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Development of glochidia of *Anodonta piscinalis* and their infection of fish in a small lake in northern Finland

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With 5 figures and 1 table in the text

Abstract

The development of glochidia in *Anodonta*, their seasonal patterns of infection in four fish species and their infection in relation to length and sex of the host individual were studied in a small hyper-eutrophic lake in Northern Finland. Glochidia development took place from June to August and they were fully developed when the water temperature began to fall in autumn. The glochidia were stored in the gill blades to be released in spring. All four fish species, the perch, roach, pike and ruffe, were infected in spring. The prevalence of infection of the perch was high throughout the infection period whereas the roach had a high prevalence of infection only at breeding season. The larger roach were infected more often than smaller ones.

Introduction

The freshwater Unionidae of the temperate zone reproduce annually (Ellis, 1978). Reproduction can be divided temporally into two stages: 1. the development of glochidia in the clam and 2. the invasion of the gills of the host fish by glochidia and their metamorphosis there. The timing of these stages is adjusted to fit the seasonally fluctuating environment.

The parasitic stage is the most critical phase in the life-cycle of the Unionidae. Only a very small proportion of the glochidia are capable to attaching themselves to the fish and going through metamorphosis successfully (Coker et al., 1921; Young & Williams, 1984). Theoretically, the adaptations that improve the survival at the most critical stage in the life cycle, in this case the parasitic stage, have highest fitness response. Unfortunately, not much is known about the details of the parasitic stage of the Unionidae.

The development of glochidia requires energy and it is probably for this reason that they are produced in summer when resources are best. It has been shown that the cost of reproduction in *Anodonta piscinalis* (Nilss.) is low, and

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the reproduction should not increase adult mortality (Haukioja & Hakala, 1978; Tuomi et al., 1983).

Glochidia infection in fish can follow one of two timing patterns:

1. The glochidia can be released in autumn to inhabit the fish throughout the winter. The benefits of this are good dispersal and the long time available for metamorphosis. This type of infection is common in the genus *Unio* (Negus, 1966) which prefer running water (Negus, 1966; Brönmark & Malmqvist, 1982).

2. The glochidia can be stored over winter in the gill blades of the female clam, to be released in the spring. Since the fish are infected for a shorter time, the time available for metamorphosis is also shorter in this case, although this can be partly compensated for by the higher water temperatures accelerating the development of the attached glochidia. The incubation of glochidia in the gill blades over winter is a cost factor for the female clams but can be minimized if the glochidia are ready in the autumn and are only stored and maintained in the gill blades throughout the winter. This type of infection is reported for species of *Anodonta* (Negus, 1966).

We study here the development of *Anodonta piscinalis* glochidia in clams in relation to water temperature and compare glochidia production and release with a detailed infection pattern for fish individuals in relation to species, sex and size of the fish.

Material and methods

Study area

The area studied, Lake Kuivasjärvi, is located in the city of Oulu (65 °N, 25 °30′E), in the northern Finland (Fig. 1). It is a small (0.84 km²), shallow (mean depth 1.9 m), almost hypereutrophic lake (Myllymaa, 1978) which is covered by ice from late October to early May.

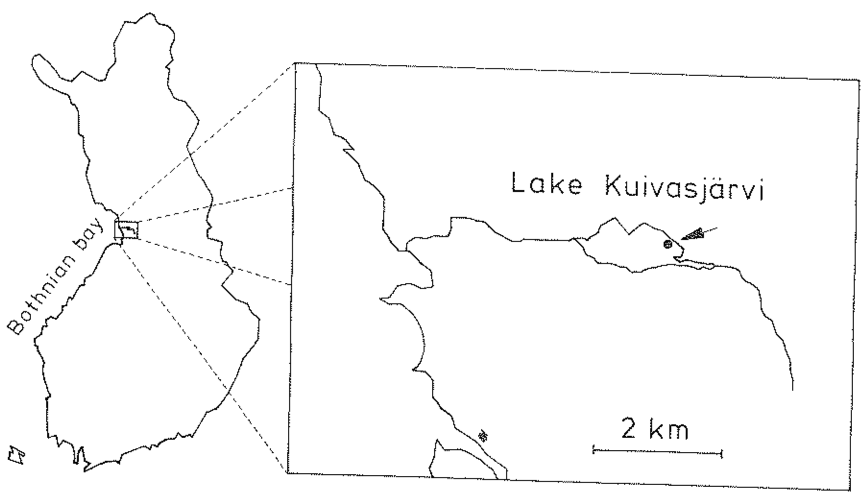

Fig. 1. Location of Lake Kuivasjärvi. The sampling site is indicated by an arrow.

