**Research Article**

**Galba truncatula** (O.F. Müller, 1774) (**Gastropoda**, **Lymnaeidae**): the colonization of new stations on acid soil by low numbers of snails

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**Abstract** – Samples of 5, 10 or 20 adult *Galba truncatula* each were introduced into 25 slope rush beds and 24 road ditches on acid soil to study the colonization of these new sites by the snail. Surveys were conducted over the following eight years to determine the area where these *G. truncatula* colonized in these new stations and to count the number of overwintering snails in April or May. Snail populations were present in about 50% of the sites where samples of 5 snails each were introduced and in all sites when each sample included 10 or 20 snails. The average area of these habitats showed a peak before decreasing in the following years and this peak was all the more late as the sample introduced into the station increased in size. In contrast, snail abundance in all cases peaked in the second or third year after introduction and gradually decreased thereafter. In all cases, a concentration of snails was noted in the most upstream zone, whereas the number of individuals decreased gradually towards the downstream zone. These results demonstrate that a small number of snails (10 or 20 adults per sample) allow the development of a new population of *G. truncatula* in new acid soil stations.

**Keywords:** Abundance / colonization / *Galba truncatula* / habitat / Haute-Vienne

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**1 Introduction**

*Galba truncatula* (O.F. Müller, 1774), also known as *Lymnaea truncatula*, is the intermediate host of the parasite *Fasciola hepatica* Linnaeus, 1758. It ensures the larval development of this digenean and is therefore an indispensable link in the transmission of the disease due to this parasite. This lymnaeid is widespread worldwide and is considered a native species in Europe and in northern, western and southern Asia. It is also found in several African countries as well as in the north-west of North America (*Seddon et al.*, 2014; *Dreyfuss et al.*, 2015) and would have been introduced into several South American countries: Argentina, Bolivia, Chile, Peru, Venezuela (*Mas-Coma et al.*, 1999, 2005, 2009; *Bargues et al.*, 2012) or in New Zealand (*Winterbourn*, 2021). *Seddon et al.* (2016) also indicate that this species is tolerant to pollution and easily colonizes temporary habitats so that there would be no significant threat for its future.

In the department of Haute-Vienne (central France), most of the populations of *G. truncatula* identified over a period of 37 years (6701 out of 7407) were observed between 160 and 400 m above sea level, while the other 706 were found between 401 and 570 m (*Dreyfuss et al.*, 2018b). On the crystallophyllian and metamorphic soils of this area, these authors noted that the frequency of snail populations and the characteristics of their habitats showed a significant decrease when the altitude increased. However, two studies have shown that the populations of this species have been in decline since the 2000s. Compared to the number of populations counted between 1976 and 1992, this decline was 34% on the grasslands of 162 farms in Corrèze, Creuse and Haute-Vienne (*Dreyfuss et al.*, 2016). Similarly, on the sedimentary soils of three departments bordering the Haute-Vienne department, *Dreyfuss et al.* (2018a) reported a 30.8% fall on the pastures of 52 farms compared to the values recorded before 1998. According to these authors, this current decrease in the number of populations is mainly due to human activity on these farms.

Given the decline in the number of populations reported by *Dreyfuss et al.* (2016, 2018a), it was useful to understand how the species could colonize new habitats. This type of study could only be carried out by studying the results of *G. truncatula* transferred from their original habitat to new areas. Experimental introductions of snail samples, each comprising 100 adult *G. truncatula*, have already been carried out between 1976 and 1980 by our team in 36 open drainage furrows (meadows) and 15 road ditches on acid soils. Introduced snails formed new populations in 9 grasslands.

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and 13 road ditches with density peaks in the second or third year post-introduction (Vareille-Morel et al., 2002). These first results quickly raised a problem as the G. truncatula samples introduced by Vareille-Morel et al. (2002) each contained 100 adult snails. We therefore wondered whether the number of molluscs introduced into each drainage furrow or each road ditch was not too high in relation to the area of habitat usually colonized by G. truncatula on acid soil. Indeed, in Haute-Vienne, the area of such habitats was less than 2 m² in 56.8% of cases (Rondelaud et al., 2011).

Under these conditions, the question arose as to whether colonization of potential new habitats could be successfully performed by using a lower number of adult G. truncatula (5, 10 or 20 per sample). In response to this problem, an eight-year experiment was carried out by introducing snails in 25 slope rush beds and 24 road ditches in the north of Haute-Vienne. All of these new habitats had a temporary source from mid-October to the following June. The number of adult snails from the previous year (overwintering molluscs) and the area colonized by the population in these new habitats were monitored during eight years in April or May, depending on the altitude of these habitats.

