

Characteristics of the peripheral blood of the *Anguillicola crassus* (Kuwahara, Niimi et Tagaki, 1974) and *Trypanosoma granulosum* Laveran et Mesnil, 1902 infested eel (*Anguilla anguilla* L.) ascending the River Rega (north-western Poland)

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The study was aimed at characterising the peripheral blood of eel (*Anguilla anguilla* L.) ascending the River Rega and infested with *Anguillicola crassus* (Kuwahara, Niimi et Itagaki, 1974) and *Trypanosoma granulosum* Laveran et Mesnil, 1902. **Material and methods.** Haematological assays were performed on 58 eel individuals caught in August and September 2001 and in July 2002. The nematode *Anguillicola crassus* was identified in the swim bladder lumen of 44 eel individuals. The mean infestation intensity amounted to 3.2, from 1 to 11 nematodes being found in a single host. Values of the basic haematological indices were determined from May-Grünwald-Giemsa (MGG)-stained blood smears. The data were subjected to statistical treatment involving the analysis of variance (ANOVA) and Kruskal-Wallis test (STATISTICA 6.0). **Results.** Microscope slides showed the eel to be infested by *Trypanosoma granulosum* Laveran and Mesnil, 1902 occurring at a prevalence of 100%. Significant differences between the months of fish capture were revealed in the mean haematocrit, MCHC, and MCV values of all the eel, regardless if they were infested with *Anguillicola crassus* or not. On the other hand, there were no significant differences in the values of the parameters studied (E, Hb, Ht, L), including the haematological indices (MCH, MCHC, MCV), between the *Anguillicola crassus*-infested individuals and those free of the parasites, throughout the period of study. The relative and absolute leukograms showed a substantial domination of lymphocytes over other blood cells of the leukocyte line. The qualitative characteristics of the peripheral blood of the *Anguillicola crassus*-affected and -free eel were similar. The results indicate that some haematological parameters could be related to the season of capture. Effects of *Trypanosoma granulosum* on the blood seems to be distinct, but requires further, detailed studies, including research on the infestation intensity and taking into account characteristics of the parasite's morphological forms.

Key words: *Anguilla anguilla*, *Anguillicola crassus*, haematological indices, *Trypanosoma granulosum*.

Introduction

Haematologic indicators of the health state of the European eel as well as the species' parasitic fauna in some water bodies of north-western Poland are known as a result of studies reported on by Orecka-Grabda [1, 2],

Wierzbicka and Orecka-Grabda [3–5], and Eiszporn-Orecka [6].

After *Anguillicola crassus* appeared in European waters, effects of the parasite on peripheral blood parameters of the European eel were analysed [7–13]. The studies failed to provide unequivocal conclusions. Determination of

the parasite's effects on blood parameters is rendered difficult by the lack of any distinct threshold between values typical of the healthy fish and those indicating pathology, and by the high variability of fish haematological indices [14, 15].

This study was aimed at obtaining a quantitative and qualitative characteristics of morphotic elements of the peripheral blood of young European eel (*Anguilla anguilla* L.), particularly with respect to the infestation of the nematode *Anguillicola crassus* inhabiting the eel's swim bladder and feeding on the blood.

Materials and methods

Some of the materials for this study were collected within the framework of a program supported by the National Board of the Polish Anglers' Association ("Qualitative and quantitative protection of the juvenile European eel, *Anguilla anguilla*, ascending the rivers Wieprza, Grabowa, Radew, and Rega from the Baltic Sea"). Haematologic assays and parasitological examination were carried out on 58 individuals caught in August and September 2001 and in July 2002. The eel length and weight data are provided in Table 1. The lumen of the swim bladder of 44 individuals was found to contain the nematode *Anguillicola crassus*. The mean infestation intensity was 3.2 and ranged from 1 to 11 nematodes in a fish. The infestation prevalence changed from 66.7% in August 2001 to 93.3% in September 2001 to 82.3% in July 2002. The swim bladders examined contained both mature *A. crassus* and juvenile and larval forms (Fig. 1).

The blood for assays was drawn from the caudal vessels. The assays were performed using haematological methods recommended to be applied in studies on fishes [17]. The haematocrit value (Ht %), haemoglobin content (Hb g%), and erythrocyte (E million/mm³) and lymphocyte (L thou./mm³) counts were determined. The following red corpuscle indices were calculated: mean haemoglobin content (by weight) per erythrocyte (MCH), mean haemoglobin concentration per erythrocyte (MCHC), and

mean erythrocyte volume (MCV). Blood smears were stained using the May-Grünwald-Giemsa (MGG) technique.

