Chapter 17

Quantitative investigation of the crowding effect of *Hymenolepis diminuta* in *Rattus norvegicus*

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Contents

Introduction

The life cycle of the rat tapeworm (*Hymenolepis diminuta*) includes two hosts (See Figure 17.5). The eggs of adults are passed out with feces from the definitive host, the rat (*Rattus norvegicus*). The eggs must be ingested by the intermediate host, the grain beetle (*Tenebrio molitor*). Inside the beetle's intestine, the embryo (oncosphere) hatches from the egg, penetrates the intestinal tract, and subsequently transforms into the larval state (cysticercoid) in the hemocoel. Beetles infected with the cysticercoid larva of *H. diminuta* are ingested by the rat. After release from the digested beetle, the cysticercoids evaginate their scoleces and attach to the wall of the small intestine of the rat.

This exercise can be completed over a six week period, a three to four week period, or a two to three hour lab period (Figure 17.1). It is suitable for almost any level biology course. At the University of Nebraska, this lab is used for our Parasitology course and results in a written scientific paper from the students. The objectives of the lab are to: 1) Introduce parasite-host relationships to the students; 2) Complete experimentally the life cycle of *Hymenolepis diminuta;* 3) Quantify and discuss the effect of variable infection intensities on populations of *H. diminuta*.

The six week long lab necessitates infecting beetles (*Tenebrio molitor*) with eggs from an *Hymenolepis diminuta*-infected stock of rats (*Rattus norvegicus*). The beetles must be maintained for three weeks after exposure to the rat feces. At three weeks, the students can cut the beetles to remove the larvae (cysticercoids) from the hemocoel and feed them to the rats.

Infections in rats must remain for at least three weeks to ensure the worms are mature and producing eggs. Directing the lab with this time schedule is the preferred hands-on introduction to a host-parasite association, a complex life cycle, data interpretation, and methods of manipulating biological material.

