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## Oxygen and Acid–Base Status of Hemolymph in the Pacific oyster *Crassostrea gigas* after Cannulation of the Adductor Muscle

Takeshi Handa<sup>†</sup>, Akira Araki and Ken-ichi Yamamoto

**Abstract** : We examined hemolymph O<sub>2</sub> partial pressure, pH, total CO<sub>2</sub> content, CO<sub>2</sub> partial pressure and bicarbonate concentration in order to evaluate the acid–base balance of the Pacific oyster *Crassostrea gigas* after pretreatment of the adductor muscle by cannulation. The hemolymph O<sub>2</sub> and acid–base properties changed just after surgery. The temporary and significantly fluctuation of hemolymph properties disappeared at 1 h after surgery in this study, and the O<sub>2</sub> and acid–base status was stable afterwards in normoxic conditions. The results in this study showed the possibility that sampling with a cannula can collect hemolymph as required. This sampling may be useful, when respiratory and endocrine function must be monitored in minimally disturbed animals without the effects of handling.

**Key words** : *Crassostrea gigas*, Pacific oyster, cannulation, hemolymph, acid–base balance, adductor muscle

### Introduction

Pacific oyster *Crassostrea gigas* is a Ostreidae bivalve classified in the Pterioidea, Pteriomorpha,<sup>1)</sup> and is widely distributed in Japan and East Asia.<sup>1)</sup> Pacific oyster inhabits the intertidal and subtidal gravel to mud bottom of brackish–water embayments, and it often forms oyster reefs.<sup>1)</sup> The Pacific oyster is an important cultured species for food, and it is cultivated in many countries. In Japan, the production volume of Pacific oyster is greatest in Hiroshima, Okayama, Hyogo, and Miyagi prefectures. Pacific oyster has been the subject of previous research in terms of anatomy and respiratory physiology. The anatomical structures of the digestive diverticula, ctenidium, and circulatory system were clarified recently.<sup>2,3)</sup> The regulation of ventilation volume, O<sub>2</sub> uptake, and ciliary movement of the ctenidium in normoxic, hypoxic, hypotonic, anathermal, and feeding conditions has been studied.<sup>4–8)</sup> However, there are few reports on the respiratory mechanism from the viewpoint of CO<sub>2</sub> dynamic phase and acid–base balance in Pacific oyster. Research into the acid–base status could contribute to efficient CO<sub>2</sub> utilization, which is related to

respiration, and calcification for the formation of the shell valves. The acid–base balance and CO<sub>2</sub> dynamic phase of Pacific oyster was useful for the evaluation of cultivation environments, and of the effects of ocean acidification and increasing CO<sub>2</sub> levels. In some marine bivalves in normoxic and normocapnic conditions, the CO<sub>2</sub> partial pressure (Pco<sub>2</sub>) of the hemolymph was 0.57–2.3 torr (mmHg).<sup>9–15)</sup> The hemolymph Pco<sub>2</sub> of Pacific oyster was supposed be low and similar to other bivalves, and, therefore, direct measurements of Pco<sub>2</sub> would be difficult. The estimation of Pco<sub>2</sub> by application of the Henderson–Hasselbalch equation is practiced in studies of acid–base balance owing to the relative ease and accuracy of the estimates.<sup>16)</sup> In the equation, the characteristic values of the CO<sub>2</sub> solubility coefficient ( $\alpha_{\text{CO}_2}$ ) and apparent dissociation constant of carbonic acid (pK<sub>app</sub>) in the hemolymph were required for experimental animals. Therefore, we determined hemolymph  $\alpha_{\text{CO}_2}$  and pK<sub>app</sub>, and estimated hemolymph Pco<sub>2</sub> and bicarbonate concentration ([HCO<sub>3</sub><sup>-</sup>]). In order to accurately measure the hemolymph properties (O<sub>2</sub> and acid–base status), hemolymph was collected from submerged Pacific oysters. We developed a hemolymph withdrawal method using a cannula. The

surgical procedure for collecting hemolymph involved cannulation of the adductor muscle, and hemolymph was collected anaerobically through the cannula from submerged Pacific oysters. This study evaluated the effect of the surgical procedures on the basis of the change of the hemolymph properties,  $O_2$  and acid-base status, in the Pacific oyster *C. gigas*. The technical knowledge proposed in this study may contribute to the advances in research on respiratory physiology and homeostasis in Pacific oyster.

