**FNR 351- AQUATIC SAMPLING TECHNIQUES**
**LABORATORY II: WATER QUALITY**

## 1. Introduction

Water, although the most important component for maintaining healthy populations of fish and other aquatic organisms, is often the most neglected factor. Fish, immature stages of amphibians, and most aquatic invertebrates are totally dependent on water: They derive oxygen from it, and they are in perpetual contact with it. Poor water quality can cause massive mortalities and is often the major factor contributing to disease in both aquaculture and wild situations.

Water quality does not remain constant over time, changing on a daily and seasonal basis (see **Figure 1**). It also varies significantly across different water systems. Thus water quality should be measured on a regular basis.

## 2. Objectives:

The main objectives of this lab are to learn how to:

a. Collect water samples for analysis of "core" water quality parameters (see **Table 1**)

b. Measure "core" water quality parameters

c. Interpret and compare water quality data obtained from different water systems

## 3. Approach:

In this laboratory you will be measuring the "core" water quality parameters which include:

a) Dissolved oxygen (DO)

b) Temperature

c) pH

d) Nitrates and nitrites

e) Total ammonia, ionized ammonia, and unionized ammonia

f) Water hardness

g) Salinity

This handout contains a detailed explanation of how to correctly measure these parameters using the respective instruments.

We will be measuring and comparing the above water quality parameters from the following water systems (due to the recent flooding event, the specific field site might have to change depending on conditions):

a) Small agricultural ditch

b) Flow-trough system

c) Recirculating system

Parameters will be measured from each of these systems by two or three students. Make sure to write down all of the values you obtain (see page 10 of this handout). The last portion of this lab will consist of a comparison and discussion of water quality differences across different water systems by all class members. You will need this data in order to answer some of the questions at the end of this handout. You will be required (and graded) to turn in pages 10 and 11 of this handout. Assignment is due Thursday January 17 during class.

**PORTABLE METERS: Dissolved Oxygen, Temperature, and Salinity**

**Dissolved Oxygen (DO, mg/L) and Temperature (ºC): YSI 55 meter**

1. IMPORTANT: These two parameters need to be measured on site!
2. DO meters need to be calibrated prior to use. Follow these steps for calibration. (In this lab you do not need to worry about this step, since the instrument has already been calibrated).
    a. Ensure that the sponge inside the instrument's calibration chamber is wet. Insert the probe into the calibration chamber.
    b. Turn the instrument on by pressing the **ON/OFF** button on the front of the instrument. Wait for the DO and temperature readings to stabilize (usually 15 minutes is required after turning the instrument on).
    c. To enter the calibration menu, use two fingers to press and release both the **UP ARROW** and **DOWN ARROW** keys at the same time. The LCD will prompt you to enter the local altitude in hundreds of feet. Use the arrow keys to increase or decrease the altitude (Hint: Our altitude is 70 feet). Example: Entering the number 12 here indicates 1200 feet.
    d. When the proper altitude appears on the LCD, press the **ENTER** key. The Model 55 should now display **CAL** in the lower left of the display, the calibration value should be displayed in the lower right of the display, and the current DO reading (before calibration) should be on the main display.
    e. Make sure that the DO reading (large display) is stable, then press the **ENTER** button. The LCD will prompt you to enter the approximate salinity of the water you are about to analyze. You can enter any number from 0 to 40 parts per thousand (PPT) of salinity (Hint: For this lab, we will be using a salinity value of 0.0). When the correct salinity appears on the LCD (zero for fresh water), press the **ENTER** key. The instrument will return to normal operation.
3. Place the probe in your water system at a depth of approximately 10 cm. Wait until the measurements stabilize to read the results.

**pH: Hach sensION 2 pH/ISE meter**

1. pH meters need to be calibrated prior to use. Follow these steps for calibration. (In this lab you do not need to worry about this step, since the instrument has already been calibrated).
    a. Turn the meter on by pressing **I/O**. Press **pH mV** until the display shows pH.
    b. Press **SETUP**. Press the **UP ARROW** three times. Press **ENTER** to toggle to the number of desired decimal places (1), and then exit to leave setup.
    c. Press **CAL**, the display will show **Standard 1?**
    d. Rinse electrode in deionized (DI) water and blot dry using a kimwipe.
    e. Place the electrode in the pH 7.0 buffer solution and press **ENTER**. The display will show **stabilizing…**
    f. When a stable pH is determined it will show **Standard 2?**
    g. Remove the electrode from the cup, rinse with DI water and blot dry. Place electrode in the 4.0 pH buffer and press **ENTER**.
    h. After the last calibration point has stabilized and the display reads **Standard 3?**, repeat using the pH 10.0 buffer.
2. Place electrode in the water sample and press read. The meter will beep once the readings have stabilized.

