

SURVEY OF THE PARATENIC HOSTS OF *Anguillicola crassus* IN LAKE VELENCE, HUNGARY

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(Received January 17, 1994)

Anguillicola crassus infection of paratenic hosts was investigated in Lake Velence, Hungary, in October 1992. A total of 155 specimens of 8 species (*Gymnocephalus cernuus*, *Lepomis gibbosus*, *Stizostedion lucioperca*, *Abramis brama*, *Alburnus alburnus*, *Cyprinus carpio*, *Pseudorasbora parva* and *Rutilus rutilus*) were examined: all species proved to be infected. There were large differences in the prevalence of infection among fishes, but the prevalence was generally higher in Perciform fishes and it was the highest in *G. cernuus* (100%). The occurrence and physical stage of larvae did not depend on the size of the fish. In *S. lucioperca* the size of third stage larvae was bigger than in other fishes, and only live larvae were found. The comparison of Lake Velence and Lake Balaton indicates that the rate of larval infection in paratenic hosts of *A. crassus* is higher in Lake Velence. This might be explained by the significant ecological differences between the two lakes (population of intermediate hosts, water depth, salinity, pH).

Key words: *Anguillicola crassus*, paratenic hosts, Lake Velence

Anguillicola crassus is a parasitic nematode of eels (*Anguilla* spp.), which originates from East Asia (Kuwahara et al., 1974). It was introduced to Europe in the early 'eighties via the import of infected eels for consumption or restocking, and spread quickly through most European countries (Peters and Hartmann, 1986). In Hungary *A. crassus* was first recorded in Lake Balaton in 1990 by Székely et al. (1991), who reported heavy infections in eels. They did not observe dead fish in the lake, but found mortality among collected, transported and stored (stressed) specimens.

This species is widely distributed in the Japanese eel (*Anguilla japonica*) living in natural waters and eel farms of Japan but causes no, or very little damage. However, the European eel (*Anguilla anguilla*) introduced to Japan develops much heavier infection by this nematode which produces

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pathological changes in the swimbladder and causes the eels to lose their appetite and become emaciated (Egusa, 1979).

By now *A. crassus* infection of the European eel (*A. anguilla*) has become widespread in natural basins and in intensive eel cultures (Moravec and Taraschewski, 1988). Some reports indicate that in intensive culture this infection can cause economic losses (Møllergaard, 1988). Repeated mortality caused by *A. crassus* in eels living in natural waters has been reported by Molnár et al. (1991, 1993).

The intermediate hosts involved in the life cycle of *A. crassus* are copepods in which second stage larvae (L₂) develop to the third stage (L₃) infective to eels. As copepods are not considered a major food item for eels, because eels of larger size feed almost exclusively on fish and on chironomids (Paulovits and Biró, 1987), it was reasonable to assume that other fish species might act as paratenic hosts (Haenen and Van Banning, 1990).

These paratenic hosts are facultatively involved in the life cycle of *A. crassus*. The parasites do not develop within them; they only accumulate there and remain infective to the definitive host only (Thomas and Ollevier, 1992).

In Europe there have been only a few reports on the occurrence of *A. crassus* larvae in various fish species other than eels (De Charleroy et al., 1990; Haenen and Van Banning, 1990; Thomas and Ollevier, 1992). These authors have reported that the transfer of L₃ from experimentally infected *Leuciscus idus* (ide), *Cyprinus carpio* (common carp), *Osmerus eperlanus* (smelt) and *Gymnocephalus cernuus* (ruffe) is possible. They mentioned that these fish species are able to act as paratenic host, and they are facultative in the life cycle of the parasite, which means that the parasites do not develop within them, only accumulate and remain infective only to the final host. The most comprehensive investigations on the paratenic hosts of *A. crassus* were conducted by Thomas and Ollevier (1992) on fishes of Belgian channels and by Székely (1994) on fishes of Lake Balaton, Hungary.

Data obtained from a survey of *A. crassus* larval infection of different freshwater fish species of Lake Velence are presented in this paper.

