Understanding Ecological Principles through Parasitological Pedagogy

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Abstract: Parasites comprise over 50% of the organisms on earth, yet they receive little attention in either lecture or laboratory components of ecology courses. This is a major shortcoming given the important role that these organisms play in ecosystem dynamics. The following exercises utilize parasites to teach students about key ecological ideas, experimental design, field-sampling methods, and parasite identification. In general, these labs are relatively easy to perform (for both instructors and students) and provide novel means of teaching (and reinforcing) traditional ecological concepts in exciting unconventional ways.

Introduction

Parasitic organisms comprise a very large component of the earth's biodiversity. Yet these organisms are often neglected in both classroom lectures and laboratory experiments. These two laboratory exercises are designed to 1) foster an appreciation for the parasitic lifestyle and its importance in ecosystems, and 2) use parasites from both field-infected and experimentally infected hosts to introduce and emphasize key ecological concepts. These laboratories are best suited for upper-level courses where class sizes are intermediate (12-18 people) and students are familiar with general laboratory techniques and the scientific method.

In both exercises, students focus on trematode parasites. These are parasitic flatworms that complete their life cycles by infecting a number of different host species (= complex life cycles). Although these parasites infect an impressive array of animal groups at latter points in development, they always infect mollusks at the first point of their life cycles. This is extremely important for our purposes, as snail populations (both aquatic and terrestrial) are relatively easy to access and most populations exhibit some degree of infection by these parasites.

The first lab exercise (Part I) is designed to serve two main purposes:1) to introduce students to a local host-parasite system, and 2) to have students consider the ecological processes underlying

patterns of parasitic infection in snail hosts. This exercise demonstrates the importance of parasitism in natural systems and emphasizes the critical role that biotic and abiotic factors play in structuring parasite communities. In addition, students learn general field-sampling methodologies and the morphological features of larval trematodes (cercariae) that can be used for taxonomic identification.

The second lab exercise (Part II) has students testing the ecological prediction that increasing animal densities reduces individual fitness through intraspecific competition for resources. Unlike the first lab exercise, this one involves experimental host infections, subsequent host dissections, and the acquisition of adult trematode parasites. By assessing parasite traits, students uncover the consequences of limiting resources (such as space, nutrients, etc.) on parasite life-history attributes such as growth, reproduction and survival. Furthermore, this lab emphasizes important components of the scientific method such as hypothesis testing, experimental design and interpretation of qualitative statistics.

Although these labs require some degree of preparation on the part of the instructor(s), we feel that these novel systems capture and maintain student interest thereby facilitating the understanding of both parasitism and key concepts in ecology.

Part I - Field study

Notes for Instructors

The first series of exercises is designed to span two laboratory periods of 2-3 hrs each. The first period should be spent at the field site introducing students to the site, the sampling design, the organisms, and the importance of environmental factors on parasite communities. In the Midwest of the United States, we often find freshwater snail communities comprised of physids, lymnaeids, and helisomes which can be distinguished based on both size and shell shape (Thorp and Covich, 2001). Because snail densities can vary widely across season and even across days (due to wind and rain events), we suggest assessing aquatic sites 2-3 days prior to collections to eliminate unexpected surprises on the collection day (such as no snails!). It is important to collect a large number of snails (n = 200-300) to ensure infected hosts and to sample from sites that differ based on environmental factors such as depth and macrophyte composition. If snail densities are generally low, have each student group collect hosts from two to three quadrats as opposed to just a single sample. Students should use dip nets to exhaustively sample each quadrat from the substrate to the surface. Oftentimes, snails are on the underside of aquatic vegetation, so be sure that students spend time investigating the plants. Once back in the lab, snails can be maintained in aerated buckets or aquaria and fed romaine lettuce.