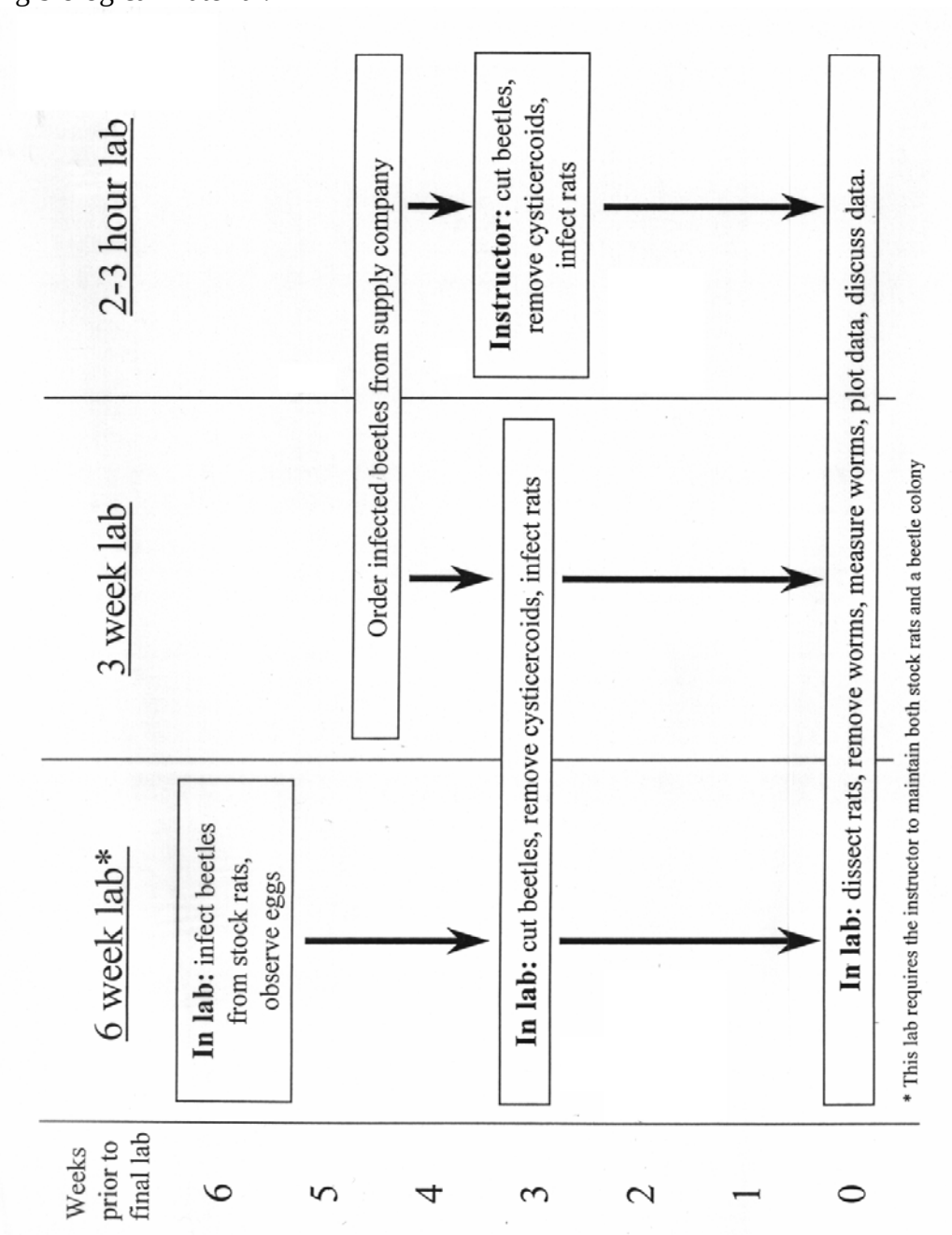

Figure 17.1. Flow chart showing various options available for conducting this laboratory exercise. The six-week lab format is preferred.

The first step introduces the students to the life cycle of *H. diminuta*, the morphology of the egg, and animal care. We have the students remove a fecal pellet from the cage of an infected rat to observe the parasite's egg. This exercise gives students an idea of the number of eggs in a drop of feces and the movement patterns of the oncosphere (embryo) inside of the egg (Figure

17.6A). The movement of the hooks on the oncosphere indicates how it burrows from the gut of the beetle into the hemocoel.

The three-week lab requires *H. diminuta*-infected beetles to be purchased from a supply company (e.g. Carolina Biological, Burlington, NC), then having the students recover the larvae from the beetles and feed them to the rats. Again a period of three weeks is optimal for worm maturation in the rats. Similar to the six-week long lab, dissecting the beetles allows the students to observe the larval stage and to understand the difficulties in gathering infective stages and successfully administering them to a host. In the six or three week lab, supplementing the lab exercise with prepared slides of the cysticercoid before dissecting the beetles should help the students find the larva and understand gross anatomy. Additionally, providing a composite of the adult prior to dissecting the rats will familiarize them with gross anatomy.

The two-three hour long lab involves the instructors purchasing *H. diminuta*-infected beetles and infecting the rats themselves prior to the scheduled lab. The student will spend the lab period dissecting the worms from the rats, measuring them and discussing the results. If time permits, the use of the prepared slides demonstrating gross anatomy of the cysticercoid and composite of the adults is recommended.

The topics in this lab are usually introduced first through a discussion of parasitic relationships, the close association between a parasite and its host and complex life cycles. After the students have a grasp of the intimate relationship between the parasite and its host, the instructor can encourage them to consider the fate of these parasites after they are ingested by the host. For example, why do we not see many cases of hyperinfestation? We can ask: Do all larvae establish in a host? If not, why not? If all the larvae establish, can they all survive to maturity? Or if all the larvae establish, is there an effect of population size on some character of the parasite? The last question will introduce the ideas of this lab.

The phenomenon of the crowding effect is defined in a given infection as the size of the worms being inversely proportional to the number of worms present per rat (Figure 17.2). The crowding effect can be measured by weight-length ratio, length, mean dry/wet weight, surface area, daily egg production, egg/proglottid, or any combination. For our junior/senior level class, we have the student measure the total length of the worms and plot the averages. In the graduate class, we have the students measure length, width/length of a single gravid proglottid, and eggs/proglottid, graph the results, and write a paper.

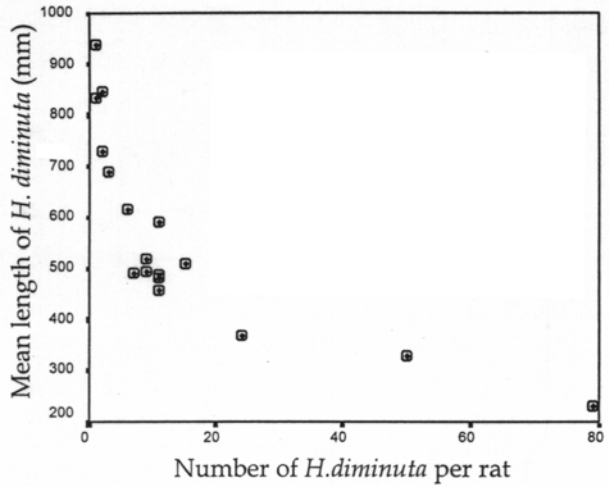

Figure 17.2. Example of actual data of the relationship between mean length and the number of *Hymenolepis diminuta* in *Rattus norvegicus*.