## Materials and Methods

### *Experimental animals and conditions*

The experiments used 29 Pacific oyster *Crassostrea gigas* (shell length:  $58.9 \pm 1.4$  mm (mean  $\pm$  SE), shell height:  $123.6 \pm 3.1$  mm, total wet weight:  $113.2 \pm 5.2$  g). The animals were obtained from a marine farm in the western sea area of Hiroshima Prefecture, Japan. After cleaning the shell valves, they were reared for 1 month at  $10^\circ\text{C}$  in aerated seawater with added cultivated phytoplankton.<sup>3-5</sup> Twenty-four hours before collecting hemolymph, the Pacific oysters were transferred to particle-free ( $>0.45 \mu\text{m}$ ) seawater. All experiments were conducted in seawater with a salinity of 32 psu, water temperature  $10^\circ\text{C}$ ,  $O_2$  saturation 99%, pH 8.18, and total  $\text{CO}_2$  content 1.3 mM/L.

### *Surgical procedures*

Hemolymph was collected from the adductor muscle using a cannula (polyethylene tubing, 0.96 mm outer diameter, 0.58 mm inner diameter, PE-50, Clay Adams). A small hole (2 mm diameter) was made adjacent to the shell valves near the adductor muscle at the posterior margin. A cannula with a stylet was inserted through the hole into the adductor muscle and was advanced 5 mm toward the center of the adductor muscle. The stylet was removed, and the end of the cannula was closed. The cannula was gently fixed to the right shell valve using denture adhesive (Kobayashi Pharmaceutical Co., Ltd.) in order to prevent any effect of the movement of the

shell valves. This surgical procedure was completed within 7 minutes. The cannulated oyster was transferred to a respiratory chamber at  $10.1 \pm 0.2^\circ\text{C}$  in normoxic conditions.

### *Hemolymph collection*

Multiple collections of hemolymph were conducted at 0 h (initial collection), 0.5 h, 1 h, 2 h, 3 h, 24 h, and 48 h after surgery (N=6). Single collection of hemolymph was conducted at 24 h after surgery (N=6). A hemolymph sample was drawn through the cannula using a gas-tight micro syringe (Model 1750, Hamilton Co.). The volume of hemolymph collected was 0.3-0.4 mL in each time.

### *Hemolymph properties analysis*

The hemolymph  $O_2$  partial pressure ( $P_{O_2}$ , torr), pH, and total  $\text{CO}_2$  content ( $T_{\text{CO}_2}$ , mM/L) were measured immediately after each collection. The pH was measured using a blood gas meter (BGM200; Cameron Instruments) with glass and reference electrodes (E301, E351; Cameron Instruments) at  $10.0 \pm 0.2^\circ\text{C}$ .  $T_{\text{CO}_2}$  was measured using a total  $\text{CO}_2$  analyzer (Capnicon 5; Cameron Instruments). The hemolymph  $\text{CO}_2$  partial pressure ( $P_{\text{CO}_2}$ , torr) and bicarbonate concentration ( $[\text{HCO}_3^-]$ , mM/L) were calculated by rearranging the Henderson-Hasselbalch equation.<sup>17</sup> In the equation, the  $\alpha_{\text{CO}_2}$  and  $\text{pK}_{\text{app}}$  of the Pacific oyster hemolymph were required. The determinations of  $\alpha_{\text{CO}_2}$  and  $\text{pK}_{\text{app}}$  were performed by *in vitro* experiments.

