Teaching Population Growth Using Cultures of Vinegar Eels, *Turbatrix aceti* (Nematoda)

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***Abstract*:** A simple laboratory exercise is presented that follows the population growth of the common vinegar eel, *Turbatrix aceti* (Nematoda), in a microcosm using a simple culture medium. It lends itself to an exercise in a single semester course.

Key words: ecology, invertebrate, little-r, population dynamics, vinegar eel

INTRODUCTION

The dynamics of population growth is a challenging topic to explore in an ecology course that is only one semester in length. Two uncomplicated options for instruction include analyzing extant data sets (Frazer, 1991) and use of computer models (Alstad, 2003; Donovan and Welden, 2002; EcoBreaker© 2004; Jungck et al., 2003). Fast-growing microbes such as bacteria, yeasts, algae, and protists, or other organisms such as fruitflies and *Lemna* (Beiswenger, 1993; Gause, 1934) also can be employed. Unfortunately, exercises based on those models are more time consuming and add the possibility of failure. This paper describes a simple, inexpensive laboratory exercise that follows population growth of the vinegar eel (*Turbatrix aceti*; Nematoda) in microcosms using a simple culture medium comprising 1.5 L of pasteurized apple cider vinegar, and a bit of decaying apple.

Goals: Students will explore population growth and the constraints that limit it. This will be accomplished by studying the growth of a population of the vinegar eel (*Turbatrix aceti*) in an environment with limited resources.

Skills: A small culture of *T. aceti* has been started. Students will follow simple instructions to collect and record the data of population growth. They will perform simple calculations to determine *little-r* for the experimental population. Students will learn the operation of the Millipore® filter apparatus.

Learning Outcomes: The basic principles of hypothesis formulation, experimentation, data collection, analysis, and presentation, as they relate to population growth, will be reinforced.

MATERIAL AND METHODS

Cultures of *T. aceti* are available from several biological supply houses. These nematodes are easy to grow and the cultures last for years with little maintenance. A culture may be started by placing ca. 1.5 L of pasteurized vinegar, two pieces of apple (ca. 2 x 2 x 4 cm), and a small aliquot (≈2 ml) of an active *T. aceti* culture into a 2-L flask, closed with a 1- or 2-hole rubber stopper to permit some gas exchange. The population growth experiment reported here ran for 13 weeks. Thus, to fit this time frame into a semester, the culture was established three weeks before the start of the fall semester. Once a week, 3 replicates of 1-ml each were removed and the number of animals per ml of culture fluid was determined using membrane filter technology (see below). However, the population density also could be determined by other direct count methods such as using a Sedgewick Rafter counting cell (Wildlife Supply Co., Saginaw, MI) or a plankton

counting chamber (Canimpex Enterprises Ltd., Halifax, Nova Scotia, Canada). In practice, the latter two methods should be less expensive then using membrane filters. Also the animals are observed directly, so the difficultly in deciding whether an object is a live *T. aceti* or is debris (e.g., dead animals and molted cuticles) is much less difficult. I used the membrane filter technique because I wanted the students to learn that technique.

Replicate samples from the *T. aceti* culture were taken using the following protocol. The culture was mixed by swirling the flask for about 15 sec. before a 1 ml aliquot was removed from the upper 5 cm using an automatic pipette. Each of these samples was filtered using Millipore® tower assembly and a 25 mm, 1.2 µm pore size, gridded filter (Figure 1), with a vacuum of <1 atm. (NB: Gridded filters are more costly, but aid when the animals are counted. Filters with larger pore sizes would probably be better as a small pore size captures more debris. Before filtration the membrane filters can be labeled along their outer edge using a ballpoint pen.) After filtration, the filters were removed from the tower assembly using flat forceps, placed over a 22 mm hole on a plastic drying rack, and weighted down with ca. 23 mm ring weight to hold the filters flat while drying. Filters were allowed to dry for 24–48 hrs. before examination. Whereas all the samples could be saved for a laboratory session later in the semester, in the results reported here, the students processed the samples within one week.

