Ann. Parasitol. Hum. Comp., 1992, *67* : n° 6, 174-179.

Mémoire.

Key-words: Ecology. Ethology. *Fasciola hepatica. Lymnaea truncatula.* Morocco.

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VERTICAL SPATIAL BEHAVIOUR PATTERNS OF *LYMNAEA TRUNCATULA* IN RELATION WITH ORIGIN OF SNAILS, INFECTION WITH *FASCIOLA 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, 1974*a*).

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 responsable 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-*

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Accepté le : 16 avril 1992.

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 (Tamrhakht) was living in an isolated conduit located along route RP 7002, near Tamsergout (Morocco). This cemented conduit was fed by the Oued Tamrhakht 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 m² aquariums (10 per aquarium), with sediment and a 10 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 molluscs 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 Tamrhakht. 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 Tamrhakht, 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.

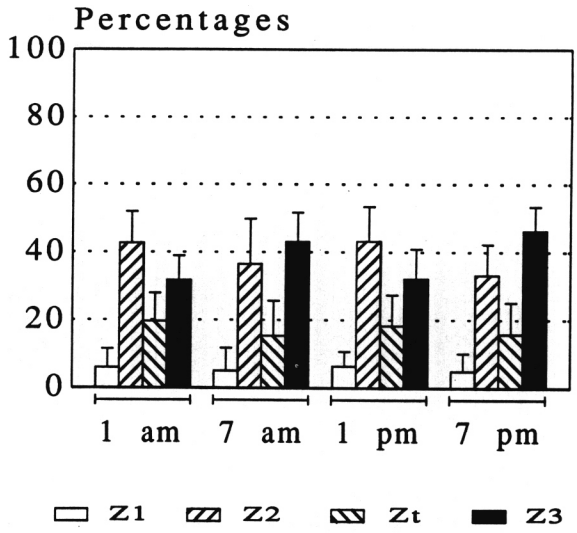


FIG. 1. — Distribution of *L. truncatula* in zones Z1, Z2, Zt and Z3 at 1 a.m., 7 a.m., 1 p.m. and 7 p.m., the eight days being grouped together. Colony A of Tassila, experiment (1). Captions. Z1, zone located at least 1 cm above the water's surface; Z2, zone located 1 cm above and below the water's surface; Zt, zone located 1 to 8 cm below the water's surface; Z3, zone beginning 8 cm below the water's surface.

The numbers of snails counted in each zone were averaged, and standard deviation established for each colony (2 aquariums) and each observation hour in experiment (1). Snail distribution during experiment (1) is given in *Figure 1* for colony A in relation to the observation hours, and in *Figure 2* for the Tassila colonies in relation to the observation dates (at 7 a.m.).

No significant difference was noted in snail distribution when the colonies A-B of Tassila were tested together in an experiment. Results obtained with these two populations were grouped together before being averaged and the standard deviation established. *Figures 3* gives, for example, the results obtained at 7 a.m. in the four zones during the eight days of observation. *Figure 4* shows the results of experimental drying of the snail habitat.

Mean values obtained from colony A in experiment (1) were compared by analysis of variance with those of colony B. The other values obtained in experiments (2) to (9) were also compared by the same statistical test with the grouped results of the experiment (1). These comparisons concerned only values obtained in zones Z1, Z2 and Z3.

