

LONG-TERM VARIATIONS IN CATION CONTENT OF TWO POPULATIONS OF ADULT MARINE CLAM (*TAPES DECUSSATUS* L. AND *T. PHILIPPINARUM*) REARED IN A COMMON HABITAT

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Abstract—1. The levels of four major cations, Na⁺, K⁺, Mg²⁺ and Ca²⁺ were measured using flame spectrophotometry over a 16-month period in two populations of adult marine clam reared in a common habitat.

2. The variations in absolute and relative values of Na⁺ and Mg²⁺ were virtually identical within each species; the relative values showed a marked increase during the period of negative energy balance previously reported, even when the external salinity decreased.

3. Calcium levels showed very irregular long-term variations, reflecting its multiple roles as a metabolic, osmotic and structural element.

4. A close relationship was observed between the levels of K⁺ and organic constituents, especially glycogen.

5. An adaptive benefit of this relationship is postulated.

INTRODUCTION

The seasonal variations in the biochemical components of temperate-water bivalves have been the object of considerable study for some time (see Gabbott, 1983, for review). In a recent series of publications a detailed study of such variations has been presented for two populations of marine clams reared in a common habitat: the Atlantic *Tapes decussatus* L. and the Indo-Pacific *Tapes philippinarum* (Beninger, 1984; Beninger and Lucas, 1984; Beninger and Stephan, 1985). These studies focused on seasonal variations in organic biochemical constituents. However, no study to date has investigated the possibility of long-term variations in the ionic content of whole bivalve tissue. Short-term studies (less than a few days) are relatively numerous (see review by Pierce, 1982) and have been undertaken to investigate osmotic behavior, being confined to observations of hemolymph and/or mantle fluid.

The present work documents the variations in the levels of Na⁺, K⁺, Ca²⁺ and Mg²⁺ over a 16-month period in the two populations of *Tapes*. The existing data concerning concurrent seasonal variation of the biochemical components offered the unique possibility of detecting any eventual link between long-term variations of cationic and organic tissue constituents.

MATERIAL AND METHODS

A complete description of the sampling site and characteristics of each population, as well as details of tissue preparation, have been given previously (Beninger and

Lucas, 1984). Aliquots of 3–5 g of pooled, homogenized tissue of each species for each monthly sampling were dried in porcelain containers for one hour at 100°C. The temperature was then increased to 300°C for 15 min and finally fixed at 550°C for 24 hr, thus ensuring complete oxidation while minimizing losses of sodium and potassium (Grove *et al.*, 1961). The containers were removed from the muffle furnace and placed in a desiccator over activated silica gel for 24 hr before weighing with a Mettler H54 precision balance in the presence of activated silica gel. The ash was prepared for cation analysis using a modification of the technique reported by Mayzaud and Martin (1975). The ash was dissolved in 5 ml of 37% HCl while gently heating the porcelain containers. The solution was flushed into a volumetric flask using a stream of demineralized distilled water and diluted to a final volume of 100 ml. Weight verification of the porcelain container confirmed complete recovery.

The solutions were analysed by flame spectrophotometry using a Beckman K-Li-Na and Ca-Mg analyser. All determinations were performed in duplicate; the reliability of the measurements was excellent.

The quantity of tissue present at each monthly sampling was expressed as ash-free dry weight (AFDW) of a standard animal (Beukema and DeBruin, 1977; Beninger and Lucas, 1984). The cation levels were expressed in two complementary ways: relative and absolute values. The relative value is defined as the percent cation content of wet tissue, thus allowing the detection of changes in the proportions of ions and water in the tissues. The absolute value is defined as the weight of a given chemical component per standard animal dry tissue weight (Ansell, 1972, 1974, 1975; Ansell *et al.*, 1980; Beninger and Lucas, 1984). The use of absolute values allows the amount of a given substance in a standard animal to be quantified and is not influenced by reciprocal effects of proportions or by growth. In the long-term study of cation content, the use of absolute values allows the distinction between changes caused by variations in the tissue content (absolute values follow AFDW curve) and when some other source of variation is present (absolute values do not follow AFDW curve).

