# Chapter 12

# Life-History Schedules in *Daphnia magna*: An Ecological Activity for Multiple Laboratory Sessions

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## Contents



## Introduction

The nature of most laboratory courses can sometimes leave students with the impression that science can be done in two or three hours (the duration of a lab session). To counter this impression, it seems important to introduce students to the nature of long-term studies early in their academic careers. This lab exercise requires students to take an active part in a three-week study that exposes them to basic ecological principles influencing population growth. In most cases, this type of hands-on exercise which requires students to 'do science' in between the standard lab meeting times allows them the opportunity to contemplate the principles that are being evaluated.

In this lab, student groups will raise populations of *Daphnia magna* at three densities in order to determine how density affects the rate at which populations grow. Students are introduced to basic ecological constructs, like survivorship, fertility schedules, life-history tables, and net reproductive rates, to measure the change in population growth that occurs among the *Daphnia* populations during a three week period. Every day, one member of each student group counts the number of live adult and baby *Daphnia* present, as well as, cleaning and feeding their populations throughout the duration of this experiment.

The culmination of this exercise is a scientific paper written by each student presenting their findings. This report makes up a significant part of their total points for the course. Thus, it strongly encourages their participation and cooperation as a part of a data-collecting team and emphasizes that the results of scientific experiments must be interpreted, described, and presented for the scientific body of knowledge to be expanded.

## Materials

**For the entire class:**

Aquaria and water source for sustaining *Daphnia* populations (see Appendix A: *Daphnia* Maintenance)
Sufficient plastic petri plates to accomodate the students' *Daphnia* counting
Black surface to place under petri dish containing *Daphnia* to aid counting
Plastic pipettes with tips cut off so that adult *Daphnia* easily fit through the opening (~1/8 inch opening)
Small fish net to collect adult *Daphnia* from source populations (a mesh size of ~1/16 inch will allow baby *D. magna* to pass through while still holding the adults)
Black Sharpies and white freezer tape for labelling centrifuge tubes
Plastic cups or some other inexpensive container for housing the baby *Daphnia* to be used by the students.
*Daphnia Food:* (See Appendix A: *Daphnia* Maintenance
*Spirulina* algae powder
Baker's Yeast
Deionized water

**For each group of 4–6 students:**

50 ml centrifuge tubes with screw on covers (7/group)
Tube holder capable of housing the seven centifuge tubes (we use the styrofoam holders that new 50 ml centrifuge tubes come in)
Three-day-old *D. magna* (60/group)

## Notes for the Instructor

Typically, a portion of one lab period is used to introduce this exercise, describe how the experiment is set-up, and explain the steps involved in data collection. The class is then divided into groups of four to six students and these groups begin the investigation at that time. Before the students get their hands on the *Daphnia* we require them to each write brief hypotheses regarding the affects they believe density will have on survivorship, fecundity, and net reproductive rates. The students are then allowed to establish their study populations. Every day during the next three weeks, one member of each group must come to the lab and collect data on *Daphnia* survival and fecundity. This student must also change the water on their populations and feed the *Daphnia*. At the end of the experiment a full lab period is devoted to strengthening the students' understanding of the ecological concepts involved, pooling the data that were collected by each of the groups into a comprehensive data set for the entire class, and describing how we expect the students to write their scientific paper.

In response to the many questions I received at the conference about maintaining *Daphnia* populations, I have attached "Appendix A: *Daphnia* Maintenance" to explain what we do with our *D. magna* populations. Maintaining sufficient numbers of these little crustaceans seems to be basically trouble-free for us and hopefully the same will be true for you. The biggest preparatory step for this experiment seems to be building up sufficient numbers of gravid females to provide the three-day old waterfleas that the students start their populations with. We begin to expand our remaining *Daphnia* population from last year about 4 months before we need the babies by sequentially subdividing our population into more aquaria. We need to have *Daphnia* populations in about 20 ten-gallon aquaria by the time we start the investigation to support the 700 students in our thirty lab sections.

Four days before the students get their hands on these critters, TAs set out enough gravid females in plastic cups of well water to provide the offspring that their students will need. One day later, the TAs collect the babies that were produced during the last 24 hours. These young *Daphnia* are partitioned into three groups and each of these groups is housed at one of the different densities at which they will be maintained by the students. Therefore, the students receive baby waterfleas that have been preconditioned to the density conditions that they will be kept at during the experiment.

