ION CONCENTRATION AND ACTIVITY
IN THE HAEMOLYMPH OF *AEDES AEGYPTI* LARVAE

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SUMMARY

The ionic composition of the haemolymph was measured in fourth instar larvae of *Aedes aegypti* (reared in 0–30% sea water, the animals' natural salinity range). Inorganic ions and free amino acids form a substantial part of the haemolymph osmotic concentration. With increasing external osmotic pressure, there is an increase in the concentration of the amino acids, not the major inorganic cations.

Measurements with ion-selective electrodes indicate that the activities of sodium, potassium and chloride can vary independently of their total concentration. The osmotic contribution of these inorganic ions is therefore not necessarily determined by their total concentration.

INTRODUCTION

The extensive studies of osmoregulation in the larvae of the mosquito, *Aedes aegypti*, during the previous five decades (Wigglesworth, 1933 *a–c*; Ramsay, 1950, 1951, 1953; Stobbart, 1959, 1960, 1965, 1967, 1971 *a–c*, 1974) have revealed that the hindgut tissues enable the insects to regulate their internal fluid environment in response to changes in the external environment. It is therefore surprising that no measurements have been made of the changes in the haemolymph composition resulting from alterations in the external medium in this classical freshwater insect. However, such measurements have been made for larvae of the salt-water mosquitos, *Aedes campestris* and *A. taeniorhynchus* (Bradley & Phillips, 1977).

The advent of easily produced ion-selective microelectrodes adds a new dimension to the study of body fluid composition (e.g. Lettau *et al.* 1977) and have been used, together with measurements of total ion, amino acid and osmotic concentrations to investigate the changes in haemolymph concentration of *A. aegypti* larvae in response to alterations in the external medium. The measurement of ionic activities is of particular importance in the present context, for it is the free ions that are available to salt pumps and largely contribute to osmotic concentrations of the body fluids.

MATERIALS AND METHODS

Mosquito larvae were raised from eggs in beakers containing water at salinities between o and 30% sea water. The composition of artificial sea water was as in Edwards (1982a). Larvae were fed with desiccated liver powder. Fourth instar larvae were used for all experiments.

Haemolymph samples were taken by puncturing blotted larvae on squares of Parafilm and immediately taking the fluid up into micropipettes. Fluid samples were immediately prepared for composition measurement. Inorganic cation concentrations were measured by flame spectrophotometry; in the emission mode for sodium and potassium and in the absorption mode for calcium and magnesium precautions were taken to use reference solutions containing an appropriate background of interfering ions. Chloride concentration was determined by potentiometric titration (Ramsay et al. 1955). Osmotic pressure was measured by depression of freezing point using a Clifton cryostat biological nanolitre osmometer. The charring reaction with concentrated sulphuric acid was used to determine total sugar concentration (Diamond & Denman, 1973) while ninhydrin staining and colorimetry was used for amino acids. Urea concentration was measured by a miniaturization of the method of Fawcett & Scott (1960). Single-barrelled liquid ion exchanger electrodes were produced, following the technique of Lettau et al. (1977), to measure sodium, potassium and chloride. Sodium ion exchange resin was synthesized (Kraig & Nicholson, 1976), commercially produced resins (Corning and Orion) were used in potassium and chloride electrodes. Electrodes were selected for use on the basis of the time course and stability of their response to the ion to be measured. For each resin used an interference characteristic was determined by exposing electrodes to solutions containing varying concentrations of potential interfering ions. Sodium resin suffers considerable interference from potassium (Na/K selectivity 2:1) but because sodium is the major monovalent cation in the haemolymph, maximum interference is less than 10%. Chloride resins had a selectivity of over 100:1 for all interfering ions tested. Potassium resins are also very good except for their responsiveness to ammonia (see Discussion). The reference electrode consisted of a glass microelectrode which, when filled with 3 M KCl, showed a resistance of about 1 MΩ. To measure haemolymph activity, larvae were punctured under oil and both ion-selective electrode and reference electrode were put into the drop exuded; the resultant potential was measured with a Keithley electrometer. Electrodes were kept in haemolymph for a period of 30 s after the 95% response point on the electrodes' time response curve. If left longer, a considerable decay in their characteristics was observed.

