Marine Biology Letters, 5 (1984) 371–381
Elsevier

MBL 00273

ACID-BASE BALANCE IN THE SEA MUSSEL, *MYTILUS EDULIS*. III. EFFECTS OF ENVIRONMENTAL HYPERCAPNIA ON INTRA- AND EXTRACELLULAR ACID-BASE BALANCE

MICHAEL I. LINDINGER[1], DARREL J. LAURÉN[2] and D. GORDON McDONALD[2,*]

Huntsman Marine Laboratory, St Andrews, New Brunswick, Canada, E0G 2X0

(Received 5 July 1984; revised version received 4 October 1984; accepted 5 October 1984)

SUMMARY

Exposure of *Mytilus edulis* L. to normoxic-hypercapnic seawater (P_{CO_2} = 6.5, 13.0, and 26.0 mmHg) for 24 h produced an elevation in hemolymph $[H^+]$, $[CA^{++}]$, $[HCO_3^-]$, $[NH_4^+]$, and P_{CO_2}. Simultaneous measurement of intracellular pH (pH_i) at a P_{CO_2} of 26 mmHg showed that pH_i followed hemolymph pH but was maintained about 0.4 pH U lower. A large (6.37 ± 0.65 mequiv. H^+/L) non-respiratory component to the intracellular acidosis developed within 0.5 h of exposure to P_{CO_2} of 26 mmHg.

At all CO_2 levels the initial drop in pH was partially compensated over the remaining 23.5 h of CO_2 exposure by increases in extracellular and intracellular bicarbonate, while P_{CO_2} remained constant. About 70 to 80% of the additional bicarbonate was produced by the dissolution of shell and body fluid stores of calcium carbonate; the remainder may have resulted from intracellular to extracellular ion exchanges and/or transepithelial ion exchange processes.

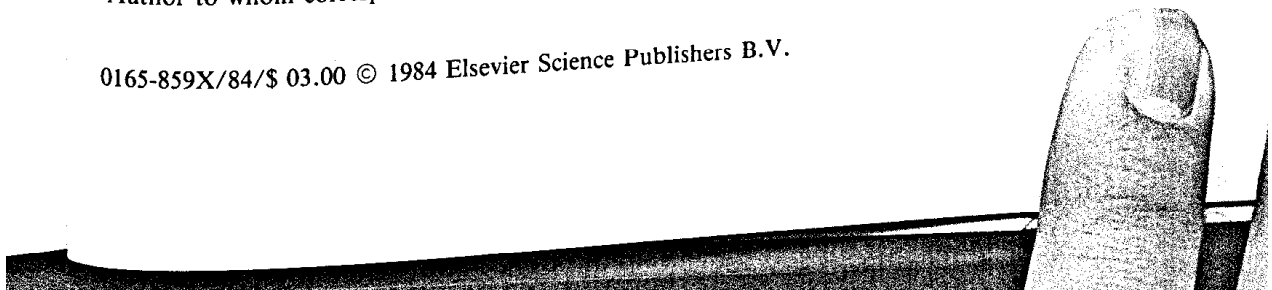
It is concluded that acid-base regulation in *Mytilus edulis* is largely passive in response to environmental hypercapnia. Furthermore, even in the presence of oxygen, CO_2 appears to exert a major effect on cellular metabolism in *Mytilus*.

Key words: *Mytilus edulis*, hypercapnia, intracellular pH, acid-base balance, shell buffering.

INTRODUCTION

Cyclic changes in salinity and degree of aerial exposure are common events for intertidal animals. Bivalves such as *Mytilus edulis* decrease the severity of such changes by closing their valves (Schoffeniels and Gilles, 1972; Shumway, 1977). Shell closure is accompanied by changes in intermediary metabolism (Hochachka, 1975; de Zwaan, 1977; Kluytmans et al., 1977), increases in hemolymph P_{CO_2} and

Present addresses: [1]Department of Medicine, McMaster University, Hamilton, Ontario L8N 3Z5.
[2]Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1.
*Author to whom correspondence should be addressed.



[Ca^{++}] (Crenshaw and Neff, 1969), and a decrease in hemolymph pH (Crenshaw and Neff, 1969; Jokumsen and Fyhn, 1982; Booth et al., 1984).

The preceding two studies (Booth et al., 1984; Walsh et al., 1984), have confirmed that shell closure results in an elevation in P_{CO_2}, i.e. a respiratory acidosis, in both extra- and intracellular compartments. Furthermore, there was a metabolic acid component to the intracellular acidosis (Walsh et al., 1984) which was restricted to that compartment and is most likely attributable to the accumulation of anaerobic end products. This is in keeping with the finding that under low O_2 conditions *Mytilus* apparently does not release acidic end-products into the hemolymph (Zurburg and Ebberink, 1981). Clearly then, shell closure produces acid-base disturbances of a relatively complex origin which somewhat complicates the analysis of possible mechanisms of acid-base regulation.

