

## Helminth community structure study on urban and forest blackbird (*Turdus merula* L.) populations in relation to seasonal bird migration on the south Baltic Sea coast (NW Poland)

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### Summary

The aim of the study was to compare helminth community structure of urban and forest blackbird populations. 24 helminth species in 98 blackbirds were found. Higher species richness was noted in the forest population of the blackbird (23 species) in comparison to the urban population (14 species). The response of the helminth fauna to a synanthropic habitat, contrary to a natural habitat, consists in a significant reduction in most parasitological parameters. Higher species richness has been noted in spring (17 species) than in autumn (14 species). Urban habitat, in contrast to the forest, may cause changes in the abundance of helminth communities in male and female blackbirds. The helminth fauna of nestlings, in spite of low species richness is characterized by a higher prevalence and intensity of infection in comparison to blackbirds feeding on their own. Helminth fauna of the blackbird seems to be a good indicator of environmental quality.

Key words: *Turdus merula*; helminths; urban; forest; migrations

### Introduction

The helminth fauna of the blackbird (*Turdus merula* Linnaeus 1758) is well known both in Poland (Machalska, 1974, 1978, 1980a, 1981; Okulewicz A., 1979a, b, c, 1981; Okulewicz J., 1982, 1992, 1993; Machalska & Okulewicz A., 1984; Okulewicz A. *et al.*, 2007; Okulewicz A. *et al.*, 2010) and in other countries (Ryšavý, 1955, 1958; Ryšavý & Baruš, 1964; Binder, 1971; Hanak & Vojtek, 1973; Schmidt, 1975; Bogdarenko & Galina, 1978; Iskova, 1979; Iskova *et al.*, 1995; Salamatina, 2000; Misof, 2005; Sitko *et al.*, 2006). The role of habitat in determining the structure of parasite communities has been stressed i.a. by Bush

(1990). Anthropogenic factors, contributing to a distortion of the natural ecosystem influence parasitic organisms as well as free-living ones. They may cause excessive development of populations of some organisms and oust others which are not adjusted well enough (Niewiadomska & Pojmańska, 2004). Parasites can be an indicator of environmental quality, which has been well documented for aquatic environment by research into parasitic fauna of fish (see e.g. Dzika, 2003). The response of bird parasites to changes in environmental quality has been studied less extensively. Several studies have been devoted to the effect of bird synanthropization on species richness and diversity of their helminths (Rutkowska, 1973; Okulewicz A., 1979a, b; Sitko & Zalesny, 2014). A comparative study on nematode fauna of blackbirds in urban and forest habitats was conducted by Okulewicz A. (1979a, b). Furthermore, thanks to the studies conducted by Machalska (1974, 1978, 1980a, b, 1981) and Machalska and Okulewicz A. (1984), digeneans, nematodes and acantocephalans, of migrating blackbirds are well known. Finally, the effect of blackbird urbanization on its helminth fauna has been proved by a comparative study conducted in the eastern part of the Czech Republic (Sitko & Zalesny, 2014). However, studies devoted to urban blackbird populations are rather few and we still do not have sufficient knowledge about the helminths of birds which show a tendency for synanthropization. The blackbird is a very good object of study because it forms two types of populations: the forest population, living in a natural habitat, and the synanthropic population, which has been associated with an urban habitat for a long time. Urban and forest blackbird populations can differ with respect to biological and ethological characteristics, including the extent of undertaking seasonal migrations, diet, ways of obtaining food and the diversity of

feeding grounds. Already in the 1970s it was noted that most forest blackbirds (ca. two thirds of the total population) migrated for winter, whereas urban blackbirds led a sedentary lifestyle, since their urge to migrate had disappeared (Graczyk, 1961).

As noted by Bush (1990), a seasonal character of environmental quality is one of the factors which can play an important role in helminth distribution. It is especially important in the case of migratory birds, as spring and autumn migrations provide opportunities for transfer of numerous parasite species (Niewiadomska & Pojmańska, 2004). For example, the following trematode species have been reported during migration time of birds: *Brachylaima arquata* in birds belonging to the genus *Turdus*: (Machalska, 1980a), *Mihajlovia migrata* in *Turdus philomelos*; *Cyclocoelum polonicum* and *Laterotrema vexans* in *T. merula* and *T. philomelos* (Sulgostowska & Czaplińska, 1987); the cestode species: *Emberizotaenia raymondi* (syn. *Ptilotolepis philomelae* J. Okulewicz, 1991) in *T. philomelos*: (Okulewicz J., 1991), and acantocephalans: *Corynosoma pyriforme* and *Sphaerostris lancea* in *T. merula*: (Machalska, 1981). Trematode species *Mossesia sittae* and *Mossesia microsoma* noted in the Turdidae of Lower Silesia had formerly been noted in the Far East (Okulewicz J., 1993). Typically “southern” species (Dogiel, 1962) have been identified among trematodes of birds in the Czech Republic, accidentally introduced from tropical regions, e.g. *Brachydistomum olssoni* brought by the common swift *Apus apus* (Sitko *et al.*, 2006) and *Morishitium elongatum*, *Mossesia microsoma*, *Psilotornus contortus*, *Euamphimerus pancreaticus*, *Mihajlovia migrata* brought by *T. merula* (Sitko & Zaleśny, 2014).

Studies on bird migration conducted on the Polish Baltic Sea coast and results of the analyses of information about returning ringed birds indicate that two migration streams of the blackbird run through the Polish coast. One runs towards the Atlantic coast of Europe and the other towards the Czech Republic and beyond, to the western and central Mediterranean Sea shores. Those two streams divide blackbirds into different “migrant” populations (J. Chruściel, unpublished data). Furthermore, Czech blackbirds presumably overwinter in Italy, while most blackbirds flying over the Polish coastal region presumably overwinter in France. Information about seasonal migration routes is necessary for parasitologists to analyze the helminth fauna structure of migratory birds.

