FISH 415 LIMNOLOGY UI Moscow

Zooplankton Identification lab

Purpose: The purpose of this lab is to familiarize you with the different zooplankton present in lakes of northern Idaho; to introduce a variety of keys to aid your identification of zooplankton; to calculate the density of zooplankton to express your results quantitatively; to compare equipment efficiencies; and to undertake phosphorus and chlorophyll a analyses.

Typically zooplankton samples are examined with an inverted microscope, to count small rotifers and nauplii (young of copepods), and with a stereo microscope to count and identify larger zooplankton. To accurately identify zooplankton to species requires the mounting of individuals followed by examination under a compound microscope. This is an exact and demanding process, and should illustrate why it is advantageous for you to obtain a species list for the area you sampled before you start counting and identification. Once you become familiar with the plankton from a region, it becomes easy to spot differences and you will need to mount far fewer specimen, or none at all. Do not become frustrated in this lab, this may be your first look at some of these organisms. Rather, enjoy their beauty and striking diversity.

Typical zooplankton counting procedure.

1) Collect a zooplankton sample and preserve (you have already done this)
2) Pour sample into 64-80µm sieve and wash to remove formalin
3) Dilute to a known volume in a calibrated beaker
4) Mix well; remove a small sub-sample with a wide mouthed pipette or a Henson-Stemple pipette
5) Place sub-sample in a counting dish
6) Add a couple drops of dilute soap solution to reduce surface tension
7) Count and identify zooplankton

For this lab we will concentrate on the larger zooplankton that you can identify easily with the dissecting microscopes (e.g. *Daphnia* and copepods). The following is a general overview of the most common pelagic zooplankton you are likely to encounter.

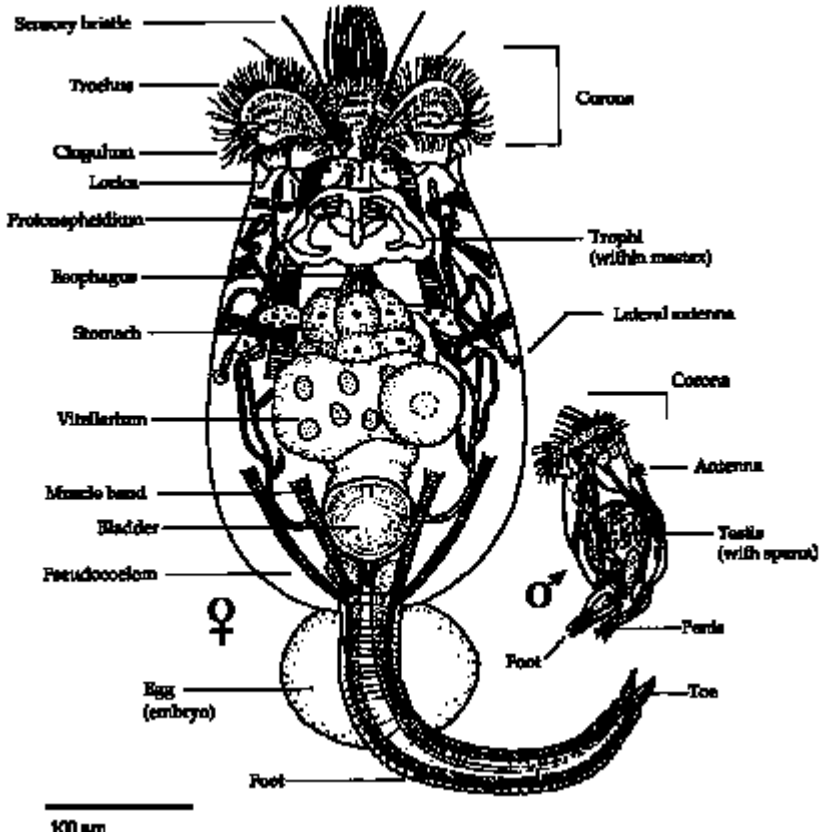
Phylum: **Rotifera** (Rotatoria)

Some of the smallest multicellular animals. Many are sessile, associated with littoral macrophytes, but most are in the open water and form a significant portion of the zooplankton community. They serve as food for may of the larger zooplankton and larval fish. General length 100 - 1000 µm (some as large as 2000µm).

