Temperature sensitivity of the oxygenation reaction of stripped haemolysates from the freshwater fishes *Labeo capensis* and *Clarias gariepinus*

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The oxygen binding properties of haemoglobin solutions of the mudfish *Labeo capensis* and the catfish *Clarias gariepinus*, stripped by gel filtration chromatography and buffered at 23°C in 0,05 M Hepes (pH 7,48), were determined at 8°C, 15°C and 23°C. The P_{50} values obtained for *L. capensis* at these respective temperatures were 0,89 (pH 7,63); 1,29 (pH 7,52) and 3,02 (pH 7,49) and those for *C. gariepinus* haemoglobin were 2,47 (pH 7,61); 3,34 (pH 7,53) and 6,30 (pH 7,49). The lower oxygen affinity of *C. gariepinus* haemoglobin may be related to the obligatory air breathing of *C. gariepinus* by means of a branchial organ which is absent in the mudfish. The purified hemolysate from *C. gariepinus* also displayed higher haem-haem co-operativity (*n*) at all three experimental temperatures compared to *L. capensis*. The heat of oxygenation (ΔH) between 8°C (pH 7,63) and 23°C (pH 7,49) calculated for *L. capensis* haemoglobin (–56,3 kJ.mol^{-1}) exceeded that of *C. gariepinus* (–43,1 kJ.mol^{-1}).

Die suurstofbindingseienskappe van hemoglobienoplossings van die moddervis *Labeo capensis* en die baber *Clarias gariepinus*, gesuiwer deur middel van jelfiltrasie-chromatografie en gebuffer by 23°C in 0,05 M Hepes (pH 7,48), is by 8°C, 15°C en 23°C vasgestel. Die P_{50}-waardes vir *L. capensis* hemoglobien by hierdie temperature was onderskeidelik 0,89 (pH 7,63); 1,29 (pH 7,52) en 3,02 (pH 7,49) en vir *C. gariepinus* hemoglobien 2,47 (pH 7,61); 3,34 (pH 7,53) en 6,30 (pH 7,49). Die laer suurstofaffiniteit van *C. gariepinus* hemoglobien kan verband hou met die teenwoordigheid van 'n brangiale orgaan by hierdie vis en die feit dat babers verpligte lugasemhalers is. So 'n orgaan is afwesig by die moddervis. Die gesuiwerde hemolisaat van *C. gariepinus* vertoon, in vergelyking met die van *L. capensis*, 'n groter mate van koöperatiwiteit (*n*) by aldrie eksperimentele temperature. Die oksigeneringswarmte (ΔH), soos bereken tussen 8°C (pH 7,63) en 23°C (pH 7,49) is hoër vir *L. capensis* hemoglobien (–56,3 kJ.mol^{-1}) as vir C. gariepinus (–43.1 kJ.mol^{-1}).

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Freshwater fish, in contrast to most marine fish or terrestrial animals, must cope with large daily or seasonal variations of oxygen supply. In arid and tropical regions oxygen availability in freshwater is largely determined by highly fluctuating temperatures coupled with unpredictable rainfall patterns. In order to satisfy the oxygen demand of the tissues, molecular, physiological and anatomical adaptations have been developed by fish to provide adequate amounts of oxygen to organs (Riggs 1979; Powers 1980; Weber & Jensen 1988; Di Prisco & Tamburrini 1992).

An adaptive anatomical feature in the genus *Clarias*, to obtain oxygen in stagnant and very poorly oxygenated waters, is the branchial respiratory tree. Each gill cavity is extended upwards as an air-chamber, having two branching structures which develop from the upper parts of the second and fourth gill arches. Both the air chambers, the lining of which may be folded, and the tree-like outgrowths have a rich blood supply – the whole complex forming an efficient 'lung' (Marshall 1965). The gills alone do not provide enough oxygen to keep the fish alive. Therefore, the clariid group of fishes has to rely on aerial oxygen uptake in both oxygen-rich and oxygen-poor water via the branchial organ (Jubb 1967). The mudfish *L. capensis* lives in the same habitat as *C. gariepinus*. It is a detritus feeder but also eats *Potamogeton pectinatus* (Schoonbee 1969). It has well-developed gills but no accessory respiratory organ.

