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ACID–BASE REGULATION IN THE FRESHWATER PEARL MUSSEL *MARGARITIFERA MARGARITIFERA*: EFFECTS OF EMERSION AND LOW WATER pH

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Summary

Freshwater pearl mussels *Margaritifera margaritifera* L. were exposed to air for 7 days, then immersed in circumneutral water (pH 7·85) or acidified water (pH 5·25) for 5 days. Mantle fluid pH and composition were monitored throughout. The mussels were observed to gape periodically when in air. Periodic gaping permitted aerial gas exchange such that mantle fluid P_{CO_2} and dissolved oxygen concentration stabilized at levels about twice and half, respectively, those of immersed mussels. During emersion, a dilute carbonate buffer equilibrium was established in the mantle fluid, involving reactions with $CaCO_3$ reserves and, simultaneously, aerial release of CO_2. Aerial CO_2 release was sufficient to shift the carbonate buffer equilibrium in the alkaline direction, resulting in a significant alkalosis of mantle fluids during air exposure. Mantle fluid characteristics returned to initial (time zero) values within 3 days of immersion in circumneutral water (pH 7·85). When immersed in acid water (pH 5·25), the mussels were able to maintain a sizeable gradient between mantle fluid pH and ambient water pH. Regulation of mantle fluid pH in acid water did not involve any isolation reaction (valve closure), but rather environmental protons were buffered at the expense of $CaCO_3$ reserves. Net calcium transfer, the difference between calcium uptake and loss, was shifted in the negative direction by decreases in water pH.

Introduction

Bivalve molluscs utilize their $CaCO_3$ reserves to buffer disturbances in haemolymph pH (reviewed by Burton, 1983). In this way, they are able to regulate their acid–base status in the face of retention of CO_2 and/or acid metabolites when the valves are closed, a behaviour commonly adopted to avoid adverse environmental

Key words: acid–base, emersion, mantle fluid, mussel.

factors (e.g. emersion, salinity changes, anoxia). Generally, when the valves are closed, the circulating levels of calcium and carbonates and P_{CO_2} increase significantly (Alyakrinskaya, 1971, 1972, 1977; Burton, 1983), although the accompanying fall in internal pH can be relatively slight (Collip, 1921). Acid–base regulation during exposure to low water pH probably involves a similar strategy for utilizing $CaCO_3$ reserves.

The freshwater pearl mussel *Margaritifera margaritifera* is relatively sensitive to water quality, including water pH (Bauer, Schrimpff, Thomas & Herrmann, 1980; Klupp, 1983). As a result, the distribution of this mussel has been reduced from its historical range by pollution (Krasowska, 1978; Young & Williams, 1983) and is threatened further by atmospheric deposition of acidic compounds. The primary response of aquatic biota to moderately low water pH (4·0–5·5) is a failure of ionic regulation (Haines, 1981; Malley & Chang, 1985). Apart from the work of Chaisemartin's laboratory on ion transport in *M. margaritifera* (Chaisemartin, 1968; Chaisemartin, Martin & Bernard, 1968), little is known of the ability of this mussel to regulate its ionic and acid–base homeostasis, especially in the face of acidic pH challenges.

The present study was undertaken to examine the ability of *M. margaritifera* to regulate acid–base homeostasis. Internal pH disturbances were induced by air emersion and immersion in acidified water. Freshwater pearl mussels seldom experience emersion naturally. Emersion was employed to provide information on the effects of valve closure, a behaviour which was expected to complicate our acid-immersion studies. The acid–base status of mussels was assessed by following changes in mantle fluid pH and composition. As well, we examined the effects of environmental pH on net calcium transfer in the animal.

Materials and methods

Freshwater pearl mussels (*Margaritifera margaritifera*) were collected in the area of the White Sea Biological Station of the Soviet Academy of Sciences, Chupa, Karelskaya ASSR (67 °N, 33 °E). The animals (12–13 cm in length, 40–50 g in mass) were held and tested at the Institute of Biology of Inland Waters, Borok, Jaroslavl Oblast, in flowing, aerated ground water (temperature 13–15 °C, pH 7·85, [calcium] 1·25–1·50 mmol l^{-1}). Each mussel was fitted with a 15–20 cm piece of polyethylene surgical tubing (PE 50), which was chronically implanted in the mantle cavity by way of the inhalant/exhalant aperture to enable sampling of mantle fluid.

