Chapter 15
Control of Fasciolosis-Transmitting Lymnaeids in the Field

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Abstract The objective of this chapter is to review the different methods for field control of host snails involved in the transmission of fasciolosis. Environmental measures such as drainage of swampy soils or cutting of vegetation in watercourses can reduce the number of snails. Synthetic molluscicides are being used less and less because of their cost, toxicity, and contamination they cause in the environment. Conversely, research on plant extracts is being developed because many of them are natural molluscicides that are less toxic and more environmentally friendly. In the nature, several groups of vertebrates and invertebrates predate lymnaeids and a control technique of *Galba truncatula* has been developed in central France using predation by the terrestrial snail *Zonitoides nitidus*. Biological control can be performed using animal species which compete with lymnaeids for food. Pathogens such as parasites and other infectious agents can also be used. Finally, an integrated liver fluke control strategy for the control of liver fluke, associating deworming of the definitive hosts and control of host snails, is analysed with a review of results provided by this type of control.

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15.1 Introduction

Fasciolosis is a parasitosis caused by fluke species of the genus *Fasciola*. If *F. hepatica* is the main cause of this disease because of its very wide distribution over the five continents, *F. gigantica* seems to be of secondary importance because it is limited to the Old World (Mas-Coma et al. 2009). When present, these parasites are common in cattle and sheep, resulting in significant economic losses in the livestock industry due to their pathogenicity (Torgerson and Claxton 1999). This parasitosis also affects people. Human fasciolosis was considered a secondary disease until the late 1990s (Chen and Mott 1990). But the situation has changed considerably in recent years due to the increasing importance of this disease in Europe, Asia, Africa, as well as the North, Central, and South Americas (Mas-Coma et al. 2009).

Both species of *Fasciola* use freshwater snails of the Lymnaeidae family as intermediate hosts. The latter ensure the larval development of these parasites (Kendall 1965). At least twenty species of this family have been reported as host molluscs for *F. hepatica* (Boray 1978; Torgerson and Claxton 1999; Hurtrez-Boussès et al. 2001; Vázquez et al. 2018) and most species of the genus *Galba* (amphibious molluscs) are involved in the transmission of this fasciolosis (Alda et al. 2021). In the case of *F. gigantica*, transmission is mainly ensured by molluscs belonging to the genus *Radix* (Spithill et al. 1999; Mas-Coma et al. 2009; Vázquez et al. 2018). Among the means that have been proposed to fight the disease, the control of host snails has long been suggested (Mehl 1932; Taylor 1964).

The control application faces three types of difficulties. The first relates to the snail's lifestyle. Most species are strictly aquatic like *Lymnaea stagnalis*, while others are semi-aquatic like *Omphiscola glabra* or amphibious with the possibility of living in water or on wetlands. The treatment of these amphibious snails is more complex because the survivors do not hesitate to take refuge on emerged areas when a molluscicide or a biological control agent is introduced into their habitat (Dreyfuss et al. 2015). The second difficulty is the type of habitat, depending on whether it remains in water during the summer or dries out. In permanent watercourses, environmental measures or biological control agents will be preferred at the expense of chemical treatment as the latter is known to have a lethal effect on associated flora and fauna as well as animal and human health if this water is used for drink (Rozendaal 1997). In drying habitats, several lymnaeid species bury themselves during the summer months (aestivation) and this process should therefore be taken into account when deciding whether to apply environmental measures or to introduce biological control agents (Dreyfuss et al. 2015). The third difficulty is the number of snail generations per year. Most aquatic species have only an egg-laying period extending from spring to mid-summer depending on the climatic and environmental conditions of their habitat. Semi-aquatic and amphibious species often have two egg-laying periods, one in the spring and the other in the autumn at the end of aestivation. But in very wet years in temperate countries, a third generation of snails (summer generation) occurs as a result of the eggs laid by the spring generation (Taylor 1964; Dreyfuss et al. 2015).

Among the techniques proposed by the authors, environmental measures are the oldest and relate to modifications of the habitat in which the host snail lives. The most well-known example is drainage which allows faster flow of excess water into lymnaeid habitats (Taylor 1964). Synthetic or natural molluscicides, which humans can introduce into snail habitats, were mainly used up to the 1970s in Western Europe (Euzeby 1971). The disadvantages caused by the use of these products have led to a gradual decrease in their use over time and their abandonment in some countries (Rozendaal 1997; Bustinduy and King 2014). Finally, three types of biological agents, i.e. predators, competitors, and pathogens, have been proposed by the authors (Taylor 1964). The development of this type of control has been much slower than the application of environmental measures or molluscicides. The main difficulty is the often incomplete elimination of snails and these agents must be used for several successive years to have complete eradication of the snail (Dreyfuss et al. 2015). Whatever treatment chosen for the snails, it is necessary to couple this control with the treatment of definitive hosts in an integrated management concept in order to eliminate the reservoir of the disease in the definitive host (Mage et al. 1989; Dreyfuss et al. 2015).

The methods for controlling host snails, reported in the paragraphs below, are not exclusive to lymnaeids. They also concern the intermediate hosts of other diseases. Many articles related to the control of host snails of *Schistosoma* spp. have been published in the literature. As many aquatic lymnaeids are part of a larger malacological population (case of *Radix natalensis* with *Bulinus* spp. and/or *Biomphalaria* spp. in Africa), many references concerning intermediate hosts of *Schistosoma* spp. have been incorporated into this chapter.

As the nomenclature of Lymnaeidae is still controversial, the species names cited in this chapter have been updated following the names of the taxa listed in MolluscaBase (2022). Species, whose names have changed, are cited by first indicating the old name, followed by the current name in brackets.

15.2 Environmental Measures

15.2.1 Drainage of Lands

Soil drainage has a long history and apparently dates back to the earliest civilizations of Mesopotamia and Iran in the 4000s BC (Valipour et al. 2020). Nowadays, it is the most widely used method for drying the environment in a wet meadow or a swamp (Euzeby 1971). According to this author, drainage is a difficult, delicate, and costly operation which is rarely performed for the sole prophylaxis of parasitoses. However, if properly implemented, it is a radical means to eliminate *Galba truncatula* because the cleaned soil does not allow the snails to live there. Three techniques are used to do this drainage (Brouwer et al. 1985; Ritzema 1994).

The most common is the digging of a network of surface swales and ditches (surface drainage) using a trench plough (Taylor 1964). To prevent the walls of these swales and ditches from being destroyed by trampling of domestic ruminants, a fence must be put in place around the network (Taylor 1964) but this is rarely applied in central France for reasons of cost (Dreyfuss et al. 2015). To overcome this difficulty, it is useful to restore the drainage network every year in October or November, i.e. during the months when the passage of tractors is still possible on the ground to do this operation (Taylor 1964). In central France, the annual cleaning of this network is carried out only by a small number of farmers, while the others clean up swales and ditches only every 2 or 3 years when the network has been completely levelled by the passage of cattle or sheep (Dreyfuss et al. 2015). This first method only limits the development of snail populations and the results obtained depend on the date where the drainage is performed in the field.