The data were subjected to statistical treatment involving the analysis of variance (ANOVA) and Kruskal-Wallis test (STATISTICA 6.0) applied to data arranged in two sets. One set was meant to test for significance of differences between dates of fish capture, without division into the *A. crassus*-infested and parasite-free individuals. The other set tested significance of differences between two groups of eel: the *A. crassus*-infested and the parasite-free individuals. The significance of difference was tested at $p \leq 0.05$ and $p \leq 0.01$.

At the same time, blood smears were examined to detect morphological changes in blood cells in the *A. crassus*-infested and nematode-free fish; the smears were also examined for the presence of blood parasites.

Results

Anguillicola crassus was found to induce slight changes in the appearance of the internal organs of those fish the swim bladders of which contained up to 6 nematodes. The changes were mostly observed in the swim bladder and were typical of anguillicolosis. The swim bladder of those eel that were more heavily infested (7–11 nematodes) was much larger. The swim bladder walls were thinner than those in both the less heavily infested eel and the nematode-free fish. The swim bladder contained sanguineous fluid and mucus.

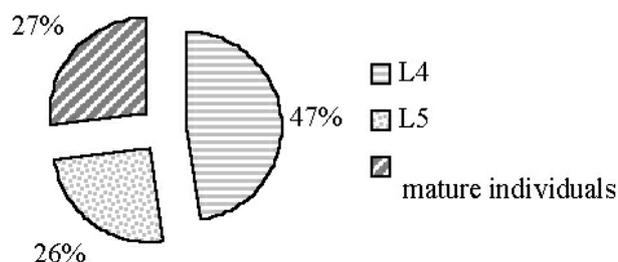


Fig. 1. Per cent contributions of juvenile (L5), larval (L4), and mature forms of the nematode *Anguillicola crassus* found in the swim bladder of eel *Anguilla anguilla*

Table 1. Dates of capture, sample sizes, and characteristics of the River Rega eel

Date of capture	N	Length (cm)		Weight (g)	
		x	S.D.	x	SD
August 2001	26	25.60	4.71	17.78	11.52
September 2001	15	24.25	3.63	12.90	6.71
July 2002	17	27.81	4.44	22.37	11.85
Total	58	25.90	4.51	17.86	11.01

N — sample size, x — means, S.D. — standard deviation

Examination of blood smears showed the eel to be infested by the protozoan *Trypanosoma granulosum* Laveran et Mesnil, 1902, occurring at a prevalence of 100% and at a variable intensity. The protozoans showed variable dimensions; typically, a single host carried both small and large forms of the parasite.

The analyses of the peripheral blood showed a significant difference in mean haematocrit (Ht), MCHC, and MCV between the eel caught in August and September 2001, regardless of whether they were or were not infested by *A. crassus* (Table 2). No significant difference in the parameters studied (E, Hb, Ht, L), including the haematological indices (MCH, MCHC, MCV) was detected between the *A. crassus*-infested and the nematode-free fish (Table 3).

Analyses of the relative and absolute leucocyte patterns showed a considerable domination of lymphocytes over other leukocytes in the blood (Table 4). The peripheral blood of the *A. crassus*-infested eel showed the percentages of lymphocytes and granulocyte series cells to average 93 and 7%, respectively. The nematode-free eel showed an identical average lymphocyte percentage, that of granulocytes being

higher by 5%. The absolute lymphocyte and granulocyte counts in the *A. crassus*-infested eel amounted to 86 and 5 thou./mm³, respectively, while the nematode-free individuals showed the absolute lymphocyte and granulocyte counts higher by 18 and by about 0.5 thou./mm³, respectively. No significant differences in mean relative and absolute counts of individual lymphocyte types between the *A. crassus*-infested and nematode-free eel were found (Table 4).

The qualitative pattern of the peripheral blood cells in the *A. crassus*-infested eel was similar to that revealed by the nematode-free individuals. Most of the eel examined showed the presence of microcytes. The nematode-infested and -free individuals featured haemolysed blood cells as well as erythrocytes differing in size and shape, including polychromatic juvenile erythrocytes. In some cases, the erythrocyte cytoplasm was unevenly stained (poorly staining erythrocytes) and was mostly condensed in the cell periphery. The erythrocyte cytoplasm showed the presence of vacuoles, often producing the so-called cytoplasmic bridges. Erythrocyte nuclei were disfigured.

