Lymnaea truncatula, intermediate host of some Plagiorchiidae and Notocotylidae species in León, NW Spain

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Abstract

To study the distribution of *Lymnaea truncatula* in the Porma river basin (León, NW Spain) and its helminth fauna, malacological samplings were carried out at 66 points in the basin and the presence of these molluscs was detected in 31. To trace the dynamics of this mollusc population and the prevalence and intensity of its infection by trematodes, malacological samplings were made at fortnightly intervals over two years at five locations, situated in the upper and middle regions of the river Porma basin. To confirm the identity of the larval stages found in the molluscs, second intermediate and/or definitive hosts, depending on the trematode species, were experimentally infected to complete the life cycles. Two different species of Plagiorchiidae and one of Notocotylidae were identified. The infection prevalence of *Plagiorchis elegans* for the 6291 specimens of *L. truncatula* examined was 2.8% and infection was observed in snails collected in 7 of the 31 sample localities. This parasite was found in all months of the year, with the highest prevalence observed in July and October. When the corrected frequency values were considered, a slightly positive relationship was observed between the infection prevalence and the latter months. The highest percentages of snails harbouring immature sporocysts were detected in March and June–July, while the highest percentages with sporocysts containing mature cercariae were observed in spring and at the end of summer–autumn, and cercarial shedding in the latter. Of the 6291 *L. truncatula* examined 0.3% were infected by sporocysts of *Opisthioglyphe ranae*. The infection was detected in only one locality, in four months of the year, with the highest prevalence in May. *Notocotylus neyrai* was found in 2.6% of the 6291 mollusc specimens checked and was collected from 12 of the 31 localities. The highest prevalence was observed in October, April and July and the corrected frequency values reveal a positive relationship between the infection prevalence and those three months. The highest percentage of molluscs harbouring rediae of *N. neyrai* with germinal mass was found in April and September–October. On the other hand, rediae with mature cercariae were present in almost every month, but we only observed cercarial shedding in October and March. The *L. truncatula* infection prevalence by *P. elegans*, *O. ranae* and *N. neyrai* increased with the size of the molluscs. Double infection was only observed in four snails: one harboured *O. ranae* and *N. neyrai* and three *F. hepatica* and *P. elegans*.

Introduction

The basommatophoran mollusc *Lymnaea truncatula* (Müller, 1774) is well-known as an intermediate host of *Fasciola hepatica* Linnaeus, 1758. This trematode is responsible for fasciolosis, a parasitic disease distributed throughout much of the world and which causes large economic losses in ruminants and zoonotic problems in man.

Within the framework of a research programme on the epidemiology of fasciolosis in León Province (NW Spain), the natural infection of *L. truncatula* by *F. hepatica* was studied for two consecutive years (Manga-González *et al.*, 1991) and egg excretion of *F. hepatica* by cattle (González-Lanza *et al.*, 1989) and sheep (Manga-González *et al.*, 1990) grazing in the study area.

L. truncatula has also been found harbouring the following species of trematode: *Echinostoma revolutum* in Lithuania (Kiseliene, 1963); *Echinostoma revolutum* and *Paramphistomum* in Hungary (Merenyi, 1978); *Paramphistomum microbothrium* (Samnaliev & Vassilev, 1981), *Echinostoma revolutum* (Vassilev & Kamburov, 1972), *Echinostoma echinatum* and *Echinoparyphium recurvatum* (Kanev, 1985) in Bulgaria; *Haplometra cylindracea* and *Notocotylus* sp. in France (Hourdin *et al.*, 1991); *Echinostoma revolutum* and *Hypoderaeum conoideum* in Ukraine (Skovronskii, 1985); *Haplometra cylindracea* in Russia (Budalova, 1986); a species of Plagiorchiidae and *Notocotylus neyrai* in Spain (Simon-Vicente & Ramajo-Martin, 1978; Simon-Vicente, 1979; Simon-Vicente *et al.*, 1985); *Cercaria truncatuloides* n.sp. (*Paramphistomum*) in the German Democratic Republic (Odening & Samnaliev, 1987).

