Effect of air exposure on the oxygen and acid–base status of hemolymph in the noble scallop *Mimachlamys nobilis*

Takeshi Handa[1†] and Akira Araki[1]

Abstract : We investigated the oxygen and acid–base status of the noble scallop *Mimachlamys nobilis* during air exposure for 24 h. The hemolymph of noble scallop was collected from the adductor muscle, and O_2 partial pressure (Po_2), pH, CO_2 partial pressure (Pco_2), and bicarbonate ion concentration ($[HCO_3^-]$) were examined during air exposure. Hemolymph Po_2 decreased from 69.5 torr (mean value) to 46.3 torr during air exposure for 6 h, and reached to 19.0 torr after 24 h. The hemolymph Po_2 of air-exposed noble scallops decreased gradually and caused progressive hypoxemia by hypoventilation of the ctenidium. Air-exposed noble scallops showed a reduction in pH and elevation of Pco_2 and $[HCO_3^-]$ of the hemolymph. In air-exposed noble scallops, the hemolymph pH decreased from 7.460 to 7.045 at 6 h and to 6.348 at 24 h. The hemolymph Pco_2 increased from 1.30 torr to 5.05 torr at 6 h and to 56.6 torr at 24 h during air exposure. The $[HCO_3^-]$ increased from 1.26 mM/L to 1.88 mM/L at 6 h and to 4.19 mM/L at 24 h. From these results, in the first 6 h of air exposure, noble scallops mainly underwent respiratory acidosis by excess accumulation of CO_2 due to hypoventilation. Meanwhile, after 24 h of air exposure, noble scallops showed mainly metabolic acidosis partially compensated by mobilized $[HCO_3^-]$ from the shell.

Key words : *Mimachlamys nobilis*, noble scallop, hemolymph, acid–base balance, air exposure, respiratory physiology

Introduction

The noble scallop *Mimachlamys nobilis* is a filibranchial bivalve classified in the Pectinidae[1]. Noble scallop is distributed on the rocky or sandy sea bottom from the littoral zone to a depth of several fathoms in southwest Japan[2,3], and noble scallop has been industrialized as a unique local product using a suspended (basket) culture system. Noble scallop has been the subject of previous research in terms of its: reproductive cycle;[4] induction of oviposition, and seedling production[5]; early food[6]; karyotype[7]; genetic variation[8]; and gametogenesis and triploid induction[9,10]. The ciliary movement of the ctenidium in hypoxic and anathermal conditions has been studied[11]. The anatomical and histological structure of the ctenidium was clarified recently[12,13]. The noble scallop was examined to reveal its hemolymph acid–base balance in normoxic condition[14]. There are, however, few reports of the effect of air exposure on the respiratory physiology from the viewpoint of CO_2 dynamic phase and acid–base balance in noble scallop. In noble scallop production, the animals are often exposed to the air for maintenance for the suspended culture and for transportation to markets as living shellfish. In this study, we examined the hemolymph oxygen and acid–base status of noble scallop 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 of the acid–base balance owing to 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 animal. Noble scallops were examined to determine the αco_2 and pKapp of the hemolymph, which was collected from the adductor muscle[14]. In this study, we used the results of a previous report to calculate the hemolymph CO_2 partial pressure, bicarbonate concentration, and buffer capacity of the noble scallop during air exposure.

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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).

Materials and Methods

Experimental animals and conditions

Noble scallop *M. nobilis* (n = 24; mean total wet weight, 53.8 g; shell height, 69.6 mm; shell length 64.1 mm) were obtained from a marine farm in Sasebo, Nagasaki Prefecture, Japan. After cleaning the shell valves, they were reared for 1 month at 24℃ in aerated seawater with added cultivated phytoplankton[16]. Twenty-four hours before collecting hemolymph, the noble scallop 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 34, water temperature 24℃, O_2 saturation 97%, pH 8.17, and total CO_2 content 2.1 mM/L.

