

## AEDES AEGYPTI: ENERGETICS OF OSMOREGULATION

By H. A. EDWARDS

*Department of Zoology, South Parks Road, Oxford OX1 3PS, England*

(Received 16 February 1982 – accepted 18 June 1982)

### SUMMARY

1. Oxygen consumption of *A. aegypti* larvae, about  $210 \mu\text{l g}^{-1}$  tissue wet weight  $\text{h}^{-1}$ , does not change when the salinity of the environment is changed.
2. The number of mitochondria in the anal papillae, a salt-absorbing epithelium, increases as the external medium is diluted.
3. There is no difference in oxygen consumption between isolated anal papillae in 0, 2 and 20% sea water. The papillae represent about 5% of body volume and their oxygen consumption is about 2% of the animal's total.
4. The theoretical minimum energy cost of osmoregulation is four orders of magnitude smaller than the measured figure for the anal papillae alone.
5. Osmoregulatory phenomena which would explain the recorded observations are discussed.

### INTRODUCTION

Many animals living in aquatic environments maintain the ionic concentrations and osmotic pressure of their body fluids at levels substantially different from those of the external medium. The maintenance of such osmotic and ionic gradients requires the expenditure of energy. Energy for osmoregulatory mechanisms is normally supplied by oxidative metabolism, and measurements of oxygen consumption could, therefore, be used as a measure of the energy used (Potts, 1954; Harvey, Haskell & Zerahn, 1967).

Theoretical calculations of the minimum energy cost for osmoregulation (Potts, 1954) indicate that if an animal is to maintain its body-fluid composition then the energy requirement increases steeply when the environment is not isotonic with the body fluids. Increases have been measured in several animals. Oxygen consumption in *Carcinus* and *Nereis* (Schlieper, 1929), *Ocypode albicans* (Flemister & Flemister, 1951) and *Palaemonetes varians* (Lofts, 1956) is at a minimum when the external medium is isotonic with the blood. *Astacus* (Peters, 1935), *Hemigraspus nudus* and *H. oregonensis* (Denhel, 1960) all show an increase in oxygen consumption as the external salinity is reduced below that of sea water.

In contrast to these findings, however, three species of good osmoregulators – *Eriocheir sinensis* (Schwabe, 1933), *Anguilla anguilla* (Raffy, 1933) and *Artemia salina* (Gilchrist, 1956) – show no change in oxygen consumption when maintained at different salinities. And *Maia* and *Hyas* show a reduction of their oxygen consumption as the medium is diluted from 100% sea water (Schlieper in Remane & Schlieper, 1958).

An increased number of mitochondria, indicating increased activity of the cells, has been observed in the osmoregulatory tissues of *Gammarus oceanicus* (Milne & Ellis, 1973) and *Fundulus* (Philpott & Copeland, 1963) when these animals are kept in dilute media.

Most of the available evidence, then, suggests that aquatic animals' energy expenditure is increased under osmotic stress. This study compares the measured and expected energy expenditure in *Aedes aegypti* larvae, which can survive when they are exposed to media between 0 and 30% sea water (Edwards, 1979). Anal papillae are one of the major sites of salt regulation in this species. These structures are easily isolated, and comparison of the effect of different salinities on these tissues alone can be considered.

#### MATERIALS AND METHODS

Eggs of *Aedes aegypti* were supplied by Shell Research, Sittingbourne. The eggs were scattered on beakers of water and put in an incubator at 28 °C. Larvae were fed desiccated liver powder. 4th instar larvae were kept for 72 h then used for experimentation.

The respiration rate of whole larvae was measured with Warburg manometers.

Anal papillae were isolated by tying a silk ligature around the terminal segment of the larvae just anterior to the siphon; the rest of the animal was then cut away. Isolation in this way maintains the tracheal supply to the papillae. The respiration rate of isolated anal papillae was measured with Cartesian divers (Holter, 1943; Zeuthen, 1943).

Mitochondrial frequency in the papilla epithelium under different osmotic stresses was calculated as the percentage of area in electron microscope sections occupied by these structures. Electron microscope material was fixed in 2.5% glutaraldehyde with 0.68% sucrose (OP 357.3 m-osmol, which is slightly hypertonic to the haemolymph) and 0.05 M phosphate buffer, pH 7.2. Specimens were embedded in Araldite. Sections were stained with uranyl acetate and lead citrate.

