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本論文（Full paper）

# The oxygen and acid–base status of hemolymph in winged pearl oyster *Pteria penguin* under the normoxic condition

Takeshi Handa† and Akira Araki

**Abstract**: We examined hemolymph $O_2$ partial pressure ($Po_2$), pH, total $CO_2$ concentration ($Tco_2$), $CO_2$ partial pressure ($Pco_2$), and bicarbonate concentration ($[HCO_3^-]$) to evaluate the acid–base balance of the winged pearl oyster *Pteria penguin* under normoxic condition. Hemolymph was collected anaerobically from the adductor muscle. Mean values for hemolymph $Po_2$, pH and $Tco_2$ were 73.4 torr, 7.598 and 2.40 mM/L, respectively. Hemolymph $Pco_2$ was calculated using the rearranged Henderson–Hasselbalch equation, yielding 1.83 torr and a $[HCO_3^-]$ of 2.33 mM/L. The non-bicarbonate buffer value (hemolymph pH–$[HCO_3^-]$ relational expression slope) was 1.99 slykes, higher than that of other marine bivalves. Thus, the winged pearl oyster hemolymph has a comparatively greater non-bicarbonate buffering capacity.

**Key words**: *Pteria penguin*, $O_2$ partial pressure ($Po_2$), $CO_2$ partial pressure ($Pco_2$), pH, apparent dissociation constant of carbonic acid (pKapp), normoxia

## Introduction

The winged pearl oyster *Pteria penguin* is a filibranchial bivalve belonging to the family Pteriidae[1]. Winged pearl oyster distribution extends from the Red Sea and Arabian Gulf throughout the tropical eastern Indo-Pacific to southern Japan, including Southeast Asia, the Philippines, Queensland round to northwest Australia, Thursday Island, southern China, and Taiwan[2]. In Japan, this species inhabits the rock reef up to 20 m depth at the tropical West Pacific area from the Kii Peninsula[1]. Winged pearl oysters have nacreous aragonite on the inner surface of its shell valves and are a principal source of half-pearls[2]. Pearl production has depended on wild-collected oysters, but recent increased pearl demand has required augmenting stocks with adult oysters cultured from artificial seedlings.

Winged pearl oysters have been studied in terms of ciliary movement[3], structure of the ctenidium and digestive organs[4], juvenile growth and survival[5], larvae and micro-algae concentrate use[6], oxidative stress[7], and anesthetic induction for nucleus implantation[8]. However, there are few respiratory physiology reports from the perspective of $CO_2$ dynamics and acid–base balance in winged pearl oysters. Understanding the acid–base status may assist in determining optimal conditions for efficient $CO_2$ use, a factor related to respiration and calcification. Knowledge of the acid–base balance of winged pearl oysters will be useful for evaluating the aquaculture environment and impacts of by the increase of $CO_2$ levels (ocean acidification). Under normoxic conditions, several marine bivalves are reported to exhibit hemolymph $CO_2$ partial pressures ($Pco_2$) within 0.9-2.3 torr[9-13] indicating a low $Pco_2$ with a narrow range of fluctuation. The winged pearl oyster is expected to behave similarly, but directly measuring $Pco_2$ is difficult due to the low $Pco_2$ value. In the respiration of aquatic animals, $Pco_2$ is often estimated using the Henderson–Hasselbalch equation owing to its ease and accuracy[14]. In the equation, for each experimental animal type, $CO_2$

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Department of Applied Aquabiology, National Fisheries University
†Corresponding author: handat@fish-u.ac.jp

solubility coefficient ($\alpha co_2$) and apparent dissociation constant of carbonic acid (pKapp) values are required. Therefore, winged pearl oyster hemolymph $O_2$ partial pressure ($Po_2$), pH, and total $CO_2$ concentration ($Tco_2$) were measured, and then $Pco_2$ and bicarbonate concentration ($[HCO_3^-]$) were evaluated using the hemolymph $\alpha co_2$ and pKapp determined in this study. These results expand understanding of bivalve respiratory physiology and provide fundamental knowledge to improve winged pearl oyster aquaculture environments.

## Materials and Methods

### *Experimental animals and conditions*

Winged pearl oysters (mean wet weight, 204 g; n = 3) were obtained from a marine farm in Kagoshima prefecture. After cleaning the shell valves, they were reared for one month at 23°C in aerated seawater with added cultivated phytoplankton[15-17]. Twenty-four hours before hemolymph collection, winged pearl oysters were transferred to particle-free seawater (> 0.45 μm). All experiments were conducted in seawater with a salinity of 30–35 psu, water temperature 23°C, $O_2$ saturation 95%, pH 8.00, and total $CO_2$ content 2 mM/L.

