

# The role of the European bitterling (*Rhodeus amarus*, Cyprinidae) in parasite accumulation and transmission in riverine ecosystems

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**Abstract** In aquatic ecosystems, fish play a key role in parasite accumulation and transmission to predaceous animals. In the present study, realized on seven populations of a small cyprinid fish species, the European bitterling *Rhodeus amarus*, we investigated (1) the role of the European bitterling as a potential intermediate or paratenic host, (2) the ability of the fish to accumulate parasites with similar final host group, and (3) its significance as a potential source of parasite infection in the ecosystem in respect to habitat characteristics. A total of 36 parasite species were recorded; 31 species (90% of all parasite specimens) were classified as endoparasites. Most of the endoparasites were found in the larval life stage, using bitterling as an intermediate or paratenic host. In particular, parasite community structure

showed significantly higher proportions of allogenic parasites in comparison with autogenic. The supposed co-occurrence of parasite species with identical final host groups showed only a weak association. The adjacent reservoir areas were a significant determinant of both the total and infracommunity parasite species richness and for the mean parasite abundance. No relationship between the distance of sampling site from the adjacent reservoir and parasite community characteristics was found. As a small-sized fish with a wide distribution range and high local abundances, the European bitterling can represent a natural prey for a wide range of piscivorous predators. Due to its susceptibility to the number of larval endoparasites, this fish species may therefore fulfill the role as important transmitter of parasites to their final hosts.

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## Introduction

Transmission and dispersal are two basic processes in the life cycle of parasites, in which the fundamental objective is to find a suitable host (Bush et al. 2001). Parasite life cycles show a variety of forms. Throughout evolution, the original direct life cycles have become more complex, where intermediate hosts play an important role (Park et al. 2007; Poulin 2007). However, a number of parasites may employ hosts in which development does not occur and that are not always obligatory for the completion of parasite life cycles. These paratenic hosts (transport hosts) are frequently used to bridge an ecological or trophic gap (Bush et al. 2001). Intermediate and paratenic hosts are probably the most important components of parasite transmission and dispersion. The main advantages in using intermediate or paratenic hosts are the protection of certain parasite stages from the external environment, the channeling of the parasite toward the final host if the intermediate/paratenic host is a prey of the definitive host and, furthermore, the transmission of the same genetic information to many different host individuals (Poulin 2007). Many trophically transmitted parasites have complex life cycles; they pass through at least one intermediate host before reproducing in their final host. In addition, the mobility of the host over its geographical range is responsible for the dispersal of the parasites (Sousa and Grosholz 1991).

In aquatic ecosystems, fish play a key role in parasite transmission to predacious animals (Zander et al. 1993; Groenewold et al. 1996; Zander 1998; Zander et al. 1999; Zander 2005; Valtonen et al. 2010). Small-sized fish figure as effective mediators between these two levels, because birds and mammals that may feed on tiny planktonic copepods or small snails are rare (Zander 1998). The importance of fish as paratenic host species has been confirmed in many ecological studies (Moravec et al. 1995; Moravec et al. 1998; Ondračková et al. 2009 etc.). The external environment, host age, and feeding

habits contribute significantly to the rate of parasite species accumulation in intermediate or paratenic hosts (Valtonen and Gibson 1997; Morand et al. 2000; Johnson et al. 2004; Dávidová et al. 2008 etc.).

In the present study realized on a small cyprinid fish, the European bitterling (*Rhodeus amarus* Bloch), we investigated (1) the role of the European bitterling as a potential intermediate and/or paratenic host for metazoan parasites, (2) the ability of the fish to accumulate parasites with similar final host group, and (3) its significance as a potential source of parasite infection within the ecosystem in respect to habitat characteristics. While studies in marine ecosystems indicate that small-sized fishes figure as intermediate or paratenic hosts for a wide range of parasite species more frequently than larger species (Zander 1998), as far as we know, similar observations in freshwater systems have not yet been published.

## Materials and methods

### Study system

The European bitterling is a small ostracophilous (deposing its eggs in the cavity of bivalves) fish species and the most common representative of the East Asian cyprinid subfamily Acheilognathinae in Europe (Holčík 1999). This species is characterized by enormous changes in its distribution and abundance over time. Although the parasite fauna of the European bitterling is relatively well known through its range of distribution (Gelnar et al. 1994; Kadlec et al. 2003; Dávidová et al. 2005, 2008), studies concerning parasites of bitterling around the Aegean Sea drainage area are still limited (Akmirza 2007).

