Influence of Population Density on Offspring Number and Size in Burying Beetles

Claudia M. Rauter

Department of Biology, University Nebraska at Omaha
Allwine Hall 114, 6001 Dodge Street, Omaha, NE 68182-0040

E-mail: crauter@unomaha.edu

Abstract: This laboratory exercise investigates the influence of population density on offspring number and size in burying beetles. Students test the theoretical predictions that brood size declines and offspring size increases when competition over resources becomes stronger with increasing population density. Students design the experiment, collect and analyze the data, and discuss the results in the context of adaptive phenotypic plasticity.

Keywords: Population density, contests, brood size, offspring size, burying beetles

Introduction

Some animals have only a few large offspring at a time, whereas others have many small offspring. This negative relationship between offspring number and size can be observed not only among species, but also within many species (Roff, 2002). Negative relationships between offspring number and size are the consequence of mechanical constraints or limited resource availability (Roff, 1992, 2002). For example, body size of a female limits the number and size of offspring she can produce. For a given body size, a female can either produce many small or a few large offspring. When resources are limited, a trade-off between offspring number and offspring size may occur. How females solve this dilemma when resources are limited, may be a function of the intensity of competition over these resources, especially when large individuals are more likely to prevail (Leips and Travis, 1999; Both, 2000; Creighton, 2005; Goubault et al., 2007). If high population density results in more intense competition over resources and large individuals are more successful in monopolizing resources, females should have fewer, yet larger offspring (Mesterton-Gibbons and Hardy, 2003). This would ensure that their offspring have a higher probability of gaining access to resources necessary for reproduction. Reciprocally, if population density is low and thus competition over resources is weak, females should have many small offspring (Mesterton-Gibbons and Hardy, 2003).

This laboratory exercise tests the theoretical predictions that offspring number declines and offspring size increases when competition over resources becomes stronger with increasing population density.

Burying Beetles

In the United States, there are 15 species of burying beetles (Coleoptera: Silphidae: *Nicrophorus sp.*; for distribution, natural history, and keys for identification of each of these species see: Anderson and Peck, 1985; Peck and Kaulbars, 1987; Ratcliffe, 1996). These species are widely distributed and occur in many habitat types (e.g. forests, prairie, mountains). The most widespread species is *N. marginatus* which can be found in open fields, prairie, open woods, or other open habitats. Most burying beetle species are active at night and reproduce in spring or early summer.

Burying beetles use carrion as a food resource for their offspring (for reviews on burying beetle biology see: Ratcliffe, 1996; Eggert and Müller, 1997; Scott, 1998). Carrion is a high quality, but rare, resource. Contests (i.e. fights) over carrion are common, especially when population density is high. The largest individual of either sex usually wins in a contest, thus gaining access to the carrion. Generally, a male and a female beetle bury the carrion. While burying the carrion, the beetles remove fur or feathers. As early as 12 hours after encountering the carrion, the female lays eggs in the surrounding soil. About two days later, the larvae hatch and crawl to the carrion. During the first two to three days on the carrion, the larvae are fed by the parents. Subsequently, the larvae feed by themselves from the carrion. The larvae of many species can survive and grow without being fed by the parents, although less well (e.g. *N. defodiens*, *N. marginatus, N. pustulatus*, and *N. vespilloides*). Larvae of *N. mexicanus, N. orbicollis,* and *N. sayi*, however, require parental feeding for survival. After the carrion has been consumed, typically 6 to 10 days

after the larvae have hatched, the larvae disperse and pupate in the surrounding soil, emerging as adult beetles four weeks hence. Beetles reach sexual maturity after an additional three more weeks. The majority of species overwinters as adults (e.g. *N. defodiens*, *N. marginatus, N. pustulatus*, and *N. vespilloides*). Some species overwinter in the third larval stage as prepupae (e.g. *N. tomentosus, N. vespillo*).

