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Effects of Fluctuating Salinity on the Behaviour of the West African Blood Clam *Anadara senilis* and on the Osmotic Pressure and Ionic Concentrations of the Haemolymph

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Abstract

Specimens of the West African blood clam *Anadara senilis* were exposed to both sinusoidal and abrupt salinity regimes. Measurements showed that haemolymph osmolality and concentrations of Na, K^+, Ca^{2+} and Mg^{2+} followed the concentrations of the external medium up to the time of shell valve closure. Shell valves closed when the seawater concentration had fallen to about 15.4‰ S and reopened at a similar salinity. The closure mechanism was effective in preventing excessive haemolymph dilution.

Introduction

Anadara senilis, the West African blood clam, is found in many open and closed lagoons along the West African coast. In the open tidal lagoons the clam beds are periodically exposed at low tides. During the rainy season there is considerable run-off from the land into these lagoons, and at such times the clams are exposed to waters of very low salinity, particularly at low tide.

In the study reported here, samples of *Anadara senilis* were exposed to a fluctuating salinity regime of near tidal periodicity. Measurements were made of tissue water content and of the total osmotic pressure and concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+} in the haemolymph. The salinity levels that induce shell valve closure and opening were determined.

Materials and Methods

Blood clams (*Anadara senilis*) were flown from Ghana to the Marine Science Laboratories (UK) and kept in seawater in the laboratory at 25°C. The apparatus used to produce fluctuating salinity regimes has been described by Davenport *et al.* (1975).

The cockles were subjected to both gradual (sinusoidal) and abrupt (square-wave) salinity changes. Maximum seawater concentrations always corresponded to 33.5‰ S, minimum concentrations to 0‰ S. Samples of mantle fluid were taken from *Anadara senilis* by prising open the valves and withdrawing the fluid from the mantle cavity with a hypodermic syringe (Pierce, 1970; Shumway, 1977). Blood samples were obtained by blotting the bivalve dry, still on the half-shell, and then slashing the mantle, foot and adductor muscles. The bivalve was then inserted into a beaker and the blood drained from the cut surfaces for 3 to 5 min. Approximately 1 ml of blood was taken from each specimen. The blood samples were centrifuged for 2 min at 8,000 x *g* to remove debris.

The osmotic concentration of all samples was measured immediately using a Halbmikro Osmometer with a reading accuracy of ± 1 mOsm. The samples were then frozen in Eppendorf tubes for ionic analyses.

Sodium, potassium, calcium and magnesium 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. Standards were made up in an artificial seawater matrix according to Perkin-Elmer (1973).

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Adductor muscles were collected every 3 h over a 24 h period for an estimation of tissue water. The posterior muscles were removed, weighed, freeze-dried and re-weighed. The total water as a percentage of the wet weight was calculated from the following formula:

$$\frac{\text{wet weight - dry weight}}{\text{wet weight}} \times 100 = \text{per cent tissue water.}$$

Percentage values for individual clams in a given salinity were averaged and standard deviations computed.

To determine the salinity levels that induce shell valve closure and opening in specimens of *Anadara senilis* exposed to conditions of fluctuating seawater concentration, the following experimental procedure was carried out.

Five clams were each attached by one valve, with dental cement, to pieces of slate glued to the floors of the compartments of a 5-chambered Perspex box supplied with seawater at 25°C by the fluctuating salinity apparatus described by Davenport *et al.* (1975). Each chamber of the box had a water volume of 200 ml and a flow-through of 70 ml min^{-1}. Movements of the shell valves of the clams were monitored by stress gauges attached by a thread to glass hooks cemented to the upper shell valves as shown in Fig. 1. The gauge resistance changes, reflecting shell valve displacement, were displayed on Smiths servoscribe chart recorders. The salinity of the water flowing over the clams was continuously monitored by platinum conductivity cells connected to a Carwyn Instruments Salinity Monitor. The 5 clams were held in flowing full seawater (33.5‰ S) for 24 h to allow them to establish a normal rhythm of shell valve movement. They were then exposed for 24 h to a sinusoidal salinity regime of 12 h near-tidal wavelength which fluctuated between full seawater and pure freshwater. During this period, therefore, they encountered lowered salinity levels on two occasions. From the timing of shell valve closure/opening derived from the stress gauge traces, and from the monitored seawater concentrations it was possible to establish the salinity levels corresponding to closure and opening.

