Effect of air exposure on the oxygen and acid–base status of hemolymph in the densely lamellated oyster, *Ostrea denselamellosa*

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

Abstract : We investigated the oxygen and acid–base status of the densely lamellated oyster, *Ostrea denselamellosa*, during air exposure for 24 h. The hemolymph O_2 partial pressure decreased from 68.0 torr (mean value) to 52.1 torr during air exposure for 18 h, and reached 42.1 torr after 24 h. The hemolymph pH decreased from 7.579 to 6.798 at 18 h and to 6.361 at 24 h. The hemolymph CO_2 partial pressure increased from 1.30 torr to 40.9 torr at 24 h during air exposure. The hemolymph bicarbonate concentration increased from 1.36 mM/L to 2.81 mM/L at 24 h. The hemolymph calcium ion concentration increased from 8.2 mM/L to 10.9 mM/L at 24 h. From these results, it was revealed that the densely lamellated oysters caused a progressive hypoxemia by hypoventilation of the gill during air exposure. The densely lamellated oysters were inhibited from releasing CO_2 from the gill by hypoventilation, and respiratory acidosis was caused due to the accumulated CO_2. The densely lamellated oysters exposed to air for a long time developed metabolic acidosis due to anaerobic metabolism partially compensated with mobilized [HCO_3^-] from the shell valve.

Key words : *Ostrea denselamellosa*, densely lamellated oyster, oxygen partial pressure, acid–base balance, air exposure

Introduction

The densely lamellated oyster, *Ostrea denselamellosa*, is a Ostreidae bivalve classified as Pterioida, Pteriomorphia[1]. The densely lamellated oyster is distributed from the Boso Peninsula southwards to the western Pacific, and it inhabits sand and gravel bottoms in bays at a water depth of 3–10 meters[1]. The densely lamellated oyster is a local specialty food of the littoral zone in the Seto Inland Sea, but it has rarely been seen recently due to a considerably decrease in its catch. The densely lamellated oyster has been the subject of previous studies in terms of the histology of its gonad[2], seedling production[3], reproduction[4], and DNA identification of the family Ostreidae[5]. The oxygen uptake and regulation of the gill ventilation volume have been studied under normoxic and feeding conditions[6]. The anatomical structures of the ctenidia were also clarified recently[7]. The densely lamellated oyster was examined to reveal its hemolymph acid–base balance under a normoxic condition[8]. There are, however, few reports on the effect of air exposure on the respiratory physiology from the viewpoint of the CO_2 dynamic phase and acid–base. In the production of bivalves, the animals are often exposed to air for maintenance in the culture and for transportation to markets as living shellfish. Research into the acid–base balance could contribute to efficient CO_2 utilization, which is related to respiration and calcification for the formation of the shell valves. The acid–base balance and CO_2 dynamic phase of the densely lamellated oyster is useful for evaluation of the cultivation and handling environments. In this study, we examined the hemolymph oxygen and acid–base status of the densely lamellated oyster and evaluated the acid–base balance and CO_2 dynamic during air exposure. The estimation of 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[9]. 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 animal. The hemolymph αco_2 and pKapp in

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densely lamellated oysters have been 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

The experiments used 27 densely lamellated oysters (mean total wet weight: 130 g). The animals were obtained from marine farms in the Seto Inland Sea. After cleaning the shell valves, they were reared for 1 month at 24°C in aerated seawater with added cultivated phytoplankton[10–12]. Twenty-four hours before collecting the hemolymph, the densely lamellated oysters were transferred to a respiratory chamber with a flow of particle-free seawater (>0.45 μm). All the experiments were conducted in seawater with a salinity of 32, water temperature of 24°C, O_2 saturation of 98%, pH of 8.18, and total CO_2 content of 1.6 mM/L.

