Estimation of α co2 and pKapp of hemolymph acid–base balance in Mytilus coruscus between 16℃ and 28℃

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Estimation of αco_2 and pKapp of hemolymph acid–base balance in *Mytilus coruscus* between 16℃ and 28℃

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Abstract: The influence of temperature on the hemolymph CO_2 solubility coefficient (αco_2) and the apparent dissociation constant of carbonic acid (pKapp) in *Mytilus coruscus* was investigated. *Mytilus coruscus* hemolymph was equilibrated with standard CO_2 gas mixtures to obtain expressions for αco_2 and pKapp as a function of temperature. The relationship between αco_2 and temperature (T) is expressed as follows: $\alpha co_2 = 138.247 - 11.253 \cdot T + 0.554 \cdot T^2 - 0.0140 \cdot T^3 + 0.000138 \cdot T^4$. The following relationship between pKapp and temperature was found: $pKapp = 6.6407 - 0.01589 \cdot T$. The parameter units are ℃ for T and μM/L/torr for αco_2. These equations enable estimation of hemolymph αco_2 and pKapp at arbitrary temperatures and simple calculation of Pco_2 and $[HCO_3^-]$.

Key words: *Mytilus coruscus,* hemolymph acid–base balance, CO_2 solubility (αco_2), apparent dissociation constant (pKapp), temperature effect, normoxia

Introduction

The hard-shelled mussel *Mytilus coruscus* is a Mytilidae bivalve classified under the order Mytiloida within the subclass Pteriomorphia[1]. In Japan, *M. coruscus* inhabits the rocky bottom of intertidal zones from Hokkaido to Kyushu[1]. Collected in the littoral zone, it is considered a premium seafood and is referred to as "Sendai-gai" in Miyagi prefecture, or "Seto-gai" in Yamaguchi prefecture.

Mytilus coruscus was previously studied in terms of larvae morphology[2], polymorphic microsatellite loci[3], microsatellite markers[4], influence of natural biofilm on the settlement mechanism[5], immune activities of hemocytes[6], hybrid molecular identification[7], light-responsive genes[8], and marine environment[9]. In the context of respiratory physiology, the response to air exposure on hemolymph acid–base balance was studied, indicating a slow, partial compensation of metabolic acidosis[10]. However, for *M. corscus* hemolymph, there are few reports evaluating CO_2 dynamics and acid–base balance with respect to the influence of temperature on the CO_2 solubility coefficient (αco_2) and apparent dissociation constant of carbonic acid (pKapp).

Under normoxic and normocapnic conditions, in blue mussel *M. edulis*, the hemolymph CO_2 partial pressure (Pco_2) was 0.9 torr[11], for Akoya pearl oyster *Pinctada fucata martensii* it was 1.7-2.3 torr[12,13], and 1.55 torr for the noble scallop *Mimachlamys nobilis*[14]. As bivalves generally have very low Pco_2 values, the same was expected for Pco_2 in *M. coruscus*; however, direct Pco_2 measurement is difficult. Estimation of Pco_2 via the Henderson–Hasselbalch equation is often used in studying acid–base balance owing to its ease and accuracy[15]. In the equation, αco_2 and pKapp values are required for the experimental animal. As temperature influences αco_2 and pKapp[15], it is an important factor in determining *M. coruscus* hemolymph αco_2 and pKapp. Therefore, the effect of temperature on *M. coruscus* hemolymph αco_2 and pKapp was investigated. For *M. coruscus*, a simple relationship between temperature, hemolymph αco_2, and pKapp allows Pco_2 calculation with ease, contributing to understanding the relationships between acid–base balance, respiratory physiology, and aquaculture environments.

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Materials and Methods

Experimental animals and conditions

These experiments used 85 hard-shelled mussel *M. corsucus* (mean wet weight: 180 g). They were 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 with seasonal variations (16-28℃) and fed cultivated phytoplankton[16-18]. 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 29 psu, O_2 saturation 96%, pH 8.0, and a total CO_2 concentration of 1.5 mM/L.

Hemolymph collection

Adductor muscle hemolymph was collected anaerobically by direct puncture with a gas-tight microsyringe (Model 1750LTN, Hamilton Co.), collecting approximately 0.3-0.4 mL. The hemolymph was used for analyses of αco_2 and pKapp in *in vitro* experiments.

