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RESEARCH ON THE INFLUENCE OF TEMPERATURE AND WATER HARDNESS ON BREATHING IN SOME FISH SPECIES

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Abstract
Fish change their energy metabolism, spending a larger quantity of energy to mitigate the stress. This paper aims is to investigate the changes of some important respiratory indices (energy metabolism, respiratory rate, number of red blood cells) for three common species in the Arges river (Carassius gibelio Bloch, Perca fluviatilis L. and Alburnus alburnus L.), exposed to different conditions of temperature and degree of water hardness. The samples of fishes were being investigated at two temperature levels (18-20ºC, and 6-8°C) and two levels of water hardness (150 and 300 mg $CaCl_2$/l water). The experimental samples regarded the presence of respiratory and hematological changes (and, where appropriate, the extent of these changes) in prussian carp, perch and bleak adapted to different temperatures (6-8°C and 18-20°C) and revealed differences in the reactivity of the three species to changing environmental conditions. Although adaptation to low temperatures caused metabolic decreases and increases in the respiratory rate in all three species, the evolution of these parameters fitted the picture described in the specialized studies. The perch had the lowest oxygen consumption and adapted to low temperatures better than the prussian carp and the bleak.

Keywords fish, respiratory indices, temperature, water hardness

1. INTRODUCTION

For aquatic organisms, abiotic factors such as temperature and water hardness are known to be crucial in growth and reproduction processes (Menni et al., 1996; Mazerolle, 2005; Lacoul and Freedman, 2006). Water temperature is a critical variable in determining the rates at which chemical, enzymatic, and biological processes take place in aquatic systems; all poikilothermic animals are susceptible to both heat and cold stress.

The thermal factor is one of the most important eco-factors to which living organisms have had to adapt. Most of the physiological processes of poikilothermic organisms are modified by changing the temperature of the environment; in the majority of cases, the rate of physiological processes increases with increasing temperature, to a maximum, and then decreases faster or slower (Precht et al., 1973). The increased temperatures tend to increase the speed of the physiological processes and, as the temperature changes, the rate of the different processes must be balanced and coordinated, the organism intervening to compensate or minimize the changes within it (Reynolds and Casterlin, 1980).

Water hardness is the concentration of all divalent cations in water. Calcium (Ca^{2+}), the most common cation in the freshwater environment, is usually expressed in terms of the amount of calcium carbonate ($CaCO_3$) present in the medium. Calcium is important for ion regulation in

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freshwater organisms (Whiteley, 1999). Increased water hardness reduces the permeability of the gill surface, subsequently protecting against whole body Na loss, which is a primary mechanism of low pH and metal-induced toxicity (McDonald et al., 1991; McDonald and Wood 1993).
Acute water hardness toxicity studies reveal a LC50 of 96 hours of: 4 630 mg/l in *Pimephales promelas*, 9500-11300 mg/l (Mount şi colab., 1997) in *Lepomis macrochirus*, 13.400 mg/l in *Gambusia affinis* (EPA, 1991). All research conducted so far reported a toxicity of calcium chloride (acute or chronic) greater than 100 mg/l, regardless of species.
The choice of three common species in the Arges river (*Carassius gibelio* Bloch, *Perca fluviatilis* L. and *Alburnus alburnus* L.) is based on their widespread, their preservation in laboratory conditions, their importance in the water ecosystems and last but not least their sensitivity to various toxic actions (Bandt - 1941, quoted by Mălăcea, 1969 classifies fish in four sensitivity groups; the selected species are classified in the last three groups: very sensitive - the perch, sensitive – the bleak and less sensitive - the prussian carp).
Therefore, our research was directed towards highlighting the action of temperature, degree of water hardness and fish size on some important respiratory indices (energy metabolism, respiratory rate, number of red blood cells). The advantage of determining the energy metabolism and the respiratory rate is the speed of the response to the action of the stressful factor on the one hand, and its non-invasive nature, on the other hand.