Therefore, we considered it worth while to study the trematode fauna in *L. truncatula*. It was hoped to establish a differential diagnosis between *F. hepatica* and other trematodes, to carry out the specific determination of these, to discover the mutual relationship of the parasite species in the molluscs, and to follow the dynamics of the infection by each trematode.

Materials and methods

In order to discover the distribution of *L. truncatula* in the Porma river basin (León, NW Spain) and its helminth fauna, malacological samplings were carried out at 66 points in the basin. *Lymnaea truncatula* was detected in 31 sites, four of which were situated on the lower part of the river (UTM coordinates: 30TTN91) and the rest in the middle and upper basin (UTM coordinates: 30TUN01 to 30TUN27). The study area has a continental climate within the Mediterranean–Atlantic transition. According to the monthly temperature and precipitation data (period 1951–1980) from one meteorological station situated in the highest part of the Porma basin and another in the lowest part, the maximum temperature oscillated between 11.5°C (February, December) and 36.4°C (June), the minimum between −15.6°C (February) and 3°C (July) and the average between −0.1°C (February, December) and 19.3°C (July). Average precipitation also varied between 16.6 mm (August) and 192 mm (December).

Fortnightly malacological samplings were made between March 1985 and March 1987 at five of these locations: Vegaquemada, Primajas, Orones, Redipollos and Cofiñal. These sites, chosen because molluscs infected by larval stages of *Fasciola hepatica* had previously been detected there, are situated in the upper and middle regions of the Porma river basin. The sampling methods for *L. truncatula* and the periodic variation of its habitats at the five localities are the same as those described by Manga-González *et al.* (1991).

In fig. 1, monthly distribution according to shell length (from mm to mm) of the 6291 molluscs examined for trematode presence is summarized. Generally, between 30 and 70 *L. truncatula* specimens were checked for each collection point and date, depending on abundance.

Dissection of individual snails was performed *in vivo* using a stereomicroscope to detect and extract the larval stages of the digenetic trematodes found in their various organs. The intensity of the infection in each mollusc was estimated, due to the great difficulty in counting parasites. So three categories were established concerning hepatopancreas infection intensity: 1) very localized and reduced infection; 2) infection that had spread to the rest of the hepatopancreas but with areas that were more or less free of parasites; 3) a very intense infection that had destroyed almost all of the hepatopancreas and that, on occasions, had spread to other organs.

Although most of the parasites found in the molluscs remain preserved in 70° alcohol in our collection, a number of fresh larval stage specimens, representative of those found in the molluscs or shed by them, were studied morphologically, drawn, measured and photographed. Moreover, the chaetotaxy technique was used on some emitted cercariae.

In order to confirm the identity of the larval stages found in the molluscs, second intermediate and/or definitive hosts, depending on the trematode species, were experimentally infected in the Institute of Parasitology of Bulgaria to complete the life cycles and obtain adult specimens.

Two laboratory mice were each fed with 30 *Notocotylus* metacercariae collected from the bottom and walls of the Petri dish. The mice were killed 18 days post-infection (p.i.) and examined for adult parasites.

In the case of the two plagiorchiid species, attempts were made to infect 40 *Culex pipiens* specimens as second intermediate hosts, and one *Mus musculus* and one *Gallus gallus domesticus* as definitive hosts with one of the species. Twenty *L. truncatula* and tadpoles were used as second intermediate hosts and three *Rana temporaria* as definitive hosts for the other species. To obtain the adult trematodes,

