Reading:


Skim Chapters 53 (Population Ecology), 54 (Community Ecology), and 55 (Dynamics of Ecosystems) in Raven et al. (2005).

I. Introduction

Ecology is the study of the interactions of organisms with each other and with their environment. These interactions often involve the transfer of energy, especially when one organism feeds on another. The interactions can be examined at several different but interrelated levels. At the level of individual organisms is physiological ecology, which considers how members of a species respond to the physical environment. Taking a wider perspective, population ecology emphasizes the environmental and biological factors that affect populations, i.e. groups of interbreeding individuals living in the same area. At a still broader level, community ecology examines the effects of interactions among populations of two or more species sharing a common habitat. At the most expansive level is ecosystem ecology, which treats the ways groups of species interact with the physical environment, and in particular the flow of energy and nutrients through biological systems.

A major goal of both population and community ecology is to learn what determines the distribution and abundance of organisms. Principles of community ecology, for example, can be used to explain why some parts of the Carleton Arboretum are dominated by hardwood forest while other parts contain oak savannahs and prairie, how periodic fires contribute to maintenance of prairie and savannah in the American Midwest, or how the reintroduction of wolves into Yellowstone National Park is likely to affect populations of elk and other herbivorous mammals.

II. Ecology of Lakes and Ponds

Within a community, species interact both with the environment and with each other. Interactions with the physical environment are referred to as abiotic while interactions among organisms are termed biotic. A familiar example of an important abiotic factor for freshwater pond communities is pH. In many lakes of the northeastern United States the decline in pH associated with acid rain has had devastating effects on the diversity and population sizes of fish and invertebrates. Many other abiotic factors can also affect pond communities, and before coming to lab you should think of several additional examples.

Biotic factors include competition, predation, and mutualism. The first two of these have attracted particular attention from ecologists studying the composition of aquatic communities.

A useful concept for understanding the organization of communities is that of trophic levels. This term describes an organism's feeding status relative to other members of the community; it is also used to describe the flow of nutrients and energy through communities.
and ecosystems. At the base of most communities are the **primary producers**, the green plants or photosynthetic protists that make their own food using the energy of the sun. In freshwater communities these would include both phytoplankton (mostly unicellular algae) floating in the water column, and algae and other plants attached to surfaces.

At the second trophic level, feeding on the primary producers, are the **primary consumers** or **herbivores**. Prominent herbivores on the phytoplankton in a pond community are a variety of crustaceans, mostly copepods and cladocerans, which together make up the zooplankton. Other herbivores that graze on attached algae include snails, tadpoles, and flatworms.

At the next highest trophic level are the **secondary consumers**, most notably the **carnivores** that feed on the herbivores. The most obvious of these predators in many (but not all) ponds are fish. Other pond predators include a variety of insects, such as dragonfly larvae (Odonata) and water boatmen (Hemiptera: Corixidae), as well as other invertebrates such as *Hydra*.

A fourth trophic category that is also important in ponds consists of the **decomposers**, which break down the organic matter in the bodies of all members of the community. In freshwater systems the dominant decomposers are fungi and especially bacteria. Many of the smaller secondary consumers that feed on phytoplankton may also eat bacteria.

Freshwater ponds and lakes offer several advantages for studying ecology. One of these is that such bodies of water, unlike many terrestrial communities, have fairly obvious boundaries, essentially the water's edge. This makes it easier to decide whether or not a given species is a member of the community and helps in identifying the many biotic and abiotic factors that may be acting in the community. A second advantage is that ponds contain well-defined assemblages of species at the different trophic levels. The trophic relationships among species can be readily arranged in a **food chain**, or more appropriately for a whole community with complex interactions, a **food web**. You will receive a food web diagram for a freshwater ecosystem in lab.

The third and most important advantage of working with pond communities is that artificial ecosystems, often referred to as **mesocosms**, can be readily created and maintained. These mesocosms can closely mimic natural ones and can be used to examine ecological questions.

