The hemolymph CO_2 partial pressure and bicarbonate concentration of the acid–base balance of *Mytilus coruscus* under resting conditions

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Abstract : We investigated the hemolymph CO_2 partial pressure (Pco_2) and bicarbonate concentration ($[HCO_3^-]$) of the acid–base balance of *Mytilus coruscus* under resting conditions. Hemolymph collected from the adductor muscle was subjected to the following measurements. Mean values for hemolymph pH and total CO_2 concentration for this state between 18℃ and 23℃ were 7.568-7.601 and 1.54-1.59 mM/L, respectively. Hemolymph Pco_2 and $[HCO_3^-]$ were calculated using the hemolymph pKapp estimated using the relational expression with temperature. Hemolymph Pco_2 and $[HCO_3^-]$ were 1.77-1.83 torr and 1.47-1.50 mM/L at 18℃ and 23℃. To verify Pco_2 and $[HCO_3^-]$, the values were calculated using pKapp obtained by *in vitro* method (tonometry). Despite the different determination methods, no statistical difference in the obtained values of Pco_2 and $[HCO_3^-]$ were observed. Non-bicarbonate buffer values (β_{NB}), which were calculated using the slope of the relational expression between pH and $[HCO_3^-]$ in hemolymph, were 0.42 slykes at 18℃, and 0.54 slykes at 23℃. The hemolymph β_{NB} of *M. coruscus* was in the range of other bivalves, and the hemolymph buffer capacity of the non-bicarbonate buffer system would reflect the Mitilid species.

Key words : *Mytilus coruscus*, hemolymph acid–base balance, Pco_2, $[HCO_3^-]$, resting condition, CO_2 dynamics.

Introduction

The hard-shelled mussel *Mytilus coruscus* is a Mytilidae bivalve inhabiting the rocky bottom of intertidal zones from Hokkaido to Kyushu in Japan[1]. *Mytilus coruscus* is referred to as "Sendai-gai" in Miyagi prefecture, or "Seto-gai" in Yamaguchi prefecture, and is a premium seafood. Investigation of *M. coruscus* was performed on the technological development of aquaculture in Miyagi prefecture[2,3], and on the production of population in Seto Inland Sea[4]. *Mytilus coruscus* is endemic to East Asia, the shores of the Yellow Sea, and Sea of Japan[5], and is commercially cultivated in China[6]. *Mytilus coruscus* was previously studied in terms of larvae morphology[7], polymorphic microsatellite loci[8], microsatellite markers[9], influence of natural biofilm on the settlement mechanism[10], hemocyte immune activities[11], hybrid molecular identification[12], light-responsive genes[13], and marine environment[14]. In the context of respiratory physiology, the relationship between hemolymph acid–base status of *M. coruscus* and air exposure are of interest. Air-exposed *M. coruscus* showed partially compensated metabolic acidosis[15]. Understanding the dynamic state of CO_2 in the hemolymph is important to evaluate the acid–base balance. Under normal conditions, marine bivalve hemolymph CO_2 partial pressures (Pco_2) range within 0.9-2.3 torr[16-20]. Under normoxic and normocapnic conditions, marine bivalves have a very low Pco_2 with a small fluctuating range. This behavior was expected for *M. coruscus*; however, directly measuring Pco_2 is difficult due to the low Pco_2 value[21]. Estimation of Pco_2 via the Henderson–Hasselbalch equation is often used in studying acid–base balances owing to its ease and accuracy[22]. In the equation, the CO_2 solubility coefficient (αco_2) and apparent dissociation constant of carbonic acid (pKapp) values are required for each experimental animal type. As temperature influences αco_2 and pKapp, Handa and Araki (2025) investigated the relation among hemolymph αco_2, pKapp, and temperature in *M. corsucus*, proposing a relational expression for these

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properties[21]. In this study, hemolymph Pco_2 was calculated using αco_2 and pKapp estimated using this relational expression[21], with the *M. coruscus* hemolymph acid–base balance evaluated under resting conditions. To validate the Pco_2 calculated using the estimated pKapp, hemolymph Pco_2 was determined using *in vitro* methods to obtain pKapp. These results assist in further understanding respiratory physiology and fundamental aspects of aquaculture environments.