Weekly salinity measurements were performed using a salinity refractometer.

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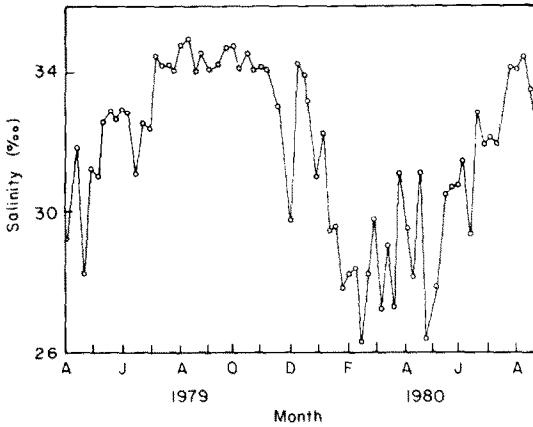


Fig. 1. Weekly variation of salinity in the external medium of the two populations studied.

RESULTS

The weekly salinity data show a marked seasonal cycle, with maxima in late summer and a minimum in late winter (Fig. 1). The amount of tissue present in a standard animal of each population (AFDW) varied in a regular fashion over the sampling period: a maximum in spring and summer 1979, a minimum in late winter and a smaller maximum in spring 1980 (Fig. 2).

The variations in cation levels are presented in absolute values (Fig. 3) and relative values (Fig. 4). The relative values were multiplied by a constant, the mean wet tissue weight of *T. decussatus* over the sampling period (3.839 g), in order to express these

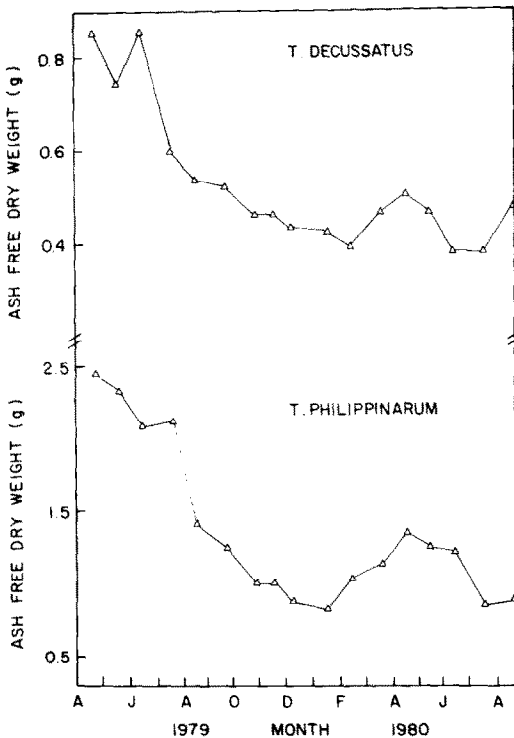


Fig. 2. Seasonal variations in the ash-free dry weight of a standard animal of each of the populations studied.

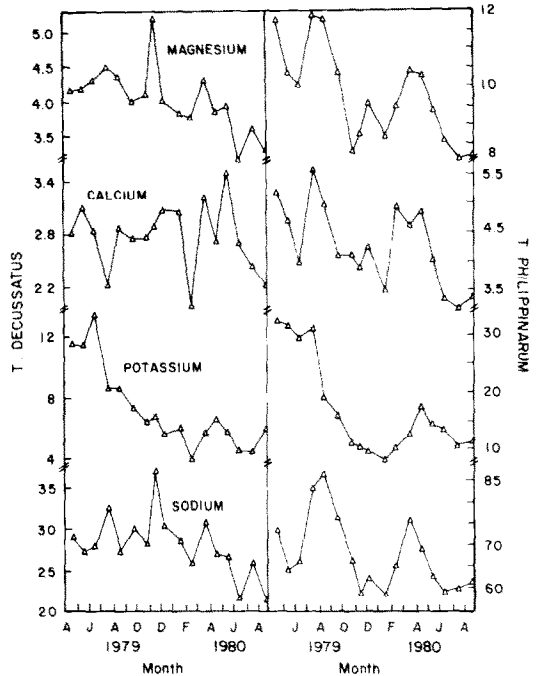


Fig. 3. Tissue levels of the four major cations in the two populations studied, expressed as mg per standard animal wet weight (absolute values: see text for details).

values in terms close to the weights of the organisms studied, rather than as percentages.

