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Survival potential, fecundity and fertility of Biomphalaria pfeifferi (Krauss, 1848) during acclimatization in the laboratory

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Survival potential, fecundity and fertility of *Biomphalaria pfeifferi* (Krauss, 1848) during acclimatization in the laboratory

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Survival potential, fecundity and fertility of *Biomphalaria pfeifferi* were investigated during the process of acclimatization in the laboratory. Thirty adult snails (average size 5 × 13 mm) were used in Experiment A (control; laboratory-bred snails) and B (acclimatizing snails from the wild). Both experiments aimed at monitoring survival and reproductive potential of snails were performed at room temperature (21–29 °C) for five weeks. The mean pH, conductivity, total dissolved solid, dissolved oxygen, water and room temperature were 7.77, 228 ps/cm, 121, 0.5 mg/L, 25.0 and 29.0 °C, respectively. The mean and standard deviation of eggs per egg-mass was 19.86 ± 0.46 and 14.88 ± 5.01, the number of eggs per snail was 53.26 ± 10.34 and 38.51 ± 10.03, the amount of egg-mass per snail was 2.68 ± 0.04 and 2.48 ± 1.09 in Experiments A and B, respectively. Results of the experiment indicate a significant difference ($p < 0.05$) between fecundity, fertility and survival of *B. pfeifferi* in Experiments A and B. Experiment A did not produce a significant difference in all the parameters tested in contrast to Experiment B, which recorded a significant difference in all the parameters tested. The results obtained indicate that acclimatization in the laboratory affects the survival and reproductive potential of *B. pfeifferi* fetched from the wild.

Sraigių *Biomphalaria pfeifferi* išgyvenamumas, vaisingumas ir vislumas buvo tiriamas vykstant jų aklimatizacijai laboratorijoje. Naudota 30 suaugusių sraigių, kurių vidutinis dydis buvo 5 × 13 mm. Eksperimente A buvo tiriami laboratorijoje išauginti kontroliniai individai, eksperimente B – laukinės sraigės, perkeltos į laboratoriją. Abu bandymai buvo vykdomi kambario temperatūroje (21–29 °C) ir truko po penkias savaites. Vidutinis vandens pH buvo 7,77, laidumas 228 pS/cm, bendras ištirpusių kietųjų dalelių kiekis 121 mg/l, ištirpusio deguonies koncentracija 0,5 mg/l, vandens temperatūra 25,0 °C, kambario temperatūra 29,0 °C. A ir B eksperimentuose gauti duomenys (vidurkis ± SD): vidutinis kiaušinėlių skaičius masėje 19,86 ± 0,46 ir 14,88 ± 5,01; vienos sraigės padėtų kiaušinėlių skaičius 53,26 ± 10,34 ir 38,51 ± 10,03; vienos sraigės padėtų kiaušinėlių masių skaičius: 2,68 ± 0,04 ir 2,48 ± 1,09. Nustatyta, kad *B. pfeifferi* vaisingumas, vislumas ir išgyvenamumas abiejuose bandymuose reikšmingai skyrėsi ($p < 0,05$). Eksperimente A tirtų parametrų skirtumų nebuvo, eksperimente B šių parametrų skirtumai patikimi. Gauti rezultatai rodo, kad aklimatizacija laboratorijoje paveikia laukinių sraigių išgyvenamumą ir dauginimosi potencialą.

Keywords: survival potential; fecundity; fertility; *Biomphalaria pfeifferi*; acclimatization process

Introduction

Pulmonate snails, the most abundant snails in West African freshwaters (Wethington and Dillon 1993), occupy a wide range of freshwater environments (Holznagel, Colgan, and Lydeard 2010). Most pulmonates, especially *Biomphalaria* spp., are bred and maintained in the laboratory for various purposes (James, Karen, and Bernard 2011). The ubiquity and relative ease of culture of pulmonates have made researchers focus attention on the behaviour, ecology and physiology of these snails at the cellular level (Norton and Bronson 2006).