Anatomy: Head with ciliar bands (corona); specialized mouthparts (trophi) for feeding; Armored (loricate) or unarmored (aloricate) body; may possess foot with one or two toes.

Growth and reproduction: Juveniles mature in hours and generation time is 1-2 days under optimal conditions.

Feeding ecology: Herbivorous/omnivorous; algae and bacteria; some large species (e.g. *Asplanchna* are carnivorous).



Modified from Thorp and Covich 2001.

Phylum: **Arthropoda**

Subphylum: **Crustacea**

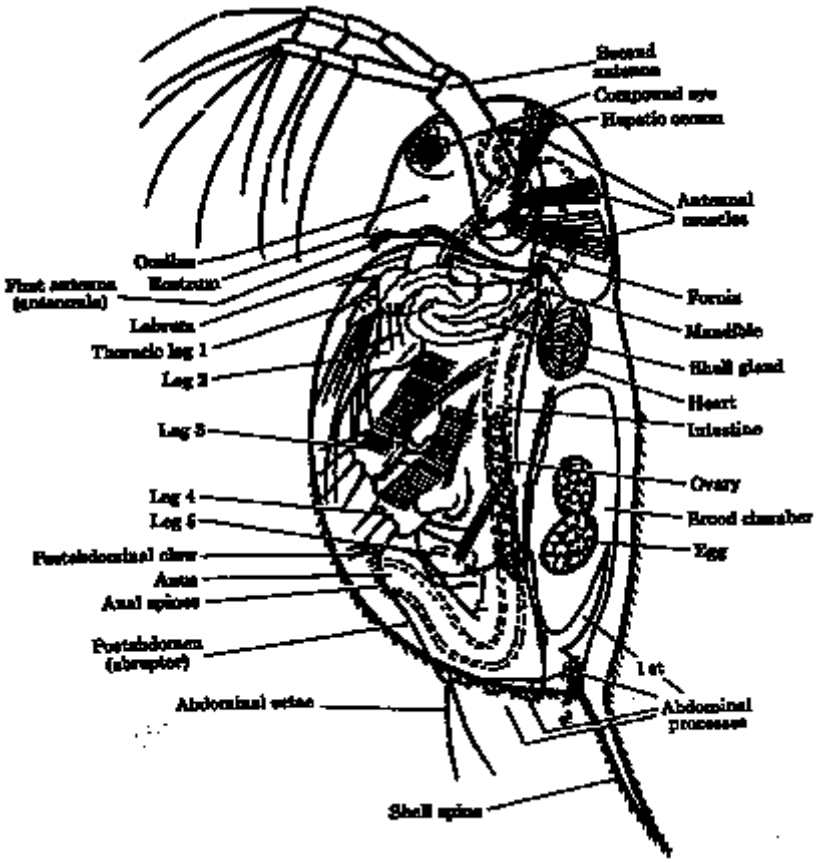
Class: **Branchipoda** “gill feet”

