# Value and shape of the Bohr Effect curve as a functional characteristic of hemoglobin in different fish species

**Article** *in* Journal of Ichthyology · January 2009

DOI: 10.1134/S0032945209010160



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ISSN 0032-9452, Journal of Ichthyology, 2009, Vol. 49, No. 1, pp. 123–127. © Pleiades Publishing, Ltd., 2009.
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# Value and Shape of the Bohr Effect Curve as a Functional Characteristic of Hemoglobin in Different Fish Species

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Received December 13, 2007

**Abstract**—Curves characterizing the Bohr effect value in fish species differing in ecology and systematic position (freshwater and marine bony fishes as well as anadromous sturgeons) were studied and described. Changes in the Bohr Effect curve shape in different species were observed. The classification that divides curves can be placed into two groups: (1a) arc-shaped with a maximum at 50% saturation, (1b) arc-shaped with the maximum shifted towards low saturation, (1c) arc-shaped with the maximum in the zone of high saturation, and (2a) straight without a pronounced maximum, (2b) straight with the maximum in the zone of hemoglobin's low saturation with oxygen, (2c) straight with the maximum in the zone of high saturation. It is shown that any influence upon fish causes changes in the curve's shape, which tends to form an arc with the maximum in the zone of 50% saturation of hemoglobin with oxygen.

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## INTRODUCTION

Many researchers devote significant attention to the Bohr effect value when studying hemoglobin functional features in different vertebrates (Gimmerih, 1963; White et al., 1968; Soccoro and Silva, 1984; Irzhak, 1975; Brix et al., 1999; Samuelsen et al., 1999; Brauner et al., 2001; Pichavant et al., 2003; Soldatov et al., 2004). It is known that the Bohr effect reflects an interaction between an affinity to oxygen and the proton dissociation of hemoglobin (its acidic features). The attachment of oxygen to heme simplifies proton dissociation of protein and is expressed in the shift of the dissociation curve at increasing acidity towards oxygen's higher partial tension.

Thanks to the Bohr effect, the saturation of blood with oxygen in respiratory organs and its emission in tissues is eased. This is why a physiological curve lies between curves of arterial and venues blood (Prossor and Braun, 1961). The Bohr effect is vividly expressed in the blood of most organisms, but its value varies between different animals. However, some authors believe that this is not related to a given organism's position in the evolutionary scale and is more likely conditioned by an animal's ecologic peculiarities—mainly its type of breathing (Korzhuev, 1949; Stroganov, 1962).

Kirschbaum (1963) showed that the highest value of the Bohr effect may obtained when comparing points of 50% saturation of hemoglobin with oxygen ($P_{50}$), while above 60% and below 40% saturation levels, the Bohr effect value decreases. Since that time, the Bohr effect value has been measured in the $P_{50}$ zone of hemoglobin saturation with oxygen by the majority of researchers. However, earlier we established that this is not exactly so with fish hemoglobins (Kamshilov, 2000). The maximum Bohr effect value may shift towards both low (from 40% to 10%) and high (70 to 80%) saturation of hemoglobin with oxygen.

In this study we attempt to show the change in the Bohr effect curve shape in different species of fish both under normal conditions and when exposed to different factors.

## MATERIAL AND METHODS

Bohr effect values were studied in fish species different in ecology and systematic position, such as freshwater bony fishes (bream (*Abramis brama* (L.), crucian carp *Carassius auratus gibelio* (Bloch), tench *Tinca tinca* (L.), pike *Esox lucius* (L.), anadromous sturgeons (Russian sturgeon *Acipenser gueldenstaedtii* Brandt, beluga *Huso huso* (L.), stellate sturgeon *Acipenser stellatus* Pallas), and marine bony fishes (toothfish *Anarhichas lupus* L., Arctic flounder *Liopsetta glacialis* (Pallas)). Blood for analysis was taken immediately after catching.

For the experiments on the effect of various stress factors on the hemoglobin of bream, fish were put into aquaria and allowed to adapt for one month and then exposed to different stressors: either acute (handling, adrenaline injection, or simply a prick), or chronic (change in temperature or light regime, and oxygen concentration reduction).

Studies were performed using erythrocytes hemolysate, and values of the Bohr effect curve were obtained graphically using curves of oxygen balance corresponding with Dill's expression Δlog(Pm)/ΔpH. Curves of oxyhemoglobin dissociation were registered using a spectrophotometer in a deoxygenated inert gas (nitrogen or helium) in balance with an air-compensated hemoglobin solution in a potassium phosphate buffer system with pH 6.6 and 7.2 and ionic strength 0.005 and 0.05 M with extinction E = 1.000 at 430 nm wavelength. Hemoglobin concentration and the presence of methemoglobin in the solution were determined using a DU-8A (Beckman) spectrophotometer.

