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Seasonal dynamics of *Proteocephalus sagittus* in the stone loach *Barbatula barbatula* from the Haná River, Czech Republic

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Abstract

The seasonal cycle of the cestode *Proteocephalus sagittus* (Cestoda: Proteocephalidae) was studied for the first time in the stone loach *Barbatula barbatula* from the Haná River, Czech Republic. A total of 180 loaches were examined monthly from January to December 2001. The parasite occurred in loaches throughout the year but infection parameters differed significantly among seasons, with the highest values of prevalence and abundance from the late winter to the early summer. Parasite recruitment took place in the winter and early spring and the worms sexually matured in the late spring and early summer. In contrast to *P. torulosus*, the gravid worms of which laid eggs only at the end of the spring/beginning of the summer, gravid worms of *P. sagittus* were also found, although in low numbers, in the autumn and early winter. The rate of infection of loach with *P. sagittus* was neither dependent on the sex nor on the size of its fish host.

Introduction

Tapeworms of the genus *Proteocephalus* Weinland, 1858 occurring in Europe represent a monophyletic (Zehnder & Mariaux, 1999; Škeříková *et al.*, 2001) and morphologically homogeneous group of species slightly differing one from another (Scholz *et al.*, 1998; Scholz & Hanzelová, 1998, 1999). They are common intestinal fish parasites and their occurrence and maturation exhibit marked seasonality (Chubb, 1982).

Proteocephalus sagittus (Grimm, 1872) was described from the stone loach, *Barbatula barbatula* of the family Balitoridae (according to Kottelat, 1997; synonym *Noemacheilus barbatula* of the family Cobitidae), but it was put into synonymy with *P. torulosus* (Batsch, 1786) by Scholz & Hanzelová (1998) on the basis of morphological similarity of both the taxa. However, Scholz *et al.* (2003) have recently resurrected *P. sagittus* on the basis of morphological and molecular data. Data on seasonal patterns in the occurrence and maturation of this tapeworm are not available (see Chubb, 1982; Scholz & Moravec, 1994). In the present paper, data based on annual observations of the occurrence and maturation of *P. sagittus* in the stone loach of a small river in South Moravia, Czech Republic, are provided and the seasonal pattern of this cestode is compared with those of congeneric species, in particular of *P. torulosus*.

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Materials and methods

Stone loaches were sampled monthly (15 specimens each month; 180 specimens in total) from January 2001 to December 2001 by electrofishing from the Haná River, a tributary of the Morava River (Danube Basin, South Moravia, Czech Republic), where the stone loach is the most dominant fish species (Prokeš & Baruš, 2002). Two other fish species, *Leuciscus cephalus* (about 1–3 specimens per sample; a total of 9 fish) and *Gobio gobio* (10 specimens per sample; a total of 120 fish), were also examined monthly for parasites. Fish were examined for metazoan parasites using standard procedures (Ergens & Lom, 1970). The size (total length – TL) of all stone loaches ranged from 53 to 140 mm and differences among median size of fish in all month samples fall into 10 mm range. Descriptive statistics of fish are presented in table 1. Juvenile tapeworms were fixed in glycerin-ammonium-picrate (Malmberg, 1956) which is also commonly used for monogenean parasites preparations, whereas others were fixed in hot 4% formaldehyde solution (Scholz & Hanzelová, 1998).

Worms were stained with iron acetocarmine, mounted as permanent preparations in Canada balsam, and divided into four groups according to the state of their maturity: (I) juvenile (scolices and unsegmented worms); (II) maturing (strobila segmented, genitalia developing); (III) mature worms (genitalia fully developed, no eggs); (IV) gravid worms with eggs. The material has been deposited in the helminthological collections of the Department of Zoology and Ecology, Faculty of Science, Masaryk University, Brno, Czech Republic (Collection no. C-1) and the Institute of Parasitology, AS CR, České Budějovice, Czech Republic (Collection no. C-33).

The values of prevalence and intensity of infection were computed according to Bush *et al.* (1997). The relationship between infection rate and fish size was tested. Firstly, the length of infected fish was tested among all samples by ANOVA. Since significant differences were not found (only for samples with more than three infected fish), the interaction of season and fish size was not confirmed. Secondly, Spearman correlations of (i) fish total length and the number of gravid specimens (stage IV) and (ii) fish size categories (step 0.5 cm) and median intensity of parasite infection were undertaken. Statistical analyses were performed using standard parametric and non-parametric methods (Statistica for Windows 6).

Results

Proteocephalus sagittus was found in 11 of 12 monthly samples (absent in September) and 48 of 180 fish (prevalence 27%) were infected. The mean intensity of infection was 4.7 worms per fish (range 1–29). All worms were located in the anterior part of the gut.

Although the cestodes were present throughout the year, except for September, their occurrence differed quantitatively among seasons (fig. 1). The prevalence increased from the beginning of the spring (March) and declined after the early summer (June). The intensity of infection increased during the late winter (February), then

Table 1. Monthly data on water temperature and total length (TL) and weight (w) of male and female *Barbatula barbatula* in the Haná River from January to December 2001.

