An Estimation of the Minimum Level of Dissolved Oxygen in Water Required for Normal Life of Fish—II

An Experiment with Carp under Carbon Dioxide Accumulation*¹

Yasuo ITAZAWA*² and Tatsusuke TAKEDA*²

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Dissolved oxygen in ambient water is considered to have effects on fish life through the influence on oxygen level in the arterial blood. The minimum requirement of ambient oxygen for carp is, based on this idea, estimated to be 70–80 mmHg in partial pressure or 45–55% in percent saturation, from the minimum level of ambient oxygen required for maintenance of normal levels of partial pressure, percent saturation and estimated concentration of oxygen in the arterial blood.

These values coincide with those estimated in the previous paper by one of the present authors and those estimated from rearing experiments of CHIBA.

The minimum level of dissolved oxygen in water required for healthy life of fish is a fundamental criterion essential for evaluation of the carrying capacity of a fish pond, estimation of the necessary water conveyance to a fish reserve, and calculation of the necessary oxygen supply during transportation of live fish. The level is an important factor to rule life of fish in the natural environment, too, because distribution and migration of fish are often controlled by the level of dissolved oxygen in the environmental water, as shown in the sardine¹⁾ and the anchovy²⁾ for example.

It is not easy to establish the criterion by rearing experiment, because it is not easy to maintain the oxygen saturation of the ambient water at various constant levels which should be much lower than air saturation. And it is defective in theoretical basis to estimate the criterion from culturists' experiences. One of the present authors proposed a method to estimate the criterion from the oxygen requirement for maintenance of the arterial oxygen content at the normal level, based on an idea that ambient level of oxygen have effects on fish life through the arterial level of it (ITAZAWA³⁻⁵⁾). At that time, however, a rough technique to puncture directly at the caudal peduncle was almost only available one to collect the blood from a fish artery. After that time, an elaborate technique for repeated sampling of blood through a cannula planted into the dorsal aorta of salmonid fish was

devised by SMITH and BELL.⁶⁾ In the present study, the minimum level of dissolved oxygen in ambient water for healthy life of the carp is estimated, based on the same idea as that in the previous one, using the cannulation technique for blood sampling.

Material and Methods

The experiments were carried out with the carp *Cyprinus carpio* weighing 555 ± 77 g, which were kept for more than ten days before experimentation in tanks of the experimental temperature, 24 to 26°C, being fed on pellets made from fish meal (38%), wheat flour (29%), soybean cake (15%), vitamins and minerals. Fish were not fed during operation and experimentation.

A fish was cannulated at the dorsal aorta, the buccal and opercular cavities, following the methods devised by SMITH and BELL,⁶⁾ HOLETON and RANDALL⁷⁾ and SAUNDERS,⁸⁾ respectively. The cannulated fish was placed in a respiration chamber of $9 \times 44 \times 12$ (depth) cm irrigated with circulating water of percent oxygen saturation more than 90% at an average velocity of about 0.9 mm/s for a day, and then offered to experimentation in the same chamber.

Oxygen pressure in the circulating water decreased gradually from 140 to 20 mmHg owing to respiration of the fish, in the course of the experi-

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*² Department of Fisheries, Faculty of Agriculture, Kyushu University 46-04, Hakozaki, Higashi-ku, Fukuoka, 812, JAPAN (板沢靖男・竹田達右: 九州大学農学部水産学科).

Fig. 1. Diagram of the experimental arrangements used to measure the respiratory parameters for estimation of the minimum requirement of ambient oxygen.

RC: respiration chamber, P: pump, F: filter made of active carbon, FI: liquid flow meter, R: reservoir, FC: floating cover, I, A, E: cannula to the buccal cavity, the dorsal aorta, and the opercular cavity, respectively.

