Effect of hypoxia on the oxygen and acid–base status of hemolymph in Akoya pearl oyster, *Pinctada fucata martensii*

Takeshi Handa [1 †] and Akira Araki [1]

Abstract : We investigated the hemolymph oxygen and acid–base status of Akoya pearl oysters, *Pinctada fucata martensii*, exposed to hypoxic seawater to elucidate the acid–base balance. Akoya pearl oysters cannulated to the anterior aorta for hemolymph collection from the submerged animals showed oxygen and acid–base disturbance of the hemolymph during environmental hypoxia for 24 h (O_2 partial pressure in seawater, Pwo_2 8 torr). The hemolymph O_2 partial pressure (Po_2) decreased from 72.2 torr to 13.6 torr, pH decreased from 7.581 to 7.129, and CO_2 partial pressure (Pco_2) increased from 0.86 torr to 3.31 torr during hypoxia. The hemolymph total CO_2 concentration (Tco_2) and bicarbonate ion concentration ($[HCO_3^-]$) were 1.93–1.95 mM/L and 1.80–1.91 mM/L, respectively, and there was no statistically significant change between pre-hypoxia and hypoxia for 24 h. When normoxic seawater was resumed after the hypoxia, the hemolymph Po_2, pH, and Pco_2 returned to their initial levels for about 3 h, and hemolymph Tco_2 and $[HCO_3^-]$ gradually increased. These results showed that Akoya pearl oysters undergo hypoxemia and respiratory acidosis in the hypoxic environments for 24 h (Pwo_2 8 torr). In post-hypoxia, most of the disturbances disappeared within 3–24 h, and the increase in hemolymph $[HCO_3^-]$ which was a secondary change compensated for respiratory disturbance.

Key words : *Pinctada fucata martensii*, hemolymph acid–base balance, Pco_2, CO_2 dynamic phase, environmental hypoxia, respiratory physiology

Introduction

The Akoya pearl oyster, *Pinctada fucata martensii*, is a filibranchial bivalve classified in the Pteriidae[1]. Akoya pearl oysters are used to produce Akoya pearls, and the process of pearl production is directly related to metabolism. The metabolism of the Akoya pearl oyster has been studied in terms of the regulation of oxygen uptake, gill ventilation volume, and ciliary movement under normoxic and hypoxic conditions[2-6]. The pearl oyster, *P. fucata martensii*, was found to maintain the level of oxygen uptake (water temperature, 20.5°C), even if the oxygen partial pressure of seawater (Pwo_2) was reduced to about 30 torr[3]. However, below 22 torr, oxygen uptake decreased[3]. A further reduction in Pwo_2 below 10.3 torr induced frequent closing of shell valves and mantle lobe, decreasing gill ventilation. Ciliary movement enabled maintenance of the initial level without stopping under environmental hypoxia[3]. The hemolymph acid–base balance of Akoya pearl oysters has been studied under the resting condition[7], prolonged air exposure[8], short-term air exposure[9], and post-cannulation to the adductor muscle[10]. Akoya pearl oysters in normoxic seawater at 20–28°C, have a hemolymph pH of 7.330–7.586; total CO_2 concentration (Tco_2) of 1.90–2.25 mM/L; CO_2 partial pressure (Pco_2) of 0.92–2.2 torr; and bicarbonate ion concentration ($[HCO_3^-]$) of 1.72–2.21 mM/L. In the air exposure, the animals showed hypoxemia and respiratory acidosis in short-term exposure (4 h)[9], and accompanied metabolic acidosis in prolonged exposure (24 h)[8]. There are, however, few reports on the effect of hypoxia on Akoya pearl oysters from the viewpoint of the CO_2 dynamic phase and acid–base balance of the hemolymph. In pearl production, Akoya pearl oysters are cultured in inner bays, and are often exposed to hypoxic seawater (oxygen-deficient water mass). We examined the effect of

Received date: 5 June 2023. Accepted date: 22 August 2023
1 Department of Applied Aquabiology, National Fisheries University, 2-7-1 Nagata-honmachi, Shimonoseki, Yamaguchi, Japan
† Corresponding author: handat@fish-u.ac.jp (T. HANDA)

hypoxia on hemolymph oxygen and acid–base status, evaluating acid–base balance and CO_2 dynamics. These results would contribute to the fundamental understanding of Akoya pearl oysters and assist environmental assessments of sea areas in which they are cultured.

The direct measurement of Pco_2 is difficult when there is only a small quantity of hemolymph sampled, because the Pco_2 of the bivalves is very low under normal conditions. Estimation of the CO_2 partial pressure by application of the Henderson–Hasselbalch equation is practiced in studies on the acid–base balance owing to its relative ease and accuracy[11]. In the Henderson–Hasselbalch equation, the CO_2 solubility coefficient (αco_2) and apparent dissociation constant (pKapp) of carbonic acid in the hemolymph are required. The hemolymph αco_2 and pKapp in Akoya pearl oysters at 20°C were previously reported[8], and we used the results to calculate the hemolymph CO_2 partial pressure and bicarbonate concentration in this study.

