Chapter 11

Experimental Evaluation of Community Structure In Aquatic Ecosystems

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Reprinted From: Bader, J. M. 1999. Experimental evaluation of community structure in aquatic ecosystems. Pages 207-222, *in* Tested studies for laboratory teaching, Volume 20 (S. J. Karcher, Editor). Proceedings of the 20th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 399 pages.

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Introduction

We use this series of investigations in our upper level Aquatic Biology laboratory which we offer each fall semester and in an abbreviated form in a summer workshop for high school teachers. In the aquatic course, I require students to write a literature review paper on community structure first so that they become familiar with the major models that have been presented and their underlying assumptions. In the summer workshop, the teachers do not have the time to do the research, so I give an introductory lecture highlighting the major features of each model. Either way, I believe that the major value of these exercises is that they force students to evaluate different hypotheses, derive predictions from each of these hypotheses, and devise methods for testing those predictions.

These exercises are most valuable if the students collect and analyze the data, but for schools that do not have access to ponds or lakes, data can be extracted from a large number of studies that have addressed the relative merits of the models. I would be happy to provide these references as well as data our students have generated for anyone who is interested.

For the first half of the semester, our students do not know how they are going to analyze their samples because they are in the process of researching their papers. Once they have submitted their papers, they understand what sort of data they need to test their predictions. Fortunately, data collection can begin immediately and samples preserved for later analysis. This way, when students have an idea of how they want to approach the subject, they can retrieve samples collected weeks earlier.

Materials

Assuming all samples will be field collected, the following materials are commonly used for work in aquatic systems.

- Van Dorn or similar water sampler for collecting phytoplankton and water for nutrient analysis
- Lugol's solution for preserving phytoplankton for counting and identification
- Sedimentation chambers or similar device (we use 12 well tissue culture plates) for counting phytoplankton
- Inverted microscope for use with sedimentation chamber
- Spectrophotometer for chlorophyll a analysis
- Membrane filtration apparatus and Millipore HA 0.45 µm filters for filtering water samples for chlorophyll a analysis
- Mortar and pestle for grinding filters
- Alkaline acetone for extracting phytoplankton pigments from filters
- Clinical centrifuge for spinning down phytoplankton homogenate

- 1 N HCl for acidifying chlorophyll extracts
- Zooplankton net for collecting fresh samples and those to be preserved
- 70% ethanol for preserving zooplankton
- Sedgewick Rafter cell or similar device for counting preserved zooplankton samples
- Compound microscope for identifying and counting zooplankton
- Oven for baking fresh filtered zooplankton samples
- HachPhosVer 3 reagent packets for determining phosphate concentrations
- HachNitraVer6 and NitriVer3 reagent packets for determining nitrate concentrations
- Nitrate and phosphate stock solutions
- Enamel pans and forceps for sorting macroinvertebrate samples
- Identification references for phytoplankton, zooplankton, and macroinvertebrates
- Fish trapping supplies such as hoops, nets, or seines

Notes for the Instructor

One of the keys which makes this series of exercises much more interesting is selecting systems that are sufficiently different from each other that they lead to very different predictions. Characteristics to consider when selecting ponds include : What is the nature of the fish populations? Are there many different species present, or perhaps just a few? Are there piscivorous fish present as well as planktivorous? Are there lots of fish? Often this information can come from people who are familiar with the system, fishermen for instance. What might you suspect about the nutrient levels? Is the system located in an urban or rural watershed? Are there likely to be significant runoffs from lawn fertilizers? Does the pond or lake have any history of algal blooms?

Normally we use three ponds that are located at our biological field station. One of the ponds is typically covered with duckweed and has significant algal blooms. Just looking at the pond suggests a system that is full of nutrients. The other two ponds that we use have no emergent vegetation and look much less eutrophic. One of the ponds has no fish, which makes it ideally suited for assessing the impact of top down forces. Such ponds are relatively rare, however, so it is more likely that ponds with fish will have to be used.

One should keep in mind that this exercise can be used in its entirety or just in part. Some may prefer to analyze just phytoplankton response to nutrient levels or the impact of fish on zooplankton populations.

Student Outline

Introduction

The field of ecology has moved well beyond the basic description of the distribution and abundance of organisms to the application, in field experiments, of the principles derived from these descriptive studies. This is especially true in aquatic ecosystems where very few pristine bodies of water remain. Most freshwater systems, from the large inland seas of the Great Lakes to rural farm ponds, are routinely managed and manipulated to control fish populations, weeds, or water levels.

