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

An Experiment with Carp Avoiding Carbon Dioxide Accumulation*¹

Tatsusuke TAKEDA*² and Yasuo ITAZAWA*²

(Received October 19, 1978)

Low P_{CO_2} in the ambient water owing to diffusion of expired carbon dioxide into nitrogen bubbles, in the present experiments, reduced P_{CO_2} in the arterial blood, increased oxygen affinity of the blood, and caused lowering from 70–80 mmHg to 30 mmHg of the minimum level of ambient oxygen required for maintenance of oxygen concentration in the arterial blood. The higher value obtained by the previous experiments, where accumulation of carbon dioxide in both water and blood was not avoided, is considered to be reliable as the criterion of ambient oxygen, because decrease of ambient oxygen is usually followed by increase of ambient carbon dioxide at an equal ratio. The present value is considered to be useful for discussion in basic physiology of carp respiration, because the effects of hypoxia on fish are clearly shown by avoiding the effects of carbon dioxide accumulation.

The minimum level of dissolved oxygen in water required for healthy life of the carp was estimated from oxygen levels in the ambient water required for maintenance of oxygen levels in the arterial blood, in the previous paper.¹⁾ Accumulation of carbon dioxide in both water and blood was unavoidable in that study, because ambient oxygen was reduced by respiration of fish. Changes of many respiratory parameters were examined, in the present study, avoiding accumulation of carbon dioxide in both water and blood.

The general aspects of gas exchange in the carp gills have been reported,²⁾ and the present paper is presented from the viewpoint of the minimum requirement of ambient oxygen.

Material and Methods

The experiments were carried out with the same fish (the carp *Cyprinus carpio*) of the same size (561 ± 38 g) at the same temperature ($24.5 \pm 0.5^\circ\text{C}$) as those in the previous study. Experimental arrangements were also similar to those described in the previous paper, except a device to avoid accumulation of carbon dioxide in both water and blood by bubbling nitrogen for reducing dissolved oxygen in the circulating water (Fig. 1). Another

difference between the present study and the previous one was examination of the mixed venous blood as well as the dorsal aortic blood. The mixed venous blood was collected from the bulbus arteriosus through a cannula following the method of HOLETON and RANDALL.³⁾

Fig. 1. Diagram of the experimental arrangements used to measure respiratory parameters of carp avoiding accumulation of carbon dioxide.

RC: respiration chamber, P: pump, F: filter, R: reservoir, Fg: gas flow meter, Fl: liquid flow meter, E₁ and E₂: gas equilibration column, I, A, V, E: cannula to the buccal cavity, the dorsal aorta, the bulbus arteriosus, and the opercular cavity, respectively.

*¹ This work was supported by a grant to Y. I. from the Ministry of Education (No. 956096). This article was read at the Ann. Meetg. of Japan. Soc. Sci. Fish., Tokyo, Apr. 1975.

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

The following respiratory parameters were added to those examined in the previous study: concentration ($C_{\bar{v},O_2}$, ml/dl) and partial pressure ($P_{\bar{v},O_2}$, mmHg) of oxygen in the mixed venous blood, minute volume of cardiac output ($\dot{Q}_G$, ml/kg, min) calculated by an equation $\dot{Q}_G = 100 \dot{V}_{O_2} / (C_{a,O_2} - C_{\bar{v},O_2})$ based on FICK principle, and partial pressure of carbon dioxide in the mixed venous blood ($P_{\bar{v},CO_2}$, mmHg). Suffix a and $\bar{v}$ mean the arterial and mixed venous blood, respectively. Other symbols are the same as those in the previous paper.