The  $\alpha_{\text{CO}_2}$  was determined using Pacific oyster hemolymph adjusted to pH 2.5 by the addition of lactic acid (Wako Pure Chemical Industries, Ltd.). The acidified sample was transferred to a tonometer flask, and equilibrated with humidified standard  $\text{CO}_2$  gas ( $\text{CO}_2$ , 15.0%;  $O_2$ , 20.9%;  $N_2$  Balance) using the equilibrator (DEQ-1; Cameron Instruments) at  $10.0 \pm 0.3^\circ\text{C}$ , and subsequently the  $T_{\text{CO}_2}$  of each equilibrated sample was measured using the total  $\text{CO}_2$  analyzer. The  $P_{\text{CO}_2}$  of the equilibrated sample was calculated from a known  $\text{CO}_2$  concentration standard gas (15%), prevailing barometric pressure, and water vapor pressure at the experimental

temperature.  $\alpha\text{CO}_2$  was calculated using the equation:

$$\alpha\text{CO}_2 = \text{Tco}_2 \cdot \text{Pco}_2^{-1}$$

For determination of the pKapp, hemolymph was transferred to a tonometer flask and equilibrated with humidified standard  $\text{CO}_2$  gases ( $\text{CO}_2$ , 0.2%, 0.5%, 1.0%, 2.0%, 5.0%, and 15%;  $\text{O}_2$ , 20.9%;  $\text{N}_2$  balance) using an equilibrator at  $10.1 \pm 0.3^\circ\text{C}$ . After equilibration, the pH and  $\text{Tco}_2$  of the sample were measured using the blood gas meter and the total  $\text{CO}_2$  analyzer. Using the sample pH,  $\text{Tco}_2$  and  $\alpha\text{CO}_2$  calculated using the above equation, pKapp was determined by rearrangement of the Henderson-Hasselbalch equation<sup>17)</sup> as follows:

$$\text{pKapp} = \text{pH} - \log \left[ \frac{(\text{Tco}_2 - \alpha\text{CO}_2 \cdot \text{Pco}_2)}{\alpha\text{CO}_2 \cdot \text{Pco}_2} \right]$$

where  $\text{Pco}_2$  was calculated from the known  $\text{CO}_2$  concentration of standard gases.

The  $\alpha\text{CO}_2$  and pKapp obtained in this study were used for the calculation of hemolymph  $\text{Pco}_2$  from measured pH and  $\text{Tco}_2$ :

$$\text{Pco}_2 = \text{Tco}_2 \cdot \left[ \alpha\text{CO}_2 \cdot (1 + 10^{(\text{pH} - \text{pKapp})}) \right]^{-1}$$

The hemolymph  $[\text{HCO}_3^-]$  was calculated from  $\text{Tco}_2$ ,  $\alpha\text{CO}_2$ , and  $\text{Pco}_2$  using the following equation<sup>18)</sup>:

$$[\text{HCO}_3^-] = \text{Tco}_2 - \alpha\text{CO}_2 \cdot \text{Pco}_2$$

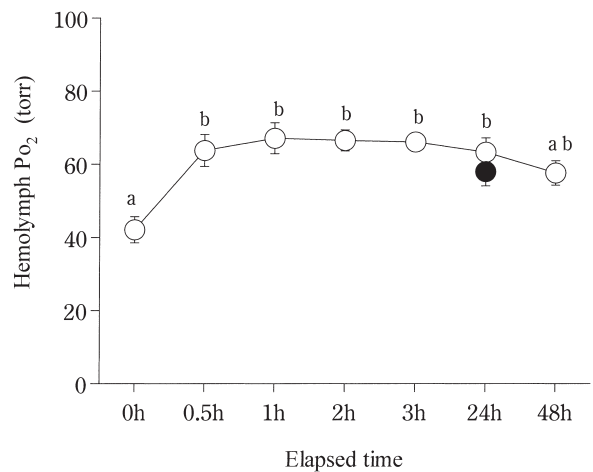
### Statistical analysis

All data are expressed as mean  $\pm$  standard error. Normality of distribution in hemolymph properties was assessed through use of the Shapiro-Wilk test. For the test of the fluctuation of the hemolymph properties in multiple collections, two-way repeated measures analysis of variance and one-way analysis of variance were used with normal distributions. Multiple comparison test used the Tukey-Kramer's test. Unpaired  $t$ -test was used for the comparison of mean values of hemolymph properties between the multiple collections and a single collection.

