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VERSITA

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Endoparasitic helminths of water frog complex in Poland: do differences exist between the parental species *Pelophylax ridibundus* and *Pelophylax lessonae*, and their natural hybrid *Pelophylax esculentus*?

M. POPIOŁEK[1], B. ROZENBLUT-KOŚCISTY[2], M. KOT[1], W. NOSAL[2], M. OGIELSKA[2]

[1]Department of Invertebrate Systematics and Ecology, Institute of Biology, Wrocław University of Environmental and Life Sciences, Kożuchowska 5b, 51-631 Wrocław, Poland, E-mail: *marcin.popiolek@up.wroc.pl*;
[2]Department of Biology and Conservation of Vertebrates, Zoological Institute, University of Wrocław, ul. Sienkiewicza 21, 50-335 Wrocław, Poland

Summary

Parasitic fauna of water frogs was mainly studied in the second half of the 20th century. However, these studies were done without differentiation into species and hybrids and pooled the 3 taxa as "water frogs" or "green frogs". The aim of this study was to make an inventory of helminth species as well as their prevalence and intensity of infection in the two parental species (*Pelophylax ridibundus* and *P. lessonae*) and the hybrid (*P. esculentus*) of water frogs from 3 big populations composed of hundreds or thousands of individuals inhabited natural and semi-natural landscapes in Poland. Eight helminth species were found: *Polystoma integerrimum*, *Diplodiscus subclavatus, Opisthoglyphe ranae, Gorgodera cygnoides*, *Haematoloechus variegatus*, *Oswaldocruzia filiformis*, *Cosmocerca ornata* and *Acanthocephalus ranae*. The results were compared with data from other, polish and European studies. Additionally we compared the level of infection among water frog taxa.

Keywords: helminth parasites; green frogs complex; *Pelophylax ridibundus*; *Pelophylax lessonae*; *Pelophylax esculentus*; Poland

Introduction

Parasitic fauna of water frogs was mainly studied in the second half of the 20th century (Combes, 1972; Combes & Sarrouy, 1971; Odening, 1955 – 56, 1957; Vojtkova, 1974, 1975; Vojtkova & Vojtek, 1975; Cedhagen, 1988; Fernandez, 1984; Galeano *et al.,* 1990). A review of helminthfauna of frogs and toads of Europe was published by Vojtkova & Roca (1993, 1994, 1996). In Poland the research were carried out by Sander (1949), Grossman & Sandner (1954), Grabda-Kazubska (1972a,b, 1978, 1980, 2000), Kozłowska (1960), Plasota (1969), Kuc & Sulgostowska 1988a,b), and Pilecka-Rapacz (1997, 1998). However, these studies were done without differentiation into the species and hybrids and pooled the 3 taxa as "water frogs" or "green frogs". For this reason and due to rapidly changing environment and increasing decline of amphibian populations, the information about water frog parasites should be updated (Pojmańska *et al.,* 2007). Furthermore, the assignment of the species composition of parasitic fauna to host species is also important from the verification of host specificity point of view.

The taxonomy of water frogs changed over time (Berger, 1969, 1983). Before 1959 all European taxa were named *Rana esculenta* after Linne (1758). Then *R.. ridibunda* was described as a separate species, whereas *R. lessonae* was described as a subspecies or a geographical form of *R.. esculenta* owing to their morphological similarity. Finally in 1967 Berger revealed that *R. esculenta* is a natural hybrid between *R. ridibunda* and *R. lessonae* (for review see Graf & Polls-Pelaz, 1989). Now the name of the genus has been changed into *Pelophylax* (Frost, 2009).

Water frog complex in Europe is composed of 3 taxa: two species *Pelophylax lessonae* (formerly *Rana lessonae* Camerano, 1882) and *P. ridibundus* (formerly *R. ridibunda* Pallas, 1771), and their hybridogenetic hybrid *P. esculentus* (formerly *R. esculenta* L.). All three taxa are very closely related and can mate and give progeny. *P. ridibundus* inhabits and hibernate in rivers, lakes and big fish ponds with running water; *P. lessonae* lives in small ponds and hibernates on land, whereas *P. esculentus* inhabits all types of water bodies and hibernates either in water or on land. In the wild they form mixed populations (L-E, *lessonae-esculentus* and R-E, *ridibundus-esculentus*) according to their ecological preferences; in rare cases all three taxa live together in the same locality (L-E-R, *lessonae-esculentus-ridibundus*) (for review see Berger, 1966; Rybacki & Berger 2001; Plötner, 2005).