List of Materials

- Metafane or ether
- One-two gallon jar
- Rats for stock infections infected and/or uninfected
- *Tenebrio molitor* (Carolina Biological Supply Co., NC)
- Microscope slides and coverglass
- Any kind of vial so the students can take the worms home
- 70% ethanol to preserve the worms
- CO^2 tank to kill the rats (or use Metafane or ether—it takes longer, but is more humane.)
- Prepared slides of cysticercoids and composite slides of the adults of *Hymenolepis diminuta* (optional, but recommended) (Carolina Biological Supply Co., NC)
- Compound microscope to observe the egg, cysticercoid, and adults of *Hymenolepis diminuta*
- Garbage bags to collect carcasses

Each group of two to three students will need:

- One container for 15 beetles
- One rat
- One - 300 ml dish to collect the worms
- One eye dropper
- Two disposable Petri dishes (60 X 15mm)
- One pair scissors
- One to two forceps
- Two probes
- Dissecting microscope
- Yard stick to measure worms
- One dissecting tray
- Small wash bottle for water (clean with no trace of chemicals)

Notes for the Instructor

Many students are nervous about handling rat feces and beetles. We make sure that the teaching assistants (TA) have practiced the lab beforehand to ensure a smooth lab. A nervous or hesitant TA can create problems like low student involvement and confidence. We explain each procedure very carefully before the students start handling the animals. The seriousness of the introduction usually inspires questions that may have otherwise gone unasked.

Safety concerns involved with this lab are few. Since the rat tapeworm has a two-host life cycle, humans can become infected only by ingesting the cysticercoid from the beetle. The students should be made aware that handling infected grain beetles must be done with attentiveness, to avoid infection. However, it should be stressed to the students that care must be taken when handling animals, and that proper cleaning of hands and labware is essential. Remind students to limit the handling of items other than those required during the experiment. Rat carcasses and beetle remains should be treated as normal biological waste. Also, freezing can kill unused cysticercoids easily.

Rats will be infected with tapeworm larvae. To check for successful infection, rats will be killed with carbondioxide (*or* ether *or* metophane). Rats will be exposed to the larvae while anesthetized with ether *or* metophane. Infection with this parasite, *Hymenolepis diminuta* does not produce any clinical conditions. All animals will be cared for and disposed of according to departmental regulations.

Infecting the beetles is relatively simple and they are easily maintained. We use plastic shoeboxes with the middle of the lid removed and replaced with mesh. We place a paper towel on the bottom of the box, add the fecal pellet and beetles, then overlay that with another paper towel. Exposing beetles to fresh rat fecal pellets insures infective *Hymenolepis diminuta* eggs and a beetle's interest in it as food! The paper towel must be splashed with water every day for three weeks. These beetles are sensitive to changes in humidity. Therefore, prior to the dissection of the beetles, we order infected beetles to accommodate high deaths or low infections. Since the beetles are infected by eating the eggs in the feces of the rat, you already must have a rat(s) infected with adults of *H. diminuta*. This can be accomplished by ordering beetles infected with *H. diminuta* and infecting some stock rats that you keep in the lab or animal facilities.

Dissecting the beetles progresses smoothly when you have the students remove the head, legs and elytra (hard outer-wing covering). Most of the students do not like this practice and will spend considerable time with forceps and scissors trying to manipulate a slippery, struggling beetle that inevitably will escape at least once! Usually, if the TA gives a quick, serious demonstration on how to dissect the beetle and open the abdomen, most of the students follow form. The larval stage, the cysticercoid, is found in the hemocoel of the beetle. There is no need to cut any of the organs. In fact, it is better that they are not cut away, in order to reduce the amount of material in the dish obstructing their view of these small cysticercoids. It is important to make sure you use glassware and eye droppers that have not been exposed to any chemicals, even alcohol. Any chemical can kill the sensitive cysticercoid. Moving the cysticercoids from the beetle dish into a dish with fresh water is the best way to handle them for the rat infections.

Infecting the rats requires the most dexterity from the students and TA. Before the rat is anesthetized, make sure the desired number of cysticercoids is in the eyedropper, and is ready to be used. Once the rat is placed in the anesthetization jar, students must watch the rat carefully, shaking the jar occasionally to test the reaction of the rat. Once sluggish, the rat should be removed from the jar. One student should hold the rat by the scruff of the neck and lay it on its back on the other hand, while the other student with the eye dropper dispenses the water with cysticercoids into the back of the rat's throat. When the rat is removed from the anesthetization jar, students have about 30 seconds of time during which the rat is stunned. After this time, the rat may start to squirm, but will not yet be fully conscious.