At the core of all management practices is the realization that populations in a freshwater environment do not exist in a vacuum, but rather they are impacted by a host of biotic and abiotic factors. In many cases, management objectives are to restore systems that have been heavily impacted by human intervention. Years of abuse in the form of pollution, over fishing, and excess nutrient loading have left many freshwater ecosystems in conditions far from their natural states. In order for these manipulations to meet management objectives, it is necessary that the underlying forces that regulate the structure of aquatic communities be understood.

Community structure rather than productivity or nutrient cycles is often used as an indicator of overall ecosystem status. Analysis of community structure as represented by species composition and biomass, may reflect the extent of human impact on aquatic systems as lake acidification, eutrophication, introduction of exotic species, and exploitation of native stocks alter the natural balance of the ecosystem, sometimes in a predictable way. Some aquatic systems are more resilient to these perturbations than others, owing to the presence of nutrient retention mechanisms, food web structures that favor phosphorus use by herbivores rather than phytoplankton, and biogeochemical processes that inhibit nutrient recycling from the sediments (Carpenter and Cottingham 1997). Many lakes show little alteration in community structure despite substantial changes in nutrient loading or species composition. On the other hand, it has been shown that the decline of a single species can lead to significant changes in the overall community (Neill 1988).

Early attempts at describing community structure were based on a linear model in which organisms at different trophic levels were linked in a food chain. Plants would be eaten by herbivores, herbivores would be eaten by primary carnivores, and primary carnivores would be eaten by secondary carnivores. Only rarely does this type of model accurately describe the complexity of community structure. The food chain concept has been replaced by the more complex food web as illustrated in Figure 11.1 for a typical aquatic community. Even this figure is an oversimplification of the interactions that typically take place between aquatic organisms and their environment, and it makes no effort to reflect the relative strength of each interaction.

Over the years, a number of models have been proposed to describe the community structure in various habitats. The keystone predator model is a classic example as illustrated by the role of the starfish *Pisaster* in the intertidal zone (Paine 1966). As illustrated in Figure 11.2, *Pisaster* feeds on a number of species found in the intertidal area. In his classic experiment, Paine removed *Pisaster* from the area and monitored the community dynamics. He found that soon after *Pisaster* was removed, the acorn barnacle *Balanus* occupied 60-80% of the area. With time, *Mytilus* and *Mitella* displaced *Balanus*. Eventually *Mytilus* took over most of the space and a community which consisted of more than a dozen species was reduced to two.

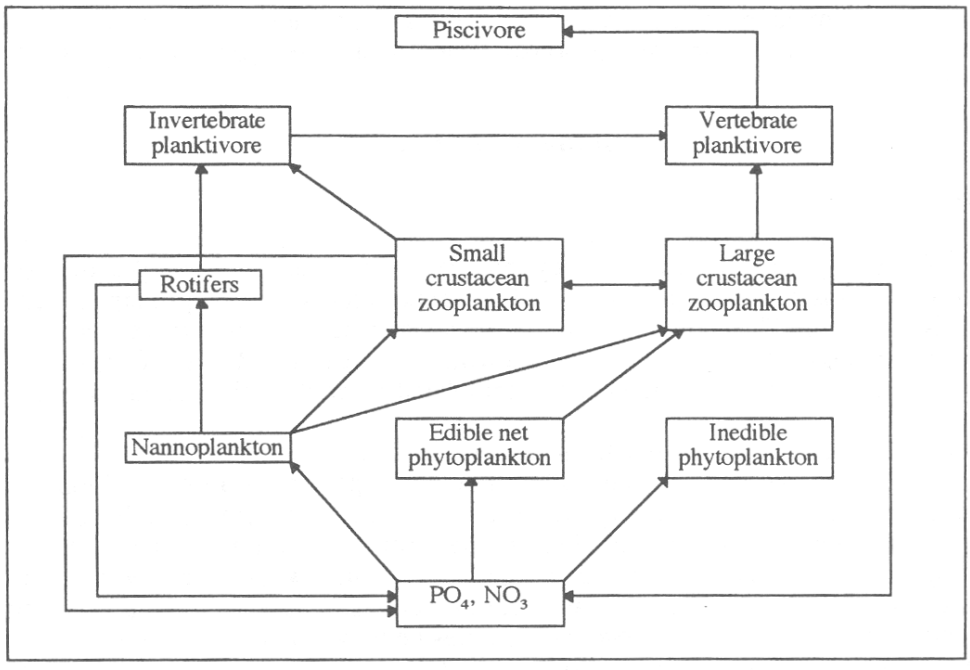

Figure 11.1. A generalized food web typical of many freshwater ecosystems. (After Carpenter et al 1985)