Experimental protocols

Analysis of αco_2 used hemolymph adjusted to pH2.5 by lactic acid (Wako Pure Chemical Industries, Ltd.) addition. The acidified sample was transferred to a tonometer flask and equilibrated with humidified standard CO_2 gas (CO_2, 5.0% or 15%; O_2, 20.9%; N_2 Balance) using an equilibrator (DEQ-1, Cameron Instruments) at experimental temperature. After equilibration, the sample Tco_2 was measured (n=41). The Pco_2 of the equilibrated sample was calculated using the known standard gas CO_2 concentration, barometric pressure, and water vapor pressure. The αco_2 was calculated using the equation:

$$\alpha co_2 = Tco_2 \bullet Pco_2^{-1}$$

For pKapp determination, the hemolymph was used immediately after collection for tonometry analysis. Hemolymph was equilibrated with humidified standard CO_2 mixes (CO_2, 0.2-5.0%; O_2, 20.9%; N_2 Balance) with the equilibrator at 16℃ (n=25), 22℃ (n=30), or 28℃ (n=30). After equilibration, the pH and Tco_2 of the sample were measured. Using the sample pH, Tco_2, and αco_2, calculated from the above equation, pKapp was determined by rearrangement of the Henderson–Hasselbalch equation[15,19] as follows:

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

where Pco_2 was calculated from the known CO_2 concentration of standard gases.

Hemolymph analysis

Tco_2 was measured using a total CO_2 analyzer (Capnicon 5, Cameron Instruments). 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. Hemolymph Pco_2 and $[HCO_3^-]$ were calculated by rearranging the Henderson–Hasselbalch equation[15,19]. The obtained αco_2 and pKapp were then used to calculate hemolymph Pco_2 from pH and Tco_2:

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

The bicarbonate ion ($[HCO_3^-]$) concentration was calculated from Tco_2, αco_2 and Pco_2, or from αco_2, Pco_2, pH, and pKapp using the equations:

$$[HCO_3^-] = Tco_2 - \alpha co_2 \bullet Pco_2$$
$$[HCO_3^-] = \alpha co_2 \bullet Pco_2 \bullet 10^{(pH - pKapp)}$$

To assess the relationship between hemolymph pH and $[HCO_3^-]$ in the experimental animals, the non-bicarbonate buffer values (β_{NB}) were calculated from the slope of the relational expression between pH and $[HCO_3^-]$.

Statistical analysis

The Kruskal–Wallis test was performed for changes in hemolymph sample properties and the calculated pKapp. Multiple comparison for all pairs used the Steel–Dwass test. 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 and Discussion

The influence of temperature on *M. coruscus* hemolymph αco_2 and pKapp was investigated, and the relationship clarified. The hemolymph αco_2 of *M. coruscus* are shown in Fig. 1. Analysis of samples over 30℃ used hemolymph collected from animals reared at 28℃, and sample analysis at 15℃ used hemolymph collected from animals reared at 16℃. The mean αco_2 are 29.1－54.3 μM/L/torr between 15℃ and 34℃. Cameron (1986) reported CO_2 solubility as a function of temperature and salinity, with solubility coefficients of 31.58－53.45 mM/L/torr at a salinity of 30 between 14℃ and 34℃[20]. The obtained hemolymph αco_2 reflected that reported by Cameron (1986)[20]. Although the information with respect to this point is limited, the hemolymph αco_2 decreased with increasing temperature in *M. coruscus*, which matched responses of other animals; hemolymph of disk abalone *Haliotis (Nordotis) discus discus* under 20℃[21] and plasma of rainbow trout *Salmo gairdneri* under 15℃[15]. In the hemolymph of *M. coruscus*, a polynomial equation is fitted to the αco_2 data (Fig. 1), with this hemolymph αco_2 equation estimating αco_2 within the temperature range. The relationship between αco_2 and temperature is expressed as follows:

$$\alpha co_2 = 138.2475 - 11.2533 \bullet T + 0.553901 \bullet T^2 - 0.01399 \bullet T^3 + 0.000138 \bullet T^4 \quad (R^2 = 0.9384)$$

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

Hemolymph pKapp at each temperature is shown with corresponding Pco_2, pH, and Tco_2 (Tables 1-3). The mean hemolymph pKapp were 6.38068 at 16℃, 6.30234 at 22℃, and 6.18990 at 28℃. These mean values enable calculation of hemolymph Pco_2 and $[HCO_3^-]$ at each temperature.