Air exposure and hemolymph collection

Six different animals were used for each duration on the air exposure. Experimental animals were subject to air exposure for 0 h (AE0h, n = 6), 6 h (AE6h, n = 6), 12 h (AE12h, n = 6), or 24 h (AE24h, n = 6). Experimental animals in the respiratory chamber were exposed to air by stopping the flow into the chamber and siphoning out the water. The temperature and humidity of the air were maintained by passing air through the experimental seawater, and by adjusting air flowed into the respiratory chamber. The hemolymph was collected anaerobically by direct puncture with a gas-tight microsyringe (Model 1750LTN, Hamilton Co.) from the adductor muscle of each animal. The volume of each hemolymph sample was 0.3–0.4 mL.

Hemolymph analysis

The hemolymph samples were immediately measured after each collection. The hemolymph oxygen partial pressure (Po_2, torr) and pH were measured using a blood gas meter (BGM200; Cameron Instruments Co., USA) with O_2 and pH electrodes (E101, E301, E351; Cameron Instruments Co., USA) at 24 ℃. Total CO_2 concentration (Tco_2, mM/L) was measured using a total CO_2 analyzer (Capnicon 5; Cameron Instruments Co., USA). Hemolymph calcium ion concentration ($[Ca^{2+}]$, mM/L) was determined with a test kit (Calcium E-test, Wako Pure Chemical Co., Japan) and a spectrophotometer (610 nm, Spectronic 20A, Shimadzu Co., Japan).

Calculation

The estimation of CO_2 partial pressure (Pco_2) by application of the Henderson–Hasselbalch equation is practiced in studies of the acid–base balance owing to its relative ease and accuracy[15]. In the equation, the CO_2 solubility coefficient (αco_2) and apparent dissociation constant of carbonic acid (pKapp) are required. The hemolymph αco_2 and pKapp of the noble scallop at 24℃ were 0.039 mM/L/torr and 6.064140, respectively[14]. The hemolymph Pco_2 of the noble scallop was calculated by rearrangement of the Henderson–Hasselbalch equation[15,17], and the bicarbonate concentration ($[HCO_3^-]$) was calculated as follows:

$$Pco_2 = Tco_2 \cdot [0.039 \cdot (1+10^{(pH-6.064140)})]^{-1}$$

$$[HCO_3^-] = Tco_2 - 0.039 \cdot Pco_2$$

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

The buffering capacity of the hemolymph *in vivo* was calculated using pH and $[HCO_3^-]$ at AE0h and AE24h for the air-exposed animals. The non-bicarbonate buffer value (β_{NB}, slykes), which is the slope of the relational expression of the hemolymph non-bicarbonate buffer system, was used from the results of pH and $[HCO_3^-]$ in the *in vitro* experiment.[14]

Statistical analysis

Data are expressed as means ± standard deviation. Kruskal–Wallis test was performed for changes in hemolymph properties over the experimental time course. 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 v.6.0 (KyensLab Inc., Japan).

Results

Noble scallops exposed to the air showed significant changes in hemolymph oxygen and acid–base properties.

The mean values of hemolymph Po_2 statistically significantly decreased from 69.5 torr to 46.3 torr with AE6h and to 44.6 torr with AE12h, and reached 19.0 torr with AE24h ($P < 0.05$, Fig. 1). The hemolymph pH decreased statistically significantly from 7.460 to 7.045 with AE6h, and reached 6.348 with AE24h ($P < 0.05$, Fig. 2). The hemolymph Tco_2 increased from 1.31 mM/L to 2.07 mM/L with AE6h, and reached 6.40 mM/L with AE24h ($P < 0.05$, Fig. 3). The calculated hemolymph Pco_2 and $[HCO_3^-]$ at AE0h were 1.30 torr and 1.26 mM/L, respectively, and these values increased statistically significantly during air exposure ($P < 0.05$, Figs. 4, 5).