### Host and parasite collection

Fish were sampled from seven locations in south-eastern Europe (Turkey, Greece and Bulgaria). *R. amarus* populations were collected in running waters (small rivers or streams) to minimize the effect of habitat type on parasite communities. All sampling sites were located within a maximum distance of 10 km from the adjacent reservoir (Table 1). Except for the Nestos River associated with the Toxotes Dam (Greece), the sampling sites

**Table 1** Characteristics of sampling sites and fish collected

Sampling site	Country	Drainage	Coordinates	Adjacent reservoir (surface area)	Distance to reservoir	Year	Water temperature (°C)	Mean SL (mm) range	Mean W (g) range
Eski Riva Stream	TU	Black Sea	N 41°00'16" E 29°26'07"	Ömerli Dam (23.9 km <sup>2</sup> )	9.5 km	2008	15.1	43 (30–70)	2.1 (0.5–8.4)
Ballica Stream	TU	Black Sea	N 41°00'02" E 29°25'08"	Ömerli Dam (23.9 km <sup>2</sup> )	7.8 km	2008	16.5	53 (39–64)	3.7 (1.1–7.4)
Kurtkoy Stream	TU	Black Sea	N 40°42'20" E 30°11'57"	Sapanca Lake (45 km <sup>2</sup> )	0.2 km	2008	15.7	41 (31–56)	1.5 (0.5–3.8)
Masukiye Stream	TU	Black Sea	N 40°43'01" E 30°08'25"	Sapanca Lake (45 km <sup>2</sup> )	0.8 km	2008	16.0	36 (25–53)	1.0 (0.3–2.9)
Nestos River	GR	Aegean Sea	N 41°05'24" E 24°46'20"	Toxotes Dam (0.25 km <sup>2</sup> )	0.1 km	2009	15.6	52 (40–65)	3.2 (1.4–6.5)
Struma River	BG	Aegean Sea	N 42°29'36" E 23°55'17"	Pchelina Dam (5.4 km <sup>2</sup> )	4.5 km	2009	14.2	46 (32–61)	2.3 (0.7–5.6)
Iskar River	BG	Black Sea	N 42°32'17" E 23°29'58"	Pasarel Dam (0.34 km <sup>2</sup> )	1.4 km	2009	14.0	48 (29–61)	2.8 (0.6–5.9)

TU Turkey, GR Greece, BG Bulgaria, SL fish standard length, W fish weight

were located upstream the reservoirs with a possibility of fish migration between the river and reservoir. Fish were collected by electrofishing during October 2008 and 2009. Water temperature varied between sampling sites from 14 to 16.5°C. A total of 239 adult individuals of *R. amarus* were collected. Fish were transported alive in riverine water to the laboratory and individually killed by spinal transection prior to dissection within 24 h of transport. Standard length (SL; to the nearest 1 mm; measurement excludes the length of the caudal fin) and total body weight (W; to the nearest 0.1 g) were measured. Sampling site details and fish characteristics are shown in Table 1.

Fish were examined under a binocular microscope for the presence of metazoan parasites according to standard methods (Ergens and Lom 1970). Collected parasites were preserved in 4% formaldehyde (Acanthocephala, Digenea, Cestoda, Crustacea), in a mixture of ammonium picrate and glycerin (Monogenea) and in a mixture of glycerin and alcohol (Nematoda). Prior to species identification, acanthocephalans were cleared in clove oil and nematodes were cleared in a glycerin–water solution. Preserved digeneans and cestodes were stained in ferric acetocarmine (IAC), dehydrated in gradual alcohol series, and mounted into “Canada” balsam (Ergens and Lom 1970). Parasites were identified using a light microscope equipped with phase-contrast, differential interference contrast (DIC) and Olympus Digital Image Analysis. In addition, the keys for parasite determination (Ergens and Lom 1970; Gussev 1985; Bauer 1987; Moravec 1994; Niewiadomska 2003) were used. The material is deposited in the helminthology collection of the Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic.