For non-normal distributions, multiple comparison test used the Friedman test. Wilcoxon rank-sum test was used for the comparison of hemolymph properties between the multiple collections and a single collection. In *in vitro* experiments, one-way analysis of variance was performed for changes in hemolymph properties using the standard  $\text{CO}_2$  gases. Statistically significant differences were set at  $P < 0.01$ .

## Results

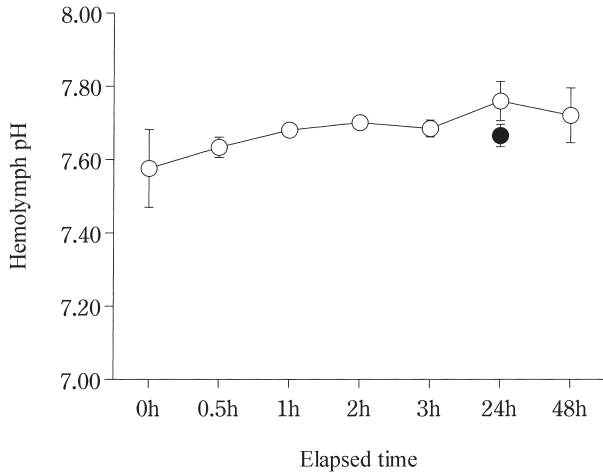
Hemolymph was collected from the adductor muscles of Pacific oyster through cannulae. The mean values of hemolymph  $\text{Po}_2$  were statistically significantly increased from 0 h to 0.5 h, and  $\text{Po}_2$  was 57.6–67.1 torr at 1 h or later (Fig. 1). The hemolymph pH and  $\text{Tco}_2$  were 7.576–7.760 and 1.36–1.50 mM/L, respectively (Figs. 2–3). In *in vitro* experiments, the hemolymph  $\alpha\text{CO}_2$  was  $59.11 \pm 0.98 \mu\text{M/L/torr}$  ( $N=9$ ), and the hemolymph pKapp was  $6.3158 \pm 0.0374$  ( $N=8$ ).  $\text{Pco}_2$  and  $[\text{HCO}_3^-]$  were calculated by substitution of the mean value of hemolymph  $\alpha\text{CO}_2$  and



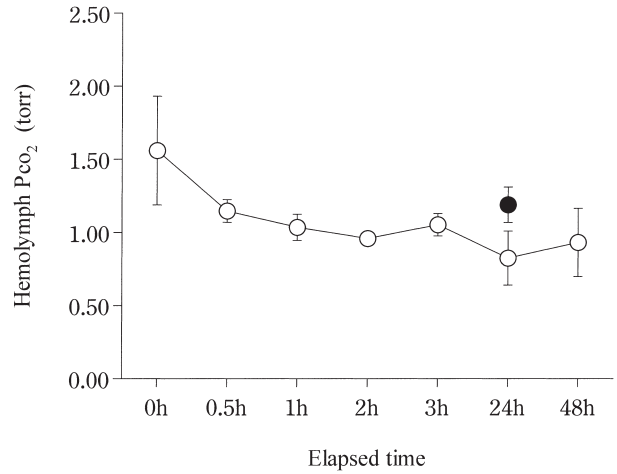
**Fig. 1.** Hemolymph oxygen partial pressure ( $\text{Po}_2$ , torr) in Pacific oyster *Crassostrea gigas* after surgical procedures (cannulation of the adductor muscle). The time indicates the time elapsed from when the surgical procedure and the transfer of the experimental animal to a respiratory chamber were completed. The values shown are means  $\pm$  SE. Each value from multiple and single collections is shown as open circles and closed circles, respectively. Different letters indicate statistically significant differences from the other values ( $P < 0.01$ ).

pKapp in the rearranged Henderson-Hasselbalch equation. The hemolymph  $P_{CO_2}$  decreased slightly from 0 h to 0.5 h and was 0.82–1.05 torr at 1 h or later (Fig. 4).  $[HCO_3^-]$  was 1.33–1.44 mM/L (Fig. 5). The fluctuation