The purpose of the present study was therefore to investigate the responses of *Mytilus* to a pure respiratory acidosis, i.e. without accompanying hypoxia resulting from valve closure. This was achieved by equilibrating *Mytilus* to elevated ambient levels of CO_2 by gassing the seawater while maintaining O_2 at normal ambient levels. To ensure large magnitude responses and thereby magnify any acid-base regulation that might occur, ambient P_{CO_2} levels of up to 26 mmHg were employed. While it is unlikely that *Mytilus* would be exposed to CO_2 levels this high in sea water (Newell, 1970; Truchot and Duhamel-Jouve, 1980), Jokumsen and Fyhn (1982) have shown that *Mytilus* may experience hemolymph P_{CO_2} levels of up to 100 mmHg during prolonged air exposure. Furthermore, we have produced conditions in vivo which may allow the differentiation between pH or P_{CO_2} as the driving force for the shift to 'anaerobic' metabolic pathways (Hochachka and Mustafa, 1972; Hochachka, 1975; de Zwaan, 1977) in mussels.

MATERIALS AND METHODS

Mytilus edulis (3.5–5.5 cm shell length; 4–8 g wet tissue mass) were collected and handled as described by Booth et al. (1984).

In order to expose *Mytilus* to normoxic hypercapnia and at the same time determine fluxes of acid (H^+) and ammonia ($NH_3^{\dagger}NH_4^+$) between animals and water (see Booth et al., 1984 for details) mussels were scrubbed clean and placed upright in 100 ml plastic beakers (3 animals per beaker). Beakers were then filled with 80–90 ml of sea water. This volume allowed for periodic water sampling without air exposing any of the animals. Additionally, one beaker containing sea water only, and one beaker containing sea water plus cleaned *Mytilus* shells served as controls. The beakers were covered with tight fitting lids through which polyethylene tubing (PE-50), carrying the CO_2 gas mixture, was led. Other small holes in the lid permitted excess gas to escape, thereby eliminating volume changes due to the bubbling action of the gas. Mussel and control beakers were bubbled with air for an 8- to 24-h control period followed by 24 h exposure to ambient P_{CO_2} of either 6.5, 13, or 26

mmHg, i.e. 1%, 2% or 4% CO_2 in air. These gases were obtained from bottled 5% CO_2 in air mixed with a Wösthoff gas mixing pump.

Duplicate beakers, each containing 3 mussels, were set up for sampling at each of the following times: $t = 0$, 0.5, 1, 4, 8, and 24 h of CO_2 exposure, such that 12 beakers containing 36 mussels, plus the control beakers, were used at each P_{CO_2}. At each sampling time, 4.0 ml of water was collected from each of the remaining beakers for flux determinations. Also, terminal hemolymph samples were drawn from the posterior adductor muscles of 6 mussels in two beakers using Hamilton gas tight syringes. Care was taken to ensure that mussels had their valves open prior to their removal from the beaker for sampling. After blood sampling, animals were dissected from their shells to determine wet and dry tissue weights. Hemolymph acid-base parameters were determined as described by Booth et al. (1984). Additionally, 200 μl hemolymph was analyzed for ammonia by colorimetric assay using a micro-modification of the salicylate hypochlorite method of Verdouw et al. (1978), and 50 μl hemolymph was acidified with 150 μl dilute (0.014 N) nitric acid for later determination of Ca^{++}. Hemolymph Ca^{++} was determined after appropriate dilution by atomic absorption spectrophotometry (Varian AA1275).

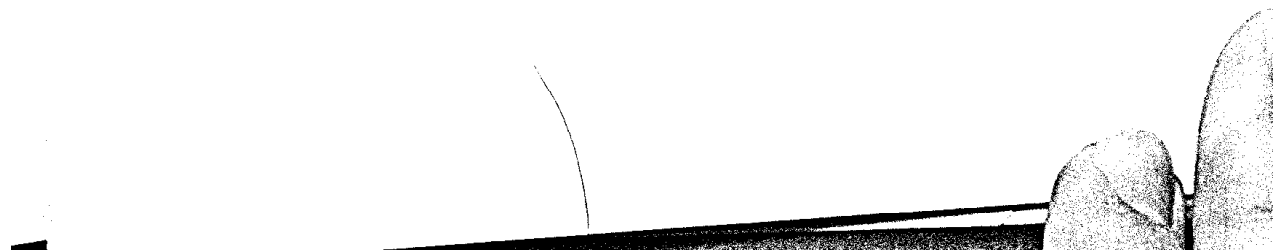
Determination of the in vitro non-bicarbonate buffer value (β_{NB}) of *Mytilus* hemolymph was performed by equilibrating 4.0 ml aliquots of hemolymph (pooled from several animals) to humidified air or 1%, 2%, and 4% CO_2 in humidified air. Following 20 min equilibration, 50 μl aliquots were used to measure pH with a Radiometer micro pH electrode coupled to a Radiometer PHM-72 pH meter, and total CO_2 (C_{CO_2}) with a Corning 965 CO_2 analyzer (refer to Booth et al., 1984 for details). Bicarbonate (HCO_3^-) and partial pressure of CO_2 (P_{CO_2}) were calculated as described by Booth et al. (1984).