Most importantly, studies on planorbids have become areas of intense research because of the role they play as hosts for larval stages of most helminths, which cause such diseases in man and animals (Mas-Coma, Bargues, and Valero 2005) as schistosomiasis and fasioliasis. *Biomphalaria pfeifferi* is one of the aquatic pulmonate snail species that serve as an intermediate host for *Schistosoma mansoni*, the causative agent of intestinal schistosomiasis, a parasitic disease affecting over 200 million people in the world (Carter Center 2011). This trematode infection continues to limit the development of rural areas of low-income countries in tropical and subtropical regions. As intermediate hosts, snails are essential for transmission of this disease. Hence, control efforts have been targeted at studying the intermediate host in the laboratory. A prerequisite for the above-mentioned studies is snail rearing in laboratory conditions, which is indispensable for maintaining their life cycle (Toledo and Fried 2010). However, rearing and maintaining snails in laboratory conditions is a difficult endeavour. *Biomphalaria* spp. are hermaphrodites and are capable of both self and

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cross-fertilization, the latter mechanism being preferable. Under ideal conditions, the egg to egg cycle may be as short as one month (Eveland and Haseeb 2011). A mature snail may lay 10,000 or more eggs/year. Eggs are laid in gelatinous masses of several eggs that are found on the walls of the laboratory aquaria. Naturally, these snails can live for more than 12 months, but their survival in the laboratory under close monitoring may or may not exceed 12 months. Since the generation time is approximately five weeks, these snails can produce several generations over a 12-month period (Ritchie, Berrios-Duran, and DeWeese 1963; Loker 2006).

Survival, fecundity and death rates are important indicators of the physiological state of the snail host (Eveland and Haseeb 2011) especially during its acclimatization. The physicochemical status of the culture water is a key to determining the survival, fecundity and fertility of freshwater snails. It has been reported that in the proper environment, snails produce larger egg-masses and higher numbers of eggs per mass (Costa, Grault, and Confalonieri 2004). Studies on various aspects of reproduction such as laying of egg masses, egg capsules and fecundity are essential for developing effective control measures (Muthiah and Sampath 2000). Among the factors that may affect the survival and reproduction of pulmonates in the laboratory are temperature, diet, crowding and parasitism (Toledo and Fried 2010).

However, there is a lack of data on the fecundity and fertility of *B. pfeifferi* during the first several weeks of their acclimatization to laboratory conditions. Besides extending the knowledge of snails' fecundity and fertility during the period of acclimatization, this study also present some information on difficulties of rearing snails under laboratory conditions. The results of this work will broaden the knowledge of this pulmonate snail, especially at the initial stage of its acclimatization to laboratory conditions, and therefore will be of great help to malacologists.

Material and methods

Sampling of snails

Adult *B. pfeifferi* snails were collected from Eleyele Reservoir, Ibadan, Nigeria (latitude: 7°25′ and longitude: 3°51′), in the second week of August, 2012. Snails were collected early in the morning between 8.00 and 10.30 am using a flat dip-net scoop as described by Demian and Kamel (1972) and employed by Benson and Morenikeji (2012). The collected snails were placed in sterile plastic containers with 47 cm^3 of Reservoir water with sterile cotton wool a little above it, and taken to the Parasitology Research Laboratory, Department of Zoology, University of Ibadan, Ibadan for identification and maintenance.

Maintenance of the snails

In the laboratory, snails were identified to the species level using the snail identification key by WHO (1971). Snails were checked for infection using the shedding method described by Frandsen and Christensen (1984), and employed by Benson (2012). Following this method, each snail was placed in a beaker half-filled with dechlorinated tap water. The beakers (each with a snail) were exposed to sunlight and left for 1 h or more to allow cercariae to emerge. Two sets of experiments were conducted during this study: Experiment A, which also was the control experiment, was carried out on laboratory-bred snails and Experiment B on acclimatizing snails from the wild. Both experiments designed to elucidate the survival and reproductive potential of snails were conducted at room temperature (21–29 °C) over a period of five weeks. In the first experiment, 30 active and healthy snails of the same species and the uniform size (average 5×13 mm) were freshly collected from the wild and were placed into one glass trough each. In the second experiment, 30 active and healthy laboratory-bred snails of the similar size were also placed in a similar trough. Each trough was interiorly covered with a polythene bag, a layer of clay and some gravel, which had been sterilized by heating in an electric cooker for at least 1 h. Then troughs were filled with 3 L of dechlorinated tap water and snails were placed into them. Well-processed lettuce (*Lactuca sativa*) leaves were used to feed the snails thrice a week. The experiments were checked for dead snails daily. When dead snails were found, they were removed and counted. The aquarium was maintained at a temperature between 25 and 29 °C.

