FACTORS AFFECTING HAEMATOLOGICAL INDICES IN FREE-LIVING FISH POPULATIONS

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Abstract

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The values of physiological indices in fish blood are significantly affected by a complex of factors. Since fishes are poikilothermic, this complex includes the influence of both biological and environmental factors. For a correct interpretation of the observed values of the various haematological indices, the knowledge and consideration is required of the most significant factors that may affect the collection, treatment and, above all, evaluation of data. In investigations of 2 - 3 annual cycles of selected indices in the blood of adults of *Salmo trutta* m. *fario*, *Thymallus thymallus*, *Chondrostoma nasus*, and partial cycles of *Leuciscus cephalus* and *Barbus barbus*, I evaluated the most important, generally occurring influences affecting their values. With respect to the rather high individual variability, values obtained from a group sample of fish should be employed on principle. One should also respect the seasonal variation in any index under study, as well as the sex of the fish, which significantly affects some of the indices (HC, HB, AST). Spawning appears to be a so-called major factor that significantly affects, above all, the values of biochemical indices. It is highly desirable to use identical procedures and standard methods in determining the values of haematological and biochemical indices in fish blood.

Blood plasma, hematological and biochemical indices, biological and environmental influences

In evaluating concrete values of fish blood indices, the basic problem is to determine whether they lie within or beyond the "physiological range". As a rule, the problem is connected with the so-called bioindicative use of the blood indices, both to estimate the condition of the fish organism and in relation to its environment and the mediated assessment of the latter (Neff 1985; Adams 1990; Wendellar Bonga 1997). The definition of the "physiological range" bearing the character of the so-called normal range or normal values, shows a number of philosophical, interpretative and determinative aspects (Vácha 1980; Messe 1990; Folmar 1993; Folmar et al. 1992; Lusková 1996). Thus, the problem of "normal" and "non-normal" in ichthyohaematology, notably with regard to the poikilothermia of fishes, is a still actual but still unresolved problem. In laboratory conditions the problem can be resolved by using a parallel, so-called unstressed sample. In natural conditions, where the use of fish blood indices could serve bioindication, a number of problems arise which complicate the evaluation of concrete data obtained in the field. In my contribution, based on several years of investigations into fish hematology and biochemistry, I wish to point out certain basic aspects that should be considered in assessing the condition of an environment as well as that of the fish organism by means of the basic haematological and biochemical blood indices.

Materials and Methods

This study is based on examinations of adult individuals from free-living populations of *S. trutta* m. *fario* (n = 504), *T. thymallus* (n = 305), *C. nasus* (n = 120), *L. cephalus* (n = 112) and *B. barbus* (n = 64), captured in streams.

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The basic haematological parameters, i.e. erythrocyte count (ER), haematocrit value (HC), haemoglobin concentration (HB), leukocyte count (LEU), leukocrit value (LC), were determined using standardized methods (Dacia and Lewis 1968; Svobodová et al. 1986). The activities of enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), creatine kinase (CK), lactate dehydrogenase (LDH), lactate (LACT), cholinesterase (CHE), glucose (GLU) and total protein (TP) in blood plasma were determined using standard optimized sets made by Boehringer, Mannheim (see Lusková 1997). Blood was sampled through cardiac puncture when the fish were in deep electronarcosis within 3 minutes after capture by electrofishing in the natural environment of a stream. The basic data enabling subsequent determination of haematological indices were obtained directly in the field. For biochemical examinations, the plasma was decanted and, after being transported to the laboratory in a cooled box, analysed the same day.

Results and Discussion

In estimating and evaluating data on the various fish blood indices, it is necessary to consider a number of circumstances and influences in order to attain an objective conclusion. The knowledge and identification of the possible impact of such deteriorating influences is a prerequisite to their elimination. As will be shown below, this is a pretentious issue, and that is why sufficient results and knowledge are still lacking in this respect.