During the inoculation with cysticercoids, students need to be careful not to squeeze the water from the eyedropper into the trachea and into the lungs. Such action may endanger the rat or waste cysticercoids. Occasionally, the rats won't swallow the water, causing the water to drool out of the mouth, loosing cysticercoids. This can be avoided by carefully pipetting the water as far back into the mouth as possible. To facilitate ingestion of the liquid, gently squeeze and release the eyedropper bulb and move the dropper up and down ensuring the liquid remains in the rat's mouth. If all else fails, put the rat back in the cage and try again after a few minutes. A smooth infection comes with practice.

If a rat should become overexposed to the Metafane or ether but is not dead, place it in its cage and put it in a dark, quiet place for a few hours. Most rats will recover. Remember that

you should **always** be gentle when infecting these rats. Using young rats is easier for this lab. Their small size makes them easy to handle and they are less aggressive than the older rats, but younger rats are more nervous. To anesthetize the rats, we use one to two gallon jars with cotton to absorb the Metafane or ether. Rats are fed *ad libitum* on rat pellets and water. Beetles are given bran after two weeks post- exposure to the eggs in the feces.

Caveats

- Causes of death in the rats post exposure are usually: destruction of tissue by the eyedropper, drowning from water in the lungs, biting the eyedropper and swallowing the glass, and/or over-exposure to the anesthetic.
- If humans ingest the eggs of *H. diminuta*, they will not become infected. However, humans can become infected by ingestion of the cysticercoids. Careful lab procedure and clean-up will avoid this issue**. Do not stick any objects in your mouth while dissecting beetles. Wash hand thoroughly after the laboratory exercise.**
- A rat that is provoked (poked too often or teased) will bite. Clean, swift, single movements are advised, to avoid handling the rat longer than is necessary.

Sources of error

- Cysticercoids are small and can remain stuck in the eyedropper after administering the larvae, resulting in a lower infection number than anticipated.
- The students are not very dexterous at cutting the rat's small intestine and will cut the long adult worms in several places. This error will result in worms that are measured smaller than their actual size, plus with all the pieces, it is difficult to decide how many worms there are in the pile. The best method for counting the number of adult worms is to count only the number of scoleces present. Scoleces are small and thin, buried in the intestine and often are removed unknowingly from the rest of the body, resulting in shorter worms.

Student Outline

He was as fitted to survive in this modern world
as a tapeworm in an intestine.
Sir William Golding (1911 - 1993)

Objectives

In this laboratory you will apply your knowledge relating to a host-parasite relationship to investigate a population phenomenon: the crowding effect. A biological phenomenon is an observable event qualified and quantified by scientific investigation.

Your objectives are:

1. To complete experimentally the life cycle of the tapeworm *Hymenolepis diminuta* and investigate the role of the crowding effect.
2. To learn how to conduct a laboratory experiment and to handle and manipulate biological material in order to obtain significant results.

3. To write a scientific paper by developing a hypothesis and making predictions based on the literature. You will also think about how to test your predictions.

Outline

1. Week 1: Discuss the life cycle of *H. diminuta*. Look at the eggs from the feces of an infected rat and set up beetle infections.
2. Week 3: Infect rats and look at slides of cysticercoids.
3. Week 6 or 7: Look at slides of adult *H. diminuta*. Dissect rats.
4. Pool and plot class data, discuss conclusions.

Introduction

Did you know that there are more kinds of organisms with a parasitic way of life than any other? This highly successful way of life is present in nearly every phylum, such as protists, arthropods, plants, and chordates. A parasite and its host are considered to have a parasitic relationship, a kind of symbiotic relationship, in which two organisms are living in a physical environment with each other. A parasite is an organism that lives **in** or **on** another organism (host) of a different species, is dependent physiologically on that organism, and imparts some degree of cost to that organism.

Many studies indicate that hyperinfestation (a host which contains a high number of parasites) is not a common situation, or if it occurs, then the host does not suffer visibly. One of the main questions of this laboratory exercise is to address the question: *Why might a host infected with a high number of parasites not harmed more than a host infected with few parasites?*

To begin to answer this question, we must think about the effect hyperinfestation has on the host AND the parasite. When a host is infected with a single parasite, the host will incur a certain cost. If we assume, that each parasite added to this host will cost the same amount as the first parasite, cases of hyperinfestation would lead to a decrease in host-health, and creates an imbalanced host-parasite interaction (Figure 17.3, dashed line) since the parasite has adversely affected its environment. On the other hand, if we assume that as more parasites are added to a host, each has less of a cost, hyperinfestation would lead to no increase in harm to the host. This latter system is considered to be a properly balanced host-parasite interaction (Figure 17.3 solid line).