Hemolymph properties and pKapp at known Pco_2 are shown in Tables 1-3. Hemolymph pH and pKapp significantly change with increasing Pco_2 at each temperature ($P < 0.05$, Kruskal–Wallis test). A polynomial equation was fitted to the mean values of pKapp and pH, yielding a relationship between pKapp and pH as follows:

$$16℃ \quad pKapp = -32.557 + 12.885 \bullet pH - 1.265 \bullet pH^2 - 0.0316 \bullet pH^3 \quad (R^2 = 0.9983)$$

$$22℃ \quad pKapp = 144.863 - 64.994 \bullet pH + 10.106 \bullet pH^2 - 0.521 \bullet pH^3 \quad (R^2 = 0.9787)$$

$$28℃ \quad pKapp = 537.058 - 241.340 \bullet pH + 36.558 \bullet pH^2 - 1.845 \bullet pH^3 \quad (R^2 = 0.8827)$$

These equations enable pKapp estimation for each temperature, with hemolymph Pco_2 and $[HCO_3^-]$ calculation by Henderson–Hasselbalch equation rearrangement.

The distribution of pKapp and corresponding pH is shown for each temperature in Fig. 2. The distribution of pKapp at 28℃ was different to that at 16℃ and 22℃ ($P < 0.05$, Steel–Dwass test). There was no significant difference in pH distribution. The linear regression was fitted to the mean values of pKapp and temperature (Fig. 3), and the relationship between pKapp and temperature expressed as follows:

$$pKapp = 6.6407 - 0.01589 \bullet T \quad (R^2 = 0.9894)$$

where T is temperature in ℃ .

This equation estimates hemolymph pKapp at arbitrary temperatures (between 16-28℃). Thus, the hemolymph Pco_2 and $[HCO_3^-]$ may be calculated for a range of physiologically relevant temperatures.

Hemolymph pH and calculated $[HCO_3^-]$ with the Pco_2 of standard gases are listed in Table 4. The non-bicarbonate buffer values (β_{NB}), obtained as a regression coefficient relating pH and $[HCO_3^-]$, were 0.40 slykes at 16℃ , 0.47 slykes at 22℃ , and 0.29 slykes at 28℃ . 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[22,23]. Therefore, the hemolymph of *M. coruscus* has a greater buffer capacity at 16℃ and 20℃ than at 28℃. For other mussels, hemolymph β_{NB} in *M. edulis* was 0.4 slykes at 12℃[11], and 0.65 slykes in *M. galloprobicialis* at 18℃[24]. The hemolymph β_{NB} of *M. coruscus* reflected other mussels, with the hemolymph buffer capacity of the non-bicarbonate buffer system expected to reflect the Mitilid species.

In *M. coruscus*, the effect of temperature on hemolymph αco_2 and pKapp were investigated, values required to calculate Pco_2 and $[HCO_3^-]$. As temperature strongly affects hemolymph αco_2 and pKapp in *M. coruscus*, the proposed equations enable estimation of hemolymph αco_2 and pKapp at arbitrary temperatures and calculation of both Pco_2 and $[HCO_3^-]$ with relative ease. Boutilier et al. (1985) described variations in plasma αco_2 and pKapp for rainbow trout in response to temperature and ionic strength, with pKapp changes influenced by plasma pH[15]. If it is possible to similarly discuss acid-base balance of the fish and the mussel, further exploration of the acid-base balance under resting conditions and relationships among temperature, pH and pKapp of *M. coruscus* is necessary.

Table 1. 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 16℃

Standard gas		Hemolymph			
CO_2 (%)	Pco_2 (torr)	pH	Tco_2 (mM/L)	pKapp	n
0.2	1.5	7.538	1.67	6.2197382	5
0.5	3.7	7.305	1.86	6.3690807	5
1.0	7.4	7.119	2.13	6.4608385	5
2.0	14.7	6.794	2.29	6.4940144	5
5.0	36.8	6.382	3.95	6.3597565	5

Water temperature, 16.0℃; Mean value of pKapp, 6.3806857.

Table 2. 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 22℃

Standard gas		Hemolymph			
CO_2 (%)	Pco_2 (torr)	pH	Tco_2 (mM/L)	pKapp	n
0.2	1.5	7.485	1.56	6.117095	6
0.5	3.7	7.297	1.66	6.331092	6
1.0	7.5	7.088	1.92	6.383487	6
2.0	14.9	6.777	2.10	6.412913	6
5.0	37.3	6.355	3.57	6.267141	6

Water temperature, 21.8℃; Mean value of pKapp, 6.3023457.