Materials and methods

Materials

A total of 155 fish were caught by net from Lake Velence (Hungary) on 15 October 1992. These fish belonged to the following 8 species: bleak (*Alburnus alburnus*), bream (*Abramis brama*), common carp (*Cyprinus carpio*), ruffe (*Gymnocephalus cernuus*), pumpkinseed (*Lepomis gibbosus*), Chinese rasbora

(*Pseudorasbora parva*), roach (*Rutilus rutilus*), and pike perch (*Stizostedion lucioperca*). The fish were immediately sent to the laboratory alive, where they were kept in aquaria until dissected for detailed light microscopic examination.

Characteristics of Lake Velence

The water surface of the lake is 25 km². The lake is shallow, with an average water depth of 1–1.6 m. Annual average water temperature of the lake is 10.8 °C. The pH of the water is high (8–9.5) and its salinity is not the same in different parts of the lake: in the average season it varies between 1,500 and 2,000 µS cm⁻¹ and in dry periods it is 2,200–2,700 µS cm⁻¹. Water is supplied by rainfall, Brook Császár and small springs. The fish population mostly comprises cyprinids and percids. Stocking of eel into the lake was started 25 years ago and finished 10 years ago. The eel population of the lake comprises only large specimens (with a length exceeding 50 cm).

Methods

The length of the fish was measured and they were dissected at the laboratory. The presence of *A. crassus* was checked in squash preparations made between two slides. Preparations from swimbladder (including the surrounding mesenteric tissues), stomach, intestine, liver, and kidney were examined under a light microscope. The total number of live and dead L₃ larvae was determined by moving the microscopic field step by step in a zigzag line at 32- to 400-fold magnification.

Statistical analysis of the data of relevant fish species was performed and "prevalence" and "mean intensity" were calculated to provide an index of the degree of dispersion of *A. crassus*.

Results

Prevalence and mean intensity

A total of 155 fish specimens of 8 species, belonging to 4 different families and 2 orders, were examined. With the exception of 2 *Alburnus alburnus*, 1 *P. parva* and 8 *S. lucioperca*, all fishes (144) were found infected with L₃ larvae, at least with one larva (Table 1). The highest prevalence (100%; Fig. 1) and mean intensity (184 larvae per infected fish; Fig. 2) were found in *G. cernuus*, followed by *Alburnus alburnus* and *R. rutilus* (Table 2). In some cases only 1 to 3 individuals (*Abramis brama*, *C. carpio*, *L. gibbosus* and *P. parva*) were examined, but they were also infected. Species belonging to Perciformes showed a higher prevalence of live larvae than most Cypriniformes.

Table 1
Anguillicola crassus larval infection of paratenic host fishes in Lake Velence

Fish species	Mean length cm	No. of non-infected fish	No. of fish infected only by dead larvae	Mean intensity	No. of fish infected by live and dead larvae	Mean intensity (live larvae)	Mean intensity (dead larvae)	No. of fish infected only by live larvae	Mean intensity	Total no. of fish
<i>Alburnus alburnus</i>	7.7	2	22	8	18	1	7	3	2	45
<i>Abramis brama</i>	8.8	0	1	16	0	0	0	0	0	1
<i>Cyprinus carpio</i>	4.4	0	1	4	0	0	0	0	0	1
<i>Gymnocephalus cernuus</i>	6.8	0	0	0	77	96	88	0	0	77
<i>Lepomis gibbosus</i>	4.5	0	1	10	0	0	0	2	1	3
<i>Pseudorasbora parva</i>	8.5	1	1	1	1	3	5	0	0	3
<i>Rutilus rutilus</i>	7.5	0	2	14	7	11	8	0	0	9
<i>Stizostedion lucioperca</i>	7.5	8	0	0	0	0	0	5	3	13

