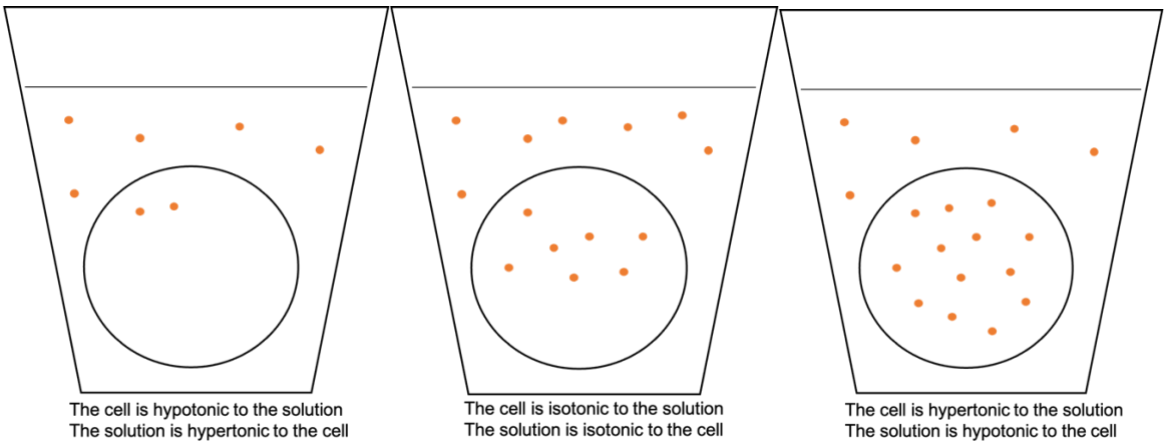
Cells and Diffusion

Part 1: Osmosis and Tonicity

We use the concept of **tonicity** to compare solutions to each other. A **hypotonic solution *contains less solutes than the solution it is being compared to***. A **hypertonic solution *contains more solutes than the solution it is being compared to***. An **isotonic solution *has the same amount of solute as the solution to which it is being compared.***



An easy way to remember this is that "hypo" means "low" (for example, "hypothermia" means "*low* temperature") so hypotonic means *lower* solute content. "Hyper" means "high" (for example "hyperactive" means "high activity"), so hypertonic means a *higher* solute content.

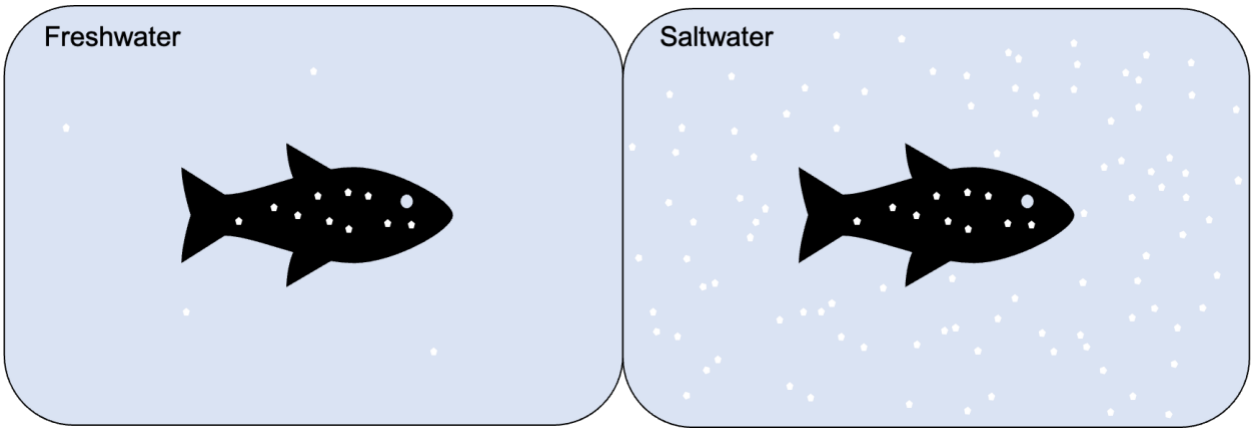
Remember that the words hypertonic and hypotonic only apply when a solution is being *compared* to another solution. You can't say a solution is hypertonic if it isn't being compared to anything.

In our next experiment, we will be looking at the impact of tonicity on osmosis. Remember that osmosis occurs when only the **solvent**--water--diffuses across the selectively permeable membrane while the **solute** does not.

