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RESEARCH ARTICLE OPEN ACCESS

Galba truncatula and *Omphiscola glabra* (Gastropoda, Lymnaeidae): present decline in populations living on sedimentary soils in central France

Gilles Dreyfuss*, Philippe Vignoles and Daniel Rondelaud

Laboratory of Parasitology, Faculty of Pharmacy, University of Limoges, 87025 Limoges, France

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Abstract – Field investigations in habitats colonized by *Galba truncatula* or *Omphiscola glabra* were carried out to determine if the number of habitats, their area and the number of overwintering snails had not changed over the past 20 years. These habitats were studied in 57 cattle- or sheep-breeding farms located in three French natural regions on sedimentary soils. Compared to the numbers of snail habitats recorded before 1998, the values observed in 2016–2017 were significantly lower, with an overall decline rate of 30% for *G. truncatula* and 38% for *O. glabra*. Variations in this decline rate were noted with the type of snail habitat and the largest decreases were observed for vernal pools in meadows. Significantly lower areas in 2016–2017 were noted in two habitat types (drainage furrows, road ditches) for *G. truncatula* and two other habitat types (drainage furrows, pond banks) for *O. glabra.* Significantly lower densities of overwintering snails were observed in 2016–2017 in *G. truncatula* habitats located in drainage furrows and road ditches, while no significant difference was noted for *O. glabra*, whatever the habitat type. Several causes were at the origin of this population decline and the most important were the present development of mechanical cleaning in road ditches, that of subsurface drainage in meadows and regular gyro-crushing of vegetation around pools in meadows. The data reported in this study confirm the decline that several authors have already noted for *O. glabra* in Western Europe. The results obtained for *G. truncatula* require reviewing the biogeographical status of this species and taking possible measures to ensure its conservation while taking into account its role as intermediate host in the *F. hepatica* cycle.

Keywords: Decline rate / *Galba truncatula* / habitat / *Omphiscola glabra* / sedimentary soils / snail density

1 Introduction

Two species of Lymnaeidae, *i.e. Galba truncatula* (O.F. Müller) and *Omphiscola glabra* (O.F. Müller), act as intermediate hosts in the transmission of human and animal fasciolosis. The former species is known to be the main snail host of *Fasciola hepatica* (Linnaeus) in Western European countries (Torgerson and Claxton, 1999; Mas-Coma *et al.*, 2009a). In contrast, *O. glabra* is an occasional intermediate host whose role was mainly demonstrated on acid soils in the French region of Limousin (Abrous *et al.*, 1999, 2000). Identifying habitats colonized by either lymnaeid, determining their area and counting overwintering snails are needed to set up an integrated control of fasciolosis on livestock and the host snail living in a farm (Mage *et al.*, 1989).

As other native plant and animal species, these lymnaeids are exposed to all kinds of threats that vary between species and populations. The causes of threats are multiple and the most common are degradation of snail habitats, predation by introduced animals, pollution or climate change (Government of South Australia, 2014). Land snails, slugs and bivalves are also vulnerable to this endangerment in the near future. One third of the malacofauna living in Ireland, for example, is, at present, under threat of extinction according to the recent Irish Red List of threatened species (National Biodiversity Data Centre of Ireland, 2016). According to the IUCN Red Lists of threatened species, the conservation status of both lymnaeids was not the same. *Omphiscola glabra* is assessed as critically endangered in the Republic of Ireland, endangered in Germany, and vulnerable in Great Britain, the Netherlands and Sweden (Prié *et al.*, 2011). This classification is based on the generalized decline in the number and size of *O. glabra* populations that several authors have reported in Western Europe (Jackiewicz, 1999; Byrne *et al.*, 2009; Welter-Schultes, 2012, 2013). In contrast, *G. truncatula* is a widespread species and no specific threats exist at the global level, as this snail is tolerant to pollution

*Corresponding author: gilles.dreyfuss@unilim.fr

Table 1. Distribution of the 57 cattle- and sheep-breeding farms studied in three French departments on sedimentary soils according to their geographical location, the type of raised ruminant and the number of lymnaeid habitats.