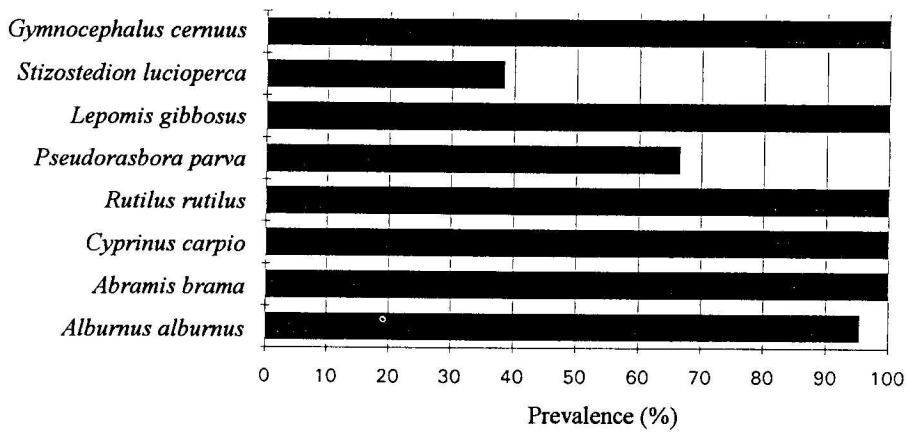


Fig. 1. Prevalence of *Anguillicola* larval infection in different fish species in Lake Velence, 1992

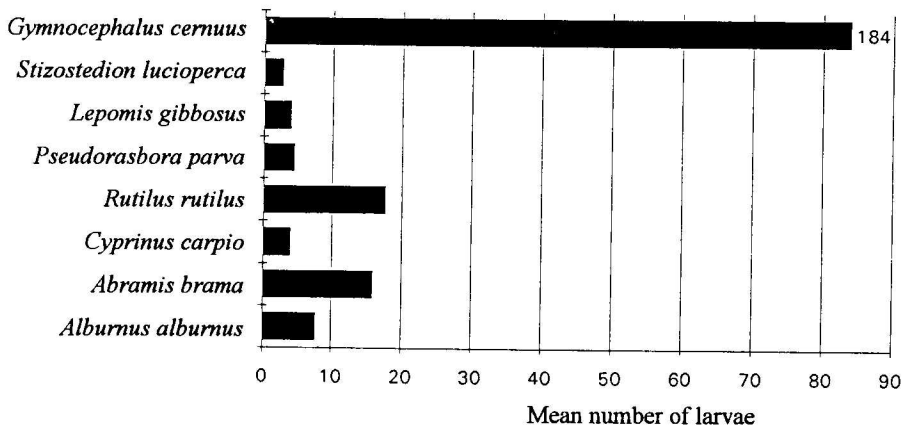


Fig. 2. Mean intensity of *Anguillicola crassus* infection in different fish species in Lake Velence, 1992

The overall mean intensity and range of L₃ larvae differed by fish species. Mean intensity was the highest in species showing the highest prevalence and was lower for other species (Table 2).

Table 2
Prevalence and mean intensity of infection by 3rd stage *Anguillicola* larvae in the fish examined

Fish species	No. of fish examined	Mean length (cm)	Prevalence (%)	Mean intensity	Range min.- max.
<i>Alburnus alburnus</i>	45	7.7	95.5	7.74	1-37
<i>Abramis brama</i>	1	8.8	100*	16	-
<i>Cyprinus carpio</i>	1	4.4	100*	4	-
<i>Gymnocephalus cernuus</i>	77	6.8	100	184	103-290
<i>Lepomis gibbosus</i>	3	4.5	100*	4	1-10
<i>Pseudorasbora parva</i>	3	8.5	66.6	4.5	1-8
<i>Rutilus rutilus</i>	9	7.5	100	17.7	7-36
<i>Stizostedion lucioperca</i>	13	7.5	38.4	2.8	1-4

*Only a few fish were examined or only one fish was infected

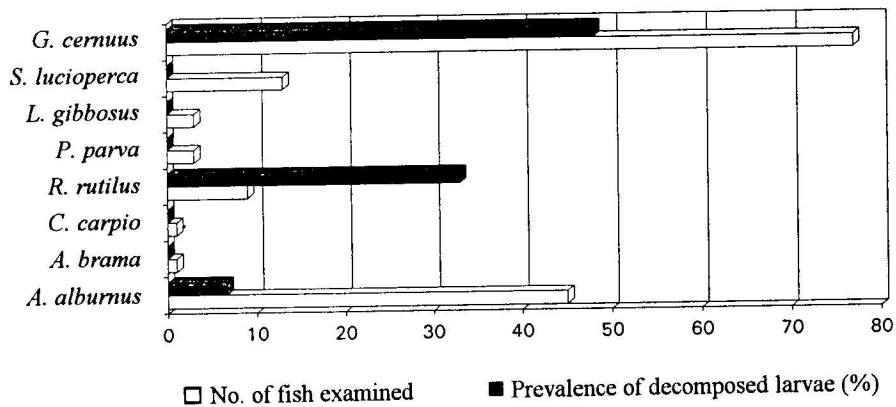


Fig. 3. Ratio of decomposed third-stage larvae of *Anguillicola crassus* in different fish species of Lake Velence, 1992