The experiment was conducted in two stages. Initially, mussels were exposed to air in a humidified, thermostatted chamber (13 °C). After 7 days, some of the air-exposed mussels were transferred to circumneutral water (pH 7·85) and others were transferred to acidified water (pH 5·25) (water temperature 13 °C in both cases). The pH of acidified water was maintained by titration with 0·1 mol l^{-1} HCl, using a Soviet BAT-15 automatic titration block.

At specific time intervals throughout the experiment, samples of mantle fluid (250 μl) were withdrawn by way of the indwelling cannulae into gas-tight syringes. During the emersion phase of the experiment, sampled fluid was replaced with an equivalent volume of air-equilibrated water. The samples were analysed immediately for pH, total CO_2 content (C_{CO_2}), dissolved oxygen concentration (D_{O_2}) and calcium concentration. Mantle fluid pH was measured at the mussel body temperature using a glass electrode (Soviet ESL-43-07) adapted by us for work with small sample volumes (50 μl) under hermetic conditions. C_{CO_2} was measured using a CO_2 electrode (Radiometer type E5036), as described by Cameron (1971). The partial pressure of carbon dioxide (P_{CO_2}) and apparent bicarbonate concentration of mantle fluid were calculated from the measured C_{CO_2} and pH values, using rearrangements of the Henderson–Hasselbalch equation for carbonic acid and tabulated values for the apparent pK of carbonic acid and solubility coefficient of CO_2 in fresh water (Boutilier, Heming & Iwama, 1984). D_{O_2} was measured at the mussel body temperature using an O_2 electrode (Radiometer type E5046). Calcium concentration was determined by atomic absorption spectrophotometry (Soviet AAS-1).

To evaluate the effect of environmental acidification on the calcium balance of the animals, mussels were acclimated to acidified waters (pH 5·25 or 6·50, [calcium] 1·25–1·50 mmol l^{-1}, temperature 13 °C) for 45 days. The animals were then transferred to 5 l of distilled water containing $CaCl_2$ (0–410 μmol l^{-1}) at the same pH. The calcium concentration of the exposure water was monitored for 8 days.

An additional study was designed to determine the effect of environmental acidification on filtration activity of the animals. In this study, 30 mg l^{-1} of finely ground clay was suspended in water and the mixture allowed to stand for 24 h. Three litres of the supernatant was then poured into each of three tanks. A control group of mussels was added to one of the tanks. Water in the second tank was titrated to pH 5·25 and a test group of mussels added. The third tank served as a control for abiotic sedimentation. Abiotic sedimentation was found to be independent of water pH in our studies. The initial turbidity (S_i) and the final turbidity after 1 h of experimentation (S_f) in all three tanks were measured after specific time intervals using a Soviet SPEKOL-10 with TK attachment. The filtration activity (FA) of control and test animals was then calculated from the formula:

$$FA = (S_i - S_f) - (S_{ai} - S_{af})/\text{mussel mass}, \quad (1)$$

where S_{ai} and S_{af} were the respective initial and final turbidities in the abiotic sedimentation control. Changes in the filtration activity of test mussels were expressed as a percentage of that of paired control animals.

The data are presented as means ($\bar{x}$) or mean differences ($\bar{d}$), with their standard deviations (S.D. or $s_{\bar{d}}$, respectively). Mean differences were calculated between individual values and their paired initial (time zero) values. The significance of mean differences was assessed using paired *t*-tests (null hypothesis: $\bar{d} = 0$).

Results

Initial (time zero) characteristics of the mantle fluid of freshwater pearl mussels are presented in Table 1. The effects of air exposure and subsequent immersion of *M. margaritifera* in circumneutral or acidified waters are summarized in Fig. 1. During the emersion test period, no significant differences were observed between mussels destined for circumneutral immersion or acid immersion. Consequently, emersion data for the two groups were pooled (Fig. 1).

Air exposure resulted in a significant increase in mantle fluid pH within 24 h (Fig. 1). Mantle fluid pH increased progressively until day 4, after which little further change was observed. This alkalosis reflected a proportionally greater increase in mantle fluid carbonates than CO_2 during emersion (Fig. 2). Emersion was accompanied by an almost fivefold increase in mantle fluid bicarbonate, and the P_{CO_2} of mantle fluid approximately doubled. During the same exposure period, mantle fluid [calcium] increased 6·5-fold. The relationship between changes in mantle fluid bicarbonate (y, mmol l^{-1}) and changes in calcium (x, mmol l^{-1}) was best described as:

$$y = 1{\cdot}78 + 2{\cdot}24x\,, \qquad (2)$$

($r = 0{\cdot}882$, $N = 18$), indicating that carbonates were mobilized with calcium in a ratio of about 2:1. Mantle fluid D_{O_2} decreased during emersion from $8{\cdot}16 \pm 0{\cdot}41$ to $3{\cdot}07 \pm 0{\cdot}44$ mg l^{-1}, equivalent to a decrease from approximately 79 % of air saturation to 30 %. As with mantle fluid pH, the major alterations in [HCO_3^-], P_{CO_2}, D_{O_2} and [calcium] occurred during the first 4 days of air exposure; little further change was observed between days 4 and 7.