Age and sex of the host are factors frequently determining the structure of a parasite community (e.g. Bush, 1990; Poulin, 1996; Zuk & McKean, 1996; Margolis & Cone, 1997; Schalk & Forbes, 1997; Isomursu *et al.*, 2006; Robinson *et al.*, 2008; Calegario-Marques & Amato, 2010). The role of testosterone has also been stressed in literature (Folstad & Karter, 1992, but see Roberts *et al.*, 2004). Blackbirds are the only thrush species showing sexual dimorphism, but body size and weight of males and females are similar (Wysocki, 2002). Only females build nests but otherwise both sexes lead a similar lifestyle. No differences have been noted in their diet, either (Glutz von Blotzheim *et al.*, 1982).

The aim of the study was to gain insight into the relation between the structure of the helminth fauna on the one hand and the habitat and seasonal migration of the blackbird from the southern coast of the Baltic Sea on the other. We assumed that in the studied geographical region the component community of helminths in the forest population of the blackbird would be richer than in the synanthropic population. Probably there would also be a significant difference between spring and autumn in the forest population of the blackbird. We also assumed that the helminth fauna of males and females would not differ significantly and that the helminth fauna of nestlings would be much less diversified than that of the blackbirds, feeding on their own. The applied method of collecting helminths took into account the principles of environmental protection. In many European countries the blackbird is a game bird but in Poland it is a protected species. However, it frequently falls prey to predators such as cats, martens and corvids (Bauer *et al.*, 2005). According to a study conducted in the Czech Republic, helminths were a probable cause of death of 41 passerines, including 9 blackbirds *T. merula* (Okulewicz A. & Sitko, 2012). As noted by Hromada *et al.* (2000) many museums continue to acquire material by collecting dead birds which died of “natural” causes. Most ringing teams, throughout their work, also find birds that die of exhaustion, and most of us find dead birds from time to time, as well (Hromada *et al.*, 2000). In this study we show that material collected from dead birds is useful and can bring measurable benefits for science. Results of the study will expand available knowledge and contribute to forming general ideas about the reduction of the helminth fauna richness of the blackbird in synanthropic habitat. They will also show that the structure of helminth community of the blackbird seems to be an indicator of environmental quality in urban areas.

## Material and methods

In the years 2006 – 2012, 98 blackbirds from the southern coast of the Baltic Sea in north-western Poland were studied (Table 1). The specimens represented forest blackbird populations from the following sites: Wiselka (53° 57' N, 14° 34' E) Goleniów (53° 33' N, 14° 49' E), Kopań (54° 27' N, 16° 25' E), Wicie (54° 30' N, 16° 28' E), Choczewo (54° 44' N, 17° 53' E), Hel (Kuźnica) (54° 44' N, 18° 34' E), Pobierowo (54° 3' N, 14° 55' E) and the urban blackbird population from the city of Szczecin (53° 26' N, 14° 32' E). Blackbird specimens from forest populations were collected mainly in spring and in autumn, while specimens of urban blackbirds were collected mainly in spring and in summer (Table 1). The sex of blackbirds feeding on their own was identified at necropsy. There were 17 (53 %) males and 12 (38 %) females and 3 (9 %) non sexed individuals among the collected urban blackbirds, and 21 (45 %) males and 18 (38 %) females and 8 (17 %) non sexed individuals among the collected forest blackbirds. Helminths were preserved in 70 – 75 % ethanol. Permanent microscope slides stained with alum carmine and

acetocarmine were prepared from trematodes and cestodes. Cestode scolices were cleared in Fora fluid. Nematodes and acantocephalans were cleared in lactic acid.

Qualitative and quantitative structure of helminth community of forest and urban blackbird populations was determined at the level of a component community and infracommunities. Structure analysis was conducted according to the terminology proposed by Bush *et al.* (1997). Basic parasitological parameters were determined, such as: prevalence (*P*), mean intensity of infection (*MI*) and range (min – max) (Bush *et al.*, 1997). Community richness was determined on the basis of the number of helminth species. A relationship between the helminth community structure and blackbird habitat was studied by comparing a group of urban blackbirds with a group of forest blackbirds during spring and summer (Table 1). A relationship between the helminth community structure and seasonal host migration was studied by comparing the helminth community structure of forest blackbirds in spring and in autumn. A relationship between the helminth community structure and the sex of blackbirds was studied by comparing groups of males and females in the urban and the forest habitat. The helminth fauna of nestlings (age 5 – 14 days) was studied by comparing them with blackbirds feeding on their own in the urban habitat (immature, adult and juvenile birds were combined together) (Table 1). Helminth species diversity, expressed by Simpson's diversity index,  $1 - D$  ( $D = \sum [n_i(n_i - 1) / N(N - 1)]$ ) was determined for the studied group of blackbirds, where  $n_i$  was a number of individuals of the species  $i$  and  $N$  was the total number of helminths in a given community (Magurran, 2004). The value of this index ranged between 0 and 1; the greater the value of the index, the greater the sample diversity. Furthermore, Berger-Parker's index of dominance was calculated ( $d = N_{max} / N$ ) where  $N_{max}$  was a number of individuals of the most abundant species and  $N$  was the total number of individuals, showing the proportion of the most common species in the community or sample, and finally, Jaccard's index of similarity ( $J = [c / (a + b + c)] \times 100$ ), determining faunistic similarity among communities, where  $a$  was the number of species present only in sample 1,  $b$  was the number of species present only in sample 2 and  $c$  was the total number of species present in both samples.

The following set of parameters was compared among various blackbird groups:

- The prevalence of helminths representing respective systematic groups (Digenea, Cestoda, Nematoda, Acanto-

cephala) expressed by the percentage share of infected hosts;

- The number of helminth species per each examined host and the number of helminth species representing respective systematic groups (Digenea, Cestoda, Nematoda, Acantocephala) per each examined host (mean  $\pm$  *SD*);

- The number of parasites expressed by the number of helminths per each examined host, including the number of helminths representing respective systematic groups (Digenea, Cestoda, Nematoda, Acantocephala) (mean  $\pm$  *SD*);

- The number of parasites belonging to species characterized by the highest prevalence per each examined host (mean  $\pm$  *SD*).

As distributions of variables deviated from normal distribution, values of parameters were transformed by logarithming. After the transformation, the distribution of the studied variables acquired the characteristics of a normal distribution (Dytham, 2011). The significance of differences was determined using a parametric test (*t*); samples were compared in pairs. Results feature parameter values which show significant differences:  $p \leq 0.05$ ,  $p \leq 0.01$  and  $p \leq 0.001$ .