The effectiveness of oxygen removal from the water (E_{w,O_2}), the effectiveness of oxygen uptake by the blood (E_{b,O_2}), the oxygen transfer factor (T_{O_2}), and the effectiveness of carbon dioxide uptake by the water (E_{w,CO_2}) were calculated following the equations by RANDALL *et al.*⁴¹

$$E_{w,O_2}(\%) = 100 \dot{V}_{O_2} / \dot{V}_G \cdot \alpha_{w,O_2} (P_{I,O_2} - P_{\bar{v},O_2})$$

$$E_{b,O_2}(\%) = 100 \dot{V}_{O_2} / \dot{Q}_G \cdot \alpha_{b,O_2} (P_{I,O_2} - P_{\bar{v},O_2})$$

$$T_{O_2} = \dot{V}_{O_2} / [0.5(P_{I,O_2} + P_{E,O_2}) - 0.5(P_{a,O_2} + P_{\bar{v},O_2})]$$

$$E_{w,CO_2}(\%) = 100 \dot{V}_{CO_2} / \dot{V}_G \cdot \alpha_{w,CO_2} (P_{\bar{v},CO_2} - P_{I,CO_2})$$

Results

Three determinations were carried out in the course of each experiment, the first one in well-aerated water, the second one at three hours after the first one, *i. e.* one hour after setting the ambient oxygen at a hypoxic level, and the third one at five

Fig. 2. Change of ventilatory frequency (VF) in the course of hypoxia experiments. Open circles: values in well-aerated water, solid circles: values at one hour after setting the ambient oxygen at a hypoxic level, crosses: values at six hours after setting the ambient oxygen at the hypoxic level, and a horizontal line: the normoxic level. The same rules apply to the following figures.

hours after the second one, *i. e.* six hours after setting the ambient oxygen at the hypoxic level. The results are shown in Figs. 2-17, representing the values by three different symbols.

Discussion

Basic aspects of physiological adjustments to hypoxic conditions in the present study were similar to those in the previous one¹¹. Gill venti-

Fig. 3. Change of minute volume of gill ventilation ($\dot{V}_G$) in the course of hypoxia experiments.

Fig. 4. Change of stroke volume of gill ventilation (V_{sv}) in the course of hypoxia experiments.

Fig. 5. Change of minute volume of cardiac output ($\dot{Q}_G$) in the course of hypoxia experiments.

Fig. 6. Change of oxygen pressures of the arterial blood (P_{a,O_2}) and the mixed venous one ($P_{\bar{v},O_2}$) in the course of hypoxia experiments.

Fig. 7. Change of carbon dioxide pressure of the arterial blood (P_{a,CO_2}) and the mixed venous one ($P_{\bar{v},CO_2}$) in the course of hypoxia experiments.

Fig. 8. Change of hematocrit value (H_{ta}) and hemoglobin concentration (H_{ba}) of the arterial blood in the course of hypoxia experiments.

lation increased according to decrease of the ambient oxygen (Fig. 3), and this increase was achieved not by increase of the amplitude of ventilatory movements (Fig. 4), but by increase of the frequency of them (Fig. 2). Increased ventilation caused decrease of oxygen utilization at the gills (Fig. 13). Partial pressures (Fig. 6), concentrations (Fig. 9) and percent saturation of oxygen

Fig. 9. Change of oxygen concentration of the arterial blood (C_{a,O_2}) and the mixed venous one ($C_{\bar{v},O_2}$) in the course of hypoxia experiments.

Fig. 10. Change of percent oxygen saturation of the arterial blood (S_{a,O_2}) in the course of hypoxia experiments.

Fig. 11. Change of percent oxygen saturation of the mixed venous blood ($S_{\bar{v},O_2}$) in the course of hypoxia experiments.

Fig. 12. Change of resting rate of oxygen consumption ($\dot{V}O_2$) in the course of hypoxia experiments.

Fig. 13. Change of percent oxygen utilization at the gills (U) in the course of hypoxia experiments.

Fig. 14. Change of the effectiveness of oxygen removal from water (E_{w,O_2}) in the course of hypoxia experiments.

Fig. 15. Change of effectiveness of oxygen uptake by the blood (E_{b,O_2}) in the course of hypoxia experiments.

Fig. 16. Change of oxygen transfer factor (T_{O_2}) in the course of hypoxia experiments.

Fig. 17. Change of the effectiveness of carbon dioxide uptake by water (E_{w,CO_2}) in the course of hypoxia experiments.