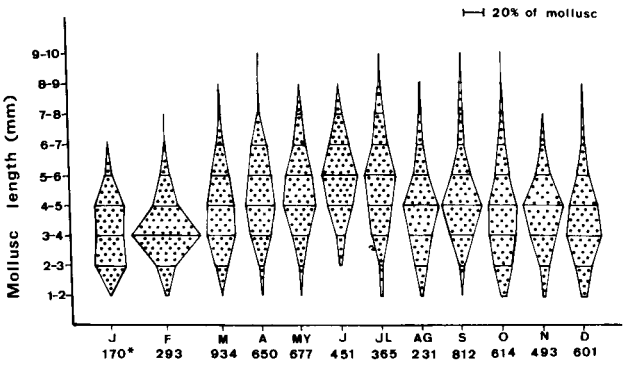

Fig. 1. Monthly distribution, according to shell length, of the molluscs examined each month. Scale value is based on monthly total examined.

one mouse and one chicken were fed 20–30 metacercariae each and killed 8 days p.i. Likewise, three frogs were each fed 50 metacercariae and killed 50 days p.i.

The prevalence figures, the corrected frequency of the prevalence (partial prevalence ÷ total prevalence) and the estimated infection intensity for the different groups of snails according to shell length (from mm to mm) were calculated taking into account all the molluscs examined (6291) from the 31 localities. The monthly values of these parameters were obtained considering all the examined snails (5476) from the five localities, where fortnightly malacological samplings were carried out for two years. Similarly, the data referring to the monthly percentage of infected molluscs, according to different stages of larval development, were calculated on the total of snails examined (6291).

In reference to the corrected frequency, only the results that indicate a positive relationship (those higher than 1.2) are presented here.

Results

When the 6291 specimens of *L. truncatula* were examined to detect digenetic trematodes, not only *F. hepatica* larval stages (Manga-González *et al.*, 1991) but also other species of Plagiorchiidae and Notocotylidae were found. However, double infections by trematodes were observed in only four of the 973 snails found infected.

The larval stages of the plagiorchiid species cannot be mistaken for those of *Fasciola hepatica*, because the cercariae of the former are of the xiphidiocercaria type and they develop within sporocysts, while those of *F. hepatica* have no stylet and develop within rediae. Moreover, plagiorchiid metacercariae develop within different second intermediate hosts, while those of *F. hepatica* do so on plants (or other supports).

In the same way, the *Notocotylus neyrai* cercariae can be distinguished from those of *F. hepatica* due to their having only one oral sucker and two or three (depending on their development) eye spots. Although both develop inside rediae, these are quite different because the *N. neyrai* rediae have a long and wide digestive tract that occupies most of the rediae, but not the typical raised collar near the anterior end and the two bulging appendages in the posterior third of the body of *F. hepatica*. Although the metacercariae of *N. neyrai* become infective, like those of *F. hepatica*, in the external medium, they have three conspicuous eyespots lacking in the case of *F. hepatica*.

Family Plagiorchiidae

On comparing the plagiorchiid larval stages found by us in *L. truncatula* with those described by Palm (1966), Grabda-Kazubska (1969), Samnaliev *et al.* (1982), Bock (1984), Genov & Samnaliev (1984) and Dimitrov *et al.* (1989), it was possible to identify two different species: *Plagiorchis elegans* and *Opisthioglyphe ranae*. This identification was experimentally confirmed later on obtaining 6 and 11 *Plagiorchis elegans* adults from one *Mus musculus* and one *Gallus domesticus*, respectively and 2, 5 and 16 *Opisthioglyphe ranae* adults recovered from 3 *Rana temporaria* specimens.

The *L. truncatula* specimens found naturally infected with these parasites harboured sporocysts in the hepatopancreas at different stages of development, that is: 1) Immature sporocysts or those with germinal mass; 2) Sporocysts with immature cercariae; 3) Sporocysts with mature cercariae; 4) Sporocysts that shed cercariae. Sometimes parasites at different stages of development were found in the same snail.