An example of a species playing a keystone role in a freshwater system is provided by Neill (1988) and illustrated in Figure 11.3. At low nutrient loadings and high nitrogen:phosphorus

ratios (N:P), *Daphnia* dominates the system. Large nutrient additions or large recruitment failures in the *Daphnia* population may open a window of opportunity for the invertebrate predator *Chaoborus* to dominate. Under relatively low nutrient conditions with a low N:P ratio and in the absence of *Chaoborus*, a community of cyanobacteria, flagellates, rotifers, and small crustacea may develop which is invaded by predatory cyclopoid copepods.

Community structure based on environmental gradients attempts to address large scale trends in plant and animal communities. Typically, gradient analysis is used in which observations of the abundance of species and nature of species associations are made along transects (for instance latitude) which are interpreted to represent gradients of environmental conditions. The observations are usually analyzed by inspection of graphs of abundance against the environmental variable, and regions are identified where species dominate or patterns of community composition occur. Marshall and Ryan (1987) used this approach when investigating the community attributes of fish and selected the environmental and fish community characteristics listed below.

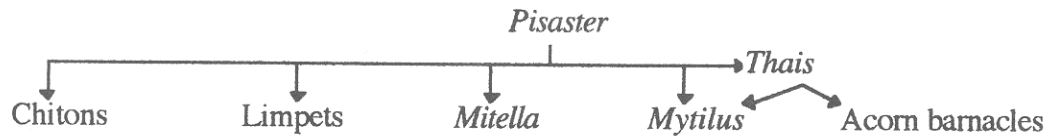

Figure 11.2. Community structure in an intertidal zone with Pisaster as a keystone species.

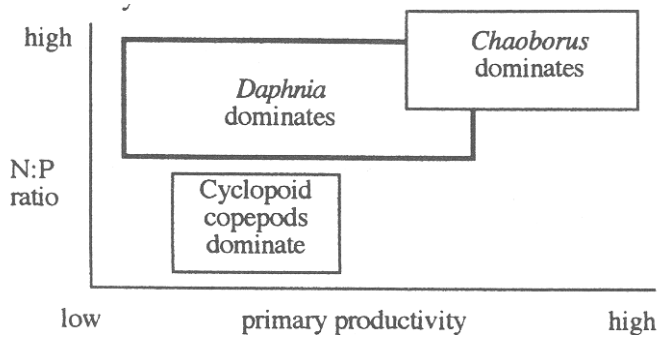

Figure 11.3. Summary of community space dominated by different ecological processes. (from Neill 1988)

• Environmental parameters	Lake mean depth
	Surface area
	Secchi depth
	Morphoedaphic index
• Community characteristics	Relative abundance
	Species diversity
	Community mean weight

Surface area and lake mean depth emerged as the best predictors of community structure. This was most probably related to thermal stratification as lakes with mean depths below a critical

value typically were not deep enough to stratify. Those that were larger and did stratify had a cold water hypolimnion that could support a cold water fish community.

Community structure based on nutrient supply is historically the most popular model of community productivity in freshwater ecosystems. This model predicts that nutrient levels control productivity in a linear fashion. There have been countless field studies that support this model in which lakes have been fertilized (with nitrogen, phosphorus, or both) and primary productivity monitored. In nearly every case, primary productivity increased with fertilization. This model was the foundation of the plan of action implemented in the Great Lakes to combat the massive fish die-offs and algal blooms that were characteristic of these lakes in the late 1960s and early 1970s. Industry and farming were depositing an enormous amount of phosphorus from detergents and nitrogen from fertilizers into tributaries feeding the Great Lakes. This nutrient loading led to tremendous phytoplankton productivity. When the algae began to die-off, the high rate of decomposition created localized oxygen deficits that caused the unsightly fish kills that the world came to associate with the Great Lakes in general and Lake Erie in particular. A drastic reduction in nutrient loading was the goal of the historic Great Lakes Water Quality Agreement (GLWQA) signed by the United States and Canada in 1972 and updated in 1978 and again more recently. The regulation was largely successful and within 20 years, Lake Erie was once again a vibrant system.

The trophic cascade hypothesis was developed in an attempt to resolve those systems in which the N:P ratios did not explain all of the variation in primary productivity. The concept of cascading trophic interactions explains the differences in productivity among lakes with similar nutrient supplies but contrasting food webs. Simply put, a rise in piscivore biomass brings decreased planktivore biomass, increased herbivore biomass, and decreased phytoplankton biomass.

