The influence of diet and isolation on growth and survival in the land snail *Bulimulus tenuissimus* (Mollusca: Bulimulidae) in laboratory

Liliane M. O. Meireles; Lidiane C. Silva; Flávia O. Junqueira & Elisabeth C. A. Bessa

Núcleo de Malacologia, Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora. Campus Universitário, 36036-330 Juiz de Fora, Minas Gerais, Brasil. E-mail: lilianemeirelesbio@yahoo.com.br

ABSTRACT. Feed influences directly growth and survival in land snails. Here, we investigate the influence of diet and isolation on growth and survival in *Bulimulus tenuissimus* (d'Orbigny, 1835) in the laboratory. Animals were maintained individually and in groups and three different types of diets: lettuce, ration and both lettuce and ration (mixed diet) for 210 days. Isolated animals, in all diet types, grew more rapidly than grouped animals. Also, snails fed with lettuce grew most slowly in isolated or grouped treatments. Within isolated or grouped treatments, growth rates were similar for the ration and the mixed diet. Mortality rate was higher in animals fed on lettuce only, in both density treatments. Thus, diet and density are important components of the population dynamics of *B. tenuissimus*.
KEY WORDS. Diet; feed; land snail; mortality.

RESUMO. **Influência da dieta no crescimento e na sobrevivência de *Bulimulus tenuissimus* (Mollusca: Bulimulidae) mantidos isolados e agrupados em condição de laboratório.** O conteúdo energético e a aglomeração intra-específica são fatores que influenciam diretamente aspectos da biologia de moluscos terrestres. Foi investigada a influência da dieta sobre o crescimento e a sobrevivência de *Bulimulus tenuissimus* (d'Orbigny, 1835) mantidos agrupados e isolados em terrários plásticos e submetidos a três tipos diferentes de dietas: alface, ração e ração e alface (RA), por um período de 210 dias. Foi verificado que os animais mantidos isolados, independentemente, do tipo de dieta oferecida apresentaram maior crescimento do que aqueles mantidos agrupados, evidenciando a influência da densidade populacional no crescimento. Moluscos alimentados com dieta alface apresentaram o menor crescimento, tanto mantidos agrupados quanto isolados. Não houve diferença significativa entre o crescimento dos animais mantidos agrupados e isolados alimentados com as dietas RA e ração. A taxa de mortalidade foi significativamente maior para os indivíduos alimentados com a dieta alface em ambas as densidades. Tais resultados evidenciam que a dieta oferecida exerceu grande influência na biologia de *B. tenuissimus*.
PALAVRAS-CHAVE. Alimentação; dieta; molusco terrestre; mortalidade.

Terrestrial pulmonate gastropods are important economically and medicinally. Many species are edible and widely reared and sold for human consumption and others are important agricultural pests as well as intermediate hosts for parasites (Mead 1979). Despite this importance, land snail studies usually emphasize morphology, taxonomy, systematics and geographic distribution, while neglecting biology and behavior (Almeida & Bessa 2000, 2001, D'ávila & Bessa 2005). Mitigating the negative impacts of land snails requires that we better understand the biology and especially that of growth and reproduction (Leahy 1984). Growth and reproduction are directly influenced by diet and population density (Raut *et al*. 1992, Furtado *et al*. 2002).

Energy content, nutritional value, texture and structure, as well as digestibility and absorption of items in the diet influence growth rates and reproductive success in molluscs (Thomas *et al*. 1983, Mcshane *et al*. 1994, Foster *et al*. 1999). Also, intra-specific grouping may also directly and indirectly influence population dynamics through their effects on fecundity, growth rates and adult size, activity and survival (Pearce 1997).

Bulimulidae is relatively species rich and widespread geographically (Breure 1979, Oliveira & Almeida 1999). However, *Bulimulus tenuissimus* (d'Orbigny, 1835) is to date the only species that is part of the parasite-host cycle of domestic animals (Thiengo & Amato 1995). This species is widespread in Brazil (Morretes 1949, Oliveira & Almeida 1999, Simone 2006), yet poorly studied, with most information on anatomy and histology (Araújo *et al*. 1960, Rezende *et al*. 1972).

To better understand the biology of this economically important species, here we investigated the influence of diet and density on growth and survival of *B. tenuissimus* in the laboratory.