Studies on air breathing Amazonian fishes (Johansen, Mangum & Lykkeboe 1978; Johansen, Mangum & Weber 1978) have revealed that low oxygen affinity haemoglobins are associated with air-breathing fish and high oxygen-haemoglobin affinity with water breathers. However, Powers, Martin, Garlick, Fyhn & Fyhn (1979) have shown that this correlation diminishes if all water and air-breathing fishes from the Amazonian area are compared. Their research indicates that there is a strong correlation between the haemoglobin-oxygen affinity and habitat. Freshwater fish living in rapidly flowing waters have haemoglobins with low oxygen affinities and fish inhabiting stagnant waters have high oxygen-haemoglobin affinity. According to Riggs (1979) the results obtained by Johansen and his group are true when a comparison is made of closely related fish inhabiting the same body of water.

This study examines the oxygen affinities of haemoglobin solutions, stripped by gel filtraton chromatography, of two Orange River fishes, *L. capensis* and *C. gariepinus*, at different temperatures. Our results, presented here, concur with the Amazonian findings of Johansen, Mangum & Lykkeboe (1978) and Johansen, Mangum & Weber (1978).

Five adult specimens each of *Labeo capensis* and *Clarias gariepinus* were collected in November 1992 from the Department of Nature Conservation's cultivation dams at the Hendrik Verwoerd Dam, Orange River, Republic of South Africa. All specimens were transported to the laboratory at Potchefstroom where, on arrival, blood was collected by cardiac puncture. Approximately 3 ml of blood was collected from each fish using 5-ml syringes flushed with 5000 units

sodium heparin (Sigma, USA) whereafter it was centrifuged at 2000 G to obtain packed red blood cells. The red cells were washed three times with two volumes of a solution containing 1,7% NaCl and 10 mM Tris-HCl (2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride, Sigma, USA), pH 7,84 at 4°C (Riggs 1981). The packed red blood cells were lysed by adding 3 volumes of 1 mM Tris-HC1, pH 8.05 (4°C) and left to cool for 1 h on ice. Thereafter the cells were centrifuged at 20 000G for 40 min at 8°C. The supernatants containing the haemoglobin were pipetted off and pooled for each species. The two haemoglobin solutions were separately stabilized against methaemoglobin formation by blowing carbon monoxide gas (50 ml.min^{-1}) over the haemoglobin solution for 45 min (Van Aardt 1992). For organic phosphate stripping and ion removal, a 0,9 cm × 15 cm column packed with Sephadex G25 and equilibrated with 0,05 M Hepes (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, Merck), pH 7,48 at 23°C, was used. One-millilitre fractions were eluted at 4°C at a rate of 0,8–1,0 ml.min^{-1}.

Oxygen dissociation curves of the stripped haemoglobin solutions, buffered with 0,05 M Hepes, were made in duplicate with the aid of a thin layer optical cell (Dolman & Gill 1978) for each fish species at 8°C, 15°C, and 23°C. According to Gill (1981) the part of the curve at PO_2 below 1 mm Hg can be determined with an error of less than 4%, which makes this method particularly suitable for high O_2-affinity haemoglobins. Immediately prior to the oxygen-binding measurements, the thin layer haemoglobin solution in the optical cell was equilibrated with air containing 4% CO_2 and the bound carbon monoxide removed from the haemoglobin molecule by exposure to a 'cold' light source (Schott, Mainz KL 150B) for 2 min (Riggs 1981). Oxygen dissociation curves and Hill-plots were made with the aid of a computer programme (Van Aardt & Naude 1990) in order to establish haemoglobin O_2-affinity (P_{50}) and oxygen binding site co-operativity (n) between 25% and 75% oxygen saturation. The heat of oxygenation, ΔH, was calculated with respect to the temperature changes at constant pH using the Van't Hoff equation (Morris & Bridges 1985). pH-measurements of the haemoglobin solutions were made at the same temperatures at which oxygen dissociation curves were constructed.

The half saturation oxygen partial pressures (P_{50}) of *L. capensis* haemoglobin were 0,89 mm Hg at 8°C (pH 7,63); 1,29 mm Hg at 15°C (pH 7,52) and 3,02 mm Hg at 23°C (pH 7,49) (Figure 1). The P_{50} values obtained for *C. gariepinus* haemoglobin were 2,47 mm Hg at 8°C (pH 7,61); 3,34 mm Hg at 15°C (pH 7,53) and 6,30 mm Hg at 23°C (pH 7,49) (Figure 2). Freshwater teleosts differ widely in the O_2-affinities of their haemoglobins. P_{50}-values as high as 22,4 mm Hg for rainbow trout (*Oncorhynchus mykiss*) and as low as 0,79 mm Hg for carp (*Cyprinus carpio*) at 20°C and pH 7,4 were reported by Weber, Wood & Lomholt (1976) and Weber & Lykkeboe (1978), respectively. According to Fyhn, Fyhn, Davis, Powers, Fink & Garlick (1979) such differences can mainly be attributed to differences in haemoglobin structure and multiplicity.