The second technique is the digging of the subsoil using a mole plough (subsurface drainage) to create simple underground conduits, reinforced or not by pottery or PVC pipes. This technique has several advantages, namely the complete disappearance of snail populations on the surface which no longer have enough moisture to live and the easy passage of agricultural machines on the ground especially when the pipes are surrounded by gravel (Euzeby 1971; Blann et al. 2009). However, the purchase of pipes, whatever their nature, is expensive, which limits the diffusion of this technique. If the farmer does not use pipes, this technique can only be used in soils with sufficient structural stability to prevent the collapse of underground conduits during the passage of ruminants (Euzeby 1971). Fencing of these networks, at least in central France, was little used (Dreyfuss et al. 2015).

The third method is to do a system of shovel-dug ditches to remove most of the runoff in a meadow. In fairly flat areas, the application of this technique creates regular areas of pasture separated from each other by parallel ditches, oriented in the direction of the steepest land slope. Even if these ditches are deeper than simple drainage ditches, fencing is necessary to prevent them from collapsing under the passage of domestic animals. All this represents a financial cost that many farmers hesitate to assume in order to clean up their pastures (Dreyfuss et al. 2015).

15.2.2 Other Environmental Measures

In watercourses, several techniques have been proposed to control freshwater snails. Removal of aquatic vegetation in the canals reduces the number of snails. This cleaning can be very effective as it can be durable and selective against specific snail species. The most relevant requirement is to target all the habitats covered by macrophytes and algae where the snails live because a single snail can rapidly recolonize the whole irrigation system (Rozendaal 1997; Sabourin et al. 2018). Similarly, rising and falling water levels and increasing flows can disturb snail habitats and food sources (Brown 1994; Rozendaal 1997; Lardans and Dissous 1998; Secor 2014). However, results vary according to the species of snails. In

Morocco, Laamrani et al. (2001) found that the three methods used for irrigation siphons (covering, regular cleaning, and increase in water speed) were effective in reducing the number of *Bulinus truncatus* while other species such as *Radix peregra* (= *Peregriana peregra*) and *Physella acuta* were little affected. In the irrigation system of Akka (southern Morocco), the repeated cleaning of the network and the elimination of aquatic vegetation resulted in a significant reduction in the number of bulinids and their egg masses, indicating that these low-cost measures are effective and sustainable (Boelee and Laamrani 2004).

Agronomic measures have also been applied in the meadows where cattle and/or sheep are grazing. On acidic soils in central France, Dreyfuss et al. (2015) noted several series of measures that 141 breeders have applied over the past 40 years to prevent the risk of fasciolosis in their farms. The first series aims to isolate or destroy the habitats of *G. truncatula*. In pastures, the habitats of the snail, located at the peripheral end of the surface drainage swales, are isolated from the rest of each meadow by barbed wire so that cattle or sheep are not in contact with this vegetation. As these habitats on acidic soils are small (less than 3 m^2 per habitat: Dreyfuss et al. 2015), their isolation represents less than 1% of the total area of the pasture. In the case of sources, the method used depends on their status. If they are temporary, the vegetation is destroyed by rotary crushing so that there was currently a drastic decrease in their number in central France. Permanent sources are fenced off and running water sometimes circulates in underground pipes to the nearest drainage ditch or stream. The second series of measures aims to prohibit the access of cattle or sheep to permanent water collections that are present on pastures (pools, small ponds). The latter are increasingly fenced off so that animals cannot access them and are often watered using mobile or fixed water tanks. Trampling of animals around these water tanks can sometimes lead to the formation of snail habitats when there is a permanent flow of water towards a ditch, but this case is rather rare in central France. Finally, many meadows that are difficult to drain and known locally to be “fluke hotbeds” have simply been transformed into ponds (Dreyfuss et al. 2015).

In order to show that these techniques have changed since the 1970s, Table 15.1 gives the number of agronomic transformations that breeders in the 141 above farms have applied in their meadows. Surface drainage is still widely applied on these pastures while subsurface drainage, usually coupled with the laying of PVC pipes, has shown a marked increase since the 1980s. Digging deeper ditches using a shovel was more recent and did not really develop until 2000. The transformation of meadows into ponds is an incidental process that remains rare in central France. The other agronomic measures have been applied in various ways from the 1980s. The use of water reservoirs was widespread in the region (73.1% of meadows). In contrast, this is not the case for the fencing of *G. truncatula* habitats (38.1% of meadows owned by 49 breeders only). The destruction of hillside sources did not really develop until the 2000s. The other two measures, i.e. the laying of underground pipes for the water flow coming from permanent sources and the fencing of water collections, only interested a small number of pastures (Dreyfuss et al. 2015).

Table 15.1 Changes in agronomical measures applied to 776 meadows by 141 breeders in central France. From Dreyfuss et al. (2015)

Agronomical measure	Frequency (%) of meadows concerned by an agronomical measure between			
	1970 and 1979	1980 and 1989	1990 and 1999	2000 and 2008
Surface drainage	99.7	96.7	82.9	59.1
Subsurface drainage	0.001	2.8	13.0	30.5
Ditches (mechanical shovel)	0	0.002	3.7	9.6
Fencing of snail habitats	0	1.4	7.6	38.2
Gyro-crushing of rushes (temporary springs)	0.001	0.001	5.6	22.5
Buried pipes (permanent springs)	0	0	1.4	4.2
Installation of watering tanks	0.003	8.6	41.1	73.1
Fencing of water collections	0	0.001	3.0	9.1
Creation of a pond	0.001	0.001	0.002	0.006

15.2.3 Consequences of Environmental Measures

The effects of these agronomic measures are really well known only for surface drainage in swampy meadows and marshes. The results depend on the date on which this operation is carried out. If drainage is done in spring (March), the density of lymnaeids (*G. truncatula* in this case) decreases hyperbolically over time. Snails of the overwintering generation suffer considerable losses due to lack of water and are unable to reproduce, which increases the decline in population during the season (Worden et al. 1963). If this measure is applied later in summer, the effects of this operation are then different on the two generations of *G. truncatula*. Spring generation snails, resulting from the reproduction of molluscs that have spent the winter, are little affected by the realization of drainage. In contrast, those of the overwintering, older generation are more sensitive to drought and die gradually (Worden et al. 1963). Finally, if the drainage is done in October or November, the remaining *G. truncatula* have time to recolonize the habitat and lay eggs so that regular maintenance can keep the snails at high and substantially constant values. On the other hand, if the cleaning is irregular (every two, 3, or 4 years), the numbers of snails decrease, sometimes up to the disappearance of the species (Rondelaud 1977b). In any case, as Euzeby (1971) underlines it, there are always a few individuals which are able to repopulate the habitat. For this reason, surface drainage applied alone cannot be sufficient to completely eradicate the population of *G. truncatula*.

Unlike surface drainage, the data on the consequences of other agronomic measures are scarce, so it is difficult at present to do a synthesis on this point. The only information available relates to the isolation of snail habitats. In this case, the prevalence of natural infection with *F. hepatica* in cattle shows a drastic drop in the seven farms where this technique was used (Dreyfuss et al. 2015). But these results come from only one region and it is difficult to generalize them without a larger study to verify their validity.

15.3 Molluscicides

Two types of products, i.e. synthetic molluscicides to which copper salts can be added, and natural substances from various plant species have been used to control aquatic snails which intervene as intermediate hosts in the life cycle of digeneans. Copper salts have been little applied in the field due to their toxicity (Taylor 1964). Synthetic molluscicides have been widely used in programmes that many countries, especially in Africa (Brown 1994), have implemented to control snail hosts of *Schistosoma* spp. Conversely, these products have been little used to control the intermediate hosts of *Fasciola* spp. Research on natural molluscicides has developed considerably since the 1990s for the host snails of *Schistosoma* spp. and from the 2000s for those of *Fasciola* spp. But most of these studies have been carried out under laboratory conditions to determine the toxicity of these products on snails, and few of them have yet been carried out in the field.