MATERIAL AND METHODS

Recently hatched snails were studied from May-November 2006 (210 days). Animals were provided by the Mollusc Biology Laboratory of the Prof. Maury Pinto de Oliveira Malacology Museum of the Federal University of Juiz de Fora. A total of 240 snails were used, of which 120 were divided into 12 groups of 10 individuals and kept in plastic terraria (12 cm wide x 9 cm deep). The other 120 snails were kept individually in plastic terraria (8 cm x 6 cm). All snails were fed *ad libitum* with: 1) lettuce (*Lactuca sativa* Linné, 1758), 2) rations enriched with calcium carbonate, 3) lettuce and rations (1+2) (hereafter called "lettuce," "rations," and "mixed").

Rations comprised cornmeal, soy flour, wheat flour, gluten, corn flour, ground bone, chicken, fish meal, calcium phosphate, salt, minerals and vitamins (Tab. I). This "flour" was sifted (through 1 mm mesh size) and enriched with calcium carbonate in proportions 3:1 (Oliveira *et al*. 1968, Bessa & Araújo 1996) and placed in plastic plates of 4 cm diameter.

Table I. Nutritional composition of the rations used in feeding *Bulimulus tenuissimus* during 210 d (values provided by the supplier).

Component	Percent (%)
Humidity (Max)	13.0
Crude Protein (Min)	20.0
Stereo extract (Min)	2.5
Dietary fiber (Max)	6.0
Minerals (Max)	10.0
Calcim (Max)	1.5
Phosphorus (Min)	0.5

Terraria were closed with cotton cloth to maintain aeration. Substrate was sterilized potting soil. Every 24 h the food was changed and the soil was humidified. Every 15 days the snails were measured (shell length) with calipers (Kanon Hardened Stainless 1/28 in 1/20 mm). Temperature (maximum, minimum) and relative humidity (Maximum-minimum thermometer, wet-dry hygrometer) were measured daily throughout the experiment (Fig. 1).

Treatments were compared by the Kruskal-Wallis tests (size) and Mann-Whitney (growth) using the software BioEstat 4.0, and significance was determined when $p < 0.05$.

RESULTS

Growth was lowest in the lettuce treatment in both, the grouped ($H = 7.22$, $p = 0.027$) and isolated ($H = 19.64$, $p = 0.001$) treatments (Tab. II). That is, the two food treatments with rations resulted in snails of the same size (grouped: $Z = 0.54$, $p = 0.58$, isolated: $Z = 0.22$, $p = 0.83$). Lettuce resulted in the smallest sizes (grouped: 2.81 mm, isolated: 2.77 mm).

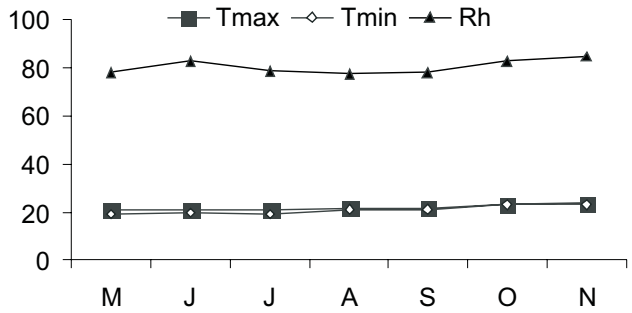

Figure 1. Average monthly maximum (Tmax) and minimum (Tmin) temperatures (°C) and relative humdity (Rh) (%) during this study (May-November 2006).

Table II. Comparisons of shell length (mean ± standard deviation) in *Bulimulus tenuissimus* kept in groups or isolated with three different diets for 210 d.

Diet treatment	Shell Length (mm)	
	Grouped	Isolated
Lettuce	2.81 ± 0.234 a A	2.77 ± 0.101 a A
Rations	15.41 ± 0.251 b A	20.00 ± 0.233 b B
Rations and Lettuce	15.60 ± 0.252 b A	20.10 ± 0.202 b B

* Treatments with different letters are different at $p < 0.05$. Upper case letters indicate comparisons among group treatment while lower case letters indicated differences between diet treatments.

The grouped and isolated treatments responded differently to diet when fed rations, but not lettuce ($Z = 1.04$, $p = 0.2976$), with respect to growth. Isolated snails were larger with rations ($Z = 4.35$, $p = 0.001$) and with the mixed diet ($Z = 4.14$, $p = 0.001$, Fig. 2).