Consider a food web including limiting nutrients and four trophic levels: piscivores such as bass, pike, or salmon; zooplanktivores, herbivorous zooplankton, and phytoplankton (Figure 1). Invertebrate planktivores like insect larvae and predatory copepods take smaller prey than vertebrate planktivores like minnows. Small crustacean zooplankton include grazers such as *Daphnia pulex* and invertebrate planktivores. The phytoplankton are divided into three functional groups : nannoplankton subject to grazing by all herbivores, edible net phytoplankters that are grazed only by larger zooplankton, and inedible algae.

According to the model, changes in the density of large piscivorous fish should result in changes in density, species composition and behavior of zooplanktivorous fish. In Wisconsin lakes containing bass or pike, spiny-rayed planktivorous fish such as bluegill replace soft-rayed minnows, which are common in the absence of piscivores. The depletion of prey fishes by salmonids stocked in Lake Michigan shows how piscivores can regulate zooplanktivorous fishes (Crowder et al 1987). Prey fish biomass declines as their predators increase in density; in contrast, prey fish productivity reaches a maximum at intermediate predator densities.

High planktivory by vertebrates is associated with low planktivory by invertebrates as well as high densities of rotifers and small crustaceans. Where planktivorous fishes are absent, invertebrate planktivores and large crustacean zooplankton predominate. Planktivorous fishes select the largest available prey and can rapidly reduce the density of zooplankters larger than 1 mm. In contrast, planktivorous invertebrates select and deplete herbivores smaller than 0.5-1 mm. Heavy planktivory by invertebrates favors large cladocerans that grow rapidly until they cannot be taken by the planktivores. At this size, these cladocerans shift energy allocation from growth to producing many small offspring. Planktivorous fishes which consume large zooplankton (including invertebrate planktivores) promote dominance by small cladocerans that grow continually, reproduce at an early age, and have small clutches of large offspring.

Differences in size structure among herbivorous zooplankton communities leads to pronounced differences in grazing and recycling rates. Herbivorous zooplankton alter phytoplankton species composition and size structure directly through selective grazing and indirectly through nutrient cycling. The relationship between phytoplankton biomass and nutrient levels has been established many times over.

Each model presented here is based on specific assumptions about the strength of interactions between trophic levels. They also produce predictions that should be true if the given model is in effect. For instance, if system A has greater nutrient levels than system B, the nutrient loading model would suggest that system A would have greater algal biomass, greater zooplankton biomass, and perhaps greater fish biomass as well. Other models would lead to different predictions given the same set of initial conditions. In this laboratory we will consider the two most popular models, nutrient loading and trophic cascade. Prior to sampling our systems for comparison, we want to develop predictions of how the trophic levels may be influenced by different environmental conditions. If we consider a lake with high nutrient levels, a lake with low nutrient levels, a lake with no fish, and a lake with an abundance of piscivorous fish, both models should give us different predictions on how the phytoplankton biomass, zooplankton biomass, macroinvertebrate biomass, herbivorous fish biomass, and piscivorous fish biomass would be affected. We will spend time to consider this question and develop predictions based on each model.

Before sampling, remember that we will be sampling only once or twice. Aquatic communities are dynamic with populations often following seasonal cycles of abundance, so the time of sampling is important. Realize that if you repeated this sampling procedure earlier or later, the species composition and biomass of all the organisms would be different. However, we are operating under the assumption that the systems we will be sampling have been undisturbed for many years, and as a result, the forces that determine community structure have been in place for some time. We are making relative measures by comparing one system to another, rather than absolute measures for a particular ecosystem.

Procedure

Each student group will be assigned one component presented below to analyze in all three of the systems. The data will be pooled in the table at the end of this exercise so that we can assemble a complete profile for each pond.

A. Nitrate-nitrogen determination

Nitrate nitrogen is the most highly oxidized state of nitrogen found in natural waters. It is also usually more abundant than the other inorganic combined forms, namely ammonia and nitrite. Nitrate nitrogen is the form most easily taken up by aquatic green plants, and it represents the end product of aerobic decomposition of organic nitrogen containing molecules. Usually nitrate nitrogen occurs in relatively small concentrations in unpolluted surface waters, the world average being about 300 µg/l NO_3-N.

High levels of nitrate in water can indicate biological wastes in the final stages of stabilization or run-off from heavily fertilized fields. Nitrate-rich waters often encourage excessive algal growths which may degrade surface waters.