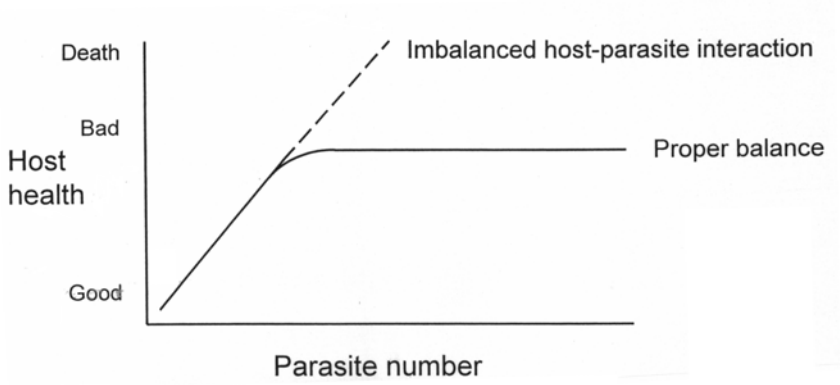

Figure 17.3. In properly balanced host-parasite interactions, the health of the host is largely independent on the number of parasites per host. Conversely, in imbalanced systems, large number of parasites can lead to the death of a host.

In this laboratory experiment, we will test whether parasites promote balanced interactions with their hosts, by measuring the crowding effect. The crowding effect is defined as the phenomenon of parasite biomass adjusting to the carrying capacity of the host (Read, 1951; Roberts, 1996). Thus, the number of eggs produced and the worm biomass present in a particular host is assumed to be constant, independent of the intensity of infection. The crowding effect is one mechanism by which parasites are believed to balance their cost to their hosts, by self-regulation.

The crowding effect has been described in nematodes [i.e., *Ascaris lumbricoides*, (Monzon, 1990)] , trematodes [i.e., *Philophthalmus nocturnus*, (Swarnakumari, 1992)] , and cestodes [i.e., *Hymenolepis nana*, (Ghazal, 1974), and *Hymenolepis diminuta*, (Read, 1963)]. Alternatively, some species of acanthocephalans (spiny headed worm) do not demonstrate the crowding effect phenomenon (Crompton, 1972; Uznanski, 1982).

Traditionally, two main hypotheses have been proposed to explain the phenomenon of the crowding effect: 1) resource limitation, (Reid, 1942; Read 1951), or 2) inhibition of worm growth by a substance(s) secreted by the worms (Roberts, 1961; Zavras, 1984; Cook, 1991). In this laboratory you will investigate whether an increase in the number of *H. diminuta*/host causes a decrease in the growth of worms. Based on your data and the literature, you will speculate what may explain the presence or absence of the crowding effect in these tapeworms.

During this lab, the presence of the crowding effect will be tested by counting and measuring the length of *H. diminuta* from hosts which are infected with variable numbers of parasites. The crowding effect will only be supported if the hosts with higher infections harbor smaller worms (Figure 17.4). This result would suggest that the parasites are limiting their size, in order to lessen damage or cost to their host.