Materials and Methods

Experimental animals

Akoya pearl oysters (n=60; shell length, 59.8 ± 3.2 mm (mean ± SD); shell height, 64.9 ± 3.1 mm; total wet weight, 35.9 ± 6.1 g) were obtained from a marine farm in Tsushima, Nagasaki Prefecture, Japan. After cleaning the shell valves, they were reared for one month at 20°C in aerated seawater containing added cultivated phytoplanktons[12]. The Akoya pearl oysters were transferred to a respiratory chamber with a flow of particle-free (>0.45 μm) seawater before the experiment. All experiments were conducted in seawater with a salinity of 31, water temperature (WT) of 20°C, O_2 saturation (Swo_2) of 97%, pH of 8.08, and total CO_2 content of 1.9 mM/L.

Animal Surgery

We operated on the shell valve and anterior aorta of the Akoya pearl oysters for hemolymph collection from the submerged animals[7,13]. Part of the left shell valve near the dorsal margin was resected in a rectangle (4 x 10 mm) with a router and a scalpel. Through the rectangular window, the cannula (polyethylene tubing, 0.97 mm outer diameter, Clay Adams PE50, Becton Dickinson Co., USA) was inserted into the anterior aorta with a stylet and advanced toward to the heart direction for 5 mm. The stylet was then removed, and the outside of the cannula was sealed. The cannula was gently fixed to the left shell valve, and the window was securely closed with dental adhesive (Kobayashi Pharmaceutical Co., Japan) and surgical superglue (Aron alpha A, Sankyo Co., Japan). The cannulated animals were returned to the respiratory chamber and allowed to rest overnight under a normoxic condition.

Experimental protocol and Hemolymph collection

The cannulated animals were induced to hypoxia. Pwo_2 was adjusted at 8 torr, because the pearl oyster decreased gill ventilation volume under Pwo_2 10.3 torr[3]. The hypoxic seawater, which was gassed continuously with N_2 in a counter-current gas exchange column, was flowed into the respiratory chamber for 24 h. After hypoxia for 24 h, the supply of hypoxic seawater was stopped, and the inflow of normoxic seawater was resumed into the respiratory chamber. The animals experienced normoxia for 24 h after the hypoxia. As a control group, the cannulated animals did not induce to the hypoxia and were examined at the same time with the hypoxic group.

Hemolymph collection was performed on different animals each time. In the hypoxic group, an initial hemolymph sample was collected through the cannula before the induction of hypoxia (pre-hypoxia, PreHy). When the experimental animals experienced the hypoxia for 24 h, the hemolymph samples were similarly collected (Hy24). After the hypoxia was finished, the hemolymph samples were collected at 3 h, 12 h, or 24 h (post-hypoxia, PostHy3, 12, 24). In the control group, hemolymph samples were collected from the cannulated animals at the same time as those in the hypoxic experiment. All hemolymph samples were anaerobically collected through the cannula which was connected to a gas-tight

microsyringe (Model 1750LTN, Hamilton Co., USA). The volume of each hemolymph sample was approximately 0.3 mL.

Hemolymph analysis and calculation

The hemolymph oxygen partial pressure (Po_2, torr), pH, and total CO_2 concentration (Tco_2, mM/L) were measured immediately after each collection. Po_2 was measured using a blood gas meter (BGM200, Cameron Instruments Co., USA) and Po_2 electrode (E101, Cameron Instruments Co., USA). The pH was measured using a blood gas meter with pH glass and reference electrodes (E301, E351, Cameron Instruments Co., USA). The Po_2 and pH electrodes were installed in a water jacket maintained at 20°C. Tco_2 was measured using a total CO_2 analyzer (Capnicon 5, Cameron Instruments Co., USA). The hemolymph CO_2 partial pressure (Pco_2, torr) and bicarbonate concentration ($[HCO_3^-]$, mM/L) were calculated by rearranging the Henderson–Hasselbalch equation[11,14]. In the equation, the CO_2 solubility coefficient (αco_2, μM/L/torr) and apparent dissociation constant of carbonic acid (pKapp) of the Akoya pearl oyster are required. Handa and Araki (2021) described the hemolymph αco_2 (40 μM/L/torr), and pKapp, Pco_2, and $[HCO_3^-]$ were calculated using the following equations[8]:

$$pKapp = 183.939 - 77.811 \bullet pH + 11.340 \bullet pH^2 - 0.5508 \bullet pH^3$$

$$Pco_2 = Tco_2 \bullet [0.040 \bullet (1+10^{(pH-pKapp)})]^{-1}$$

$$[HCO_3^-] = Tco_2 - 0.040 \bullet Pco_2$$

where the units of the parameters are torr for Pco_2, and mM/L for Tco_2 and $[HCO_3^-]$.

For assessment of the relationship between hemolymph pH and $[HCO_3^-]$ of the experimental animals, the non-bicarbonate buffer value (β_{NB}, the slope of relational expression) used 0.46 Slykes[8]. The hemolymph calcium concentrations ($[Ca^{2+}]$, mM/L) were determined with a test kit (Calcium E-test, Wako Pure Chemical Co., Japan) and a spectrophotometer (Spectronic 20A, Shimadzu Co., Japan).

