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Temporal and spatial distribution of glochidial larval stages of European unionid mussels (Mollusca: Unionidae) on host fishes

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Abstract. Glochidia are the larval stage of freshwater unionid mussels that parasitize the fins and gill apparatus of fish. A total of 22 fish species were examined for the presence of glochidia whose distribution on individual hosts was studied on three common fish species, the roach *Rutilus rutilus* (L.), perch *Perca fluviatilis* L. and bitterling *Rhodeus sericeus* (Pallas). Between 1997 and 1999, the fish were obtained from the rivers Morava and Kyjovka and surrounding water pools in the Czech Republic. The glochidia of two genera, *Unio* and *Anodonta*, were found. *Anodonta* glochidia were observed on 10 fish species, *Unio* glochidia on 17 fish species. There was a difference in spatial distribution of glochidia on the body of the host fish. *Unio* glochidia were predominantly located on the gills, whereas most *Anodonta* glochidia were found on the fins, with the highest numbers of glochidia were observed on the margin of the pectoral fins. For the gill apparatus, *Unio* glochidia were found predominantly on the second and third arch. *Anodonta* glochidia were predominantly found during winter and spring (November–May), whereas *Unio* glochidia were more abundant during May and June. The number of glochidia was positively correlated with fish length in perch highly infected by *Anodonta* glochidia and perch infected by *Unio* glochidia. Of the three fish species, the highest occurrence of parasites was found on perch with fewer observed on roach. In spite of the close relationship between bitterling and unionid mussels, glochidiosis was rare on this fish species.

Glochidia are the parasitic larval stage of unionid mussels that attach to the fish host after release from the adult mussel. They encyst within the epidermis of the host fish and become surrounded by a thin hyaline membrane of host origin (Wood 1974, Silva-Souza and Eiras 2002). Glochidia remain on the host fish until metamorphosis is completed, the duration of which is dependent on water temperature. The glochidia of different genera are released at different times of the year (Bauer 1994).

Fish are important in the dispersal of unionid mussels. Generally, attachment of the parasitic stage (glochidium) of the mussel to fish or amphibians is required before transformation to the free-living juvenile stage can occur (Lefevre and Curtis 1910). Dartnall and Walkey (1979) claimed that glochidial attachment is theoretically a random process. Glochidia attach mainly onto fish living in the same habitat as mussels, but only few successfully metamorphose from the larval to juvenile stages. If glochidia attach onto an unsusceptible host, they may form a cyst, but are released in a few hours or days and subsequently die (Meyers et al. 1980).

Glochidia attach to the gills, fins and the body of fish. According to Davis and Fuller (1981), glochidia occupy different niches depending on whether an attachment hook and thread are present. Berrie and Boize (1985) and Pekkarinen and Englund (1995) have reported that glochidia with reduced or absent hooks attach to the gill filaments of fish, while glochidia of species with well-developed hooks attach to harder tissues such as fins, scales and skin.

The aim of the present study was to document the host specificity of glochidia on several species of fish, and to investigate the spatial and temporal distribution of the glochidia of four species of freshwater mussels: *Anodonta anatina* (L., 1758), *Anodonta cygnea* (L., 1758), *Unio tumidus* Philipsson, 1788 and *Unio pictorum* (L., 1758), on their fish hosts in Central Europe.

Three fish species, perch *Perca fluviatilis* L., roach *Rutilus rutilus* (L.), and bitterling *Rhodeus sericeus* (Pallas), studied in greater detail, were common in all of the localities in the study area. These hosts differ significantly in their biology with respect to the close relationship between bitterling and freshwater mussels. The presence of the bitterling is associated with freshwater mussels due to their importance for fish reproduction. Female fish spawn into gill cavity of the mussel, where young fish spend part of their life. Bitterling is a shoaling species and feeds entirely on phytoplankton or detritus, whereas perch are "sit and wait" predators, moving longer distances only occasionally. Young perch feed on plankton whereas older fish are predominantly predators. Roach are generally stationary fish and migrate only during the spawning season. Their diet consists predominantly of zooplankton and macrovegetation (Baruš and Oliva 1995).

MATERIALS AND METHODS

A total of 2,494 specimens of 22 species of fish, including esocids, percids and cyprinids, were examined for the presence of unionid glochidia (Table 1). Temporal and spatial distributions of glochidia were examined on the perch, roach

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and bitterling. A total of 692 perch, 763 roach and 657 bitterling were collected in April–November 1997 and March–November 1998 (Table 1). No samples were collected from July to August 1997 due to flooding in the study area. Other fish species were examined for either the presence or absence of glochidia in the months when the highest occurrence was expected in 1998 and 1999 (April to June and during November).