Materials and Methods

Preparation Before Class

In summer, two months before the students conduct the experiment, approximately 30 burying beetles are collected using pitfall traps baited with decaying ground beef. A wide-mouth jar (at least 200 ml.), plastic bucket, or similar container can be used as pitfall trap. The trap is filled with about 2 to 5 cm of moist soil or leaves which provide hiding places and humidity for the trapped beetles thus reducing mortality due to aggressive interactions among the beetles or heat stress, respectively. As bait any well-aged meat (e.g. ground beef, chicken liver, or fish exposed to room temperature for two to three days) will attract beetles. The bait is placed in a small container (e.g. 30 ml plastic cup) that can be closed tightly with a lid to keep beetles and flies off the bait. To release the smell of the bait, small holes are punched into the container or the lid. The container with the bait is placed onto the soil or leaves in the trap. The trap is buried in the soil with the rim of the trap level with the surface of the ground. A rain cover or sun shield should be placed over the trap. A plastic plate secured with three large nails can serve as a rain cover and sun shield. A one-foot square piece of one-inch mesh screen wires placed over the pitfall trap and staked down securely will keep scavengers out. Instead of burying the traps, they can be hung, at least 3 feet above ground, from tree branches or stakes. Trapping rates are improved by avoiding dense vegetation, with the placement of traps in prairies, open woods, or less dense parts of forests. Additional information on trapping of burying beetles can be found in Ratcliffe (1996), Trumbo (1996), and Bedick et al. (2004). Traps should be checked daily, especially on hot or rainy days. Beetles caught in the pitfall traps are transferred to containers filled with moist peat or soil and transported to the lab.

The beetles are sexed in the lab by comparing the tarsi of the front legs and the last segments of the abdomen (Figure 1). Ten to 15 pairs of beetles are placed individually into clear plastic containers of sufficient size to accommodate a mouse carrion (e.g. 17 × 12 × 6 cm). The containers are filled with about 2 cm of moist peat and supplied with a previously frozen mouse available from pet food supply sources. Mice weighing approximately 20 to 30 g work best. Burying beetles rear very small broods on lighter mice and they have difficulties keeping larger mice carrion clean of bacteria and mold. The containers with the beetles and the mouse carrion are kept in a darkroom. Containers are checked daily for eggs that are easily visible on the bottom of the containers. The day after the first eggs are visible, the beetles and the carrion are removed. The peat is carefully searched for eggs using forceps. Eggs are transferred onto a small piece of wet paper towel. The paper towel with the eggs is afterwards placed into a container that can hold a mouse (e.g. 17 × 12 × 6 cm), filled 1 cm deep with moist peat and containing a previously frozen mouse. To facilitate consumption of the carrion by the larvae, a small incision with scissors is made in the back of the mouse. The containers with the eggs are kept in a dark room at room temperature. About two days later, the larvae hatch. When the larvae have consumed all or most of the carrion and are crawling along the container walls, they are transferred to a new container of the same size filled to the top with moist peat. No more than 15 larvae are placed in a single container (17 × 12 × 6 cm). If smaller or larger containers are used, the number of larvae should be adjusted accordingly. The containers are kept in a darkroom at room temperature until the new beetles emerge about four weeks later. The rearing of larvae without the parents reduces transfer of mites and parasites carried by all field-caught beetles. Parasites and mites introduced by field-caught beetles can cause problems with laboratory exercises (e.g. brood failure). This procedure works well for burying beetles that overwinter as adults and the larvae can be reared without parents (e.g. *N. defodiens*, *N. marginatus, N. pustulatus*, and *N. vespilloides;* Peck and Kaulbars, 1987; Trumbo, 1992; Eggert and Müller 1997).

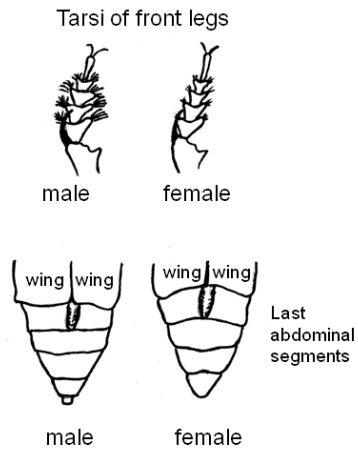

Figure 1. Dorsal view of tarsi of front legs and last segments of abdomen of adult female and male burying beetles, *Nicrophorus pustulatus*.