Statistical analysis

The data are expressed as means ± standard deviation. Kruskal–Wallis test was performed for changes in the hemolymph properties over the experimental time course. The multiple comparison of all pairs used the Steel–Dwass test. Differences between the control group and hypoxic group were compared using the Mann–Whitney U test. Statistically significant differences were set at $P < 0.05$. All analyses were performed with the statistical software Kyplot v. 5.0 and 6.0 (KyensLab Inc., Japan).

Results

Akoya pearl oyster in the control group did not show significant changes in hemolymph properties. ($P > 0.05$, Figs. 1–6). In the hypoxic group, the experimental animals were exposed to the hypoxic seawater for 24 h (Pwo_2, 8 torr), and hemolymph properties were statistically significantly changed. The mean values of hemolymph Po_2 decreased from 72.2 torr to 13.6 torr during hypoxia ($P < 0.05$, Fig. 1). The hemolymph pH decreased from 7.581 to 7.129 during hypoxia ($P < 0.05$, Fig. 2). The hemolymph Tco_2 was 1.93–1.95 mM/L between PreHy and Hy24, and there was no significant change ($P > 0.05$, Fig. 3). The calculated hemolymph Pco_2 and $[HCO_3^-]$ at PreHy were 0.86 torr and 1.91 mM/L, respectively. The hemolymph Pco_2 increased during hypoxia, reaching 3.31 torr at Hy24 ($P < 0.05$, Fig. 4). The hemolymph $[HCO_3^-]$ was 1.80–1.91 mM/L between PreHy and Hy24, and there was no significant change ($P > 0.05$, Fig. 5). After the period of hypoxic condition, the inflow of normoxic seawater into the respiratory chamber was resumed, and hemolymph Po_2 and pH increased to their initial levels for 3–24 h ($P < 0.05$, Figs. 1–2). The hemolymph Pco_2 decreased to the initial level and was maintained for 3–24 h ($P < 0.05$, Fig. 4). The hemolymph Tco_2 and $[HCO_3^-]$ gradually increased after hypoxia, reaching 2.39 mM/L and 2.34 mM/L at PostHy24, respectively ($P < 0.05$, Figs. 3, 5). Hemolymph Tco_2 and $[HCO_3^-]$ of the hypoxic group between PostHy3 and PostHy24 were higher than that of the control group (P

< 0.05, Figs. 3, 5). Hemolymph [Ca^{2+}] did not show significant changes during each phase ($P > 0.05$, Fig. 6). The progress of change in the acid–base balance of the experimental animals is summarized in a pH-[HCO_3^-] diagram (Fig. 7). The hemolymph pH of the hypoxic animals (Hy24) decreased with increasing Pco_2, but hemolymph [HCO_3^-] did not change significantly, and the point at Hy24 followed along the non-bicarbonate buffer line. After hypoxia, the hemolymph [HCO_3^-] at PostHy24 was higher than the initial value, and this point was located above the non-bicarbonate buffer line (Fig. 7).

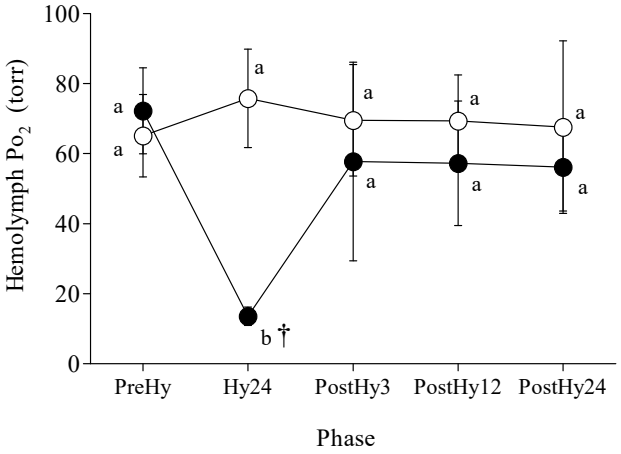

Fig. 1 Effect of hypoxia (Pwo_2 8 torr) on the hemolymph O_2 partial pressure (Po_2, torr) in the Akoya pearl oyster, *Pinctada fucata martensii*. Hemolymph of the anterior aorta was collected *via* cannula from each experimental animal. Solid circle: hypoxic group; open circle: control group (no induced hypoxia). PreHy: pre-hypoxia (Pwo_2 155 torr); Hy24: exposure to hypoxic seawater for 24 h (Pwo_2 8 torr); PostHy3–24: post-hypoxia for 3 h, 12 h, or 24 h (resumption of exposure to normoxic seawater, Pwo_2 155 torr). The values are means ± SD ($n = 6$ in each plot). Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test). The dagger indicates a significant difference from the control group and hypoxic group ($P < 0.05$, Mann–Whitney U test).