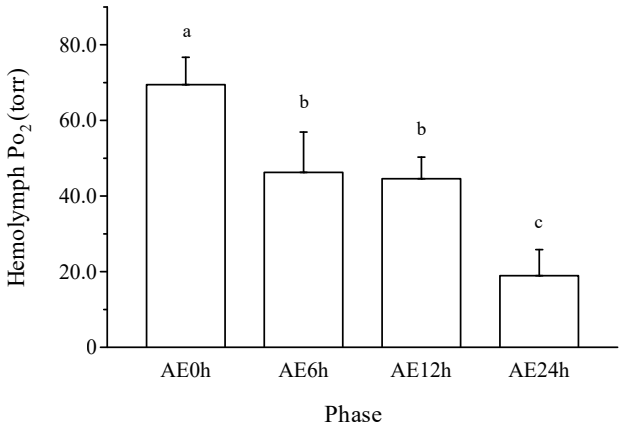

Fig. 1 Effect of air exposure on the hemolymph O_2 partial pressure (Po_2) in the noble scallop *Mimachlamys nobilis* during air exposure. AE0h: air exposure for 0 h (control); AE6h: air exposure for 6 h; AE12h: air exposure for 12 h; AE24h: air exposure for 24 h. Hemolymph from the adductor muscle was collected from each experimental animal ($n = 6$ in each symbol). Values are means ± SD. Different lowercase letters (a, b, c) indicate statistically significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

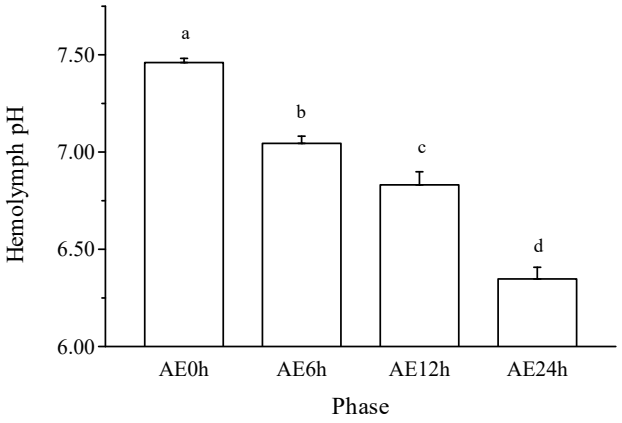

Fig. 2 Effect of air exposure on the hemolymph pH in the noble scallop *Mimachlamys nobilis* during air exposure. AE0h: air exposure for 0 h (control); AE6h: air exposure for 6 h; AE12h: air exposure for 12 h; AE24h: air exposure for 24 h. Hemolymph from the adductor muscle was collected from each experimental animal ($n = 6$ in each symbol). Values are means ± SD. Different lowercase letters (a, b, c, d) indicate statistically significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

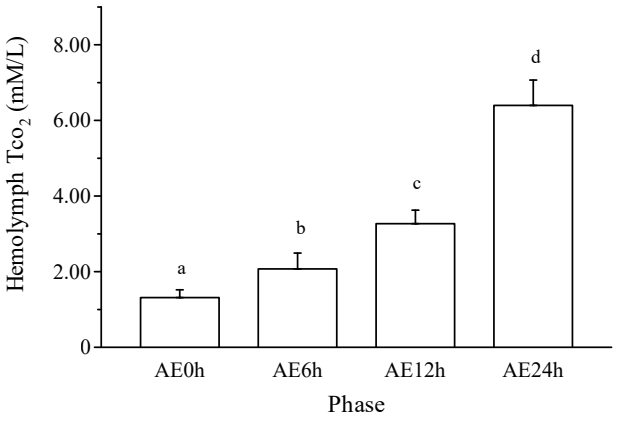

Fig. 3 Effect of air exposure on the hemolymph total CO_2 concentration (Tco_2) in the noble scallop *Mimachlamys nobilis* during air exposure. AE0h: air exposure for 0 h (control); AE6h: air exposure for 6 h; AE12h: air exposure for 12 h; AE24h: air exposure for 24 h. Hemolymph from the adductor muscle was collected from each experimental animal ($n = 6$ in each symbol). Values are means ± SD. Different lowercase letters (a, b, c, d) indicate statistically significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