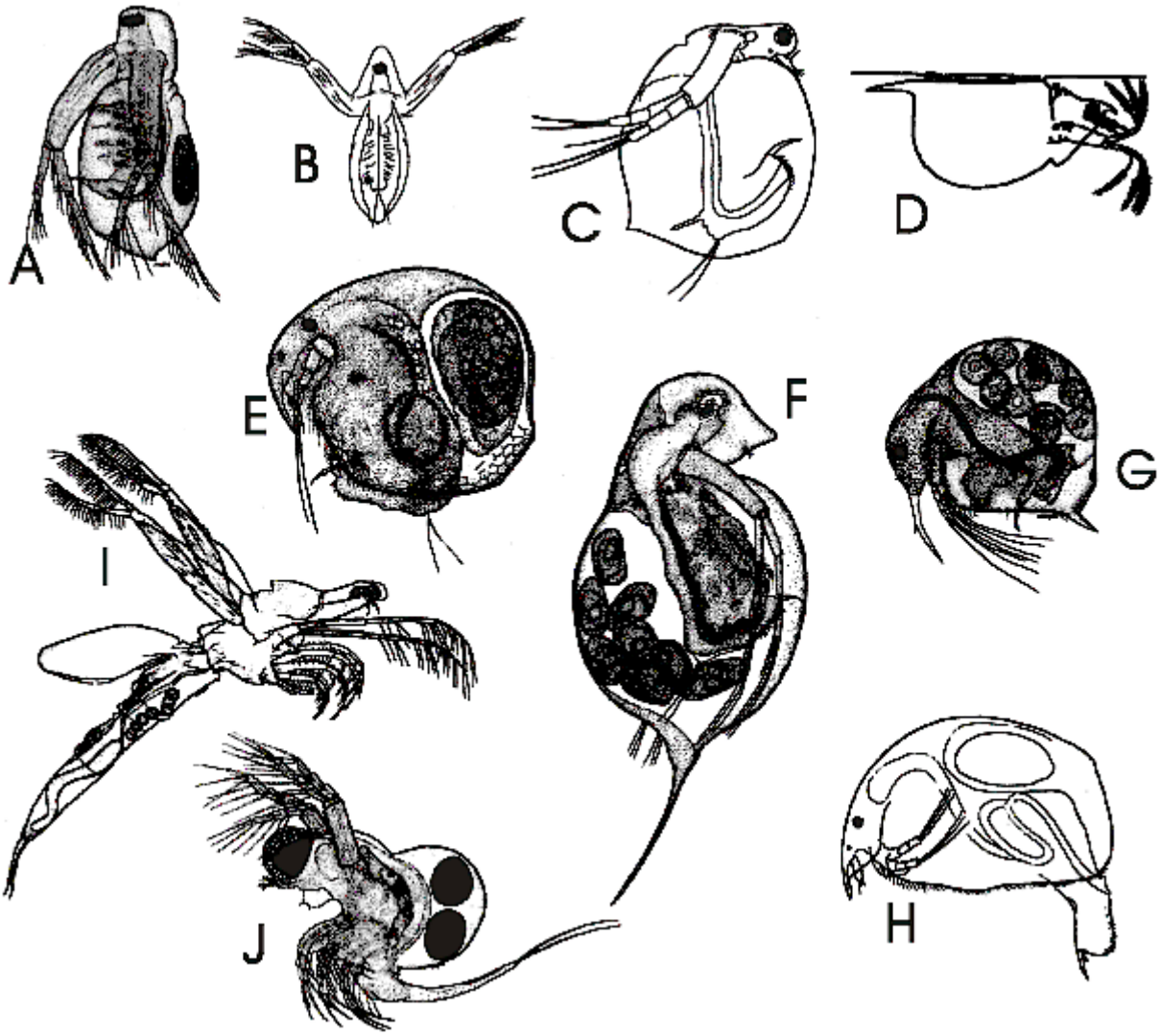
Order: **Cladocera** “water fleas”; very important component of zooplankton communities; predominantly filter feeders (e.g. *Daphnia*), but some are predatory (e.g. *Leptodora, Ployphemus and Bythotrephes*)

Anatomy: Body enclosed in a laterally compressed bivalve carapace; five trunk appendages adapted for filtering and gas exchange.

Growth and reproduction: Emerge in spring from resting eggs (ephippia). Females are primarily parthenogenic and carry developing young in a dorsal brood pouch. Stress (temperature, starvation, crowding) induced formation of males and subsequent sexual reproduction produces ephippia (not in all populations).

Feeding ecology: Predominantly herbivorous - small algae; can consume blue green algae but not the preferred food. Some feed on sediment. Predaceous cladocerans capture other zooplankton with modified trunk appendages.





Cladocera:

A & B - ***Diaphanosoma***: 0.8-1.2 mm; rectangular body and head; large swimming antaennae; six pairs of legs distinct

C - ***Ceriodaphnia***: 0.4-1.0 mm; oval body; bent head separated from the back by a crease ending in eye; small spine on upper rear end of body.

D - ***Scapholeberis***: 0.5-1.0 mm; ventral edge of carapace straight, ending in spine.