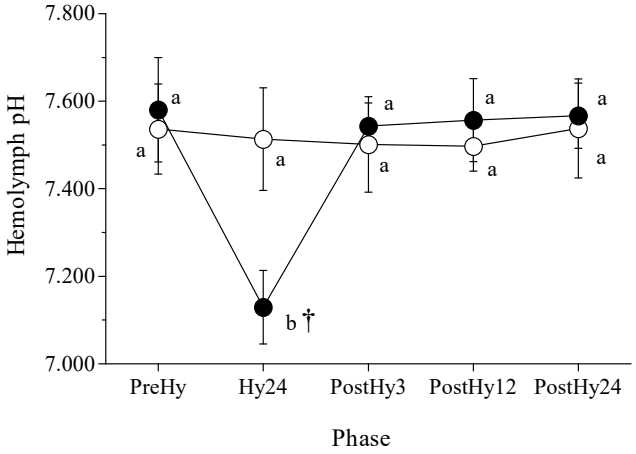

Fig. 2 Effect of hypoxia (Pwo_2 8 torr) on the hemolymph pH in the Akoya pearl oyster, *Pinctada fucata martensii*. Hemolymph from the anterior aorta was collected *via* cannula from each experimental animal. Solid circle: hypoxic group; open circle: control group (no induced hypoxia). PreHy: pre-hypoxia (Pwo_2 155 torr); Hy24: exposed to hypoxic seawater for 24 h (Pwo_2 8 torr); PostHy3–24: post-hypoxia for 3 h, 12 h, or 24 h (resumption of exposure to normoxic seawater, Pwo_2 155 torr). The values are means ± SD ($n = 6$ in each plot). Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test). The dagger indicates a significant difference from the control group and hypoxic group ($P < 0.05$, Mann–Whitney U test).

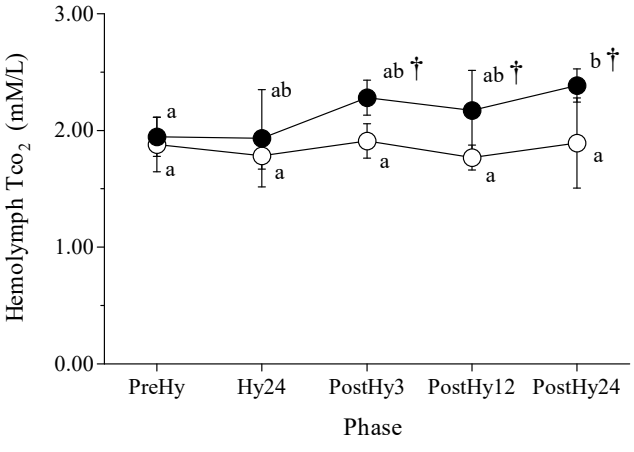

Fig. 3 Effect of hypoxia (Pwo_2 8 torr) on the hemolymph total CO_2 concentration (Tco_2, mM/L) in the Akoya pearl oyster, *Pinctada fucata martensii*. Hemolymph from the anterior aorta was collected *via* cannula from each experimental animal. Solid circle: hypoxic group; open circle: control group (no induced hypoxia). PreHy: pre-hypoxia (Pwo_2 155 torr); Hy24: exposed to hypoxic seawater for 24 h (Pwo_2 8 torr); PostHy3–24: post-hypoxia for 3 h, 12 h, or 24 h (resumption of exposure to normoxic seawater, Pwo_2 155 torr). The values are means ± SD ($n = 6$ in each plot). Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test). The daggers indicate a significant difference from the control group and hypoxic group ($P < 0.05$, Mann–Whitney U test).

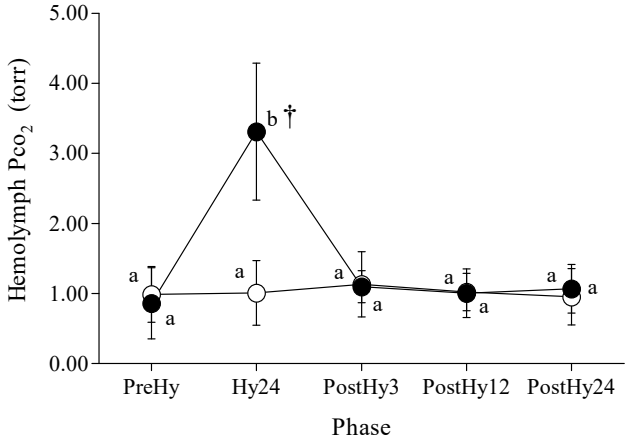

Fig. 4 Effect of hypoxia (Pwo_2 8 torr) on the hemolymph CO_2 partial pressure (Pco_2, torr) in the Akoya pearl oyster, *Pinctada fucata martensii*. Hemolymph from the anterior aorta was collected *via* cannula from each experimental animal. Solid circle: hypoxic group; open circle: control group (no induced hypoxia). PreHy: pre-hypoxia (Pwo_2 155 torr); Hy24: exposure to hypoxic seawater for 24 h (Pwo_2 8 torr); PostHy3–24: post-hypoxia for 3 h, 12 h, or 24 h (resumption of exposure to normoxic seawater, Pwo_2 155 torr). The values are means ± SD (n = 6 in each plot). Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test). The dagger indicates a significant difference from the control group and hypoxic group ($P < 0.05$, Mann–Whitney U test).