### *Hemolymph collection and analysis*

From each winged pearl oyster, hemolymph (0.3–0.5 mL) was collected anaerobically from the adductor muscle twice successively by direct puncture. Hemolymph $Po_2$, pH and $Tco_2$ were measured immediately from the first collected sample. The $Po_2$ and pH were measured using a blood gas meter (BGM200; Cameron Instruments Co., Texas) with $O_2$ and pH electrodes (E101, E301, E351; Cameron Instrument Co.) at 23°C. $Tco_2$ was measured using a total $CO_2$ analyzer (Capnicon 5; Cameron Instrument Co.). The remaining hemolymphs from first collection and the second-collection hemolymph were used for *in vitro* experiments.

For the Henderson–Hasselbalch equation[18] the winged pearl oyster $\alpha co_2$ and pKapp are required. The following relative expression, obtained in a previous study[19], was used to calculate $\alpha co_2$:

$$\alpha co_2 = 182.3717 - 24.3932 \cdot T + 1.6396 \cdot T^2 - 0.0492 \cdot T^3 + 0.000536 \cdot T^4$$

where T is temperature (℃).

For hemolymph pKapp determination, hemolymph was transferred to a tonometer flask and equilibrated with humidified standard $CO_2$ gases ($CO_2$, 0.2, 0.5, 1.0, 2.0 and 5.0%; $O_2$, 20.9%; $N_2$ Balance) using an equilibrator at 23°C. After equilibration, sample pH and $Tco_2$ were measured using a blood gas meter and a total $CO_2$ analyzer. With the sample pH, $Tco_2$, and $\alpha co_2$ calculated by the above equation, pKapp was determined by rearrangement of Henderson–Hasselbalch equation[14,18] as follows:

$$\mathrm{pKapp} = \mathrm{pH} - \log\left[(Tco_2 - \alpha co_2 \cdot Pco_2) \bullet (\alpha CO_2 \cdot Pco_2)^{-1}\right]$$

where $Pco_2$ is calculated from known $CO_2$ concentration of standard gases.

The obtained $\alpha co_2$ and pKapp were then used to estimate hemolymph $Pco_2$ and $[HCO_3^-]$ from the measured pH and $Tco_2$:

$$Pco_2 = Tco_2 \cdot \left[\alpha co_2 \cdot (1+10^{(\mathrm{pH} - \mathrm{pKapp})})\right]^{-1}$$

$$[HCO_3^-] = Tco_2 - \alpha co_2 \cdot Pco_2$$

The non-bicarbonate buffer value ($\beta_{NB}$, slykes) and relational expression for the hemolymph non-bicarbonate buffer were calculated using pH and $[HCO_3^-]$ from the *in vitro* experiment.

### *Statistical analysis*

Normality of the hemolymph variables was assessed using the Shapiro–Wilk test. The Kruskal–Wallis test was performed for changes in hemolymph properties using the standard gases. Statistically significant differences were set at $P < 0.05$. All analyses were conducted using the statistical software Kyplot ver. 6.0.2 (KyensLab Inc., Japan)

## Results

Mean hemolymph values for $Po_2$, pH, and $Tco_2$ were 73.4 torr, 7.598, and 2.40 mM/L, respectively (Table 1). Hemolymph $\alpha co_2$ was estimated to be 40 μM/L/torr. The hemolymph pKapp at known $CO_2$ partial pressures (standard gases) and corresponding measured pH and $Tco_2$ values are shown in Table 2. There was no significant difference in pKapp values ($P > 0.05$). The mean pKapp was calculated as 5.99878. Hemolymph $Pco_2$ and $[HCO_3^-]$ were estimated by substituting the $\alpha co_2$ and pKapp values into the rearranged Henderson-Hasselbalch equation as follows:

$$Pco_2 = Tco_2 \cdot [0.040 \cdot (1+10^{(pH-5.99878)})]^{-1}$$

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

where equation parameter units are torr for $Pco_2$, mM/L for $Tco_2$ and $[HCO_3^-]$, and mM/L/torr for $\alpha co_2$.

This yielded hemolymph $Pco_2$ and $[HCO_3^-]$ values of 1.83 torr and 2.33 mM/L (Table 1). The relation between pH and $[HCO_3^-]$ is shown in Fig. 1. The $\beta_{NB}$, obtained as the regression coefficient relating pH and $[HCO_3^-]$, was 1.99 slykes.