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The narrow littoral zone of the lake has a very dense population of *Anodonta piscinalis* (NILSS.). The distribution of *Anodonta* is restricted to the littoral zone partly because the sediment there is sand, which this species prefers (BOYCOTT, 1936), and because the deeper parts of the lake are covered by 0.5—1 m of mud and suffer from anoxia during winter (MYLLYMAA, 1982).

The most common fish species inhabiting the lake are perch (*Perca fluviatilis* (L.)), roach (*Rutilus rutilus* (L.)), ruffe (*Gymnocephalus cernuus* (L.)), pike (*Esox lucius* L.) and crucian carp (*Carassius carassius* (L.)). The fish populations suffered from anoxic conditions in winter during the period covered by this research. Migration of fish to the lake from the Bothnian Bay, part of the Baltic Sea, is known to occur for breeding purposes in spring. This may partly compensate for the losses encountered by the fish populations during the winter.

Anodonta

16 samples of about 20 *Anodonta piscinalis* specimens each were collected from May 1980 to May 1981 including weekly samples in July—August. Samples were collected

Table 1. *Anodonta piscinalis* and four fish species sampled in Lake Kuivasjärvi between May 1979 and April 1981.

	Anodonta studied (samples)	Perch		Roach		Ruffe		Pike	
		%	(n)	%	(n)	%	(n)	%	(n)
1979 V–VI	*	95.0	(20)	42.1	(19)	–	–	100	(6)
VII	*	0	(20)	–	–	0	(2)	0	(15)
VIII	*	0	(30)	0	(31)	0	(11)	0	(1)
IX	*	0	(30)	0	(28)	–	–	0	(5)
X	*	0	(20)	–	–	–	–	0	(5)
XI	*	0	(21)	0	(22)	0	(3)	0	(8)
XII	*	0	(21)	0	(22)	–	–	0	(14)
1980 I	*	0	(6)	–	–	–	–	–	–
II	*	–	–	–	–	–	–	–	–
III	*	85.0	(20)	10.0	(10)	37.5	(8)	–	–
IV	*	89.5	(19)	6.3	(16)	81.0	(21)	–	–
V	20 (1)	51.3	(39)	67.5	(40)	100	(3)	55.5	(27)
VI	60 (3)	20.0	(10)	0	(10)	–	–	–	–
VII	83 (4)	0	(20)	–	–	0	(4)	0	(6)
VIII	81 (4)	0	(16)	–	–	0	(20)	0	(5)
IX	40 (2)	*	*	*	*	*	*	*	*
X	40 (2)	*	*	*	*	*	*	*	*
XI	19 (1)	*	*	*	*	*	*	*	*
XII	20 (1)	*	*	*	*	*	*	*	*
1981 I	17 (1)	*	*	*	*	*	*	*	*
II	20 (1)	*	*	*	*	*	*	*	*
III	20 (1)	*	*	*	*	*	*	*	*
IV	20 (1)	*	*	*	*	*	*	*	*
Total	440 (22)	25.8	(292)	18.7	(198)	28.8	(72)	20.2	(103)

* indicates cases samples were not taken, and – cases in which no fish could be caught. Monthly prevalences (%) of glochidia infection on gills of the fish are given.

monthly from November 1980 to April 1981 when the lake was covered with ice (Table 1).

The maximum length of the clams was measured in the laboratory to the nearest mm, the occurrence of glochidia in the outer gill blades recorded and the body and outer gill blades weighed separately to the nearest 0.01 g.

The length adjusted mass of the female gill blades was calculated to observe the development of the glochidia. The weekly differences in the measured gill masses were tested with ANCOVA using the length of the individual as a covariate. Both measures were ln-transformed. Length adjusted means were calculated and ANCOVA performed with the MGLH module of SYSTAT statistical package.

Fish

Fish samples were collected monthly between May 1979 and August 1980 using gill-nets and fish traps, but despite this effort it was not possible to catch all the fish species each month. A total of 292 perch and 198 roach, 104 pike and 80 ruffe were studied (Table 1).