We provide the students with several forms on which to record information. This practice increases the efficiency and consistency of the groups. The first of these forms we call the "Work Responsibility Sheet" and it lists the names of the students in each group and the dates they are responsible for taking care of the *Daphnia.* One copy of this form is provided for the instructor as a means of insuring student accountability. A second form, entitled "*Daphnia* Life History Schedule Data Sheet," is where the students record their findings each day. Seven copies of this form are necessary for each group—one for each centrifuge tube housing *Daphnia*. All seven copies of this page are stored with the *Daphnia* populations. The final sheet, called "Pooled *Daphnia* Raw Data," is used by the students to summarize their data at the end of the experiment and is also used to display the pooled results from the entire class. Each student requires one copy of this sheet plus an additional sheet is necessary for each group.

The outcome of this experiment has been relatively consistent over the years, although there is some year-to-year variance in the actual values that are obtained. The results we typically see in this experiment are that:

1. The high density populations show increased mortality early in the experiment and offspring are produced a day or two earlier in these populations.
2. *Daphnia* raised at a low density tend to survive longer and produce more offspring than those at higher densities.
3. The intermediate density populations will have intermediate values for fecundity and survivorship.

These outcomes demonstrate that the density under which an organism lives influences the rate at which they reproduce and survive; furthermore, this effect can be extended into future population sizes when the net reproductive rate of a population is considered. Apparently, the stresses associated with high density conditions trigger individuals in dense populations to reproduce early in their life and this early reproduction is associated with increased mortality. These conditions lead to low $R_O$ values. The low density population shows just the opposite effect, adults survive longer, reproduce later, and produce more offspring yielding a much higher net reproductive rate. The intermediate density populations typically show values in between these two extremes, indicative of a correlation between density, survival, and reproduction.

I have intentionally avoided a description of what the students are required to present in their scientific paper. I am sure we each have our own preferences when it comes to what we think the students should stress in a paper and, therefore, it seems best that this step of the project be left up to your insights and expertise about your students' writing talents and weaknesses. However, I do feel strongly that the students gain a much greater appreciation of the principles being studied when they are challenged with a writing exercise similar to the one we use.

In the way that we use this experiment it is necessary that students have access to their populations seven days a week so that data can be collected each day. This practice may be restrictive on commuter campuses and at smaller institutions where there isn't a large crop of TAs to monitor the use of laboratory facilities each day. This inconvenience could probably be avoided by limiting data collection to every second or third day, but I haven't tried this so I'm unsure how it may influence *Daphnia* care or data interpretation. I really can't imagine it being much of a issue, though.

Other alterations of this experiment also seem plausible (although I haven't personally tested any of these, either). It seems likely that you could maintain constant densities across the experimental populations and vary the amount of food that is provided to the three populations to determine how much of an influence nutrient availability has on life-history patterns relative to other factors associated with crowding. In the same manner, the quality of the environment could be manipulated by introducing trace amounts of pollutants into the different populations and then assessing the influence such a perturbance has on survivorship and fecundity. In some systems, it has been shown that the presence of a predator influences life-history phenomena and this could be tested by placing dragonfly larvae or small fish among some *Daphnia* populations in such a way that the predator can't actually consume the waterfleas. Another variation on this theme I'd like to work with, especially in upper-level classes, would have the students designing their own experiments with waterfleas. This would provide them the opportunity to "do science" beginning with the projects conception. Whatever the case may be, I hope that your experiences with this type of hands-on, long-term experiment as a teaching tool are as rewarding for you as they have been for me.

## Student Outline

### Purpose

The purpose of this experiment is to help you better understand the role age-specific survival and fecundity play in the population growth (or decline) of organisms. Furthermore, this investigation will allow you to experience long-term experiments (relative to most experiments done in an introductory Biology teaching lab) first-hand.