The low haemoglobin oxygen affinity found in *C. gariepinus* compared with that of mudfish haemoglobin could be related to the obligatory air breathing occurring in *C. gariepinus*. The concentration of oxygen in air is high and constant.

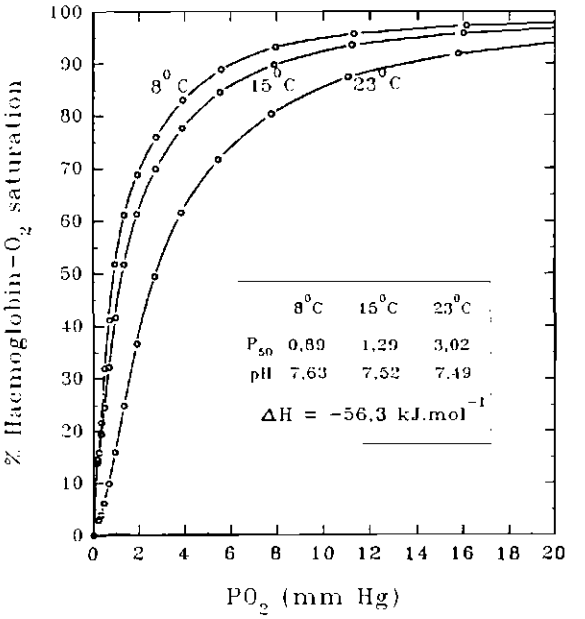

Figure 1 Oxygen dissociation curves obtained from haemoglobi solutions of *Labeo capensis* stripped by gel filtration chromatogra phy and buffered in 0,05 M Hepes (pH 7,48) at 23°C. Measurement were made at 8°C (pH 7,63); 15°C (pH 7,52) and 23°C (pH 7,49 showing the effect of temperature on oxygen–haemoglobin affinity.

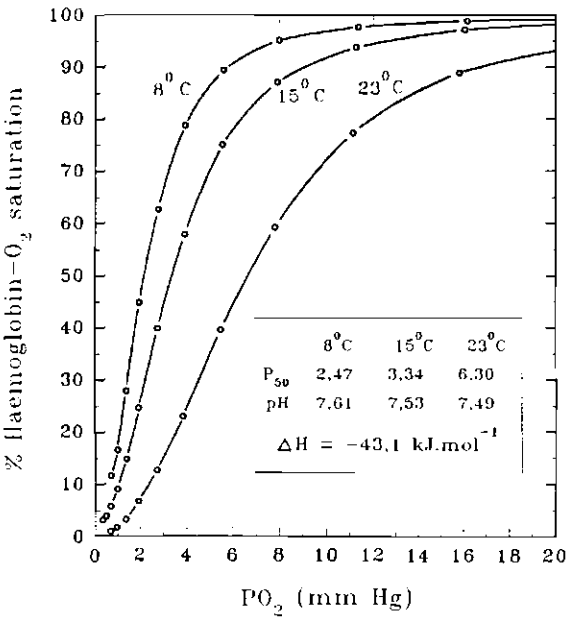

Figure 2 Oxygen dissociation curves obtained from haemoglobin solutions of *Clarias gariepinus* stripped by gel filtration chromatography and buffered in 0.05 M Hepes (pH 7,48) at 23°C. Measurements were made at 8°C (pH 7,61); 15°C (pH 7,53) and 23°C (pH 7,49), showing the effect of temperature on oxygen–haemoglobin affinity.