When air-exposed mussels were transferred to circumneutral water (pH 7·85), all the measured variables returned to their initial (time zero) values within 3 days (Fig. 1). Readjustment of mantle fluid pH in circumneutral water was accomplished by a rapid wash-out of accumulated carbonates and CO_2 by inhalant water (Fig. 2). At the time of immersion, there was little difference between inhalant water pH (7·85) and mantle fluid pH ($8{\cdot}01 \pm 0{\cdot}01$). Consequently, mantle fluid pH during the wash-out phase remained elevated, when compared with initial pH values. The subsequent return of mantle fluid pH to initial values resulted from

Table 1. *Characteristics of mantle fluid of* Margaritifera margaritifera *held in water at pH 7·85 and at 13 °C*

pH	$7{\cdot}73 \pm 0{\cdot}05$
C_{CO_2} (mmol l^{-1})	$5{\cdot}39 \pm 0{\cdot}55$
[HCO_3^-] (mmol l^{-1})	$5{\cdot}21 \pm 0{\cdot}55$
P_{CO_2} (mmHg)	$2{\cdot}8 \pm 0{\cdot}3$
D_{O_2} (mg l^{-1})	$8{\cdot}16 \pm 0{\cdot}41$
[calcium] (mmol l^{-1})	$1{\cdot}66 \pm 0{\cdot}37$

1 mmHg = 133·3 Pa.

an increase in fluid P_{CO_2} at a constant $[HCO_3^-]$. This increase in P_{CO_2} probably reflected the recovery of aerobic processes that were suppressed during the emersion period.