The length (mm) and weight (g) of each fish was measured, the gonads examined to determine the sex and developmental stage, and the gill arches cut away on one side of the fish and the attached glochidia counted unter a dissection microscope.

The data on the perch and roach were extensive enough for detailed examination of the infection patterns. The sex and size bound prevalences of infection (X^2-test and Mann-Whitney U-test, respectively) was compared between these species. In the tests where infection was involved only the data for the infection period was used (March to June). Statistical analysis were performed using the NPAR and TABLES modules of the SYSTAT statistical package.

Results

Development of glochidia in relation to temperature

Fully developed glochidia were observed on the outer gill blades of many of the clams upon inspection for the first time in the third week of May, the prevalence of clams with glochidia being 17 % (Fig. 2). No glochidia nor eggs were found in the three samples collected in June. The first eggs and developing glochidia were observed in early July, the mean proportion of clams with developing glochidia in July being about 50 %. The glochidia developed quickly from July onwards and they seemed to be fully developed in August. The colour and fullness of gill blades did not change markedly during the winter. The proportion of clams in December and March samples carrying glochidia was 20 % or lower, as also in the sample collected in May.

Comparison of the weekly length adjusted means for the mass of the gill blades containing glochidia starting from the third week of May 1980 (Fig. 3) confirmed the visual observations on the development of the glochidia. The mass of the gill blades with glochidia increased rapidly in summer reaching its peak in late August. The differences in the masses compared in terms sampling date were statistically significant (ANCOVA, length as a covariate, gills with ke was covered with ice

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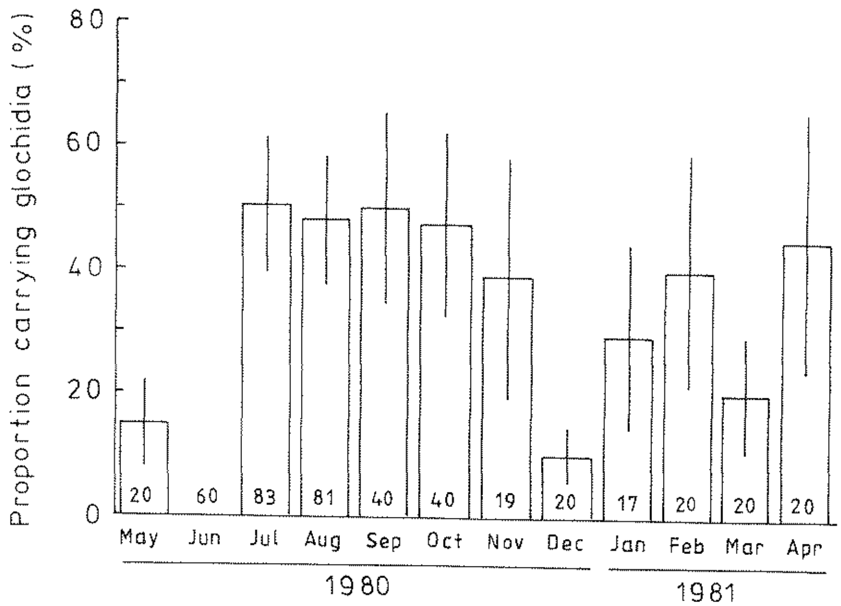

Fig. 2. *Anodonta piscinalis* recovered from Lake Kuivasjärvi between May 1980—April 1981. Monthly proportion of clams carrying glochidia (%). The bars show 95% confidence intervals. The number of clams studied in each month is depicted in figure.