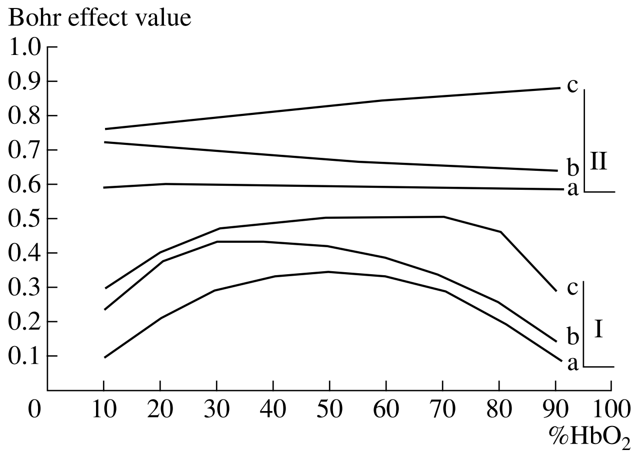


**Fig. 1.** Pattern of different shapes of the Bohr effect value.

## RESULTS

In Fig. 1, curves characterizing the Bohr effect value in fish species differing in ecology (freshwater and marine bony fishes as well as anadromous sturgeons) were analyzed. Curves fell into two groups: (1a) arc-shaped with a maximum of 50% saturation, (1b) arc-shaped with the maximum shifted towards low saturation, (1c) arc-shaped with the maximum in the zone of high saturation, and (2a) straight without a pronounced maximum, (2b) straight with a maximum in the zone of hemoglobin's low saturation with oxygen, (2c) straight with a maximum in the zone of high saturation.

The study of Bohr effect curves in freshwater bony fish caught in the wild yielded following results: (1) The maximum Bohr effect value was found in the hemoglobin of pike, followed by crucian carp, bream, and tench (Fig. 2). The Bohr effect in pike is approximately 7 times higher than that of tench. (2) At the same time, the hemoglobins of studied fish differ in terms of the shape of the Bohr effect curves belonging to the following groups: (1a) bream; (1b) pike; (2b) tench, crucian carp.

All anadromous sturgeons (Fig. 3) had the following pattern of Bohr effect curve shape change: all curves

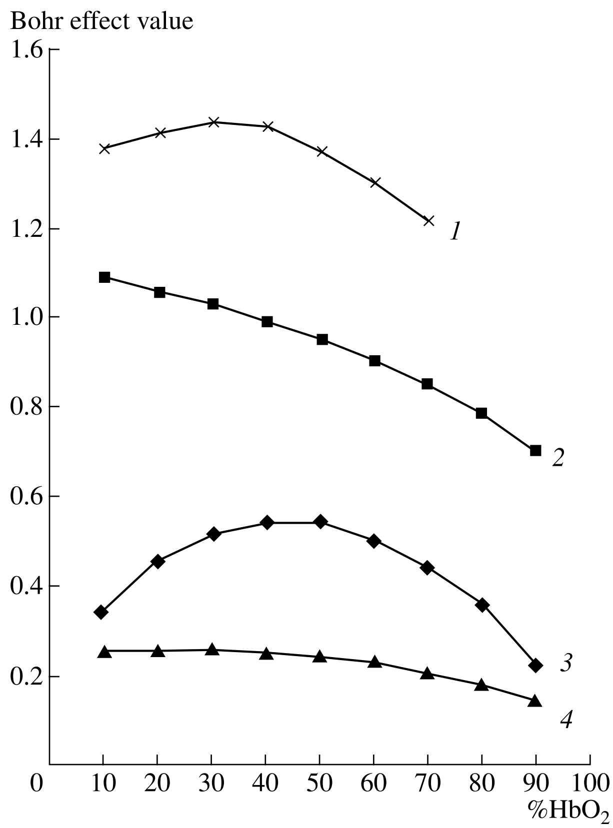


**Fig. 2.** Shape of Bohr effect curve values in different freshwater bony fishes: (*1*) pike, (*2*) crucian carp, (*3*) bream, (*4*) tench.