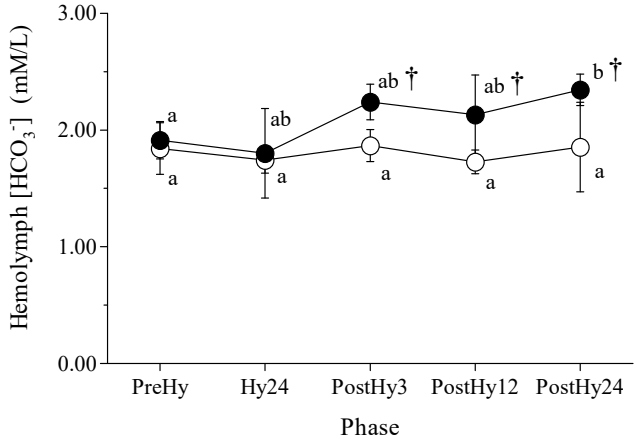

Fig. 5 Effect of hypoxia (Pwo_2 8 torr) on the hemolymph bicarbonate concentration ($[HCO_3^-]$, mM/L) in the Akoya pearl oyster, *Pinctada fucata martensii*. Hemolymph from the anterior aorta was collected *via* cannula from each experimental animal. Solid circle: hypoxic group; open circle: control group (no induced hypoxia). PreHy: pre-hypoxia (Pwo_2 155 torr); Hy24: exposing to the hypoxic seawater for 24 h (Pwo_2 8 torr); PostHy3–24: post-hypoxia for 3 h, 12 h, or 24 h (resumption of exposure to normoxic seawater, Pwo_2 155 torr). The values are means ± SD (n = 6 in each plot). Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test). The daggers indicate a significant difference from the control group and hypoxic group ($P < 0.05$, Mann–Whitney U test).

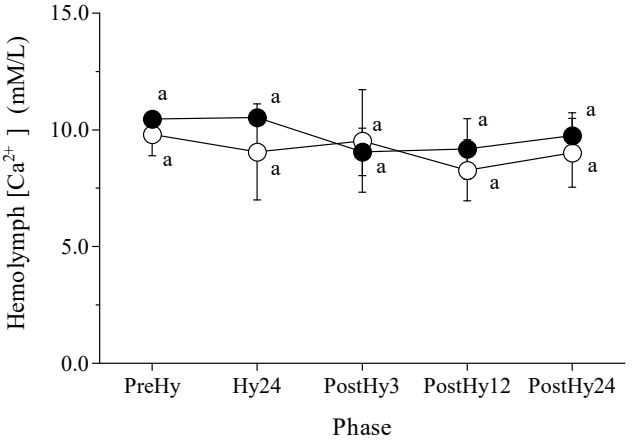

Fig. 6 Effect of hypoxia (Pwo_2 8 torr) on the hemolymph calcium ion concentration ($[Ca^{2+}]$, mM/L) in the Akoya pearl oyster, *Pinctada fucata martensii*. Hemolymph from the anterior aorta was collected *via* cannula from each experimental animal. Solid circle: hypoxic group; open circle: control group (no induced hypoxia). PreHy: pre-hypoxia (Pwo_2 155 torr); Hy24: exposure to hypoxic seawater for 24 h (Pwo_2 8 torr); PostHy3–24: post-hypoxia for 3 h, 12 h, or 24 h (resumption of exposure to normoxic seawater, Pwo_2 155 torr). The values are means ± SD (n = 4-6 in each plot). Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test). There were no significant differences between the control and hypoxic groups.

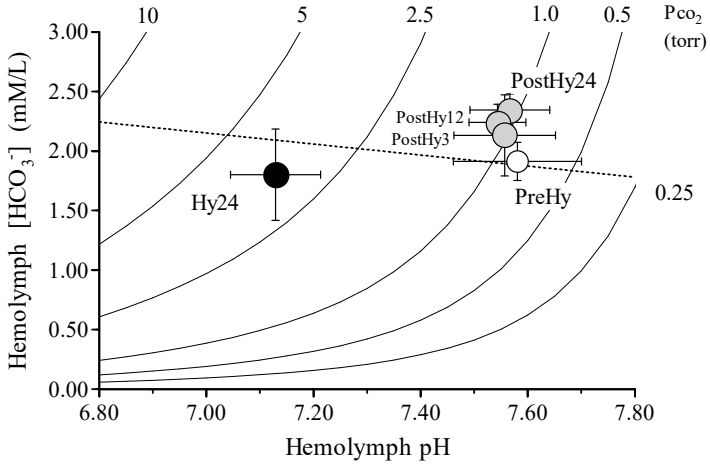

Fig. 7 Hemolymph pH-$[HCO_3^-]$ diagram of Akoya pearl oysters, *Pinctada fucata martensii*. Hemolymph from the anterior aorta was collected *via* cannula from each experimental animal. PreHy: pre-hypoxia (Pwo_2 155 torr, white circle); Hy24: hypoxia for 24 h (Pwo_2 8 torr, black circles); PostHy3–24: post-hypoxia for 3, 12, or 24 h (Pwo_2 155 torr, grey circle). The values are means ± SD (n = 6 in each). The Pco_2 isopleths are derived from rearranging the Henderson–Hasselbalch equation. The dashed line is the nonbicarbonate buffer line: $[HCO_3^-] = 5.77 - 0.463 \cdot pH$. The non-bicarbonate buffer value (β_{NB}, 0.46 slykes), which is the slope of the relational expression, was described in a previous study [8].