In drawing up the checklist of helminths of the blackbird in Poland original works were used, as well as keys (Gibson *et al.*, eds., 2002, Jones *et al.*, eds. 2005), catalogues of parasitic fauna of Poland (Sulgostowska & Czaplińska, 1987; Czapliński *et al.*, 1992; Okulewicz, 1997; Sulgostowska, 1997), the internet database Fauna Europaea (de Jong, 2013), the monograph by Pojmańska *et al.* (2007), and finally, also the results of the authors' own research (see Table 6). The compiled checklist of helminths includes information on synonyms featured in literature and main references. Data from the checklist has been used for discussion on helminth species typically found in different migratory populations of the blackbird: the "southern" population whose seasonal migration route runs through southern Poland (S Poland) and the Czech Republic, and the "northern" population migrating through northern Poland (N Poland).

## Results

Twenty-four helminth species were found (9 Digenea, 8 Cestoda, 5 Nematoda and 2 Acantocephala). Species richness of the helminth fauna of forest blackbirds was higher than species richness of urban blackbirds (Table 2). In the forest blackbird population 23 helminth species were noted

Table 1. Material. Number of examined blackbirds in the urban and the forest habitat

Phenological season	Urban habitat		Forest habitat	Total
	Nestlings	Blackbirds feeding on their own	Blackbirds feeding on their own	
Spring (III – V)	4	21	18	43
Summer (VI – VIII)	15	4	2	21
Autumn (IX – XI)	0	1	24	25
Winter (XII – II)	0	6	3	9
<b>Total</b>	<b>19</b>	<b>32</b>	<b>47</b>	<b>98</b>

Table 2. Helminths of the blackbird in the forest and the urban habitat on the southern coast of the Baltic Sea. The table features information on the occurrence (+) or absence (-) of helminth species. *N* - number of individuals, *P* - prevalence; *MI* - mean intensity

Helminth species or higher taxonomic group	Forest blackbird population N=47*				Urban blackbird population N=32*				Nestlings N= 19	Location in the host	
	Males N=21		Females N=18		Males N=17		Females N=12				
	MI (min-max)	P	MI (min-max)	P	MI (min-max)	P	MI (min-max)	P			
<i>Brachylaima</i> sp.	2.1		1.0		3.1		7.0		-		intestine
<i>Leucochloridium perturbatum</i> Pojmańska, 1968	6.4		28.7 (2-70)		-		-		-		cloaca
<i>Morishitium elongatum</i> (Harrach, 1921) <sup>s</sup>	4.2		3.5 (2-5)		+		-		-		air sacks
<i>Tamerlania zarudnyi</i> Skryabin, 1924 <sup>a</sup>	2.1		2.0		+		-		-		kidney
<i>Psilotornus confertus</i> Machalska 1974 <sup>s</sup>	2.1		1.0		+		-		-		intestine
<i>Prosthogonimus ovatus</i> (Rudolphi, 1803) <sup>s</sup>	2.1		3.0		+		-		-		bursa Fabricii
<i>Leyogonimus postgonoporus</i> (Neiland, 1951) <sup>s</sup>	2.1		1.0		+		-		-		intestine
<i>Lutztrema attenuatum</i> (Dujardin, 1845)	29.8		11.2 (1-48)		+		18.9	12.3 (1-60)	+		liver, gall bladder
<i>Zonorchis petiolatus</i> (Railliet, 1900)	21.3		1.9 (1-3)		+		12.5	2.0 (1-5)	+		gall bladder
<i>Digenea</i> gen. sp. <sup>s</sup> very young specimens	2.1		25.0		?		-	-	-		bursa Fabricii
<i>Digenea</i> gen. sp. <sup>s</sup>	-		-		-		3.1	1.0	+		intestine
<i>Dilepis undula</i> (Schrank, 1788)	53.2		8.9 (1-33)		+		46.9	7.5 (1-43)	+	47.4	14.0 (2-34)
<i>Passerilepis crenata</i> (Goeze, 1782)	4.2		3.5 (3-4)		+		3.1	1.0	+		intestine
<i>Fernandezia spinosissima</i> (von Linstow, 1894) <sup>s</sup>	-		-		-		6.2	3.0 (2-4)	+		intestine
<i>Sobolevitaenia unicoloronata</i> (Fuhrmann, 1908)	8.5		14.2 (1-52)		+		9.4	4.3 (1-10)	-		intestine
<i>Monopylidium caenodex</i> (Mettrick & Beverley-Burton, 1962) <sup>s</sup>	8.5		2.2 (1-3)		+		18.8	9.7 (3-24)	+		intestine
<i>Spasspasskya dubinini</i> (Bauer, 1941) <sup>s</sup>	2.1		8.0		+		3.1	1.0	+		intestine
<i>Variolepis farciniosa</i> (Goeze, 1782) <sup>s</sup>	2.1		3.0		+		3.1	3.0	-		intestine
<i>Monorcholepis dujardini</i> (Krabbe, 1869) <sup>a</sup>	2.1		7.0		?		-	-	-		intestine
<i>Cestoda</i> gen. sp.	29.8		7.6 (1-19)		+		34.4	25.0 (1-61)	+	15.8	4 (1-7)
<i>Eucoleus contortus</i> (Creplin, 1839) <sup>s</sup>	2.1		3.0		+		-	-	-		oesophagus
<i>Baruscapillaria ovopunctata</i> (Linstow, 1837)	2.1		5.0		-		3.1	40.0	+		intestine
<i>Capillaria</i> sp.	10.6		4.2 (2-7)		+		6.2	2.5 (1-4)	+		intestine
<i>Porrocaecum ensicaudatum</i> (Zeder, 1800)	51.1		3.4 (1-30)		+		21.9	4.7 (1-19)	+	26.3	5.8 (1-22)
<i>Cardiofilaria pavlovsky</i> Strom, 1937	8.5		5.7 (4-7)		+		-	-	+		intestine
<i>Plagiorhynchus (Prosthorhynchus) cylindraceus</i> (Schrank, 1788)	44.7		6.4 (1-26)		+		28.1	4.8 (1-14)	+		body cavity
<i>Sphaerostris lancea</i> (Westrumb, 1821)	10.6		5.6 (1-17)		+		-	-	+		intestine
<i>Acantocephala</i> gen. sp.	10.6		2.0 (1-5)		-		12.5	3.7 (1-8)	+		intestine