**Salinity (PPT): VITAL SINE SR-6 Refractometer (Figure 2A)**

Calibration:

1. Prepare a known sample of salt solution and place a few drops on the measurement prism using the same procedure described below. Use the small flat blade screwdriver included in the kit to adjust the value you see on the refractometer to the known salinity of the sample. (In this lab you do not need to worry about this step, since the instrument has already been calibrated).

To take a reading:

2. Place a few drops of your water sample on the measurement prism. Ensure that enough solution is added to the prism in order to cover the entire prism. Close the prism so that the liquid spreads across the entire surface of the prism without air bubbles or dry spots (see (see **Figure 2B**). Allow the sample to remain on the prism for approximately 30 seconds.
3. While holding the instrument under a light source, look through the eyepiece. The salinity concentration is determined by the intersection of the boundary of the light and dark fields (known as the shadowline) on the printed scale (see **Figure 2C**). The left side of the scale indicates the specific gravity and the right side the salinity in parts per thousand (PPT).
4. If the scale appears out of focus, the eyepiece may be adjusted by rotating the knurled portion. The instrument also features an eye guard to prevent stray light from entering the eyepiece and causing reflections. It may be necessary to adjust the position of the light source to maximize the contrast of the shadow-line. Under normal conditions, optimal contrast is obtained by holding the instrument underneath and perpendicular to a light source.
5. Once a reading has been taken, wipe dry with a clean cloth (do not wash or rinse) or kimwipe and place the instrument in the supplied plastic case.

**SPECTROPHOTOMETER (HACH): Nitrate, Nitrite, and Nitrogen/Ammonia (mg/L) (see Figure 3 for a refresher of sources and fate of nitrogenous compounds).**

**Nitrate**

1. Press **HACH PROGRAMS**.
2. Select Program 355 N, Nitrate HR. Press **START**.
3. Fill a round sample cell with 10 mL of your water sample.
4. Add the contents of one NitraVer Reagent Powder Pillow.
5. Cap and shake and press the **TIMER** icon. Press **OK**. A 1-minute reaction period will begin. Shake the cell vigorously until the timer beeps.
6. When the timer beeps, press **OK**. A 5-minute reaction will occur. An amber color will develop if nitrate in present.
7. When the timer beeps again, fill a second round sample cell with 10 mL of DI water (This will be the blank).
8. Place the blank in the Hach and press **zero**. The display will show .000 mg/L $NO_3$-N.
9. Wipe off the sample cell and put into the holder. Press **read**. Results will appear in mg/L $NO_3$-N

**Nitrite**

1. Press **HACH PROGRAMS.**
2. Select Program 371 N, Nitrite LR. Press **START**.
3. Fill a round sample cell with 10 mL of your water sample.
4. Add the contents of one NitraVer 3 Reagent Powder Pillow (this is the test sample).
5. Cap and shake to dissolve for 20 seconds. A pink color will develop if nitrite is present.
6. Press the **TIMER** icon. Press **OK.** A 20-minute time period will begin.
7. When the timer beeps, fill a second sample cell with 10 mL of sample (this is the blank).
8. Wipe the blank (always use a kimwipe for wiping) and place into the cell holder.
9. Press **ZERO**. The display will show .000 mg/L $NO_2^-$ - N.
10. Wipe the test sample and put into the holder.