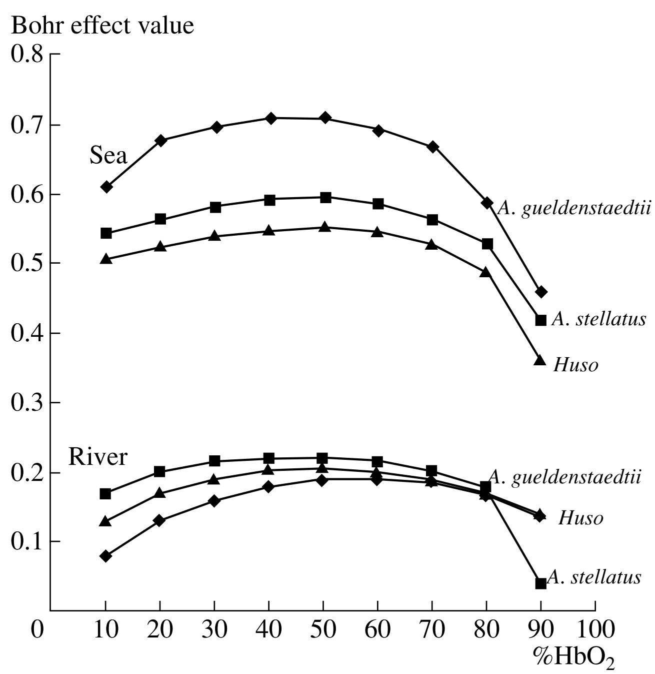


**Fig. 3.** Shape of Bohr effect curve values in different anadromous sturgeons species in the marine and river periods of life.

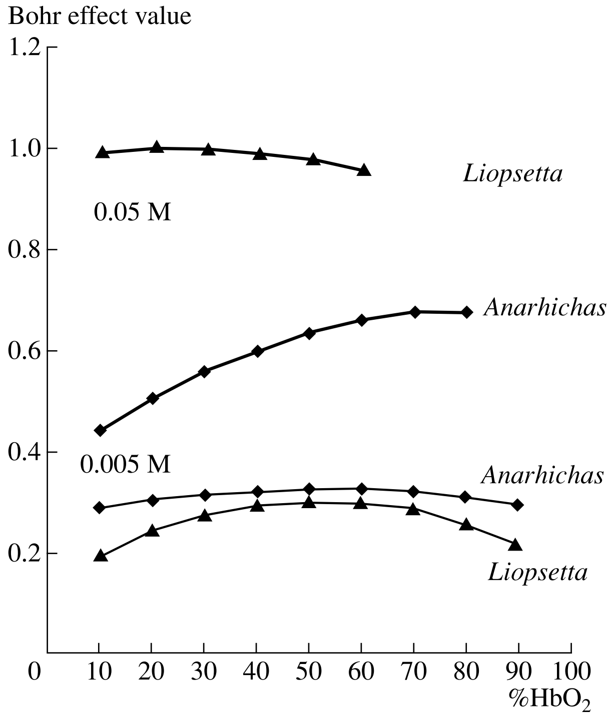


**Fig. 4.** Shape of Bohr effect curve values in two species of marine bony fishes at different molarities of the buffer solution.

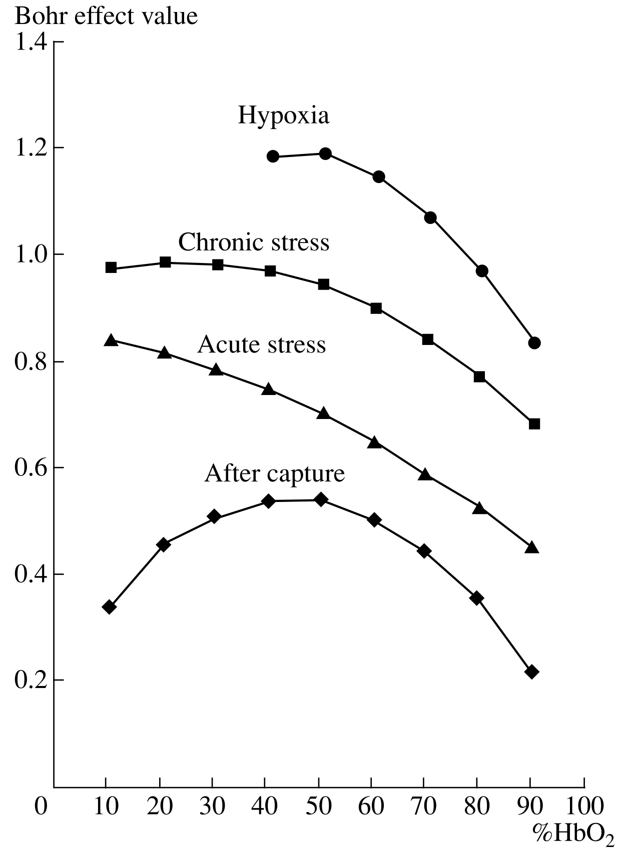


**Fig. 5.** Change in Bohr effect curve shapes in hemoglobin of bream exposed to different stressors.

are arc-shaped with a maximum in the zone of hemoglobin's 50% saturation with oxygen and belong to group 1a. It is necessary to note that an approximately three fold increase of the Bohr effect was noted in anadromous sturgeons in their marine period of life.

