Chapter 15

Aquatic Food Web Interactions: Microcosms as Lake Models

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Contents

Introduction

Natural communities contain many species arrayed in a *food web*, a concept first described by Charles Elton (Elton 1927) that includes species from each *trophic level*. A food web begins with species that provide energy to the entire community – the *autotrophs*. Here, plants or algae fix light energy through photosynthesis and store it in chemical bonds of organic molecules that make up living organisms. This determines the energy and nutrient base of the entire community. From this point, other organisms – *heterotrophs* – transfer energy and other nutrients throughout the food web by eating other organisms. Thus, herbivores such as grasshoppers or the aquatic water-flea *Daphnia* eat plants or algae, and predators eat herbivores or other predators. Omnivores present an interesting case in that they eat both plants or algae as well as predators; we will ignore omnivores although they are quite interesting. Consequently, food webs can be highly reticulated and provide a basic framework for understanding communities. Energy transfer between trophic levels is inefficient (10-20%) while nutrient transfer is reasonably efficient. The trophic or food-transfer activities describe the trophic structure of the community, leading to important concepts such as the *trophic pyramid* which indicates the existence of biomass or energy by trophic level in a community.

There is much current research on these types of questions for a wide variety of natural communities (Carpenter et al. 1987, Lancaster & Drenner 1990, Polis & Winemiller 1996). Nutrients such as phosphorus are well known to limit phytoplankton community production (Schindler 1974). Landmark papers by Hrbacek et al. (1961) and Brooks and Dodson (1965) clearly demonstrated the effects of planktivorous fish on the entire plankton assemblage in lakes. The introduction of planktivorous fish, particularly an exotic species, into a lake impacts both the zooplankton community and phytoplankton densities and species composition as well.

What regulates the dynamic interactions in food webs? Is total biomass in trophic levels of natural communities regulated by the total resources (e.g., P, N) available to each trophic level (a *bottom-up explanation*)? Or is predation at higher trophic levels more important for determining the biomass at each trophic level (a *top-down explanation*)? Two related questions about food chains address this issue: (a) First, what regulates the number of trophic levels found in natural communities? Elton (1927) explained the restricted number of trophic levels in natural communities using an energetics argument since the total energy coming into a community can only support a limited number of trophic levels because of energy loss in each transfer. (See Pimm 1982 for an alternate explanation.) (b) Second, what regulates the total biomass (primary or secondary production) within trophic levels? For example, just focus on responses by primary producers: Are nutrient and energy resources needed to support photosynthesis most important (bottom-up) or is the impact from herbivores the driving force (top-down)? In other words, why is the world green? This is just the question posed by Hairston, Smith and Slobodkin (1960) in a classic paper that is now referred to as the HSS hypothesis. If there is constant feeding pressure from herbivores on plants or algae (primary producers), you might expect that herbivores would reach levels at which they eat available green biomass nearly as fast as it is produced, leaving very little green material in either terrestrial or aquatic ecosystems. This would be best described as a brown world. HSS's proposed solution to this problem for a three trophic level food chain is that predators reach sufficient densities to reduce herbivore levels to the degree that production of green plant or algal material increases above that expected if there were just two trophic levels (primary producers and herbivores). In other words, interactions between trophic levels are important regulating factors in this view of the world.

If the HSS view of the regulation among trophic levels of communities is true, we can make the following set of testable predictions for a 1, 2, and 3 trophic level food chain, shown as two graphs that indicate the relative biomass of each trophic level (Figure 15.1). (See also Fretwell 1977, Oksanen et al. 1981, Power 1990, and Schmitz 1993). If the community contains only plants, the total net primary production is determined by those factors that affect photosynthesis: Availability of light, water, and mineral nutrients (e.g., N, P, K, Fe). Increased nutrient or water levels increase the final total primary production. In Figure 15.1b, herbivores are added to the community and no predators exist. Here, the primary producer biomass is reduced to a low level compared to Figure 15.1a because of feeding pressure by herbivores for the same level of nutrients available to primary producers. This also means that total herbivore biomass is determined by the amount of food available to it, as no other factors contribute to its regulation. Now, look at relative biomass of these same compartments in a 3-trophic level food chain (Figure 15.1c). Here a predator level feeds on herbivores, lowering their total biomass in the community relative to that seen in Figure 15.1b. Because there are fewer herbivores after predation, primary producer biomass increases relative to that seen in Figure 15.1b Primary production may even reach levels set by the availability of resources for photosynthesis (Figure 15.1a) if herbivore levels are reduced enough by predators. In the 3-trophic level community, predator biomass is determined by the abundance of herbivores as prey. Note that the HSS view of the world recognizes regulation of trophic levels because of the feeding impact of higher trophic levels – a top-down view – but that some trophic levels are regulated by the availability of resources – a bottom-up view. These top-down vs. bottom-up relationships alternate as one goes up the food chain in the HSS model.

