Case Sensitivity
   Exact Match           Roseobacter
   Exact Match           Alphaproteobacteria
Fall 2021 Alternative assignments/assessments/activities

Pre-Lab Assignment #3

Cavicchioli et al. 2019 (posted on Blackboard)

1. _____ organisms use the sun’s energy in the top _____ meters of the water column whereas marine life in deeper zones uses _____ and _______ chemicals for energy.

2. Why specifically are phytoplankton important in terms of primary production and global oxygen levels?

3. How does changing sea ice levels potentially impact primary production in the ocean?

4. Define any three of the terms listed in the MARINE (OCEAN) environment of figure 1 (excluding the terms light, heat, wind, and rain).

5. Describe the effects of aerosols on the environment that the paper discusses and how they are related to microorganisms.

*For these next two questions, please do not copy the figure captions.*
6. Write a brief (4-5 sentences) description on Figure 3 of the paper and describe how some of the processes might link back to microbes.

7. Write a brief (4-5 sentences) description on Figure 2 of the paper and describe how some of the processes might link back to microbes.

Pre-Lab Assignment #3
Read: Cavicchioli et al. 2019
(Answers in Quotes are taken directly from Cavicchioli et al. 2019)

1. ______ organisms use the sun’s energy in the top _____ meters of the water column whereas marine life in deeper zones uses ______ and _______ chemicals for energy.

Answer: Phototrophic microorganisms use the sun’s energy in the top 200 m of the water column, whereas marine life in deeper zones uses organic and inorganic chemicals for energy.

2. Why specifically are phytoplankton important in terms of primary production and global oxygen levels?

“Marine phyto-plankton perform half of the global photosynthetic CO2 fixation (net global primary production of ~50PgC per year) and half of the oxygen production despite amounting to only ~1% of global plant biomass30. In comparison with terrestrial plants, marine phytoplankton are distributed over a larger surface area, are exposed to less seasonal variation and have markedly faster turnover rates than trees (days versus decades).”

3. How do sea ice levels potentially impact primary production in the ocean?

“The global sea ice (Sea Ice Index) is declining, leading to higher light penetration and potentially more primary production; however, there are conflicting predictions for the effects of variable mixing patterns and changes in nutrient supply and for productivity trends in polar zones34.”

4. Define any three of the terms listed in the MARINE (OCEAN) environment of figure 1 (excluding the terms light, heat, wind, and rain).
5. Describe the effects of aerosols on the environment that the paper discusses and how they are related to microorganisms.

“Aerosols affect cloud formation, thereby influencing sunlight irradiation and precipitation, but the extent to which and the manner in which they influence climate remains uncertain. Marine aerosols consist of a complex mixture of sea salt, non-sea-salt sulfate and organic molecules and can function as nuclei for cloud condensation, influencing the radiation balance and, hence, climate. For example, biogenic aerosols in remote marine environments (for example, the Southern Ocean) can increase the number and size of cloud droplets, having similar effects on climate as aerosols in highly polluted regions. Specifically, phytoplankton emit dimethylsulfide, and its derivate sulfate promotes cloud condensation. Understanding the ways in which marine phytoplankton contribute to aerosols will allow better predictions of how changing ocean conditions will affect clouds and feed back on climate. In addition, the atmosphere itself contains ~10^22 microbial cells, and determining the ability of atmospheric microorganisms to grow and form aggregates will be valuable for assessing their influence on climate.”

*For these questions, please do not copy the figure captions.

6. Write a brief (4-5 sentences) description on Figure 3 of the paper and describe how some of the processes might link back to microbes.

7. Write a brief (4-5 sentences) description on Figure 2 of the paper and describe how some of the processes might link back to microbes.