2. MATERIALS AND METHODS

The biological material used in this paper is represented by samples belonging to three species of fishes in the Arges River and its adjacent marshes: *Carassius gibelio* (Bloch), *Perca fluviatilis* L. şi *Alburnus alburnus* L..
Fish adaptation to laboratory conditions was conducted for two weeks in glass aquariums with a capacity of 100 l, under natural photoperiodic conditions and the oxygen dissolved in water was not below 80% (by using water aeration devices) of the maximum possible at the respective temperature and pressure. Feeding during this period was *"ad libitum"* once a day, at around 10; during the experiments fish were not fed to avoid the additional influence of the food factor (Hoar, 1942), thus allowing a better interpretation and comparison of the results. For the low temperature samples, the fish were put in refrigerators, in 25 l aquariums, using artificial lighting (10 hours/day). After acclimatization in the laboratory, fish were separated into lots and placed in the experiments. The lots that met the necessary criteria (less than 5% mortality rate during the first week, and no mortality rate in the second week of acclimatization, if the mortality rate in the first week was between 5 and 10%) were divided into experimental samples (about 10 samples/experimental lot).
In the experimental samples on bleak (16.46 ± 0.85 g) and perch (28.72 ± 2.36 g) we followed respiratory indices at two thermal levels (18-20º C, and 6-8° C). The samples of prussian carp were the most comprehensive, the respiratory indices being investigated at two fish size (C_0: 6.8±3.2g and C_1: 26.25±1.52g), two thermal levels (18-20º C, and 6-8° C) and two degrees of water hardness (150 and 300 mg $CaCl_2$/l water).
Measurements were made at the same period of time (10-12) to avoid the effect of daily dynamics of energy metabolism and respiratory rate (Picoş and Năstăsescu, 1988).
Each lot was a witness in itself, the oxygen consumption and the respiratory rate being established under standard conditions.
We mention that the purpose of the work was not to highlight the respiratory changes during the acclimation, but its result.

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Oxygen consumption was determined by two methods - the classical Winkler method (confined space), and the use of Hanna HI 9146 oximeter (Picoş and Năstăsescu, 1988). Respiratory rate was measured during fish confinement to achieve Winkler method (Picoş and Năstăsescu, 1988); there were successive determinations of this index (with a chronometer) to get three close values (their arithmetic mean showed the respiratory rate at the time).

The oxygen consumption and the respiratory rate were determined for all experimental samples, at intervals of 24, 48, 72, 96, 168 and 336 hours (where allowed by the surviving animals).

The number of red blood cells was determined using Thoma counting chambers, following the method described by Picoş and Năstăsescu (1988) with blood from the caudal artery - after two weeks of experiment.

The results were interpreted statistically using SPSS 13.0 program for Windows, in accordance with the specialized studies.

3. RESULTS AND DISCUSSIONS

The passage of a poikilothermic organism from higher to lower temperature results in a decrease of its oxygen consumption according to the so-called "Krogh curve" as a consequence of the direct kinetic effect of the new temperature on the reactions involved; after stabilization, which can take hours or days (Kinne, 1964, quoted by Marinescu, 2000), the oxygen consumption reaches the value corresponding to the new temperature.

The values of oxygen consumption in prussian carps, determined at the temperature of 6-8° C are about 30% lower than those at 18-20° C in group C_1 (with the average weight of 28.38 g) and 40% in the group C_0 (with an average weight of 7.2 g). Decreases in the metabolic rate as a result of the decrease in temperature were also noted by Prosser (1973) - quoted by Marinescu, 2000 - in the so-called "thermal shocks". There are no significant differences in energy metabolism in the 14 days of investigations (after acclimatization), both in the C_0 generation and in the C_1 generation.

The average values of energy metabolism in prussian carps of different sizes are in accordance with the "law of the talies" (Marinescu, 2000), the smaller animals having higher metabolism.

In the case of the other two studied species - perch and bleak - there are no significant changes in the energy metabolism during the whole experiment, if the fish are kept at a constant temperature of 18-20° C and 6-8° C, respectively.

After a prior acclimation of the fish at temperatures of 18-20° C and 6-8° C, the average values of energy metabolism (expressed by oxygen consumption) show that, best adapted to the low temperatures, the species *Perca fluviatilis*, at which shows a reduction of energy metabolism by only 19.29% compared to the control value recorded at room temperature; the reductions of this metabolic index in the other two species studied are 38.12% for *Carassius gibelio* and 43.66% for *Alburnus alburnus*.