The aim of this study was to make an inventory of helminth species, as well as their prevalence and intensity of infection in two parental species (*P. ridibundus* and *P.*

lessonae) and the hybrid (*P. esculentus*) of water frogs from 3 big populations composed of hundreds or thousands of individuals inhabited natural and semi-natural landscapes in Poland. For this reason studies performed by us may serve as references for those carried out on water frog populations inhabiting areas with higher human impact or facing other environmental difficulties. We also focused on genetic and environmental differences in susceptibility of infection between the water frog taxa.

Material and methods

Study area

(1.) Słowiński National Park (SNP), of total area of 18 247 ha, is situated at the southern shore of the Baltic Sea. It is composed of the system of small rivers, shallow seaside lakes, bogs, peat bogs, meadows, forests, and moving dunes. The frogs (*P. esculentus*, *P. lessonae* and *P. ridibundus*) were collected in the south-east part of the Gardno Lake (N 54°40', E 17°8') with temporarily occurring slightly brackish waters and along the shores of the Dołgie Duże Lake (N 54°41', E 17°11'). The lakes are eutrophic with great diversity of water plants. The invertebrate fauna of the Gardno Lake was described by Guttowa (1958) and Strzelecki and Półtorak (1971).

(2.) Nadmorski Landscape Park (NLP) of total area of 18 804 ha (39.6 % of land along the seashore and 60.4 % of the Gulf of Puck). The frogs (*P. esculentus* and *P. lessonae*) were collected in two nature reserves: Bielawa peat bogs (N 54°48', E 18°16') and ditches in natural marshes of the Mechelińskie Łąki located at the Gulf shore (N 54°37', E 18°30').

(3.) Landscape Park of the Barycz River Valley (BRV) of total area of 87 040 ha in south-west Poland. Frogs were collected in the water bird reserve "Milicz Ponds" that is composed of forests, fields, meadows and various fish ponds (about 65 % of the area). The reserve is composed of 5 fishpond complexes composed of approximately 100 ponds with a total area of 77 km^2. It is one of the most valuable regions in Europe with high biodiversity and water management system that has been almost unchanged since the Middle Ages. The frogs (*P. esculentus* and *P. ridibundus*) were collected in two complexes: Stawno (N 51°32', E 17°21') and Potasznia (N 51°32', E 17°29'). The invertebrate fauna in the region is rich, but not well studied.

Hosts identification

We analyzed 733 randomly selected adults of both sexes collected in 2001 and 2002 from water bodies inhabited by mixed populations of water frog taxa (*P. esculentus*/*P. lessonae* and *P. esculentus*/*P. ridibundus,* respectively). The frogs analysed in this study were also used in several other projects (e.g. Ogielska *et al.,* 2004; Socha & Ogielska, 2010). The localities and numbers of animals in the samples are summarized in Table 1. The sex was identified according to external characters. For taxonomical identification of frogs we used 4 morphometric characters: snout-vent length (SVL), tibia length (T), first toe length (DP - *digitus primus*) and *callus internus* length (CI). Two biometrical indices DP/CI and T/CI, traditionally used in taxonomy of East European water frogs (Berger, 1966; Plötner, 2005), were calculated. All frogs were measured with a calliper to the nearest 0.01 mm. To support morphometric analysis, the specimens were additionally checked by chromosome analysis on metaphase plates stained with actinomycine D/DAPI according to Ogielska *et al.* (2004).

Parasitological dissection

The specimens were subjected to the standard method for helminthological dissection (Niewiadomska, 2003). The parasites were rinsed by physiological salt solution, counted and conserved in 70 % ethanol. Fixed trematodes and acanthocephalans were stained with borax carmine and differentiated by acid ethanol, dehydrated in series of ethanol, cleared in carnation oil and mounted on slides in the Canada balsam. Nematodes were cleared in glycerol. The prevalence and mean intensity of infection were calculated according to Bush *et al.* (1997).