Table 2. Haematological parameters of the peripheral blood of *Anguillicola crassus*-infested and nematode-free eel with respect to the date of capture. Prevalence of *Trypanosoma granulosum* infestation was 100%

Parameter	August 2001		September 2001		July 2002	
	x	S.D.	x	S.D.	x	S.D.
E [million/mm ³]	1.85	0.34	1.85	0.26	2.06	0.66
Hb [g%]	10.26	2.94	12.42	4.04	11.29	3.24
Ht [%]	43.25*	7.60	35.73*	7.72	39.35	7.32
L [thou./mm ³]	100.2	29.70	80.01	31.16	90.2	37.85
MCH [µg]	55.81	11.44	60.92	13.31	54.26	15.87
MCHC [%]	24.70*	6.51	34.19*	4.91	27.50	6.51
MCV [µ3]	273.02*	45.25	186.84*	34.15	201.50	52.82

*difference between August and September 2001 was significant

Table 3. Haematological parameters of the *Anguillicola crassus*-infested and nematode-free eel. Prevalence of *Trypanosoma granulosum* infestation was 100%

Parameter	<i>A. crassus</i> -infested eel (N = 44)		<i>A. crassus</i> -free eel (N = 12)	
	x ± S.D.		x ± S.D.	
E [million/mm ³]	1.93 ± 0.44		1.85 ± 0.43	
Hb [g%]	1.07 ± 3.27		11.07 ± 4.16	
Ht [%]	39.95 ± 8.01		40.69 ± 9.46	
L [thou./mm ³]	90.20 ± 32.39		99.70 ± 42.40	
MCH [µg]	57.88 ± 13.15		49.31 ± 7.16	
MCHC [%]	28.12 ± 6.93		28.00 ± 8.98	
MCV [µ3]	213.48 ± 49.50		214.48 ± 53.77	

Irregularities among granulocytes involved mainly uneven maturation of the nuclei and cytoplasm. Lymphocytes showed a high morphological variability resulting from a high diversity of both the nuclei and the cytoplasm.

Discussion

The haematocrit (Ht) values as well as MCHC and MCV showed significant differences between the eel caught in August and September 2001.

On the other hand, haematological parameters showed little difference between those eels hosting the nematode *A. crassus* in their swim bladders and the individuals which were parasite free, the difference being non-significant. The results are compatible with those reported by Palíkova and Navrátil [11]. The changes observed by those authors were more distinct when viewed in relation to the seasons of fish capture than to the *A. crassus* infestation. Results reported by Palíkova and Navrátil [11] as well as the data collected in this study show a potential of the eel blood morphotic elements

to vary seasonally.

As the fish haematological parameters are variable and dependent on a multitude of factors (i.a., age, sex, diet), it is difficult to attain a homogenous set of data. Studies on anguillicolosis-affected eel from the Szczecin Lagoon [12] showed the condition to be important for the erythrocyte count, haemoglobin content, leucocyte content, and percentages of various leucocyte series cells. At the same time, the study referred to revealed differences in haematological parameters related to the extent of *A. crassus* infestation. This allowed to conclude that the blood response in the form of variations in the values of the parameters under study was proportional to the parasite abundance. The difference between the minimum and the maximum erythrocyte count was up to a million in the peripheral blood, the haemoglobin level dropping to as low as about 7 g%. It should be mentioned that the infestation prevalence in the study referred to was 70% and the intensity varied from 1 to 35 individuals of the nematode *A. crassus* in a single swim bladder.

The eel caught in Lake Ińsko [13] showed

Table 4. Quantitative characteristics of the peripheral blood leucocytes in *Anguillicola crassus*-infested and nematode-free eel. Prevalence of *Trypanosoma granulosum* infestation was 100%