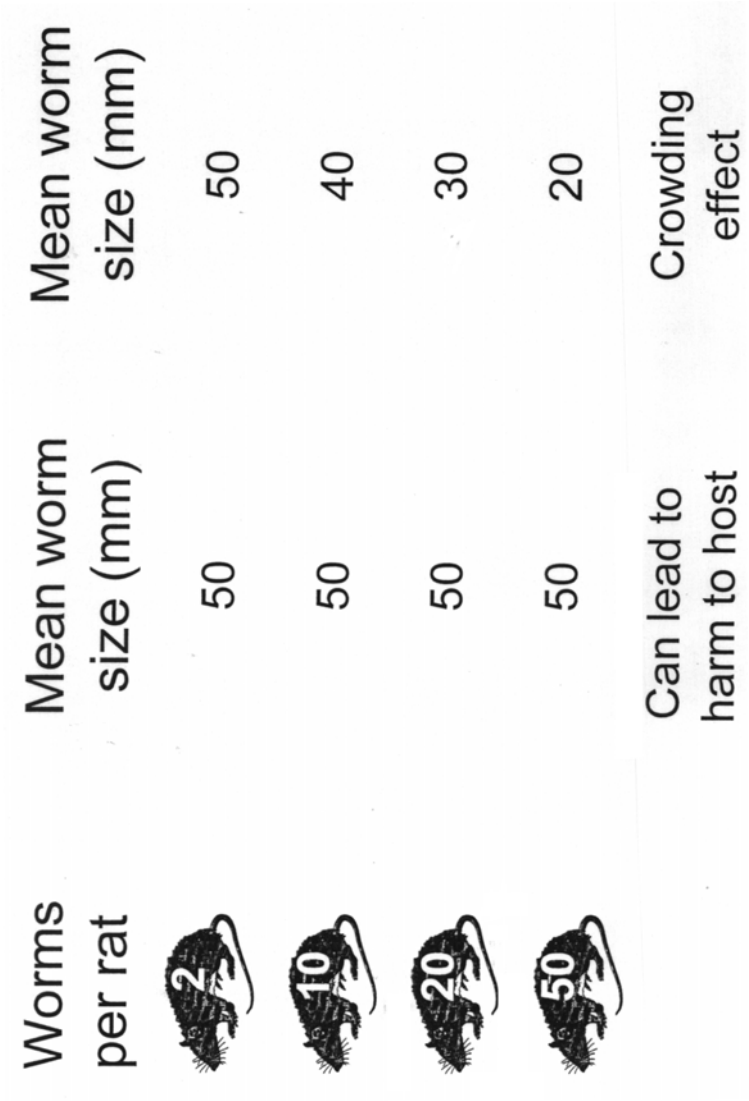

Figure 17.4. Diagrammatic representation of how the crowding effect will be tested in rats infected with a variable number of parasites. The column on the right shows the outcome if the crowding effect is present.

Hymenolepis diminuta is a tapeworm which requires two hosts (Figure 17.5). Within the intermediate host, a parasite will develop to some extent, but will only reach sexual maturity within the definitive host.

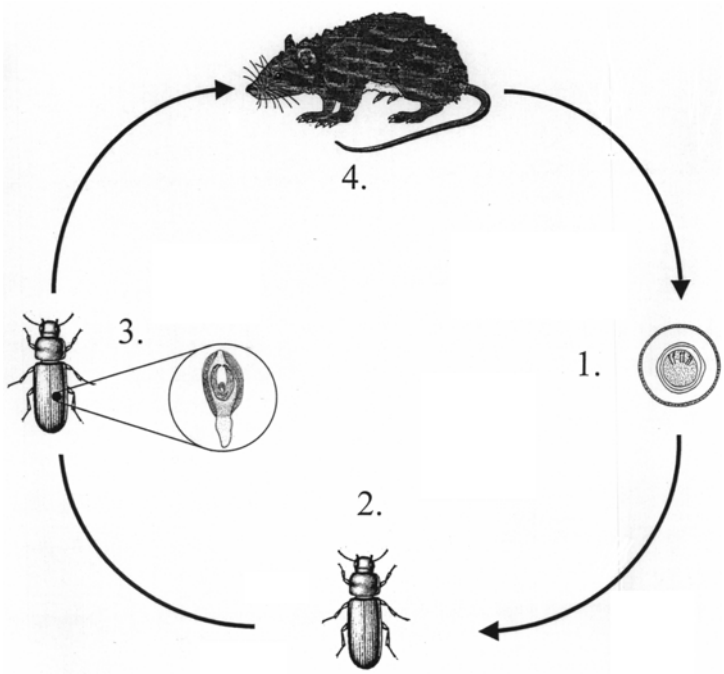

Figure 17.5. Life cycle of *Hymenolepis diminuta*. 1. Eggs are passed out with rat's feces, 2. Eggs are ingested by *Tenebrio molitor* beetles (intermediate host). Inside the insect's intestine, the oncosphere hatches from the egg, penetrates the intestinal tract and, 3. subsequently transforms into the cysticercoid in the hemocoel. 4. The beetle, infected with the cysticercoid larva of *H. diminuta* is ingested by the rat, *Rattus norvegicus* (definitive host). As the cysticercoids are released from the digested beetle, they evaginate their scoleces and attach to the wall of the small intestine of the rat.

Procedure

Step 1: (If your instructor already has infected beetles, go to step 2.) Obtain one fresh fecal pellet from the cage of a rat infected with *Hymenolepis diminuta*. Soak the pellet in some water until you can macerate it with a wooden stick. Completely homogenate this mixture by stirring. Remove one drop of this mixture and place it on a slide with a cover glass. Use low power (approximately 10X) on your microscope to find eggs then switch to higher power (approximately 40X) to examine the eggs of *Hymenolepis diminuta* (Figure 17.6A).

1. Describe the movement of the hooks of the oncosphere in the egg (Figure 17.6A)
2. What is the function of the hooks on the oncosphere?