The results of the studies on marine fish species are shown in Fig. 4. In a molar buffer solution of 0.005 M, the maximum value of the Bohr effect curve of toothfish and flounder exists in the area of $P_{50}$, though the first appearance of the curve is practically straight (group 2a), and the second is arched (group 1a). When the molar buffer solution is increased to 0.05 M, the Bohr effect value increases, and the form of the curve changes. In the hemoglobin of toothfish, the curve acquires a straight form with a maximum in the area of 80% saturation of hemoglobin with oxygen (group 2c); in flounder, on the other hand, the curve has a maximum in the area of 20% saturation (group 2b).

Research into the hemoglobin of bream that are exposed to difference stress influences reveals changes in the form of the curve and an increase in the value of the Bohr effect. In Fig. 5 it is shown that under the influence of acute stress, the arched Bohr effect curve characteristic of fish taken from their natural environment (immediately after catching) changes into an inclined straight line with a saturation maximum in the area of 10% (group 2b), while the effect's value increases by 1.5 times. In cases of prolonged (chronic) stress, the curve again starts to become arc-shaped and the effect's value in this case increases by more than 2 times as the curve becomes increasingly more arc-shaped. Another factor is the effect of hypoxia. The effect of hypoxia is expressed in the curve starting to become arc-shaped but failures of the deoxygenation process of bream's hemoglobin occur and the Bohr effect increases by 2.5 times when compared with the initial or natural value.

## DISCUSSION

Curves of hemoglobin-oxygen balance in a potassium phosphate buffer system show a logical shift to the right when the pH of the buffer solution decreases from 7.2 to 6.6 (Prosser, 1973). At the same time, the dissociation curves of many animal species stay invariant and may be transformed into each other using some coefficient. Invariance means that the balance constant remains in a simple relation with hydrogen proton concentration in the environment, in compliance with the Le Chatelier principle (Irzhak, 1975).

The shift of the oxygen curve to the left in the event of the environment's acidification provides evidence for the presence of the Bohr effect (i.e., inverse relation between pH and $P_{50}$) (Hochachka and Somero, 1973). It is established that such a dependence of the Bohr effect from saturation is observed in human whole blood when the level of $CO_2$ is kept constant. If the partial pressure of $CO_2$ changes, the dependence takes on a different characteristic: The Bohr effect has the biggest value at a small concentration of oxyhemoglobin, decreases to medium values at 50% oxyhemoglobin, and becomes minimal at 70–80% oxyhemoglobin (Hochachka and Somero, 1973; Shilov, 1985).

It is thought that having hemoglobin with a strongly pronounced Bohr effect is favorable under the presence of a highly intensive metabolism; however, cases where excessive sensitivity of hemoglobin to negative modulation by hydrogen ions becomes unfavorable are known (Hochachka and Somero, 1973). P. Hochachka and J. Somero (1973) showed that in some very active fish, blood pH may decrease so much that the Bohr effect strongly suppresses the binding of oxygen by hemoglobin in the gills due to extremely high lactate concentrations during intensive muscular work. It is known that fish sometimes die of asphyxiation during intensive motile activity (Hochachka and Somero, 1973).

Hana (1987) notes a high affinity of hemoglobin in the blood of the snakehead *Ophiocephalus argus warpachowskii* to oxygen, a pronounced Bohr effect, and an absence of Rute's effect. The breathing features of this animal's blood allows them to extract oxygen from the air without significant losses of oxygen through gills during hypoxia.

It is proven experimentally (Hochachka and Somero, 1973) that a reduction in pH may decrease the ability of hemoglobin to bind to oxygen. This effect of pH on oxygen capacity of hemoglobin is called Rute's effect. Strongly pronounced Rute's effect often accompanies a high Bohr effect. A decrease in affinity to oxygen due to the effect of an acidic environment distorts dissociation curves: they stop being S-like and become situated sloping at small angles towards the $PO_2$ axis (Irzhak, 1975). Such curves cannot be transformed into each other (Prosser and Brown, 1961; Irzhak, 1975). It is known that together with the Bohr effect there is an opposite phenomenon—simplification of $CO_2$ output during oxygenation and $CO_2$ intake during deoxygenation of hemoglobin (Holdaine's effect). Revealing the essence of Holdaine's effect, Irzhak (1975) has shown that blood free of oxygen binds more carbon dioxide than arterial blood.