For the second period, snails should be transferred into small containers (individually) and placed under a fluorescent light source approximately 3 hrs before the lab begins to initiate parasite release from infected hosts. When students arrive, they can gather in groups of two and scan containers using a dissecting microscope. The parasite larvae (=cercariae) can be distinguished from other organisms in the water by their numbers (usually hundreds to thousands of individuals) and their general morphology (please see key). Once students have identified infected snails, they should make wet mounts of the parasite larvae and use the taxonomic key to assign the larvae to particular parasite groups. Unfortunately, we can not provide a key as part of this paper due to copyright

issues. However, we have provided the citation for a comprehensive and relatively inexpensive larval and adult trematode guide at the end of this section (Schell, 1985). It is not necessary for students to recognize the complete internal anatomy of cercariae in order to assess parasite communities – broad differences in larval attributes such as the shape of the tail, the presence/absence of eyespots, and the presence/absence of collar spines should provide sufficient resolution for differentiating communities. In addition to determining parasite species, students should record snail size to determine if 'gigantism' occurs in infected hosts. This is a typical pattern that we see in snail populations where infected hosts tend to be larger than uninfected individuals (see suggested references for further details). Once the parasite has been identified and snail size has been recorded, students should fill in the appropriate spaces on their data sheets (see below). After all snails have been assessed, students should hand in their data sheets to the instructor. Instructors can then summarize the data and hand these results back to the students for use in a scientific report.

Further background on parasite communities, 'gigantism' and cercarial identification can be found in the following publications:

Parasite communities:

Kuris, A.M. and K.D. Lafferty. 1994. Community structure – larval trematodes in snail hosts. Annual Review of Ecology and Systematics, 25:189-217.

Sousa, W.P. 1993. Interspecific antagonism and species coexistence in a diverse guild of larval trematode parasites. Ecological Monographs, 63:103-128.

Gigantism:

Gerard, C. and A. Theron. 1997. Age/size- and time-specific effects of *Schistosoma mansoni* on energy allocation patterns of its snail host *Biomphalaria glabrata*. Oecologia, 112:447-452

Sandland, G.J. and D.J. Minchella. 2003. Effects of diet and *Echinostoma revolutum* infection on energy allocation patterns in juvenile *Lymnaea elodes* snails. Oecologia, 134:479-486.

Sorensen, R.E. and D.J. Minchella. 2001. Snail-trematode life-history interactions: past trends and future directions. Parasitology, 123:S1-S16.

Cercarial identification:

Schell, S.C. 1985. Trematodes of North America, north of Mexico. University of Idaho Press, Moscow, 263 pages.

Snail identification:

Thorp, J.H. and A.P. Covich. 2001. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, 1056 pages.

Materials

Field sampling (for each group of two to three students):

- one 5-gallon bucket (for collecting and maintaining snails)
- one aerator (for maintaining snails after collection)
- one thermometer
- one tape measure
- three dipnets (net size: 18 cm x 12 cm)
- one meter stick

Suggested equipment for each student:

- Insect repellant
- Rubber boots
- Rain gear (optional)
- Waders (optional)

Laboratory assessment of snails and parasites (for each pair of students):

- One compound and one dissecting microscope
- One taxonomic key
- Digital calipers
- Glass slides and cover slips
- Glass pipettes and rubber bulbs
- Petri dishes

Student Outline

Background information for field study

Purpose – To gain an appreciation for a local parasite community and to understand the factors responsible for differences in parasite community patterns at a single sampling site.

Digenean trematodes are some of the most common and most important parasites found in local aquatic habitats throughout North America. These species have complex life cycles that involve as few as two and as many as four different hosts all of which harbour a different larval stage of the parasites (Figure 1). In all cases, trematodes use snails as first-intermediate hosts. Within infected snails, these parasite undergo asexual reproduction which allows for dramatic amplification – this means that a snail infected with a single free-living larval stage (=miracidium) can generate thousands of larval clones (=cercariae) that are subsequently released into the water. Depending on the life cycle of the trematode, these larvae (cercariae) can either infect another intermediate host or they can infect a definitive host where they reach reproductive maturity. As part of this lab, we will be assessing and classifying trematode infections in snails collected from two sites at a single pond/wetland. We will discuss reasons for differences (or lack of differences) between parasite communities in different snail species from the two areas.