15.3.1 General Principle of Use

Among these substances, there is no perfect product to eliminate snails. According to Levêque (1990), three criteria must be taken into account for the selection of a product: (i) the molluscicide must be fully effective against the target snail, (ii) it must not be toxic to humans and the upper vertebrates, and (iii) it must be of an attractive price and easy to use under operational conditions. To these criteria are added three other requirements relating to the aquatic environment. The first is not to use pesticides which produce toxic and persistent degradation products that accumulate in trophic chains. Second, the molluscicide must not affect fish at different stages of their life. Third, it must not cause a long-term imbalance of ecosystems under normal conditions of application.

Spreading programmes must also take into account two elements which are specific to amphibious snails, i.e. the density of the population and the nature of its habitat. First, the molluscicide should preferably be applied in spring or late summer when the population density is at its highest and snails are active. Second, these substances are easier to use in stagnant water because it is possible to maintain a constant concentration of molluscicide at any point and to obtain a longer persistence, resulting in an increased efficiency of the molecule. The spreading must also involve a large perimeter around the main site. On the other hand, these requirements are not very reproducible in running water where dilution problems arise. In addition, prior destruction of aquatic and/or hygrophilous vegetation on the application site is necessary because it interferes with the dispersion of the product.

15.3.2 Chemically Defined Substances

Many products have been used to control different species of freshwater snails in their natural habitats (see review by Euzeby 1971, for example). From this list, only a few have been studied to determine their efficacy against lymnaeids in the field. Table 15.2 lists the main products which were widely used during the twentieth century, their mode of application and their toxicity to snails and other zoological groups. This synthesis comes from several sources: Euzeby (1971), Pécheur (1974), Gayral and Cavier (1977), McCullough et al. (1980), Rondelaud (1988), Haseeb and Fried (1997), Torgerson and Claxton (1999), and World Health Organization (2002, 2017).

Copper sulphate is the oldest. However, it should be avoided because of its toxicity when cattle and/or sheep are present on pastures. The product is very active on snails but its penetrating power is low in muddy environments. It is toxic to fish and corrosive to the material used. Another copper salt, cupric chloride, was used at a sublethal dose (0.1 mg/L) by Rondelaud (1986, 1988) in wild watercress beds on acidic soils with a spray in March. According to this author, this toxic at a sublethal

Table 15.2 Main molluscicides used during the twentieth century to eliminate the snail, their mode of application and their toxicity. LC_{90}, lethal concentration at 90%

Substances	Mode of application	LC_{90} (g/L) at 24 h		Toxicity	
		Adult snails	Young snails	Fish	Mammals
Copper sulphate $CuSO_4$	Powder (3.5 g/m^2) or aqueous solution (3%)	0.8–4.2	0.3	Yes	Yes
Cupric chloride $CuCl_2$	Aqueous solution (0.1 mg/L)	0.5–3.1	0.2	Yes	Yes
Sodium pentachlorophenate	Aqueous solution (10% or 15%)	1–4	0.2	Yes	Yes
Calcium cyanamide	Liquid solution (125 or 250 L/ha), powder (300 kg/ha) or pearled form (500 kg/ha)	2–4	0.5	?	?
N-trityl-morpholine (Frescon™)[a]	Concentrated at 16.5% (3 L/ha, diluted in 400 L of water)	0.02–0.06	0.03	Yes	Yes
Niclosamide (Bayluscide™)	Powder (2.5–10 kg/ha) or aqueous solution (0.2–1%)	0.1–0.3	0.05	Yes	Yes

[a]This product is not manufactured at the present time

dose disturbs the reproductive activity of *G. truncatula* so that egg-laying occurs later in the spring and that juveniles born from these eggs can be eaten by a predatory snail, *Zonitoides nitidus*, in June. Despite its low concentration, this salt is also toxic to fish, at least to fry, and mammals.

A comparative study with the four other products listed in Table 15.2 was carried out by Pécheur (1974) on the Belgian habitats of *G. truncatula*. According to this author, sodium pentachlorophenate caused the complete elimination of snails after a single application when water was stagnant. But this product is highly toxic to vertebrates and especially to fish. Calcium cyanamide has excellent molluscicidal properties at a rate of 300 kg/ha when the treatment is applied in March–April on stagnant water habitats. The pearly form of this product is preferable to powder because the pearls are not retained by the plant cover and fall to the bottom of the habitat where the snails are located. Frescon® (N-trityl-morpholine) was a good molluscicide while its ovicidal action was low. Only the eggs located at the periphery of snail egg masses were destroyed. According to Pécheur (1974), the toxicity of this product at the doses used was null for fish and other vertebrates, while other authors reported relative toxicity. Finally, niclosamide gives good results in muddy conditions and stagnant waters, but it is toxic to fish and other vertebrates. Although these products were widely used between 1970 and 1990, this is no longer the case today, at least for snail habitats located in temperate countries. All these products are toxic to the macrofauna found in the snail habitats as well as to fish and other vertebrates.

The World Health Organization (2017) currently recommends that niclosamide be used as a 25% emulsifiable concentrate or 70% wettable powder. Other formulations have been used in China such as 50% wettable powder of niclosamide ethanolamine salt (Yang et al. 2010), 4% niclosamide ethanolamine salt powder (He et al. 2007), or a niclosamide derivative, called 2′,5-dichloro-4′--nitrosalicylanilide-quinoid salt (Xia et al. 2014). The toxicity of niclosamide to non-target plant and animal species was assessed by Andrews et al. (1983). The product is capable of killing freshwater molluscs and free larval forms of parasites (miracidia, cercariae). But it is toxic to fish and amphibians (frogs and toads). According to Dawson (2003), aquatic plants and agricultural crops do not appear to be negatively affected by the concentrations of niclosamide used to control lamprey or freshwater snails. Mayflies (*Hexagenia* sp.) are relatively resistant to the effects of niclosamide exposure. In addition, this author concludes that there is a minimal risk to humans and the environment provided that the product is properly applied and supervised. As niclosamide can be used safely in the presence of livestock or poultry when applied at the indicated doses (World Health Organization 2017), application of this product has been carried out in several countries such as Brazil (Coelho and Caldeira 2016), China (Chen 2003; He et al. 2007, 2017; Dai et al. 2008, 2015; Xia et al. 2014), Egypt (Ismail et al. 2019), Kenya (Kariuki et al. 2013), and Morocco (Khallaayoune et al. 1998; Belkacemi and Jana 2006; Barkia et al. 2011). Despite this, the use of niclosamide has gradually decreased in several countries such as Brazil where application of the product was stopped in 2002, mainly due to increasing global pressure to preserve the environment. In addition, the presence of snails (*Oncomelania hupensis*) probably resistant to niclosamide (Cao et al. 2012) poses a new problem in countries where this molluscicide has been used for several decades.