The cadmium reduction method of nitrate determination is a procedure adequate for most survey work. In the low range nitrate test (less than 800 mg/l), cadmium metal is used to reduce the nitrates to the nitrite form. The nitrite ions react with sulfanilic acid to produce an intermediate diazonium salt which forms a red-orange color complex with chromotropic acid in

direct proportion to the nitrate concentration in the sample. Most assays are similar and the one used here is based on Eckblad (1978).

1. Prepare duplicate water samples by filling two clean 50 ml conical tubes to the 25 ml mark. For best results, the test should be performed with the sample at room temperature. Also prepare a set of nitrate-nitrogen standards by diluting the stock solution (100 µg/ml). A suggested range of values would be 10, 50, 100, 200, and 400 µg/l NO_3-N.

2. Add the contents of one NitraVer 6 Nitrate Reagent packet to each sample. Immediately cap and shake for exactly 3 minutes. Allow the sample to stand undisturbed for 30 seconds. A deposit of unoxidized cadmium metal may be present after completion of this step.

3. Add the contents of one NitriVer 3 Nitrite Reagent packet to each sample, and shake for 30 seconds. A pink color will develop if nitrate is present. Allow at least 10 minutes for proper color development, but do not wait more than 20 minutes before taking the reading.

4. Transfer 3-4 ml of each sample to a clean standard cuvet with a Pasteur pipet and determine the absorbance at 500 nm. Use 3-4 ml of the original water sample to adjust the spectrophotometer to 0 absorbance. If samples from more than one pond are being analyzed, either use separate blanks for each pond or else make a composite blank by mixing equal volumes from each pond.

5. To determine the amount of NO_3-N in your samples, create a standard curve by plotting absorbance vs. known NO_3-N concentration (10, 50, 100, 200, 400 µg/l) and drawing the best fitting straight line through the points. Alternatively, a linear regression can be obtained by plotting the points with a graphing calculator or computer.

6. Use the standard curve to determine the amount of nitrate nitrogen in your samples.

7. If one considers the replicate samples to be independent samples from the same population (pond), then the non-parametric Kruskal-Wallis or Mann-Whitney test can be used to identify significant differences between ponds.

B. Phosphate-Phosphorus determination

Intense ecological interest in phosphorus stems from its major role in metabolism in the biosphere. In comparison to the relatively rich supply of other major nutritional and structural components of the biota (C, N, O, S), phosphorus is least abundant and commonly limits biological productivity in aquatic ecosystems.

Phosphorus occurs in a number of inorganic and organic compounds in both particulate and dissolved forms. Differentiation of forms is based on their reactivity with molybdate, ease of hydrolysis, and particle size. In this procedure, acidic ammonium molybdate reacts with orthophosphate to produce a yellow phosphomolybdate complex. Ascorbic acid then reduces this complex, giving an intense blue color.

It must be stressed that when one is analyzing for phosphorus in the µg range, laboratory contamination from dust and detergents will often produce higher concentrations than the lake or river water itself. Clean glassware with phosphate-free detergents (i.e. Liqui-Nox). Ideally, all

glassware used for phosphate determination should be acid washed and stored in a dilute acid (1-5%) solution.

Because of the reported possibility of phosphorus adsorption onto polyethylene, samples for phosphorus analysis should be collected in acid-washed glass bottles. Samples should be refrigerated immediately, and analysis should be completed within a few hours. If analysis must be delayed, any filtration (to separate soluble reactive phosphorus, PO_4, from total phosphate) must be done immediately and samples stored frozen. This procedure is based on that presented by Eckblad (1978).