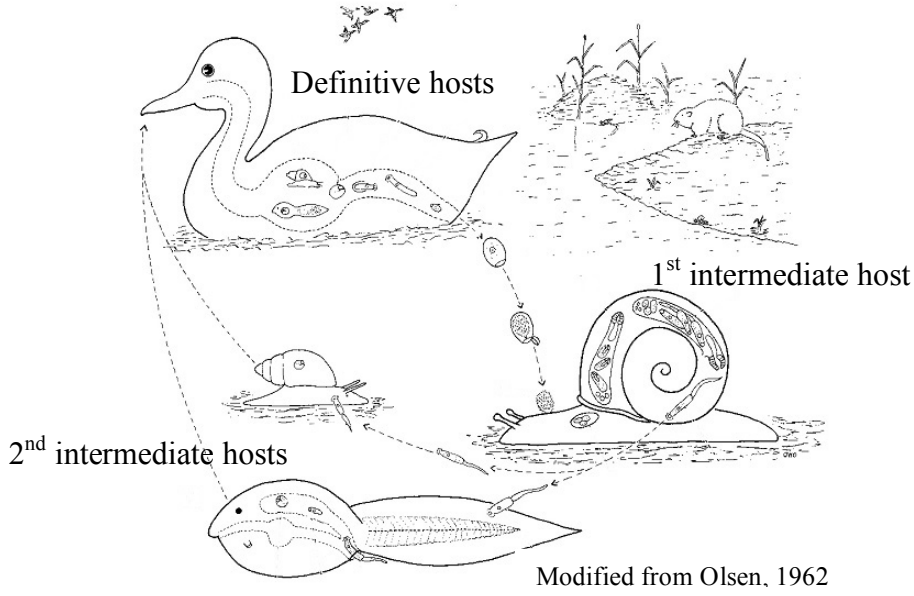


Figure 1. An example of a complex parasite life cycle. Three successive hosts are required to complete the life cycle (first-intermediate, second-intermediate and definitive host). At several points in the life cycle, several hosts may be utilized. For example, either the snail or the tadpole can serve as second intermediate hosts in the life cycle.

Procedures

Field sampling

1) Divide the class into groups of two to three students.
2) Each group is responsible for a tape measure, three dipnets, a meter stick and a thermometer.
3) Upon arriving at the field site, each group will set up a 10 m x 10 m plot. Snails will be exhaustively sampled (collect all snails) within this defined area.
4) As two members of the group sample snails, the third member will record environmental parameters such as water depth, temperature, and also a general description of the vegetation types.

   *Be sure to note the presence of any bird or mammal feces in the collection area – parasites are transmitted to snails via larvae that hatch from eggs released in the definitive host feces.
5) All snails will be placed into containers and returned to the laboratory.

DATA SHEET

Temperature of plot: _____

Depth of plot: _____

Number of snail species collected: _____

_____

Specify the locations of snails within the plot (surface, on the macrophytes, on the substrate?)

_____

List general types of vegetation observed:

_____

Any indications of animal activity? For example, direct or indirect observations of birds, muskrats, turtles, frogs, etc?

Laboratory assessment of trematode communities in snails

1) Students will remove individual snails from under fluorescent lights.
2) Using dissecting and compound microscopes in combination with taxonomic keys, each pair of students will attempt to identify the trematode species being released from each snail collected from their 10m x 10m plot.
3) Focus on the following tasks and fill in the data sheets:
   a) Notice all of the parasite larvae in the water and imagine the sheer numbers of parasites that may be released from an infected snail over its lifetime. Why might selection favor the production of such high numbers of cercariae?
   b) Compare the morphology and behavior of cercarial species. What can this tell you about 1) the means of transmission to the next host in the life cycle?, and 2) the type of host that these cercariae may infect?
   c) Measure all the snails (both infected and uninfected) from each plot using digital calipers. Is there any correlation between snail size and infection status? Why might such a correlation exist?
   d) After attempting to identify the parasites released from infected snails,
   e) take 5 infected snails and crush them using the Petri plates provided. What do you see? Create a wet mount of the parasite found within the snail – identify the structures present on/within these larvae.