French department	Cantons *	Number of farms		Number of snail habitats (first series of surveys)	
		Cattle	Sheep	*Galba truncatula*	*Galba truncatula*
Eastern Charente	Chasseneuil sur Bonnieure	3	3	37	34
	La Rochefoucauld	3	2	41	23
Southern Indre	Argenton sur Creuse	3	2	19	13
	Saint Gaultier	9	3	143	76
Southern Vienne	Lussac les Châteaux	1	9	67	37
	Montmorillon	5	14	176	86
Totals	–	24	33	483	269

* French administrative subdivision grouping several municipalities.

and is known as a colonizing species in many temporary habitats (Seddon *et al.*, 2015).

As no study had been carried out in France before 2013 to assess the conservation status of both lymnaeid populations, field investigations were carried out from 2013 to 2016 to determine the number and size of *G. truncatula* and *O. glabra* populations in 162 cattle- or sheep-breeding farms located on acid soils in the Limousin region (Dreyfuss *et al.*, 2016). According to these authors, an overall decline of 34% in the number of *G. truncatula* habitats and 23.4% in *O. glabra* habitats has been noted over the past 20 years. The size of lymnaeid populations was also affected because overwintering snails were significantly fewer in five habitat types for *G. truncatula* and three only for *O. glabra.* In contrast, the distribution of these habitats depending on their area did not show any significant change over time (Dreyfuss *et al.*, 2016). In view of these preliminary findings, it was interesting to check their validity in several other French natural regions through the following two questions: did the lymnaeid habitats located on sedimentary soils also show a decline in their number? Did their characteristics (habitat area, size of snail populations) show any change over time? To answer these questions, a comparative study between the results coming from two series of investigations was performed. The first was carried out from 1986 to 1997 in 57 farms located on sedimentary soils in three French departments, *i.e.* Charente, Indre and Vienne. The second series was performed in 2016 and 2017 in the same farms.

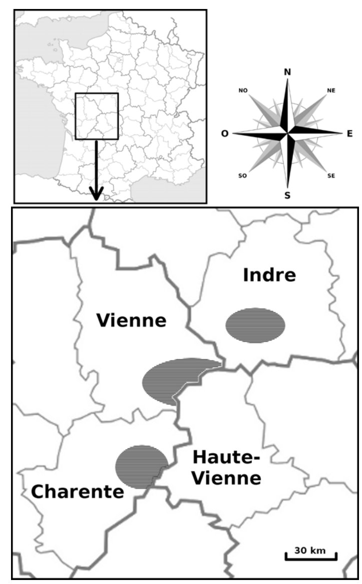

Fig. 1. Location of the departments surveyed in central France (upper map) and zones (hatched areas) in which the 57 farms are located (lower map).

2 Materials and methods

2.1 Farms investigated

From 1986 to 1997, an outbreak of fasciolosis has been detected in cattle or sheep by veterinarians in 57 farms located in three departments of central France: Charente (11 farms), Indre (17) and Vienne (29). These farms are located in natural regions close to the cristallophyllian and metamorphic soils of Limousin (Tab. 1 and Fig. 1), and their altitude ranges from 80 to 145 m above sea level. Because of the nature of soils, the pH of running water ranges from 6.8 to 8.1 and the level of dissolved calcium is generally between 28 and 41 mg/L (unpublished data). All permanent pastures present in these farms are hygro-mesophilous and are subjected to an alternate regime of grazing and mowing. An open drainage network is generally present in most pastures; in the others, the evacuation of rainwater is only ensured by one or two furrows. All are subjected to a mild oceanic climate modulated by moist winds from the Atlantic Ocean. According to years, average annual rainfall ranged from 750 to 850 mm, while the average annual temperature ranged from 11.5 °C to 12.5 °C on most pastures (Observatoire Régional de l'Environnement Poitou-Charentes, 2017).