Cells are essentially just a solution of water and various solutes (salt, glucose, etc.) inside a semi-permeable membrane. If a cell is either hypertonic or hypotonic to its environment, it will either lose or gain water, and this may cause the cell to either shrivel up or burst.

On a simplistic level, you can even think of organisms as a solution of water and solutes inside a selectively-permeable membrane of skin or other outer coverings (though most animals have skin that is less permeable than the plasma membrane of a cell). Maintaining the correct balance of water and solutes within the body is a very important part of maintaining vital life processes.

Two real-world examples of this are saltwater and freshwater fish. A freshwater fish has a higher concentration of solutes inside its body than the water outside its body, while a saltwater fish has a lower concentration of solutes inside its body than the very salty water outside its body (salt is the solute dissolved in the water).



In this experiment, we will look at the impact of tonicity on osmosis. You will start with dialysis bags that contain a 10% salt solution. Salt cannot pass through the selectively permeable dialysis membrane, so the only molecule moving in this experiment will be water.

The 10% salt dialysis bag will function as a cell or organism being placed into environments with different levels of dissolved solutes. We will place the bag into solutions with 0% (distilled water), 10%, and 20% salt solutions and measure the amount of water gained or lost by the bag.

While you are waiting to weigh your bags, discuss and answer the following questions with your lab group:

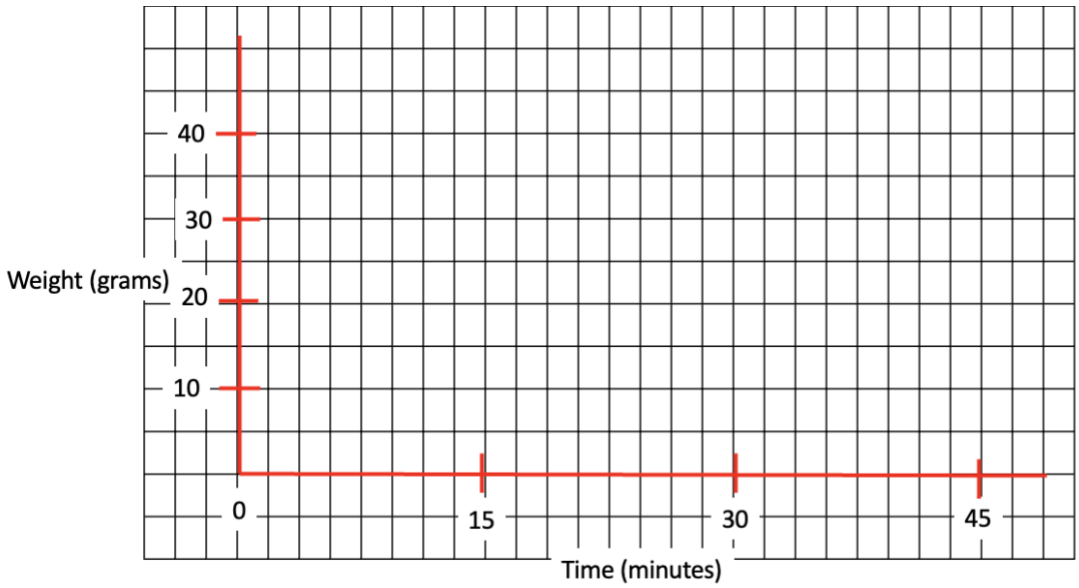
1. Which bag do you expect to gain the most weight? Which bag do you expect to gain the least weight? Why?

2. Are there any bags that you don't expect to change in weight? Why?

3. Based on your prior knowledge, how do you think large gains or losses of water would impact living organisms?

Procedure:

1. Obtain a section of soaked dialysis tubing and tie one end shut (like a water balloon). Tie the knot as close as possible to the end of the tubing.
2. Open the untied end of the dialysis bag and half-fill it with a **10% salt solution** (red).
3. Gently squeeze the bag to expel air.
4. Twist the open end of the dialysis bag and tie a knot as close as possible to the end of the tubing.
5. Tie a piece of thread around one of the knots. Make sure it is long enough to drape over the edge of the beaker so you can lift the bag out of the liquid.
6. Prepare two other bags in the same manner, all with the **10% salt solution** (red).
7. Thoroughly rinse the outside of each bag with water. Blot excess water off of each bag with a paper towel.
8. Weigh each bag one at a time and record the weights in a table.
9. Fill three beakers, one with each of the following solutions: **deionized water (0% salt)** (yellow), **10% salt** (red), and **20% salt** (blue). Place one dialysis bag in each beaker.
10. After ***15 minutes***, individually remove each of the bags from its beaker. Blot off any excess solution with a paper towel.
11. Record the weight of each bag. Only remove one bag at a time to ensure that the correct bag goes back in the same beaker.
12. Repeat this procedure (weighing the bags every 15 minutes) twice more for a total of four weight recordings (starting weight, 15 minutes, 30 minutes, and 45 minutes).
13. When you have finished, cut each dialysis bag and pour the contents of the bag and the beaker down the sink. Throw away the empty dialysis bags.
14. Create a **line graph** in your notebook showing the changes in weight over time.



Your line graph should be set up similarly to the image above (you may need to adjust the numbers on the Y-axis to match your data)

While looking at the results of your experiment, discuss and answer the following questions with your lab group:

1. Do the results of your experiment support your prediction? Why?

2. How do the different environments into which we placed our dialysis bags relate to different environmental conditions in the real world?

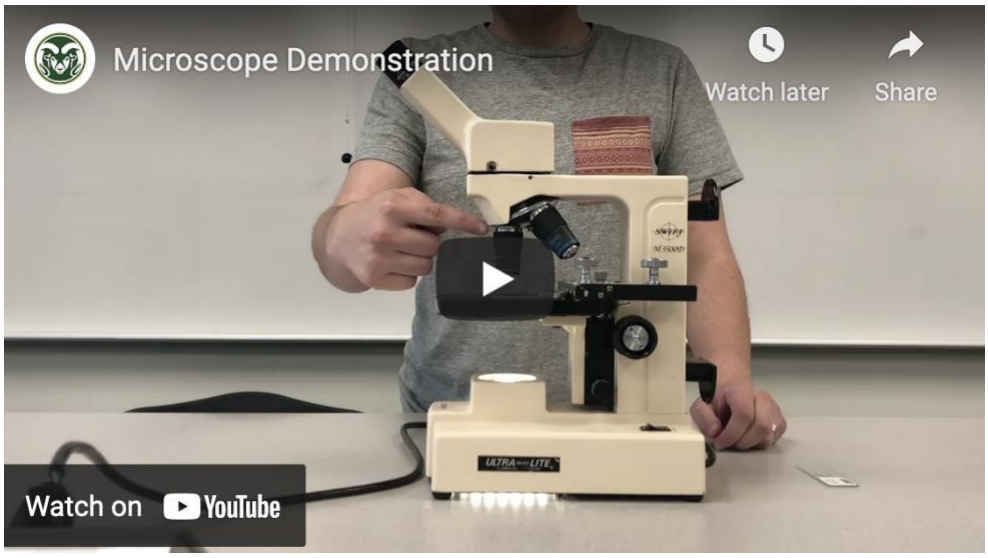
3. How do you think the water loss or gain shown in this experiment would impact a real living cell or living organism?

4. Why do you think freshwater and saltwater fish are able to live in environments that have a much higher or lower level of dissolved solutes than their bodies? Brainstorm some adaptations fish might have to deal with these conditions.

Part 2: Cells and Microscopes

Before you begin this activity, your TA will review how to use a compound microscope and you will practice using a microscope to view a newsprint slide.

Remember the basic rules and guidelines of microscopy:



Rules of Microscopy:

1. Always use two hands to carry the microscope, one on the base and one on the arm.
2. Always start on low power
3. Always look from the side at objective lens when changing from one power to another
4. Use only fine adjustment knob when using the longest (highest magnification) objective lens

Basic Microscope Procedure:

1. Plug the microscope in.
2. Switch microscope on (check that light is functional).
3. Lower the stage all the way (using objective knob)
4. Start with the lowest (4x) objective lens. You will know the lens is in place when you hear a click.
5. Put the slide on the stage (label right side up). Gently place the slide into the stage clips.

6. Use the mechanical stage control to position the object on the slide over the light source.
7. Raise the stage as high as it will go without touching the lens (using adjustment knobs).
8. Use the coarse adjustment knob to obtain clarity (except at highest magnification).
9. Use the fine focus adjustment knob to get the image as focused as possible.
10. Once the image is focused and centered on lower magnification, practice using the higher objective lenses (10x and 40x).