DATA SHEET

Site:_____

Snail species	Size	Infected?	Parasite species

Part II – Experimental study

Notes for instructors

This is a powerful lab for demonstrating density-dependent effects on the fitness of individual organisms. Unlike the first exercises, this lab requires the use of vertebrates, specifically day-old chickens. Thus, it will be critical to insure that all university animal-care requirements are satisfied prior to undertaking this exercise. Day-old chickens (preferably leghorn breeds) can be ordered via a university farm or via a number of reliable sites on the internet (including the Murray McMurray Hatchery – www.mcmurrayhatchery.com). It is important to look into ordering birds well before infection, as egg incubation (up to 3 weeks) may be required before hosts are available. For this lab we suggest ordering two to three chickens for each pair of students in the class.

The easiest way to acquire *Echinostoma revolutum* metacercariae is by contacting the authors of this paper. We continually cycle these parasites in the laboratory and have a large number of metacercarial cysts for distribution. Once cysts have been acquired, they can be used immediately or stored in the refrigerator for a number of weeks. Prior to oral inoculation, cysts should be added to well water (or tap water that has been aged for approximately a week) and separated into aggregates of 25 and 125 metacercariae using forceps and a dissecting microscope. Then, cyst aggregates can be fed to the birds via a Pasteur pipette. This can be accomplished by gently inserting the thumb and the index finger (of your left hand) into each side of the beak – this forces the beak open. The pipette can then be inserted into the mouth and the contents can be discharged SLOWLY on to the tongue. Once chickens have been infected, we mark the birds with non-toxic ink to differentiate the individuals that received the different parasite doses. Hosts can then be maintained on NON-MEDICATED chicken feed for two weeks. After that time, chickens should be euthanized (using a protocol approved by your university) and the intestines removed immediately before the lab.

Each pair of students should receive from two to three intestines (remember which students are receiving intestines from high-dose and low-dose chickens!) and students can begin performing the dissection following the procedures presented in the student outline. This species of echinostome tends to reside in the most posterior end of the intestine (primarily in the final segment). Adult worms are easy to spot using a dissecting microscope as they tend to be a darker orange/red than the rest of the intestine and they move extensively. For the sake of yourself and other individuals in the class, dissuade students from necropsying the cecae of the intestine (see figure below) as these sacks contain strong odors and few (if any) worms. For this exercise we require students to write a lab report following traditional scientific format. We have included the instructions for this report at the end of this section.

Further background on the effects of parasite densities and intraspecific competition on the fitness of individual worms can be obtained from the following publications:

Adult trematodes:

Yao, G., J.E., Huffman and B. Fried. 1991. The effects of crowding on adults of *Echinostoma caproni* in experimentally infected golden hamsters. Journal of Helminthology, 65:248-254.

Larval trematodes:

Brown, S.P., J. De Lorgeril, C. Joly, and F. Thomas. 2003. Field evidence for density- dependent effects in the trematode *Microphallus papillorobustus* in its manipulated host, *Gammarus insensibilis*. Journal of Parasitology, 89:668-672.

Sandland, G.J. and C.P. Goater. 2000. Development and intensity dependence of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas)*. Journal of Parasitology, 86:1056-1060.

