VERTICAL SPATIAL BEHAVIOUR PATTERNS OF *LYMNAEA TRUNCATULA* IN RELATION WITH ORIGIN OF SNAILS, INFECTION WITH *FASCIOILA HEPATICA*, AND EXPERIMENTAL ENVIRONMENT

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**Summary**

Ethological studies were carried out on three Moroccan populations of *Lymnaea truncatula* of which two of them were living in a flooding irrigation system (Tassila, Oued Massa valley) and the third in a nonirrigated habitat. Most of the Tassila snails were found in the deep water zone. Two factors significantly influenced the presence of snails in this zone: the water current velocity, and parasitic infection of the molluscs. In the presence of other factors (lack of food, the presence of the snail *Physa acuta*), the *L. truncatula* were recovered more frequently in zones outside of the water and just beneath the water's surface. Despite the modifications noted in snail distribution, *Bulinus truncatus* did not appear to be a competitor. Lastly, habitat drying resulted in partial or complete burrowing of 50% of snails.

**RÉSUMÉ : Comportement spatial de *Lymnaea truncatula* selon l'origine des mollusques, leur infection avec *Fasciola hepatica* et un environnement expérimental.**

Des études éthologiques ont été réalisées sur des limnées tronquées de trois colonies marocaines vivant dans un réseau d'irrigation submergé (Tassila, vallée de l'Oued Massa) pour les deux premières et dans un habitat non irrigué pour la troisième. La plupart des limnées de Tassila se rencontrent dans la zone immergée profonde. Deux facteurs influent de manière significative sur la présence des limnées dans cette zone (vitesse du courant, parasitisme des mollusques). En présence d'autres facteurs (absence de nourriture, présence de physes), les *L. truncatula* fréquentent plus les zones émergées et la strate superficielle du milieu immergé. Malgré les modifications constatées dans la répartition des limnées, les bulins ne semblent pas être des compétiteurs. Enfin, la sécheresse de l'habitat se traduit par l'enfouissement partiel ou total de 50% des limnées.

**INTRODUCTION**

It has been known for a long time that *Lymnaea truncatula* is an amphibious species (Thomas, 1883; Mehl, 1932). During the winter months, these snails remain under water. They progressively emerge with the approaching summer and permanently interact in zones outside of the water from June until the summer dry period when they become attached to some supporting structure. With the return of the post-summer rainy season, the snails go progressively underwater in October-November (Rondelaud, 1974a).

We desired to know whether *L. truncatula*’s amphibious behaviour patterns were the same in an irrigation system.

Initial observations carried out in the irrigation canals of Tassila, Oued Massa valley in the province of Agadir (Morocco) demonstrated that the snails remained at the water's bottom when there was running water, and even in the middle of summer when the water became stagnant, most of the snails remained underwater (Moukrim, 1991). We undertook this experimental study to determine which factors were responsible for this snail’s more aquatic behaviour in the Tassila irrigation system.

**MATERIALS AND METHODS**

*Snails*

These observations were performed on three colonies of *L. truncatula*. Two populations originated from the Tassila irrigation system located in the Oued Massa valley (Morocco). The first colony was living in a secondary canal next to a « K »-shaped outlet from the main canal (colony A) and was constituted of uninfected snails at the time of the experiment. The second was living in a traditional ditch (« seguia ») near the road between Tassila and Ifenhtar (colony B); snails collected in February and March were uninfected but those collected in May harboured parthenitae of *Fas*.
ciola hepatica (with rediae and independent cercariae visible underneath the snail’s transparent shell). The L. truncatula of these two colonies shared their habitats with two other freshwater species, Bulinus truncatus and Physa acuta. The third colony (Tamrhaikt) was living in an isolated conduit located along route RP 7002, near Tamsergoult (Morocco). This cemented conduit was fed by the Oued Tamrhaikt river. This last population was only constituted of uninfected L. truncatula which were often observed out of the water.