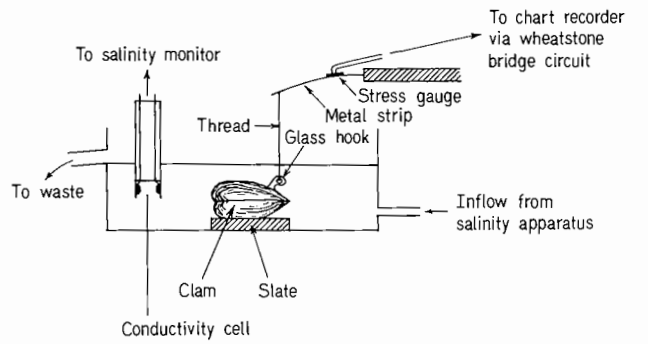


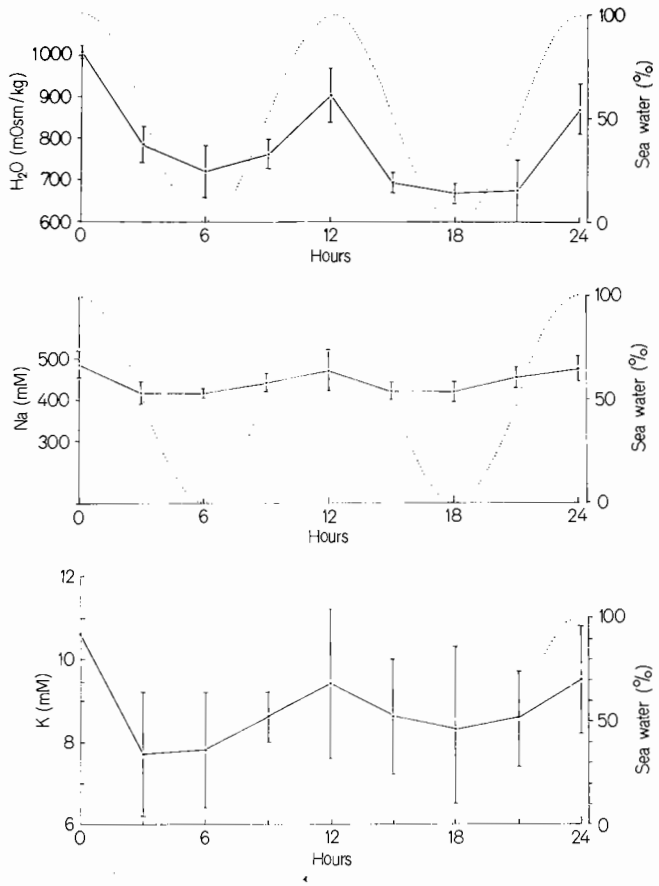
Fig. 1. Apparatus for determining threshold seawater concentrations for shell valve closure and opening in *Anadara senilis*

Results

Changes in Haemolymph Constituents

Fig. 2 shows the changes in total osmotic and ionic concentrations of the haemolymph of *Anadara senilis* exposed to a sinusoidal salinity regime. It may be seen that the haemolymph osmolality fell rapidly during the first 3 h of exposure to falling salinity, but did not change significantly during the following 6 h. The lowest mean osmolality recorded was about 720 mOsm kg^{-1} H_2O, about 28.5% below the initial haemolymph concentration. During the period between 9 and 12 h when the external salinity was rising, the haemolymph concentration rose also, but the peak value (mean 910 mOsm kg^{-1} H_2O) was significantly lower than the haemolymph concentration at the start of the experiment. A similar pattern of osmolality change occurred during the second 12 h period, with a drop in concentration between Hours 12 and 15 followed by a 6 h period of steady haemolymph osmolality. These sustained levels were rather lower than those recorded during the first 12 h period; the lowest mean haemolymph osmolality recorded was 670 mOsm kg^{-1} H_2O (33.5% below the initial value). The haemolymph concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} showed a pattern of change similar to the osmolality values.

Fig. 3 shows the changes in haemolymph osmolality and ionic concentrations exhibited by clams exposed to abrupt salinity fluctuations. During the periods of freshwater exposure the haemolymph osmolality fell to a mean of 860 mOsm kg^{-1} H_2O (14.6% below the initial concentration) at Hour 9 and to a mean of 880 mOsm kg^{-1} H_2O at Hour 21. When seawater became available again after freshwater exposure, the haemolymph osmoconcentrations rose to peaks of 970 mOsm kg^{-1} H_2O at Hour 12 and 1000 mOsm kg^{-1} H_2O at Hour 24, which did not differ significantly from the haemolymph osmolality at the start of the experiment. As for the sinusoidal salinity regime, individual ionic concentrations in



