

Effect of Salinity Fluctuation on the Osmotic Pressure and Na^+ , Ca^{2+} and Mg^{2+} Ion Concentrations in the Hemolymph of Bivalve Molluscs

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Abstract

Specimens of *Chlamys opercularis*, *Modiolus modiolus*, *Mytilus edulis*, *Crassostrea gigas*, *Scrobicularia plana* and *Mya arenaria* were exposed to both gradual (sinusoidal) and abrupt (square-wave) salinity fluctuations and measurements made of osmotic, Na^+ , Mg^{2+} and Ca^{2+} concentrations in the hemolymph and where applicable in the mantle fluid. In both sinusoidal and square-wave regimes fluctuating between 100 and 50% seawater (100% = ca. 32‰ S), the hemolymph Na^+ , Mg^{2+} , Ca^{2+} and osmotic concentrations followed the concentrations of the external medium in *Chlamys opercularis*. The hemolymph and mantle fluid osmotic Na^+ , Mg^{2+} and Ca^{2+} concentrations of *Modiolus modiolus*, *Mytilus edulis*, *Crassostrea gigas* and *S. plana* followed those of the external medium as long as the molluscs' shell valves remained open. There were no changes in the ionic or osmotic concentrations of the hemolymph or mantle fluid of any of these species during periods of shell-valve closure. The hemolymph osmotic, Na^+ and Mg^{2+} concentrations of wedged-open *Modiolus modiolus*, *Mytilus edulis*, *C. gigas* and *S. plana* followed those of the external medium. Hemolymph Ca^{2+} concentrations showed a damped response in *C. gigas* and *Mytilus edulis*. The hemolymph osmotic, Na^+ , Ca^{2+} and Mg^{2+} concentrations of *Mya arenaria* fluctuated in a similar manner to the external medium, but were damped. Wedged-open *Mytilus edulis* exposed to fluctuating salinity and supplied with a constant supply of 10 mM Ca^{2+} showed greater changes in hemolymph ionic and osmotic concentrations than *M. edulis* exposed to the same salinity fluctuation without a constant Ca^{2+} supply. *Chlamys opercularis* and *Modiolus modiolus* survived in a 50% seawater minimum sinusoidal salinity fluctuation for 10 days; wedged-open *M. modiolus* survived only 3 days. Burrowing had no effect on the osmotic, Na^+ , Mg^{2+} or Ca^{2+} concentrations of the hemolymph of *Mya arenaria* or *S. plana* exposed to fluctuating salinities. All of the species studied were shown to be osmoconformers.

Introduction

Animals of the littoral zone, estuaries and tidepools experience large and sometimes rapid fluctuations in salinity caused by tidal fluctuation, rain and freshwater run-off. Many marine invertebrates can only withstand slight changes in the osmotic concentration of the external environment. Repeated fluctuations in salinity may subject organisms to considerable stress (Webb, 1958). In these areas of varying salt concentration, animals must have either a high tolerance level or some functional method of preventing their internal medium from fluctuating widely. The reaction and tolerance of molluscs to changes in salinity have been studied by numerous authors

(see Robertson, 1964; Schoffeniels and Gilles, 1970; Stickle and Ahokas, 1973). The body fluids and tissues of most marine bivalve molluscs were thought to be isosmotic with the surrounding seawater and the concentration of mineral salts of the extracellular fluid normally close to that of the environment (Potts and Parry, 1964; Prosser, 1973). Bedford and Anderson (1972) have now shown *Rangia cuneata* to be an osmo-regulator in low salinities and Pierce (1970) showed that four species of *Modiolus* were always slightly hyperosmotic to the external environment over the non-lethal salinity range. Several other workers have studied ionic regulation of the hemolymph in bivalves (Yazaki, 1932; Topping and Fuller, 1942; Fingerman and

Table 1. *Chlamys opercularis*, *Modiolus modiolus*, *Mytilus edulis*, *Crassostrea gigas*, *Scrobicularia plana* and *Mya arenaria*. Na⁺, Ca²⁺, Mg²⁺ concentrations in hemolymph (hl) and mantle fluid (mf). Osmotic concentrations of Menai Strait seawater are also given

	Osmolality (mOsm/Kg H ₂ O)	Na ⁺ (mM)	Ca ²⁺ (mM)	Mg ²⁺ (mM)
Menai Strait seawater	965 ± 5	465 ± 7	10.0 ± 0.4	52.1 ± 1.0
<i>C. opercularis</i>				
hl	970 ± 5	470 ± 12	10.2 ± 0.7	53.0 ± 1.1
<i>M. modiolus</i>				
hl	980 ± 11	471 ± 17	10.2 ± 0.9	54.1 ± 0.7
mf	970 ± 9	465 ± 9	10.0 ± 0.9	53.2 ± 0.9
<i>M. edulis</i>				
hl	980 ± 4	468 ± 8	10.4 ± 0.8	58.0 ± 1.2
mf	955 ± 9	455 ± 11	10.2 ± 0.9	55.0 ± 1.4
<i>C. gigas</i>				
hl	980 ± 11	478 ± 6	10.2 ± 0.6	55.4 ± 1.0
mf	960 ± 13	460 ± 8	10.1 ± 0.1	54.2 ± 1.1
<i>S. plana</i>				
hl	975 ± 14	470 ± 17	10.2 ± 1.3	52.5 ± 1.6
mf	960 ± 11	462 ± 11	9.8 ± 1.4	51.4 ± 1.1
<i>M. arenaria</i>				
hl 100% seawater acclimated	1010 ± 19	478 ± 19	10.2 ± 1.0	53.0 ± 1.7
60% seawater acclimated	583 ± 13	284 ± 15	7.9 ± 1.0	37.0 ± 1.1
20% seawater acclimated	210 ± 16	112 ± 14	2.4 ± 0.7	11.2 ± 2.0

Each value is mean ± standard error for 25 separate determinations.

of permeability, which was clearly high in *C. opercularis*.

Response of *Modiolus modiolus* to Fluctuating Salinity

Normal *Modiolus modiolus* placed in a fluctuating salinity system survived in both sinusoidal and square-wave profiles varying between 50 and 100% seawater for 10 days. In contrast, wedged-open individuals exposed to the same programs died by the third day. Valve closure occurred at approximately 80% seawater in a 50% seawater minimum sinusoidal profile (visual observation). From Fig. 5 it can be seen that until the valves closed, the Na⁺, Ca²⁺, Mg²⁺ and osmotic concentrations of the hemolymph closely followed those of the external medium with a time lag presumably dependent on the molluscs' characteristic salt and water permeability. After the valves had closed, the hemolymph osmolality and ion concentrations continued to fall but very gradually. When the molluscs reopened, at about 80% seawater, the con-

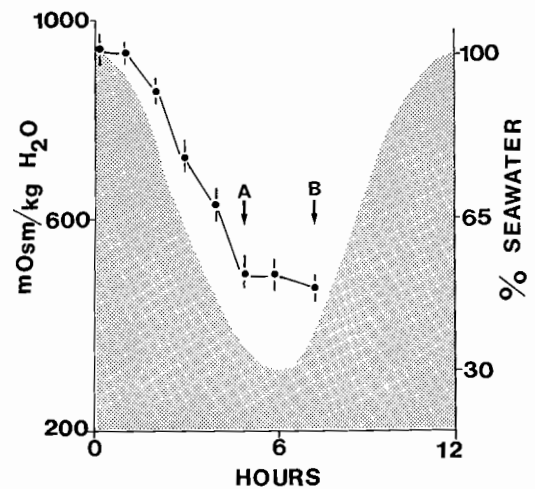


Fig. 3. *Chlamys opercularis*. Changes in hemolymph osmolality during exposure to 30% seawater minimum sinusoidal salinity regime. Arrows A and B indicate 50 and 100% mortality, respectively. Stippled area represents changes in external medium. Each point is mean of 3 scallops. Error bars represent 95% confidence limits

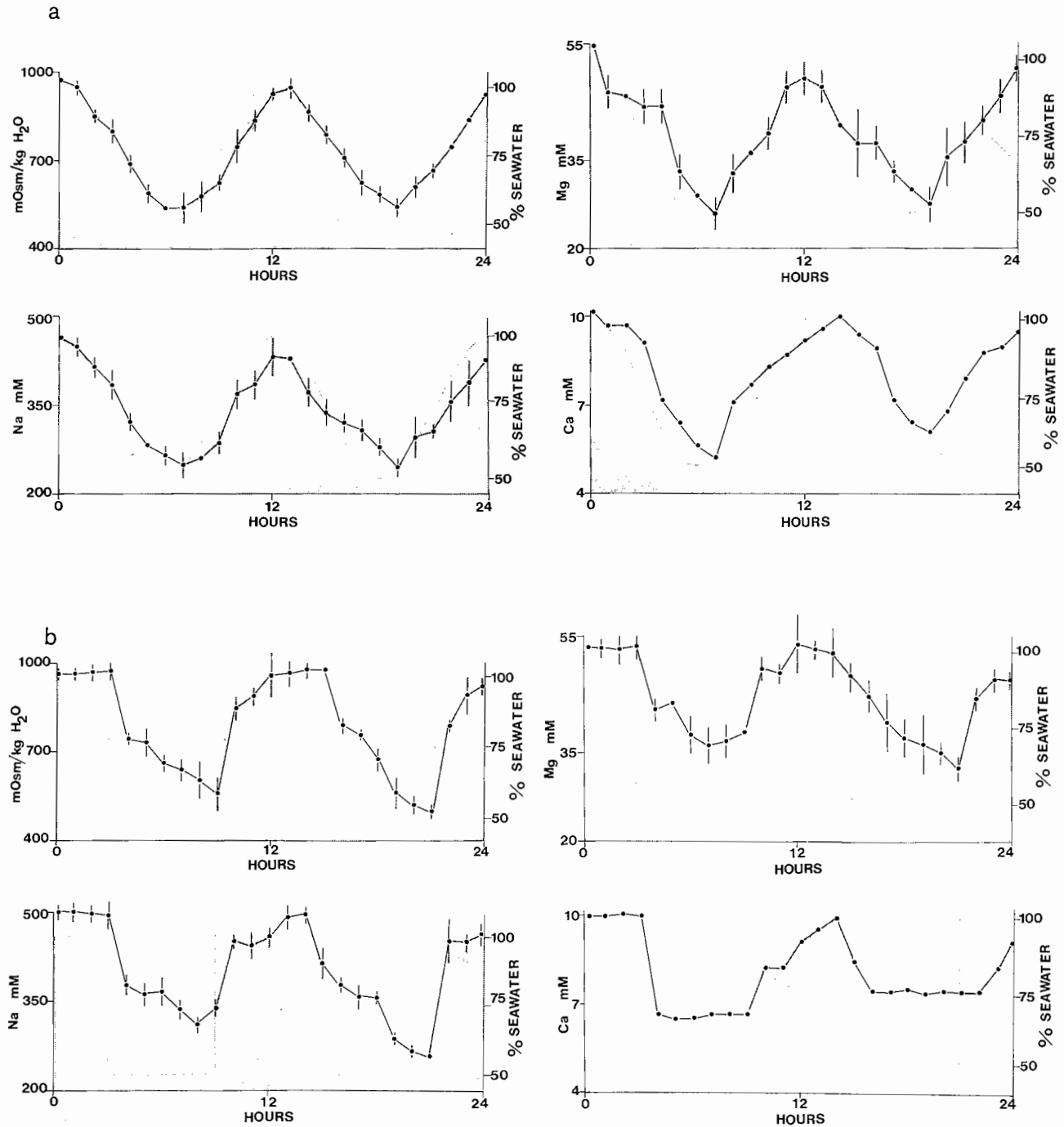


Fig. 4. *Chlamys opercularis*. Changes in hemolymph osmolality, and Na⁺, Mg²⁺ and Ca²⁺ concentrations during exposure to (a) 50% seawater minimum sinusoidal salinity regime and (b) 50% seawater minimum square-wave salinity regime. Stippled areas represent changes in external medium. Each point is mean of 3 scallops. Error bars represent 95% confidence limits

Fairbanks, 1956; Bedford and Anderson, 1972) and have reported ion concentrations in bivalves (for reviews see Hayes and Pelluet, 1947; Robertson, 1949; Prosser, 1973).

If a physiological mechanism of regulation is absent, it is necessary for the organism to develop some behavioral method if it is to survive in the estuarine environment. Such mechanisms include shell closure, secretion of mucus, burrowing and withdrawal of sensitive body parts (Kinne, 1971). In bivalves this temporary protection is normally accomplished by shell valve closure and this phenomenon has been interpreted by some authors as active regulation (Fingerman and Fairbanks, 1956; Freeman and Rigler, 1957). These authors based their assumptions on data obtained from bivalves capable of shell-valve closure, so it was not clear whether the bivalves were actually regulating their osmotic concentration or simply shutting themselves off from the external environment. A feature of virtually all previous work is that data was obtained from steady-state salinity experiments. This approach, however, has attracted recent criticism from Stickle and Ahokas (1973, 1975), Zachary and Haven (1973) and Davenport *et al.* (1975).