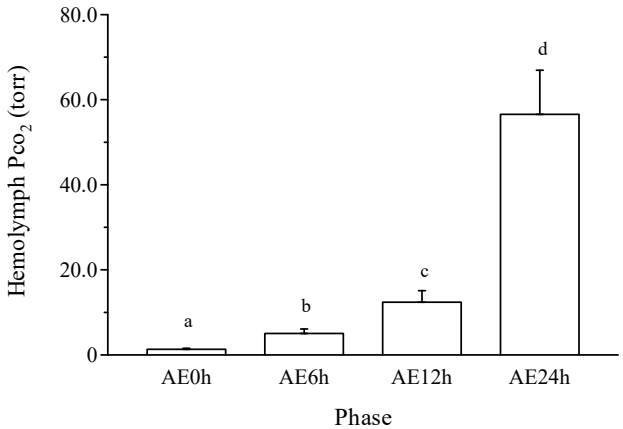

Fig. 4 Effect of air exposure on the hemolymph CO_2 partial pressure (Pco_2) in the noble scallop *Mimachlamys nobilis* during air exposure. AE0h: air exposure for 0 h (control); AE6h: air exposure for 6 h; AE12h: air exposure for 12 h; AE24h: air exposure for 24 h. Hemolymph from the adductor muscle was collected from each experimental animal ($n = 6$ in each symbol). Values are means ± SD. Different lowercase letters (a, b, c, d) indicate statistically significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

The hemolymph Pco_2 was 5.05 torr after AE6h, 12.4 torr after AE12h, and 56.6 torr after AE24h. The hemolymph [HCO_3^-] was 1.88 mM/L after AE6h, 2.79 mM/L after AE12h, and 4.19 mM/L after AE24h. The hemolymph [Ca^{2+}] increased gradually during air exposure, and the hemolymph [Ca^{2+}] was 8.9 mM/L after AE12h and 10.1 mM/L after AE24h (Fig. 6). The hemolymph [Ca^{2+}] with AE12h and AE24h was higher than the concentrations at AE0h and with AE6h ($P < 0.05$). The progress of change in acid–base balance in experimental animals is

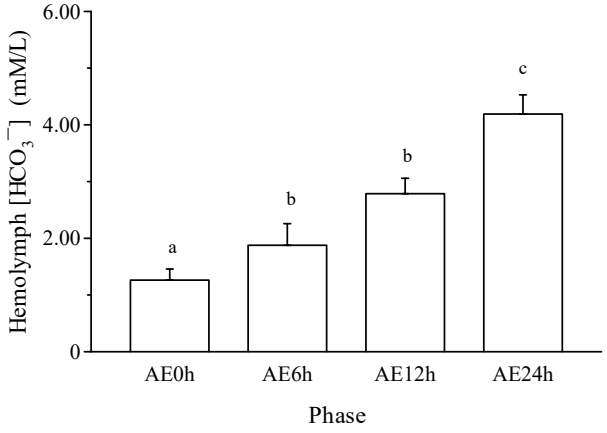

Fig. 5 Effect of air exposure on the hemolymph bicarbonate concentration ([HCO_3^-]) in the noble scallop *Mimachlamys nobilis* during air exposure. AE0h: air exposure for 0 h (control); AE6h: air exposure for 6 h; AE12h: air exposure for 12 h; AE24h: air exposure for 24 h. Hemolymph from the adductor muscle was collected from each experimental animal ($n = 6$ in each symbol). Values are means ± SD. Different lowercase letters (a, b, c) indicate statistically significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