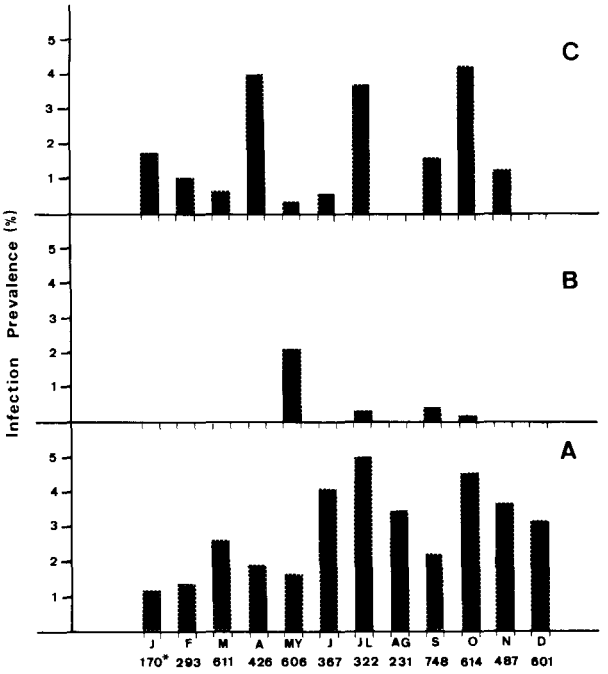

Fig. 2. Monthly prevalence of infection by: A) *Plagiorchis elegans*, B) *Opisthioglyphe ranae*, C) *Notocotylus neyrai*. *Number of molluscs examined.

Plagiorchis elegans *(Rudolphi, 1802) Braun, 1902*

The infection prevalence of this trematode for the 6291 specimens of *L. truncatula* examined was 2.8%. The highest proportion (55.9%) of the snails which were found harbouring parasites showed an infection intensity belonging to category 1 (see Materials and methods), followed by the molluscs with infection intensity category 2 (37.3%).

This parasite was observed in snails collected in seven of the 31 sample localities, in three of which (Primajas, Cofiñal and Redipollos) fortnightly malacological samplings were made. The highest infection prevalence (10.6%) was detected in *L. truncatula* from Cofiñal. Moreover, according to the corrected frequency value (3.59), there is a positive relationship between the infection prevalence of the molluscs and that locality.

Taking into account the results obtained from the helminthological studies of 5476 *L. truncatula* specimens (collected fortnightly at the 5 sampling points), we found the parasite in all months of the year, although the highest values for infection prevalence (fig. 2A) were observed in July (5.0%) and in October (4.6%). When the corrected frequency values were considered, a slightly positive relationship was observed between the infection prevalence and the latter months (1.68, 1.54, respectively) and November (1.25).

Infection intensity was very variable throughout the year but categories 3 and 1 were predominant in April and October, respectively.

When snail size was taken into consideration, we observed a clear increase in infection prevalence with mollusc length (fig. 3A). The corrected frequency values reveal a positive relationship between the infection prevalence of the molluscs and the groups with a shell length of 8–9 mm (4.45), 7–8 mm (3.13) and 6–7 mm (2.80).

When the different larval development stages of *Plagiorchis elegans* found throughout the year in *Lymnaea truncatula* were considered (fig. 4), immature sporocysts or those with germinal mass were observed almost every month, although the highest percentages of snails harbouring these types of stages were detected in March and June–July. Likewise, the highest percentages of molluscs with sporocysts containing mature cercariae were observed in spring (March–April) and at the end of summer–autumn, and cercarial shedding in the latter period.

Double infection with *F. hepatica* and *P. elegans* was observed in three *L. truncatula* specimens collected at different times of the year in Redipollos.