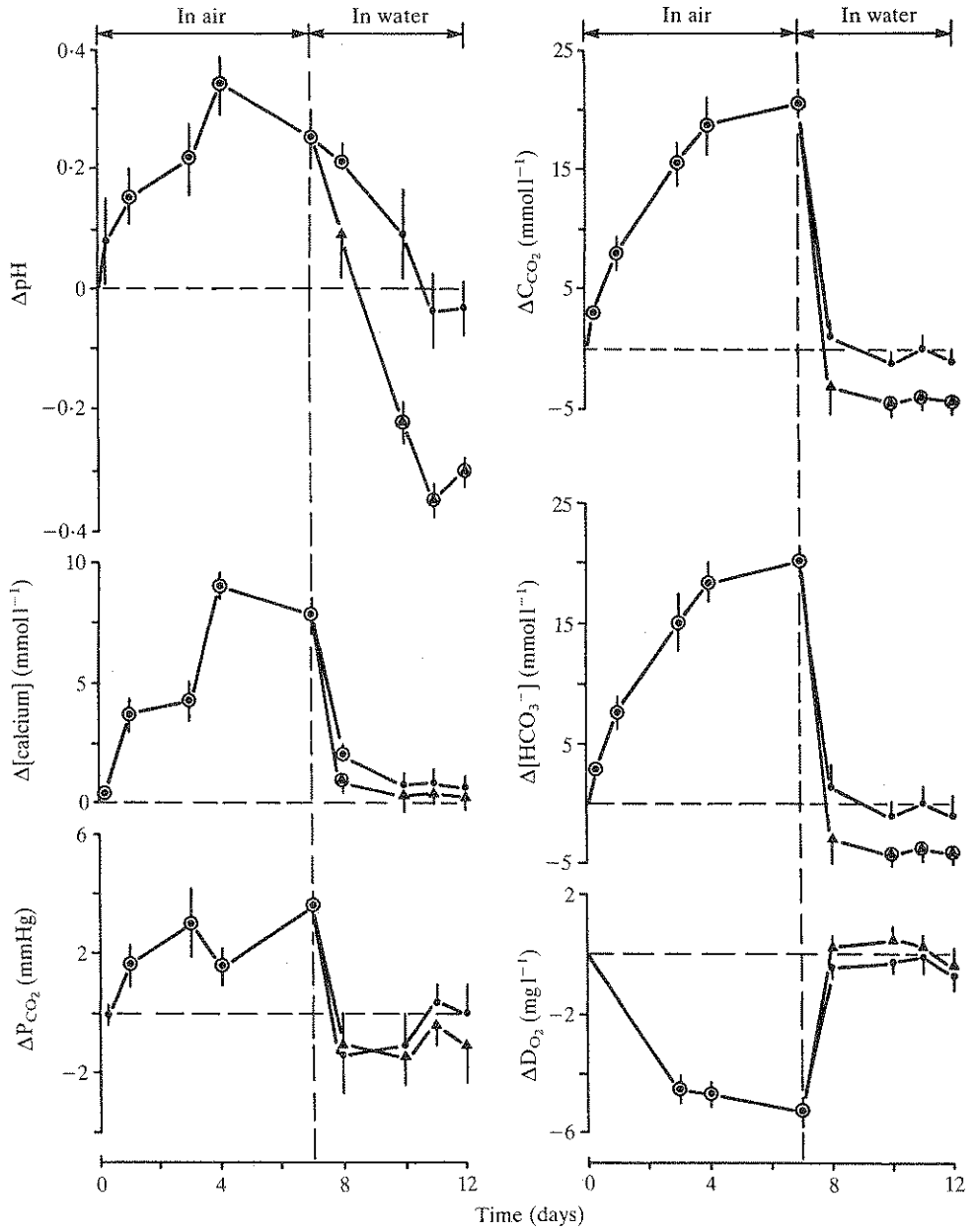

Fig. 1. Changes in the mantle fluid characteristics of *Margaritifera margaritifera* during air exposure and subsequent immersion in circumneutral water (pH 7·85, ●) or acidified water (pH 5·25, ▲) ($N = 4$). Circled entries are significantly different from zero ($P \leq 0{\cdot}05$). Mean differences ± $s_{\bar{d}}$.

