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ORIGINAL PAPER

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Seasonal dynamics of *Posthodiplostomum cuticola* (Digenea, Diplostomatidae) metacercariae and parasite-enhanced growth of juvenile host fish

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Abstract The seasonal dynamics of *Posthodiplostomum cuticola* metacercariae in 0+ juvenile fish, *Rutilus rutilus*, *Scardinius erythrophthalmus* and *Abramis bjoerkna*, was studied on the floodplain of the Dyje River, Czech Republic. Prevalence and mean abundance of *P. cuticola* were significantly higher in *R. rutilus* than in *S. erythrophthalmus* or *A. bjoerkna*. A seasonal pattern of parasite infection with maximum values in autumn was evident in all three species. No effect of overwintering on the *P. cuticola* infection was detected. Parasite-induced growth was found for all three fish species investigated; the fish standard length and body weight of parasitized individuals were significantly higher than those of unparasitized fish from July to October. In April, no difference was found. The maximum enhanced growth of parasitized fish was found in months with low zooplankton densities, while the difference was lower when food was abundant.

Introduction

Freshwater digeneans often show seasonal changes in prevalence and abundance related to cercarial emergence with an increase in water temperature (Chubb 1979). In nature, peak cercarial release corresponds to the mass propagation of cyprinid fish, which enhances intensive infection of the second intermediate host and hence parasite distribution caused by fish dispersal, as well as the maintenance of high parasite abundance (Vladimirov 1960). Young-of-the-year (0+) juvenile fish represent convenient hosts for studying the seasonal dynamics in long-lived parasites. Parasites of 0+ juvenile fish must have been acquired in the year of the study, which excludes the confusing effects of older infections (Dönges 1964). Further, for parasites that use fish as an intermediate host, the infection of juvenile fish provides the best opportunity to be transmitted to the piscivorous final host (Fisher and Kelso 1988).

Here, we studied the infection of *Posthodiplostomum cuticola* (von Nordmann, 1832) in fish intermediate hosts. The metacercariae of *P. cuticola* encyst in the fins, skin and muscle of cyprinid fish. During their development, fish tissue surrounds encysted metacercariae with melanin, which leads to clearly recognizable black spots (Dönges 1964). *P. cuticola* has been reported as pathogenic for fish hosts, and the infection may be lethal, especially during the first months of fish life (Lucký 1970). The negative or neutral impact of parasites on fish host survival and growth has been demonstrated for most parasite-fish host systems, both in aquaculture and natural populations (Lyayman and Sadkovskaya 1952; Harrison and Hadley 1982; Sindermann 1987). On the other hand, there are several studies that detected an increase rather than decrease in the growth of parasitized hosts. A growth-enhancing effect on the fish host has especially been described in cestodes: plerocercoids of the pseudophyllidean *Schistocephalus solidus* infecting *Gasterosteus aculeatus* (Milinski 1985; Arnott et al. 2000) and *Ligula intestinalis* infecting *Rutilus rutilus* (Museth 2001; Loot et al. 2002). In fish infected with digenean larvae, increased growth has only been recorded in *Phoxinus phoxinus* experimentally infected with a low number of *Diplostomum phoxini*, although the effect appeared to cease with high infections (Ballabeni 1994).

In the present study, we examined the seasonal dynamics of a *P. cuticola* metacercarial infection of 0+ juvenile fish during their first months of life. We also investigated the effect of *P. cuticola* infection on the

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Table 1 Seasonal dynamics of water temperature (°C), zooplankton density (expressed in number of individual Cladocera, Copepoda and Rotatoria per 1 l of water) and phytoplankton (expressed in μg of chlorophyll *a* per 1 l of water)

	May	June	July	August	October	April
Mean temperature (°C)	22	19	25	22	14.5	11.2
Zooplankton (n/l)	47.5	5.1	14.4	45.6	11.9	86.2
Chlorophyll *a* (μg/l)	3.9	3.0	100.6	126.7	130.9	56.1