The alternating effects of regulating forces among trophic levels in communities is called a trophic cascade. Predators reduce herbivores so that primary producers can increase. In more productive environments, more trophic levels can be supported (Elton 1927, Fretwell 1977, Oksanen et al. 1981). What happens if we add a second predator level (top predator) to the food chain that preys only on the other predator type (primary predator)? Based on the trophic cascade hypothesis of food web regulation, Figure 15.1d illustrates the new predictions relative to 1, 2, and 3 trophic level communities. In this four trophic level community, the top predator reduces the abundance of primary predators. Because there are fewer primary predators, herbivore abundance increases, leading to more regulating pressure on primary producers. Primary producer abundance again decreases relative to the 3-trophic and 1-trophic level communities. Carefully study Figure 15.1 before proceeding so that you clearly understand the basic premise of the trophic cascade hypothesis and how it compares to strictly bottom-up regulation of trophic structure in communities. For comparison, sketch a parallel set of relationships to that of Figure 15.1 for 1, 2, 3, and 4-trophic level communities if only bottom-up regulation is taking place. On a similar note, what happens to the 4-trophic level community if the top predator is removed or greatly reduced in number?
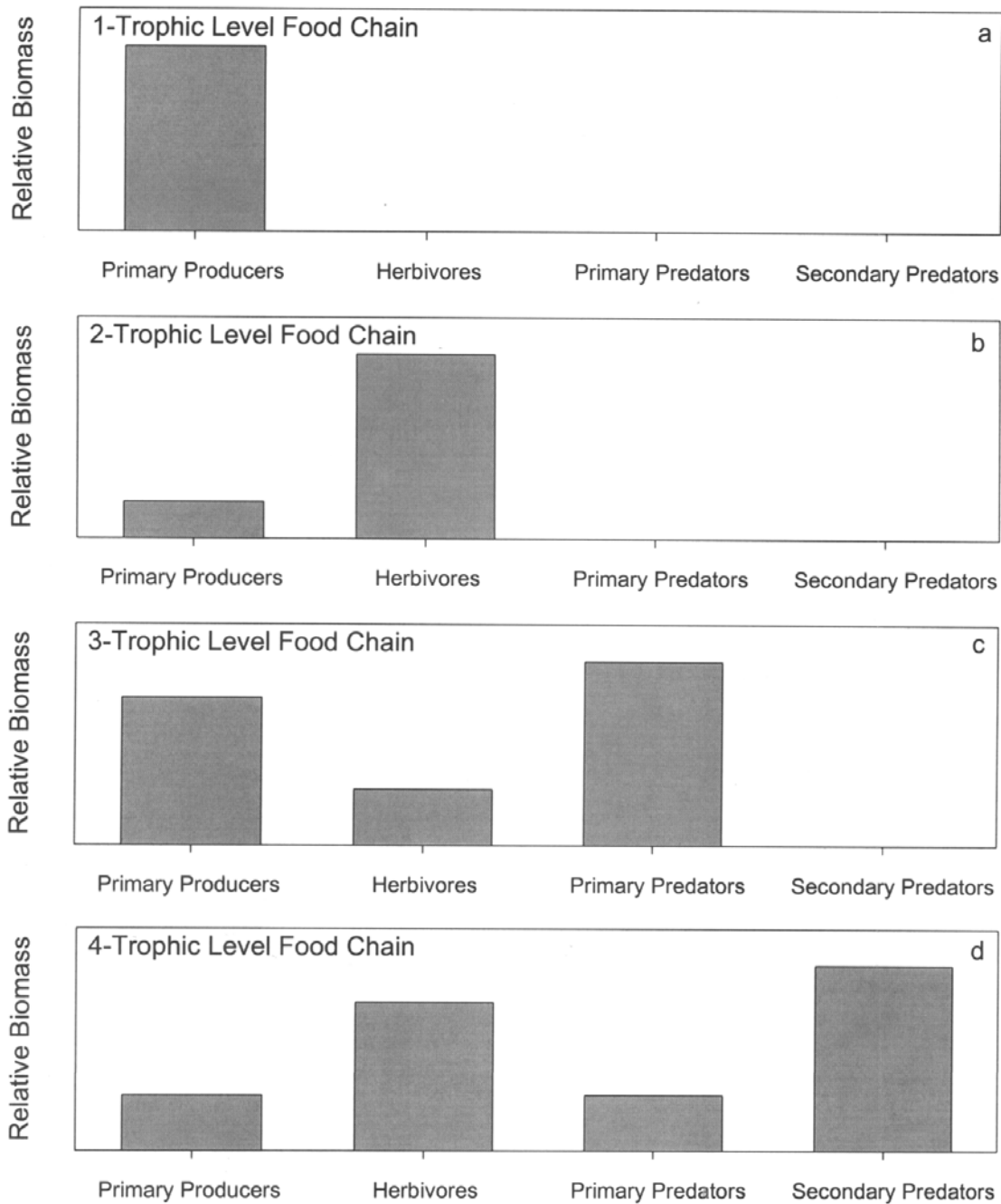
Figure 15.1. Relative biomass of primary producers, herbivores, primary predators, and secondary predators in (a) one, (b) two, (c) three, and (d) four trophic level food chains.