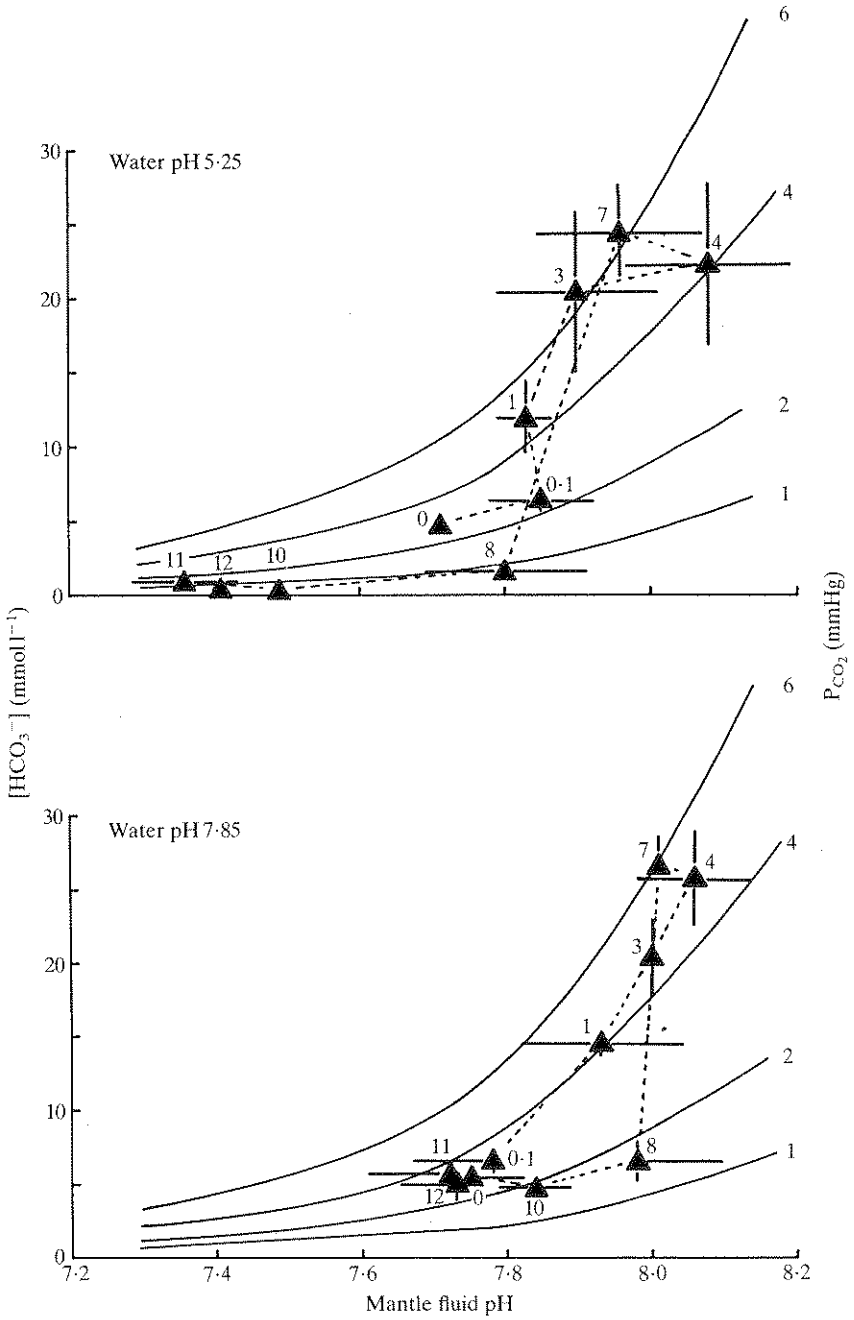

Fig. 2. Relationship between pH, HCO_3^- concentration and P_{CO_2} of the mantle fluid of *Margaritifera margaritifera*, during air exposure and subsequent immersion in circumneutral water (pH 7·85) or acidified water (pH 5·25) ($N = 4$). The study day is indicated beside each entry. Animals were emersed on day 0 and immersed on day 7. Mean values ± S.D.

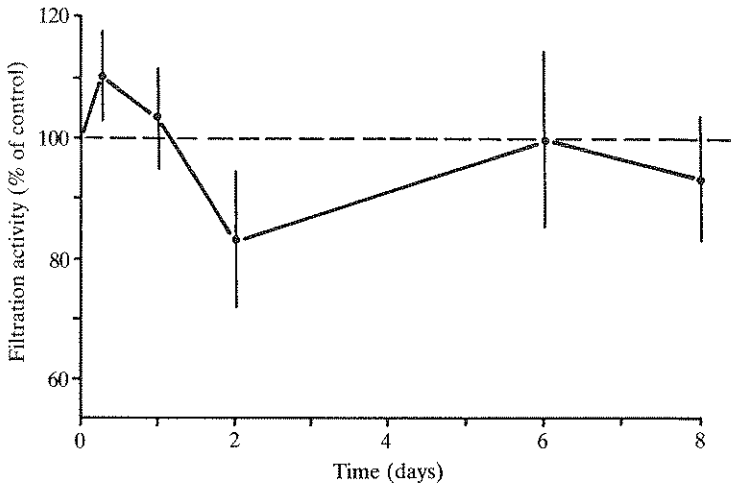

Fig. 3. Filtration activity of *Margaritifera margaritifera* in acid water (pH 5·25) relative to that of animals in circumneutral water (pH 7·85). Mean values ± S.D. ($N = 4$).

Immersion of air-exposed mussels in acidified water (pH 5·25) resulted in a significant acidosis of mantle fluids, relative to the initial (time zero) value (Fig. 1). Because of the large difference between inhalant water pH (5·25) and mantle fluid pH (7·96 ± 0·08) at the time of acid immersion, the wash-out phase in these animals was accompanied by reductions in mantle fluid pH, as well as in fluid $[HCO_3^-]$ and P_{CO_2} (Fig. 2). Mantle fluid $[HCO_3^-]$ of acid-immersed mussels was reduced both by wash-out with inhalant water and through its buffering of inhalant water pH. As a result, mantle fluid $[HCO_3^-]$ decreased in acid water to a new steady-state value approximately 4·6 mmol l^{-1} lower than the initial value. Mantle fluid pH reached a new steady-state value approximately 0·3 units lower than the initial value within 3 days of immersion. As with mussels transferred to circumneutral water, the readjustment in pH was accomplished by an increase in P_{CO_2} at a constant $[HCO_3^-]$. In the case of mussels transferred to acidified water, however, the increase in P_{CO_2} probably resulted from both a re-activation of aerobic processes and the buffering of inhalant water pH by endogenous carbonates.

Air-exposed *M. margaritifera* did not hermetically close their valves. Visual observations revealed that the mussels gaped periodically when in air. Similarly, the mussels did not isolate themselves from their external environment when immersed in acidified water. The filtration activity of mussels in acid water (pH 5·25) did not differ significantly from that of animals in circumneutral water (pH 7·85) (Fig. 3).

Mussels that had been acclimated to acid water (pH 5·25, [calcium] 1·25–1·50 mmol l^{-1}) for 45 days experienced a net calcium loss of 0·03 μmol $h^{-1} g^{-1}$ when immersed in water containing 324 μmol l^{-1} Ca^{2+} at pH 5·25. This negative calcium balance varied only slightly during the 8-day exposure (Fig. 4).