1. Prepare duplicate water samples by filling two clean 50 ml conical tubes with 25 ml of sample. For best results, the test should be performed with the samples at room temperature. Also prepare a set of phosphate standards by diluting the stock solution (50 µg/ml). A suggested range of values would be a reagent blank, 5, 10, 50, 100, and 200 µg/l PO_4-P, although our experience indicates that the lower limit of detection with this reagent is 10 µg/l. For greater sensitivity at low phosphate levels, see Wetzel and Likens (1991). The reagent blank is to be sure there is no phosphate residue in the glassware (see above) and to correct for turbidity (see Note below).
2. Add the contents of one PhosVer3 Reagent packet to each sample. Immediately cap and shake to mix. A blue color will develop if phosphate is present. Wait at least 2 min for full color development but do not wait more 10 minutes before taking a reading. Prepare the spectrophotometer during this time.
3. In cases where phosphate levels are low, greater sensitivity can be achieved by using a cuvet with a longer pathlength. If that is the case, transfer 7 ml of each sample to a clean rectangular (20 mm path length) cuvet with a Pasteur pipet and determine the absorbance at 700 nm. Use 7 ml of the original water sample to adjust the spectrophotometer to 0 absorbance. If samples from more than one pond are being analyzed, either use separate blanks for each pond or else make a composite blank by mixing equal volumes from each pond.
4. To determine the amount of PO_4-P in your samples, create a standard curve by plotting absorbance vs. known PO_4-P concentration (5, 10, 50, 100, and 200 µg/l) and drawing the best fitting straight line through the points. Alternatively, a linear regression can be obtained by plotting the points with a graphing calculator or computer.
5. Use the standard curve to determine the amount of PO_4-P in your samples.
6. If one considers the replicate samples to be independent samples from the same population (pond), then the non-parametric Kruskal-Wallis or Mann-Whitney test can be used to identify significant differences between ponds.

Note: The PhosVer 3 Reagent may cause some turbidity depending on a large number of factors. It is recommended that a reagent blank be run on each lot by adding the contents of one packet to 25 ml demineralized water. This should be read using demineralized water as a blank. The value found should be subtracted from the final test readings.

C. Enumeration and biomass of phytoplankton
Sample collection

Phytoplankton in the open water of a lake or stream often is sampled by means of water bottles such as the Van Dorn sampler. These samplers are lowered open to a specific depth and then are closed by means of a weighted messenger that is dropped along the cable to trip the closing mechanism.

Whenever possible, phytoplankton species, particularly delicate species of flagellated algae, should be examined while alive. Algae may be kept for several hours without appreciable deterioration when kept cold during transportation to the laboratory.

Normally, samples are preserved for long term storage. The best preservative is Lugol's solution, added to samples to yield a 1% final concentration. The adsorption of iodine from Lugol's solution by the cell also promotes settling when the sedimentation-inverted microscopy technique is used.

Quantitative enumeration

If the system is very productive and there are lots of phytoplankton in the sample, they may be viewed directly by adding 1 ml to a Sedgwick-Rafter cell as described below for zooplankton. More commonly, however, it is necessary to concentrate the sample for easier counting. Sedimentation chambers, developed by Utermöhl in the 1930s, are the most common means for concentrating phytoplankton samples. However, these are too expensive unless you are a professional phytoplanktologist. We will use 12-well tissue culture plates as our sedimentation chambers.

As stated above, viewing live samples is always preferable to working with preserved samples. However it may not be feasible to do so. In many cases, the Lugol's solution which is used to preserve the algal cells and make them heavier so they will sink to the bottom of a counting chamber stains the cells and gives them an unnatural color that makes them more difficult to identify. Sometimes, using material that has been collected with a zooplankton net yields a sample that is easier to work with.

1. Thoroughly mix your phytoplankton sample by mixing. Transfer 5 ml of the sample to each of four sample wells.
2. Replace the cover and allow the samples to rest undisturbed. The Lugol's solution will make the cells heavier, so they will settle more quickly. Wait for at least 30 min and preferably a few hours or overnight.
3. After settling, carefully view the samples with an inverted microscope. Scan the entire bottom of each of the four wells and identify the phytoplankton present to the genus level.
4. Calculate the number cells/ml.
5. If one considers the replicate samples to be independent samples from the same population (pond), then the non-parametric Kruskal-Wallis or Mann-Whitney test can be used to identify significant differences between ponds.

Evaluation of biomass

A number of different methods have been employed to estimate the biomass of phytoplankton populations including measures of fresh and dry weight, cell volumes, and organic carbon. Measurements of the concentrations of photosynthetic pigments can be used to estimate the composite biomass of phytoplanktonic populations. The methodology for measuring pigments is relatively direct and accurate and can be performed on algae separated from the water as well as *in vivo*. Pigment concentrations of algae can vary widely depending on metabolism, light, temperature, nutrient availability, and many other factors. Chlorophyllous pigments degrade to relatively stable phaeophytin products, which interfere with the spectrophotometric or fluorometric determinations of chlorophyll. Phaeophytin concentrations, however, can be estimated separately on the same samples for which chlorophyll is determined. Thus pigment

analysis can yield a sensitive approximation of algal biomass, but because of physiological variability, interpretation of the data must be done with care. We will use the procedure described by Wetzel and Likens (1991).