Survival and reproductive potential test

In order to check fecundity, the number of eggs per egg-mass, eggs per snail and egg-masses per snail were counted on a weekly basis (Calow 1998; Costa, Grault, and Confalonieri 2004) from each experiment. The fertility of the eggs was assessed through the snail hatch rate and the percentage of fertile eggs (Costa, Grault, and Confalonieri 2004). To calculate the survival rate for each week, the formula $\mathrm{Sr} = \frac{\mathrm{Na}}{\mathrm{Tn}} \times 100$ was employed, where Sr = survival rate, Na = number of alive snails at the end of each week, Tn = total number of snails at the beginning of each week.

Statistical analysis

Differences in survival rate, fecundity between the two experiments were compared using Student's *t*-test. Fertility (hatchability) was compared by χ^2 test. The difference was considered significant when *p*-values were lower than 0.05.

Results

Table 1 presents data on the fecundity of *B. pfeifferi* in Experiment A and in Experiment B. Fecundity parameters are defined by the number of eggs per egg mass per week, the number of eggs per snail per week and the number of egg masses per snail per week. The results for each fecundity parameter through weeks (from first to fifth week) given in Table 1 are statistically not significant ($p > 0.05$).

Table 1. Fecundity, fertility and survival of *Biomphalaria pfeifferi* in Experiment A (control) and B.

Time (weeks)	Number of eggs/egg mass A	Number of eggs/snail B	Number of egg masses/snail A
First	19.28	11.69	50.13
Second	20.65	13.62	49.57
Third	20.24	16.33	55.68
Fourth	19.03	15.46	54.36
Fifth	20.09	17.29	56.56

There was a significant difference ($p < 0.05$) recorded in the fecundity, fertility and survival of *B. pfeifferi* between Experiments A and B (Table 2). The mean number of eggs per egg mass in Experiment A was 19.86 ± 0.46, while the mean of the same parameter in Experiment B was 14.88 ± 5.00.

The number of eggs/egg mass of *B. pfeifferi* recorded during the fifth week of acclimatization in Experiment A was much higher compared to that in Experiment B (Figure 1). The number of eggs/snails of *B. pfeifferi* in Experiment A was initially higher. However, after the fourth week, the number of eggs/snail recorded in Experiment B was continuously higher than that obtained in Experiment A (Figure 1). In Experiment B, the survival rate of snails was low but increased gradually until it attained 100% in the fifth week. Meanwhile, in Experiment A it was fluctuating (Figure 2). The hatchability in Experiment B was also low in week one but increased and attained 100% in the third week, while in Experiment B, it was 100% throughout the experiment (Figure 3).

Physicochemical parameters of the water used in the experiments were measured. The mean pH, conductivity, total dissolved solid, dissolved oxygen, water and room temperature were 7.77, 228 ps/cm, 121, 0.5 mg/L, 25.0 and 29.0 °C, respectively.