The metabolic variations recorded in the experimental variants are similar with the results of Woynárovich (1963, cited by Precht et al., 1973) who report a faster decrease in oxygen consumption in juveniles of *Silurus glanis* and *Cyprinus carpio*, which prefer warm waters, compared to other species: *Esox lucius*, *Lucioperca lucioperca*, which adapts well to cold water.

Brenda Moffit and Larry Crawshaw (1983) - quoted by Marinescu, 2000, also reported a decrease in the metabolism and frequency of breathing movements in carp under conditions of gradual decrease in temperature.

In the case of acclimatized prussian carps at 6-8 ° C (figure 1), the average values of the respiratory rate show a decrease of 33-34% compared to those determined at 18-20° C. After the end of the acclimation period at low temperatures, the respiratory rate within the two groups of prussian carps

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(with an average weight of 7.2 g, respectively 28.38 g) did not show significant changes during the experiment (14 days).

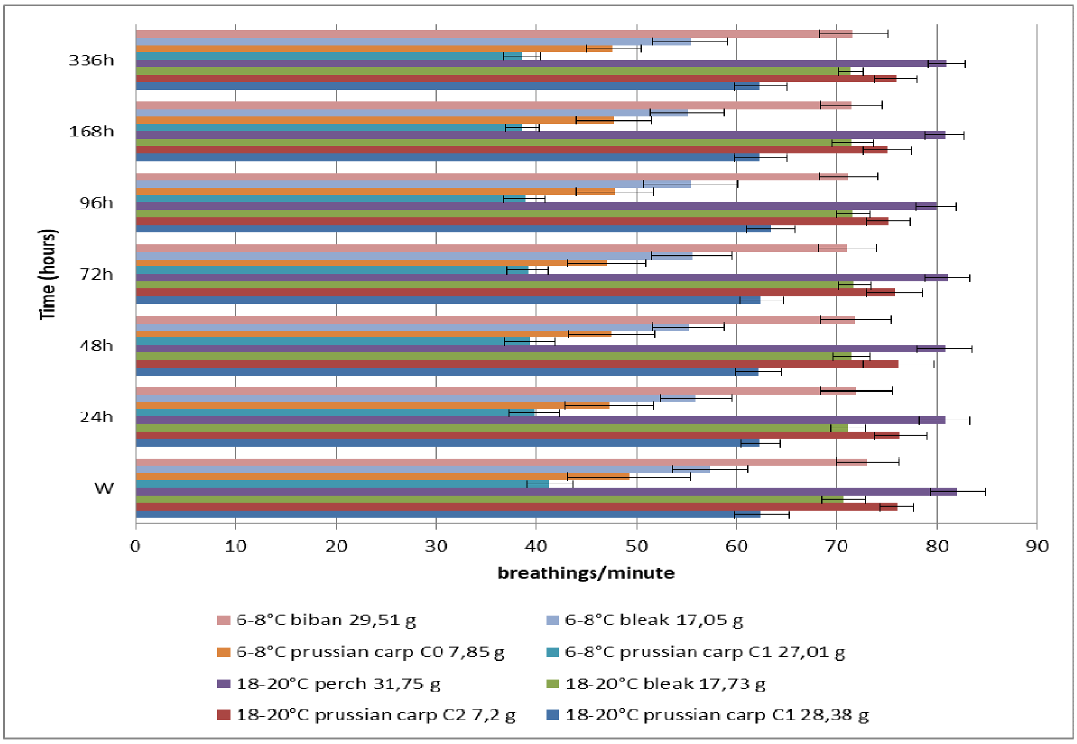

Figure 1. Mean values of respiratory rate and standard deviation at fish maintained at two thermal levels (means and standard deviation)