Now that you have explored osmosis and diffusion using models, you will observe how differences in concentration gradient can impact real cells.

You will view the following cells:

•human cheek cell

•*Elodea* (a freshwater plant) cell

•red onion cell

•*Chlorella* (freshwater algae) cell

Each of these cells will be exposed to the following three conditions:

•distilled water (100% pure water)

•20% salt solution

•regular tap water

Remember that tap water is not 100% pure water and contains a moderate amount of dissolved solutes.

Before you begin, discuss and answer the following questions with your lab group. It may help to use a table, mind map, or diagram to organize your answers.

1. Which cellular structures do you expect to find in each of these cells? What are the functions of each of these cellular components?

2. Consider the environment that each of these cell types would naturally be found in. How might this impact what kind of concentration gradient each cell is are adapted to?

3. What do you predict will happen to each of these cells in each of the conditions listed? Justify each of your predictions.

Begin by creating a wet mount of each cell type using *regular tap water* and the procedures provided below. Each member of your lab group should create one of the wet mounts.

How to Create a Cheek Cell Wet Mount:

1. Place a drop of water on a microscope slide.
2. Use the flat end of a toothpick to gently scrape the inside of your cheek.
3. Swirl the toothpick in the drop of water on the slide. (Dispose of the toothpick in the container of bleach solution).
4. Use a dropper to put half a drop of methylene blue stain on the wet mount.
5. Balance a cover slip on the slide at the edge of the liquid at a 45-degree angle.
6. When the liquid has spread across the edge of the cover slip, gently lower the cover slip on to the specimen.

How to Create An Elodea Leaf Wet Mount:

1. Place a drop of liquid in the center of the slide.
2. Place a small piece of specimen (*Elodea* leaf) in the liquid. Cells are easier to view with a very thin piece of Elodea.
3. Balance the cover slip on the slide at the edge of the liquid at a 45-degree angle
4. When the liquid has spread across the edge of the cover slip, gently lower the cover slip on to the specimen.

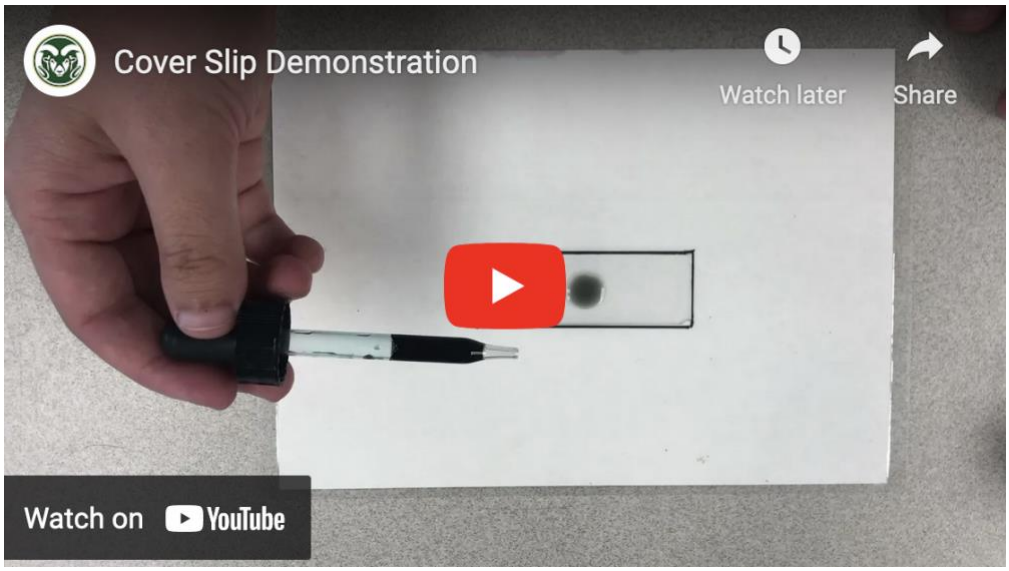
How to Create a Red Onion Wet Mount:

1. Place a drop of liquid in the center of the slide.
2. Using forceps, remove a small, thin piece of the red or purple pigmented layer of a red onion. Place the piece of onion skin in the liquid
3. Balance the cover slip on the slide at the edge of the liquid at a 45-degree angle.