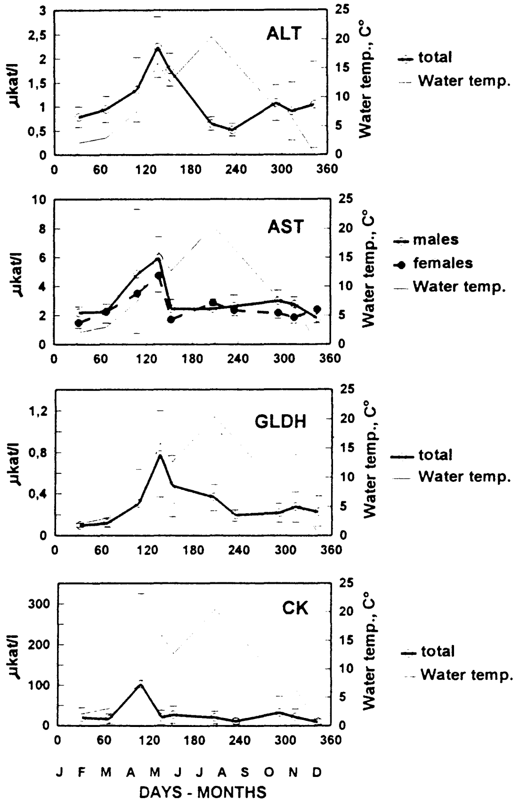

Fig. 1.
Dynamics of enzyme activities (ALT, AST, GLDH and CK) in the blood plasma in the course of the year (mean and SD) in *Chondrostoma nasus*. The marked increase in April and May is due to spawning.

Obtaining material and blood sampling

Essentially, two procedures are possible. Either the blood samples are taken immediately after the capture of the fish, or the fish are transported live to be treated in the laboratory. The former alternative is pretentious as to labour and organization and, therefore, it is less frequently used. In case that the fish are transported to the laboratory, they become "acclimatized" to the laboratory environment after some time prior to blood sampling. In that case, one cannot exclude any possible response of the organism to handling and the altered environmental conditions, all of which may become reflected in the values measured. Thus, it is necessary to distinguish between values obtained by the two above procedures. In the case of fish species that are the object of culture it is necessary to state whether the individuals examined came from natural conditions or from a culture (Van Vuren and Hamilton 1978; Folmar et al. 1992).

In the case of some of the indices, problems may arise from the use of different methods of determination, due to which the data obtained cannot be compared nor pooled (e.g. Alexander and Ingram 1980; Lusková 1995). Therefore, an agreement on the standardization of methods is highly desirable. In our conditions, a foundation has been laid by the methods of Svobodová et al. (1986) however, it is necessary to actualize them on the basis of the recent data.

Pertinence to species and population

The particular blood indices show varying levels of specificity. For example, the erythrocyte count showed marked differences in different species: its average in *S.trutta* m.*fario* was 1.09 T/l (SD=0.24), in *T. thymallus* 1.29 T/l (SD=0.25), in *C. nasus* 1.83 T/l (SD=0.45). The specific differences between the biological and physiological characteristics distinctly affect the seasonal dynamics of the blood indices. In brown trout, spawning takes place during autumn months (October and November) when the water temperatures drop to 3-6 °C. The spawning of grayling takes place in spring at water temperatures of 6-10 °C, similarly in the nase when they increase to 7-12 °C. Therefore, the spawning period of the latter two species is shorter (3-5 days), followed by intensive food intake. That is why there is a significant difference in enzyme activities in the blood of brown trout and grayling or nase in their annual course.