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 28℃

Standard gas		Hemolymph			
CO_2 (%)	Pco_2 (torr)	pH	Tco_2 (mM/L)	pKapp	n
0.2	1.5	7.196	1.02	5.924225	6
0.5	3.7	7.091	1.14	6.197768	6
1.0	7.3	6.830	1.29	6.227287	6
2.0	14.6	6.589	1.50	6.307682	6
5.0	36.6	6.277	2.53	6.292557	6

Water temperature, 28.0℃ ; Mean value of pKapp, 6.1899038.

Table 4. Mean values of hemolymph pH and bicarbonate concentration ($[HCO_3^-]$) of *Mytilus coruscus* with known Pco_2 standard gases at 16-28℃

Standard gas	16°C		22°C		28°C	
CO_2 (%)	pH	$[HCO_3^-]$ (mM/L)	pH	$[HCO_3^-]$ (mM/L)	pH	$[HCO_3^-]$ (mM/L)
0.2	7.538	1.59	7.485	1.49	7.196	0.97
0.5	7.305	1.66	7.297	1.50	7.091	1.01
1.0	7.119	1.75	7.088	1.60	6.830	1.03
5.0	6.382	2.04	6.355	1.99	6.277	1.24

The non-bicarbonate buffer value (β_{NB}), 0.40 slykes at 16℃ ; 0.47 slykes at 22℃ ; 0.29 slykes at 28℃.

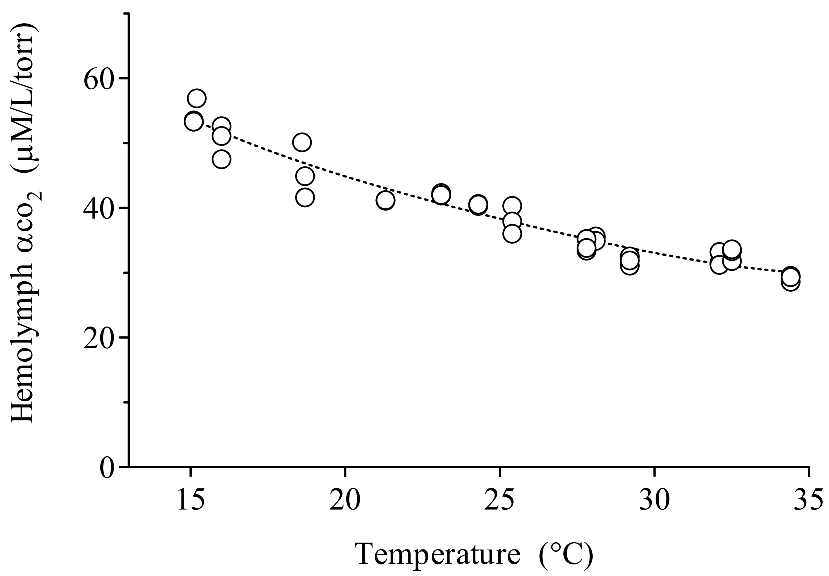

Fig. 1. Influence of temperature on CO_2 solubility coefficient (αco_2) for *Mytilus corsucus* hemolymph. Data is the calculation value. The dotted line is fitted to the data and the equation: $\alpha co_2 = 138.2475 - 11.2533 \bullet T + 0.553901 \bullet T^2 - 0.01399 \bullet T^3 + 0.000138 \bullet T^4$ ($R^2 = 0.9384$, n=41).