		Female fish					Male fish					Total			
			TL (mm)		W (g)			TL (mm)		w (g)			TL (mm)		w (g)	
Date	Water temp. (°C)	n	mean	range	mean	range	n	mean	range	mean	range	mean	range	mean	range
23.1.	0.2	6	113	(92–140)	11.4	(5.4–21.9)	9	107	(92–124)	8.9	(6–14.7)	109	(92–140)	10.0	(5.4–21.9)
19.2.	1.1	8	100	(90–107)	6.4	(4.7–7.5)	7	102	(95–115)	6.6	(4.7–9.8)	101	(90–115)	6.5	(4.7–9.8)
19.3.	8.4	11	104	(85–120)	8.0	(4.1–12.7)	4	92	(85–97)	4.9	(4.2–5.6)	101	(85–120)	7.2	(4.1–12.7)
23.4.	5.9	3	94	(93–95)	5.7	(4.8–7)	12	107	(95–127)	7.7	(5.2–12.9)	103	(93–127)	7.1	(4.8–12.9)
22.5.	14.6	14	115	(92–135)	9.6	(5.1–13.8)	1	97	(97–97)	6.3	(6.3–6.3)	115	(92–135)	9.5	(5.1–13.8)
18.6.	15.2	12	106	(71–120)	7.7	(2.8–10.8)	3	107	(104–109)	7.7	(5.6–8.8)	106	(71–120)	7.7	(2.8–10.8)
23.7.	15.1	12	112	(90–122)	10.1	(4.4–12.8)	3	100	(96–105)	6.4	(6.2–6.6)	110	(90–122)	9.5	(4.4–12.8)
20.8.	19.2	11	109	(90–125)	8.5	(4.8–11)	4	113	(105–115)	9.2	(8.2–9.7)	110	(90–125)	8.7	(4.8–11)
25.9.	11.2	8	113	(94–127)	11.2	(4.7–14.8)	7	108	(87–116)	8.0	(4–9.3)	111	(87–127)	10.0	(4–14.8)
22.10.	11.1	8	106	(74–120)	9.6	(2.7–13.7)	7	116	(69–125)	11.0	(2.3–13.8)	110	(69–125)	10.2	(2.3–13.8)
15.11.	0.8	8	96	(53–111)	6.8	(1–10.8)	7	95	(73–100)	5.5	(2.1–6.1)	96	(53–111)	6.2	(1–10.8)
12.12.	0.1	7	96	(85–111)	6.1	(4.5–9.6)	8	96	(62–106)	5.8	(1.5–7.7)	96	(62–111)	6.0	(1.5–9.6)

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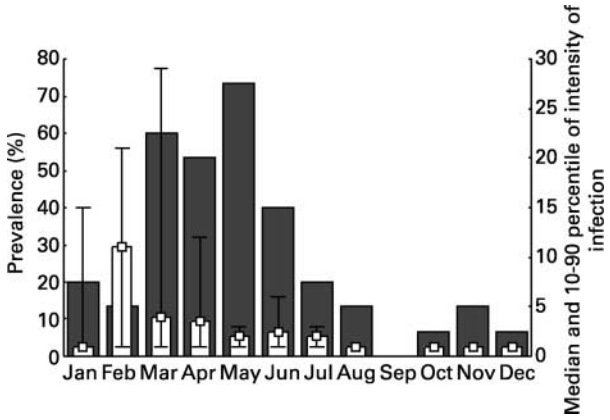

Fig. 1. The prevalence (bars, in % of hosts) and intensity of infection of *Proteocephalus sagittus* in *Barbatula barbatula* from January to November 2001.

decreased and only a few parasites were present in the autumn and beginning of the winter (from September to December).

A marked seasonal pattern was observed in the maturation of *P. sagittus* (fig. 2). Recruitment took place in the winter and early spring (from January to March/April) when only juvenile parasites were found; adult (mature and gravid) tapeworms prevailed in other samples (fig. 2). In contrast to juveniles, adult parasites were not abundant in any samples, i.e. the mean intensity of infection was low. Mean intensity of adult cestodes was 3 tapeworms. Gravid tapeworms with ripe eggs occurred from the late spring (April, May) until August, representing 33.5% of cestodes found (fig. 2); they were also found in October, November and December but in very low numbers.

Significant differences in the infection rate were found neither between males and females nor between fish of different sizes (binomial and Mann-Whitney test – data not shown).