In this study, a simulated estuarine salinity regime was used to investigate what changes in the sodium, magnesium, calcium and osmotic concentrations of the hemolymph and mantle cavity fluid would occur in the natural environment of 6 species of bivalves representing the sublittoral [*Chlamys opercularis* (L.) and *Modiolus modiolus* (L.)], littoral [*Mytilus edulis* L. and *Crassostrea gigas* (Thunberg)] and estuarine [*Scrobicularia plana* (da Costa) and *Mya arenaria* L.] environments. The importance of shell-valve closure and burrowing as means of temporary protection from a noxious external environment was also investigated.

Materials and Methods

Molluscs were collected locally from the Anglesey coast. *Chlamys opercularis* and *Modiolus modiolus* were kept at 8°C in aquaria supplied with running seawater pumped from the Menai Strait (salinity approximately 32%). *Mya arenaria* were acclimated to 20, 60 and 100% seawater at 15°C for 4 weeks prior to use. Menai Strait seawater was diluted with distilled water to give the appropriate salinity. All other species were kept at 15°C in aquaria with running seawater pumped from the Menai Strait.

The apparatus used to produce fluctuating salinity regimes has been described by Davenport *et al.* (1975). Hoyeaux *et al.* (1976) have shown that some species of bivalves are capable of temporarily isolating themselves from the external environment by closing the shell valves. In *Chlamys opercularis* and *Mya arenaria* such complete isolation is anatomically impossible, but the closure phenomenon was investigated by the use of both normal specimens of *Mercenaria mercenaria*, *Scrobicularia plana*, *Cardium edule*, *Mytilus edulis*, *Crassostrea gigas* and *Modiolus modiolus* and specimens kept open with small plastic wedges inserted ventrally between the shell valves and cemented to one valve with dental cement. Such wedges allowed the molluscs to open at will, but prevented them from closing completely.

All specimens were subjected to both gradual (sinusoidal) and abrupt (square-wave) salinity changes following the patterns shown in Fig. 1. Maximum seawater concentration was always 100% (approximately 32‰ S); minimum seawater concentration varied between programs (see relevant figures). Individuals used in burrowing experiments were placed in sand-filled Plexi-glass boxes (Fig. 2), and remained in the burrows for 3 days prior to use. *Mya arenaria* were placed in burrows according to the methods described by Van Dam (1935). *Scrobicularia plana* burrowed within minutes of being placed on the surface of the sand. Interstitial salinities were not measured, as Freeman and Rigler (1957) state that as far as the salinity relations of these molluscs are concerned the external medium is represented by the water above the sand or mud. Experiments with *Chlamys opercularis* and *Modiolus modiolus* were carried out at 8°C; experiments with all other species were carried out at 15°C.

In addition, some wedged-open *Mytilus edulis* were exposed to a 30‰ seawater minimum square-wave salinity profile while the Ca²⁺ concentration was maintained at 10 mM. This was accomplished by adding a solution of CaCO₃-CaCl₂ to seawater to give a final concentration of 10 mM Ca²⁺ and a salinity of 10‰ (approximately 30‰ seawater). The pH of the seawater was not affected.

A total of 169 *Chlamys opercularis*, 313 *Modiolus modiolus*, 698 *Mytilus edulis*, 313 *Crassostrea gigas*, 500 *Scrobicularia plana* and 650 *Mya arenaria* were examined. Samples of 3 to 4 ml of mantle fluid were taken from *Modiolus modiolus*, *Mytilus edulis*, *Crassostrea gigas* and *Scrobicularia plana* by prising open the valves and withdrawing the fluid from the mantle cavity with a hypodermic syringe (Pierce,

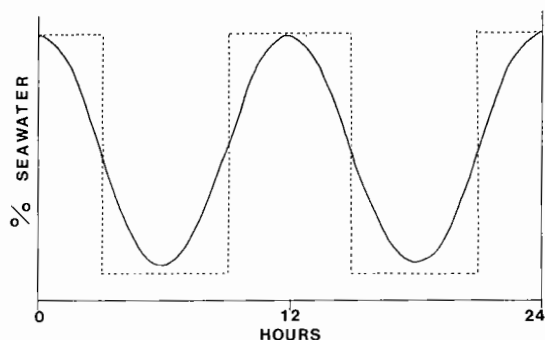


Fig. 1. Patterns of salinity fluctuations. Maximum seawater concentration = 100% (ca. 32‰ S)

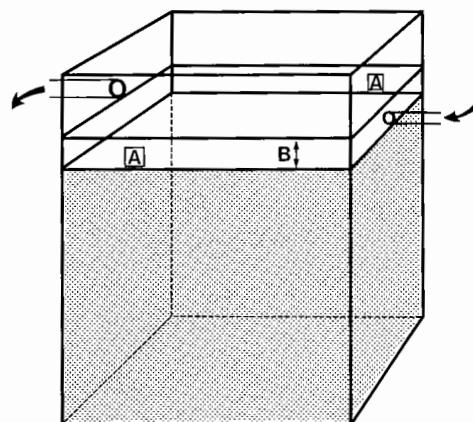


Fig. 2. Burrowing chamber. Stippled area represents depth of sand. Curved arrows indicate direction of water flow. A and B show location of air supply and water depth, respectively

1970). Hemolymph samples were obtained by blotting the bivalve dry, still on the half-shell, and then slashing the mantle, pallial blood sinus and adductor muscles. The anterior end of the mollusc was then inserted into a beaker and the hemolymph drained from the cut surfaces for 5 to 10 min (Pierce, 1970). Approximately 0.5 ml of hemolymph was taken from each specimen. Since *Chlamys opercularis* is not capable of completely isolating itself from the external environment, the mantle cavity fluid was assumed to be identical with the external medium. All fluid samples were centrifuged for 2 min at 8,000 $\times g$ to remove debris.

The osmotic concentration of all samples was measured immediately, using a Halbmikro Osmometer with a reading accuracy of ± 1 mOsm. Three determinations were made upon each sample and the mean value calculated. The samples were then frozen in Eppendorf tubes for ionic analyses.

Sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations were determined using a Pye Unicam atomic absorption spectrophotometer. Lanthanum oxide at a final concentration of 0.02% was added to samples for the determination of calcium and magnesium to prevent interference by other cations and certain anions (Stickney, 1971). Dilutions were 1/4000 for Na^+ and Mg^{2+} and 1/100 for Ca^{2+} . Standards were made up in an artificial seawater matrix according to Perkin-Elmer (1973).

Results

For comparative purposes, analyses were made of the hemolymph and mantle fluid of molluscs which had been kept for at least 2 weeks (4 weeks in the case of *Mya arenaria* acclimated to dilute seawater) in running seawater from the Menai Strait. Table 1 shows the Na^+ , Ca^{2+} , Mg^{2+} and osmotic concentrations of Menai Strait seawater and of the hemolymph of *Chlamys opercularis*, *Modiolus modiolus*, *Mytilus edulis*, *Crassostrea gigas*, *Scrobicularia plana* and *Mya arenaria*. In all cases, the hemolymph was only slightly more concentrated than the surrounding seawater.

Response of *Chlamys opercularis* to Fluctuating Salinity

Fig. 3 shows the changes in hemolymph osmolality of *Chlamys opercularis* during exposure to a 30% seawater minimum sinusoidal salinity fluctuation. All specimens became moribund at approximately 50% seawater. However, the scallops tolerated a 50% seawater minimum sinusoidal fluctuation for up to 10 days. In the sinusoidal and square-wave regimes shown in Fig. 4a and b, respectively, fluctuating between 100% and 50% seawater, the hemolymph Na^+ , Ca^{2+} , Mg^{2+} and osmotic pressure values followed the concentration of the external medium. In all cases there was evidence of a time lag between hemolymph and seawater concentrations; obviously this was a diffusion effect, the lag depending upon the level

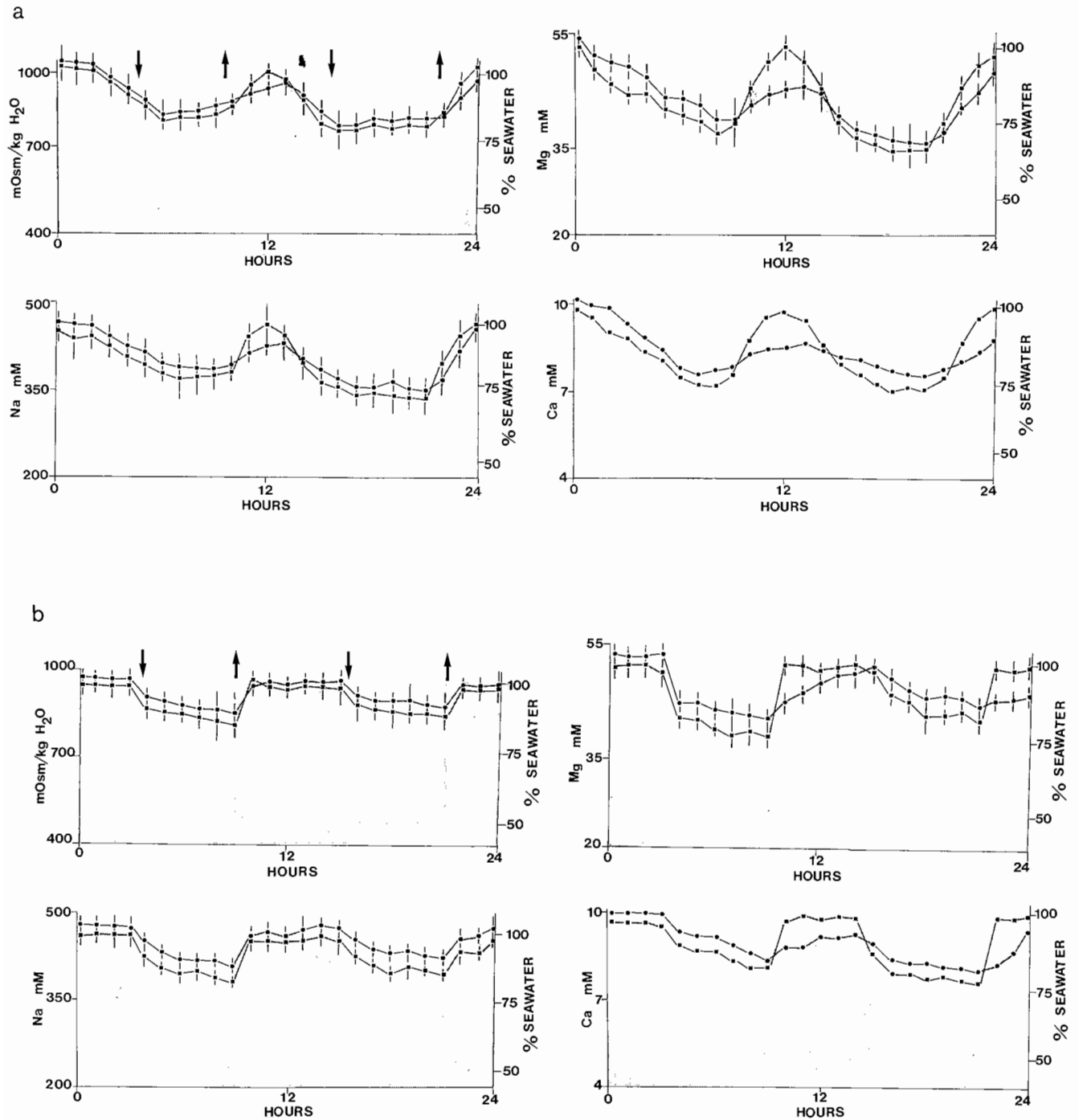


Fig. 5. *Modiolus modiolus*. Changes in hemolymph (circles) and mantle fluid (squares) osmolality and Na⁺, Mg²⁺ and Ca²⁺ concentrations during exposure to (a) 50% seawater minimum sinusoidal salinity regime and (b) 50% seawater minimum square-wave salinity regime. Stippled areas represent changes in external medium. Arrows indicate points of shell-valve closure (↓) and opening (↑). Each point is mean of 3 bivalves. Error bars represent 95% confidence limits

centrations began to rise again in an obviously passive manner. Because of the rapid changes in salinity in the square-wave profiles, it was not possible to determine the exact seawater concentration at which the molluscs closed and opened. It seems clear, however, that in this case as well, a slight concentration decline occurred after closure. The results for wedged-open *M. modiolus* (Fig. 6) show that the hemolymph osmotic and ionic concentrations changed passively with the external medium, similarly to *Chlamys opercularis*.