\* - *N* includes non-sexed individuals, <sup>s</sup> - helminths noted only in spring, <sup>a</sup> - helminths noted only in autumn

Table 3. A comparison of infection with helminths between blackbirds in the forest and the urban habitat, in spring and in summer. Mean values (mean  $\pm$  SD) refer to each examined host. Dominant helminth species are given next to the values of Berger-Parker's index

Set of parameters		Forest habitat (N = 20)	Urban habitat (N = 25)	<i>p</i>
Prevalence of helminths:	Total prevalence	100 %	84 %	0.0173*
	Digenea	65 %	28 %	0.0055**
	Cestoda	90 %	76 %	0.1021
	Nematoda	65 %	40 %	0.0457*
	Acantocephala	90 %	28 %	< 0.0001***
Number of species:	Total number of helminths	4.05 $\pm$ 1.53	2.12 $\pm$ 1.34	< 0.0001***
	Digenea	1.00 $\pm$ 0.95	0.32 $\pm$ 0.55	0.0032**
	Cestoda	1.30 $\pm$ 0.78	1.12 $\pm$ 0.82	0.1576
	Nematoda	0.80 $\pm$ 0.68	0.40 $\pm$ 0.49	0.0225*
	Acantocephala	0.95 $\pm$ 0.38	0.28 $\pm$ 0.45	< 0.0001***
Number of individuals: (groups)	Total number of helminths	35.45 $\pm$ 31.57	19.76 $\pm$ 22.14	0.0066**
	Digenea	10.10 $\pm$ 18.66	3.16 $\pm$ 11.37	0.0083**
	Cestoda	14.40 $\pm$ 13.90	11.60 $\pm$ 17.38	0.0640
	Nematoda	3.90 $\pm$ 6.94	3.12 $\pm$ 8.43	0.0944
	Acantocephala	7.05 $\pm$ 7.20	1.88 $\pm$ 4.07	< 0.0001***
Number of individuals: (species)	<i>L. attenuatum</i>	4.0 $\pm$ 10.54	2.84 $\pm$ 11.76	0.1193
	<i>D. undula</i>	8.70 $\pm$ 8.50	4.28 $\pm$ 8.92	0.0079**
	<i>P. ensicaudatum</i>	2.65 $\pm$ 6.53	1.32 $\pm$ 3.78	0.1180
	<i>P. cylindraceus</i>	6.25 $\pm$ 7.11	1.32 $\pm$ 3.44	< 0.0001***
Simpson's index (1 - <i>D</i> )		0.870	0.855	
Berger-Parker's index		0.247	0.216	
		<i>D. undula</i>	<i>D. undula</i>	

Significance levels: \* -  $p \leq 0.05$ , \*\* -  $p \leq 0.01$ , \*\*\* -  $p \leq 0.001$

and 14 helminth species were noted in the urban blackbird population. The highest difference was noted in the case of the Digenea. Species with the highest prevalence included *D. undula* and *Plagiorhynchus cylindraceus* in both the forest and the urban blackbird population, and *Lutztrema attenuatum* and *Porrocaecum ensicaudatum* in the forest population.

Higher values of parasitological parameters were noted in forest blackbirds in comparison to urban blackbirds (Table 3). Statistically significant differences were discovered in the species richness of helminths, in the prevalence of helminths, including digeneans, nematodes and acantocephalans and in the number of helminths including the number of the digeneans and acantocephalans of forest and

Table 4. A comparison of infection with helminths between the spring and autumn in the forest blackbird population. Mean values (mean  $\pm$  SD) refer to each examined host. Dominant helminth species are given next to the values of Berger-Parker's index

Set of parameters		Spring (N = 18)	Autumn (N = 24)	<i>p</i>
Prevalence of helminths:	Total prevalence	100 %	92 %	0.0742
	Digenea	67 %	38 %	0.0282*
	Cestoda	89 %	67 %	0.0374*
	Nematoda	67 %	58 %	0.2891
	Acantocephala	94 %	33 %	< 0.0001***
Number of species:	Total number of helminths	3.95 $\pm$ 1.00	2.33 $\pm$ 1.70	0.0002***
	Digenea	1.06 $\pm$ 0.97	0.50 $\pm$ 0.76	0.0206*
	Cestoda	1.33 $\pm$ 0.82	0.79 $\pm$ 0.64	0.0121*
	Nematoda	0.78 $\pm$ 0.63	0.71 $\pm$ 0.68	0.3530
	Acantocephala	1.0 $\pm$ 0.33	0.33 $\pm$ 0.47	< 0.0001***
Number of individuals: (groups)	Total number of helminths	37.33 $\pm$ 32.52	10.38 $\pm$ 13.59	< 0.0001***
	Digenea	11.11 $\pm$ 19.41	3.54 $\pm$ 9.34	0.0206*
	Cestoda	14.61 $\pm$ 14.42	3.75 $\pm$ 5.64	0.0007***
	Nematoda	3.89 $\pm$ 7.19	1.75 $\pm$ 2.44	0.1185
	Acantocephala	7.72 $\pm$ 7.28	1.33 $\pm$ 3.52	< 0.0001***
Number of individuals: (species)	<i>L. attenuatum</i>	4.44 $\pm$ 11.02	3.21 $\pm$ 9.24	0.2114
	<i>D. undula</i>	8.28 $\pm$ 8.50	1.96 $\pm$ 4.43	0.0007***
	<i>P. ensicaudatum</i>	2.89 $\pm$ 6.84	0.88 $\pm$ 0.93	0.1720
	<i>P. cylindraceus</i>	6.94 $\pm$ 7.16	0.42 $\pm$ 1.08	< 0.0001***
Simpson's index (1 - <i>D</i> )		0.873	0.829	
Berger-Parker's index		0.223	0.319	
		<i>D. undula</i>	<i>L. attenuatum</i>	

Significance levels: \* -  $p \leq 0.05$ , \*\* -  $p \leq 0.01$ , \*\*\* -  $p \leq 0.001$

Table 5. A comparison of infection with helminths between nestlings and blackbirds feeding on their own in the urban habitat