11. Press **READ**. Results will appear in mg/L $NO_2^-$ - N.

**Total Ammonia, Unionized Ammonia, and Ionized Ammonia**

1. Press **HACH PROGRAMS.**
2. Select Program 385 N, Ammonia Salic. Press **START**.
3. Fill a sample cell (that was previously cleaned) to the 10 mL mark with the water sample you wish to measure.
4. Fill another sample cell with DI water (this will act as the blank).
5. Press the **TIMER** icon. Press **OK**. A 3-minute reaction period will begin.
6. Add the contents of one Ammonia Salicylate powder Pillow to each cell. Cap and shake until all of the powder has been dissolved.
7. When the timer beeps, add the contents of one ammonia cyanurate reagent to each cell. Shake to resolve the reagent.
8. Press the **TIMER** icon. Press **OK**. A 15-minute reaction time will begin.
9. When the timer beeps, place the blank into the cell holder.
10. Press **ZERO**. The display should read 0.00 mg/L $NH_3$–N.
11. Wipe the sample and place it into the cell holder.
12. Press **READ**. Results will appear in mg/L $NH_3$–N.
13. This program is good for .01 to .50 mg/L $NH_3$–N. If the sample is over range, dilute and repeat the experiment.
14. Based on your temperature and pH values, use **Table 2** to calculate the percentage of unionized ammonia in your sample.

**LaMotte Kit (Model PHT-DR-LI): Total Hardness (mg/L) (see Figure 3 for a refresher of sources and fate of nitrogenous compounds).**

1. We will be using a titrator method for measuring water hardness.
2. Fill the test tube to the 12.9 mL line with your water sample.
3. Add 5 drops of **HARDNESS REAGENT # 5** and mix.
4. Add 5 drops of **HARDNESS REAGENT # 6** and mix. A red color will develop.
5. Fill the **DIRECT READING TITRATOR** with **HARDNESS REAGENT # 7**. Insert the titrator in the center hole of the test tube cap.
6. While gently swirling the tube, slowly press the plunger to titrate the sample until the red color changes to blue.
7. Read the test result where the plunger tip meets the titrator scale.
8. Results are expressed as total hardness in mg/L (or parts per million) calcium carbonate ($CaCO_3$). Each minor division on the titrator scale equals 4 mg/L $CaCO_3$.
9. Example: plunger tip is 3 minor divisions below line 80. Test result is 80 plus (3 divisions x 4) equals 92 mg/L.
10. If the plunger tip reaches the bottom line on the titrator scale (200 mg/L) before the endpoint color change occurs, fill the titrator and continue the titration. When recording the test result, be sure to include the value of the original amount of reagent dispensed (200 mg/L).

**Table 1.** Recommended sampling containers and storage procedures for water samples.

| Variable | Container | Volume (ml) | Handling procedure | Analyze within |
|---|---|---|---|---|
| All | Clean or new | | | ASAP |
| Oxygen | Glass stoppered glass | 300 | Fill totally, 4° in dark | 6 hours |
| Temperature | N/A | N/A | N/A | None; must do on site |
| pH | Polyethylene | 100 | 4° C in dark | 6 hours |
| Ammonia; nitrate; nitrite | Polyethylene or glass (NOT $HNO_3$-washed) | 500 | Acidify with 1 ml concentrated $H_2SO_4$/L (to pH <2.0); store on ice or freeze 4° C | 24 hours<br>24 hours |
| Metals | Polyethylene, $HNO_3$-washed | 500 | Acidify with analytical $HNO_3$ to pH <2.0; freeze if analysis delayed | 24 hours |
| Pesticides, other organochemicals | Glass- or Teflon-stoppered glass, hexane-washed (no plastics) | 500 | Fill totally | 24 hours |
| Solids (dissolved; suspended; settleable) | Glass or plastic | 500 | 4° C | |
| Cyanides | Glass or plastic | 100 | Add 0.2 ml of 10 M NaOH, to pH 12 | 24 hours |
| Algae | Glass or plastic | 100 | Fresh chilled, or add Lugol's iodine to color of weak tea or add 10% formalin 1 : 1 | 24 hours |
| Summary (agents unknown) | Polyethylene; $HNO_3$-washed | 500 | Add $HNO_3$ to pH <2.0 | 24 hours |
| | Glass or plastic; (2 samples) | 500 | Freeze one | 24 hours |
| | Glass; hexane-washed | 500 | Fill totally | 24 hours |
| | Glass; hexane-washed | 500 | Fill totally, 4° C in dark | 6 hours |

From Langdon, 1988; Hill, 1983; Boyd, 1979. Suggested time intervals should be considered liberal estimates. Samples may be less stable under some conditions.
$HNO_3$ = nitric acid; $H_2SO_4$ = sulfuric acid; NaOH = sodium hydroxide.