The consequences of the trophic cascade to understanding communities also highlights the importance of indirect interactions in natural communities. Predators can have significant positive and negative impacts on primary producers, even though they do not feed on plants or algae directly. Such effects are mediated by the effects of predators on intervening trophic levels, and for this reason are considered indirect.

Is there evidence for trophic cascades in natural communities? Are there practical implications of such a hypothesis for conservation biology or wildlife management? The answer to both questions is yes, for both aquatic and terrestrial ecosystems (Carpenter et al. 1987, Lancaster & Drenner 1990, Shapiro 1995, Polis and Winemiller 1996). Power (1990), for example, showed that fish predators in the Eel River in California had big impact on productivity of algal mats attached to boulders. Similar responses were observed in the food web in the Great Salt Lake, Utah, when unusual but natural events shifted the system from a predominantly 3 trophic level system to a four-trophic level system. Responses at each trophic level corresponded to the trophic cascade hypothesis (Wurtsbaugh 1992). Old field and grassland communities respond in similar fashion, in the face of invertebrate spider or praying mantis predators (Schmitz 1993, Moran et al. 1996). Food web alterations which employ a top-down approach for improving water quality or restoring entire lake systems is termed *biomanipulation,* a promising and powerful tool for lake management (Shapiro 1995). Biomanipulation involves the addition of a piscivorous fish such as largemouth bass to directly control the planktivorous fish community and to indirectly control phytoplankton abundance (e.g., Carpenter et al. 1987). Finally, in a wildlife management context, consider the effects of dwindling numbers of top predators in most natural systems. Loss of these species could have similar effects, although these responses have not been well studied because of the rarity of top predators in most natural ecosystems. **In addition, herbivores in agricultural settings are limited by a suite of invertebrate predators according to predictions of the trophic cascade hypothesis.** The removal of this natural enemy complex by insecticidal control of pest herbivorous insects may have unexpected consequences because indirect trophic links are disrupted.

Major groups of aquatic organisms such as phytoplankton and zooplankton typically exhibit rapid reproductive rates (for example, many algae divide every 1-2 days), consequently the outcomes of these ecological interactions become apparent within a few days, in contrast to weeks, months or years for higher organisms in terrestrial ecosystems. The following exercise can be conducted within 7-10 days, thus allowing further manipulations to be introduced or additional experiments to be conducted throughout the semester. For a larger scale version of this experiment which served as a basis for this exercise, see Lancaster and Drenner (1990). For further reading, we recommend Carpenter (1988), Lampert and Sommer(1997), Moss (1998), and Wetzel (1983).

Set-up

Equipment and Supplies (See Apendix A for addresses of suppliers.)