### Objectives

1. Define and be able to pronounce and use the terms highlighted in bold text.
2. Given raw data for fecundity and survivorship, determine $l_x$ and $m_x$.
3. From $l_x$ and $m_x$ data, construct a life table and calculate $R_0$. From a value of $R_0$ determine whether a population is increasing or decreasing in size.
4. Explain why $R_0$ is a measure of the average number of female offspring produced by a female in her lifetime rather than a measure of total (males and females) offspring produced.
5. Explain why there is generally a trade-off between fecundity and survival.
6. Explain how fecundity schedules generally differ between organisms with type I survivorship curves and organisms with type III survivorship curves. Explain what features of the organisms' environments favor one or the other of these strategies.
7. Explain why *Daphnia magna* make a good study organism for this study.

### Introduction

Animals and plants vary widely in *fecundity* (reproductive output) and in life spans. Individual carp may produce one-half a million eggs annually while most primates produce no more than a half dozen offspring in their lifetimes. The life span of *Euglena* is measured in days and that of *Sequoia* in centuries. Variation in reproductive output and life span occurs not only over such widely separated taxa, but also within closely related species and different populations of the same species. For instance, fecundity and survivorship vary from year-to-year in the carabid beetle, *Agonum fuliginosum*. One study found that in one summer females produced an average of from 7 to 9.5 eggs per female and survival to the end of the breeding season was 5%. During another summer, fecundity declined to an average of four eggs per female, but survival was as high as 68%.

In this lab, we examine relationships between survival, fecundity, and density in the waterflea (Kingdom: Animalia, Phylum: Arthropoda). *Daphnia magna* is a freshwater invertebrate that feeds on algae such as *Chlamydomonas*.

Using *D. magna* of known ages, we will measure the rate at which they die and reproduce as they age. *D. magna* live 1–2 months so we can study these variables over much of their life span in this experiment. Another variable, density, will be introduced by raising different numbers of *D. magna* in a standard volume of pond water. This will allow us to assess the affects of density on fecundity and mortality and on$R_0$ values for the different populations.

## The Life Table

To examine patterns of death and birth, a *life table* for a *cohort* (group of equal aged individuals) of *D. magna* will be constructed. To do this, data for two separate schedules must be collected. The first of these is called the *survivorship schedule*. It records the number of individuals surviving to each age. The number surviving to a particular age, $x$, is recorded as the proportion $l_x$, of organisms that survive from birth to age $x$. If time is measured in days, and after one day 80% of the *D. magna* are still alive then $l_1$=0.8; if 50% of the original number survive to age two days, then $l_2$ = 0.5, and so on. The term $l_x$ is often referred to as *age-specific survivorship*.

The most convenient way to examine survivorship data is graphically through the construction of a *survivorship curve*. The three basic types of survivorship curves are shown in Figure 12.1. *Type I* survivorship is typical of modern human societies in which most death is the result of senescence (old age). For a *type II* survivorship curve the probability of death is about equal at every age. When plotted on a semilog scale ($l_x$ on a logarithmic scale and $x$ on a normal scale) type II survivorship appears as a straight line. Some species of birds have survivorship curves that approximate type II. In *type III* survivorship most individuals die soon after birth, and a few live to reproductive ages. Many species of organisms, such as oysters, salmon, and dandelions, have survivorship curves which approximate type III.