**Table 2.** Percentage of the total ammonia nitrogen (TAN) that is present as unionized ammonia (UIA) at various temperature-pH combinations in freshwater.

| Temperature | pH | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (°C) | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 8.5 | 10.0 |
| 0 | 0.00827 | 0.0261 | 0.0826 | 0.261 | 0.820 | 2.55 | 7.64 | 20.7 | 45.3 |
| 1 | 0.00899 | 0.0284 | 0.0898 | 0.284 | 0.891 | 2.77 | 8.25 | 22.1 | 47.3 |
| 2 | 0.00977 | 0.0309 | 0.0977 | 0.308 | 0.968 | 3.00 | 8.90 | 23.6 | 49.4 |
| 3 | 0.0106 | 0.0336 | 0.106 | 0.335 | 1.05 | 3.25 | 9.60 | 25.1 | 51.5 |
| 4 | 0.0115 | 0.0364 | 0.115 | 0.353 | 1.14 | 3.52 | 10.3 | 26.7 | 53.5 |
| 5 | 0.0125 | 0.0395 | 0.125 | 0.394 | 1.23 | 3.80 | 11.1 | 28.3 | 55.6 |
| 6 | 0.0136 | 0.0429 | 0.135 | 0.427 | 1.34 | 4.11 | 11.9 | 30.0 | 57.6 |
| 7 | 0.0147 | 0.0464 | 0.147 | 0.462 | 1.45 | 4.44 | 12.8 | 31.7 | 59.5 |
| 8 | 0.0159 | 0.0503 | 0.159 | 0.501 | 1.57 | 4.79 | 13.7 | 33.5 | 61.4 |
| 9 | 0.0172 | 0.0544 | 0.172 | 0.542 | 1.69 | 5.16 | 14.7 | 35.3 | 63.3 |
| 10 | 0.0186 | 0.0589 | 0.186 | 0.586 | 1.83 | 5.56 | 15.7 | 37.1 | 65.1 |
| 11 | 0.0201 | 0.0637 | 0.201 | 0.633 | 1.97 | 5.99 | 16.8 | 38.9 | 66.8 |
| 12 | 0.0218 | 0.0688 | 0.217 | 0.684 | 2.13 | 6.44 | 17.9 | 40.8 | 68.5 |
| 13 | 0.0235 | 0.0743 | 0.235 | 0.738 | 2.30 | 6.92 | 19.0 | 42.6 | 70.2 |
| 14 | 0.0254 | 0.0802 | 0.253 | 0.796 | 2.48 | 7.43 | 20.2 | 44.5 | 71.7 |
| 15 | 0.0274 | 0.0865 | 0.273 | 0.859 | 2.67 | 7.97 | 21.5 | 46.4 | 73.3 |
| 16 | 0.0295 | 0.0933 | 0.294 | 0.925 | 2.87 | 8.54 | 22.8 | 48.3 | 74.7 |
| 17 | 0.0318 | 0.101 | 0.317 | 0.996 | 3.08 | 9.14 | 24.1 | 50.2 | 76.1 |
| 18 | 0.0343 | 0.108 | 0.342 | 1.07 | 3.31 | 9.78 | 25.5 | 52.0 | 77.4 |
| 19 | 0.0369 | 0.117 | 0.368 | 1.15 | 3.56 | 10.5 | 27.0 | 53.9 | 78.7 |
| 20 | 0.0397 | 0.125 | 0.396 | 1.24 | 3.82 | 11.2 | 28.4 | 55.7 | 79.9 |
| 21 | 0.0427 | 0.135 | 0.425 | 1.33 | 4.10 | 11.9 | 29.9 | 57.5 | 81.0 |
| 22 | 0.0459 | 0.145 | 0.457 | 1.43 | 4.39 | 12.7 | 31.5 | 59.2 | 82.1 |
| 23 | 0.0493 | 0.156 | 0.491 | 1.54 | 4.70 | 13.5 | 33.0 | 60.9 | 83.2 |
| 24 | 0.0530 | 0.167 | 0.527 | 1.65 | 5.03 | 14.4 | 34.6 | 62.6 | 84.1 |
| 25 | 0.0569 | 0.180 | 0.566 | 1.77 | 5.38 | 15.3 | 36.3 | 64.3 | 85.1 |
| 26 | 0.0610 | 0.193 | 0.607 | 1.89 | 5.75 | 16.2 | 37.9 | 65.9 | 85.9 |
| 27 | 0.0654 | 0.207 | 0.651 | 2.03 | 6.15 | 17.2 | 39.6 | 67.4 | 86.8 |
| 28 | 0.0701 | 0.221 | 0.697 | 2.17 | 6.56 | 18.2 | 41.2 | 68.9 | 87.5 |
| 29 | 0.0752 | 0.237 | 0.747 | 2.32 | 7.00 | 19.2 | 42.9 | 70.4 | 88.3 |
| 30 | 0.0805 | 0.254 | 0.799 | 2.48 | 7.46 | 20.3 | 44.6 | 71.8 | 89.0 |