Elevator Pitch Assignment

- 2–3-minute speech that summarizes the key findings from a scientific paper that you have found
- Over 3 minutes will result in a zero on the assignment
- Sign-up for a time slot on Blackboard and paste in your paper’s title. IT CANNOT BE THE SAME AS SOMEONE ELSE’S! So, please check what others have listed before you choose a source.
- Must relate to the topic of this class (marine microbiology, bacterioplankton, microbial ecology in the ocean, etc.)
- Must have a brief (1-2 sentences) of each section:
• Introduction (give title of your chosen paper)

• Objectives of investigation

• Methods

• Results

• Discussion

- Can bring speaker’s notes, but I would suggest memorizing what you want to say for the sake of time

**Elevator Pitch Rubric**

3 min or less – 3 pts  
Over 3 min – 2 pts

**Introduction**  
Relevant/concise – 4 pts  
Short/choppy or too long/Rambling – 3 pts

**Objectives of investigation**  
States objectives – 3 pts  
Unclear objectives – 2 pts

**Methods**  
Concise and relevant to results shared – 4 pts  
Not relevant to results shared – 3 pts

**Results**  
State main point that matches the objective – 3 pts  
Main point doesn’t tie back to objective/unclear – 2 pts

**Discussion**  
Communicate the importance of the result – 3pts  
Weakly communicate importance of result – 2 pts
Poster and Presentation Guidelines

Each poster presentation should be around 6-7 minutes in length, 3 minutes provided for questions afterwards

• Poster should have all the sections listed in the example poster given, and they should be thoroughly discussed and presented. Credit will be decided based on the level of concise yet thorough completion of each section, attention to detail, providing all the necessary figures/tables (at least one temperature plot and the Minimal Media Plate matrix), and the quality of the presentation of each section to the class.

  Introduction – 20 points
  Research Objectives – 10 points
  Methods (Methodology) – 15 points
  Results – 20 points
  Conclusions (Discussion) – 20 points
  Future Directions – 10 points

References (Do not need to specifically list in the presentation, just need to be on the poster) – 5 points

Post-Journal Club Assignment

By: Catie S. Cleveland

Due 11/10/21 At the Beginning of Class

*****PLEASE PRINT OUT FOR CLASS*****

*****NOT EMAIL*****

1. Write a 1.5-2 page “review” paper of the second journal club papers that we discussed in lab. Feel free to bring in ideas that we had in the discussion.

2. Remember that a review paper is a synthesis of sources and material.
3. Please make sure you are citing the references as you discuss them (ex: (Cleveland et al. 2020), (Cleveland and Cleveland 2020) for two authors, or (Cleveland 2020) for one author).

4. Give it a title, write your name under the title

5. Use 12pt font

6. Reasonable font (Calibri, Times New Roman, Arial, etc.)

7. 1.5 line spacing (same spacing that this document has)

**Microbial Morphology: Can you identify it?**

**Other microbial shapes:**

*Trichodesmium (Cyanobacteria)*

Puff
What are we looking at today?

**Exercise 1:**

1. Do some independent research on our organisms (listed on the board)
   - What are their different morphologies?
   - Are they autotrophs or heterotrophs? If they are autotrophs, what pigments do they have?
   - What type of organism are they (protist, bacteria, etc.)
   - What size are they on average (individual cells, not colonies)?

**Exercise 2:**

Move around to the stations at the benches and either use the microscope or look at the photos to characterize this organism based on your outside research and the given information above.
FOR MOTILE ORGANISMS: If you see that an organism is moving, observe it for 30 seconds to 1 minute and write down 3 movements that it is doing (example: whipping flagella, swimming straight, etc.) Please do not just use the example movements. Try to make your own observations.

1. Which organism is this?

1. What is its morphology?

1. Is it motile or non-motile (N/A for photos)?

1. What color is it?

1. Give a 1-2 sentence description of it below.
**Instructor Notes on this Lab Activity:**

Materials:

- Compound microscopes
- Pasteur pipettes
- Cultures (amount and type of organism decided by instructor)
- Regular slides (and depression slides for motile organisms)
- Slide covers

1. Find images on showing and labeled with general microbial morphologies for students to reference.
2. It is helpful to give a chalk talk on the board prior to this lab about the importance of microbial morphology and why it is useful for studying bacterioplankton specifically.
3. When this lab was deployed, the instructor had cultures of *Trichodesmium*, *Crocosphaera*, Diatoms, and Dinoflagellates. So, the students referenced photos of these organisms’ morphologies in class. Depending on what organisms are available for the students to identify, reference photos of more complex morphologies can be included in this activity at the instructor discretion under the “other microbial shapes” section. Photos can be substituted for actual microorganisms at some stations if not enough live cultures are available to the instructor.
4. If fluorescence microscopes are available, this can be helpful for seeing small *Cyanobacteria*. This would also allow the instructor to talk about the importance of pigment morphologies if desired.
5. Labeled stations should be set up with 1 microscope, 1 pasteur pipette, some slides, some coverslips, and one culture flask (that does not need to be kept afterwards). The students should make the wet mounts themselves unless the organism is particularly difficult to handle.
Fall 2021 Final Lab Report Rubric and Guidelines
(Note, in this version of the course, the salinity experiment was not performed, and the minimal medium experiment did not work- if those are added, this rubric will need modification)