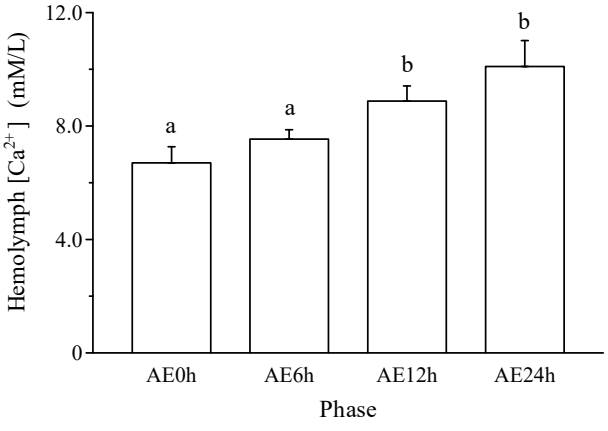

Fig. 6 Effect of air exposure on the hemolymph calcium ion concentration ([Ca^{2+}]) in the noble scallop *Mimachlamys nobilis* during air exposure. AE0h: air exposure for 0 h (control); AE6h: air exposure for 6 h; AE12h: air exposure for 12 h; AE24h: air exposure for 24 h. Hemolymph from the adductor muscle was collected from each experimental animal ($n = 6$ in each symbol). Values are means ± SD. Different lowercase letters (a, b) indicate statistically significant differences ($P < 0.05$, Steel–Dwass multiple comparison test).

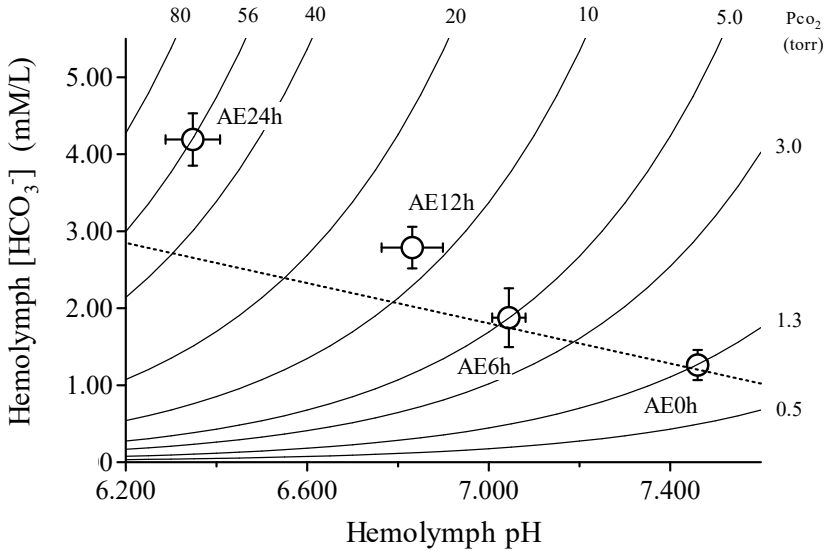

Fig. 7 The hemolymph pH-[HCO_3^-] diagram of the air exposure for 0 h (AE0h, control), 6 h (AE6h), 12 h (AE12h), and 24 h (AE24h) in the noble scallop *Mimachlamys nobilis* during air exposure. Values are means ± SD. The Pco_2 isopleths are derived from rearranging the Henderson–Hasselbalch equation. The dashed line is the non-bicarbonate buffer line, which was expressed using the results of *in vitro* experiment (Handa & Yamamoto, 2016)[14]: [HCO_3^-] = 10.86 – 1.305 · pH ($R^2 = 0.939$).

summarized in a pH-[HCO_3^-] diagram (Fig. 7). The hemolymph [HCO_3^-] of air-exposed animals increased with decreasing pH. The point after AE6h followed along the non-bicarbonate buffer line, which indicated the relationship between pH and [HCO_3^-]. The points with AE12h and AE24h were located above the non-bicarbonate buffer line (Fig. 7). The β_{NB} representing the slope of the non-bicarbonate buffer line (dashed line in the diagram) was 1.30 slykes. The hemolymph buffering capacity *in vivo* was 2.63 slykes, which was calculated using the hemolymph pH and [HCO_3^-] at AE0h and AE24h.