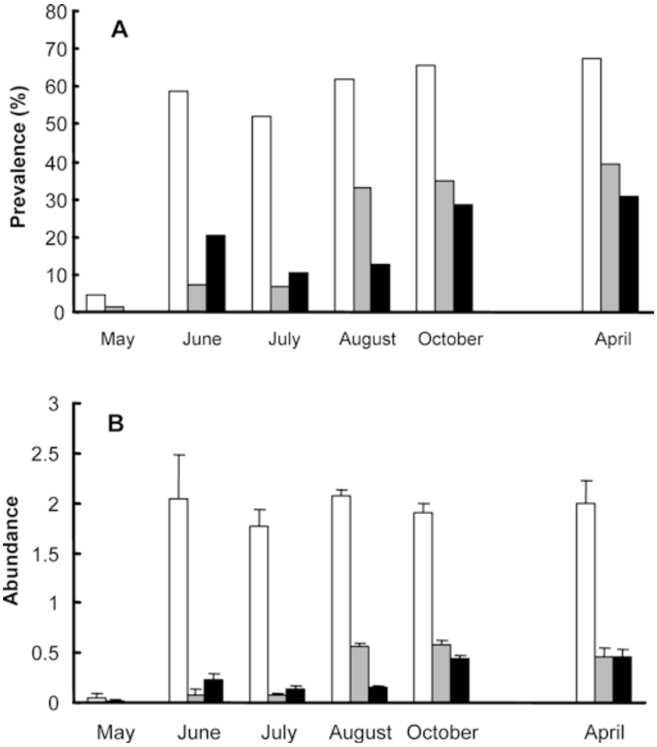

Fig. 1 Seasonal changes in **A** prevalence and **B** abundance of *Posthodiplostomum cuticola* infection of *Rutilus rutilus (white bars)*, *Scardinius erythrophthalmus* (*grey bars*) and *Abramis bjoerkna* (*black bars*). Error bars are standard error

growth and overwinter survival of 0+ juvenile fish. We compared differences in standard length and body weight between infected and uninfected fish. We also studied the relationship between host food density and host growth between parasitized and unparasitized fish.

Materials and methods

From May to October 2001 and in April 2002, age 0+, and age 1 (April 2002), juvenile *Rutilus rutilus* (L.), *Scardinius erythrophthalmus* (L.), and *Abramis bjoerkna* (L.), were collected in a single pond "Čapí Střední" on the floodplain of the Dyje River, Danube River basin, Czech Republic. The fish were sampled monthly by dip-netting (40 cm diameter, 145 cm long pole, mesh size 0.5 mm) in May and June and using beach seine (5 m long, mesh size 1 mm) from July to October and in April. For a detailed description of the study site see Halačka et al. (1998). Water temperature, phytoplankton (expressed in μl of chlorophyll *a* per 1 l of water) and zooplankton (expressed as the number of individual Cladocera, Copepoda and Rotatoria, the main components of food in juvenile fish, per 1 l of water) were recorded during every sampling using standardized methods (Table 1). All captured fish were preserved in 4% formaldehyde. In the laboratory, fish species and the number of *P. cuticola* metacercariae were recorded; fish standard length (SL; to the nearest 0.01 mm) and fish body weight (to the nearest 0.01 g) were measured.

The level of parasite infection expressed as prevalence (the proportion of infected fish of a given species in a sample as a percentage) and mean abundance (the mean number of parasites per hosts, infected and uninfected, in a sample) were assessed according to Bush et al. (1997). In analyses of the seasonal dynamics of parasite infection levels, the abundances of *P. cuticola* were compared between months using nonparametric Mann-Whitney U-tests (MW) and parasite prevalences were compared between months with χ^2-tests.

Differences in growth between parasitized and unparasitized fish hosts were analyzed using nonparametric Mann-Whitney U-tests. Variance in mean standard length and body weight of infected and uninfected fish among months and fish species were analysed using the nonparametric Friedman test. To account for interspecific differences in body size, standardized difference (D_S, in %) of the mean standard length and body weight between infected and uninfected fish of all three species was calculated to ascertain its relation to the zooplankton abundance, a limiting factor in juvenile fish growth. D_S was calculated as 100*(1–SL of unparasitized fish/SL of parasitized fish).