(6) 32-gallon plastic trash cans
(2) 5-gallon plastic buckets
150 μm-mesh plankton net [Aquatic Research Instruments]
(2) 500 mL plastic sample bottles
(12) 10-gal aquaria
Submersible pump and garden hose (*optional*)
(20) fathead minnows (available at any bait shop)
Large zooplankton [Carolina Biological Supply] (*optional*)
Graduated beaker, ≥1000 mL

35 μm-mesh plankton net [Aquatic Research Instruments]
(12) 300-mL glass sample jars (graduated)
10% sucrose-formalin solution (80 g sucrose/100 mL of 37% formaldehyde solution; dilute to 1000 mL with tap water).
Vacuum pump or sink aspirator
Compound microscopes equipped with 4 and 10X objectives (one per student)
Dissecting microscopes (one per pair of students)
Hensen-Stempel pipette (at least one) [Wildlife Supply Co.]
Sedgewick-Rafter counting cells (one per pair of students) [Wildlife Supply Co.]
Palmer counting cells (one per pair of students) [Wildlife Supply Co.]
Micropipette capable of sampling 0.1 mL (at least one)
Computer access to SAS, PC-SAS or equivalent statistical software [SAS Institute, Inc.]

Procedures

1. Secure six 32-gallon trash cans in the bed of a pick-up truck with rope.

2. Fill trash cans with water from a nearby pond, using 5-gallon buckets.

3. Fill two 500 mL plastic sample bottles with the plankton collected from ≥20 tows of a 150 μm-mesh plankton net from a dock or shore location near deep water. (Larger zooplankton will concentrate in deeper water during the day.)

4. Transport the pond water to the lab. Fill the 12 aquaria using buckets or a submersible pump and garden hose. Fill each tank in 2.5-gallon increments to ensure that the initial distribution of the water is uniform among the 12 tanks. Fill one additional tank to serve as a holding tank for any extra fish. (This tank should be aerated.)

5. Thoroughly mix the plankton samples and add equal volumes to each tank to compensate for the low zooplankton densities found in the littoral zones of lakes. *Optional: In addition to the plankton sample collected at the pond, add an equal amount large zooplankton [Carolina Biological Supply]. This will guarantee the presence of large zooplankton in the tanks.*

6. Randomly assign the each of the following treatment combinations to the tanks:
    (1) No phosphorus addition and no fish
    (2) Phosphorus addition and no fish
    (3) No phosphorus addition with fish
    (4) Phosphorus addition with fish

    Replicate each treatment combination three times, resulting in a total of 12 tanks.

7. Add two fathead minnows to each of the six tanks designated as "fish addition" treatments. Place the remaining fish in the extra holding tank.

8. Add 150 mL of phosphorus stock solution (See Appendix B for formulation) to each of the six tanks designated as "phosphorus addition" treatments.

9. Cut strips of thick black plastic (i.e. thicker than a typical garbage bag, available in most hardware stores) ca. 20 cm wide and tape them to the bottom third of the four tank sides. This will create a darker area in the bottom tanks to provide some refuge for zooplankton.

10. Allow tanks to stand undisturbed for 7-10 days. Replace dead fish in the experimental tanks with fish from the holding tank as needed. Fish starvation will be a major problem if the experiment is allowed to run for more than 10-14 days.

11. *Optional: Circulate the tank water using a undergravel filtration system (with the filters removed) or an equivalent system. This will keep the water from stagnating.*

Lab Exercise

Experimental Design

The experimental design of the study is a 2 X 2 factorial design (with & without phosphorus addition and with & without fish). This will result in four treatment combinations: (1) No phosphorus addition and no fish, (2) Phosphorus addition and no fish, (3) No phosphorus addition with fish, and (4) Phosphorus addition with fish (Figure 15.2). Each treatment combination will have three replicates, resulting in a total of 12 tanks. Each treatment should be randomly assigned to a tank.

Table 15.1. 2 X 2 factorial design with four treatment combinations.

	No Fish	Fish
No Phosphorus		
Phosphorus		

Tank Sampling

Divide the class into two groups. One group will sample and count the zooplankton; the other will sample and count the phytoplankton.

Zooplankton
a. Mix the tank thoroughly.

b. Remove 4 L from tank with a graduated beaker.

c. Pour the entire 4 L through a 35 μm-mesh plankton net.

d. Rise the material retained in the net into a 300 mL sample jar with a small amount of tap water (50-75 mL) from a plastic squirt bottle.

e. Double the sample volume to 100-150 mL with 10% sucrose-formalin solution. *Note: If the final sample volume exceeds approximately 150 mL, the zooplankton density may be too low to obtain an accurate count.*

f. Repeat for each tank.