4. When the liquid has spread along the edge of the cover slip, gently lower the cover slip on to the specimen.

How to Create a Chlorella Algae Wet Mount:

1. Agitate the algae culture provided to suspend the algae. Place a small drop of algae culture in the center of the slide.
2. Add a drop of liquid to the algae culture.
3. Balance the cover slip on the slide at the edge of the liquid at a 45 degree angle.
4. When the liquid has spread across the edge of the cover slip, gently lower the cover slip on to the specimen.



As you view each specimen, create a labeled sketch of the visible structures in your lab notebook.

Next to your sketch, be sure to label the magnification (40x, 100x, 400x) at which you made your sketch.

Discuss and answer the following questions in your lab group:

1. What do these cells have in common? How do they differ?

2. What do the organisms these cells come from have in common? How do they differ?

3. What advantages and disadvantages do you think being part of a multicellular organism has for a single cell?

4. Think about your prior knowledge about where plants and animals obtain energy from. How do the structures found in plant and animal cells reflect their differences in energy acquisition?

Create another wet mount for each cell type, but this time using the dropper bottle with *20% salt solution* instead of tap water. Note that you will need to view the human cheek cell quickly, as the salt will react with the methylene blue and crystalize.

View each of the new wet mounts, then discuss and answer the following questions with your lab group:

1. How did the appearance of these cells change (or not change) when exposed to 20% salt solution? If desired, you may draw the different cell appearances rather than describing them.

2. Why do you think these changes occurred? What is the tonicity of each of these cells compared to the 20% salt solution and how is water moving in relation to the cells?

3. Did there seem to be any relationship between the environment that each cell type is adapted to and how they responded to the 20% salt solution?

4. Based on your observations, do you think the starting concentration of solutes inside each of these cells was more or less than 20%?

Create one final wet mount for each cell type, this time using the dropper bottle with *distilled water*.

View each of the new wet mounts, then discuss and answer the following questions with your lab group:

1. How did the appearance of these cells change (or not change) when exposed to distilled water? If desired, you may draw the different cell appearances rather than describing them.

2. Why do you think these changes occurred? What is the tonicity of each of these cells compared to the distilled water and how is water moving in relation to the cells?

3. Did there seem to be any relationship between the environment that each cell type is adapted to and how they responded to the distilled water?

Part 3: Real World Applications

For your final activity, you will apply your new knowledge to two real-world issues. Your group can either work through this entire activity together, or split the questions among group members, then share and discuss your findings with the entire group. Note that every group member is responsible for knowing about all aspects of these issues, not just the part that they researched.

Issue 1: Road Salts

Review the following paper: Road Salts, Human Safety, and the Rising Salinity of our Fresh Waters by William D. Hintz, Laura Fay, and Rick A. Relyea.

Note that you do not need to read or understand the entire paper in order to gain useful information from it. Start by reading the abstract, "In a nutshell" section, and reviewing the figures (graphs, diagrams, graphics, etc.) and figure captions. If you are confused about a term or concept used in the paper, you can use the internet to learn more about it or ask your TA for help.

Using the information in the paper, discuss and answer the following questions with your group:

1. What impact does salinization have on organisms living in freshwater lakes and streams? Based on what you have learned in this week's lab, why do you think this occurs?

2. Salinization of freshwater is particularly damaging when it occurs in concert with drought that reduces the water volume of lakes, reservoirs, etc. Why is this?

3. What are some solutions to this issue suggested in the paper? If interested, you can do further research to see if you can find other solutions that have been proposed or put into use.