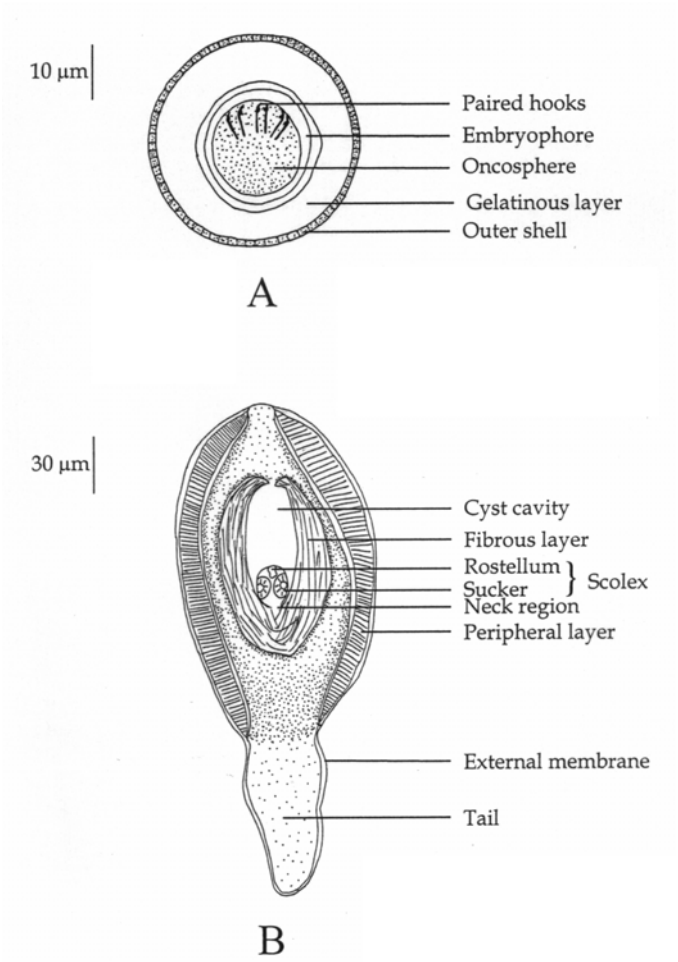

Figure 17.6. Diagrammatic representations of *Hymenolepis diminuta* egg (A), and cysticercoid (B). [A is modified from Voge (1960).]

Step 2: Infecting the beetles: Working in groups of two or three, obtain a container to house the beetles. Attach a label to your container with your names and the date. Place a paper towel on the bottom of the container. Place one to three fresh fecal pellets from the cage of one of the infected rats on the paper towel, then sprinkle some water on the towel. Place 15 adult beetles (*Tenebrio molitor*) in the container with the paper towel and the feces. Cover the container and sprinkle the paper towels with water daily for three weeks.

1. Why must the eggs be fed to a beetle rather than the rat?
2. What would happen if you feed these eggs to a rat?
3. What would happen if you ingested some of these eggs? Why?
4. How many days will pass while the developing cestode is in the beetle? What is it developing into?
5. What would happen if your rat ate the beetle after one week?

Step 3: Working with the same partner as for infecting beetles, select a rat and attach a label to the cage with your names and date. Remove a fresh fecal pellet, add water and homogenize the mixture. Take one drop of the mixture with an eye dropper and place it

on a slide and cover it with a cover glass. Observe the slide under the microscope to check for the presence of eggs. This step should take approximately 20 minutes.

1. Is there any sign of infection?
2. Why is this step important?

Step 4: Working continuously for about 40 to 90 minutes, begin dissecting the beetles: Select one beetle from your colony. Remove the head, elytra (hard outer-wing covering), and legs. Place the thorax in a small dish with some water. Use a couple of probes to tease apart the abdomen to expose the viscera. Tease apart the viscera. The cysticercoids are found in the hemocoel of the beetle. It is best to shake all the organs to release any cysticercoids (Figure 17.6B). Once the contents have settled, use the dissecting scope to look for cysticercoids. Collect the cysticercoids in a separate dish filled with water.

1. How many cysticercoids did you collect from a single beetle? How does your number compare to other students? What might cause the difference in density of infection between beetles?
2. Do the cysticercoids appear to be moving? How large are they compared to the beetle?

Step 5: Infecting the rats: Prepare an eye dropper with the appropriate number of cysticercoids before anesthetizing the rats. Once the cysticercoids have been placed in the eye dropper, the rats must be infected immediately. The rats will be placed in a container that will lightly anesthetize the rat. When the rat appears sluggish, remove it from the jar and place the eye dropper **gently** in back of the rat's tongue so the water with the larvae will wash down the rat's esophagus. When finished, place the rat in its cage and label the number of cysticercoids that were fed to the rat. Make sure the rat has enough food and water. Return the cage to its proper spot.