Data analyses

Characteristics of parasite infection (prevalence and mean abundance) were calculated according to Bush et al. (1997). Prevalence was defined as the percentage of fish infected by a given parasite species in a sample; mean abundance as the average number of parasites per host (infected and non-infected); infracommunity richness as the number of parasite species per individual fish host. Frequency of occurrence was calculated as the percentage of sampling sites with

the presence of particular parasite species. For data analyses, parasite species were classified in respect to location on the fish body (ecto- and endoparasites), maturity stage (larval, subadult, and adult), and form of parasite colonization (autogenic/allogenic). Larval endoparasites comprised a group of species for which European bitterling served as an intermediate or paratenic host. Larval stages of parasites that naturally mature in this species (*P. tomentosa*) were excluded from the analyses regarding the importance of European bitterling as a source of infective stages for subsequent hosts. Autogenic/allogenic parasite species were characterized according to Esch and Fernández (1993): autogenic species are those that mature in vertebrates inhabiting aquatic ecosystems (fish, amphibian, and aquatic reptiles), and allogenic species are those that mature in terrestrial vertebrates (birds and mammals). Allogenic species are expected to have a greater colonization potential than parasites using aquatic vertebrates as final hosts. Groups of final host for particular larval endoparasite species (Table 2) were based on literature data (Bauer 1987; Moravec 1994; Sudarikov et al. 2002; Sitko et al. 2006). In avian parasites, only orders serving as final host for more than one parasite species in the sample were used in the analyses.

General linear model (GLM) followed by a Tukey's post hoc test was used to test differences in fish standard length and parasite abundance among sampling sites. The number of parasite species observed was independent of sampling effort (the number of fish individuals per each location) (Spearman rank correlation  $r_s = 0.36$ ,  $P = 0.43$ ). For data analyses, parasite abundance data were  $\log(x + 1)$  transformed. Paired t-tests were used to compare the proportion of ecto-/endoparasites, larval/adult endoparasites, and auto-/allogenic species in parasite communities within particular sampling sites. Arcsin( $\sqrt{x}$ ) transformation was used for percentage data. The relationship between parasite abundance/species richness and geomorphological characteristics (reservoir area, distance to reservoir) was tested by a Spearman rank correlation test. The co-occurrence of parasite species with the same final host group was tested by a Mantel test, comparing association matrices of parasite species x individual fish host and parasite species x potential final host. Twenty-one parasite species were used for this analysis, excluding parasites with unclear definitive host group (parasites

not identified to species level) and extremely rare species (mean abundance  $<0.01$ ). All analyses were performed using Statistica 9.1 for Windows (StatSoft, Inc. (2010), version 9.1. [www.statsoft.com](http://www.statsoft.com)) and PAST software (Hammer et al. 2001).

## Results

A total of 36 parasite species (including parasites not determined to species level) were found. The species composition of European bitterling parasite fauna was characterized by the dominance of endoparasites (31 species, 90% of all parasite specimens), involving cestodes, digeneans, nematodes, and acanthocephalans. Very low species richness was found in the ectoparasite community, composed of monogeneans (4 species) and sporadically occurring crustaceans. However, the abundance of ectoparasites was high in the Bulgarian sites of the Struma and Iskar Rivers (Fig. 1a; Table 3). Therefore, parasite community structure showed no difference in the proportion of endo- and ectoparasite individuals (paired t-test,  $t_6 = 1.9$ ,  $P = 0.112$ ; Fig. 1a) among particular sampling sites.

Three species parasitized European bitterling at all 7 sites: host-specific monogenean *Dactylogyrus bicornis* and *Gyrodactylus rhodei*, and generalist larval digenean *Paryphostomum radiatum*. The overall prevalence and abundance of individual parasite species was relatively low. None of the species reached more than 50% prevalence; a maximum prevalence of 41% was found for monogenean *G. rhodei*. Similarly, 19 parasite species showed a very low mean abundance ( $<0.1$ ), in contrast to only four species with a mean abundance of 1.0 or higher (Table 2).