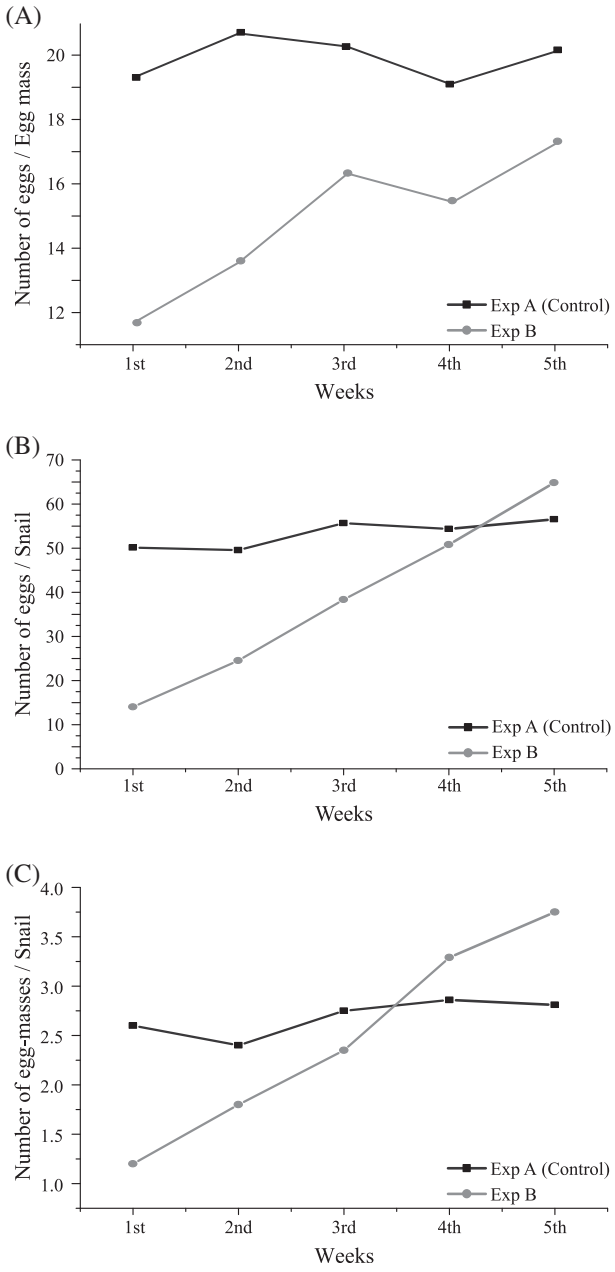

Figure 1. Changes in the reproductive biology of *Biomphalaria pfeifferi* before and after acclimatization to laboratory conditions. (A) number of eggs/egg mass; (B) number of eggs/snail; (C) number of egg masses/snail.

Discussion

The population growth rate of a species is determined by the fertility and hatchability of its eggs, if the species is oviparous (Aziz and Raut 1996). The present study provides the understanding of the survival, fecundity and

Table 2. Number of egg-masses, eggs per snail and its mean standard deviation laid by *Biomphalaria pfeifferi* during acclimatization.

	Number of egg-masses		Number of eggs per snail		Number of eggs per egg-mass	
	Exp. A	Exp. B	Exp. A	Exp. B	Exp. A	Exp. B
Mean standard deviation	19.86 ± 0.46	14.88 ± 5.01	53.26 ± 10.34	38.512 ± 410.03	2.68 ± 0.04	2.48 ± 1.09
One population *t*-test	65.35	14.87*	37.03*	4.25*	32.20	5.29*
Independent *t*-test	−4.76*		−1.61*		−0.43	

Notes: Experiment A (control; laboratory-bred snails) and Experiment B (acclimatizing snails from the wild). *Refers to the parameters showing significant differences ($p < 0.05$).

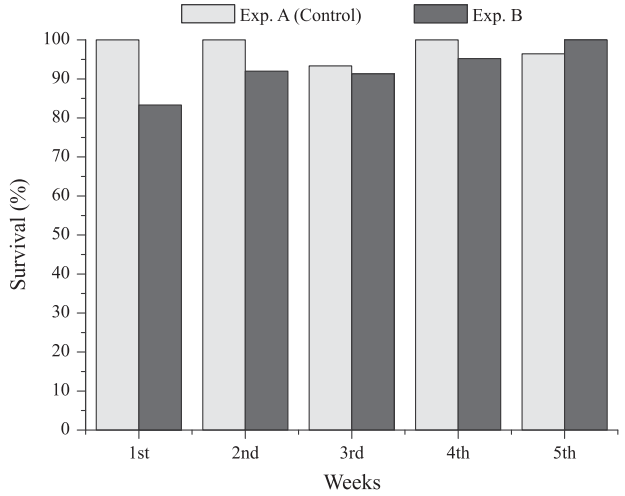

Figure 2. Survival (%) of *Biomphalaria pfeifferi* during acclimatization.

