アコヤガイのヘモリンパ液の酸塩基平衡に及ぼす大気への短期曝露の影響

Effects of short-term air exposure on the oxygen and acid–base status of hemolymph in the akoya pearl oyster, *Pinctada fucata martensii*

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Abstract : We investigated the hemolymph oxygen and acid–base status of akoya pearl oysters, *Pinctada fucata martensii*, exposed to air for a short time (4 h) to elucidate the acid–base balance and CO_2 dynamics. The hemolymph O_2 partial pressure (Po_2) in air-exposed akoya pearl oysters decreased from 88.7 torr (mean value) to 29.4 torr at 1 h, and the low Po_2 continued for the next 3 h during air exposure. The hemolymph pH decreased from 7.586 to 7.082 during air exposure for 1 h and reached 6.851 at 4 h. The hemolymph CO_2 partial pressure increased from 0.9 torr to 4.4 torr at 1 h and reached 7.3 torr after 4 h of air exposure. The hemolymph bicarbonate concentration and calcium ion concentration at 0 h (control) were 1.9 mM/L and 9.0 mM/L, respectively, and these properties did not significantly change during air exposure. From these results, it was determined that the akoya pearl oysters had hypoxemia caused by hypoventilation at an early phase of the short-term air exposure. The akoya pearl oysters inhibited the discharge of CO_2 by hypoventilation, and respiratory acidosis was caused due to the excessive accumulation of CO_2. Bicarbonate was not mobilized from the shell valve into the hemolymph during the short-term air exposure.

Key words : hemolymph acid–base balance, oxygen status, respiratory physiology, short-term air exposure, akoya pearl oyster, *Pinctada fucata martensii*

Introduction

The akoya pearl oyster, *Pinctada fucata martensii*, is a filibranchial bivalve classified in the Pteriidae, and is endemic to Japan[1]. Akoya pearl oysters are used for the production of akoya pearls. 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 filtration rate under hypoxic, anathermal, and different feeding conditions[2-6]. The hemolymph acid–base balance of akoya pearl oysters has been studied under resting conditions[7], prolonged air exposure[8], and post-cannulation to the adductor muscle[9]. Akoya pearl oysters in normoxic and normocapnic seawater at 20-28°C, have a hemolymph pH of 7.330-7.568; total CO_2 concentration (Tco_2) of 1.90-2.25 mM/L; CO_2 partial pressure (Pco_2) of 1.0-2.2 torr; and bicarbonate ion concentration ($[HCO_3^-]$) of 1.72-2.21 mM/L. Under prolonged air exposure (24 h), akoya pearl oysters showed hypoxemia and metabolic acidosis with partial compensation[8]. Similar results have been found in other bivalves (blue mussel, *Mytilus edulis*; noble scallop, *Mimachlamys nobilis*; Pacific oyster, *Crassostrea gigas*; and Asian clam, *Corbicula fluminea*)[10-13]. There are, however, few reports on the effect of short-term air exposure on the respiratory physiology from the viewpoint of the CO_2 dynamic phase and acid–base balance in akoya pearl oysters. During pearl production, akoya pearl oysters are often exposed to the air for the preparation of nucleation and surgical operation[14]. Therefore, research into the effect of short-term air exposure may contribute to the elucidation of the acid–base balance in the handling of the animals.

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

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its relative ease and accuracy[15]. In the equation, the CO_2 solubility coefficient (αco_2) and apparent dissociation constant (pKapp) of carbonic acid in the hemolymph are required for the experimental animals. The hemolymph αco_2 and pKapp in akoya pearl oysters 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 and conditions

Akoya pearl oysters (n = 66; mean total wet weight, 54 g) were obtained from a marine farm in Tsushima, Nagasaki Prefecture, Japan. After cleaning the shell valves, they were reared for 2 months at 20°C in aerated seawater with added cultivated phytoplankton[16]. Twenty-four hours before collecting the hemolymph, the akoya pearl oysters were transferred to a respiratory chamber with a flow of particle-free (>0.45 μm) seawater. All experiments were conducted in seawater with a salinity of 32, water temperature of 20°C, O_2 saturation of 99%, pH of 8.18, and total CO_2 content of 1.9 mM/L.