2 Materials and methods

2.1 Stations studied

The 25 slope rush beds and the 24 ditches concerned by this experiment are located on the following municipalities: Berneuil, Blond, Breuilaufa, Châteauponsac, Mézières-sur-Issoire, Peyrat-de-Bellac, Rancon, Saint-Junien-les-Combes, Saint-Ouen-sur-Gartempe and Villefavard (Fig. 1). The altitude of these 49 stations ranged from 187 m to 376 m. No snails have been observed in these sites since the 1970s.
Table 1. Main characteristics of the 49 stations in which adult *Galba truncatula* were introduced. A temporary source is present at each station.

<table>
<thead>
<tr>
<th>Type</th>
<th>Number</th>
<th>Area of the water collection in March (m²)</th>
<th>Beginning of the experiment</th>
<th>Number of snails introduced per station in March</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope rush beds</td>
<td>11</td>
<td>1.5–2.7</td>
<td>2006, 2007 or 2008</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.6–2.4</td>
<td>2006 or 2007</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.2–2.2</td>
<td>2008 or 2009</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.3–8.4</td>
<td>2009 or 2010</td>
<td>5</td>
</tr>
<tr>
<td>Road ditches</td>
<td>7</td>
<td>6.2–10.5</td>
<td>2010 or 2011</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.3–9.2</td>
<td>2009 or 2010</td>
<td>20</td>
</tr>
</tbody>
</table>

Each rush bed was located in the mesophilous zone of a swampy meadow. No predatory snail: *Zonitoides nitidus* (O.F. Müller, 1774) was living in these beds so that the transition to June-July (Rondelaud et al., 2006) after their introduction were not exposed to the predation of *Galba truncatula*. These stations were small in size, as evidenced by the area of the water collection from 5.3 to 10.5 m² (Tab. 1). The clumps of rushes were more numerous there with a predominance of *Juncus effusus* L. (Chèvremont, 2008) distributed around the source and along the stream. The surroundings of the source were largely covered by algal flora and sometimes mosses, while the source itself was generally free of vegetation, except for the epiphytic algae. In the case of road ditches, the temporary source was at the upstream end (21 ditches) or at the lateral position (3) of the water collection. The snail *Z. nitidus* was absent. The area of these water collections was much larger with values ranging from 5.3 to 10.5 m² (Tab. 1). The clumps of rushes were more numerous there with a predominance of *Juncus effusus* L. 1753. A few clumps of sedges were also present. As in the slope rushes, there was also a strong development of the algal flora in spring.

In both habitat types, predators other than *Z. nitidus* were also present during the eight years of the study. Tadpoles of *Bufo bufo* (Linnaeus, 1758) and *Rana temporaria* Linnaeus, 1748, have been seen irregularly in seven and two road ditches, respectively. Juveniles of *Triturus helveticus* (Razoumovsky, 1748) were sometimes noted in low numbers in five ditches and three slope rush beds. No bird prints were observed at these 49 stations during their summer drying. Among the invertebrates, dytiscid larvae were often present in 17 slope rush beds and 11 road ditches. The presence of *Nepa cinerea* Linnaeus, 1758, has only been observed in a few ditches. No larvae of Diptera Scionymidiae were noted during the study. With the exception of dytiscid larvae which are permanently present in the stations where they live, the presence of other species has been very irregular in the sites and over time.

At these stations, the sediment consisted of sand and gravel in the case of rush beds, and a mixture of mud, sand and rare silt in the road ditches. The subsoil was granite in all cases (Chèvremont, 2008). Running water was generally present from the beginning of October until the end of May or the beginning of June with a maximum from January to the end of April. The pH of the water was between 5.8 and 6.8, while the dissolved calcium ion concentration was less than 20 mg/L (Guy et al., 1996). The continental climate is strongly attenuated by the wet winds coming from the Atlantic Ocean. Depending on the year, the average annual rainfall ranged from 800 to 1000 mm, while the average annual temperature varied from 10.5 °C to 11.5 °C according to the altitude of the stations (Rondelaud et al., 2011; Vignoles et al., 2017).

2.2 Experimental protocol

The two populations of *G. truncatula* at the origin of transplanted snails lived in two open drainage furrows located in the same marshy meadow on the commune of Berneuil, department of Haute-Vienne. A total of 515 snails, measuring 5 mm or more in height and belonging to the overwintering generation, were collected between 2006 and 2011 to be introduced at the end of March in any of the 49 new stations. This range of sizes was chosen for this experiment because the snails become adults from a height of 4.5 mm (Kendall, 1953) or 4.42 mm (Smith, 1981) and can therefore lay eggs.