Statistical analysis

To show the possible differentiation between an intensity of infection of water frog taxa and between the studied sites we applied the Chi-square test, and the nonparametric tests of Mann-Whitney and Kruskal-Wallis. The normality of distributions of analyzed variables was verified with the Shapiro-Wilk test. Statistical analyses were performed at $p \leq 0.05$, with the use of statistical software package Statistica (version 9.0 PL).

Table 1. Sample sizes and localities of collection of three water frog taxa

Host	Site of collection			Total
	Słowiński National Park	Nadmorski Landscape Park	Barycz River Valley	
Pelophylax esculentus	148	25	116	289
Pelophylax lessonae	13	68	--	81
Pelophylax ridibundus	13	--	350	363
Total	**174**	**93**	**466**	**733**

Results

Review of species

The helminth fauna of water frogs from the 3 localities was represented by 8 species belonging to Monogenea, Digenea, Nematoda and Acanthocephala (Tab. 2). The list of species together with short comments concerning already existing data is provided below.

MONOGENEA

***Polystoma integerrimum* (Froelich, 1791)**

Host: *Pelophylax esculentus.*

Site of location: urinary bladder.

Locality, prevalence and mean intensity of infection (range): SNP: 4.0 %; 2.5 (from 1 to 5 individuals).

Comments: *Polystoma integerrimum* is the monogenic parasite of amphibians and was found in *Bufo viridis* and typically in *Rana arvalis* and *R. temporaria* where its prevalence was respectively 5.5 % and 10.5 % in Warszawa (Sandner, 1949), 14.4 % and 10.9 % in Wrocław and Barycz River Valley (Paul, 1934), and 12 % and 3 % in Łódź (Szulc, 1962). Madel (1983) reported its presence in Germany and Vojtkova (1989) in the Czech Republic. Although *Polystoma integerrimum* was also described in water frogs by Dawes (1946), André (1912, 1913), Layman (1933) and Grossman and Sandner (1954) doubted whether it was possible.

DIGENEA

***Gorgodera cygnoides* (Zeder, 1800)**

Hosts: *Pelophylax esculentus, P. lessonae, P. ridibundus.*

Site of location: urinary bladder.

Locality, prevalence and mean intensity of infection (range): SNP: *P. esculentus* 5.4 %, 2.1 (1 – 5); NLP: *P. lessonae* 4.4 %, 1.7 (1 – 3); BRV: *P. ridibundus* 0.3 %, 7 (7).

Comments: *G. cygnoides* is a common parasite species of anuran amphibians in Poland (Grabda-Kazubska, 1972a, Kuc & Sulgostowska, 1988a,b). The prevalence values reported for water frogs from other localities at the Baltic Sea shore (5.9 %, Grossman-Pojmańska & Sandner, 1958) were similar to ours in NLP and SNP, but lower than in other localities in Poland: the Białowieski NP (15 %, Grossman & Sandner, 1954), the Łódź region (21 %, Szulc, 1962), the Warszawa region (27 %, Sinicyn, 1905), and very high values from Wrocław and Lower Silesia (33 – 65 %, Sander, 1949 and 22 %, Paul, 1934).

***Diplodiscus subclavatus* (Pallas, 1760)**

Hosts: *Pelophylax esculentus, P. lessonae, P. ridibundus.*

Site of location: distal portion of small intestine and large intestine.

Locality, prevalence and mean intensity of infection (range): NLP: *P. esculentus* 4.0 %, 2.0 (2), *P. lessonae* 1.5 %, 1.0 (1); BRV: *P. ridibundus* 0.3 %, 1.0 (1).