Materials

For each pair of students:

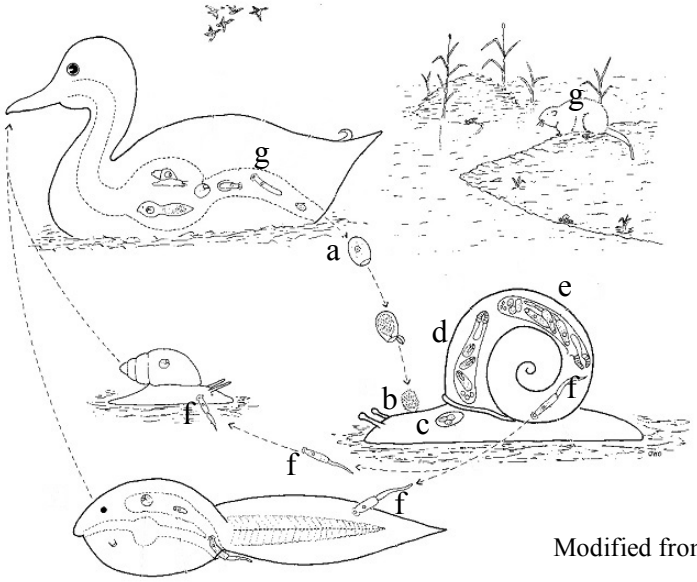
- One balance (to milligrams)
- One compound and one dissecting microscope
- Two counters
- One set of digital calipers and one ruler
- Eight dissecting pins
- Four sets of forceps
- Two pairs of scissors
- Glass slides and coverslips
- Latex gloves

Student Outline

Background information for experimental component:

Purpose – To determine the effect that increasing parasite densities can have on the establishment and development of a trematode parasite in its definitive-host bird.

The trematode *Echinostoma revolutum* has a complex life cycle involving a specific snail species (*Lymnaea elodes*) as first intermediate host, numerous snail species as second intermediate hosts, and birds as definitive hosts (Figure 2). When parasites co-occur at high densities in an environment with limited resources (space, nutrients, etc.), it can have important consequences for the establishment and development of individual parasites. This study is designed to investigate the effects that different parasite densities (and competitive interactions) have on both the establishment and the life-history of *E. revolutum* in bird definitive hosts.



Modified from Olsen, 1962

Figure 2. The *Echinostoma revolutum* life cycle – all parasite stages are indicated: a =egg, b = miracidium, c = sporocyst, d = mother rediae, e = daughter rediae, f = cercariae, g = adult.

Procedures

Preparing for dissection:

1) Two weeks prior to this laboratory, instructors infected leghorn chickens with a dose of either 25 metacercariae or 125 metacercariae. Parasites have been developing in the intestinal tracts of the chickens.
2) Each group should obtain two to three intestinal tracts recently removed from the birds by your instructors.
3) Split the intestine of the bird into five (relatively) equal sections as seen in Figure 3A.
4) Select the anterior portion first, then cut the intestine with scissors (Figure 3B) and carefully scan the area for moving orange/red worms.

