Lab 1:
Introduction, Formulating Questions and Hypothesis, pH, Salinity and Oxygen Content of Seawater

Introduction

An important aspect of any scientific discipline is the search for answers to properly formulated questions. In this class, questions will center on the structures and related functions of marine organisms and how these organisms relate to each other and to the remainder of their environment. For each lab activity, we will develop questions and try to answer them by the appropriate experimental procedures.

Our experiences and biases shape our perception of the world. Sometimes, some of these experiences and biases will be in direct conflict with information and concepts we find in our experiments. Resolving these conflicts and understanding how science actually operates will require a variety of critical-thinking skills, which this course shall help to acquire:

- Identifying critical issues
- Distinguishing fact from opinion and belief from knowledge
- Evaluating questions
- Relating causes to effects and reasoning from observation to explanation
- Recognizing underlying assumptions, clichés, stereotypes, and biases
- Distinguishing between relevant and irrelevant information
- Recognizing and judging the reliability, adequacy, and accuracy of data
- Drawing justifiable conclusions supported by facts
- Determining the validity of an argument
- Applying conclusions to new contexts

These skills are not unique to science but are common to problem-solving approaches in many human activities. Mastering these kinds of skills will also prove helpful in solving every-day problems. In scientific studies, investigations usually fall into one of two general categories of problem solving: descriptive and experimental approaches. Both are useful, and you will encounter both approaches during this lab course. Each investigational approach begins with a question to which an answer is sought. After that, though, the two approaches, descriptive and experimental, become quite different.

The Experimental Approach

The experimental approach is usually applied to what-if questions. To resolve these questions, the what-if scenario should be experimentally accessible, i.e. the “if” condition should be able to be experimentally controlled and monitored to observe the “what” effect. A simple example would be a question such as How will the oxygen concentration of seawater change (“what”) under intensified global warming (“if” = increased water temperature)? This question can be investigated in an experimental approach if seawater temperature (“if” condition) can be changed in a controlled manner and oxygen concentrations can be measured under the changed conditions (“what” effect). The experimental approach generally assumes the following format:

1. A specific **question** is formulated.
2. A **hypothesis** is developed as a preliminary, tentative answer to the question based on the information available at the time. If no information is available, the hypothesis is often little more than an educated guess. But don’t become enamored of your hypothesis; you must be ready to discard or modify your hypothesis as new information arises.
3. An **experimental procedure** is a devised to test, prove or disprove the hypothesis. As to the design of any experiment, any experiment is only as good as its control.
4. The process of running the experimental procedure (short: experiments) will provide experimental **results**, usually some time of data that may need further treatment to improve their usefulness.
5. From the experimental results, some **conclusions** may be drawn. The conclusions should either support or reject the hypothesis, providing at least a partial answer to the question that initiated the process. The conclusion derived from a single experiment may deny, but can

never absolutely prove the hypothesis. This is the principal reason why conclusions in science must remain tentative and are subject to being modified as additional information becomes available (“There is no absolute truth in science”!).

The Descriptive Approach

The descriptive approach typically deals with questions for attributes, such as: What color is it? How many are there? Descriptive approaches usually do not aim to explain a fact but rather gather information on something we don’t know enough about to develop an experimental approach. Often, new fields of research start with descriptive approaches.

1. The process is again initiated with the formulation of a specific **question** to be answered.
2. However, it is pointless to attempt a tentative answer, or hypothesis, to this type of question. Instead, the question leads directly to the experimental procedure (measuring activity).
3. **Results** are gathered, summarized, and treated as in the experimental approach.
4. From the results, a description of the system under study is created. As there is no hypothesis, there can be no hypothesis testing (proving – disproving). This approach usually concludes with a “This is the way it is” statement.

Experimental Work

The pH of Seawater

Acid rain has become a critical problem in some parts of the world, and many freshwater habitats are severely affected. Acidification of lake water can result in dramatic changes in the living communities, both in the plankton and the benthos, and some severely affected lakes in southern Sweden are almost completely deprived of their natural inhabitants because only few species can survive under acid conditions. Acid rain is produced when sulfur and nitrogen compounds from the burning of fossil fuels mix with water vapor in the atmosphere. Eventually, these acids are washed out of the atmosphere by precipitation and accumulate in ponds, lakes, and streams.

Background information: The pH of a solution is a measure of its hydrogen ion (H^+) concentration (more correctly: H_3O^+). Values on the pH scale range from 0 to 14. The pH value itself is the negative log of the H^+ concentration: A solution with a pH of 7.0 contains 10^{-7} mol H^+ ions per liter; a solution with a pH of 9.0 contains 10^{-9} mol H^+ per liter. Thus, solutions with a *lower* pH contain *more* H^+ ions and are more acid than solutions with a higher pH. A solution with a pH of less than 7 is considered acidic, a solution with a pH of more than 7 basic or alkaline. Pure water has a pH of 7.0 (neutral); the pH of seawater is slightly above 8, whereas lake water usually exhibits a pH slightly below 7.