Among the endoparasites, 30 species were found in the larval (or subadult) life stage using *R. amarus* as an intermediate or paratenic host. Only 2 species also parasitized bitterling in the adult stage (nematode *Pseudocapillaria tomentosa*, acanthocephalan *Acanthocephalus anguillae*), both with low abundances (Table 2). Accordingly, the proportion of larval endoparasites in the parasite community was significantly higher compared with the proportion of adult endoparasites at all the studied sites (paired t-test,  $t_6 = 17.9$ ,  $P < 0.001$ ; Fig. 1b). Nineteen

**Table 2** List of metazoan parasites of the European bitterling, frequency of occurrence (F, in %), and mean abundance (A)

Parasite species	Endoparasite/ Ectoparasite	Life stage	Final host	Autogenic/ Allogenic	Sampling site	F (%)	A
<b>Cestoda</b>							
<i>Ligula intestinalis</i>	Endoparasite	Plerocercoid	Birds (Po, Pe, Ci, An, La)	Allogenic	B, K, M	42.9	0.23
<i>Neogryporhynchus cheilancristrotus</i>	Endoparasite	Metacestode	Birds (Ci)	Allogenic	B	14.3	0.01
Pseudophyllidea fam. sp.	Endoparasite	Plerocercoid	n.a.	n.a.	I, E	28.6	0.01
<b>Monogenea</b>							
<i>Dactylogyrus bicornis</i>	Ectoparasite	Adult	Fish	Autogenic	S, I, N, B, E, K, M	100	0.50
<i>Gyrodactylus rhodei</i>	Ectoparasite	Adult	Fish	Autogenic	S, I, N, B, E, K, M	100	0.93
<i>Gyrodactylus laevis/ prostae</i>	Ectoparasite	Adult	Fish	Autogenic	S, I, B, E, K, M	85.7	0.15
<i>Gyrodactylus vimbi</i>	Ectoparasite	Adult	Fish	Autogenic	B, I	28.6	0.10
<b>Digenea/Trematoda</b>							
<i>Apharyngostrigea cornu</i>	Endoparasite	Metacercariae	Birds (Ci)	Allogenic	B	14.3	<0.01
<i>Apophallus muehlingi</i>	Endoparasite	Metacercariae	Birds (La)	Allogenic	K	14.3	0.18
<i>Clinostomum complanatum</i>	Endoparasite	Metacercariae	Birds (Po, Ci, La)	Allogenic	I, B, E, K	57.1	0.74
<i>Diplostomum</i> spp.	Endoparasite	Metacercariae	Birds	Allogenic	S, N, B, K, M	71.4	0.21
<i>Holostephanus</i> sp.	Endoparasite	Metacercariae	Birds	Allogenic	E	14.3	0.01
<i>Ichthyocotylurus</i> spp.	Endoparasite	Metacercariae	Birds (La)	Allogenic	E, K, M	42.9	5.38
<i>Metagonimus yokogawai</i>	Endoparasite	Metacercariae	Birds (Pe, Ci, La), Mammals	Allogenic	K	14.3	0.02
<i>Metorchis xanthosomus</i>	Endoparasite	Metacercariae	Birds (Po, Pe, La)	Allogenic	K, M	28.6	2.60
<i>Monilipher spinulosus</i>	Endoparasite	Metacercariae	Birds (Po)	Allogenic	K, M	28.6	0.99
<i>Paracoenogonimus ovatus</i>	Endoparasite	Metacercariae	Birds (Po, Pe, Ci, La), Mammals	Allogenic	K	14.3	0.03
<i>Paryphostomum radiatum</i>	Endoparasite	Metacercariae	Birds (Po, Pe, La)	Allogenic	S, I, N, B, E, K, M	100	0.94
<i>Petasiger</i> sp.	Endoparasite	Metacercariae	Birds	Allogenic	N	14.3	<0.01
<i>Posthodiplostomum brevicaudatum</i>	Endoparasite	Metacercariae	Birds (Ci)	Allogenic	S, K, M	42.9	0.03
<i>Posthodiplostomum cuticola</i>	Endoparasite	Metacercariae	Birds (Ci)	Allogenic	S	14.3	0.01
<i>Tylodelphys clavata</i>	Endoparasite	Metacercariae	Birds (Po)	Allogenic	N, B, E	42.9	2.36
<i>Tylodelphys podicipina</i>	Endoparasite	Metacercariae	Birds (Po)	Allogenic	K	14.3	0.04
Cyathocotylidae fam. sp.	Endoparasite	Metacercariae	n.a.	n.a.	K, M	28.6	0.11
Digenea sp. 1	Endoparasite	Metacercariae	n.a.	n.a.	I, B, E, K, M	71.4	1.09
Digenea sp. 2	Endoparasite	Metacercariae	n.a.	n.a.	N	14.3	0.29