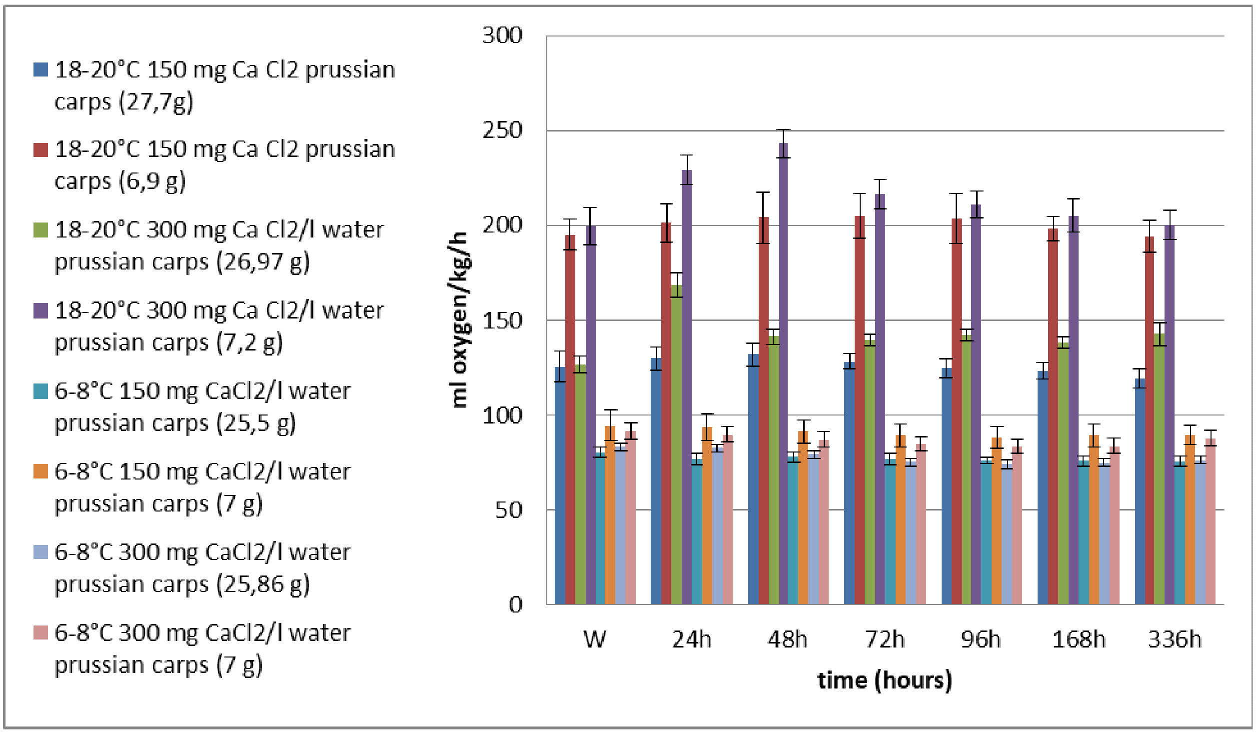

Figure 2. Influence of water hardness on energy metabolism in different size prussian carps (means and standard deviation)

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The average values of respiratory rhythm in fish acclimated to low temperatures are significantly lower than those recorded in fish acclimated to room temperature (18.82% lower in bleak and 20.97% lower in perch). From the data graphically represented in figure 1, it appears that neither the frequency of the respiratory movements changes significantly during the 14 days of experience in any of the investigated species.

The changes presented fit in the "pattern" of the reaction of the teleostei to the decrease of the temperature, which consists of bradycardia, the increase of the respiratory rate (this parameter does not increase, however, to the decreases of the temperature below 15 °C - fact found also by Gehrke and Fielder, 1988 in the species *Leiopotherapon unicolor*) and reducing oxygen consumption.

In the case of prussian carps introduced in the solution of $CaCl_2$ in the quantity of 150 mg/l water, there are no significant changes in oxygen consumption at both thermal levels for both weight categories (figure 2).

Calcium chloride at a concentration of 300 mg/l water, however, produces significant changes for the significance threshold $p < 0.05$ in fish maintained at room temperature. These changes occur 24 hours after the fish are introduced into the solution and are maintained throughout the experiment. The strongest metabolic stimulation is recorded after 48 hours, on prussian carps with an average weight of 7.2 g (221.79% more compared to the values determined before the fish were introduced into the solution). The values recorded at the end of the experiment are higher than those of the control for the larger-sized prussian carps (112.55% compared to the control values) and insignificantly different from the beginning of the experiment, in the small fish.

Respiratory rate in the prussian carps maintained at room temperature increased in both $CaCl_2$ concentrations investigated (figure 3). At the end of the experiment, the average values of this physiological index are higher than the values determined before the introduction of fish in solutions (110.11%, respectively 119.72% for the fish of the C_1 generation and 103.37% and respectively, 103.75% for those of the C_0 generation).