This exercise poses a specific problem related to the issue of acid rain in marine systems: Is acid rain a serious problem for marine ecosystems? The research **question** we formulate for this exercise is: *Is seawater more sensitive to pH stresses than freshwater?* Determine whether this question calls for an experimental or a descriptive approach. If it calls for an experimental approach, formulate a **hypothesis** for this exercise, state the hypothesis in your lab manual and reason (with information that you have) why you come to this hypothesis.

Experiment:

Variations of pH can easily be studied using an electronic pH meter. This instrument is convenient to use and rapidly provides accurate results. The instructor will discuss the operation of the instrument. Since pH sensors need some time to equilibrate, the instruments will be turned on 30 mins prior to the class with the probe in pH 7 buffer. Each research group will be provided with one water sample. We will study the effect of acidification on seawater samples of different salinity (marked on your water sample bottle) from Biscayne Bay, Lake Maule, and “Deep Station” (4 miles offshore Haulover Inlet); for comparison, one group will use distilled water. After the experiment, we will compare the results for the different water samples. In your lab journal, note the origin and salinity of the water samples of the other groups and formulate your hypothesis as to the effect of salinity on the sensitivity of seawater to pH stresses.

1. Measure 100 ml of provided water sample with a volumetric flask. In your lab manual, discuss why you are using a volumetric flask instead of a measuring cylinder.
2. Place 100 ml of seawater sample in a 150 ml beaker, place beaker on magnetic stirrer and add stir bar into sample. Following the directions provided by the instructor, determine the pH of your sample. Turn off the stirrer prior to taking the pH reading. Record the pH in your lab journal in a table as given below.
3. With a Gilson Pipetman, add 100 µl of provided 0.1 M HCl (hydrochloric acid) to the sample and turn stirrer on. After ca. 2 mins, turn off stirrer and take pH reading. Record result in your table in your lab journal.
4. Continue to add 0.1 M HCl in 100 µl steps, stir, and take pH reading
5. After completion of your test series, take your table to the central lab computer and type your results in the opened Excel spreadsheet. After all groups have entered their results, the Excel sheet will display a graph showing the pH of the water samples in response to the added amount of hydrochloric acid. Take a printout of the Excel sheet, include it in your lab journal, and discuss the results in your lab journal. Which water sample resisted pH change best? Consult your lecture notes on pH buffering to explain why. Conclude your lab journal entries with a conclusion as to the previously formulated hypothesis and explain your conclusions in the light of your results and what you have learned in this class. Do the results support or deny the hypothesis? Do your results provide a solution to the stated problem, i.e. is acid rain a serious problem to marine ecosystems?

Table 1: pH of seawater sample (100 ml) with addition of 0.1 M HCl

Volume 0.1 M HCl added	100 µl	200 µl	…	1000 µl
pH of sample				

The Oxygen Content of Seawater

All heterotrophic organisms, animal and bacteria, need oxygen to live. This is also the case for those heterotrophic organisms living in lakes and oceans. For their survival, the amount of dissolved oxygen in the water is crucial. Severe fish kills have been reported from highly eutrophied (too much plant nutrients added) lakes and coastal systems. In these systems, the high nutrient load caused a mass development of algal plankton that could not be kept down by the grazing activity of zooplankton. At some point, the algae have utilized all dissolved nutrients and cannot continue to grow. Since the majority of their biomass is not grazed, the algae will die off and settle to the sediment, where they are degraded by bacteria. Bacterial degradation consumes oxygen, and in such cases, the bacterial oxygen demand is so high that the oxygen concentration in the water column becomes too low to support animal life. Fish that cannot escape the lake or bay die, and motile benthic animals such as snails and starfish have been reported to crawl out of the water in search for oxygen.

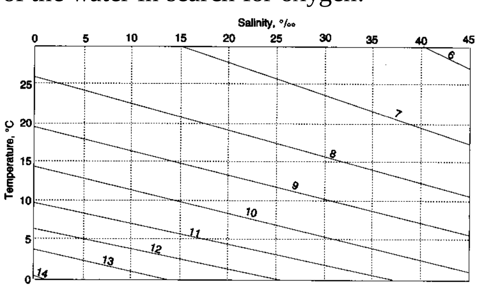

Fig. 1: Temperature-salinity-O_2 solubility diagram for water. Saturated dissolved O_2 values (ppm) are represented by the diagonal lines

The concentration of dissolved oxygen (DO) is greatest in well-mixed waters near the surface and decreases with depth. Phytoplankton production (or macroalgal production in coastal areas) can increase the dissolved oxygen concentration by their photosynthesis. Yet, even in these oxygen-rich areas, the amount of O_2 is small, rarely exceeding 8 parts per million (ppm). In contrast, air-breathing animals obtain O_2 from an atmosphere that is 21% (or 210,000 ppm) oxygen. Since the supply of O_2 in seawater is limited, even small changes in DO availability often affect the well-being of marine organisms.