Table 3
Prevalence and mean intensity of decomposed larvae in different fish species

Fish species	No. of fish examined	Mean length (cm)	No. of fish infected by decomposed larvae	No. of decomposed larvae	Prevalence (%)	Mean intensity	Range min. - max.
<i>Alburnus alburnus</i>	45	7.7	3	4	6.6	1.3	1-2
<i>Abramis brama</i>	1	8.8	0	0	0	0	0
<i>Cyprinus carpio</i>	1	4.4	0	0	0	0	0
<i>Gymnocephalus cernuus</i>	77	6.8	37	45	48	1.2	1-2
<i>Lepomis gibbosus</i>	3	4.5	0	0	0	0	0
<i>Pseudorasbora parva</i>	3	8.5	0	0	0	0	0
<i>Rutilus rutilus</i>	9	7.5	3	11	33	3.6	1-7
<i>Stizostedion lucioperca</i>	13	7.5	0	0	0	0	0

Appearance and identification of Anguillicola crassus larvae

According to the description of Moravec and Taraschewski (1988), all larvae found in or around the swimbladder and in fresh preparations from other inner organs of the different fish species were determined as L₃ larvae of *A. crassus*. A rounded head end with 4 papillae, 2 amphids and 2 vertically oriented labia which surround a split-shaped mouth opening, the darkly coloured body and the conical, short tail could be distinguished in the light microscope.

In the majority of cases freely located live L₃ larvae were found, whose viability could easily be detected by their movements. Less frequently dead larvae covered only by a thin layer of connective tissue were detected. However, encapsulated live and dead larvae in granules were also found.

In fish species with a high prevalence and mean intensity of infection encapsulated wormlike structures of different shape and size were found which could not be positively identified as dead L₃ larvae, but which we assumed to be degenerated larvae. In *G. cernuus* these degenerated larvae were very common and were found in 48% of all infected *G. cernuus*. In one *R. rutilus* 7 such larvae were counted while only 3 out of 45 *Alburnus alburnus* contained degenerated larvae (1–2 larvae per fish). In other fish species we could not detect degenerated larvae (Table 3, Fig. 3).

The reproductive organs of female fish were more infected than those of males. In the ovaries of females the number of unencapsulated live larvae was high, but in other organs the rate of infection was approximately the same in both sexes.

Only 5 out of the 13 *S. lucioperca* specimens examined were infected, and only by live larvae. These larvae were bigger than those found in other fishes.

Discussion

The present study shows that a great variety of freshwater fish species can carry *A. crassus* larvae. This part of the life cycle has not been noticed in Asia, the original region of the nematode, and has been studied in Europe only for four years.

This is the first attempt to survey the prevalence of *Anguillicola* larval infection in paratenic fish hosts species in Lake Velence, Hungary.

In comparison with the study of Haenen and van Banning (1990), in this study more species were found infected and the numbers of larvae found in the various species were in a broader range.

Considering only the more frequently infected species, infection was not restricted to a certain size class. Already young fry (length: 2.9 cm) were infected

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Prevalence and mean intensity of decomposed larvae in different fish species

Fish species	No. of fish examined	Mean length (cm)	No. of fish infected by decomposed larvae	No. of decomposed larvae	Prevalence (%)	Mean intensity	Range min. - max.
<i>Alburnus alburnus</i>	45	7.7	3	4	6.6	1.3	1-2
<i>Abramis brama</i>	1	8.8	0	0	0	0	0
<i>Cyprinus carpio</i>	1	4.4	0	0	0	0	0
<i>Gymnocephalus cernuus</i>	77	6.8	37	45	48	1.2	1-2
<i>Lepomis gibbosus</i>	3	4.5	0	0	0	0	0
<i>Pseudorasbora parva</i>	3	8.5	0	0	0	0	0
<i>Rutilus rutilus</i>	9	7.5	3	11	33	3.6	1-7
<i>Stizostedion lucioperca</i>	13	7.5	0	0	0	0	0

by *A. crassus* larvae, but the number of larvae in older fishes was higher. One of the reasons for dispersion in older fish might be the individual variability in prey selection.