### III. Ecological Regulation in Freshwater Systems

As noted above, the aquatic community living in freshwater systems can be quite complex, with many species at each trophic level. This complexity can be simulated in a mesocosm, in our case a 15 liter aquarium. A major question in ecology has been how community and ecosystem structure (which species are present and in what abundance) is regulated.

One possible form of regulation is **bottom-up regulation**. This refers to the effect of nutrients on primary producers, with manifold effects on other trophic levels. Remember that all non-photosynthesizing species in freshwater systems obtain their energy from plants, directly by eating plants or indirectly by eating something that ate plants. For example, an increase in nutrient availability may boost primary productivity (the total amount of energy stored by plants and algae). This increase in productivity should increase the total biomass of producers in the ecosystem. An increase in plant biomass could increase the biomass of herbivores and also predators. Furthermore, the increase in primary productivity may affect the number of species of herbivores and/or predators, as well as the relative abundance of the individual specie present.

Another form of regulation is **top-down regulation**. Here, the structure of the system is
largely determined by top-predators, the species at the top of the food chain. As an example, assume four main trophic levels: primary producers, herbivores, and predators. If predation increases in this system, one might expect that the density of herbivores will decrease. With fewer herbivores, plant biomass might be expected to increase since fewer plants will be eaten. As you can see, in top-down regulation a trophic level is strongly affected by the next level up in the food chain – i.e., a species is affected by who eats it.

In lab you will be testing for the presence of top-down regulation. In particular, you will examine the effects of the presence or absence of fish in freshwater mesocosms. A direct way to test the significance of fish predation in pond communities, and how the fish affect the zooplankton, is to perform controlled experiments. As described in the next section, one such experiment has been set up for you using aquatic mesocosms. During the lab period, you and your classmates will sample the zooplankton from the tanks quantitatively then carefully identify the organisms present and record some information about them, especially their size.

When you analyze these data graphically after lab you will be able to describe the community structure of the zooplankton assemblage, as well as the relative biomass community. Community structure refers to the number of species present (the species richness) and, most importantly for our purposes, the absolute and relative abundances of each species or category of species. We will also look at the distribution of different size classes of zooplankton. Measurements of community structure make it possible to compare different communities directly, to see whether they contain the same total number of individuals, to note how much they overlap in species diversity, and to determine which species or types of individuals are the most dominant. In addition, biomass estimations will allow you to examine the effects of predators on the size of the producer level.

The obvious, general hypothesis you will be testing in this experiment is that freshwater systems are top-down regulated. However, by using the natural history information described above, as well as the food web diagram, you should be able to formulate more specific and interesting hypotheses that address a) the expected overall effect of fish on the producer and zooplankton communities, b) whether particular species or types of zooplankton are likely to be more affected than others and why, and c) whether the presence or absence of fish predators has indirect effects on zooplankton community structure by altering competitive interactions in the food web.

IV. Experimental Design

The mesocosms you will be studying were created in 15 liter polycarbonate aquaria, placed in pairs in an environmental room in Hulings hall. Approximately 8 weeks before the lab the tanks were filled with water, 1 liter of washed sand, and 500 ml of topsoil (simulates sediment into a pond and provides a source of nutrients) and 250 ml of lake sediment (provide nutrients as well as an inoculation of decomposers). The biotic components of the ponds were then established by inoculating each pond with 4 liters of pond water green with phytoplankton and an additional 3 liter sample of water containing a mixture of zooplankton collected in Lyman Lakes and other ponds within the Carleton Arboretum. The concentrated zooplankton samples were collected by towing a 153-µm plankton net.

Once the zooplankton and phytoplankton were well established, about 4-6 weeks after the tanks were first filled, fish were added. This top link in the chain was added in the form of one small (3-5 cm) sunfish (genus Lepomis) introduced into a randomly chosen tank in each pair. (Why was it important to choose which tank received
This protocol provides guidelines for sampling one tank.

**Three sampling steps:**
- A. **Remove large organisms** (e.g., fish, insects, tadpoles)
- B. **Sample zooplankton** from **non**-agitated tank
- C. **Sample phytoplankton** from agitated tank

**A. Removal of large organisms**

Before you sample your tank, remove all large organisms that you can catch. This includes fish and insects. Fish should be put into the labeled fish tank and insects should be segregated by type and set aside for later identification and counting (see D below).