Whole-body pH_i was determined for mussels exposed to ambient P_{CO_2} of 26 mmHg using the DMO (5,5-dimethyloxazolidine-2,4-dione) distribution technique as described by Walsh et al. (1984). Utilizing these values of pH_i and assuming that the P_{CO_2} in the intra- and extracellular fluids is equal, intracellular $[HCO_3^-]$ was estimated by manipulation of the Henderson–Hasselbalch equation:

$$pH_i = pK_1 + \log\ (HCO_{3_i}^-/\alpha CO_2 \cdot P_{CO_2}) \quad (1)$$

where pK_1 and αCO_2 at experimental temperature (12°C) and salinity (29‰) are 6.114 (no units) and 0.0574 mmol · l per mmHg, respectively (Booth et al., 1984). Intracellular $[HCO_3^-]$ calculated in this way yields values within 0.01 mequiv./l of that estimated using the Donnan ratio (see Walsh et al., 1984).

The intracellular metabolic acid load (ΔH_m^+), or base deficit (Davenport, 1974), was estimated by equation 1 of Walsh et al. (1984), using measurements of pH_i, estimates of $[HCO_3^-]_i$ and a whole-body non-bicarbonate buffer value of −9.63 mequiv. per pH per kg, i.e. 9.63 slykes (Walsh et al., 1984). Hemolymph base excess (BE) or negative base deficit (Davenport, 1974) was similarly determined by equation 1 of Walsh et al. (1984) using extracellular measurements of pH and HCO_3^-.



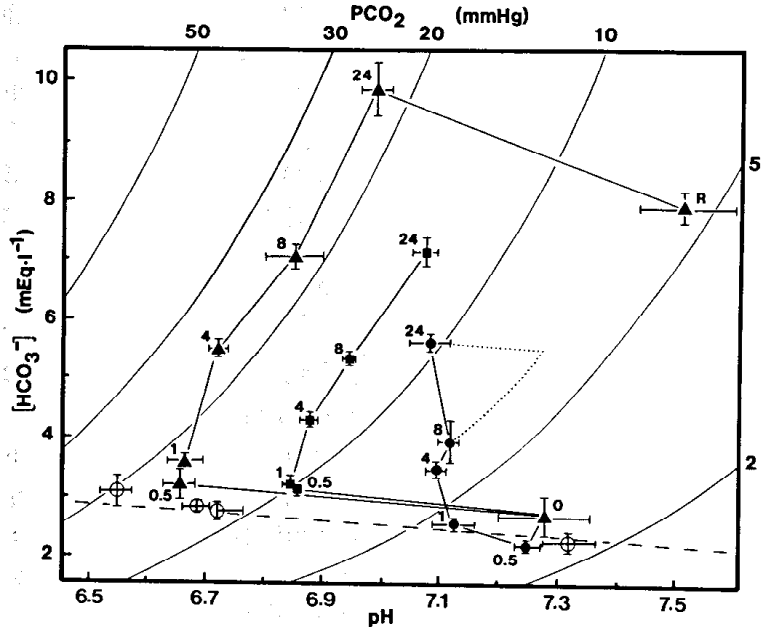


Fig. 1. A pH-HCO_3^- diagram showing changes in hemolymph pH, P_{CO_2} and [HCO_3^-] in *Mytilus edulis* during exposure to ambient seawater of P_{CO_2} = 6.5 mmHg (●), 13 mmHg (■), and 26 mmHg (▲). The hemolymph non-bicarbonate buffer line (slope = 0.622 slykes) as determined in vitro is indicated by the open circles and dashed line. The number by each point indicates the duration of CO_2 exposure in hours; R indicates 24 h recovery in the same water gassed with normocapnic air. Values are means ± SE for 6 animals at each CO_2 level.

RESULTS

Hemolymph

The hemolymph non-bicarbonate buffer value (β_{NB}) as determined in vitro was found to be 0.622 slykes. This value is about 2.2-fold greater than that for sea water (0.283 slykes) and about 1.6-fold greater than that found by Booth et al. (1984) for *Mytilus* hemolymph. This discrepancy between these hemolymph buffer values may reflect the difference in the range of P_{CO_2} employed, i.e. 0.2–26 mmHg (present study), vs. 0.2–5.0 mmHg (Booth et al., 1984), and perhaps indicates that the actual non-bicarbonate buffer line may be curvilinear over the whole range. A straight line representation (Fig. 1) is used for clarity.

Changes in hemolymph acid-base state of *Mytilus edulis* in response to environmental hypercapnia are shown in Fig. 1. It should be noted that control values for pH are 0.3 U lower than reported in the preceding paper (Booth et al., 1984). This is most likely due to the stresses of DMO infusion (Walsh et al., 1984). Initial changes ($t = 0$ to $t = 0.5$ h) in pH and [HCO_3^-] closely followed the in vitro buffer line, but from 0.5 h to 24 h, pH was compensated by an increase in [HCO_3^-]. In mussels exposed to a P_{CO_2} of 6.5 mmHg, hemolymph pH continued to decrease despite the elevation of hemolymph [HCO_3^-]. This was due to a 3-fold increase of hemolymph P_{CO_2} (above the ambient P_{CO_2} level) which may have resulted from CO_2