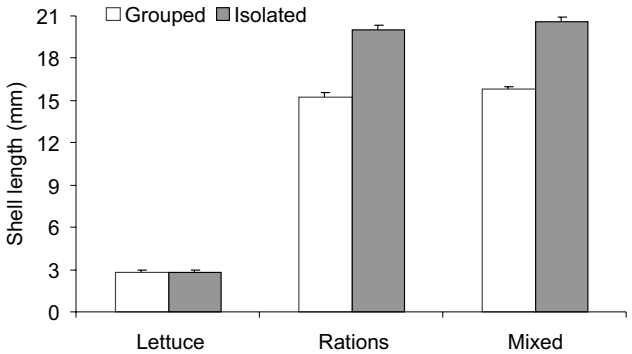

Figure 2.Comparisons of shell length (mm) among diet and grouping treatments in *Bulimulus tenuissimus* kept in groups and isolated and maintained on a diet of lettuce, rations and mixed after 210 d.

The lettuce diet was associated with greater mortality in both density treatments (82.5%). Mortality was lower for grouped snails fed with rations (52.5%) and with the mixed diet (60%). However, these differences were not significant ($Z = 2.77$, $p = 0.25$). Mortality was greater in isolated snails fed lettuce than either rations ($Z = 10$, $p = 0.031$) or mixed ($Z = 15.07$, $p = 0.0012$, Fig. 3).

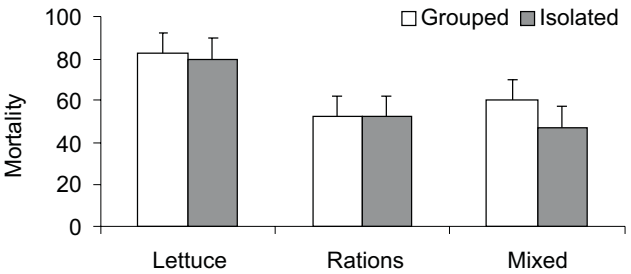

Figure 3. Comparison of mortality rates (as a percent) in *Bulimulus tenuissimus* kept in groups and isolated with three diet treatments (lettuce, rations and mixed), at the end of the experiment.

DISCUSSION

Greater growth in the ration and lettuce treatment is probably due to the greater quality of the combined diet. Clearly, lettuce is relatively low quality as shown by the decreased growth in both density treatments fed on lettuce. The high nutritional quality of the artificial diet in the laboratory (proteins, lipids, minerals and carbohydrates) favors growth and reproduction, with greater fecundity and earlier reproduction (Bessa & Araújo 1996).

Apparent preferences for a particular plant in the diet does not necessarily imply that this preference provides the nutrition necessary for development and reproduction, and may merely imply greater availability (Frantz & Mossmann 1989). Thus, in the laboratory, where animals only have access to the diet offered, it is necessary to complement the diet to assure development and growth. For example, weight was greater and mortality rate lower in *Helix aspersa* (Müller, 1774) (Helicidae) fed on a diet enriched with 16.25% crude protein (Soares *at al*. 2002). Artificial diets may be much better than plant diets to increase growth in cultured snails (Ribas 1986). Artificial diet associated with plants resulted in great grown and earlier sexual maturity than artificial food without plants, or plants alone, in *Achatina fulica* Bowdich, 1822 (Achatinidae) (Monney 1994). Here, as seen by the rapid growth and low mortality, the rations provided were sufficient to supply dietary needs of these animals in the absence of plants.

Grouped animals grew less and suffered higher mortality rates, indicating that population density interferes with growth and survival in *B. tenuissimus* and other terrestrial snails (Dan & Bailey 1982, Almeida & Bessa 2000). These density effects are generally related to competition, either for limited resources or due to interference that may result in damage to the individuals involved. For these animals, food and microclimate may be the most important limiting resources, which would explain the effect of density (Pearce 1997). Other studies have also shown increased mortality with increased density in terrestrial molluscs (Lazaridou-Dimitriadou & Daguzan 1981, Almeida & Bessa 2000).

Diet and population density, therefore, influence growth and survival in *Bulimulus tenuissimus*. The lack of minerals may have been the most important cause of lower growth rates and higher mortality rates found here. We demonstrate here that the use of an artificial diet with plant material can be advantageous for cultivating snails in the laboratory and will result in better development and survival.

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