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TOTAL BODY CALCIUM AND HAEMOLYMPH CALCIUM CONCENTRATIONS IN THE CRAYFISH *AUSTROPOTAMOBIUS PALLIPES* (LEREBOULLET)

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SUMMARY

Variations in the concentration of calcium in the haemolymph and of total body calcium in *Austropotamobius pallipes* have been investigated. Haemolymph calcium concentration was independent of both body size and sex. During premoult the calcium concentration of the haemolymph rose from 11·8 mM/l to 15·0 mM/l. After the moult the calcium concentration remained high during stages A and B but declined to the intermoult level during stage C. Total body calcium was directly proportional to fresh body weight. No difference was apparent between calcium content of male and female crayfish of similar body weight.

INTRODUCTION

Data concerning the concentration of calcium in the haemolymph are available for several species of freshwater crayfish. Sourie & Chaisemartin (1961) measured calcium concentration in the haemolymph of *Astacus* (= *Austropotamobius*) *pallipes* and observed an increase in the concentration of calcium in the haemolymph with increasing body size; from 7·0 mM – Ca/l in small animals (34 mm) to 17·5 mM – Ca/l in large animals. Male specimens were found to have higher calcium levels in the haemolymph than females. Chaisemartin (1967) examined three species of European freshwater crayfish (including *Austropotamobius*) from soft water and hard water habitats. The concentration of calcium in the haemolymph was again observed to increase with body size, and soft water animals had higher values than those from hard waters. Andrews (1967), however, found no significant correlation between body size and the concentration of calcium in the haemolymph of *Orconectes limosus*, values for female crayfish being similar to those for males during the winter months but higher during the summer. In immature specimens of the migratory crab, *Eriocheir*, the calcium concentration of the haemolymph is also independent of body size (De Leersnyder, 1967). These immature stages live in freshwater habitats. In mature *Eriocheir* the calcium concentration of the haemolymph was greater in females than in males. There are, then, marked differences between the data of Sourie & Chaisemartin (1961) and Chaisemartin (1967) on the one hand and Andrews (1967) and de Leersnyder (1967) on the other. This investigation seeks to clarify the situation in

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Austropotamobius by measuring the concentration of calcium in the haemolymph of crayfish of both sexes over a wide range of body size.

It is well established in aquatic crustacea that the concentration of calcium in the haemolymph rises during the premoult period (D). The freshwater crayfish *Austropotamobius* conforms to this pattern (Chaisemartin, 1967). After the moult, haemolymph calcium concentration falls below the intermoult level, returning to normal only in the late postmoult phase (C_1) (Chaisemartin, 1967). A similar situation exists in *Eriocheir* (de Leersnyder, 1967) and in the marine crab *Carcinus maenas* (Robertson, 1937, 1960), but not in the lobster *Panulirus argus* (Travis, 1955). In this investigation results will be presented for the concentration of calcium in the haemolymph of *Austropotamobius* throughout the intermoult cycle.

Data are available for the total body calcium and that of various parts of the exoskeleton of *Austropotamobius* (Chaisemartin, 1962*a*, *b*, 1967). Somewhat surprisingly, Chaisemartin found that total body calcium was higher in crayfish from soft waters (0·135 mM – Ca/l) than from hard waters (2·35 mM/l). This was apparently true in *O. limosus* and *Astacus astacus* as well as in *Austropotamobius* but not of *Gammarus pulex* from the same waters (Vincent, 1963, 1969). Total calcium content in crayfish is known to vary on a seasonal basis related to the moulting cycle (Chaisemartin, 1962*a*, 1967). During the premoult period calcium is withdrawn from the exoskeleton and the greater part lost to the external medium, only a small amount being stored in the gastroliths (Chaisemartin, 1962*a*, 1964, 1967; McWhinnie, 1962; Travis, 1960). Consequently, during stage D there is a decrease in total calcium content of the body (Chaisemartin, 1967; Greenaway, 1974*a*). At the moult further net loss of calcium occurs in the cast exoskeleton, the soft crayfish containing only 10–20% of its total content at the start of the preceding intermoult (Chaisemartin, 1964, 1967; Greenaway, 1974*a*). Calcification of the new exoskeleton is completed rapidly, the full calcium content being restored by stage C_4.