RESULTS AND DISCUSSION

In larvae exposed to 2–30% sea water the sodium level in the haemolymph was maintained at a constant value of about 110 mM (Fig. 1), which is close to Ramsay's (1953) figure of 113 mM for larvae in 85 mM NaCl. Animals raised in distilled water had a significantly reduced level of sodium in the blood, of around 60 mM. Above 30% sea water the regulation of haemolymph sodium broke down and there was great
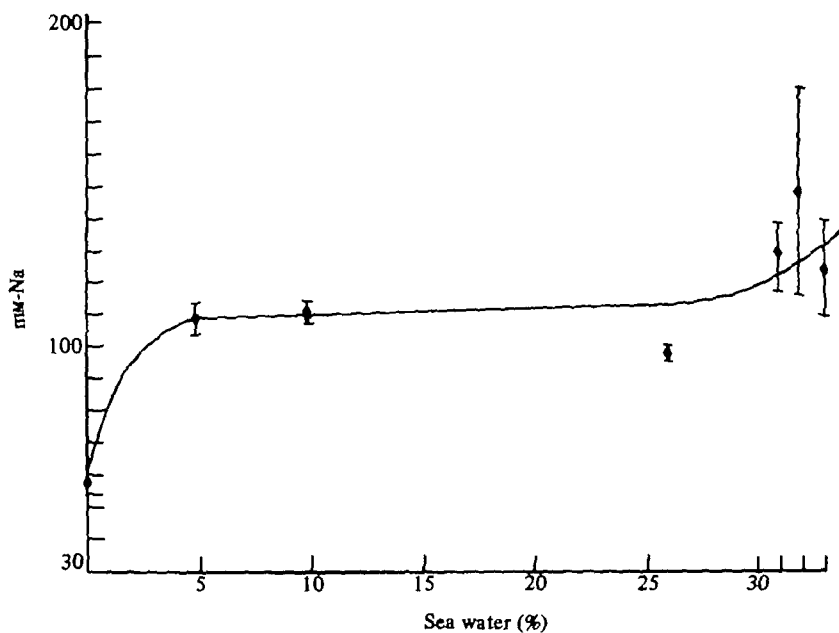
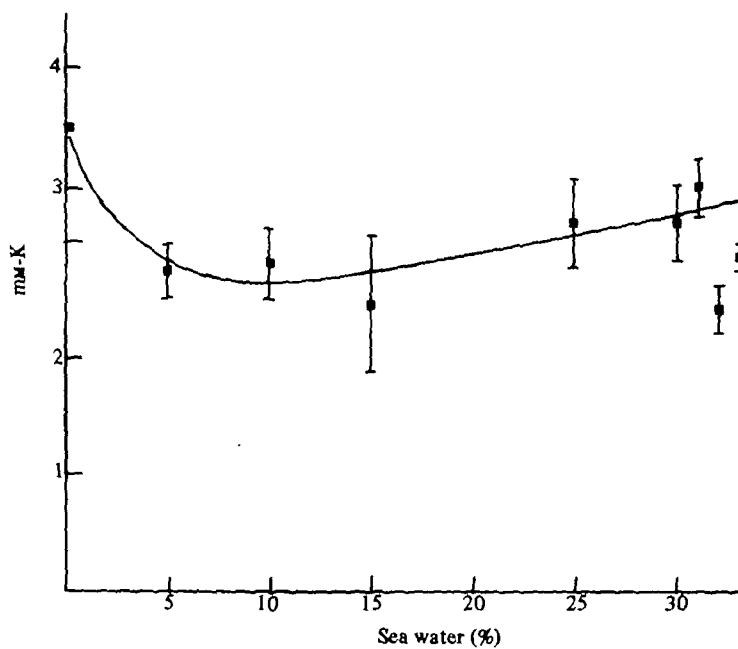Fig. 1. Haemolymph sodium concentration in larvae raised at different salinities. s.e. indicated.
$n = 5$.