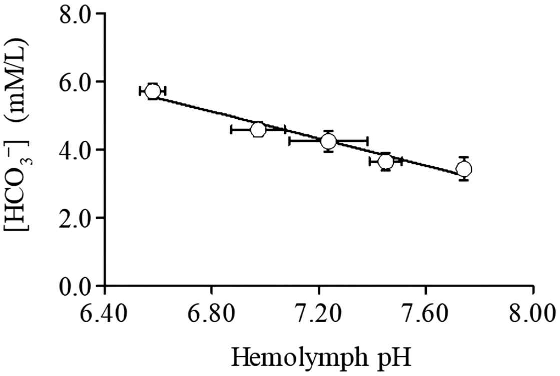

**Fig. 1**. Relationship between pH and $[HCO_3^-]$ of the hemolymph in winged pearl oyster *Pteria penguin*. Values are means ± SD. Solid line fitted to the data and the equation: $[HCO_3^-] = 18.67 - 1.99 \cdot pH$ ($R^2 = 0.9620$).

**Table 1**. Hemolymph $O_2$ partial pressure ($Po_2$), pH, total $CO_2$ concentration ($Tco_2$), and calculated $CO_2$ partial pressure ($Pco_2$) and $[HCO_3^-]$ of the winged pearl oyster (*Pteria penguin*) under normoxic condition

| | | Mean | SD | n |
|---|---|---|---|---|
| $Po_2$ | torr | 73.4 | 8.95 | 3 |
| pH | | 7.598 | 0.052 | 3 |
| $Tco_2$ | mM/L | 2.40 | 0.60 | 3 |
| $Pco_2$ | torr | 1.83 | 0.66 | 3 |
| $[HCO_3^-]$ | mM/L | 2.33 | 0.58 | 3 |

Water tempearture, 23°C.

**Table 2**. Mean values of measured pH, total $CO_2$ concentration ($Tco_2$) and calculated apparent dissociation constant of carbonic acid (pKapp) of the hemolymph in the winged pearl oyster (*Pteria penguin*) with known $Pco_2$ standard gases

| Standard gas | | Hemolymph | | |
|---|---|---|---|---|
| $CO_2$ | $Pco_2$ | pH | $Tco_2$ | pKapp |
| % | torr | | mM/L | |
| 0.2 | 1.48 | 7.741 | 3.50 | 5.98510 |
| 0.5 | 3.70 | 7.449 | 3.61 | 6.07330 |
| 1.0 | 7.39 | 7.235 | 4.28 | 6.10815 |
| 2.0 | 14.8 | 6.973 | 4.62 | 6.14258 |
| 5.0 | 37.0 | 6.578 | 5.76 | 6.11997 |

Water temperature, 23°C.

## Discussion

The hemolymph oxygen and acid–base status was examined to evaluate the acid–base balance of the winged pearl oysters under normoxic condition yielding a mean hemolymph $Po_2$ value of 73.4 torr. This is the first reported measurement of winged pearl oyster hemolymph $Po_2$. In marine bivalves, hemolymph $Po_2$ of the adductor muscle were 53.2–62.0 torr in the Pacific oyster *Crassostrea gigas*[12,20], 64.7 torr in the densely lamellated oyster *Ostrea denselamellosa*[21], 53.5–58.9 torr in the akoya pearl oyster *Pinctada fucata martensii*[22], and 69.5 torr in the noble scallop *Mimachlamys nobilis*[24]. The winged pearl oyster hemolymph $Po_2$ is within the range of other bivalves. In *M. edulis*, the adductor muscle comprises a large fraction of the total hemolymph volume, and the hemolymph samples collected from the adductor muscle contain a mixture of pre- and post-branchial hemolymph from various regions of the circulatory system[9]. The winged pearl oyster hemolymph, which was collected from the adductor muscle, would circulate around various regions and perfuse to the adductor muscle.

The measured pH for winged pearl oyster hemolymph was 7.598. Hemolyph pH values reported for other marine bivalves include 7.55 in *Mytilus galloprovincialis*[10], 7.544–7.568 in *P. fucata martensii*[13,23], 7.414–7.52 in *C. gigas*[12,20], 7.442 in *M. nobilis*[24], and 7.563 in black lip pearl oyster *Pinctada margaritifera*[25] at 18–26°C. The winged pearl oyster hemolymph yielded a slightly higher pH than that of other bivalves. The hemolymph $Tco_2$ evaluated for the winged pearl oyster was 2.40 mM/L. For other bivalves, hemolymph $Tco_2$ reported include 2.21–2.25 mM/L in *P. fucata martensii*[13,23], 1.87 mM/L in *C. gigas*[12], 1.50 mM/L in *M. nobilis*[24], and 2.04 mM/L in *P. margaritifera*[25]. The winged pearl oyster hemolymph $Tco_2$ was higher than that of other bivalves. The $Tco_2$ represents the $CO_2$ concentration which physically and chemically dissolved. As the winged pearl oyster yielded a higher concentration of hemolymph $CO_2$, it may have a greater proportion of available $CO_2$ and/or bicarbonate than other bivalves.