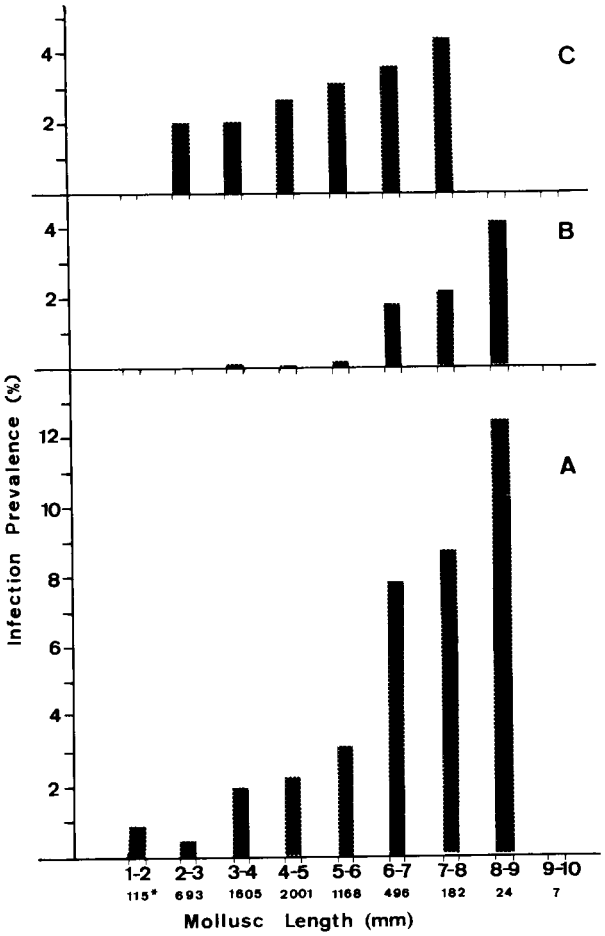

Fig. 3. Prevalence of infection according to mollusc shell length, by: A) *Plagiorchis elegans*, B) *Opisthioglyphe ranae*, C) *Notocotylus neyrai*. *Number of molluscs examined.

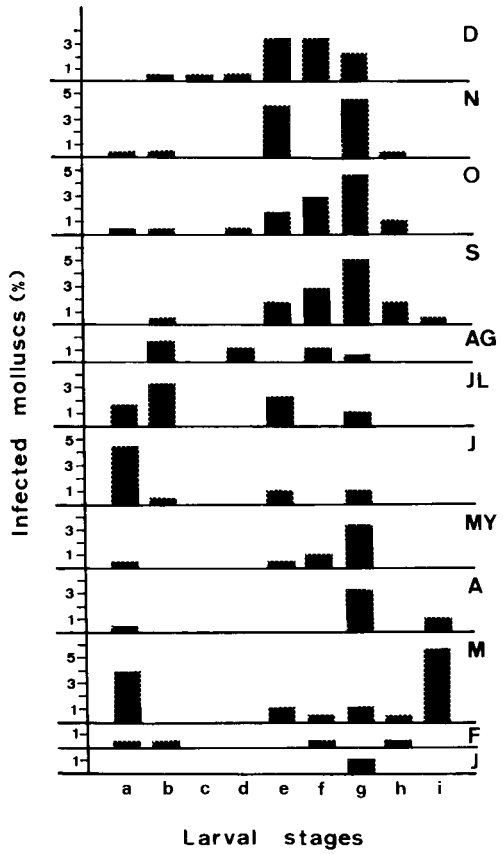

Fig. 4. Monthly percentage of molluscs infected (on the total of them) by *Plagiorchis elegans* according to different development stages harboured. a=Immature sporocysts or those with germinal mass (Sgm); b=Sgm+Sporocysts with immature cercariae (Sic); c=Sgm+Sporocysts with mature cercariae (Smc); d=Sgm+Sic+Smc; e=Sic; f=Sic+Smc; g=Smc; h=Smc+sporocysts that shed cercariae (Ssc).

Opisthioglyphe ranae *(v. Frölich, 1791) Looss, 1907*

Of the 6291 *L. truncatula* specimens examined 0.3% were infected by sporocysts of *O. ranae*. The infection was only detected in the locality of Vegaquemada. It seems that, according to the corrected frequency value (5.89), there is a positive relationship between the mollusc infection prevalence by this trematode and this locality.

O. ranae was found in only four months of the year (fig. 2B) with the highest values of infection prevalence (2.1%) and intensity in May. When the corrected frequency values were considered, a positive and a slightly positive relationship were detected between the mollusc infection prevalence and May (6.68) and September (1.25), respectively.