Introduction – 20 points

Introduce the topic of environmental microbiology/marine microbial ecology (6 points)

Introduce the topic of the importance of cultivation (2 point)
This will be required and emphasized for the final lab report. *
No mention of it – 0 points

What type of organism is US3C007? – (2 point)
No mention – 0 points

Where was US3C007 isolated? (2 point)
No mention – 0 points

What is the type of system? (2 points)
Marine environment – (2 point)
No mention of environment type – 0 points

What is the motivation for this investigation? (6 points)
They provide a generic motivation without any context – 3 points
No mention of motivation – 0 points

Methods – 20 points

Sterile/aseptic technique mentioned? – 2 points

Carbon/Nutrient stocks – 6 points
Why did we make these/what experiment are they for? – 2 points
Concentration of stock/volume of water – 2 points
Filtering mentioned – 2 points

Minimal media – 6 points
Volume of stock to each well – 1 point
The matrix format written or in a table (recommended in a table) – 2 points
Repeated the plate 3 times – 1 point
The volumes of culture/base medium to each well – 2 points

Temperature – 6 points
Type of media (Artificial Seawater) used – 2 points
Volume of media/volume of culture inoculated – 2 points
Incubation conditions (temperatures) – 2 points

Results – 20 Points

*Note: All figures must have the appropriate figure captions. The captions provide your reader with the information needed to interpret the figure. Think of it like a map legend.

● Temperature Growth Curve with growth rates on plot and thorough, appropriate figure caption – 4 points

● Section for Minimal Media Results: no tables necessary since this did not work. Just explain why all your wells were negative in terms of cell density, provide your results from the experiment, and give the results
of your controls. Remember, you are stating and explaining how you got your results in this section and will be interpreting them in the discussion. – 8 points

- Section for Temperature Results: Give the results of the temperature experiment. Provide the equations for how you calculated the growth rates. What did we find? What are the growth rates? What are the cell concentrations at the beginning and end of the experiment? Reference your figure(s) from R in this section. Remember, you are stating and explaining how you got your results in this section and will be interpreting them in the discussion. – 8 points

Discussion - 20 Points

Here is where you will interpret your data and address trends that you notice. I am not looking for a specific answer, but I want you to form conclusions based on the data and to explain why you believe this to be true. You should use this discussion section to tie the results from our experiments into the bigger picture. How do the results from the temperature experiment connect to the ocean environment? What do these results mean in the context of ecology? How does this relate to other Roseobacters? How do the results connect to biogeochemistry? Basically, you should be connecting your temperature results back to the primary literature sources to make sense of the trends that we see.

For our minimal media data, we cannot draw conclusions about the organism because there was an experimental failure, but we can discuss things like: Why did this not work? What were some likely causes of the organism not growing? What would we do differently next time? What is happening with the controls? Why are the controls important in this case? ETC.

Future Directions – 10 points

If you could continue, what experiments would you like to do? What direction would you take this research?

References – 10 points

- APA format
- Listed in alphabetical order
- All information that is taken from a primary literature source is cited
Minimal Media Experiment:
Checklist for each day of the minimal media experiment:
  o Artificial Seawater (~18 mL, ~1.5mL to each well, 6 wells per plate), verify that organisms will actually grow in it before using
  o Base Media (no nutrients) (~10 mL [account for a little extra], 1.5mL to each well, 3 wells per plate)
  o 2 x 96 deep well u-bottom plates
  o 2 x autoclaved plate lids wrapped in foil
  o mL pipette and mL tips
  o 20 uL pipette and tips
  o 0.2-2 uL pipette and tips
  o The culture (take a count of the seed culture before using it to make sure that it is healthy)
  o PCR hood wheeled over to the undergraduate teaching lab (get someone to help you with this. It is very heavy. I used a cart and slid it off the bench onto the cart, but it is very difficult to do alone.)
  o Falcon tubes of prepared nutrient stocks for each class, check if allowed to store in the classroom fridge.
  o Remind students to bring their goggles, lab coat, and to wear long pants to lab