Fig. 2. Haemolymph potassium concentration in larvae raised at different salinities.
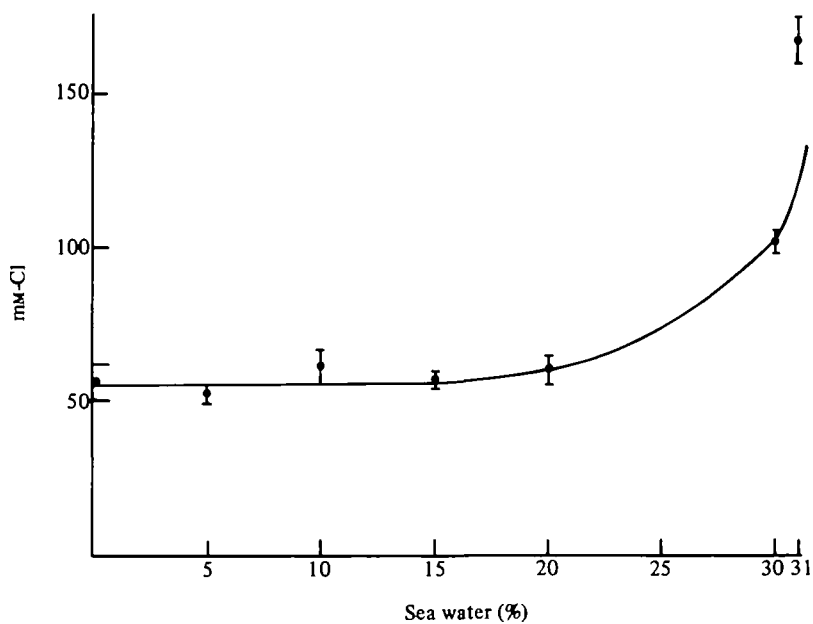s.e. indicated. $n = 5$.

Fig. 3. Haemolymph chloride concentration in larvae raised at different salinities. s.e. indicated.
$n = 5$.

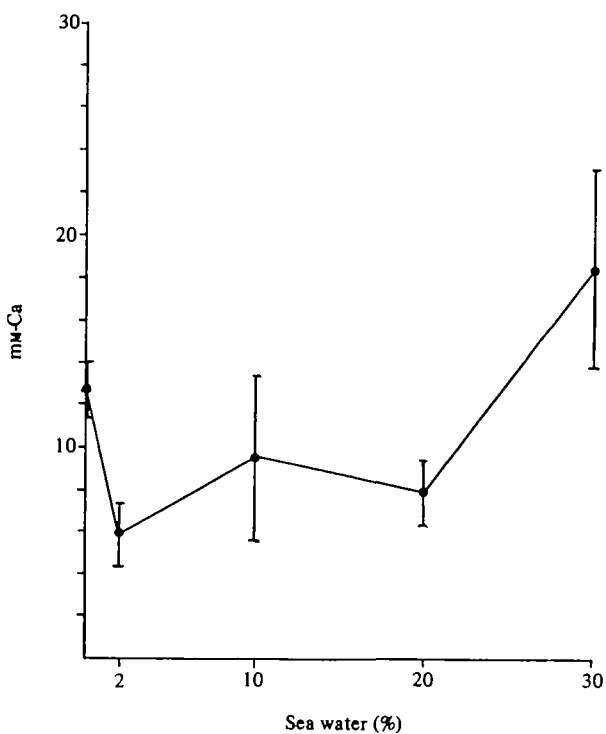
Fig. 4. Haemolymph calcium concentration in larvae raised at different salinities. s.e. indicated.
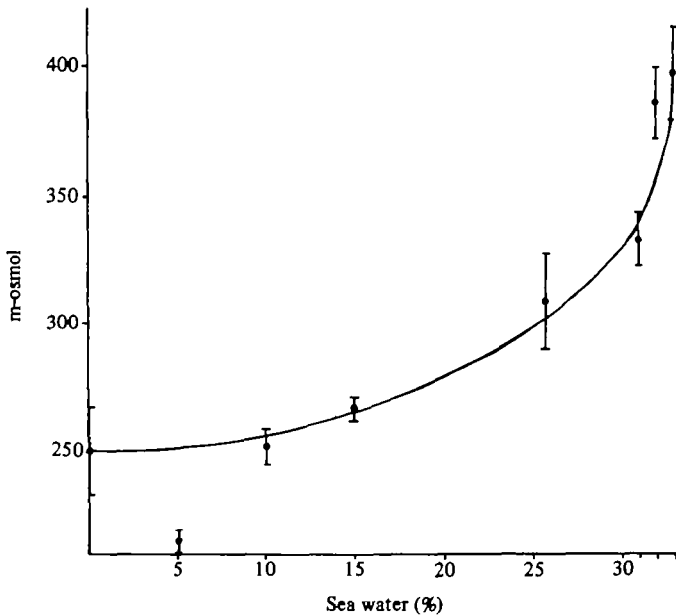$n = 5$.