Results

Seasonal dynamics in *P. cuticola* infections

A total of 8,443 juvenile *R. rutilus* ($n=2{,}526$), *S. erythrophthalmus* ($n=3{,}510$) and *A. bjoerkna* ($n=2{,}407$) were examined over a period of the first year of fish life from May to October (0+ juvenile fish) and in April (1+ juvenile fish). Infection parameters (prevalence and abundance) differed among fish species with the highest values found in roach. Significant differences in the prevalence of infection were observed among months in all three hosts, *R. rutilus* ($\chi^2_5=54.52$, $P<0.001$), *S. erythrophthalmus* ($\chi^2_5=70.10$, $P<0.007$) and *A. bjoerkna* ($\chi^2_5=40.00$, $P<0.001$). The dynamics of parasite infection levels in the first months of fish host life is shown in Fig. 1.

The seasonal dynamics in the prevalence and abundance of *P. cuticola* infection of 0+ juvenile *R. rutilus* (which was high throughout the study period except for May) showed a rapid accession of parasite infection from May to June ($\chi^2_1=72.54$, $P=0.001$ for prevalence;

MW, U = 504, $P < 0.001$ for abundance) and reached a maximum in October (65.5%). The infection level did not differ significantly in parasite prevalence ($\chi^2_1 = 0.34$, $P = 0.559$) and abundance (MW, U = 25,783.0, $P = 0.408$) after winter; i.e. between October and April the next year (Fig. 1).

Parasite infection in *S. erythrophthalmus* showed a significant increase in August ($\chi^2_1 = 71.77$, $P < 0.001$ for prevalence; MW, U = 291,889.0, $P < 0.001$ for abundance) compared to that in July. Similarly to the situation in *R. rutilus*, maximum prevalence (34.6%) and abundance (0.58) were found in October and the infection level did not significantly differ in parasite prevalence ($\chi^2_1 = 0.37$, $P = 0.541$) and abundance (MW, U = 9,114.5, $P = 0.940$) in April the following year (Fig. 1).

No infection in juvenile *A. bjoerkna* was recorded in May, but 20.3% of fish were parasitized in June. The parasite prevalence decreased in July ($\chi^2_1 = 5.92$, $P = 0.015$) and reached its maximum 28.3% in October ($\chi^2_1 = 51.96$, $P < 0.001$). Similarly, the maximum abundance (0.44) was found in October (MW, U = 162,921.0, $P < 0.001$). After winter, neither prevalence ($\chi^2_1 = 0.33$, $P = 0.564$) nor abundance (MW, U = 34,942.5, $P = 0.642$) differed significantly from October (Fig. 1).

Host size of infected and uninfected fish

Differences in fish standard length and body weight between parasitized and unparasitized fish were found in all three host species, *R. rutilus*, *S. erythrophthalmus* and *A. bjoerkna* (Fig. 2). In May and June, there was no difference in the mean standard length between parasitized and unparasitized individuals for all fish species. From July to August, when the fish became more heavily infected, the standard length and body weight of parasitized fish were significantly higher than in unparasitized fish, with the exception of *A. bjoerkna* (August sample). *A. bjoerkna* is a fish with batch spawning. In July, three cohorts were discriminated using a length-frequency distribution, but only the youngest cohort (fish that hatched in late July) was captured during our August sampling. The age of these fish corresponded to the age of fish sampled in June, when no significant difference in the standard lengths of parasitized and unparasitized fish was found (Fig. 2). In fish that survived the first winter (April 2002), no significant differences in fish standard length and body weight between infected and uninfected fish were found (Fig. 2).

No significant difference in the standardized differences in fish length between infected and uninfected fish was found among fish species (Friedman test, $\chi^2_2 = 3.5$, $P = 0.174$) and months (Friedman test, $\chi^2_3 = 7.0$ $P = 0.072$). Similar results were obtained for body weight (Friedman test, $\chi^2_2 = 3.5$ $P = 0.174$ for comparison among fish species and $\chi^2_3 = 6.6$, $P = 0.086$ for comparison among months).

Effect of food density

The difference in fish standard length between parasitized and unparasitized fish was higher when zooplankton density was low (Fig. 3). The maximum difference in fish length between infected and uninfected *S. erythrophthalmus* and *A. bjoerkna* reached 22.4% and 18.9%, respectively, when zooplankton density was lower than 20 individuals l^{-1}. On the other hand, when zooplankton density was higher than 80 individuals l^{-1}, the difference between parasitized and unparasitized fish was never higher than 3.8% (Fig. 3). A similar pattern was found for fish host weight (data not shown).