Response of Mytilus edulis to Fluctuating Salinity

As can be seen from Fig. 7, which shows the changes in body fluids of *Mytilus edulis* in a sinusoidal salinity regime (both 0 and 30‰ seawater minimum), the mussels closed the shell valves when the seawater concentration dropped to approximately 44.3‰ and the hemolymph osmolality to about 700 mOsm. As the salinity continued to decline, the mussels remained closed but reopened when the salinity increased to approximately 37‰ seawater. During this period of shell-valve closure, the mantle fluid and hemolymph ionic and osmotic concentrations remained constant. When the salinity changes were repeated, the valve closure and opening occurred at similar salinity values and the hemolymph and mantle fluid concentrations again remained constant during closure.

When placed in a square-wave profile (both 0 and 30‰ seawater minimum) the mussels closed between Hours 3 and 4 when the osmotic concentration of the hemolymph was approximately 840 to 880 mOsm, and re-opened between Hours 9 and 10 when the salinity increased, as can be seen in Fig. 8.

Experiments with wedged-open mussels showed a time lag in their response to salinity change. In the 30‰ seawater minimum sinusoidal regime, the hemolymph osmolality fell to 600 mOsm during the first cycle and 480 mOsm during the second cycle (Fig. 9a). The 0‰ sinusoidal regime gave a minimum hemolymph osmolality of 520 mOsm during the first cycle and 440 mOsm for the second cycle (Fig. 9b). It can be seen in Fig. 10a that mussels placed in the 30‰ seawater minimum square-wave profile had a low hemolymph osmolality of 680 mOsm in the first cycle and 740 mOsm in the second. There was a low of 360 mOsm for hemolymph in the first cycle of the 0‰ minimum square-wave profile and 240 mOsm in the second (Fig. 10b).

In all cases, the hemolymph Na^+ concentration closely followed the total osmotic concentration of the hemolymph, as can be seen in Figs. 7-10. In normal mussels, the Mg^{2+} and Ca^{2+} ion concentrations of the mantle fluid also followed those of the external environment when the animals were open (Figs. 7 and 8). The Mg^{2+} level of the hemolymph followed that of total osmolality. However, the Ca^{2+} levels of hemolymph dropped rapidly during the first cycle but only rose slowly or remained constant during the second cycle. In wedged-open mussels, both Ca^{2+} and Mg^{2+} concentrations in the hemolymph fluctuated in a damped but similar manner to those of the external environment, as shown in Figs. 9 and 10.

Calcium Enrichment Experiment

Mytilus edulis placed in the 30‰ seawater minimum abrupt profile, when the Ca^{2+} concentration remained constant (Fig. 11), showed significantly lower osmotic, Na^+ , and Mg^{2+} concentrations than those individuals subjected to the same profile without a constant Ca^{2+} supply (Fig. 7a). The hemolymph Ca^{2+} level remained below that of full seawater during the periods of low salinity, but was significantly higher than the Ca^{2+} level of mussels not given a constant Ca^{2+} supply.

Response of Crassostrea gigas to Fluctuating Salinity

Crassostrea gigas placed in a 30‰ seawater minimum sinusoidal salinity profile closed at 61‰ seawater with a hemolymph osmolality of approximately 700 mOsm (Fig. 12). As the salinity continued to decline, the oysters remained closed and re-opened when the external salinity reached approximately 42‰. During periods of shell closure the mantle fluid and hemolymph ionic and osmotic concentrations remained constant. The oysters' response during the second cycle was similar to that of the first.

When exposed to a 30‰ seawater minimum square-wave regime, the oysters closed between Hours 3 and 4 when the osmotic concentration of the hemolymph was approximately 800 mOsm (Fig. 12b). In both the sinusoidal and square-wave profiles, the Na^+ concentration closely followed the osmotic concentration of the hemolymph as can be seen in Fig. 12. The Ca^{2+} concentrations in the hemolymph (Fig. 12) showed a damped response and the hemolymph Mg^{2+} concentration was damped only in the sinusoidal profile (Fig. 12a).

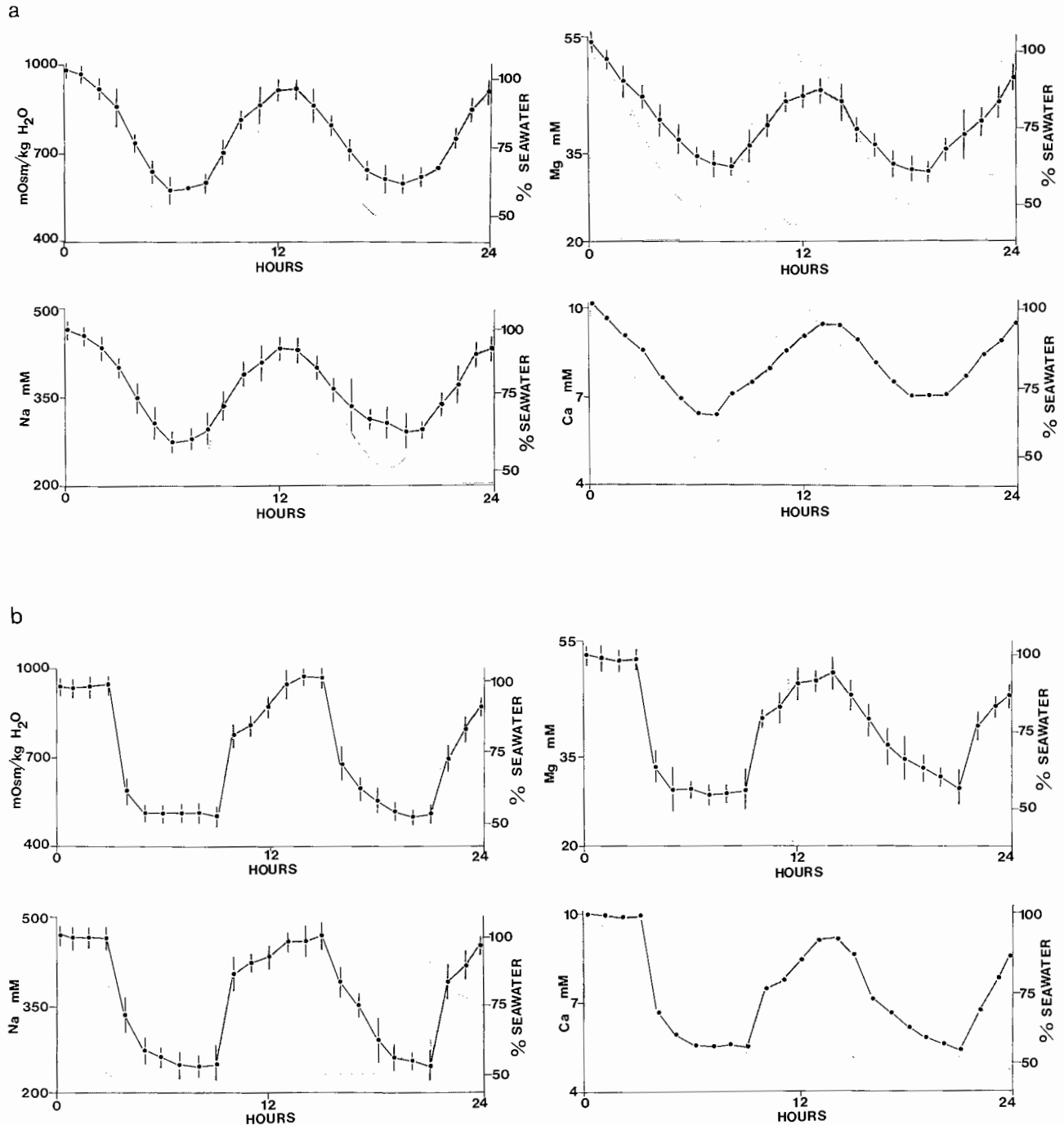


Fig. 6. *Modiolus modiolus*. Changes in hemolymph osmolality and Na⁺, Mg²⁺ and Ca²⁺ concentrations of wedged-open bivalves exposed to (a) 50% seawater minimum sinusoidal salinity regime and (b) 50% seawater minimum square-wave salinity regime. Stippled areas represent changes in external medium. Each point is mean of 3 bivalves. Error bars represent 95% confidence limits

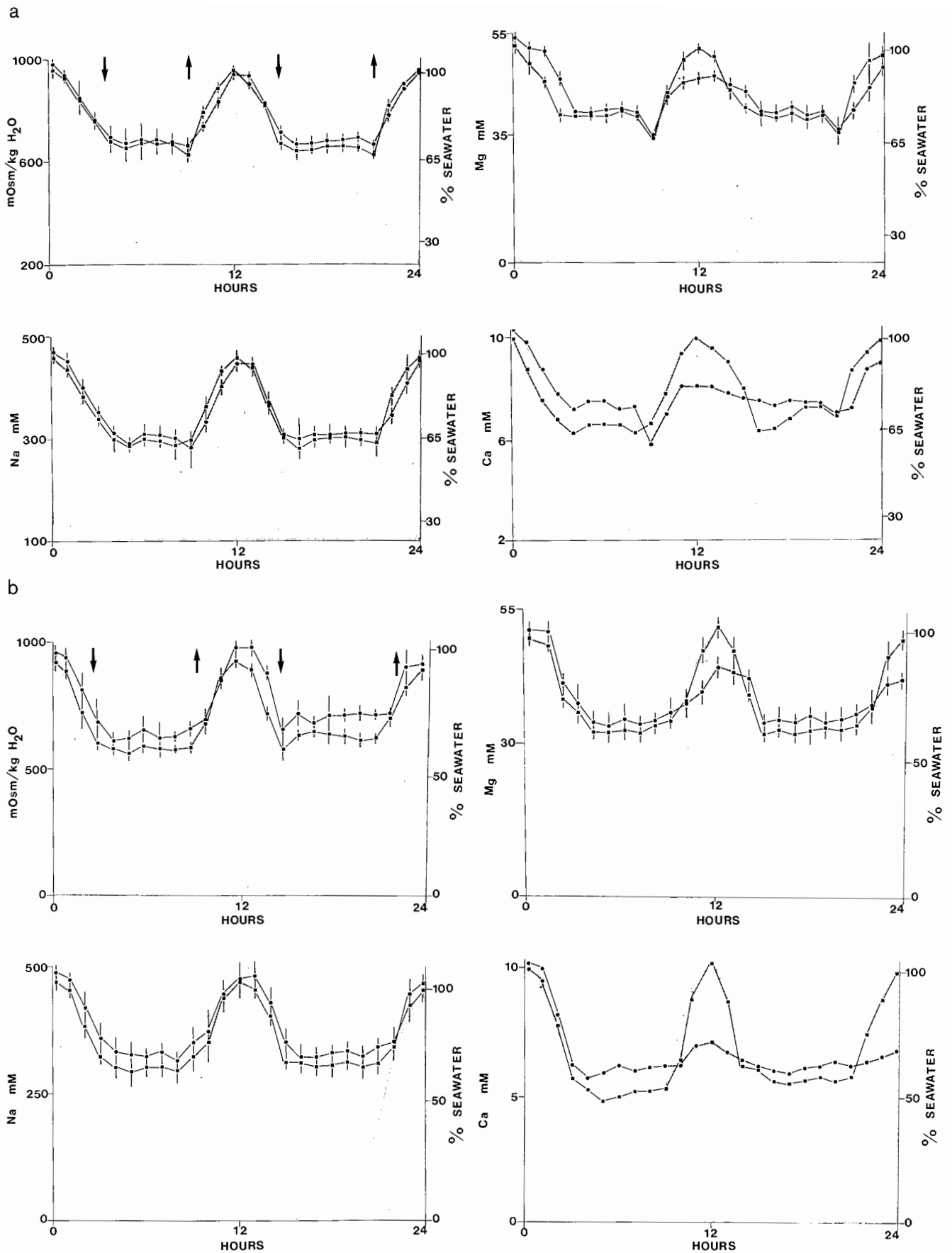


Fig. 7. *Mytilus edulis*. Changes in hemolymph (circles) and mantle fluid (squares) osmolality and Na⁺, Mg²⁺ and Ca²⁺ concentrations during exposure to (a) 30% and (b) 0% seawater minimum sinusoidal salinity regime. Stippled areas represent changes in external medium. Arrows indicate points of shell-valve closure (↓) and opening (↑) (from Bettison, unpublished). Each point is mean of 3 mussels. Error bars represent 95% confidence limits