Materials and Methods

Experimental animals and conditions

These experiments used 67 hard-shelled mussels *M. corsucus* (mean wet weight: 186 g) collected from the coast of the Seto Inland Sea in the eastern area of Yamaguchi prefecture. After cleaning the shell valves, the mussels were reared in water temperatures of 18°C or 23°C, and fed cultivated phytoplankton[23-25]. Twenty-four hours before hemolymph collection, the mussels were transferred to particle-free (> 0.45 μm) seawater. All experiments were conducted in seawater with a salinity of 28 psu, O_2 saturation 96%, pH 7.9, and a total CO_2 concentration of 1.8 mM/L.

Surgical procedures

Hemolymph was collected from the adductor muscle by cannula (polyethylene tubing, 0.96 mm outer diameter, 0.58 mm inner diameter, PE-50, Clay Adams). A small hole (2 mm in diameter) was made adjacent to the shell valves near the adductor muscle. A cannula with a stylet was inserted through the hole into the adductor muscle and advanced 0.3–0.5 cm towards the center of the adductor muscle. The stylet was removed, and the end of the cannula closed. The cannula was fixed to the left shell valve with denture adhesive (Kobayashi Pharmaceutical Co., Ltd.) to prevent influences due to movement of the shell valves. The cannulated mussel was transferred to a respiratory chamber and allowed to recover in resting conditions at 18°C or 23°C.

Experimental procedures

In vivo experiment

After allowing the cannulated mussel to recover, a hemolymph sample was drawn through the cannula using a gas-tight micro syringe (Model 1750LTN, Hamilton Co.). The hemolymph volume collected was 0.2-0.4 mL. Hemolymph pH and total CO_2 concentration (Tco_2, mM/L) were measured immediately at 18°C or 23°C (n=6 at each temperature). Hemolymph Pco_2 and bicarbonate concentration ($[HCO_3^-]$, mM/L) were calculated by rearranging the Henderson–Hasselbalch equation[22,26]. For this equation, αco_2 and pKapp were required for *M. coruscus*. Handa and Araki (2025) reported on the relation of hemolymph αco_2, pKapp, and temperature of *M. corsucus*, proposing a relational expression for these properties[21]. Hemolymph Pco_2 and $[HCO_3^-]$ were calculated using the hemolymph pKapp estimated using the relational expression[21], and then validated against pKapp values obtained using the *in vitro* method.

In vitro experiment

In vitro determination of pKapp was performed on hemolymph drawn from the adductor muscle through cannula. The hemolymph sample 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 (DEQ-1, Cameron Instruments Co., USA) at 18°C (n=25) and 23°C (n=30). After equilibration, the sample pH and Tco_2 were measured. Using these, the pKapp was determined by rearrangement of the Henderson–Hasselbalch equation[22,26], enabling hemolymph Pco_2 and $[HCO_3^-]$ calculation.

Hemolymph analysis

The hemolymph pH and Tco_2 were measured immediately after each collection. The pH was measured using a blood gas meter (BGM200, Cameron Instruments) with pH glass and reference electrodes (E301, E351, Cameron Instruments). The pH electrodes were installed in a water jacket and maintained at experiment temperatures (18°C or 23°C). Tco_2 was measured using a total CO_2 analyzer (Capnicon 5, Cameron Instruments).

Calculation

Hemolymph Pco_2 and $[HCO_3^-]$ were calculated by rearranging the Henderson–Hasselbalch equation[22,26]:

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

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

$$[HCO_3^-] = \alpha co_2 \bullet Pco_2 \bullet 10^{(pH - pKapp)}$$

where pH and Tco_2 are measured hemolymph properties, with units of torr for Pco_2 and mM/L for Tco_2 and $[HCO_3^-]$.

The αco_2 was estimated using the polynomial equation in the previous study[21]:

$$\alpha co_2 = 138.2475 - 11.2533 \bullet T + 0.553901 \bullet T^2 - 0.01399 \bullet T^3 + 0.000138 \bullet T^4$$

where T is the temperature, and units used are μM/L/torr for αco_2 and ℃ for T.

The pKapp was estimated using the relational expression (derived by linear regression)[21]:

$$pKapp = 6.6407 - 0.01589 \bullet T$$

where T is temperature in ℃.