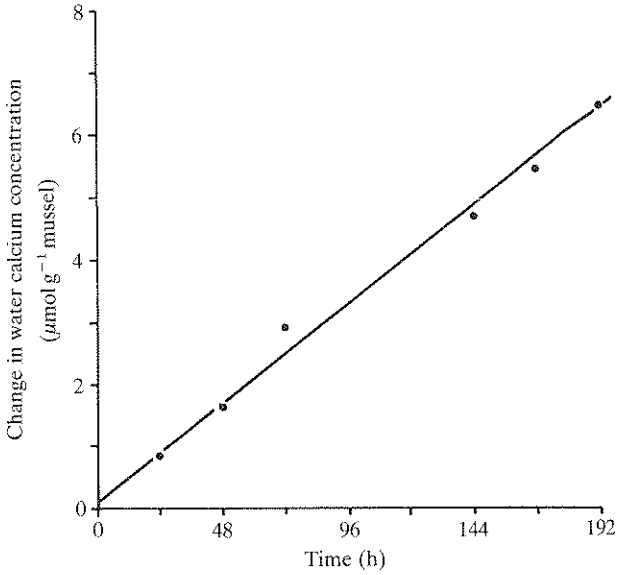

Fig. 4. Temporal changes in environmental calcium concentration during exposure of *Margaritifera margaritifera* to water containing 324 μmol l^{-1} $CaCl_2$ at pH 5·25 ($N = 4$). Best-fit regression: $y = 0{\cdot}150 + 0{\cdot}033x$ ($r = 0{\cdot}996$).

Table 2. *Calcium transfer in* Margaritifera margaritifera *as a function of the environmental calcium concentration at water pH 6·50 and at 13 °C*

Water calcium concentration (μmol l^{-1})	Increase in water calcium concentration (μmol h^{-1} g^{-1})
0	0·03 ± 0·01
20	0·02 ± 0·01
40	0·02 ± 0·01
179	0·01 ± 0·01
410	0·00 ± 0·01

$N = 6$.

Similar studies conducted at pH 6·50 indicated that environmental calcium had to exceed 179 μmol l^{-1} Ca^{2+} before *M. margaritifera* were able to establish a neutral calcium balance (Table 2). Interpolation of the data in Table 2 indicates that at pH 6·5 and 324 μmol l^{-1} calcium, the mussels experienced a net calcium loss of about 0·004 μmol h^{-1} g^{-1}, almost an order of magnitude less than the loss at the same environmental calcium concentration at pH 5·25. Thus, the net transfer of

calcium at a given ambient calcium concentration would appear to decrease as water pH decreases.

Discussion

Emersion of *Margaritifera margaritifera* was accompanied by an elevation of mantle fluid P_{CO_2} and a decline in D_{O_2}, indicating that external gas exchange was reduced in air. Whether the observed decrease in mantle fluid D_{O_2} was sufficient to cause a shift to fermentation metabolism is unclear from the present study. Bivalves typically are capable of fermentation metabolism (de Zwann & Wijsman, 1976). During emersion, the eventual stabilization of D_{O_2} at a level much lower than normal might reflect adoption of fermentation metabolism and/or periodic renewal of mantle cavity O_2 stores by inward diffusion of atmospheric O_2 when the mussels gaped. Periodic gaping has been found to allow relatively high rates of aerial O_2 consumption (20% of aquatic rate) in emersed *Corbicula fluminea* (McMahon, 1979; McMahon & Williams, 1981).

CO_2 retention during emersion, together with accumulation of acid metabolites, might be expected to cause a mixed respiratory–metabolic acidosis. Decreases in mantle fluid and/or haemolymph pH have been recorded during valve closure in other bivalve species (Dugal, 1939; Crenshaw & Neff, 1969; Crenshaw, 1972; Fyhn & Costlow, 1975). In contrast, our data indicate that air exposure of *M. margaritifera* leads to a significant alkalosis of mantle fluids. As discussed below, this alkalosis can be explained in terms of the establishment of a dilute carbonate buffer equilibrium involving reactions of $CaCO_3$ and, simultaneously, aerial release of CO_2 (Fig. 2).

Air exposure of the freshwater pearl mussel resulted in mobilization of carbonates and calcium into the mantle fluid in a ratio of approximately 2:1. This is consistent with the buffering action of $CaCO_3$ (Burton, 1983), originating from the shell (Dugal, 1939) and/or from concentrations elsewhere in the body (Istin & Girard, 1970). The observed increase in concentrations of mantle fluid carbonates and calcium during air exposure is in general agreement with previous studies of emersed bivalves (Dugal, 1939; Crenshaw & Neff, 1969; Alyakrinskaya, 1972).