Fish were collected by electrofishing from 15 localities in South Moravia, Czech Republic. The studied localities included the river Morava (50 m width) and the river Kyjovka (5 m width), 7 oxbow lakes (separated from the river Morava after channelization) with a surface area of 0.5 to 12 hectares, and 6 gravel pits (artificial ponds created during dyke construction in the floodplain of the river Dyje), with a surface area of 0.2 to 1.4 hectares. Perch, roach and bitterling were abundant in all the localities, especially in the river Morava, oxbow lake MNV and gravel pit Rohlík (Jurajda 1995, Halačka et al. 1998). Other localities were used to obtain the highest number of fish species as possible. The most common species of adult mussels found were *Unio pictorum* and *Anodonta anatina*. *Unio tumidus* and *A. cygnea* were less abundant, with *A. cygnea* found rarely in river localities (Thick 1996).

The fish were examined for glochidia with the aid of a stereomicroscope (magnification × 25.2). Glochidia were removed from host tissue and fixed in a 4% formaldehyde solution for later study. These parasites were identified according to Pekkarinen and Englund (1995).

To investigate seasonal changes in glochidial infection, epidemiological characteristics such as abundance, prevalence and intensity of infection were used according to Bush et al. (1997). Prevalence was calculated as a relative number of infected fish in the whole fish sample. Mean abundance was calculated as the mean number of parasites per fish in the whole fish sample. Intensity of infection was the number of parasites on an individual host fish and mean intensity of infection was calculated as the mean number of parasites per infected fish. Spearman's correlation was used to determine the relationships between fish length and number of glochidia.

To study the spatial distribution of glochidia on the gill apparatus, each gill arch was divided into 3 segments (dorsal – D, medial – M, ventral – V), 3 areas (distal – d, central – c, proximal – p) and cartilage, 2 hemibranchs (anterior – A, posterior – P) and 2 hemibranch areas (outer – out, inner – in), according to Gelnar et al. (1990) (Fig. 1). Only the left side of the gills was examined since no difference in the number of glochidia between the left and right side of the gill apparatus had been observed by previous authors (Paling 1968, Wootten 1974, Fustish and Millemann 1978, Cunjak and McGladdery 1991). The fins were divided into inner and marginal (fin edge) sections. The fin area was measured in perch and roach using image analysis software (Lucia 4.21). Altogether, 30 fish of each species were used to measure fin area and the average obtained from all fins was used. The relative area of each fin to the total fin area was counted. The number of glochidia on a certain fin was multiplied by the relative area of this fin. This calculation gave the number of glochidia per area unit of each fin. Those numbers were divided by the lowest of them. This ratio describes how many times a particular fin was more parasitized than the least parasitized one. A similar method using the fin perimeter has been used by Dudgeon and Morton (1984). Wilcoxon matched pair tests were used to assess spatial distribution of glochidia on gills.

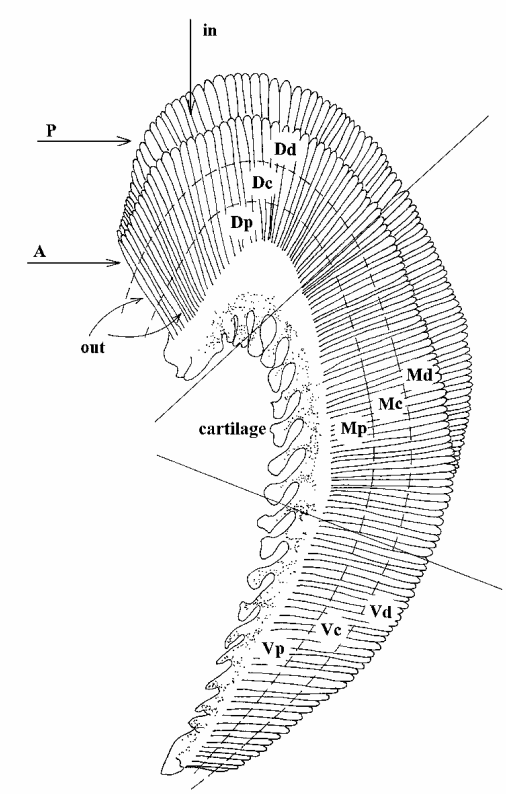

Fig. 1. Division of the gill arch according to Gelnar et al. (1990). 3 segments (dorsal – D, medial – M, ventral – V), 3 areas (distal – d, central – c, proximal – p) and cartilage, 2 hemibranchs (anterior – A, posterior – P) and 2 hemibranch areas (outer – out, inner – in).