The prevalence of infection rose with increasing host size (fig. 3B). The corrected frequency values reveal a positive relationship between the infection prevalence of the molluscs and the groups with a shell length of 8–9 mm (14.37), 7–8 mm (7.58) and 6–7 mm (6.24).

In May we found a higher number of molluscs harbouring this parasite at different development stages (fig. 5).

Family Notocotylidae

The notocotylid larval stages found by us in *L. truncatula* correspond to *Notocotylus neyrai* according to Simón-Vicente *et al.* (1985). Moreover, the larval identification was confirmed on obtaining three and five adult worms from each of the experimentally infected mice.

Notocotylus neyrai *González-Castro, 1945*

The *L. truncatula* specimens found naturally infected with *N. neyrai* contained rediae at different stages of development in the hepatopancreas as follows: 1) rediae without internal differentiation or with germinal mass; 2) rediae with rediae inside; 3) rediae harbouring immature cercariae; 4) rediae containing completely mature monostoma cercariae; 5) rediae that shed cercariae. On several occasions rediae at different stages of development were found in the same mollusc.

N. neyrai was found in 2.6% of the 6291 mollusc specimens collected from 12 of the 31 localities, in four of which (Vegaquemada, Cofiñal, Redipollos and Orones), fortnightly malacological samplings were made. The highest values for infection prevalence and corrected frequency were detected in the snails collected from localities at a lower altitude above sea level. Nevertheless, it should be pointed out that relatively few molluscs were examined from these habitats. When taking into account the five points in which fortnightly malacological samplings were made, the highest infection prevalence was observed in Vegaquemada (5.0%).

With reference to intensity, categories 2 and 3 were predominant in October.

N. neyrai was found in all months of the year except August and December (fig. 2C). Nevertheless, the prevalence and intensity of infection did not follow any clear pattern. The highest value for infection prevalence was observed in October (4.2%) although April and July showed similar percentages for infection (3.9 and 3.7, respectively). When the corrected frequency values were considered, a positive relationship was observed between the mollusc infection prevalence and October (2.7), April (2.5) and July (2.3). The highest proportion (53.3%) of the snails which were found harbouring parasites showed an infection intensity belonging to category 1, followed by the molluscs with infection intensity category 2 (35.7%).

A clear influence of mollusc size on the infection prevalence was observed (fig. 3C). This parameter value rose with length, reaching the maximum (4.4%) in molluscs measuring 7–8 mm in length. The corrected frequency values revealed a positive relationship between the infection prevalence of the molluscs and the groups with a shell length of 7–8 mm (1.68), 6–7 mm (1.39) and 5–6 mm (1.21).

The degree of larval stage development of *Notocotylus neyrai* in the molluscs did not follow any clear pattern throughout the year (fig. 6). We found rediae with germinal mass in almost all months, although the highest percentage of molluscs harbouring this stage was found in April and September–October. On the other hand, rediae with mature cercariae were present in almost every month, but we only observed cercarial shedding in October and March.

A double infection with *O. ranae* and *N. neyrai* was found in only one *L. truncatula* specimen from Vegaquemada (July).

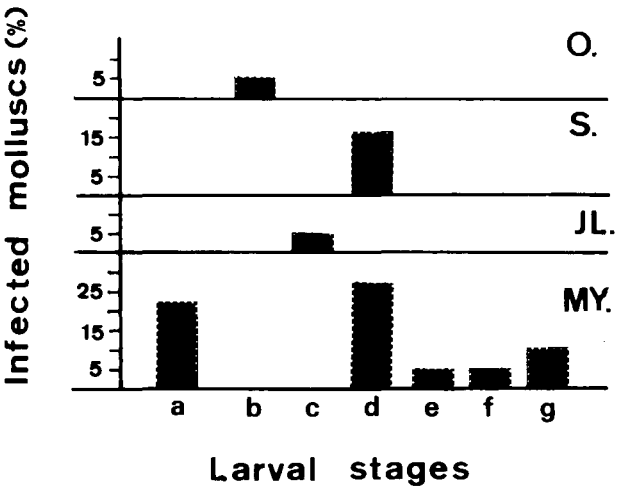

Fig. 5. Monthly percentage of molluscs infected (on the total of them) by *Opisthioglyphe ranae* according to different development stages harboured. a=Immature sporocysts or those with germinal mass (Sgm); b=Sgm+Sporocysts with immature cercariae (Sic); c=Sgm+Sporocysts with mature cercariae (Smc); d=Sic; e=Sic+Smc; f=Smc; g=Sporocysts that shed cercariae (Ssc).