In these 57 farms, no change in livestock farming was noted between the two series of investigations, at least over the past twenty years.

2.2 Protocol of investigations

From 1986 to 1997, we have investigated the entire area of each farm to examine water holes in permanent meadows and road ditches, which lined them, for snail presence. This was also done on the banks of evacuation systems, *i.e.* ponds, streams and rivers. The total area investigated on these 57

Table 2. Number of habitats with *Galba truncatula* or *Omphiscola glabra* in the 57 farms before 1998 and in 2016–2017 in relation to their type.

Type of snail habitat	Number of habitats (decline rate in %)			
	Galba truncatula		*Omphiscola glabra*	
	Before 1998	2016–2017	Before 1998	2016–2017
Drainage furrows	135	96 (28.8)	74	53 (28.3)
Road ditches	174	139 (20.1)	105	71 (32.3)
Vernal pools	71	23 (67.6)	42	11 (73.8)
Pond banks	61	49 (19.6)	31	19 (38.7)
Stream banks	42	27 (35.7)	17	11 (35.2)
Total	483	334 (30.8)	269	165 (38.6)

farms was 54.6 km^2. We have studied these water holes in March or April because all were waterlogged. In each hole where *G. truncatula* or *O. glabra* was living, the population was evaluated by counting adults (more than 4 mm shell height for *G. truncatula*, 12 mm for *O. glabra*), as all belonged to overwintering generations. Each snail count was carried out by two persons for 30–40 min (snail habitats in permanent meadows) or a single person for 15–20 min (road ditches, pond banks, stream banks). The area of each habitat with alive snails was then determined. Measurement of areas occupied by *G. truncatula* or *O. glabra* was easy in the case of furrows, ditches, ponds and streams. The habitats located around/in vernal pools in meadows were mapped and their area was determined according to their geometrical shape and their dimensions.

We re-investigated these 57 farms in 2016 or 2017 (i) to redetect snail habitats, (ii) to identify lymnaeid species, (iii) to measure the area of each snail habitat and (iv) to count overwintering snails. This was done in March or April on the entire area of each farm. The farmer was also asked to determine the cause of lymnaeid disappearance when the habitats of either species have not been found.

2.3 Types of snail habitats

We have considered five types of snail habitats for either lymnaeid. The first two were located in meadows: (i) surface drainage furrows and (ii) vernal pools ($<$20 m^2 in surface area) with or without a temporary spring. The other three types were (iii) road ditches when waterlogged during winter and spring, (iv) small streams, and (v) the banks of large ponds near their inlet. The vegetation around the drainage ditches and permanent pools was mainly composed of plants belonging to the Poaceae family, with rare clumps of rushes. On the other hand, the rushes were more abundant in the road ditches as on the banks of streams and ponds.

In the drainage furrows, vernal pools and road ditches, summer drying generally occurred from mid-June to the end of November. In contrast, its length was shorter in habitats on stream and pond banks: from late June up to mid-October.

2.4 Parameters studied

These parameters were (i) the number of habitats occupied by snails, (ii) the area of each habitat and (iii) the density of overwintering snails per m^2 of habitat. To determine the distribution of *G. truncatula* habitats in relation to their size, we have classified individual values as follows: up to 1 m^2, from 1.1 to 2 m^2, from 2.1 to 3 m^2, 3.1 to 4 m^2, and $>$ 4 m^2. In the case of *O. glabra*, the areas were classified into the following five categories: up to 2 m^2, from 2.1 to 5 m^2, from 5.1 to 10 m^2, from 10.1 to 20 m^2, and $>$ 20 m^2. Similarly, the distribution of habitats in relation to snail densities was studied using four categories for *G. truncatula* ($\leq$10 snails/m^2, from 10.1 to 25 /m^2, from 25.1 to 40 /m^2, and $>$ 40 /m^2) and four others for *O. glabra* ($\leq$5 snails/m^2, from 5.1 to 10 /m^2, from 10.1 to 15 /m^2, and $>$ 15 /m^2). These area and density categories were determined during other snail investigations on acid soils (Rondelaud *et al.*, 2011; Vignoles *et al.*, 2017). This distribution of habitats into classes according to their area and their density in snails was preferred here to the calculation of mean areas and mean densities because these classes enable to limit the influence of extreme dispersions and more easily detect any change between the two periods of investigations.