Table 2. Prevalence and intensity of infection of helminths in water frog host taxa at the studied sites

Locality	Host	Indices of infection	*P. integerrimum*	*G. cygnoides*	*D. subclavatus*	*O. ranae*	*H. variegatus*	*O. filiformis*	*C. ornata*	*A. ranae*
Słowiński National Park (SNP)	*P. esculentus*	(%)	4.0	5.4	--	--	20.3	1.4	2.7	8.9
		int.	2.5	2.1	--	--	5.7	1.0	1.3	1.3
	P. lessonae	(%)	--	--	--	--	15.4	--	--	7.7
		int.	--	--	--	--	2.5	--	--	1.0
	P. ridibundus	(%)	--	--	--	--	7.7	--	--	15.4
		int.	--	--	--	--	1.0	--	--	1.0
Nadmorski Landscape Park (NLP)	*P. esculentus*	(%)	--	--	4.0	4.0	20.0	4.0	--	24.0
		int.	--	--	2.0	2.0	2.4	3.0	--	2.5
	P. lessonae	(%)	--	4.4	1.5	4.4	13.2	7.4	4.4	26.5
		int.	--	1.7	1.0	5.3	2.4	1.6	1.7	1.2
	P. ridibundus	--	--	--	--	--	--	--	--	--
Barycz River Valley (BRV)	*P. esculentus*	(%)	--	--	--	0.9	--	0.9	--	0.9
		int.	--	--	--	1.0	--	1.0	--	1.0
	P. lessonae	--	--	--	--	--	--	--	--	--
	P. ridibundus	(%)	--	0.3	0.3	--	0.6	0.3	--	1.1
		int.	--	7.0	1.0	--	3.5	3.0	--	5.3

Abbreviations: (%) - prevalence; int. – mean intensity of infection

Comments: *Diplodiscus subclavatus* was the rarest species recorded in our study although it was usually reported as a common one. Grossman-Pojmańska & Sandner (1958) noted it in 52.8 %, Szulc (1962) in 22.4 %, Sinicyn (1905) in 17.4 %, and Plasota (1969) in 100 % individuals. Sandner (1949) recorded it in 10 – 33 % and Paul (1934) in 50 % infected water frogs in Lower Silesia, whereas we found none in the Barycz River Valley. *D. subclavatus* is a common parasite species of amphibians in Poland, both anuran (Grabda-Kazubska, 1972a; Kuc & Sulgostowska, 1988a,b; Grabda-Kazubska & Lewin, 1989; Pilecka-Rapacz, 1998) and newts (Bertman, 1994), as well as in other European countries (Vojtkova & Roca 1994; Düşen & Öz, 2006). It was also recorded in reptiles: *Lacerta agilis* (Lewin, 1992), *Natrix natrix* (Bertman, 1993) and *Vipera berus* (Lewin & Grabda-Kazubska, 1997).

***Opisthioglyphe ranae* (Froelich, 1791)**
Hosts: *Pelophylax esculentus, P. lessonae.*
Site of location: small intestine.
Locality, prevalence and mean intensity of infection (range): NLP: *P. esculentus* 4.0 %, 2.0 (2), *P. lessonae* 4.4 %, 5.3 (2 – 12); BRV *P. esculentus* 0.9 %, 1.0 (1).
Comments: *Opisthioglyphe ranae* was a rare species in our sample although its prevalence is usually high in water frogs: 36 % in the Białowieski NP (Grossmann & Sandner, 1954); 51.3 % in the Łódź region (Szulc, 1962); 78.2 % in the Kampinoski NP (Kuc & Sulgostowska, 1988b); 82.8 % in Mazury (Grabda-Kazubska, 1972a), and 57.5 % in the Barycz River Valley (Paul, 1934). Sandner (1949) recorded it in 65 %, and Kuc & Sulgostowska (1988a) in 83.9 % of *P. ridibundus* whereas we did not found it any individual of this taxon. *O. ranae* is one of the most common trematodes in amphibians of Poland, as well as in *Natrix natrix* (Grabda-Kazubska, 1972a; Kuc & Sulgostowska, 1988a,b; Pilecka-Rapacz, 1998).