Air exposure and hemolymph collection

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 = 9). The other experimental animals were exposed to air for 18 h or 24 h. The humidity and temperature of the air were maintained by passing air through the experimental seawater, and adjusted air flowed into the respiratory chamber (24°C). After exposure to air for 18 h or 24 h, hemolymph was collected from the adductor muscle (AE18h, n = 6; AE24h, n = 6). The inflow of experimental seawater was resumed into the respiratory chamber after exposing the experimental animals to air for 24 h, and the animals were immersed in seawater. Hemolymph of the immersed animals was collected at 24 h after immersion in seawater (Im24h, n = 6). 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 the 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 24°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[9,13]. In the equation, the CO_2 solubility coefficient (αco_2, μM/L/torr) and apparent dissociation constant of carbonic acid (pKapp) of the densely lamellated oysters were required. Handa et al. (2018) previously reported that the hemolymph αco_2 and pKapp of the densely lamellated oyster were 38.7 μM/L/torr and 6.10825, respectively[8]. The hemolymph Pco_2 and $[HCO_3^-]$ were calculated using the following equations:

$$Pco_2 = Tco_2 \bullet [0.0387 \bullet (1 + 10^{(pH - 6.10825)})]^{-1}$$

$$[HCO_3^-] = Tco_2 - 0.0387 \bullet Pco_2$$

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 the hemolymph pH and $[HCO_3^-]$ of air-exposed animals, the non-bicarbonate buffer value (β_{NB}, the slope of relational expression) used 1.29 slykes, which was described in the 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. The Kruskal–Wallis test was performed to measure changes in the hemolymph properties over the experimental time course. The multiple comparison of all pairs was performed using 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).

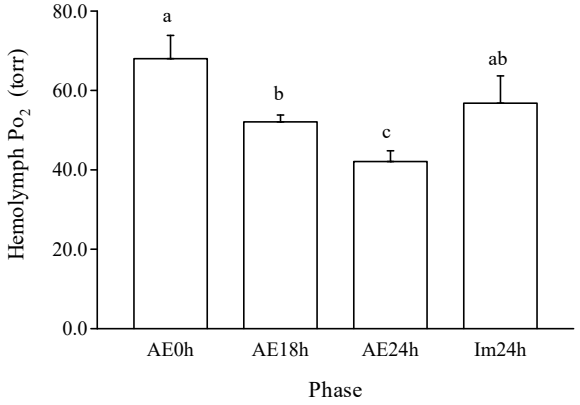

Fig. 1 Effect of air exposure on the hemolymph oxygen partial pressure (Po_2, torr) in the densely lamellated oyster, *Ostrea denselamellosa*. AE0h: air exposure for 0 h (control); AE18h: air exposure for 18 h; AE24h: air exposure for 24 h; Im24h: immersion for 24 h after air exposure, respectively. Hemolymph from the adductor muscle was collected from each experimental animal (n = 9 in AE0h, n = 6 in the other each column). Values are means ± SD. Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

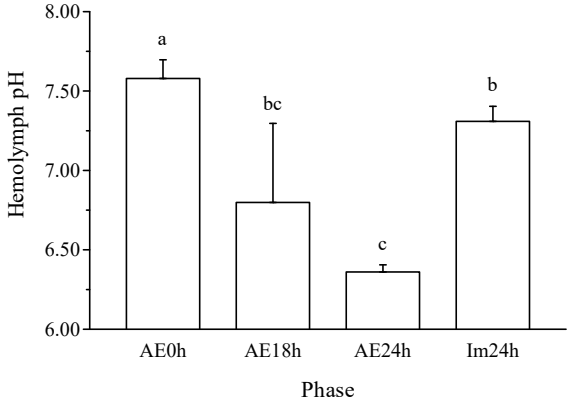

Fig. 2 Effect of air exposure on the hemolymph pH in the densely lamellated oyster, *Ostrea denselamellosa*. AE0h: air exposure for 0 h (control); AE18h: air exposure for 18 h; AE24h: air exposure for 24 h; Im24h: immersion for 24 h after air exposure, respectively. Hemolymph from the adductor muscle was collected from each experimental animal (n = 9 in AE0h, n = 6 in the other each column). Values are means ± SD. Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