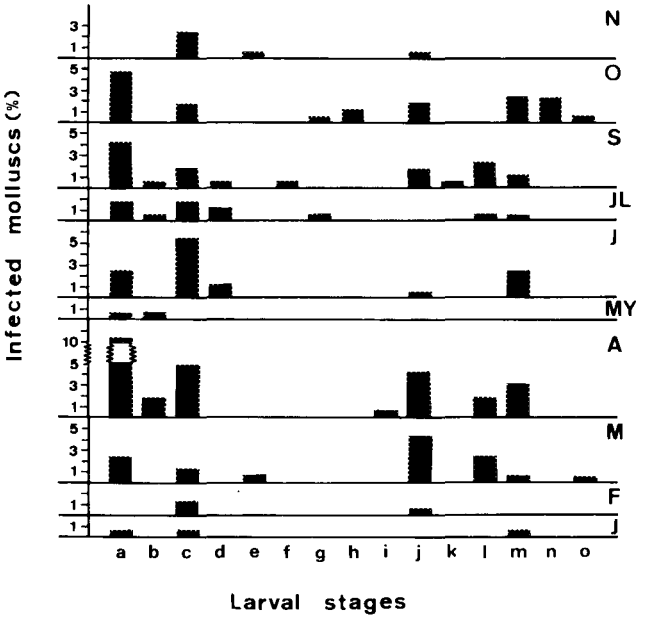

Fig. 6. Monthly percentage of molluscs infected (on the total of them) by *Notocotylus neyrai* according to different development stages harboured. a=Rediae without internal differentiation or with germinal mass (Rgm); b=Rgm+Rediae with rediae inside (Rr); c=Rgm+Rediae harbouring immature cercariae (Ric); d=Rgm+Rediae containing completely mature cercariae (Rmc); e=Rgm+Rr+Ric; f=Rgm+Rr+Rmc; g=Rgm+Ric+Rmc; h=Rgm+Rmc+Rediae that shed cercariae (Rsc); i=Rr; j=Ric; k=Rr+Rmc; l=Ric+Rmc; m=Rmc; n=Rmc+Rsc; o=Rsc.

Discussion

According to the literature this is the first time that both species of plagiorchiid have been recorded in *L. truncatula* in Spain, although Simón-Vicente & Ramajo-Martín (1978) and Simón-Vicente (1979) found xiphidiocercariae of plagiorchiid specimens in the same species of mollusc collected in Salamanca (Spain).

O. ranae and *N. neyrai* have been found in different definitive hosts in Spain (González-Castro, 1945; Combes & Gerbeaux, 1970; Simón-Vicente *et al.*, 1985; Climent *et al.*, 1987; amongst others), while *Plagiorchis elegans* has not been quoted in our country according to our information. As can be deduced from our results, the infection prevalence of *L. truncatula* by *P. elegans* (2.8%) was quite similar to that of *N. neyrai* (2.6%) and higher than that of *O. ranae* (0.1%).

These prevalence values are much lower than those obtained by Manga-González *et al.* (1991) for infection of the same mollusc by *Fasciola hepatica* (11.4%). Nevertheless, it should be remembered that the data for *F. hepatica* refer only to the fortnightly samplings carried out at the five localities in which it had previously been detected.

According to Simón-Vicente (1979) *L. truncatula* from Salamanca were more frequently infected by notocotylid and fasciolid trematodes than by plagiorchiids. This fact does not coincide with our observations.