***Haematoloechus variegatus* (Rudolphi, 1819)**
Hosts: *Pelophylax esculentus, P. lessonae, P. ridibundus.*
Site of location: lungs.
Locality, prevalence and mean intensity of infection (range): SNP: *P. esculentus* 20.3 %, 5.7 (1 – 25), *P. lessonae* 15.4 %, 2.5 (2 – 3), *P. ridibundus* 7.7 %, 1.0 (1); NLP: *P. esculentus* 20.0 %, 2.4 (1 – 4), *P. lessonae* 13.2 %, 2.4 (1 – 6); BRV: *P. ridibundus* 0.6 %, 3.5 (2 – 5).
Comments: *P. ridibundus* was the less infected species in our sample, as was also reported by Kuc & Sulgostowska (1988a) in the same host species (1.4 %). We also noted very low infection in *P. ridibundus* from the Barycz River Valley, although Paul (1934) recorded much higher prelevance of *H. variegatus* (27.9 %) in 68 water frogs from the same region. *H. variegatus* is a common parasite of amphibians in Poland (Grabda-Kazubska, 1972a; Kuc & Sulgostowska, 1988a,b) and was the most prevalent and of the highest infection intensity (1–25) also in our sample. In water frogs in Poland it was recorded in the Kampinoski NP (prevalence 11.4 % according to Plasota, 1969), environs of Warsaw (prevalence 35.6 % according to Kuc & Sulgostowska, 1988b), the Łódź region (prevalence 14.9 % according to Szulc, 1962), and Białowieski NP (prevalence 9 % according to Grossman & Sandner, 1954).

NEMATODA
***Oswaldocruzia filiformis* (Goeze, 1782)**
Hosts: *Pelophylax esculentus, P. lessonae, P. ridibundus.*
Site of location: small intestine.
Locality, prevalence and mean intensity of infection (range): SNP: *P. esculentus* 1.4 %, 1.0 (1); NLP: *P. esculentus* 4.0 %, 3.0 (3), *P. lessonae* 7.4 %, 1.6 (1 – 2); BRV: *P. esculentus* 0.9 %, 1.0 (1), *P. ridibundus* 0.3 %, 3.0 (3).
Comments: *O. filiformis* is not a common nematode species in water frogs and was formerly noted only in the Łódź region by Kozłowska (1960) and in Lower Silesia by Paul (1934). So far *O. filiformis* was described in *P. esculentus* and *P. lessonae,* but not in *P. ridibundus* (Pojmańska *et al.,* 2007) in Poland. However, we recorded this species in all 3 taxa of water frogs, similarly to Vojtkova & Roca (1994). *O. filiformis* is found in other amphibian species in Poland, typically in *Bufo bufo* (Grabda-Kazubska, 1972a), but accidentally also in fishes, as in *Salmo trutta* m. *fario* (Popiołek *et al.,* 2004). It was also recorded in reptiles: *Anguis fragilis* (Düşen *et al.,* 2010) in Turkey.

***Cosmocerca ornata* (Dujardin, 1845)**
Hosts: *Pelophylax esculentus, P. lessonae.*
Site of location: distal portion of small intestine and large intestine.
Locality, prevalence and mean intensity of infection (range): SPN: *P. esculentus* 2.7 %, 1.3 (1 – 2); NLP: *P. lessonae* 4.4 %, 1.7 (1 – 2).
Comments: In our sample *Cosmocerca ornata* was recorded for the first time in *P. lessonae* in Poland. *C. ornata* was reported in the Łódź region (prevalence 20.7 %) and Turew (35.5 %) by Kozłowska (1960), and in the Kampinoski NP (23 %, Kuc & Sulgostowska, 1988b). *C. ornata* was not reported in the Barycz River Valley neither by us nor by Paul (1935). *C. ornata* is a common parasite of amphibians in Poland, typically in *Rana teporaria* (Grabda-Kazubska, 1972a; Kuc & Sulgostowska, 1988b; Grabda-Kazubska & Lewin, 1989), also recorded in *Vipera berus* (Lewin & Grabda-Kazubska, 1997). It was also recorded in reptiles: *Anguis fragilis* (Düşen *et al.,* 2010) in Turkey.