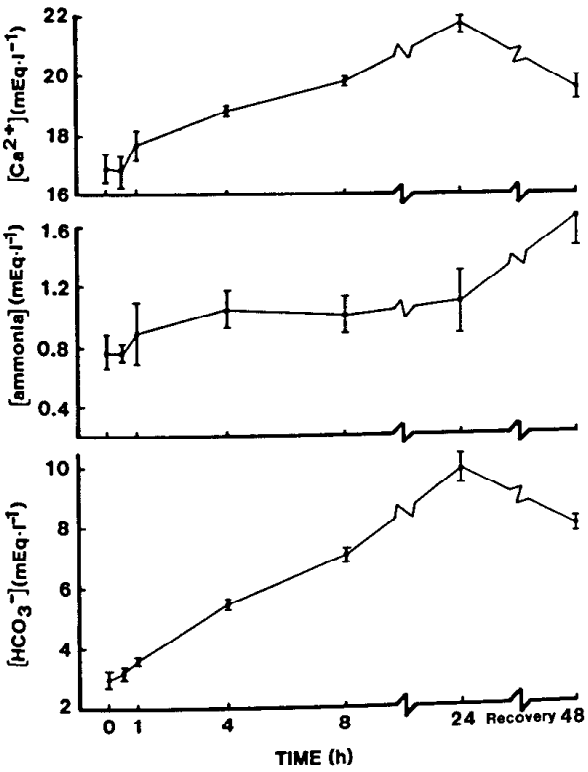


Fig. 2. Changes in $[Ca^{++}]$, [ammonia] and $[HCO_3^-]$ of hemolymph of *Mytilus edulis* ($n=6$) during 24 h exposure to ambient sea-water of $P_{CO_2}=26$ mmHg and subsequent 24 h recovery in the same water bubbled with normocapnic air. Values are means ± SE.

retention due to recent valve closure and/or a diminished rate of ventilation. In these animals, extrapolation of hemolymph $[HCO_3^-]$ at 24 h to the ambient P_{CO_2} of 6.5 mmHg (see dotted lines, Fig. 1) indicates that hemolymph pH would have been fully compensated for by the rise in $[HCO_3^-]$ were it not for this increase in hemolymph P_{CO_2}. At the higher P_{CO_2} levels (13 and 26 mmHg) hemolymph P_{CO_2} remained constant at 13 and 24 mmHg, respectively, during this period of pH compensation. These animals remained in equilibrium with the ambient P_{CO_2} (see Fig. 3). Thus the amount of pH compensation in the extracellular compartment at the end of 24 h CO_2 exposure was 66 and 71% at an ambient P_{CO_2} of 13 and 26 mmHg, respectively.

Mussels kept at ambient $P_{CO_2}=26$ mmHg for 24 h were allowed to recover for 24 h in the same ambient water bubbled with normocapnic air (Fig. 1). Removal of ambient CO_2 was accompanied by decreases in hemolymph P_{CO_2} and $[HCO_3^-]$ and an increase in pH, such that the animals became slightly alkalotic with respect to control values. However, both hemolymph P_{CO_2} and $[HCO_3^-]$ remained significantly elevated after 24 h recovery (Fig. 1).

Figure 2 shows the time course of changes in hemolymph Ca^{++}, ammonia, and HCO_3^- during exposure to sea water at a P_{CO_2} of 26 mmHg and subsequent recovery. The increase in hemolymph $[HCO_3^-]$ was significantly correlated to the

TABLE I

The changes in *Mytilus* hemolymph [Ca^{++}], [ammonia], [HCO_3^-] and base excess (BE) following 24 h exposure to ambient P_{CO_2} of 6.5, 13 and 26 mmHg.

P_{CO_2} for (mmHg)	[Ca^{++}] (mequiv./l)	[NH_4^+] (mequiv./l)	([Ca^{++}] + [NH_4^+]) (mequiv./l)	BE (mequiv./l)	% BE accounted by Ca^{++} and NH_4^+
6.5	1.37 ± 0.28	0.640 ± 0.036	2.01 ± 0.29	2.57 ± 0.14	78.2
13	2.77 ± 0.22	0.125 ± 0.045	2.90 ± 0.20	4.13 ± 0.23	70.2
26	4.86 ± 0.46	0.322 ± 0.14	5.18 ± 0.48	6.41 ± 0.45	80.8

Values are means ± SE, $N = 6$.

rise in hemolymph [Ca^{++}] + [ammonia] ($r = 0.9926$, $n = 18$, $P < 0.01$) at all ambient P_{CO_2} levels. Furthermore, at all P_{CO_2} levels employed the increase in hemolymph Ca^{++} and ammonia accounted for 70–80% of the base excess, with the ammonia component contributing to less than 5% of this amount (Table I).