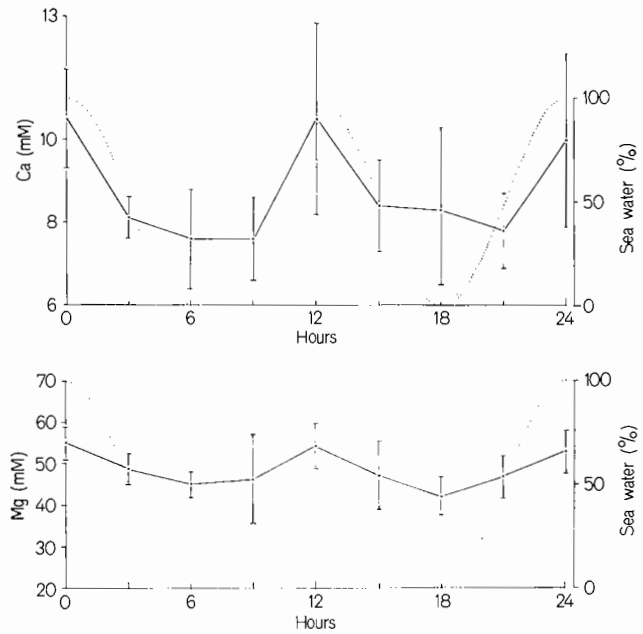
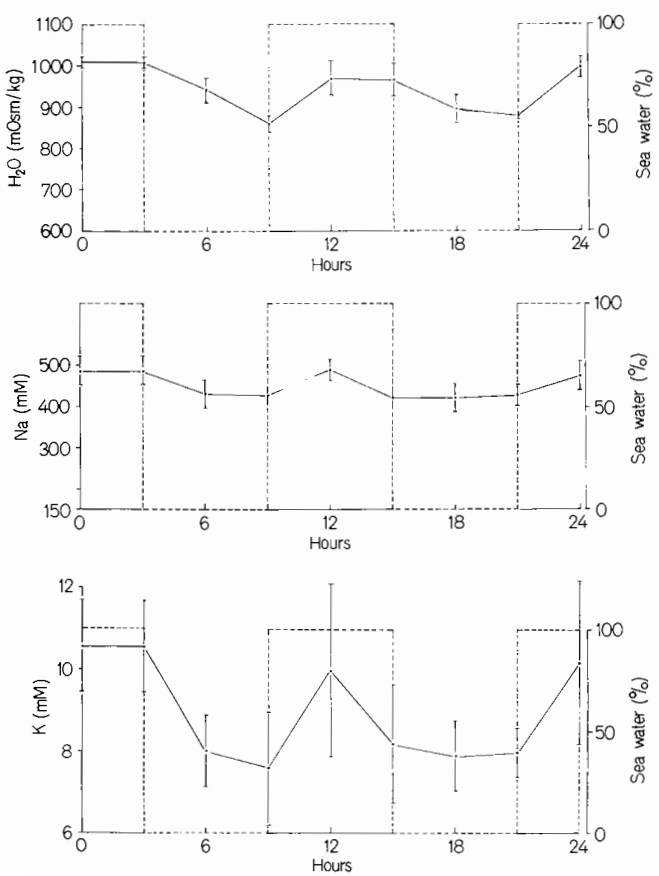


Fig. 2. *Anadara senilis*. Changes in haemolymph osmolality and Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations during exposure to 0‰ seawater minimum sinusoidal salinity regime. Each point is mean ± 95% confidence limits



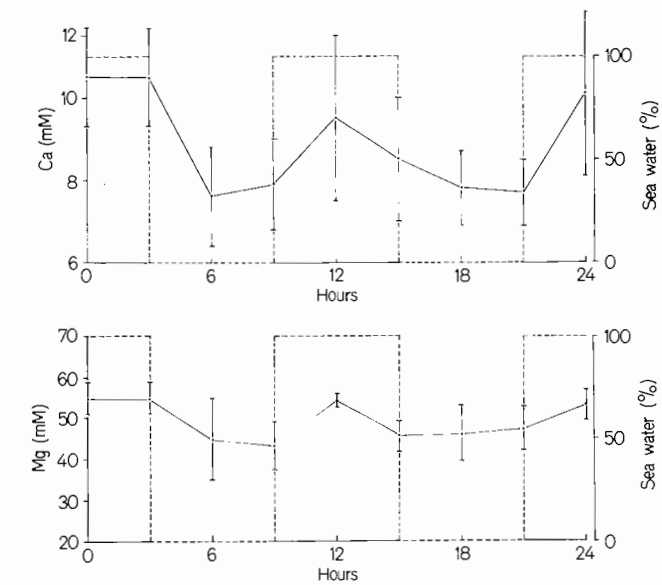


Fig. 3. *Anadara senilis*. Changes in haemolymph osmolality and Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations during exposure to 0‰ seawater minimum abrupt (square wave) salinity regime