15.3.3 Plant Substances

Much of the research on natural molluscicides has been done to find products of plant origin capable of exerting toxic activity on the host snails of *Schistosoma* spp. As these products are potentially biodegradable in the environment, their possible use in the field can prevent long-term pollution (Kashyap et al. 2019). The idea of using plants to control disease-carrying molluscs dates back to the 1930s when Archibald (1933) and then Wagner (1936) used the fruits of *Balanites* in Sudan and South Africa. Numerous trials were subsequently carried out over the world which led Kloos and McCullough (1982, 1987) to review them to identify the most effective plant species against snails, particularly in tropical countries. Contrary to the host snails of schistosomes, research on plants with a molluscicidal effect on Lymnaeidae was rare until 1990 and only became important from the 2000s. Most of these studies focused on the screening of numerous plant species to determine their toxicity to snails. Lethal concentrations (LC) of these products and identification of active substances were then studied on several species of lymnaeids (Thakur et al. 2019; Chaturvedi et al. 2021). However, information on the application of these products in the field is still scarce today.

Medina and Woodbury (1979) carried out tests on 200 plant species from Puerto Rico to identify those which had a molluscicidal effect on *Galba cubensis* and *Pseudosuccinea columella*. Of the 30 species selected by these authors, the aqueous extracts from 16 of them were highly toxic, killing all snails in a range of 25 to 200 ppm. *Solanum nodiflorum* was then selected for their further studies because all parts of the plant are toxic (Medina and Ritchie 1980). The effects of other plants have also been studied on these snails. Tests were performed by Cruz-Reyes et al. (1989) with three concentrations (5, 25, and 50 ppm) of an aqueous extract of *Piqueria trinervia* on five species of lymnaeids, including *G. cubensis* and *P. columella*. A 100% mortality was obtained for all species at 50 ppm after 6 h of exposure. The same percentage was obtained at 25 ppm after 24 h, and mortality of 60% to 100% was observed at 5 ppm after 24 h of exposure. A hydroethanolic extract of *Sapindus saponaria* fruit with concentrations ranging from 20 to 100 mg/L was effective against *G. cubensis* with a LC_{50} of 39.8 mg/L and a LC_{90} of 67.9 mg/L for 72 h of exposure (Abreu Guirado et al. 2019). In Brazil, an aqueous extract of the latex of *Euphorbia splendens* var. *hislopii* at 5 mg/L killed 97.4% of *P. columella* in an irrigation ditch after 24 h of exposure (Vasconcelos and Amorim 2003a, b).

Table 15.3 lists several plant species that the authors have cited since the 2000s for their high molluscicidal power against *G. truncatula* and *Radix acuminata* (= *R. rufescens*). This activity depends on the plant species studied, the segment of the plant, and the degree of ripeness in the case of fruits. But it also depends on the

Table 15.3 Molluscicidal activity of some plant-derived substances against *Galba truncatula* and *Radix acuminata* from the 2000s, with indication of the solvent used to obtain the extract and the length of contact between the substance and snails. Only the highest activities of these plants are listed in this table. Ac., acetate; LC_{50}: lethal concentration at 50%; LC_{90}: lethal concentration at 90%

Snail and plant species	Segment of the plant (solvent; exposure time)	Values (mg/L)		References
		LC_{50}	LC_{90}	
Galba truncatula				
Hammada scoparia	Leaf (methanol; 48 h)	28.9	69.9	Mezghani-Jarraya et al. (2009)
Solanum nigrum	Immature fruit (methanol-H_2O; 48 h)	3.9	7.4	Hammami et al. (2011)
Atriplex inflata	Leaf (Ethyl Ac.; 48 h)	5.9	8.8	Hamed et al. (2015)
Capparis spinosa	Leaf (methanol; 48 h)	3.5	29.9	Njeh et al. (2015a)
Solanum elaeagnifolium	Seed (methanol; 48 h)	1.1	2.2	Njeh et al. (2015b)
Citrullus colocynthis	Leaf (Ethyl Ac.; 48 h)	11.7	15.9	Chawech et al. (2017)
Clematis flammula	Flower (Ethyl Ac.; 48 h)	11.6	46.4	Khanous et al. (2017)
Radix acuminata[a]				
Alstonia scholaris	Stem bark (H_20; 96 h) Bark (purified; 96 h)	138.3 70.6	– –	Singh and Singh (2003)
Bauhinia variegata	Leaf (ethanol; 96 h) Leaf (purified; 96 h)	14.4 5.9	– –	Singh et al. (2012)
Euphorbia hirta	Latex (–; 24 h)	7.3	–	Yadav and Singh (2011)
Lantana indica	Leaf (acetone; 96 h)	1.3	–	Chauhan and Singh (2009)
Mimusops elengi	Bark (ethanol; 96 h) Bark (purified; 96 h)	15.0 7.2	– –	Singh et al. (2012)
Myristica fragrans	Mace (ethanol; 96 h) Nutmeg (ethanol; 96 h)	28.6 36.9	– –	Jaiswal and Singh (2009)
Nerium indicum	Bark (ethanol; 96 h)	0.94	–	Singh and Singh (1998)
Piper nigrum	Fruit (ethanol; 96 h) Fruit (purified; 96 h)	6.2 3.7	– –	Srivastava et al. (2009)

[a](= *Radix rufescens*)

solvent used to obtain the extract, as Njeh et al. (2015a) or Khanous et al. (2017) have shown this by producing ethyl acetate, methanol, and hydro-methanol extracts from the same plant. The association of rutin, ellagic acid, and/or taraxerol with the latex of *Jatropha gossypifolia*, its bark or its leaves at sublethal doses results in a significant reduction in the fecundity of *R. acuminata*, hatchability of eggs, and survival of young individuals (Yadav and Singh 2014). The incorporation of various concentrations of natural molluscicides in baits consisting of starch and agar resulted in higher toxicity to snails than that of the same raw molluscicides. Storage of these baits for up to 4 weeks resulted in a marked reduction in their toxicity (Tiwari and Singh 2007). In addition, seasonal variations in toxicity of their active ingredients have been reported (Agrahari and Singh 2012).

Few studies have been carried out on natural molluscicides and their effect against *Radix natalensis* and *R. peregra* (= *Peregriana peregra*). Vassiliades (1984) showed that *Euphorbia tirucalli* (the whole plant) and *Jatropha curcas* (the seed almond) killed 100% of *R. natalensis* at concentrations of 0.3 g/L and 0.1–0.2 g/L, respectively. In Kenya, 100 or 50 mg/L of powdered sun-dried or freeze-dried berries of *Solanum aculeatum* killed more than 60% of *R. natalensis* in the laboratory (Mkoji et al. 1989). Exposure of this snail for 24 and 48 h to dried fruit and leaf powders or raw water extracts of *Solanum nigrum* powders revealed high molluscicidal activity with an average LC_{50} of 17.7 mg/L (Ahmed and Ramzy 1997). Essential oils from leaves of three Azorean species: *Hedychium gardnerianum*, *Juniperus brevifolia*, and *Laurus azorica*, showed molluscicidal activity on both juveniles and adults of *R. peregra*, with a LC_{50} varying between 15.4 and 44.6 mg/L for juveniles and 45.3 to 54.6 mg/L for adult snails (Teixeira et al. 2012). The essential oils of *Cuminum cyminum*, *Foeniculum vulgare*, and *Petroselinum crispum* (Apiaceae) at 50 mg/L were also very active against the eggs and adults of *R. peregra*. The estimated LC_{50}s ranged from 13.7 to 46.5 mg/L for a 48-h exposure and the old fruits of *P. crispum* had the most significant molluscicidal activity (Sousa et al. 2017).