RESULTS

Glochidia of the genus *Anodonta* Lamarck, 1799 were observed on 10 fish species (8 from Cyprinidae and 2 from Percidae) from March to June and October to November. Glochidia of *Unio* Philipsson, 1788 were observed on 17 fish species (15 from Cyprinidae, 1 from Esocidae, and 1 from Percidae) from April to October. The presence of glochidia of both genera was observed on 8 fish species and only 1 specimen of *Gobio gobio*, which was infected by glochidia of both genera at the same time. Glochidia were absent on 3 fish species, *Aspius aspius*, *Leucaspius delineatus* and *Leuciscus leuciscus* (Table 1). The intensity of infection of glochidia was generally low on most fishes. More than 10 glochidia per fish were observed only on *Rutilus rutilus*, *Leuciscus idus*, *Scardinius erythrophthalmus*, *Gobio gobio* and *Perca fluviatilis*. The greatest number of glochidia on a single fish was 1,244 for *Anodonta* and 362 for *Unio* on a perch.

Table 1. Fish species examined during 1997–1999, including the number of fish caught (n) fish standard length (mean SL ± 1.0 SE), months when *Anodonta* and *Unio* glochidia were observed, the maximum value of intensity of infection (max ii) in parenthesis and prevalence (Pr, %).

Fish species	n	SL (cm)	*Anodonta*		*Unio*	
			month (max ii)	Pr (%)	month (max ii)	Pr (%)
E s o c i d a e						
Esox lucius L.	9	9.6 ± 2.6	–	0	V (1)	11.1
C y p r i n i d a e						
Rutilus rutilus (L.)	763	7.8 ± 2.2	III–V, XI (55)	6.4	V, VI, IX (142)	5.5
Leuciscus leuciscus (L.)	8	8.9 ± 2 .9	–	0	–	0
Leuciscus idus (L.)	3	41.6 ± 2.4	–	0	V (38)	66.7
Leuciscus cephalus (L.)	36	11.6 ± 4.7	V, XI (4)	5.6	V, VI (6)	16.6
Scardinius erythrophthalmus (L.)	54	5.6 ± 2.6	XI (35)	31.5	V, VI (9)	7.4
Aspius aspius (L.)	3	47.2 ± 2.1	–	0	–	0
Leucaspius delineatus (Heckel)	12	4.2 ± 0.5	–	0	–	0
Pseudorasbora parva (Temminck et Schlegel)	5	5.7 ± 0.4	–	0	VI (2)	20.0
Gobio gobio (L.)	49	8.0 ± 1.8	IV, V (2)	4.1	V (21)	4.1
Gobio albipinnatus Lukash	34	5.4 ± 1.0	XI (2)	11.8	–	0
Barbus barbus (L.)	14	10.5 ± 1.8	–	0	VI (1)	7.1
Alburnus alburnus (L.)	70	9.0 ± 2.9	V, XI (4)	15.7	V, VI (1)	4.3
Blicca bjoerkna (L.)	44	7.7 ± 2.9	IV, XI (1)	9.1	V (1)	4.5
Abramis brama (L.)	18	11.8 ± 5.9	–	0	VI (4)	22.2
Abramis sapa (Pallas)	1	25.7	–	0	VI (4)	100.0
Rhodeus sericeus (Pallas)	657	4.5 ± 0.8	IV, XI (1)	0.3	VI, IX (2)	1.0
Carassius auratus (L.)	12	18.0 ± 8.0	–	0	V (1)	8.3
Cyprinus carpio L.	5	52.7 ± 3.1	–	0	V (1)	40.0
Tinca tinca (L.)	1	32.4	–	0	V (2)	100.0
P e r c i d a e						
Gymnocephalus cernuus (L.)	4	6.5 ± 1.0	IV, XI (15)	75.0	–	0
Perca fluviatilis L.	692	8.3 ± 2.3	III–VI, X, XI (1,244)	24.7	IV–VII, IX, X (362)	14.5

The seasonal distribution of glochidia was studied on perch and roach in 1997 and 1998. Bitterling was not included in the analysis due to a low numbers of glochidia. Clear differences in the seasonal occurrence of *Anodonta* and *Unio* were observed. *Anodonta* occurred on perch in spring (April and May) and autumn (October and November) in 1997 and from March to May and in November in 1998 (Fig. 2). The highest prevalence and abundance was recorded in the early spring. A similar pattern was observed for roach, except that no infected roach were observed in autumn 1997 and in May 1998 (Fig. 3). *Unio* was found on perch from May to June and also following extensive flooding in the study localities in September and October 1997 and from April to July in 1998 (Fig. 3). The highest numbers of glochidia on fish were observed in May and June. For roach, *Unio* was recorded in similar months as perch, except in October 1997 and April 1998 when no infected fish were observed (Fig. 2). *Anodonta* was found predominantly during the colder part of the year. Conversely, *Unio* occurred during late spring and early summer. Perch was significantly more infected by both glochidia than roach (Mann-Whitney, $P<0.01$) (Table 1). Clear differences between the occurrence of glochidia of both genera and water temperature were observed. More than 93% of *Anodonta* were recorded when water temperature was below 12°C, with a maximum 1,244 glochidia on a single fish occurring at 2.9°C. On the other hand, 97% of *Unio* were recorded at temperatures between 14 and 24°C, with a maximum of 362 glochidia on a single fish at 20°C.