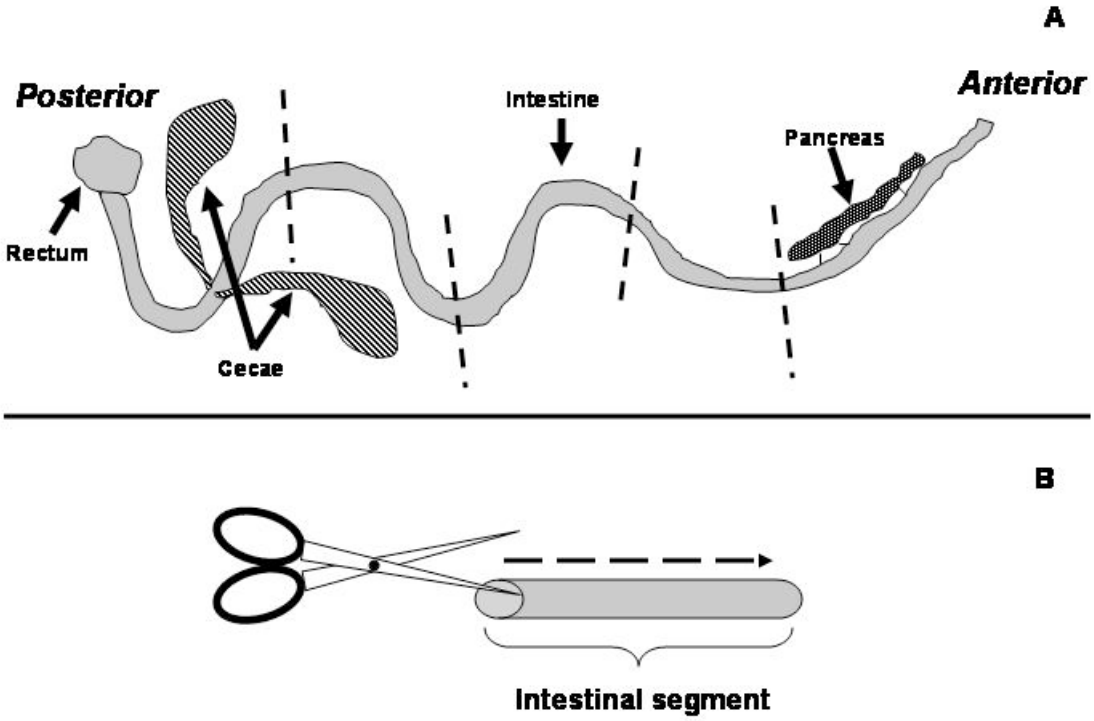


Figure 3 A. Key features of the chicken intestine and suggested cut points for separating the tract into 5 segments. **B.** Methodology for cutting each intestinal segment using scissors.

When you locate the intestinal area with E. revolutum, perform the following tasks and fill in your data sheet:

1) Estimate (using calipers or a ruler) the length of the intestine where worms are found.
2) Carefully remove the worms from the intestine, place them into a Petri plate and count them.
3) Scan the intestinal tract for evidence of pathology. If intestinal lesions are present, report them.
4) Dab the worms dry and weigh them.

5) Using digital calipers, measure the lengths and widths of 10 worms per intestine. Be sure to assess the width of the parasite at their *widest* points.
6) Using a dissecting microscope, assess the worms for evidence of reproductive maturity. Are testes well developed? Are there any eggs in the uterus of the worms? If eggs are present, tease the individuals apart and count them (refer to Figure 4 for general adult-echinostome morphology).
7) Fill in the datasheet provided with the information that you have acquired during the dissection.

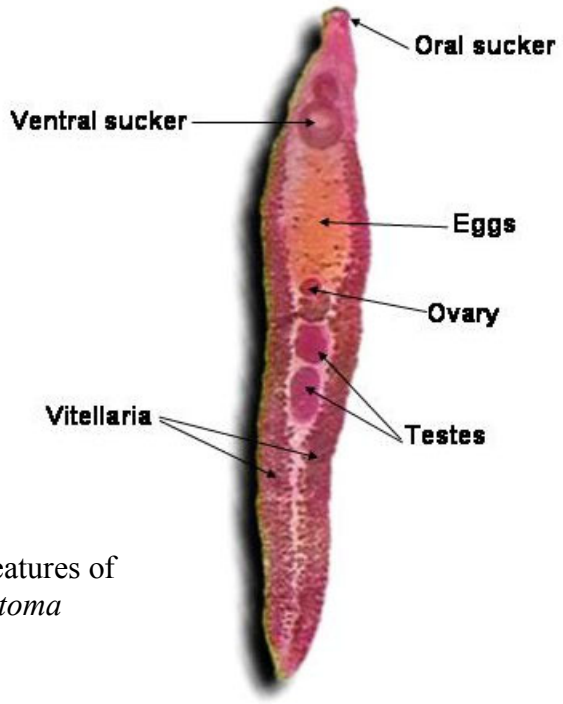


Figure 4. Key morphological features of an adult *Echinostoma revolutum*.