The numbers of sites occupied by snails in the 57 farms before 1998 and in 2016 or 2017 were compared using Fisher's exact test. The distributions of these sites according to their area or their density of overwintering snails were compared using the same test. In each case, we used the fisher.multcomp of the RVAideMemoire package (R Core team, 2016) as a post-hoc test to do pairwise comparisons. All these analyses were performed using the R 3.3.0 software (R Core Team, 2016).

3 Results

3.1 Number of snail populations

Of the 483 habitats of *G. truncatula* investigated before 1998 (Tab. 2), only 334 were found in 2016 or 2017, corresponding to an overall decline of 30%. However, this decline in the number of snail habitats was not uniform and varied according to the habitat type. The largest decline rate was observed for habitats in vernal pools (67%), followed by those on brook banks (35%) and in drainage furrows (28%). The lowest decreases were observed for habitats in road ditches (20%) and on pond banks (19%). A significant difference ($p < 0.01$) between the decline rates of the five types of habitats was noted. The decrease in the number of habitats in pools was significantly higher than those observed in drainage furrows, road ditches and pond banks. The differences between the other habitat types were not significant.

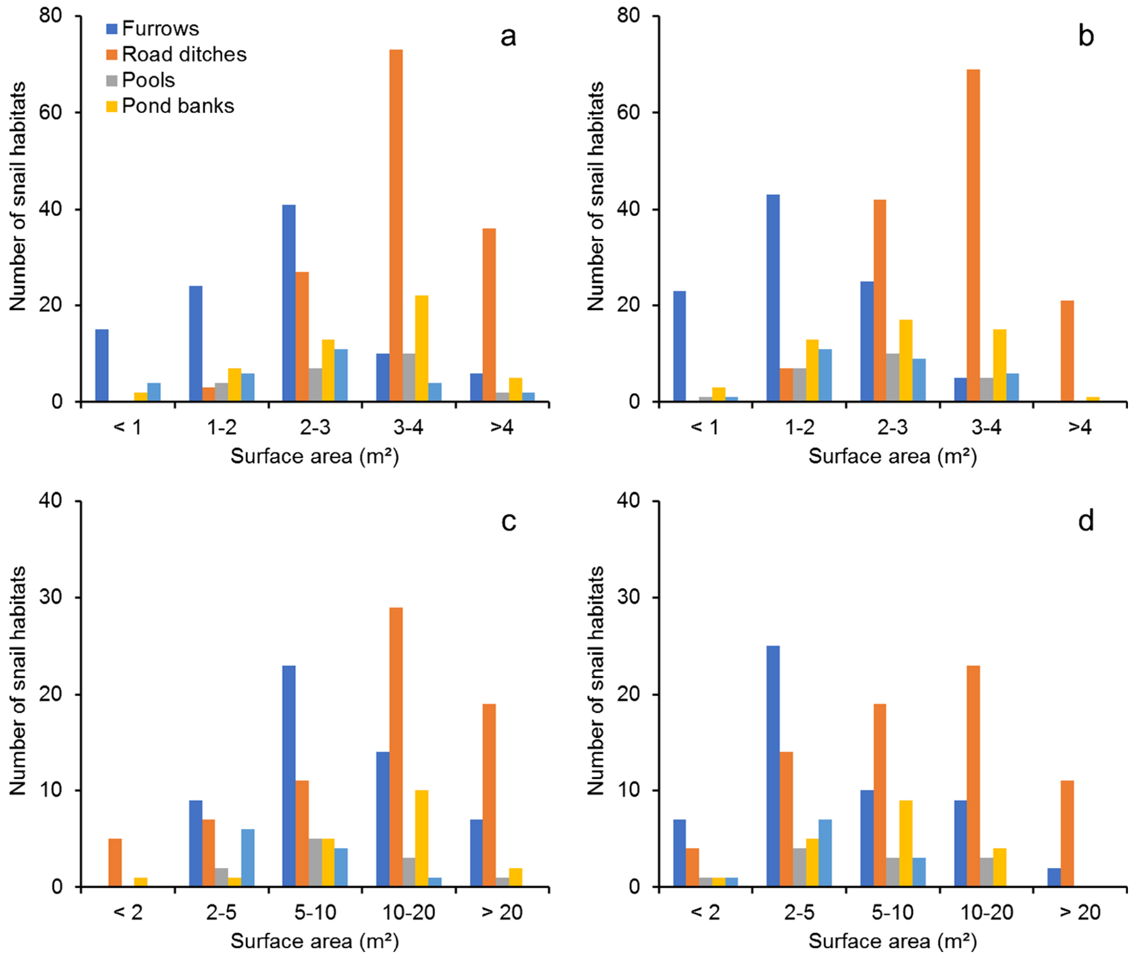

Fig. 2. Distribution of 334 populations for *Galba truncatula* and 165 for *Omphiscola glabra* observed in the 57 farms according to the surface area of their habitat in March or April: *G. truncatula* before 1998 (a) and in 2016–2017 (b), *O. glabra* before 1998 (c) and in 2016–2017 (d). The total number of populations in 2016–2017 is given in Table 2 for each habitat type.