The relationship between the intensity of infection of *Anodonta* and *Unio* glochidia and host size was studied on perch and roach. Separately were analyzed two groups of perch. The first group was one sample of perch which had a significantly higher mean intensity of infection of glochidia (220 specimens) than the second group (48 specimens) (Mann-Whitney, $P<0.01$). The number of glochidia was positively correlated with fish length in the perch group highly infected by *Anodonta* and perch infected by *Unio* (Spearman's correlation, both $P\leq0.01$) (Table 2).

The spatial distribution of glochidia on host fish species was studied on the fins and gills of perch, roach and rudd (*Scardinius erythrophthalmus*). A group of highly infected perch and rudd had sufficient numbers of *Ano-*

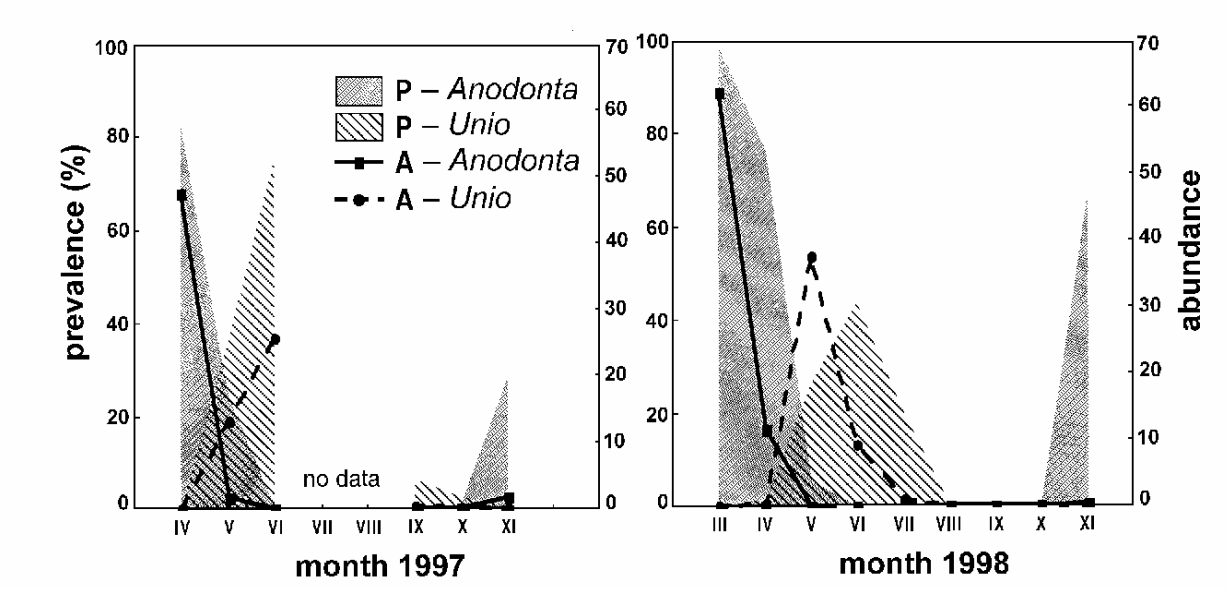

Fig. 2. Seasonal dynamics of prevalence (P, shaded areas connected to left scale) and abundance (A, lines connected to right scale) of *Anodonta* and *Unio* on perch (*Perca fluviatilis*) during 1997 (April to November) and 1998 (March to November).