From Emerson et al., 1975, *Journal of the Fisheries Research Board of Canada, 32*, p. 2382. Reprinted by permission.

**Figure 1.** Dynamic changes in water quality parameters. Shown are diurnal fluctuations in dissolved oxygen, temperature, pH, and unionized ammonia (A) and temperature changes in relation to depth (B) in ponds.

**A**

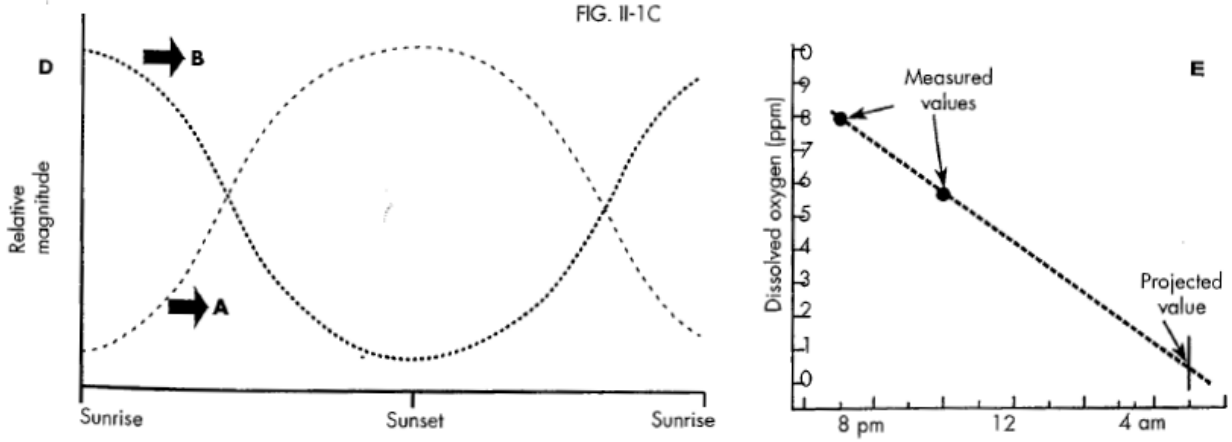



Fig. II-1—cont'd. C, Rawson's oxygen saturation values at various temperatures and altitudes. D, Diurnal fluctuations in water quality variables in a pond. Dissolved oxygen, temperature, pH, and percentage of unionized ammonia usually increase during the day (*curve A*); dissolved carbon dioxide levels usually increase overnight (*curve B*). E, Extrapolation method for estimating nighttime decline in dissolved oxygen in a pond.