Cameron[26] reported $CO_2$ solubility as a function of temperature and salinity, and a solubility was 39.24–42.33 μM/L/mmHg at 22–24°C and 30–35 psu. The winged pearl oyster hemolymph $\alpha co_2$ (40 μM/L/mmHg) is within the range noted in the previous study. The mean hemolymph pKapp value obtained in this study was 5.99878. The apparent dissociation constant of carbonic acid is equal to the pH at which it most effectively functions as a buffer[29]. The hemolymph pKapp of marine bivalves were 6.114 in *M. edulis*[27], 5.9835 in *P. margaritifera*[25], 5.8191–5.9958 in *P. fucata martensii*[13,23], 6.0734 *in C. gigas*[12], and 6.1083 in *O. denselamellosa*[21]. This shows that the winged pearl oyster hemolymph buffers similarly to *P. margaritifera* and *C. gigas* and at a lower pH than that for *M. edulis* and *O. denselamellosa*. Using the $\alpha co_2$ and pKapp obtained in this study, $Pco_2$ and $[HCO_3^-]$ were calculated. The mean values of hemolymph $Pco_2$ and $[HCO_3^-]$ were 1.83 torr and 2.33 mM/L. Comparing to other marine bivalves, hemolymph $Pco_2$ and $[HCO_3^-]$ were 0.9 torr and 1.8 mM/L in *M. edulis*[9], 1.1–2.2 torr and 1.37–1.78 mM/L in *C. gigas*[11,12], 1.15 torr and 1.62 mM/L in *M. galloprovincialis*[10], and 1.0–1.69 torr and 2.11-2.21 mM/L in *P. fucata martensii*[13,23]. Thus, the winged pearl oyster hemolymph has a greater capacity for available $CO_2$ and $[HCO_3^-]$ than the other bivalves.

The $\beta_{NB}$ of winged pearl oyster hemolymph (1.99 slykes) was higher than that of *M. edulis* (0.4–0.62 slykes)[9,27], *M. galloprovincialis* (0.65 slykes)[10], *M. coruscus* (0.44 slykes)[28], *C. gigas* (0.73–0.88 slykes)[11,12], *P. margaritifera* (0.53 slykes)[25], *P. fucata martensii* (0.46–1.45 slykes)[13,22,23], and *M. nobilis* (1.30 slykes)[24]. The $\beta_{NB}$ represents the capacity of the non-bicarbonate buffer system (for example, protein buffering) and quantifies the non-bicarbonate component of hemolymph buffering. Therefore, the winged pearl oyster hemolymph has larger non-bicarbonate buffer capacity than that reported for other bivalves. This indicates that fluctuating $CO_2$ levels are less likely to affect winged pearl oyster hemolymph pH than other bivalves.

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# 正常酸素状態におけるマベのヘモリンパ液の酸素・酸塩基状態に関する研究

半田岳志，荒木　晶

**要旨**: 正常酸素状態におけるマベ*Pteria penguin*の酸塩基平衡を明らかにするため，ヘモリンパ液の酸素分圧 ($P_{O_2}$)，pH，全炭酸含量 ($T_{CO_2}$)，二酸化炭素分圧 ($P_{CO_2}$)，および炭酸水素イオン濃度 ($[HCO_3^-]$) について調べた。ヘモリンパ液の$P_{O_2}$，pH，$T_{CO_2}$ は，それぞれ73.4 torr，7.598，2.40 mM/Lだった (平均値)。ヘモリンパ液の$P_{CO_2}$ はヘンダーソン・ハッセルバルヒの式を利用して1.83 torrと算出された。ヘモリンパ液の $[HCO_3^-]$ は 2.33 mM/Lだった。また，ヘモリンパ液の非重炭酸緩衝価 ($\beta_{NB}$) は 1.99 slykesで他の海産二枚貝より大きいことから，マベのヘモリンパ液は非重炭酸緩衝系の緩衝容量が大きいと判断された。