Most snails used in these experiments were collected in February-March and corresponded to the overwintering generation of the species. The snails infected by F. hepatica were collected in May, and belonged to the second yearly generation. At the time of collection, the shell height was 5 to 8 mm. They were transported to the laboratory under isothermal conditions and placed in standard breeding containers for at least 48 hours before being subjected to the experimentation.

Experimental protocol

The standard experiment (experiment 1) consisted of placing 20 uninfected L. truncatula from colony A in 0.66 m³ aquariums (10 per aquarium), with sediment and a 1 cm layer of stagnant water which originated from Oued Massa river. A similar experiment was performed with 20 uninfected L. truncatula and water originating from colony B. Each aquarium was divided into four zones according to those defined by Rondelaud and Vincent (1974): zone Z1 (located at least 1 cm above the water’s surface), zone Z2 (1 cm above and below the water’s surface), zone Z3 (a deep water zone, beginning 8 cm below the water’s surface), and zone Zt (a transition zone located 1 to 8 cm below the water’s surface).

Preliminary observations have shown that the first snail deaths were observed beginning at day 10 when the L. truncatula originating from the irrigation system were maintained in aquariums in the presence of other freshwater mollusks like P. acuta. Snail counts were thus performed every six hours (at 1 a.m., 7 a.m., 1 p.m. and 7 p.m.) for the first 9 days to determine snail aggregation in each zone. Laboratory mean temperature was 22° C (extremes of 14° and 26° C); the natural photoperiod was used with the aquariums protected from direct sunlight. The snails were fed lettuce ad libitum.

In addition to the standard experiment (experiment 1), several experiments were performed to investigate the influence of nine factors. The first concerned the snail’s geographic origin; this study (experiment 2) was made with the L. truncatula from the third colony originating from Tamrhaikt. The other factors concerned colonies A and B of Tassila: the presence of running water, with a volume of 5 l/min and a velocity of 5 cm/sec for 9 days (experiment 3), the depth of the water layer, i.e. 2 cm (4), the absence of superficial sediment (5), the absence of food (6), the presence of B. truncatus (10 per aquarium, in addition to the lymnaeid snails) (7), the presence of P. acuta according to the same protocol (8), parasitic infection of the snails by F. hepatica (9), and drying of the superficial sediment (10).

Experiment (2) was carried out with 40 uninfected snails from Tamrhaikt, and experiment (9), with 40 infected snails collected in May in colony B of Tassila. Experiments (1), (3), (4), (5), (6), (7), (8) and (10) were performed only in February-March on the uninfected L. truncatula from Tassila using 20 snails per colony in each experiment.

Excluding the infected L. truncatula of experiment (9), all the other snails were dissected on the 10th day of the experiment to search for any trematode or nematode infection.

Processing of data

The data obtained during this study corresponded to the numerical counts obtained from day 2 to day 9. Counts from the first day were not considered because of their wide variation.

Results

Excluding the infected snails from experiment (9), snail examination at day 10 in experiments (1) to (8) was negative. No parasites were found in these L. truncatula. Surviving snails in experiment (10) were also uninfected.

Behaviour patterns of L. truncatula from Tassila

Snail distribution of colony A is given in Figure 1 in relation to the four zones and observation hours. Low percentages of L. truncatula were found in zones Z1 and Zt, regardless of the time, and their differences were insignificant. However, snail distribution in zones Z2 and Z3 dif-
ferred significantly depending on the hour the count was performed. The percentages calculated in zone Z3 at 1 a.m. and 1 p.m. decreased in relation to those at 7 a.m. and 7 p.m. The inverse was found in zone Z2. Daily displacement, then, occurred between the water’s surface and the bottom of the habitats.

Figure 2a gives the distribution of these L. truncatula at 7 a.m. in relation to the observation dates, from D2 to D9. The snails were encountered sporadically in zone Z1 (0-15 %) and were principally found in the three other zones. In zones Z2 and Z3, varying numbers were observed until day 6; subsequently, however, the percentages became more constant with mean values ranging between 40 % and 45 % in zone Z2, and between 35 % and 45 % in zone Z3. Zone Z1 was poorly colonized by 5-25 % of snails. The distribution of snails from colony B in the four zones was the same, as demonstrated in Figure 2b. Comparison of the distributions was insignificant.