Discussion

Seasonal fluctuations in prevalence and abundance are common in many helminths infecting freshwater fish. In larval digeneans, seasonality is triggered by water temperature which affects the emergence of cercariae from the snail intermediate host (Chubb 1979). In *P. cuticola*, the optimum temperature for parasite development in the first intermediate host is 10°C (Vladimirov 1960), which corresponds to the April temperature in our study site. Because development in the snail host takes 4–8 weeks (Vladimirov 1960), maximum cercarial release was expected in May and early June. Indeed, the first peak of prevalence and abundance in juvenile *R. rutilus* and *A. bjoerkna* was observed in June, which corresponded well to the cercarial emergence in late May. After this initial increase, parasite prevalence and abundance in 0+ juvenile *R. rutilus* remained high throughout the study period until October. Shukhgalter and Chukalova (2002) recorded similar dynamics in adult *A. brama*. However, they reported a decrease in the parasite level in *A. brama* during the winter months, which was not observed in juvenile fish in the present study.

The seasonal dynamics of *P. cuticola* infection in juvenile *S. erythrophthalmus* and *A. bjoerkna* showed a slightly different pattern. In both species, the infection level was lower than in *R. rutilus*. Relatively low parasite infections in *S. erythrophthalmus* could be caused by low fish host abundance in a sample in May and June and extremely high dominance (over 70%) of the youngest cohort (which was not exposed to the high abundance of cercariae in May) in the July sample. In August, fish of all cohorts were infected with *P. cuticola* metacercariae, which was reflected in a significant increase in parasite prevalence and abundance in *S. erythrophthalmus*. In *A. bjoerkna*, due to the absence of older juveniles in the August sample, the maximum parasite prevalence and abundance was recorded in October. Nevertheless, a similar increase in parasite infection in *A. bjoerkna* in August would have probably occurred if older juveniles had been captured.