The main problems with these natural molluscicides are the lack of field experiments and the lack of studies on non-target flora and fauna. In addition, the use of these products in the field would require a lot of labour and relatively skilled workers in many cases. Treatments should be applied regularly and arable land may be required for growing these plants (Hammond et al. 1994).

15.3.4 Long-Term Effects

They are known only for chemically defined molluscicides. Their effect on lymnaeids varies with the date of treatment (Worden et al. 1963). If applied in the spring, prior to the period of snail reproduction, the number of molluscs remains low and no growth in numbers like that seen in the control population is observed. If spreading is made in September, there is a drop in numbers, followed by their maintenance at low values. The density of snails can be modified depending on the nature of the habitat. Van den Bruel (1968) and Pécheur (1974) noted that the results obtained with the same molluscicide are not always overlapping from one *G. truncatula* habitat to another.

To be effective, chemical control must allow the complete elimination of the snail population. If a few individuals remain alive, this is sufficient to repopulate the habitat due to the high multiplying power of the species. It is therefore easy to imagine that the presence of shelters will allow snails to escape the action of treatment. Similarly, spreading over a small area often results in re-colonization of the habitat by snails coming from neighbouring untreated sites (Ximenes 1991).

15.4 Biological Control by Predation

15.4.1 Species of Predators

Several taxa of vertebrates and invertebrates are natural predators of freshwater molluscs, particularly Lymnaeidae. According to Dillon (2004), mammals, birds, turtles, salamanders, fish, crustaceans, insects, predatory molluscs, and leeches can feed on these snails. Several authors such as Pelseneer (1928), Mehl (1932), Michelson (1957), Taylor (1964), and Brown (1994) have reviewed the various groups of vertebrates and invertebrates involved in the predation of the snail hosts of *Schistosoma* spp. or *Fasciola* spp.

The predatory action of several groups of species resulted in a strong reduction in the number of lymnaeids. The malacophagous action of domestic ducks has been reported in several articles (Samson and Wilson 1973; Hull 2017, for example). If these birds are present in a meadow, the number of snails strongly decreased. According to SuSin (2001), a density of 5 to 10 ducks per ha in continuous grazing for a period of 1 to 2 months in irrigated rice significantly reduced the number of snails from 5/m^2 to less than 1 snail/m^2. However, not all palmipeds have the same ability to eat snails because it depends on their type of diet (Euzeby, 1971). Small waders, starlings, thrushes, and blackbirds incorporate *G. truncatula* in their diet but no figures have yet been reported to determine the amount of snails they can eat. According to Taylor (1964), the action of these birds also depends on the type of habitat in which *G. truncatula* lives because the predation of snails would be low or null in rush meadows.

The introduction of snail-eating fish like *Astatoreochromis alluaudi* (Cichlidae) into small dams in western Kenya and ponds in Cameroon has raised much hope. But the results were not satisfactory. According to Slootweg et al. (1993, 1994), no significant reduction in the number of bulinids was seen after the introduction of *A. alluaudi* in the water bodies of Cameroon. Other authors reported positive results in the elimination of snails when *Mylopharyngodon piceus* or *Trematocranus placodon* were used (Chiotha et al. 1991; Ben-Ami and Heller 2001), while the effects of native fish on snail reduction were limited in water bodies in the lower basin of the Senegal River (Arostegui et al. 2019). This variability in results may be explained by the fact that native or introduced fish species are only partially molluscivorous (Brown 1994).

The introduction of the crayfish *Procambarus clarkii* into irrigation canals in Egypt has resulted in a strong reduction and sometimes complete disappearance of snails (Ibrahim et al. 1995; Sleem and El-Hommossany 2008). Similar results were also obtained in Kenya (Mkoji et al. 1999). However, the introduction is not without danger because this crayfish also consumes aquatic plants (hence a partially molluscivorous diet) and undermines the earthen banks by digging burrows (Brown 1994). Another side effect could be observed due to the activity of many crayfish species as second intermediary hosts in the life cycle of *Paragonimus* spp.

The prawn *Macrobrachium vollenhovenii* is also capable of reducing the abundance and lifespan of snails in the Senegal River basin (Sokolow et al. 2015; Swartz et al. 2015) but this prawn is also partially molluscivorous (Jimoh et al. 2011). Predation by the water bug, *Sphaerodema urinator*, has been used to control host snails that transmit schistosomosis (Younes et al. 2017). According to Brown (1994), the only completely molluscivorous invertebrates are leeches of the Glossiphoniidae family and marsh flies of the Sciomyzidae family. Predation of *Glossiphonia complanata* was higher when leeches fed on juvenile *Stagnicola emarginata* (= *Ladislavella emarginata*).than on juveniles of *Physa gyrina* (Brönmark 1992). The Sciomyzidae had raised great hope to eliminate the intermediate hosts of *Fasciola gigantica* in the Hawaiian Islands in the 1950s. These flies had been released in these islands to eliminate the local host snail, but the results were not successful (Berg 1964). According to Knutson and Vala (2011), first, second, or third stage larvae often eat a limited part of the snail's body, i.e. the foot in most cases.

There are few aquatic predators of *G. truncatula* in central France. According to Rondelaud (1979), predation of adult dytiscids and their larvae was significantly greater than that of hirudinids and hemipterids. Sciomyzidae larvae were more common in small stagnant water collections such as pools and were not specific in the choice of their prey (Dreyfuss et al. 2002). On emerged areas, numerous terrestrial predators are present in the habitats of *G. truncatula* at the onset of summer drying. The larvae of *Lampyris noctiluca* (glowworm) are already known to be molluscivorous (Taylor 1964). The carabids *Anisodactylus binotatus*, *Platysma nigrita*, and *Poecilus cupreus* leave many empty and broken shells of *G. truncatula* at the end of their predation, while the shells remain intact in the case of other carabid species. This predation of prey occurred in the 2–3 weeks preceding the summer drying out of *G. truncatula* habitats (Rondelaud 1976). A land mollusc, *Zonitoides nitidus*, also consumes *G. truncatula* at the onset of summer drying (Rondelaud 1975). This ability of *Z. nitidus* was at the origin of biological control by predation that Rondelaud et al. (2006) developed for populations of *G. truncatula* living on acidic soils in central France.

15.4.2 Control of Galba truncatula

In central France, *Z. nitidus* is common in swampy meadows and along rivers on acidic soils. On sedimentary soils, it is only found on the banks of streams and rivers (Didier 1986). Its peculiarity comes from its diet. From September to May, it feeds on plant fragments as shown by the analysis of its intestinal content under a stereomicroscope (Rondelaud et al. 2006). From the month of June, the adult predates at the expense of other pulmonates and bivalves which live in the same habitat as it. Predation first affects *G. truncatula* and *Omphiscola glabra*, then *Succinea* sp. When the first cracks form in the drying soil, the *Pisidium* population pays its tribute. Finally, towards the end of July, *Z. nitidus* does not hesitate to eat its own congeners, either alive or dead (adults die during this period). According to Didier and Rondelaud (1989a, b), *Z. nitidus* needs this supply of animal proteins in order to ensure its reproduction (it lays in July). In addition, this snail has chitinase, which allows it to partially destroy areas in the shell of its victims.