MATERIALS AND METHODS

Materials and methods were as described previously (Greenaway, 1972). It was important to determine accurately the stage of the intermoult cycle which each of the experimental crayfish had attained. Intermoult, early premoult and late postmoult animals were separated by calcium balance experiments. Postmoult stages showed a positive calcium balance whilst premoult animals lost calcium to the medium. Late premoult and early postmoult stages were readily identified by external physical characteristics.

Animals used for total calcium measurements were washed briefly in distilled water, dried with paper tissue and killed by placing them in an atmosphere of CO_2. They were then dried to constant weight at 105 °C and ashed in platinum crucibles at 450 °C. The ash was dissolved with analytical grade HCl and the resultant solution diluted suitably for analysis. Measurements of calcium and magnesium concentrations were made with an E.E.L. 240 atomic absorption spectrophotometer.

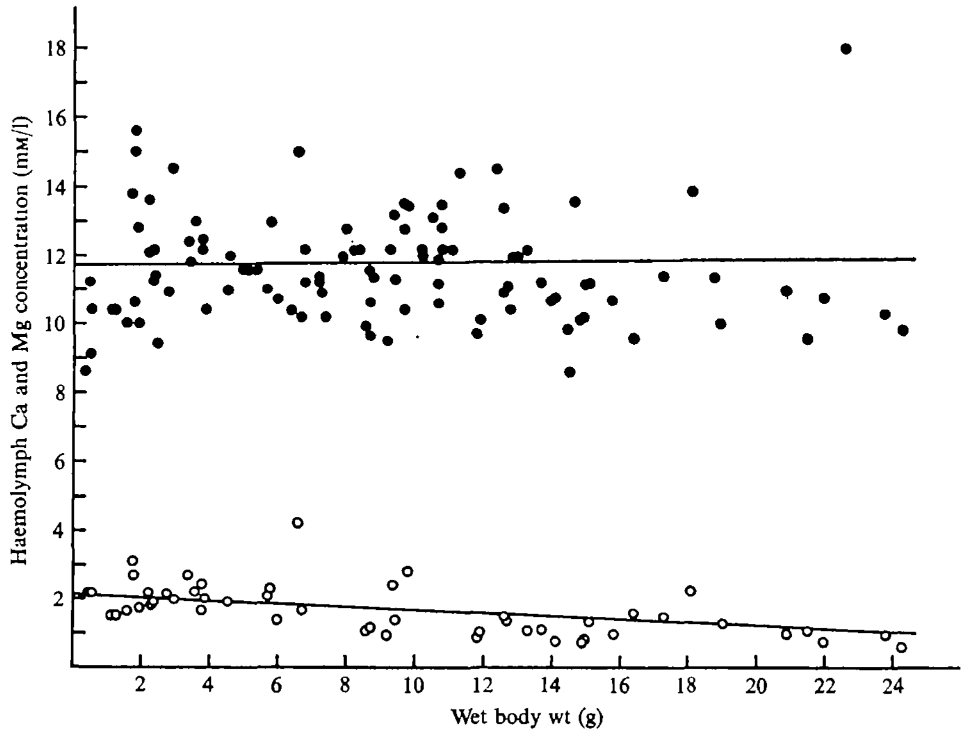

Fig. 1. The relationship between the concentration of calcium and magnesium in the haemolymph and body size. Regression lines were calculated by the least squares method. No distinction is made between values for male and female crayfish. ●, Values for calcium concentration in the haemolymph; ○, values for magnesium concentration in the haemolymph.

RESULTS

Haemolymph calcium in intermoult crayfish

The effect of body size

In Fig. 1 the relationship between body size (wet weight) and the calcium concentration of the haemolymph is shown for a sample of the crayfish *Austropotamobius* covering the normal size range found in the field. There was no correlation between calcium concentration and body weight (correlation coefficient = 0·03). The mean calcium concentration in the haemolymph of all the animals examined was 11·74 mM/l, which agrees quite well with the value published earlier for 10 g animals (Greenaway, 1972).

A few measurements of the concentration of magnesium in the haemolymph of *Austropotamobius* are shown in Fig. 1. The values varied between 4·2 and 0·56 mM – Mg/l, little correlation with body weight being apparent (correlation coefficient = 0·46). Andrews (1967) found the concentration of magnesium in the haemolymph of *O. limosus* to be independent of body size.