Sea water

Sea-water acid-base changes at an ambient P_{CO_2} of 26 mmHg are shown in Fig. 3. During the first 30 min of CO_2 exposure the pH of sea water containing shells or mussels was rapidly titrated in an acid direction along the sea-water buffer line. Following this initial reduction in pH, sea water containing mussels showed an in-

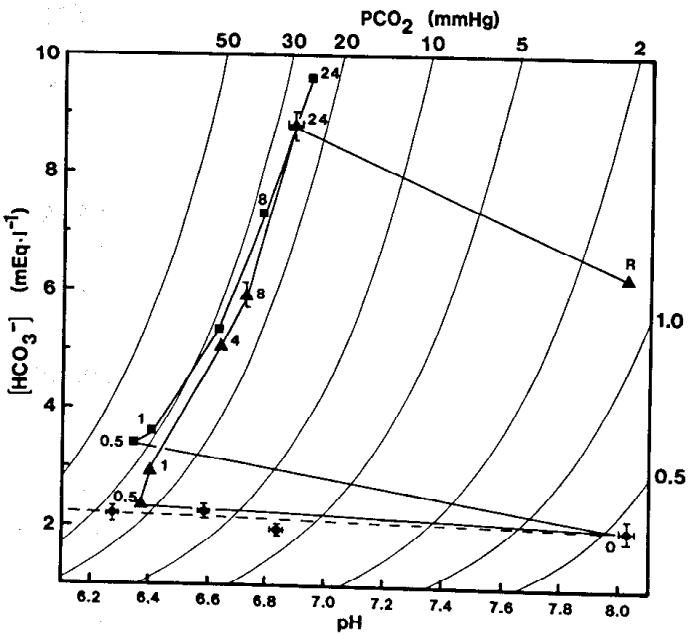


Fig. 3. A pH-HCO_3^- diagram showing changes in sea-water pH, P_{CO_2} and [HCO_3^-] during gassing with hypercapnic air of $P_{CO_2} = 26$ mmHg. (●) sea water alone ($n = 6$); (■) sea water + 6 cleaned hemishells ($n = 1$); (▲) sea water + 3 *Mytilus* ($n = 3$). The sea-water non-bicarbonate buffer line (slope = 0.283 slykes) is indicated by the dashed line. The number by each point indicates the duration of CO_2 exposure. Values are means ± SE.

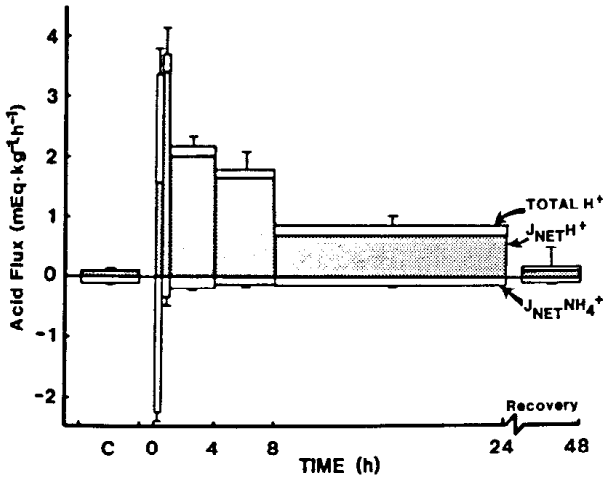


Fig. 4. Total H^+ flux, net H^+ flux and net ammonia flux between *Mytilus* ($n=6$) and ambient sea-water prior to and during exposure to ambient P_{CO_2} of 26 mmHg. Net H^+ flux = total H^+ flux − ammonia flux (signs considered). Values are means ± SE.

crease in pH and $[HCO_3^-]$ upwards along the 26 mmHg P_{CO_2} isopleth. These changes paralleled those which occurred in sea water containing cleaned *Mytilus* shells (Fig. 3) and in *Mytilus* hemolymph (Fig. 1).

The titratable alkalinity (TA) of sea-water controls remained unchanged (2.18 ± 0.09 mequiv. H^+/l, pH 4.00 endpoint) with increasing CO_2 exposure, but the TA of sea water containing shells or mussels increased 2-fold at $P_{CO_2}=6$ mmHg, 3-fold at $P_{CO_2}=13$ mmHg, and 4-fold at $P_{CO_2}=26$ mmHg. In sea-water containing mussels this increase in TA is also apparent as an increase in the apparent net H^+ influx (OH^- or HCO_3^- − efflux, its functional equivalent) as seen in Fig. 4. The rate of this apparent H^+ flux decreased exponentially with time at all CO_2 exposures. Ammonia efflux from mussels into sea water (Fig. 4) was also increased with the onset of CO_2 exposure but returned to control levels within 8 h.