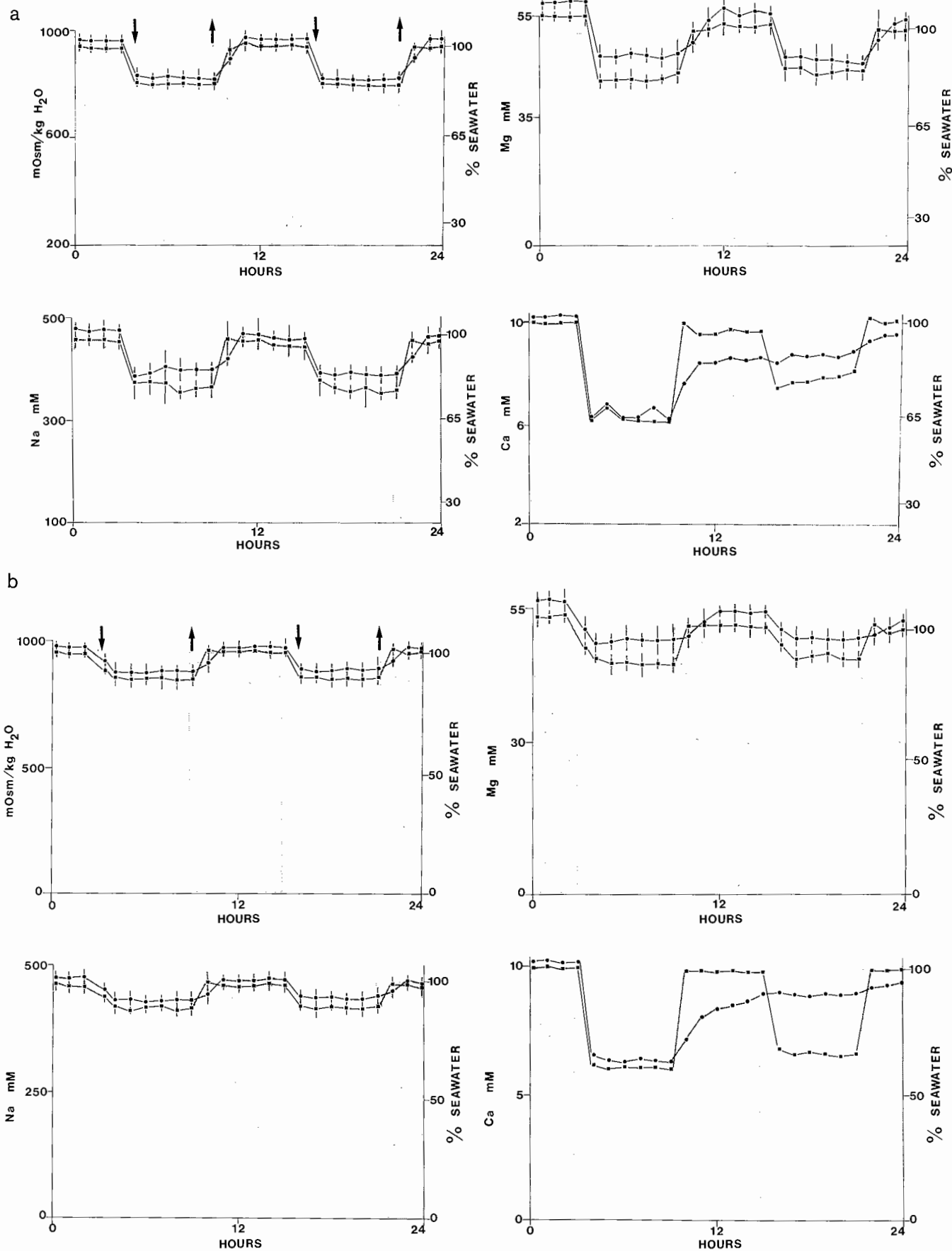


Fig. 8. *Mytilus edulis*. Changes in hemolymph (circles) and mantle fluid (squares) osmolality and Na^+ , Mg^{2+} and Ca^{2+} concentrations during exposure to (a) 30% and (b) 0% seawater minimum square-wave salinity regime. Stippled areas represent changes in external medium. Arrows indicate points of shell valve closure (\downarrow) and opening (\uparrow) (from Bettison, unpublished). Each point is mean of 3 mussels. Error bars represent 95% confidence limits

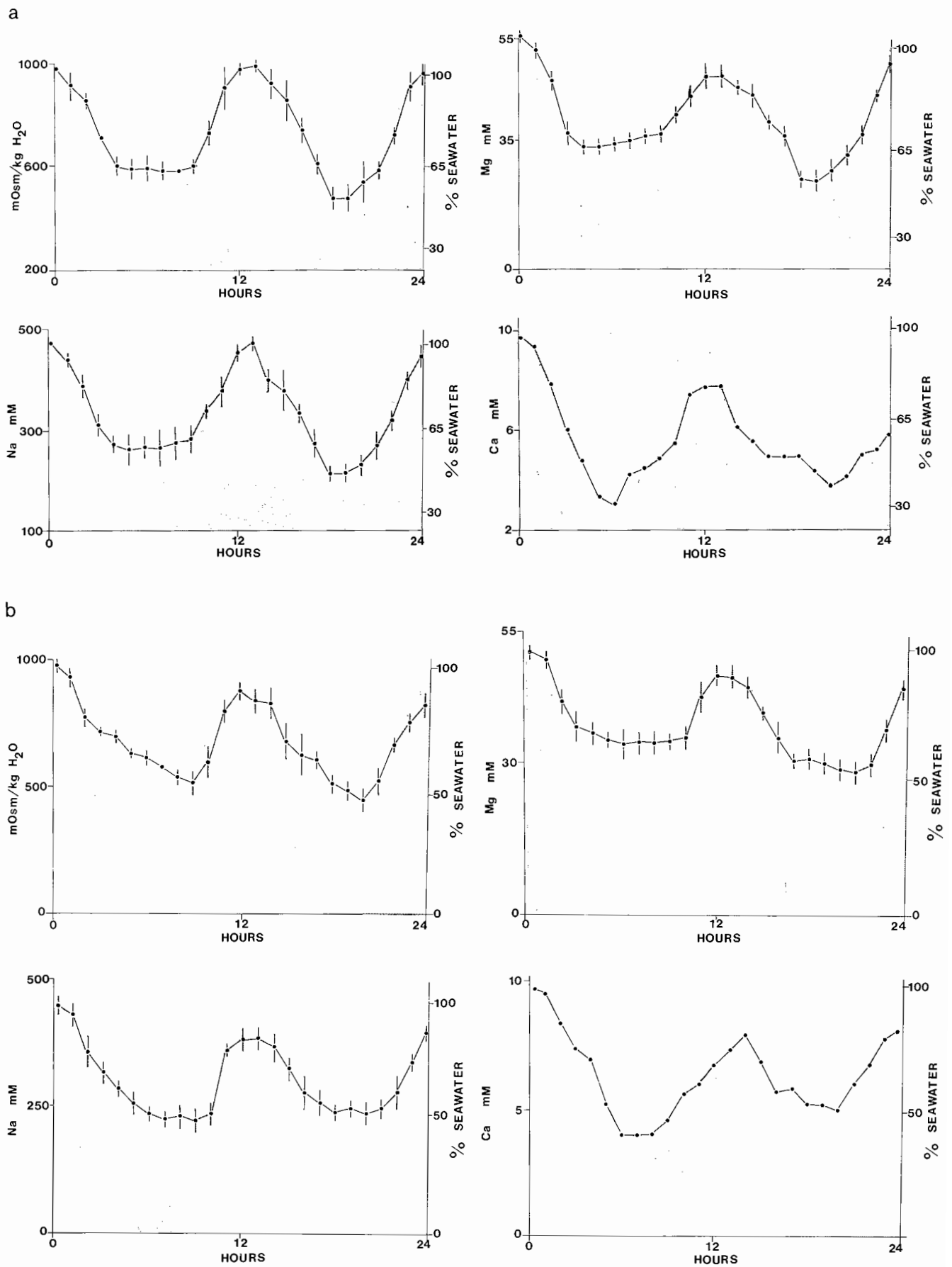


Fig. 9. *Mytilus edulis*. Changes in hemolymph osmolality and Na⁺, Mg²⁺ and Ca²⁺ concentrations of wedged-open mussels exposed to (a) 30% and (b) 0% seawater minimum sinusoidal salinity regime. Stippled areas represent changes in external medium. Each point is mean of 3 mussels. Error bars represent 95% confidence limits

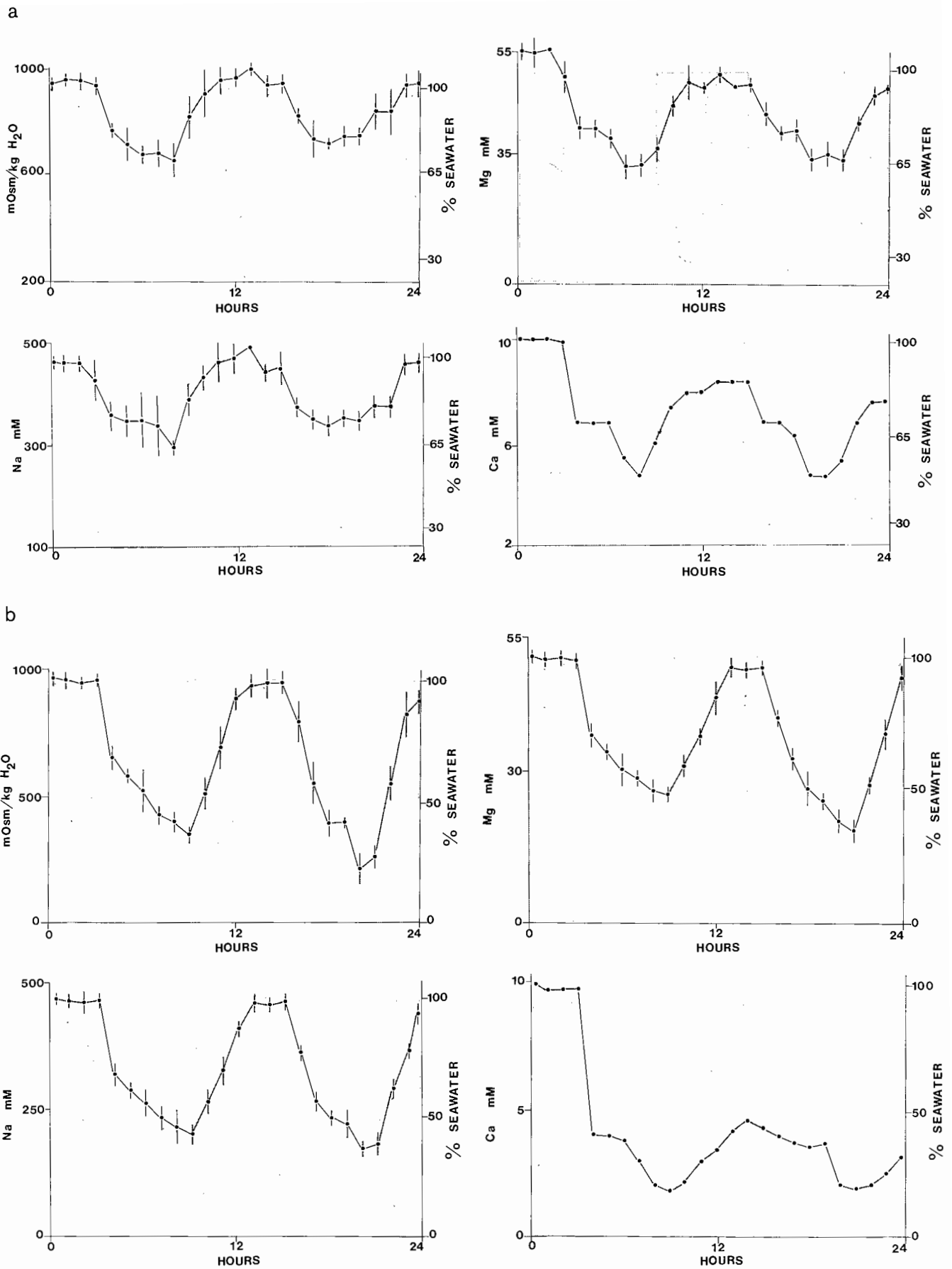


Fig. 10. *Mytilus edulis*. Changes in hemolymph osmolality and Na⁺, Mg²⁺ and Ca²⁺ concentrations of wedged-open mussels exposed to (a) 30% and (b) 0% seawater minimum square-wave salinity regime. Stippled areas represent changes in external medium. Each point is mean of 3 mussels. Error bars represent 95% confidence limits