Fig. 5. Haemolymph osmotic pressure for larvae raised at different salinities. s.e. indicated.
$n = 5$.

variation between individual larvae; the mean sodium concentration rose sharply
(Fig. 1). Potassium levels in the larval haemolymph were low over the whole range
0–33% sea water, values falling in the range 2–4 mM (Fig. 2), somewhat below
Ramsay's (1953) estimate of 3·9–6·9 mM which were recorded from larvae kept in
pure solutions of KCl.

Chloride concentration was more variable between cultures than sodium but the
relationship to increased concentration of the external medium was, however, remark-
ably constant. Between 0 and 15% sea water chloride concentration was at a plateau
of approximately 50 mM, although Wigglesworth (1938) showed an increase from 34
to 51 mM approximately over the external range 0–2 mM.

Haemolymph calcium concentration lay between 6 and 18 mM; there was great
variation between individual animals (Fig. 4). The mean value of 11 mM was lower than
the values for Simuliid larvae, 35–65 mM (Gordon & Bailey, 1976), but comparable
with those of other dipteran larvae (*Tipula paludosa*, 12·3 mM, *Chironomus* sp.,
12·5 mM *Eristalomyia*, 10·0 mM Duchateau from Florkin & Jeuniaux, 1974). Calcium
can be regarded as a significant but not major osmotic factor. Calcium adds to the
osmotic pressure but does not appear to be responsible for osmotic pressure changes.
Magnesium levels varied little, being between 6 and 8 mM and showing no significant
change over the range 0–30% sea water. Values are below those quoted for most
dipteran larvae (Florkin & Jeuniaux, 1974) but about the same as those for Simuliid
larvae (Gordon & Bailey, 1976). Magnesium forms a constant part of the haemolymph
osmotic pressure.