The newly emerged beetles are sexed (Figure 1) and can then either be used to establish a laboratory colony (see below) or for laboratory exercises. Beetles used for this laboratory exercise are randomly assigned to the low-population density treatment or the high-population density treatment. Low-population density beetles are placed individually in containers (about 17 × 12 × 6 cm; much smaller containers do not work as well with the high-population density treatment) filled with 1 cm of moist peat. Beetles assigned to high-density population treatment are kept in groups of four beetles in containers of the same size. Beetles are kept in these containers for at least three weeks at a 15:9 h light:dark cycle at room temperature, and are fed wet cat food or decapitated mealworms twice weekly.

To establish a laboratory colony, the newly emerged beetles are maintained either individually or in single-sex groups. Up to six beetles can be kept in containers of about 17 × 12 × 6 cm. For smaller or larger containers the number of beetles should be adjusted. The containers are filled with 2 to 3 cm of moist peat or soil. The beetles are kept at 15:9 h light:dark cycle at room temperature (i.e. summer conditions) and fed wet cat food twice weekly. Small pieces of liver, fly maggots, decapitated mealworms, or dead crickets are also suitable as food. Three to six weeks after emergence of the adult beetles, the next generation of beetles is produced by placing a male and female beetle in a container able to hold a mouse, filled about 1 cm with moist peat or soil, and containing a previously frozen mouse. The parent beetles are removed when the larvae are about five days old, to prevent that the parent beetles eat the larvae after the carrion has been consumed. Dispersing larvae are transferred to new containers filled with moist peat or soil for pupation.

In Class (Week 1)

Introduced to the ecological concept of reproductive strategies including the trade-off between offspring number and size, students are asked to design an experiment that tests whether population density influences offspring number and size in burying beetles. While designing the experiment, the students are directed to consider: 1) the null hypothesis and alternative hypotheses, 2) the factor they have to manipulate (i.e. population density), 3) the measurements they should take (i.e. number and weight of larvae), 4) the factors they must control for or measure (e.g. carrion size, room temperature, light cycle, number of parents providing care), and 5) sample size. Students work in small groups to draft the experimental design that is subsequently subject to review and criticism by the whole class. Once the class has decided on an experimental design, all students establish together a protocol for the experiment and prepare data sheets. With the protocol set and the data sheets ready, the students start the experiment.

The experiment begins by filling 40 containers that are able to hold a mouse (e.g. 17 × 12 × 6 cm) with about 2 cm of moist peat and by placing a previously frozen mouse on top of the peat. Female and male beetles are added to these prepared containers as follows: ten containers receive each one high-density female and one high-density male; into ten additional containers one high-density female and one low-density male are placed; to ten containers one low-density female and one high-density male are added and the last ten containers receive each one low-density female and one low-density male. The containers with the beetles are maintained in a darkroom at room temperature for the next two weeks. It takes approximately two weeks from the time the females encounter the carrion until the larvae have consumed the carrion and are dispersing, i.e. crawling along the container wall. To simplify the system, males are removed two days after the matings have been initiated. To avoid cannibalism of dispersing larvae by the females, the female beetle is removed when the larvae are five days old. If no dark room is available, a black cloth can be placed over the containers.

In Class (Week 3)

Two weeks after the experiment has been started, the larvae of each brood are counted and the weight of the whole brood is determined. Average larval weight for each brood is calculated by dividing the weight of the whole brood by the number of larvae in the brood. Several minutes are required to count the larvae of a brood and to weigh the whole brood. During a short break the instructor compiles the data that the students will use to calculate means and standard errors, graph the results, and conduct statistical analyses in class.

At the end of class, all the larvae are collected and used to start a new laboratory colony or added to a previously established laboratory colony of burying beetles. Burying beetles are also regularly used for additional behavioral laboratory exercises such as studies of mating systems or conflicts between individuals over resources (Trumbo, 1996; Scott, 2003).

Results and Discussion

This laboratory exercise allows students to design and conduct an experiment and to perform statistical data analyses. I use this laboratory exercise as the first laboratory exercise in my behavioral ecology course for junior and senior biology majors to reinforce the concept of trade-offs and to prepare the students for their lab research project in the second part of the course. The week between the first and second part of the experiment is used to introduce the students to statistical analyses, in particular to linear regression analyses as well as to one-tailed and two-tailed t-tests (e.g. Sokal and Rohlf, 1995). Using linear regression analysis, students can test for the trade-off between number and size of offspring, while the one-tailed t-test allows to test whether high-density females have fewer, but heavier offspring than low-density females.