Relative values are statistically non-independent. Moreover, as the independence of the absolute values is uncertain (the distribution of these ions in the intra- and extracellular fractions is not known with cer-

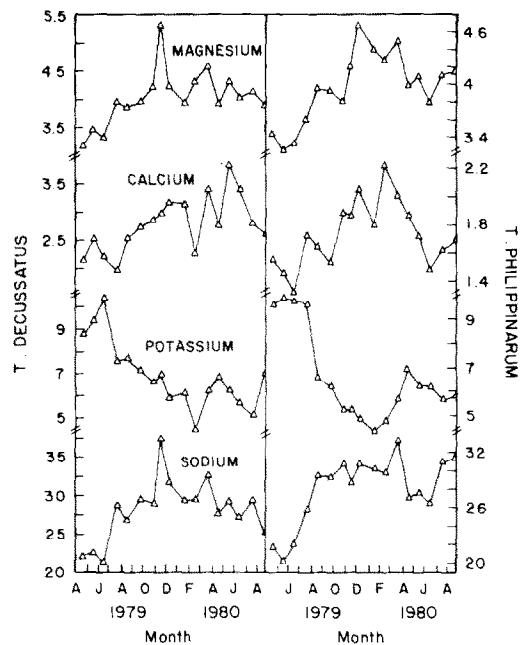


Fig. 4. Tissue levels of the four major cations in the two populations studied, expressed as % wet tissue weight \times mean wet tissue weight of *T. decussatus* (relative values: see text for details).

tainty in the two species studied), as well as being necessarily linked to the amount of tissue present, statistical correlations between cation levels and between cations levels and standard animal AFDW were not performed.

Sodium–magnesium

The long-term variations in the levels of Na^+ and Mg^{2+} were identical, although these variations were different for the absolute and relative values in *T. philippinarum* (Figs 3 and 4). In addition the pattern of variation of the absolute values was quite different for each species, whereas the pattern of variation of the relative values was rather similar in both species.

In *T. philippinarum*, a very regular variation gave rise to absolute value minima in mid-summer 1979 and 1980 and in the intervening winter. Maxima were observed in late summer 1979 and early spring 1980. This contrasts with the irregular long-term variations in the *T. decussatus* Na^+ and Mg^{2+} levels.

In both species the relative values of Na^+ and Mg^{2+} showed a steady increase in the summer of 1979 and the values remained at an elevated level for the rest of the sampling period.

Calcium

A marked difference in the long-term variations of the Ca^{2+} absolute values is evident in the two populations studied. In *T. philippinarum*, the variations in these values closely followed those of Na^+ and Mg^{2+} , while this was not true for *T. decussatus* (Fig. 3). In both populations the long-term variations in Ca^{2+} relative values showed considerable irregularity (Fig. 4).

Potassium

The variations in the levels of K^+ ions follow the AFDW very closely and the curves of absolute and relative values are virtually identical within each species (Figs 2–4). The potassium cation is the only one of the four measured which demonstrates these properties.

DISCUSSION

As previously mentioned, most studies of bivalve osmoregulation are short-term, taking place over the course of a few hours or days (Schoffeniels and Gilles, 1972; Baginski and Pierce, 1977; Shumway, 1977; Shumway *et al.*, 1977). It is not known at present whether *T. decussatus* and *T. philippinarum* are osmoconformers or osmoregulators; however, marine molluscs are essentially osmoconformers with a certain capacity for osmoregulation (Schoffeniels and Gilles, 1972; Mahasreh and Pora, 1981). The mechanisms involved in bivalve osmoregulation are complex and incompletely understood. In addition to mineral ions, organic molecules (especially free amino acids) participate in osmoregulation (Pierce and Greenberg, 1972; Pierce, 1982) and may constitute up to 50% of osmo-active elements (Schoffeniels and Gilles, 1972).