Discussion

We examined the hemolymph acid–base status of noble scallop *M. nobilis* to evaluate the effect of air exposure on acid–base balance. Noble scallops showed oxygen and acid–base disturbance during air exposure. The hemolymph Po_2 decreased gradually from 69.5 torr to 19.0 torr with AE24h. The air-exposed noble scallops were unable to ventilate the ctenidium, which inhibited the uptake of oxygen. The oxygen that remained inside the body was consumed and the hemolymph Po_2 decreased, and the air-exposed noble scallops experienced hypoxemia. In some marine and freshwater bivalves, the hemolymph and pericardial fluid showed reductions of oxygen partial pressure during air exposure. In blue mussel *Mytilus edulis*, the hemolymph Po_2 decreased from 60.5 torr to 15 torr during air exposure for 1 h[18]. In king scallop *Pecten maximus*, the hemolymph Po_2 decreased from 118.7 torr to 57.5 torr during air exposure for 2 h, and reached 28.1 torr after 24 h[19]. In Asian clam *Corbicula fluminea*, the pericardial fluid Po_2 decreased from 60.9 torr to 42 torr during air exposure for 8 h, and reached 21.8 torr after 24 h[20]. In boreal clam *Anodonta grandis simpsoniana*, the pericardial fluid Po_2 decreased from 90 torr to 30 torr during air exposure for 24 h[21]. In noble scallop in this study, Po_2 decreased gradually during air exposure and animals experienced progressive hypoxemia. Air-exposed noble scallops may show hypoxemia in the early period, as observed for other bivalves.

Noble scallops showed a reduction in pH and elevation of Pco_2 in the hemolymph. The hemolymph pH decreased from 7.460 to 7.045, and the hemolymph Pco_2 increased from 1.30 torr to 5.05 torr with AE6h. In some marine and freshwater bivalves, the hemolymph and pericardial fluid showed a reduction in pH and Pco_2 increased during air exposure[18-25]. The noble scallops were inhibited from releasing CO_2 from the ctenidium by hypoventilation with AE6h, and CO_2 accumulated gradually in the hemolymph. Therefore, the initial cause of acidosis should be respiratory acidosis by hypoventilation of the ctenidium. In noble scallops exposed to air over a prolonged period, the hemolymph pH decreased extremely to 6.348 with AE24h. The results of biochemical studies on anaerobic metabolism[26-30] suggested that air exposure in this study was sufficient to force anaerobic metabolism in noble scallops. Although we did not measure the anaerobic end-products, noble scallops exposed to air for a long time should undergo metabolic acidosis due to anaerobic metabolism with hypoxemia. Noble scallops exhibited increased hemolymph [HCO_3^-] and [Ca^{2+}] during air exposure for 12 h and 24 h. The increased [HCO_3^-] and [Ca^{2+}] during air exposure seemed to be mobilized from $CaCO_3$ crystals in the shell of noble scallops. In marine and freshwater bivalves, acidosis during air exposure induces increases in [HCO_3^-] and [Ca^{2+}] of the hemolymph or pericardial fluid[18,20-23,25]. Research using radiolabeled markers indicated that the source of increased calcium is the shell valve[31]. The increase in acidic end-products of anaerobic metabolism may dissolve the shell valve of noble scallops, and bicarbonate and calcium ions were mobilized into the hemolymph during air exposure in this study. The mobilized bicarbonate seemed to be effective for buffering acidosis in the hemolymph of noble scallop.

According to the pH-[HCO_3^-] diagram of the hemolymph (Fig. 7), the hemolymph [HCO_3^-] and Pco_2 increased considerably with the reduction in pH. Wood et al. (1977) expounded on the pH-[HCO_3^-] diagram from blood [32]. 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. If a decrease in pH is due solely to an increase in non-volatile acid, the blood will be titrated along a constant Pco_2 isopleth. In noble scallops, the point after AE6h followed along the non-bicarbonate buffer line. It was considered that noble scallops exposed to air in the first 6 h mainly experienced respiratory acidosis due to hypoventilation. Noble scallops after AE12h and AE24h showed hemolymph acidosis and high [HCO_3^-]. If hemolymph acidosis at AE12h and AE24h was due solely to an increase in acidic end-products of anaerobic metabolism (simply metabolic acidosis), hemolymph [HCO_3^-] was consumed for buffering the acid, and the points for AE12h and AE24h should move along the constant Pco_2 isopleth below the non-bicarbonate buffer line. However, the hemolymph [HCO_3^-] increased, and the points for AE12h and AE24h were located above the non-bicarbonate buffer line. The increased hemolymph [HCO_3^-] was mobilized from the shell dissolved by the increment of the acidic end-products, and the mobilized [HCO_3^-] compensated for the metabolic acidosis. Therefore, noble scallops with AE12h and AE24h mainly underwent metabolic acidosis and partially compensated by the mobilized [HCO_3^-].