**Table 2** continued

Parasite species	Endoparasite/ Ectoparasite	Life stage	Final host	Autogenic/ Allogenic	Sampling site	F (%)	A
Nematoda							
<i>Agamospirura</i> sp.	Endoparasite	Larva	Reptiles, Amphibia	Autogenic	M	14.3	0.01
<i>Camallanus</i> sp.	Endoparasite	Larva	Fish	Autogenic	M	14.3	<0.01
<i>Eustrongylides</i> sp.	Endoparasite	Larva	Birds (Pe, Ci)	Allogenic	K	14.3	<0.01
Philometridae fam. sp.	Endoparasite	Larva	Fish	Autogenic	B	14.3	<0.01
<i>Pseudocapillaria tomentosa</i>	Endoparasite	Adult/larva	Fish	Autogenic	S, I, B, E, K, M	85.7	0.25/0.14
<i>Raphidascaris acus</i>	Endoparasite	Larva	Fish	Autogenic	S, I, K	42.9	0.04
<i>Spiroxyis contortus</i>	Endoparasite	Larva	Reptiles	Autogenic	E, M	28.6	0.02
Acanthocephala							
<i>Acanthocephalus anguillae</i>	Endoparasite	Adult/ subadult	Fish	Autogenic	S, I	28.6	0.01/0.01
<i>Pomphorhynchus laevis</i>	Endoparasite	Subadult	Fish	Autogenic	S, I	28.6	0.04
Crustacea							
<i>Ergasilus sieboldi</i>	Ectoparasite	Adult	Fish	Autogenic	I	14.3	0.03

Avian final host: *Po* Podicipediformes, *Pe* Pelecaniformes, *Ci* Ciconiiformes, *An* Anseriformes, *La* Lariformes, *n.a.* not available; Sampling sites: *S* Struma, *I* Iskar, *B* Ballica, *E* Eski Riva, *K* Kurtkoy, *M* Masukiye, *N* Nestos

endoparasite species were classified as allogenic, with avian or mammalian final host, and eight as autogenic, with fish, amphibian, or reptilian final host (Table 2). Parasite community structure showed a significantly higher proportion of allogenic parasites in comparison with autogenic at particular sampling sites (paired *t*-test,  $t_6 = 4.6$ ,  $P = 0.004$ ; Fig. 1c), with no autogenic endoparasites in the Nestos River and a higher proportion of autogenic endoparasites compared with allogenic in the Struma and Iskar Rivers (Fig. 1c).

The majority of endoparasite species/individuals in *R. amarus* were parasites with avian final hosts (all cestodes and digeneans, one nematode species). On the other hand, parasites having fish, amphibians, or reptiles as final hosts (nematodes and acanthocephalan) occurred with a low frequency and abundance, with the exception of *P. tomentosa* (Table 2). The co-occurrence of parasite species with an identical final host group showed statistically significant but modest association (Mantel test,  $r = 0.14$ ,  $P = 0.033$ ).

Mean parasite abundance and infracommunity richness differed significantly between sampling sites (GLM,  $F_{6,232} = 24.2$ ,  $P < 0.001$  and  $F_{6,232} = 44.0$ ,  $P < 0.001$ , respectively) with maximum abundance

and parasite species richness found in the Kurtkoy Stream (Table 3). Although fish standard length varied between sampling sites ( $F_{6,232} = 24.8$ ,  $P < 0.001$ ), neither parasite abundance nor infracommunity richness were affected by fish size (Spearman rank correlation,  $P > 0.05$  in all tests). The adjacent reservoir area was a significant determinant for the total and infracommunity species richness ( $r_s = 0.86$ ,  $P = 0.01$  and  $r_s = 0.88$ ,  $P = 0.009$ ) and for the mean parasite abundance ( $r_s = 0.80$ ,  $P = 0.03$ ). No relationship between the distance of sampling site from the adjacent reservoir and parasite community characteristics was found ( $P > 0.05$ ).