**B**

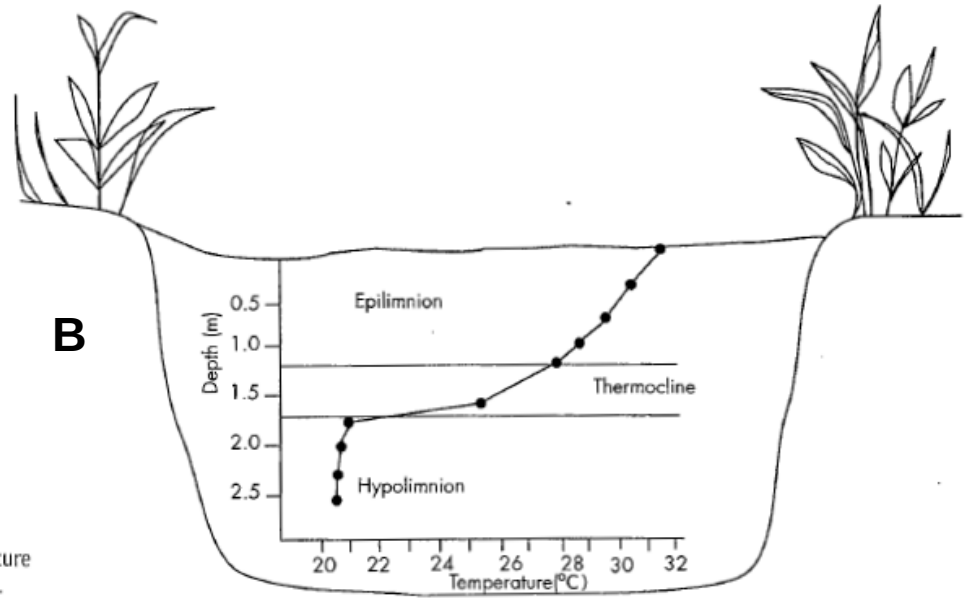
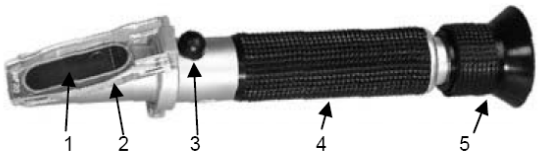



Fig. II-3 Typical temperature zones in a stratified pond.

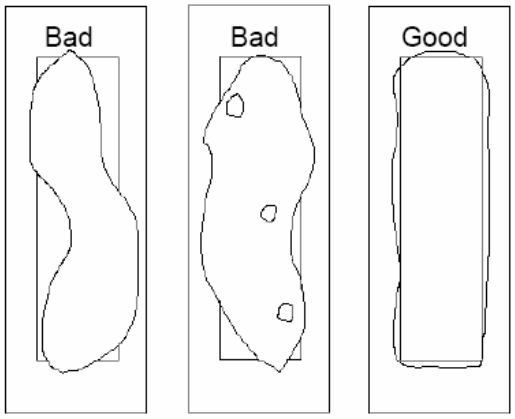
**Figure 2.** Salinity measurements using the VITAL SINE SR-6 Refractometer.

***Description***

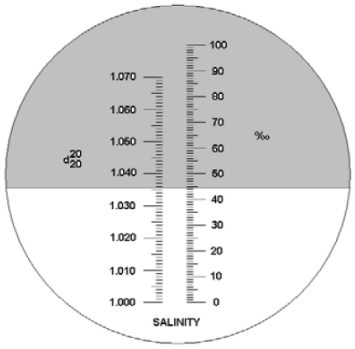
1. Prism
2. Cover Plate
3. Adjustment Screw
4. Mirror Tube
5. Eyepiece




**A. Portable refractometer.**




**B. How to place sample on refractometer.**

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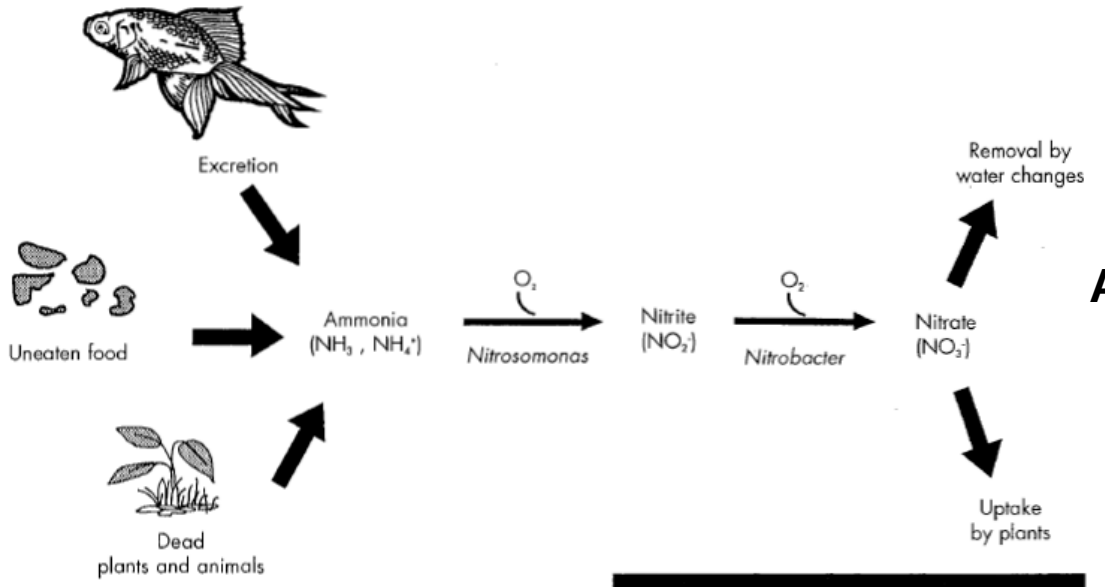
**C. What you should see in the refractometer.**