**B. Zooplankton Sampling**

Zooplankton are (mostly) small crustaceans that swim about in the water. We will assume that they will not be present in the sediment. Furthermore, it is important not to disturb the sediment to reduce the amount of debris in the sample.

We will take a sample of water from an unagitated tank and pass it through a 100 µm filter, then concentrate the filtrate with a centrifuge. Remember to use a glass pipet or plastic transfer pipet when pipetting the zooplankton (a p1000 pipet tip can damage the organisms).

1. **Collect Water Sample**
   a. Without disturbing the sediment, collect a 2000 ml sample of water from the tank in two 1000 ml plastic beakers.
   b. Remove any visible debris and animals from the sample with a fiberglass screen.
2. Filter the Zooplankton Sample
   a. Obtain a zooplankton filter and screw the cap on the bottom (not too tightly).
   b. While holding the filter over a plastic tub, put a funnel in the top of the filter and pour the entire water sample through the filter.
   c. Use extra water to rinse out the beakers – pour the rinse water into the filter. Remember that we want **everything** in the sample (even stuff you can't see) to go into the filter.
   d. Tip the filter to pour off extra water that is in the bottom of the filter (make sure the water drains out through the filter screen).
   e. While holding the filter over a 500 ml beaker, carefully unscrew the bottom cap and allow the contents to drain into the beaker. Using a squirt bottle of 70% ethanol, rinse the inside of the filter into the beaker. Also rinse the inside of the cap into the beaker. Remember that there are tiny zooplankton potentially lining the entire inside of the filter, including the screw threads. Try to limit the total sample volume in the beaker to 50ml.

3. Concentrate the Zooplankton Sample
   a. Distribute the contents of the beaker into 3 or 4 (or more if needed) 15-ml conical tubes. Using a small amount of ethanol rinse out the bottom of the beaker into a tube.
   b. Place tubes into a clinical centrifuge and spin at ½ maximum speed for 5 seconds. Make sure the centrifuge is balanced!
   c. Carefully pipet out most of the supernatant from the tubes – leave approximately 2 ml in each tube.
   d. Use a plastic transfer pipet with the tip cut off to resuspend the content of the tube and pipet contents of all tubes into a small beaker. Add 2 ml of wetting/thickening agent (= glycerol + detergent). This solution thickens the sample and slows down evaporation from the surface.

4. Identify and count zooplankton
   a. Using a plastic pipet, transfer approximately 2 ml of the concentrated sample to a Sedgewick-Rafter counting cell. Your sample should fill the cell but not be so much that water will spill out.
   b. Place counting cell on a dissecting microscope stage. Use either a black or clear stage background (not white!).
   c. Illuminate the sample with a dissecting scope lamp. Light from a sideways angle is best.
   d. Slowly scan each column in the grid and record the identity of **every** animal you see with a short line or "tic mark" in the appropriate cell of Table 1 below. For each cladoceran or copepod you record, you **must also** enter a "tic mark" in Table 1 to indicate its size category.
      
      Identify your zooplankton using the drawings and **information in** Figure 1 below. This figure illustrates zooplankton species likely to be encountered in our mesocosms. Pay particular attention to the key features indicated by the arrows on the drawings and not just to size; members of large genera like *Daphnia* or *Simocephalus* start off as small, miniature versions of the adult shape. Most of the species you will encounter are small crustaceans (primarily cladocerans and copepods), although you may also find rotifers and *Hydra.*
It is very important to try to identify each animal in your sample accurately. Note that preserved specimens often come to rest in different orientations than in life or in the illustrations and are thus harder to recognize. It is therefore essential that you use a dissecting needle to turn individuals over or spread them out to give you a different or better view.

Note, too, that not all species are present every year or at all times of the year. Some are only seasonally abundant and some disappear from the water column for months at a time due to various abiotic factors. Your lab instructor will tell you which of the organisms listed in Table 1 you can expect to encounter during the lab period. If you think you have found any other species in your sample, you must ask your TA or instructor to verify your identification before entering a "tic mark" on the table!