**Behaviour patterns of L. truncatula from Tamrhakht**

When the experiment was repeated with snails from Tamrhakht (Fig. 3a), opposite results were obtained from those of the experiment (1). Thirty to 35 % of the snails were found in zone Z1 beginning from day 6, and 40-65 % in zone Z2. The deep zone was colonized by 5-10 % of the snails at the end of the experiment. There were significant differences (P = 0.01) in distribution in the three zones, as compared with the experiment (1).

**Effect of some factors on behaviour patterns of L. truncatula from Tassila**

When there was running water (Fig. 3b), almost all of the snails were found in zone Z3 beginning on day 4 of the experiment. Comparison of the percentages with those of the experiment (1) demonstrated that the differences were all significant (P = 0.01).

When the water layer was 2 cm in depth (Fig. 3c), the obtained percentages were found to be higher than those of experiment (1). Sixty to 65 % of the snails were found in zone Z3 (Z3 in this case represented the 1 cm layer of water below Z2) beginning at day 6 of the experiment, and zone Z2 by 30-35 % of the snails. Colonization of zone Z1 was very sporadic, as with the standard experiment. Comparison of the distributions demonstrated a significant difference in zone Z3 (P = 0.01) and was insignificant in the other two zones.
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tion of Tassila snails at the bottom of the habitat. This finding has also been observed in 2-meter-wide earth ditches (« seguias ») and ordinary ditches when running water was present (Moukrim, 1991). We believe the presence of running water and its periodicity (in general, every 46 days in the Tassila system) are the two factors responsible for snail distribution at the bottom of the habitat. Additional studies in the field and under laboratory conditions are still necessary to determine the effects of periodicity on this distribution, since environmental factors play a major

DISCUSSION

A review of the literature demonstrates the lack of information concerning the spatial behaviour patterns of L. truncatula in habitats with running water. Most of our knowledge comes from observations carried out on populations living in marshland (Mehl, 1932; Bednarz, 1960; Taylor 1964; Moens, 1974; Rondelaud, 1974a, b). These studies have demonstrated this snail’s highly amphibious nature, since it often lives temporarily underwater when conditions are favorable. The distribution of Tamrhakht snails showed that this colony’s behaviour was identical to that reported by the authors with marshland populations.

However, the results obtained on the Tassila snails showed that these populations’ amphibious behaviour was rather limited. Ecologically and ethologically, these colonies have to be distinguished from populations which live permanently on banks or in temporary biotopes such as those found in marshland. Movement of the snails (from Tassila) on the underwater sediment and migration towards the water’s surface throughout the day concord with the daily activity cycle described by Rondelaud and Vincent (1974) in snails living in marshland. The snail’s nocturnal rest period(s) occurs underwater. Movement towards the surface is related to the requirement of regularly supplying oxygen to the lungs (Russel-Hunter, 1953; Hunter, 1957); displacement of these snails to obtain air, however, probably occurs less frequently than with snails living outside of the water, since the former’s cutaneous respiration plays a greater role (Lambert, 1990).

According to our results, the presence of running water is one of the factors which influences the distribution of Tassila snails at the bottom of the habitat. This finding has also been observed in 2-meter-wide earth ditches (« seguias ») and ordinary ditches when running water was present (Moukrim, 1991). We believe the presence of running water and its periodicity (in general, every 46 days in the Tassila system) are the two factors responsible for snail distribution at the bottom of the habitat. Additional studies in the field and under laboratory conditions are still necessary to determine the effects of periodicity on this distribution, since environmental factors play a major

In the absence of sediment (results not shown), there was greater colonization of zone Z1 (5-15 % of the total number beginning from day 6). On the other hand, the results from the other zones were similar to those of the experiment (1), the differences being statistically insigniﬁcant.