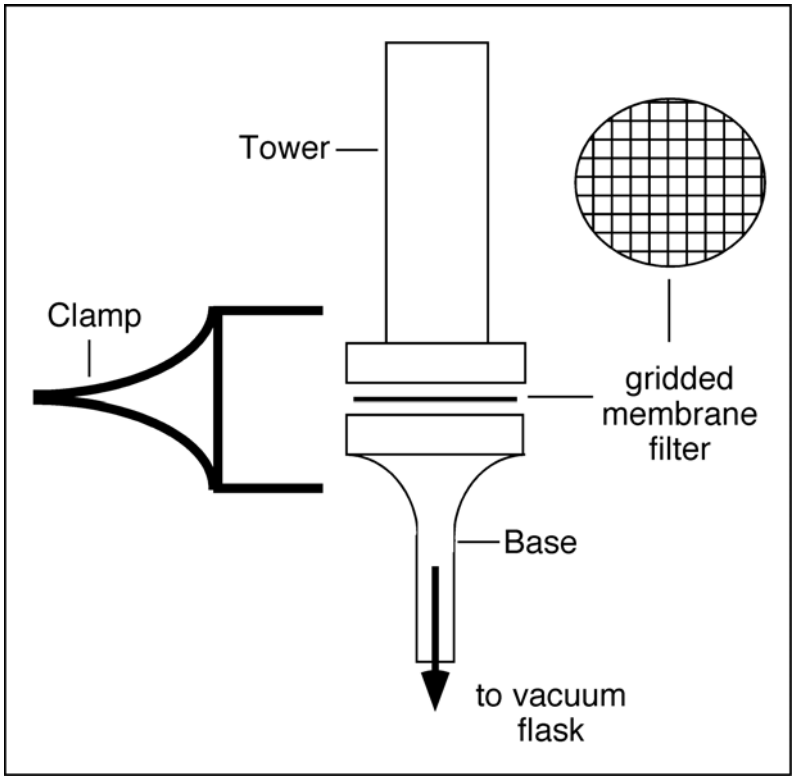

Figure. 1. *Schematic view of a Millipore filter apparatus.*

While all of the results discussed here were done without staining the *T. aceti*, one student tested various concentrations of four stains in an attempt to improve visibility of the *T. aceti* on the filter. The stains that were tested were Rose Bengal (at 0.01, 0.05, and 1%), Methylene Blue (0.3%), and Nigrosin (for the negative stain in bacteriology). The best results were obtained by adding one drop of Nigrosin per 10 mL of culture fluid for 5–10 mins. before filtration. Thus, these staining techniques require the removal of larger volumes of the culture fluid.

To examine the filters for *T. aceti*, 1–2 small drops of immersion oil were placed on a clean microscope slide and, using flat forceps, a dry filter was positioned over the drop of oil so that the grid lines of the filter paper are aligned as shown in Figure 2. Then a second drop of immersion oil was put on the top of the filter, a coverglass was placed on the filter, and gentle pressure was applied to the coverglass (Figure 3). This step distributed the oil over the filter and eliminated most of the air bubbles. Excessive pressure can crack the coverglass. (NB: The outcome of this is that the filter paper becomes clear. At this

point I ask the students to consider the physics of this interesting optical phenomenon.) The last step before examining the filters for *T. aceti* is to have the students wipe off any oil that may have gotten onto the bottom of the slide.

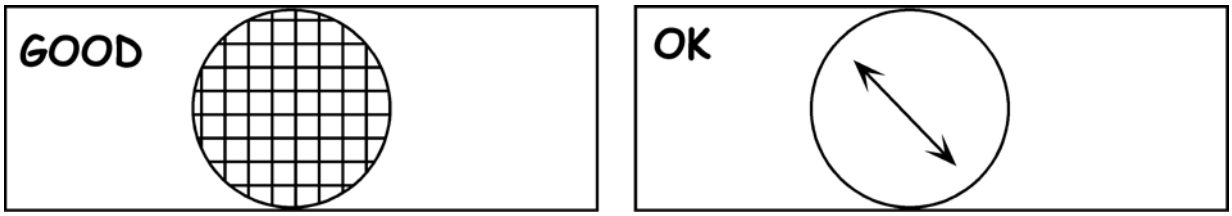

Figure. 2*. Convenient (left) & inconvenient (right) positioning of a filter on a microscope slide.*

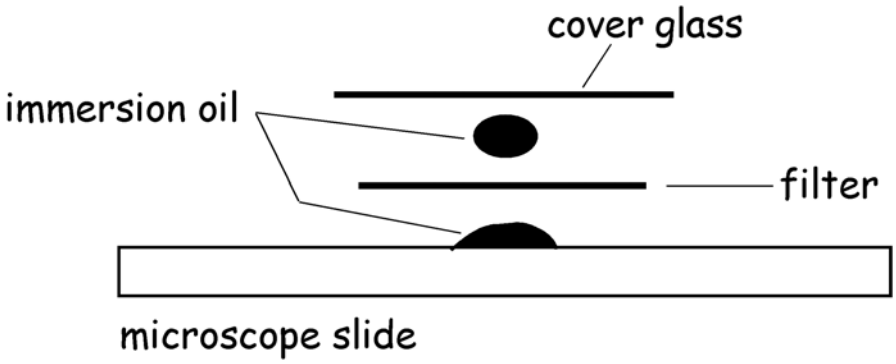

Figure. 3. *Preparation the membrane filter for observation.*

Examination of the filter should be done in a systematic manner and in a way that precludes double counting animals. Sometimes the *T. aceti* will have moved outside of the filtration area before the drying process was completed. Thus, the students will need to examine the area surrounding where the filtration took place to make sure that they count all the animals. However, in a subsequent trial I found that adding 1–2 drops of full strength formalin to fluid to be filtered 1 minute before starting the filter pump killed the *T. aceti* thus preventing the migration.

