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Comparison of affinity of total Hb to O_2 between 2 subspecies of Japanese char, Gogi, *Salvelinus leucomaenis imbrius* and Nikkoiwana, *S. l. pluvius*

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Abstract Total Hbs were compared for affinity to O_2 among the 2 subspecies of the Japanese char, *Salvelinus leucomaenis pluvius*, called 'Nikkoiwana' and *S. l. imbrius*, called 'Gogi'. At pH7.8, O_2 saturation curve was a little steeper for Nikkoiwana than for Gogi, but the P_{50} value was almost at the same level (13 and 12torr, respectively). At pH7.0, however, O_2 saturation curve was slightly steeper for Gogi than for Nikkoiwana, and the P_{50} value was about 78 and 92torr, respectively. This suggests the differences in dissolved oxygen concentration between the water environments to which the two subspecies had adapted.

Key words: char, Gogi, hemoglobin, Nikkoiwana, oxygen affinity

INTRODUCTION

In the Chugoku Mountain Chains, 2 subspecies of Japanese char *Salvelinus leucomaenis* (PALLAS) (called "Iwana"), *S. l. pluvius* (HILGENDORF) (called "Nikkoiwana") and *S. l. imbrius* (JORDAN et MCGREGOR) (called "Gogi"), are distributed (Miyadi et al., 1976). Gogi has been described to incline to live in a rather benthic life style (Miyadi et al., 1976). Besides, Gogi is well known to share much longer reaches in the river with other freshwater fishes, e.g., *Oncorhynchus masou masou* (BREVOORT), *Zacco temmincki* (TEMMINCK et SCHLEGEL) and *Plecoglossus altivelis* TEMMINCK et SCHLEGEL, than Nikkoiwana and "Yamatoiwana" (*S. l. japonicus* OSHIMA, another subspecies of Japanese Char) (Kimura, 1989). These reports, together with our field observation of Gogi living in rather stagnant waters at considerably high temperatures in drought seasons (unpublished data), suggest that Gogi be adapted to more oxygen-poor environments than other subspecies.

To prove the above-mentioned hypothesis, in this study, oxygen affinity of total hemoglobins (Hbs) were compared at different pH conditions between the 2 subspecies.

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MATERIALS AND METHODS

Char samples

Char samples were kindly provided by Mr. F. Nishimura, Culture Association of Yamame in Yoshiwa Village, Hiroshima Prefecture. Four individuals were sacrificed for each subspecies. The total length, body length and body weight were in the ranges of 250-305mm, 225-275mm and 182-306g, respectively, for Gogi and in the ranges of 315-395mm, 275-350mm and 282-576g, respectively, for Nikkoiwana.

Preparation of total Hb solution

Blood samples were obtained from anal vein with a syringe, suspended in an adequate amount of saline (0.15 M NaCl solution) and centrifuged at 1,600×g. A pellet of red blood cells was washed twice with saline and punctured by dialysis against a buffer of 1mM Tris-HCl, pH 7.5. The supernatant after centrifugation at 10000×g of the lysate was used as total Hb solutions.

Examination of oxygen affinity

Total Hb solutions were dialysed against a buffer of 1mM Tris-HCl, containing 0.1M NaCl, pH 7.0 or pH 7.8. After centrifugation, the solutions were examined for affinity to O_2 as follows. O_2-Hb equilibrium curves were determined at pH 7.0 and 7.8 by measuring the O_2 content of Hb solutions tonometered against air and standard gas mixtures of known composition. All the gas mixtures have 0.3% of CO_2 (about 2 mmHg). Tonometry was performed in a glass chamber (about 60 ml) at 15℃. Equilibration time in the tonometers was 15 minutes except for the gas mixture having the lowest oxygen pressure (1 hour). Hb solution was removed from the tonometers while the equilibration gas continued to flow. The time elapsed between Hb solution sample removal and transfer to the O_2 content analyzer was maximally 30 seconds. O_2 content was measured by Lex-O_2-Con Aparatus (Lexington Instruments Corporation U.S.A.). Measurements were performed at 5 different oxygen tensions above and below the predicted P_{50} value. The measured O_2 contents were corrected for physically dissolved O_2. O_2 capacity (i.e. 100% O_2 saturation) was obtained subtracting physically dissolved O_2 from O_2 content of Hb solution equilibrated with air. The P_{50} values and other characteristics of the equilibrium curves were calculated according to the Hill equation.

RESULTS

O_2 dissociation curve of total Hb (Fig. 1)

O_2 dissociation curve of total Hb of Gogi was slightly steeper than that of Nikkoiwana at pH 7.0. P_{50} value of Hb was about 78 and 92 torr for Gogi and Nikkoiwana, respectively.

O_2 dissociation curve of total Hb of Nikkoiwana was a little steeper than that of Gogi at pH 7.8. However, P_{50} value of Hb was almost at the same level (about 12 and 13 torr for Gogi and Nikkoiwana, respectively).