1) Make sure that you are checking the positive control as the experiment proceeds. After the 2 weeks have passed for the first plate incubation, make sure that you see growth in the artificial seawater positive control. If there is no growth (verify what growth should look like based on the growth rate of the organism being used), the experiment should be started again with new media.
2) I suggest running this plate on the flow cytometer when someone will be around to watch it. It takes a long time to run, but the instructor should make sure that someone will be there to ensure that it does not stop in the middle of the run since it is a big plate.
3) Pipette ~1.5 mL of Base media to each necessary well (see plate matrix) BEFORE the beginning of the lab. The students won’t have time to do this. Verify that this is an appropriate dilution for your given cell count.
4) Make sure your lecture is no longer than ~20-30 minutes. Otherwise, the students won’t have time to add all the nutrient stocks and culture. Do a demonstration for them first on how to use the pipette, how to be sterile in the hood, etc.
5) For the nutrient stocks, use tape on the lid of the falcon tube for the students to write on. This will make the process of them gathering their assigned nutrients much easier on minimal media days.
6) Make sure to thoroughly explain what the controls are on the first day of the experiment.
7) You have one positive control, which is the artificial seawater inoculated with the culture to make sure that culture is fine. The first negative control is the Base media with no nutrients added and the culture. The second negative control is the artificial seawater with no culture added.
8) Use a tray (maybe an autoclave tray if available) to carry everything over
9) Assign students to the plate matrix prior to class
10) Change the order of students each week so that the same students are not going last for every minimal media plate transfer.

Temperature Experiment:
Checklist on what to bring to lab:
  o Culture (take a count before to make sure it is healthy)
  o Small Erlenmeyer flasks filled with 50mL of sterile artificial seawater before the start of lab, use a tray to carry them to the classroom
  o 20 uL pipette and tips (for culture, calculate what cell concentration to dilute to)

1) Make sure that once the students inoculate the flasks, that the flasks remain upright and don’t fall over. This can be difficult since there are so many. You will need a tray to carry them back to the Thrash lab.
2) Remind the students of the sterile and aseptic technique that they have learned
3) Explain the process of the experiment to the students since they don’t see what happens behind the scenes: incubating for _____ days at ___ number of temperatures, replicates at each temperature, taking daily counts on the flow cytometer, etc.

4) Check the daily counts for contamination/expected growth

Morphology and Microbial Characterization Lab (Made by Catie Cleveland, contact cselevel@usc.edu if you have any questions):

Note: Organisms can be switched out depending on what is available to the instructor. The main goal of this lab activity is to get the students to observe and think about microbial morphology

Checklist:
- Diatoms (Pseudo-nitzchia or other types)
- Dinoflagellates (Alexandrium)
- Crocosphaera species
- Trichodesmium erythraeum and thiebautii
- Bucket of ice to keep diatoms and dinoflagellates semi-cool
- Pasteur pipettes for each station
- Tube racks for the cultures in tubes
- Microscope slides (also need a few depression slides for the motile organisms)
- Cover slips
- Microscopes for each organism at stations (cabinet under benches)
- Morphology guide (post or print for students)

1) Have students follow the Morphology guide instructions
2) Let them work in groups of 2 to walk around to the labeled stations (station A, B, etc.) and identify the organisms
3) Some can work on identification while some can work on the web search assignment portion
4) Each group needs to make their own slides
5) Help them spot the motile Dinoflagellates, they are fast! Dinoflagellates may concentrate at the bottom of the bottle, so shake them up every now and then for the students.
6) Highlight the importance of morphology and microbial characterization. I drew a diagram on the board with different types of morphology based on their guide, and asked them “What are some different cell shapes? What are some colony shapes?” Etc.
7) Help them with using the microscopes.
8) Make sure the students know which organisms are bacteria and which are not.
Example student carbon plate results for LSUCC0117.