Experimental procedure

Different animals were used for each duration of air exposure. The experimental animals in the respiratory chamber were exposed to air by stopping the flow into the chamber and siphoning out the water. When the air exposure started (0 h), hemolymph was collected from the adductor muscle as a control (AE0h, n = 11). Other experimental animals were exposed to air for 1 h, 2.5 h, or 4 h. The temperature and humidity of the air were maintained by passing the air through the experimental seawater, and the adjusted air flowed into the respiratory chamber (20°C). After exposure to air for 1-4 h, hemolymph was collected from the adductor muscle (AE1h, AE2.5h, AE4h, n = 11 in each). The inflow of experimental seawater was resumed into the respiratory chamber after exposing the experimental animals to air for 4 h, and the animals were immersed in seawater. Hemolymph of the immersed animals was collected at 1 h or 4 h after immersion in seawater (Im1h, Im4h, n = 11 in each). The hemolymph was collected anaerobically by direct puncture with a gas-tight microsyringe (Model 1750LTN, Hamilton Co., USA) from the adductor muscle of each animal. The volume of each hemolymph sample was 0.3–0.4 mL.

Hemolymph analysis and calculation

The hemolymph oxygen partial pressure (Po_2, torr), pH, and total CO_2 content (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[15,17]. 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 were 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]:

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

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

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

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, which was described in a previous study[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. Statistically significant differences were set at $P < 0.05$. All analyses were carried out with the statistical software Kyplot v. 5.0 and 6.0 (KyensLab Inc., Japan).

Results

Akoya pearl oysters exposed to the air for a short time (4 h) showed significant changes in the hemolymph oxygen and acid–base properties. The mean hemolymph Po_2 showed a significant decrease from 88.7 torr at 0 h to 29.4 torr at 1 h, and the low Po_2 continued during the short-term air exposure ($P < 0.05$, Fig. 1). The hemolymph pH decreased from 7.586 to 7.082 at 1 h, reaching 6.851 at 4 h ($P < 0.05$, Fig. 2). The hemolymph Tco_2 was between 1.92 mM/L and 2.34 mM/L for 4 h, but there was no significant change ($P > 0.05$, Fig. 3). The calculated hemolymph Pco_2 and $[HCO_3^-]$ at 0 h were 0.92 torr and 1.88 mM/L, respectively (Figs. 4-5). The hemolymph Pco_2 increased during short-term air exposure, reaching 7.3 torr at 4 h ($P < 0.05$, Fig. 4). The hemolymph $[HCO_3^-]$ slightly increased to 2.05 mM/L at 4 h, but did not significantly change ($P > 0.05$, Fig. 5). The hemolymph $[Ca^{2+}]$ at 0 h (control) was 9.0 mM/L, and there was no significant change ($P > 0.05$, Fig. 6). When the experimental animals were immersed in seawater after air exposure for 4 h, the hemolymph Po_2 and pH increased, and the Pco_2 decreased ($P < 0.05$, Fig. 1-2, 4). There was no significant difference between the control and immersed animals in terms of hemolymph Po_2, pH, and Pco_2. The changes in Tco_2, $[HCO_3^-]$, and $[Ca^{2+}]$ from 0 h to 8 h were not significant ($P > 0.05$, Figs. 3, 5, 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 Pco_2 of the air-exposed animals increased with decreasing pH, but the hemolymph $[HCO_3^-]$ did not change significantly, and the points between 1 h (AE1h) and 4 h (AE4h) followed along the non-bicarbonate buffer line. In the immersed animals, the points during immersion (Im1h, Im4h) approached the control values (AE0h) and were located near the non-bicarbonate buffer line (Fig. 7).

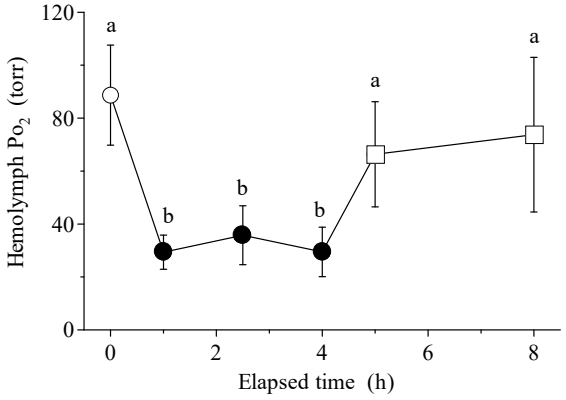

Fig. 1 Effect of air exposure on the hemolymph oxygen partial pressure (Po_2, torr) in the akoya pearl oyster, *Pinctada fucata martensii*. Open circle: air exposure for 0 h (control); solid circle: air exposure for 1-4 h; open square: immersion for 1 and 4 h. The values are means ± SD ($n = 11$ in each plot). Hemolymph from the adductor muscle was collected from each experimental animal. Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