Three techniques of snail control were used. The first consists of mowing the hygrophilous vegetation at the onset of summer drying and depositing it on the habitats of *G. truncatula*, which maintains humidity and attracts local predators. If necessary, *Z. nitidus* is introduced at a rate of 10 per m^2 of watercress bed and 20 per m^2 in other types of habitats. The second technique is not to mow the natural environment but a higher number of predators are introduced (60/m^2). The third technique consists of two steps: (i) a solution of cupric chloride (8 L to 0.1 mg/L) is poured into each habitat in April during the spring mating of *G. truncatula* and (ii) 20 Z. *nitidus*/m^2 are introduced or not at the beginning of summer drying (Ximenes 1991). These three techniques have been applied in 122 valley-bottom meadows, 59 hillside rush beds around temporary or permanent sources, 11 trampled areas, 37 river or pond banks, and 134 wild watercress beds (Rondelaud et al. 2006). Table 15.4 gives the survival rates of *G. truncatula* in September in several habitat types after 1, 2, 3, and 4 year(s) of control with *Z. nitidus*. Mowing of the hygrophilous vegetation in June and its deposition on the lymnaeid habitats resulted in the removal of *G. truncatula* after 2 years of control in the habitats located in swampy meadows and around temporary sources. In the case of wild watercress beds, 3 years

Table 15.4 Percentage of alive *Galba truncatula* counted in September in several types of habitats on acidic soils after a control of 1, 2, 3, and 4 years with *Zonitoides nitidus*. From Rondelaud et al. (2006)

Habitat type	Number of habitats	Percentage of alive *Galba truncatula* counted in September after a control of			
		1 year	2 years	3 years	4 years
Swampy meadows					
Controls	9	94.3	90.2	92.7	96.5
Treated with mowing	78	11.5	0.1	0	0
Treated without mowing	14	91.2	87.3	95.3	88.7
Rush beds with temporary source					
Controls	3	78.5	69.6	81.3	73.6
Treated with mowing	20	5.1	0.6	0	0
Treated without mowing	4	81.7	63.2	56.5	43.7
Rush beds with permanent source					
Treated with mowing	1	29.1	22.5	17.5	9.3
Treated without mowing	2	61.3	47.6	32.1	15.4
Wild watercress beds					
Treated with mowing	15	39.6	7.2	2.3	0

were necessary. In contrast, around the permanent sources, there was only a gradual decrease in the number of *G. truncatula* during the 4 years of control. In treated habitats without mowing, the populations of *G. truncatula* remained stable as controls (swampy meadows) or decreased slightly over time (both types of rush beds). A combination of predatory molluscs: *Z. nitidus* + *Oxychilus draparnaudi*, allowed the elimination of *G. truncatula* in a single year (rushes with temporary source) or 2 years (swampy meadows, wild watercress beds). Finally, in the 117 watercress beds treated with cupric chloride and *Z. nitidus*, no lymnaeid was observed in September after a single year of control, while in the 17 others, 2 years were required to eliminate *G. truncatula* (Rondelaud et al. 2006).

With the exception of a few owners who have introduced *Z. nitidus* into their watercress beds each year, the use of this zonitid to control *G. truncatula* has not become widespread in cattle and sheep farms in the French department of Haute Vienne. The reasons for this situation are probably the complexity of the techniques used to apply this control in the field by non-specialists and the difficulty for selecting the date of this control at the end of June due to the frequent rains (Rondelaud et al. 2006).

15.4.3 Long-Term Effects of this Control

Table 15.5 indicates, for each habitat type, the number of sites that were recolonized by *G. truncatula* in the years following the last application of biological control. In habitats located in swampy meadows and on river banks, most of them were re-invaded in the third or fourth post-control year. For areas around temporary sources and watercress beds, the re-colonization of most habitats occurred 6 or 7 years after the last application of control. In both cases, several habitats have not been recolonized by *G. truncatula* at the end of investigations (2005) and this fact can be easily explained by the location of these habitats at the peripheral end of the surface drainage networks.

The predator *Z. nitidus* showed numerical variations after its introduction into the habitats of *G. truncatula*. In swampy meadows, predators decreased in numbers for 2 years and then showed constant values. In the other three types of habitats, the predator populations disappeared during the first year (areas around sources), second year (river banks), or third year (watercress beds) after the last application of control. More surprising are the numerical variations that *O. glabra* presents in the treated habitats (*G. truncatula* and *O. glabra* often live in the same swampy meadows but each species occupies its own habitat: Vareille-Morel et al. 1999). Indeed, the number of snails per population and the area of habitat increased from the last application of biological control until the third year after. Subsequently, there was a gradual decrease in numbers while the habitat area did not change over time. As the extension of *O. glabra* occurs after the disappearance of *G. truncatula*, this finding suggests that there is probably competition for food between the two species. An

Table 15.5 Number of habitats recolonized by *Galba truncatula* in the years following the last application of biological control. n, total number of habitats studied. From Rondelaud et al. (2006)

Number of years after the last year of biological control (1978)	Number of *Galba truncatula* habitats recolonized			
	Swampy meadows ($n = 83$)	Rush beds with temporary source ($n = 25$)	Pond and river banks ($n = 21$)	Watercress beds ($n = 26$)
3	21	0	3	1
4	33	2	10	2
5	11	5	2	3
6	4	8	1	2
7	3	4	2	4
8	0	1	2	7
9	0	1	1	3
10	5	1	0	0
12	2	0	0	0
15	2	0	0	0
No re-colonization in 2005	2	3	0	4

argument supporting this interpretation is provided by Dreyfuss et al. (2006). According to these authors, when adults of both species are raised together in the laboratory, there is competition between the two lymnaeids, which causes the death of many *G. truncatula*.

As most field experiments were carried out on 12 farms over a 25-year period, it was interesting to determine the impact of this control on the prevalence of natural *F. hepatica* infection in cattle. Blood samples were therefore taken during the 4 years of control (1975–1978) on the ruminants from these herds and analysed serologically for antibodies against *F. hepatica*. In the first year, the frequency of infected animals was 11.7% (out of a total of 1514 cattle). In subsequent years, the prevalence fell to 0.26%, 0.15%, and 0.11%, respectively (Rondelaud et al. 2006).

15.5 Biological Control by Competition

Biological control of the host snails of schistosomes and other digeneans has been considered in recent decades as an alternative to molluscicides. Several groups of organisms have been proposed to control the host snails, but very few have proven their efficacy in the field. Competitive snails can be considered the most effective biological control agents and numerous promising laboratory studies and field experiments have been carried out, mainly in the Caribbean. Two species of Caenogastropoda snails belonging to the families of Ampullariidae (*Marisa cornuarietis*) and Thiaridae (*Melanoides tuberculata*) succeeded in eliminating or reducing the populations of schistosome-transmitting snails, especially *Biomphalaria glabrata* in several different habitats in Saint Lucia, Martinique, and Guadeloupe (Pointier et al. 2011). Three other species belonging to Physidae

(*Aplexa hypnorum*, *Physella acuta*) and Tateidae (*Potamopyrgus antipodarum*) can also be cited due to their competition with *G. truncatula*.