E - ***Chydorus***: 0.3-0.5 mm; round body, indistinct head with ‘beak’

F - ***Daphnia***: 1-3 mm; oval body; head with ‘beak’ and possibly a helmet; tail spine.

G - ***Bosmina***: 0.2-0.8 mm; round body; head with ‘elephant trunk’ small spine on lover rear end of body.

H - ***Alona***: 0.5-1.0 mm; oval body with arrow shaped head region.

I - ***Leptodora***: 5-10 mm; elongate body; large swimming antennae; raptorial legs; carapace surrounding eggs only

J - ***Polyphemus***: 0.5-1.5 mm; small body with large brood pouch and eye; raptorial legs.

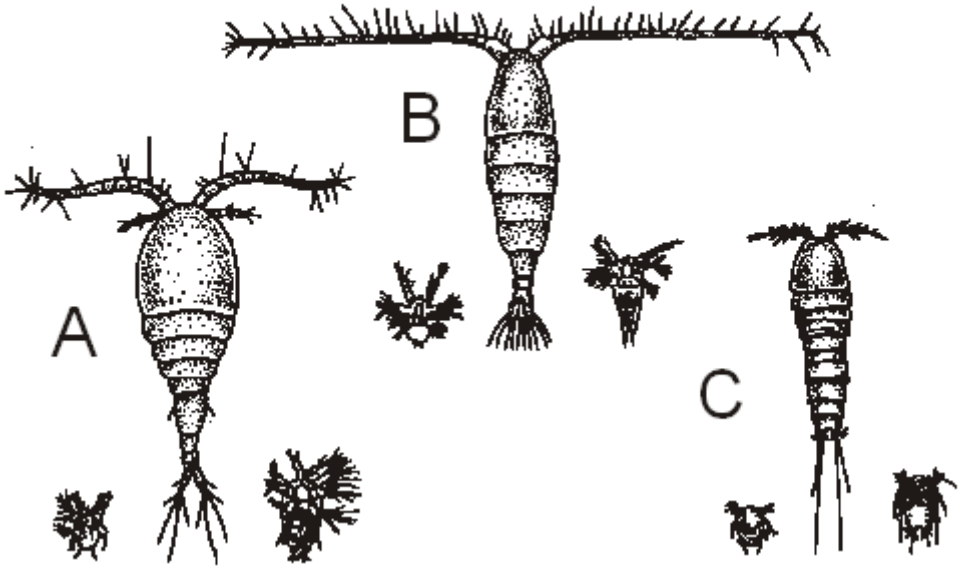
Class: **Copepoda**

The largest and one of the most important class of crustacean zooplankton. Littoral, benthic, and pelagic species are common. Occur in fresh and saltwater. Three general groups in freshwaters 1) Calanoida, 2) Cyclopoida, 3) Harpacticoida. Strong escape responses.

Anatomy: Cylindrical, segmented body consisting of a cephalothorax (metazome) and an abdomen (urosome). The metasoime includes 2 pairs of antennae (the first being long an conspicuous), mandibles and swimming legs.

Growth and reproduction: Reproduce sexually. Eggs hatch into larvae (nauplii) which pass thorugh 6 instars before metamorphosing into juveniles (copepodites). Five copepodite stages are required before reaching sexual maturity.

Feeding ecology: Omnivorous, cannibalistic. Some establish a feeding current to direct food towards them.



Copepods

A - **Cyclopoid**: 0.5 - 4.0 mm; short antennae; tear drop shaped body; eggs in 2 sacs