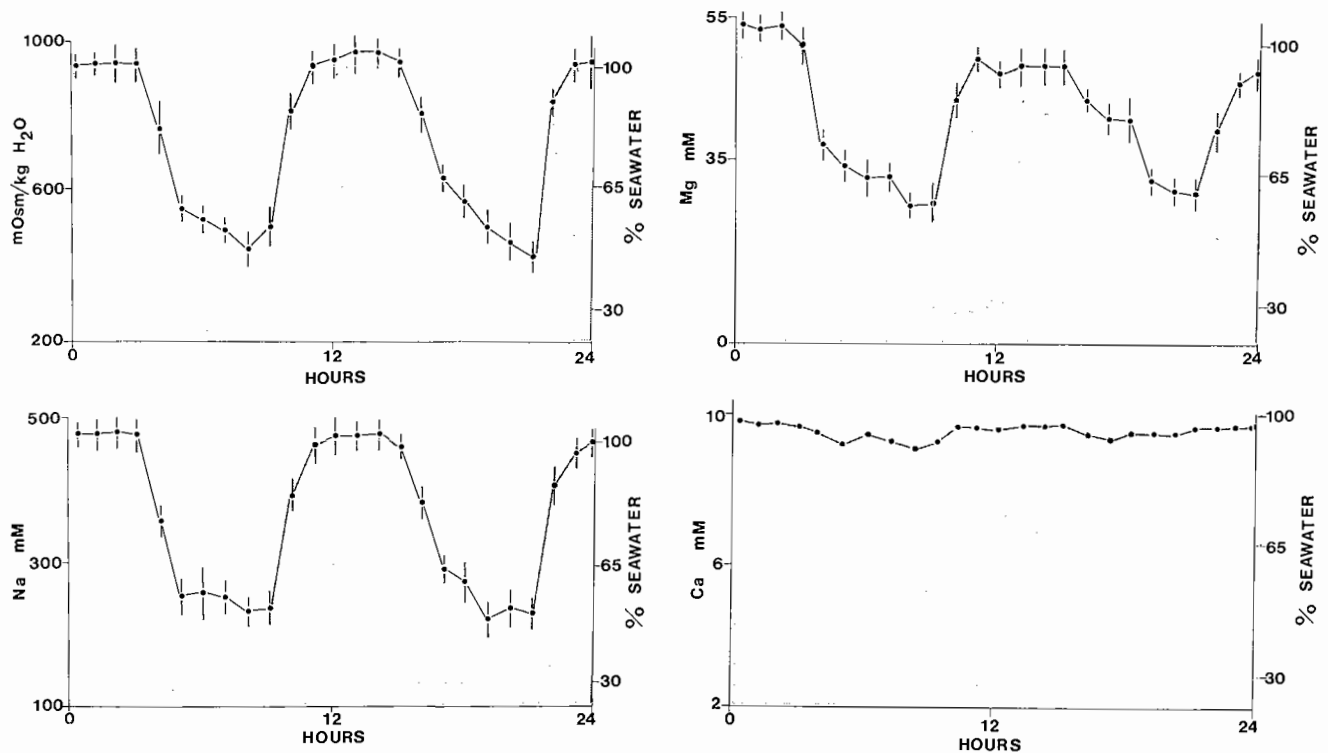


Fig. 11. *Mytilus edulis*. Changes in hemolymph osmolality and Na^+ , Mg^{2+} and Ca^{2+} concentrations of wedged-open mussels exposed to 30% seawater minimum sinusoidal salinity regime, and supplied with a constant 10 mM Ca^{2+} supply. Stippled areas represent changes in external medium. Each point is mean of 3 mussels. Error bars represent 95% confidence limits

When wedged-open individuals were used, the total osmotic and ionic concentration of the hemolymph passively followed the seawater concentration in both the sinusoidal and the square-wave profiles (Fig. 13). As can be seen in Fig. 13, the concentrations of Ca^{2+} and Mg^{2+} showed a lagged response and the Na^+ concentration closely followed the osmotic concentration of the hemolymph.

Response of *Scrobicularia plana* to Fluctuating Salinity

When placed in a 30% seawater minimum sinusoidal salinity profile, *Scrobicularia plana* remained open during the first cycle and closed when the external seawater concentration reached approximately 40% seawater during the second cycle. When placed in a 30% seawater minimum square-wave regime, the bivalves closed when the hemolymph reached an osmotic concentration of 880 mOsm during both cycles of the program. In fluctuating salinities, the Na^+ , Mg^{2+} , Ca^{2+} and osmotic concentrations of the hemolymph and mantle fluid of non-burrowed *S. plana*

followed those of the external environment as long as the bivalves remained open (Fig. 14).

The Na^+ , Mg^{2+} and osmotic concentrations of the hemolymph of wedged-open *Scrobicularia plana* followed those of the external medium, as shown in Fig. 15, but showed a damped response. The osmotic concentration of the hemolymph reached a low of 460 mOsm in individuals in the sinusoidal regime and 420 mOsm in individuals in the square-wave regime. The Ca^{2+} concentration in the sinusoidal regime followed that of the external medium during the first cycle, but maintained a constant level of approximately 9 mM during the second cycle. The Ca^{2+} concentration of the hemolymph of wedged-open *S. plana* placed in a 30% seawater minimum square-wave profile dropped rapidly during the first cycle, and after Hour 4 remained at a mean concentration of 3.9 mM.

The hemolymph of burrowed *Scrobicularia plana* showed the same responses to fluctuating salinities as normal, non-burrowed ones in both the sinusoidal and square-wave profiles, as can be seen in Fig. 14.

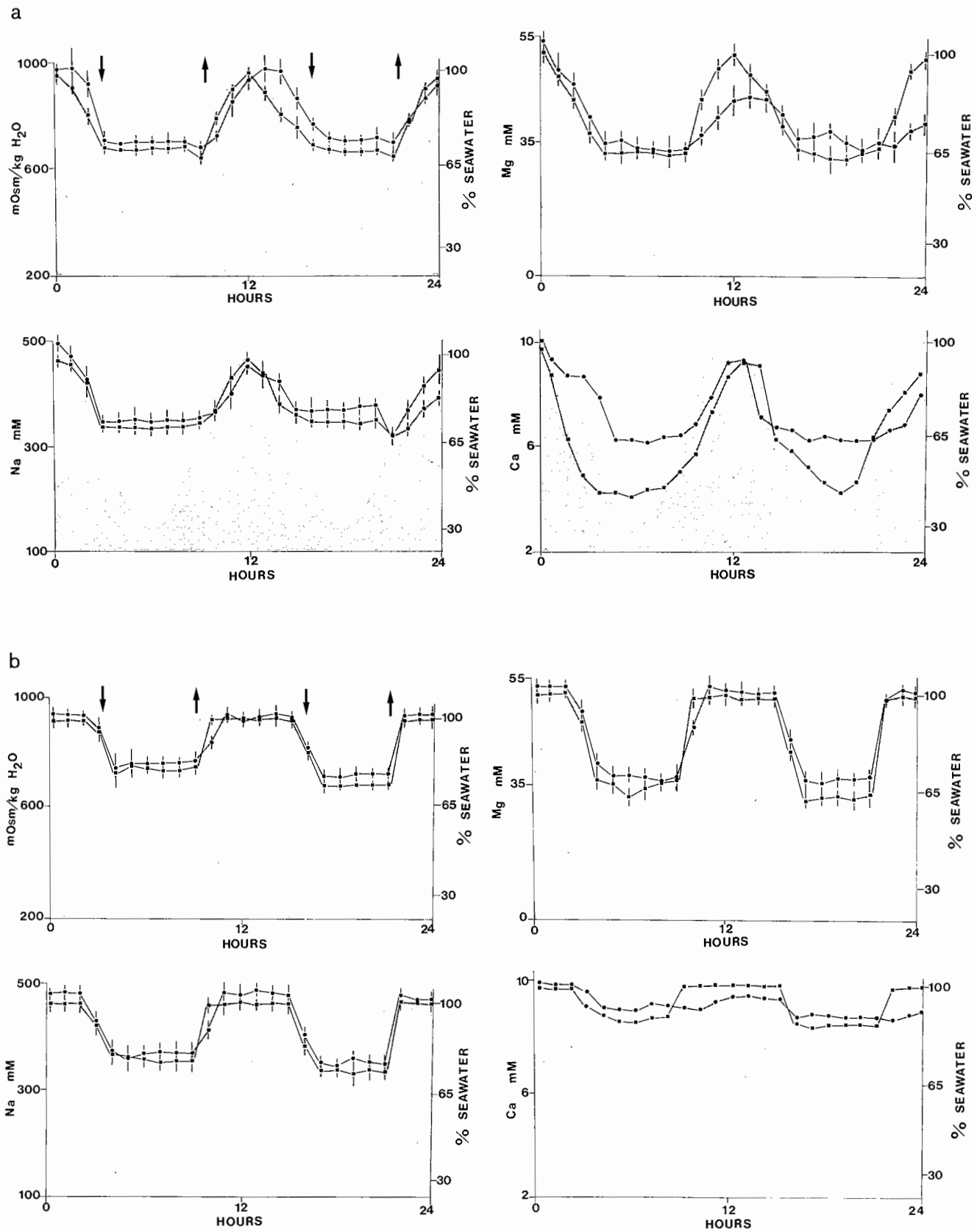


Fig. 12. *Crassostrea gigas*. Changes in hemolymph (circles) and mantle fluid (squares) osmolality and Na⁺, Mg²⁺ and Ca²⁺ concentrations during exposure to (a) 30% seawater minimum sinusoidal salinity regime and (b) 30% seawater minimum square-wave salinity regime. Stippled area represents changes in external medium. Arrows indicate points of shell valve closure (↓) and opening (↑) (from Bettison, unpublished). Each point is mean of 3 oysters. Error bars represent 95% confidence limits

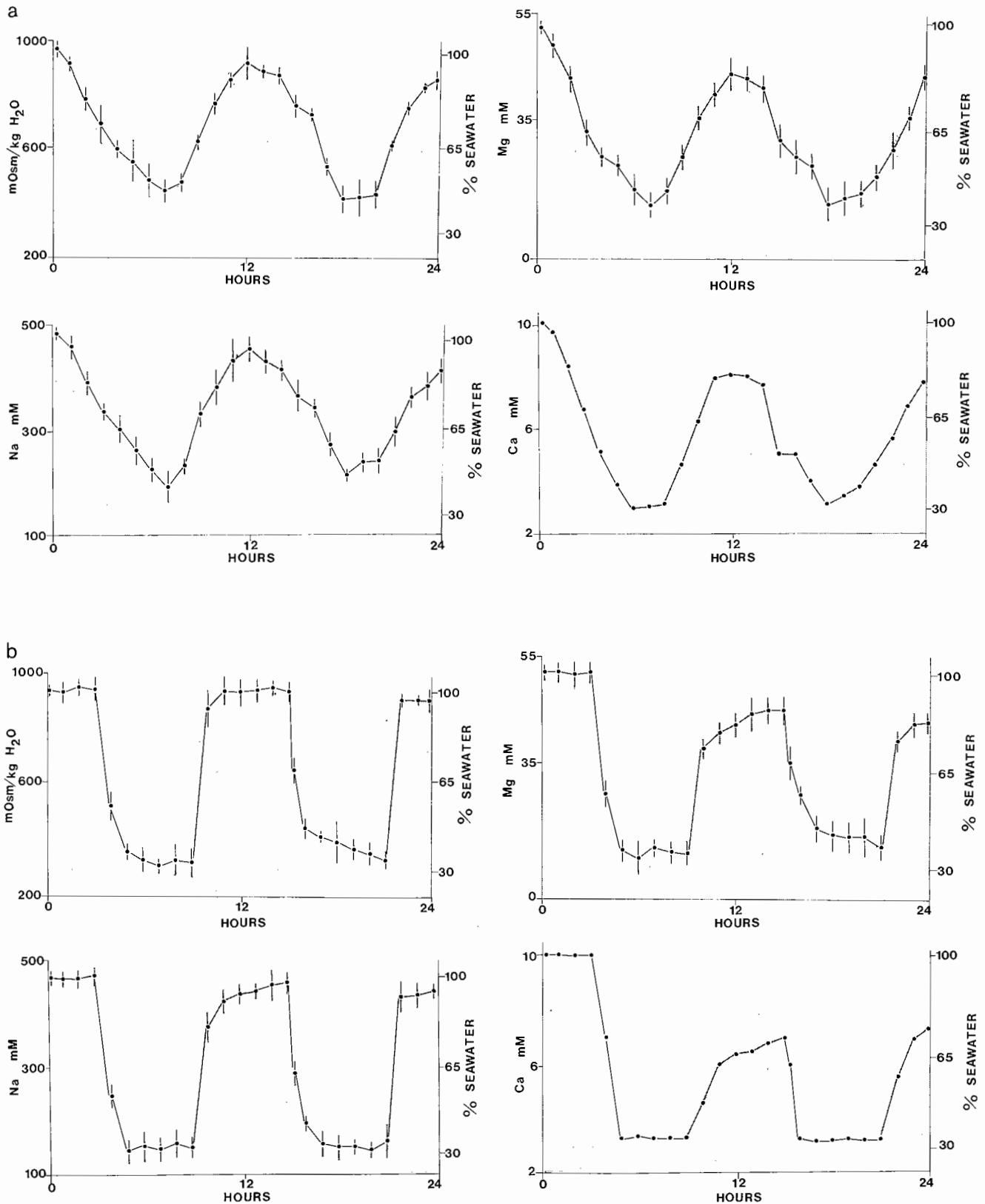


Fig. 13. *Crassostrea gigas*. Changes in hemolymph osmolality and Na^+ , Mg^{2+} and Ca^{2+} concentrations of wedged-open oysters exposed to (a) 30% seawater minimum sinusoidal salinity regime and (b) 30% seawater minimum square-wave regime. Stippled areas represent changes in external medium. Each point is mean of 3 oysters. Error bars represent 95% confidence limits