(Figs. 10 and 11) in both the arterial and mixed venous blood decreased in hypoxic conditions. Hematocrit value and hemoglobin concentration of the arterial blood increased in severely hypoxic fish (Fig. 8). The minimum level of P_{i,O_2} required for maintenance of P_{a,O_2} was 60–70 mmHg in the present study, and not much different from that in the previous one, 70–80 mmHg. The minimum requirement of P_{i,O_2} for maintenance of P_{v,O_2} , 50–60 mmHg, was a little lower than that for maintenance of P_{a,O_2} (Fig. 6). The minimum level of P_{i,O_2} required for maintenance of E_{w,O_2} was about 40 mmHg (Fig. 14).

The main difference between the results of the present experiments and those of the previous ones was lowering of the minimum levels of P_{i,O_2} required for maintenance of C_{a,O_2} . The level obtained by the present experiments, about 30 mmHg (Fig. 9), was much lower than the level obtained by the previous ones, 70–80 mmHg. The minimum requirement of P_{i,O_2} for maintenance of C_{v,O_2} was the same as that for maintenance of C_{a,O_2} .

The main difference of conditions between the two series of experiments was P_{CO_2} in the ambient water. P_{i,CO_2} was controlled under the detectable level, in the present experiments, through diffusion

of expired carbon dioxide into nitrogen bubbles. The very low P_{i,CO_2} is considered to have enabled carbon dioxide in the mixed venous blood to diffuse into the water through the gills. Increased ventilation in hypoxic fish must have accelerated the elimination of carbon dioxide from the blood. P_{a,CO_2} and P_{v,CO_2} decreased with decrease of P_{i,O_2} , and got to about 1 mmHg, in the present experiments (Figs. 7), while P_{a,CO_2} increased with decrease of P_{i,O_2} , and reached 4–6 mmHg, in the previous ones. This difference should bear a very important meaning for the oxygen affinity of the blood, because the oxygen dissociation curve of carp blood is known to shift remarkably between two P_{CO_2} conditions, 0–1 mmHg and 4–6 mmHg^{*1, *2}

The low critical value of P_{i,O_2} required for maintenance of C_{a,O_2} obtained in the present study is considered to be achieved by the larger oxygen affinity of the blood owing to the lowered P_{a,CO_2} . From the viewpoint of basic physiology of carp respiration, the present value of the critical P_{i,O_2} , 30 mmHg, should be more important than the value obtained in the previous study, because the effects of hypoxia on fish are more clearly shown in the present experiments avoiding the effects of

carbon dioxide accumulation. However, the previous value, 70–80 mmHg in partial pressure or 45–55% in percent saturation, is considered to be more reliable in estimation of the minimum level of dissolved oxygen in water required for healthy life of the carp, because decrease of the ambient oxygen both in fish farm and natural environments are usually followed by increase of carbon dioxide in the water at the equal molar ratio.

The shift of the critical level of ambient oxygen by presence of carbon dioxide may imply that removal of carbon dioxide as well as supply of oxygen should be considered in fish culture and transportation of live fish.

References

- 1) Y. ITAZAWA and T. TAKEDA: *Bull. Japan. Soc. Sci. Fish.*, **35**, 323–327 (1979).
- 2) Y. ITAZAWA and T. TAKEDA: *Respir. Physiol.*, **35**, 263–269 (1978).
- 3) G. F. HOLETON and D. J. RANDALL: *J. Exp. Biol.*, **46**, 297–305 (1967).
- 4) D. J. RANDALL, G. F. HOLETON, and E. DON STEVENS: *J. Exp. Biol.*, **46**, 339–348 (1967).

^{*1} Y. ITAZAWA, I. HITOTSUMATSU, and T. TAKEDA: Ann. Meetg. Kyushu Branch of Japan. Soc. Sci. Fish., Nagasaki, Feb. 1974.

^{*2} T. TAKEDA and Y. ITAZAWA: Ann. Meetg. of Japan. Soc. Sci. Fish., Tokyo, Apr. 1975.