1. Water samples must first be filtered. The amount of sample required will vary with the productivity of the system. While 200 ml may be adequate for productive systems, as much as 1 liter may required for oligotrophic waters. We will filter 500 ml through a 0.45 µm membrane filter (e.g. Millipore HA). Filter approximately 50 ml at a time and replace the filter as often as necessary.

2. Place the filters into a mortar and add approximately 3-4 ml of 90% alkaline acetone. Grind the samples thoroughly for 45 seconds, decant the homogenate into a graduated 15 ml conical tube. If some parts of filters still remain, add another 3 ml to the tissue grinder and grind for another 15 seconds. It is best to keep the acetone volume to a minimum so that it does not dilute the pigment. Combine the homogenates and record the total volume to the nearest 0.1 ml.

3. Centrifuge at maximum speed in a clinical centrifuge (~ 1000 x g) for 5 min. If possible, use a refrigerated centrifuge. Repeat the centrifugation if the supernatant is cloudy.

4. If the chlorophyll extracts are likely to be dilute, as in an unproductive system, increase the sensitivity of the assay by transferring 7 ml to a 20 mm light path length (rectangular) cuvet. (A 13 x 100 mm glass culture tube would be fine if working with a Spectronic 20.) Make a blank with 90% alkaline acetone.

5. Measure the absorbance at 750 and 665 nm using the 90% alkaline acetone as a blank.

6. Add 0.1 ml of 1 N HCl per ml of extract directly to the cuvet, cover, and invert to mix. Allow the tube to stand for 5 min.

7. Remeasure the absorbance in the acidified samples at 750 and 665 nm.

Calculations

$$\text{Chl a } (\mu g/l) = \frac{(k)(F)(E_{665_o} - E_{665_a})(v)}{(V)(Z)}$$

where

E_{665_o} = turbidity-corrected absorption at 665 nm before acidification
= $A_{665_o} - A_{750_o}$, where A = absorption value

E_{665_a} = turbidity-corrected absorption at 665 nm after acidification
= $A_{665_a} - A_{750_a}$

k = absorption coefficient of chlorophyll a = 11.0

F = factor to equate the reduction in absorbency to initial chlorophyll concentration
= 1.7:0.7, or = 2.43

R = maximum ratio of $E_{665_o} : E_{665_a}$ in the absence of phaeopigments, = 1.7

v = volume of extract in ml

V = volume of water filtered in liters

Z = length of light path through cuvet or cell in cm

$$\text{Phaeopigments } (\mu g/l) = \frac{(k)(F)[R(E_{665_a}) - E_{665_o}](v)}{(V)(Z)}$$

D. Enumeration and biomass of zooplankton

Sample collection

Various types of nets or traps have been used to concentrate (filter) zooplankton from large volumes of water. The most common means is a plankton net. Whenever taking a sample, be sure to note the length (depth) of the tow. This will be necessary when calculating the volume of water sampled. For species identification and enumeration, samples may be preserved in enough neutralized formalin to reach a final concentration of 4%. However, 70% ethanol works just as well and is less noxious.

Quantitative enumeration of individuals

Once the sample is obtained from the lake and is concentrated, the organisms must be counted to determine the abundance and relative body size of each species present. When the number of individuals is relatively small, it is best to settle the entire sample and count all the organisms with an inverted microscope.

Usually, however, there are too many individuals to count them all, so several subsamples should be counted. Follow the procedure below to determine the number of organisms per liter.

1. Mix the sample container thoroughly so that all the organisms are suspended. Transfer a sample to a Sedgwick-Rafter cell with a Pasteur pipet and cover with a cover glass. The Sedgwick-Rafter cell holds exactly 1 ml of sample.

2. Count all the organisms in the subsample. Identify the cladocerans to at least genus level and species if possible. Identify the rotifers to genus level. Group the copepods as calanoid, cyclopoid, or harpacticoid depending on the length of the antennae relative to body length. Use the references that are available and the expertise(?) of the instructor to assist in the identification. For statistical accuracy, you should count enough 1 ml samples so that you total at least 200 individuals.

3. To determine the number of zooplankton/l :

   a. Determine the average number/ml based on the subsamples = N

   b. Measure the volume (in ml) of the concentrated sample = V_s

   c. Determine the volume of lake water filtered in liters (area of net opening x height of water column sampled) = V_f

$$\#/l = \frac{(N)(V_s)}{V_f}$$

Evaluation of biomass

The mass of an organism may be estimated from its volume or it can be determined directly by weighing. Many workers estimate average dry weight biomass of zooplankters from estimates of average length and regressions of length versus weight.