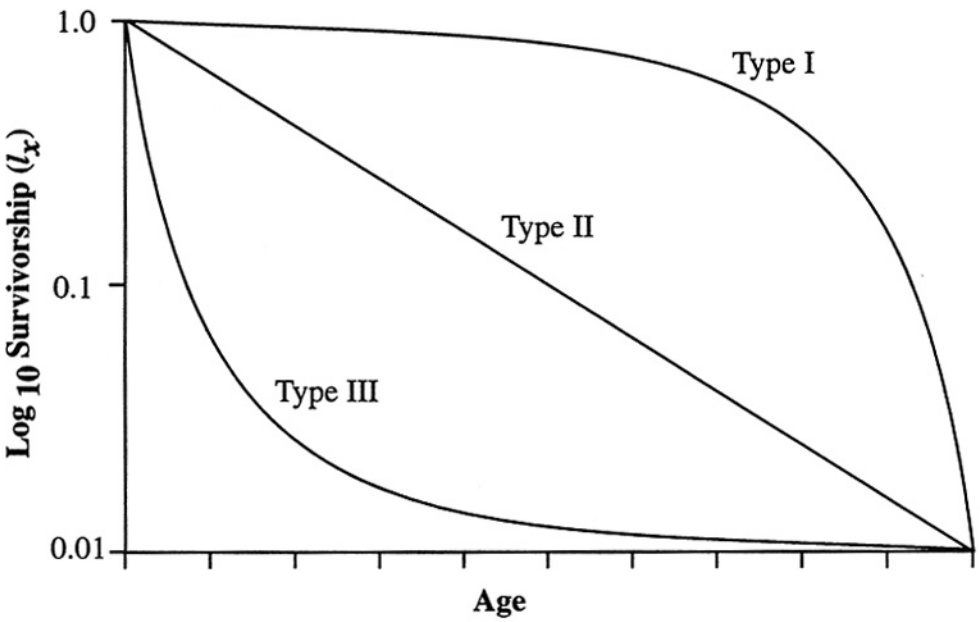


**Figure 12.1**. Examples of the three types of survivorship curves.

The other schedule that will be constructed is called a fertility schedule. It consists of *age-specific birth rates* (the average number of female offspring, $m_x$, born to each female at age x). Again, consider a hypothetical example: if one-day-old *D. magna* have no offspring, $m_1$=0; if two-day-old have on average 3 female offspring, $m_2$ =3; and so on through the entire life span.

From a survivorship schedule and a fertility schedule one can construct a life table. The life table simply tells us the probability that an individual will survive to a given age, say 7 days, and the number of offspring a one-week-old individual produces on average. By multiplying these two

values, $l_7$ and $m_7$, we can derive an expected reproductive contribution from one-week-old individuals. This product incorporates the individual's chances of surviving to the age of one week and the number of offspring it will produce once it gets to that age. One can derive this product for every age. Adding them all up gives a value known as the *net reproductive rate*, $R_0$. The equation for $R_0$ is simply:

$R_0 = \sum l_x m_x = l_1 m_1 + l_2 m_2 + \ldots + l_n m_n$, where $n$ is the life span of the organism.

The value $R_0$ gives the average lifetime reproductive contribution of individuals in a population. Remember that this is an average or expected value. Some will live to the maximum age and reproduce many times while others will not.

An example of a life table is shown below (from Wilson and Bossert, 1971). In a population of mice 50% survive to their first breeding season (age = 1 year), at which time they give birth to an average of three female offspring. By year two, 25% of the original cohort are still alive, and these produce an average of three female babies. By the third year 12.5% are left and three females are produced per survivor. No adults live to a fourth breeding season. The life table follows:

**Table 12.1.** Mouse life table from Wilson and Bossert (1971)

| $x$ (years) | $l_x$ | $m_x$ | $l_x m_x$ |
|---|---|---|---|
| 0 | 1.0 | 0 | 0 |
| 1 | 0.5 | 3 | 1.5 |
| 2 | 0.25 | 3 | 0.75 |
| 3 | 0.125 | 3 | 0.375 |
| 4 | 0 | 0 | 0 |
| | | $R_0 = \sum l_x m_x =$ | **2.625** |

An $R_0$ of 2.625 means that the average number of female offspring produced by a single female in her entire lifetime is 2.625. Remember that because this is an *average* and that it reflects the fact that an entire lifetime for some females amounts to a couple of months with no reproduction, while others last through one or two years. Another way to think of $R_0$ is as the expected number of female offspring a newborn female may have over her lifetime.

You may have noticed that in this example only females were counted; nothing was said about males. It turns out that for sexually reproducing organisms in which populations are composed of roughly equal numbers of both sexes, *only female offspring are counted* when determining fertility schedules (as for the mice above). Males are generally ignored since a single male can fertilize many females. Hence, $R_0$ is actually the average number of daughters born to a female during her lifetime.

The value, $R_0$, indicates whether a population is growing, is declining in size, or is stable. A population is stable if, on average, each female replaces herself during her lifetime, or in other words, if each female produces an average of one daughter during her lifetime. In this case, $R_0$ would be equal to 1.0. Similarly, a population is increasing in size if each female more than replaces herself; that is, $R_0$ is greater than 1.0. When $R_0$ is less than 1.0 each female is not replacing herself

and the population is decreasing in numbers. When viewed in these term, it is clear why $R_O$ is called the *net replacement rate*.