The pKapp was obtained via *in vitro* experiments and rearrangement of the Henderson–Hasselbalch equation[22,26] as follows:

$$pKapp = pH - \log [(Tco_2 - \alpha co_2 \bullet Pco_2) \bullet (\alpha co_2 \bullet Pco_2) - 1]$$

where units include mM/L for Tco_2, mM/L/torr for αco_2, and torr for Pco_2.

This pKapp was then used to calculate hemolymph Pco_2 and $[HCO_3^-]$ via the rearranged Henderson–Hasselbalch equation[22,26].

To assess the *M. coruscus* hemolymph buffer value, the non-bicarbonate buffer values (β_{NB}, slykes) were calculated using the slope of the relational expression between pH and $[HCO_3^-]$ in hemolymph.

Statistical analysis

The Mann–Whitney *U* test was performed to evaluate hemolymph Pco_2 and $[HCO_3^-]$ calculated via different pKapp determination methods. Statistically significant differences were set at $P < 0.05$. All analyses were carried out using the statistical software Kyplot 6.0 (KyensLab Inc., Japan).

Results

Hemolymph was collected from the adductor muscle of *M. coruscus* via cannula. Experiment water temperatures were 17.8℃ and 23.3℃. At these temperatures, αco_2 and pKapp were estimated using the relational expression. Values of αco_2 and pKapp at 17.8℃ were 48.4 μM/L/torr and 6.357858, and 40.5 μM/L/torr and 6.270463 at 23.3° C. The mean values of hemolymph pH and Tco2 in resting condition were 7.568-7.601 and 1.54-1.59 mM/L between 17.8℃ and 23.3℃, respectively (Table 1). The αco_2, pKapp, pH, and Tco_2 were then substituted into the rearranged Henderson–Hasselbalch equation, and hemolymph Pco_2 and $[HCO_3^-]$ calculated. At 17.8℃, hemolymph Pco_2 and $[HCO_3^-]$ were 1.77 torr and 1.50 mM/L, and 1.83 torr and 1.47 mM/L at 23.3℃ (Table 1). For the *in vitro* experiments under the known Pco_2 of standard gases, the hemolymph pKapp and corresponding pH and Tco_2 values are shown in Table 2-3. The mean values of all pKapp were 6.34817476 at 17.8℃ and 6.27451764 at 23.3℃. Hemolymph Pco_2 and $[HCO_3^-]$ which were calculated using the mean pKapp obtained by *in vitro* experiment were 1.74 torr and 1.50 mM/L at 17.8℃, and 1.85 torr and 1.47 mM/L at 23.3 ℃ (Table 4). There was no significant difference observed in values of hemolymph Pco_2 and $[HCO_3^-]$ between the two methods used to obtain pKapp ($P > 0.05$, Mann–Whitney *U* test). Hemolymph pH and calculated $[HCO_3^-]$ values are shown in Table 5. Hemolymph β_{NB} were 0.42 slykes at 17.8℃, and 0.54 slykes at 23.3℃.

Table 1. Hemolymph acid–base status for *Mytilus corusucus* under resting conditions

	WT 17.8°C		WT 23.3°C	
	Mean	SD	Mean	SD
pH	7.601	0.023	7.568	0.031
Tco_2 (mM/L)	1.59	0.06	1.54	0.09
Pco_2 (torr)	1.77	0.07	1.83	0.08
$[HCO_3^-]$ (mM/L)	1.50	0.06	1.47	0.09

WT: water temperature. SD: Standard deviation (n=6 at each temperature).

Table 2. Mean values of measured pH, total CO_2 content (Tco_2) and calculated apparent dissociation constant of carbonic acid (pKapp) of *Mytilus corusucus* hemolymph with known Pco_2 standard gases at 18°C

Standard gas		Hemolymph		
CO_2 (%)	Pco_2 (torr)	pH	Tco_2 (mM/L)	pKapp
0.2	1.5	7.486	1.59	6.1592448
0.5	3.7	7.292	1.77	6.3464156
1.0	7.4	7.106	2.03	6.4372888
2.0	14.7	6.781	2.19	6.4700960
5.0	36.8	6.369	3.77	6.3311269

Water temperature, 17.8°C; Mean value of pKapp, 6.34817476 (n=5 at each Pco_2).