Unlike the intermediate hosts of schistosomes, there is much less work on the effects of these competitors on Lymnaeidae. Under laboratory conditions, *M. cornuarietis* can compete with *Lymnaea caillaudi* (= *Radix natalensis*), a planorbid and a bulinid by feeding on aquatic plants in the environment. The competitor is also capable of predatory activity by consuming egg masses, newborns, juveniles, and adults of the three snail species (Demian and Lutfy 1965, 1966). The introduction of *M. cornuarietis* in 1972 into an ornamental pond resulted in the disappearance of *P. columella* and a planorbid (Peebles et al. 1972). This result was confirmed by Nguma et al. (1982) who reported the disappearance of *R. natalensis* and other pulmonates in a small man-made dam 12 months after the introduction of *M. cornuarietis*. No pulmonate was found in subsequent snail samples collected by the authors over the next 2.5 years (Nguma et al. 1982). The omnivorous diet of *M. cornuarietis*, together with its large body mass, high reproductive efficiency and often high density, means that these snails can rapidly alter the structure of the macrophytic community, with consequent disturbances in nutritional balance, turbidity, and trophic structure of water bodies. The use of *M. cornuarietis* for such purposes is no longer encouraged at this time due to the adverse environmental effects of the species (Barker 2016). The role of *M. tuberculata* as a competitor of pulmonate gastropods is still controversial. In several Caribbean islands, this species has significantly reduced the populations of the planorbid *Biomphalaria glabrata* (Perera et al. 1990; Pointier and Augustin 2000; Pointier 2001) while the results are negative in irrigation canals in Sudan (Madsen et al. 1988). In Kenya, *M. tuberculata* lives in equilibrium with *R. natalensis* and other freshwater pulmonates (Mkoji et al. 1992). However, the presence of *M. tuberculata* in Tunisia causes a decrease in the number of annual generations in *G. truncatula* (2 instead of 4 in the control habitat) so that the authors conclude that there is competition between the thiarid and the lymnaeid (Ghouaidia and Hammami 2013).

The competition of *P. acuta* has already been reported by Michelson (1957) and Graber and Euzéby (1975). This species is able to exclude *G. truncatula* from its habitat when both species live in the same environment (Rondelaud 1978). Another species, *A. hypnorum*, is also able to compete with lymnaeids. If it is introduced into a ditch colonized by *G. truncatula*, its presence ultimately results in a displacement of the *G. truncatula* population upstream of the water collection and a progressive decrease in the size of the lymnaeid population over the 7 years of the study (Rondelaud et al. 2016). The competition of these two physids was only observed in small habitats of *G. truncatula* with stagnant or low-flowing waters and it is questionable whether this competition between physids and native species exists in other types of habitats. Although *P. acuta* was considered a competitor in Egypt (El-Hassan 1974), populations of this species often co-existed with planorbids in other African countries (Brown 1994). In addition, this competition with *G. truncatula* is not exclusive and has been observed with other species of freshwater snails. Several authors have reported that the presence of *P. acuta* in several countries resulted in competition with native snail species such as *Physastra* *variabilis* (syn. *Glyptophysa variabilis*) in New Zealand (Winterbourn 1980), *Bulinus tropicus* in South Africa (Brackenbury and Appleton 1993), or *Glyptophysa gibbosa* in South Australia (Zukowski and Walker 2009). In the USA, *A. hypnorum* also competes for food and habitat type with *Stagnicola elodes* (= *Ladislavella elodes*) and *Physa gyrina* (Brown 1982).

The invasive snail *Potamopyrgus antipodarum* is known to be a potential competitor with native snails such as two hydrobiid species in a freshwater spring in the USA (Richards et al. 2001) or another hydrobiid in Australia (Sardiña et al. 2015). In the presence of another competitor such as *P. acuta*, the growth and reproductive performance of both snail species in New Zealand are influenced more by the density of conspecifics than the presence and density of the other species (Cope and Winterbourn 2004). However, this competitor also acts on Lymnaeidae as reported by Rondelaud (1977a) for *G. truncatula* in several temporary habitats on French acidic and sedimentary soils. When there is a high concentration of *P. antipodarum* in habitats, *G. truncatula* cannot reproduce and there is a gradual decrease in the initial number of lymnaeid snails. If *G. truncatula* lays its eggs, the number of young individuals issuing from these masses is low. In addition, the presence of the competitor in the submerged zone forced the lymnaeid to emerge earlier in the season and daily migrations showed a short immersion time of the snails. The use of large groups of *Potamopyrgus* allows therefore to obtain an effective competition against *G. truncatula* during the immersion of habitats.

15.6 Biological Control with Pathogens

Like other animal groups, Lymnaeidae are sometimes infected by parasites, bacteria, and/or viruses. They can also host commensal species. As the list of these pathogens includes many species, the information presented in the paragraphs below mainly concerns the amphibious snails of the genus *Galba*.

Within Oligochaeta, *Chaetogaster limnaei* has an unusual parasitic relationship with freshwater pulmonate snails. Two subspecies are known: *C. limnaei limnaei* is an ectosymbiont and is found inside the mantle cavity of the snail, while *C. limnaei vaghini* is a parasite and lives in the snail's kidney. This oligochaete infects most species belonging to the Lymnaeidae family such as *G. truncatula* (Muñiz-Pareja and Iturbe-Espinoza 2018), *Lymnaea stagnalis* (Buse 1971), *P. columella* (Martins and Alves 2010), or *R. natalensis* (Fashuyi and Williams 1977; Ibrahim 2007). Most authors consider *C. l. limnaei* as an ectosymbiont because the animal consumes various small organisms (algae, rotifers, miracidia, trematode cercariae) that it manages to suck on its host (Michelson 1964; Fried et al. 2008). This efficacy of *C. l. limnaei* led Muñiz-Pareja and Iturbe-Espinoza (2018) to consider this oligochaete as a controller of *Fasciola hepatica* during experimental infections of snails. In France, *C. l. limnaei* was found in six populations of *G. truncatula* on acidic soils (out of a total of 317 populations studied). The infection of three *C. l. limnaei*-carrying populations with *F. hepatica* resulted in the death of snails between day 14 and day 21[t] post-exposure at 20 °C (Dreyfuss et al. 2015). Under these conditions, one can wonder whether this oligochaete would not be an ectoparasite which weakens the snail by its presence and causes the death of *G. truncatula* when the snail is subjected to an experimental infection with *F. hepatica.*

Terrestrial nematodes are capable of infecting molluscs of the Lymnaeidae family and can be used to control intermediate hosts of trematodes. Morley (2010), in his review, identified 18 species of nematodes which use lymnaeids as auxiliary intermediate hosts. One of these species, *Phasmarhabditis hermaphrodita*, is commercially available as a biological control agent for slugs and land snails. Among nematodes listed by Morley (2010), *Angiostrongylus cantonensis*, *Muellerius capillaris*, *Cystocaulus ocreatus*, *Elaphostrongylus rangiferi*, *Syngamus trachea*, and *Angiostrongylus dujardini* have the widest range of lymnaeids as intermediate hosts: 11, 8, 4, 4, 4, and 3 species, respectively. *Galba truncatula* may be infected with *C. ocreatus*, *E. rangiferi*, *M. capillaris*, *Neostrongylus linearis*, *S. trachea*, and *Varestrongylus sagittatus* (Morley 2010). The first stage (L1) larvae of these nematodes enter the snail, transform into second stage larvae (L2) and then into third stage larvae (L3) which exit from the snail before being ingested by the definitive host. But all these species of lymnaeids do not support the larval development of these nematodes from L1 to L3. Several *N. linearis* reached the L3 stage in *G. truncatula* subjected to monospecific infections while *M. capillaris* did not exceed the L2 stage (Hourdin et al. 1991). On the other hand, if *G. truncatula* is subjected to co-infections with *F. hepatica* and *M. capillaris*, or vice versa, L3 larvae of *M. capillaris* have been observed in snails with an abortive *F. hepatica* infection and the formation of these L3 in this case would be due to a phenomenon of facilitation (Hourdin et al. 1990).