The mobilized [HCO_3^-] of air-exposed noble scallops may enhance the buffering capacity of the non-bicarbonate buffer system in the hemolymph. The buffer value as a measure for the buffering capability is defined as the change in base or acid form of the buffer system per change in pH[33,34]. The β_{NB} is the buffer value of the non-bicarbonate buffer system (mainly protein residues). The buffering capacity *in vivo*, which was calculated using the hemolymph pH and [HCO_3^-] at AE0h and AE24h, was 2.63 slykes, and the capacity was 2-fold higher than the non-bicarbonate buffer capacity (β_{NB} 1.30 slykes). Therefore, air-exposed noble scallops may enhance the buffering capacity of the non-bicarbonate buffer system in the hemolymph with the mobilized [HCO_3^-]. Byrne et al. (1991) reported that the resulting base mobilized (primarily bicarbonate) functions to increase the apparent "non-bicarbonate" buffering capacity almost 17-fold over that of isolated hemolymph (β_{NB} 0.99 slykes) of Asian clam during air exposure for 72 h[20]. This source of readily available buffering power compensates for the low inherent buffering capacity of native hemolymph[20]. Therefore, the noble scallops in this study enhanced the buffering capacity of the hemolymph using mobilized base (bicarbonate) and provided a partial metabolic compensation for acidosis during air exposure. Duncan et al. (1994) reported that the king scallops develop progressive and uncompensated respiratory acidosis during air exposure for 24 h[19]. The hemolymph β_{NB} of king scallops was 3.88 slykes[19], and was higher than noble scallops in this study (β_{NB} 1.30 slykes, buffering capacity *in vivo* 2.63 slykes). Noble scallops had lower buffering capacity of the non-bicarbonate buffer system than king scallops, and underwent metabolic acidosis between AE6h and AE24h, though with the partial compensation.

From the results in this study, the noble scallops in the early phase of air exposure were hypoxemia and mainly respiratory acidosis (within 6 h). In prolonged air exposure, the animals showed severe metabolic acidosis though partially compensated by the mobilization of bicarbonate from the shell. Noble scallops are often reared as a local specialty product, and they experience exposure to the air for maintenance of suspended culture and for transportation to markets as a living shellfish. When the air-exposed noble scallops are returned to the seawater within 6 h, they should not experience severe acidosis.

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ヒオウギガイのヘモリンパ液の酸塩基平衡に及ぼす大気曝露の影響

半田岳志・荒木晶

和文要旨：ヒオウギガイ*Mimachlamys nobilis*のヘモリンパ液の酸素分圧は，大気曝露前に69.5torr（平均値）を示したが，曝露6時間後に46.3 torr，24時間後に19.0 torrにまで減少した．ヘモリンパ液pHは曝露前に7.460を示したが，曝露6時間後に7.045，24時間後に6.348にまで低下した．ヘモリンパ液の二酸化炭素分圧は曝露前に1.30 torrを示したが，曝露6時間後に5.05 torr，24時間後に56.6 torrにまで増加した． 重炭酸イオン濃度は曝露前に1.26 mM／Lを示したが，曝露6時間後に1.88 mM／L，24時間後に4.19 mM／Lにまで増加した．これらの結果から，ヒオウギガイは大気に曝露されると進行性の低酸素血症を引き起こすとともに，二酸化炭素の過剰蓄積による呼吸性の酸性血症を示し，その後に部分代償性の代謝性酸性血症を呈することが明らかとなった．