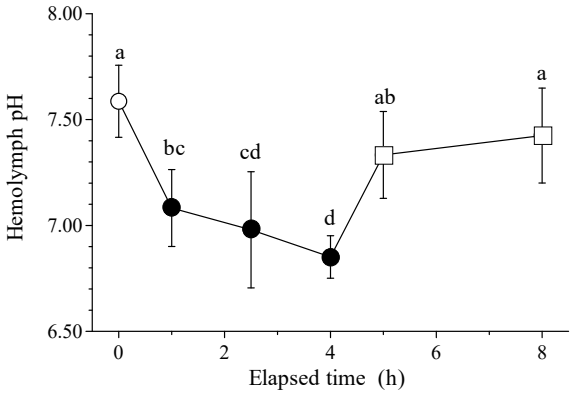

Fig. 2 Effect of air exposure on the hemolymph pH of the akoya pearl oyster, *Pinctada fucata martensii*. Open circle: air exposure for 0 h (control); solid circle: air exposure for 1-4 h; open square: immersion for 1 and 4 h. The values are means ± SD ($n = 11$ in each plot). Hemolymph from the adductor muscle was collected from each experimental animal. Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

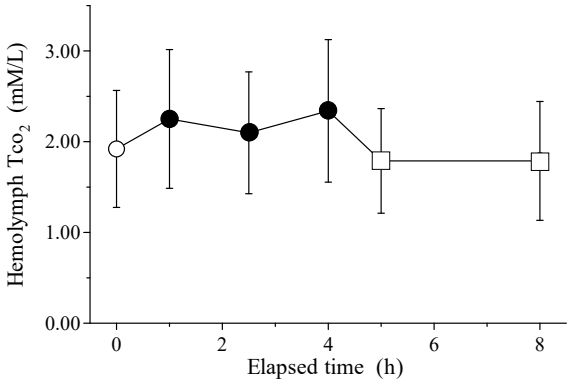

Fig. 3 Effect of air exposure on the hemolymph total CO_2 concentration (Tco_2, mM/L) of the akoya pearl oyster, *Pinctada fucata martensii*. Open circle: air exposure for 0 h (control); solid circle: air exposure for 1-4 h; open square: immersion for 1 and 4 h. The values are means ± SD (n = 11 in each plot). There were no significant differences for each value ($P > 0.05$, Steel–Dwass multiple comparison test).

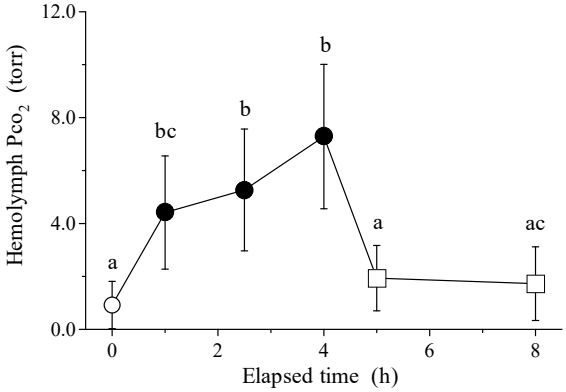

Fig. 4 Effect of air exposure on the hemolymph CO_2 partial pressure (Pco_2, torr) of the akoya pearl oyster, *Pinctada fucata martensii*. Open circle: air exposure for 0 h (control); solid circle: air exposure for 1-4 h; open square: immersion for 1 and 4 h. The values are means ± SD (n = 11 in each plot). Hemolymph from the adductor muscle was collected from each experimental animal. Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

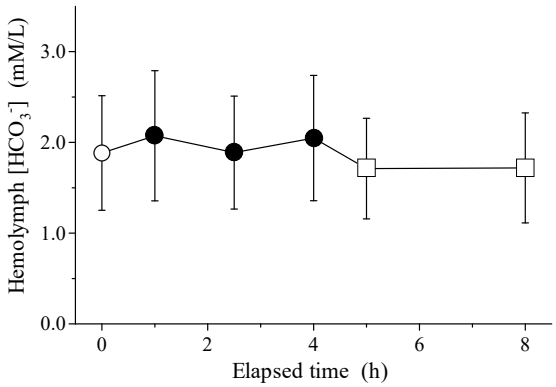

Fig. 5 Effect of air exposure on the hemolymph bicarbonate concentration ($[HCO_3^-]$, mM/L) of the akoya pearl oyster, *Pinctada fucata martensii*. Open circle: air exposure for 0 h (control); solid circle: air exposure for 1-4 h; open square: immersion for 1 and 4 h. The values are means ± SD (n = 11 in each plot). There were no significant differences for each value ($P > 0.05$, Steel–Dwass multiple comparison test).