Discussion

We examined the hemolymph oxygen and acid–base status of Akoya pearl oysters under hypoxic environment to elucidate the acid–base balance and CO_2 dynamics. The experimental animals in this study were cannulated to the anterior aorta for hemolymph collection from submerged animals. Hemolymph properties of the cannulated control group animals did not significantly change. In the hypoxic group, the cannulated animals were exposed to hypoxic seawater, and the hemolymph oxygen and acid–base status showed significant changes. These changes were caused by the hypoxic exposure, and not due to surgery.

Akoya pearl oysters decreased Po_2 from 72.2 torr to 13.6 torr during hypoxia for 24 h, and showed a hypoxemia in this study. In some marine bivalves, the hemolymph showed reductions in the oxygen partial pressure during environmental hypoxia or air exposure. The hemolymph Po_2 decreased from 108 torr to 8 torr during environmental hypoxia for 8 h in the blue mussel, *Mytilus edulis*[15]. The hemolymph Po_2 decreased during air exposure for 2 h from 118 torr to 57 torr in the king scallop, *Pecten maximus*[16]; and from 88 torr to 29 torr for 1 h in the pearl oyster, *P. fucata martensii*[9]. In this study, hypoxemia would be caused in the early time of the environmental hypoxia, and Akoya pearl oysters experienced the hypoxemia for about 24 h. The respiratory responses were reported in the pearl oyster, *P. fucata martensii*, under hypoxic conditions[3]. When Pwo_2 was less than 10.3 torr at 20.5°C, the movements of the mantle lobe and shell valves increased, and the gill ventilation volume decreased[3]. The oxygen uptake decreased to below 0.75-fold of the initial level under Pwo_2 10.3 torr[3]. Although the Akoya pearl oysters showed hypoxemia in this study, the animals could show slight gill ventilation and oxygen uptake even if the environmental oxygen tension decreased[3].

The hemolymph pH decreased from 7.581 to 7.129, and Pco_2 increased from 0.86 torr to 3.31 torr during hypoxia for 24 h. The hypoxic Akoya pearl oysters showed acidosis with an elevation of Pco_2 in the hemolymph. CO_2 was accumulated gradually in the hemolymph during hypoxia for 24 h. The accumulated CO_2 hydrates to carbonic acid, and carbonic acid dissociates to bicarbonate and hydrogen ions. The concentration of hydrogen ions gradually increased in the hemolymph, and the hemolymph pH continued to decrease during hypoxia for 24 h. In the case of air exposer, Akoya pearl oysters showed mixed acidosis (respiratory and metabolic acidosis) with the increase in the hemolymph [HCO_3^-] and [Ca^{2+}] under prolonged exposure[8]. Because acidic end-products, which were increased by anaerobic metabolism, dissolved the shell valves ($CaCO_3$ crystal), the bicarbonate and calcium ions were mobilized to the hemolymph[8,17]. In this study, the hemolymph [HCO_3^-] and [Ca^{2+}] of the Akoya pearl oysters did not change significantly during hypoxia for 24 h. Therefore, the shell valves of the experimental animals were not dissolved by the acidic end-products, and anaerobic metabolism hardly progressed. The hemolymph acidosis in this study should be derived from the excessive CO_2 accumulation.

When the inflow of normoxic seawater was resumed to the respiratory chamber, Akoya pearl oysters showed increases in the hemolymph Po_2 and pH, and a decrease in Pco_2 within 3–24 h. The experimental animals would accelerate O_2 uptake and discharge excessive accumulated CO_2 from the gills (the respiratory compensation action) under a normoxic condition after hypoxia. The animals could exchange hemolymph gases by diffusion from the surface of the soft body. Changes in the hemolymph Po_2, pH, and Pco_2 at PostHy3–24 were not significant between the hypoxic and control group. Therefore, the effect of hypoxia on the hemolymph Po_2, pH, and Pco_2 almost disappeared for 3–24 h under a normoxic condition. On the other hand, the hemolymph Tco_2 and [HCO_3^-] of the hypoxic group increased at PostHy24 from the PreHy values, and were higher than the control group. The hemolymph Tco_2 increased with the elevation of [HCO_3^-] at PostHy24. The increase in [HCO_3^-] was a secondary change and a metabolic compensation response. The experimental animals might reabsorb bicarbonate at the renal region or absorb it from the seawater, though there was no result of ion

transport evaluation in this study.