Intracellular acid-base state

Changes in *Mytilus* whole-body intracellular acid-base parameters during CO_2 exposure at 26 mmHg are shown in Fig. 5. A marked depresson of pH_i occurred during the initial 30 min of exposure. This decrease (−0.70 pH U) was similar to that observed in extracellular fluids (−0.64 pH U) but was much larger than can be attributed purely to an increase in intracellular P_{CO_2}. With the much higher non-bicarbonate buffering power of intracellular fluids relative to hemolymph (9.63 vs. 0.622 slykes, solid line, Fig. 5) an increase in P_{CO_2} to 26 mmHg would have depressed pH_i by less than 0.2 U (Fig. 5). Thus, there was a very rapid onset, non-respiratory acidosis in the intracellular compartment. The non-respiratory contribution to the intracellular acidosis, i.e. base deficit, calculated from pH_i and $[HCO_{3i}^-]$ was 6.37 ± 0.65 mequiv./l at $t=0.5$ h. This metabolic acid load declined with time but was still substantial by 24 h (2.43 ± 0.65 mequiv./l). As in the ECF, pH_i was par-

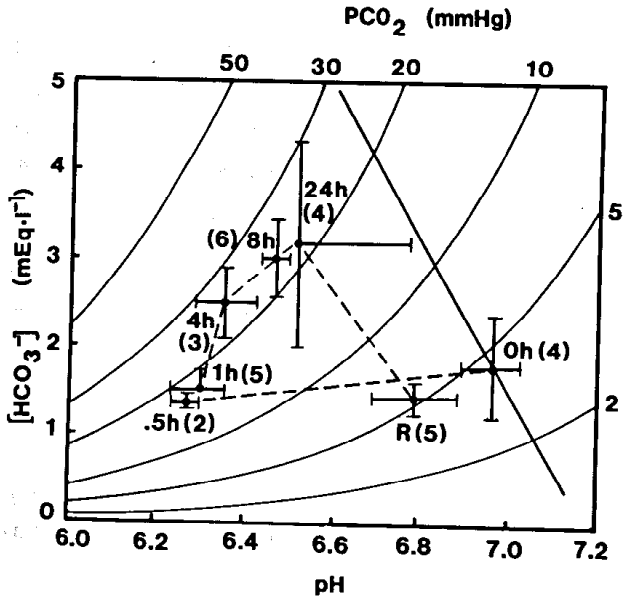


Fig. 5. A pH-HCO_3^- diagram showing changes in intracellular fluid (ICF) pH, P_{CO_2} and $[HCO_3^-]$ during exposure of *Mytilus edulis* to sea-water of $P_{CO_2}=26$ mmHg. The ICF non-bicarbonate buffer line (slope = 9.63 slykes, Walsh et al., 1984) is indicated by the solid line. The time course of CO_2 exposure in hours is indicated. Values in brackets (*n*) represent the number of animals at each point. Values plotted are means ± SE.

tially compensated (54%) by an increase in intracellular fluid $[HCO_3^-]$. Following 24 h recovery from 4% CO_2 exposure, intracellular acid-base state returned to near-control values (Fig. 5).

DISCUSSION

For many aquatic animals the regulation of acid-base balance is thought to be largely dependent upon active transepithelial ion exchanges between the ECF and the environment, i.e. Cl^-/HCO_3^- and Na^+/H^+ exchanges (Maetz, 1974; Gilles, 1975; Evans, 1982; Heisler, 1982). However, in the present study we have shown that the passive dissolution of carbonates by CO_2 appears to account for the great majority of the buffering of H^+ loads (Fig. 1, Table I). This is based on the finding that the increases in hemolymph $[Ca^{++}]$ (Fig. 2), arising from $CaCO_3$ dissolution – see equation 6 of Booth et al. (1984), accounted for most of the base excess during normoxic hypercapnia with ammonia production contributing a small additional amount (Table I). Nevertheless, there remains a significant amount (i.e. 20 to 30%) of the base excess which cannot be attributed to either of these sources.

Three additional possibilities can be suggested for the unexplained base excess: dissolution of other carbonate salts ($MgCO_3$ or Na_2CO_3); intracellular/extracellular H^+/HCO_3^- transmembrane exchange processes and transepithelial ion exchange processes. The first is unlikely since lamellibranch shells consist of almost pure

$CaCO_3$ (Potts, 1954). The second, on the surface, also seems unlikely given that the typical pattern in acid-base regulation is to defend the intracellular compartment at the expense of the extracellular compartment, rather than the other way around. However, the fact that *Mytilus* so rapidly developed an intracellular acidosis means that this possibility cannot be ruled out. The final possibility of transepithelial acid-base excretion must also be considered, particularly in view of the fact that it would not be possible, with the methods employed in this study, to detect any significant H^+ excretion even if it had occurred. The action of high ambient CO_2 on exposed shell surfaces (Fig. 3) clearly produces an apparent transepithelial H^+ uptake (Fig. 4) which would mask any real H^+ excretion/HCO_3^- uptake that might occur. An interesting observation in this regard was the large initial elevation in ammonia excretion at the onset of hypercapnia (Fig. 4). Ammonia, excreted as NH_4^+ would, of course, be equivalent to H^+ excretion (or HCO_3^- uptake) and this may, indeed, be an important mechanism for acid excretion in *Mytilus*. However, an equally likely explanation is that ammonia was excreted primarily as NH_3, passing outward on a transepithelial pH gradient which favored diffusion. Since sea water is less well buffered than hemolymph, the high CO_2 exposure titrated sea water to a lower pH (6.367 vs. 6.654 at $t = 0.5$ h at 26 mmHg P_{CO_2}) and this, by itself, could have accounted for the marked increase in ammonia excretion. With continued hypercapnia, the addition of base to the sea water, due to dissolution of the external shell surface, reduced the pH difference between sea water and hemolymph and the excretion of ammonia decreased accordingly (Fig. 4).