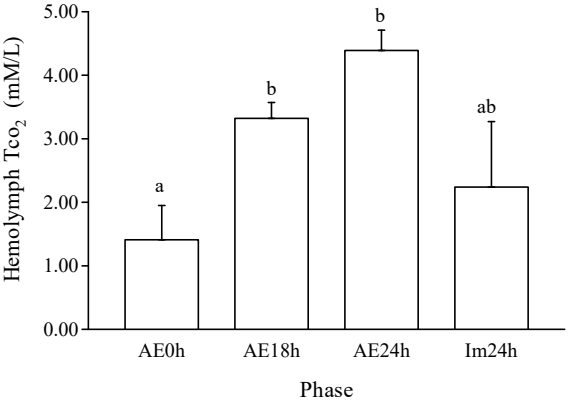

Fig. 3 Effect of air exposure on the hemolymph total CO_2 concentration (Tco_2, mM/L) in the densely lamellated oyster, *Ostrea denselamellosa*. AE0h: air exposure for 0 h (control); AE18h: air exposure for 18 h; AE24h: air exposure for 24 h; Im24h: immersion for 24 h after air exposure, respectively. Hemolymph from the adductor muscle was collected from each experimental animal (n = 9 in AE0h, n = 6 in the other each column). Values are means ± SD. Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

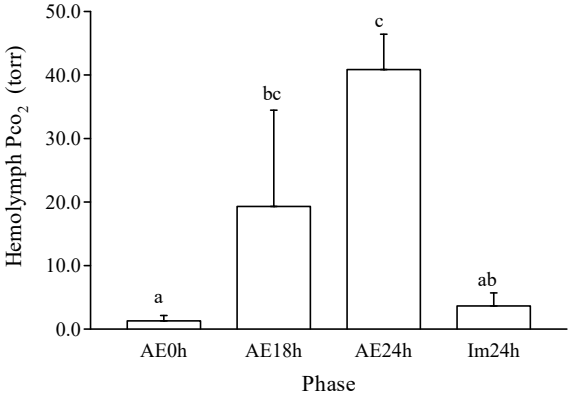

Fig. 4 Effect of air exposure on the hemolymph CO_2 partial pressure (Pco_2, torr) in the densely lamellated oyster, *Ostrea denselamellosa*. AE0h: air exposure for 0 h (control); AE18h: air exposure for 18 h; AE24h: air exposure for 24 h; Im24h: immersion for 24 h after air exposure, respectively. Hemolymph from the adductor muscle was collected from each experimental animal (n = 9 in AE0h, n = 6 in the other each column). Values are means ± SD. Different lowercase letters indicate significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

Results

The densely lamellated oysters exposed to air showed significant changes in the hemolymph oxygen and acid–base properties. The mean values of hemolymph Po_2 significantly decreased from 68.0 torr to 52.1 torr with AE18h and reached 42.1 torr with AE24h (Fig. 1). The hemolymph pH significantly decreased from 7.579 to 6.798 with AE18h, and reached 6.361 with AE24h (Fig. 2). The hemolymph Tco_2 increased from 1.41 mM/L to 3.32 mM/L with AE18h, and reached 4.39 mM/L with AE24h (Fig. 3). The calculated hemolymph Pco_2 and $[HCO_3^-]$ at AE0h were 1.30 torr and 1.36 mM/L, respectively. The hemolymph Pco_2 and $[HCO_3^-]$ significantly increased during air exposure, reaching 40.9 torr and 2.81 mM/L after AE24h, respectively (Figs. 4, 5). The hemolymph $[Ca^{2+}]$ increased gradually during air exposure, and the hemolymph $[Ca^{2+}]$ increased from 8.2 mM/L to 10.9 mM/L with AE24h (Fig. 6). When the experimental animals were immersed in seawater after air exposure, the hemolymph Po_2 and pH increased, and Pco_2 and $[Ca^{2+}]$ decreased at Im24h. The hemolymph Tco_2 and $[HCO_3^-]$ gradually decreased at Im24h. The progress of change in the acid–base balance in the experimental animals is