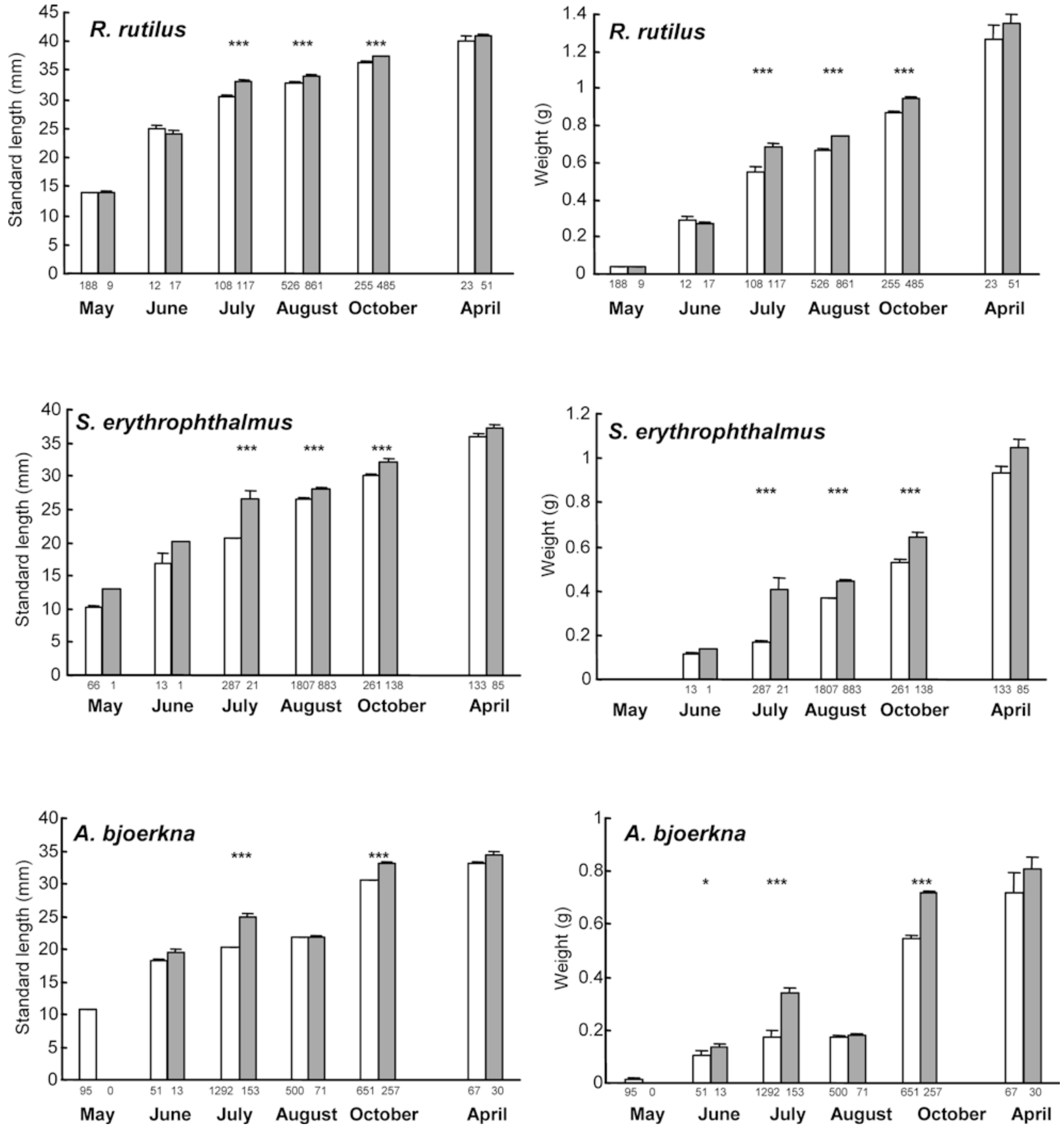

Fig. 2 Differences in standard length (*left column*) and body weight (*right column*) between unparasitized (*white bars*) and parasitized (*grey bars*) fish in each month of sampling. The total number of fish for each sample is indicated. *Error bars* are standard error. *Asterisks* indicate significant differences (Mann-Whitney U-test, * $P < 0.05$, *** $P < 0.001$)

During the first growing season, juvenile fish face the competing energy demands of maximizing somatic growth and energy storage to enable their survival during the first winter (Prost and Parkinson 2001). Generally, parasites utilise the energy reserves of their hosts; this is most obvious in species that undergo a detectable growth or development within their fish host (Ward et al. 2000). Although a negative effect of parasites on overwinter survival of fish hosts has been described in many parasite-host systems (Lemly and Esch 1984; Coleman and Travis 1998; Johnson and Dick 2001), no evidence of a decline in *P. cuticola* abundance and prevalence after the first winter was found for any of the three host species in the present study. The lack of a negative effect of the metacercarial infection on 0+ juvenile fish winter survival could be explained by sufficient body size and energy storage of parasitized fish before winter. Indeed, we found that the standard length and body weight of all three species were significantly higher in parasitized than in unparasitized fish in

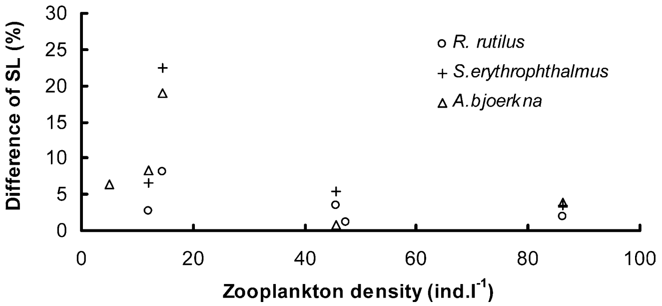

Fig. 3 Relationship between zooplankton density (expressed as the number of individuals per 1 l of water) and standardised difference (in %) in the mean standard length (*SL*) between parasitized and unparasitized fish hosts *R. rutilus*, *S. erythrophthalmus* and *A. bjoerkna*

October. The increase in body weight cannot be accounted for by the weight of the metacercariae themselves, because their mass is less than 0.0001 g (Ondračková, unpublished data). Instead, the larger size of infected fish may represent an adaptive response that promotes host survival during the winter and larger fish may reduce the starvation-induced mortality risk over the winter, similarly to fish infected with larval cestodes (Milinski 1985; Barber et al. 2000; Loot et al. 2002).