Set of parameters		Nestlings (N = 19)	Blackbirds feeding on their own (N = 32)	<i>p</i>
Prevalence of helminths:	Total prevalence	63 %	84 %	0.0518
	Digenea	0 %	28 %	0.0004***
	Cestoda	63 %	78 %	0.1333
	Nematoda	26 %	31 %	0.3511
	Acantocephala	0 %	34 %	< 0.0001***
Number of species:	Total number of helminths	0.89 ± 0.79	2.09 ± 1.35	0.0008***
	Digenea	0.00 ± 0.00	0.38 ± 0.66	0.0008***
	Cestoda	0.63 ± 0.48	1.06 ± 0.75	0.0311*
	Nematoda	0.26 ± 0.44	0.31 ± 0.45	0.3987
	Acantocephala	0.00 ± 0.00	0.34 ± 0.48	< 0.0001***
Number of individuals: (groups)	Total number of helminths	8.63 ± 13.32	16.38 ± 20.65	0.0401*
	Digenea	0.00 ± 0.00	2.81 ± 10.64	0.0023*
	Cestoda	7.11 ± 9.77	9.31 ± 15.96	0.4014
	Nematoda	1.53 ± 4.91	2.44 ± 7.56	0.2856
	Acantocephala	0.00 ± 0.00	1.81 ± 3.86	0.0005***
Number of individuals: (species)	<i>L. attenuatum</i>	0.00 ± 0.00	2.31 ± 10.61	0.0163*
	<i>D. undula</i>	6.63 ± 9.96	3.50 ± 8.03	0.1391
	<i>P. ensicaudatum</i>	1.53 ± 4.91	1.03 ± 1.04	0.3746
	<i>P. cylindraceus</i>	0.00 ± 0.00	1.34 ± 3.34	0.0027**
Simpson's index (1 - <i>D</i> )		0.368	0.865	
Berger-Parker's index		0.773	0.215	
		<i>D. undula</i>	<i>D. undula</i>	

Significance levels: \* -  $p \leq 0.05$ , \*\* -  $p \leq 0.01$ , \*\*\* -  $p \leq 0.001$ 

urban blackbirds. Simpson's diversity index was slightly higher for the forest blackbird population than for the urban blackbird population. Helminth communities of both the forest and the urban population were dominated by *D. undula*. Faunistic similarity expressed by Jaccard's index amounted to 26 %.

Higher values of parasitological parameters were noted in spring (Table 4). Seventeen helminth species were found in spring and fourteen were found in autumn. Statistically significant differences were found regarding species richness of helminths, total prevalence of helminths and prevalence and number of individuals representing the Digenea, Cestoda and Acantocephala, including the number of *D. undula* and *Plagiorhynchus cylindraceus*. Biodiversity of the helminth community expressed by Simpson's diversity index was higher in spring than in autumn. Berger-Parker's index of dominance was higher in autumn than in spring and *Lutztrema attenuatum* was a dominant species. The faunistic similarity between the spring and autumn in the forest population (Jaccard's index) was 22 %.

A comparison of helminth community structure of males and females showed that it differed between the urban and the forest population (Table 2). In the forest population no statistically significant differences were discovered between males and females with respect to the analyzed set of parameters. Differences which were the closest to the estimated level of significance referred to the prevalence of cestodes (males: 62 %; females: 83 %;  $p = 0.064$ ) and the number of *D. undula* – for males:  $5.81 \pm 6.07$  (mean  $\pm$  SD) per one examined host; for females:  $4.06 \pm 8.55$ ;  $p = 0.070$ . The faunistic similarity of helminth communities of

males and females in the forest population equaled 26 %. A comparison of males and females in the urban blackbird population revealed significant statistical differences with respect to the number of helminths per host – males:  $10.65 \pm 17.05$  (mean  $\pm$  SD) per every examined host; females:  $25.33 \pm 23.11$ ;  $p = 0.042$ , including the number of cestodes (males:  $2.88 \pm 3.61$  per every examined host; females:  $18.08 \pm 21.75$ ;  $p = 0.011$ ). Differences which were the closest to the estimated level of significance referred to the prevalence of cestodes (males: 71 %; females: 92 %;  $p = 0.066$ ) and the number of cestodes species (males:  $0.88 \pm 0.68$ ; females:  $1.25 \pm 0.60$ ;  $p = 0.059$ ) and also the number of nematodes (males:  $0.47 \pm 0.98$  per every examined host; females:  $5.50 \pm 11.62$ ;  $p = 0.061$ ). The faunistic similarity of helminth communities of males and females in the urban population equaled 23 %.

Prevalence of helminths in nestlings in the urban blackbird population was equal to 63 % while in the group of blackbirds feeding on their own 84 % ( $p = 0.0518$ ). Helminths found in nestlings included only 2 species of helminths (Table 2). In nestlings there were not found any Digenea or Acanthocephala. A comparison of the helminth community structure of nestlings and blackbirds feeding on their own revealed statistically significant differences with respect to a few parameters (Table 5). Simpson's index was lower in nestlings than in blackbirds feeding on their own and Berger – Parker's index was higher. The faunistic similarity between nestlings and females was higher than the faunistic similarity between nestlings and males, Jaccard's index: 19 % and 16 % respectively.

Table 6. The occurrence of helminth species in two migratory blackbird (*Turdus merula*) populations: “southern” population, whose migration route runs through S Poland and the Czech Republic and “northern” population, whose migration route runs through N Poland. The table features information on the occurrence (+) or absence (-) of helminth species. The Table is simultaneously the checklist of helminth species of the blackbird in Poland (syn. – synonyms used in literature)