Guidelines for determining size class:

1. Measure the longest body dimension, excluding the elongated spine on Daphnia or appendages such as swimming antennae. (See Figure 1)

2. If the specimen is not as wide as one of the mm scale marks (i.e. less than the width of the actual black line itself), put it in the < 0.5 mm category.

3. If the specimen is wider than one of the mm scale marks but spans less than half the distance between adjacent marks, put it in the 0.5 mm category.

4. If the specimen fills more than half the distance between two marks but does not stretch across both of the marks themselves, put it in the 1.0 mm category. (See "a" in the diagram below.)

5. If the specimen extends completely across adjacent marks -- including the widths of the lines -- but less than halfway to the next mark, put it in the 1.5 mm category. (See "b" in the diagram below.)

5. Enter data on computer spreadsheet

As directed by your lab instructor, count zooplankton in your mesocosm(s). When you finish, total the tic marks in each line of Table 1. After your lab group has completely counted all of the zooplankton, determine the combined totals for the group. Enter these values on the appropriate lines in the class Excel spreadsheet. These results, representing pooled data from different pairs of tanks, will be posted on Collab by the end of the lab week.
C. Phytoplankton Sampling

The primary producers in our tanks are comprised of both phytoplankton and filamentous algae. Much of the phytoplankton settles onto the bottom of the tank in calm water. Therefore, to sample all algae we need to agitate the tank contents to suspend all algae in the water. Then, relative amount of algae will be estimated by measuring the amount of chlorophyll-a in the water sample. This should be done twice for each tank, with the chlorophyll values averaged to get a single value for the tank.

1. Collect algal Sample
   a. Using a plastic spatula/scraper, scrape all sides of the tank to free attached algae.
   b. Using your hand, agitate the contents of the tank thoroughly. You should scoop your hand through all the sediment.
   c. Wait 5 minutes for the sediment to settle.
   d. After 5 minutes, collect two 500-ml samples of water with 1000ml plastic beakers.
   e. Remove large debris (if any) from each sample.

2. Filter algal sample
   a. Place a new Whatman GF/C filter paper into a clean filter holder – the filter disk should sit on top of the flat surface with concentric ridges and grooves.
   b. Carefully place the white sealing ring on top of the filter so that it is perfectly centered. Screw the filter cap on firmly.
   b. Fill a clean 50- or 60-ml syringe with water from your algal sample beaker. Make sure to thoroughly agitate/swirl the beaker to resuspend all sediment. Purge any air from your syringe and record exactly how much water is in your syringe.
   c. Screw the syringe onto the cap of the filter and slowly push water through the filter. Filter a known amount of water through the filter. Stop filtering water when there is significant resistance in the filter (if you push too hard the filter paper will rip in the filter) – you may need to refill your syringe (record the amount in the syringe each time!).
   d. Record the total amount of water that you filtered (i.e., how much water you pushed through the filter with the syringe).
   e. Working over a mortar, carefully remove the cap of the filter. Using tweezers, place the filter paper and sealing ring into the mortar.
   f. Using a glass pipet, rinse the cap into the mortar with 5 ml of 95% ethanol – this will capture any algae in the cap.
   g. Use tweezers to remove the sealing ring from the mortar this step is important!
   g. Use the pestle to thoroughly grind the filter so that pieces are no longer visible.
   h. Pour the mortar contents into a labeled 15 ml conical tube.
   i. Again using 5 ml of 95% ethanol, rinse the pestle into the mortar, swirl the mortar contents, and then add the mortar contents to the conical tube.
   j. Centrifuge at high speed in the collectible (but still highly useful) clinical centrifuge for 10 minutes.