## Discussion

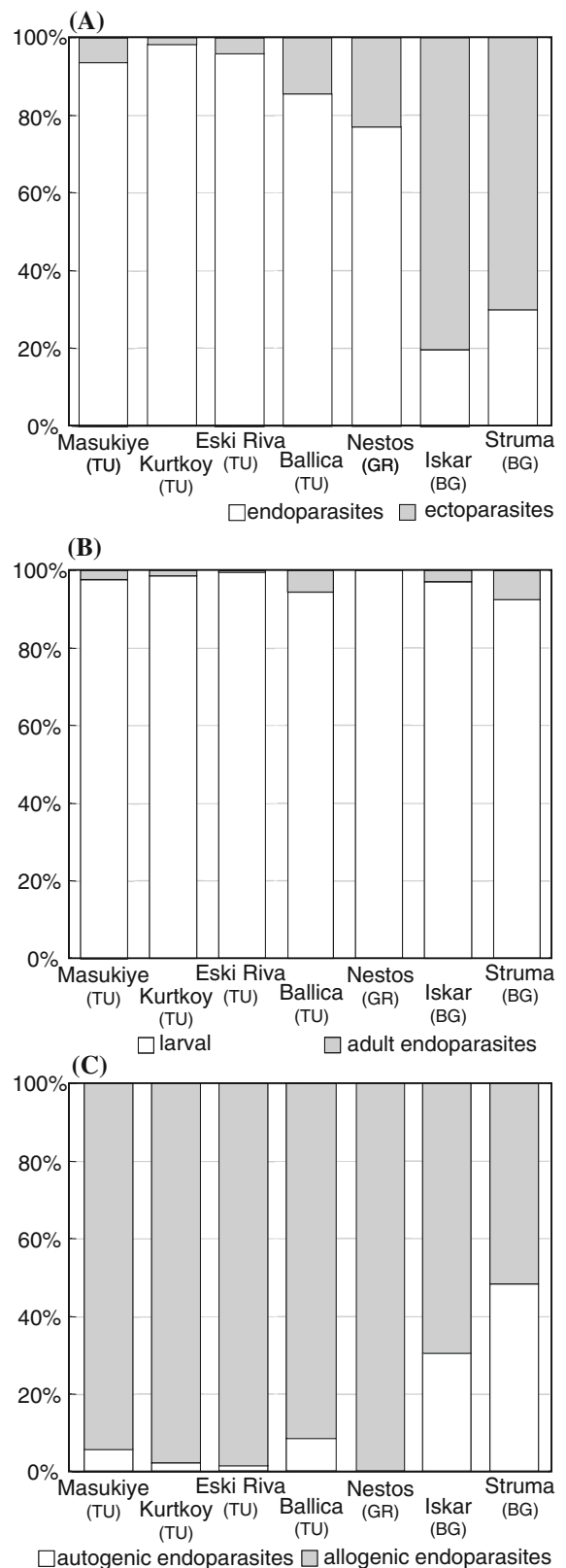
The present study focused on a freshwater cyprinid fish, the European bitterling, and its potential role in parasite accumulation and transmission in riverine ecosystems. *R. amarus* is a widely distributed and abundant small-sized fish and hence may represent a suitable intermediate host for many parasite species. Moreover, due to inhabiting diverse environments including rivers, backwaters, artificial canals, and

**Fig. 1** Proportion of **a** endoparasites and ectoparasites, **b** adult and larval endoparasites in particular sampling sites of the European bitterling (TU Turkey, GR Greece, BG Bulgaria)

estuarine waters in many European countries (Kozhara et al. 2007), *R. amarus* may be potentially parasitized by relatively high number of parasite species. Our study sites were restricted to the area of southeastern Europe where bitterling populations have been locally present throughout the Quaternary (Bohlen et al. 2006) and where, therefore, host–parasite interactions might have persisted for a long time.

Generally, the diversity of metazoan parasites of European bitterling in the southeastern European region is very high. A total of 36 parasite species were recorded, 31 of which were categorized as endoparasites. The composition of parasite fauna in European bitterling showed that this host species was predominantly parasitized by larval parasites using fish as an intermediate host, while the abundance of ectoparasites or adult endoparasites was relatively low. The sporadic occurrence of ectoparasitic species with wide host range (*Gyrodactylus laevis/prostae*, *G. vimbi*, *Ergasilus sieboldi*, see Table 2) indicates the low importance of European bitterling as a reservoir for ectoparasite infections. In this study, it was found that only two endoparasitic species parasitized European bitterling in the adult stage. The occurrence of the nematode *Pseudocapillaria tomentosa* in bitterling was previously described by Dávidová et al. (2005, 2008). However, the presence of acanthocephalan *Acanthocephalus anguillae* in the European bitterling was recorded for the first time. The infrequent occurrence of adult endoparasites passively transmitted (via ingestion) to the fish host is related to the bitterling feeding strategy. This fish species is considered to be strictly detritophagous and phytophagous, seldom feeding on annelids, chironomids, or crustaceans (Przybylski 1996), which are potential intermediate hosts for fish endoparasites.