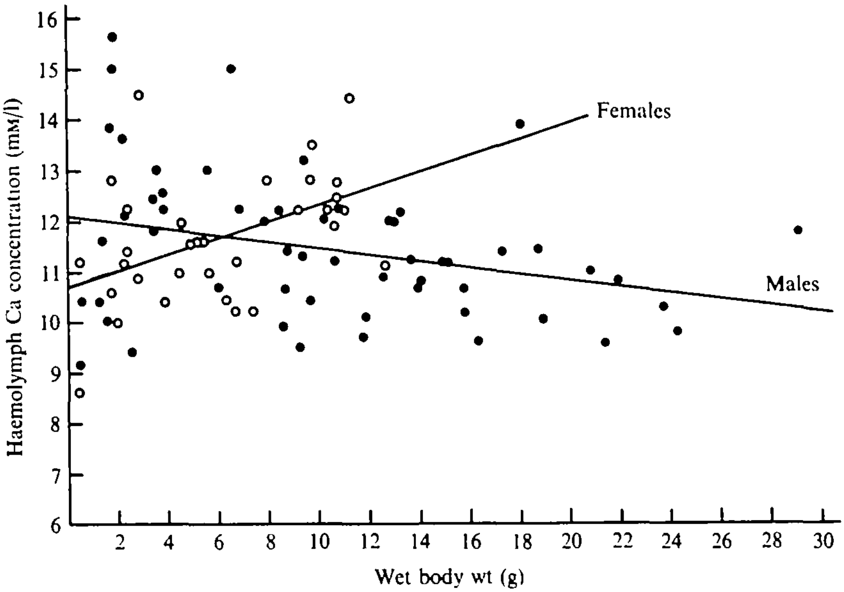

Fig. 2. The effect of sex on the concentration of calcium in the haemolymph. ●, Values for male animals; ○, values for female animals. Regression lines were calculated by the least-squares method.

The effect of sex

Data for the concentration of calcium in the haemolymph of male and female crayfish have been plotted separately (Fig. 2). As in the field, values for male crayfish cover a wider range of body size than those for females. No significant difference is apparent between the two sets of data. Sourie & Chaisemartin (1961), however, found male crayfish to have a greater concentration of calcium in the haemolymph than did females.

Haemolymph calcium concentration during the intermoult cycle

The concentration of calcium in the haemolymph was measured at each of the clearly discernible stages of the moult cycle (Fig. 3), using animals kept either in large volumes of artificial tap water or in small volumes which were changed regularly to avoid fluctuations in the calcium content of the medium. During the premoult stage (D) the haemolymph calcium concentration was significantly higher ($P < 0{\cdot}01$) than the intermoult (C_4) level of about 11·7 mM/l, reaching 15·0 mM – Ca/l by stage D_3 and remaining close to this level during D_4. Chaisemartin (1967) recorded rather higher values at the corresponding stage. Immediately after the moult the calcium concentration of the haemolymph reached a maximum of 15·6 mM/l (stage A). This declined slightly during stage B but remained above the intermoult level until calcification had been completed. McWhinnie (1962) has provided data for the total calcium concentration in the haemolymph of *O. virilis*. Her values of 3·4 mg/ml blood = 76·1 mM – Ca/l) for crayfish at the premoult stage and 13·55 mg/ml (= 333·8 mM – Ca/l) for premoult 'eyestalkless' animals are so high that an error in measurement must be suspected.

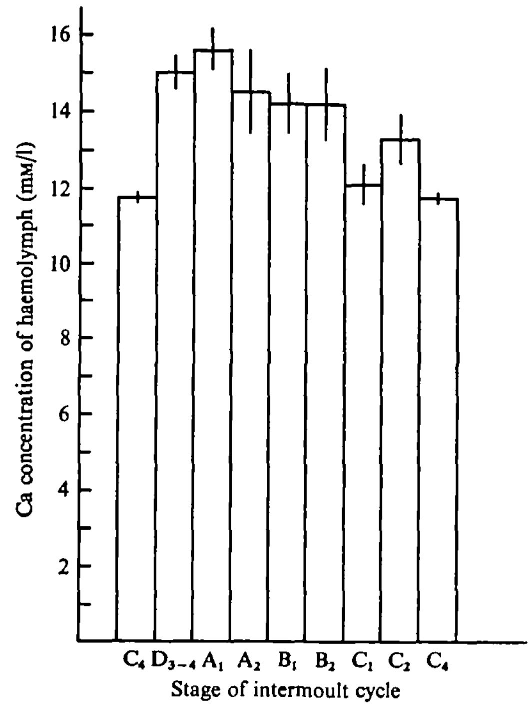

Fig. 3. Variations in the concentration of calcium in the haemolymph during the inter-moult cycle. Vertical lines represent standard errors.