Oxyhemoglobin is more acidic than deoxyhemoglobin (Korzhuev, 1964; White et al., 1968). That is why hemoglobin becomes a better buffer when it is emitting oxygen to the tissues. In lungs or gills, on the other hand, as a result of oxygenation there arises a lower partial pressure of carbon dioxide and a concentration gradient favorable to its passage from erythrocytes through the plasma into the environment.

Hemoglobin affinity to oxygen in fish is dependent on upon a given species' ecological peculiarities, including the degree of its motility (Luk'yanenko et al., 1991). The majority of data available at the moment are from studies of the Bohr effect in hemoglobins of higher and lower mammals. Normal hemoglobins of these groups are characterized by value of the Bohr effect fluctuating from 0.4 to 0.6, while hemoglobin's affinity to oxygen in these animals differs to a significantly larger degree. A much higher diversity of the Bohr effect is seen in amphibians and fish. Such values of the Bohr effect as 0.8 or 1.2 are not rare and signify a rapid shift of oxyhemoglobin dissociation curves to the right appearing at the event of the environment's acidification. It is thought that species inhabiting stagnant waters have a less pronounced Bohr effect than species inhabiting running water (Prosser and Brown, 1961; Prosser, 1973). In a few species of fish, for example, the shark *Squalus acanthias*, no Bohr effect is observed. The Bohr effect is also not present in the myxine *Myxine glutinosa* (Prosser and Brown, 1961). Specific differences between hemoglobins in terms of Bohr effect value let us judge the ecological specificity of this protein.

The shape of Bohr effect curve is directly related to the shape of the oxyhemoglobin dissociation curve shape. If the hemoglobin oxygen balance curves are invariant, then the Bohr effect curve would be arc-shaped with maximum in the area of 50%, as is observed in anadromous sturgeons. Correspondingly, the less the oxygen balance curves differentiate from each other, the greater the Bohr effect curves will be distinguished from the arc. If we stop using Dill's expression, then the curve characterizing the Bohr Effect lies between the curves of oxygen balance of arterial and venous blood and its shape becomes directly dependent on the species of the fish and the ecological conditions in which this species lives. Even the most short-term impact on the organism would cause changes in the hemoglobin ionic environment and decrease pH due to emission of acid metabolites into the blood that change the concentration of organic phosphates and lead to a shift of the oxygen balance curve and change of its shape. At the same time, the shape of the Bohr effect curve value changes, becoming a sloping straight line. In the case of a long-term effect of a stressor on the organism, some unstable equilibrium is achieved and the Bohr effect curve rapidly tends to become arc-shaped again. The internal reserves of the organism are in a critical state due to the strengthening of metabolic processes that lead to blood acidification by their products, on one hand, and to intensive spending of ATP, on the other hand. If acidification causes a shift of the dissociation curve to the right, then ATP loss acts in an opposite direction causing both an increase of the Bohr effect value and a change of its curve shape. At the same time, heterogeneity of hemoglobin is of importance as some of its components may have different functional features (absent or vice-versa, pronounced Bohr effect) that start acting at the start of certain conditions. Thus, two components were isolated from bream hemoglobin, one from crucian carp, six from Russian sturgeon, seven from stellate sturgeon, and five from beluga (Luk'yanenko et al., 1991).

It should also be noted that different fish species' hemoglobin are differently resistant to various environmental factors. For example, sturgeon hemoglobin may be kept for months at temperatures 5–8°C and not form methemoglobin. An absolutely different picture is observed in freshwater bony fishes, where hemoglobin turns into methemoglobin in 5–6 days at the same conditions of storage. Presence of even the slightest quantities of methemoglobin in a sample leads to an increase of hemoglobin affinity to oxygen as well as a change of the Bohr effect.

## CONCLUSIONS

(1) Studies of the value of the Bohr effect have yielded two main forms of curves characterizing this effect: (1a) arc-shaped with a maximum in the area of 50% saturation, (1b) arc-shaped with a maximum shifted towards low saturation, (1c) arc-shaped with a maximum in the zone of high saturation, (2a) straight without a pronounced maximum, (2b) straight with a maximum in the zone of hemoglobin's low saturation with oxygen, (2c) straight with a maximum in the zone of high saturation.

(2) Acute stress leads to a change of the Bohr effect curve shape (bream hemoglobin) turning it from arc-shaped into a sloped straight line that tends to become arc-shaped after continuation of the effect or chronic stress.

## ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 06-04-48282.

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