Helminth species or higher taxonomic group	Main reference	Type of blackbird population	
		“Southern” Czech Republic/ S Poland	“Northern” N Poland
<b>Digenea: 16 species</b>			
<i>Lutztrema attenuatum</i> (Dujardin, 1845); syn.: <i>Brachylecithum attenuatum</i> (Dujardin, 1845); <i>Lutztrema monenteron</i> (Price et McIntosh, 1935)	Sitko <i>et al.</i> (2000), Machalska (1980b), Rząd <i>et al.</i> (2011)	+	+
<i>Morishitum elongatum</i> (Harrach, 1921); syn.: <i>Cyclocoelum polonicum</i> Machalska 1980 <i>Leucochloridium paradoxum</i> Carus, 1835	Machalska (1980a, 1980b), Rząd <i>et al.</i> (2011)	+	+
<i>Leucochloridium perturbatum</i> Pojmańska, 1969	Machalska (1980b)	+	+
<i>Macyella postgonoporus</i> Neiland, 1951; syn.: <i>Leyogonimus postgonoporus</i> (Neiland 1951)	Pojmańska (1969), Machalska (1980b), Rząd <i>et al.</i> (2011)	+	+
<i>Prosthogonimus ovatus</i> (Rudolphi, 1803)	Okulewicz J. & Wesolowska (2003), Rząd <i>et al.</i> (2011)	+	+
<i>Psilotorus confertus</i> Machalska 1974	Machalska (1980b)	+	+
<i>Urogonimus macrostomus</i> (Rudolphi, 1802); syn.: <i>Leucochloridium macrostomum</i> (Rudolphi, 1802)	Machalska (1974), Rząd <i>et al.</i> (2011)	+	+
<i>Zonorchis petiolatus</i> (Railliet, 1900) syn.: <i>Lyperostomum petiolatum</i> Railliet, 1938, <i>Lyperostomum</i> sp.; <i>Lyperostomum turdia</i> Ku, 1900; <i>Oswaldoia petiolata</i> (Railliet, 1900)	Machalska (1978, 1980b)	+	+
<i>Tamerlania zarudnyi</i> Skrjabin, 1924 <i>Collyricloides massanae</i> Vaucher, 1969 <i>Euamphimerus pancreaticus</i> Baer, 1960 <i>Mossesia microsome</i> (Singh, 1962); syn.: <i>Pleuropsolus microsome</i> Singh, 1962 <i>Urotocus rossiensis</i> (Mühling, 1898)	Sitko <i>et al.</i> (2006); This study Okulewicz <i>et al.</i> (2010) Okulewicz J. (1982) Okulewicz J. (1993)	+	+
<i>Brachylaima arquata</i> (Dujardin, 1845) <i>Laterotrema vexans</i> (Braun, 1901)	Okulewicz J. (1992) Machalska (1980b) Machalska (1980b)	+	-
<b>Cestoda: 8 species</b>			
<i>Dilepis undula</i> (Schränk, 1788)	Czapliński <i>et al.</i> (1992); Okulewicz J. & Wesolowska (2000)	+	+
<i>Soboleviaenia unicoloronata</i> (Fuhrmann, 1908)	Czapliński <i>et al.</i> (1992)	+	+
<i>Passerilepis crenata</i> (Goeze, 1782)	This study	+	+
<i>Fernandesia spinosissima</i> (von Linstow, 1894)	Salamatin <i>et al.</i> (2010)	+	+
<i>Monorcholepis dijudardi</i> (Krabbe, 1869)	This study	+	+
<i>Variolepis farcinosa</i> (Goeze, 1782)	This study	+	+
<i>Spaspasskiya dubinini</i> (Bauer, 1941)	Salamatin <i>et al.</i> (2007)	+	+
<i>Monopylidium caenodex</i> (Mettrick & Beverley-Burton, 1962)	Salamatin <i>et al.</i> (2007)	-	+

<i>Porrocaecum ensicaudatum</i> (Zeder, 1800)	Okulewicz A. (1979a, 1979 b), Machalska & Okulewicz A. (1984)	+	+
<i>Porrocaecum semiteres</i> (Zeder, 1800)	Okulewicz A. (1979a, 1979b), Machalska & Okulewicz A. (1984)	+	+
<i>Syngamus merulae</i> Baylis, 1926	Okulewicz A. (1979a, 1979b), Machalska & Okulewicz A. (1984)	+	+
<i>Baruscapillaria ovopunctata</i> (von Linstow, 1873); syn.: <i>Capillaria ovopunctata</i> (Linstow, 1873)	Okulewicz A. (1979a, 1979b, 1997), Machalska & Okulewicz A. (1984)	+	+
<i>Eucoleus contortus</i> (Creplin, 1839); syn.: <i>Thomix contorta</i> (Creplin, 1839)	Okulewicz A. (1979a)	+	+
<i>Capillaria similis</i> (Kowalewski, 1903); syn.: <i>Thomix similis</i> (Kowalewski, 1903)	Okulewicz A. (1979a, 1979b); Machalska & Okulewicz A. (1984)	+	+
<i>Cardioflaria pavlovskiy</i> Strom, 1937	Okulewicz A. (1979a, 1979b), Machalska & Okulewicz A. (1984)	+	+
<i>Splendidofilaria mavis</i> (Leiper, 1909); syn.: <i>Ornithofilaria mavis</i> (Leiper, 1909)	Okulewicz A. (1981)	+	+
<i>Anchotheca exilis</i> (Dujardin, 1845); syn.: <i>Capillaria exile</i> (Dujardin, 1845)	Okulewicz A. (1979a, 1979b), Okulewicz A. (1997)	+	-
<i>Microtetrameres</i> sp.	Okulewicz A. (1979a, 1979b)	+	-
<i>Echinuria uncinata</i> (Rudolphi, 1819)	Okulewicz A. (1979a, 1979b)	+	-
<i>Oxyspirura chabaudi</i> Baruš, 1965	Okulewicz A. <i>et al.</i> (2007)	+	-
<i>Physaloptera (Pseudophysaloptera) soricina</i> (Baylis, 1934)	Okulewicz A. (1979c)	+	-
<b>Acantocephala: 3 species</b>			
<i>Plagiorhynchus (Prosthynchus) cylindraceus</i> (Goeze, 1782)	Machalska (1981)	+	+
<i>Sphaerostris lancea</i> (Westrumb, 1821)	Machalska (1981)	+	+
<i>Corynosoma piriforme</i> (Bremer in Rudolphi, 1819)	Machalska (1981)	-	+