As the density of these snails per m² of habitat did not exceed 40 individuals in March or April in 86.9% of cases on acidic soils of Haute-Vienne (Rondelaud et al., 2011), samples of 5, 10 or 20 adult *G. truncatula* from either of the two original populations were introduced to these new stations according to the protocol presented in Table 1. These snails were placed at one time at the end of March at the border between water and wet soil in the case of rush beds, while they were placed in the most upstream portion of the water collection present in each road ditch. The method of introduction used for ditches is due to the fact that *G. truncatula* habitats are often located at the periphery of a river system (Moens, 1991; Vareille-Morel et al., 1999). The choice of the end of March for snail introduction took into account the date (mid-April) when the first egg-masses are laid by the overwintering *G. truncatula* in Haute-Vienne (Granet, 1996; Vareille-Morel et al., 1998).

In stations where the introduction of *G. truncatula* resulted in the development of a population, habitat characteristics were monitored over an eight-year period after the introduction of snails. The area of each habitat was first calculated by mapping the site according to its geometric shape and determining this value by the appropriate mathematical formula. The over-
wintering snails were then counted by sight as they were then in the adult stage and were all over 6 mm high. No survey was conducted at these stations in 2015. These surveys were performed at the end of March, in April or in early May (depending on the altitude of the stations and, consequently, the climatic conditions) during the other years from 2007 to 2019.

The rush beds were surrounded by barbed wire to avoid trampling during the grazing of cattle or sheep and no vegetation mowing was carried out during the eight years of the experiment. In 2016 and 2017, five beds were destroyed by rotary crushing due to the change of ownership in two farms. In the road ditches, no fencing was installed and vegetation in most ditches was mown by rotary crushing each year or every two years in September. From 2016, several ditches were cleared, resulting in the disappearance of the snails.

### 2.3 Parameters studied

The first was the frequency of new habitats colonized by *G. truncatula*, while the other two were the area of each habitat and the density of overwintering snails per m² of habitat in April or May. As the introduction of *G. truncatula* into new stations took place over several years (see Tab. 1), the results of each parameter were expressed in relation to the serial number of the post-introduction year.

Individual values for the habitat area or the density of snails were reduced to an average, framed by a standard deviation, taking into account the type of station (rush beds or ditches), the sample size (5, 10 or 20 snails) and the serial number of the year after introduction. First, the normality of these values was first analyzed using the Shapiro-Wilk test (Shapiro and Wilk, 1965). As their distributions were not normal, the Kruskal-Wallis test was used in a second step to establish statistical significance levels. In a third step, we have also used the r pgirmess package as a post-hoc test for pairing comparisons. The various analyses were performed using software R 3.3.0 (R Core Team, 2016).

In order to analyze the distribution of *G. truncatula* in these new habitats, the numbers of snails were also expressed in percentages relative to the length of the sites they colonize. The values used for this study are those of the third year post-introduction at stations where samples of 20 snails have been implanted because these values are the highest.

### 3 Results

#### 3.1 Number of populations

Table 2 shows the number of populations recovered over the eight years of the experiment. At the end of the first year, snails were observed in five of the 11 rushes in which samples of 5 *G. truncatula* each were implanted. On the other hand, the introduction of 10 or 20 snails in the other 14 beds was successful with the development of populations in all cases. The same type of result was found in roadside ditches at the end of the first year: five sites (out of 10) colonized by *G. truncatula* when the sample consisted of 5 molluscs, and populations in the other 14 ditches when 10 or 20 snails were introduced.

Several populations of *G. truncatula* disappeared during the experiment due to the destruction of their habitat: a total of five in the case of rush beds out of the 19 noted at the end of the first year and five in the case of ditches out of a total of 19 (Tab. 2). The disappearance of one population in a rush bed during the second year was not explained, while the other four beds were destroyed by rotary crushing during the seventh or eighth year. In the case of ditches, one population was destroyed in the second year by the laying of pipes on another segment of the ditch located further upstream. Another population disappeared in the fourth year due to pollution by diesel oil. The other three ditches were cleared using a shovel in the seventh or eighth year, resulting in the disappearance of the snails.

#### 3.2 Surface area colonized by snails

In rush beds where samples of 5 *G. truncatula* have been implanted (Fig. 2a), this area peaked during the second year post-introduction (at 4 m²) before gradually decreasing until the end of the experiment (at 2.6 m²). The same variation in this area was also observed in the other two types of beds but the peak was later in the third year. No significant difference between the mean values of these peaks was noted. The differences between the areas noted during the eighth year of the experiment were also not significant.

In road ditches (Fig. 2b), the same variation as in rush beds was observed, but the peaks were later and the mean values were higher. The peak was thus noted in the fourth year (at 4.6 m²) at the sites where samples of 5 snails were introduced. In contrast, it
occurred in the fifth year (at 5.8 m$^2$) and the sixth year (at 7.6 m$^2$) in the ditches where samples of 10 and 20 snails, respectively, have been implanted. The difference between mean peak values was significant ($H = 13.87, p < 0.001$) with higher values at sites where samples of 20 $G. truncatula$ were introduced. Significantly higher averages in the ditches, where samples of 20 snails were introduced, were noted when peaks were compared ($H = 16.95, p < 0.001$) or at the end of the experiment ($H = 12.90, p < 0.001$).