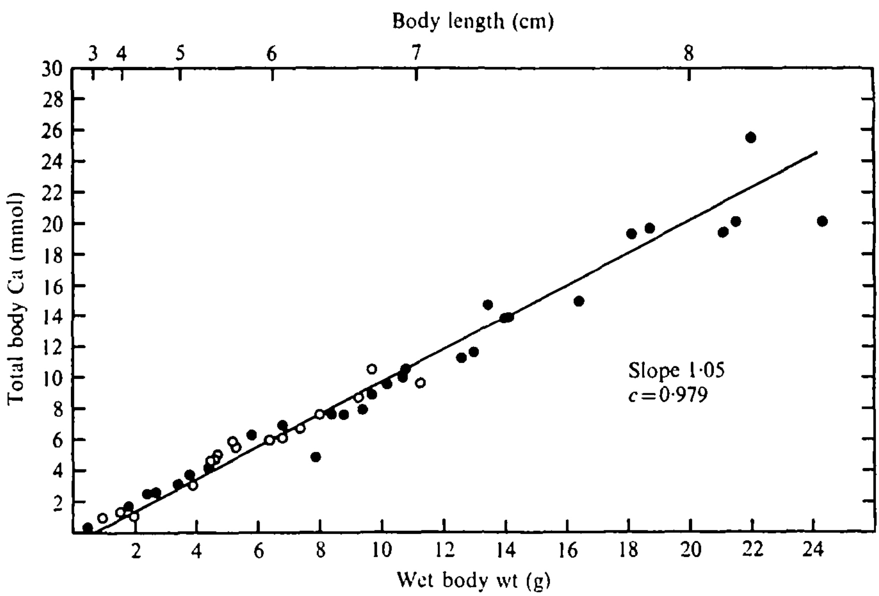

Fig. 4. The relationship between total body calcium and body size. ● represents values for male animals and ○ values for female animals.

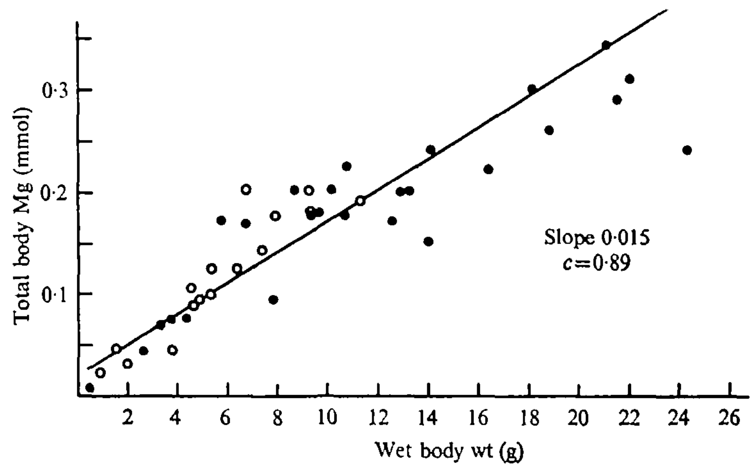

Fig. 5. The relationship between total body magnesium and body size in intermoult crayfish. ● represents values for male animals and ○ values for females.

Total calcium content at the intermoult stage

The total calcium content of *Austropotamobius* has been measured in a sample of animals covering the whole of the size range available at the collecting site (Fig. 4). Total body calcium is related in a linear fashion to body size (wet weight) and length, there being fairly close agreement between the present data and that of Chaisemartin (1967) for *Austropotamobius*. It is apparent that there is little difference between total calcium contents of similar sized crayfish of different sex. In consequence, the regression line has been calculated using the combined data for male and female crayfish. Chaisemartin (1962*a*, *b*) has compared the calcium content of selected parts of the exoskeleton in male and female crayfish. In each region examined the calcium content of the portions of male exoskeleton was about 10% greater than in the female. Values for the total calcium content were not given, but presumably were about 10% greater in male than in female crayfish of comparable size.

Values for the total magnesium content of the body are shown in Fig. 5. The calculated ratio for total calcium to magnesium is largely independent of body size. The mean value of 53:1 may be related to the ratio of Ca:Mg in the water, but no evidence is available on this point. The Ca:Mg ratio of the cast exoskeleton, although lower (44:1) did not differ significantly from the above value ($P > 0{\cdot}5$). The ratio in the haemolymph varied with body size (mean 7·8:1) but was always lower than in the exoskeleton.