ACANTHOCEPHALA
***Acanthocephalus ranae* (Schrank, 1788)**
Hosts: *Pelophylax esculentus, P. lessonae, P. ridibundus.*
Site of location: small intestine.
Locality, prevalence and mean intensity of infection (range): SNP: *P. esculentus* 8.9 %, 1.3 (1 – 3), *P. lessonae* 7.7 %, 1.0 (1), *P. ridibundus* 15,4 %, 1.0 (1); NLP: *P. esculentus* 24.0 %, 2.5 (1 – 5), *P. lessonae* 26.5 %, 1.2 (1 – 2); BRV: *P. esculentus* 0.9 %, 1.0 (1), *P. ridibundus* 1.1 %, 5.3 (1 – 16).
Comments: *A. ranae* was a common species in our sample

and was noted in the 3 frog taxa from all localities. According to Kuc & Sulgostowska (1988a), 69.7 % *P. ridibundus* individuals were infected, whereas in our study we recorded prevalence 15.4 % for the same species in the Słowiński NP. Sandner (1949) reported from 16 to 59 %, and Paul (1934) in 23.5 % infected water frogs. *A. ranae* is known from all frog species in Poland. Styczyńska (1958) noted it in 50 % water frogs from the Drużno Lake, Plasota (1969) from 4.5 % in the Kampinoski NP, and Grossman & Sandner (1954) in 11 % from the Białowieski NP. *A. ranae* is a common parasite of amphibians in Poland (Grabda-Kazubska, 1972a; Kuc & Sulgostowska, 1988b) and other localities in Europe (Vojtkova & Roca, 1996; Düşen & Öz, 2004, 2006; Düşen, 2007; Düşen *et al.,* 2009; Düşen & Oğuz, 2010; Düşen, 2011). It was rarely recorded in a duck *Anas platyrhynchos* (Sulgostowska, 1997) and in *Vipera berus* (Lewin & Grabda-Kazubska, 1997).

Comparison of prevalence of infection between water frog taxa

General prevalence of infection differed among the studied taxa. *Pelophylax lessonae* was the most infected species (55.5 % out of 81 individuals), *P. esculentus* was infected in 27.7 % (out of 289 individuals), and *P. ridibundus* was the less infected species (3.3 % out of 365 individuals).

Pelophylax esculentus was the only taxon that shared the same populations with the other 2 taxa (*P. lessonae* and/or *P. ridibundus*) in all localities and therefore we were able to compare infection in relation to co-existing parental species under the same environmental conditions. The highest prevalences (44.0 %; mean infection intensity: 2.4) were recorded in *P. esculentus* from L-E (*lessonae-esculentus*) mixed population in the Nadmorski Landscape Park (NLP) and from L-E-R (*lessonae-esculentus-ridibundus*) mixed population (35.1 %; mean infection intensity: 4.2) in the Słowiński National Park (SNP). The lowest values (2.6 %, mean infection intensity: 3.0) was noted in the Barycz River Valley (BRV). The differences were statistically important (χ^2=51.8; d=2; p=0.000). The diversity of helminths also differ: we found 6 species (*P. integerrimum*, *G. cygnoides*, *H. variegatus*, *O. filiformis*, *C. ornata*) in L-E-R population from SNP, 5 species (*O. ranae, D. subclavatus, H. variegatus*, *O. filiformis*, *A. ranae*) in L-E from NLP, and only 2 species (*H. variegatus, A. ranae*) in R-E (*ridibundus-esculentus*) population in BRV. Two species (*O. filiformis*, *A. ranae*) were found in *P. esculentus* from all types of populations, but we noticed differences in prevalences only for *A. ranae.* The highest prevalence (24 %) was recorded in L-E from NLP, then 8.9 % in L-E-R from SNP, whereas the lowest (0.9 %) was noted in R-E from BRV (χ^2=44.02; d=2; p=0.000). Similarly, intensity of infection was the highest in L-E and the lowest in R-E (Kruskal-Wallis test: H=45.2; p=0.000).

