Chapter 12

Trail-following in Snails: A Behavior and Statistical Laboratory Exercise

Sandra V. Millen

Department of Zoology
6270 University Blvd.
University of British Columbia
Vancouver, British Columbia V6T 2A9

Sandra Millen is a lecturer in invertebrate zoology at the University of British Columbia, Vancouver, B.C. Canada. For 15 years she has been running labs in an introductory second year course and an advanced third year course. The labs for the third year course have a variety of themes on which the students run experiments, do the appropriate statistics on their data, and write up a weekly lab report. The snail lab is flexible enough to be modified for any year level, and is one of Sandra's favorites, as her own research is on the shell-less snails, the nudibranchs.

INTRODUCTION

Many species of snails have been shown to have the ability to make use of short distance chemoreception to detect the trails laid by snails of their own species, and extract chemical information from these trails. Snails may follow con-specific trails because they are gregarious (Raftery 1982), wish to return home (Cook & Cook 1975), find a mate (Dinter 1974) or locate a well-fed individual who may know a good food source (Pratt 1976). Snails rarely follow the trails of other species unless they are predators and the trail is that of potential prey. However, some snails do not distinguish between the trails of closely related species and their own species (Trott & Dimock 1978). This ability of snails to detect and follow trails may be used in a variety of lab exercises. The objective of the exercises listed here is both to teach the students about snails, especially their locomotory and chemosensory abilities, and to generate data which can be statistically tested. The statistical portion of the exercise can teach the need for a large sample size and the normality of large variance in biological experiments as well as the tests themselves.

MATERIALS

One (or two) snail species that moves well (ie. fast) on a glass surface. Land snails can be used if the surface is damp. Marine and pond snails need to be covered by water. A good marine snail is *Littorina* sp. If starved animals are to be used, withhold food for one week prior to their use.

Aquarium tanks (10 gal.)
tracing paper
masking tape
pencils (2 colors)
calibrated map wheel for measuring the snails trail

Statistical Tests

These can be obtained from biological statistics texts.

Binomial test - for sample sizes under 25

Chi-square test - for sample sizes over 25.

F test - for homogeneity of variances

t - test - requires homogenous variances

Mann-Whitney U test - for non-homogenous variances

(other non-parametric tests can be substituted)

METHODS

Tape tracing paper to the bottom of a clean aquarium tank. If the snail is aquatic, add just enough water to cover the snail. Balance the aquarium between two stools or chairs, on a lab bench if possible, so that the students can get under the aquarium to trace the snails movements. Place a snail (marker) in the center of the aquarium and trace its path as it moves. The trail should be more than 15 cm long and not go up the sides of the aquarium.

To obtain coincidence distances, start a second snail (tracker) facing onto the marker's trail. Trace the trackers trail (t) with a second colored pencil for 15 cm after it contacts the markers trail (m).

$$\text{coincidence index} = \frac{\text{distance tracker follows marker}}{15 \text{ cm}} \times 100\,\%$$

Examples:

Section A utilizes advanced statistics, section B, simple statistics. The A section can be done and only the means compared (or means and variances) to simplify the statistical tests.

Section A: To see if snails can detect if the trail was laid by a fed or a starved snail, test fed (m) with starved (t) vs. starved (m) with starved (t). To see if snails can distinguish sex pheromones in the trail, test male (m) with male (t) vs. male (m) with female (t) (or vice versa). This test is only useful if you can sex live animals (and they are not hermaphroditic). Some littorines have a red penis behind the right tentacle which can be easily spotted. Most land snails, however, are hermaphroditic. To see if snails can distinguish the trails of related snails, test species 1 (m) with species 1 (t) vs. species 1 (m) with species 2 (t).

These experiments will give paired sets of coincidence indices. An F test will tell you if the variances are homogenous or not. If they are use a t-test, if they are not, use a Mann-Whitney U test, to see if there are statistically significant differences between the two sets of data. Because variances tend to be rather high, you will need a large number of runs to get enough data to be significant. Pooling class data is recommended.

Section B: These experiments can be used to quickly generate data which can be tested by much simpler statistical tests: the binomial and chi-square tests. Use the binomial when the sample size is under 25, the chi-square when it is larger.

Run a marker snail across a tank as far as you can, tracing the trail and marking its direction. Start a tracker snail facing the trail and score it as follows:

+ = turns the same direction as marker trail

- = turns in opposite direction

o = no response

r = retreat

Remove the tracker snail as soon as it has moved enough to be scored. The one long marker trail can be used to score a number of tracker snails. Any of the three paired combinations of snails suggested in Section A can be used as markers and trackers. Once the data has been obtained, first test to see if trail following occurs (+ and - vs. o and r). If the answer is positive, indicating snails do follow trails, pose the question, can they sense the direction of the trail? (+ vs -). Students should work in pairs and each pair can do enough runs to obtain data for statistical testing.

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