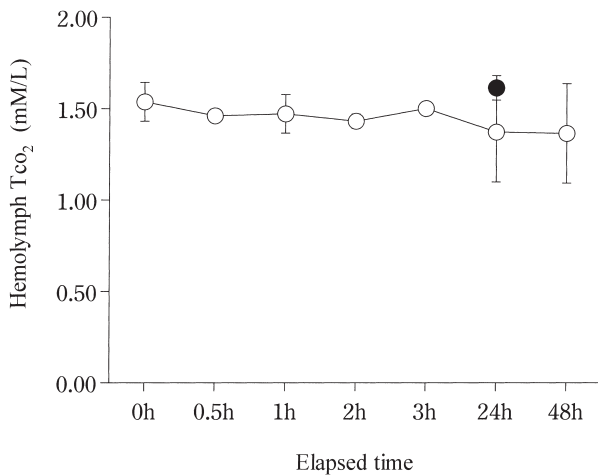
of hemolymph properties in the multiple collection were not statistically significant, except for  $P_{O_2}$  at 0 h. There was no significant difference in the multiple collections and the single collection (Figs. 1–5).



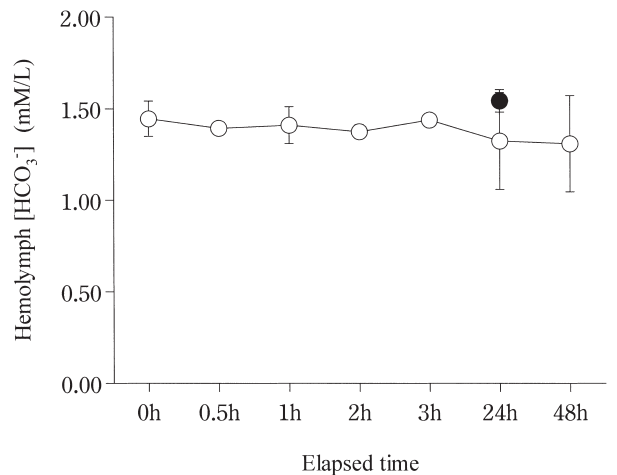
**Fig. 2.** Hemolymph pH in Pacific oyster *Crassostrea gigas* after surgical procedures (cannulation of the adductor muscle). The time indicates the time elapsed from when the surgical procedure and the transfer of the experimental animal to a respiratory chamber were completed. The values are shown means ± SE. The symbols are the same as in Fig. 1. There were no statistically significant differences in each value.



**Fig. 4.** Hemolymph  $CO_2$  partial pressure ( $P_{CO_2}$ , torr) in Pacific oyster *Crassostrea gigas* after surgical procedures (cannulation to the adductor muscle). The time indicates the time elapsed from when the surgical procedure and the transfer of the experimental animal to a respiratory chamber were completed. The values are shown means ± SE. The symbols are the same as in Fig. 1. There were no statistically significant differences in each value.



**Fig. 3.** Hemolymph total  $CO_2$  content ( $T_{CO_2}$ , mM/L) in Pacific oyster *Crassostrea gigas* after surgical procedures (cannulation of the adductor muscle). The time indicates the time elapsed from when the surgical procedure and the transfer of the experimental animal to a respiratory chamber were completed. The values are shown means ± SE. The symbols are the same as in Fig. 1. There were no statistically significant differences in each value.



**Fig. 5.** Hemolymph bicarbonate concentration ( $[HCO_3^-]$ , mM/L) in Pacific oyster *Crassostrea gigas* after surgical procedures (cannulation of the adductor muscle). The time indicates the time elapsed from when the surgical procedure and the transfer of the experimental animal to a respiratory chamber were completed. The values are shown means ± SE. The symbols are the same as in Fig. 1. There were no statistically significant differences in each value.