Typical results from this exercise are shown in Figures 2, 3, and 4. Average larval weight decreases with increasing brood size (equation for linear regression line: $Y = 368.8 - 6.4\ X$; $F_{1,69} = 47.51$; $P < 0.0001$; Figure 2). Brood size is significantly smaller for high-density females than for low-density females (one-tailed t-test: $t = 2.3$; d.f. = 67; $P = 0.01$; Figure 3), but the larvae of high-density females are heavier than those of low-density females (one tailed t-test: $t = -1.8$; d.f. = 56; $P = 0.04$; Figure 4). These results illustrate the trade-off between offspring size and number, corroborating the findings of Creighton (2005) in *N. orbicollis*. These results also support the prediction of the theoretical model by Mesterton-Gibbons and Hardy (2003) that brood size should decrease when contests over resources become more frequent.

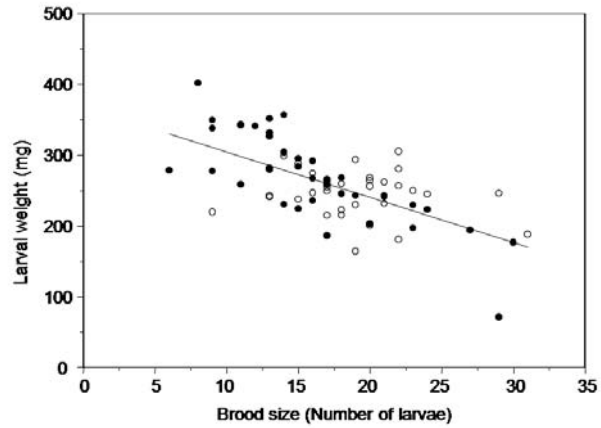

Figure 2. Average larval weight in relationship to brood size in *N. putsulatus*. Open circles: low-population density beetles. Filled circles: high-population density beetles.

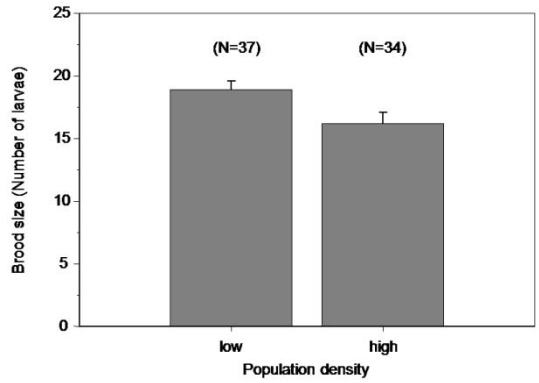

Figure 3. Brood size in relationship to population density in *N. pustulatus*. Means and standard errors are shown. Sample sizes are given in parentheses.

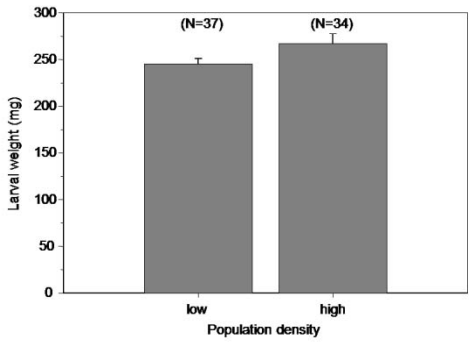

Figure 4. Average larval weight in relation to population density in *N. pustulatus*
. Means and standard errors are shown
. Sample sizes are given in parentheses.

Students discuss in class the importance of the ability of females to adjust offspring number and size in relation to environmental conditions so that they maximize their fitness. This discussion introduces the students to the concept of phenotypic plasticity (i.e. capacity of a genotype to produce different phenotypes depending on the environmental condition the genotype experiences) and its adaptive value (i.e. the consequences for the animals' fitness).

This laboratory exercise can be simplified by increasing the guidance and providing the basic experimental design. On the other hand, the rigor of this exercise can be raised by increasing the complexity of the experimental design. In a separate offering of this exercise, the experimental design was made more complex by adding additional levels of population density and including the weight of the mouse as covariate in the experiment.

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