DATA SHEET
Intestine number:
Length of intestine occupied by worms:
Total weight of worms:

Worms	Length	Width	Egg number
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Do you see any lesions in the intestine? If 'yes' approximately how many?

Written Assignment

Using the following guidelines, write a lab report based on the experimental component (competition study).

1) Introduction (three paragraphs)
   a) In the first paragraph outline the background to the study. Why is it important that we assess intraspecific competition in parasite species?
   b) In the second paragraph, outline some of the previous work that has been done in this area and the results that have been reported. Two references have been provided for you.
   c) In the third paragraph, state the parasites and hosts used in this study, then list the objectives of the study - these should follow from the background and results (from previous studies) mentioned in the first two paragraphs.

2) Materials and Methods (please use full sentences, *not bullets*!).
   Describe the three main tasks:
   a) Infecting the chickens (your instructors will provide details).
   b) Dissecting the intestine.
   c) Assessing the parasite life-history parameters (weight, length, width, numbers of eggs).

3) Results
   a) Create a table using the data listed above. Please label the table with a caption that appears ABOVE the actual table itself. Be sure that your titles are descriptive – the reader should be able to comprehend the components of the table without actually seeing it
   b) Create one or two figures using the data listed above. The figure must represent data that are important within the objectives of the study. These data include parasite sizes (mean + standard error) (length/width), weights, eggs, numbers of parasites recovered and the proportion of parasites recovered. Be sure that all graph axes are clearly labeled (this includes units and a descriptive figure caption that appears below the figure itself).
   c) This time, the results section must contain a description of the patterns that you observe in each of the figures. For example: In chickens that were exposed to low intensities of metacercariae, adult worms had larger lengths than echinostomes acquired from chickens exposed to high intensities of metacercariae (Figure 1).
   d) The results should be described in this section – NOT interpreted.

4) Discussion
   a) For the discussion, you should attempt to interpret the results that you have described – what are the factors that may drive the observed patterns? If you use other sources to gain insight into these factors (which you should), then a citation is required.
   b) Be sure to compare the patterns that you observe to those that have been shown in the past – are your results the same or different? If similarities (or differences) exist, why might this be the case?
   c) Please conclude the discussion with a summary sentence or two. Abruptly finishing a discussion without reminding the reader of the study's overall importance is undesirable.

5) Literature Cited
   a) This section is a list of the full citations mentioned in the body of the report. Here is a list of the papers that we provided for you (please follow the same general format for any additional references that you include):

Sandland, G.J. and C.P. Goater. 2000. Development and intensity dependence of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas)*. Journal of Parasitology, 86:1056-1060.

Yao, G., J.E. Huffman, and B. Fried. 1991. The effects of crowding on adults of *Echinostoma caproni* in experimentally infected golden hamsters. Journal of Helminthology, 65:248-254.

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About the Authors

Gregory Sandland received his B.Sc from University of Victoria (1996), M.Sc. from University of Lethbridge (1999) and his Ph.D. from Purdue University (2005). He is currently a post-doctoral researcher at Purdue University where he taught a course in parasite ecology. His research focuses on the degree to which host genetic variability influences resistance to parasitic infection and the role that interspecific competition plays in modulating parasite virulence.

Jillian Detwiler received her B.A., B.S. (2002) and M.S. (2004) from the University of Nebraska-Lincoln. She is currently a Ph.D. candidate at Purdue University where she taught a course in parasite ecology in partial fulfillment of the Graduate Assistance in Areas of National Need (GAANN) fellowship. Her research investigates the effects of host specificity and host vagility on parasite population structure.

Dennis Minchella is a professor in the Department of Biological Sciences at Purdue University where he teaches first-year biology and upper-level discussion courses. His research program focuses on the population genetics of host-parasite interactions and the effects that parasites have on host life-history strategies.