## Discussion

We carried out repeated collections of hemolymph from the adductor muscle of the Pacific oyster through cannulae and measured hemolymph  $P_{O_2}$ , pH, and  $T_{CO_2}$ . The hemolymph  $\alpha_{CO_2}$  and  $pK_{app}$  were determined by *in vitro* experiments using a tonometer and  $CO_2$  standard gases. The  $\alpha_{CO_2}$  and  $pK_{app}$  obtained in this study were used for the calculation of hemolymph  $P_{CO_2}$  and  $[HCO_3^-]$ . The  $P_{O_2}$ , pH,  $T_{CO_2}$ ,  $P_{CO_2}$ , and  $[HCO_3^-]$  of hemolymph in the multiple collections were stable by 1 h after the surgical procedure. The hemolymph properties in the multiple collections were not significantly different from those in the single collection. These findings indicated the  $O_2$  and acid-base status were not greatly influenced at 1 h after the surgical procedure in these experimental conditions.

The hemolymph  $P_{O_2}$  in Pacific oyster just after surgery (0 h) was significantly lower than that at 0.5 h or later because the experimental animals were exposed to air and closed shell valves during surgery. When marine bivalves<sup>9,11,19-21)</sup> and freshwater bivalve<sup>22)</sup> close their shell valves or are exposed to the air, the  $O_2$  partial pressure of body fluid decreased rapidly but pH decreased slowly. Therefore, the hemolymph in this study appeared to undergo temporary mild hypoxemia just after surgery.  $P_{CO_2}$  just after surgery (0 h) was slightly higher than that after 0.5 h and later. Handa and Yamamoto (2011) indicated that akoya pearl oyster *Pinctada fucata martensii* increased hemolymph  $P_{CO_2}$  during cannulation surgery of the anterior aorta, and the possibility of inhibition of  $CO_2$  discharge during the surgery.<sup>11)</sup> In some bivalves, the discharge of  $CO_2$  was inhibited and accumulated during air exposure.<sup>9,11,19-21)</sup> The accumulated  $CO_2$  titrated toward acidity and reduced the hemolymph pH in akoya pearl oyster *P. fucata martensii*, and the akoya pearl oysters showed temporary respiratory acidosis during air exposure for the surgery.<sup>11)</sup> However, the Pacific oyster did not show acidosis, although temporary mild hypoxemia was shown in this study. The experimental temperature in this study was 10°C, which was lower than the previous study (28°C) in akoya pearl

oyster.<sup>11)</sup> The time required for the surgical procedure was 7 minutes in Pacific oyster adductor muscle and 15 min in akoya pearl oyster anterior aorta. In this study,  $CO_2$  production and accumulation in Pacific oyster should be lower because of the differences of the experimental conditions. Therefore, the Pacific oyster showed only temporary mild hypoxemia without respiratory acidosis in this study.

In the Pacific oyster, a temporary and significantly fluctuation in hemolymph properties disappeared at 1 h after surgery in this study, and the  $O_2$  and acid-base status were stable afterwards in normoxic conditions. Pacific oyster required 1 h for a process to return to preoperative levels or a stable state. Hemolymph collection through a cannula from the adductor muscle was useful for research on the respiratory physiology of Pacific oyster *C. gigas*. The results in this study showed the possibility that sampling with a cannula can collect body fluids under various conditions. This sampling may be useful, for example, when the hemolymph containing gases and hormones must be monitored in minimally disturbed animals without the effects of handling. The technical knowledge proposed in this study may contribute to the advances in research on homeostasis and environmental tolerance in Pacific oyster.

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## References

- 1) Hayami I: Ostreidae. *In*: Okutani T (ed) Marine Mollusks in Japan. The second edition, Tokai University Press, Tokyo, 1182-1185 (2017)
- 2) Yamamoto K, Handa T, Kondo M: Trial of corrosion casting to the digestive diverticula of the Pacific oyster *Crassostrea gigas*. *J Nat Fish Univ*, 51, 95-104 (2003)
- 3) Yamamoto K, Handa T: Anatomical structure of