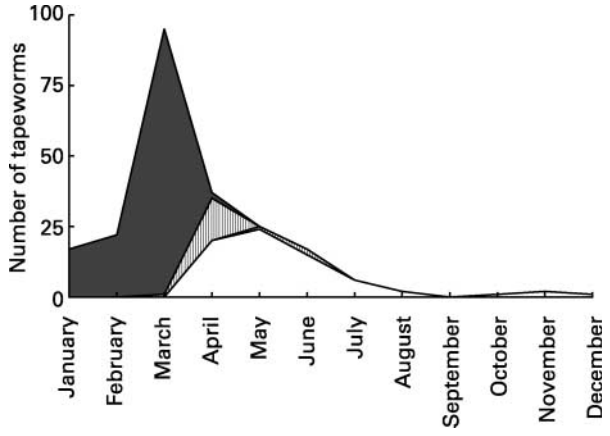

Fig. 2. Proportions of developmental stages of *Proteocephalus sagittus* in *Barbatula barbatula* from January to December 2001. ■, juveniles (stage I); ▥, maturing (II); ▨, mature (III); □, gravid (IV).

Discussion

The data obtained suggest that the life-span of *P. sagittus* is shorter than one year, similar to that in most other species of *Proteocephalus* in the Holarctic (see Chubb, 1982 for review). Under temperate conditions, recruitment of these cestodes takes place from the autumn, parasites grow and mature in spring and eggs are released from gravid worms from late spring to summer (Chubb, 1982). Differences exist between individual species and populations from different geographical regions in the time of the absence of tapeworms from their definitive hosts (usually in late summer/early autumn), the recruitment period and the length of the egg-release period (Chubb, 1982).

The present study is the first to deal with the seasonality of *P. sagittus* but the pattern of its occurrence and maturation corresponds in general to those observed in congeneric species as briefly summarized above. This is also valid for the most similar species, *P. torulosus*, a parasite of cyprinid fish in the Holarctic (Wagner, 1917; Kennedy & Hine, 1969; Wierzbicka, 1978; Scholz, 1989; Scholz & Moravec, 1994; Pietrock *et al.*, 1998; Scholz & Hanzelová, 1999).

A distinct difference between *P. sagittus* and *P. torulosus* exists in the presence of gravid tapeworms of the former species in the fish definitive hosts also in late autumn (October, November) and in December. Although the number of the parasites found was very low, the presence of gravid specimens in this season is unusual among species of *Proteocephalus*, including *P. torulosus* (see Chubb, 1982 for review).

In the latter species, gravid cestodes were never observed in late autumn and early winter; last egg-producing adults were found in August in Germany (Wagner, 1917), in July and August in England (Kennedy & Hine, 1969), mid-August in Karelia, Russia (Ieshko *et al.*, 1976), and in May and June, respectively, in two cyprinid fishes (chub and barbel) from two South Moravian rivers situated fairly near from the Haná River (Scholz, 1989; Scholz & Moravec, 1994).

Another difference between seasonal patterns in the maturation of *P. sagittus* and *P. torulosus* from the Czech Republic is in the longer period of recruitment in the latter species because juvenile *P. torulosus* were also observed in late summer (Scholz, 1989; Scholz & Moravec, 1994), whereas those of *P. sagittus* appeared as late as in winter. However, the present study was limited to only one year and the unusual seasonal pattern in the distribution of juvenile and gravid tapeworms of *P. sagittus* should be confirmed in other localities.

Chubb (1967, 1982) suggested that the timing of maturation of fish cestodes, which is considered to be temperature controlled, determines seasonal periodicity. Kennedy & Hine (1969) questioned the direct influence of temperature on the cestode maturation and egg production, emphasizing the importance of temperature-dependent fish host resistance to new infections. The period of parasite sexual maturation may also be influenced by fish spawning and host hormone levels that may provide the stimulus to egg production (Kennedy & Hine, 1969).

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Although *P. sagittus* exhibited quantitative changes in the occurrence throughout the year and a marked seasonality in its maturation, no seasonal changes in the localization of tapeworms in the fish intestine were observed and all parasites were located in the anterior part of the gut. In *P. torulosus* from chub and barbel, tapeworms were found not only in the anterior but also in the middle third of the gut, presumably due to a shift of sexually mature worms towards the posterior end of the digestive tract (Scholz, 1989; Scholz & Moravec, 1994).

The tendency of *P. torulosus* to prevail in larger fish hosts was found in chub (Scholz, 1989) and barbel (Scholz & Moravec, 1994). In the present study, a significant relationship between the size of stone loaches and their infection with *P. sagittus* was not found. Fish smaller than 7.5 cm were not infected but they formed only a small proportion of samples examined.

The present data indicate that *P. sagittus* differs slightly from other species of *Proteocephalus*, including *P. torulosus*, in its seasonal pattern of the occurrence and maturation. However, more data from other localities and geographical regions are necessary to confirm the differences between the ecology of *P. sagittus* and other species of *Proteocephalus* observed in this study.

In the Haná River, where *P. sagittus* occurred fairly frequently in the stone loach, no *Proteocephalus* tapeworms were found in chub (*Leuciscus cephalus*) and gudgeon (*Gobio gobio*). Taking into account that this cyprinid fish is one of the most suitable definitive hosts of *P. torulosus* (see Scholz & Hanzelová, 1998), its absence in the locality where *P. sagittus* is common represents further, ecologically-based, evidence of the validity of *P. sagittus*, which has been resurrected just recently from the synonyms of *P. torulosus* (see Scholz *et al.*, 2003).

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