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-

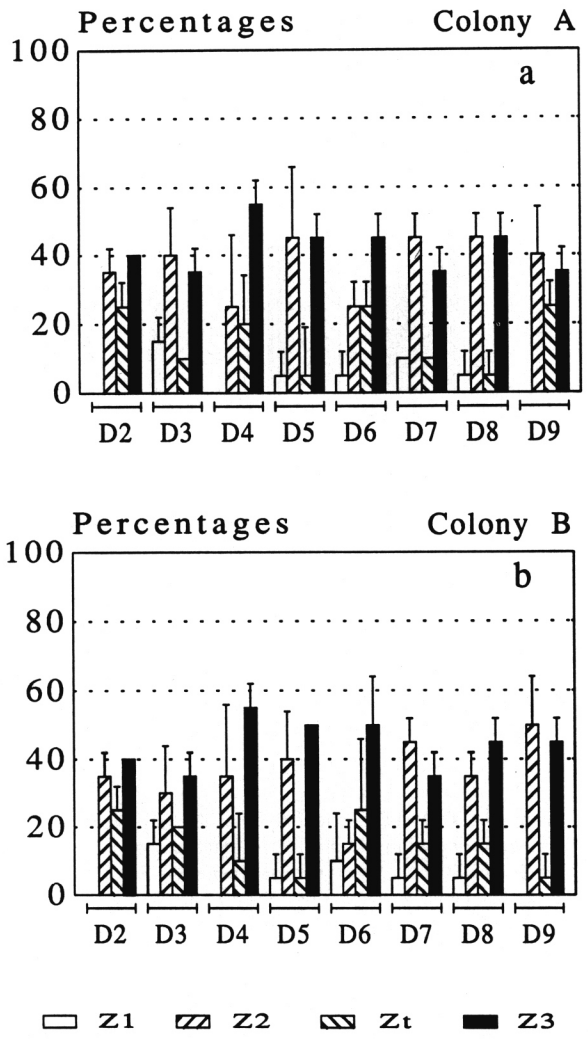


FIG. 2. — Distribution of *L. truncatula* at 7 a.m., in zones Z1, Z2, Zt and Z3 during the eight days of experiment (1). Colony A from Tassila (*2a*) and colony B (*2b*). Captions. D2 to D9, days after the beginning of experiment; Z1, zone located at least 1 cm above the water's surface; Z2, zone located 1 cm above and below the water's surface; Zt, zone located 1 to 8 cm below the water's surface; Z3, zone beginning 8 cm below the water's surface.

fered 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 the 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 2*a 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 Zt 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 2*b. Comparison of the distributions was insignificant.

Behaviour patterns of L. truncatula *from Tamrhakht*

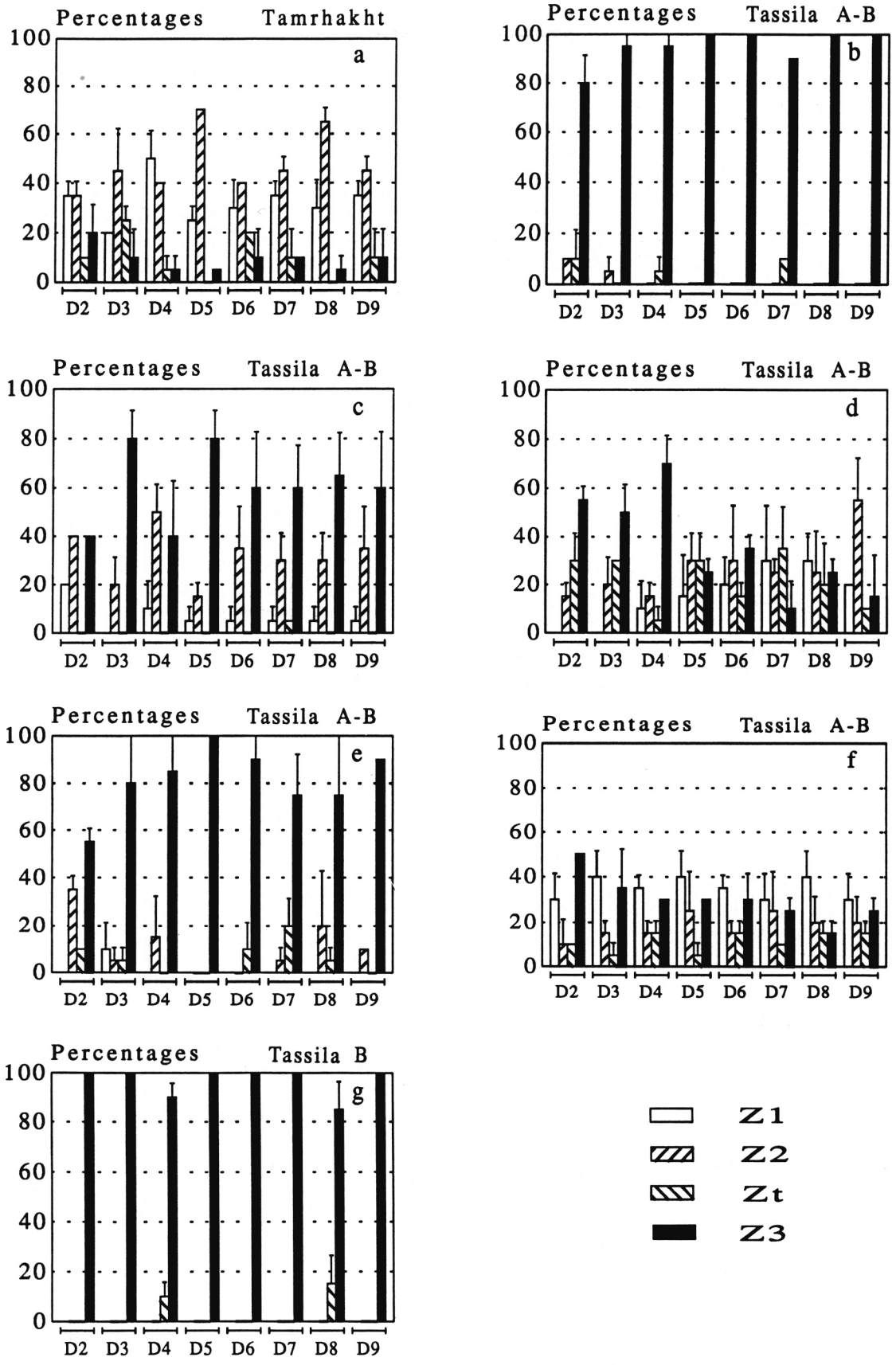
When the experiment was repeated with snails from Tamrhakht (*Fig. 3*a), opposite results were obtained from those of the experiment (1). Thirty to 35 % of the snails were found in zone Z1 beginning from day 6, and 40-65 % in zone Z2. The deep zone was colonized by 5-10 % of the snails at the end of 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. 3*b), 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. 3*c), 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 two other zones.