In several studies that report the enhancement in host growth and/or condition by infections of larval cestodes, the growth enhancement in parasitized fish hosts resulted from a parasite-mediated change in fish foraging behavior, increased food conversion efficiency and/or reduced activity (Arnott et al. 2000). In larval digeneans, Ballabeni (1994) showed that juvenile minnow with a low infection of *Diplostomum phoxini* metacercariae tended to grow more quickly than uninfected fish and fish with a high infection. The growth of highly infected fish did not differ from that of uninfected individuals (Ballabeni 1994). Crowden and Brown (1980) found that *Leuciscus leuciscus* infected with *Diplostomum spathaceum* spent a longer time foraging than uninfected conspecifics, but suggested that parasitized fish only compensated for the loss of feeding efficiency caused by the parasite infection.

Our findings clearly demonstrate that in wild 0+ juvenile fish populations, parasitized fish grow faster and reach a larger body size than uninfected fish. This was not demonstrated in May and June, when fish were infected for a short period and growth enhancement in parasitized fish was not yet manifest. However, from July the length and weight of parasitized fish were significantly higher than those in unparasitized individuals.

Larval digeneans, though encysted in host tissue, use host-derived energy for the maintenance of their vital functions. Consequently, heavily infected individuals must spend more time foraging to attain the same nutritional benefit as less-parasitized and unparasitized individuals (Barber et al. 2000). *P. cuticola* appears to be able to manipulate host behavior by increasing its motivation to forage or reducing its activity. The difference in host body size between parasitized and unparasitized juvenile cyprinids suggests that an increased food intake of infected fish outweighed a possible parasite-mediated energy loss. The maximum enhanced growth of parasitized individuals was found in months with low zooplankton densities, whilst the difference was lower when food was abundant. It was shown that many fish compromise between feeding and predator avoidance, and that they forage in risky areas when the need for food is increased or when foraging in places with predators is much more rewarding (Milinski 1985). Parasitized fish are in general less prone to predator presence than unparasitized fish (Milinski 1985; Godin and Sproul 1988).

Thus, at low food densities, infected fish may spend more time foraging than uninfected fish. This apparently risk-prone behavior was formulated into the “manipulation hypothesis”. This suggests that any infection-associated change in host behavior that enhances the rate of transmission to the next host in the parasite life cycle should be strongly selected during parasite evolution because of the major selection pressures involved (parasites that are not transmitted die) (Moore and Gotteli 1990).

In the case of *P. cuticola*, parasite induced growth may be one of the parasite adaptations to enhance transmission. Previous studies have demonstrated that fish with black spot disease caused by the metacercariae of *Crassiphialla bulboglossa* showed a tendency to occupy the front of shoals (Ward et al. 2002), which provides considerably better foraging opportunities than other shoal positions (Krause et al. 1992). Consequently, the fish in the front section were larger than those at the rear (Ward et al. 2002). Because shoaling behavior is typical for juveniles of all three cyprinids studied, a similar reason of enhanced growth in parasitized fish in our study could be considered. Further, the black spots that surround metacercariae and present conspicuous marks that highlight the fish body in the water environment may represent an efficient way to attract the attention of bird predators. This corresponds to adaptive parasite-induced manipulation of the host to increase parasite trophic transmission (Lafferty 1992; Lafferty and Morris 1996).

Despite many references to the pathogenic effect and increased mortality caused by the metacercariae of *P. cuticola* in juvenile fish (Lyayman and Sadkovskaya 1952; Dönges 1964; Lucký 1970), a positive effect, such as the enhanced growth of parasitized fish, and consequently unaffected winter survival, has been found in the present study. According to our knowledge, this highly significant increase in the somatic weight and standard length of parasitized hosts has not been recorded for larval digeneans infecting a fish host. We suggest that the increased growth of parasitized individuals is caused by parasite-induced foraging behavior, a parasite strategy of host manipulation that enhances their probability of transmission. Thus, larval parasites are not simply inert cysts waiting for transmission, but they may greatly alter intermediate host behavior leading to higher transmission rates (Holmes and Bethel 1972).

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