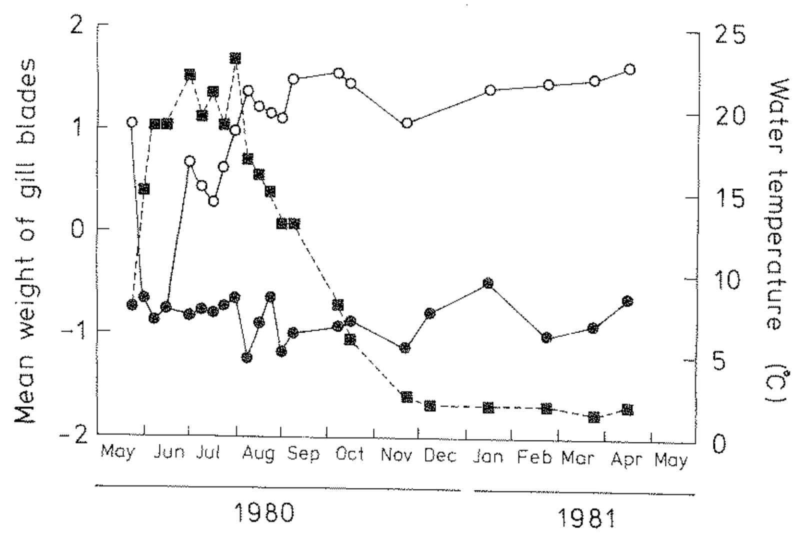

Fig. 3. Length adjusted mass of gill blades of *Anodonta piscinalis* carrying glochidia (open circles) and not doing so (closed circles). The dotted line denotes the water temperature.

glochidia $F_{1,17} = 13.83$, $P < 0.001$), but no such trend was observed in the mass of the gill blades not containing glochidia, although the mass differences were statistically significant when compared by sampling date (ANCOVA, length as a covariate, gills without glochidia $F_{1,21} = 6.38$, $P < 0.001$).

These results obviously coincide with the changes in water temperature. Water warmed up rapidly in spring and the maximum water temperature was reached in the first week of August, after which the temperature decreased steadily (Fig. 3).

Glochidia infection in the fish

The fish were infected with glochidia in spring and early summer. In 1979 sampling was started in May, but only a few fish were caught (although all of them were infected) (Table 1). The data of May and June were therefore combined, resulting in 95 % of the perch, 42 % of the roach and all the pike captured being infected. No glochidia were found on any of the fish species from July onwards to the end of the year.

In spite of many attempts, no samples were obtained in January and February 1980, the only fish caught being the six uninfected perch. The prevalence of infection in perch was high from March to June (85 %, 90 %, 51 %, 20 %, respectively) (Table 1), but that in the roach only in May (10 %, 6 %, 68 %, 0 %, respectively). Pike samples were obtained only in May, when the prevalence of infection was 56 %. Infected ruffe were found whenever fish of this species were caught. In April, when a sufficient number of ruffe were caught, the prevalence of infection was 81 % (Table 1). In June two perch out of ten contained glochidia, while in July—August all the fish samples were free of glochidia.

The intensity of glochidia infection between March and June was higher in the perch than in the roach when the years were combined (Mann-Whitney U-test, U = 6875, $P < 0.001$).

The females of the both perch and roach fish were longer than males (Fig. 4), the difference was statistically significant in both species (Mann-Whitney U-test, U = 5322, $P < 0.001$ for perch and U = 472.5, $P < 0.001$ for roach). Only in the roach did the prevalences of glochidia infection differ statistically significantly between the sexes, being higher in the females (X^2-test, $X^2 = 3.75$, P = 0.053 and Yates corrected $X^2 = 11.68$, P = 0.001 for perch and roach respectively). The infected roach were also statistically significantly longer than the non-infected ones (Mann-Whitney U-test, U = 471, $P < 0.001$) whereas the infected perch were not (Mann-Whitney U-test, U = 993.5, P = 0.104).

Since the female roach were in general bigger and their prevalence of glochidia infection higher, the effect of sex and length on infection was evaluated. The infected female roach were statistically significantly longer than the not-infected ones (Mann-Whitney U-test, U = 365.5, P = 0.014). The differences among males were not tested as there were only three infected males.

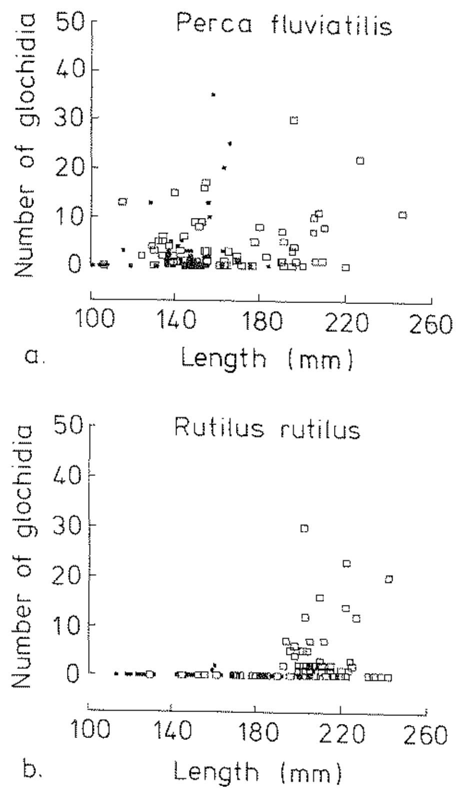

Fig. 4. Numbers of glochidia on a) *Perca fluviatilis* and b) *Rutilus rutilus* in March—June in relation to length of the fish. Open squares = females, closed squares = males.