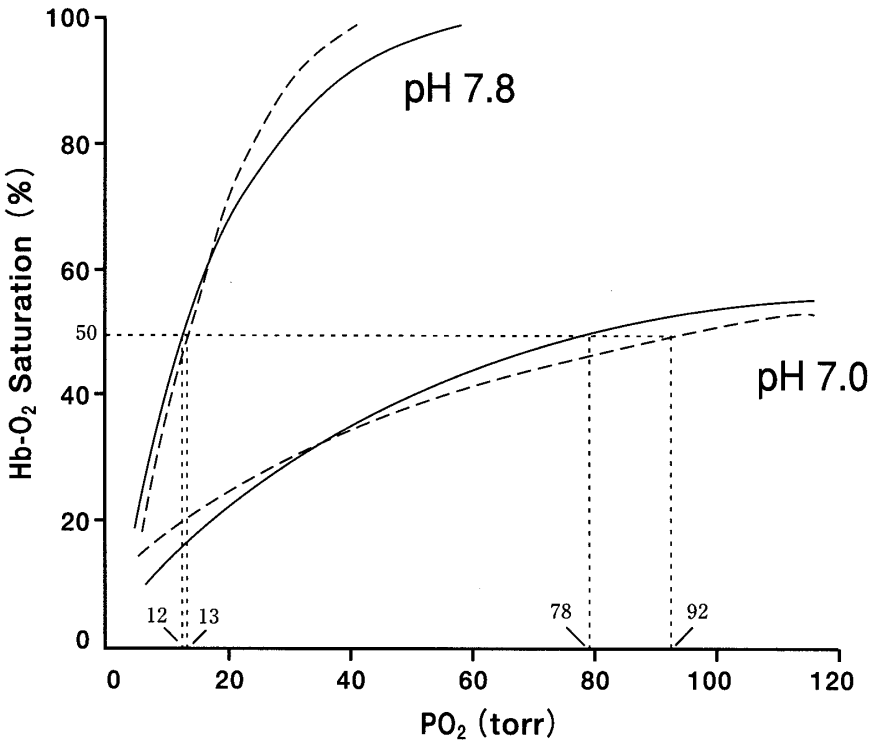

Fig. 1 Oxygen dissociation curves of char hemoglobins. Solid line, Gogi; broken line, Nikkoiwana.

DISCUSSION

Gogi has been sometimes observed to be alive and even to show an appetite for bait organisms in pool-like environments in connection to but almost completely isolated from the main flow of a river in drought seasons in our field studies (unpublished data). The observation supposes that Gogi could adapt to more stagnant waters than Nikkoiwana with showing a much higher oxygen affinity. To the present study, whereas P_{50} value was a little lower for Gogi than for Nikkoiwana at pH 7.0, no remarkable differences in P_{50} value of total Hb were observed between Gogi and Nikkoiwana at pH 7.8. This suggests a higher O_2 affinity of Gogi Hb at pH 7.0. This might reflect that Gogi can do well by a smaller amount of oxygen consumption than Nikkoiwana at the same level of activity in the field, considering a general pH range of 6.6-7.2 for rivers in Japan (Hirayama, 1968). In addition, Gogi Hb is considered to be much weakly subjected to a Bohr effect than Nikkoiwana Hb because O_2 dissociation curve of total Hb was steeper for Gogi than Nikkoiwana at pH 7.0 and it was steeper for Nikkoiwana than Gogi at pH 7.8 in this study. However, there have been no studies on a comparison of oxygen consumption rate between these subspecies.

In this study, affinity of char Hb to O_2 was measured at 15℃. Considering a common distribution of Gogi in waters at a little higher temperature in summer (23℃) than Nikkoiwana (Imanishi et al., 1979), however, there may be some differences in effects of temperature on O_2 dissociation curve of Hb between the two subspecies.

In this study, a total Hb was compared for O_2 affinity between the two subspecies. Chars have been reported to possess many Hb components with a variety of electrophoretic properties (Yoshiyasu and Fumoto, 1972). Therefore, an examination of O_2 affinity should be carried out at

each Hb component level.

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日本産イワナ2亜種，ゴギとニッコウイワナ全ヘモグロビンの酸素親和性の比較

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要　旨　日本産イワナ2亜種，ゴギとニッコウイワナの全ヘモグロビンの酸素親和性を比較した。pH7.8 では，酸素解離曲線の傾きはゴギよりニッコウイワナの方が急であったが，P_{50} 値はほぼ同レベルであった（それぞれ12及び 13torr）。しかし，pH7.0 では，傾きはニッコウイワナよりゴギの方が急で，P_{50} 値はそれぞれ92及び 78torr であった。すなわち，ゴギ Hb の方が弱い Bohr 効果を示した。これらの結果は，2亜種間で適応してきた棲息環境の溶存酸素濃度に違いがあることを示唆する。

キーワード：イワナ，ゴギ，ヘモグロビン，ニッコウイワナ，酸素親和性

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