## Discussion

Presented study results confirm that the helminth fauna structure is related to factors characterizing the natural and the synanthropic habitat. These results coincide with results obtained by Sitko and Zaleśny (2014) who studied the helminth fauna of blackbirds in the eastern part of the Czech Republic. The authors showed that urbanization led to a significant reduction in the helminth fauna of the blackbird since the hosts adjusted themselves to the synanthropic habitat (Sitko & Zaleśny, 2014). An identical trend noticed in studies on various blackbird populations of the Baltic Sea Coast and the Czech Republic justifies a generalization that urbanization leads to a reduction in the species diversity of the helminth fauna of a bird species living in a synanthropic habitat in comparison to a bird species living in its natural forest habitat (Sitko & Zaleśny, 2014). A similar tendency showing a difference between the intensity of infection in forest and city habitats was revealed by studies on blood parasites of the blackbird (Hatchwell *et al.*, 2000; Bentz *et al.*, 2006; Geue & Partecke, 2008). The helminth fauna of the blackbird is well-known in Poland (Table 6), and it can provide a good model for studying the connection between the helminth fauna structure of an avian host and the host's adjustment to life in an urban habitat when more extensive research is conducted in the future. The checklist of helminths of the blackbird in Poland, featuring 40 species (16 Digenea, 8 Cestoda, 13 Nematoda and 3 Acantocephala), systematizes available knowledge on the helminth fauna of this host (Table 6).

A difference between helminth communities of the forest and urban habitat is clearly characterized by species richness expressed by the number of helminth species. Ten helminth species were found only in the forest habitat and one species was found only in the urban habitat (Table 2). Another 13 species were common for both the forest and the urban blackbird population. Apart from the above striking difference, helminth communities of the forest and the urban blackbird population were characterized by a certain similarity. It was reflected by the presence of species with higher infection parameters than in the case of the remaining helminths. In both populations, such species included: *Lutztrema attenuatum*, *Dilepis undula*, *Porrocaecum ensicaudatum* and *Plagiorhynchus cylindraceus* (Table 2). The same tendency could be seen in study results obtained by Sitko and Zaleśny (2014). The above helminths are typical for and common among birds of the genus *Turdus*. *D. undula* occurs all over Poland and its presence has been confirmed in many bird species, i.a. *Corvus* spp., *Pica pica*, *T. merula* and *T. philomelos* (Pojmańska *et al.*, 2007). *Porrocaecum ensicaudatum* is a cosmopolitan species, characteristic for *Passeriformes*. It has been encountered in many bird species in Poland, i. a. those belonging to the genera *Corvus* and *Turdus* (Okulewicz A., 1997). As for *Plagiorhynchus cylindraceus*, in Poland it has been found in birds of the genera *Corvus* and *Turdus* (Sulgostowska, 1997). The habitat of

the host plays an important role in forming the structure of helminth community of the blackbird. The prevalence and species richness were higher in all helminth groups in the forest habitat than in the urban habitat. This phenomenon can be explained by a probably lower availability of intermediary hosts in urban areas and different dietary habits of blackbirds in the forest and the urban areas: in the latter blackbirds frequently feed on human food waste having no access to dietary components available to their forest counterparts (Sitko & Zaleśny, 2014). Those differences are especially noticeable in the case of the digeneans and acantocephalans. The above tendency was noted both in our study and in the study by Sitko and Zaleśny (2014). The number of nematode species per each examined host and the prevalence of nematodes differed between the studied habitats and the differences were statistically significant (Table 3). A similar tendency was noted in the study by Okulewicz A. (1979b). Helminth communities of both the urban and the forest blackbird population are strongly dominated by nematodes. *Leucochloridium perturbatum* is a dominant species in forest blackbirds from the eastern part of the Czech Republic, and *D. undula* is a dominant species in forest blackbirds in that region (Sitko & Zaleśny 2014). *Leucochloridium perturbatum* is a frequent parasite of passerines and waders in the Holarctic Region and in North Africa (Sitko *et al.*, 2006). The difference between the forest blackbird population in Poland and in Czech Republic is interesting and is probably due to a difference in the intermediary host density, i.e. snails of the genus *Succinea* (Pojmańska, 1969). Similarly to our study, Sitko and Zaleśny (2014) noted high parameters of infection with *D. undula* in both habitat types. Study results suggest that the structure of the component helminth community of the blackbird may find application in future studies focusing on the evaluation of the quality of urban ecosystems to find out how much of a natural character they have retained and to what extent they have been degraded due to urbanization. The response of the helminth fauna to environmental changes facilitates the evaluation of changes taking place in the urban habitat in comparison to the forest habitat, the latter being the natural habitat of the blackbird.

The phenological season and the seasonal migration of blackbirds connected with it play a crucial role in forming the helminth fauna structure in the blackbird. This is reflected by the occurrence of some species only in spring or only in autumn (Table 2). A higher species diversity in spring than in autumn is caused, among other things, by the fact that birds return from the regions where they overwintered bringing parasites which they acquired there. Seasonal bird migrations provide an opportunity for transfer of many parasite species, therefore, when the presence of a parasite is noted in a migratory bird it cannot be certain whether the parasite belongs to native fauna of Poland (Niewiadomska & Pojmańska, 2004). This can be exemplified by *Michajlovia migrata* found in *T. philomelos* in Poland, encountered only twice during the spring migration season (Sulgostowska & Czaplińska, 1987, Pojmańska