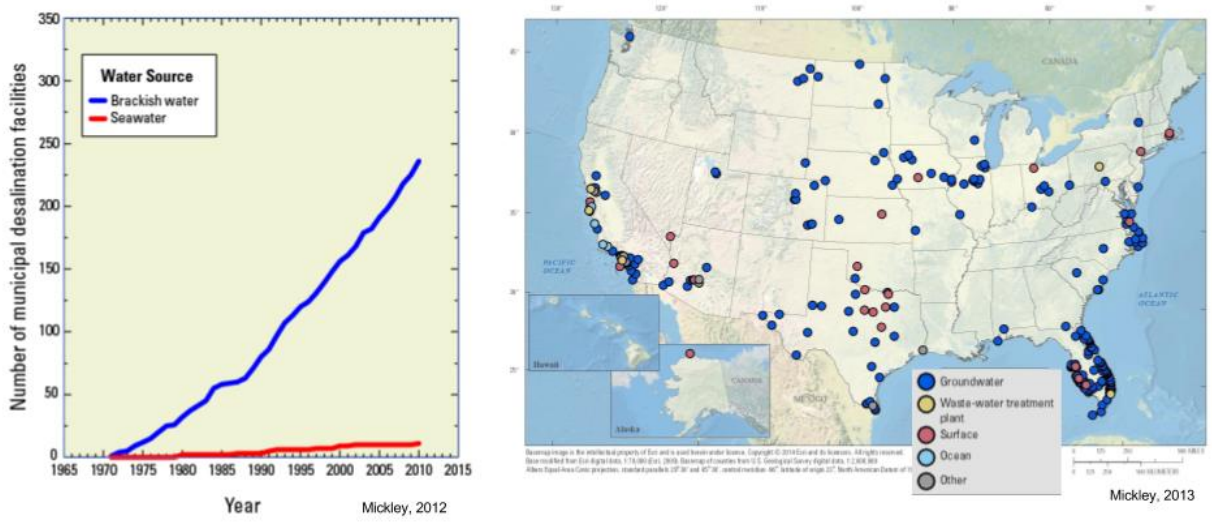
4. Why would it be a problem to simply stop using road salts altogether?

Issue 2: Reverse Osmosis and Desalination

Due to drought, pollution and salinization of water sources, increased demand for water, and other factors, water scarcity is becoming a more common issue in much of the world. One possible solution is **desalinization**, or ***removing salts from***

brackish (saltier than freshwater but less salty than saltwater) or seawater to create clean, freshwater.

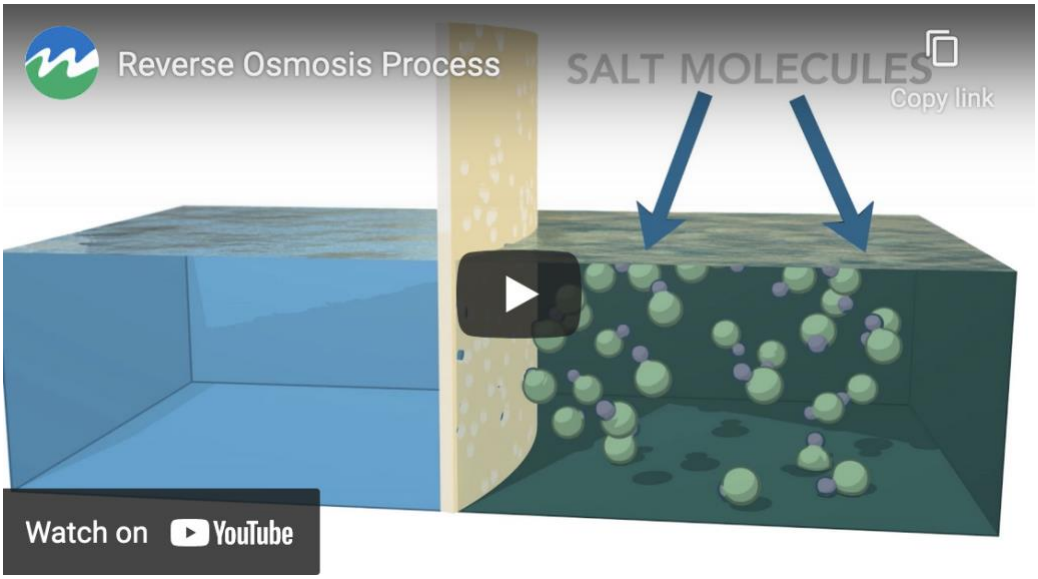
The following [graph from the United States Geological Survey] shows the change in use of desalination in the United States in recent years.



Use of desalination technology in the United States

Source: United States Geological Survey

One of the main methods of desalination is reverse osmosis. Watch the short video below to get an overview of how this technology works.



Using the information provided above, discuss and answer the following questions with your group:

1. How has the use of desalination in the United States changed in recent years?

2. Compare and contrast the processes of reverse osmosis and osmosis. What is different about these processes and what is similar?

3. Based on your knowledge of osmosis, concentration gradients, etc., would you predict that the reverse osmosis process uses a small or large amount of energy? Why?

4. Make a list of pros and cons of using reverse osmosis to make freshwater from brackish water or saltwater. If you are having trouble coming up with a list, it may help to do some additional research on your lab computer or personal device.