To determine haemolymph composition, cation concentrations were measured by flame photometry, chloride was measured by potentiometric titration (Ramsay, Brown & Croghan, 1955) and osmotic pressure was measured with a Clifton cryostat biological nanolitre osmometer. In the steady state fluid and salt input will equal output; the rate of urine production was assumed to be the same as the drinking rate. The osmotic movement through the body wall was ignored because measurements of osmotic permeability showed that such movement would be less than 1% of the drinking rate. Drinking rate was determined from the uptake of media labelled with [<sup>14</sup>C] inulin. The osmotic permeability of the body wall was measured using the method of Nicolson & Leader (1974). The surface area of larvae was calculated from measurements of length and diameter, assuming them to be right cylinders. Ion activities in the haemolymph were measured using the ion-selective resin electrodes described by Lettau *et al.* (1977). Activities in the bathing sea-water solutions were calculated using the Davis (1962) equation with the Guggenheim convention:

Table 1.

Salt	g/l in s.w.	Molarity
NaCl	23.991	0.403
KCl	0.742	0.001
CaCl <sub>2</sub> .6H <sub>2</sub> O	2.240	0.0106
MgCl <sub>2</sub> .6H <sub>2</sub> O	10.893	0.0539
Na <sub>2</sub> SO <sub>4</sub>	4.012	0.0282
NaHCO <sub>3</sub>	0.197	0.0234

$$\text{Davis equation } \log y = -Z^2 \cdot 0.5 \frac{\sqrt{I}}{1+I} - 0.31,$$

$$\text{Guggenheim convention } y = (y_2 \cdot I)^2 = (y_1)^4,$$

where  $I$  = ionic strength,  $Z$  = valency and  $y$  = activity coefficient.

The composition of the artificial sea water used in all experiments was as shown in Table 1.

#### RESULTS AND DISCUSSION

*Aedes aegypti* larvae can survive salinities between 0 and 30% sea water indefinitely. The oxygen consumption of larvae raised at 0, 10, 20 and 30% sea water was measured and showed no significant differences. Transferring larvae raised in 20% sea water to distilled water and vice versa did not cause an alteration of respiration rate. Small changes in salinity between 0 and 10% sea water, closer to the animal's ecological range, and unphysiological salinities between 30 and 80% sea water did not affect oxygen consumption either. Blocking the osmoregulatory organs by covering the anal papillae and anus with beeswax resin mixture was also without effect. In short, the oxygen consumption remained constant over a range of salinities from 0 to 80% sea water.

The rate of oxygen consumption was between 100 and 400  $\mu\text{l O}_2 \text{ g}^{-1}$  tissue wet wt  $\text{h}^{-1}$  (mean = 210,  $n = 430$ ). This result represents the pooling of results from all manometry experiments and therefore much of the variation stems from the different cultures used. The variation in oxygen consumption quoted here is much larger than that found in individual experiments but does represent the range within this species, which is comparable with that from other freshwater insect larvae: *Chironomus plumosus* 280  $\mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (Edwards, 1958), *Anatopyma varia* and *A. nebulosa* between 100 and 400  $\mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$  and plecopteran larvae between 100 and 900  $\mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (Nagel, 1974). Much of the variation in oxygen consumption in *Chironomus* can be attributed to size differences between individuals (Edwards, 1958).

Anal papillae were isolated from larvae reared in 0, 2 and 20% sea water, and their oxygen consumption was measured with Cartesian divers (Table 2). The two major tracheal trunks in the siphon were seen to pump for several hours in isolated preparations and this was used as an indicator of the viability. The viability of preparations was also indicated by their constant rate of oxygen consumption (regression better than 0.95 in all cases) over the period of measurement, indicating that metabolic substrates and hormonal supplies, if necessary, were not significantly depleted during this time.