Discussion

The details of the parasitic pattern of the Unionidae are moulded by the three functions that the parasitic stage serves. Firstly, the time spent attached to a fish must be long enough for metamorphosis. Secondly, the young clams should leave the fish at the beginning of the new growing season in order to minimize first year mortality. Thirdly, the parasitic stage is also used for dispersal of the clams. This is especially important for species living in running water.

The length of the growing season for the Unionidae is regulated by temperature (NEGUS, 1966; ÖKLAND, 1963), and the present results indicate that the development of *Anodonta piscinalis* glochidia is also closely limited by temperature. Producing the glochidia in July—August, the time of highest water temperatures in the present area (see Fig. 3), is energetically most advantageous and may be the only possibility for achieving a high output at a low cost.

In the *Anodonta* population of Lake Kuivasjärvi the glochidia are stored in the outer gill blades for the whole winter, but infected fish were observed only between March and June, the overall prevalence being highest in May (64.8 %, both years combined). This suggests that the release of glochidia from the clams is not synchronous, but rather glochidia may be released from March to May at least. The moment of invasion of the fish may be influenced by individual factors, i.e. the movement of fish around the clams.

The glochidia of *Anodonta piscinalis* are attached to fish for less than four weeks (Ellis, 1978), and this is confirmed by the present data. The last time the glochidia were observed in the clams was the third week of May, while infected fish were found in May and June but not in July. This indicates that metamorphosis of *Anodonta piscinalis* may be completed in four weeks, the time elapsing between the last observation of glochidia in the clams and the last observation of infected fish, at least at the end of May and in June when the water temperature has already increased. It is very probable that metamorphosis needs a longer time in March—April, while the lake is covered by ice and water temperatures are low as Ellis (1978) also states. The reason for this would appear to be slower metabolism in both the fish host and the attached glochidia. This would also have the effect of synchronizing the detachment of the young clams with the beginning of the new growing season, in that the glochidia that become attached earlier remain on the fish longer than those that become attached later. This could partly explain why some infected fish are already observed in March.

Another question is what factor induces the emergence of glochidia in March or earlier, at the time when water temperatures are still very low (Fig. 3). The only factor which changes drastically at this time of the year at the latitude concerned is the increasing light which can penetrate through the ice. Schulman (1989), for example, suggests that the increasing light activates the maturation of the monogenean parasite *Gyrodactylus* in the minnow of lakes on the Kola Peninsula.

According to the present data, *Anodonta* glochidia can infect several host species. All the four fish species studied here were infected, and infection started at the same time in all cases, although the prevalences and intensities were different. Unfortunately we have not studied the success of infection in different fish species. Engel & Wächtler (1989) observed a similar wide host spectrum in *Unio crassus*, and also differences between the hosts in the survival of the glochidia. It is possible that clams may have no mechanism for identifying fish species at the time of the release of glochidia and these may also attach themselves to species on which glochidia are less likely to survive. The low prevalences of *Anodonta* glochidia on the gills of the roach in March, April and June may be connected with to the possibility that the roach may not be the most favoured host for *Anodonta*, so that only decreased resistance due to