Therefore, there was no need to develop a high affinity haemoglobin in the catfish. This contrasts with mudfish which are often exposed to very low oxygen concentrations in the water, where the gills are the only gas exchange organs. A high affinity haemoglobin thus gives a high degree of oxygen

saturation of the blood at a relatively low partial pressure of oxygen in the water. This type of molecular adaptation of the respiratory pigment in fishes has been developed in a number of fish species living in a variety of habitats (Powers 1980; Weber & Jensen 1988). The synthesis of a certain type of haemoglobin from the gene pool to overcome an ecologically induced problem can manifest itself phenotypically on a seasonal or longer time base. Both the mudfish (Du Toit, Hattingh & Schabort 1973) and the catfish (Hattingh & Du Toit 1973) have multiple haemoglobins that can be synthesized under experimental or seasonal variability of temperature and oxygen. How these different haemoglobins function in *L. capensis* and *C. gariepinus* is not known. From our experiments it is clear that a mixture of mudfish haemoglobin components has a much higher oxygen affinity than the multiple haemoglobins of the catfish *C. gariepinus*.

The heat of oxygenation (ΔH) between 8°C (pH 7,63) and 23°C (pH 7,49) calculated for the stripped hemolysate of *L. capensis* (–56,3 kJ.mol^{-1}) indicates a higher temperature sensitivity for oxygen binding than in *C. gariepinus* (–43,1 kJ.mol^{-1}). Since pH decreased with increasing temperature, this difference could be due, at least in part, to a larger Bohr effect in *L. capensis*. The ΔH values for the haemoglobins of both *L. capensis* and *C. gariepinus* agree, with the exception of highly stenothermic species (Johansen & Weber 1976), with those calculated by Powers *et al.* (1979) and Powers (1980) for various neotropical and temperate zone fishes.

The heat of oxygenation, ΔH, at a pH between 7,49 and 7,63 calculated for both *L. capensis* and *C. gariepinus* haemoglobin indicated that these haemoglobin solutions were moderately sensitive to changes in temperature. In general haemoglobins are far more sensitive to temperature changes at high pH (above 8,0). At pH 9,0 maximal ΔH values of –45,9 kJ.mol^{-1} and –61,0 kJ.mol^{-1} have been found respectively for Antarctic fish and human haemoglobin A, which functions at a constant internal thermal environment (Powers, *et al.* 1979). However, caution must be taken with our samples of mixed haemoglobin. It is known that a haemoglobin found in such a mixture may be temperature insensitive but pH sensitive (tuna) or not sensitive to either pH or temperature (trout) (Riggs 1979).

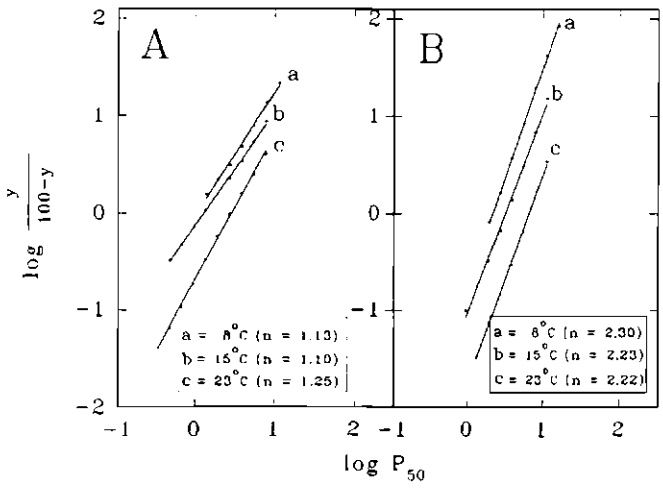

Figure 3 Hill-plots of haemoglobin mixtures of *Labeo capensis* (A) and *Clarias gariepinus* (B) stripped by gel filtration chromatography and buffered in 0,05 M Hepes (pH 7,48) at 23°C. Measurements were made at 8°C; 15°C and 23°C showing the effect of temperature change on the co-operativity of oxygen binding (n) in haemoglobin

Co-operativity of oxygen binding for the haemoglobins of both species was relatively insensitive to temperature (Figures 3A & 3B). The n-values for stripped *L. capensis* haemoglobin were 1,13 at 8°C; 1,10 at 15°C, and 1,25 at 23°C (Figure 3A). As was the case for *L. capensis*, the n-values for *C. gariepinus* varied little with change in temperature (Figure 3B). The n-values for the latter species were 2,30 at 8°C; 2,23 at 15°C, and 2,22 at 23°C.

Although this study has shown that there is a distinct difference between the oxygen affinities of the stripped haemoglobins of *C. gariepinus* and *L. capensis*, experiments should be carried out to measure the functions of the different haemoglobin components contained in these haemoglobin mixtures. Only then can predictions be made regarding the ecological adaptiveness of the various haemoglobins present in the blood of the two freshwater fishes studied.

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