The value of *N. neyrai* infection prevalence found by us (2.62%) in *L. truncatula* falls between those of *Notocotylus* sp. given by Hourdin *et al.* (1991) in the same species of snail in France (1–9%). Nevertheless, our figures are much lower than those obtained by Simón-Vicente *et al.* (1985) in Salamanca (4–50%).

When the dynamics of monthly prevalence infection for *P. elegans* and *N. neyrai* studied in this paper were compared with that found by us (Manga-González *et al.*, 1991) for *F. hepatica* in the same molluscs, it seems that, generally, three peaks can be considered in these three species of trematodes throughout the year. The maximum values for each peak, in decreasing order, were detected in July, October and March for *P. elegans* and October, April and July for *N. neyrai* and October–November, July and February for *F. hepatica*.

According to our results, *L. truncatula* infection prevalence by *P. elegans*, *O. ranae* and *N. neyrai* increased with the size of the molluscs. This agrees with the observations by Hourdin *et al.* (1991) in their studies on *L. truncatula* infection with *Notocotylus* sp. and with *H. cylindracea*, and by Simón-Vicente (1979) in the same species of molluscs harbouring *N. neyrai*, Plagiorchiidae sp. and other species of trematodes.

The peak periods for *L. truncatula* infection by *P. elegans* seem to be March and June–July, because it was in these months when the highest rate of molluscs harbouring immature sporocysts was observed. Nevertheless, the most appropriate periods for the infection of the second intermediate hosts (*Culex pipiens*) could be spring (March–April) and autumn, because the highest number of molluscs shedding cercariae was detected in these seasons.

When the results obtained by us for *O. ranae* are taken into account, it appears that infection of the second intermediate hosts (or definitive hosts in the abbreviated life cycle) may occur in May since it was in this month when we observed the highest number of *L. truncatula* specimens shedding cercariae. This seems to agree with Grabda-Kazubska (1969) who found numerous young specimens of *Opisthioglyphe rastellus* in young *Rana terrestris* and *R. temporaria* frogs just after metamorphosis in July.

Our observations of *N. neyrai* infections coincide only partially with the results obtained by Simon-Vicente *et al.* (1985). These authors pointed out May–June as the months in which parasitism was higher, while in the Porma basin the highest value for infection prevalence was observed in October followed by April and July. May is also the month in which Hourdin *et al.* (1991) found the highest rate of *L. truncatula* harbouring *Notocotylus* sp.

According to our results, mollusc infection by *N. neyrai* mainly occurs in April and September–October, since the highest percentages of molluscs harbouring rediae with germinal mass were found in these months. In the same way, the most appropriate months for the infection of the definitive host could be October and March, due to the highest percentages of molluscs shedding cercariae being detected in these months. Our observations do not coincide with those of Simon-Vicente *et al.* (1985) because for these authors it is at the end of February when young rediae of *N. neyrai* are harboured by the molluscs, while cercarial shedding is quicker and more constant at the end of spring.

Taking into account the results of this paper and those of Manga-González *et al.* (1991), *F. hepatica* cercarial shedding by *L. truncatula* examined occurs in September–December, which seems to indicate a delay when referring to the time of year in which cercarial shedding of the three species of trematodes, was observed.

According to our data, it seems that the presence of one trematode species in a mollusc could prevent establishment of another species since only 0.4% of the 973 snails found infected harboured two species of trematode at the same time. Hourdin *et al.* (1991), in a study on natural infection in France of *L. truncatula* by the plagiorchiid species *Haplometra cylindracea* (Zeder, 1800) and by *Notocotylus* sp., never found specimens with double infections.

Antagonism phenomena have also been observed in *L. truncatula* (=*Galba truncatula*) between *F. hepatica* and *Paramphistomum microbothrium* and between the latter and *Echinostoma lindoense* (Samnaliev *et al.*, 1978) and between *Haplometra cylindracea* Müller and *Fasciola* (Moukrim & Rondelaud, 1992).

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