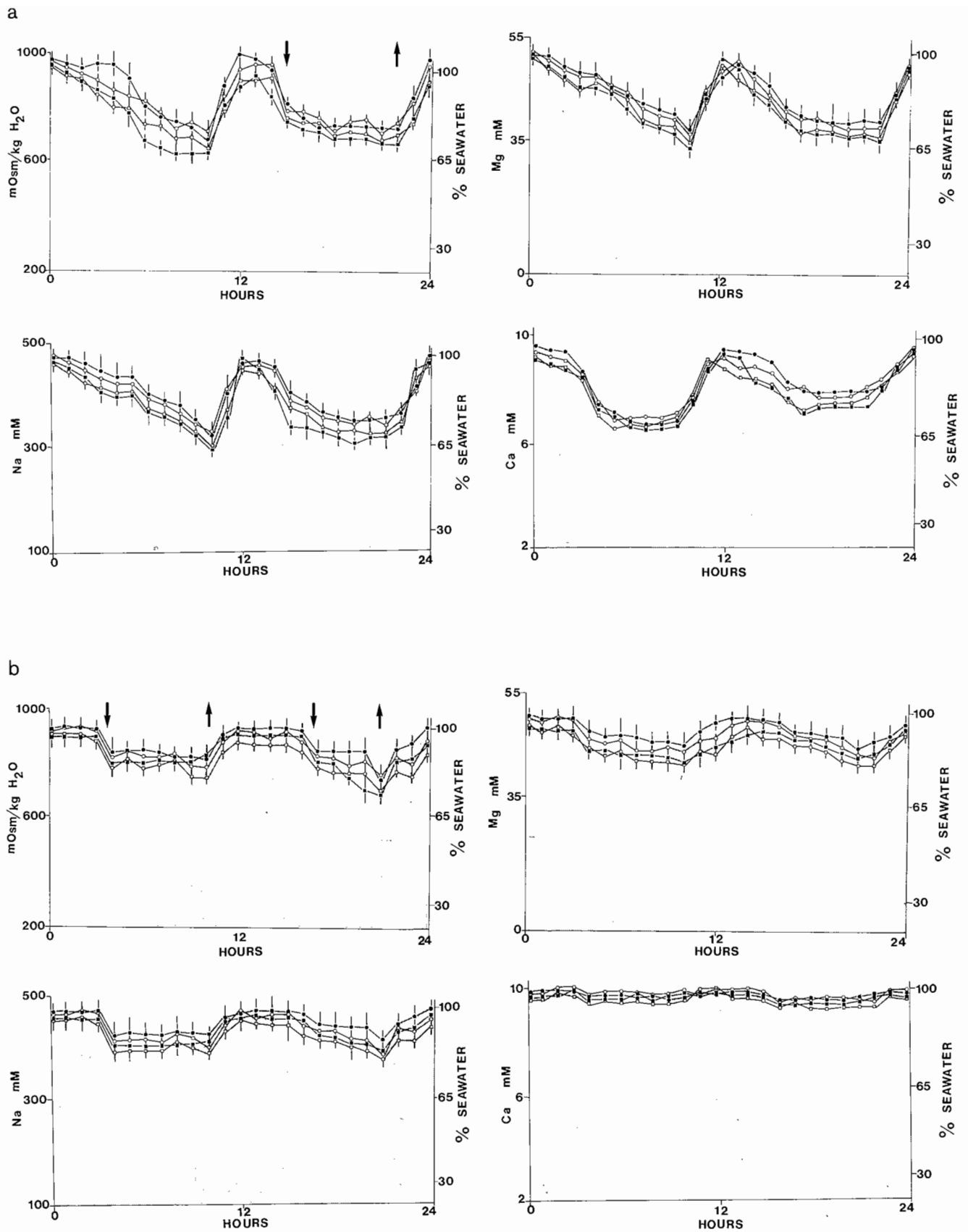


Fig. 14. *Scrobicularia plana*. Changes in hemolymph and mantle fluid osmolality and Na⁺, Mg²⁺ and Ca²⁺ concentrations of burrowed and non-burrowed bivalves exposed to (a) 30% seawater sinusoidal salinity regime and (b) 30% seawater minimum square-wave regime. Filled circles: non-burrowed-hemolymph; open circles: burrowed-hemolymph; filled squares: non-burrowed-mantle fluid; open squares: burrowed-mantle fluid. Stippled areas represent changes in external medium; arrows indicate points of shell valve closure (↓) and opening (↑). Each point is mean of 3 bivalves. Error bars represent 95% confidence limits

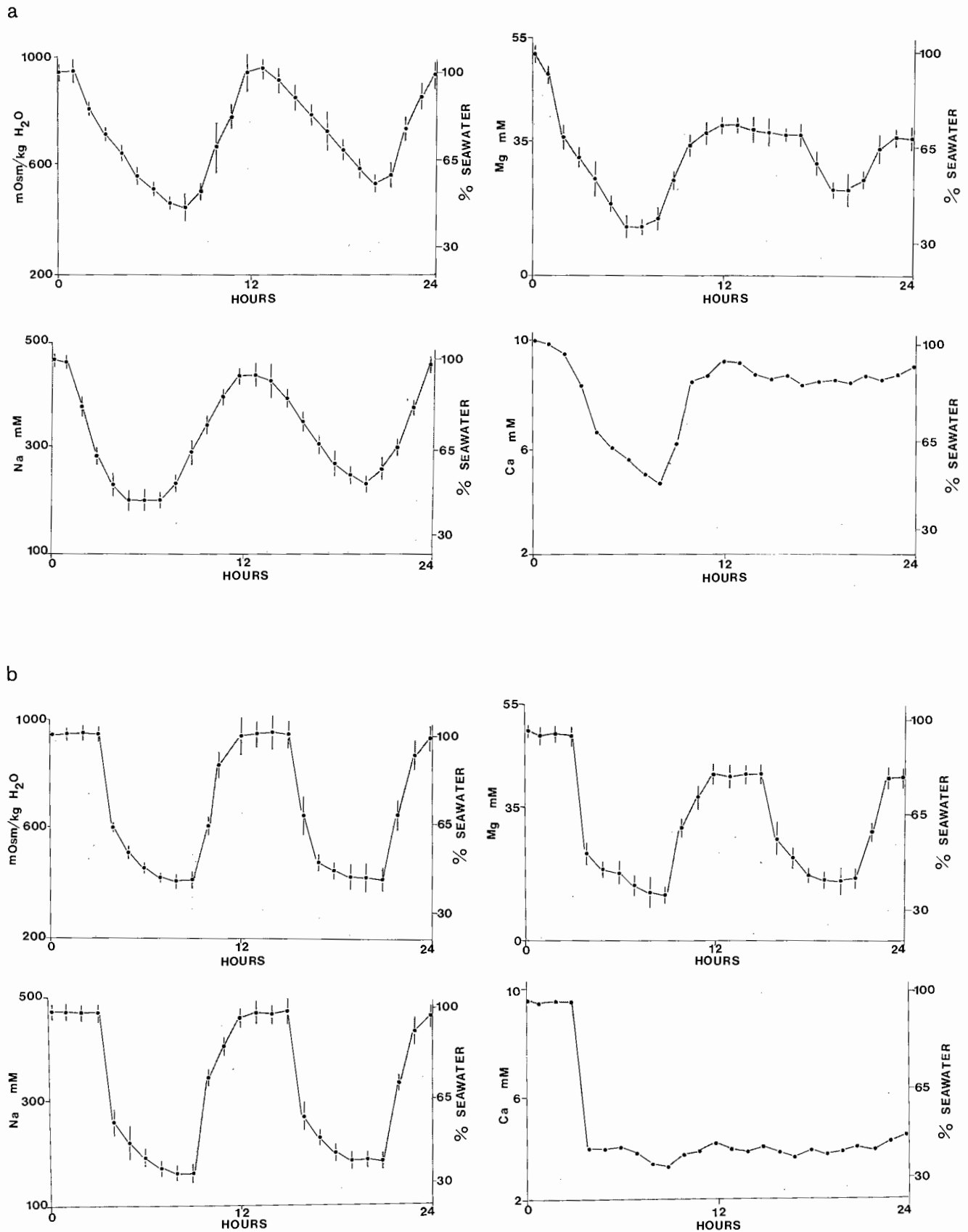


Fig. 15. *Scrobicularia plana*. Changes in hemolymph osmolality and Na⁺, Mg²⁺ and Ca²⁺ concentrations of wedged-open bivalves exposed to (a) 30% seawater minimum sinusoidal salinity regime and (b) 30% seawater minimum square-wave regime. Stippled areas represent changes in external medium. Each point is mean of 3 bivalves. Error bars represent 95% confidence limits

Response of Mya arenaria to Fluctuating Salinity

When placed in a 20% seawater minimum sinusoidal regime the Na^+ , Ca^{2+} , Mg^{2+} and osmotic concentrations of the hemolymph of free *Mya arenaria* acclimated to 60% seawater (salinity approximately 20‰; approximately 580 mOsm) followed those of the external environment but, as shown in Fig. 16, exhibited a damped response. The hemolymph osmolality never dropped below 420 mOsm and never rose higher than 900 mOsm. The rate of increase in hemolymph osmolality in increasing salinities was slightly lower than the rate of decrease in decreasing salinities. The hemolymph Na^+ , Mg^{2+} and Ca^{2+} levels followed the same pattern of change as hemolymph osmolality.

Non-burrowed *Mya arenaria* acclimated to 20% (salinity approximately 6‰; approximately 200 mOsm) seawater showed a damped response to fluctuations in salinity when placed in a 20% seawater minimum abrupt profile, as seen in Fig. 17. The hemolymph osmolality attained a high of 540 mOsm and a low of 269 mOsm. Variations in the Na^+ and Mg^{2+} levels in the hemolymph were similar to those of osmolality. The Ca^{2+} level remained fairly constant, with a mean concentration of 2.9 mM.

Non-burrowed *Mya arenaria* acclimated to 100% seawater (Fig. 18) showed a very damped response to salinity fluctuation when placed in both 30% seawater minimum sinusoidal and square-wave salinity profiles. In the 30% minimum sinusoidal profile, the hemolymph osmolality reached a low of 690 mOsm during the first cycle and 790 mOsm during the second cycle. The hemolymph Na^+ , Mg^{2+} and Ca^{2+} concentrations showed a pattern of change similar to that of hemolymph osmolality. In the 30% seawater minimum square-wave profile, the hemolymph reached a low concentration of 800 mOsm during the first cycle and 840 mOsm during the second cycle. Again, the hemolymph Na^+ , Mg^{2+} and Ca^{2+} concentrations showed a similar pattern of change to that of osmolality.

After 3 weeks of acclimation to 20% seawater the hemolymph osmolality of *Mya arenaria* was still 160 mOsm above that of the external medium, even though the molluscs were actively feeding during this time. After 4 weeks acclimation, the hemolymph (215 mOsm) was nearly isosmotic with the surrounding seawater (190 mOsm). Individuals acclimated to 100% seawater reached and maintained a hemolymph osmolality 55 mOsm above that of the surrounding seawater after only 1 day.

There was no significant difference between the hemolymph concentrations of

burrowed and non-burrowed individuals subjected to the same salinity program, as can be seen in Figs. 16-18.

Discussion*Sublittoral Bivalves - Chlamys opercularis and Modiolus modiolus*

Brand and Roberts (1973) showed that scallops are incapable of long-sustained tonic closure. Similarly, Coleman and Trueman (1971) found that *Modiolus modiolus* was not able to retain water in the mantle cavity during exposure to air because water seeped through the byssal opening, which may be quite large. Thus, it follows that the mussels are also incapable of preventing the entry of water from the external medium. This seepage would explain the continued slight and gradual decrease in osmotic and ionic concentration of the mantle fluid and hemolymph which occur in *M. modiolus* despite valve closure (Fig. 5).

