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Letter to Editor

Selected Immunological and Haematological Indices in Breams *(Abramis brama)* Inhabiting Various Aquatic Ecosystems

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Abstract

Physiological parameters in fish provide grounds for conclusions as to the physiological status of the organism. The status is directly related to inner and outer factors, biotic and abiotic influences which act on the organism. Present studies aimed at defining the set of immunological and haematological indices in breams, which form fish populations in Dabie Lake and Szczecin Bay, whose ecosystems differ in water cleanness. Immunological studies in breams involved determining selective defensive functions of neutrophilic granulocytes, expressed by the index of phagocytosis of a standard bacterial strain (Ipg), the percentage of phagocytes which ingested the bacteria (%gp), NBT index, the amount of formazan and the index of myeloperoxidase activity (WA MPO). Haematological studies included determination of absolute leukocyte number and the differential leukocyte pattern. Bacteriological and physicochemical studies were also performed on water samples of reservoir sites from which the fish originated.

Results of examination of water samples from Dabie Lake and Szczecin Bay demonstrated a significant level of water pollution in the ecosystems. A definitely higher level of water pollution, particularly in respect to bacterial contamination, was observed in Dabie Lake. Comparison of immunological and haematological results demonstrated significant differences between the two studied bream populations, i.e. those originating from Dabie Lake and the other, originating from Szczecin Bay. Such differences were disclosed both in immunological and in haematological indices.

Keywords: breams, leukocytes, neutrophilic granulocytes, environmental conditions.

Introduction

Similar to the situation in humans and higher animals, physiological parameters in fish provide grounds for conclusions as to the physiological status of an organism. The status is directly related to outer and inner factors as well as to biotic and abiotic influences which affect an organism. The status reflects several functions of the organism, shaped either in conditions of the properly maintained water habitat or altered by adaptive or protective reactions to agents which contaminate the water reservoir. Due to the extensive biological and chemical variability of water ecosystems, investigation and definition of the physiological status of the inhabiting fish becomes increasingly important for appropriate evaluation of changes which may take place in most individual fish

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which inhabit a given water reservoir. Apart from cognitive aspects, the problem remains important for practical reasons, e.g. due to its impact on ichthyopathological diagnosis. Equally important is access to the specific database for the fish population of a specific water reservoir. Present studies aimed at determining immunological and haematological indices in breams, which form fish populations in Dabie Lake and in Szczecin Bay, ecosystems which differ in the extent of water pollution.

Material and Methods

Material

A total of 227 breams *(Abramis brama)* were examined, 640 g to 3540 g body weight, originating from two regions of Dabie Lake (80 breams) and from five regions of Szczecin Bay (147 breams). On the basis of clinical, anatomopathological, parasitological, and microbiological tests the breams were regarded as healthy, as they showed no lesions or signs which would suggest infective, invasive or environmentally-induced diseases.

Diagnostic Studies in the Fish

Evaluation of physiological status was based on determination of selected immunological and haematological parameters. In parallel to examination of the breams, microbiological and chemical testing of the water samples was performed, obtained from the sites where the fish originated.

Immunological and Haematological Studies on the Fish

Immunological tests performed in the bream included estimation of:

a. index of phagocytosis of the standard strain of bac teria by neutrophilic granulocytes (Ipg) using a modified technique of Brzuchowska [1, 10];

b. percentage of neutrophilic granulocytes (%gp) which were capable of ingesting the bacteria, according to the technique of Deptuta [1];

c. nitrotetrazolium blue reduction index (NBT index) and amount of formazan (in mg) per ml blood, estimated as described by Raman and Poland [4] and by Sychlowy and Lucas [12], modified by Siwicki et al. [7];

d. index of myeloperoxidase activity (WA MPO) in neutrophilic granulocytes, estimated by a modified tech nique of Graham [9, 13].

Haematological tests on breams involved estimation of an absolute number and of a differential pattern of leukocytes using the routine techniques applied in laboratory diagnosis of fish [10].

Bacteriological and Physicochemical Testing of Water

Microbiological tests on water samples included determination of total number of psychrophilic and of mesophilic bacteria per ml water, the most probable number (MPN) of *coli* group bacteria and of faecal type *coli* group bacteria per 100 ml water and determination of titres of *coli* group bacteria and of faecal type *coli* group bacteria, using the techniques defined by norms legally binding in the country [3].