The control of *Fasciola*-infected snails by another digenean has been the subject of few investigations. *Echinostoma audyi* has been proposed by Hoa et al. (1970) to control *F. gigantica* because the rediae of the former digenean predate on the sporocysts of *F. gigantica* within the snail. This control method was applied in the field by Estuningsih (1991, 1998) and Suhardono et al. (2006). According to the latter authors, the introduction of duck faeces containing *Echinostoma revolutum* eggs and cattle faeces with *F. gigantica* eggs into rice fields resulted in a decrease in the number of *Radix rubiginosa* infected with *F. gigantica* compared to control rice fields. According to Lim and Heyneman (1972), locally available parasites could be used to control the number of host snails in the field. For these methods to work, these authors indicated that the definitive host of the parasite intended for control must be present in sufficient numbers and be easily maintained and infected in order to produce enough parasites to overwhelm the snails. For such a control zone, a specific antagonist system must be put in place because a single system is not applicable in all cases (Lim and Heyneman 1972; Torgerson and Claxton 1999).

No bacteria capable of killing molluscs have yet been reported in Lymnaeidae. On the other hand, several bacteria have been tested against the vector snails of schistosomes. *Bacillus thuringiensis kurstaki* has negative effects on snail populations through molluscicidal activity and by preventing the hatching of *Biomphalaria alexandrina* eggs (Gamalat et al. 2011). *Brevibacillus laterosporus* has also been reported as a pathogen against juveniles of *Biomphalaria glabrata* (de Oliveira et al. 2004). A preliminary study suggested the potential pathogenicity of *Bacillus brevis* towards *Biomphalaria pfeifferi* and *Bulinus truncatus* (Singer et al. 1994), but this has never been tested in the field. Finally, a new bacterium, *Paenibacillus glabratella*, affected both adult snails and embryonic stages of *B. glabrata*, and has caused significant mortality. This bacterium invades most snail tissues and proliferates, causing high lethality, and can be transmitted both vertically and horizontally to other snails causing their death within 30 days. This latter bacterium may be promising as a biological control agent to limit the transmission of schistosomosis and other parasitoses in the field (Duval et al. 2015). To these pathogens, we must add the discovery of an iridovirus in a French population of *G. truncatula* after the sudden occurrence of numerous deaths of snails and the examination of their corpses by transmission electron microscopy (Barthe et al. 1984). Field investigations were then carried out by Rondelaud and Barthe (1992) in 11 French populations of *G. truncatula* to determine the prevalence of this viral infection. Prevalence ranged from 3% to 87% and was rather constant in eight snail populations. The three others showed variations in frequency over time (from 23% to 69%). *Iridovirus sp.* is a common infection in snails living in central France, suggesting an equilibrium between the virus and its host (Rondelaud and Barthe 1992). Further studies are still needed to determine whether this virus affects other lymnaeid species such as *O. glabra* that live in the same grasslands as *G. truncatula* and whether it can be proposed as a biological control agent.

Several species of fungi have been used to study their activity on the intermediate hosts of *Schistosoma* spp. Contact of *B. alexandrina* with sublethal concentrations of an extract of *Aspergillus fumigatus* for 24 h before, during, or after exposure of snails to *Schistosoma mansoni* resulted in a significant reduction in the rate of infection and a decrease in the total number of cercariae shed by surviving snails (Gamalat et al. 2013). The same type of contact between *B. alexandrina* and sublethal concentrations of a filtrate of *Aspergillus terreus* or *Penicillium janthinellum* caused adverse effects on the gonad of snails with degeneration or deformation of the ova and spermatozoa (Saad et al. 2014). Development of *B. glabrata* eggs was significantly slowed down when hyphae or conidia of *Beauveria bassiana* and *Metarhizium anisopliae* were present (Duarte et al. 2015). Conversely, no report on the effects of these fungi on Lymnaeidae is yet available in the literature.

15.7 Integrated Liver Fluke Control Strategy

No single control strategy such as drainage or application of a molluscicide can effectively reduce the transmission of fasciolosis. The objective of this strategy is therefore to use the various prophylactic means available to eradicate fasciolosis in some farms and limit the frequency of the disease in most other farms (Mage and Rondelaud 1983). An integrated parasite control programme is therefore needed to control this parasitosis. Several studies were conducted to test a strategic integrated control programme to reduce the prevalence of fasciolosis in central France (Mage et al. 1989, 1995; Vignoles et al. 2016) and in Peru (Claxton et al. 1998; Raunelli and Gonzalez 2009).

The first step in this strategy is to determine the presence of the parasite in cattle by coproscopy or serology. The topography of the land, bearing capacity of the soil, runoff circulation, and pasture management must also be analysed. The snail habitats are isolated in a second step with a fence and deworming of the definitive host is carried out using an anthelmintic. The third step is the choice of the technique to eliminate the populations of snails. The isolation of habitats must be carried out in the absence of any other possibility. Very swampy areas must be transformed into ponds. Surface drainage must be carried out when the areas to be treated are large. Small areas such as rush beds should be treated with molluscicide or predatory snails.

A first trial took place in a cattle farm in the department of Corrèze (central France). In 1986, the herd consisted of 30 bovines and the coproscopies for *F. hepatica* were all positive. *G. truncatula* was only found in one area where cattle waited before entering stalls. The various measures in the concept were applied to the herd and the waiting area. The *G. truncatula* disappeared at the end of 1988 and the coproscopies performed after the cleaning of the waiting area were all negative (Mage et al. 1989). Further trials were carried out in the summer meadows in mountains. Three dairy farms in the French department of Cantal have simultaneously applied fasciolicide to 164 cattle and eliminated the sources of infection present on these pastures. At the end of the second year, the percentage of cattle naturally infected with *F. hepatica* was only 11.1% at the time of grazing, whereas it was 57.4% in the 10 farms which used only fasciolicide alone as therapeutic prevention (Mage et al. 1995). Another trial was performed on two farms between 2011 and 2014 by isolating the habitats of *O. glabra* from the rest of the pastures, as the area of these habitats on acidic soils is generally small. Isolation of *O. glabra* in both farms resulted in a decrease in the prevalence of snail infections in 2012 and the disappearance of *F. hepatica* larval forms in snails in 2013 and 2014. In cattle, the prevalence decreased gradually to its negativation in 2014. These results show that it is possible to safeguard the populations of the host snail during an integrated control in livestock while interrupting the development cycle of the parasite (Vignoles et al. 2016).

In Peru, a first control programme involving two doses of triclabendazole together with the use of niclosamide was evaluated for a single year. This double treatment did not significantly reduce the overall parasite burden, although there was a significant reduction in the number of intermediate host snails after the application of molluscicide (Claxton et al. 1998). Another study carried out by Raunelli and Gonzalez (2009) in the same country over a 2-year period involved three fasciolicide treatments per year, strategically timed according to the epidemiological cycle of the disease, and environmental control activities aimed at irrigation ditches and paddock drainage. The application of this integrated control led to a decrease in the prevalence of *F. hepatica* in cattle (from 63% to 14%), a 38% increase in the live weight of cattle, and a 75% increase in milk production over the 2 years of the study.

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