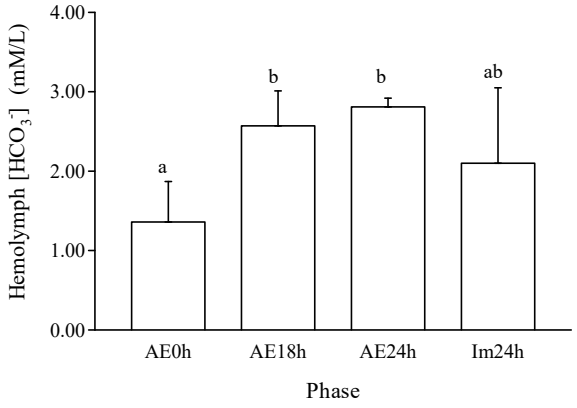

Fig. 5 Effect of air exposure on the hemolymph bicarbonate concentration ($[HCO_3^-]$, mM/L) in the densely lamellated oyster, *Ostrea denselamellosa*. AE0h: air exposure for 0 h (control); AE18h: air exposure for 18 h; AE24h: air exposure for 24 h; Im24h: immersion for 24 h after air exposure, respectively. Hemolymph from the adductor muscle was collected from each experimental animal (n = 9 in AE0h, n = 6 in the other each column). Values are means ± SD. Different lowercase letters indicate significant differences (P < 0.05, Steel–Dwass multiple comparison test).

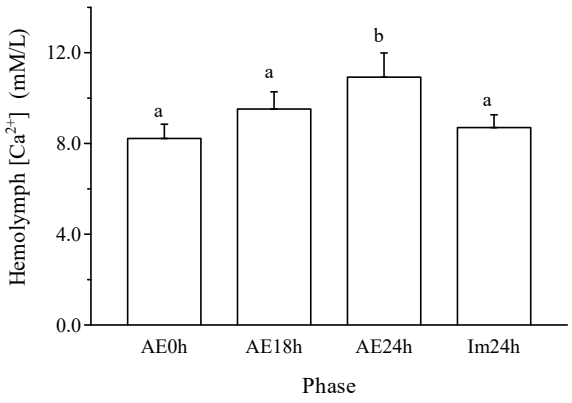

Fig. 6 Effect of air exposure on the hemolymph calcium ion concentration ($[Ca^{2+}]$, mM/L) in the densely lamellated oyster, *Ostrea denselamellosa*. AE0h: air exposure for 0 h (control); AE18h: air exposure for 18 h; AE24h: air exposure for 24 h; Im24h: immersion for 24 h after air exposure, respectively. Hemolymph from the adductor muscle was collected from each experimental animal (n = 9 in AE0h, n = 6 in the other each column). Values are means ± SD. Different lowercase letters indicate significant differences (P < 0.05, Steel–Dwass multiple comparison test).

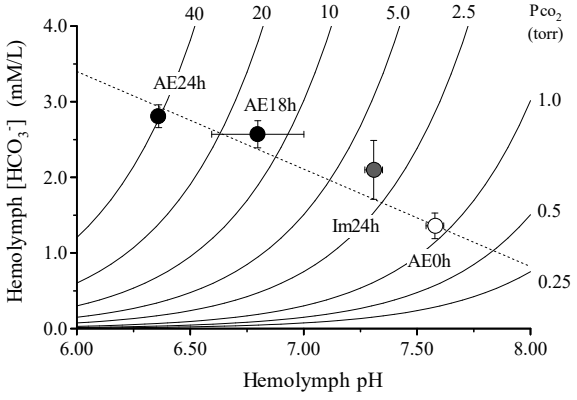

Fig. 7 Hemolymph pH-$[HCO_3^-]$ diagram of the air-exposed densely lamellated oyster, *Ostrea denselamellosa* (black circles, AE18h, AE24h, n = 6 in each), immersion after air exposure (gray circle, Im24h, n = 6), and control (white circle, AE0h, n = 9). Values are means ± SE. The Pco_2 isopleths are derived from rearranging the Henderson–Hasselbalch equation. The dashed line is the non-bicarbonate buffer line: $[HCO_3^-] = 11.137 - 1.29 \bullet pH$. The non-bicarbonate buffer value (β_{NB}, 1.29), which was the slope of the relational expression, was used as the value of the hemolymph, as described in our previous study[8].

summarized in the pH-[HCO_3^-] diagram (Fig. 7). The hemolymph [HCO_3^-] and Pco_2 of the air-exposed animals increased with decreasing pH. The points after AE18h and AE24h followed along the non-bicarbonate buffer line. The point after Im24h approached the value at AE0h, and was located near the non-bicarbonate buffer line.