The Na^+ , Ca^{2+} , Mg^{2+} and osmotic concentrations of the hemolymph of *Chlamys opercularis* and wedged-open *Modiolus modiolus* behaved in a similar manner in both sinusoidal and abrupt regimes. It should be noted, however, that the rate of drop in hemolymph concentration in the square-wave regimes was significantly greater in wedged-open *M. modiolus* (Fig. 6) than in *C. opercularis* (Fig. 4b). Three possibilities present themselves as explanations for this difference. First, although *C. opercularis* is incapable of complete closure, it can adduct its valves for a short period. Short-term adduction may decrease the water flow through the mantle cavity to a lower level than in wedged-open *M. modiolus*. Second, there is the possibility that ciliary action is greater in *M. modiolus*, thereby maintaining a high flow rate through the mantle cavity, whereas the cilia of *C. opercularis* may be paralysed by lower concentrations of seawater. Third, it may simply be the case that the permeability and surface area to volume ratio in *M. modiolus* are such as to allow a more rapid exchange of salts and water than occurs in *C. opercularis*.

It is quite clear from the investigation reported here that both species can survive quite considerable dilutions of seawater in fluctuating regimes. This contrasts with the results obtained in conventional steady-state salinity experiments. Pierce (1970), using constant salinities, reported an 80% seawater lower lethal limit for *Modiolus modiolus*. In a preliminary steady-state experiment it was found that *Chlamys opercularis* also

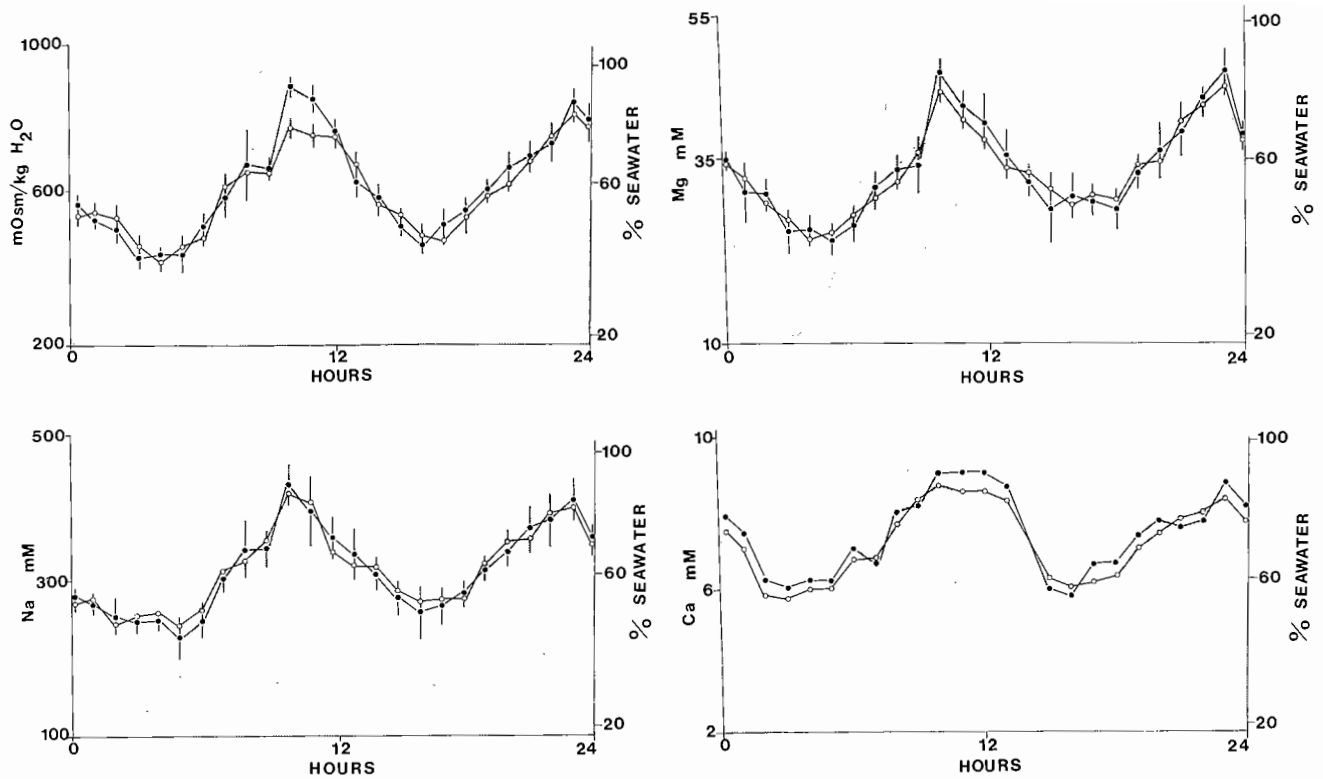


Fig. 16. *Mya arenaria*. Changes in hemolymph osmolality and Na^+ , Mg^{2+} and Ca^{2+} concentrations of 60% seawater-acclimated burrowed (open circles) and non-burrowed (filled circles) clams exposed to 20% seawater minimum sinusoidal salinity regime. Stippled areas represent changes in external medium. Each point is mean of 3 clams. Error bars represent 95% confidence limits

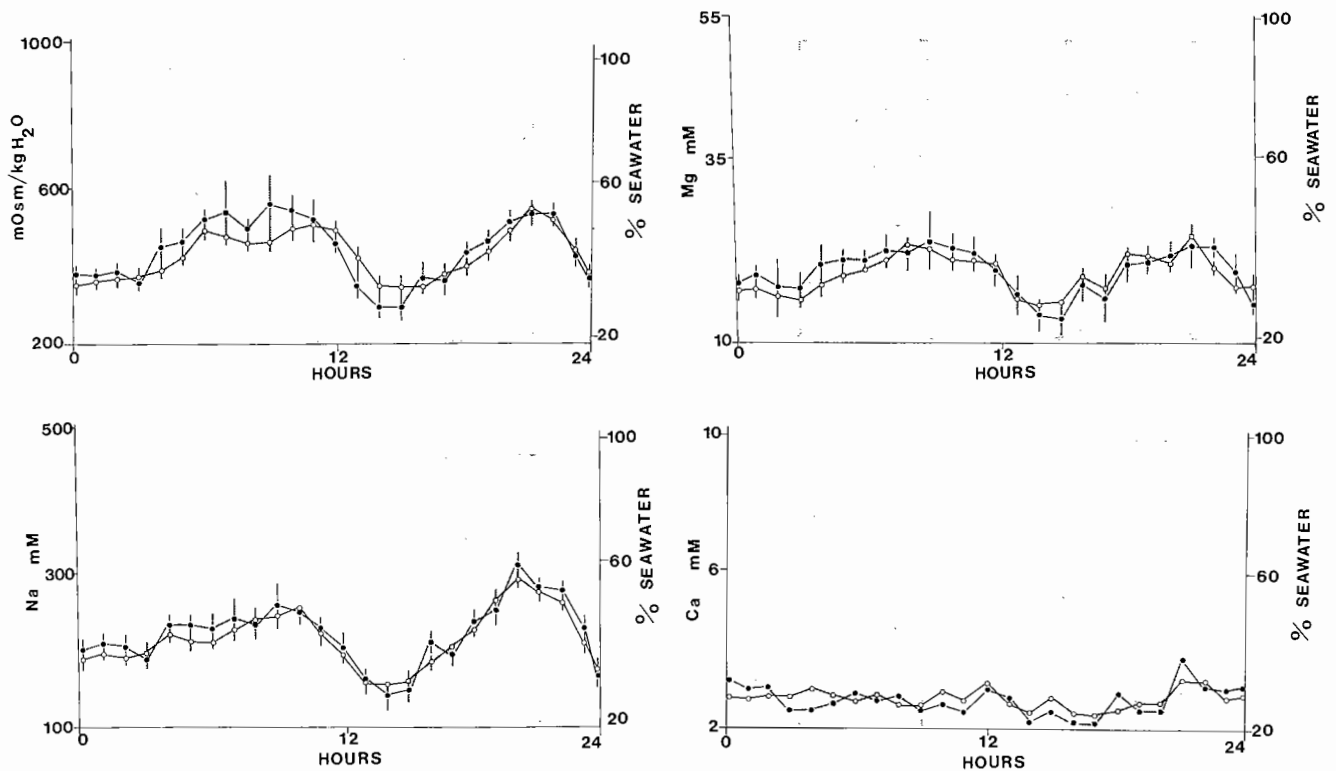


Fig. 17. *Mya arenaria*. Changes in hemolymph osmolality and Na^+ , Mg^{2+} and Ca^{2+} concentrations of 20% seawater-acclimated burrowed (open circles) and non-burrowed (filled circles) clams exposed to 20% seawater minimum square-wave salinity regime. Stippled areas represent changes in external medium. Each point is mean of 3 clams. Error bars represent 95% confidence limits

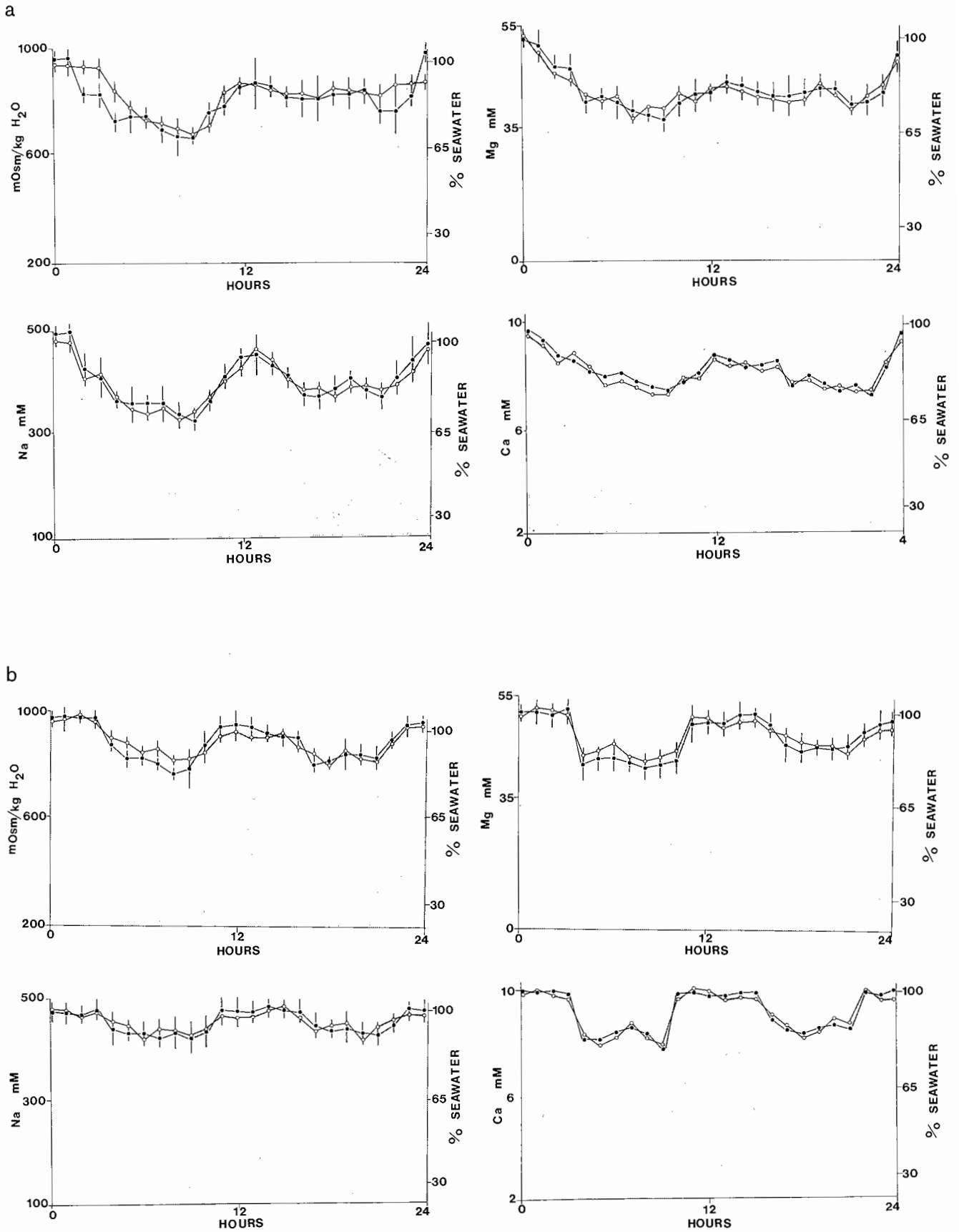


Fig. 18. *Mya arenaria*. Changes in hemolymph osmolality and Na⁺, Mg²⁺ and Ca²⁺ concentrations of 100% seawater-acclimated burrowed (open circles) and non-burrowed (filled circles) clams exposed to (a) 30% seawater minimum sinusoidal salinity regime and (b) 30% seawater minimum square-wave salinity regime. Stippled area represents changes in external medium. Each point is mean of 3 clams. Error bars represent 95% confidence limits

had a lower lethal limit of 80% seawater. Continually low seawater concentrations are not likely to be encountered by either of these essentially sublittoral, open-sea species, so the constant salinity experiment is essentially an academic exercise. Exposure to fluctuating salinity regimes is also largely artificial, although useful as a comparative basis for work on more euryhaline species. There may be some environmental significance for *M. modiolus*, however, since Coleman and Trueman (1971) showed that some beds of horse mussels may be exposed at low spring tides for brief periods. Despite the byssal aperture which prevents complete protection against the effects of aerial exposure or brief freshwater influence, *M. modiolus* does exhibit some preadaptation to short-term unfavorable environmental conditions. *C. opercularis* shows no such preadaptation, but needs none given its sublittoral distribution and well developed swimming ability.