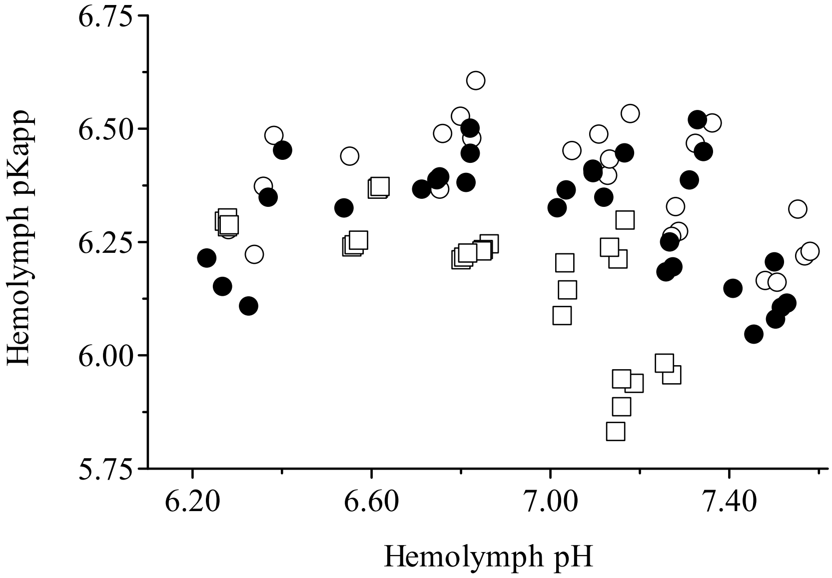

Fig. 2. The pKapp distribution and corresponding pH of the hemolymph in *Mytilus corsucus* at 16℃, 22℃ and 28℃. Data is the calculation value. Open circle, 16℃ (n=25); solid circle, 22℃ (n=30); open square, 28℃ (n=30). The pKapp distribution at 28℃ was different to that at 16℃ and 22℃ ($P < 0.05$, Steel–Dwass multiple comparison test).

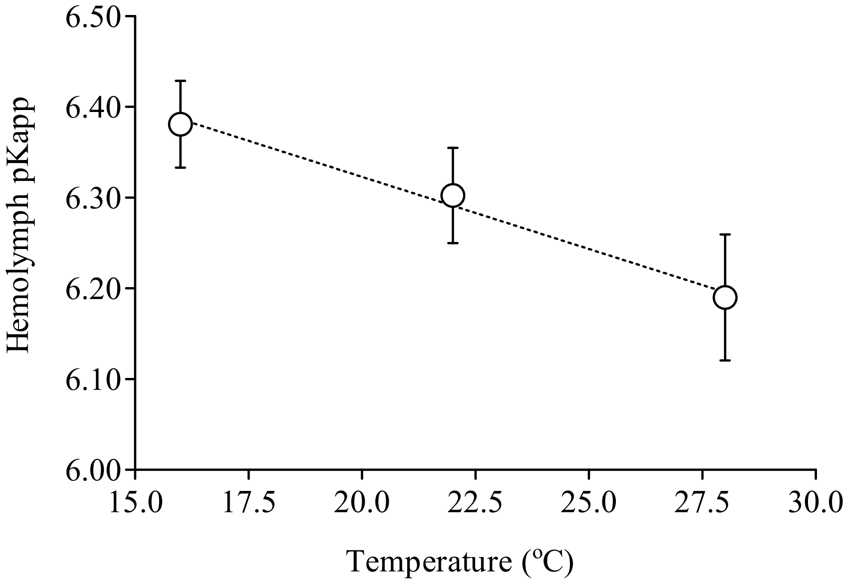

Fig. 3. Influence of temperature on the apparent carbonic acid dissociation constant (pKapp) of *Mytilus corsucus* hemolymph between 16℃ and 28℃. Data are Mean ± standard error. The dotted line is fitted to the data and the equation: pKapp = 6.6407 – 0.01589 • T (R^2 = 0.9894).

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水温16℃から28℃のイガイにおける
ヘモリンパ液の二酸化炭素溶解度と炭酸解離恒数の推定

半田岳志，荒木　晶

和文要旨：イガイの呼吸機能，特に酸塩基平衡を評価するため，イガイ閉殻筋から採取したヘモリンパ液を用いて，二酸化炭素溶解度（αco_2）と炭酸解離恒数（pKapp）に及ぼす温度の影響について調査した。各実験温度において，イガイのヘモリンパ液を二酸化炭素標準ガスと平衡させ，pHと全炭酸含量を測定し，温度（T）とαco_2あるいはpKappとの関係を分析したところ，以下の関係式を得た。$\alpha co_2 = 138.247 - 11.253 \bullet T + 0.554 \bullet T^2 - 0.0140 \bullet T^3 + 0.000138 \bullet T^4$，$pKapp = 6.6407 - 0.01589 \bullet T$（$\alpha co_2$: μM/L/torr; T: ℃）。これらの式により，任意の温度でイガイのヘモリンパ液におけるαco_2とpKappの推定が可能となった。これら推定値を用いれば，微量なヘモリンパ液の二酸化炭素分圧や炭酸水素イオン濃度を任意の温度で把握することができるだろう。