- Ctenidia of the Pacific oyster *Crassostrea gigas*. *J Nat Fish Univ*, **61**, 190–210 (2013)
- 4) Yamamoto K, Adachi S, Tamura I, Aramizu T and Koube H: Effects of hypoxia and water temperature on ciliary movement of gills 5 bivalvia, *Mytilus edulis*, *Atrina pectinata*, *Pinctada fucata martensii*, *Chlamys nobilis* and *Crassostrea gigas*. *J Nat Fish Univ*, **44**, 137–142 (1996)
  - 5) Yamamoto K, Handa T: Effect of hypoxia on ventilation in the Pacific oyster *Crassostrea gigas*. *Aquaculture Sci*, **59**, 1–4 (2011)
  - 6) Yamamoto K, Handa T: Effect of low salinity on ventilation in the oyster *Crassostrea gigas*. *Aquaculture Sci*, **59**, 5–8 (2011)
  - 7) Yamamoto K, Handa T: Effect of hypoxia on oxygen uptake in the Pacific oyster *Crassostrea gigas*. *Aquaculture Sci*, **59**, 199–202 (2011)
  - 8) Yamamoto K, Handa T: Ventilation in the Pacific oyster *Crassostrea gigas* with feeding. *Aquaculture Sci*, **59**, 203–206 (2011)
  - 9) Booth CE, McDonald DG, Walsh PJ: Acid–base balance in the sea mussel, *Mytilus edulis*. I. Effects of hypoxia and air–exposure on hemolymph acid–base status. *Mar Bio Lett*, **5**, 347–358 (1984)
  - 10) Michelidis B, Ozounis C, Paleras A, Portner HO: Effects of long–term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Mar Ecol Prog Ser*, **293**, 109–118 (2005)
  - 11) Handa T, Yamamoto K: The blood acid–base balance in the pearl oyster, *Pinctada fucata martensii*, after the surgery. *J Nat Fish Univ*, **60**, 57–61 (2011)
  - 12) Handa T, Yamamoto K: The acid–base balance of the hemolymph in the pearl oyster *Pinctada fucata martensii* under normoxic conditions. *Aquaculture Sci*, **60**, 113–117 (2012)
  - 13) Handa T, Yamamoto K: Estimation of CO<sub>2</sub> partial pressure and bicarbonate concentration in the hemolymph of the black–lip pearl oyster *Pinctada margaritifera*. *J Nat Fish Univ*, **63**, 181–188 (2015)
  - 14) Handa T and Yamamoto K: Estimation of CO<sub>2</sub> partial pressure and bicarbonate concentration in the hemolymph of the noble scallop *Mimachlamys nobilis*. *J Nat Fish Univ*, **64**, 188–194 (2016)
  - 15) Handa T, Araki A, Yamamoto K: Acid–base balance of the hemolymph in hard–shelled mussel *Mytilus coruscus* in normoxic Conditions. *J Nat Fish Univ*, **65**, 39–46 (2017)
  - 16) Boutilier RG, Iwama GK, Heming TA, Randall DJ: The apparent pK of carbonic acid in rainbow trout blood plasma between 5 and 15°C. *Resp Physiol*, **61**, 237–254 (1985)
  - 17) Davenport HW: Fundamental equation. In: The ABC of acid–base chemistry 6th edition. University of Chicago Press, Chicago, 39–41 (1974)
  - 18) Heisler N: Acid–base regulation, Interrelationships between gaseous and ionic exchange. In: Boutilier RG (ed) Vertebrate gas exchange, Comparative & environmental physiology 6, Springer–Verlag Berlin Heidelberg, 211–251 (1990)
  - 19) Jokumsen A, Fyhn HJ: The influence of aerial exposure upon respiratory and osmotic properties of haemolymph from tow intertidal mussels, *Mytilus edulis* L. and *Modiolus modiolus* L. *J Exp Mar Biol Ecol*, **61**, 189–203 (1982)
  - 20) Walsh JP, McDonald DG, Booth CE: Acid–base balance in the sea mussel, *Mytilus edulis*. II. Effects of hypoxia and air–exposure on intracellular acid–base status. *Mar Biol Lett*, **5**, 359–369 (1984)
  - 21) Littlewood DT, Young RE: The effect of air–gaping behavior on extrapallial fluid pH in the tropical oyster *Crassostrea rhizophorae*. *Comp Biochem Physiol*, **107A**, 1–6 (1994)
  - 22) Byrne RA, Shipman BN, Smatresk NJ, Dietz TH, McMahon RF: Acid–base balance during emergence in the freshwater bivalve *Corbicula fluminea*. *Physiol Zool*, **64**, 748–766 (1991)