A comment needs to be made about the *D. magna* that we will be using in this experiment. Most reproduction in this species, and all that we will be measuring during this lab, occurs through *parthenogenesis* (the reproduction of offspring from an unfertilized egg). Males are absent from *Daphnia* populations except during periods of environmental stress. Only during these times of stress does the production of males lead to sexual reproduction. When environmental conditions are again favorable, only females are produced and this occurs through parthenogenesis. All the *Daphnia* present at the beginning of this experiment are female, and all their offspring are female as a result. Thus, all offspring are counted when determining fertility schedules. In a parthenogenetically asexual organism like *Daphnia,* $R_O$ gives the average total number of progeny. In a sexual organism, $R_O$ includes about half of all progeny produced by an average female (if sex ratios at birth are approximately equal).

## Life History Strategies

Many organisms, it seems, pay a survival cost for producing many offspring. When energy and resources are devoted to reproduction early in life, fewer resources are left for survival. On the other hand, channeling many resources to survival leaves fewer for reproduction. Thus, it appears that organisms have a finite amount of energy and resources that they allocate to reproduction and survival. Investing more in one means less to the other. This is known as the *life-history trade-off*.

What determines whether organisms should tilt their energy and resource expenditures in favor of survival or reproduction, by that I mean, what *life-history strategies* have evolved and why? One answer to this question is that resources should be spent in a way that yields the highest $R_O$. But this does not really answer the question since $R_O$ depends on survival and fecundity schedules. An organism could produce many young early in life, but this would lessen its chances of survival to later reproductive seasons when it could produce additional young. Conversely, refraining from reproduction at early ages is not advantageous if it is not compensated later by a longer reproductive life or a greater expected reproductive potential.

The way in which animals and plants balance the demands of survival and reproduction has been the subject of much research in ecology. In general, what has been found is that in stable, high-density populations where competition for food, space, or light is keen, organisms channel much of their energy and resources into surviving the rigors of this struggle. In this situation, organisms tend to delay reproduction to later ages and to produce few offspring. However, they tend to invest heavily in each offspring to give their offspring high chances of survival. Such organisms typically have a type I survivorship curve. Examples include most primates and perhaps most mammals.

At the other extreme are organisms that are often faced with low population densities and abundant resources. In this situation, competition is not intense. Population density may be kept low by predation, or density may be temporarily low in a changing environment in which new areas often open up for colonization when the already-colonized areas become inhospitable. Consequently, organisms adapted to this type of environment devote much of their energy and resources to reproduction. They produce many offspring, but invest relatively little in each one. These organisms usually have type III survivorship curves. Any individual offspring has little chance of surviving to breeding age, but by producing large numbers of offspring the parent is assured of leaving at least a few successful ones. Examples include many fish, marine invertebrates, most insects and many plants.

## Long-Term Studies

As you can imagine, studying the life-history of a species can take a very long time. Following a *cohort* of elephants, for instance, would take the lifetime of the researcher. Even studying the life-history of a short-lived organism like *Daphnia* takes some time. There are many types of ecological studies besides those on life-history that require a long-term time commitment. In fact, the vast majority of science requires running studies over long periods of time. Furthermore, the procedures for collecting data are often repetitive and tedious. For example, archaeologists may have to spend months on their hands and knees meticulously extracting a fossil or artifact form the earth. A plant ecologist may have to spend weeks counting, collecting and cataloging the plants from study plots. But this is all part of the process of science, and scientists do not mind doing it because we realize it is necessary to answer the questions that intrigue us. And of course, sometimes the tedium is offset by the nature of the study, like being outdoors.

The nature of laboratory courses can sometimes leave students with the impression that science can be done in two or three hours—the duration of a lab session. This investigation will expose you to the nature of long-term studies, to counter this impression. Therefore, one of the purposes of this experiment is to serve as an introduction to long-term studies. You will be required to come in periodically (usually every four or five days) to collect data on the *Daphnia* during the next three weeks. By the end of this experiment you should understand and appreciate the individual responsibility and commitment required to perform a scientific experiment. Doing good science takes time!

## Procedure

You will be following 3 cohorts of *Daphnia magna*, each at a different density, for approximately 3 weeks. As a group you will be keeping track of survivorship and fecundity daily. Class data will be pooled, and these data will be used to construct a life table and calculate $R_O$. Survivorship curves will also be derived. Each density treatment will be analyzed separately. These data will be used to write a paper, in the format of a scientific paper.