In the case of *O. glabra* (Tab. 2), we noted a total of 165 habitats with alive snails in 2016–2017 out of the 269 detected before 1998, so that the overall rate of decline between these two periods was 38%. The highest decline rate was recorded in vernal pools (73%) and the lowest in drainage furrows (28%). In the other three habitat types, the rates of decrease ranged from 32% to 38%. Although the decrease in the number of habitats located in vernal pools was higher than those observed in the other habitat types, the difference between these decline rates was not significant.

No significant correlation between these decreases in the number of habitats occupied by snails and the department, where the farms are located, or the type of ruminants (cattle or sheep) was noted.

3.2 Area of snail habitats

Figure 2 shows the distribution of 334 habitats for *G. truncatula* and 165 for *O. glabra* according to their area before 1998 and in 2016–2017. Compared to values noted before 1998, the surface areas noted in 2016 or 2017 (Fig. 2a, b) were significantly decreased in *G. truncatula* habitats located in drainage furrows ($p < 0.001$) and road ditches ($p < 0.05$). In the other three habitat types, the differences between these two periods were not significant. In the case of *O. glabra* (Fig. 2c, d), significantly lower values for snail habitats in drainage furrows ($p < 0.001$) and on pond banks ($p < 0.05$) were noted in 2016–2017, while the differences between the other three habitat types were not significant.

3.3 Number of overwintering snails

Figure 3 shows the distribution of snail densities according to the number of overwintering snails counted before 1998 and in 2016–2017. In the case of *G. truncatula* (Fig. 3a, b), snail densities recorded in 2016–2017 were significantly lower in habitats located in drainage furrows ($p < 0.001$) and road ditches ($p < 0.05$) than values noted before 1998, while differences between the other three habitat types were not significant. In contrast, no significant differences in snail densities between 1998 and 2016–2017 were noted for *O. glabra*, whatever the habitat type (Fig. 3c, d).