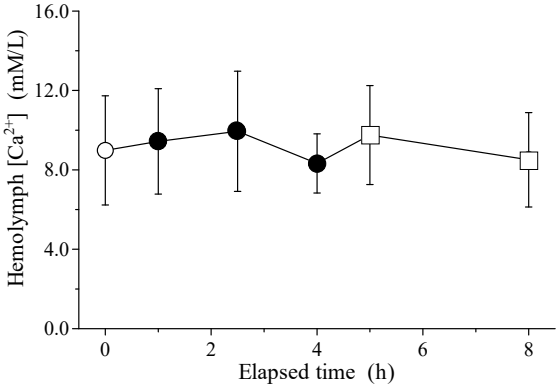

Fig. 6 Effect of air exposure on the hemolymph calcium ion concentration ($[Ca^{2+}]$, mM/L) of the akoya pearl oyster, *Pinctada fucata martensii*. Open circle: air exposure for 0 h (control); solid circle: air exposure for 1-4 h; open square: immersion for 1 and 4 h. The values are means ± SD (n = 11 in each plot). There were no significant differences for each value ($P > 0.05$, Steel–Dwass multiple comparison test).

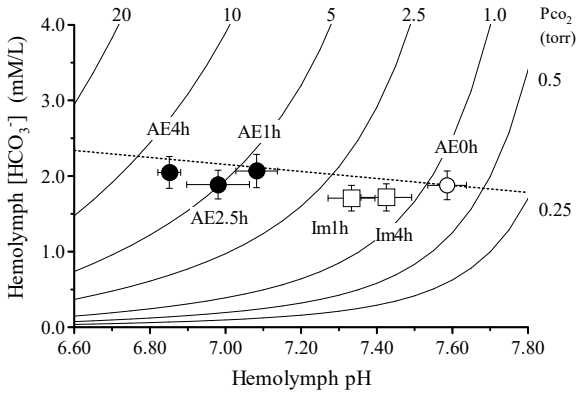

Fig. 7 Hemolymph pH-$[HCO_3^-]$ diagram of the air-exposed akoya pearl oyster, *Pinctada fucata martensii*. AE0h: air exposure for 0 h (control, open circle); AE1h: air exposure for 1 h; AE2.5h: air exposure for 2.5 h; AE4h: air exposure for 4 h (solid circles). Im1h: immersion for 1 h after air exposure; Im4h: immersion for 4 h after air exposure (open squares). The values are means ± SE (n = 11 in each). The Pco_2 isopleths are derived from rearranging the Henderson–Hasselbalch equation. The dashed line is the non-bicarbonate 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 during short-term air exposure. The akoya pearl oysters showed oxygen and acid–base disturbance at an early phase of the air exposure. The hemolymph Po_2 had already decreased from 88.7 torr to 29.4 torr at 1 h of air exposure and continued at a low level for 4 h. The air-exposed akoya pearl oysters were unable to ventilate their gills, and oxygen uptake was interrupted. The oxygen remaining inside the body was consumed, and the hemolymph Po_2 decreased. The akoya pearl oysters underwent hypoxemia during air exposure for 1 h. In marine and freshwater bivalves, the hemolymph and pericardial fluid showed reductions in the oxygen partial pressure during air exposure. The hemolymph Po_2 decreased after 8 h air exposure from 108 torr to 8 torr in the blue mussel, *Mytilus edulis*[10]; from 118.7 torr to 57.5 torr after 2 h air exposure in the king scallop, *Pecten maximus*[18]; and from 69.5 torr to 46.3 torr after 6 h air exposure in the noble scallop, *Mimachlamys nobilis*[11]. In the Asian clam, *Corbicula fluminea*, the pericardial fluid Po_2 decreased after air exposure for 8 h from 60.9 torr to 21.8 torr[13]. In this study, the hemolymph Po_2 of the akoya pearl oysters dropped during the early phase of air exposure, and they continuously experienced hypoxemia.