The maximum values of the frequency of respiratory movements were recorded after 24 hours in the case of larger fish (18.32% and 27.17% compared to the control values) and after 48 hours in the case of small fish (14.41% and 15.87% compared with the control values).

The respiratory rate of the prussian carps introduced into the water with different hardness at low temperature registered a slight decrease compared to the control values (with a maximum of 10% after 14), a decrease observed from the first 24 hours after exposure. At this temperature of experimentation, there are no significant differences between the fish of different sizes.

Calcium chloride had no lethal effects during the test (14 days) at any of the concentrations experienced (150 and 300 mg/l respectively), a finding that is similar with the data from the literature, the lethal effects appearing at more than 10 times larger (Mount et al., 1997).

At the temperature of 6-8° C the average number of red blood cells decreases compared to the one registered at 18-20° C in all three species (the decrease of this index is 11-15%) –figure 4.

The mean values of the number of erythrocytes of the prussian carps maintained both at room temperature and at low temperatures (6-8° C) in waters with different hardnesses (150 and 300 mg $CaCl_2$/l water) do not differ significantly from the control values.

Decreased number of erythrocytes in fish exposed to low temperatures has been reported by numerous researchers (Frankel et al., 1966; De Wilde and Houston, 1967; Houston and De Wilde 1968; Huggurgs and De Wilde 1969; Cameron, 1970; Precht et al., 1973).

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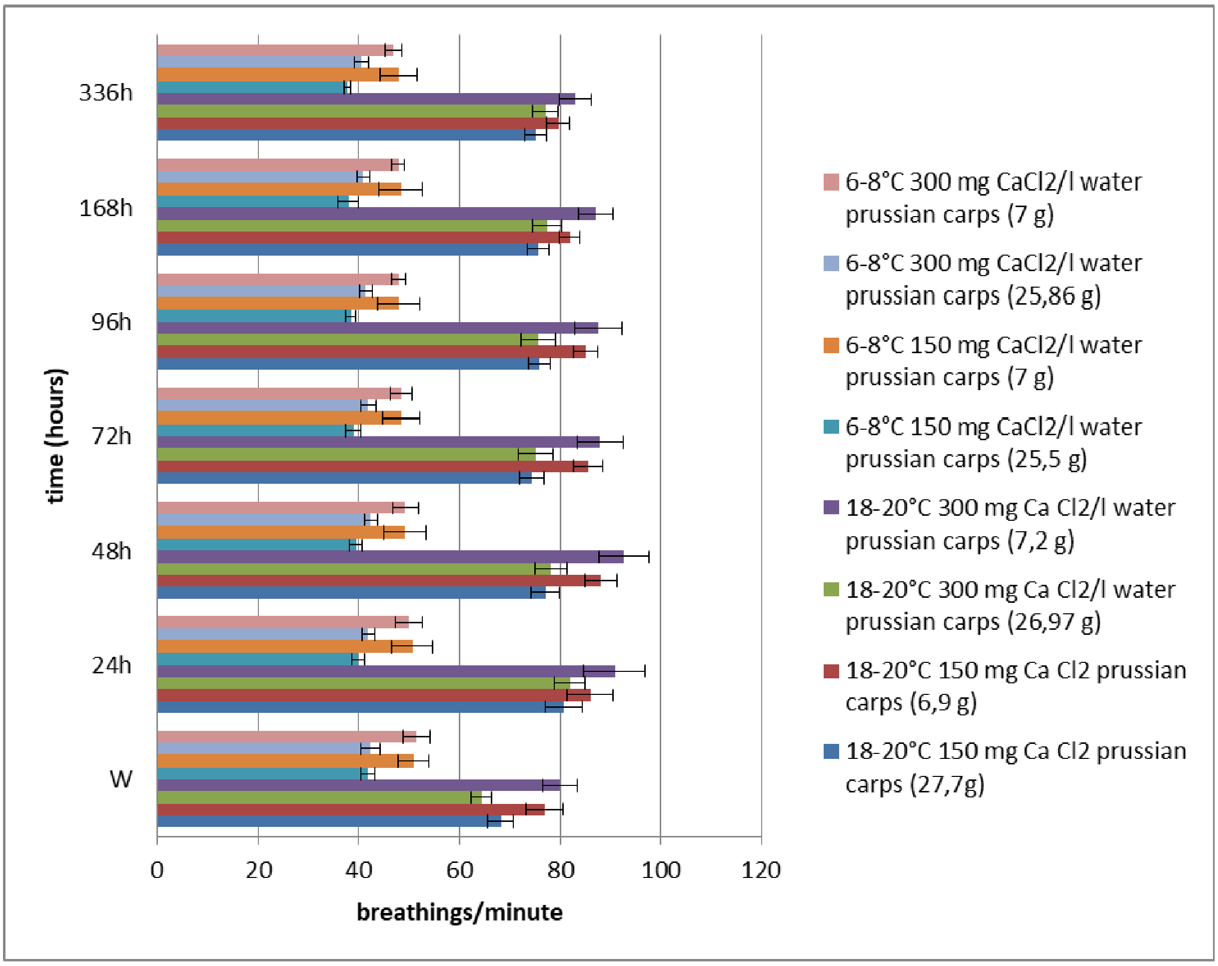