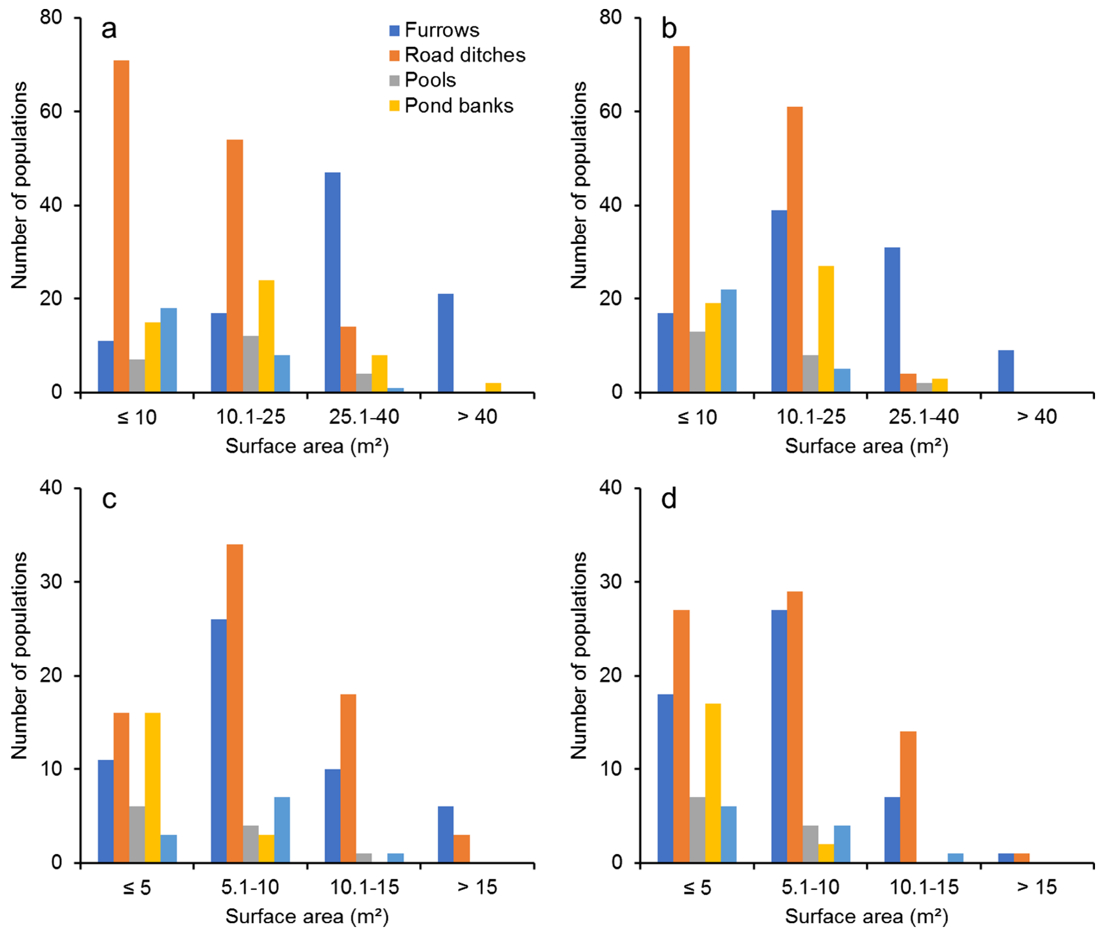

Fig. 3. Distribution of 334 populations for *Galba truncatula* and 165 for *Omphiscola glabra* observed in the 57 farms according to the density of their overwintering snails in March or April: *G. truncatula* before 1998 (a) and in 2016–2017 (b), *O. glabra* before 1998 (c) and in 2016–2017 (d). The total number of populations in 2016–2017 is given in Table 2 for each habitat type.

Table 3. Number of snail habitats disappeared in 2016–2017 in relation to the cause of population decline and the type of habitat. A/B, sites inhabited by *Galba truncatula*/sites inhabited by *Omphiscola glabra*.