Although the relative values used in the present study reflect changes in the proportions of cations and water in the tissues, it is immediately obvious that the variations in the relative values of the four

cations studied do not correspond to the seasonal variations in salinity of the external medium (Figs 1 and 4). Consequently the long-term behavior of these ions appears to be influenced by factors other than external salinity. These factors will be considered in the present discussion.

Sodium–magnesium

It is evident that the long-term variations of Na^+ are quite similar to those of Mg^{2+} within each species (Figs 3 and 4), both in absolute and relative values. In *T. philippinarum* most of these variations in absolute values may be attributed to seasonal variations in the amount of tissue (Fig. 2). In *T. decussatus* the absolute values show a less regular variation, indicating that the variation of Na^+ and Mg^{2+} levels influences the long-term variations of these ions more so than in *T. philippinarum*.

In both species studied the relative values of Na^+ and Mg^{2+} showed a progressive increase starting in mid-summer of 1979, remaining at an elevated level throughout the following winter and summer. This observation is particularly interesting since the sum of these two ions represents the majority of the major cations and these values remain elevated even when the external salinity decreases (Fig. 1). This observation is at variance with the osmoconformity of marine bivalves. Indeed decreases in the levels of these ions (as well as of Ca^{2+}) have been reported in the hemolymph of seven bivalve species exposed to salinity decreases over a 12-hr period (Shumway, 1977; Shumway and Youngson, 1979). Concurrent observations have shown that the percent water content of the tissues of these two populations presented long-term variations similar to those of Na^+ and Mg^{2+} , suggesting that these variations were due to an increase in tissue water content during periods of nutritional deficiency (Beninger and Lucas, 1984). An increase in the proportion of ash in starved zooplankton has been observed by Mayzaud (1976) and he suggests this is due to an increase in alkaline metals. An increase in sodium levels in liver and muscle has also been reported in food-deprived American eels (Moon, 1983). It may thus be proposed that the increase in Na^+ and Mg^{2+} relative values observed in the two populations studied is due to an increase in the levels of these ions in the tissues, resulting in a subsequent partial increase in tissue water content.

The physiological mechanism of these variations can be inferred from osmoregulatory studies on *Mytilus edulis* (Natochin *et al.*, 1979). Under normal conditions, Na^+ diffuses passively into the cells when the external medium is hypertonic. This could account for the increase in Na^+ and Mg^{2+} relative values observed in the summer of 1979, where the external salinity increased to a maximum in late August. In a hypotonic medium Na^+ is actively excreted from the cells of *M. edulis* (Natochin *et al.*, 1979). Given the apparent negative energy balance of the two populations studied during the winter of 1979 and the inability of these populations to reconstitute energy reserves the following summer (Beninger and Lucas, 1984), it is likely that the Na^+ level remained elevated due to passive retention, even while the external salinity decreased.

Calcium

The long-term variations in Ca^{2+} absolute and relative values are more irregular than the corresponding variations in Na^+ and Mg^{2+} , although the absolute values demonstrate some similarity in *T. philippinarum* (Figs 3 and 4). It is known that Ca^{2+} plays an important role in mollusc shell formation (Wilbur, 1972) and the irregular long-term variations probably reflect the multiple roles of this ion (metabolism, osmoregulation, skeletal element). In particular, the irregularity in the absolute-level long-term variations in both species suggests that shell and tissue formation are not always simultaneous; this has previously been suggested for *T. decussatus* on the basis of allometric data (Arnaud and Raimbault, 1963).