Table 3. Mean values of measured pH, total CO_2 content (Tco_2) and calculated apparent dissociation constant of carbonic acid (pKapp) of *Mytilus corsucus* hemolymph with known Pco_2 standard gases at 23°C

Standard gas		Hemolymph		
CO_2 (%)	Pco_2 (torr)	pH	Tco_2 (mM/L)	pKapp
0.2	1.5	7.485	1.56	6.095000
0.5	3.7	7.297	1.66	6.307600
1.0	7.5	7.088	1.92	6.358205
2.0	14.9	6.777	2.10	6.382874
5.0	37.3	6.355	3.57	6.228949

Water temperature, 23.3°C; Mean value of pKapp, 6.27451764 (n=6 at each Pco_2).

Table 4. Comparison of hemolymph CO_2 partial pressure (Pco_2) and bicarbonate concentration [HCO_3^-] calculated using the mean pKapp and the estimated pKapp in *Mytilus corusucus*

	WT 17.8°C		WT 23.3°C	
	Mean	SE	Mean	SE
Pco_2 (torr)				
using the mean value of pKapp	1.74	0.03	1.85	0.03
using the estimated pkapp	1.77	0.03	1.83	0.03
[HCO_3^-] (mM/L)				
using the mean value of pKapp	1.50	0.03	1.47	0.04
using the estimated pkapp	1.50	0.03	1.47	0.04

WT: water temperature. SE: standard error (n=6 at each temperature).
Mean values of pKapp: 6.34817476 at 17.8°C; 6.27451764 at 23.3°C. See detailed in Tables 3-4.
The relational expression with temperature[21] is shown in the section of materials and methods.
There is no significant difference observed in values of hemolymph Pco_2 and [HCO_3^-] between the two methods ($P > 0.05$, Mann–Whitney U test).

Table 5. Mean values of hemolymph pH and bicarbonate concentration ([HCO_3^-]) of *Mytilus coruscus* with known Pco_2 standard gases at 18-23°C

Standard gas	WT 17.8°C		WT 23.3°C	
CO_2 (%)	pH	[HCO_3^-] (mM/L)	pH	[HCO_3^-] (mM/L)
0.2	7.486	1.52	7.485	1.50
0.5	7.292	1.59	7.297	1.51
1.0	7.106	1.67	7.088	1.62
5.0	6.369	1.98	6.355	2.07

WT: water temperature. Data are mean values (n=5-6 at each temperature).
Non-bicarbonate buffer value (β_{NB}) of 0.42 slykes at 18°C; 0.54 slykes at 23°C.

Discussion

The hemolymph acid–base balance of *M. coruscus* was investigated under resting conditions, and the hemolymph Pco_2 and [HCO_3^-] calculated using the different methods of pKapp determination was compared. *Mytilus coruscus* hemolymph pH at 17.8°C was 7.568, and 7.601 at 23.3°C. In the other marine bivalves, hemolymph pH was 7.65 in *M. edulis* at 12°C[16], 7.55 in *M. galloprovincialis* at 18°C[17], 7.442 in *Mimaclamys nobilis* at 24°C[27], 7.36-7.414 in *Crassostrea gigas* at 15-23°C[18,19]. The hemolymph pH of *M. coruscus* was almost identical to *M. edulis* and *M. galloprovincialis*. The hemolymph Tco_2 of *M. coruscus* was 1.59-1.54 mM/L at 17.8-23.3°C. Mean hemolymph Tco_2 values for other marine bivalves are 1.87 mM/L in *C. gigas* at 23°C[19], 2.04 mM/L in *P. margaritifera* at 26°C[28], and 1.9-2.1 mM/L in *P. fucata* martensii at 28°C[20]. Tco_2 in *M. coruscus* was less than that for the Pterioida species.

The hemolymph Pco_2 and [HCO_3^-] were calculated by Henderson–Hasselbalch equation rearrangement. In the equation, animal-specific αco_2 and pKapp values are required. Handa and Araki (2025) reported the influence of temperature on *M. coruscus* hemolymph αco_2 and pKapp, and proposed the relational expressions for arbitrary temperatures (between 16-28°C)[21]. At the experimental temperatures used in this study, the values of αco_2 and