Osmotic pressure rose slowly over the first half of the range and then increased
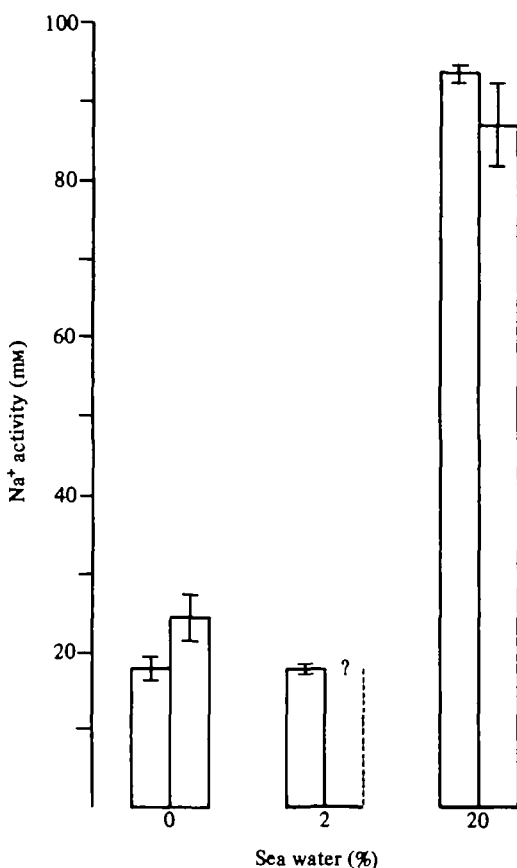
Fig. 6. Haemolymph activity of sodium in larvae reared in 0, 2 and 20% s.w. Pre- and post-calibration results, first and second bars respectively. s.e. indicated. $n = 16, 6, 6$.

rapidly (Fig. 5). In distilled water osmotic pressure of the haemolymph was around 250 m-osmol, comparable with that of other freshwater insect larvae, for example 256 m-osmol in *Simulium venustum* (Gordon & Bailey, 1976). Richards & Meier (1974) found an increase of 30 m-osmol between early and late fourth instar larvae which would account for much of the variability in the results (Fig. 5) which were recorded from fourth instar larvae of all temporal ages.

The increase in osmotic pressure was greater than the change in total osmotic concentration of the measured ions (Figs. 1–4). It was also greater than any changes which are likely to occur in the concentration of sugars; which are present at c. 210 μM in 0% sea water (Edwards, 1982b). It could, however, have been due to a change in the level of amino acids, which are present at about 40–100 mM in 0% sea water (Edwards, 1982b). Further, the level of free amino acids in the haemolymph rises with salinity to parallel the increase in osmotic pressure (Edwards, 1982b).

Measurements of ion activity in the haemolymph over a range of external salinities are summarized in Figs. 6–8. Over the external range of 2–20% sea water, there was a large increase in sodium activity (Fig. 6) whereas total concentration of sodium showed
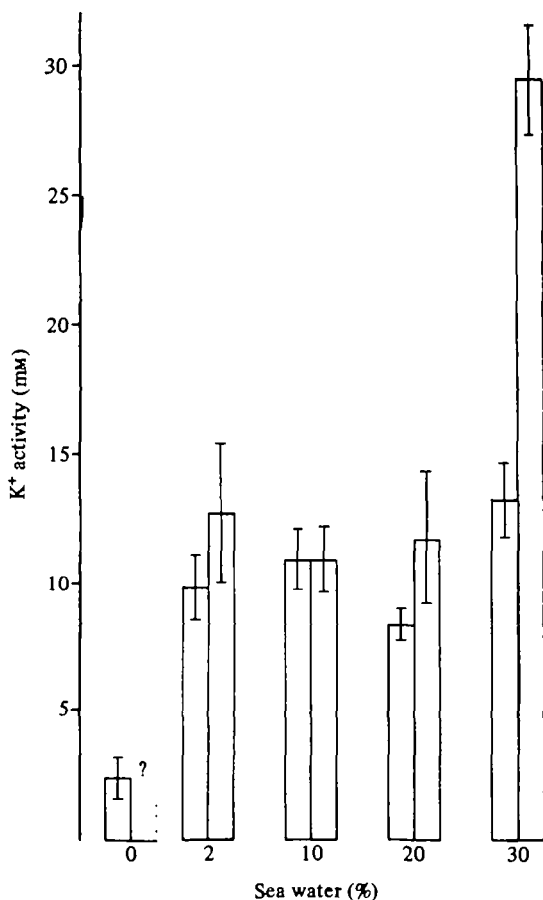
Fig. 7. Haemolymph potassium activity of larvae reared in ó–30% s.w. Pre- and post-calibration results, first and second bars respectively. s.e. indicated. n = 7, 12, 10, 14, 5.