Larval survival rate in the various fish species is still not known, but the high prevalence of encapsulated dead larvae in comparison with that of live larvae in Cypriniformes, similar to what was observed for fishes of Lake Balaton (Székely, 1994), may indicate a special host reaction which decreases the survival rate of larvae. On the other hand, *S. lucioperca* harboured only live larvae, suggesting that the longevity of *A. crassus* larvae might differ in different species of fishes. The high intensity of live larvae in the reproductive organs of females may also indicate that the longevity of larvae might differ in the various organs.

The bigger (overgrown) larvae seen in the swimbladder wall of *S. lucioperca* indicate that in some fish species L₃ larvae may reach a larger size than usual: they grow bigger and their intestine becomes darker with or without reaching the L₄ stage (less motile, dark-coloured intestine). In the study of Thomas and Ollevier (1992) these grown larvae were seen in the swimbladder of *P. fluviatilis*, *S. lucioperca* and *G. cernuus*. All these fishes infected by grown larvae belong to Perciform fishes. The high prevalence of live and overgrown larvae might indicate that the survival rate of larvae in these fishes is higher than in Cypriniformes.

Fishes belonging to physoclist (closed swimbladder) and physostome (open swimbladder) species were equally represented in this study. There were large differences in prevalence and mean intensity among the fish species, and the prevalence of infection was generally higher in physoclist fishes. The swimbladder wall of *G. cernuus* showed the highest rate of, in agreement with what was reported by Thomas and Ollevier (1992). It is not clear whether the difference in swimbladder structure or some other physiological or ecological difference plays a role in these findings.

Other aspects in which the fishes differed were feeding habit, although most fish feed on plankton during at least some period of their life (Lazzaro, 1987). The most heavily infected species in Lake Velence, the ruffe (*G. cernuus*), is a benthic fish. This is consistent with the results of a Swedish study on a benthic paratenic host of *Anguillicola*, the black goby (*Gobius niger*; Höglund and Thomas, 1992). Both the ruffe and the black goby feed on worms, insect larvae and zooplankton, and look for food in the mud. On the other hand, free-living L₂ larvae sink to the bottom, and the prevalence of infection in copepod intermediate hosts is the highest in the bottom region. These data indicate that a benthic mode of life (in deeper water layers) exposes the fish to a higher risk of becoming infected with *A. crassus* larvae. Most Perciformes are benthic fishes but the Cypriniformes involved in this study are mostly planktivorous species. This might be a reason why the rate of infection is lower in these fishes.

Due to their bottom-dwelling mode of life and the restricting effect of mouth size, eels prefer rather small fish inhabiting the bottom of lakes (Sinha and Jones, 1967; De Nie, 1988). A benthic mode of life enhances the probability of infection and of transferring L₃ larvae to the eel.

Paratenic hosts accumulate *A. crassus* larvae and contribute to their transfer to the definitive host but are not essential for the completion of the life cycle (Ryzhikov, 1964). Thomas and Ollevier (1992) suggested in their study that some species like *Alburnus alburnus* and *R. rutilus* were only occasionally infected and could therefore be considered accidental hosts. Our study has proved that these species are not accidental hosts, because the prevalence and intensity of infection in these fishes are considerable. Our findings are supported by the results of Székely (1994) who, calculating with the feeding habits of eels in Lake Balaton, found that the bleak (*Alburnus alburnus*) was the most important paratenic host for *A. crassus* in that lake.

There is a wide range of possible paratenic hosts with high infection rates in Lake Velence and Lake Balaton. A comparison between the two lakes revealed that the rate of infection is higher in Lake Velence than in Lake Balaton (Székely, 1994). This result is rather surprising because Lake Balaton has a significantly bigger eel population than does Lake Velence, though the rate of *A. crassus* infection of eels is the same in both lakes. It seems that in Lake Velence the larvae can find more intermediate hosts and have better chances of further development. This contradictory phenomenon might be explained by the significant ecological differences between the two lakes, such as shallow and eutrophic water, temperature or salinity.

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