Table 2. *Oxygen consumption of isolated anal papillae*

% s.w. of medium	0	2	20
$O_2$ nl prep. <sup>-1</sup> h <sup>-1</sup>	7.29	11.45	10.41
S.E.	1.76	3.53	1.50
n	4	3	4

Table 3. *Composition of blood, urine and medium*

s.w. (%)	Osmotic permeability	Na (mM)	Na <sub>a</sub> (mM)	Cl mM	Cl <sub>a</sub> (mM)	m-osmol	Vol. (l <sup>-1</sup> mg <sup>-1</sup> h <sup>-1</sup> )
Blood							
0	$5.76 \times 10^{-3}$	60	15	90	35	250	—
2	$7.00 \times 10^{-3}$	80	17.5	90	83	250	—
20	$1.20 \times 10^{-3}$	110	91	95	83	280	—
Urine							
0	—	4	3.55	4.5	4.15	12	$1.8 \times 10^{-4}$
2	—	9.5	8.4	10.76	9.6	19.6	$3.55 \times 10^{-4}$
20	—	95	72.6	107.6	83.1	196	$2.55 \times 10^{-4}$
Medium							
Larval surface area (m <sup>2</sup> )							
0	$7.67 \times 10^{-6}$	1*	1*	1*	1*	1*	
2	$7.67 \times 10^{-6}$	9.5	8.4	10.76	9.6	19.6	
20	$9.00 \times 10^{-6}$	95	72.6	107.6	83.1	196	

$T = 293^\circ\text{C}$ .

For the purpose of calculations *A. aegypti* is assumed to be an efficient osmoregulator (Potts, 1954) and therefore urine concentration (U) equals that of the medium (M). Drinking rate equals urine production in the steady state.

\* A small but finite value is inserted here to avoid taking the natural log of zero in calculations.

Thermodynamic analysis of osmoregulation (Potts, 1954) generates equations describing the theoretical minimum energy cost of regulation for a known osmotic gradient. Using values from Table 3 in Potts' (1954) analysis of the minimum energy cost of osmoregulation, the minimum osmoregulatory work done by mosquito larvae in 0, 2 and 20% sea water has been calculated. Substituting Potts's equation (4) in equation (10) ( $V$  for  $PA[B-M]$ ) and using the measured concentrations):

$$\begin{aligned}
 \text{work} &= 5.90 \times 10^{-6} \text{ J h}^{-1} \quad \text{in } 0\% \text{ s.w.} \\
 &= 6.44 \times 10^{-6} \text{ J h}^{-1} \quad \text{in } 2\% \text{ s.w.} \\
 &= 1.73 \times 10^{-6} \text{ J h}^{-1} \quad \text{in } 20\% \text{ s.w.}
 \end{aligned}$$

If one substitutes ion activities for ion concentrations, then

$$\text{work} = 6.00 \times 10^{-6} \text{ J h}^{-1} \quad \text{in } 0\% \text{ s.w.};$$

the values at 2% + 20% s.w. remain unchanged.

Substituting in Potts's equation (5) which includes permeability:

$$\begin{aligned}
 \text{work} &= 6.68 \times 10^{-5} \text{ J h}^{-1} \quad \text{in } 0\% \text{ s.w.} \\
 &= 5.30 \times 10^{-5} \text{ J h}^{-1} \quad \text{in } 2\% \text{ s.w.} \\
 &= 1.73 \times 10^{-6} \text{ J h}^{-1} \quad \text{in } 20\% \text{ s.w.}
 \end{aligned}$$

Osmotic gradient and permeability are the major factors in determining the energy cost of regulation.

From measurements of their oxygen consumptions and the calorific value of their respiratory substrates the energy consumption of larvae can be calculated. Glucose is assumed to be the substrate, and the following calculations are for 100% efficiency of conversion. The calorific value of glucose is  $2867 \text{ kJ mol}^{-1}$ , and six mol of oxygen ( $\text{O}_2$ ) are used to oxidize 1 mol of glucose. Therefore,  $477.9 \text{ kJ}$  are associated with the consumption of 1 mol  $\text{O}_2$ . The oxygen consumption of larvae at  $25^\circ\text{C}$  is  $210 \text{ ml kg}^{-1} \text{ h}^{-1}$  and correcting the  $\text{O}_2$  molar volume from S.T.P. to this temperature, it transpires that  $4.10 \text{ J kg}^{-1} \text{ h}^{-1}$  are available to the larvae.

