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Correlational modeling of Roman snail (*Helix pomatia.* L.) oviposition behavior

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Abstract

Roman snail reproductive behavior was monitored in the natural environment from mating till oviposition. The correlational modeling among snail size, time between these two moments, number of dug nests, and clutch size revealed strong relationships only between the former and the latter features. However, we suggested that this relationship biologic significance must be assessed in connection with other factors such as local climate or substratum physico-chemical properties than limited to these two factors. We found that Roman snails can sometimes migrate on larger distances to lay their eggs than was previously reported. These findings are discussed using the data presented in the malacogical literature as guiding marks.

Keywords: snail, egg laying, behaviour, embryo, ontogenetic development

1. Introduction

Like most terrestrial gastropods, Roman snails (*Helix pomatia*) are hermaphrodite species that have both male and female organs in one common genital apparatus [1]. As a result, their chances to mate increase considering that usually their average distance of migration reaches 3.5–6.0 m [2] whereas they may copulate with any other potential mating partner they encounter [3]. Although this species reproductive biology and behavior are very well known [4-6] **there are still several issues far from being understood. Thus, when** seeking a suitable place for egg-laying the **snails were often observed leaving** unfinished nests and starting to dig new ones [7]. On the other hand, discrepant data are found in the malacological literature about relationships between snail`s size, time from mating till oviposition, and clutch size with most studies pointing to a complex phenotypic and genotypic background that rules them [8,9].

As a result, we investigated *Helix pomatia* reproductive behavior between mating and oviposition from a novel perspective, namely the assessment of these relationships from mathematical point of view using simple correlations as guiding marks to quantify their biologic significance.

In spite of numerous attempts to domesticate it, Roman snail (*Helix pomatia*) proved unsuited for a rentable rearing under controlled environment in specialized farms [10]. The increasing demand from the international market for the snail meat was met through their harvesting from the wild and finally conducted to a significant decrease of natural populations in the last decades [11]; therefore, *Helix pomatia* was put on the Red list of endangered animals (IUCN Invertebrate Red Data Book, 1983, 632 pp., International Union for Conservation of Nature and Natural Resources) and its gathering from the wild was prohibited or controlled through severe legislation in force in many countries from the Western Europe [12].

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As a result, the afferent pressure dropped on the wild populations from the Eastern Europe`s countries that actually represent the main major suppliers of Roman snail meat [13,14]. Nevertheless, most studies monitored Roman snail reproductive behavior just to understand its physiological background and genetic determination [10,15-17] and only a few of them approached this issue aiming to quantify their importance for human nutrition [18,19]. Contextually, this study approached snail oviposition behavior aiming to found novel information usable as the base for establishing of well-organized monitoring systems that would allow a more efficient and sustainable exploitation of wild populations from Romania.

2. Material and Method

2.1. Snails gathering. Twenty snails were randomly gathered from a garden placed in Timişoara (Timiş county; Latitude: 45.675°N; Longitude: 21.215°E). Next, they were taxonomically classified according to the shell shape, appearance, and color as *Helix pomatia* [20] whereas the presence of hardened and turned-out aperture lip marked the sexual maturity [18]. Then, the snail size was assessed in connection with its conchological features. Since *Helix pomatia* has a globular shell, almost equal in width and depth [20] only the shell width was taken into account in order to assess the snail size. This feature was established using a vernier caliper as the largest diameter measured from the left to the right part of the shell [21]. After that, each snail shell was counted with black acrylic paint in order to allow its facile identification. Finally, they were released into their native habitat aiming to observe their behavior since mating till oviposition. The following parameters were closely monitored during this period the time from mating till oviposition, the clutch size, and the number of dug nests. As in favorable areas the nest density was high, they were individually marked using counted wooden plates.

2.2.Statiscal analysis. Parameters analysis used statistical tests available on line for free at web site: http://faculty.vassar.edu/lowry/VassarStats.html.

First, data were assessed from descriptive point of view as mean values including standard errors (X ± SE). Next, a simple correlation analysis (Pearson correlation) was conducted to quantify the relationships between the snails` size, the time from mating till oviposition, the clutch size, and the number of dug nests. Only values of correlation coefficients (*r*) higher than ± 0.50 demonstrated strong relationships whereas the lower ones pointed to the lack of significant correlation between them.

3.Results and discussion

The mean shell width (33.44±1.51 mm) fitted to the lower values of the interval considered standard for this species: 34-51 mm [20]. In areas with wet, light-textured, and rich soil a high number of nests per unit area was observed, sometimes exceeding 15 nests/sqm. Interestingly, the Roman snail dug on average 1.85±0.34 nests, but not all of them were finished. Similarly, in laboratory conditions *Theba pisana* specimens were observed digging four to six “sham” holes before finally laying their eggs [22]. This behavior, also reported to *Helix aspersa* [23,24], is rather unusual since nest-digging behaviour normally precedes oviposition [22]. Contextually, snails seeking a suitable place for egg-laying were observed first feeling the ground through lips and inferior tentacles and only then starting to dig a nest. Since these organs are intimately involved in snail olfaction and taste [25,26] we inferred that these mollusks might be able to detect the most suited places for oviposition by tasting the soil. As a result, they stop digging the nest when the substratum physicochemical properties are not optimal for clutch incubation.