| Carbon Source          | Plate 1 | Plate 2 | Plate 3 |
|------------------------|---------|---------|---------|
| Threonine              | NA      | +       | +       |
| Tyrosine               | NA      | +       | +       |
| Valine                 | NA      | +       | -       |
| Arginine               | -       | +       | +       |
| Cysteine               | -       | -       | -       |
| Histidine              | -       | -       | -       |
| Isoleucine             | -       | -       | -       |
| Leucine*               | +       | +       | +       |
| Lysine*                | +       | +       | +       |
| Methionine*            | +       | +       | +       |
| Phenylalanine          | +       | -       | -       |
| LSUCC0117 + ASM        | +       | +       | NA      |
| LSUCC0117 + ASM        | +       | +       | NA      |
| LSUCC0117 + ASM        | +       | +       | NA      |
| LSUCC0117 + ASM        | +       | +       | NA      |
| Glutamine              | +       | -       | -       |
| Dextrose               | -       | -       | -       |
| Ribose                 | -       | -       | -       |
| Pyruvate               | -       | -       | -       |
| Citrate                | -       | -       | -       |
| Glutamate*             | +       | +       | +       |
| Acetate                | -       | -       | -       |
| Succinate*             | +       | +       | +       |
| a-ketoglutaric acid    | +       | +       | -       |
| Urea                   | +       | -       | -       |
| Glycine                | -       | -       | -       |
| Choline chloride salt  | -       | -       | -       |
|                          | A1 | A2 | A3 |
|--------------------------|----|----|----|
| Cyanate potassium salt   | -  | -  | +  |
| Sucrose *                | +  | +  | +  |
| Fructose                 | +  | +  | -  |
| Glucose                  | +  | +  | -  |
| Ornithine                | +  | +  | -  |
| Serine*                  | +  | +  | +  |
| Folic Acid*              | +  | +  | +  |
| L-Tryptophan             | -  | -  | -  |
| LSUCC0117 + ASM-C        | +  | +  | -  |
| LSUCC0117 + ASM-C        | +  | +  | -  |
| LSUCC0117 + ASM-C*       | +  | +  | +  |
| LSUCC0117 + ASM-C        | -  | +  | -  |
| ASM-C                    | -  | -  | -  |
| ASM-C                    | -  | -  | -  |
| ASM-C                    | -  | -  | -  |
| ASM-C                    | -  | -  | -  |
| LSUCC0117 + ASM          | -  | +  | NA |
| LSUCC0117 + ASM          | -  | +  | NA |
| LSUCC0117 + ASM          | +  | +  | NA |
| LSUCC0117 + ASM          | +  | +  | NA |

*Indicates sources that were positive for all three transfers. ASM is an abbreviation for Artificial Seawater Medium.
Appendix 29: Example informal essay submissions

Student #1 Essay #1—"I am an animal science, pre-vet major. My goal is to eventually earn my DVM and a PhD in either small animal soft tissue surgeries or exotics. I am very interested in research, particularly in studies that better our understanding of animals… I haven’t heard much about CURE labs other than the fact that they are a great way to get your feet wet in undergraduate research. My only worry for this course is that I won’t be able to keep up. However, I know I will try my best to be as successful as possible. I am very excited to be a part of a real research lab making new discoveries instead of doing “cookie cutter” labs and getting the same results as thousands of students before me. I hope to obtain a better understanding of how research is conducted in real life as well as establish connections that I can use one day whether it be for undergraduate research or for my honors college thesis. I think that research experience applies to my goals because it will teach me to look at things from a scientific standpoint. Having a better understanding of how scientific research works may allow me to conduct my own research as a veterinarian."

Student #1 Essay #2—“Overall, I felt that this class greatly enhanced my understanding of the scientific world and how research is conducted. Although it was somewhat more difficult, I feel that this class was a better opportunity than its regular lab counterpart. It was far more interesting to observe and record data that had not been found before as opposed to doing a lab done by thousands of bored students before me. I enjoyed learning lab techniques like the culture transfers as well as how to build presentations for the class. It was very interesting and a challenge to go in depth in such a niche area of research, and I feel that it better prepared me for both future lab classes and any research I may do in the future. I struggled with the communication aspect, particularly presenting, but I feel that I improved my public speaking abilities and built more confidence than I had before.”