Visual observations showed that *M. margaritifera* periodically opened their valves when in air. Gaping during emersion allows for aerial release of CO_2, evolved when acid metabolites are buffered by $CaCo_3$ or produced as an end-product of aerobic metabolism (Wijsman, 1975; de Zwaan & Wijsman, 1976; Booth & Mangum, 1978). In the present study, aerial release of CO_2 was insufficient to maintain mantle fluid P_{CO_2} at normal levels. Aerial CO_2 release was sufficient, however, to shift the carbonate buffer equilibrium in the alkaline direction (Fig. 2). This interpretation is supported by the results of Wijsman (1975) and Booth & Mangum (1978). Their studies demonstrate the existence of a relationship between the degree of valve closure and the pH of mantle fluids and haemolymph in *Mytilus edulis* and *Modiolus demissus*. With a total hermetization, the mantle fluids and haemolymph acidified. With a partial hermetization, such as

occurred in our study, there was a tendency towards alkalinization of the mantle fluids and a decrease in the degree of haemolymph acidification.

Immersion in acid water (pH 5·25) caused a significant acidosis of the mantle fluids of *M. margaritifera*. The mussels were able to establish a sizeable gradient, however, between the levels of acidity of mantle fluid and ambient water. At apparent steady state, the H^+ concentration of mantle fluid was only 0·7 % of that of ambient water. Maintenance of this pH gradient did not involve an isolation reaction (continual valve closure) by the bivalves; filtration activity of the mussels was unaffected by immersion in water at pH 5·25. Rather, protons entering the mantle cavity with inspired water were neutralized, largely through reactions with $CaCO_3$.

M. margaritifera relied primarily on utilization of its $CaCO_3$ reserves to regulate acid–base homeostasis during both emersion and acid immersion. An isolation reaction (continual valve closure) was not evoked by either stimulus. During emersion, periodic gaping permitted aerial gas exchange to a limited degree, but one might reasonably expect evaporative water loss to be greater than during complete hermetization. During acid immersion, the lack of an isolation reaction permitted relatively tight regulation of acid–base homeostasis while maintaining normal filtration activity, but at the expense of the mussel's calcium reserves. Net calcium transfer, the difference between calcium uptake and loss, was shifted in the negative direction by decreases in water pH. Thus, regulation of mantle fluid pH in poorly mineralized, acid water resulted in a gradual depletion of the animal's calcium reserve, which is emitted together with exhalant water. For adult mussels in the present study (live mass 40–50 g), a net daily loss of 32 μmol Ca^{2+}, equivalent to 4 mg $CaCO_3$, in poorly mineralized, acid water (calcium 324 μmol l^{-1}, pH 5·25) was probably of minimal biological significance. Moreover, in natural conditions where significant quantities of calcium enter bivalves during feeding (Timmermans, 1969; Romanenko, Arsan & Solomatina, 1982), ingested calcium would partially compensate for the calcium losses associated with acid–base regulation. Nonetheless, environmental acidification can be expected to play a negative ecological role in the distribution of *M. margaritifera*. The effect of water pH on net calcium transfer probably has substantial impact on early life stages when intensive valve growth takes place.

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References

ALYAKRINSKAYA, I. O. (1971). Several adaptations of *Mytilus galloprovincialis* (Bivalvia) and *Planorbis corneus* (Gastropoda) to the absence of water. *Zool. Zh.* **50**, 648–656.

ALYAKRINSKAYA, I. O. (1972). Biochemical adaptations of aquatic molluscs to air environment. *Zool. Zh.* **51**, 1630–1635.

ALYAKRINSKAYA, I. O. (1977). On adaptations to the conditions of drying of the White Sea littoral bivalves. *Zool. Zh.* **56**, 1110–1112.

BAUER, G., SCHRIMPFF, E., THOMAS, W. & HERRMANN, R. (1980). Zusammenhänge zwischen dem Bestandsrückgang der Flussperlmuschel (*Margaritifera margaritifera*) im Fichtelgebirge und der Gewasserbelastung. *Arch. Hydrobiol.* **88**, 505–513.

BOOTH, C. E. & MANGUM, C. P. (1978). Oxygen uptake and transport in the lamellibranch mollusc *Modiolus demissus*. *Physiol. Zool.* **51**, 17–32.

BOUTILIER, R. G., HEMING, T. A. & IWAMA, G. K. (1984). Physicochemical parameters for use in fish respiratory physiology. In *Fish Physiology*, vol. 10A (ed. W. S. Hoar & D. J. Randall), pp. 403–430. New York: Academic Press.