3. Measure absorbance
   a. Without disturbing the pellet, pipet 1 ml of supernatant into a square spectrophotometer cuvette.
   b. Measure the absorbance at three wavelengths: 649nm, 664nm, and 750 nm. Make sure the blank the machine at each wavelength with 95% ethanol.
4. Calculate amount of Chlorophyll-a

The following equation is used to calculate the chlorophyll a content, which is a good estimator of relative amount of algae in the sample. You might note that the equation is similar to that used last week, except that a third absorbance value ($A_{750}$) is added - $A_{750}$ controls for turbidity in the sample (i.e., tiny floating sediment). Use the following two equations to calculate the amount of chlorophyll-a in your tanks.

\[
C_a (\text{chl a concentration in cuvette [ug/ml]}) =
13.7(A_{665} - A_{750}) - 5.76(A_{649} - A_{750})
\]

And finally, to calculate chlorophyll content in each tank sample, you need to take into account the amount tank water passed through the filter and the amount extraction liquid (i.e., 10 ml of 95% ethanol).

\[
\text{Chl a Content} = C_a \times \frac{\text{(Ethanol volume)}}{\text{(volume of H}_2\text{O filtered)}}
\]

Now average the chlorophyll a content values for the two samples from each tank. Record chlorophyll a content in units of µg chlorophyll per ml tank water (µg/ml).

V. Hand-in Assignment

The details for the write-up will be discussed during lab.
**Figure 1. Guide to common zooplankton in experimental ponds**

| **Cladocerans:** | Key Features | Relative size
|------------------|--------------|----------------|
| Chydorus | round outline; dark | ![Symbol](symbol1.png)
| Bosmina | "trunk"-like beak; small & rounded | ![Symbol](symbol2.png)
| Ceriodaphnia | small head; distinctly rounded body; blunt tail; brownish pigmentation | ![Symbol](symbol3.png)
| Scapholebris | ventral surface appearing as dark, nearly straight line in side view; body dark; two short posterior spines | ![Symbol](symbol4.png)
| Diaphanosoma | small head; slim body; rather delicate looking with fringed antennae | ![Symbol](symbol5.png)
| Simocephalus | like a large, overly fat Daphnia but with an undersized head; short, blunt posterior projection | ![Symbol](symbol6.png)
| Daphnia | large, helmet-like head; long posterior spine (caution: tail spine may be broken in preserved specimens) | ![Symbol](symbol7.png)

| **Copepods:** | | |
| cyclopoid copepod | tapered cylindrical body, usually seen in side view (a); *cyclopoid* type swimming antennae shorter than head and thorax; *calanoid* type antennae longer; cyclopoid females may have conspicuous paired egg sacs (b); size and color vary widely with species | ![Symbol](symbol8.png)
| calanoid copepod | | ![Symbol](symbol9.png)

| copepod nauplius | Ostracods | look like "shrimp in a clam;" carapace often looks "fuzzy" and may be patterned; size very variable | ![Symbol](symbol10.png)
| ostracod | |

*Note: Sizes of Cladocerans are for adults; juveniles have the same shapes but are smaller.*

Length of *Simocephalus* silhouette corresponds to actual size of 2.7 mm; *Chydorus* silhouette corresponds to 0.4 mm. When judging length *clad*do not include tail spines or antennae.

Table 1. Zooplankton results

Tank # ____  Microcosm **WITH** Fish

<table>
<thead>
<tr>
<th>Identification</th>
<th>Size category for cladocerans (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladocerans (&quot;water fleas&quot;)</td>
<td></td>
</tr>
<tr>
<td>Chydorus</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Bosmina</td>
<td>0.5</td>
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<tr>
<td>Ceriodaphnia</td>
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<tr>
<td>Scapholebris</td>
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<tr>
<td>Diaphanosoma</td>
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<tr>
<td>Daphnia</td>
<td>&gt; 2.5</td>
</tr>
<tr>
<td>Simocephalus</td>
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<td><strong>Total Cladocerans:</strong></td>
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<table>
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<tr>
<td>calanoid type &gt;50% red</td>
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<tr>
<td>gray or &lt;50% red</td>
<td>0.5</td>
</tr>
<tr>
<td>cyclopid type</td>
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<td>Copepod nauplius</td>
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<th>Isopods:</th>
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<tr>
<td>Aquatic insect larvae</td>
<td>Dragonflies (Odonata):</td>
<td>Corixidae (water boatmen):</td>
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<td></td>
<td>Water Scorpions (Hemiptera:Nepidae):</td>
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<tr>
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