Figure 3. Influence of water hardness on respiratory rhythm in different size prussian carps (means and standard deviation)

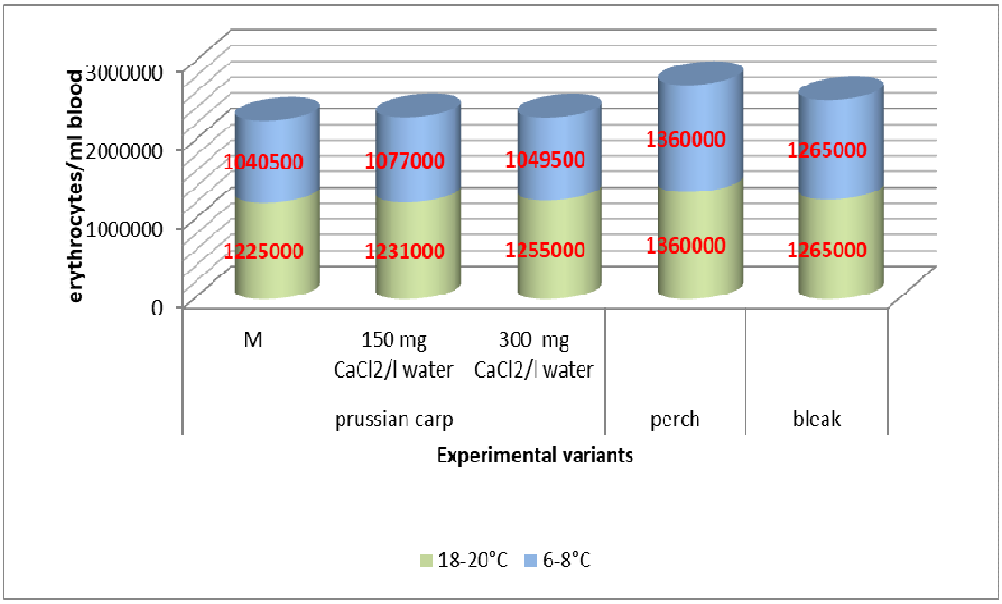

Figure 4. The average values of the number of erythrocytes after two weeks from the setting of the experiments

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4. CONCLUSIONS

The experimental samples regarded the presence of respiratory and hematological changes (and, where appropriate, the extent of these changes) in prussian carp, perch and bleak adapted to different temperatures (6-8° C and 18-20° C) and revealed differences in the reactivity of the three species to changing environmental conditions.

Although adaptation to low temperatures caused metabolic decreases and increases in the respiratory rate in all three species, the evolution of these parameters fitted the picture described in the specialized studies. The perch had the lowest oxygen consumption and adapted to low temperatures better than the prussian carp and the bleak.

There were not many physiological changes in fish immersed in water of different hardness degree, although the metabolic stimulations in the early hours of exposure were recorded for larger prussian carps. There were no significant variations in the number of red blood cells depending on water hardness.

The fish adapted to temperatures of 6-8°C recorded significant decreases in the number of red blood cells for the significance threshold $p<0.05$, without large variations between the species.

The perch adapted to low temperatures better than the prussian carp and the bleak.

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