### *Set-up*

Each class is divided into four to five groups of students. Each group follows the same procedure. Split up today's work among the group members so that the procedure is done quickly and efficiently.

1. Each group will receive a manila folder containing data recording sheets and the schedule sheet. Think of a clever name for your group and label your folder (note: the staff reserves the right to edit group names judged to be in poor taste). You will have a "*Daphnia* Life History Data Sheet" on which to record the names of your group members, their phone numbers, and the dates each person is responsible for collecting data. Each group must also fill out a second copy of this sheet for your instructor to use as a reference page.
2. Obtain seven 50 ml plastic centrifuge tubes and make a white tape label for each tube. The information on each label should include the group name and the density of *Daphnia*. The tubes should be labeled as follows:
   Four centrifuge tubes - 1 *D. magna* per 2.0 ml of water
   Two centrifuge tubes - 1 *D. magna* per 1.0 ml of water
   One centrifuge tube - 1 *D. magna* per 0.5 ml of water

**3.** Fill each tube with between 6 and 8 ml of the aerated well water that is provided in the large container. Throughout this experiment, use *aerated well* water whenever you add water to one of these tubes. *NEVER USE TAP WATER!*

**4.** Three-day-old *Daphnia* will be provided by your instructor. Place these *Daphnia magna* into the labeled containers as follows:

The tubes labeled 1 *D. magna* per 2.0 ml of water each receive 5 *Daphnia*.
Those labeled 1 *D. magna* per 1.0 ml of water get 10 *Daphnia* each.
The tube labeled 1 *D. magna* per 0.5 ml of water receives 20 *Daphnia*.

These three-day-old *Daphnia* were obtained by placing egg-laden females in aerated well water and collecting all the offspring that appeared during the next 24 hours. The young were then raised separately from the adults for the past three days. Transfer the appropriate number of young *Daphnia* from their "nursery" to your centrifuge tube with a clean plastic eye-dropper. Transfers should be made carefully so as not to injure the *Daphnia*. Be careful to insure that the tip of the eye-dropper in submerged in the water before releasing the *Daphnia* into your centrifuge tubes. Air bubbles can get trapped under the *Daphnia*'s carapace if you allow them to be exposed to the air.

**5.** Adjust the volume of water in each tube to 10 ml by adding or removing sufficient water with an eye-dropper. Obtain the "*Daphnia* Food" from your instructor and add one drop to each centrifuge tube. Screw the tops on the tubes loosely—*Daphnia* need oxygen for cellular respiration, just as we do!

**6.** Place your group's *Daphnia* populations in their holders and set them on the shelf marked for your lab period. Place your manila folder with the data sheets and schedule on the shelf with your centrifuge tubes.

*Daily Procedures*

During the next three weeks you must feed and change water on the *Daphnia* every day, as well as collect survival and fecundity data. The total procedure may take an hour or more the first time you do it, but will go more quickly as you become more proficient at the procedures and as the population of *Daphnia* declines. Each time you come in to take care of your populations, you must obtain your *Daphnia* and data sheets from the shelf and do the following:

1. Gently pour the contents of one of your centrifuge tubes into a petri dish. Set this petri dish on a black background. Add *6–8 ml* of aerated well water to the empty centrifuge tube. Using a plastic pipette, remove and count *only* the adults from the petri dish. Put the adults back into the centrifuge tube, transferring as little of the old water back as possible. Discard any dead adults. *Record* the number of live adults for this tube on your data sheet. If you have too many adults the first day the count is done, (i.e., you count 21 for a tube that should only contain 20), discard extras into the appropriately labeled aquarium. Be sure your count is accurate!
2. *Now count* the number of *baby Daphnia* that remain in the petri dish. Young *D. magna* are easily distinguished from adults by their small size. *Record* the number of babies for this tube on your data sheet. Place these babies in the aquarium labeled "BABIES" located in the counting area. *DO NOT* put the babies back in your tubes!!!!
3. *Adjust the volume of water in the centrifuge tube* containing the adults to re-establish the original density. In order to maintain the same density throughout the experiment, the water volume decrease as adults die. For instance, if two individuals die in a tube that originally contained 10 *Daphnia* (i.e., 1 *Daphnia* per 1.0 ml water) you must decrease the volume of water by 2 ml—one milliliter for each *Daphnia*. If you need to take out water, be careful not to remove *Daphnia* in

the process. If you need to add water, use an eye-dropper to do so. If no deaths have occurred, keep the same water volume.