The theoretical minimum energy cost of osmoregulation is small. From simple parameters it is possible to demonstrate that the efficiency of the osmoregulatory system would have to be 1% or less to account for the measured oxygen consumption.

Assuming the following values:

larvae length = 7 mm

larvae radius = 0.5 mm (volume =  $5.49 \text{ mm}^3$ )

papilla length = 1 mm

papilla radius = 0.15 mm (volume =  $0.28 \text{ mm}^3$  (because there are four papillae))

oxygen consumption of whole larvae = wt in mg  $\times \text{O}_2$  consumption  $\text{nl mg}^{-1} \text{ h}^{-1}$   
=  $500 \text{ nl h}^{-1}$

Measured oxygen consumption of four isolated papillae =  $10 \text{ nl h}^{-1}$ . Then papillae represent 5% of body volume and their oxygen consumption is 2% of the animal's total. Therefore, the metabolic rate of the papillae is less than the rest of the body tissue. Osmoregulation is very cheap compared to the total energy costs of the animal; of the regulatory tissues only the anal papillae  $\times (4.10 \text{ J kg}^{-1} \text{ h}^{-1})$  have been considered and these alone exceed the theoretical value for regulation in distilled water, the most extreme case, by four orders of magnitude. Even if the regulatory system is very inefficient, any changes would be masked by the 'background' metabolism of the tissue.

Osmoregulation may require only a small percentage of the animal's total oxygen consumption, and any changes in the oxygen consumed by regulatory tissues may then be difficult to detect. Electron microscope studies of the anal papillae show a characteristic salt-transporting epithelium which interestingly enough is a syncytium. Mitochondrial counts show a large increase where larvae are kept in dilute media: mitochondria occupy 8% of the area of sections in 20% sea-water animals and 30% of cell area in 0% sea-water animals.

It is paradoxical that even though the number of mitochondria changes so much, there is no statistically significant change in oxygen consumption. In isolated frog skins there is a direct relationship between oxygen consumption and salt transport – one molecule of oxygen being consumed for every 18 sodium ions transported (Zerahn, 1956). However, in *Hyalophora cecropia* midgut the uptake of oxygen is independent of potassium transport (Harvey, Haskell & Zerahn, 1967).

Styczynska-Jurewicz (1970) gives evidence to show that if animals are adapted to different salinities then there is no difference in their oxygen consumption. Mosquito larvae maintain the osmotic gradient across their body wall against changes in salinity of the external medium by increasing the level of free amino acid in the haemolymph (Edwards, 1982). Schoffeniels (1973) proposed a model for osmoregulation in which the energy supply was switched between salt uptake in dilute media and amino acid synthesis in more concentrated media by a salt-sensitive enzyme. Such a model would explain the changes in amino acids in the haemolymph and why salinity does not affect oxygen consumption. An alternative explanation, strongly suggested by the present results, is that as osmoregulation is always such a small part of the animal's total energy budget then the changes in mitochondrial number are not associated with osmoregulation directly. Rather it may be that in more dilute media, mitochondria become less efficient and therefore their numbers are increased to maintain the energy supply for all functions.

Mosquito larvae have a very low osmotic permeability, so reducing the energy cost of osmoregulation, an advantage accruing from air breathing which does not require contact of the permeable respiratory surface with the water.

This work was carried out while I was in receipt of an S.R.C. Case studentship: my thanks to the S.R.C., my supervisor Dr J. E. Treherne and to I.C.I. my industrial sponsors. I thank Dr N. J. Lane and B. J. Harrison of the A.R.C. Unit in the Zoological Laboratory, Downing Street, Cambridge, and Mr S. Young of the British Antarctic Survey for their assistance.