In aquatic ecosystems, fish frequently serve as intermediate hosts for parasites with piscivorous final hosts (Zander et al. 1993; Groenewold et al. 1996; Zander 1998; Zander et al. 1999; Zander 2005; Valtonen et al. 2010). Many parasites require diverse hosts at different trophic levels for the completion of their life cycles, and their transmission is often



**Table 3** Epidemiological characteristics of parasite communities in the European bitterling

Sampling site	n Fish	Total species richness	Infracommunity richness	Mean parasite abundance						
				Total	Ectoparasites	Endoparasites	Larval endoparasites	Adult endoparasites	Allogenic endoparasites	Autogenic endoparasites
Eski Riva Stream	30	12	2.5 (0–6) <sup>a</sup>	31.0 ± 59.4 <sup>b</sup>	1.3 ± 1.7	29.8 ± 58.9	29.7 ± 58.9	0.1 ± 0.4	21.9 ± 60.0	0.1 ± 0.4
Ballica Stream	30	14	2.2 (0–5) <sup>a</sup>	9.3 ± 18.6 <sup>a</sup>	1.3 ± 1.6	7.9 ± 18.7	7.7 ± 18.7	0.3 ± 0.4	6.6 ± 18.2	0.03 ± 0.2
Kurtkoy Stream	40	20	6.0 (2–10) <sup>c</sup>	43.3 ± 36.8 <sup>c</sup>	0.8 ± 1.7	42.5 ± 37.0	42.2 ± 36.7	0.3 ± 0.8	41.1 ± 36.6	0.1 ± 0.3
Masukiye Stream	40	16	3.7 (1–9) <sup>b</sup>	20.6 ± 26.5 <sup>b</sup>	1.3 ± 1.4	19.3 ± 25.7	19.0 ± 25.6	0.3 ± 0.8	17.3 ± 25.4	0.1 ± 0.4
Nestos River	36	7	1.5 (0–4) <sup>a</sup>	5.0 ± 9.0 <sup>a</sup>	1.1 ± 1.8	3.8 ± 9.0	3.8 ± 9.0	–	1.9 ± 2.9	–
Struma River	33	11	1.5 (0–4) <sup>a</sup>	2.7 ± 2.6 <sup>a</sup>	1.9 ± 2.2	0.8 ± 1.2	0.8 ± 1.1	0.1 ± 0.2	0.4 ± 0.7	0.3 ± 0.7
Iskar River	30	13	2.2 (0–7) <sup>a</sup>	5.9 ± 6.4 <sup>a</sup>	4.8 ± 5.5	1.2 ± 2.6	1.1 ± 2.4	0.03 ± 0.2	0.8 ± 2.3	0.2 ± 0.4

Different letters (a, b, c) indicate groups with a significant difference between them using Tukey's multiple comparisons post hoc test after the GLM ( $P < 0.05$ )

dependent on a predator–prey relationship (Valtonen et al. 2010). In this study, the majority of parasites in *R. amarus* were found in the larval stage (digeneans, cestodes, nematodes, and acanthocephalans, (see Table 2), which suggests that the European bitterling could act as an important intermediate or paratenic host in freshwater habitats. This finding corresponds to a previous study indicating that the European bitterling seems to be a suitable intermediate host for a wide spectrum of metazoan parasites (Dávidová et al. 2008). Similar studies applied to marine ecosystems have proved that small-sized fish such as gobies or sticklebacks are often parasitized with larval stages of metazoan parasites and may figure as their transmitters (Zander 1998, 2007).