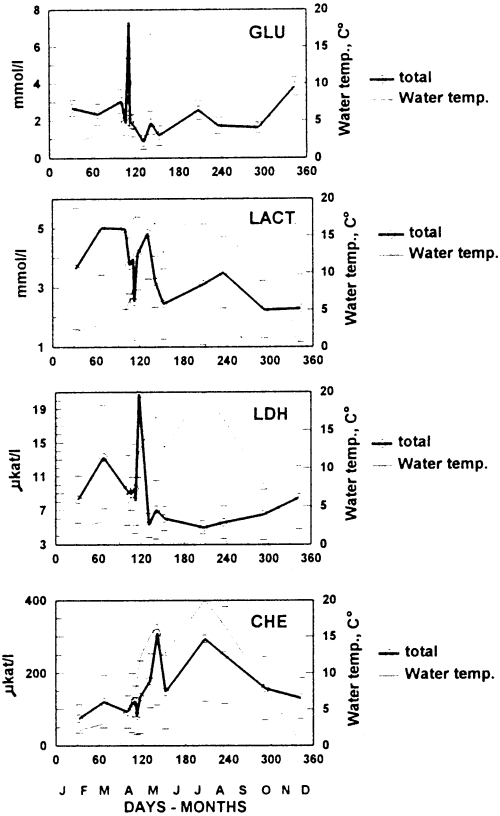

Fig. 2.
Seasonality in CHE, LDH and influence of increased water discharges on GLU and LACT.

The differences in the values of haematological parameters that would result from the pertinence to different populations is masked by possible differences in environmental conditions. Therefore, this problem has been mentioned only occasionally and, as a rule, with ambiguous conclusions (McCarthy et al. 1973). I have encountered with this problem when evaluating haematological parameters obtained from brown trout populations living in the Bílý potok Stream and the Svratka River. I have found significant differences in the case of HB ($P=1.87 \cdot 10^{-9}$) and LC ($P=2.8 \cdot 10^{-12}$). Certain differences were apparent even in other hematological parameters. In the literature, differences between confined and free-living populations have been pointed out (Van Vuren and Hattingh 1978; Folmar 1993).

Individual variability, sex

The high level of individual variability, particularly of some of the fish blood indices, distinctly complicates the definition of their "physiological normal values". The variability of an index under evaluation may be measured by the variation coefficient (VC). In the species under study, the lowest variation levels have been found in ER, HC and HB whose VC varied between 16 and 25 %. LEU and LC showed distinctly higher levels of variation (VC between 60 and 100 %). In TP the value of VC did not exceed 30 %. The values of VC

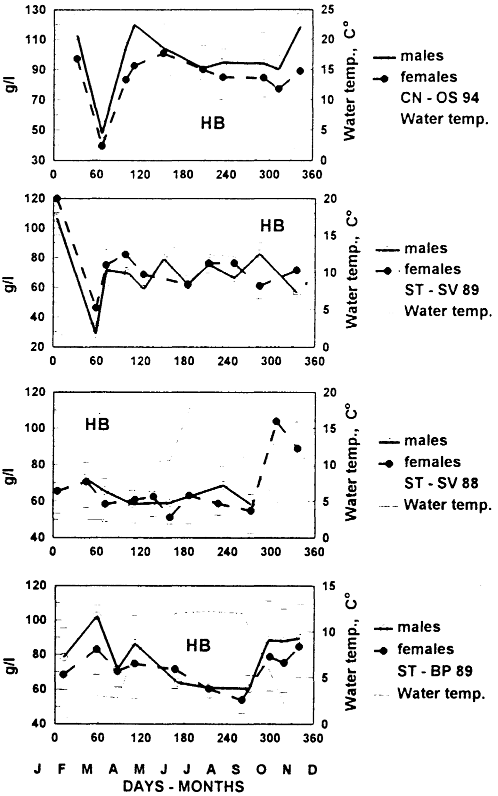

Fig. 3.
Influence of increased water discharges on HB and "normal" annual dynamics of these indices. A significant drop in haemoglobin content in *Chondrostoma nasus* and *Salmo trutta m. fario* caused by increased discharges of cold water from melting snow, lasting for several days.

in the activities of ALT, AST and GLDH varied between 50 and 100 %. The highest variability has been observed in CK activities, the VC attaining 279 %.

The influence of sex has been observed in some of the blood indices. The mean values of haematocrit were invariably higher in the males than in the females of the species under study. Also, the mean values of haemoglobin content were always higher in the males. Of the enzyme activities under study, an unequivocal difference was observed in AST activity, the mean value of which was also higher in the males. In the cases of the remaining blood indices, higher mean values were observed in the males or, on the contrary, in the females, or they did not differ in the two sexes at all.