When the peaks obtained in the rush beds and road ditches are compared, no significant difference between these means was noted, regardless of the sample size.

### 3.4 Distribution of snails in their habitat

Figure 4 shows the distribution of $G. truncatula$ relative to the length of its habitat from the upstream source to the downstream end. The values are those that our team noted in the third year post-introduction in six rush beds and seven ditches where samples of 20 snails were implanted. In both types of stations, there was a concentration of snails over the first three metres in the upstream zone of the habitat: 65.2% of $G. truncatula$ were counted in the case of rush beds (Fig. 4a) and 55.4% in the case of ditches (Fig. 4b). The remaining molluscs were distributed over a length of 8 m in the case of rush beds and 10 m in the case of ditches with a gradual decrease in snail numbers going towards the downstream zone.

### 4 Discussion

The literature reports that a minimum number of individuals is required for a population to develop in a new site and remain viable with a probability of 100% over a specified period of time (Shaffer, 1981, 1987; Wang et al., 2019). According to Reed et al. (2003), this minimum viable population (MVP) size would be very specific and would depend on the environmental and biological characteristics of the species being studied. The figures put forward by the authors show a large variation according to species. Robinson and Vath (2015) reported the “50/500” rule: a minimum population size of 50 individuals is needed to control inbreeding and a minimum of 500 individuals is needed to reduce genetic drift. Other authors such as Lehmkuhl (1984) and Thomas (1990) estimate that a MVP of 500 to 1000 is an average for terrestrial vertebrates when inbreeding or genetic variability is ignored. When inbreeding effects are included, MVP estimates for many species are in the thousands. For example, Traill et al. (2007) reported that an average of 4169 individuals is required to have MVP in many species. Although the present study is limited in time (only eight years), our results showed that a minimum number of $G. truncatula$ ranging from 5 to 20 adults per sample allowed the development of new populations in some stations on acid
be a preferential selfer (Trouvé et al., 2003, 2005) as it is known to live on the periphery of a river system (Moens, 1991) such as the upstream end of open drainage furrows (Vareille-Morel et al., 1999). An argument supporting this latter assumption is provided by Figure 3: 65.2% of snails in the case of rush beds and 55.4% in the case of ditches were counted on the first three metres of each water collection.

In stations where a population of *G. truncatula* has developed, the density of snails showed a peak in the second or third year post-introduction before gradually decreasing thereafter. These results are consistent with the report by Vareille-Morel et al. (2002) and indicate that this variation in snail abundance is independent of the number of lymnaeids contained in each sample. The most valid hypothesis for commenting on these results is to admit that the number of snails in each habitat would be progressively regulated by biotic or environmental factors, and that such regulation would begin in the second or third year after introduction. The distribution of numbers throughout the habitat (Fig. 4) showed a concentration of snails in the upstream zone of each rush bed or each ditch, while the number of individuals decreased sharply by going towards the downstream zone of the habitat. If one excludes the possible dispersion of some snails by swimming on the surface of the water or by rafting (Kappes and Haase, 2012), the displacement of *G. truncatula* towards the downstream seems to be limited to eight metres in the case of rush beds and to ten metres in the case of ditches. As *G. truncatula* is able to migrate against the current to the upstream end of its habitat in winter and spring (Rondelaud et al., 2005a, b), the downstream dispersion of individuals was smaller. The vegetation present in both types of stations cannot be incriminated to explain this result because the abundance of macrophytes is quite low throughout the water collections studied. As for the location of habitats on the periphery of a river system, the limited dispersion of individuals downstream appears to be a specific trait of *G. truncatula*. Further studies are still needed to clarify this dispersal of snails downstream throughout the year in order to determine the area from which the downstream migrating individuals leave and the means used by these snails to move.

This ability of *G. truncatula* to colonize new habitats with small snail samples can be largely explained by the genetic structure of this species. The mollusc is known to be a preferential selfer in its habitat (Trouvé et al., 2003, 2005; Meunier et al., 2004a), which in theory allows the colonization of a site from a single individual. In addition, the low level of polymorphism and the high heterozygote deficiency observed in this species (Meunier et al., 2004b; Hurtrez-Boussès et al., 2006) may be factors that explain *G. truncatula’s* ability to colonize.

In conclusion, a sample of 10 or 20 adult *G. truncatula* allowed the development of a new population on acid soil when introduced into a potential new station provided there was a temporary water source. This population showed a peak in the second or third year post-introduction before gradually returning to lower values in subsequent years. These results show that a small number of snails allow the development of a new population of *G. truncatula* in new stations on acid soil.
References