Phytoplankton

Note: Phytoplankton samples should be concentrated prior to enumeration. Therefore, the instructor should collect the samples 24 h before the lab and concentrate the samples immediately before the lab. The procedure for concentrating the samples is given below in the phytoplankton enumeration section.

a. Mix the tank thoroughly.

b. Remove 100 mL from the tank with a graduated cylinder.

c. Pour the sample into a 125 ml glass sample jar.

d. Add 1 mL of Lugol's solution (Appendix B) to preserve the sample.

e. An additional qualitative sample should be collected so that students can observe living material when learning the various algal genera present. (Color is a helpful taxonomic indicator!).

Enumeration

Zooplankton

a. Mix the concentrated samples thoroughly and remove 1ml of sample with a Hensen-Stempel pipette. Empty the contents of the pipette into a Sedgwick-Rafter counting cell.

b1. *Small Zooplankton* (primarily rotifers). Count an entire SR-cell (i.e., 1 mL) for each sample with a compound microscope at 100X (total magnification). Count only the small zooplankton and ignore the larger animals at this point.

b2. *Large Zooplankton* (e.g., daphnids, calanoid copepods, cyclopoid copepods, large rotifers). Count one entire Petri dish filled with 20-30 mL for each sample with a dissecting microscope at 40X (total magnification). Count only the large zooplankton and ignore the small zooplankton.

c. It may be difficult to identify easily each organism to species, or even genus. Simply place each counted organism into defined taxonomic categories such as *Daphnia* A, *Daphnia* B, *Bosmina*, *Ceriodaphnia*, calanoids, cyclopoids, *Asplanchna*, *Keratella*, *Brachionus*, etc. (Appendix C). Focus on the dominant species.

d. Calculate the number of organisms per liter of tank water using the following formulas for each taxonomic category.

Organisms/L of tank water = (organisms per mL of concentrate) (1000) / (concentration factor)

where:

concentration factor = volume of tank water filtered (mL) / volume of concentrate (mL)

For example:

If 4 L of tank water were filtered, the concentrate volume was 100 mL, and 10 *Daphnia* were counted in the entire l mL SR-counting cell, then there are 250 *Daphnia* per liter of tank water.

Concentration factor = 4,000 mL/100 mL = 40
Daphnia/L of tank water = [(10)(1000)]/40 = 250

Phytoplankton

      a. Settle organisms to the bottom of the sample jar by allowing the jar to stand undisturbed overnight. (Note: Samples can also be concentrated using a large volume centrifuge.)

      b. Concentrate the organisms by reducing the volume of each sample to a known volume (start by reducing to 5 or 10 mL) with a pipette attached to trap and a vacuum pump or a sink aspirator. Do not to disturb the organisms on the bottom of the jar when removing the liquid off the top of each sample jar.

      c. Mix the concentrated samples thoroughly and remove 0.1 mL of sample with a pipette. Empty the contents of the pipette into a Palmer counting cell.

      d. Count 5 to 10 random fields of view for each sample with a light microscope at 100X (total mag.). If necessary, adjust the sample volume (i.e. the initial 5-10 mL) to increase or decrease the phytoplankton density in the counting cell. For example, the samples from tanks without fish may have lower phytoplankton densities and the sample volume may need to be reduced to ≤5 mL. Aim for about ten organisms per field of view.

      e. It may be especially difficult to identify each organism to species, or even genus for the phytoplankton. Simply place each counted organism into well defined taxonomic categories such as alga A, B, C, etc. However, some species can be identified to genera using the recommended keys (Appendix C). Focus on the dominant species.

      f. Use the formulas given in the zooplankton enumeration section to calculate the number of organisms per liter of tank water for each taxonomic category.

Additional Analyses (*optional*)

Total Phosphorus

      The class may choose to quantify the difference in phosphorus concentrations between the no phosphorus addition and phosphorus addition treatments. The students will learn: (1) One of the most common water chemistry procedures used in aquatic ecology, (2) How to develop and use a standard curve, and (3) How to use standard water chemistry equipment (e.g., spectrophotometer, pipettes). Lind's (1985) spectrophotometric procedure is widely accepted in the field of limnology/aquatic ecology. Some instructors may choose to use prepared reagents from Hach® and analyze the color change using a spectrophotometer or a Hach® colorimeter (see Appendix D).