While a base excess developed in the ECF compartment during exposure to normoxic hypercapnia, at an ambient P_{CO_2} of 26 mmHg, a large base deficit evolved rapidly in the intracellular compartment (Fig. 5). This indicated that hypoxia is not necessary for the intracellular accumulation of metabolically produced H^+, a finding consistent with the observation that aerobic and anaerobic metabolism may occur simultaneously in *Mytilus edulis* (Livingstone and Bayne, 1974; de Zwaan and de Bont, 1975).

The source of the intracellular H^+ cannot be identified with the methods employed in this study and may have arisen by: CO_2-induced inhibition of ATP synthesis by both aerobic and anaerobic pathways; the accumulation of acidic end products; the transfer of H^+ from the ECF into the ICF; or a combination of any of the above. Although the present study does not allow differentiation of these possible mechanisms it is apparent that CO_2 may, in fact, be a much more powerful effector of cellular metabolism than hypoxia. Clearly, then, normoxic hypercapnia may serve as a useful tool for investigating metabolic control in the marine bivalves.

ACKNOWLEDGEMENT

The authors thank the director, Dr. Tom Moon, and staff of the Huntsman Marine Laboratory for their technical support and assistance. We thank Dr. Pat

Walsh for supplying the labelled DMO and Dr. A.P. Farrell for providing the CO_2 gases. We also thank Dr. C.E. Booth for his critical reading of the manuscript. This work was funded by a Natural Sciences and Engineering Council of Canada operating grant (U0005) to DGM.

REFERENCES

Booth, C.E., McDonald, D.G. and Walsh, P.J., 1984. Acid-base balance in the sea mussel, *Mytilus edulis*. I. Effects of hypoxia and air-exposure on hemolymph acid-base status. Mar. Biol. Lett. 5, 347–358.

Crenshaw, M.A. and Neff, J.M., 1969. Decalcification at the mantle-shell interface in molluscs. Am. Zool. 9, 881–885.

Davenport, H.W., 1974. The ABC of acid-base chemistry, 6th edition. The University of Chicago Press, Chicago, 124 pp.

Evans, D.H., 1982. Mechanism of acid extrusion by two marine fishes: the teleost *Opsanus beta*, and the elasmobranch *Squalus acanthias*. J. Exp. Biol. 97, 289–299.

Gilles, R., 1975. Mechanisms of ion and osmoregulation. In: Marine Ecology, Vol. II, Physiological Mechanisms, Part 1, pp. 259-347. Editor: O. Kinne. John Wiley and Sons, Toronto.

Heisler, N., 1982. Transepithelial ion transfer processes as mechanisms for fish acid-base regulation in hypercapnia and lactacidosis. Can. J. Zool. 60, 1108–1122.

Hochachka, P.W., 1975. An exploration of metabolic and enzyme mechanisms underlying animal life without oxygen. In: Biochemical and Biophysical Perspectives in Marine Biology, Vol. 2. Editors: D.C. Malins and J.R. Sargent. Academic Press, New York.

Hochachka, P.W. and Mustafa, T., 1972. Invertebrate facultative anacrobiosis. Science 178, 1056–1060.

Jokumsen, A. and Fyhn, H.J., 1982. The influence of aerial exposure upon respiratory and osmotic properties of haemolymph from two intertidal mussels, *Mytilus edulis* L. and *Modiolus modiolus* L. J. Exp. Mar. Biol. Ecol. 61, 189–203.

Kluytmans, J.H., Bont, A.M.T. de, Janus, J. and Wijsman, T.C.M., 1977. Time dependent changes and tissue specificities in the accumulation of anaerobic fermentation products in the sea mussel *Mytilus edulis* L. Comp. Biochem. Physiol. 58B, 81–87.

Livingstone, D.R. and Bayne, B.L., 1974. Pyruvate kinase from mantle tissue of *Mytilus edulis* L. Comp. Biochem. Physiol. 48B, 481–497.

Maetz, J., 1974. Aspects of adaptation to hypo-osmotic and hyper-osmotic environments. In: Biochemical and Biophysical Perspectives in Marine Biology, Vol. 1, pp. 1–167. Editors: D.C. Malins and J.R. Sargent. Academic Press, New York.

Newell, R.C., 1970. Biology of intertidal animals. Logos Press Ltd., London.

Potts, W.T.W., 1954. The inorganic composition of the blood of *Mytilus edulis* and *Anodonta cygnea*. J. Exp. Biol. 31, 376–385.

Schoffeniels, E. and Gilles, R., 1972. Ionoregulation and osmoregulation in Mollusca. In: Chemical Zoology, Vol. VII, pp. 393–420. Editors: M. Florkin and B.T. Scheer. Academic Press, New York.

Shumway, S.E., 1977. Effect of salinity fluctuation on the osmotic pressure and Na^+, Ca^{2+} and Mg^{2+} ion concentrations in the hemolymph of bivalve molluscs. Mar. Biol. 41, 153–157.