#### REFERENCES

- DENHEL, P. A. (1960). Effect of temperature and salinity on the oxygen consumption of two intertidal crabs. *Biol. Bull. mar. biol. Lab. Woods Hole* **122**, 208-227.
- EDWARDS, R. W. (1958). The relation of oxygen consumption to body size and to temperature in the larvae of *Chironomus riparius* Meigen. *J. exp. Biol.* **35**, 383-395.
- EDWARDS, H. A. (1979). *Aedes aegypti*: salt, water and cells. Ph.D. thesis, University of Cambridge.
- EDWARDS, H. A. (1982). Free amino acids as regulators of osmotic pressure in aquatic insect larvae. *J. exp. Biol.* **101**, 153-160.
- FLEMISTER, L. J. & FLEMISTER, S. C. (1951). Chloride ion regulation and oxygen consumption in the crab *Ocyropsis albicans* (Bosq). *Biol. Bull. mar. biol. Lab. Woods Hole* **101**, 259-273.
- GILCHRIST, B. M. (1956). The oxygen consumption of *Artemia salina* (L.) in different salinities. *Hydrobiologica* **8**, 54-63.
- HARVEY, W. R., HASKELL, J. A. & ZERAHN, K. (1967). Active transport of potassium and oxygen consumption in the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **46**, 235-248.
- HOLTER, H. (1943). Technique of the Cartesian Diver. *Compt. Rend. Carlsberg* **24**, 399-478.
- LETTAU, J., FOSTER, W. A., HARKER, J. E. & TREHERNE, J. E. (1977). Diel changes in potassium activity in the haemolymph of the cockroach *Leucophaea maderae*. *J. exp. Biol.* **71**, 171-186.
- LOFTS, B. (1956). The effects of salinity changes on the respiratory rate of the prawn *Palaemonetes varians* (Leach). *J. exp. Biol.* **33**, 730-736.
- MILNE, D. J. & ELLIS, R. A. (1973). The effect of salinity acclimation on the ultrastructure of the gills of *Gammarus oceanicus* (Segerstrale, 1947) (Crustacea: Amphipoda). *Z. Zellforsch. Mikrosk. Anat.* **139**, 311-318.
- NAGEL, B. (1974). The basic picture and ecological implications of the oxygen consumption/oxygen concentration curve of some aquatic insect larvae. *Proc. 5th Int. Symp. Chironomidae Entomologicae tidrift* **95**, Supplementum Stakh.
- NICOLSON, S. W. & LEADER, J. P. (1974). The permeability of the cuticle of the larva of *Opifex fuscus* Hutton (Diptera Culicidae). *J. exp. Biol.* **60**, 593-603.

- LETERS, H. (1935). Über den Einfluss des Salzgehaltes im Aussen medium auf den Bau und die Funktion der Exkretions organe dekapoder Crustaceen *Potamobius fluviatilis*, *Homarus vulgaris*. *Z. Morph. Okol. Tiere* **30**, 355-381.
- PHILPOT, C. & COPELAND, D. (1963). Fine structure of chloride cells from three species of *Fundulus*. *J. Cell Biol.* **18**, 389-404.
- POTTS, W. T. W. (1954). The energetics of osmoregulation in brackish and freshwater animals. *J. exp. Biol.* **31**, 618-630.
- RAFFY, A. (1933). Recherches sur le métabolisme respiratoire des poikilothermes aquatiques. *Ann. inst. oceanog., Paris* **13**, 257-391.
- RAMSAY, J. A., BROWN, R. H. J. & CROGHAN, P. C. (1955). Electrometric titration of chloride in small volumes. *J. exp. Biol.* **32**, 822-829.
- REMANE, A. & SCHLIEPER, C. (1958). Die Biologie des Brackwassers. In *Die Binnengewässer* (ed. A. Thieneman), Band XXII. Stuttgart: E. Schweizerbartsche Verlagsbuchhandlung.
- SCHLIEPER, C. (1929). Einwirkung niederer Salzkonzentrationen auf marine Organismen. *Z. Verg. Physiol.* **9**, 478-514.
- SCHOFFENIELS, E. (1973). Amino acid metabolism and cell volume regulation. In *Comparative Physiology, Locomotion, Respiration, Transport and Blood* (ed. L. Bolis, K. Schmidt-Nielsen and S. H. P. Maddrell). Amsterdam: North-Holland.
- SCHWABE, E. (1933). Über die Osmoregulation verschie dener Krebse (Malacostracen). *Z. Vergleich. Physiol.* **19**, 183-236.
- STYCZYNSKA-JUREWICZ, E. (1970). Bioenergetics of osmoregulation in aquatic animals. *Pol. Arch. Hydrobiol.* **17**, 295-302.