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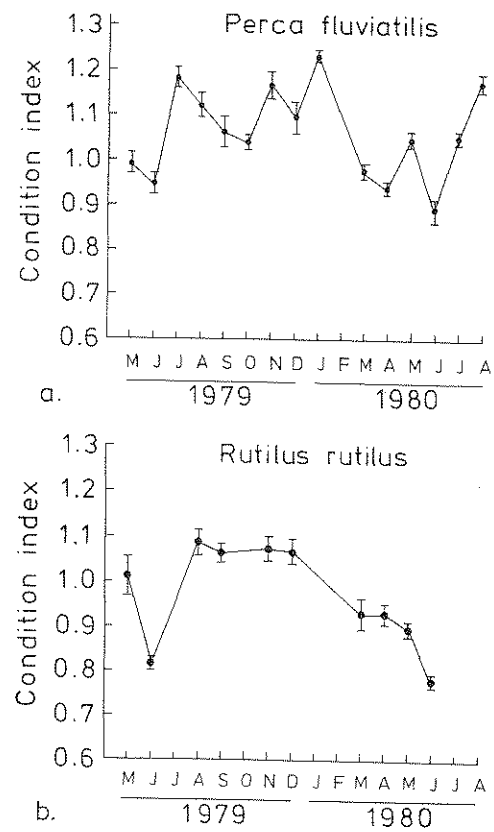

Fig. 5. Variations in Fulton's condition coefficient (weight 100 (g)/length^3(cm)) with time in a) *Perca fluviatilis* b) *Rutilus rutilus*. The bars show 95 % confidence intervals.

spawning stress in May made attachment possible. The spawning behaviour of the roach, schooling in the littoral zone where also the glochidia are released, may also cause the increased infection. It is clearly seen in the present fish material that the condition of both the perch and especially the roach (see Fig. 5) is lowest during spawning.

The detailed infection patterns differ in the roach and perch. Glochidia infection in the roach is associated with size rather than sex, the bigger ones more often being infected (Fig. 4 b), whereas the infection pattern in the perch is not associated with size or sex (Fig. 4 a). There are also temporal differences in the prevalences and intensities of infection (Table 1).

The higher breeding stress in the large roach during the short breeding season in May would explain the size dependence of the infection. The breeding season for the perch is longer, and therefore the differences in breeding stress are not likely to be so great during the period of glochidia infection. The fact that the prevalences are high for the whole infection period, together with the above, may also indicate that on average the perch is the preferred host for *Anodonta* rather than the roach.

The production of glochidia in the clam can be regarded as the controlled and optimized stage in reproduction, while the attachment of the glochidia to the fish and their metamorphosis can be said to make up the stochastic stage. This results in a steady output of glochidia from year to year but a variable juvenile survival due to the stochastic nature of the parasitic stage. The resulting variation in juvenile survival is partly compensated for by the long reproductive lifespan of *Anodonta* (up to 10 reproductions). We agree with the view of Haukioja & Hakala (1978), which is also supported by our results, that the life history strategy of *Anodonta* resembles bet hedging (Stearns 1976), a strategy in which the variation in juvenile survival is compensated for by a higher number of offspring and a long reproductive life span.

This opens up some interesting speculation. Evidently a high fitness response would result from all the adaptations which improve the survival of the glochidia during the parasitic stage. Since host fish species that are availabale change markedly within the geographical distribution of *Anodonta* (spatially, temporally and geographically) phenotypic plasticity in the details of parasitic stage could be expected to be an important factor affecting the total fitness of the individual. Also, because the host fish community can change rather quickly, selection can be expected to favour individuals that maintain or increase the potential to adapt to different host communities and environments.

Summary

The glochidia moved to the outermost gill blades of *Anodonta piscinalis* (Nilss.) in July, were fully developed in August and remained in the gill blades throughout the winter (Fig. 3). This development coincided with the occurrence of warm water temperatures (Fig. 3).

Glochidia were observed on the gills of the roach and perch from March to June, the combined prevalence of infection on roach being 42 % in May and June 1979, and 10 %, 6 %, 68 % and 0 % respectively in March, April, May and June 1980 (Table 1). In the perch the prevalence of infection in May—June 1979 combined was 95 %, and that in March, April, May and June 1980 85 %, 90 %, 51 %, 20 % respectively. The pike showed heavy infection in May (64 %) and the ruffe was also infected in spring.

The infection pattern in relation to length and sex of the host was random in the perch (Fig. 4 a), but the larger roach were infected more often than the smaller ones (Fig. 4 b).

Differences in infection pattern between the perch and roach may be due to differences in breeding stress.

The reproduction of *Anodonta piscinalis* can be divided into two stages: 1. The production of glochidia in the clam, which forms the controlled, optimized stage, and 2. the attachment of glochidia on fish and their metamorphosis, a stochastic stage. Losses at the stochastic stage of reproduction are compensated for by the long reproductive life span of this species.

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