Blood cell type	Relative leucogram [%]		Absolute leucogram [thou./mm ³]	
	<i>A. crassus</i> -infested x ± S.D.	<i>A. crassus</i> -free x ± S.D.	<i>A. crassus</i> -infested x ± S.D.	<i>A. crassus</i> -free x ± S.D.
Granuloblasts	1.47 ± 1.89	1.39 ± 2.11	0.89 ± 1.28	0.92 ± 1.51
Progranulocytes	1.57 ± 1.57	0.91 ± 0.56	1.41 ± 1.35	0.78 ± 0.43
Granulocytes and metagranulocytes	1.41 ± 1.89	0.92 ± 1.73	1.18 ± 1.21	0.75 ± 1.32
Rod-shaped heterophiles	1.45 ± 1.88	3.31 ± 6.83	1.16 ± 1.50	3.02 ± 4.97
Segmented heterophiles	0.93 ± 1.18	3.21 ± 7.55	0.59 ± 0.62	2.47 ± 5.66
Granulocytes total	6.87 ± 7.02	7.25 ± 11.76	5.23 ± 4.33	5.86 ± 8.54
Lymphocytes	93.13 ± 7.02	92.87 ± 11.82	85.66 ± 41.28	103.71 ± 51.84

the *A. crassus* infestation prevalence to be higher, amounting to 88.7%, the infestation intensity ranging from 1 to 15 parasites per fish. Haematological assays of those eel showed their erythrocyte count as well as Hb and Ht values to be depressed as a result of the presence of *A. crassus*. The erythrocyte haemoglobin concentration varied and showed a tendency to be reduced in the more heavily infested eel. Analysis of the erythrocyte-related indices confirmed the irregularities in haemoglobin saturation of the red blood cells. The MCV values showed a tendency towards an increase in erythrocyte size with increasing number of nematodes in the swim bladder. Observations on the basic haematological parameters and indices allowed to conclude on erythrocyte susceptibility to haemolysis, confirmed by the analysis of blood smears. Irregularities in the structure and maturation of blood cells were reported as well [13]. The young eel caught in the Rega showed similar changes in their blood.

Effects of the number of nematodes on the peripheral blood haematological parameters were demonstrated also by Höglund et al. [10]. Their study showed a reduction in the haemoglobin content in the eel containing more than 10 *A. crassus* nematodes in the swim bladder lumen. The haematocrit was at its lowest in that eel group as well. As shown by Boon et al. [7-9], there were no differences in mean haematocrit value of the eel carrying *A. crassus* in their swim bladder and those with the swim bladder free of the parasites.

The River Rega eel leucocyte pattern showed a distinct predomination of lymphocytes over other leucocyte series cells. Similar results were reported by Paliková and Navrátil [11] as well as by Rząd and Pilecka-Rapacz [12, 13]. Neutrophilous granulocytes and lymphocytes belong to the teleost fish immune system cells [18]. In the opinion of Stosik and Deptuła [18], research on counts of neutrophilous granulocytes and their protective function in healthy and disease-affected fishes has been so far incomplete and failed to provide a comprehensive approach to the problem. Changes observed in the thrombocyte and leucocyte

counts are strongly related to the form of bacterial or parasitic disease [18].

The present authors' own observations as well as reports on the eel blood containing the protozoans *Trypanosoma* sp. provide evidence that effects of those parasites on the eel blood parameters should not be treated lightly. The presence of trypanosomes does not leave the host unaffected and may be an underlying cause of changes in the eel peripheral blood, such as erythrocyte degradation [19]. The protozoans *Trypanosoma* sp. were present in the blood of all the eel individuals examined. During their study carried out in 1970–1973 in two lakes of north-western Poland, Wierzbicka and Orecka [5] found *T. granulosum* to occur at prevalences of 24 and 68% in the eel from lakes Siecino and Dąbie, respectively. The *Trypanosoma* sp. invasion prevalence in the eel caught in Lake Ińsko [13] was 24% as well.

Boon et al. [9] pointed out to a simultaneous infestation of the eel by *A. crassus* and by *T. granulosum* present in the peripheral blood. Concerning the *A. crassus* infestation, the results reported by Boon et al. [9] were in agreement with earlier studies [7, 9] and confirmed that the nematodes produced no changes in the haematocrit of the wild eel. On the other hand, the authors quoted wondered about the simultaneous occurrence of a *T. granulosum* infestation and a haematocrit reduction. It is noteworthy that, in the study described by Boon et al. [9], 62.5% of the eel of 23.7 g mean individual weight contained the two parasitic species at the same time. The peripheral blood trypanosomes show a high variability of their morphological characters. The causes underlying the variability may be sought, e.g., in the stage of the parasitaemia, including multiple infestation facilitated by intermediate hosts, or may be viewed as a result of competition for food [20].

Effects of *T. granulosum* on the fish blood require further studies, including the determination of infestation intensity and the characteristics of morphological forms of the trypanosomes.

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Wpłynęło 13 lipca 2006

Zaakceptowano 23 kwietnia 2007