Physicochemical tests on water samples included determination of pH, total suspension, BZT_5, ChZT ($KMnO_4$ and $K_2Cr_2O_7$), dissolved oxygen, ammonium nitrogen, nitrate nitrogen, nitrite nitrogen phosphates, water hardness, contents of chlorides, sulphates, dissolved substances. Determination of the variables [2] as well as evaluation [5] and qualification [6] of water were conducted as specified by the norms currently binding in the country.

Results of immunological studies (Table 1) and of haematological tests (Table 2) were subjected to statistical analysis using Student's *t* test at p = 0.05 and were presented in means (X) plus minus standard deviation (SD), calculated for the entire period of studies on breams fished of Dabie Lake and Szczecin Bay. Results of bacteriological tests on water samples (Table 3) were presented in the form of mean values while results of physicochemical testing were given in the form of appropriate water classes (Table 4).

Table 1. Immunological indices in breams fished of Dabie Lake and Szczecin Bay.

Water reservoir	Ipg	%gp	Indeks NBT	Amount of formazan (mg/ml blood)	WA MPO
Dąbie Lake	2.20 ± 0.38	66.26 ± 13.55	1.38 ± 0.31	12.17 ± 2.21	1.03 ± 0.13
Szczecin Bay	3.37 ± 0.40**	51.23 ± 6.04*	5.48 ± 0.43**	11.91 ± 1.50*	1.90 ± 0.28**
Reference values determined for breams inhabiting fresh waters of Lubuskie voivodship [8]	1.73 ± 0.31	23.39 ± 4.18	0.63 ± 0.11	6.12 ± 1.10	1.17 ± 0.09

Explanations: * decrease, ** increase in index value, "both significant" as compared to the value of the index established for the fish of Dabie Lake.

Table 2. Haematological indices in breams fished of Dabie Lake and Szczecin Bay.

Water reservoir	Leukocyte number	Leukocyte differential pattern				
		Lymphocytes	Granulocytes			Monocytes
			neutrophilic	basophilic	acidophilic	
Dąbie Lake	39.05 ± 4.31	72.26 ± 5.99	17.79 ± 2.97	1.86 ± 0.62	3.93 ± 1.21	4.16 ± 2.08
Szczecin Bay	37.54 ± 4.92	78.62 ± 5.24	13.32 ± 2.14*	3.15 ± 0.25**	1.75 ± 0.19*	3.16 ± 0.39*
Reference values determined for breams inhabiting fresh waters of Lubuskie voivodship [8]	78.31 ± 6.13	80.23 ± 3.27	12. 17 ± 0.89	1.14 ± 0.09	1.56 ± 0.08	4.90 ± 0.42

Explanations: * decrease, ** increase in index value, "both significant" as compared to the value of the index established for the fish of Dabie Lake.

Table 3. Results of bacteriological tests and class of water samples taken from Dabie Lake and Szczecin Bay.

Water reservoir	Water temperature (°C)	Psychrophilic bacteria per ml	Mesophilic bacteria per ml	MPN of *coli* group bacteria per 100 ml	MPN of faecal type *coli* group bacteria per 100 ml	*coli* titre	Faecal type *coli* titre
Dąbie Lake	11.7	20.625	2.735	≥ 2.400	700	≤ 0.04	0.1
Szczecin Bay	12.5	16.550	6.749	15	16	7	7
Instruction of Minister of Environment. Prot. Nat. Res. For. of 5th Nov., 1991 [6]	0-26	Not specified	Not specified	Not specified	Not specified	Not specified	1.0-0.01
*Instruction of Minister of Health Soc. Wel. of 04.09.2000 [5]	Not specified	Not specified	Not specified	10.000 (suggestion < 500)	1.000 (suggestion < 100)	Not specified	Not specified

Explanations: denotes bacteriological conditions which should be fulfilled by water in baths.

Table 4. Results of physicochemical tests on water samples taken from Dabie Lake and Szczecin Bay, expressed in class criteria.