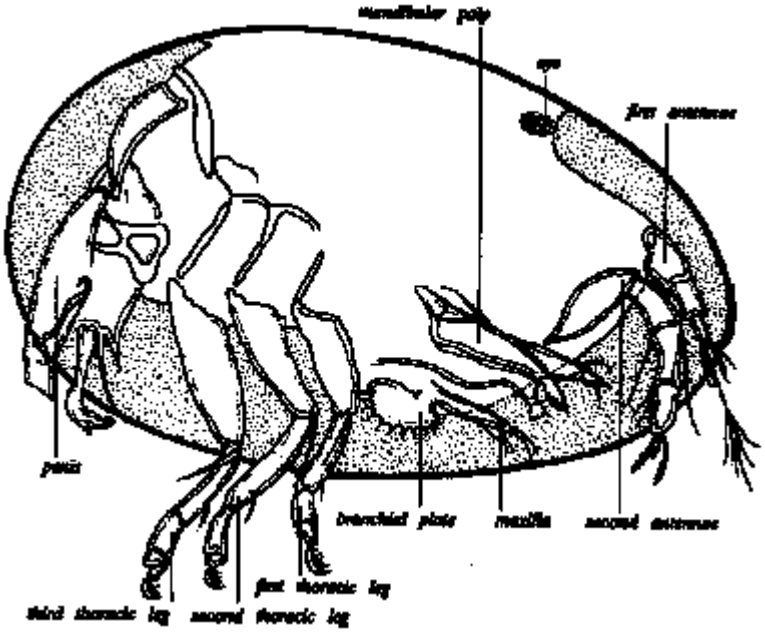
B - **Calanoid**: 1.0-3.5 mm; long antennae; cigar shaped body; eggs in 1 sac

C - **Harpacticoid**: 0.3-1.0 mm; very short antennae; straight body; eggs in 1 sac

naupliar stages shown with each - very difficult to distinguish species in naupliar stage

Class: **Ostracoda**

‘ seed shrimp’ Usually benthic but can accumulate to great densities just above sediment-water interface. Head and body enclosed in a thick bivalve carapace with an opaque outer wall. Carapaces preserve well in sediments and can be used in paleoecological studies. Omnivorous and scavengers.



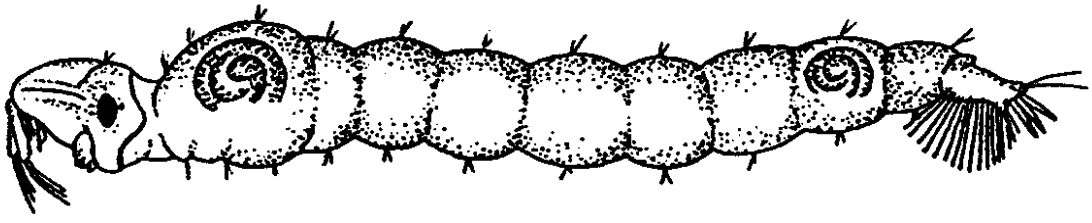
modified from Thorp and Covich 2001 - Generalized sketch of a male entocytherid.

Subphylum: **Uniramia**

Class: **Insecta**

Order: **Diptera**

Larvae of the Phantom midge *Chaoborus* are frequently found in some plankton samples, especially those collected at night, or those from below the chemocline in deep stratified lakes. They have long slender, almost transparent bodies with a pair of posterior and anterior airbladders. *Chaoborus* preys on other zooplankton and can have a strong impact on the species composition. It can tolerate low oxygen concentrations and can thus ‘hide’ from fish.



Lab 2 Assignment

For this laboratory prepare a **manuscript-style** write up (no abstract or introduction) which focuses on a synthesis of the profile and biological data. Use the references discussed and given in class as a starting point (see the course reference web page). Prepare a methods section in which you relate what was done, what samples were collected (use the data spreadsheet to help guide your write up); a results section; and a discussion section. In the discussion you should concentrate on synthesizing the data and showing the connectedness between the chemical, physical and biological parameters. **As well** you should put your findings in context by comparing them to the literature - i.e., what have other researchers found in similar systems? This will give you and the reader a feel if your study lake is 'normal' or 'abnormal'. Include a reference section as well. (See how to write a lab report on the course web page).

You will have TP, temperature, conductivity, light and oxygen profiles as well as Chl a measurements - your profiles are already done. In addition you will have biological (zooplankton) data from a Schindler trap series, a series with the closing plankton net from the bottom to the thermocline and from the thermocline to the surface; and whole water column hauls with a plankton net; (this will allow you to compare the efficiency of the various devices - and to relate the distribution of the zooplankton to the chemical parameters (profiles). Given the wealth of data, you should attempt some statistical test e.g. t-test to compare gear or distributions.