1. Why was the rat given a dropper of cysticercoids instead of feeding the rat a beetle infected with *H. diminuta* cysticercoids?
2. What is the purpose of feeding the rats variable numbers of cysticercoids?

Step 6: Dissecting the rats: Select a fresh fecal pellet from your rat; place it in a dish with some water and homogenize it. Put a drop of the homogenized solution on a slide; cover the drop with a coverglass; and check the slide for eggs. Are the worms in the rat producing eggs? Kill your rats following the instructor's instructions. Place your rat on a dissecting pan and make a lateral incision on the abdomen. Once you have the visceral organs exposed, remove the small intestine, taking care not to cut any holes in the intestine. Discard the carcass and rinse away any blood or feces. Once you have isolated the small intestine, see if you can observe any worms inside. Now, slit the intestine lengthwise. Be careful not to cut any worms that may be present. Remove the adult tapeworms. When you have removed a few worms, look at them under a dissecting microscope. Working with your partner or partners, measure and record the length and number of worms. After finishing the worm measurements, dispose of the rat as instructed. Be sure to wash your hands.

1. What do the worms look like (Figure 17.7)? How do they feel? How do they move? Can you see the scolex? Can you see the sucker moving?
2. Where in the intestine were most of the adult worms located?
3. Since this complex life cycle occurs naturally, where do you expect to find all the components that make up this life cycle?
4. Look at the worms collected from other students. How do your worms appear in overall size compared to the other worms collected? How many larvae did those students administer?

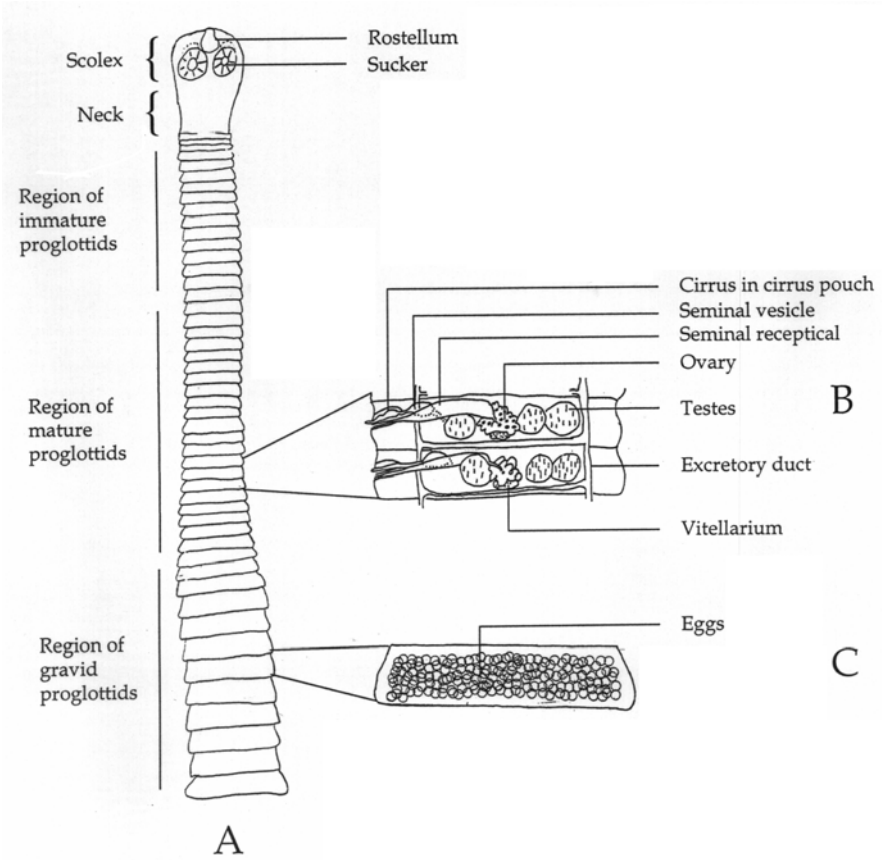

Figure 17.7. Diagrammatic drawings of a *Hymenolepis diminuta* adult (A), mature proglottid (B), and gravid proglottid (C).

Step 7: Organizing and interpreting the results: Pool the data with the rest of the class and complete the following table. Make a graph of the class data (number of worms and the mean length) on the graph provided.

1. What is the trend?
2. How might you explain this trend?
3. Discuss some advantages for the tapeworms to continue the crowding effect phenomenon.
4. Is this the best method to measure the crowding effect? Why or why not?

Acknowledgments

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Appendices

Appendix A: Data Table for measurements of worms.

Number of worms per rat	Range of total body length (mm)	Mean body length (mm)

Appendix B: Graph paper for visual representation of worm data.