Intertidal Bivalves - Mytilus edulis and Crassostrea gigas

The results indicate that both *Mytilus edulis* and *Crassostrea gigas* are osmoregulators which exert control over the concentrations of their body fluids by means of well developed behavioral mechanisms. As long as the molluscs remain open, the osmotic and ionic concentrations of the hemolymph and mantle fluid follow the same pattern of change as that of the external medium. There is, however, a damping which is clearly a function of permeability. Once the shell valves are closed, the mollusc is isolated from the external environment. Loosanoff (1952) found that as long as the valves of *Crassostrea virginica* remained open, the changes in salinity of their body fluids followed changes in salinity of the external environment. Figs. 7 and 12a of the present paper indicate that *M. edulis* and *C. gigas*, when placed in a sinusoidal salinity regime, close when the external concentration of seawater reaches approximately 50%, and the osmotic concentration of the hemolymph is about 700 mOsm. For *M. edulis* this was true in both the 30 and 0% seawater minimum profiles. Several other workers, using steady-state experiments, found that pumping stopped and shell valves closed at approximately 50% seawater. Anderson and Prosser (1953) found that *Mercenaria mercenaria* stopped pumping below 50% seawater and *Modiolus demissus* stopped pumping below 35% seawater. Ricci (1939) gives a value of 50% seawater for shell-

valve closure in *Mytilus galloprovincialis* and Hopkins (1936) gives 33 to 41% seawater as the lower limit for effective pumping in *C. gigas*. Loosanoff and Smith (1949) give a value of approximately 12% S for cessation of pumping in *C. virginica* acclimated to 27% S (47% of its acclimation salinity). It is not possible to estimate the exact concentration of seawater at the time of closure when using abrupt profiles because of the rapid drop in salinity (4.5 min to fall from 100 to 20% seawater).

All of the molluscs placed in sinusoidal profiles closed at 50% seawater and all those placed in square-wave profiles closed when the hemolymph concentrations were between 800 and 880 mOsm. This suggests that the bivalves were reacting to the absolute concentration of the external medium rather than the rate of salinity change. The high osmotic concentrations in individuals from square-wave regimes is probably due to the fact that the hemolymph does not have enough time to become diluted before the molluscs close. The fact that the animals are reacting to a concentration rather than a rate of change is in contrast with the theory of Milne (1938) and Basindale (1943), who stated that the range of salinity fluctuation may not be as critical as the rate of change.

Fingerman and Fairbanks (1956) stated that *Crassostrea virginica* has a limited ability to osmoregulate. This idea was based, however, on hemolymph analyses only 4 to 8 h after transferring oysters adapted to 17% S into salinities from 10 to 36%. Also, Galstoff (1964) reported that if maintained for several hours, a change of 10% S will reduce the amount of time that *C. virginica* will remain open. Since Fingerman and Fairbanks did not wedge any specimens open, it is probable that the mechanism of shell-valve closure isolating the bivalves from their environment gave the impression that they were osmoregulators.

There is no evidence of ionic regulation for Na^+ , Ca^{2+} or Mg^{2+} in *Mytilus edulis* or *Crassostrea gigas*. The ion concentrations show the typical marine bivalve trend; the concentrations of Na^+ in the hemolymph and seawater are almost equal, while Ca^{2+} and Mg^{2+} are slightly more concentrated in the hemolymph than in the surrounding seawater (Table 1). Gilles (1972) reports that *M. edulis* and *Glycymeris glycymeris*, when placed in low salinities, regulate K^+ ions to maintain the concentration at the value it has in the hemolymph of normal seawater individuals. It is not known, however, whether the maintenance of the K^+ con-

centration at a given level is important in the osmoregulation process.

As shown in Figs. 7-10 and Figs. 12 and 13, Ca^{2+} and Mg^{2+} exhibit a more damped response to fluctuating salinity than does Na^+ . Webb (1940) and Conway (1956, 1960) have shown that small ions pass at a faster rate than large ions through cell membranes. Thus, in a system of passive equilibrium, as exhibited by *Mytilus edulis* and *Crassostrea gigas*, the larger Ca^{2+} and Mg^{2+} ions would not be expected to permeate at the same rate as the smaller Na^+ ions. Also, the cell walls and the intercellular cement are stabilised and made generally less permeable by the presence of calcium and, to a lesser extent, magnesium (Robertson, 1941; Tucker, 1970).

As shown in Figs. 7 and 8, the Ca^{2+} level in *Mytilus edulis* hemolymph drops rapidly during the first cycle of salinity change, while during the second cycle it remains steady or increases gradually at a rate quite different from Na^+ . This difference in the behaviour of Ca^{2+} is not seen in *Crassostrea gigas* (Fig. 12). It is known that under certain conditions calcium is taken up by the body fluids from the shell in other animals (see Tucker, 1970) and that it is reversibly bound by cephalin and protein molecules which make up the cell membrane structure. These two factors suggest a change in concentration of calcium ions different from that of other cations which are not bound to the same extent. When *M. edulis* is subjected to dilute media, free Ca^{2+} ions in the body fluid may combine with molecules of the cell membranes, so reducing the permeability of the cell wall. This binding may partially explain the rapid decrease and slow increase in Ca^{2+} concentration in the hemolymph in fluctuating regimes.

As seen in Fig. 13, when the molluscs are wedged open, the total osmotic and ionic concentrations of *Crassostrea gigas* drop much more rapidly than those of *Mytilus edulis* (Figs. 9 and 10). This may be caused by the decrease in calcium ion concentration in the external environment and a difference in the response of the two species to a fall in the Ca^{2+} concentration. The pumping of seawater by lamellibranchs is accomplished primarily by the beating of cilia on the lateral epithelium of the gill filaments. In marine organisms, lack of calcium in the external medium causes the cessation of ciliary activity (Gray, 1924; Pantin, 1926; Robertson, 1941; Schlieper, 1966). More recently, Papero and Murphy (1975) have shown that changes in Ca^{2+} affect the basal rate of lateral cilia in *M. edulis*. For example, a change in calcium

ion concentration from 10 to 0 mM resulted in a decline from a mean of 8.3 beats/sec to a mean of 2.7 beats/sec within 30 min, and they also found that a Ca^{2+} concentration of 10 mM (approximate concentration in seawater at 34‰ S) is required to maintain the basal rate of beating. An interesting species difference was reported by Ghiretti (1966), who found that 60‰ seawater depressed activity of excised gills of *M. edulis* but not those of *C. virginica*.

The data here seem to support this finding. Wedged-open *Mytilus edulis* given a constant supply of Ca^{2+} while in a fluctuating salinity regime yielded hemolymph osmotic, Na^+ and Mg^{2+} concentrations intermediate to *M. edulis* not given a constant Ca^{2+} supply and to *Crassostrea gigas*. A lack of ciliary activity could account for lack of water movement throughout the mantle cavity of *M. edulis*; hence the high osmotic concentration. It is also possible that *M. edulis* is generally less permeable to water than is *C. gigas*.

Estuarine Bivalves - Scrobicularia plana and Mya arenaria

The results indicate that *Scrobicularia plana* and *Mya arenaria* are osmoconformers with varying capabilities for behavioural osmotic control, which are not linked in either species to the burrowing habit. The idea that the ability to burrow is a protection against the effects of external salinity is valid for forms such as annelids and coelenterates, but in bivalves, valve closure and siphon retraction are likely to be more effective mechanisms and this is clearly demonstrated by the present study. In *S. plana* and *M. arenaria*, burrowing is probably most effective and significant as protection against desiccation and predation.

It has been suggested by Freeman and Rigler (1957) that *Scrobicularia plana* is an osmoregulator in low salinities. These authors found that, in 30‰ seawater, the osmotic pressure of the hemolymph was significantly higher than that of the external medium. The data was obtained, however, from bivalves which were able to close their valves and it was not clear whether they were showing active control or were resisting dilution by valve closure. The data presented here show that, as long as the bivalves remain open, the Na^+ , Mg^{2+} , Ca^{2+} and osmotic concentrations of the hemolymph approximate to the external environmental concentrations. The data derived from wedged-open *S. plana* again strongly sup-

port the conclusion that the mollusc is an osmoconformer, since the Na^+ , Mg^{2+} and osmotic concentrations of the hemolymph passively change with the concentration of the external medium in both the 30% seawater minimum gradual and abrupt profiles (Figs. 14 and 15).

The Ca^{2+} data derived from wedged-open *Scrobicularia plana* are, however, somewhat puzzling. Intact individuals, allowed to close the valves and retract the siphons at will, gave results for hemolymph Ca^{2+} concentrations which were perfectly explicable in terms of passive diffusion. This is particularly noticeable in the 30% seawater minimum sinusoidal regime shown in Fig. 14a, where the bivalves did not close in the first 12 h cycle, but did so in the second. Here the bivalves' hemolymph calcium changed gradually with the external salinity in the first cycle and remained high in the second when valve closure occurred. In the wedged-open individuals, unexpected results were obtained. The hemolymph calcium levels in the sinusoidal regime shown in Fig. 15a were much the same as for free specimens capable of closure, whereas in the abrupt regime hemolymph calcium shown in Fig. 15b declined rapidly during the first exposure to dilute seawater but then remained low during the remainder of the program, despite the later exposures to full seawater.

Either result above may be explained by a reduction in the bivalves' permeability to calcium, but in the case of *Scrobicularia plana* exposed to the 30% seawater minimum sinusoidal regime the onset of impermeability would have to occur at high salinities, while for specimens in the square-wave profile it would need to happen during the first exposure to dilute seawater. An alternative hypothesis would be to assign the observed abrupt-regime calcium concentrations to the consequences of a low salinity reduction in permeability, but to explain the high Ca^{2+} concentration of the second cycle by postulating some active retention of calcium.

Further speculation would have little value in the absence of more data, but these strange patterns of hemolymph Ca^{2+} change merit some discussion, especially as they occur against a background of straightforward passive changes in the Na^+ , Mg^{2+} and osmotic concentrations. It seems unlikely, however, that they have relevance for the normal, free bivalves.

Mya arenaria acclimated to extreme salinities (20 and 100% seawater) tended to maintain their hemolymph osmotic concentration at a level never more than 300 mOsm above or below the osmolality

of the acclimation water. The hemolymph osmolality of *M. arenaria* acclimated to 60% seawater varied with the fluctuating salinity, but the molluscs tended to conform to high salinities at a faster rate than to low salinities. In all cases, the tendency was for the mollusc to conform to the external medium, usually at a very slow rate. There was no evidence of regulation for any of the ionic species studied in *M. arenaria*. The hemolymph Na^+ , Mg^{2+} , Ca^{2+} and osmolality all showed similar patterns of change in individuals acclimated to 100 and 60% seawater. Specimens acclimated to 20% seawater had a consistently low hemolymph Ca^{2+} level and the hemolymph Mg^{2+} also showed a damped response to salinity change.

Mya arenaria is not capable of completely closing the shell valves or fully withdrawing the siphons. The edges of the mantle are, however, completely joined except for a small aperture near the anterior end through which the foot can be protruded, and the musculature at the tip of each siphon is modified to form a sphincter (Chapman and Newell, 1956). The combination of the joined mantle edges and the sphincter muscles at the siphon tips appears to enable *M. arenaria* to isolate itself from the external environment and is comparable to the mechanism of shell-valve closure in *Modiolus modiolus*, *Mytilus edulis* and *Crasostrea gigas*.

Mya arenaria conforms quickly to waters of high salinity (1 day in 100% seawater) and very slowly (4 weeks in 20% seawater) to waters of low salinity. This may be an adaptive mechanism designed to conserve energy in a fluctuating environment. This phenomenon is not present in *Scrobicularia plana* which, although tolerant of wide salinity variations, is found most commonly at mid-tide level (Milne, 1938; Freeman and Rigler, 1957). *M. arenaria* penetrates to the upper estuarine regions (Green, 1968), where fluctuations in salinity are harsh. It has been shown by Matthiessen (1960) that the rate of feeding of *M. arenaria* decreases with decreasing salinity and that the highest rate of feeding is in waters of high salinities. This implies a decrease in the activity of the siphons and in the amount of water pumped through the mantle cavity by ciliary action in low salinities.

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