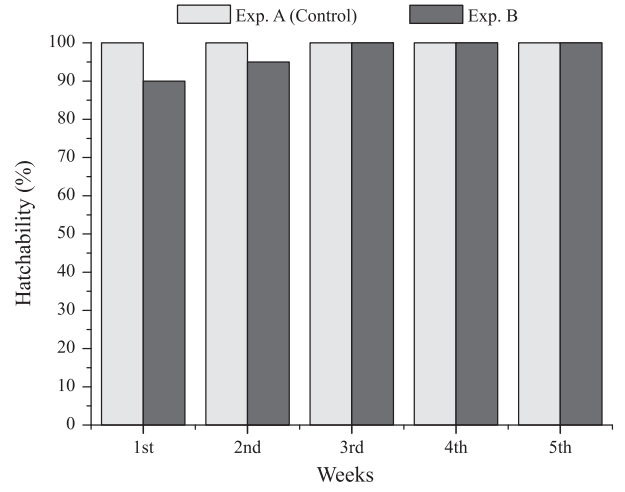

Figure 3. Hatchability (%) of *Biomphalaria pfeifferi* during acclimatization.

fertility of *B. pfeifferi* snails during their acclimatization in the laboratory immediately after fetching them from the wild.

The mean number of eggs per egg-mass recorded in Experiment A was not significant through the five weeks of monitoring, while in Experiment B there was a significant difference found. The survival and reproductive potential parameters obtained in Experiment B were lower compared to the values obtained in the control. This may be due to the physiological changes brought about by the acclimatization process in the laboratory, since these snails were obtained from the wild. The mean values observed in Experiment B although low, were either higher or comparable to those reported by other authors. Rozemberg, Rey, and Pieri (1992) recorded lower and higher values for *B. glabrata*, and *B. straminea* while studying the fecundity of these snails. *Biomphalaria* spp. snail eggs/egg-mass averages have been known to show great variations during culture in the laboratory.

There was no significant change in the number of eggs produced by snails in the control group throughout the course of the experiment. However, in Experiment B, there was a significant difference recorded in the number of eggs produced. In addition, the values in this study are much higher than those reported by Michelson (1961), Scherrer, Chquiloff, and Freitas (1976) and Rozemberg, Rey, and Pieri (1992). However, our results agree with the findings obtained by Scherrer, Chquiloff, and Freitas (1976) and Rozemberg, Rey, and Pieri (1992). The change of mean number of egg mass per snail per week was not significant in control experiments but was significant in Experiment B. Mortality was observed in both experiments, but it was higher in Experiment B when compared to the control. This may be due to a complete change in the physicochemical nature of the culturing water in the laboratory. Fertility of the eggs laid was very high in both experiments. The little drop in fertility observed in Experiment B during the first two weeks may also be due to the effect of the physicochemical condition on the biology of snails (Sturrock and Sturrock 1972; James, Karen, and Bernard 2011).

The fecundity, fertility, survival and death rates are important indicators of the physiological state of the snail host (James, Karen, and Bernard 2011), and the physiological state of snails is predetermined by the physiochemical nature of rearing conditions. The obtained pH, conductivity, total dissolved solid, dissolved oxygen, water and room temperature are within the optimum range for the rearing of *B. pfeifferi*. However, room temperature has its biggest effect on the rate of egg production, the time required for the development of embryo and the hatchability of eggs. It seems that all other factors being similar, temperature plays a significant role in regulating the physiology of snails (Sturrock and Sturrock 1972).

Conclusion

The results of the present study clearly indicate that the acclimatization process in the laboratory affects the survival and reproductive potential of *B. pfeifferi* fetched from the wild. The survival and fecundity parameters, which were initially low, increased gradually and became very high at the end of the fifth week. The results of this study will be very useful to researchers in the field of malacology who culture *B. pfeifferi* and other pulmonate moluscs in the laboratory. The biological processes or mechanisms involved in the acclimatization of pulmonates require further investigation. Also, there is a need for further comparative studies on acclimatization among pulmonates in order to identify the best parameters for laboratory culture.

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