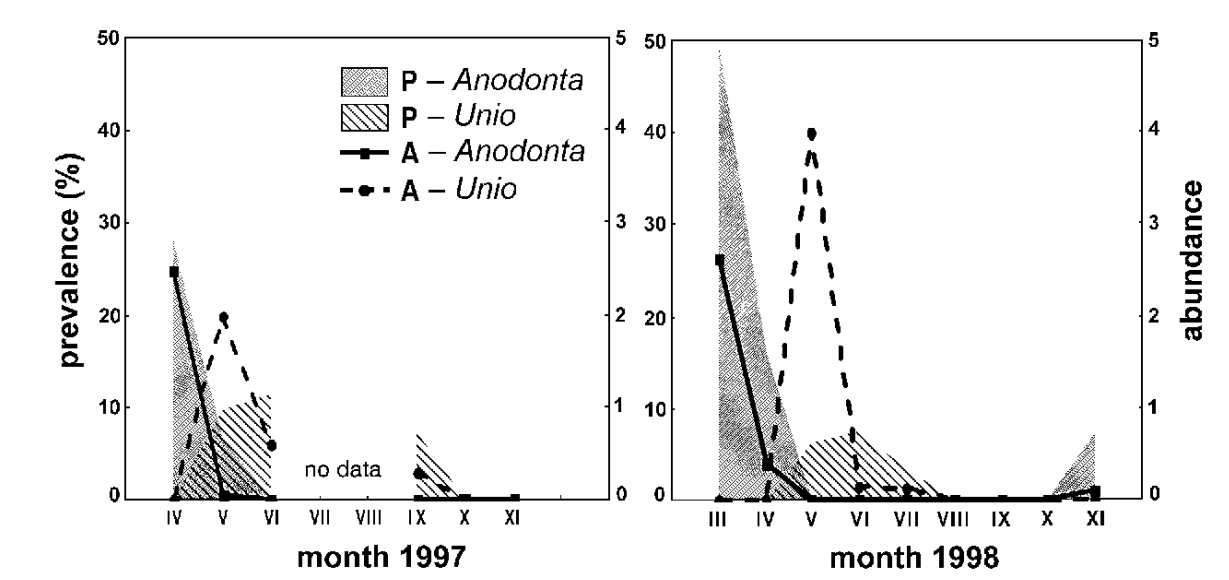

Fig. 3. Seasonal dynamics of prevalence (P, shaded areas connected to left scale) and abundance (A, lines connected to right scale) of *Anodonta* and *Unio* on roach (*Rutilus rutilus*) during 1997 (April to November) and 1998 (March to November).

donta to describe the spatial distribution of glochidia over the entire fish body. Significantly more *Anodonta* were recorded on the fins than on the gills of the perch and roach (Wilcoxon, both $P<0.001$). Perch had significantly more glochidia on the gills compared to roach (Mann-Whitney, $P<0.001$). Conversely, *Unio* occurred in significantly higher numbers on the gill apparatus compared to the fins for both fish species (Wilcoxon, both $P<0.001$). No significant difference in distribution of *Unio* on the fins and the gills was observed between roach and perch (χ^2 test, $P>0.05$).

For fish with a high intensity of infection of *Anodonta*, the majority of glochidia were situated on the fins of perch and rudd compared to the gills and the rest of the fish body (Wilcoxon, both $P<0.001$). Compared to perch, rudd had significantly more glochidia attached to the body compared to the gills (Wilcoxon, $P<0.001$). On the body of both fish species, glochidia were attached to the eyes, mouth, gill covers, scales and the rest of body surface. Relatively more glochidia were observed on the gill covers and in the mouth in perch and rudd, respectively. No glochidia were attached to the

eyes of rudd and there was a significant difference in the glochidial distribution between perch and rudd (χ^2 test, $P<0.001$).

Anodonta parasitizing the fins of perch was mainly situated on the marginal area of the fin at both low and high intensities of infection (χ^2 test, both $P<0.001$). On roach, significantly more glochidia were attached to the margin of the fins (χ^2 test, $P<0.001$), except for pectoral fins, where more *Anodonta* occurred on the inner area (χ^2 test, $P<0.001$).

For all host fish, most *Anodonta* were found attached to the pectoral fins, with the anal fin parasitized the least. Perch with a low intensity of infection had 6.7 times more glochidia on the pectoral fin compared to the anal fin and almost 14 times more in heavily infected perch. For roach, the pectoral and caudal fins were 11 times more infected compared to the anal fin (Tables 3, 4).

The spatial distribution of *Anodonta* over the gill apparatus was studied on perch with both low and high intensity of infection and on perch and roach with *Unio*. Glochidia were not distributed evenly (Table 5). *Anodonta* and *Unio* were mainly attached to the second and third gill arch on perch that had a low intensity of infection. The second gill arch was the most infected in perch with a high intensity of *Anodonta*. In roach, the fourth gill arch had the fewest glochidia. Glochidia of both genera occurred more frequently on the medial segment. A similar pattern for the medial and ventral segment was recorded in perch with low intensity of glochidia. *Anodonta* was recorded more often on the proximal and distal gill area in heavily infected perch, with no preference observed for less infected perch. Conversely, *Unio* was found more often on the central gill area. Significantly more *Anodonta* was found attached to the gill cartilage compared to *Unio* (χ^2 test, $P<0.001$). All glochidia were mostly attached to the outer compared to the inner gill surface. The number of glochidia between hemibranchs differed only in perch with low intensity of infection of *Anodonta* (Table 5).