Consider your data carefully - there are a number of ways you can synthesize it to pare it down to a minimum number of figures and tables (The goal is not to generate an endless stack of figures, but rather a well thought-out, coherent report). Consider using appendices for any data/calculation examples not immediately relevant to your main report or which you want to submit as supporting documentation.

Beware - you can not do an adequate job if you start the night before - plan accordingly. Be sure to seek help if you have questions or problems. The assignment will be due in 2 weeks and data will be posted by late afternoon of the analysis lab or the following morning.

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Laboratory report grading criteria

Cover / Title (/2)

2 - Clear, succinct title
1 - vague, long title
0 - no title

Methods and Materials (/5)

5 - Contains information that is effective, quantifiable, and concise which allows the experiment to be repeated; is written so that all information inherent to the report can be related to this section; identifies all sources of data to be collected and instruments used; identifies sequential information in an appropriate chronology; gives brief summary of how data was analyzed; does not contain unnecessary, wordy descriptions (references common methods appropriately).
4 - as above, but missing identity of some equipment; contains unnecessary information, and/or wordy descriptions within the section - common methods not referenced.
3 - presents information in format that is replicable; all information in the report can be related to this section; however, fails to identify some sources of data and/or presents sequential information in a disorganized, difficult pattern.
2 - presents information in format that is marginally replicable; parts of the basic design must be inferred by the reader; procedures not quantitatively described; some information in later parts of report can not be anticipated by reading the Methods and Materials section.
1 - presents information so poorly or in such a non-scientific way that it cannot be replicated.

Results (/5)

5 - Presents correct/accurate summary data in graphs / tables; where possible, means and standard deviations are presented to show variation among samples; is written in parallel order to methods; summarizes main aspects of data in narrative with reference to figures/tables for support.
4 - same as above but some mistakes in data analysis - i.e. numbers/calculation are not correct.
3 - mistakes in calculations; data not well summarized in tables or figures; some data missing; section not parallel to methods.
2 - reader is left to infer major trends on own; narrative and data (figures/tables) not integrated; data/figures missing; incorrect calculations.
1 - results contains tables of raw data, poorly written narrative that does not use reference to figures and/or table for support, poorly summarized data, if any.

Discussion (/5)

5 - Purpose and findings of the research are summarized; student draws inferences that are consistent with the data and scientific reasoning and relates/contrasts these to other findings of other studies; student explains expected results and offers explanations and/or suggestions for further research for unexpected results; student presents data honestly; distinguishes fact and implication; and avoids overgeneralizing; accepts or rejects hypothesis.
4 - same as 5 above, but information not related/contrasted to findings of others.
3 - same as 4 above but student overgeneralizes
2 - Student summarizes the purpose and finding of the research; student explains expected results but fails ignores unexpected results.
1 - Student may or may not summarize the results, but fails to interpret their significance to interested audiences

References (/5)

5 - all references cited included and in proper format; current primary literature
4 - all references cited; format not consistent.
3 - references cited in text but not included in list.
2 - few primary references; references not ordered
1 - some web references

Figures (/5)

5 - Proper figures including labeled axes; units, complete figure captions.
4 - Axis labels/units missing, incomplete figure captions.
3 - same as 3 above, no figure captions
2 - Figures out of order, lack figure caption
1 - some figures included, no labels, caption or units

Tables (/5)

5 - descriptive table captions, row and column headings, data well organized only presenting necessary information such as depths means and standard errors.
4 - as 5 above but not including standard errors.
3 - descriptive table caption missing
2 - same as 3 above, data not well organized, superfluous data included
1 - same as 2 above; missing crucial data.

Organization (Scientific format demands) (/5)

5 - All materials placed in correct sections; organized logically within each section; runs parallel among sections.
4 - All material placed in correct sections; organized logically within each section, but may lack parallelism among sections.
3 - Material placed in right sections, but not well organized within sections; disregards parallelism.
2 - Some materials are placed in wrong sections or are not adequately organized wherever they are placed.
1 - Material placed in wrong sections or not sectioned; poorly organized wherever placed.

FISH 415 LIMNOLOGY UI Moscow

Chlorophyll *a* Analysis: Spectrophotometric Method for Ethanol Extraction

1. Filter **X** mL of lakewater through a 47 mm glass fiber filter (GF/C - Whatman). Stop the vacuum pump just as the last bit of water leaves the filter tower to avoid rupturing cells. Also be sure that the vacuum is not higher than 15 inches of Hg, for the same reason.