Cause of population decline	Number of disappeared habitats: A/B *				
	Furrows (39/21)	Road ditches (35/34)	Pools (48/31)	Streams (12/12)	Ponds (15/6)
Mechanical cleaning	2/1	13/11	1/2	0/0	1/0
Subsurface drainage	4/5	0/0	8/5	0/0	0/0
Vegetation gyro-crushing	13/9	11/9	11/10	3/2	4/1
Cattle trampling	1/0	0/0	19/11	3/2	0/1
Periodic drying out of ponds*	0/0	0/0	0/0	5/5	8/4
Straightening of stream or pond banks	0/0	2/1	0/0	1/0	0/0
Not determined	19/6	9/13	9/3	0/3	2/0

* Usually every three years.

4 Discussion

On sedimentary soils, both lymnaeid species have shown a current decrease in the number of their populations over time. Moreover, this decline rate varied according to snail species and habitat type. The disappearance of 149 habitats for *G. truncatula* and 104 for *O. glabra* over the past 20 years is due to several causes which are presented in Table 3. The most frequent cause in drainage furrows, road ditches and vernal pools was vegetation gyro-crushing, as this technique was frequently used by 27 farmers between 1998 and 2016–2017 to destroy rush clumps around ditches, pools and in road ditches. As the technique was generally applied in early July, the absence of vegetation around/in snail habitats had a negative effect on the survival of snails during summer drying. Another cause was the mechanical cleaning of road ditches, as this technique is increasingly used in grasslands and road ditches by herders and/or local administrative authorities in central France (Rondelaud *et al.*, 2009; Dreyfuss *et al.*, 2015). Cattle trampling also was the main cause for the disappearance of snail habitats in vernal pools, as these water bodies are still used by animals for their water needs, despite the presence of artificial reservoirs in the pastures since the 2000s (Rondelaud *et al.*, 2009; Dreyfuss *et al.*, 2015). The other causes listed in Table 3 are of minor importance. On the other hand, the cause of snail disappearance was not specified for 37 habitats for *G. truncatula* and 22 for *O. glabra* in drainage furrows, road ditches and vernal pools. We suggest that this snail disappearance might be due to the effects of global warming from the 2000s because the latter is known to act on animal or plant biodiversity (Root *et al.*, 2003; Stewart, 2009). In addition, this increase in temperature has a disruptive effect on snails which intervene as intermediate hosts in the transmission of several helminthoses (Mas-Coma *et al.*, 2008, 2009b). An argument supporting this approach is the total drying of many pools occurred in recent years in the French departments of Indre and Vienne, whatever the time of the year (D. Rondelaud, personal observation). In the present study, the disappearance of populations over the past 20 years was greater in *O. glabra* (38%) than in *G. truncatula* (30%). This finding disagreed with the report by Dreyfuss *et al.* (2016) on the populations of the two lymnaeids living on acid soils. According to these authors, the overall decline in the number of habitats was 34% for *G. truncatula* and 23% for *O. glabra* between 1976–1992 and 2013–2016. To explain this difference, the most reliable hypothesis is that *O. glabra* populations would be less resistant to unfavourable conditions than those of *G. truncatula* when they live on sedimentary soils. This hypothesis is supported by the following observations: (i) the populations of *O. glabra* were more numerous on the cristallophyllian soils of central France than in the surrounding sedimentary regions (Vignoles *et al.*, 2017) and this distribution is consistent with the report by Boycott (1936), as the species prefers to live on slightly acid soils; (ii) the habitats of *O. glabra* were larger than those of *G. truncatula* (Fig. 2) in the studied areas and the vegetation around them was often grassy, which protects them poorly from the effects of the sun during summer drying, even if *O. glabra* is able to withstand this drying by burying in the soil (Rondelaud *et al.*, 2003); (iii) the amphibiosis of *G. truncatula* allows it to adapt to degraded life conditions, while *O. glabra* is more aquatic (Taylor, 1965).