Seasonal differences, annual cycle

In central European conditions, seasonality is characterized by a cycle of four seasons of the year. The dynamics of an index examined in the course of the year, caused by the year, caused by the action of decisive seasonal characteristics (in our conditions, chiefly temperatures and photoperiod), is considered here as seasonality. I have estimated the seasonality of the blood indices in the species under study on the basis of 2 - 3 annual cycles. The most marked seasonality was observed in LEU and TP, which indices are positively correlated with the variation in water temperature in the course of the year. Practically no seasonality was observed in ER and HC (Fig. 4). The seasonality of enzyme activities is masked by the distinct effect of spawning. Nevertheless, a rather marked increase in activity levels was observed, above all, in protein metabolism enzymes (ALT, AST and GLDH) in spring and autumn seasons. An exceptional seasonality, depending upon water temperature, was exhibited by CHE the course of which closely followed the variation in water temperatures (Fig. 1, 2).

Some of the seasonality phenomena may cause stress to the fish organism. Increased discharges of cold water from melting snow, lasting for several days, were followed by a marked drop in the levels of HB and GLU in nase in the Oslava River (Fig. 3, 2). A similar response was observed in brown trout in the Svratka River in 1993 and 1994. The investigations on seasonality have permitted me to fully understand and define the effect of spawning on the dynamics of the various indices.

Spawning

The reproduction of fishes and notably the spawning process itself show essential effects on various biological manifestation of adults. On the basis of several years of investigations, I have characterized the spawning as a so-called major factor in regard of extent to which it affects the blood characteristics, particularly the enzyme activities (Lusková 1996). Haematological indices respond with some delay and the effect of spawning is usually masked by other factors (such as seasonality, dynamics of haematopoiesis), so that it cannot be unequivocally determined as a rule. Quite a different situation is found in the biochemical indices under study. In connection with the spawning, enzyme activities are quite significantly increased, as observed e.g. in the nase (Fig. 1). The changes are so marked that this phenomen must be considered in evaluating the values measured as well as in defining the limits of the physiological ranges. Although spawning is a natural physiological process, its consequences show a heavy stress effect in fishes, including increased mortality.

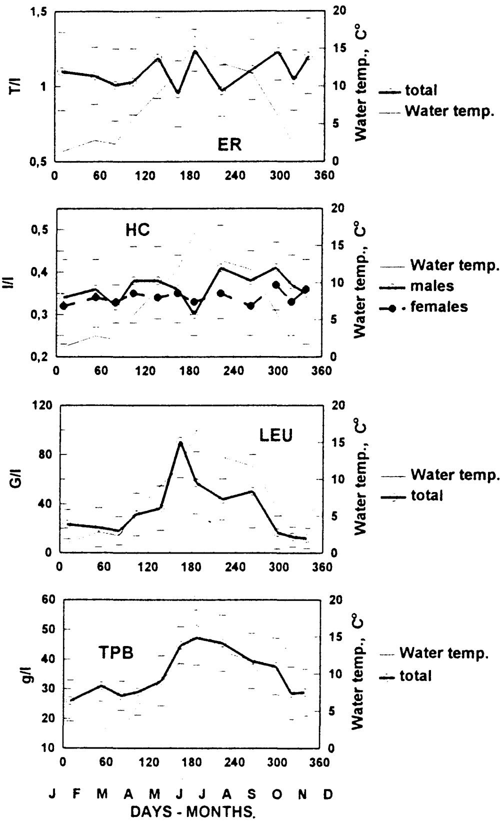

Fig. 4.
Annual dynamics of erythrocyte and leukocyte count, haematocrit and protein content in the blood plasma of *Salmo trutta*.