Chlorophyll a

Summing the algal densities for each taxonomic category may reflect algal biomass. However, a treatment with a high density of small algae may have a lower overall biomass than a treatment with a low density of large algae. Determination of the concentration of the chlorophyll *a* pigment indirectly reflects the actual algal biomass (i.e. higher chlorophyll *a* concentrations indicate greater algal biomass). Fluorometric and spectrophotometric methods are described in Appendix D. Both methods use a standard to determine the actual concentration. However, the tests can be conducted without using standards and will yield "relative" concentration results. For example, if the absorption reading for treatment A is twice as high as that of treatment B, then treatment A has approximately twice as much algal biomass.

Turbidity

Turbidity in water is the presence of suspended solids (including plankton), which reduce the transmission of light. Less turbid tanks will therefore have greater water clarity. Turbidity can be determined using a turbidimeter (Hach®) or spectrophotometer using procedures described in Lind (1985). *Note: It is important to measure turbidity before the plankton samples are collected from the tanks. The plankton sampling will disturb the fish waste and detritus on the bottom of the tanks; thus confounding the turbidity readings.*

Data Analysis and Interpretation

The data collected in this exercise should be statistically analyzed by a completely randomized two-way analysis of variance (ANOVA) using SAS/STAT (SAS Institute 1985) or equivalent statistical software. This analysis will enable the students to assess the main effects due to phosphorus addition or fish addition and the interaction effects due to the combination of phosphorus and fish addition. We recommend that the analysis focus on the densities of the dominant phytoplankton and zooplankton species since significant density differences of rare species may be difficult to detect with three treatment replicates. [Note that the data obtained from any of the optional analyses (i.e. total phosphorus, turbidity, chlorophyll *a*) can also be statistically analyzed with the same program.]

SAS Program

Use the following program to analyze the data for each dominant species in each group, i.e. zooplankton or phytoplankton (note that the program includes sample data illustrated below):

```
OPTION  LS=72;
DATA A;
INPUT TANK NUTTRT$ FISHTRT$ VALUE;
CARDS;
1  P  F        1000
2  NP NF       100
3  NP F7       50
4  P  NF       200
5  P  NF       250
6  NP F        700
7  NP NF       175
8  P  F 1100
9  NP NF       125
10 NP  F       775
11 P    F      950
12 P    NF     275
RUN;
PROC SORT; BY NUTTRT FISHTRT;
PROC MEANS; BY NUTTRT FISHTRT; VAR VALUE;

PROC GLM;
CLASS NUTTRT FISHTRT;
MODEL VALUE=NUTTRT FISHTRT NUTTRT*FISHTRT;
RUN;
```

SAS Output

The output is the result of an analysis of the data shown above:

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NUTTRT	1	110208.3	110208.3	43.18	0.0002
FISHTRT	1	1435208.3	1435208.3	562.37	0.0001
NUTTRT*FISHTRT	1	20833.3	20833.3	8.16	0.0212

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NUTTRT	1	110208.3	110208.3	43.18	0.0002
FISHTRT	1	1435208.3	1435208.3	562.37	0.0001
NUTTRT*FISHTRT	1	20833.3	20833.3	8.16	0.0212

Interpretation of the Output

There are three probability values ("p-values") generated by the SAS analysis for each species, which are used to assess the significance of phosphorus effects, fish effects, and interaction effects. It is common practice in aquatic ecology to employ a 0.05 level of significance (or α-level) in evaluating each of these potential effects. Initially, there are two outcomes of immediate interest to this exercise. First, a significant phosphorus effect and/or fish effect (this may be positive or negative for each treatment) may be found. One would expect that every phytoplankter would respond positively to phosphorus addition because it is so often a limiting nutrient in fresh waters, however increasing the phosphorus level may alter competitive interactions among the phytoplankton, resulting in a negative effect of addition for some species but not others. Ultimately, such competition will lead to a shift in the composition of the algal community, particularly as greater periods of time enhance species differences as the communities diverge. Similarly, fish may have a significant negative effect on larger cladocerans (e.g., *Daphnia*) because they are more visible and therefore more likely to be eaten.