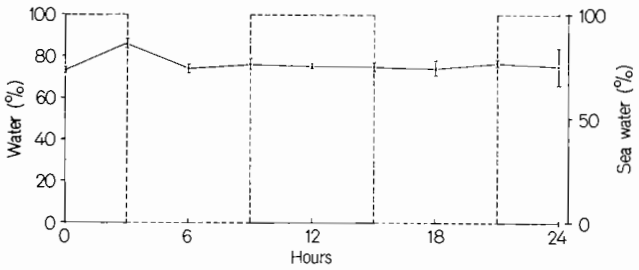


Fig. 4. *Anadara senilis*. Changes in tissue water content during exposure to abrupt (square wave) salinity regime

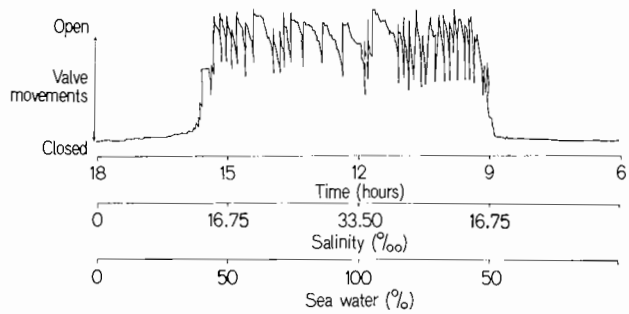


Fig. 5. *Anadara senilis*. Example of shell valve movements of clams exposed to fluctuating seawater concentrations

Table 1. *Anadara senilis*. Closure/opening concentrations in 100 to 0% seawater sinusoidal regime of 12 h wavelength (100% = 33.5‰ S)

Clam no.	Closure (1)	Opening (1)	Closure (2)	Opening (2)
1	41	49	40	49
2	37	41	47	47
3	51	48	52	49
4	49	43	47	43
5	49	35	49	34
Mean ± 95% confidence limits	45.4±7.5	43.2±7.1	47.0±5.5	44.4±7.8

Table 2. *Anadara senilis*. Analysis of variance: seawater concentrations at which valves open and close. For this analysis percentages were changes into arcsin values

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between opening and closure concentrations	3	12	4
Between clams	4	53	13.3*
Residual	12	119	9.92*
Total	19	184	

*Not significant at P = 0.05.

the haemolymph showed a pattern of change similar to the osmolality values. Overall it would appear that abrupt salinity changes induce smaller fluctuations in haemolymph concentrations than does exposure to sinusoidal salinity regimes, and that the clams are able to regain their initial haemolymph osmolality at high environmental salinity levels in the abrupt regimes but not in the sinusoidal salinity profiles.

Tissue Water Content

Fig. 4 shows changes in tissue water content of clams exposed to abrupt salinity fluctuations. No significant changes in tissue water content were shown by individuals in either gradual or abrupt salinity regimes.

Shell Valve Movements

Fig. 5 shows a typical stress-gauge trace reflecting shell valve movements. Table 1 summarises the results of this experiment.

In the fluctuating salinity regime, the clams closed their valves when the seawater (sw) concentration fell to a mean value of 46.2 ± 3.6% sw. The valves reopened when the concentration of water rose above a mean value of 43.8 ± 4.1% sw. An analysis of variance (Table 2) showed that differences between seawater concentrations corresponding to valve opening and closure were not significant.

Discussion and Conclusions

The stress gauge experiments show that *Anadara senilis*, like many species of bivalves (Holyeaux *et al.*, 1976; Shumway, 1977), is capable of closing its shell valves in order to isolate itself from a low-salinity environment. Our studies show that *A. senilis* keeps its shell valves open in the salinity range of 15 to 33.5‰. In this species, burrowing deeper down into the muddy bottom may also be a habit which helps the avoidance of exposure to freshwater, but the present study did not investigate this.

While the shell valves remain open, *Anadara senilis* is unable to regulate its internal concentration to counteract changes in the external medium. Thus, the osmotic concentration and ionic concentration of the haemolymph tend to follow changes in the external medium.

Since the maximum drop in haemolymph concentration observed was a mean fall of 33.5% below initial haemolymph osmo-

lality during the second 12 h period of the sinusoidal salinity regime, and since the valve closure mechanism operates at seawater concentrations about 34% below maximum, it would appear that almost perfect osmoconforming occurs when the valves are open, and there is no sign of a damped internal osmolarity fluctuation of the type predicted by Spaargaren (1974). Shell valve closure, however, appears to be an effective mechanism in preventing excessive dilution of the haemolymph and, in this respect, the present results are similar to those obtained by Shumway (1977) for estuarine species such as *Mytilus edulis* and *Crassostrea gigas* from temperate areas.

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