In 2016–2017, the mean area of habitats colonized by *G. truncatula* was significantly lower in drainage ditches and road ditches. The same finding was noted for *O. glabra*, but for habitats in drainage ditches and pond banks. These results disagreed with the observations of Dreyfuss *et al.* (2016) in habitats on acid soils because they did not note any significant variation in the habitat areas of each lymnaeid considered separately. This difference between our results and those of the above authors is difficult to explain. The dates on which habitats were investigated in 2016 or 2017, and, consequently, weather conditions can not alone explain this discrepancy because these area-reduced habitats were noted throughout the survey period (March or April) for the two years. The most valid explanation is to relate this difference to properties (moisture, texture, drying conditions) of soils present in sedimentary or acid regions and local climatic conditions. Indeed, the average annual temperature in the studied areas varied from 11.5 °C to 12.5 °C (Observatoire Régional de l'Environnement Poitou-Charentes, 2017), whereas it was between 10 °C and 11 °C on acidic soils in Limousin (CRPF Limousin, 2010). Similarly, annual precipitation ranged from 750 to 850 mm depending on the area in the sedimentary regions and from 900 to 1000 mm in most communes of Limousin.

Contrary to lymnaeid habitats on acid soils, the numbers of overwintering *G. truncatula* in sedimentary regions have significantly decreased in two types of habitats (drainage furrows, road ditches). On the other hand, no significant variation in the numbers of *O. glabra* was recorded between the two study periods, whatever the habitat type. These results disagreed with the observations of Dreyfuss *et al.* (2016) on acid soils, as these authors reported a decline in numbers in five habitat types for *G. truncatula* and only three types for *O. glabra*. Two explanations have already been proposed by Dreyfuss *et al.* (2016) to explain this decrease: (i) the occurrence of iridovirosis in the case of *G. truncatula* (this epizooty has not yet been observed in *O. glabra*), as that noted in Limousin (Barthe *et al.*, 1984; Rondelaud and Barthe, 1992); (ii) the development of vegetation surrounding the habitat at more advanced stages in response to favourable climatic and hydrological conditions, leading to a limitation or loss of algal cover on which the snails feed (Moens, 1981, 1991). But this decline in snail numbers in several populations may also be simply a consequence of human activity (repeated passages of tractors, vegetation gyro-crushing, ...) or of animals (cattle trampling, ...) especially on pastures, as already reported for snail populations living on acid soils (Rondelaud, 1977).

At the present time, it is difficult to determine the consequences that the disappearance of several snail populations may have on the prevalence of fasciolosis in a farm. Two facts support this assertion. First, the use of triclabendazole to treat cattle against this parasitosis has become widespread in central France from the 2000s, which led to a gradual decrease in the prevalence of the disease in ruminants and, consequently, in the number of snails naturally infected with *F. hepatica* (Dreyfuss *et al.*, 2016). Second, most farmers involved in the present study used rotational grazing for their ruminants in relation to vegetation growth throughout the year, which does not allow for an accurate study because the habitats disappeared over the past 20 years were located on different pastures and were often adjacent to other habitats with alive *G. truncatula* or alive *O. glabra*.

In central France, the two species of lymnaeids show a general decline in the number of their populations, whatever the geological nature of the subsoil. In habitats on sedimentary soils, there is also a reduction in area for two habitat types and a decrease in the number of overwintering snails for two other habitat types. The data reported in this study confirm the decline that several authors such as Byrne *et al.* (2009), Prié *et al.* (2011) or Welter-Schultes (2013) noted for *O. glabra* in other European countries. On the other hand, the results obtained for *G. truncatula* in the report by Dreyfuss *et al.* (2016) and in this study require reviewing the biogeographical status of this species and taking possible measures to ensure its conservation while taking into account its role as intermediate host in the *F. hepatica* cycle.

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