FIG. 3. — Distribution of *L. truncatula* at 7 a.m., in zones Z1, Z2, Zt and Z3 during the eight days of the experiment. Graph 3*a* corresponds to distribution of *L. truncatula* from Tamrhakht (to study snail's geographic origin). The graphs 3*b* to 3*f* correspond to distribution of *L. truncatula* (colonies A and B) observed after the following modifications in the environment in Tassila snails: *i*) running water (3*b*); *ii*) 2 cm thick layer of water (3*c*); *iii*) absence of food (3*d*); *iv*) presence of *Bulinus truncatus,* in addition to the *L. truncatula* of Tassila (3*e*); and *v*) presence of *Physa acuta,* with the *L. truncatula* of Tassila (3*f*). Graph 3*g* corresponds to distribution of *L. truncatula* from Tassila (colony B) after infection with *F. hepatica.* Captions. D2 to D9, days after the beginning of the experiment; Z1, zone located at least 1 cm above the water's surface; Z2, zone located 1 cm above and below the water's surface; Zt, zone located 1 to 8 cm below the water's surface; Z3, zone beginning 8 cm below the water's surface.


Percentages
Tamrhakht
a
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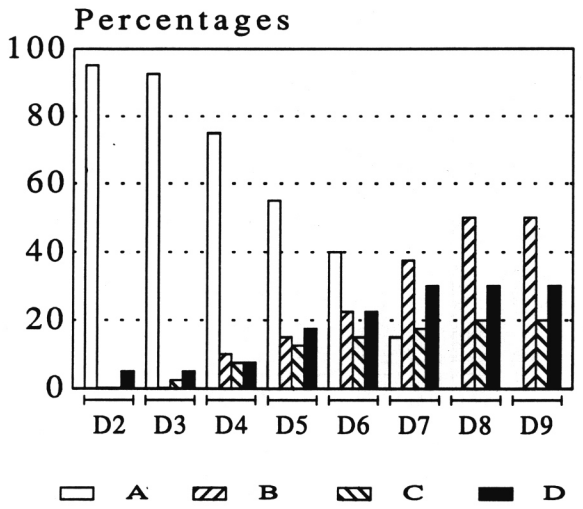

FIG. 4. — Behaviour of *L. truncatula* from Tassila during the 8 days of habitat drying (cumulative percentages). Captions. D2 to D9, days after the beginning of the experiment; A, *L. truncatula* with normal displacements; B, permanent fixation of the snail onto the substrate without burrowing; C, complete burrowing of *L. truncatula*; D, incomplete burrowing of snails, with the tip of the shell visible.

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. 3*d) 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. 3*e) 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 3*f. 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. 3*g) resulted in the almost exclusive colonization of zone Z3, and sometimes 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 burrowing (50 %), or were incompletely buried (20 %), leaving the tip of the shell visible. However, all buried snails were living at the end of experiment whereas most fixed snails were dead.

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, 1974*a, 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 ot 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 responsable 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

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 cercariae. 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 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 responsable for this burrowing behaviour and the survival rate of snails buried in the superficial sediment.

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