Pelophylax lessonae individuals were collected at the Baltic Sea shore from 2 regions localized about 80 km apart. The prevalence of infection was higher in the NLP than in the SPN (44.0 % *vs.* 30.8 %), but the difference was not statistically important. However, there was a great difference in number of helminth species that infected *P. lessonae* from the two localities. We noticed 6 species in NPK (*G. cygnoides*, *D. subclavatus, H. variegatus*, *O. filiformis*, *C. ornata, A. ranae*) and only 2 species in SNP (*H. variegatus* and *A. ranae*). The prevalences of *H. variegates* were similar in both localities (NLP: 13.3 %; 2.4 *vs.* SNP: 15.4; 2.5), whereas the prevalence of *A. ranae* was higher in NLP than in SNP (26.5 % *vs.* 7.7 %), but not statistically important (χ^2=2.14; d=1; p=0.143).

Pelophylax ridibundus **-** the third species of the analyzed frogs was found only in locality of SPN and BRV. Because of significant differences in sample sizes from both localities, a comparison of the level of infection may be burdened with some error. Since, however, in the case of *P. ridibundus*, sample size is also a reflection of the occurrence frequency of this species in these two very different environments, it was decided to show the basic parasitological indicators. The overall prevalence of infection in *P. ridibundus* SPN was higher than in the BRV (21.1 % vs. 4.6 %), while the average value of intensity was as vice versa (SPN vs. 1.0. BRV 2.1). In the SPN was recorded only two species of parasites (*H. variegatus* and *A. ranae*), while in frogs of BRV as many as five (*G. cygnoides, D. subclavatus, H. variegatus, O. filiformis* and *A. ranae*). Prevalence of infection of individual helminths species (including two occurring in both places) proved to be significantly higher in SNP than in the BRV.

The only locality where we were able to analyze an environmental impact on susceptibility of the three water frog taxa to helminth infection was the L-E-R population in SPN. Both prevalence and intensity of infection were similar for *P. esculentus* and *P. lessonae* (35.1 % and 4.2 *vs.* 30.8 % and 3.0) and lower for *P. ridibundus* (23.1 % and 1.0). *P. esculentus* individuals were invaded by six helminth species (*G. cygnoides*, *D. subclavatus, H. variegatus*, *O. filiformis*, *C. ornata, A. ranae*), whereas *P. lessonae* and *P. ridibundus* only by two (*H. variegatus* and *A. ranae*). We particularly focused on *P. esculentus* individuals collected in high numbers from two sites within SNP (Gardno and Dołgie Duże lakes), but we did not found differences in prevalences and intensities of infection between them when tested by χ^2. The composition of helminth species hosted by *P. esculentus* from the Gardno and Dołgie Duże lakes did not differ as well.

Discussion

The helmintofauna of water frogs from the three localities studied by us was rather poor and not numerous (Tab. 2) and was represented by 8 species, out of 48 described so far in water frogs from Poland (Grabda-Kazubska, 1972a). However, this information does not tell us about differences among the two parental species (*Pelophylax ridibundus* and *P. lessonae*) and hybrids (*P. esculentus*), because it pools all the water frog taxa together.

The most interesting question in our study is whether

prevalences, infection intensities and diversity of parasite species differ in relation to localities (environment) or water frog taxa (genetics). A good example comes from a comparison between two different sites: Nadmorski Landscape Park (NLP) and the Barycz River Valley (BRV). In NLP the values of prevalence, infection intensities and diversity of helminth species were high for the parental species *P. lessonae* and the hybrid *P. esculentus*. On the other hand, the extremely low values for the parental species *P. ridibundus* and the hybrid were recorded in BRV, most probably owing to procedures used in the carp culture, where one of the cyclic procedures from at least several decades has been manuring with lime that certainly influence the diversity and quantity of the plankton and benthos invertebrate fauna, and thereby can eliminate some of the intermediate hosts of the helminth species.

We do not observe such a clear effect of genetics of particular water frog taxa for susceptibility of helminth infection. An environmental influence may be ruled out only in the Słowiński National Park (SNP), where the two parental species and the hybrid inhabit the same sites. However, we observed only slight differences in intensities of infection among the taxa, although the hybrid *P. esculentus* seems to be more exposed probably as a result of a higher migratory activity in comparison to the parental species (Berger, 1983).

These two data sets show that the influence of environmental cues on the helminth infection of water frogs is out of question, whereas their genetics is only slightly involved.

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