little change (Fig. 1). This indicates that there is less free sodium in larvae raised in more dilute media; the activity of sodium ions is presumably reduced by association with large anionic molecules, for example, large proteins. A reduction in activity of the ions in the haemolymph of these animals must reduce the energy cost of living; it is better to 'hold' ions in an associated state than to lose them to the external medium from where they have to be pumped back. Osmotic pressure is determined by the free ion concentration rather than the total concentration; thus, in effect, a reduction of activity will reduce the osmotic influx of water which would become large in dilute media because of the increased osmotic gradient. Reducing haemolymph activity reduces the need to produce urine to remove excess water. In more concentrated media, e.g. 20% sea water, the osmotic gradient is not so great and haemolymph ion activity can be allowed to rise.

In all media with the exception of 0% sea water, the measured potassium activity (Fig. 7) was much higher than the concentration (Fig. 2). Metabolic waste or compounds containing ammonia could be responsible for much of this activity since Corning potassium resin is particularly sensitive to ammonia (M. M. Civan, personal
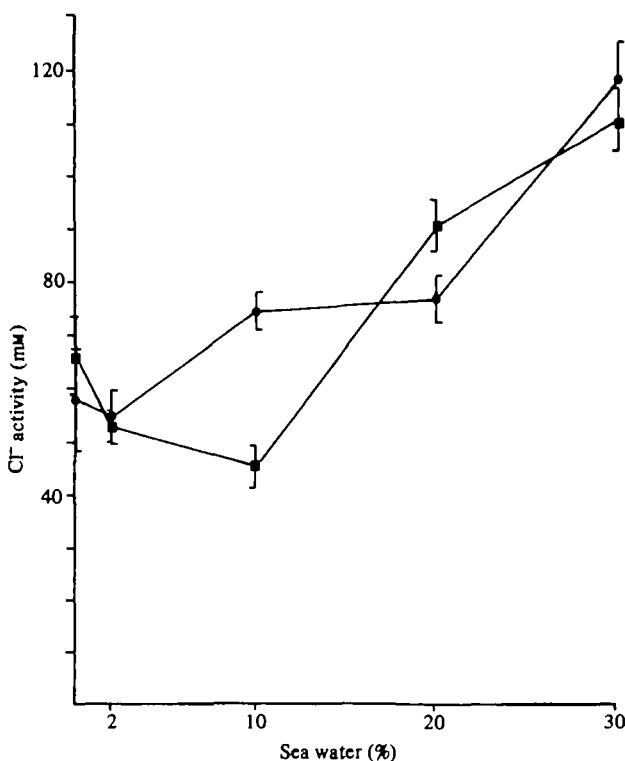
Fig. 8. Chloride activity in the haemolymph of larvae from 0–30 % s.w. rearing medium. Squares, results based on pre-calibrations; Dots, those based on post-calibrations of electrodes. s.e. indicated. $n = 12, 13, 12, 14, 10.$

communication). The apparent potassium activity rose with increased salinity as well as the amino acid levels. The potassium resin may respond to this change either directly or indirectly to the ammonia mobilized during synthesis of these compounds. Oehme & Simon (1976) describe an alternative resin which is far less sensitive to ammonia, which would allow more accurate measuremnts of potassium activity to be made.

Chloride activity (Fig. 8) followed much the same pattern as chloride concentration (Fig. 3). In more dilute media the activity was fairly constant; in more concentrated media there was a fairly large increase. The chloride activity coefficient is much higher than the sodium activity coefficient at low concentration making chloride the most important ion osmotically. The study of ion activity adds a new dimension to salt balance and osmoregulatory studies which warrants further and more detailed study.

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