Larval stages of parasites with avian final host dominated in all bitterling endoparasite communities, including most of the digenean and cestode species, and one nematode species. Birds represent the most numerous group of warm-blooded vertebrates and harbor the highest number of helminth parasites (Sitko et al. 2006). The highest diversity of avian parasites was found in Turkey, at sampling sites located close to large reservoirs. The host specificity of larval digenean parasites (metacercariae) and some cestodes (e.g. *Ligula intestinalis*) is generally low; they are able to parasitize a wide range of fishes, and therefore, the probability of infecting a particular host species such as bitterling is high. Moreover, two digenean parasite species with mammalian definitive host were also found to parasitize European bitterling (see Table 2). However, the importance of this fish species as a parasite transmitter to the local mammalian community is disputable because of very low parasite abundance and frequency of occurrence. The same pattern was observed for nematodes using cold-water vertebrates as definitive hosts. Similarly as for mammalian parasites, the importance of bitterling as a reservoir for nematode infection seems to be only marginal.

Many ecological studies indicate that the occurrence of parasites with complex life cycles primarily reflects the type of habitat, the size of the location, and the presence of suitable first intermediate or final hosts (Valtonen and Gibson 1997; Morand et al. 2000; Smith 2001; Ondračková et al. 2004; Dávidová et al. 2008; Gam et al. 2008 etc.). In particular, the occurrence of larval and adult digeneans reflects the type of habitat. Lentic habitats (gravel pits, oxbows



or reservoirs) represent a more suitable environment for the completion of digenean life cycles than riverine habitats. In lentic waters, specific conditions encourage the presence of snails (higher water temperature, lower water velocity, and the presence of vegetation) and final hosts, especially birds (Ondračková et al. 2004; Dávidová et al. 2008). Therefore, we expected that the size of the adjacent reservoir area and the distance of the sampling site to the reservoir would affect parasite species richness and/or parasite abundance in *R. amarus*. Although all European bitterling populations were collected in running waters, the sampling sites were located near a reservoir with the possibility (except for the Greek population of *R. amarus*) of migration to that area. The impossibility of bitterling migration to the reservoir could explain why the lowest parasite species richness was observed in the Nestos River population. The distance of sampling site from the adjacent reservoir did not affect the parasite communities of European bitterling. All sampling sites were at a distance of 0.1–9.5 km from the adjacent reservoir area, which might be too short to demonstrate any significant changes in the parasite species richness. However, the size of the adjacent reservoir area (an example of a lentic water body) was found to be a significant determinant for the total and infracommunity species richness and also for the mean parasite abundance.

In general, lentic habitats represent more suitable environments for the reproduction and dissemination of larval digeneans (Dávidová et al. 2008), as lentic waters and adjacent water bodies are typical foraging habitat for many bird species. The heterogeneity in the parasitism of intermediate fish hosts can be strongly influenced by the distribution of definitive hosts. Larger reservoirs are expected to be more attractive for a higher diversity of aquatic vertebrates, serving as a final host for many parasite species. Moreover, larger reservoirs could also offer more potential niches for first intermediate hosts (e.g. snails) in parasites with complex life cycles. Corresponding to our results, a positive correlation between the species richness of allogenic parasites and pond surface area was found by Fellis and Esch (2005), who compared parasite communities of *Lepomis macrochirus* in a series of freshwater ponds. Similarly, a positive association between location area, and the abundance of a single digenean species

in fish was found in several water bodies in the floodplain of one small European lowland river (Ondračková et al. 2004). The interconnection between lentic waters, such as reservoirs, and river channels may then play an important role in the occurrence and abundance of many parasite species, especially those belonging to the group of allogenic parasites.

Due to the high diversity of allogenic parasite species, we tested the co-occurrence of different endoparasite species with an identical final host group. Andersen and Valtonen (1992) noted a high percentage of co-occurrence for two allogenic species, the larval cestodes *Diphyllbothrium ditremum*, and *Schistocephalus solidus*, both maturing in fish-eating birds. Although our results showed that the co-occurrence of parasite species with the same definitive host group was found more frequently than the co-occurrence of species with a different definitive host group, this relationship was not very strong. This modest association may be explained by the relatively high number of parasite species occurring at low frequencies.

In conclusion, our results show that the European bitterling figures as an important intermediate and/or paratenic host for a number of parasite species, predominantly those with bird definitive hosts. As a small-sized and fast-maturing fish, with a wide distribution range and high local abundances in various habitats, the European bitterling represents a natural and frequent prey for a wide range of piscivorous animals including fishes, reptiles, birds, and mammals. Therefore, in addition to its natural role in the food web, it may play a significant role as a transmitter of a variety of metazoan parasites to their final hosts.

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