Student #2 Essay #1—“I am currently majoring in Human Movement Science with a concentration in Kinesiology Pre-med. As of now, I plan to minor in Biology as I hope to continue my education by attending medical school. After medical school, my goal is to finish as a pediatric anesthesiologist. During my years at [high school], my science classes were always based on the simple science experiments. I never had the chance to learn about real research. When I think about the term “research,” the first thing that comes to mind is a lab rat spinning on a wheel and a scientist with big, frizzy hair. As I was receiving emails about my classes before school started, I learned that my … class was a “CURE” lab, and I never heard of “CURE” before. I am very excited to be a part of current research that nobody has ever done before, which also makes me nervous at the same time. I have never done lab research, so jumping straight into a current research project scares me because it may be a lot of hard work for me to handle for my first time. After I attended my first lab, my nerves were gone. I learned that it is okay to mess up because there is no certain outcome that should happen with current research. Once this semester is finished, I hope to expand my excitement about research, and obtain a student job… for my undergraduate research project. I am very confident that the research hours I will have under my belt going into medical school will help me push through and finish with a high amplitude of experience as I earn my Doctorate.”

Student #2 Essay #2—“I was very intimidated about CURE research. Now that I am going into the last week of this class, I am very honored to have been able to work with it. I have many friends who are enrolled in [the regular section], and all they do is write charts down and work with results that have already been found. Knowing that I could’ve been stuck with working in those labs makes me even more happy that I was a part of the CURE lab. Working with a new bacterium was a great experience. I definitely believe that this CURE lab has prepared me for all types of research that will come up for my future lab classes. Not only did I learn how to conduct the experiments in the lab, I was able to learn how to create a well-organized and professional poster along with a lab report. I learned the rights and wrongs of presenting in front of people and received feedback of my presentation by being videoed. I would recommend to any biology student to take a CURE lab at least once. It has prepared me for the future and developed my knowledge and skills in ongoing research. After completing every experiment and characterizing LSUCC0135, I truly feel like a researcher, and I have been a part in helping define such a cool bacterium that is very close to my home. I would love to thank CURE for giving me this great opportunity!”
Appendix 30: Example student poster

Characterization of Carbon Substrate Usage, Temperature, and Salinity of Coastal Louisiana Isolate LSUCC0135

Names removed
CURE Students in the Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, 70808

Introduction

The results of this study highlight the potential of the global oceanic formation of their ability to cycle essential elements through the oceans. In the open ocean, isotope ratios of carbon, nitrogen, and silicon are used to indicate the efficiency of the biological pump. The isotopic composition of the carbon compounds produced by marine microorganisms reflects the nutrient input, the metabolic pathways, and the oceanic circulation patterns. The aim of this study is to investigate the potential of coastal Louisiana isolates to cycle carbon substrates.

Methods

Carbon Substrate Usage

The carbon substrates used in this study were acetate, glucose, and glycerol. The isolates were grown in a series of media containing these substrates. The growth of the isolates was monitored by measuring the optical density at 600 nm.

Temperature

The isolates were tested for growth at temperatures ranging from 4°C to 37°C. The isolates showed optimal growth at 25°C and 30°C.

Salinity

The isolates were tested for growth at salinities ranging from 0% to 35%. The isolates showed optimal growth at 25% salinity.

Results

Figure 1: Growth curves of LSUCC0135 grown on acetate, glucose, and glycerol at 25°C and 30°C. The isolates showed optimal growth at 25°C and 30°C.

Discussion

The results show that the isolates are capable of utilizing a variety of carbon substrates. The isolates showed optimal growth at 25°C and 30°C. The isolates are also capable of surviving in a wide range of salinities, with optimal growth at 25% salinity.

Future Directions

Further studies are needed to investigate the metabolic pathways of the isolates and their potential for carbon cycling in the ocean. The isolates could be used in biotechnological applications for the production of biofuels and other valuable products.

References

1. Mohn, M., Guatte-Bewley, G., and Dugan, M.A. (2018) The isolation and characterization of a new marine isolate. J. Bacteriol. 4, 1-10.

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