Secondly, the ANOVA may reveal a significant interaction effect, that is between the two treatments, phosphorus and fish. A significant interaction is evident not only from the p-value in the output, but also from a plot of density (of any dominant alga or zooplankter) versus phosphorus addition, with and without fish (Fig. 2). If there is a significant interaction, the slope of the two response plots will differ enough to result in the two plots intersecting one another (i.e they are not parallel), perhaps not within the gradient of concentrations used in the exercise. In other words, the response of organism *y* to phosphorus or fish addition is influenced by a change in the other factor (i.e. phosphorus or fish). The power of the factorial design of this experiment lies with its ability to reveal these kinds of interactions.

In addition to these more obvious *direct effects*, a number of *indirect effects* should be evident, that is, effects that demonstrate or support the trophic cascade hypothesis. For example, the addition of phosphorus may have a significant positive effect on zooplankton as a result of an increase in edible algae, or fish addition may have a positive effect on phytoplankton density by eliminating the larger, more efficient zooplankton grazers. Other indirect effects are certainly possible and of great interest in the study of aquatic food webs and how they function. See chapter 10 in Underwood (1997) for a detailed discussion of interpreting results from a factorial experiment.
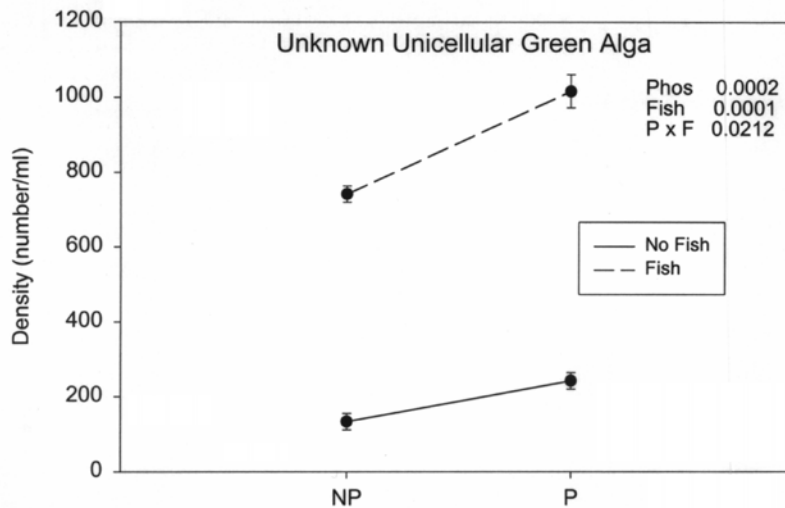
Figure 15.2. Hypothetical plot of density for organism *y* (an unknown unicellular green alga) versus phosphorus addition, with and without fish.

Additional Exercises

The following variations on the preceding exercise were proposed by Limnology students at the University of Nebraska-Lincoln:

- Substitute the visual feeding planktivore (i.e. fathead minnow) with a pump filter feeder such as gizzard shad (supplied by local game commission).

- Add complexity to the system by doubling the number of tanks and introducing aquatic macrophytes (e.g., *Elodea*) to provide a refuge for zooplankton, as well as periphyton (attached micro-community), etc.

- After 7-10 days, introduce a piscivore (larger aquaria would be necessary) or simply remove the minnows and monitor recovery.

- Substitute phosphorus addition with nitrogen addition or some other potentially limiting nutrient (and/or alter Total-N:Total-P ratios).

- Conduct the same experiment at several different times during the growing season, such as spring, summer, and fall, and compare results.

- Overlay the experimental design with two different light levels (using shade cloth), to examine the bottom-up effects.

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Appendix A - Equipment and Material Suppliers

Aquatic Research Instruments
P.O. Box 2302
Flathead Lake, MT 59911
800-320-9482
email: ari@cucafe.com
[phytoplankton nets, zooplankton nets, etc.]

Wildlife Supply Company
301 Cass St.
Saginaw, MI 48602-2097
800-799-8301
email: goto@wildco.com
[Sedgewick-Rafter counting cells, Palmer counting cells, Hensen-Stempel pipettes, etc.]
Note: Disposable Palmer cells are reusable and very inexpensive.

Hach Company
P.O. Box 608
Loveland, CO 80539-0608
800-227-4224
email: orders@hach.com
[phosphorus standards and reagents]

Carolina Biological Supply
2700 York Road
Burlington, NC 27215
800-334-5551
www.carolina.com
[living *Daphnia*]

SAS Institute, Inc.
SAS Campus Drive
Cary, NC 27513-2414
919-677-8000
www.sas.com
[SAS Statistical Computer Software]

Appendix B - Formulations

Phosphorus Stock Solution: Dissolve 0.2197 g of potassium dihydrogen phosphate (KH_2PO_4) in distilled water. Then dilute to 1.0 liter [*See the Total-P procedure in Lind (1985), beginning on page 79*].