BURTON, R. F. (1983). Ionic regulation and water balance. In *The Mollusca*, vol. 5 (ed. A. S. M. Saleuddin & K. M. Wilbur), pp. 291–352. New York: Academic Press.

CAMERON, J. N. (1971). Rapid method for determination of total carbon dioxide in small blood samples. *J. appl. Physiol.* **31**, 632–634.

CHAISEMARTIN, C. (1968). Place de la function rénale dans la régulation de l'eau et des sels chez *Margaritana margaritifera* (Unionidés). *C. R. Séances Soc. Biol. Filiales* **162**, 1193–1195.

CHAISEMARTIN, C., MARTIN, P. N. & BERNARD, M. (1968). Homéoionémie chez *Margaritana margaritifera* L. (Unionidés), étudiée à l'aide des radioéléments ^{24}Na et ^{36}Cl. *C. R. Séances Soc. Biol. Filiales* **162**, 523–526.

COLLIP, J. B. (1921). A further study of the respiratory processes in *Mya arenaria* and other marine mollusca. *J. biol. Chem.* **49**, 297–310.

CRENSHAW, M. A. (1972). The inorganic composition of molluscan extrapallial fluid. *Biol. Bull. mar. biol. Lab., Woods Hole* **143**, 506–512.

CRENSHAW, M. A. & NEFF, J. M. (1969). Decalcification at the mantle–shell interface in molluscs. *Am. Zool.* **9**, 881–885.

DE ZWAAN, A. & WIJSMAN, T. C. M. (1976). Anaerobic metabolism in bivalvia (Mollusca). Characteristics of anaerobic metabolism. *Comp. Biochem. Physiol.* **54**B, 313–324.

DUGAL, L.-P. (1939). The use of calcareous shell to buffer the product of anaerobic glycolysis in *Venus mercenaria*. *J. cell. comp. Physiol.* **13**, 235–251.

FYHN, H. J. & COSTLOW, J. D. (1975). Anaerobic sampling of body fluids in bivalve molluscs. *Comp. Biochem. Physiol.* **52**A, 265–268.

HAINES, T. A. (1981). Acidic precipitation and its consequences for aquatic ecosystems: a review. *Trans. Am. Fish. Soc.* **110**, 669–707.

ISTIN, M. & GIRARD, J. P. (1970). Carbonic anhydrase and mobilisation of calcium reserves in the mantle of lamellibranchs. *Calcif. Tissue Res.* **5**, 247–260.

KLUPP, R. (1983). Das Perlmuschelproblem. *Fischwirt.* **33**, 34–36.

KRASOWSKA, E. (1978). Skoja perlorodna, *Margaritifera margaritifera* L. (Mollusca, Bivalvia) – gatunek chroniony. *Przegl. Zool.* **12**, 48–54.

MCMAHON, R. F. (1979). Tolerance of aerial exposure in the Asiatic freshwater clam, *Corbicula fluminea* (Müller). *Proc. 1st int.* Corbicula *Symp. 1977*, 227–241.

MCMAHON, R. F. (1983). Ecology of an invasive pest bivalve, *Corbicula*. In *The Mollusca*, vol. 6 (ed. W. D. Russel-Hunter), pp. 505–562. New York: Academic Press.

MCMAHON, R. F. & WILLIAMS, C. J. (1981). Aerial oxygen consumption in the introduced freshwater clam, *Corbicula fluminea* (Müller). *Am. Zool.* (Abstr.) **21**, 983.

MALLEY, D. F. & CHANG, P. S. S. (1985). Effects of aluminium and acid on calcium uptake by the crayfish *Orconectes virilis*. *Archs environ. Contam. Toxicol.* **14**, 739–747.

ROMANENKO, V. D., ARSAN, O. M. & SOLOMATINA, V. (1982). *Calcium and Phosphorus in the Vital Activity of Hydrobiots*. Kiev: Naukova Dumka.

TIMMERMANS, L. P. M. (1969). Studies on shell formation in molluscs. *Neth. J. Zool.* **19**, 417–523.

WIJSMAN, T. C. M. (1975). pH fluctuation in *Mytilus edulis* L. in relation to shell movements under aerobic and anaerobic conditions. *Proc. 9th europ. mar. Biol. Symp. 1975*, 139–149.

YOUNG, M. & WILLIAMS, J. (1983). The status and conservation of the freshwater pearl mussel *Margaritifera margaritifera* Linn. in Great Britain. *Biol. Conserv.* **25**, 35–52.