RESULTS AND DISCUSSION

The results of this exercise for the class in 2002 are shown in (Figure 4). Results for the class in 2003 were similar. As seen by the error bars (±1S.D.), there was considerable variation in the population density estimates among the three replicate samples for the first six weeks of this exercise. Nevertheless, there was a clear region of exponential growth that lasted through week three. Thereafter growth slowed and the population entered a stabilized phase where it appears to have achieved a carry capacity of about 320 *T. aceti* per mL. Because the exercise was stopped after 13 weeks it is not known how long the stationary phase lasts. However, two stock cultures (ca. 500 mL each) in my laboratory have had robust populations for more than two years without the addition of either fresh vinegar or apple. After data collection was stopped the students were given the raw population numbers and were given the following assignment.

1. Plot population size as a function of time twice, once when both axes use an arithmetic scale and again with the population size scale being logarithmic.
2. Using a histogram format, plot the estimated value of *little–r*, from week to week according to the following equation:

   $N_t = N_o e^{rt}$. Solve for *r*: $\ln N_t = \ln N_o + r \bullet t$

   Thus, $r = (\ln N_t - \ln N_o) / t$ (equation 1)

   Here r = the intrinsic rate of natural increase, t = the time that the population has been

growing, and N_o starts and N_t ends a period to be calculated.

3. Examine these two figures and discuss the following questions.
   A. Did exponential growth occur in this culture? If so where and for how long?
   B. Compare the two graphs and discuss which one best illustrated exponential growth.
   C. Did the culture reach a carrying capacity? If so, when?
   D. In a essay of about 250 words, describe how this exercise demonstrates population growth and the effects of limiting resources on population growth.
   E. Develop an abstract that you would use in a manuscript for publication.
   F. Offer suggestions to improve this laboratory exercise.

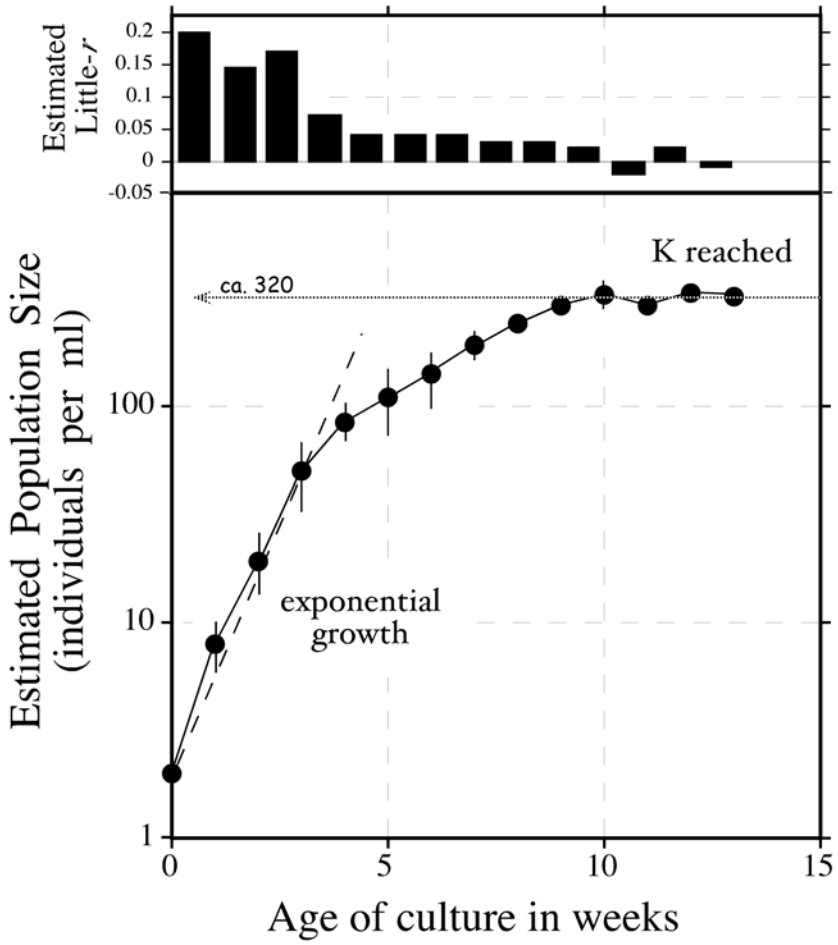

Figure. 4. *Growth of a batch culture of the vinegar eel (*Turbatrix aceti*; Nematoda) over a 13-week period. Upper panel: week-by-week estimates of the value little-r (i.e., 0–1, 1–2, … 12–13); Lower panel: estimates of the population size as individuals per mL (solid circles). (Lines = error bars as ±1S.D.; error bars are not indicated when they fall within the circle.) (K = carrying capacity of this population.)*

Alternative Enrichment Exercises:

Variations of this exercise including the following: (1) diluting the vinegar to different concentrations; (2) monitoring the pH of the system; (3) monitoring the level of yeast and/or bacteria in the culture; (4) varying incubation temperatures among replicate cultures; (5) comparing cultures incubated in the light vs. the dark; (6) altering the procedures (e.g., buffer the medium, add various natural chemicals or potentially toxic agents).

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