DISCUSSION

As with another freshwater crayfish, *O. limosus* (Andrews, 1967) no correlation has been found between haemolymph calcium and body size in *Austropotamobius*: a conclusion which contrasts with that of Chaisemartin (1967) for these species and for *A. astacus*. Although the measurements of the latter authors were statistically sound, those of Sourie & Chaisemartin (1961) were performed during the moulting period (May to July) when the calcium concentration of the haemolymph would be fluctuating. The observations of Chaisemartin (1967), on the other hand, were made du

Table 1. *The concentration of calcium in the haemolymph of some freshwater crustacea*

Species	Water Ca conc. (mM/l)	Haemolymph Ca conc. (mM/l)	Source
Austropotamobius pallipes	0·6–1·1	8·6–18·0	Present study
	0·135	6·5–16·5	Chaisemartin (1967)
	2·45	7·9–12·8	
Astacus astacus	0·135	6·5–15·2	Chaisemartin (1967)
	2·45	8·0–12·0	
Orconectes limosus	0·135	6·5–13·5	Chaisemartin (1967)
	2·45	7·9–11·3	
	—	17·5–20·5 (mM/kg H_2O)	Andrews (1967)
Potamon niloticus	—	12·7	Shaw (1959)
Eriocheir sinensis	—	11·5	de Leersnyder (1967)
Gammarus pulex	0·038–0·045	5·85	Vincent (1969)
	2·5–2·75	7·65	

The calcium concentration of the water represents the concentration at the collecting sites.

intermoult when the crayfish were in calcium equilibrium. It can only be concluded that the French populations of *O. limosus* and *Austropotamobius*, investigated by Chaisemartin, differed fundamentally from those currently examined in England and previously in Germany by Andrews.

The minimum recorded values for calcium concentration at the crayfish collection sites (0·6 mM – Ca/l) were well above the level required to saturate the calcium uptake mechanism (0·4 mM/l) (Greenaway 1974*b*) and it was not therefore possible to compare total body calcium and haemolymph calcium concentration of crayfish from hard and soft waters as achieved by Chaisemartin (1967). He found these two parameters to be elevated in soft water populations, although this was not true of total body calcium in *G. pulex* (Vincent, 1963). Differences in calcium content between hard and soft water populations of freshwater crustaceans, therefore, seem to be peculiar to crayfish.

The maximum concentration of calcium in the haemolymph was recorded at stages A_{1-2} whereas Chaisemartin (1967) found the concentration of calcium at stage A to be lower than the intermoult level. Andrews (1967) found the haemolymph calcium to be higher than normal for a week after the moult in *O. limosus*. *Carcinus*, however, shows a reduced concentration of calcium in the haemolymph after the moult (Robertson 1937, 1960) and in *Panulirus* the concentration falls to the intermoult level at ecdysis (Travis, 1955). It is clear, therefore, that there is no interspecific uniformity amongst aquatic decapods regarding the behaviour of calcium in the haemolymph during the postmoult stage. One might, however, expect intraspecific uniformity in this respect.

The rise in total calcium concentration of the haemolymph in premoult and postmoult stages of *Austropotamobius* are not matched by a rise in ionized calcium (Greenaway, 1974*a*, *b*). It is likely, therefore, that the recorded rise in total calcium concentration at this time is a passive phenomenon caused by the increased amounts of organic, calcium-binding materials in the haemolymph.

The concentration of calcium in the haemolymph varies seasonally (Andrews, 1967; Sourie & Chaisemartin, 1961) due to changes in the calcium metabolism brought about by the moulting process. The value of such seasonal measurements is dubious, as measurements made at clearly defined stages of the intermoult cycle give a more accurate picture of the normal cycle of variation in the concentration of calcium in the haemolymph.

Some published values for the concentration of calcium in the haemolymph of freshwater crustacea are given in Table 1. These values are similar to those found in marine crustacea, e.g. *Carcinus* 12·4 mM – Ca/l (Robertson, 1937), suggesting that little or no reduction in the level of haemolymph calcium appears to have occurred during the colonization of fresh water. In contrast, the calcium concentration of the haemolymph of freshwater molluscs is reduced, e.g. *Limnaea stagnalis* 4·9 mM – Ca/l (Greenaway, 1970), *Anodonta* 6·0 mM – Ca/l (Potts, 1954) and *Viviparus viviparus* 5·7 mM – Ca/l (Little, 1965).

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