According to the pH-[HCO_3^-] diagram of the hemolymph (Fig. 7), the Akoya pearl oysters had a reduced pH with the elevation of Pco_2 during hypoxia for 24 h. Wood et al. (1977) expounded the pH-[HCO_3^-] diagram from the blood[18]. If a decrease in pH is due solely to a change in Pco_2, the blood would be simply titrated along the non-bicarbonate buffer line, and the point of the pH value moves on this line[18]. The decrease in pH is determined by simple respiratory acidosis. In metabolic acidosis, a decrease in pH is due solely to an increase in non-volatile acid, and the blood will be titrated along a constant Pco_2 isopleth and decreased [HCO_3^-][18]. The decrease in pH is determined by simple metabolic acidosis. In this study, the Akoya pearl oyster showed a reduction in pH and elevation in Pco_2, and the point at Hy24 was slightly below the non-bicarbonate buffer line, but it moved along the line (Fig. 7). The Akoya pearl oysters did not increase hemolymph [HCO_3^-] and [Ca^{2+}], and did not produce acidic end-products (anaerobic metabolites) under hypoxemia in this study. During the 24 h exposure to hypoxic conditions in this study, Akoya pearl oyster experienced the respiratory acidosis. The points at PostHy3–24 approached PreHy, and the respiratory acidosis was alleviated considerably. The increase in [HCO_3^-] at PostHy3–24 was a secondary change due to respiratory disturbance, and worked on the respiratory acidosis as a metabolic compensation. The experimental animals recovered the hemolymph pH sufficiently to the pre-hypoxic level. Hemolymph [HCO_3^-] did not recover to normal levels, despite the recovery of Po_2, pH and Pco_2 after 24 h. The hemolymph [HCO_3^-] recovery needed over 24 h. The completion of the metabolic compensation action would require longer period than the respiratory compensation action.

In this study, the Akoya pearl oysters showed oxygen and acid–base disturbance (hypoxemia and respiratory acidosis) due to environmental hypoxia for 24 h (Pwo_2, 8 torr; WT, 20°C). When the Akoya pearl oysters were returned to normoxic seawater, hypoxemia and respiratory acidosis disappeared within 24 h. This acidosis was compensated by discharging the excessive CO_2 and by the increment of [HCO_3^-] which was a secondary change caused by respiratory disturbance. Therefore, the respiratory disturbance was classified as compensated respiratory acidosis. Yamamoto et al. (1999) reported the oxygen uptake of the pearl oyster at different water temperatures under normoxic and hypoxic conditions. The pearl oyster showed an oxygen uptake per wet weight of 0.43 mL/min/kg at 20.5°C and 0.46 mL/min/kg at 27.2°C, and there was no significant difference[3]. The pearl oyster maintained the oxygen uptake till 22 torr at 20.5°C, but decreased it with the lowering of Pwo_2 at 27.2°C[3]. Thus, the effect of hypoxia on the hemolymph acid–base balance of Akoya pearl oysters would be greater at higher water temperatures. Akoya pearl oysters for pearl production are frequently reared in the culture ground of inner bays, and often experience hypoxia (oxygen-deficient water mass). The phenomenon of oxygen-deficient water mass includes hypoxia and anoxia, and the dissolved oxygen levels (DO) were proposed in hypoxia between 0.025 mL/L (0.036 mg/L) and 2.5 mL/L (3.6 mg/L), and in anoxia under 0.025 mL/L (0.036 mg/L)[19-22]. In this study, Akoya pearl oysters underwent severe hypoxia for 24 h (Pwo_2, 8 torr ≈ DO, 0.38 mg/L; WT, 20°C), and showed hypoxemia and respiratory acidosis. Although most hemolymph properties recovered from the effect of the hypoxia relatively early in returned to normoxic seawater, the effect of the hypoxia on the animals was prolonged as hemolymph [HCO_3^-] needed over 24 h. When hypoxic conditions (oxygen-deficient water mass) are repeated or continued over several days, Akoya pearl oysters may fall into an irreparable situation because of the prolonged insufficient compensation.

Acknowledgments

We would like to express our sincere gratitude to Dr. Ken-ichi Yamamoto, Professor Emeritus, for securing the experimental animals for this study.

References

1) Hayami I: Pteriidae. *In*: Okutani T (ed) Marine Mollusks in Japan. The second edition, Tokai University Press, Kanagawa, 1179–1181 (2017)

2) Numaguchi K: Effect of water temperature on the filtration rate of Japanese pearl oyster *Pinctada fucata martensii*. *Aquaculture Science*, **42**, 1–6 (1994)

3) Yamamoto K, Adachi S, Koube H: Effects of hypoxia on respiration in the pearl oyster *Pinctada fucata martensii*. *Aquaculture Science*, **47**, 539–544 (1999)

4) Yamamoto K: Effects of water temperature on respiration in the pearl oyster *Pinctada fucata martensii*. *Aquaculture Science*, **48**, 47–52 (2000)