The air-exposed akoya pearl oysters showed a reduction in the pH and elevation of Pco_2 in the hemolymph. In some marine bivalves, the hemolymph showed a reduction in the pH and Pco_2 increased during air exposure[10,11,18]. The hemolymph pH of the blue mussel, *M. edulis*, decreased from 7.65 to 7.24, and the Pco_2 increased from 0.8 torr to 3.3 torr[10]. In the king scallop, *P. maximus*, the hemolymph pH and Pco_2 changed from 7.36 to 7.11 and from 1.0 torr to 5.8 torr, respectively, during air exposure for 1-8 h[18]. The hemolymph pH and Pco_2 of the noble scallop, *M. nobilis*, which was exposed to air for 6 h, changed from 7.460 to 7.045 and from 1.30 torr to 5.05 torr, respectively[11]. In this study, the hemolymph pH decreased from 7.586 to 7.082, and Pco_2 increased from 0.92 torr to 7.3 torr during air exposure for 1-4 h. The akoya pearl oysters were unable to ventilate their gills during the short-term air exposure and the discharge of CO_2 was inhibited. The air-exposed animals accumulated CO_2 gradually in the hemolymph even during short-term air exposure. The accumulated CO_2 hydrates to form carbonic acid in the fluid, and carbonic acid dissociates to bicarbonate and hydrogen ions. The concentration of the hydrogen ions gradually increased in the hemolymph, and the hemolymph pH continued to decrease during air exposure for 4 h. Under a prolonged air exposure (24 h), the akoya pearl oysters experienced hypoxemia mixed acidosis (respiratory and metabolic acidosis), which increased the hemolymph [HCO_3^-] and [Ca^{2+}] because the increased acidic end-products by anaerobic metabolism dissolved the shell valve ($CaCO_3$)[8]. In this study, during short-term air exposure (4 h), the hemolymph [HCO_3^-] and [Ca^{2+}] of the akoya pearl oysters did not significantly change. Therefore, the shell valves of the experimental animals were not dissolved by the acidic end-products, and anaerobic metabolism hardly progressed. Acidosis, which was caused during the short-term air exposure, would be mainly derived from the accumulation of CO_2.

When the experimental animals were immersed in seawater for 1-4 h, they showed an increase in the hemolymph Po_2 and pH, and a decrease in Pco_2. The immersed animals would resume gill ventilation and accelerate O_2 uptake and discharge CO_2 at the gill. The immersed animals could exchange hemolymph gases by diffusion from the surface of the soft body. In the immersed animals, the hemolymph properties approached the initial level (0 h) after about 1 h, and the effect of short-term air exposure almost disappeared within 4 h.

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 short-term air exposure. Wood et al. (1977) expounded the pH-[HCO_3^-] diagram from the blood[19]. 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[19]. 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^-][19]. The decrease in pH is determined by simple metabolic acidosis. In this study, the akoya pearl oysters showed a reduction in pH and elevation in Pco_2, and the points at 1-4 h (AE1h, AE2.5h, and AE4h) followed along the non-bicarbonate buffer line (Fig. 7). The akoya pearl oysters did not produce acidic metabolites under hypoxemia in this study, and [Ca^{2+}] did not mobilize from the shell dissolution and increase in the hemolymph. Therefore, the akoya pearl oysters during short-term air exposure experienced respiratory acidosis, and not metabolic acidosis. When the experimental animals were immersed in seawater, the akoya pearl oysters discharged the excessive accumulated CO_2, and the hemolymph Pco_2 was reduced. The points at Im1h and Im4h approached AE0h (control), and moved along the non-bicarbonate buffer line, and respiratory acidosis in the akoya pearl oysters decreased considerably.

In this study, the akoya pearl oysters experienced hypoxemia and respiratory acidosis even after short-term air exposure (for 4 h). Akoya pearl oysters are abundantly reared for pearl production, and they experience exposure to the air during preparation and the surgical operation of nucleation. When the air-exposed akoya pearl oysters are returned to seawater, the hypoxemia and respiratory acidosis rapidly improved in about 1 h, and the effect of the short-term air exposure on the oxygen and acid–base status disappeared within 4 h.

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アコヤガイのヘモリンパ液の酸塩基平衡に及ぼす大気への短期曝露の影響

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和文要旨：アコヤガイ *Pinctada fucata martensii* のヘモリンパ液酸素分圧は，大気曝露前に 88.7 torr（平均値）を示したが，曝露 1時間後に 29.4 torrへ減少し，大気曝露 4時間まで低い酸素分圧を示した．ヘモリンパ液の pH は曝露前に 7.586を示したが，曝露 1時間後に 7.082，4時間後に 6.851にまで低下した．二酸化炭素分圧は曝露前に 0.9 torrを示したが，曝露 4時間後に 7.3 torrにまで増加した．炭酸水素イオン濃度は曝露前に 1.9 mM / Lを，カルシウムイオン濃度は曝露前に 9.0 mM / Lを示したが，供試貝を大気に 4 時間曝露してもヘモリンパ液中の炭酸水素イオンとカルシウムイオン濃度は有意に変動しなかった．これらの結果から，大気に曝露される時間が短くても（1〜4時間）、アコヤガイは低酸素血症と酸性血症を示すことが明らかとなった。また，この酸性血症は，二酸化炭素の過剰蓄積による呼吸性アシドーシスと判断され，殻体からの炭酸水素イオンの動員による代償作用は認められなかった．