DISCUSSION

Unionid larvae are sometimes observed on atypical hosts, where they are released before finishing metamorphosis and die (Bauer and Vogel 1987). In this study, differences in susceptibility to glochidia were observed in different host species. Jokela et al. (1991) recorded that *Perca fluviatilis* was most susceptible to *Anodonta anatina*. Fewer parasites were observed on *Esox lucius*, *Gymnocephalus cernuus* and *Rutilus rutilus*, which probably became more susceptible during times of spawning stress. Berrie and Boize (1985) found *Gasterosteus aculeatus* L. and *P. fluviatilis* to be the most susceptible to *Unio* in the river Thames, with *R. rutilus* and *Alburnus alburnus* also acting as a host. Aldridge (1997) experimentally studied the intensity of infection and the duration of glochidial attachment on bitterling compared with *R. rutilus*, *Scardinius erythrophthalmus*, *P. fluviatilis* and *G. aculeatus*. Glochidia of *A. anatina* attached to bitterlings, but were all lost within five days. On the other hand, much higher intensities of infection of *Anodonta* were observed on the other fish species in the experiment (650 *A. anatina* on *P. fluviatilis*) and for periods of more than 50 days. These results suggest that bitterling avoid glochidiosis or lose glochidia shortly after glochidia attachment. Other observations indicate that fish can develop an immune response to glochidia that protects them against infection (Bauer 2001, Jansen et al. 2001). Although there is no evidence for such immunological protection for bitterling, their close association with mussels would make such a response adaptive, and this is a possible explanation for the low prevalence and intensity of infection of bitterlings by glochidia. The marked failure of bitterlings to host glochidia appears to suggest that a mutualistic symbiosis between bitterling and mussels does not occur (Smith et al. 2004).

In the present study, *Anodonta* glochidia were observed on 10 fish species from 2 families and *Unio* glochidia on 17 fish species from 3 families. The fish examined were not caught in sufficient numbers throughout the study period, and therefore the susceptibility of only certain host species could be examined. When comparing the perch, roach and bitterling, the perch *P. fluviatilis* appeared to be the most susceptible host to all glochidia. It is probable that the roach *R. rutilus* is also a suitable host for both genera of glochidia. The life cycle of the bitterling *Rhodeus sericeus* includes an unusual spawning symbiosis with freshwater mussels. Adult fish spawn inside living unionid mussels, where fish embryos develop for approximately 1 month (Wiepkema 1961). In spite of this, in our study bitterling seemed to be innately resistant to the glochidia of both mussel genera.

Developed glochidia are released from the host and develop into mature mussels in the substratum. Dartnall and Walkey (1979) observed glochidia of *Anodonta cygnea* on *G. aculeatus* in Great Britain from December until May. Prevalence reached 100% during winter and decreased in May, with no more than 20 glochidia per fish in 86% of cases of glochidiosis. Jokela et al. (1991) observed glochidia of *Anodonta anatina* on perch during the winter until June. Paling (1968) studied the glochidia of *A. cygnea* on the brown trout, *Salmo trutta* L., caught from November until January in Great Britain. Jansen (1991) studied the occurrence of the glochidia of *Anodonta grandis simpsoniana* Lea, 1861 on the yellow perch *Perca flavescens* (Mitchill) in North America. The number of parasitic larvae increased from October to May, when 100% of fish were infected, after which followed a rapid decrease in the abundance. Intensities of infection varied from 6 to 49 glochidia per fish (Jansen 1991). In contrast, *Unio* attach to fish in the warmer months of the year and metamorphosis is completed before the end of summer (Pekkarinen 1993).

Table 2. The relationship between fish size and glochidial infection on perch, *Perca fluviatilis*, and roach, *Rutilus rutilus*. Perch with low [*P. fluviatilis* (L)] and [high *P. fluviatilis* (H)] intensity of infection by *Anodonta* glochidia were analysed separately. Numbers of fish examined (n, n_1, n_2), standard length (SL, mean ± SE), Spearman's correlation coefficients and *P* values are given. Positive correlations are highlighted.