1. Collect a sufficient number of organisms by doing 2 to 3 total vertical hauls. It is extremely important that the samples be clean without a significant amount of algae, mud, or other organic matter. A pure zooplankton sample is ideal, although it is not always possible to obtain. Be sure to note the depth of each haul so that the amount of water filtered can be calculated.
2. Filter the pooled sample (it is best to filter approximately 50 ml at a time because the filters clog rapidly. Use as many filters as necessary, usually 2 to 3). Dry the filters at 60°C for 2 to 3 days.
3. Weigh the filters to the nearest mg and divide by the total volume of water filtered. Express the biomass as mg/l.

E. Macroinvertebrates

Aquatic macroinvertebrates are those that are large enough to be seen by the naked eye and commonly includes insect larvae, various arthropods, snails, and clams. In streams, the species composition and abundance of macroinvertebrates has long been used as indicators of pollution status.

1. If transects or sampling stations have been established previously, sample at regular intervals along those lines. If they have not been established, it is permissible to chose a representative number of sampling locations that represent the variety of habitats present in the system.
2. Use a sediment sampler (Ekman dredge or strong dip net for instance) to collect your sample. An alternative method that we commonly use is to place a cylinder or box of 0.5 m^2 at a uniform depth, something in the neighborhood of 0.5 m. We then use a dip net to suspend the material from the sediment in the water column within the sampling cylinder or box. We continue to sample the volume of water in the cylinder until we are confident that we have obtained all of the organisms present.
3. In the laboratory, pour some of your sample into an enamel pan and sort the macroinvertebrates into major groups such as dragonfly larvae, beetle larvae, adult beetles, amphipods, isopods, etc. Preserve these samples in 70% alcohol.
4. After identifying and counting the specimens, determine the number of each group per m^2.
5. Since the samples from the same population (pond) are independent, then the non-parametric Kruskal-Wallis or Mann-Whitney test can be used to identify significant differences between ponds.

F. Fish

Fish biomass is more difficult to determine than the other components of the food web. A number of options are available, but most are logistically difficult or labor intensive. Perhaps the best method to yield reliable estimates of biomass is a mark-recapture approach where fish are caught, marked by clipping a fin and released. Sampling continues at a later date and the fish are identified to species, weighed, measured, scales are taken for possible age analysis, and it is

noted whether or not the fish was caught in the marking session. To determine the population size, use the formula :

$$N = \frac{MC}{R}$$

Where: N = population estimate
M = total number marked
C = total number recaptured
R = number of marked fish recaptured

The fish may be captured with a seine net or a hoop net. Minnow traps may also be suitable for some species. In some systems it is not possible to use nets because of obstructions or heavy vegetation. An electro-shocker is probably the only way to obtain a reasonable sample in such systems.

An alternative is removal sampling as described by Brower et al. (1990). The general idea is that fish are captured and removed on successive sampling dates and as the population size decreases, so will the catch. If removing the fish is not an option, they can be marked and released, but not counted in subsequent samplings if they are caught again.

Discussion

1. Identify the limitations to this type of analysis. Consider the problems associated with seasonality in population and biomass levels. How can this be accounted for or overcome?
2. Which data do you consider reliable and which do you have less faith in? Why?
3. Would correlation/regression analysis be a suitable approach to identifying relationships within ponds?
4. Revisit your predictions in light of the information you have gathered. Is there any compelling evidence in support of the nutrient loading model? the trophic cascade model?

Appendix - Recipes

Lugol's solution

Dissolve 20 g KI in 200 ml distilled water. Add 20 ml concentrated glacial acetic acid. Dissolve 10 g I_2 and dissolve by stirring. You may leave out the acetic acid if desired, but the iodine takes longer to dissolve and never completely goes into solution. If you are going to leave out the acetic acid, stir the KI/I_2 mixture for at least 20 min on a magnetic stirrer. Store in an amber bottle or a bottle wrapped in foil. Some people keep the solution in the refrigerator when not in use.

Alkaline acetone
100 ml dH_2O, 900 ml acetone, 2 drops concentrated NH_4OH

Results

Table 11.1. Physical, chemical, biological profiles of the study sites.

	System A	System B	System C
Physical characteristics Surface area Mean depth Volume			
Nutrients Nitrate (µg/l) Phosphorus (µg/l)			
Phytoplankton Species composition (cells/ml) Biomass (µg/l Chl a)			
Zooplankton Species composition (#/l) Biomass (mg/l dry weight)			
Macroinvertebrates Species composition (#/m^2)			
Fish Species composition Biomass (kg/m^2)			

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