The absence of food (Fig. 3d) lead to greater colonization of zone Z1 (20-30 % of the snails beginning at day 6), and decreased numbers in zone Z3 (10-35 %). The remaining snails were distributed between zone Z2 (25-55 %) and zone Zt (10-35 %). The differences were insignificant except for zone Z1 (P = 0.01).

The introduction of B. truncatus into the aquariums (Fig. 3e) did not modify the distribution of lymnaeid snails from Tassila. The deep zone was colonized by 75 % to 95 % of the snails beginning at day 6, and zone Z2 by 0 to 20 %. No snails were found in zone Z1. When compared to the experiment (1), significant differences were found in zones Z2 and Z3 (P = 0.01).

If P. acuta was introduced into the environment, it principally sought out zones Zt and Z3. The repercussion on L. truncatula distribution is demonstrated in Figure 3f. Thirty to 40 % of the total number were found in zone Z1 beginning at day 6, and 15-25 % in zone Z2. Only 15-30 % of the snails were encountered in zone Z3 during the same period. When compared to the experiment (1), there were significant differences in snail distribution in the three zones when considered separately (P = 0.01).

Infection of the snails by F. hepatica (Fig. 3g) resulted

in the almost exclusive colonization of zone Z3, and some­
times zone Zt. Comparison of the percentages with those of experiment (1) demonstrated that the differences were significant (P = 0.01).

Lastly, habitat drying (Fig. 4) lead to complete burrowing of 30 % of the snails beginning at day 2. The other snails became permanently fixed onto the substrate without bur­
rowing (50 %), or were incompletely buried (20 %), leav­
ing the tip of the shell visible. However, all buried snails were living at the end of experiment whereas most fixed snails were dead.

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role in the installation and continued presence of snails in Belgian marshland (Moens, 1981).

The results concerning water level, and the absence of sediment or food indicate that these are minor factors which affect snail distribution.

It is more difficult to interpret zone frequentation in the presence of other molluscs. In the presence of B. truncatus, snail colonization of the deep water zone was greater than in the experiment (1). B. truncatus did not act as a true competitor and it can even be assumed that the presence of this mollusc favored colonization of the deep zone by the snails which were probably searching for food. In the presence of P. acuta, the results were reversed, since the snail was encountered more frequently in zones outside of the water and in the superficial water stratum. In this case, there was true competition for food sources, however, it can not be excluded that the newborn snails were eaten and that survivors fled towards safer zones, since P. acuta is capable of consuming newborn snails in the field and under experimental conditions (Rondelaud, 1978).

Snails infected by F. hepatica are more aquatic than healthy snails (Rondelaud and Vincent, 1974). This aptitude is greatest at the end of the prepatent period, just before the first shedding of cercaria. During our experiments, the results confirmed the work done by these authors, excluding the fact that all infected snails were found in the deep water zone. This minor discordant finding must be related to the geographical origin of the snails in question, since the Tassila molluscs were already more aquatic than their homologous counterparts living in temporary habitats. Snail distribution in experiments (1) to (8) was variable during the first days and became more stable after the 5th or 6th day. These changes in zone frequentation were observed with the three colonies tested and may be explained by the fact that these snails were discovering a new habitat to which they were not accustomed.

Snail burrowing in sediment during environmental drying was, however, a new finding which has never been reported before by previous authors. During the summer in the temporary habitats, the snail became anchored to protected areas, especially the upper portion of retraction cracks, but no burrowing was ever found (Patzer, 1927; Kendall, 1949; Roberts, 1950; Rondelaud and Morel-Vareille, 1975). This represents, then, a particular characteristic of snails which colonize flooding irrigation canals. The existence of this process in the field (Moukrim, 1991) lends credence to this hypothesis. To verify the latter, further studies must be undertaken to determine which factors are responsible for this burrowing behaviour and the survival rate of snails buried in the superficial sediment.

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