No.	Type of test		Class assignment	
			Dąbie lake	Szczecin bay
1	Temperature	°C	I	I
2	PH		II	I
3	Total suspension	mg/L	beyond classes	beyond classes
4	BZT_5	mg O_2/L	II	II
5	ChZT ($KMnO_4$)	mg O_2/L	II	II
6	ChZT ($K_2Cr_2O_7$)	mg O_2/L	II	II
7	Dissolved oxygen	mg O_2/L	I	I
8	Ammonium nitrogen	mg NH_4/L	II	II
9	Nitrate nitrogen	mg NO_3/L	III	I
10	Nitrite nitrogen	mg N-NO_2/L	III	beyond classes
11	Phosphates	mg PO_4/L	III	III
12	Total hardness	mval/L	II	II
13	Chlorides	mg Cl/L	beyond classes	beyond classes
14	Sulphates	mg SO_4/L	I	II
15	Dissolved substances	mg/L	beyond classes	beyond classes

Class assignment according to the decree of the Minister of Environmental Protection, Natural Resources and of Forestry of 5 November, 1991 [6],

Results

Results of bacteriological tests on water samples taken from D^bie Lake and from Szczecin Bay (Table 3) indicate that the ecosystems are heavily polluted, whether they have been classified in sanitary respect as belonging to class I of water cleannes (Dabie Lake), to the respective II class (Szczecin Bay) or exhaust the requirements of swimming water. The detected extensive degradation of water biotope in the studied aquatic systems has been documented by the number of psychrophilic and mesophilic bacteria, content (NPL) of *coli* type bacteria and faecal *coli* type bacteria, titre of *coli* type bacteria. It has also been confirmed by physicochemical tests on the water samples (Table 4).

Comparison of immunological-haematological test values manifested significant differences between the two studied bream populations originating, respectively, from Dqbie Lake and from Szczecin Bay. The differences were detected both in the immunological and in the haematological indices. In the case of immunological indices, they were noted in Ipg index, NBT and WA MPO indices, which were higher in breams originating from Szczecin Bay, and in the same breams, in %gp as well as formazan levels, which were lower than those in breams from D^bie Lake. Among haematological results, the differences were noted in the leukocyte differential pattern, related to neutrophilic and acidophilic granulocytes and to monocytes, the proportion of which in the fish from Szczecin Bay was lower than in breams originating from D^bie Lake. In the case of proportion of basophilic granulocytes, the reverse situation was detected.

Discussion

In the conducted medico-veterinary and laboratory examination of the fish it is significant that the studied breams have shown no alterations, which would suggest development of infectious, parasitic or environmentally induced pathology. Thus, the observed status of immunological and haematological variables in breams originating from Dqbie Lake and Szczecin Bay has not been demonstrated to reflect a reaction to activity of pathogenic agents or an ongoing pathological process. It should, however, be added that differences observed, i.e., in the data presented in Tables 1 and 2, between data obtained for the same species of fish inhabiting clean fresh-water reservoirs (of the first, sporadically the second class of water cleanness) have resulted most probably from adaptation of fish organisms to physical, chemical and biological conditions in the aquatic biotope [8]. It should be stressed that, according to the literature data [11], biotic and abiotic elements, which constitute a risk for environment or contaminate it are not neutral to organisms which inhabit the biotope and which develop in the ecosystem under pression of such factors. As demonstrated previously [11], the originally disturbed physiological functions of the animals may become normalised after some time. However, particularly following exposure to low concentrations of noxious agents, the organism's metabolic-functional balance stabilises at a level distinct from that observed before exposure. This might explain differences noted within the examined parameters in the fish of the same species but originating from distinct environments. Maintenance of the new metabolic-functional balance, expressed in the persistence or higher activity of enzymes and systems controlling cell turnover, provides potential for mobilising mechanisms which inactivate or eliminate noxious agents and for reparative processes [11]. The few available literature data [11] indicate that fish can manifest high adaptive capacity, particularly after prolonged exposure to an environment contaminated with toxic/noxious agents, present in concentrations which do not induce clinical signs.

Conclusions

Values of immunological and haematological indices defined in breams from Dąbie Lake and Szczecin Bay, distinct in respect to water cleanness, significantly differed between the two groups of fish. The presented above results of immunological and haematological studies seem to represent physiological parameters of breams inhabiting Dąbie Lake and Szczecin Bay. The argument seems to be strengthened by the good health condition of the fish, defined by medico-veterinary examination. The breams have seemed fully adapted to environmental conditions in ecosystems of their origin.

In view of the general lack of uniformity of environmental conditions in various water reservoirs, the need should be strongly stressed for formulating reference data for selected species of fish in specific ecosystems, which would permit objective evaluation of health status in the fish and of changes taking place in their biotope.

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