Discussion

We examined the hemolymph oxygen and acid–base status of air-exposed densely lamellated oysters. The densely lamellated oyster showed oxygen and acid–base disturbance during air exposure. The hemolymph Po_2 decreased during air exposure, and reached 42.1 torr from 68.0 torr with AE24h. The air-exposed densely lamellated oysters were unable to ventilate the gill, and this interrupted the oxygen uptake. The oxygen remaining inside the body was gradually consumed and the hemolymph Po_2 further decreased, and the air-exposed densely lamellated oyster experienced hypoxemia. In some marine and freshwater bivalves, the hemolymph and pericardial fluid showed reductions in the oxygen partial pressure during air exposure. The hemolymph Po_2 decreased during air exposure within 8 h in the blue mussel, *Mytilus edulis*[14]; king scallop, *Pecten maximus*[15]; and noble scallop, *Mimachlamys nobilis*[16]. In the Asian clam, *Corbicula fluminea*[17], the pericardial fluid Po_2 decreased during air exposure for 8 h. In this study, the hemolymph Po_2 in densely lamellated oysters gradually decreased, and these animals experienced progressive hypoxemia. The air-exposed densely lamellated oysters also showed hypoxemia in the early period, as observed in the other bivalves.

The air-exposed densely lamellated oysters showed a reduction in the pH and elevation of Pco_2 in the hemolymph. The hemolymph pH decreased from 7.579 to 6.798, and the hemolymph Pco_2 increased from 1.30 torr to 19.3 torr with AE18h. In some marine and freshwater bivalves, the hemolymph and pericardial fluid showed a reduction in pH and Pco_2 increased during air exposure[14–23]. The densely lamellated oysters were inhibited from releasing CO_2 from the gill by hypoventilation with AE18h, and CO_2 accumulated gradually in the hemolymph. Therefore, the reduction in pH with AE18h should include respiratory acidosis by hypoventilation. In densely lamellated oysters exposed to air over a prolonged period, the hemolymph pH decreased immensely to 6.361 with AE24h. The results of biochemical studies on anaerobic metabolism[24–28] suggested that air exposure in this study was sufficient to force anaerobic metabolism. Although we did not measure the anaerobic end-products, densely lamellated oysters exposed to air for a long time should undergo metabolic acidosis due to anaerobic metabolism with hypoxemia. Densely lamellated oysters gradually increased the hemolymph [HCO_3^-] and [Ca^{2+}] during air exposure for 24 h. The increased [HCO_3^-] and [Ca^{2+}] seemed to be mobilized from $CaCO_3$ of the shell valve. In marine and freshwater bivalves, acidosis during air exposure induces increases in [HCO_3^-] and [Ca^{2+}] in the hemolymph or pericardial fluid[14, 17, 18, 20, 21, 23]. Crenshaw and Neff (1969) reported that research using radiolabeled markers showed that the source of increased calcium is the shell valve[29]. The increase in the acidic end-products of anaerobic metabolism would have dissolved the shell valve of the densely lamellated oysters in this study, and bicarbonate and calcium ions were mobilized into the hemolymph from the shell valve during air exposure. The mobilized bicarbonate seemed to be effective for buffering acidosis in the hemolymph. Therefore, the densely lamellated oysters in this study developed metabolic acidosis with partial compensation.

When the experimental animals were immersed in seawater, they showed an increase in hemolymph Po_2 with Im24h. The immersed animals could resume gill ventilation, and rapidly discharged CO_2 from the gill and by diffusion from the surface of the soft body. Aerobic metabolism resumed in the immersed animals, and the production of anaerobic acidic end-products stopped. The increased [HCO_3^-] during air exposure was consumed to compensate for acidosis, and [HCO_3^-] gradually approached the initial level (AE0h). As a result, the densely lamellated oysters were reducing acidosis during

Im24h. The increased [Ca^{2+}] during air exposure was decreased by re-calcification within 24 h in the immersed animals. Silverman et al. (1983) reported that the freshwater mussel, *Ligumia subrostrata*, releases shell calcium under hypoxic conditions, and it reclaims Ca^{2+} as calcium phosphate concretions in the gill tissue[30]. The densely lamellated oysters seemed to reclaim surplus Ca^{2+} as a calcium phosphate concretion for 24 h, although there were no results of histological analysis in this study.