Lugol's Fixative: Dissolve 5 g of iodine, 10 g of potassium iodide, and 10 mL of glacial acetic acid in 100 mL deionized or distilled water. Add 1 mL of this solution per 100 mL of phytoplankton sample.

Appendix C - Taxonomic Keys

Algae

Green Algae, Blue-green Algae, Dinoflagellates, etc.

Prescott, G.W. 1962. Algae of the Western Great Lakes Region. Wm. C. Brown Co. Pub., Dubuque, IA. 977 pp.

Prescott, G.W. 1970. How to Know the Freshwater Algae. Wm. C. Brown Co. Pub. Dubuque, IA. 348 pp.

Whitford, L.A. and G.J. Schumacher. 1973. A Manual of Fresh-water Algae. Sparks Press. Raleigh, NC. 324 pp.

Diatoms

Hustedt, F. 1930. Bacillariophyta. In: A. Pascher (Ed.), Die Süsswasser-flora Mitteleuropas. Heft 10. Gustav Fischer Verlag, Jena. 466 pp.

Patrick, R. and C.W. Reimer. 1966. The Diatoms of the United States. Vol. I. Academy of Natural Sciences of Philadelphia Monograph no. 13. 688 pp.

Patrick, R. and C.W. Reimer. 1975.The Diatoms of the United States. Vol. II. Part I. Academy of Natural Sciences of Philadelphia Monograph no. 13. 688 pp.

Invertebrates

Kudo, R.R. 1966. Protozology. 5th Edition. Charles C. Thomas Pub. Springfield, IL. 1174 pp.

Patterson, D.J. 1992. Free-living Freshwater Protozoa: A Color Guide. CRC Press. Boca Raton, FL. 220 pp.

Pennak, R.W. 1989. Fresh-water Invertebrates of the United States. 3rd Edition. John Wiley & Sons, Inc. New York, NY. 628 pp.

Thorp, J.H. and A.P. Covich. 1991. Ecology and Classification of North American Freshwater Invertebrates. Academic Press. New York, NY. 911 pp.

Ward, H.B. and G.C. Whipple. 1959. Fresh-water Biology. 2nd Edition. John Wiley & Sons, Inc. New York, NY. 1248 pp.

Appendix D - Additional Procedures

Chlorophyll *a*

[Note: an alternative method can be found in Lind (1985), pg. 129]

(1)   Collect whole water sample of known volume, typically 1 L, and keep cool. (Do *not* place the sample directly on ice because cells will lyse!)

(2)   Filter a known volume of sample (The concentration of algae in the sample will dictate the volume, typically >100 mL, to obtain a green or brown "lawn" on the filter.), using a Whatman GF/C or similar porosity filter.

(3)   Place the filter into a standard test tube containing 10 mL of 90% ethanol. Cover the test tube to prevent evaporation.

(4)   Place tubes in 78° C water bath for 5 minutes (keep covered from the light).

(5)   Remove tubes from bath and cool *in the dark* at room temperature. (Read the samples the same day.)

(6)   Decant extract into a test tube suitable for the fluorometer. Read at 665 nm (no turbidity correction necessary) or at 663 nm in a spectrophotometer [with a turbidity correction at 750; see Lind (1985)]. **Note:** Due to the high sensitivity of a fluorometer, dilution of the extract with 90% ethanol may be required.

(7)   *If* acidification is needed, acidify with 0.3 mL of 2N HCl. Mix the sample and allow it to stand for 5-30 minutes before rereading at 665 nm (fluorometer) or 665 *and* 750 nm [spectrophotometer; see Lind (1985)]. **Note:** An acidification step may not be necessary. See Turner Designs literature.

(8)   Compare the fluorometric reading with a standard curve run using chlorophyll *a* from Sigma Chemical Co. and/or spectrophotometric values derived using a standard formula.

Total Phosphorus

We recommend use of the molybdate method outlined in Lind (1985), beginning on page 79. It may be necessary to modify the standards (pg. 81) to assure that the lake Total-P concentration falls within the range of standards used to establish the standard curve.