5) Yamamoto K, Handa T, Yamashita I: Particle transport on the gills of the pearl oyster *Pinctada fucata martensii*. *Aquaculture Science*, **50**, 309–314 (2002)

6) Yamamoto K, Handa T, Matsubara T: Change on ventilation volume in the pearl oyster *Pinctada fucata martensii* associated with feeding. *Aquaculture Science*, **58**, 447–451 (2010)

7) Handa T, Yamamoto K: The acid–base balance of the hemolymph in the pearl oyster *Pinctada fucata martensii* under normoxic conditions. *Aquaculture Science*, **60**, 113–117 (2012)

8) Handa T, Araki A: Effect of air exposure on the acid–base balance of hemolymph in akoya pearl oyster *Pinctada fucata martensii*. *Journal of Shellfish Research*, **40**, 499–504 (2021)

9) Handa T, Araki A: Effect of short-term air exposure on the oxygen and acid–base balance of hemolymph in the akoya pearl oyster *Pinctada fucata martensii*. *Journal of National Fisheries University*, **71**, 35-42 (2022)

10) Handa T, Araki A, Yamamoto Ken-ichi: Hemolymph acid–base balance of akoya pearl oyster *Pinctada fucata martensii* with cannulated adductor muscle in normoxic conditions. *Journal of National Fisheries University*, **69**, 9-15 (2020)

11) Boutilier RG, Iwama GK, Heming TA, Randall DJ: The apparent pK of carbonic acid in rainbow trout blood plasma between 5 and 15°C. *Respiratory Physiology*, **61**, 237–254 (1985)

12) Yamamoto K, Handa T, Nakamura M, Kitukawa K, Kita Y, Takimoto S, Nishikawa S: Effects of ozone-produced oxidants on respiration of the pearl oyster, *Pinctada fucata martensii*. *Aquaculture Science*, **47**, 241–248 (1999)

13) Handa T, Yamamoto K: The blood acid–base balance in the pearl oyster, *Pinctada fucata martensii*, after the surgery. *Journal of National Fisheries University*, **60**, 57-61 (2011)

14) Davenport HW: Fundamental equation. *In*: The ABC of acid–base chemistry 6th edition. University of Chicago Press, Chicago, 39–41 (1974)

15) Booth CE, McDonald DG, Walsh PJ: Acid–base balance in the sea mussel, *Mytilus edulis*. I. Effects of hypoxia and air-exposure on hemolymph acid–base status. *Marine Biology Letters*, **5**, 347–358 (1984)

16) Duncan P, Spicer JI, Taylor AC, Davies PS: Acid–base disturbances accompanying emersion in the scallop *Pecten maximus*. *Journal of Experimental Marine Biological and Ecology*, **182**, 15–25 (1994)

17) Crenshaw MA, Neff JM: Decalcification at the mantle-shell interface in molluscus. *American Zoology*, **9**, 881-885 (1969)

18) Wood CM, McMahon BR, McDonald D: An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus*. *Journal of Experimental Biology*, **69**, 173–185 (1977)

19) Yanagi T: A summary of symposium of oxygen-deficient water mass. *Coastal Oceanography*, **26**, 141-145 (1989)

20) Karim MdR, Sekine M, Ukita M: Simulation of eutrophication and associated occurrence of hypoxic and anoxic condition in a coastal by in Japan. *Marine Pollution Bulletin*, **45**, 280-285 (2002)

21) Yanagi T: Hypoxia – The chemical and biological consequences of the mechanisms of its generation, maintenance, variability and disappearance. *Oceanography in Japan*, **13**, 451-460 (2004)

22) Oh SJ, Moon S, Yoon YH, Kim DH, Kang IkJ, Shimasaki Y, Biomonitoring system to assist in early detection of oxygen-deficient sea water using shell valve movements of Pacific oyster, *Crassostrea gigas*. *Journal of Faculty of Agriculture Kyushu University*, **66**, 217-225 (2021)

アコヤガイのヘモリンパ液の酸塩基平衡に及ぼす低酸素の影響

半田岳志・荒木　晶

和文要旨：アコヤガイ*Pinctada fucata martensii* を酸素飽和度約5％の低酸素海水に24 時間曝露すると，前大動脈から採取したヘモリンパ液の酸素分圧は72.2 torr（平均値）から13.6 torr，pH は7.581 から7.129 にまで低下し，二酸化炭素分圧は0.86 torrから3.31 torr に増加した．低酸素海水に曝露したアコヤガイを酸素飽和度約100％の正常海水に戻すと，ヘモリンパ液の酸素分圧，pH，二酸化炭素分圧は3～24 時間以内に曝露前の値にまで回復した。カルシウムイオン濃度は，統計的に有意な変動を示さなかった．これらの結果から，アコヤガイは酸素飽和度約5％の低酸素海水に24 時間曝露されると，低酸素血症と呼吸性アシドーシスを呈することが明らかとなった。酸素飽和度約100％の正常海水にアコヤガイを戻すと，ヘモリンパ液の酸素分圧，pH，二酸化炭素分圧に及ぼす低酸素の影響は24 時間以内に消失することが示された。