According to the pH-[HCO_3^-] diagram of the hemolymph (Fig. 7), the densely lamellated oysters reduced pH with the elevation of [HCO_3^-] and Pco_2 during air exposure. Wood et al. (1977) expounded the pH-[HCO_3^-] diagram from blood[31]. If a decrease in pH is due solely to a change in Pco_2, the blood will be simply titrated along the non-bicarbonate buffer line, and the point of the pH value moves on this line[31]. 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^-][31]. The decrease in pH is determined by simple metabolic acidosis. In this study, the densely lamellated oysters showed respiratory and metabolic acidosis (mixed acidosis), and increased Pco_2 and mobilized [HCO_3^-] into the hemolymph from the shell valve, which was dissolved by the acidic end-products (non-volatile acid). As a result, hemolymph [HCO_3^-] did not decrease during air exposure, and the points at AE18h and AE24h followed along the non-bicarbonate buffer line (Fig. 7). Although the acid–base disturbance was shown in the air-exposed densely lamellated oysters, the mobilized [HCO_3^-] contributed as a compensatory response. Acidosis with compensation during air exposure was reported in some marine bivalves such as the akoya pearl oyster[18], Pacific oyster[21], and black-lip pearl oyster[23]. These bivalves increased the hemolymph [HCO_3^-] and [Ca^{2+}] during air exposure for 12–48 h, and the mean values were located above the non-bicarbonate buffer line in the pH-[HCO_3^-] diagram of the hemolymph. The non-bicarbonate buffer line represents the relation between the pH and [HCO_3^-] of the non-bicarbonate buffer system in an *in vitro* experiment. The β_{NB} (non-bicarbonate buffer value), which is the slope of the relational expression, is the buffer capacity of the non-bicarbonate buffer system (mainly protein residues)[32,33]. The non-bicarbonate buffer values were reported as 1.29 slykes in the densely lamellated oyster[8], 0.88 slykes in the Pacific oyster[21], 0.53 slykes in the black-lip pearl oyster[23], and 0.46 slykes in the akoya pearl oyster[18], and the β_{NB} of the densely lamellated oyster was higher than these bivalves. Therefore, in comparison with these bivalve hemolymphs, the densely lamellated oyster had a greater buffer capacity in the non-bicarbonate buffer system of the hemolymph, and the points at AE18h and AE24h did not move above the non-bicarbonate buffer line in the pH-[HCO_3^-] diagram. When the experimental animals were immersed in seawater, the densely lamellated oysters discharged the excessive accumulated CO_2, and consumed bicarbonate for buffering hydrogen ion (H^+), and the hemolymph Pco_2 and [HCO_3^-] reduced. Though the hemolymph pH did not return to the initial level with Im24h, the densely lamellated oysters decreased the mixed acidosis considerably.

In this study, the densely lamellated oysters in the early phase of air exposure showed hypoxemia and respiratory acidosis. In prolonged air exposure, the animals showed severe respiratory and metabolic acidosis (mixed acidosis) partially compensated by the mobilization of bicarbonate from the shell. Densely lamellated oysters are often reared as a local specialty product, and they experience exposure to the air for the maintenance of aquaculture and for transportation to markets as a living shellfish. When the air-exposed densely lamellated oysters are returned to seawater, the hypoxemia and severe acid–base disturbance would improve within 24 h, and the recovery of the acid–base status to the initial level required over 24 h.

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イタボガキのヘモリンパ液の酸塩基平衡に及ぼす大気曝露の影響

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和文要旨：イタボガキ*Ostrea denselamellosa*のヘモリンパ液の酸素分圧は，大気曝露前に68.0 torr（平均値）を示したが，曝露18時間後に52.1 torr，24時間後に42.1 torrにまで減少した．ヘモリンパ液pHは曝露前に7.579を示したが，曝露18時間後に6.798，24時間後に6.361にまで低下した．二酸化炭素分圧は曝露前に1.30 torrを示したが，曝露 24時間後に40.9 torrにまで増加した．炭酸水素イオン濃度は曝露前に1.36 mM ／ Lを示したが，曝露24時間後に2.81 mM ／ Lにまで増加した．カルシウムイオン濃度は曝露前に8.2 mM ／ Lを示したが，曝露24時間後に10.9 mM ／ Lにまで増加した．これらの結果から，イタボガキは大気に曝露されると進行性の低酸素血症を示し，呼吸性と代謝性の混合性酸性血症を引き起こすとともに，殻体からヘモリンパ液に動員した炭酸水素イオンによって酸性血症を部分的に代償することが明らかとなった．