Truchot, J.-P. and Duhamel-Jouve, A., 1980. Oxygen and carbon dioxide in the marine intertidal environment: diurnal and intertidal changes in rockpools. Respir. Physiol. 39, 241–254.

Verdouw, H., van Echteld, C.J.A. and Dekkers, E.M.J., 1978. Ammonia determination based on indophenol formation with sodium salicylate. Water Res. 12, 399–402.

Walsh, P.J., McDonald, D.G. and Booth, C.E., 1984. 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.

Zurburg, W. and Ebberink, R.H.M., 1981. The anaerobic energy demand of *Mytilus edulis*. Organ specific differences in ATP-supplying processes and metabolic routes. Mol. Physiol. 1, 153–164.

Zwaan, A. de, 1977. Anaerobic energy metabolism in bivalve molluscs. Oceanogr. Mar. Biol. Ann. Rev. 15, 103–187.

Zwaan, A. de and de Bont, A.M.T., 1975. Phosphoenolpyruvate carboxykinase from adductor muscle tissue of the sea mussel *Mytilus edulis* L. J. Comp. Physiol. 96, 85–94.

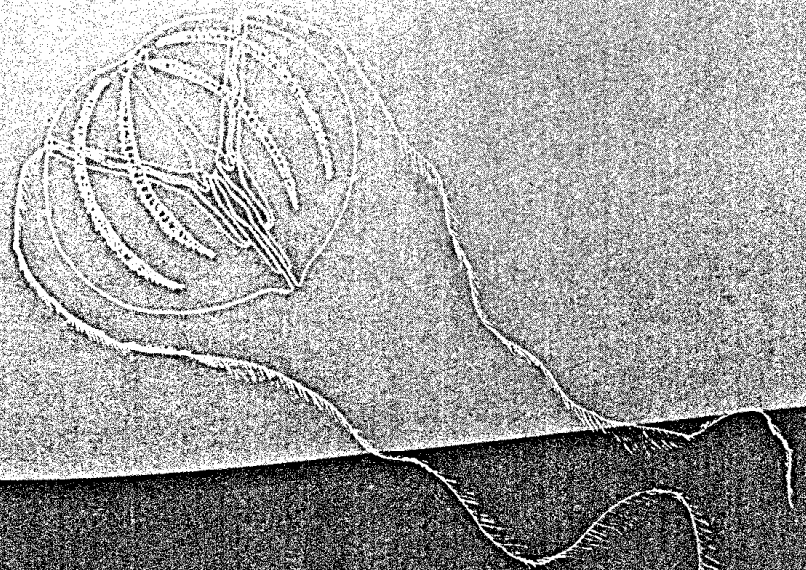
Volume 5, Number 6
December 1984

ISSN 0165-859X
MBLED7 5(6) 315-388 (1984)

MARINE BIOLOGY LETTERS

FISHERIES·OCEANOGRAPHY

Physiology, biochemistry and biophysics of marine organisms and populations



ELSEVIER

AIMS AND SCOPE

Marine Biology Letters will provide a medium for the **rapid** publication of essentially short, definitive articles on marine organisms, which are concerned with biophysical, biochemical and physiological processes as they affect the individual or the population.

Specific areas to be included are: sensory and motor neural mechanisms; respiratory physiology and energy metabolism; aspects of nutrition and reproduction; homeostatic mechanisms and hormonal control; experimental embryology and cytology; biochemical and population genetics. Articles on multi-disciplinary studies will also be considered and occasional reviews will be published following invitation by the Editors-in-Chief.

EDITORIAL POLICY

Marine Biology Letters will **not** be an appropriate medium for purely descriptive papers such as works on descriptive morphology, descriptive ecology or natural product chemistry. However, where the main purpose is to analyse or explain function, descriptive matter will not be excluded if it forms an integral part of the contribution. It is envisaged that the average paper will be in the region of 6–8 printed pages in length, but papers will not be rejected on account of brevity.

Contributions may be sent to either of the Editors-in-Chief who will arrange for them to be considered.

Subscription information:
The *Subscription price* for Volume 5 (6 issues) is Dfl. 225.00 (US $ 84.90), postage included. The Dutch guilder price is definitive. The U.S. dollar price is subject to exchange rate fluctuations and is only given as a guide. Journals are automatically sent by air to the U.S.A., Canada and India at no extra charge, and by surface delivery to all other countries. Airmail rates for other countries are available on request. Subscription orders can only be entered by calendar year (Jan.–Dec.) and should be sent to: Elsevier Science Publishers, Journals Department, P.O. Box 211, 1000 AE Amsterdam, The Netherlands; telex 18582 ESPA NL or to your usual subscription agent. Claims for missing issues should be made within three months of publication, otherwise they cannot be honoured free of charge. The Publisher reserves the right to issue additional volumes during the course of the year. Such volumes will be invoiced before publication and delivered after receipt of payment. In the United States and Canada: For further information concerning this or any other Elsevier Science Publishers journal, contact Elsevier Science Publishers, Journal Information Center, 52 Vanderbilt Avenue, New York, NY 10017; tel. (212) 370-5520.

Advertisements: apply to the Publisher.

Printed in The Netherlands.