4. Make written comments about circumstances involved with this tube in the "Notes" column of your data sheet if appropriate.
5. *Repeat* procedures 1–4 for the other 6 centrifuge tubes.
6. *Feed your Daphnia.* Obtain the "*Daphnia* Food" from the location to be described by your instructor (it is in well-marked dropper bottles). Be sure the cap on the food bottle is tight and *shake it well.* Dispense *one drop* into each centrifuge tube. NOTE: OVERFEEDING IS DELETERIOUS *to the health of Daphnia*. *Return the food to its original location.* It is important that the food remain cool or it will spoil. Screw the caps back onto the centrifuge tube loosely.
7. As your *D. magna* populations die off, you may want to *combine some centrifuge tubes.* Combine *Daphnia* from different tubes of the *same density* whenever the number of *Daphnia* in a tube reaches *two*. This avoids very low water volumes. Do not mix different density treatments (i.e., you shouldn't combine a 1 *Daphnia* per 1.0 ml tube with a 1 *Daphnia* per 0.5 ml tube).

**Assignment**

You will be required to write up an assignment based on the data collected. More information on the assignment will be given to you when this experiment is completed.

## Acknowledgements

I am indebted to Dr. Dennis Minchella, the previous instructors, and staff of Biology 122: Diversity, Ecology, and Behavior at Purdue University who nurtured the development and evolution of this laboratory exercise. Much of the information included in the "Student Outline" section of this chapter was taken directly from our Biology 122 laboratory manual.

## Literature Cited

Wilson, E. O., and W. H. Bossert. 1971. A primer in population biology. Sinauer Assoc., Inc., Sunderland, Massachusetts, 192 pages.

## APPENDIX A
## *Daphnia Maintenance*

Feeding can be done at various rates and has an effect on how quickly the culture grows. The following recipe describes a good nutrient source for the waterfleas.

3 heaping tablespoons of *Spirulina* algae powder
2 packets of Baker's Yeast
500 ml deionized water
Combine ingredients in a blender and blend until well mixed. Keep food refrigerated after mixing. NOTE: The amount of yeast and algae powder is somewhat variable and can be increased or reduced depending upon whether you are merely maintaining stock cultures or attempting to expand the populations.

A maintenance level feeding rate consists of 20 ml of *Daphnia* food every 2–3 days for each aquarium containing *D. magna*. When the cultures are being expanded the feeding rate is increased to 50–100 ml of food every other day depending upon the density of critters in the tank. These filter feeders will clear the cloudiness caused by feeding in about two days.

If you overfeed the culture there will be an accumulation of "scum" that forms on the bottom of the tank. This exceeds the natural accumulation of algae and dead *Daphnia*, and it builds up rather quickly. Experience is the best teacher when trying to balance the health of the culture and the need for extra food when you are expanding the size of your populations. When the cultures are merely being preserved for future uses you can go as long as two weeks between feedings.

Once a tank appears to be teeming with these crustaceans, you should remove about two-thirds of the adults for founding new populations (if you're getting too many *Daphnia*, they make wonderful fish food). If the density of *Daphnia* in your aquaria is not monitored, there is the possibility the culture will "crash," in which case, *all* the *Daphnia* will die. Knowing when a tank is approaching this point may take a little practice and, regretfully, a couple of crashes. A foul odor is a good sign that the population is getting too dense

Our aquaria have a rich algal fauna in the water column, attached to the glass surfaces and a good layer of organic matter on the bottom and the waterfleas seem to do fine. However, these critters are very sensitive to chlorinated water and excessive mineral concentrations. To counter this, we start by letting the water sit in the aquaria for a day or two before adding the *Daphnia*; a dechlorinating water conditioner (for fish) can be used if you're in a bind. After the culture is started and water begins to evaporate, you can replenish the volume with either distilled or deionized water so that the mineral concentration remains tolerable to the crustaceans. We have also found that aeration through air stones aids the culturing of *Daphnia*. A slow stream of bubbles is sufficient.