   X - is any amount of water that i) you can filter through the filter; ii) imparts a distinct green color to the filter. It will vary from 50 mL for very green water such as in aquaculture ponds to in excess of 2 L for water from an ultra-oligotrophic lake.
   Be sure to record X!

2. At this point, chlorophyll *a* may be extracted immediately or the filter paper may be stored in a dark freezer. (Frozen filters can be stored for up to 1 month, but ideally should be analyzed within a few weeks).

3. For extraction, roll the filter and place each in a separate 10 mL centrifuge tube. Add 10.0 mL of 95% ethanol (EtOH). Macerate (grind to a pulp) the filters, cap the tubes and store in a dark refrigerator for 18-24 hr. Shake the tubes vigorously 1 hr after adding EtOH. make sure all of the filter paper is in the EtOH after shaking.

4. After the extraction period, centrifuge at 5,000 rpm for 10 minutes to separate the chlorophyll *a*/EtOH solution and the glass fibers.

5. Read the optical density (absorbance) in a 1 cm cell against 95% EtOH blanks at 750, 665 and 649 nm (the spectrophotometer in the lab will correct for the EtOH blanks).

6. Chlorophyll *a* is calculated from:

$$\text{Chlorophyll } a \ (\mu g/L) = \frac{v\,[\,13.7\,(665 - 750) - 5.76\,(649 - 750)\,]}{(V)\,(L)}$$

where
   750 is the absorbance for turbidity (this is a correction for any turbidity remaining in the samples from filter particles or other small particles which can interfere with the spectrophotometer. Ideally this reading should be close to zero)
   649 is the absorbance for chlorophyll b
   665 is the total chlorophyll

   v is the volume of the EtOH extractant in ml (10)
   V is the volume of lakewater filtered in litres (**X**) (remember 1L = 1000 mL)
   l is the length of the light path through the cell, in cm (1)

You do not have to worry about converting units on any of the measurements, as the constants "13.7" and "5.76" take care of this. Output is in µg/L.

A variety of Chl a extraction methods exist. We use EtOH because it is relatively safe in a classroom setting.

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TOTAL PHOSPHORUS ANALYSIS

1. You have already taken 35 ml samples of lake water and added 0.3 g potassium persulphate to each sample.

2. The samples have been autoclaved at 30 p.s.i. for 20 minutes. Autoclaving (high pressure producing high temperatures) in the presence of potassium persulphate digests the sample so that all phosphorus is liberated into solution; hence the analysis is for 'total phosphorus'.

3. Prepare your phosphorus calibration standards of 0, 25, 50, 100, 200 and 400 µg/L from the 1000 µg/L stock standard.

4. Add 3.5 ml of mixed reagent to each vial containing 20 ml of sample (including your standards).

5. Let stand for at least 10 minutes (for the blue color to develop) and read absorbance at 885 nm in a 1 cm cell in a light spectrophotometer.

6. Obtain absorbance values for your standards and plot a standard curve (absorbance vs [P]).

7. If the relationship is linear, proceed with the analysis of your samples. Otherwise, re-run the standards and if the relationship is not linear, re-make the standards that appear out of line.

NOTES:

The mixed reagent is made from a "pre-mixed reagent" as follows:
a. in a 125 ml (or larger flask), add 1.2925 g L-ascorbic acid to 25 ml of distilled deionized water.
b. when dissolved, add 100 ml of pre-mixed reagent and mix well.
c. the solution should be a pale yellow; it only lasts for a few hours and should be kept in the dark.

Pre-mixed reagent is made as follows:

a. add 3 g ammonium molybdate into 330 ml of distilled deionized water
b. mix well
c. add 0.07 g of antimony potassium tartrate
d. mix well
e. add 28 ml of concentrated sulphuric acid
f. allow to cool and store in a dark bottle in the fridge.

To make the phosphorus stock solution:
1) add 0.4394 g of KH_2PO_4 in 1000 mL of H_20 = 100 ppm