ment of 5 to 8 hours, by covering the free surface of water in the reservoir (Fig. 1). Pressure of carbon dioxide in the water increased from 0 to 5 mmHg, also owing to respiration of the fish, in the course of the experiment. In the course of each experiment, six or less determinations were carried out on the following respiratory parameters: pressures of oxygen (P_{O_2} , mmHg) and carbon dioxide (P_{CO_2} , mmHg) and pH of the dorsal aortic blood as well as the inspired and expired water. These parameters were determined with a gas analyzing system, IL Model 113-01, keeping the electrodes at the same temperature as that of the fish. Oxygen concentration of the blood (C_{a,O_2} , ml/dl) was determined with a C_{O_2} determining instrument, LEX- O_2 -CON. Hematocrit of the blood (Ht_a , %) was determined by centrifugation of blood sample in a capillary tube at 11,000 rpm for five minutes, and the hemoglobin concentration (Hb_a , g/dl) by cyanmethemoglobin method. Suffix 'a' in the above symbols means the aortic blood. Ventilatory frequency (VF, strokes/min) was determined by visual observation. Rate of oxygen consumption ($\dot{V}_{O_2}$, ml/kg, min), minute volume of gill ventilation ($\dot{V}_G$, ml/kg, min), stroke volume of gill ventilation (V_{sv} , ml/kg) and percent oxygen utilization (U , %) were calculated by the following equations.

$$\dot{V}_{O_2} = \alpha_{w,O_2}(P_{I,O_2} - P_{E,O_2})f/Wt$$

$$\dot{V}_G = \dot{V}_{O_2}/\alpha_{w,O_2}(P_{I,O_2} - P_{E,O_2})$$

$$V_{sv} = \dot{V}_G/VF$$

$$U = 100(P_{I,O_2} - P_{E,O_2})/P_{I,O_2}$$

α_{w,O_2} : capacitance coefficient of oxygen in water (ml/ml, mmHg). suffix w, i, o, i, or E means water, water flowing into or out of the respiration chamber, inspired or expired water, respectively, f: flow rate of water irrigating the respiration chamber (ml/min).

Wt: weight of fish (kg).

The volume of blood sample taken from a single fish was 0.36 ml at each time and 2.16 ml or less in the whole course of an experiment. Effects of the repeated sampling of blood from a single fish on Hb_a and C_{a,O_2} are shown in Fig. 2. Hb_a decreased a little in the beginning of the course of each experiment and then was maintained at a constant level (Fig. 2-top). This decrease of Hb_a is considered not to be an effect of blood loss, but to be an effect of blood sampling. C_{a,O_2} also decreased a little following the decrease of Hb_a (Fig. 2-middle). But estimation indicated that the value had been maintained at a roughly constant level, when the effect of Hb_a change was eliminated (Fig. 2-bottom).

Fig. 2. Change of Hb_a , an apparent value of C_{a,O_2} , and an estimated value of C_{a,O_2} eliminating the effect of Hb_a change.

Results of two experiments with two different fish are shown in open and solid circles.

Results

Results are shown in Figs. 3 to 14, putting results of 10 experiments carried out with 10 different fish together in each figure of each respiratory parameter.

VF increased, slowly from the very early stage

Fig. 3. Change of ventilatory frequency (VF) in the course of hypoxia experiments. The normoxic level of VF is shown in a horizontal line. The same rule applies to the following figures.

Fig. 4. Change of minute volume of gill ventilation ($\dot{V}_g$) in the course of hypoxia experiments.

Fig. 5. Change of stroke volume of gill ventilation (V_{sv}) in the course of hypoxia experiments.

Fig. 6. Change of percent oxygen utilization (U) in the course of hypoxia experiments.

Fig. 7. Change of hematocrit of the dorsal aortic blood (Ht_a) in the course of hypoxia experiments.

Fig. 8. Change of hemoglobin concentration of the dorsal aortic blood (Hb_a) in the course of hypoxia experiments.

Fig. 9. Change of partial pressure of oxygen in the dorsal aortic blood (P_{a,O_2}) in the course of hypoxia experiments.

of hypoxia (P_{I,O_2} of about 100 mmHg), and then rapidly at P_{I,O_2} under 50 mmHg (Fig. 3). $\dot{V}_G$ also increased in the similar pattern to that of VF (Fig. 4), but $V_{s,r}$ did not show a clear tendency to increase until P_{I,O_2} dropped under 20 mmHg (Fig. 5). U started to decrease when $\dot{V}_G$ started to increase (Fig. 6).

Fig. 10. Change of partial pressure of carbon dioxide in the dorsal aortic blood (P_{a,CO_2}) in the course of hypoxia experiments.

Fig. 11. Change of pH of the dorsal aortic blood (pH_a) in the course of hypoxia experiments.