"Physiological values"

The "normal physiological values" of haematological indices are of importance in assessing the health of the individuals examined with respect to bioindication and biomonitoring of their environment. It is difficult to define the "physiological normal values" above all, due to the strong effects of the external environment on the fish organism. Defining the "reference physiological values" on a number of data obtained from the fish species under study (Lusková et al. 1975; Lusková 1996, 1997). I have concluded that it is necessary to respect, above all, the annual physiological dynamics and variation in the values of the individual indices. For this reason, I have been inclined to use, in ichthyohematology, the term "physiological reference range" or "physiological normal value". The term "normal value" or "norm" are usually connected with knowledge obtained from warm-blooded animals which, due to internal thermoregulation and homeostasis, show a markedly more stable internal environment. Despite a number of problems connected with defining "normal" and "different" fish blood indices, a number of authors have pointed out the need to define certain ranges or limits (e.g. Blaxhall 1972; Confield et al. 1994; Miller et al. 1983). On the basis of my own (Hlavová 1993, 1993a; Lusková 1995, 1996) and literary data (Pickering 1985; McCarthy et al. 1973). I have concluded that establishing the limits that would define the "normality" of concrete parameter should be considered to be a process in which, by means of standard methods, one will obtain increasingly stable and, thus, generally more valid limits of "normality".

Bioindication

The response of an organism to a stimulus, usually evaluated as a stress, is the essence of bioindication. For our purpose pertaining to fishes one may accept that the stress is defined as a condition in which the dynamic equilibrium of animal organisms, called homeostasis, is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli, commonly defined as stressors (Chrousos and Gold 1992), as interpreted by Wendelaar Bonga (1997). Essentially, bioindication is based on the identification of a response to the action of stressor which produce effects with a possible or actual influence on the homeostatic equilibrium and call forth behavioural and physiological responses of the organism. Identification of a stress is the most important and most complicated part of bioindication. Therefore, in spite of all the problems, there are continuing efforts to define ranges within which the values of an index can be considered physiologically normal. The values of fish blood indices are exposed to numerous effects of both intrinsic and extrinsic factors, some of which have been discussed above. All this tends to complicate the identification of a stress, in the sense of the response to it, which, to a considerable extent, is thus an arbitrary decision (see also Wendelaar Bonga 1997). Maximum knowledge of the effects of the so-called natural factors enables to identify the stress, and the attempts to quantify it.

Nejvýznamnější faktory ovlivňující ukazatele krve u divoce žijících populací ryb

Hodnoty ukazatelů v krvi ryb jsou významně ovlivněny komplexem různých činitelů. Vzhledem k poikilotermii ryb se výrazně uplatňují vedle biologických i vlivy vnějšího prostředí. Správná interpretace zjištěných hodnot jednotlivých ukazatelů vyžaduje znalost a respektování nejvýznamnějších činitelů uplatňujících se v průběhu sběru materiálu, při jeho zpracování a zejména při hodnocení. Při výzkumu 2-3 ročních cyklů vybraných hematologických (ER, HC, HB, LEU, LC, TPB) a biochemických (AST, ALT, CK, GLDH, CHE, LDH, LACT, GLU) ukazatelů v krvi adultních jedinců druhů *Salmo trutta* m. *fario*, *Thymallus thymallus*, *Chondrostoma nasus* a dílčích cyklů u druhů *Leuciscus cephalus* a *Barbus barbus* byly vyhodnoceny nejvýznamnější obecně se uplatňující vlivy na jejich hodnoty. Vzhledem ke značné individuální variabilitě je nutno zásadně používat hodnot získaných u skupinového vzorku ryb. Je nutno respektovat sezonnost hodnoceného ukazatele (nejvýraznější u LEU, LC, TPB, GLU, CHE), a příslušnost k pohlaví, která se průkazně projevuje u některých ukazatelů (HC, HB, AST). Jako tzv. velký faktor ovlivňující významně především hodnoty biochemických ukazatelů se projevuje tření. Vysoce potřebné je používání shodných pracovních postupů a standardních metod pro zjišťování hodnot hematologických a biochemických ukazatelů krve ryb. Významný problém představuje vymezení tzv. „fyziologických normálních hodnot“ což podmiňuje možnosti bioindikačního využití jednotlivých ukazatelů krve ryb.

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