pKapp at 17.8℃ were estimated as 48.4 μM/L/torr and 6.357858, and 40.5 μM/L/torr and 6.270463 at 23.3℃. Hemolymph Pco_2 and [HCO_3^-] in *M. coruscus* were calculated as 1.77-1.83 torr and 1.47-1.50 mM/L, respectively. Hemolymph Pco_2 and [HCO_3^-] of other marine bivalves are reported: 0.9 torr and 1.8 mM/L in *M. edulis* at 12℃[16]; 1.15 torr and 1.62 mM/L in *M. galloprovincialis* at 18℃[17]; 1.13 torr (0.15 kPa)-2.18 torr and 1.37-1.78 mM/L in *C. gigas* at 15-23℃[18,19]; 1.0 torr and 2.21 mM/L in *P. fucata* martensii at 20℃[20], 1.50 torr and 1.98 mM/L in *P. margaritifera* at 26℃[27]. Hemolymph Pco_2 and [HCO_3^-] in *M. coruscus* are in the same range as other marine bivalves.

In *in vitro* experiments, the mean pKapp were determined 6.34817476 at 17.8℃ and 6.27451764 at 23.3℃. The hemolymph Pco_2 and [HCO_3^-] calculated using the mean pKapp were 1.74-1.85 torr and 1.47-1.50 mM/L, respectively. Comparing with the values of hemolymph Pco_2 calculated via *in vivo* and *in vitro* experiments, no significant difference was observed ($P > 0.05$, Mann–Whitney *U* test). Along with Pco_2, no statistically significant difference in hemolymph [HCO_3^-] was observed, despite the different methods of pKapp determination. Therefore, the relational expression which shows the relationship between pKapp and temperature is practical for the calculation of Pco_2 and [HCO_3^-].

The non-bicarbonate buffer values (β_{NB}), obtained as a regression coefficient relating pH and [HCO_3^-], were 0.42 slykes at 17.8℃ and 0.54 slykes at 23.3℃. The hemolymph β_{NB} of *M. coruscus* was 0.40 slykes at 16℃, 0.47 slykes at 22℃, and 0.29 slykes at 28℃[21]. *Mytilus coruscus* increased hemolymph β_{NB} with rising temperature, but at 28℃ the value decreased to half that of β_{NB} at 23.3℃. The non-bicarbonate buffer value was determined by the buffer capacity of the non-bicarbonate buffer system (for example, protein buffer system), and used to quantify buffering of the solution component[29,30]. *Mytilus coruscus* hemolymph has a greater buffer capacity at 23℃, but this capacity diminishes at temperatures over 28℃. In other bivalves, hemolymph β_{NB} in *M. edulis* was 0.4 slykes at 12℃[16], and 0.65 slykes in *M. galloprobicialis* at 18℃[17], 0.46 slykes in *P. fucata martennsii* at 20℃[20], and 0.73-0.88 slykes in *C. gigas* at 15-23℃[18,19]. *Mytilus coruscus* hemolymph β_{NB} lies in the range of other bivalves, and the hemolymph buffer capacity of the non-bicarbonate buffer system reflects the Mitilid species[21].

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安静状態のイガイ*Mytilus coruscus*酸塩基平衡における ヘモリンパ液の二酸化炭素分圧と重炭酸イオン濃度

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和文要旨：我々は，安静状態におけるイガイ*Mytilus coruscus* のヘモリンパ液の酸塩基平衡を調べた。水温18℃でのヘモリンパ液 pH は7.601±0.023（平均値±標準偏差），全炭酸含量（Tco_2）は1.59±0.06 mM/Lを示した。水温23℃ではpH 7.568±0.031，Tco_2 1.54±0.09 mM/Lを示した。ヘモリンパ液の二酸化炭素分圧（Pco_2）と重炭酸イオン濃度（$[HCO_3^-]$）は，温度との関係式から推定された炭酸解離恒数（pKapp）を使用して計算された。Pco_2と$[HCO_3^-]$ は水温18℃で1.77±0.07 torrと1.50±0.06 mM/L，水温23℃で1.83±0.08 torrと1.47±0.09 mM/Lを示した。推定したpKappを使い算出したPco_2を検証するため，本研究の*in vitro*実験で決定したpKappを用いてPco_2を計算した。異なる方法で算出した2つのPco_2に統計的な有意差は認められなかった。これらのことから，イガイヘモリンパ液のpKappを温度との関係式から推定することは，Pco_2と$[HCO_3^-]$の算出に有効と判断された。イガイヘモリンパ液の非重炭酸緩衝価（β_{NB}）は18℃で0.42 slykes，23℃で0.54 slykesであり，他のイガイ類の緩衝能をよく反映していた。