The clutch size also ranged between 21-58 eggs, with an average of 39.35±3.52 eggs/clutch. These values fitted to those in Romanian literature for the Roman snail (*Helix pomatia*): 20 - 60 eggs/clutch [20]. Descriptive statistical analysis also revealed a high variability of clutch size (28.27%). In addition, simple correlation analysis revealed a strong positive correlation (r = 0.74) between the shell great diameter and the clutch size.

Since shell width is usually considered as a direct indicator of snail size [27] our results pointed to a direct relationship between clutch and snail size. Nonetheless, divergent information are reported in malacological literature in respect to this issue. Thus, recent studies revealed to *Helix pomatia* no correlations between the parent size and the number of eggs [28]. Similar relationships were also recorded to *Trochoidea seetzeni* [29]. Oppositely, strong correlations between shell and clutch size were found to many terrestrial snails such as both subspecies of *Helix aspersa* [30,31], *Cernuella virgata* [32], *Ovachlamys fulgens* [33], *Oreohelix cooperi* [34], *Achatina fulica* [35], or *Theba pisana* [32].

On the same lines, *Arianta arbustorum* displayed within the same populations an allometric scaling between clutch and shell size pointing to a size-specific fecundity [36]. At the first glance, larger snails are able to mobilize higher amounts of nutrients and energy in reproduction and as a consequence, they are expected to lay more eggs than the smaller ones. There is direct relationship between clutch size and hatchlings survival rate since large clutches are less prone to dehydration [37]. On the other hand, predation risks are often associated to laying of more smaller clutches at multiple sites [38]. Furthermore, although soil calcium content is a key element in land snail reproduction [39] several other factors as altitude, food availability, or temperature may also affect clutch size [40-42]. Contextually, interpopulational differences within the same species in relationships between shell and clutch size require an integrate asssessment of the effects of shell size, local climate, and substratum type.

The average interval from mating till oviposition was of 34.70±3.67 days. Albeit all the snails had already reached reproductive adulthood this moment was very difficult to predict therefore both cases when eggs were laid 15 days and 7 weeks after mating were observed. To understand this behavior it is important to know that *Helix pomatia* individuals are hermaphrodite with mutual fertilization at copulation [1] that can have many partners and can store sperm for several months before fertilizing the oocytes [43]. In addition, multiple matings before oviposition were recorded to several species of terrestrial land snails [17, 44]. Furthermore, the influence of nutrition [45], climatic factors [46], and individual reproductive status [11] must also not be neglected. As a result, further studies are required to account for the physiological background of these findings.

Our study showed no correlation between the shell size and either the number of dug nests (r = 0.23) or the interval mating – oviposition (r = - 0.22). In addition, strong relationships were not found between the clutch size and neither the number of dug nests (r = 0.30) nor the interval mating – oviposition (r = 0.30). Furthermore, three cases were noted when marked snails migrated long distances (≈60 m) in order to lay their eggs. Previous studies reported travel distances of up to 15 m in the search for adequate soil conditions considering them as fixed cost of egg laying [1].

In our opinion, two possible phenomena might explain this behavior: the natural competition for seizing the most suited place for egg-laying, on one hand, and the instinct for colonizing new areas, on the other hand. In respect to the latter issue the seasonal and lifetime spatial movements of this species were found to be associated especially to feeding areas whereas no evidence of age-dependent dispersal was found [47]. Nonetheless, other factors might influence this behavior. Thus, a reverse relationship was found between the number of eggs cannibalized and distance to the batch of hatching snails pointing to egg cannibalism as a population regulating factor [36].

Overall, our findings reinforce the assumption that land snail reproduction is regulated by multiple divergent factors whereas the intriguing relationships that characterize their behavior during reproductive season are still far from being understood. As a result, further studies are required to enlighten the ethological and physiological background of these processes and their potential biologic and ecologic significance. The value of such information is high since they may be used to control efficiently the gathering Roman snails for the wild, to assess the moment when a natural population must be totally protected, and to predict its evolution on long-term.

4.Conclusions

The following conclusions derive from the mathematical moddeling of wild Roman snail (*Helix pomatia*) reproductive behavior: (1) number of eggs tends to increase proportionally with snail size; (2) no significant correlations are found between the shell size and either the number of dug nests or the interval mating – oviposition; (3) clutch size is not significantly influenced neither by the number of dug nests nor by the interval mating – oviposition; (4) competition for seizing the most suited place for egg laying and the instinct of colonizing new habitats constitute potential factors that induce snail migration after mating.

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