The amount of O_2 that can be held in solution in water decreases with increased temperature because of an increase in the kinetic energy of the water molecules. The salinity of water also has a bearing on its ability to hold gases in solution owing to the varying ability of freshwater and seawater to form hydrogen bonds. Figure 1 illustrates the relationship between water temperature, salinity, and O_2 solubility.

The standard technique to determine dissolved oxygen in water is the *Winkler titration method*. It is based on the chemical reaction of manganese, which after addition of iodine combines with the dissolved oxygen in the water to form an oxygen-manganese complex; the complex forms a visible precipitate, the dissolved oxygen is “fixed” in this precipitate. The precipitate is then dissolved by sulfuric acid (that’s why most people doing the *Winkler* method have wholes in their lab coats), which frees iodine in an amount proportional to the original amount of dissolved O_2. The iodine is colored with starch (blue), and sodium thiosulfate in known concentration is added until all iodine is reduced (blue color disappears). From the volume of added thiosulfate solution, the original oxygen concentration can be calculated.

The *Winkler* method is still the most accurate way to measure dissolved oxygen concentrations in water. Due to the hazard of working with concentrated acid and the quite laborious nature of the technique, we will not perform oxygen analyses by the *Winkler* titration. Modern electronic oxygen sensors provide us with a convenient, fast, and reasonably precise alternative to measure DO. In this lab, we will use a polarographic oxygen sensor directly linked to a Windows computer system (SensorLink) to measure the dissolved O_2 concentration in our water samples. The instructor will explain the instrument, and measurements will be performed on your samples under the instructor’s supervision. Since only one such system is available, groups have to perform their measurements upon availability, planning their measurements with other groups and using the “waiting time” to perform the pH experiment.

Experiment:

The first part of this exercise is to measure the oxygen concentration in the water samples from the different sampling locations (Maule Lake, Biscayne Bay, Deep Station). The research **question** for this exercise, thus, is: *What is the dissolved oxygen concentration of the water samples provided?* Obviously, there is no reasonable **hypothesis** to propose and this exercise demonstrates a descriptive approach.

The second part of this exercise addresses the solubility of oxygen in relation to water temperature. For this study, the instructor placed water samples of each station at different temperatures (refrigerator, room temperature, oven). You will determine the oxygen content of the water samples of “your” station (each research group is assigned to one of the three stations or the distilled water control and will perform both the pH and oxygen experiments on water samples from this station) at the different temperatures and plot DO concentration versus water temperature. After all groups reported their results, we will discuss the differences in oxygen concentrations in the different samples and see if there is a relation to the samples’ salinity. Discuss in your lab journal if this part of the exercise is a descriptive or experimental approach. If you conclude that it is an experimental approach, formulate your **hypothesis** that you will test by the performed experiment.

1. The instructor will demonstrate the SensorLink oxygen probe system
2. Take your room temperature water sample to the SensorLink station; all water samples for the oxygen experiments are already filled in special glass bottles (BOD bottles; Biological Oxygen Demand bottles that have a special bottle neck to accommodate most oxygen sensors directly; the bottle is sealed against oxygen exchange with the air after the probe has been inserted). Take your oxygen reading, note temperature of your sample and oxygen concentration in your lab journal.
3. Repeat the measurements with your temperature samples by taking one sample at a time (to minimize change in temperature due to exposing the bottles to room temperature). Note the sample temperature and oxygen content in your lab journal.
4. After all samples are measured, type your results in the Excel spreadsheet on the central lab computer.
5. After all groups have typed their results into the Excel spreadsheet, the Excel page will display a graph showing the oxygen concentration of the four different water samples in relation to water temperature. Take a printout of the Excel sheet, include it in your lab journal, and discuss the results in your lab journal. The oxygen concentration in which water sample was most/least sensitive to temperature changes? Is there a relationship between the sensitivity of DO to temperature changes and the salinity of the water sample? Do the results support your hypothesis you developed before? Do your results comply with the expectations derived from figure 1? Also discuss the oxygen concentration in the room temperature samples. Is there a regional difference in DO concentrations among the three sampled stations? Are these differences related to seawater salinity? What other reasons can explain the differences in DO concentrations among the sampled stations?