Fig. 12. Change of apparent value of oxygen concentration of the dorsal aortic blood (C_{a,O_2}) in the course of hypoxia experiments.

Fig. 13. Change of percent oxygen saturation of the dorsal aortic blood (S_{a,O_2}) in the course of hypoxia experiments.

Fig. 14. Change of resting rate of oxygen consumption ($\dot{V}_{O_2}$) in the course of hypoxia experiments.

Ht_a decreased a little in the middle of the course of each experiment and then increased in severely hypoxic conditions (Fig. 7). Hb_a followed the tendency of Ht_a change (Fig. 8). P_{a,O_2} showed a wide fluctuation, but a tendency to decrease at P_{I,O_2} under 70–80 mmHg was observed, roughly speaking (Fig. 9). P_{a,CO_2} showed a tendency to increase at P_{I,O_2} under 50–60 mmHg (Fig. 10). The pH_a was maintained at an almost fixed level, about 7.9, until it sharply dropped at P_{I,O_2} under 30–40 mmHg (Fig. 11).

C_{a,O_2} decreased gradually from the early stage of hypoxia without showing a critical point (Fig. 12). S_{a,O_2} was maintained at the normal level until P_{I,O_2} decreased to 70–80 mmHg (Fig. 13).

$\dot{V}_{O_2}$ increased in hypoxic conditions until it began to decrease at P_{I,O_2} of about 50 mmHg, and then dropped to the values below the normal level at P_{I,O_2} under 20 mmHg (Fig. 14).

Discussion

The carp is considered to adjust the physiological

conditions to decreased levels of the ambient oxygen. Increases of VF and $\dot{V}_g$ are the adjustments appearing from the early stage of the environmental hypoxia. The increase of $\dot{V}_g$ is achieved mainly by increase of VF, and the contribution of $V_{a,v}$ is small. Decrease of U is unavoidable when $\dot{V}_g$ increases.

Increases of Ht_a and Hb_a are also considered to be physiological adjustments to the decreased oxygen level. Slight decreases of Ht_a and Hb_a in the midst of the course of the experiments are considered not to be effects of decreased P_{i,o_2} , but to be effects of blood sampling as the decrease of Hb_a in Fig. 2. The decrease of C_{a,o_2} in the midst of the course of the experiments is considered to be a result of the Hb_a decrease owing to blood sampling, because factors determining C_{a,o_2} , i. e. P_{a,o_2} , P_{a,co_2} , pH_a and Hb_a , did not change until P_{i,o_2} decreased under 70–80 mmHg, except Hb_a . The value of C_{a,o_2} estimated by the elimination of the effect of Hb_a change was maintained at a roughly constant level until P_{i,o_2} decreased to 70–80 mmHg (Fig. 15). P_{a,o_2} and S_{a,o_2} were also maintained at P_{i,o_2} above 70–80 mmHg. The maintenance of P_{a,o_2} and S_{a,o_2} as well as the estimated C_{a,o_2} is considered to be a homeostasis attained through the increase of $\dot{V}_g$.

Fig. 15. Change of estimated value of oxygen concentration of the dorsal aortic blood (C_{a,o_2}) calculated eliminating effects of Hb_a change in the course of hypoxia experiments.

$\dot{V}_{o_2}$ did not decrease but rather increased in hypoxic conditions, until it dropped in severe hypoxia. The increase of $\dot{V}_{o_2}$ is considered to be caused by the increased activity of gill ventilation. The pH_a was maintained at about 7.9, in spite of increased P_{a,co_2} , until it dropped drastically in severe hypoxia. Homeostasis by strong buffering systems of the blood is observed in the maintenance of pH_a .

The minimum level of dissolved oxygen in the ambient water required for healthy life of the carp is estimated to be 70–80 mmHg in partial pressure or 45–55 % in percent saturation, based on critical P_{i,o_2} required for maintenance of normal levels of P_{a,o_2} , S_{a,o_2} and estimated C_{a,o_2} . These values, 70–80 mmHg and 45–55 %, are just the same as those estimated in the previous paper⁶⁾ and those proposed through rearing experiments by CHIBA⁹⁾ with juvenile carp of 0.54–3.40 g. These values are considered to be reliable as a rough criterion of ambient oxygen level for carp life, although it may be modified according to conditions of fish and water such as age and nutrition of fish, temperature and P_{co_2} of water.

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