Fish species	Glochidia	Effect of fish size			
		n	SL (cm)	Spearman	*P*
P. fluviatilis (L)	*Anodonta*	121	8.1 ± 1.9	0.109	0.236
P. fluviatilis (H)	*Anodonta*	38	6.7 ± 1.1	0.411	**0.010**
P. fluviatilis	*Unio*	101	8.9 ± 1.8	0.385	**<0.001**
R. rutilus	*Anodonta*	45	8.6 ± 1.6	–0.0048	0.755
R. rutilus	*Unio*	34	8.5 ± 1.6	0.048	0.788

Table 3. *Anodonta* glochidia on perch, *Perca fluviatilis*, with high (H) and low (L) intensity of infection. Relative ratio of fin area, number of glochidia observed, and relative attractiveness of fins (see Materials and Methods for further details).

Fin	Relative ratio of fin area	Total no. of glochidia		Relative fin attractiveness	
		(L)	(H)	(L)	(H)
Caudal	0.21	438	1,182	4.7	7.0
First dorsal	0.17	411	718	3.5	3.4
Second dorsal	0.12	177	459	1.1	1.5
Anal	0.08	242	437	1.0	1.0
Pectoral	0.23	576	2,157	6.7	14.0
Ventral	0.19	447	1,045	4.4	5.6
Total	1.00	2,291	5,998		

Table 4. *Anodonta* glochidia on roach, *Rutilus rutilus*. Relative ratio of fin area, number of glochidia observed and relative attractiveness of fins (see Materials and Methods for further details).

Fin	Relative ratio of fin area	Total no. of glochidia	Relative fin attractiveness
Caudal	0.35	50	10.9
Dorsal	0.17	35	3.8
Anal	0.10	16	1.0
Pectoral	0.19	94	11.0
Ventral	0.19	29	3.5
Total	1.00	224	

Table 5. The distribution of *Anodonta* and *Unio* glochidia on the gill apparatus of perch and roach; sum (Σ) and relative numbers (%) of glochidia per gill part. Numbers significantly higher than expected are highlighted in bold (Wilcoxon matched pair test, $P<0.05$). Asterisks denote significant difference within highlighted numbers ($P< 0.05$).

Fish species (glochidium)	No. of fish	Sum and relative number of glochidia	Gill arch number				Segment			Gill area				Surface		Hemi-branch	
			1	2	3	4	D	M	V	p	c	d	cartilage	in	out	A	P
P. fluviatilis (L) (*Anodonta*)	131	Σ	146	**208**	**235**	111	162	**273**	**227**	223	171	201	125	59	**527**	252	**312**
		%	20.9	29.7	33.6	15.8	24.5	41.2	34.3	31.0	23.8	27.9	17.3	10.1	89.9	44.7	55.3
P. fluviatilis (H) (*Anodonta*)	38	Σ	**329**	**516***	**380**	119	395	**566**	379	**417**	279	**371**	273	101	**1249**	635	708
		%	24.5	38.4	28.3	8.8	29.5	42.2	28.3	31.1	20.8	27.7	20.4	7.5	92.5	47.3	52.7
P. fluviatilis (*Unio*)	100	Σ	314	**528***	**451**	332	**530**	**742***	423	415	**687***	**585**	16	155	**1537**	845	803
		%	19.3	32.5	27.8	20.4	31.3	43.8	24.9	24.4	40.3	34.4	0.9	9.2	90.8	51.3	48.7
R. rutilus (*Unio*)	37	Σ	**92**	**83**	**73**	23	72	**155**	58	89	**139**	50	6	6	**270**	135	122
		%	34.0	30.6	26.9	8.5	25.3	54.4	20.3	31.3	48.9	17.7	2.1	2.2	97.8	52.5	47.5

D – dorsal, M – medial, V – ventral segment; d – distal, c – central, p – proximal area; A – anterior, P – posterior hemibranch; out – outer, in – inner hemibranch areas (Fig. 1).

Berrie and Boize (1985) observed the larvae of *Unio pictorum* and *U. tumidus* parasitizing fish in the river Thames in Great Britain from April to August with a peak in June and July.

In the present study, *Anodonta* was caught during low water temperatures in spring, when prevalence and abundance were the highest. In spring, glochidia were observed till May; in autumn, glochidia appeared in October and November. The release of glochidia from maternal shells probably occurs over winter and larvae stay attached to the fish until spring. Our results suggest that the occurrence of parasitic stage of *Anodonta* was restricted by a water temperature of 14°C, which appeared to be the upper limit. During winter, it was not possible to catch fish due to ice cover and therefore the occurrence of *Anodonta* on fish remains unknown.