Potassium

Like other animals bivalves have a strong transmembrane gradient of potassium, which is concentrated in the cells (Natochin *et al.*, 1979; Hochachka and Somero, 1984). The intercellular volume is quite small in bivalves: approximately 25% of total volume in *Mytilus edulis* (Bricteux-Grégoire *et al.*, 1964b) and in *Ostrea edulis* (Bricteux-Grégoire *et al.*, 1964a). The K^+ levels determined in the present study are thus essentially measures of intracellular potassium.

In both populations studied, the long-term variations in K^+ relative and absolute values closely follow the AFDW variations (Figs 2-4). It is thus evident that the K^+ levels do not merely follow the variations in the amount of tissue but the proportion of K^+ per unit tissue closely follows that of the organic matter. Furthermore the data of Table 1 demonstrate that this variation in the proportion of K^+ is due to changes in the amount of K^+ and not merely to changes in the amount of water present in the animal, as was observed in *Mytilus edulis* by Natochin *et al.* (1979). Indeed the K^+ ion is the only cation to demonstrate such a close link to the amount of organic matter present. Intracellular K^+ is known to be electrostatically retained by non-diffusable organic anions and protein (Shaw, 1955a,b; Robertson,

1957), so that variations in total organic content may be accompanied by similar variations in K^+ levels. Previous observations suggest that such variations may present an adaptive benefit. In various mussel species the activity of pyruvate kinase, a key enzyme in the conversion of glycogen to lipid (via acetyl Co-A), has been found to be directly proportional to the K^+ concentration (Zhuchikhina and Skulskij, 1979). The previously reported peak in glycogen levels of these two populations corresponds to the K^+ maxima of the present study while the K^+ minima correspond to the glycogen minima. It has been proposed that bivalve glycogen is converted to lipid during gamete maturation (Reid, 1969; Walne, 1970; Gabbott and Stephenson, 1974; Gabbott, 1975; Barber and Blake, 1981) and indeed the peak and decline in K^+ and glycogen levels corresponds to the spawning period in the two populations studied (Beninger and Lucas, 1984). The K^+ and glycogen minima are evident at the end of the spawning period and during the sexually inactive winter period.

These considerations are obviously hypothetical but the peculiar long-term variations of K^+ observed in the present study suggest an interesting subject for further research.

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Table 1. Tissue levels of the four major cations in the two populations studied, expressed as % dry tissue weight

	Na^+		K^+		Mg^{2+}		Ca^{2+}	
	Td	Tp	Td	Tp	Td	Tp	Td	Tp
1979								
April	2.98	2.70	1.18	1.19	0.43	0.43	0.29	0.19
May	3.19	2.49	1.33	2.57	0.49	0.40	0.36	0.18
June	2.85	2.85	1.37	1.27	0.44	0.43	0.29	0.17
July	4.57	3.46	1.20	1.29	0.63	0.49	0.31	0.23
August	4.27	5.09	1.21	1.13	0.61	0.69	0.40	0.29
September	4.80	5.07	1.16	1.07	0.64	0.68	0.44	0.27
October	5.01	5.42	1.13	0.92	0.73	0.67	0.49	0.33
November	6.53	4.87	1.21	0.89	0.92	0.72	0.51	0.32
December	5.66	5.79	1.05	0.91	0.75	0.88	0.57	0.39
1980								
January	5.46	5.78	1.14	0.83	0.73	0.85	0.58	0.34
February	5.34	5.20	0.81	0.83	0.78	0.75	0.41	0.39
March	5.37	5.46	1.00	0.93	0.75	0.74	0.56	0.33
April	4.48	4.32	1.11	1.10	0.64	0.64	0.45	0.30
May	4.73	4.26	1.01	0.97	0.70	0.63	0.62	0.27
June	4.64	4.12	0.96	0.96	0.68	0.59	0.58	0.23
July	5.52	5.73	0.94	1.04	0.77	0.76	0.52	0.30
August	3.76	5.70	1.06	1.06	0.58	0.75	0.39	0.31

Td, *Tapes decussatus*; Tp, *Tapes philippinarum*.

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