*et al.*, 2007). This digenean species has recently been found in *T. merula* in a forest area in the Czech Republic (Sitko & Zaleśny, 2014). On the basis of study results reported by Dogiel (1962), we were able to select helminth species which infected blackbirds only in the regions where they overwintered (“southern species”) and were transferred to breeding grounds during the spring migration. There were two: *Morishitium elongatum* and *Psilotornus confertus*. Those digenean species, as well as *Leyogonimus postgonoporus* (Okulewicz J. & Wesołowska, 2003) had previously been noted in Poland during the spring migration of turdid species *T. merula*, *T. philomelos* and *Turdus iliacus* (Machalska, 1974, 1980, 1981; Sulgostowska & Czaplińska 1987). We did not find any of the “southern species” in blackbirds from the urban population (Table 2). The same result was reported by Sitko and Zaleśny (2014). The reasons for the low biodiversity of helminths in spring in the urban habitat in comparison to the forest habitat were probably sedentary lifestyle and the fact that urban blackbirds did not undertake seasonal migration. Spending winter in the city, urban blackbirds were not exposed to an invasion of “alien” helminth species. According to ornithological studies of many years focusing on average migration of the urban blackbird population from Szczecin indicated that ca. 20 % blackbirds migrated for winter, 30% were nomads (i.e. travelled short distances without going in any specific direction) and 50 % did not migrate for winter at all (Zyskowski & Wysocki, 2011). Other reasons for a higher species diversity in spring included seasonal changes in the blackbird diet and greater availability of feeding grounds in spring than in summer. Probably, those were the reasons why differences were noted in the number of *D. undula* and *Plagiorhynchus cylindraceus* between spring and autumn and the dominance of *Lutztrema attenuatum* in autumn. According to Okulewicz A. (1979b), one of the reasons why extensive and intense invasions of parasitic helminths persisted was the fact that blackbirds ate large amounts of food before they undertook autumn migration. In autumn, blackbirds largely feed on vegetative food, i.e. berries (Bauer *et al.*, 2005), which is conducive to infection with helminths with simple development cycles. In the present study, no statistically significant differences were found between spring and autumn as for parameters of infection with nematodes (Table 4). Okulewicz A. (1979b) studied the correlation between the nematode fauna and the season and observed seasonal differences comparing summer months to winter months. The author stressed the fact that the seasonal infection dynamics of *T. merula* resulted from changes in the diet of birds. A maximum prevalence and intensity of occurrence of *Porrocaecum ensicaudatum* in *T. merula* was noted by the author during summer months, whereas minimum prevalence was noted by her during winter months (Okulewicz A., 1979b). Studies on the occurrence of nematodes of *T. merula* during spring and autumn migrations revealed the presence of 6 nematode species (see Table 5) (Machalska & Okulewicz A., 1984). Similarly to our study, *Porrocaecum ensicaudatum* was characterized

by the highest prevalence among nematodes (Machalska & Okulewicz A., 1984).

In future studies, additional information can be obtained by analyzing seasonal migration routes of blackbirds. As it is assumed that blackbirds migrating through northern Poland (“northern” population) and blackbirds migrating through southern Poland in the direction of the Czech Republic (“southern” population) belong to two different migration streams, their helminth fauna structures should be studied separately in future. The analysis of the helminth occurrence in the “southern” and “northern” population allowed for a preliminary selection of species that probably occur in one of those migratory blackbird populations but not the other (Table 6). The trematodes: *Collyricloides massanae*, *Euamphimerus pancreaticus*, *Mossesia microsoma*, *Urotocus rhositensis* and nematodes: *Aonchotheca exilis*, *Microtetrameres* sp., *Echinuria uncinata*, *Oxyspirura chabaudi* and *Physaloptera* (*Pseudophysaloptera*) *soricina* characterized the “southern” blackbird population. The trematodes: *Brachylaima arquata*, *Laterotrema vexans*, cestode species *Monopylidium caenodex*, and the acantocephalan species *Corynosoma piriforme* characterized the “northern” blackbird population (Table 6). It can be assumed that the differences were due to different migration routes of the blackbird. An analysis of the relation between the helminth occurrence and blackbird migration routes is rendered difficult by a comparatively low prevalence of the above listed helminths. Therefore, the present study requires a continuation as it indicates perspectives which should be verified by analyzing a larger amount of material.

No statistically significant differences were revealed between males and females in the forest population with respect to the studied parameters. Similar results were obtained by Sitko and Zaleśny (2014) for the blackbird and Calegaro-Marques and Amato (2010) for the rufous-bellied thrush *Turdus rufiventris* in the metropolitan region of Porto Alegre (Brasil). So far, no insight has been gained into differences concerning the diet of male and female blackbirds (Glutz von Blotzheim *et al.*, 1982). Preliminary results of studies on blackbird behaviour indicate that during the breeding season females feed under the cover of trees much more frequently than males (Wysocki D. unpublished). Males, whose task is to protect their territory, feed mainly on lawns. However, we cannot exclude the possibility that they become infected with certain parasites present in the ground cover under shrubs. Such differences in feeding habits do not exist in the forest, where males and females feed in the same places. In our study, noteworthy differences were discovered between males and females in the urban blackbird population. Contrary to the forest blackbird population, in the urban population the difference between males and females with respect to the infection with cestodes was statistically significant. Furthermore, parameters of infection with nematodes were higher in the case of females than in the case of males. A similar result was obtained by Okulewicz A. (1979b), who noted that the intensity of infection with nematodes of adult females of *T. merula* in the surroundings of Wrocław

was twice as high as in the case of males. Despite the fact that the results coincide they should be confirmed by studying a larger amount of material. If the frequent character of the observed phenomenon will be found its conditions ought to be studied.

The infection of birds with helminths is tightly connected with the type of consumed food and the manner of feeding, since transmission via food is the most frequent way in which helminths invade the organism of a host. This is connected with differences in parameters of infection between nestlings and blackbirds that feed on their own. The occurrence of cestodes and nematodes was noted in urban nestlings but no digeneans and acantocephalans species were found in this age group. Two helminth species were noted: *D. undula* and *Porrocaecum ensicaudatum*. Blackbird nestlings do not look for food on their own but are fed by their parents. Both parents participate in the process (Bauer *et al.*, 2005). Food brought to the nest consists mainly of invertebrates, and intermediary and paratenic hosts of helminths can occur among them. Older blackbirds, able to look for food independently, have a much broader feeding spectrum than nestlings and search for food in diversified habitats. Parameters of nestling infection (*D. undula* and *P. ensicaudatum*) were higher than those of blackbirds feeding on their own. A similar result with respect to the high intensity of nestling infection with *P. ensicaudatum* in comparison to adult blackbirds was obtained by Okulewicz A. (1979b). Okulewicz A. (1979b) found representatives of those nematodes in *T. merula* and *T. philomelos* nestlings which were only two days old, whereas 55.5 % of nestlings which were 1 – 5 days old were infected with *P. ensicaudatum*. The developmental cycle of both *D. undula*, and *P. ensicaudatum* involves the participation of intermediary hosts: earthworms (Oligochaeta). As noted by Okulewicz A. (1979b), the youngest nestlings of *T. merula* are fed mainly with soft, easily digestible food, i.e. earthworms (Korodi Gal, 1967), which explains the high intensity of infection with the above mentioned helminth species.

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