Unio glochidia were observed attached to fish predominantly from May to July, with a peak in June. Almost 97% of *Unio* were found when the water temperature was 14 to 24°C, which could indicate the beginning and end of *Unio* parasitic stage. The occurrence of *Unio* on fish in September and November in 1997 indicated that widespread flooding in July may have influenced the release of glochidia. During flooding, glochidia remained in the marsupia of the maternal shell, apparently to be released 2 months later after flooding ceased. Glochidia of both genera were observed in May, when the abundance of *Anodonta* decreased and that of *Unio* increased.

A positive relationship between the intensity of glochidia infection and perch length has been observed by Jokela et al. (1991) for *A. anatina*. A similar relationship has been reported by Dartnall and Walkey (1979) on *G. aculeatus* parasitized by glochidia of *A. cygnea*. Tedla and Fernando (1970) observed a negative correlation between number of glochidia of *Lampsilis radiata* (Gmelin, 1791) and length of yellow perch (*Perca flavescens*). Bigger fish have a larger fin area and filter more water through the gills (Bauer and Vogel 1987), resulting in a theoretically higher chance of infection. Older and larger fish could, in addition, develop antibodies against glochidia, thus enabling their rejection before metamorphosis is finished. Therefore more glochidia are observed on young fish (Young and Williams 1984).

During this study a positive correlation between the number of glochidia and fish length was observed for perch with a high intensity of *Anodonta* and *Unio*. Perch with a high intensity of infection were caught in a single late-autumn sample. A positive relationship between the number of *Anodonta* and fish length on the autumn perch may indicate that primary attachment occurred and some glochidia would have been probably rejected. In that sample, we may have recorded the beginning of glochidial release from maternal shells.

Glochidia are not randomly distributed on the host body. Berrie and Boize (1985) observed 97.8% of *Unio* on the gills, 0.8% on the fins and 1.4% on the body surface. *Anodonta cygnea* larvae parasitizing *G. aculeatus* attached predominantly to the fins (48.4%), with most on the caudal and pectoral fins (Dartnall and Walkey 1979). Jansen (1991) determined that glochidia of *A. grandis simpsoniana* on *P. flavescens* were attached mainly on marginal parts of the fins, soft filaments of the gills and on the head between bones on the softer parts of the head. According to Threlfall (1986), 80% of glochidia of *Anodonta cataracta* Say, 1817 on *G. aculeatus* attached to the fins were on pectoral fins.

In the present study, *Anodonta* was attached predominantly to the fins, while *Unio* was mainly on the gills. Fewer glochidia were on the scales, the area around and inside the mouth, eyes and the gill covers. A total of 72% of *Anodonta* glochidia attached to the fins, 16% to the gills and 12% on the other parts of the body. *Unio* attached to perch and roach on the gills in more than 95% of fish. Most *Anodonta* glochidia were attached to the marginal parts of fins. In perch and roach the highest density of aggregation occurred on the pectoral and the caudal fins, respectively. Glochidia are sucked into the mouth with the respiratory current and attach to the first appropriate place such as the mouth, gills and gill cover margins. After release from the buccal cavity they may still attach to pectoral fins, which are the first fins exposed to the respiratory current. Other fins may be infected with free glochidia in the water column or as they are released directly from the maternal shell. Mussel larvae on the head and gill covers were attached to the soft connective tissue between bones and to the nares and eyes. On the fish body, glochidia were attached mainly on the scale margins where they clamp onto tissue.

According to Wootten (1974), glochidia do not choose the site of attachment, but clamp at the first possible place; thus the spatial distribution on the gill apparatus is significantly influenced by the route of the respiratory current (Paling 1968). An assumed preference for attachment to the second gill arch has been observed by Wootten (1974) on *Gymnocephalus cernuus*, where most glochidia were found on the ventral segment, distal area and always on the outer surface of gill filaments. The medial segment, anterior hemibranch and second arch were mainly infected in the study on *Lepomis gibbosus* (L.) by Hanek and Fernando (1978). Tedla and Fernando (1970) observed the same pattern on *P. flavescens*. Jansen (1991) recorded no significant preference of any gill arch on *P. flavescens* where most of glochidia were attached on the filaments.

In this study, glochidia on perch were found mainly on the second gill arch. On roach, glochidia were attached uniformly among the first three gill arches and less on the fourth arch. All glochidia were attached mainly on the medial segment and outer gill surface. *Anodonta* was more often situated on the proximal and distal areas, whereas *Unio* was found on the central

area. There was no difference in the distribution of glochidia between the anterior and posterior hemibranch, except for the perch highly infected by *Anodonta*, where the posterior hemibranch was more infected. *Anodonta* was more often observed on the cartilage. Thus, our study supports the views of Paling (1968), Wootten (1974) and Dartnall and Walkey (1979), in which the spatial distribution of glochidia is affected by respiratory current and host fish behaviour and habitat use rather than active choice of attachment site.

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