**Figure 3.** Major sources of nitrogen input and removal in a culture system (A) and changes in ammonia and nitrogenous compounds in a recirculating system with a biofilter (B).



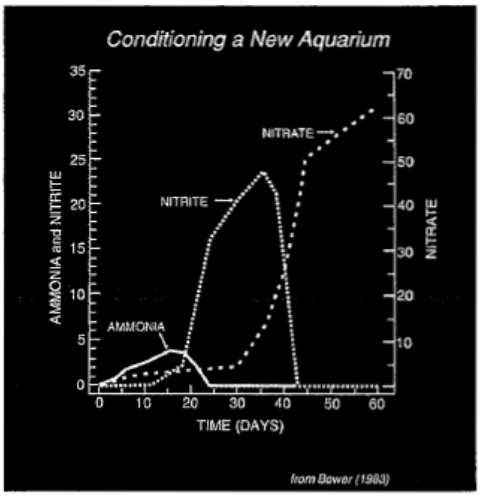




Fig. II-4 A, Major sources of nitrogen input and removal in a culture system. B, Typical ammonia and nitrite concentrations present during new tank syndrome if fish are added on day 0. Time required to establish an active filter and reduce ammonia and nitrite to nontoxic levels are ~3 weeks in freshwater aquaria (Carmignani & Bennett, 1977) and ~2 months in marine aquaria at ~20° to 22° C (Bower & Turner, 1981). Time required to establish the filter increases considerably at lower temperatures.

(*B* from Bower, 1983.)

**FNR 351- AQUATIC SAMPLING TECHNIQUES**
**LABORATORY II: WATER QUALITY**

**Collector's Name:** _____

**Time and Date of Water Collection:** _____

**General Weather Conditions:** _____

**Type of Water System Studied:** _____

**Brief Description of Water System Studied:** _____

_____

_____

**Data collected:**

| Parameter | Measured Value |
|---|---|
| Dissolved Oxygen (mg/L) | |
| Temperature (ºC) | |
| pH | |
| Salinity (PPT) | |
| Water Hardness (mg/L) | |
| Nitrite (mg/L) | |
| Nitrate (mg/L) | |
| Total Ammonia (mg/L) | |
| Unionized Ammonia (mg/L) | |
| Ionized Ammonia (mg/L) | |

**Summary of values from all water systems:**

| Parameter | Agricultural Ditch | Recirculating System | Flow-trough System |
|---|---|---|---|
| Dissolved Oxygen (mg/L) | | | |
| Temperature (ºC) | | | |
| pH | | | |
| Salinity (PPT) | | | |
| Water Hardness (mg/L) | | | |
| Nitrite (mg/L) | | | |
| Nitrate (mg/L) | | | |
| Total Ammonia (mg/L) | | | |
| Unionized Ammonia (mg/L) | | | |
| Ionized Ammonia (mg/L) | | | |

Answer the following questions based on your own data and that collected by others during this laboratory exercise. Use extra paper if needed.

1. Based on data obtained from your own water sample, choose one water quality parameter and discuss in detail including: a) factors that can affect it; and b) what can happen to aquatic organisms if value is above normal and below normal.

2. Choose one parameter and compare across the different water samples. Briefly discuss the factor(s) that could explain the differences observed.

3. Based on all the water systems studied, which one is LESS likely to support a healthy population of fish? Why?

4. What was the most interesting thing you learned?